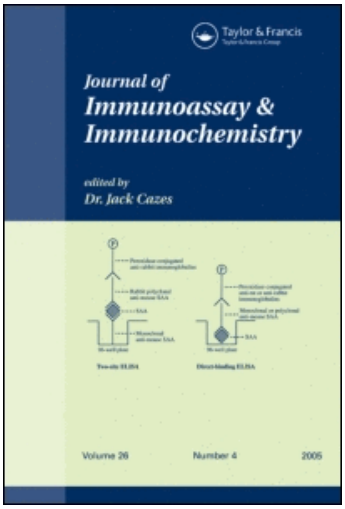

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LARGE SCALE SCREENING PROGRAMME FOR SELECTION OF ANTISERA FOR RADIOIMMUNOASSAY OF HUMAN PARATHYROID HORMONE

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ABSTRACT

A large-scale, three-phase screening programme has been devised for the rapid selection of antisera which might be of potential use in clinical radioimmunoassays for human parathyroid hormone. A total of 122 sera from 169 guinea pigs and 6 rabbits immunized with bovine parathyroid hormone, and 12 guinea pigs immunized with human parathyroid hormone were assessed relative to reference antisera. Pre-determined criteria for the three phase programme were imposed by the requirement for antisera that could be used at dilutions greater than 10^{-5} in order to ensure continuity of supplies for wide-spread distribution and by the limited availability of human parathyroid hormone for testing purposes. Of the sera tested, only 5 were selected as having high titre and sensitivity for low concentrations of human parathyroid hormone. The 5 antisera were further evaluated for amino- and carboxyl-region specificities for the human parathyroid hormone in comparison with antiserum 211/32, widely distributed for use in radioimmunoassay for clinical purposes. The selected antisera appear to be of high affinity with good recognition of the whole or carboxyl-region parts of the human parathyroid molecule.

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INTRODUCTION

Antisera for the clinical immunoassay for parathyroid hormone (PTH) were first made available in 1971 as a result of a collaboration between the Wellcome Research Laboratories and Dr J. O'Riordan (Middlesex Hospital, London) with MRC support, and the active encouragement of the MRC Clinical Endocrinology Committee. Of several antisera produced in guinea pigs, one in particular (AS 211/32) proved to be a very satisfactory immunoassay reagent and was used in laboratories around the world until commercial supplies were exhausted in 1977.

The success of antiserum 211/32 and the appearance of published reports from different laboratories on the successful development and clinical application of radioimmunoassays based on antisera raised to bovine PTH (bPTH) in guinea pigs (as reviewed in reference 1, 11, 12, 13, 14 and 15) were stimuli to initiate further series of immunizations of guinea pigs with bPTH. In the period between 1971 and 1977 a further 169 guinea pigs have been immunized with bovine PTH (bPTH) at the Wellcome Research Laboratories. Six rabbits were also immunized. The batches of immunogen used had been partially or highly purified, and were supplied either by Dr J. O'Riordan, with

M.R.C. support or by the National Institute for Biological Standards and Control (NIBSC). In addition, 12 guinea pigs were immunized with partially purified human PTH (hPTH) supplied by Dr J. O'Riordan. Some animals died during the immunization programme; 114 guinea pig and 8 rabbit bleeds were available for testing. Although some of the sera from the immunized animals have been screened in various laboratories for the presence of antibodies to PTH, there has been no systematic and comparative examination of all these antisera with reference to known antisera, and in particular to AS 211/32 which has been very widely used in assays for clinical purposes.

This paper describes a three-phase screening procedure devised for the 122 sera, and the selection and laboratory characterization of five antisera likely to be suitable for certain clinical assays.

MATERIALS AND METHODS

1) Immunization Programme

Groups of six to 30 random-bred guinea pigs were immunized according to schedules based on subcutaneous or multiple intradermal primary injections of partially or highly purified bPTH in Freund's complete adjuvant. Amounts corresponding to approxi-

mately 50 µg of bPTH were used for primary injections. One to five booster injections of half the primary dose were administered subcutaneously at intervals of not less than one month (Table 1) The guinea pigs were anaesthetised and exsanguinated 10 to 14 days after the final injection. After separation of the blood the serum was stored at -20°C after addition of sodium azide to a concentration of 0.1%.

Six rabbits were immunized with highly purified bPTH using the multiple intradermal route for the primary injections and the intramuscular route for boosters. Test bleeds were obtained from each of the surviving rabbits 10 to 14 days after each booster injection and treated as above.

2) Immunoassay

The system used is based on that described by Segre and his colleagues (1) with modifications developed at NIBSC and described elsewhere (2) or as specified, where relevant, in the text of this report.

