# A Specific and Sensitive Radioimmunoassay for Human Choriogonadotropin

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A specific and sensitive radioimmunoassay for human choriogonadotropin (hCG) has been developed using rabbit antiserum to chemical analogs of beta subunit of human chorionic gonadotropin prepared by controlled reduction and S-alkylation of its disulfide linkages. The assay was highly specific for hCG as the binding of [$^{125}$I]-hCG to the antibody was not affected by standard human lutropin, by human male serum, postpartum serum from women, serum from post-menopausal women and human menopausal gonadotropin (Pergonal). The assay was highly sensitive, the minimal detection limit in terms of highly purified hCG (L-129, 12 500 IU/mg) being 1 ng/ml or 0.2 ng/tube (or 12 mlU/ml in terms of WHO 2nd international reference preparation of hCG). Using this assay we were unable to detect any immunoreactive hCG in human tissues like lung, liver and colon. The high specificity, sensitivity, accuracy and reproducibility of the assay make this a highly desirable radioimmunoassay for human choriogonadotropin.

Ever since radioimmunoassay for human choriogonadotropin (hCG) was developed [1], numerous attempts have been made to increase the specificity of hCG assay. Human choriogonadotropin, like other glycoprotein hormones, lutropin (LH), follitropin (FSH) and thyrotropin (TSH), consists of two dissimilar subunits designated $\alpha$ and $\beta$ [2]. Their $\alpha$-subunits are almost identical in amino acid sequences, differing only in the type of monosaccharides present and the structure of oligosaccharide units located at specific amino acid residues in the polypeptide chains [3]. Due to this extensive structural homology, antisera raised against native hCG invariably cross-react with human LH (hLH), human TSH (hTSH) and human FSH (hFSH). With the recognition that the $\beta$-subunits of glycoprotein hormone confer hormonal specificity and that the free $\beta$-subunit of hCG is a much more specific antigen than hCG, *anti*-hCG-$\beta$ sera have replaced *anti*-hCG sera in radioimmunoassays for

hCG. Although *anti*-hCG-$\beta$ sera are able to discriminate hCG from hLH to a greater extent than anti-hCG sera [4], they have about 10 to 30% cross-reaction with hLH. Because of this cross-reaction it is difficult to make accurate determinations of hCG in serum when the levels of the hormone are low. While the immunological cross-reactivity of anti-hCG sera with other glycoprotein hormones hFSH and hTSH is primarily due to the identical $\alpha$-subunit, that with hLH however is also the result of considerable structural homology in their $\beta$-subunits.

During the course of our studies on the structure-activity relationships in hCG-$\beta$, among the many chemically modified derivatives of hCG-$\beta$ tested, DS$_5$-hCG-$\beta$, the reduced and S-carboxyamidomethylated derivative in which 5 out of 6 disulfide bonds had been modified did not show cross-reactivity in the [$^{125}$I]-hLH-*anti*-hLH radioimmunoassay (RIA) system [5, 6]. It did, however, effectively inhibit the binding [$^{125}$I]-hCG to hCG antibodies [5, 6]. This observation relating to the selective destruction of hCG/hLH determinants by chemical modification

of hCG-$\beta$ and retention of hCG determinants in DS$_5$-hCG-$\beta$ suggested that immunization of rabbits against DS$_5$-hCG-$\beta$ might yield an antiserum specific to hCG. Such an antiserum was indeed produced in rabbits and shown to be highly specific to hCG by immunological and biological criteria [7]. Further, the affinity and titer of the antibody was high. Hence, the present work was undertaken to establish RIA using this antiserum for measuring hCG. The characteristics of this RIA and the results of its application to various clinical samples are described below.

## Materials and Methods

Highly purified hCG (L-129, 12500 IU/mg) was prepared from a crude commercial preparation of hCG with a potency of 3100 IU/mg (Organon, West Orange, New Jersey) essentially according to Bahl [8] except that a pH of 7.4 was maintained throughout the purification. Human LH (LER 960) was obtained from the National Pituitary Agency through the Hormone Distribution Officer of the National Institute of Arthritis, Metabolic and Digestive Diseases, the National Institutes of Health, Bethesda, USA. Pergonal (75 IU of FSH + 75 IU of LH/ampoule) was purchased from Serono Laboratories, USA. Carrier free Na$^{125}$I was obtained from the Radio-Chemical Center, Amersham. Bovine serum albumin (BSA) and bovine gamma globulin were purchased from Sigma Chemical Co., St. Louis, USA. All other chemicals were of certified analytical grade.

