Human Chorionic Gonadotropin

I. PURIFICATION AND PHYSICOCHEMICAL PROPERTIES*

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SUMMARY

A highly active preparation of human chorionic gonadotropin has been obtained by simple methods of column chromatography on diethylaminoethyl Sephadex and Sephadex G-100. The preparation appeared homogeneous by disc and immunoelectrophoresis, ultracentrifugation, and end group analysis. It assayed at 12,000 i.u. per mg by the rat prostrate gland and accessory sex organ method. The \( \varepsilon_{20, v} \) value was found to be 2.89 S. A molecular weight of 59,000 \( \pm \) 4,000 was determined by gel filtration on Sephadex G-150. However, the sedimentation equilibrium method gave a value of 47,000 \( \pm \) 3,000. The molecular weight of the reduced carboxamidomethylated human chorionic gonadotropin, determined by gel filtration, was 30,000 \( \pm \) 2,000, indicating that human chorionic gonadotropin was probably composed of two polypeptide chains. The minimal molecular weight, computed from the chemical composition, was 27,000. The amino acid and carbohydrate composition of the hormone has been determined.

Human chorionic gonadotropin is a glycoprotein hormone discovered by Aschheim and Zondek in 1927 (1). Since then, several investigators have attempted to purify the hormone from pregnancy urine (2-4) and from urine of patients with trophoblastic tumors (5, 6). Got and Bourillon (2) obtained a highly active preparation with a biological activity of 12,000 i.u. per mg from pregnancy urine. This preparation was homogeneous by free boundary, starch, and immunoelectrophoresis and by ultracentrifugation. Their procedure used adsorption on benzoic acid, extraction, and fractional precipitation with ethanol, adsorption on kaolin, and either chromatography on Decalco and Dowex 2 ion exchange resin or starch electrophoresis. Another preparation of HCG was obtained by Reisfeld and Hertz (5) from the urine of patients with trophoblastic tumors. Although this preparation had biological potency higher than that of Got and Bourillon (2), it showed heterogeneity on free boundary electrophoresis. The isolation procedure involved adsorption on kaolin, DEAE-cellulose chromatography, and adsorption on BaCO\(_3\). More recently, Wilde and Bhagshawe (6) have reported the isolation of HCG from a similar source. Their preparation was immunologically homogeneous and also assayed biologically at 14,000 i.u. per mg and immunologically at 5,900 i.u. per mg. They extracted HCG from urine with benzoic acid and subjected the crude material thus obtained to DEAE-cellulose chromatography, column electrophoresis, gel filtration, and, finally, DEAE-Sephadex chromatography. Van Hall et al. (3) have been able to obtain an electrophoretically heterogeneous and immunologically homogeneous preparation of HCG from normal pregnancy urine with a biological activity of 18,800 i.u. per mg. Their procedure involved ethanol fractionation, column chromatography on carboxymethyl Sephadex, and gel filtration on Sephadex G-200 or G-100.

Although some physicochemical properties of these preparations have been reported (2-6), no detailed structural investigations have been carried out. Present investigation was undertaken with the object of isolating, on a large scale, a preparation of HCG which would be suitable for studies on the primary structure of the hormone. A simple procedure has been devised yielding a highly active preparation of HCG, homogeneous by ultracentrifugation and by disc and immunoelectrophoresis. Studies on the molecular weight, end group analysis, and amino acid and carbohydrate composition of the hormone are reported.

MATERIALS AND METHODS

HCG, Lot 90679, of potency 3200 i.u. per mg, was purchased from Organon, Inc. (West Orange, New Jersey). Ferritin and alkaline phosphatase were purchased from Mann. Crystalline ovalbumin was obtained from Calbiochem. Bovine serum albumin was supplied by Nutritional Biochemicals. Ribonu-

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† The abbreviations used are: HCG, human chorionic gonadotropin; ICSH, interstitial cell-stimulating hormone.
clase, pepsin, leucine aminopeptidase, and diisopropyl fluorophosphate-treated carboxypeptidase were procured from Worthington. DEAE-Sephadex A-50, Sephadex G-100, and G-150 were products of Pharmacia.