Tracer Labelled Ligand

The batch of bPTH used was prepared collaboratively in 1975 by Dr J.A. Parsons (MRC National Institute for Medical Research), Dr J.M. Zanelli (NIBSC) and Dr J.S. Woodhead (University Hospital, Cardiff)

Table 1 Immunization Programme and Titres of Antisera Produced

Species of animal	No. of animals/ group	Immunogen used*	Route of primary immunization	Secondary injections (boosters)		Number of sera obtained for testing	Relative ratios for binding of ^{125}I-bPTH for test serum and reference antiserum, both at a final dilution of 1/18,000*					
				Route	Number		A	B	C	D	E	F
Guinea pigs	15	Unpurified TCA.bPTH for all groups	Subcutaneous for all groups	sc	4	10	1	-	2	3	1	3
	25			sc	5	12	-	1	-	3	3	5
	30			sc	5	12	1	3	1	3	1	3
	30			sc	4	21	1	0	0	1	4	15
Guinea pigs	20	Highly purified bPTH	Subcutaneous	sc	4	11	-	-	2	-	5	4
Guinea pigs	19	TCA.bPTH mixed with highly purified bPTH (10% w/w)	Subcutaneous	sc	3	17	3	-	2	3	2	7
Guinea pigs	10	Highly purified bPTH for all groups	Multiple intradermal for all groups	sc	1	5	-	-	-	-	1	4
	10			sc	2	8	-	-	-	-	3	5
	10			sc	3	10	-	-	1	2	4	3
Guinea pigs	6	Unpurified TCA.hPTH	Subcutaneous for both groups	sc	4	3	-	-	-	-	-	3
	6			sc	4	5	-	-	-	-	-	5
Rabbits	6	Highly purified bPTH	Multiple intradermal	im	1	4 survivors	-	-	-	-	-	4
				im	2	2 survivors	-	-	-	-	-	2
				im	3	2 survivors	-	-	-	-	-	2

* as described in text

from 5 kg of bovine parathyroid glands using the phenol extraction and trichloroacetic acid (TCA) precipitation method (3). The TCA bPTH was purified by gel chromatography (Ultragel AcA 54 (LKB), Woodhead and Zanelli, unpublished method). The final material had high biological activity (4000 International Units/mg) in in vivo and in vitro bioassay systems. Chemical characterization by amino acid composition analysis and end-terminal analysis (Edman degradation) carried out by Dr H. Keutmann (Massachusetts General Hospital, USA) confirmed the material as intact 1 - 84 bovine PTH, approximately 95% pure.

This batch, Number 100375, has proved consistently useful for radio-iodination in a number of international laboratories and has also been provided routinely by NIBSC to the majority of Supra-regional Assay Service and research laboratories in the United Kingdom between 1976 and 1981 (in ampoules coded 76/568, 77/602, 78/622 and 80/509).

Other samples of highly purified bPTH, prepared under similar conditions and assessed by the same criteria were also used as indicated in the text.

Radioiodination

The peptide was labelled with ^{125}I by the Chloramine T method (4) modified as described previously

(2) and with a 20 second oxidation time. The tracer labelled ligand was rapidly frozen on dry ice in aliquots, stored at -40°C and re-purified on Biogel P30 prior to addition to an assay.

Standard and Test Peptides

For characterizing assay systems in terms of intact bovine and human extracted hormone the WHO First International Preparation of Parathyroid Hormone, Bovine, for Immunoassay, ampoule code 71/324, and NIBSC Research Standard human parathyroid hormone for immunoassay, ampoule code 75/549 were used. Residues of bPTH or hPTH stock solutions prepared from these or similar ampouled research preparations were pooled and "snap frozen" aliquots stored at -40° for displacement and cross-reactivity tests.

Specificity studies were carried out using synthetic fragments of the human peptide: 1-34 (5), 44-68 and 53-84 generously provided by Dr. H. Keutmann and Dr. M. Rosenblatt (Massachusetts General Hospital, Boston, USA) or purchased from Bachem Fine Chemicals (Torrance, California, USA). Solutions were prepared and aliquots stored at -40°.

A biological fluid, derived from plasma by ultra-filtration during haemodialysis of a patient with hyperparathyroidism secondary to renal failure (6)

was included in the final phase of this study for comparison with results obtained with the small synthetic h53-84 fragment of hPTH. This fluid, hereafter referred to as "diafiltrate", was made available in sufficient quantity for chemical analysis and for ampouling (code number 78/618, each ampoule contained the lyophilized residue of 1 ml of fluid) for inclusion in a recently completed international collaborative study (7). This collaborative study, organized on behalf of the World Health Organization (WHO) by NIBSC, established an ampouled sample of highly purified hPTH as the first international standard of hPTH for immunoassay (8). For purposes of comparison and characterization, three different ampouled samples of hPTH and two different ampouled samples of biological fluids of human origin were included in the study; the diafiltrate was one of the latter two substances. Information on the diafiltrate was obtained from 34 different immunoassay systems, 5 in vitro bioassays and high performance liquid chromatography, provided from the 29 expert laboratories from 12 countries who participated in the WHO study. Details are published elsewhere (7, 9) but in summary, the diafiltrate was shown to consist of a heterogeneous mixture of carboxyl region cleavage products together with a small amount of intact and amino-region hPTH.

Separation Procedure

Separation of antibody-bound and free tracer-labelled ligand was assessed by two different systems:

i) Dextran-coated charcoal (2) was used for antiserum dilution curves.

ii) Second antibody (donkey anti guinea pig globulin, Wellcome Reagents AS 691) with the addition of polyethylene glycol 6000 (PEG) to accelerate precipitation (2) was used for the final cross-reactivity and specificity assessments.

3) Antiserum Dilution Curves

All sera were tested at 5 dilutions (final dilutions 1/2000, 1/6000, 1/18,000, 1/54,000 and 1/162,000) by incubation with labelled bPTH (approximately 4000 counts/min/tube, equivalent to approximately 1-2 x 10^{-15} moles, or 10-20 pg) for 3 days at 4^{o}C. Dextran-coated charcoal was used for separation of bound and free tracer-labelled ligand. A reference antiserum (Wellcome Reagents AS 262) was included at the same 5 dilutions in all test batches for between-assay comparison.

