International Standards for human Prolactin: calibration by international collaborative study

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ABSTRACT

Three ampouled preparations of purified human prolactin were assessed by 20 laboratories in eight countries for their suitability to serve as International Standards for the estimation of human prolactin in serum. Bioassays (pigeon crop sac assays and NB2 cell assays) were carried out in two laboratories, radioreceptor assays by one laboratory and radioimmunoassays by 17 laboratories.

By physicochemical analysis the preparations appeared similar. Each preparation contained small amounts of contaminants and/or prolactin variants. No major differences among the three preparations were detected by immunoassay although, in one radioreceptor assay system, one of the preparations was found to differ from the other two.

On the basis of all the available information, the Expert Committee on Biological Standardization of the World Health Organization (ECBS) in 1986 established the preparation in ampoules coded 83/562 as the Second International Standard for Prolactin and in October 1988 established the preparation in ampoules coded 84/500 as the Third International Standard for Prolactin. A value of 0.053 IU (53 mIU) prolactin activity/ampoule was assigned to both the Second and Third IS on the basis that this unitage would, insofar as possible, maintain continuity of the IU defined by the First International Reference Preparation of Prolactin, human, for Immunoassay (coded 75/504).

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INTRODUCTION

The first International Reference Preparation (IRP) of human Prolactin, for Immunoassay (code number 75/504) was established in 1978 (Gaines Das & Cotes, 1979) at a time when purified human prolactin was scarce. The IRP was widely used in research, in clinical diagnosis and for the calibration of commercial assay kits for prolactin until the stocks became exhausted.

A batch of ampoules of purified prolactin (coded 81/541) was prepared by the procedures established by the Expert Committee on Biological Standardization (ECBS) of the World Health Organization (WHO) for International Biological Standards (29th Report ECBS, Annex 4, 1978) and assessed in an international collaborative study for its suitability to serve as a replacement for the IRP. As reported to the ECBS (WHO/BS/83.1401, 1983), the results of this study showed that 81/541 behaved anomalously in certain immunoassay systems and it was therefore not recommended for establishment as an International Standard (IS).

An IS is a preparation to which an international unit (IU) has been assigned on the basis of an international collaborative study involving different assay systems in different laboratories. An IRP serves the same function as an IS, but may have been established either without a full international collaborative study or where such a study had shown the preparation to be not entirely suitable to serve as an IS. The ECBS has agreed (34th Report, ECBS, 1984) that only one category of reference material, the International Standard, will now be established to define the IU. Current IRPs will remain until either discontinued or replaced by a new IS.

There has been a continuing need for an IS for human prolactin since immunoassays of human prolactin are extensively used in clinical medicine. Purified hormone is scarce, heterogeneous, somewhat unstable and difficult to characterize—particularly by bioassay procedures. An IS for human prolactin was therefore urgently sought and as an interim measure ampoules of 81/541 were distributed (without official status) with a nominal content of 50 mIU/ampoule.
(a consensus figure based on results from the earlier collaborative study).

In response to the WHO request (34th Report ECBS, 1984), further preparations of prolactin were obtained, ampouled and examined in an international collaborative study, the results of which are now reported.

The aims of the study were: (a) to assess several preparations of purified human prolactin for their suitability to serve as International Standards for the estimation of human prolactin in serum or plasma, (b) to select one or more of these preparations as the IS for human prolactin, and (c) to assign a unitage to the selected preparation(s) which, as far as possible, maintained continuity of the previously defined IU for human prolactin.

Participants in the study

Twenty-five laboratories in nine countries agreed to take part in the study. Data were received from 20 laboratories in eight countries; these are listed below. Throughout this report each laboratory is identified by a number from 1 to 22 which does not correspond to the alphabetical order of listing.

Dr R. Aston, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.
Dr L. Binet and Ms C. Mentha, Serono Diagnostics, Centre Industriel, Case Postale 10, CH-1267 Coinsins, Switzerland.
Dr B. David and Dr C. Fallais, Institut National des Radioelements, B-6220 Fleurus, Belgium.
Dr R. Edwards, NE Thames Region Immunoassay Unit, St Bartholomew's Hospital, 51–53 Bartholomew Close, London EC1A 7BE, U.K.
Dr E. Endert, Academisch Ziekenhuis, University Hospital, F-2-106, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.
Mr K. Ferguson and Professor S. L. Jeffcoate, Department of Endocrinology, Chelsea Hospital for Women, Dovehouse Street, London SW3 6LT, U.K.
Dr B. J. Green, Dr W. Barnes and Ms M. Butler, Diagnostics Division, d-93J, Abbott Laboratories, Abbott Park, North Chicago, IL 60064, U.S.A.
Miss J. F. Grove-White, Quality Assurance, Amersham International plc, Forest Farm, Whitchurch, Cardiff CF4 7YT, U.K.
Professor J. B. Josimovich and Ms A. C. Smith, Department of Obstetrics and Gynecology, College of Medicine and Dentistry, 100 Bergen Street, Newark, NJ 07103, U.S.A.
Dr P. Kelly, Laboratory of Molecular Endocrinology, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec, Canada H3A 1AI.