**Purification of HCG**

All steps of purification were carried out at 4° unless otherwise specified. Eluates from columns were monitored for protein content by measuring absorbance at 280 nm (7) with a Beckman DU spectrophotometer and for carbohydrate content by the phenol-sulfuric acid method (8).

**FIG. 1. Chromatography of crude HCG on DEAE-Sephadex A-50.** A 4.84-g sample of crude HCG in 25 ml of 0.02 M Tris-phosphate buffer, pH 8.7, was applied to a column (5 X 100 cm) of DEAE-Sephadex A-50 in 0.04 M Tris-phosphate buffer. The column was eluted in a stepwise discontinuous gradient. Elution was started with 0.04 M Tris-phosphate buffer, pH 8.7, and was changed to 0.1 M NaCl and 0.2 M NaCl-0.04 M Tris-phosphate buffer, pH 8.7, and finally 0.2 M NaCl-0.04 M Tris-phosphate, pH 9. Fractions of 10 ml were collected. ↓, where buffer changes were made; — — , fractions pooled; — — — , absorbance at 280 nm; — — — — , absorbance at 490 nm, phenol-sulfuric acid test for carbohydrates.

**FIG. 2. Chromatography of HCG from Step 1 on DEAE-Sephadex A-50.** A 1.2-g sample of partially purified HCG in 5 ml of 0.04 M Tris-phosphate buffer, pH 8.3, was applied to another column of DEAE-Sephadex A-50 (2.5 X 100 cm), previously equilibrated with the above buffer containing 0.1 M NaCl. The column was initially eluted with 400 ml of a linear gradient between 0.1 M NaCl and 0.2 M NaCl in 0.04 M Tris-phosphate buffer. Following the gradient, the elution was continued with 0.2 M NaCl-0.04 M Tris-phosphate buffer. Fractions of 8.5 ml were collected. Fig. 2 indicates the fractions pooled.

**FIG. 3. Gel filtration of HCG on Sephadex G-100.** A 1.0-g sample of HCG from Step 2, in 5 ml of 0.05 M sodium phosphate buffer, pH 7.5, was applied to a column of Sephadex G-100 (2.5 X 100 cm). The column was eluted with the same buffer and 6.5-ml fractions were collected. — — , absorbance at 280 nm; — — — , absorbance at 490 nm, phenol-sulfuric acid test for carbohydrates; — — — , fractions pooled.

**Step 1:** Chromatography on DEAE-Sephadex—A column of DEAE-Sephadex A-50 (5 X 100 cm) was equilibrated in a 0.04 M Tris-phosphate buffer, pH 8.7. A solution of 4.84 g of commercial HCG in 25 ml of 0.02 M Tris-phosphate buffer, pH 8.7, was applied to the column. The elution of the column was initiated with 0.04 M Tris-phosphate buffer, pH 8.7, followed by a series of buffers of increasing pH and NaCl concentration in a discontinuous stepwise gradient. Fractions of 10 ml were collected. The elution diagram shown in Fig. 1 represents the fractions pooled and the location of various buffer changes.

**Step 2:** The fraction between 6 and 6.75 liters in the previous step was dialyzed extensively against distilled water and lyophilized; recovery, 1.2 g. The resulting residue was dissolved in 5 ml of 0.04 M Tris-phosphate buffer, pH 8.3, and was applied to another column of DEAE-Sephadex A-50 (2.5 X 100 cm), previously equilibrated with the above buffer containing 0.1 M NaCl. The column was initially eluted with 400 ml of a linear gradient between 0.1 M NaCl and 0.2 M NaCl in 0.04 M Tris-phosphate buffer. Following the gradient, the elution was continued with 0.2 M NaCl-0.04 M Tris-phosphate buffer. Fractions of 8.5 ml were collected. Fig. 2 indicates the fractions pooled.