4) Displacement of tracer (^{125}I bPTH) with bPTH and hPTH

Sera selected on the basis of titre for displacement studies were tested initially with 3 doses of bovine and human PTH ($1x10^{-9}$, $3.3x10^{-10}$/$1.1x10^{-10}$ moles/L; 10, 3.3 and 1.1 ng/ml) in a 3+3 day delayed tracer addition assay system. The antiserum dilution was selected according to the test for titre already carried out and was that expected to give 20-40% binding of tracer. Separation was by second antibody with PEG.

Antisera showing significant displacement of tracer by bovine and human PTH on this initial test were further assessed using a full dose-range of bovine and human PTH, ie 10 two-fold dilutions of a stock solution containing $2x10^{-9}$ moles/L (20 ng/ml) down to 3.9×10^{-12} moles/L (0.039 ng/ml). The working dilution of the antiserum was further adjusted, if necessary, to give 25-30% binding of tracer in the 3+3 day delayed tracer addition system. Separation was by 2nd antibody with PEG.

As reference antiserum AS 262 does not show significant cross-reactivity with hPTH, antiserum AS 211/32 was used at a final dilution of 1/500,000 for comparative purposes in these tests.

5) Assessment of Regional Specificity using Fragments of Human PTH

Antisera selected for further study on the basis of displacement studies with hPTH were tested for binding of radioiodinated h1-34 PTH (prepared and evaluated as described previously (2)). The antisera were also screened using 4 widely spaced doses of amino-(1-34) and carboxyl-(53-84) region fragments of human PTH, ie at 2.5×10^{-8}, 2.5×10^{-9}, 2.5×10^{-10} and 2.5×10^{-11} moles/L or 100, 10, 1.0 and 0.1 ng/ml in the 3+3 assay design with radioiodinated bPTH (1-84) as tracer. Full dose-response curves, using two fold dilutions of hPTH fragments were carried out for each antiserum once the appropriate specificity and dose range had been indicated.

RESULTS

1) Initial Tests for Binding of Radioiodinated bPTH -Antiserum Dilution Curves

The per cent tracer bound values derived from the dilution curve for each serum were compared with the reference serum AS 262, and antisera were grouped according to the ratio of test to reference at the 1/18,000 dilution (Examples of one antiserum from each of the groups as described below are shown in

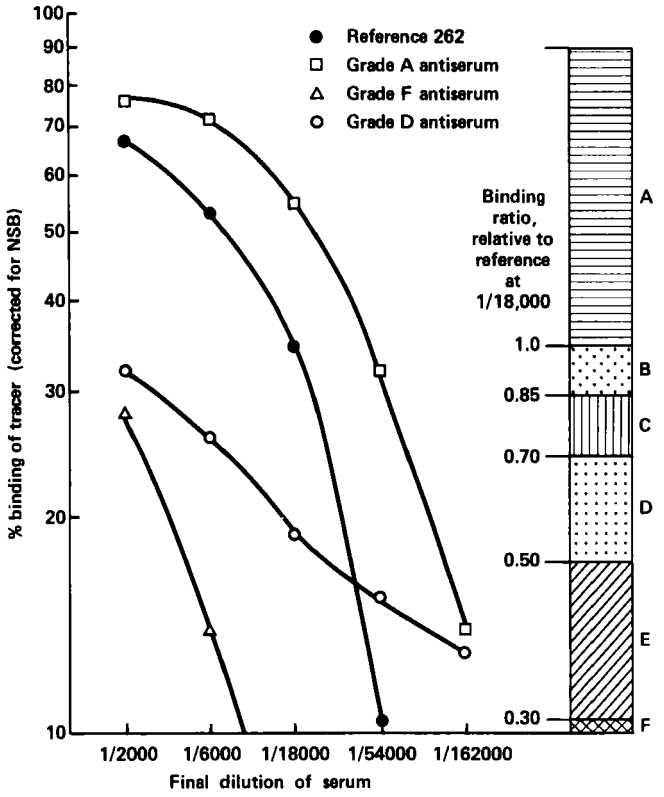


Fig. 1

Figure 1). The binding of tracer was typically between 30 and 40% at this dilution of the reference antiserum.

The six antisera which showed a higher binding of tracer than the reference antiserum 262, at the same dilution of 1/18,000, were classified as A. Antisera with tracer binding at 1/18,000 dilution which was less than that of the reference antiserum AS 262, were assigned to groups as follows - Group

B, ratios of 0.85 to 0.99 relative to the reference (4 antisera), Group C, 0.7 to 0.84 (8); Group D, 0.5 to 0.69 (15); Group E, 0.3 to 0.49 (24) and F, a ratio of less than 0.3 relative to reference (65 sera), (Figure 2). The 89 test bleeds in Groups F and E together with 5 test bleeds from Group D were rejected from the study at this stage. These included the sera from the animals immunised with hPTH. The 10 antisera remaining in Group D were retained for further study as some of the dilution curves gave tracer binding values that were greater than those of the reference antiserum 262 when compared at a final dilution of 1/162,000. (An example is shown in Figure 1).

2) Displacement of Tracer by b and h PTH

Out of 28 sera from Groups A, B, C and some from Group D, 14 showed displacement of tracer with the 3 doses of bPTH, but only 8 showed recognition of hPTH at the relatively low doses used. Examples of an antiserum (number A1) with good cross reactivity, and one (number A4) with poor cross reactivity with hPTH are shown in Figure 3 in comparison with antiserum 211/32.