Dr D. L. Kleinberg, New York University Medical Center, School of Medicine, 550 First Avenue, New York, NY 10016, U.S.A.
Dr H. G. Kwa and Dr A. A. Verstraeten, Antoni van Leeuwenhoekhuis, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.
Dr N. Leonard, Dr F. Conlan and Ms N. Myciw, Becton Dickinson Immunodiagnostics, Mountain View Avenue, Orangeburg, NY 10962, U.S.A.
Dr S. S. Lynch and Professor W. R. Butt, Department of Clinical Endocrinology, Birmingham and Midland Hospital for Women, Snowell Green Lane, Sparkhill, Birmingham B11 4HL, U.K.
Dr N. Norman, Hormone Laboratory, Akker Hospital, N-0514 Oslo 5, Norway.
Dr J. V. Patzke, Corning Medical, Corning Glass Works, 333 Coney Street, E. Walpole, MA 02032, U.S.A.
Mr B. Rafferty and Dr D. Schulster, National Institute for Biological Standards and Control, Blanche Lane, Potters Bar, Herts EN6 3QG, U.K.
Professor L. H. Rees and Dr J. G. F. Gilbert, Department of Chemical Endocrinology, St Bartholomew's Hospital, 51–53 Bartholomew Close, London EC1A 7BE, U.K.
Dr G. A. Scassellati and Dr F. Lunghi, Diagnostics Division, Sorin Biomedica SpA, 13040 Saluggia (VC), Italy.
Dr W. Wagner and Ms M. Scherf, Q C Dept, Mallinckrodt Diagnostica GmbH, von Hevesy Strasse 1–3, 6057 Dietzenbach 2, F.R.G.

MATERIALS

Ampouled materials used in the study, summarized in Table 1, are described below.

Characterization and ampouling of the purified prolactin preparations

Three different human pituitary extracts containing prolactin were obtained as candidates for an IS and used in the preparation of ampoules. Throughout the filling procedure solutions were maintained at 4°C. The filled ampoules were then pre-frozen to −35°C and after freeze-drying were further desiccated in vacuo, filled with pure dry nitrogen and sealed by glass fusion according to the procedures used for international biological standards (29th Report, ECBS, Annex 4, 1978). A few ampoules of each preparation were stored severally at 4, 20, 37 and 45°C so that stability could be assessed on the basis of accelerated thermal degradation.

The contents and contaminants in these candidate materials were assessed. Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE)
**TABLE 1. Preparations supplied to participants in the collaborative study**

<table>
<thead>
<tr>
<th>Candidate prolactin preparation and ampoule code</th>
<th>Code in collaborative study</th>
<th>Nominal unitage/ampoule and ampoule content</th>
</tr>
</thead>
<tbody>
<tr>
<td>83/562</td>
<td></td>
<td>50 mIU; 3 μg 'h prolactin', 0-4 μg moisture, and buffer residues</td>
</tr>
<tr>
<td>83/573</td>
<td>Y</td>
<td>50 mIU; 3 μg 'h prolactin', 3-13 μg moisture, and buffer residues</td>
</tr>
<tr>
<td>84/500</td>
<td></td>
<td>50 mIU; 2.2 μg 'h prolactin', 90-115 μg moisture, and buffer residues</td>
</tr>
<tr>
<td>81/541</td>
<td>E</td>
<td>50 mIU; 2.5 μg 'h prolactin', 140-205 μg moisture, and buffer residues</td>
</tr>
</tbody>
</table>

**Accelerated degradation samples of 83/562 (IS) stored:**
- continuously at −20 °C: Content assumed identical to 83/562
- 396 days at 20 °C: T
- 396 days at 45 °C: L  

**Accelerated degradation samples of 83/573 stored:**
- 133 days at 20 °C: B,G
- 133 days at 45 °C: A,F  

**Accelerated degradation samples of 84/500 stored:**
- continuously at −20 °C: Content assumed identical to 84/500
- 84 days at 4 °C: K
- 84 days at 20 °C: S

**Other samples containing prolactin (non-purified):**
- 71/167: N
- 75/523: M
- 75/524: P
- 81/557: Q
- 83/560: H,R  

**Chelsea Kit Working Standard**
- D: 3 mIU; freeze-dried residue of amniotic fluid

**1st International Reference Preparation for Prolactin for immunoassay (code 75/504)**
- IRP: 650 mIU/ampoule

**International Reference Preparation for Human GH (coded 66/217)**
- Z: 350 mIU/ampoule

*These ampoules were supplied without further coding.

