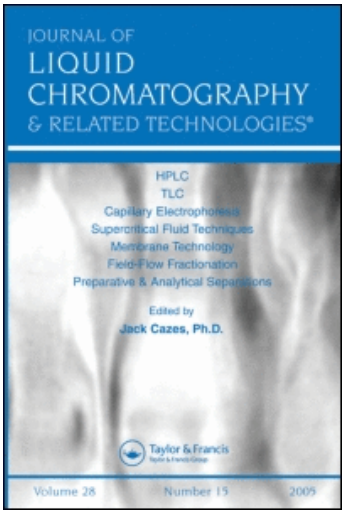

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A Method for Measuring the Binding Affinity and Capacity of Growth Hormone Binding Protein in Human Serum Using FPLC to Separate Bound and Free Ligand

C. A. M. Roelen[a]; G. H. Donker[a]; J. H. H. Thijssen[a]; M. A. Blankenstein[a]
[a] Department of Endocrinology, Academic Hospital Utrecht, GA Utrecht, The Netherlands

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A METHOD FOR MEASURING THE BINDING AFFINITY AND CAPACITY OF GROWTH HORMONE BINDING PROTEIN IN HUMAN SERUM USING FPLC TO SEPARATE BOUND AND FREE LIGAND

C. A. M. ROELEN, G. H. DONKER,
J. H. H. THIJSSEN, AND M. A. BLANKENSTEIN
Department of Endocrinology
Academic Hospital Utrecht
P. O. Box 85500
3508 GA Utrecht, The Netherlands

ABSTRACT

A method was developed for the measurement of growth hormone binding protein (II-GHBP) in human serum. Iodinated human growth hormone (hGH), freshly purified on Sephacryl 200-HR, was incubated overnight at room temperature with human serum in the presence of different concentrations of radioinert hGH. Free and bound hGH were separated on a Superose 12 column by a fully automated FPLC system. The column was eluted with 0.05 M phosphate buffer, pH 7.5, containing 0.15 M NaCl. Concentrations of bound and free hormone were calculated from the elution pattern. Binding affinity and capacity were calculated by Scatchard plot analysis of the binding data. The data were corrected for endogenous hGH in the samples.

The intra-assay variation in the percentage bound GH was 4% whereas the interassay variation in binding capacity was found to be 12%. The II-GHBP levels in serum from healthy female and male volunteers were 1350 ± 530 (mean $\pm$ S.D.; $n = 29$) and 900 ± 205 fmole/l ($n = 22$) respectively ($p < 0.01$). The affinity constant (K_a) differed between serum from female (0.31 ± 0.07 nM^{-1}) and male subjects (0.43 ± 0.11 nM^{-1}; $p < 0.01$). The advantage of the present method over published methods for II-GHBP assay is that binding capacity and affinity are determined. The disadvantage is that, even with the automated FPLC, the method is slow since 6 FPLC runs are required for every sample. Current efforts are directed to improve this disadvantage.

INTRODUCTION

In 1964 Hadden *et al* and Collip *et al* suggested the presence of a specific binding protein for growth hormone in human plasma (1-3). Recently a specific, high affinity, low capacity binding protein for human growth hormone (hGH) has been characterized (4,5). The physiologic function of this growth hormone binding protein (GHBP) is not completely known, but the binding protein is known to affect biologic action and metabolism of hGH (6-15). Leung *et al* reported similarity of the GHBP to the extracellular domain of the hGH receptor (16). Smith *et al* (17) stated that mouse serum GH binding protein had the extracellular domains of the mouse GH receptor, but contained modified transmembrane domains. In 1990, Baumann *et al* (18) described a second binding protein with a lower affinity and a higher binding capacity for human growth hormone. As the first discovered binding protein eluted in the second peak using Sephadex G-100 gel filtration chromatography, this protein was termed (peak)II-GHBP. The lower affinity binding protein, eluting in the first peak, was termed (peak)I-GHBP. No functional relationship between II-GHBP and I-GHBP has been reported.

Previously, slow and cumbersome gel filtration techniques have been used in investigations of normal and abnormal regulation of the II-GHBP. Herington *et al* (5) introduced gel filtration on AcA 44 mini-columns to achieve a more rapid and simpler separation. However, this technique only incompletely separates bound from free hGH and requires continuous fraction collection. Baumann *et al* (19) presented a DEAE-cellulose gel filtration which in their hands is rapid and readily applicable to large series of samples. Using this technique however, we could not achieve significant displacement of bound ^{125}I-hGH by unlabeled hGH. To overcome this problem, we devised a method using automated Superose 12 gel filtration to separate bound and free ligand.