During this stage of the study it was found that dextran-coated charcoal separation as used during

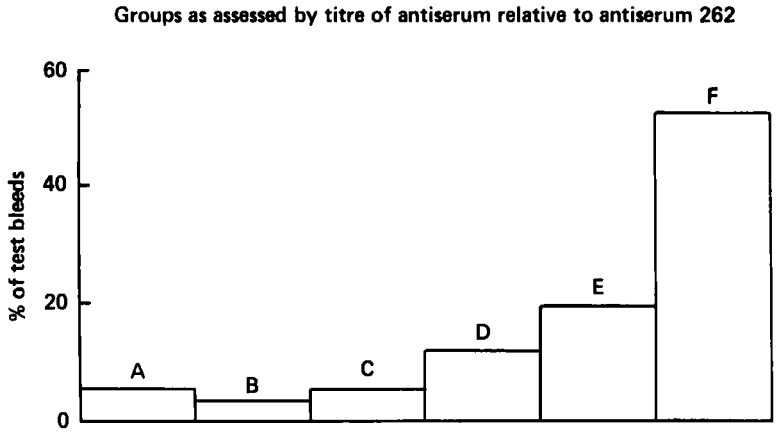


Fig. 2

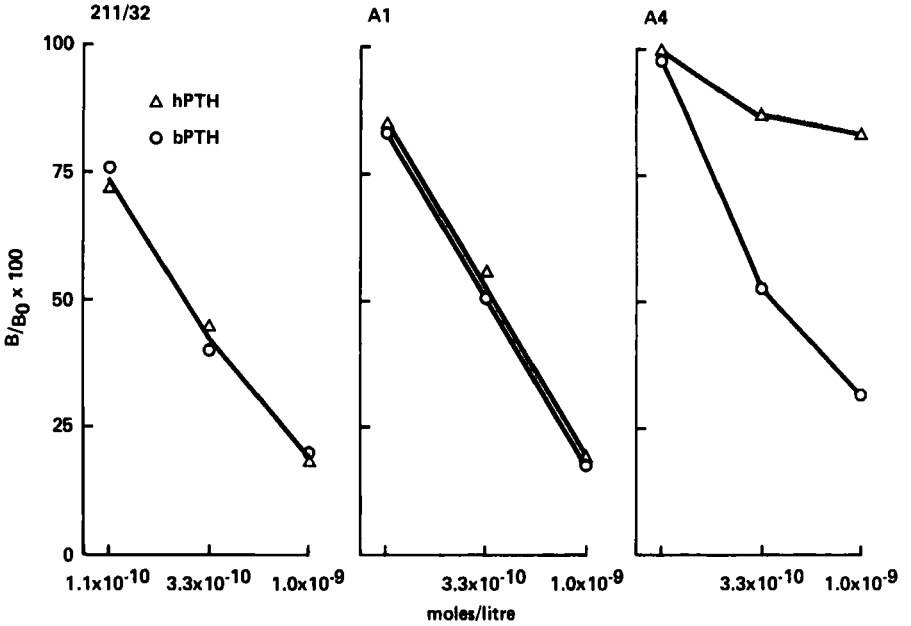


Fig. 3

Table 2

Comparison of dextran coated charcoal and second antibody separation systems on non-specific binding (NSB) and specific binding at zero dose (B_o) (incubation period 3 days at 4°C).

Antiserum	Final Dilution	Dextran coated charcoal % NSB	Dextran coated charcoal % B_o corrected for NSB	Second antibody % NSB	Second antibody % B_o corrected for NSB
AS 262	1/18,00		42		55
	1/54,000	10.1	13	5.2	35
	1/162,000		10		19
AS 211/32	1/200,000	12.0	37	5.3	51
	1/500,000		22		29
A1	1/160,000	10.7	24	5.4	53
	1/500,000		13		36
C4	1/20,000		17.5		67
	1/100,000	10.6	11.8	5.1	56
	1/500,000		10.4		53
	1/1,000,000		not detectable		31

the initial screening tended to give a higher non-specific binding (NSB) and lower binding at zero dose (B_o) than did the PEG accelerated 2nd antibody separation as shown in Table 2. The NSB was typically between 9 and 13%, although this included a fraction of 5 - 7% tracer adsorbed to soda glass incubation tubes. Adsorption could be reduced to 1 - 2% by the use of neutral borosilicate glass tubes. With PEG/2nd antibody separation, the NSB was 4 - 7% and

the lower values could be ensured by the use of neutral borosilicate glass incubation tubes. As the difference in NSB did not account for the higher % binding of tracer at zero dose (B_o) when separation of bound and free was by second antibody precipitation, it was usually necessary to adjust the final dilution of anti-PTH serum when changing from dextran charcoal to 2nd antibody separation although the degree of adjustment varied with different antisera presumably reflecting differences in the affinity. Examples of the apparent difference in titre, as a result of dextran-coated charcoal adsorbtion of free tracer, and PEG/2nd antibody precipitation of bound tracer for different dilutions of antisera A1 and C4, in comparison with AS 262 and AS 211/32 are given in Table 2. A further two batches of bPTH were radioiodinated and used in these experiments to test the possibility that different tracers would have different NSB and B_o values in the different separation systems. The different tracers did not provide significant changes in either of the separation systems.