*Different samples of these ampoules were also supplied without further coding (as 83/573 and 81/541 respectively).

*Amploue contents included the residues left after freeze-drying 1 ml buffer containing 5 mg lactose, 1 mg human serum albumin and 0.63 mg ammonium formate.

*Amploue contents included the residues left after freeze-drying 1 ml buffer containing 2 mg mannitol, 10 mg glycine and 2.5 mg sodium bicarbonate.

*See Gaines Das & Cotes (1979) for further details.


*Received and distributed in screw-cap vials.

(15%) (Plate) revealed the presence of prolactin variants and isohormones.

For each ampouled candidate preparation (containing approx. 3 μg prolactin), immunoassay showed less than 10 ng human growth hormone (hGH) per ampoule (assuming 2 IU of First IRP for hGH equivalent to 1 mg hGH), less than 0.03 ng human adrenocorticotropic hormone per ampoule, less than 15 ng human luteinizing hormone (hLH) per ampoule (assuming 1 mIU of First IRP for LH equivalent to 0.15 ng LH), less than 1 ng human follicle-stimulating hormone (hFSH) per ampoule (assuming 5 mIU of highly purified FSH equivalent to 1 μg FSH) and less than 5 ng arginine vasopressin (AVP) per ampoule (assuming 480 IU of First IS for AVP equivalent to 1 mg AVP).

**Ampoules coded 83/562**

Prolactin used for ampoules coded 83/562 was prepared from highly purified, freeze-dried pituitary extract (batch AFP-2480C isolated by Dr A. F. Parlow and Dr B. Shome, Torrance, CA, U.S.A.). This material was donated by the National Institute...
for Arthritis, Metabolism and Digestive Diseases, Bethesda, MD, U.S.A., via Dr S. Raiti and the National Pituitary Agency (Baltimore, MD, U.S.A.). Radioimmunoassays had indicated contamination of about 1% by weight with hGH; hFSH 2-10 mIU/mg, hLH about 100 mIU/mg and human thyroid-stimulating hormone about 30 mIU/mg.

The bulk powder (3-0 mg) was dissolved in 1 litre of a buffer (pH 6.8) containing 0.1% human serum albumin, 0.5% lactose in 0.01 mol ammonium formate/l (buffer ALAF) sterilized by filtration through a polycarbonate (Unipore, Bio-Rad Laboratories, Richmond, CA, U.S.A.) membrane of mean pore diameter 0.8 µm. Some 940 ampoules (code number 83/562) were filled according to the procedures described above (mean weight 1.002 g, range ±0.005 g). Moisture determined in two ampoules of 83/562 after lyophilization and secondary desiccation was 4.4 µg/ampoule in one and below the level of detectability in the other.

**Ampoules coded 81/541**

Prolactin used for ampoules coded 81/541 was prepared from the same extract as that used to prepare ampoules coded 83/562. Bulk powder (10.4 mg) was dissolved in 4 litres of a buffer (pH 7.5) containing 1% glycine, 0.2% mannitol and 0.25% bicarbonate (buffer GMB), and sterilized by filtration through a polycarbonate (Unipore) membrane of mean pore diameter 8 µm. Ampoules were filled with 1 ml solution (mean weight 1.002 g, range ±0.002 g) and coded 81/541. Moisture determined in three of these ampoules was 140, 165, and 205 µg/ampoule. Ampoules coded 81/541 were examined in a previous international collaborative study (WHO/BS/83.1401, 1983).

**Ampoules coded 83/573**

Prolactin in ampoules coded 83/573 was prepared from a highly purified pituitary extract donated by KabiVitrum, Sweden (Dr L. Fryklund). The material (batches M/R 23 and 25a) was provided as a frozen solution in 0.01 mol ammonium bicarbonate/l (assessed as 1.06 mg prolactin/ml). The frozen stock solution (12 ml) was dissolved in 4 litres buffer ALAF. Ampoules (coded 83/573) were filled according to the above procedures (mean weight 1.002 g, range 0.003 g). Moisture determinations for eight ampoules ranged from 2.6 to 12.8 µg/ampoule (mean 6.5 µg/ampoule).

**Ampoules coded 84/500**

Prolactin in ampoules coded 84/500 was prepared from highly purified pituitary extract, kindly donated by Professor W. R. Butt and Dr S. S. Lynch (Birmingham, U.K.). The material (batch H-Prol SKF2) was provided as a frozen solution in 0.1 mol ammonium acetate/l (pH 8.5). Radioimmunoassay indicated a potency of 39.5 IU human prolactin/mg and approx. 5 µg GH/mg extract. The bulk solution (35.46 ml; 10 mg protein) was dissolved in 4 litres buffer ALAF that had been sterilized by filtration through 0.4 µm Millipore HA filters (Millipore, Bedford, MA, U.S.A.). Ampoules (coded 84/500) were filled according to the procedures described above (mean weight 1.002 g, range ±0.002 g). Moisture determinations on three ampoules were 90, 105 and 115 µg/ampoule.