The aim of the present study was to evaluate the Superose 12 gel filtration technique and to determine values for the affinity constant and maximal binding capacity of the II-GHBP by Scatchard plot analysis of plasma samples of healthy male and female human subjects.

MATERIALS AND METHODS

Briefly, the method we devised for the measurement of the binding capacity and affinity of II-GHBP consists of the following steps: incubation of serum with iodinated hGH and increasing amounts of unlabeled hGH, separation of bound and free ligand with FPLC gel filtration, quantification of bound and free hGH and Scatchard plot analysis of the binding data.

Iodination of hGH

Unlabeled hGH was purchased from UCB Bioproducts (Braine l'Alleud, Belgium). Solubilized in 15 μl 0.01 M phosphate buffer aliquots of 5 μg of hGH were stored in polypropylene cups at -20 °C until iodination. HGH was radiolabeled in these cups by the Chloramine-T oxidation method, using 1 mCi of $Na^{125}I$ in 10 μl diluted NaOH purchased from Amersham (Buckinghamshire, England), 15 μg Chloramine T (Sigma Chemical Company, St. Louis, USA) in 5 μl 0.01 M phosphate buffer (pH 7.5) and 10 μl 0.01 M phosphate buffer (pH 7.5). The reaction time was 1 minute; the reaction was terminated by the addition of 240 μg sodium meta-bisulphite (BDH Chemicals Ltd., Poole, England) in 100 μl 0.01 M phosphate buffer (pH 7.5). After iodination ^{125}I-hGH was separated from the free iodide on a Sephadex G-25 M column obtained from Pharmacia (Uppsala, Sweden), which was eluted with 0.01 M phosphate buffer (pH 7.5) containing 0.1% BSA and 0.15 M NaCl. Sixteen fractions of 1.0 ml were collected, The average incorporation of ^{125}I was 91.3% ± 1.9% (range 88% - 94%, n = 9). The mean specific activity obtained after iodination was 165 μCi/μg ± 15 μCi/μg. The peak fraction was isolated and fractionated in aliquots of 50 μl to which 100 μl of the elution buffer was added. These solutions were stored at -20 °C until use.

Specimens

Serum samples of 51 healthy blood donors were obtained from the blood bank in Utrecht: 29 were samples from female donors, aged 22 to 61 years and 22 samples from male donors, aged 24 to 64 years. In all serum samples, the hGH concentration was measured by radio-immuno assay as described by the manufacturer (Oris Industry Company, Gif-sur-Yvette, France: lower detection limit 0.25 ± 0.02 ng/ml, intra-assay CV 7.7%, inter-assay CV 11%, reference values ≤ 20 mE/l). The mean serum hGH concentration was 2.2 mE/l (range 0.4 - 18 mE/l). No abnormal hGH concentrations (hGH > 20 mE/l) were found in the specimens we analysed (n = 51). The hGH concentration was used to correct specific activity of the tracer in each particular experiment.

Purification of ^{125}I-hGH and Incubation of Serum Specimens

Five hours before use, ^{125}I-hGH was purified on a 60 x 1 cm column, containing Sephacryl 200 High Resolution (Pharmacia, Uppsala, Sweden) eluted with a 0.01 M phosphate buffer (pH 7.6), containing 0.15 M NaCl and 0.25% BSA at a flow rate of 0.25 ml/min. Fractions of 1.0 ml were collected and counted for 1 minute in a Packard autogamma counter. The monomeric ^{125}I-hGH peak was isolated and its peak fraction was used for incubation with the human serum samples.

Serum samples of 240 µl were incubated with a fixed amount of ^{125}I-hGH (10,000 -20,000 cpm) and different final concentrations of unlabeled hGH (10, 30, 60, 100, 300 µg/l and 10 mg/l). The concentrations of radioinert ligand were verified by radio-immuno assay. The samples were diluted with demineralized water to a total volume of 300 µl. To select optimal incubation conditions, the binding percentages after different incubation times at room temperature were determined.

Gel Chromatography

The incubation mixtures were analyzed on a 30 x 1 cm Superose 12 column in a FPLC-system using a P-3500 pump and a liquid chromatography controller (LCC-500). The column was rinsed with the elution buffer (0.15 M NaCl, 0.05 M phosphate buffer (pH 7.5)) for 30 minutes before each sample analysis. After application of the serum sample, the column was eluted with 24 ml of elution buffer at a flow rate of 0.4 ml/min. After 60 fractions had been collected, the column was washed with demineralized water for 45 minutes. The entire chromatography process was performed automatically at room temperature.