### Serum samples

Serum samples were obtained from pregnant women who came into clinics. Sera were also obtained from normal adult healthy male subjects, from women in post-partum stage (around one month after parturition) and from post-menopausal women.

### Tissue extracts

Tissues removed from human subjects at autopsy were immediately frozen in dry ice, transported to the laboratory and stored frozen at $-70\,°$C. They were extracted and assayed within a day after their removal from the body. Two extraction procedures were chosen. In the first procedure frozen tissues

were minced into small pieces before homogenization in a Potter-Elvehjem tissue grinder in 0.1 M sodium borate buffer, pH 8.4. The homogenates were centrifugated at $10000 \times g$ for 15 min at 4 °C and the supernatants were assayed for hCG by the RIA. The second procedure was essentially that of Yoshimoto *et al.* [9] with minor modifications. Briefly, the tissues were extracted with 5 volumes of glacial acetic acid using a Waring Blendor. The homogenates were kept in a shaking water bath at 80 °C for 30 min before centrifugation at $10000 \times g$ for 15 min at 4 °C. The supernatants were lyophilized. The lyophilized materials were suspended in 0.1 N HCl and kept stirred for 30 min at 20 °C followed by centrifugation at $5000 \times g$ for 15 min at 4 °C. The pH of the supernatants were adjusted to 7.5 to 8.0 using NaOH. The extracts were adjusted to 1 gm tissue/ml and then assayed in duplicate by the RIA.

### Production of antiserum

The details of the preparation of DS$_5$-hCG-$\beta$ and DS$_6$-hCH-$\beta$ by controlled and complete reduction and S-alkylation of hCG-$\beta$ have been described elsewhere [6]. Briefly, hCG was dissociated into $\alpha$- and $\beta$-subunits using 8 M urea and the subunits were separated by chromatography on DEAE-Sephadex [8]. The $\beta$-subunit was further purified by second urea treatment and chromatography on Sephadex-G 100. Residual contamination with hCG (as indicated by radioreceptor assay) in hCG-$\beta$ was removed by treating hCG-$\beta$ with an immunoadsorbent of anti-hCG-$\alpha$ serum prepared by coupling the $\gamma$-globulin fraction of this serum to CNBr-activated Sepharose [10]. Subsequently the hCG-$\beta$ subunit was reduced partially (5 out of 6 disulfide bonds to prepare DS$_5$-hCG-$\beta$) with dithioerythritol followed by S-alkylation of the free SH groups with iodoacetamide [6]. The DS$_5$-hCG-$\beta$ and DS$_6$-hCG-$\beta$ preparations were then desalted on a column of Sephadex-G-25. The DS$_5$-hCG-$\beta$ was treated with an immunoadsorbent of *anti*-LH-serum to remove any unreduced hCG-$\beta$ [5]. The purified DS$_5$-hCG-$\beta$ was conjugated to hemocyanin and the resulting conjugate was used as an immugen to generate antibodies in rabbits by periodic intramuscular injections. The antigen was emulsified with Freund's complete adjuvant before each injection. The rabbits were boosted either with DS$_5$-

hCG-$\beta$ or DS$_6$-hCG-$\beta$. Hence the antiserum will be hereafter referred to as DS$_5$, DS$_6$-hCG-$\beta$-hem serum. Periodically samples of blood were drawn from the ear vein and serum collected. 200 µl of serum at various dilutions (1:10 to 1:10000) was incubated with [$^{125}$I]-hCG for 1 h at 37 °C followed by the addition of 100 µl of 1% bovine gamma globulin solution and 1 ml of 10% ammonium acetate in ethanol. The tubes were kept for 1 h at 4 °C, then centrifuged, supernatants decanted, residual liquid removed by wiping with filter paper strips and finally pellets were counted for antibody bound radioactivity. The dilution of the antiserum binding 20% of the [$^{125}$I]-hCG (40 − 50 µCi/µg) was chosen for RIA. The radioimmunoassay was routinely conducted with 1:5000 diluted *anti*-DS$_5$, DS$_6$-hCG-$\beta$-hem serum.