**Other (non-purified) samples containing prolactin**

Hyperprolactinaemic serum in ampoules coded 81/557 and 83/560 was obtained from a 40-year-old female patient after excision of a pituitary tumour and kindly provided by Professor L. H. Rees and Dr I. G. F. Gilbert (St Bartholomew's Hospital, London, U.K.). Ampoules (coded 81/557) were prepared from 1 ml of a serum pool (collected over a 13-month period (1980/81)) diluted with 150 ml buffer GMB. Each ampoule contained 6.7 µl serum/ml buffer solution and was filled as described above. Ampoules (coded 83/560) were prepared from 70 ml serum from this patient (seven samples obtained at different times and pooled) dissolved in 1 litre buffer GMB. Each ampoule contained 70 µl serum/ml buffer giving a concentration of prolactin sufficient to permit use of this material as a non-purified serum standard. The ampouled solution was freeze-dried and sealed as described above. Tests at the National Institute for Biological Standards and Control (NIBSC) for hepatitis B antigen and human immunodeficiency virus antibodies found these preparations to be negative.

Sera in ampoules coded 75/523, 75/524 and 71/167 were selected to typify samples which would be presented for clinical evaluation. These were from a normal female subject (coded 75/523), from the same subject after administration of thyrotropin-releasing hormone (coded 75/524) and from a serum pool collected from patients treated with phenothiazine (coded 71/167).

Amniotic fluid in vials coded D was kindly provided by Mr. K. Ferguson and Professor S. L. Jeffcoate as an example of a non-purified working standard, and stored at NIBSC at −20 °C before their distribution. An amniotic fluid pool was centrifuged, filtered and freeze-dried in screw-cap vials at Chelsea Hospital for Women (London, U.K.) and used there as a working standard in Prolactin Kits provided by Chelsea Hospital.
Ampoules of hGH standards

The International Reference Preparation of Growth Hormone, Human, for Immunoassay (66/217) was included amongst the samples sent to participants. The International Standard for human Growth Hormone for Bio assay (coded 80/505; Bangham, Gaines Das & Schulster, 1985) was included in physicochemical studies.

METHODS

Design of the collaborative study

Each participant received a selected subgroup of the preparations including coded duplicates of one or more preparations in order to assess the consistency of recovery and the variability of the estimates made. Where possible, participants also included their own house standard and the First IRP (75/504). Laboratories undertaking immunoassays were asked to perform at least two independent assays beginning with fresh ampoules of each preparation and different batches of tracer. All raw data with assay details were returned to NIBSC for analysis. Subsequently, a subset of participants examined thermal degradation samples which had been stored for longer periods of time (described in Table 4 with results).

Assay systems used and assays contributed

Pigeon crop sac in-vivo bioassays (Nicoll, 1967) were carried out by laboratory 1, and NB2 cell in-vitro bioassays (Tanaka, Shiu, Gout et al. 1980) by laboratories 1 and 20. Radioreceptor assays (Shiu, Kelly & Friesen, 1973) were carried out by laboratory 10. Seventeen laboratories carried out radioimmunoassays using various polyclonal antisera and procedures. Most laboratories used a phosphate buffer (varying from 0.02 to 0.10 mol/l, pH 7.5) with 0.5–2.5% bovine (or human) serum albumin; incubation times were 18 h or longer except in laboratories 3, 11 and 13 (3–5 h); temperatures were either 4°C or room temperature and all separation methods involved a second antibody. Six laboratories (2, 5, 7, 13, 16 and 18) were able to include the First IRP (code number 75/504) in their assays. Laboratories 2, 5, 6, 18 and 22 also carried out assays at a later date to examine accelerated degradation samples of the candidate materials stored for up to 3 years at elevated temperatures. For these assays each participant received a set of 8 to 14 ampoules coded by letter only; times and temperatures of storage for these samples are shown with results. In laboratory 7 a different diluent was used in one assay and this assay has been identified as 7.1 and treated separately.

Physicochemical determinations

Electrophoresis of samples (20 µg) of the bulk prolactin candidate materials was performed by the method of Laemmli (1970) using 15% SDS-PAGE. Materials for electrophoresis and isoelectric focusing were from BDH (Poole, Dorset, U.K.) unless otherwise stated. The samples were compared with the IS for human Growth Hormone (coded 80/505; Bangham et al. 1985) which contains no albumin carrier protein and was used as a molecular weight marker. Gels were stained with Coomassie Blue and ultra-sensitive silver stain (Marshall, 1984).