Data Analysis

The bound (B) and free (F) ^{125}I-hGH were calculated by integrating the corresponding peaks of the Superose 12 elution pattern. B and F were used for Scatchard plot analysis. As the Scatchard plot yielded a curvilinear pattern it was resolved by the method of Rosenthal (20). Throughout this paper, results are given as mean ± SD (n). The significance of differences was evaluated with the non-parametric Wilcoxon test for two samples. Statistical significance was concluded when a p-value of less than 0.05 was found.

RESULTS

Purification of ^{125}I-hGH

The elution of the Sephacryl 200 HR (S-200 HR) column yielded 2 peaks, as illustrated in Figure 1A. The first peak corresponded with monomeric ^{125}I-hGH and the second peak with free ^{125}I. After prolonged storage of the

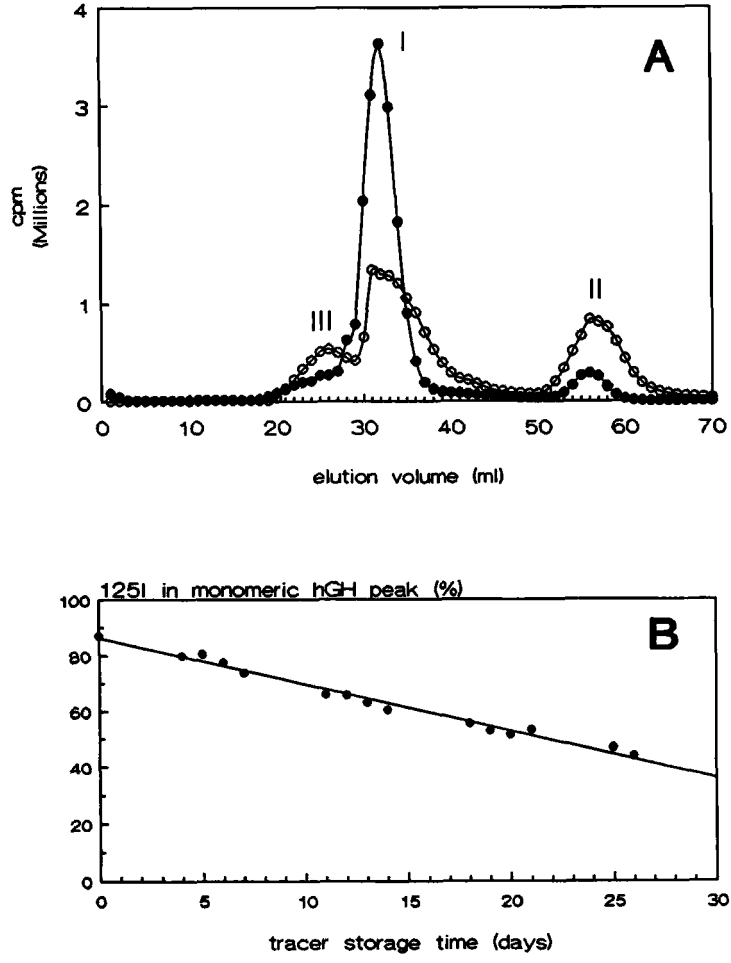

FIGURE 1. Effect of storage on the quality of ^{125}I-hGH tracer.
A) Sephacryl 200SR elution pattern of freshly prepared tracer (●) and tracer stored for 4 weeks at -20°C (○). Elution was with 0.01 M phosphate buffer pH 7.5 containing 0.25% BSA, at 0.25 ml/min. Peak I represents monomeric ^{125}I-hGH; whereas peaks II and III represent free iodide and aggregates formed upon storage respectively.
B) Percent monomeric ^{125}I-hGH in tracer solutions as a function of storage time at -20°C.

^{125}I-hGH solutions, a third peak of higher molecular mass than peak I appeared in the elution pattern of the S-200 HR column, the peak of monomeric hGH broadened and the free ^{125}I increased. The effect of tracer storage time on the percentage of radio-activity in the monomeric hGH peak is shown in Figure 1B. Based on the progressive decrease of the tracer quality upon storage, it was decided to purify the tracer prior to each experiment.

A single serum specimen was incubated four times with tracer of different iodination sessions and after different storage time. The maximal binding capacity (MBC) after 2 days of tracer storage was 796 fmol/ml. The MBC after 13, 20 and 27 days of storage was 705 fmol/ml, 805 fmol/ml and 869 mol/ml respectively.