### Radioiodination and radioimmunoassay

The radioiodination of highly purified hCG was performed by the chloramine-T procedure of Greenwood *et al.* [11] with minor modifications. To 10 µg of hCG in 10 µl of water was added 20 µl of 0.5 M sodium phosphate buffer, pH 7.2 followed by 0.5 mCi of Na$^{125}$I (10 µl). Reaction was initiated by the addition of 10 µl of a 1 mg/ml solution of chloramine-T in 0.1 M phosphate buffer pH 7.2 followed 45 s later by 10 µl of a 2 mg/ml solution of sodium metabisulfite to stop the reaction. The reaction mixture was loaded onto a Sephadex G-75 column (1.0 × 30 cm) to separate [$^{125}$I]-hCG from free iodide. The peak fraction was frozen immediately and stored frozen. Tubes employed for collecting the effluent from the column contained 0.5 ml of 2% BSA to prevent denaturation of [$^{125}$I]-hormone. The specific activity of [$^{125}$I]-hCG ranged between 40 − 50 µCi/µg.

The radioimmunoassay was performed essentially as per the procedure of Bellisario and Bahl [12]. Briefly, 200 µl of serial dilutions of standard hCG (100 ng/ml to 0.195 ng/ml), or serum samples or tissue extracts were incubated with 200 µl of 1:5000 diluted *anti*-DS$_5$, DS$_6$-hCG-$\beta$-hemserum and 200 µl of [$^{125}$I]-hCG (50000 cpm) for 1 h at 37 °C followed by 16 h at 4 °C. All dilutions were done in 0.1 M sodium borate buffer pH 8.4 containing 0.5% BSA (hereafter referred to as RIA buffer). At the end of the incubation 100 µl of 1% bovine gamma globulin prepared in RIA buffer were added to all

tubes followed by 1.4 ml of 10% ammonium acetate in ethanol (resulting in 6.6% of final concentration of ammonium acetate and 66% of final concentration of ethanol). The tubes after vortexing were kept at 4 °C for 1 h followed by centrifugation at 2000 × $g$ for 15 min at 4 °C. The supernatants were then decanted, and the traces of liquid on the walls of the tubes were removed by wiping with paper strips. The pellets were counted for antibody bound radioactivity. Suitable controls without antiserum (for standards) and without antiserum but with sera or tissue extracts (for unknown sera and tissue extracts) were also routinely included in the assay. The results were expressed as B/B$_0$ × 100 and plotted against log dose of hCG standards. B$_0$ represents cpm radioactivity bound in the absence of any competing antigen and B represents cpm radioactivity bound in the presence of various levels of competing standard hCG or unknown sample. The radioimmunoassay data were also analyzed by a computer, Wang Model 2200, using program RIADS-2200, based on the method developed by Rodbard and Lewald [13].