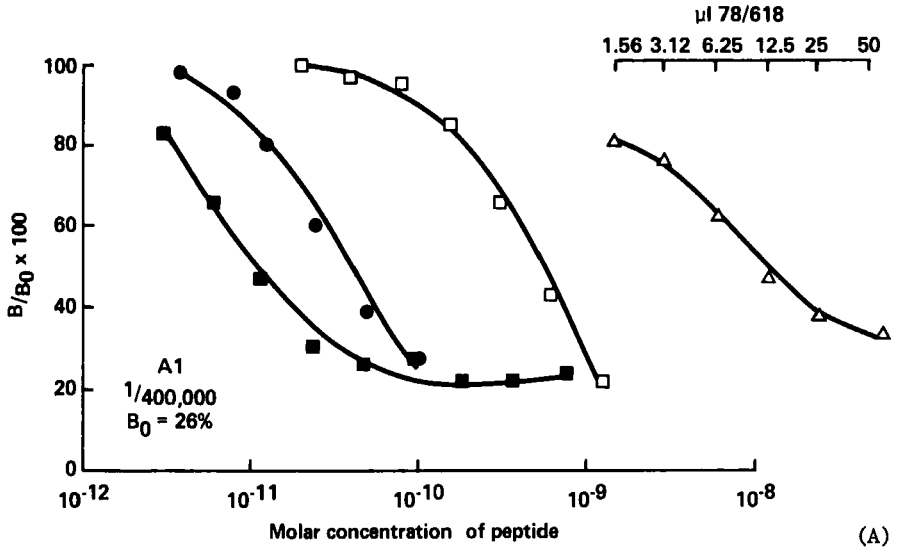
Out of eight antisera tested, three were eliminated from the programme at this stage as being uneconomical in that the antiserum could not be used at a dilution greater than 1/150,000 (or insufficiently sensitive to hPTH). Of the five remaining antisera

tested with bPTH and hPTH to cover the complete dose response range, three sera (A1, A2, A3) gave computed log dose responses that were parallel and two (C3, C4) showed significant non-parallelism between bPTH and hPTH when tested by analysis of variance. In all cases, the antisera showed greater recognition of intact bovine PTH than of intact hPTH, the ratios varying from approximately 1.5 (antiserum A1) to approximately 10 (antiserum C4).

3) Assessment of Regional Specificity using Synthetic hPTH Fragments

None of the five antisera selected on the basis of recognition of hPTH showed significant binding of the radioiodinated h 1-34 PTH synthetic fragment (prepared and evaluated as described elsewhere (2)). Three antisera (A1, A2, C4) showed displacement of radioiodinated bPTH (intact hormone) by approximately a ten-fold molar excess of h 1-34 PTH. (Figure 4 a, b, e).

These three antisera also required molar concentrations of the h53-84 synthetic fragment which at the 50% tracer displacement point were respectively ten-fold (antiserum C4), six-fold (A2) and four-fold (A1) lower than the molar concentrations of intact hPTH (Figure 4e, b and a). Two antisera A3 and C3



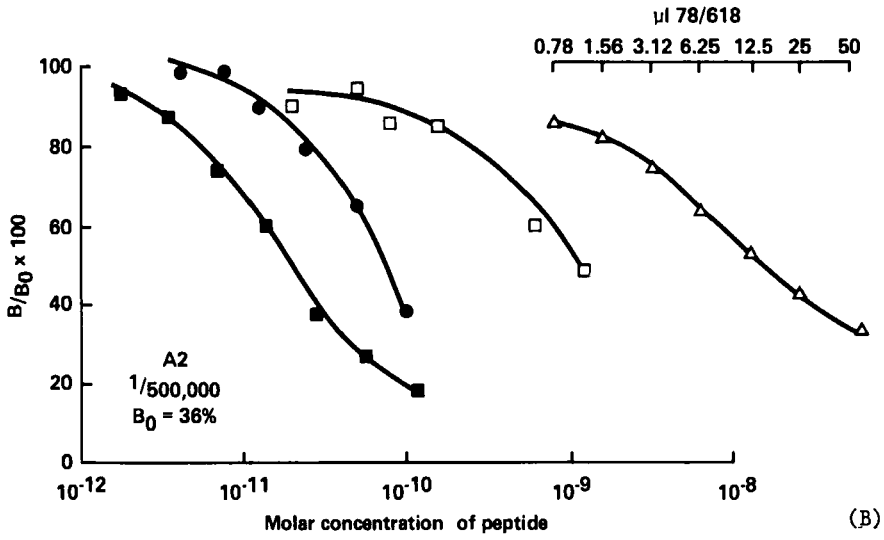
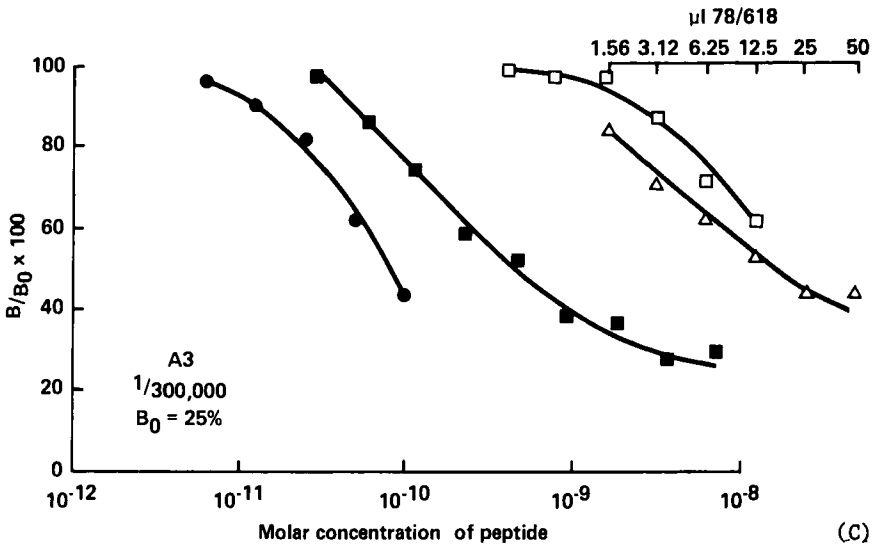