To further evaluate the integrity of the monomeric hGH peak, eluted from the S-200 HR column, this peak was run on the Superose 12 column. A single peak was observed with a K_{av} corresponding to that of monomeric growth hormone. We concluded therefore that S-200 HR gel filtration proved to be an efficient purification technique for iodinated growth hormone.

Serum Incubation

Human serum samples were incubated immediately after purification of the tracer. Equilibrium was reached after 2 hours of incubation at room temperature and did not change for up to 40 hours (Figure 2). For practical reasons an overnight incubation (16 - 18 hours) at room temperature was chosen as standard condition. Maximally, 7 serum samples were incubated from each subject; since the chromatography of one sample takes 1 hour and 45 minutes, the minimum and maximum incubation periods were 16 and 27 hours respectively. As shown in Figure 2, equilibrium is unaffected by such variation of incubation times. To ascertain this, serum samples from six subjects were incubated with iodinated hGH in duplicate. One of the serum samples was chromatographed at the beginning of the gel filtration series and the other aliquot was run at the end i.e. 10 hours later. The results of the second measurements were 101.8 ± 1.5% of those of the first experiments.

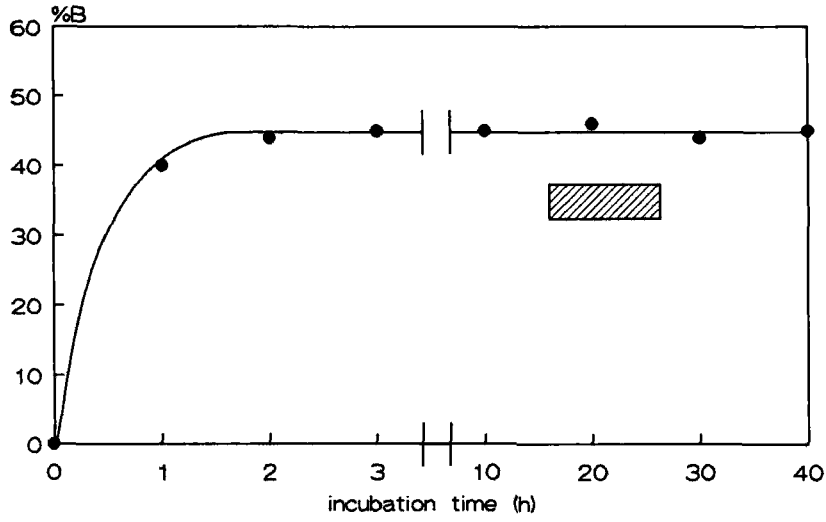

FIGURE 2. Binding percentage of ^{125}I-hGH in plasma as a function of incubation time at room temperature. The interval in which the analysis was performed is indicated by the hatched block.

Gel Chromatography

Elution of the Superose 12 column yielded 2 peaks; peak I appeared to be the ^{125}I-hGH-BP complex and peak II the free monomeric ^{125}I-hGH (Figure 3). The percentage of bound tracer of each serum sample was determined by integration of the first peak (fraction 25 - 29). Incubation with increasing amounts of radio-inert hGH resulted in a gradual decrease of the binding percentage due to displacement of ^{125}I-hGH by unlabeled hGH. Incubation with 1 mg hGH/l resulted in subtotal and incubation with 10 mg hGH/l in total displacement of labeled hGH. After incubation with 10 mg hGH/l a binding percentage (%B) 14.1 ± 3.1%, (n = 51) was still found; we tentatively attributed this to binding of ^{125}I-hGH to the lower affinity I-GHBP. The B/F ratio obtained by incubation with 10 mg hGH/l was used for the correction of the Scatchard plot (see data analysis).

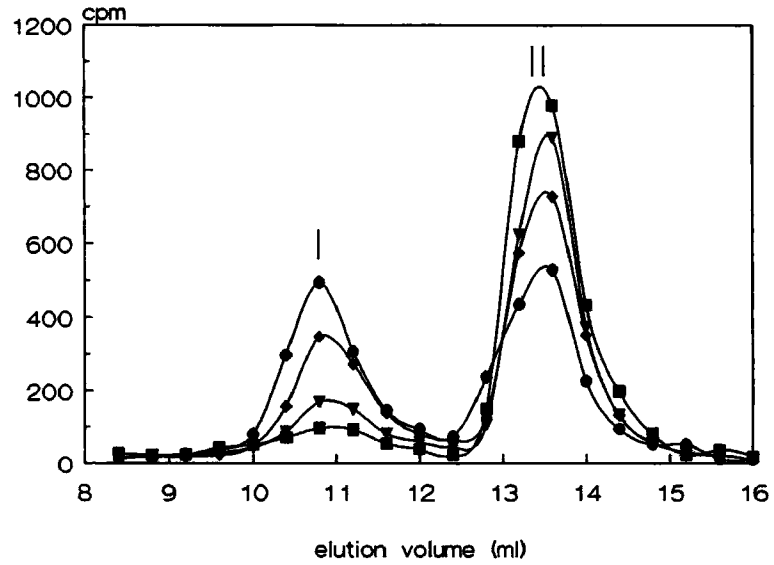

FIGURE 3. Superose 12 elution profile of a serum sample incubated with ^{125}I-hGH alone (○) and displacement of ^{125}I-hGH from serum growth hormone binding protein by 10 (♦); 30 (▾) and 100 (■) ng/ml radioinert hGH.