## Results

### Specificity of the radioimmunoassay

The specificity of the present radioimmunoassay for hCG was evaluated in a number of ways. When serum samples from adult human male volunteers and from post-partum women were assayed for hCG, there was no detectable hCG (data not shown). These samples are known to have normal physiological levels of hLH which apparently did not interfere in the assay. In an extension of this study serum samples and urinary concentrates containing high levels of hLH were also tested in the present RIA. It was found that neither post-menopausal women's sera nor Pergonal, a commercial preparation of human menopausal urinary gonadotropin had any detectable hCG (data not shown). The sera were always assayed in duplicate or triplicate and at various doses (*e.g.* normal serum and 1:2, 1:4, 1:8, 1:16 and 1:32 or higher dilutions). In order to know whether the present RIA offers any advantage over the conventional RIA using *anti*-hCG-$\beta$ serum with regard to the interference by hLH, both the RIAs were performed with hLH as standard. As indicated in Fig. 1, hLH inhibits the
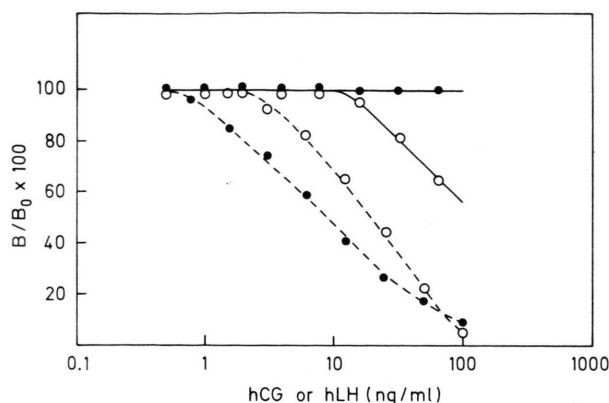
Fig. 1. Comparison of the radioimmunoassays using *anti*-hCG-$\beta$ and *anti*-DS$_5$, DS$_6$-hCG-$\beta$-hem sera. RIAs were performed using [$^{125}$I]-hCG-*anti*-hCG-$\beta$ and [$^{125}$I]-hCG-*anti*-DS$_5$, DS$_6$-hCG-$\beta$-hem systems. In both systems hCG and hLH were employed separately as standards in the dose range indicated in the figure. See text for conditions of assay. Results are express as B/B$_0$ × 100 vs log dose of the competing antigen. The value of B$_0$ ranged from 7000−8000 cpm corresponding to 20% to 22% binding. The *anti*-hCG-$\beta$ and *anti*-DS$_5$, DS$_6$-$\beta$-hem sera were used at 1:10000 and 1:5000 dilutions respectively. ○---○ standard hCG with *anti*-hCG-$\beta$ serum; ○—○ standard hLH with *anti*-hCG-$\beta$ serum; ●---● standard hCG with *anti*-DS$_5$-hCG-$\beta$-hem serum and ●—● standard hLH with *anti*-DS$_5$-hCG-$\beta$-hem serum.

binding of [$^{125}$I]-hCG-$\beta$ to *anti*-hCG-$\beta$ serum at 20 ng/ml or higher concentrations. However, in the RIA using *anti*-DS$_5$, DS$_6$-hCG-$\beta$-hem serum, hLH did not interfere at the level of 1−100 ng/ml tested (Fig. 1).

*Sensitivity of the RIA*

The radioimmunoassays were routinely performed using 1:5000 diluted *anti*-DS$_5$, DS$_6$-hCG-$\beta$-hem serum. The sensitivity of the RIA was high as the minimal detection limit was 0.2 ng/tube or 1 ng/ml (using a highly purified hCG preparation, L-129 as standard). Using WHO international reference preparation, this amounted to 12 mIU/ml. The precision of measurement of each point on the standard curve is given in Table I. The standard deviations about each point indicates that the present RIA is characterized by good precision and high sensitivity with a reproducible standard curve.

*Measurement of hCG in serum samples*

Initially, the RIA was characterized by measuring hCG in three pools of sera containing high, middle

and low levels of hCG. Each of the samples was assayed at 4 to 5 dose levels and in triplicate at each dose level. Assay was repeated for each sample on different days in order to determine intra-assay and inter-assay variations. Results were expressed as coefficient of variation $\left(\dfrac{\text{S.D.}}{\text{mean}} \times 100\right)$. The intra-assay variations for each of the samples are indicated under Table II. The low pool samples was assayed at 1:2, 1:4, 1:5 dilutions, the middle pool at 1:5, 1:10, 1:20 dilutions, and high pool at 1:20, 1:40, 1:50, 1:100 dilutions. The intra-assay and inter-assay coefficients of variation were determined both from these three pools of sera and from sera from pregnant women at various gestational ages.