**Step 3:** Gel Filtration on Sephadex G-100—The major fraction from Step 2 (Fig. 2) was subjected to further purification by gel filtration. After dialysis of this fraction against several changes of distilled water, it was lyophilized; recovery, 1.0 g. A solution of the residue in 5 ml of 0.04 M sodium phosphate buffer, pH 7.5, was applied to a column of Sephadex G-100 (2.5 X 100 cm) previously equilibrated with the same buffer. The column was eluted with 0.04 M sodium phosphate buffer, pH 7.5. Fractions of 6.5 ml were collected. HCG was eluted from the column in a single peak (Fig. 3). All fractions under the peak were pooled. The combined fractions were dialyzed extensively against water and lyophilized; recovery, 0.92 g.

**Bioassay of HCG**

Biological activity of the purified HCG was estimated by the rat prostate and accessory sex organ method (9). Gonadotropic activity of the various fractions from DEAE-Sephadex in the first step of the purification scheme described above was...
tested by the ovarian hyperemia response in immature female Sprague Dawley rats (10).

Disc and Immunoelectrophoresis2

Analytical disc electrophoresis was conducted on the purified HCG as well as on all other fractions obtained from DEAE-Sephadex chromatography (Step 1) described above. The disc electrophoresis technique of Davis (11) was used in these studies with slight modification in the staining and destaining procedure. The gels were stained by a 0.05% solution of Coomassie brilliant blue R 250 in 12% trichloracetic acid (12) (Colab Laboratories, Inc., Chicago, Illinois).

Immunoelectrophoresis was performed on the LKB apparatus model 6800 A with ionagar No. 2 in barbituric acid buffer, pH 8.6; ionic strength, 0.08 (13). Rabbit antiserum prepared against commercial HCG was used in these studies.

Molecular Weight Determinations

Ultracentrifugation—Sedimentation velocity and equilibrium runs were made in a Spinco model E analytical ultracentrifuge. A 0.9 to 1% solution of HCG in 0.02 M acetate-buffer-0.08 M NaCl, pH 5.5, or 0.04 M sodium-phosphate buffer, pH 7.5, was used for sedimentation velocity runs at 59,780 rpm. Sedimentation equilibrium determinations were carried out in a short column by low (14) and high speed equilibrium (15) methods. In the low speed runs three concentrations of HCG from 3 to 5 mg per ml in 0.1 M sodium-phosphate buffer, pH 7, were used. The high speed runs were made with 0.1 to 0.3 mg per ml of HCG solutions in 0.05 M sodium-phosphate buffer, pH 6.5. A value of 0.727 was assumed for the partial specific volume (2).

Gel Filtration—A column (2 x 107.5 cm) of Sephadex G-150 was packed at 4° in 0.04 M sodium-phosphate buffer, pH 7.5. A total hydrostatic head of 17.0 cm was maintained during the packing as well as the elution of the column. The column was calibrated by using a 1.5-ml solution of a mixture of proteins of known molecular weights containing 3 to 6 mg per ml of individual proteins (16-18). A constant flow rate of 4.3 ml per hr was maintained during the elution and 1-ml fractions were collected in graduated tubes. The elution volume (Ve) of ferritin (300,000), 102 ml, was taken as the void volume (Vo) of the column. The location of the various proteins was established either by measuring the absorbance of the eluate at 280 mp or by enzymatic activity (Fig. 4). A 1.5-ml sample of HCG (3 mg) was run separately under identical conditions. The molecular weight of the hormone was determined by reference to a plot of Ve/Vo and log molecular weight of the standard proteins (Fig. 5). The values for the molecular weights of the reference proteins were taken from the published literature (16-18). The molecular weight of the reduced and carboxamidomethylated HCG was also determined in the same manner. The preparation of this derivative is described in the following paper (19).