The intra-assay binding percentage of samples without radio-inert hGH was found to be 48.3 ± 0.5% (n = 5; CV 1%); the intra-assay binding percentage after addition of 100 μg hGH/l to a serum sample of the same subject was 20.7 ± 0.9% (n = 4; CV 5%). To determine the intertest reproducibility, maximal binding capacities of several assays on a single specimen were compared; thus the inter-assay maximal binding capacity was 860 ± 103 fmol/ml (n = 4 ; CV 12%).

Binding Capacity and Affinity of II-GHBP

Scatchard plot analysis of the binding data yielded a curvilinear plot, due to the presence of the second binding protein (I-GHBP) for human growth

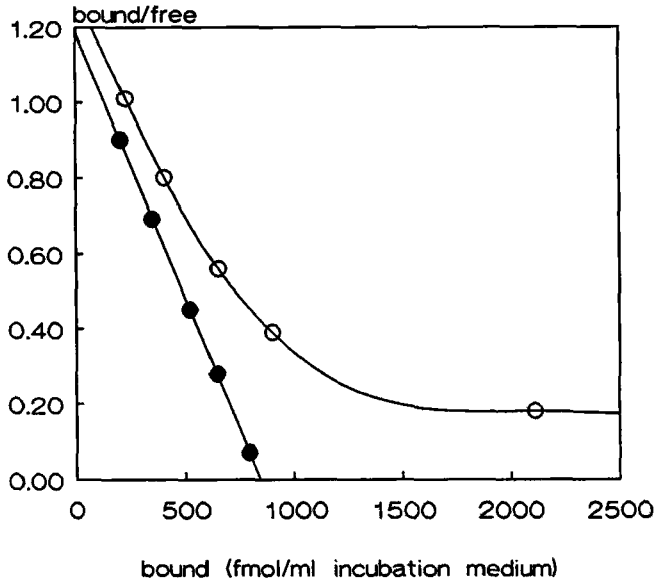

FIGURE 4. Scatchard plot before (○) and after (●) graphic correction (see text). MBC 842 fmol/ml serum; K_a 0.47 nM^{-1}.

hormone. The affinity of I-GHBP is much lower than that of the II-GHBP. Using the correction method, described in the materials and methods section, we obtained linear Scatchard plots for the binding of hGH to II-GHBP (Figure 4).

The results of Scatchard plot analysis are shown in Figure 5. Statistical evaluation by the Wilcoxon's test for two pairs showed a significant difference in maximal binding capacity (MBC) between female and male subjects ($p<0.01$). The MBC for female sera was 1350 ± 530 fmol/ml ($n = 29$) and the MBC for male sera was 900 fmol/ml ± 205 fmol/ml ($n = 22$). A significant difference was also found between the affinity constant (K_a) of female and male subjects ($p < 0.01$): the K_a for female sera was 0.31 ± 0.07 nM^{-1} and the K_a for male sera was 0.43 ± 0.11 nM^{-1}.

Although the differences between the different age groups were small and a large variation was observed, women aged 36 to 45 years had lower II-