Table I. Characteristics of the hCG radioimmunoassay system using *anti*-DS$_5$, DS$_6$-hCG-$\beta$-hem serum. See text for details of the assay.

| Precision<br>Dose of the standard<br>hCG (L-129, 12500 IU/mg)<br>[ng/ml] | B/B$_0$ × 100<br>(Mean ± S.D.) |
| --- | --- |
| 100 | 9.57 ± 0.405 |
| 50 | 17.15 ± 0.710 |
| 25 | 27.78 ± 1.18 |
| 12.5 | 46.40 ± 6.45 |
| 6.25 | 59.20 ± 0.41 |
| 3.125 | 76.30 ± 2.17 |
| 1.56 | 84.10 ± 1.9 |
| 0.78 | 96.30 ± 3.15 |
| 0.39 | 98.16 ± 1.15 |
| 0.195 | 97.90 ± 1.60 |
| Sensitivity<br>(Minimal detection limit) | 1.5 ng/ml |
| ED$_{50}$ | 9.2 ng/ml |

Table II. Intra- and Inter assay coefficient of variation of the radio-immunoassay using *anti*-DS$_5$, DS$_6$-hCG-$\beta$-hem serum. See the text for details of the assay.

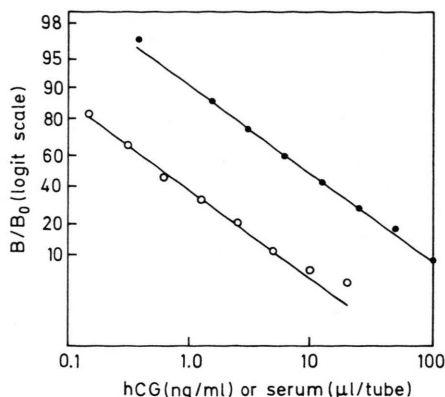| | hCG [ng/ml] | C.V. [%] |
| --- | --- | --- |
| Intra-assay coefficient of variation (C.V.) | | |
| Low pool sample | 4.25 | 11.2 |
| Middle pool sample | 29.50 | 13.6 |
| High pool sample | 1385.00 | 6.96 |
| Inter-assay coefficient of variation (C.V.) | | |
| Sample A | 9055 | 6.94 |
| Sample B | 8625 | 8.56 |
| Sample C | 5155 | 6.70 |
| Sample D | 2433 | 16.60 |

Fig. 2. Parallelism between the radioimmunoassay plots obtained with standard hCG (L-129, 12500 IU/mg ●—●), and a pooled serum sample (○—○) containing hCG. RIA was performed as described in the text using [$^{125}$I]-hCG and *anti*-DS$_5$, DS$_6$-hCG-$\beta$-hem system. Results were expressed as B/B$_0$ × 100 (logit scale) *vs* log dose hCG (ng/ml) or volume of serum used for the assay.

As indicated in Table II, these values ranged from 6.9% to 16.6%.

In order to further characterize the RIA with regard to realiability, serial dilutions of a pooled serum sample of hCG (at 1:10 to 1:5120 dilutions) were assayed in triplicate. The inhibition curve obtained was compared to the standard inhibition curve obtained with hCG. As indicated by Fig. 2, the immunoreactive material in the serum gave an inhibition curve parallel to that given by standard hCG indicating that hCG like material was being measured in the serum sample.

Subsequently, sera collected from pregnant women at various stages of gestation were assayed for hCG levels. Assays were always conducted in duplicate or triplicate for each sample and at more than one dose level. The results (not presented here) are generally in agreement with the reported circulatory levels of hCG during gestation in women [1, 14].

*Application to tissues extracts*

Extracts of tissues removed at autopsy from two apparently "normal" human subjects were assayed for possible presence of hCG-like material in view of the recent reports that such a material was present in many normal, non-cancerous human tissues [9, 15, 16]. The results indicated that no cross-reactive material was detectable at the levels (data not

shown). In contrast, when an extract of human term placenta was used as a control, an expected quantity of hCG was found to be present (data not shown).

**Discussion**

The existing radioimmunoassays based on *anti*-hCG-$\beta$ for the quantitation of hCG in serum samples suffer from a drawback in that hLH interferes in the assays to varying extent [17]. Owing to the structural homology between hCG-$\beta$ and hLH-$\beta$ [5] it is not surprising to find such a cross-reaction. The specificity of an RIA is a function of the specificity of the antibody or that of the antigen used to raise the antibody. HCG or hCG-$\beta$ does not generally elicit such specific antibodies. Consequently, in order to prepare specific antigen(s) from hCG-$\beta$, two approaches have been taken. Since one of the major differences between hLH-$\beta$ and hCG-$\beta$ is the presence of a unique 30 amino acid residue peptide at the carboxy-terminus of the latter, one approach involved the use of this peptide as an antigen to elicit specific antibodies. The second approach was based on the use of the chemically modified analogs of hCG-$\beta$ as antigens [23]. The *anti*-carboxyterminal peptide sera were found to vary in their specificity, sensitivity and in their ability to neutralize hCG depending on the length of the peptide used as an antigen. Peptides of 35 amino acid residues (residues 111—145) or longer yielded antisera which were able to neutralize the biological activity of hCG [18] and thus appear to have potential in fertility regulation. Peptides shorter than 35 amino acid residues (residues 116—145 or less) generate specific antibodies of low titer and lack the ability to neutralize hCG. In general, the specific antisera for hCG and therefore, the RIA based on these antisera are not sensitive enough to detect early pregnancy.