COOH- and NH2-terminal Analyses

COOH-terminal analysis was conducted with carboxypeptidases A and B, essentially according to the procedure of Fraenkel-Conrat (20). The results obtained by the enzymatic approach were further confirmed by the hydrazinolysis procedure of Akabori (21, 22), with slight modifications. NH2-terminal analyses were performed by the use of leucine aminopeptidase and Sanger’s reagent (20). The details of these investigations will be described elsewhere.
### Amino Acid Composition of HCG

Samples of 1 to 1.2 mg of HCG were heated at 105° with 1 ml of thrice glass-distilled constant boiling HCl in evacuated sealed tubes for varying time intervals. The acid was removed by evaporation in a rotary evaporator with repeated additions of water. The resulting residue was dissolved in 2.5 ml of sodium-citrate buffer (0.2 M Na⁺), pH 2.2. A 1-ml aliquot was applied to each column of a Spinco amino acid analyzer equipped with an accelerated system (23).

Half-cystine content was determined independently as cysteic acid (24). Tryptophan in the intact protein was determined spectrophotometrically (25) or on an amino acid analyzer after alkaline hydrolysis of the protein (26).

### Determination of Free Sulfhydryl Groups

Spectrophotometric titration with p-chloromercuribenzoate was performed by the method of Boyer (27). The presence of any free sulfhydryl groups was also checked by carboxamidomethylation of the protein. A sample of 1.633 mg of the hormone, in 1 ml of 0.05 M sodium-phosphate buffer, pH 8.4, and 3 M urea, was treated with 3 mg (~300 M excess) recrystallized iodoacetamide. The reaction mixture was incubated for 6 to 7 hours at 37°, followed by dialysis against distilled water. Finally, the dialysate was dried and hydrolyzed with 6 N HCl as described above. The hydrolysate was analyzed by a Spinco amino acid analyzer.

### Carbohydrate Composition

#### Identification of Monosaccharides

After acid hydrolysis of HCG, sialic acid, glucosamine, galactosamine, and neutral sugars such as galactose, mannose, and fucose were identified by paper, thin layer, and gas-liquid chromatography. Solvent systems for paper chromatography of neutral sugars were as follows: A, n-butyl alcohol-ethanol-water, 4:1:5 (v/v, upper phase) (28); B, pyridine-ethyl acetate-water, 2:5:7 (v/v, upper phase) (29); C, n-butyl alcohol-pyridine-0.1 N HCl, 5:3:2 (v/v) (30). Solvent C was also used for sialic acid. Glucosamine and galactosamine were identified on Whatman No. 1 paper with (D) ethyl acetate-pyridine-n-butyl alcohol-ethylic acid-water, 10:10:5:1:5 (v/v) (31). Thin layer chromatography of neutral sugars was carried out on cellulose plates in (E) pyridine-ethyl acetate-water, 2:1:2 (v/v) (32). For sialic acid, Silica Gel G plates were used in (F) i-butyl alcohol-ethanol-water-N\(_2\)H\(_4\)OH, 50:70:40:1 (33). Spray reagents for the detection of sugars included ammoniacal silver nitrate (34), periodate-acetylacetate (35), thiobarbituric acid (36), and potassium permanganate-sulfuric acid (33).

Paper electrophoresis of sugars was carried out in borate buffer, pH 9.2, in a sandwich type apparatus (37). The paper electropherograms were sprayed with p-anisidine (37).

Samples for paper and thin layer chromatography of neutral sugars were prepared as follows. A 0.5- to 1-ng sample of HCG was hydrolyzed at 100° for 6 to 8 hours with 1 ml of 1 N HCl in an evacuated sealed tube. The hydrolysate was neutralized with Dowex 1-CO\(_{-}\)-2 and filtered. The residue was washed with about 10 ml of water and the filtrate and washings were evaporated to dryness. The residue was then examined by thin layer and paper chromatography. For hexosamines, a 0.5- to 1-ng sample of HCG was hydrolyzed with 2 N HCl at 105° for 4 hours in an evacuated sealed tube. The hydrolysate was freed of amino acids and neutral sugars by the method of Doaa (38) and examined for hexosamines by thin layer and paper chromatography. Sialic acid was removed by acid hydrolysis of 0.5 to 1 mg of HCG with 250 μl of 0.06 N H\(_2\)SO\(_4\) at 80° for 1 hour or with Vibrio cholerae neuraminidase (39). The sialic acid was removed by dialysis and identified by paper and thin layer chromatography.