Statistical analysis

Bioassays using the pigeon crop sac were analysed by the standard method for parallel line assays taking wet weight of crop as the response.

For the immunoassays, NB2 cell assays and radioreceptor assays, the response data were transformed to logits using fitted asymptotes and analysed as multiple parallel line assays (using the computer program WRANL; Gaines Das & Tydeman, 1982).

A common weight for the estimates of log potency from each laboratory was determined as follows. For each preparation examined a 'total' interassay variability was determined from all estimates of log potency made by the laboratory. Further, for each preparation examined in duplicate, variability of estimates was determined both as the between-assay variation of the observed estimates of potency of the coded duplicate in terms of the identical material and also as the deviation of the observed estimates from their expected potency of 100%. If these estimates of variability were similar, an average variability was calculated and the weight was taken to be its reciprocal; otherwise a weight was obtained as the reciprocal of the medial variability.

Log potency estimates, with their weights as described above, were examined for heterogeneity using a chi-squared test before being combined as weighted or unweighted geometric means; each combination is described in the text as it occurs. The confidence intervals for weighted geometric means were determined using the sum of the weights of the estimates combined. Those for unweighted geometric means were calculated using the variance of the log potency estimates combined.

Estimates of the relative activity (to ampoules of the same preparation stored continuously at −20°C) remaining in the ampoules of the various purified prolactin preparations after storage at elevated temperatures were used to fit an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay (Kirkwood & Tydeman,
1984) and hence were used to predict the degradation rate of the standard stored at \(-20\,^\circ\text{C}\).

**RESULTS**

**Physicochemical determinations**

Polyacrylamide gel electrophoresis (Plate) showed that all three prolactin samples contained many separable bands when visualized by silver staining. However, the sample of the bulk material used for preparation of the Second IS 83/562 had least contamination with high molecular weight or dimeric material and 83/573 contained more separable bands than either of the other two samples.

Isoelectric focusing confirmed that all three pituitary prolactin samples (83/562, 83/573 and 84/500) were heterogeneous, not dissimilar from each other and that fewer bands were evident in the sample of the bulk material used for preparation of the Second IS 83/562.

**Bioassays in vivo**

The ampouled preparation (coded 83/562) was compared with the IRP of ovine prolactin by pigeon crop sac bioassay. Slopes of log dose–response lines for these preparations in this assay did not differ significantly. Estimated prolactin content was 30 mIU (95% limits 9–87 mIU) per ampoule of 83/562.

**NB2 cell in-vitro bioassay**

Estimates made by NB2 cell assay in laboratory 20 of the purified prolactin preparation in ampoules coded 83/562 and the serum in ampoules coded 83/560, in terms of 81/541 (assumed to contain 50 Units per ampoule), were some six or more times greater (approximately 200 and 300 Units per ampoule respectively) than such estimates made in laboratory 1. The participant in laboratory 20 commented that cell cultures became alkaline with 81/541 and suggested that this may have contributed to the decreased activity of this preparation. However, both laboratories found that in this system the activity in ampoules 83/562 was approximately half that in ampoules of 83/560. Preparations of 84/500 and 83/573 were also examined in laboratory 20 and found to have prolactin-like activity approximately the same as that of 83/562 (ampoule/ampoule).

**Radioreceptor assays**

Preparations 83/562, 83/573, 84/500, 83/560 (one assay only), 81/541 and local standards of ovine and human prolactin were compared in two assays in laboratory 10. The slope of the log dose–logit response line for 83/560 was greater than the slopes for the other preparations in the one assay including this preparation. In both assays, 81/541 tended to have steeper log dose–logit response lines than the other ampouled prolactin preparations. The activity for the total contents of an ampoule of each of 81/541, 83/562, 84/500 and 83/560 was found to be very similar while that of 83/573 was some two times greater.

**Radioimmunoassays**

The log dose–logit response lines for the various preparations (including accelerated degradation samples) did not, insofar as could be determined, deviate consistently from linearity or parallelism in the

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>83/562 and J</th>
<th>84/500 and C</th>
<th>83/573 and Y</th>
<th>83/560 H and R</th>
<th>81/541 and E</th>
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<td>2</td>
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<tr>
<td>7.1</td>
<td>27.2*</td>
<td>24.1*</td>
<td>35.6*</td>
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<td>60.4</td>
<td>31.8</td>
<td>83.3</td>
</tr>
</tbody>
</table>

Geometric mean of assays omitting estimates marked*  
52.8  53.2  64.3  30.8  58.9

*Analysis of variance of log potency estimates from all assays shows no significant difference between laboratories if these estimates are omitted.
majority of laboratories. However, in four laboratories (2, 8, 14 and 17) the line for 81/541 differed from lines for the other preparations. Results for the hGH preparation Z did not indicate any substantial cross-reactivity in these systems.