The second approach of using a chemically modified form of hCG-$\beta$ for immunization yielded promising results. It was earlier shown by us that among the various derivatives of hCG-$\beta$ prepared and tested, DS$_5$-hCG-$\beta$ did not interfere in the [$^{125}$I]-hLH-*anti*-hLH RIA system [5]. The rabbit antibodies to this analog of hCG-$\beta$ were specific to hCG as shown by the lack of cross-reactivity with other hormones like hCG-$\alpha$, hTSH, hPRL, hFSH in

the RIA [7]. The immunogeneity of these derivatives in which the tertiary structure has been affected to a great extent was reduced considerably when compared to that of native hCG-$\beta$ subunit. Hence, the derivative was conjugated to key hole limpet haemocyanin and the conjugate was used as immunogen. The boosting was done with the derivative alone in order to increase the antibody population against the antigenic determinant(s) on the hormone rather than against the haemocyanin. Because of the reasonably high titers achieved now, it was considered useful to characterize the *anti*-DS$_5$, DS$_6$-hCG-$\beta$-hem-[$^{125}$I]-hCG RIA system further and apply it to the measurement of hCG in blood and tissues. The minimal detection limit of the RIA was $10-12$ mIU/ml which is much lower than what has been achieved in RIA using *anti*-carboxy-terminal peptide sera. The sensitivity of the assay, if neeed, can be further enhanced by using [$^{125}$I]-hCG of higher specific radioactivity $(80-100 \mu Ci/\mu g)$ than the one employed here $(40-50 \mu Ci/\mu g)$. Matsuura *et al.* [19] using antisera H-93 and H-114 which were raised against the natural carboxy-terminal peptide isolated from hCG-$\beta$ subunit and the synthetic N-$\alpha$-acetyl triaconatpeptide analogous to the carboxy-terminal region (residues $116-145$) respectively reported a minimal detection limit of 19.4 and 31.5 ng/ml respectively.

The specificity of the present RIA for hCG/hCG-$\beta$ is indicated by the fact that hLH in any form either as standard (up to 100 ng/ml), in serum (normal male serum or post-menopausal women's serum) or as a urinary extract (Pergonal, up to 50 mIU of hLH and hFSH) did not interfere in the assay. From our preliminary studies, it appears that the antibody to DS$_5$, DS$_6$-hCG-$\beta$-hem is directed against an antigenic site in the N-terminal region [23]. Apparently, this seems to be the molecular basis underlying the specificity of the *anti*-DS$_5$, DS$_6$-hCG-$\beta$.

Using RIAs involving antisera to carboxy-terminal peptides, hCG or hCG-like materials have been detected in a variety of non-trophoblastic and non-cancerous tissues including human pituitary glands [9, 15, 16]. Although McManus *et al.* [20] could not detect hCG or hCG-like materials in normal human tissues, these authors and others [21] using peroxidase labelled antibodies or immunofluorescence techniques have been successful in locating such materials on many tumor cell membranes. We have examined liver, colon and lung tissues from two apparently "normal" individuals (accident victims) with our RIA and have not found hCG or hCG-like material at the tissue levels tested. It may be added that the negative results are not due to the two procedures used for the extraction of the tissues since the human term placenta extracted similarly gave values which were in agreement with those reported by others [22]. Based on these limited studies, one cannot be sure of the presence or absence of hCG or hCG-like materials in normal human tissues. This question can only be resolved unequivocally by examining a large pool and by the demonstration of hCG or hCG-like material by further physicochemical and biological characterization. The present RIA system involving *anti*-DS$_5$, DS$_6$-hCG-$\beta$-hem serum offers a considerable advantage in specificity, sensitivity and reproducibility over the other RIA systems using *anti*-hCG-$\beta$ or *anti*-peptide sera. Consequently, not only it should prove to be highly useful in the early detection of pregnancy but also in the early diagnosis and the treatment of certain malignancies.