Fig. 4

● Intact hPTH
■ 53-84 hPTH
□ 1-34 hPTH
△ diafiltrate 78/618



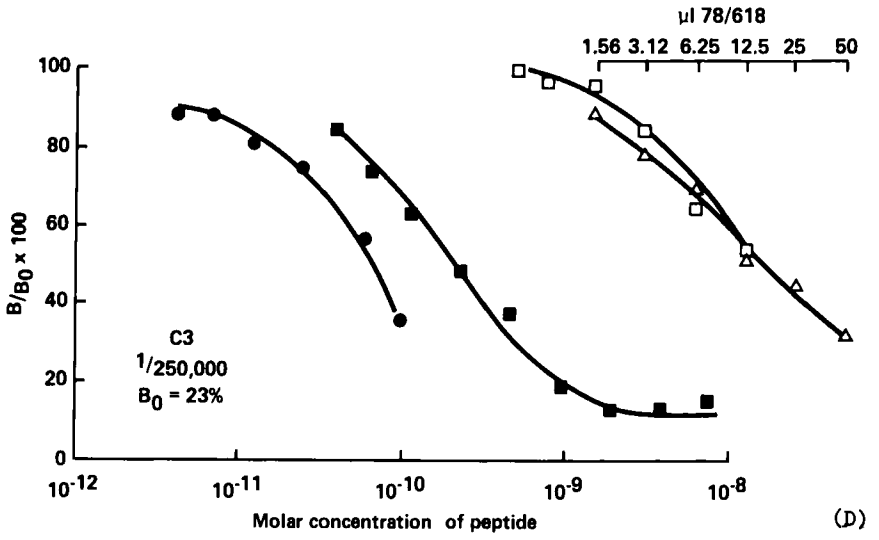


Fig. 4 (continued)

● Intact hPTH
■ 53-84 hPTH
□ 1-34 hPTH
△ diafiltrate 78/618

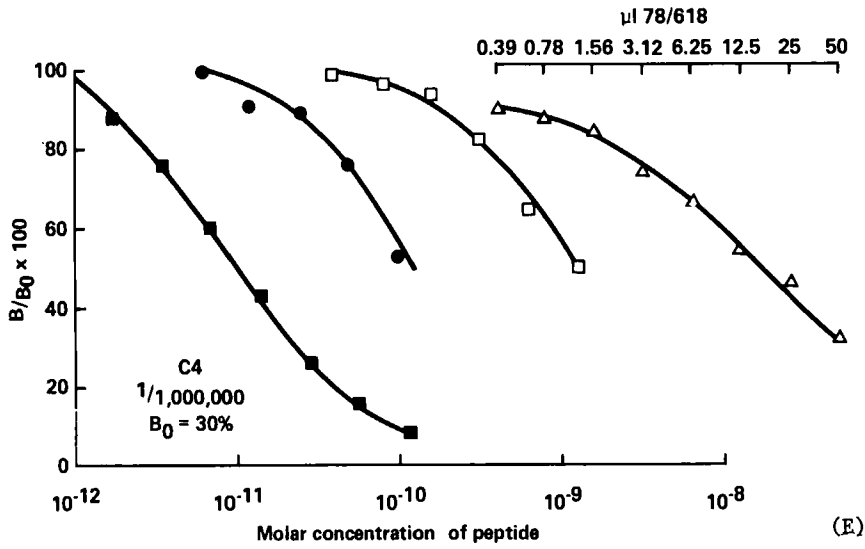


Fig. 4 (continued)

● Intact hPTH
■ 53-84 hPTH
□ 1-34 hPTH
△ diafiltrate 78/618

respectively required four-fold and ten-fold molar excess of the synthetic h53-84 fragment (Figure 4d and 4c). The log dose response curves for the intact hPTH and the synthetic h53-84 peptide fragment were not necessarily parallel (Figure 4 a - e).

The relevance of the specificity assessment study using the synthetic carboxyl-terminal peptide fragment to the potential application of these five antisera in clinical use was assessed in a preliminary study using the diafiltrate, ampoule code 78/618.