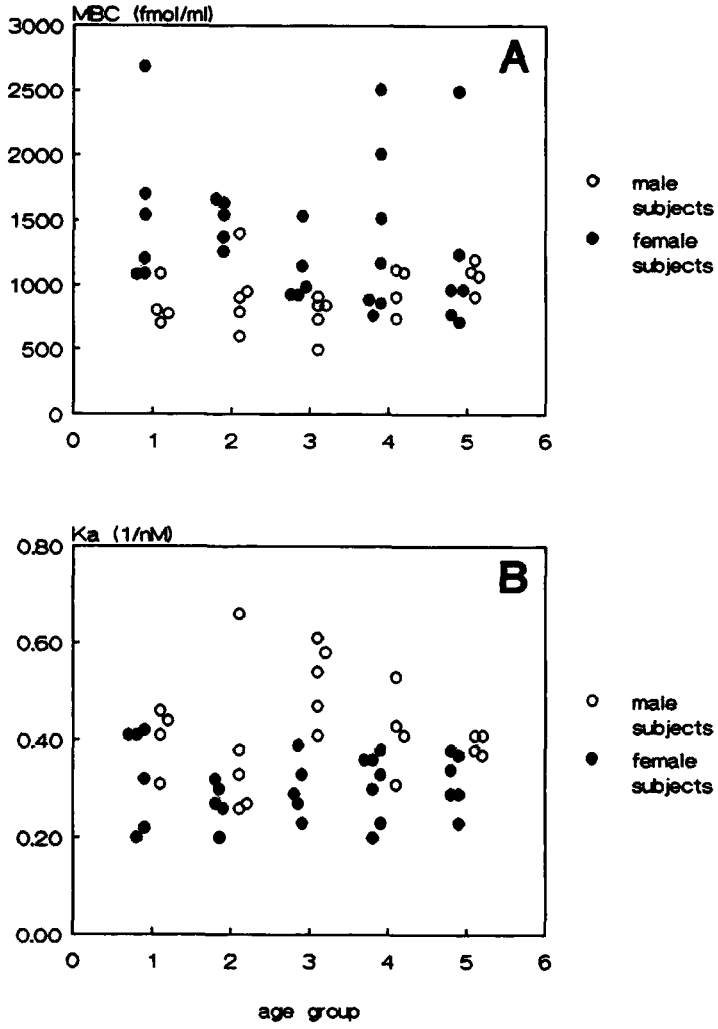

FIGURE 5. Binding capacity (A) and affinity (B) of GHBP-I in serum of healthy human subjects of different age groups: 1: ≤ 25 y; 2: 26 - 35 y; 3: 36 - 45 y, 4: 46 - 55 y; 5: ≥ 56 y.

GHBP levels than women aged 20 to 35 years ($p<0.03$); a statistically significant difference in MBC was also found between women aged 20 to 35 years and women aged $\geq$ 55 years ($p < 0.05$). II-GHBP levels of men aged 20 to 35 years were not singificantly lower than that of men aged 36 to 45 years or aged $\geq$ 55 years.

Women aged 20 to 35 and 36 to 45 years were found to have higher II-GHBP levels than men of comparable age ($p<0.03$ and $p<0.01$ respectively). The affinity constant of male subjects aged $\geq$ 36 years was higher than that of female subjects ($p<0.03$). Within both sexes, the K_a was found to be independent of age.

DISCUSSION

After the isolation of a growth hormone-binding protein by Baumann *et al* (4) and Herington *et al* (5), much research concerning this binding protein (II-GHBP) has been performed. Gel chromatographic separation of free and bound ligand is one of the most essential methods, used in the studies published thus far. Most gel chromatographic methods are time consuming and cumbersome e.g. on large columns containing Sephadex G-100 or G-200. More rapid gel filtration techniques, such as AcA 44 minicolumns, only partially separate free and bound ligand (5). Tar *et al* (21) described gel filtration on a HPLC Protein Pak 300sw column as a rapid and reproducible method for measuring both growth hormone-binding proteins (21).

As reported here Superose 12 gel filtration in a FPLC-system provides a rapid sample analysis; 45 minutes after application of the sample to the column, sample analysis is completed. Under such circumstances, dissociation of the ^{125}I-hGH-II-BP complex during gel filtration, a problem mentioned earlier by Baumann *et al* (19), is of minor importance.

Although sample analysis is relatively rapid, this technique is not readily applicable to large series of plasma samples since Scatchard plot analysis requires multiple incubations for each specimen and extensive washing and re-

equilibration of the column is necessary between the runs. The FPLC-system automatically applies serum samples to the Superose 12 column, collects the fractions of interest, washes and re-equilibrates the column, so little manpower has to be invested in Scatchard plot analysis. However, automatic functioning of the FPLC-system requires incubation mixture volumes of 300 μl or more. Reliable Scatchard plot analysis thus requires 1.5 - 2.0 ml of serum. If only smaller serum volumes are available, the incubation mixture volume can be adapted, but in those cases manual injection of the samples on the column is necessary. Superose 12 gel filtration yields excellent separation of free ^{125}I-hGH and the ^{125}I-hGH-II-BP complex. With the current method separation of II-GHBP and I-GHBP complexes is not achieved.