Three preparations, 81/541, 83/573 (both purified prolactin) and 83/560 (serum), together with ampoules identical to them except for code were, insofar as possible, included in each assay so that within- and between-assay variability could be assessed. For each of these three preparations, estimates of the activity of the 'coded duplicate' relative to the identical material were in good agreement with the expected values of 100%. The only exception was laboratory 22 in which there was evidence of considerable assay drift. For each coded duplicate preparation within each laboratory the estimate of between-assay variation was similar to the estimate of within-assay (between ampoules) variation. Estimates of variation for the different preparations were not similar, with that for 81/541 being greatest and that for 83/560 being least in six of the 15 laboratories providing estimates for all three preparations ($P<0.05$ if all orderings were equally likely). Analysis of variance of the logarithms of the variances showed significant ($P<0.05$) differences between the variances of these coded duplicates. However, estimates of variability both within and between assays differed more between laboratories (some 40-fold from smallest to largest) than within laboratories (tenfold in the most extreme case and less than threefold in the majority).

### Calibration of the ampouled prolactin preparations

Laboratory geometric mean estimates for the various prolactin preparations in terms of the First IRP are shown in Table 2. In laboratory 5 and laboratory 7.1, the purified prolactin preparations appeared to contain substantially less prolactin; however, the prolactin estimates were not less for the serum preparations 83/560 and 81/557 when assayed in these systems. If estimates from these two systems are omitted, estimates from the remaining systems were homogeneous for each of the preparations except 81/541.

### Comparison of the prolactin preparations with one another

Comparison of 83/562 and purified prolactin preparations 83/573 and 84/500 gave results which were homogeneous in the 17 assay systems included in this study (Table 3). Estimates of the prolactin content of the serum preparation 83/560 in terms of 83/562 were homogeneous if results from laboratories 5 and 7-1 were omitted. Estimates of the prolactin content of

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**Table 3. Laboratory geometric mean estimates of the prolactin concentration of various prolactin preparations expressed as mIU of the IS, 83/562, per ampoule**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>83/573 and Y</th>
<th>84/500 and C</th>
<th>83/560 H and R</th>
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<td>25.5</td>
</tr>
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<td>11</td>
<td>60.1</td>
<td>52.5</td>
<td>28.4</td>
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<td>12</td>
<td>54.7</td>
<td>49.9*</td>
<td>33.5*</td>
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<tr>
<td>13</td>
<td>65.0</td>
<td>54.7</td>
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<td>54.7</td>
<td>38.1</td>
<td>32.1</td>
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<td>55.1</td>
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<td>64.1</td>
<td>54.9</td>
<td>31.2</td>
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<td>28.8</td>
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<td>18</td>
<td>61.5</td>
<td>52.0</td>
<td>34.7</td>
</tr>
<tr>
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<td>51.6*</td>
<td>50.9*</td>
<td>34.0*</td>
</tr>
<tr>
<td>21</td>
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</tr>
<tr>
<td>22</td>
<td>75.2</td>
<td>51.9</td>
<td>22.1*</td>
</tr>
<tr>
<td>22*</td>
<td>51.2*</td>
<td>45.2*</td>
<td>20.5*</td>
</tr>
<tr>
<td>Weighted geometric mean</td>
<td>62.85</td>
<td>53.72</td>
<td>30.27</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>61.0–64.8</td>
<td>52.1–55.4</td>
<td>29.3–31.3</td>
</tr>
<tr>
<td>Unweighted geometric mean of all estimates</td>
<td>64.01</td>
<td>52.77</td>
<td>32.71</td>
</tr>
</tbody>
</table>

*Indirect estimate, omitted from weighted mean.

**Likely to be substantially underestimated due to assay drift; omitted from means.

*Not assayed

*Contributed excessively to heterogeneity chi-squared, omitted from weighted geometric mean.

*Estimates from later stability study, not included in calculation of means.

81/557 in terms of 83/562 were homogeneous if results from laboratories 5, 7.1 and 6 were omitted.

### Calibration of the serum samples and amniotic fluid

The prolactin concentrations of the serum samples 71/167, 75/524 and 75/523 were expressed in terms of the IRP (75/504) and 83/562. Except in laboratory 5 and 7.1 there was good agreement of estimates in terms of the two standards. The mean estimates for each sample in terms of the First IRP (75/504) were 0.11, 0.98 and 0.76 for 75/523, 75/524 and 71/167 respectively. These are in good agreement with the values obtained in previous studies (Gaines Das & Cotes, 1979).
### TABLE 4. Laboratory and overall unweighted geometric mean estimates of remaining prolactin-like reactivity in the various degradation samples expressed as per cent of activity of the sample of the same material stored continuously at —20 °C. Means for coded duplicates are based only on assays which also included degradation samples. All results are from immunoassays except where otherwise noted.