#### Quantitative Estimation of Sugars

Neutral sugars were estimated by gas-liquid chromatography as their trimethylsilyl ethers\(^4\) and alditol acetates (40). A 0.5- to 1-ng sample of HCG in 50 μl of water was hydrolyzed with 50 μl of a 40% suspension of Dowex 50-H\(^{+}\), 50 to 100 mesh, in a sealed tube. After the addition of 25 μg of erythritol in 25 μl of water to the hydrolysate, it was passed through a column of charcoal (0.50 x 0.25 cm) overlying a layer of mixed bed resin (0.50 x 0.25 cm) composed of equal amounts of Dowex 50-X2 and Dowex 3, 50 to 100 mesh (41). Erythritol was used as an internal standard. The column was washed with 6 to 8 ml of 5% ethanol and the eluate was evaporated to dryness. The residue was dissolved in 100 μl of water and a 75-μl aliquot was evaporated to dryness. Finally, the residue was treated with 20 μl of silylating reagent prepared by mixing pyridine-hexamethyldisilazane-trimethylchlorosilane, 10:2:1, v/v. After shaking the reaction mixture for 10 min, a 1-μl aliquot was applied to a Varian Aerograph model 1500 gas chromatograph, equipped with dual flame ionization detectors and a matrix temperature programmer. A linear temperature program from 115-175° with 2° per min increase was used. The column (1 inch x 9 feet) was packed with 5% SE-52 on Chromosorb W, 60 to 80 mesh. The neutral sugars were also determined by quantitative paper chromatography (42).

The hexosamines were determined by heating a 0.4- to 0.8-mg sample of glycopeptide or glycoprotein with 1 ml of 2 N HCl for 4 to 6 hours at 105° in an evacuated sealed tube. The acid was removed carefully in a rotary evaporator with several additions of water. After dissolving the residue in 1.1 ml of sodium citrate buffer (0.2 M Na⁺), pH 2.2, 1 ml of the resulting solution was applied to a short column (0.9 x 12 cm) of the Spinco amino acid analyzer. Standards of glucosamine and galactosamine were treated in the same manner in the presence of bovine serum albumin approximately equal to the peptide content of the sample. The hydrolysate was carried out for two time intervals and the hexosamine content was determined by extrapolation to zero time (43).

Sialic acid was assayed by thiobarbituric acid method of Warren (44) and by the resorcinol method (45).

### Results

#### Purification of HCG—Chromatography on DEAE-Sephadex

A-50 resulted in the isolation of several fractions designated as 1 to 12 in Fig. 1. All other fractions showed biological activity when tested qualitatively by the ovarian hyperemia response in immature rats except Fraction 1. Disc electrophoresis of these fractions at pH 8.3 indicated that the fractions which eluted at greater elution volumes also showed greater electrophoretic mobility on polyacrylamide gel (Fig. 6). Sialic acid content of the DEAE-Sephadex fractions followed the same pattern. Fraction 8 from the DEAE-Sephadex column was taken for further purification by repeating DEAE-Sephadex column chromatography with a continuous linear gradient (Step 2).

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Finally, the resulting material was subjected to gel filtration on Sephadex G-100 (Step 3).

The highly purified material thus obtained showed a biological activity of 12,000 i.u. per mg (10) and an immunoassay of 5,900 i.u. per mg (46). The value for the absorbance at 278 nm of an aqueous solution of the protein at a concentration of 1 mg per ml was found to be 0.388.