Baumann *et al* (11) studied age and sex dependency of the II-GHBP. This study, however, was based on binding percentages (%B) of labeled hGH in plasma samples incubated without excess unlabeled hGH. They found a great variation in %B for both male and female subjects of different ages, but they concluded there was no significant difference in %B between men and women; only neonates had a significantly lower %B. Daughaday and Trivedi (13) noted a prominent, age-based variation in the concentration of II-GHBP. The II-GHBP levels increased in childhood and adolescence; the II-GHBP concentration was found to be lower in the elderly (60 - 70 years). Silbergeld *et al* (22) reported a progressive increase in serum II-GHBP levels with increasing age from early childhood to young adulthood, maximal values of II-GHBP concentration were found in young adults. Tar *et al* (21) found higher hGH-binding in adults than in children, though they were not able to detect a sex difference.
Herington *et al* (5) found an affinity of 0.32 $\pm$ 0.06 nM^{-1} for the II-GHBP. Baumann *et al* (4) reported a K_a of 0.2 - 0.3 nM^{-1} and a binding capacity of 20 ng/ml (= 910 fmol/ml).

Thus far, no studies concerning age and sex dependency of the affinity and binding capacity of the II-GHBP are reported. In our study we found a higher maximal binding capacity (MBC) for female than for male subjects (Figure 5A); this difference was mainly present in the age groups 1, 2 and 3 (i.e. $\leq$ 45 years). The MBC of postmenopauzal controls was lower than that of

premenopauzal women. In age group 4 a marked variation of the MBC was found. Two controls in group 4 had a MBC > 2000 fmol/ml. The findings suggest an oestrogen influence on II-GHBP levels, as is true for most of the other hormone binding proteins in human plasma.

The affinity constant (K_a) also showed a significant difference; in the older age groups (3, 4 and 5, i.e. > 36 years) male subjects had higher K_a-values than female subjects, however the hGH concentration was not statistically different. No difference in K_a between premenopauzal and postmenopauzal female controls could be found (Figure 5B).

We conclude that Superose 12 gel filtration in a FPLC-system is a technically sophisticated and highly effective method for the study of growth hormone binding properties of human plasma. Furthermore we suppose an oestrogen influence on the concentration and possibly on the conformation of the growth hormone-binding protein, though further research is necessary to verify this hypothesis.

REFERENCES

1. Hadden, D. R., and Prout, T. E., A growth hormone binding protein in normal human serum, Nature, 202, 1342, 1964.

2. Collipp, P. J., Kaplan, S. A., Boyle, D. C., and Shimizu, C. S. N., Protein-bound human growth hormone, Metabolism 13, 532, 1964.

3. Collipp, P. J., and Kaplan, S. A., Interaction of ^{14}C-labeled human and bovine growth hormone with serum proteins, Biochim. Biophys. Acta 117, 416, 1966.

4. Baumann, G., Stolar, M. W., Amburn, K. D., Barsano, C. P., and DeVries, B. C., A specific growth hormone-binding protein in human plasma: initial characterization, J. Clin. Endocrinol. Metab. 64, 134, 1986.

5. Herington, A. C., Ymer, S., and Stevenson, J. L., Identification and characterization of specific binding proteins for growth hormone in normal human sera, J. Clin. Invest. 77, 1817, 1986.

6. Baumann, G., Stolar, M.W., and Buchanan, T. A., The effect of circulating growth hormone-binding protein on metabolic clearance, distribution and degradation of human growth hormone, J. Clin. Endocrinol. Metab. 64, 657, 1987.

7. Baumann, G., Shaw, M. A., and Winter, R. J., Absence of the plasma growth hormone-binding protein in Laron-type dwarfism, J. Clin. Endocrinol. Metab. 65, 814, 1987.

8. Baumann, G., Amburn, K. D., and Shaw, M. A., The circulating growth hormone (GH)-binding protein complex: a major constituent of plasma GH in man, Endocrinology 122, 976, 1988.

9. Baumann, G., and Shaw, M. A., The second growth hormone-binding protein (peak I) in human plasma: characterization and partial purification (Abstract), Endocrinology 122(suppl), 304, 1988.

10. Baumann, G., and Shaw, M. A., Immunochemical similarity of the human plasma growth hormone-binding protein and the rabbit liver growth hormone receptor, Biochem. Biophys. Res. Commun. 52, 573, 1988.

11. Baumann, G., Shaw, M. A., and Amburn, K. D., Regulation of plasma growth hormone-binding proteins in health and disease, Metabolism 38, 683, 1989.

12. Daughaday, W. H., and Trivedi, B., Absence of serum growth hormone-binding protein in patients with growth hormone receptor deficiency (Laron dwarfism), Proc. Natl. Acad. Sci. USA 84, 4636, 1987.