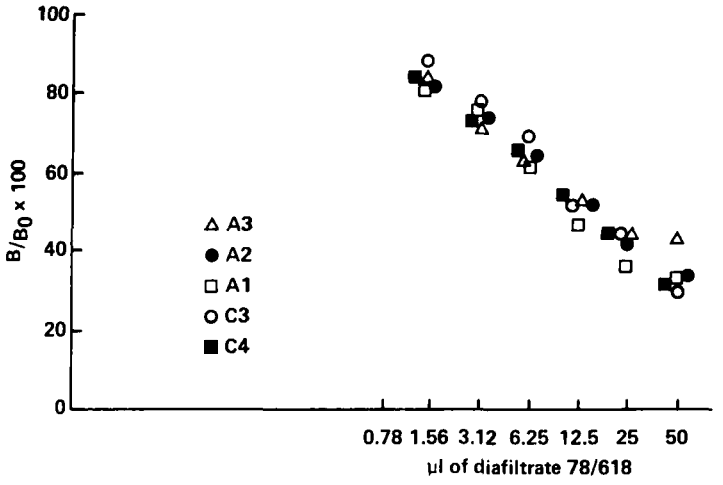


Fig. 5

The log dose response curve given by two-fold dose intervals of this material ranging from 0.87 µl to 50 µl was between 15% and 70% displacement of tracer in all five antisera, and in all cases, the 50% displacement point approximated to 15 µl (Figure 5). A potency value for this biological fluid in terms of intact hPTH or the h1-34 or h53-84 synthetic fragment in each antiserum assay system could not be computed as dose response curves were not parallel, but the comparison of values in "mole equivalents" of the intact hPTH or fragments at 50% tracer displacement are given in Table 3.

One antiserum (A1) in which intact b and hPTH were approximately equipotent and which showed parallel

Table 3

Comparison of the doses of the intact hPTH, synthetic h1-34 P and h53-84 PTH peptides and the secondary hyperparathyroid plasma diafiltrate required to give 50% displacement of tracer.

Antiserum Code No.	Molar Concentrations hPTH 1-84	hPTH 1-34	hPTH 53-84	µl of Plasma diafiltrate
A1	$4x10^{-11}$	$5.6x10^{-10}$	$1.1x10^{-11}$	12
C3	$6.5x10^{-11}$	$1.6x10^{-8}$	$2.2x10^{-10}$	16
A2	$7.5x10^{-11}$	$1.2x10^{-9}$	$2x10^{-11}$	16
A3	$8x10^{-11}$	$2x10^{-8}$	$4.6x10^{-10}$	17
C4	$1.2x10^{-10}$	$1.2x10^{-9}$	$1x10^{-11}$	16

displacement curves was used in an assay comparison of plasma immunoreactivity detectable in samples from 68 elderly patients with no biochemical evidence of primary or secondary parathyroid disease. Clinical details on these patients have been published elsewhere (10). All samples were assayed simultaneously using the antiserum A1 in comparison with antiserum AS 211/32. Results (Figure 6) show that whereas approxi-

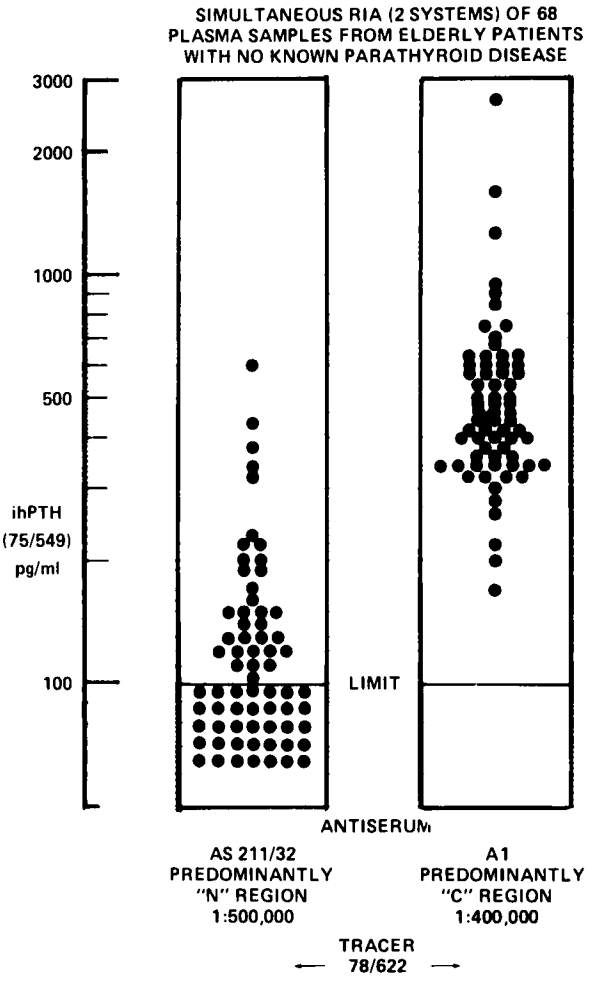


Fig. 6

mately 50% of the patients had detectable levels of immunoreactive PTH (iPTH) using AS 211/32, iPTH could be detected in all samples using antiserum A1 which recognised predominantly carboxyl region PTH.

4) Correlation between Immunization Programme and Production of Antisera

The immunization programme and titres of antisera produced are summarized according to the groupings given in Table 1. The guinea pigs immunized with a crude (2% pure) extract of bovine parathyroid glands mixed (to 10% by weight) with highly purified bPTH produced the greatest proportion of antisera to bPTH - 3 in group A, 2 in group C, 3 in group D, 2 in group E, and 7 in group F. Three out of five antisera (A1, A2 and A3) selected for further assessment were from these guinea pigs.

DISCUSSION

There is no clear indication that the purity of the immunogen, route of immunization or immunization-time schedule has a noticable effect upon the frequency, titre or specificity of antisera produced. Although one group (see Table 1) immunized with a mixture

of crude extract of bovine parathyroid glands and highly purified bPTH yielded the greatest proportion of potentially useful antisera, further studies would be needed to eliminate other factors such as genetic characteristics of groups of out-bred guinea pigs.