#### Preparation in ampoules coded 83/562

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Storage temperature and time of storage for each preparation</th>
<th>4°C (coded duplicate)</th>
<th>20°C 45°C</th>
<th>4°C 1042 days</th>
<th>37°C 1042 days*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td>596 days</td>
<td>396 days</td>
<td>1042 days</td>
<td>1042 days*</td>
</tr>
<tr>
<td>2*</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
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<td>6</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>94</td>
<td>54</td>
<td>94</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>91</td>
<td>80</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Geometric mean</td>
<td></td>
<td>—</td>
<td>91</td>
<td>52</td>
<td>98</td>
</tr>
</tbody>
</table>

#### Preparation in ampoules coded 84/500

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Storage temperature and time of storage for each preparation</th>
<th>4°C (coded duplicate)</th>
<th>20°C 45°C</th>
<th>4°C 1042 days</th>
<th>37°C 1042 days*</th>
</tr>
</thead>
<tbody>
<tr>
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<td>84 days</td>
<td>84 days</td>
<td>692 days</td>
<td>692 days</td>
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<tr>
<td>2*</td>
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<td>7</td>
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<td>90</td>
<td>91</td>
<td>—</td>
<td>—</td>
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<tr>
<td>14</td>
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<td>113</td>
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<td>—</td>
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<td>66</td>
</tr>
<tr>
<td>22*</td>
<td></td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>65</td>
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<tr>
<td>Geometric mean</td>
<td></td>
<td>—</td>
<td>99</td>
<td>68</td>
<td>100</td>
</tr>
</tbody>
</table>

#### Preparation in ampoules coded 83/573

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Storage temperature and time of storage for each preparation</th>
<th>4°C (coded duplicate)</th>
<th>20°C 45°C</th>
<th>4°C 746 days</th>
<th>37°C 746 days</th>
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</thead>
<tbody>
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<td>2</td>
<td></td>
<td>133 days</td>
<td>133 days</td>
<td>746 days</td>
<td>746 days</td>
</tr>
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<td>73, 78</td>
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<td>69, 70</td>
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<td>99</td>
<td>94</td>
</tr>
<tr>
<td>22*</td>
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<td>108, 87</td>
</tr>
<tr>
<td>Geometric mean</td>
<td></td>
<td>—</td>
<td>95</td>
<td>77</td>
<td>101</td>
</tr>
</tbody>
</table>

---

Note:

- Estimates unreliable due to assay drift.
- Radioreceptor assay.
- Of the 1042 days, 81 were at 45°C.
- Contributed for the supplementary study using a different method.
- In view of the excessive estimate for samples stored at 45°C, this estimate was arbitrarily taken to be 100 and other estimates taken relative to it giving combined results for this lab of 95, 86 and 73 for samples stored at 4, 20 and 37°C respectively, which were used to obtain the overall g.m.
- Taking into account the values for the coded duplicate.

Vials of amniotic fluid, coded D, were compared with 83/562 in 11 laboratories and with the IRP (75/504) in five of these. Estimates for the prolactin content of D in terms of the IRP (75/504) ranged from 1.78 to 2.98 mIU/vial (geometric mean of laboratory means 2.36 mIU/vial). Estimates in terms of 83/562 ranged from 1.96 to 2.97 mIU/vial, omitting estimates from laboratories 5, 7.1 and 14 (4.2, 4.5 and 5.1 mIU/vial respectively), with a weighted geometric mean of 2.39 mIU/vial.
Stability studies

Predicted losses of activity based on the accelerated degradation samples stored for relatively short times (described in Table 1) were imprecise. Subsequently a subset of participants examined samples of the purified prolactin preparations which had been stored at elevated temperatures for up to 3 years. All results are summarized in Table 4. For each of the three prolactin preparations, both for samples stored for some 2 or more years at 4 °C and for 'coded duplicates' (i.e. samples stored continuously at −20 °C), activities relative to samples stored continuously at −20 °C were calculated; these activities were not detectably different from one another. This indicates that substantial losses of activity are not likely. Insofar as the data lend themselves to prediction, 83/562 appears to be more stable than 83/573 which appears more stable than 84/500. The predicted yearly loss at −20 °C is less than 0.1% for each preparation; however, the loss detected is dependent upon the assay system used, so it may not be assumed that these results will apply in all assay systems.