Homogeneity and Molecular Weight of HCG—Ultracentrifugation (Fig. 7), disc electrophoresis (Fig. 8), and immunoelectrophoresis (Fig. 9) all showed a high degree of homogeneity of the preparation. Treatment with carboxypeptidase A and B released 1 mole of serine per 30,000 g of HCG and traces of valine and threonine (less than 0.15 mole of each). Hydrazinolysis of the protein yielded 0.6 mole of serine per 30,000 with less than 0.15 mole each of glycine and threonine. The low recovery of COOH-terminal amino acid by this procedure is not surprising because other investigators have had similar results (47). NH2-terminal amino acids with intact or sialic acid-free HCG could not be identified either with leucine aminopeptidase or by Sanger's procedure (20).

Gel filtration on Sephadex G-150 gave a value of 59,000 ± 4,000 for the molecular weight of HCG and 30,000 ± 2,000 for the reduced carboxamidomethylated HCG. However, assuming a partial specific volume of 0.727 (2), the molecular weight, determined by low and high speed sedimentation equilibrium methods, was 46,400 ± 3,000 and 47,700 ± 3,000 respectively. The $s_{20,w}$ value was found to be 2.89 S.

Fig. 6. Disc electrophoresis on polyacrylamide gel of Fractions 2 to 12, from DEAE-Sephadex column (Step 1). Sample size, 10 to 25 μg; →, junction of the spacer and separation gels (12).

Fig. 7. Schlieren patterns of sedimentation velocity run at 59,780 rpm. A 1% solution of HCG in 0.04 M sodium phosphate buffer, pH 7.5, was used. Pictures were taken (from left to right) at 15-min, 30-min, 1-hour, 2-hour, and 3-hour time intervals.

Fig. 8. Disc electrophoresis of HCG on polyacrylamide gel. 1, crude HCG; 2, purified HCG; +, the junction of the spacer and the separation gels (12).

Fig. 9. Immunoelectrophoresis of crude and purified HCG. LKB electrophoresis apparatus 6800 A was used with ionagar No. 2 in barbital buffer, pH 8.6; ionic strength, 0.08. Electrophoresis was performed at 4.5 ma per slide for 3 hours. All incubations for development of precipitin curves were done at room temperature for 16 to 40 hours. Rabbit antiserum against crude HCG was used. 1 and 2, crude HCG; 3 and 4, purified HCG.
**Amino Acid and Carbohydrate Composition of HCG**—Table I describes the chemical composition of HCG. The amino acid data were computed on the basis of 3 residues of histidine. The weight of the polypeptide unit calculated from its amino acid content was 18,000. Since the polypeptide constitutes 66% of the molecule, the minimal molecular weight of HCG would be 27,000. HCG showed a remarkably high content of proline, cystine, and serine. No free sulphydryl group was discovered, either by direct spectrophotometric titration with p-chloromercuribenzoate or by carboxamidomethylation with iodoacetamide and subsequent hydrolysis of the product. The carbohydrate content of the hormone was about 33%. The carbohydrate moiety was composed of 9 residues each of mannose and galactose, 1 of fucose, 11 residues of N-acetylglucosamine, 3 of N-acetylgalactosamine, and 8 residues of sialic acid per 27,000 (Table II).

**Table I**

*Amino acid and carbohydrate composition of HCG*

Amino acid residues were calculated on the basis of 3 histidine residues.