The predetermined criteria (titre, recognition of concentrations of hPTH within the clinical assay range) have indicated that five antisera are likely to be useful in clinical assay systems when utilised with suitable methodology and with the appropriate reagents and standards. All five antisera have predominant specificities directed towards the carboxyl region of the PTH molecule although the potency of the synthetic 53-84 fragment relative to the intact hormone varied considerably. It was disappointing that not one of the five selected antisera was similar to antiserum 211/32 in having predominant specificity for the amino-region of hPTH. This result was not surprising when considered in the light of experience of many other laboratories who have published reports on the development and application of heterologous radioimmunoassay systems using antisera raised in guinea pigs to bPTH (as reviewed in references 1, 11, 12, 13, 14, 15 and as evidenced by the 29 laboratories participating in the WHO international collaborative study, (7) and further details to be published separately).

Recent reviews on clinical applications of immunoassays for parathyroid hormone (11, 12, 13, 14, 15) have demonstrated that heterologous assay systems directed towards the carboxyl terminal region of PTH are still the most useful in the differential diagnosis of hypercalcaemia and in the diagnosis and follow up of primary and secondary hyperparathyroidism. For more detailed understanding of the factors influencing secretion, metabolism and clearance of parathyroid hormone and its products, clinical research groups are now developing region-specific immunoassays. These systems depend on methods in which antisera are either purified by affinity chromatography or are raised to synthetic hPTH peptide fragments, and the same peptides, or related analogues now available commercially, are used for preparing radioiodinated ligand for the radioimmunoassay systems and as standard in both radioimmunoassay and immunoradiometric assay systems (16, 17, 18, 19).

The equipotency of the diafiltrate known to contain a heterogeneous mixture of carboxyl region cleavage products when tested with each of the five antisera was remarkable. The results with the diafiltrate were all the more remarkable when compared with results obtained with the small synthetic h53-84 peptide so often used by laboratories in "defining"

the specificity of their immunoassay systems. There were obvious slope differences between the dose-response curves for diafiltrate and other antigens tested with most of the antisera, and attempts to calibrate the diafiltrate in terms of mole or mass equivalents of either hPTH or various synthetic fragments at the 50% displacement level failed to yield homogeneous results. The latter finding is not unexpected in view of the results of a recent international collaborative study organised on behalf of the World Health Organisation (8), in which the potency of the diafiltrate varied more than 300-fold within the group of 29 laboratories using 34 different immunoassay systems and a common reference preparation of intact hPTH.

It has been generally agreed that antiserum 211/32 has predominantly amino-region PTH specificity and does not show significant displacement of bovine 1-84 PTH tracer by the carboxyl region h53-84 PTH fragment (as reconfirmed in this report). There are, however, at least two published reports demonstrating that antiserum 211/32 can recognise the h53-84 PTH fragment (20, 21). In one case this was shown to be due to the use of a tracer prepared from a commercial batch of bovine PTH that lacked part of the amino-region (21). It is interesting to note

that in an early international collaborative study (22) to characterize and calibrate the NIBSC research standard, hPTH, for immunoassay (code 75/549) and in the recent WHO study (7), several laboratories used the same antisera (including 211/32) but did not necessarily report the same relative potencies for other ampouled b or hPTH preparations assayed simultaneously. It is clear that several factors combine to determine the specificity of an assay for PTH; these factors include the dilution at which an antiserum is used, the batch of bPTH used for radioiodination and the differences in techniques used for preparation and purification of tracer, the incubation reagents and conditions and the methods used for separation of bound and free tracer.

In view of the many differences in immunoassay systems, it is recommended that users of any of the five antisera, selected as a result of the study reported in this paper, carry out tests of titre, sensitivity and specificity in their own laboratory and optimise an assay system using their own reagents and their own methodology.

This systematic laboratory study points out some of the methodological artefacts of heterologous radioimmunoassay systems. It thus emphasises the need for cautious and careful characterization and

standardization of both the reagents and the experimental conditions under which they are used and the need for relevant reference materials to control for inter-assay variation.

The screening programme devised has proved rapid and effective, yet economical on time and reagents. The criteria for rejection or inclusion of antisera at each phase were pre-determined and rigidly adhered to. The criterion for high titre (greater than 10^{-5}) may have certainly caused the rejection of some potentially useful antisera at the first phase, yet ensured that the antisera finally selected would be available in sufficient quantity for long term continuity in wide spread use. The semi-quantitative classification scheme, expressed in terms of a reference antiserum, does provide a rational basis for subsequent re-assessment of rejected antisera.

The criteria used in the second phase for selecting antisera on the basis of tracer displacement under the final experimental assay conditions but using only three doses of bovine and human PTH covering a clinically relevant 10 fold dose interval also proved efficient yet economical in use of hPTH.

The third phase of screening, using only four doses of synthetic hPTH peptide fragments to cover a 1000 fold dose interval was designed to take account

of expected radioimmunoassay phenomena, such as incomplete displacement of tracer, and non-parallelism resulting in log-orders of magnitude of variation in doses of synthetic peptides. This strategy also proved reasonably economical with expensive reagents yet provided sufficient information for more detailed final assessment.

In conclusion, the three-phase screening programme for 122 sera has been practical and successful in selecting five antisera for more detailed evaluation. The final laboratory characterization has confirmed that at least three of these sera are high affinity antisera which could be used in overnight assays instead of the 3 + 3 day systems. All five sera have good recognition of either the whole or carboxyl-region parts of the hPTH molecule. The five antisera are now available for distribution on a trial basis.

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