DISCUSSION

With the exception of estimates from laboratories 5 and 7.1, estimates in terms of the First IRP (75/404) for the prolactin content of these more recently ampouled preparations are in good agreement. Estimates of the prolactin content of serum 83/560 in terms of 83/562 were homogeneous with the omission of a limited number of estimates. Although there were few laboratories for which data were available, potency estimates for the serum samples in terms of the purified preparations showed agreement between laboratories as good as that given by estimates in terms of the First IRP, subject to the omissions noted above.

Selection of the International Standard

Comparisons among the purified prolactin preparations 83/562, 83/573 and 84/500 did not suggest that these preparations differed markedly from one another. When estimates of prolactin concentration (in terms of the First IRP, 75/504) for these preparations were analysed, those for 83/562 showed smaller within- and between-laboratory variances than those for 83/573 or 84/500; however, these differences were not significant (F test assuming estimates were independent). Calibration of the serum samples 71/167, 75/523 and 75/524 in terms of these three preparations gave, for each serum sample, similar between-laboratory variances of estimates. Between-laboratory variance for estimates of the prolactin concentration of serum 81/577 in terms of 83/562 was smaller than that for estimates in terms of 83/573, although the difference was not significant. On the basis of remaining prolactin-like activity of the degradation samples examined in this study, the preparation in ampoules coded 83/562 appeared to be more stable than either of the other two purified preparations. Various prolactin variants are known (Nyberg, Roos & Wide, 1980; Meuris, Svoboda, Christophe & Robyn, 1984) and all three preparations could be separated into many components. However, results of the physicochemical analysis suggested that 83/562 was largely free of dimers and polymers and higher molecular weight material and that the preparation in ampoules coded 83/573 contained the most contaminants.

On the basis of these results, the preparation in ampoules coded 83/562 was established as the Second IS for human Prolactin (37th Report, ECBS, 1987). Recently stocks of this have become depleted and the preparation in ampoules coded 84/500 has been established as the Third IS for human Prolactin (39th Report, ECBS, 1989). The other preparation (83/573) is very similar to 83/562 and 84/500 in these assay systems and it may also be useful as a reference material. The serum sample coded 83/560 may be of interest as a research reagent in the development and characterization of assay systems.

Assignment of unitage to the various ampouled preparations

In view of the noted variations in behaviour of 81/541 in different assay systems and of the desirability of maintaining continuity with the established IU for prolactin, considerable weight is given to the calibration of the various materials in terms of the First IRP. The Second IS for human Prolactin was assigned a unitage of 53 mIU (37th Report, ECBS, 1987). This agrees with calibration in terms of 81/541, if 81/541 is taken to contain 60 mIU per ampoule. This value also maintains reasonable continuity of unitage with the First IRP based on the results of calibration of serum samples in this study. It was also agreed that the preparation in ampoules coded 83/573 contained, on the basis of this study, a unitage of 63-3 mIU per ampoule, and the preparation in ampoules coded 83/560 contained a unitage of 30-2 mIU per ampoule.

It has recently been agreed by the ECBS of WHO at its meeting in 1988 that the material in ampoules coded 84/500 be established as the Third IS for human Prolactin also with a unitage of 0-053 IU (53 mIU) per ampoule since stocks of the Second IS are becoming depleted.
Use of the International Standards for human Prolactin

The IS is intended for calibration of national and laboratory standards. Stocks of the Second IS are now low; requests for the Third IS for human Prolactin should be addressed to the National Institute for Biological Standards and Control, P.O. Box 1193, Potters Bar, Herts EN6 3QH, U.K., with a brief outline of the intended scientific use for it.

ACKNOWLEDGEMENTS

Grateful thanks are due to Dr A. F. Parlow, Dr L. Fryklund, Professor W. R. Butt and Dr S. Lynch for supplying the candidate materials; Dr D. Miller (Centre for Applied Microbiology and Research, Porton Down, Salisbury, Hants, U.K.) for hGH determinations; Professor L. H. Rees and Dr I. G. F. Gilbert of St Bartholomew's Hospital for the generous supply of the prolactinaemic blood sample; Dr P. J. Campbell for ampouling; all the participants in the collaborative study; Mr N. Sutcliffe for invaluable technical assistance with the electrophoresis; and Ms A. Richardson and Ms F. Forrester for secretarial assistance.

REFERENCES


DESCRIPTION OF PLATE

Electrophoresis on 15% sodium dodecyl sulphate-polyacrylamide gels. Prolactin (PRL; 20 µg) samples were obtained from the bulk material supplied. They were compared with 20 µg of the ampouled International Standard for human GH (code no. 80/505) (which contains no albumin carrier protein) and which is known to contain dimeric (40–44 kDa), monomeric (22 kDa and 20 kDa) and cleaved (14 kDa) forms. Gels were stained (Fig. 1) with Coomassie Blue and (Fig. 2) with ultra-sensitive silver stain. The origin is indicated by the arrows at the top.

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