<table>
<thead>
<tr>
<th>Amino acid and sugars</th>
<th>6-hr hydrolysis residues</th>
<th>24-hr hydrolysis residues</th>
<th>48-hr hydrolysis residues</th>
<th>No. of amino acid residues</th>
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<tr>
<td>Lysine</td>
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<tr>
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<td>Glycine</td>
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<tr>
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<td>3.92</td>
<td>4.08</td>
<td>3.96</td>
<td>4</td>
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</table>

| Tryptophan            | 1                       |                           |                           |                           |
| Galactose             | 9                       | (5.3 ± 0.3%)              |                           |                           |
| Mannose               | 9                       | (5.3 ± 0.3%)              |                           |                           |
| Fucose                | 1                       | (6.6 ± 0.05%)             |                           |                           |
| N-Acetylgalactosamine | 11                      | (8.9 ± 0.3%)              |                           |                           |
| N-Acetylglucosamine   | 3                       | (2.1 ± 0.1%)              |                           |                           |
| Sialic acid           | 8                       | (9.0 ± 0.3%)              |                           |                           |

* See Table I for details.

**DISCUSSION**

The present investigation was aimed at isolating, on a large scale a highly active and homogeneous preparation of HCG which would be suitable for structural studies. The results reported in this, as well as a subsequent communication (19), indicate that the procedure of purification described above yields a product suitable for structural investigations. Although the preparation of HCG thus obtained is homogeneous by various physicochemical criteria, it apparently shows less biological activity than that reported recently by Van Hell et al. (3).

This discrepancy between the two preparations may be due to the differences in the method of assay or in their sialic acid content. Since sialic acid has a profound effect on the biological potency of HCG, it is quite conceivable that this preparation might be slightly deficient in sialic acid. A loss of 0.3% sialic acid has been found to drop the biological potency from 18,800 to 11,800 i.u. per mg (3). Whether this degraded product is of placental origin or is an artifact introduced during the process of isolation by the partial cleavage of labile glycosidic bonds involving sialic acid is hard to say at present. It may be noted that there were several fractions, obtained from the DEAE-Sephadex column in the first step of purification described above, which possessed higher electrophoretic mobility on acrylamide gel and also showed higher sialic acid content. It may be possible to obtain out of these fractions, after further purification, a preparation with higher biological activity than that reported here. Since these fractions form such a small percentage of the starting material, they were not selected for structural studies reported in the subsequent paper.

A molecular weight of 59,000 ± 4,000 was obtained for HCG by gel filtration. Ultracentrifugation, however, gave a value of 47,000 ± 3,000 (average of low and high speed sedimentation equilibrium values). A high value by gel filtration is consistent with the results with other glycoproteins (16). Another reason...
TABLE III
Carbohydrate composition of HCG and ovine ICSH

<table>
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<th></th>
<th>HCG</th>
<th>Ovine ICSH</th>
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<tr>
<td></td>
<td>g/100 g</td>
<td>g/100 g</td>
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<tr>
<td>Total hexose</td>
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<tr>
<td>Galactose</td>
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<td>Mannose</td>
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<tr>
<td>Fucose</td>
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<tr>
<td>Total hexosamine</td>
<td>11.0</td>
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<tr>
<td>N-Acetylglucosamine</td>
<td>8.9 ± 0.3</td>
<td>6.0</td>
</tr>
<tr>
<td>N-Acetylgalactosamine</td>
<td>2.2 ± 0.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>9.0 ± 0.3</td>
<td>0.5 (0.37 ± 0.01)</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>31.3</td>
<td>14.4</td>
</tr>
</tbody>
</table>

a Walborg and Ward (50).
b Papkov et al. (48).

3. The minimal molecular weight of 27,000 derived from the chemical composition is consistent with the above results. HCG has biological properties similar to interstitial cell-stimulating hormone and, therefore, one would expect structural similarity between them. Recently, ovine ICSH has been purified and its chemical composition has been determined (50). A comparison between the two hormones in Table II indicates a similarity in their relative amino acid content. Both contain a remarkably high proportion of proline and cysteine residues. The carbohydrate content of HCG, on the other hand, is much higher than ICSH. HCG has 9.0% sialic acid (Table III) while ICSH has almost negligible amount of 0.5% sialic acid (50). It is interesting to note that, whereas sialic acid is essential for the hormonal activity of HCG, it is not required for the activity of ICSH (51). Similarly, HCG has 5.3% galactose as against 0.8% in ICSH.

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