[1] D. P. Goldstein, T. Aono, M. L. Taymor, R. Jochelson, R. Todd, and E. Hines, Am. J. Obst. Gynecol. **102**, 110 – 114 (1968).
[2] N. Swaminathan and O. P. Bahl, Biochem. Biophys. Res. Comm. **40**, 422 – 427 (1970).
[3] R. Bellisario, R. B. Carlsen, and O. P. Bahl, J. Biol. Chem. **248**, 6796 – 6809 (1973).
[4] G. T. Ross, Am. J. Obst. Gynecol. **129**, 795 – 805 (1977).
[5] O. P. Bahl, M. R. Pandian, and R. D. Ghai, Biochem. Biophys. Res. Commun. **70**, 525 – 532 (1976).
[6] R. D. Ghai, T. Mise, M. R. Pandian, and O. P. Bahl, Endocrinology **107**, 1556 – 1563 (1980).
[7] M. R. Pandian, R. Mitra, and O. P. Bahl, Endocrinology **107**, 1564 – 1571 (1980).

[8] O. P. Bahl, J. Biol. Chem. **244**, 567 – 574 (1969).
[9] Y. Yoshimoto, A. R. Wolfsen, and W. D. Odell, Science **197**, 575 – 576 (1977).
[10] P. Cuatrecausas and C. B. Anfinsen, In Methods in Enzymology, W. B. Jakoby (Ed.) Academic Press, **Vol. 22**, pp. 345 – 378 (1971).
[11] F. C. Greenwood, W. M. Hunter, and J. S. Glover, Biochem. J. **89**, 114 – 123 (1963).
[12] R. Bellisario and O. P. Bahl, J. Biol. Chem. **250**, 3837 – 3844 (1975).
[13] D. Rodbard and J. E. Lewald, Acta Endocr. **64**, (Suppl. 147) 79 – 98 (1976).
[14] K. G. Post, A. K. Roy, L. L. Cederquist, and B. B. Saxena, J. Clin. Endocrinol. Metab. **50**, 169 – 175 (1980).

[15] H. C. Chen, G. D. Hodgen, S. Matsuura, L. J. Lin, E. Gross, L. E. Reichert, S. Birken, R. E. Canfield, and G. T. Ross, Proc. Natl. Acad. Sci (US) **73**, 2885–2889 (1976).
[16] G. D. Braunstein, J. Rasor, and M. E. Wade, New Engl. J. Med. **293**, 1339–1343 (1975).
[17] J. L. Vaitukaitis, G. T. Ross, G. D. Braunstein, and P. L. Rayford, Rec. Prog. Horm. Res. **32**, 289–321 (1976).
[18] V. C. Stevens, Immunological Aspects of Reproduction and Fertility (J. Hearn. Ed.) MTP Press, London 1980.
[19] S. Matsuura, M. Ohashi, H. C. Chen, and G. D. Hodgen, Endocrinology **104**, 396–401 (1979).

[20] L. H. McManus, M. A. Naughton, and Martinex-Fernandez, Cancer Res. **36**, 3476–3479 (1976).
[21] H. F. Acevedo, M. Shliftrin, G. R. Pouchet, M. Rachsham, In Proceedings of the Third International Symposium on Detection and Prevention of Cancer, H. E. Nilbert (Ed.), Marcel Dekker, New York 1977.
[22] H. Bohn, In Proteins of Biological Fluid (H. Peeters, Ed.), **Vol. 24,** pp. 117, Pergamon Press, Oxford, New York 1976.
[23] O. P. Bahl and K. Muralidhar, In Immunological Aspects of Infertility and Fertility Regulation (D. S. Dhindsa and G. F. B. Schumacher, eds.) Elsevier North Holland Inc. New York, pp. 225 (1980).