13. Daughaday, W. H., Trivedi, B., and Andrews, B. A., The ontogeny of serum GH binding protein in man: a possible indicator of hepatic GH receptor development, J. Clin. Endocrinol. Metab. 65, 1072, 1987.

14. Moore, J., Vandlen, R., MacKay, P., and Spencer, S. A., Serum clearance of human growth hormone bound to growth hormone binding protein (Abstract), Endocrinology 122(suppl), 121, 1988.

15. Mannor, D. A., Shaw, M. A., Winer, L. M., and Baumann, G., Circulating growth hormone-binding proteins inhibit growth hormone (GH) binding to GH receptors but not in vivo GH action, Clin. Res. 36, 870A, 1988.

16. Leung, D. W., Spencer, S. A., Cachianes, G., Hammonds, R. D., Collins, C., Henzel W. J., Barnard, R., Waters, M. J., and Wood, W. I., Growth hormone receptor and serum binding protein: purification, cloning and expression, Nature 330, 537, 1987.

17. Smith, W. C., Kuniyoshi, J., and Talamantes, F., Mouse serum growth hormone (GH) binding protein has GH receptor extracellular and substituted transmembrane domains, Mol. Endocrinol. 3, 984, 1989.

18. Baumann, G., and Shaw, M. A., A second, lower affinity growth hormone-binding protein in human plasma, J. Clin. Endocrinol. Metab. 70, 680, 1990.

19. Baumann, G., Shaw, M. A., and Amburn, K. D., A rapid and simple assay for growth hormone binding protein activity in human plasma, Acta Endocr. 119, 529, 1988.

20. Rosenthal, H., A graphic method for the determination and presentation of binding parameters in a complex system, Anal. Biochem. 20, 525, 1967.

21. Tar, A., Hocquette, J.-F., Souberbielle, J.-C., Clot, J.-P., Brauner, R. and Postel-Vinay, M.-C., Evaluation of the growth hormone-binding proteins in human plasma using high pressure liquid chromatography gel filtration, J. Clin. Endocrinol. Metab. 71, 1202, 1990.

22. Silbergeld, A., Lazar, L., Erster, B., Keret, R., Teper, R., and Laron, Z., Serum growth hormone binding protein activity in healthy neonates, children and young adults: correlation with age, height and weight, Clin. Endocrinol. 31, 295, 1989.

23. Eshet, R., Laron, Z., Pertzelan, A., Arnon, R., and Dintzman, M., Defect of human growth hormone receptors in the liver of two patients with Laron-type dwarfism, Isr. Med. Sci. 20, 8, 1984.

24. Ymer, S., Stevenson, J. L., and Herington, A. C., Identification of a rabbit liver cytosolic binding protein for human growth hormone, Biochem. J. 221, 617, 1984.

25. Ymer, S., and Herington, A. C., Evidence for specific binding of growth hormone to a receptor-like protein in rabbit serum, Mol. Cell. Endocrinol. 41, 153, 1985.

26. Baumann, G., and Shaw, M. A., The circulating growth hormone binding proteins: partial purification and structural characterization by affinity cross-linking (Abstract), Clin. Res. 34, 949A, 1986.

27. Spencer, S. A., Hammonds, R. G., Henzel, W. J., Rodriguez, H., Waters, M. J., and Wood, W. I., Rabbit liver growth hormone receptor and serum binding protein, J. Biol. Chem. 263, 7862, 1988.

28. Baumann, G., Shaw, M. A., and Merimee, T. J., Low levels of high affinity growth hormone-binding protein in African pygmies, N. Engl. J. Med. 320, 1705, 1989.

29. Baumbach, W. R., Horner, D. L., and Logan, J. S., The growth hormone binding protein in rat serum is an alternatively spliced form of the rat growth hormone receptor, Genes Dev. 3, 1199, 1989.

30. Laron, Z., Klinger, B., Erster, B., and Silbergeld, A., Serum GH binding protein activities identifies the heterozygous carriers for Laron type dwarfism, Acta Endocr. 121, 603, 1989.

31. Snow, K. J., Shaw, M. A., Winer, L. M., and Baumann, G., Diurnal pattern of plasma growth hormone binding protein in man, J. Clin. Endocrinol. Metab. 70, 417, 1990.

32. Baumann, G., Growth hormone binding proteins (Review), Trends Endocrinol. Metab. 2, 342, 1990.

33. Spencer, S.A., Leung, D.W., Godowski, P.J., Glenn Hammonds, R., Waters, M.J. and Wood, W.I., Growth hormone receptor and binding protein, Recent Progress in Hormone Research, 46, 165, 1990.

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