Synthesis and Secretion of Human Chorionic Gonadotropin Subunits by Cultured Human Malignant Cells*

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The synthesis and secretion of human chorionic gonadotropin (hCG) subunits have been studied by pulse-chase techniques in JAR choriocarcinoma cells, which produce both the α and β subunits of hCG "eutopically," that is, as part of their expected repertoire of gene products, and in three cell lines that produce either α or β subunit "ectopically." JAR cells contain two intracellular forms of the α subunit (M₀ = 15,000 and 18,000) and of the β subunit (M₀ = 18,000 and 24,000) but do not accumulate fully processed, "mature" α (M₀ = 22,000) or β (M₀ = 34,000) subunits during chase of pulse-labeled cells. Mature subunits, however, are secreted into the culture medium during this time. Thus, secretion of mature subunits appears to occur rapidly after processing of the intracellular forms. Two cell lines that ectopically secrete a but not β subunit, ChaGo bronchogenic carcinoma cells and HeLa S₁ cervical carcinoma cells, also contain the M₀ = 15,000 and 18,000 intracellular forms of α subunit and appear to accumulate mature α subunit intracellularly. The CBT cell line, derived from a glioblastoma multiforme, produces the M₀ = 18,000 and 24,000 intracellular forms of β subunit, with no evidence for a subunit synthesis or secretion. These four cell lines should provide "biologic reagents" for the further study of α and β subunit processing and secretion.

The production of the α and β subunits of the glycoprotein hormone human chorionic gonadotropin (hCG) by human malignant cells is often unbalanced in vivo (1-3) and in vitro (4-6), with a subunit production frequently predominating. During normal pregnancy, also, there is an excess production of α over β by the placenta, and the synthesis of β appears to be rate-limiting in the production of complete hCG (2). It is now known that, in the placenta, the mRNAs for the α and β subunits are separate and that translation of the α and β mRNAs in cell-free systems is differentially sensitive to Mg²⁺ ion concentration (7). Thus, from both in vitro and in vivo studies, there is considerable evidence suggesting that there is unbalanced synthesis and secretion of α and β subunits of hCG in both normal and malignant cells. There have also been reports of biochemical differences between hCG subunits produced by normal trophoblastic cells and those secreted by malignant cells (8-10). Moreover, the effects of a number of drugs on the eutopic secretion of hCG subunits by trophoblastic cells are different from the effects on ectopic production by non trophoblastic cells (5, 6, 11-13). Very little is known, however, about the control of hCG secretion by trophoblastic and non trophoblastic cells or about the processing of intermediate glycosylated forms prior to secretion. The studies reported here were undertaken to characterize and to compare the synthesis and secretion of hCG subunits in eutopic and ectopic hCG subunit-producing cell lines.

Experimental Procedures

Cell Culture Lines—JAR choriocarcinoma cells (obtained from Dr. Roland Pattillo, Medical College of Wisconsin (14)) were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. ChaGo bronchogenic carcinoma cells and CBT glioblastoma cells (from Dr. Alan Rabson, National Cancer Institute) were grown in RPMI 1640 medium with 20% fetal bovine serum. HeLa S₁ cultures (American Type Culture Collection) were grown in Eagle's minimal essential medium with 10% fetal bovine serum.

Radioactive Labeling and Preparation of Cell Lysates—Late log or early confluent cultures (grows in 100-mm diameter Petri dishes) were incubated at 37°C for various times with 100 μCi/ml of [³⁵S]methionine (500 to 700 Ci/mmol, New England Nuclear Corp.) in methionine-free medium. In some experiments, the radioactivity was "chased" by removing the labeling medium, washing the cells three times with complete medium, and incubating for various times in the presence of complete medium. Tunicamycin at various doses was added to some culture plates 16 h prior to a radioactive pulse. After incubation, the medium was removed, and the cells were washed with phosphate-buffered saline solution (0.01 M sodium phosphate containing 0.14 M NaCl, pH 7.2) and lysed by the addition of 2 to 5 ml of phosphate-buffered saline containing 1.0% Triton X-100, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (lysis buffer), followed by sonication. Culture medium was brought to the same concentration of lysis buffer by the addition of a concentrated solution. Cell lysates were clarified at 100,000 X g for 1 h at 0°C. The 100,000 X g pellet contained no detectable hCG subunits, as determined by SDS-PAGE followed by fluorography (see below), and was discarded. Cell lysates and media were stored at −70°C until analyzed.

Immunoprecipitation and SDS-PAGE—Detection of hCG-specific polypeptides in cell lysates and media was performed with rabbit antisera directed against complete hCG, hCG-α, or hCG-β (see miniprint supplement). In some experiments, immunoprecipitations were carried out with an antiserum raised in rabbits to a synthetic polypeptide representing the 15 COOH-terminal amino acids of the hCG-β subunit (see miniprint supplement). Serum from nonimmunized

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The abbreviations used are: hCG, human chorionic gonadotropin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; FSH, follicle-stimulating hormone; TSH, thyroid-stimulating hormone; LH, luteinizing hormone.

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rabbis was used as a control. Cell lysates or media were incubated with immune or nonimmune rabbit serum (1:5000 final dilution) at 4°C for 10 h. Immunoprecipitates were washed three times in lysis buffer, dissolved in electrophoresis buffer containing 0.062 M Tris HCl (pH 6.7), 1% SDS, 10% glycerol, and 2.5% beta-mercaptoethanol, heated at 100°C for 5 min, and layered on 5 to 20% linear gradient gels (unless otherwise indicated) prepared by the method of Laemmli (15). Electrophoresis was carried out for 16 h at 35 V in a Bio-Rad model 220 slab gel apparatus. Radioactivity was visualized by the fluorographic method of Bonner and Laskey (16). The following 14C-labeled molecular weight standards (New England Nuclear Corp.) were employed: phosphorylase b (92,500), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and cytochrome c (12,500). Molecular weights of the hCG-specific polypeptides were determined from logarithmic plots based on the migration of the molecular weight standards.

RESULTS

Eutopic Production of hCG Subunits by JAR Choriocarcinoma Cells—We have previously reported (17) that JAR choriocarcinoma cells contain glycosylated "precursors" of hCG-a and hCG-beta with intracellular half-lives of ≥2 h. The precursor of the a subunit had an apparent molecular weight of 18,000 (by SDS-PAGE) and contained high mannose core, asparagine-linked carbohydrate units. Mature a and beta subunits secreted by JAR cells were M, 22,000 and 34,000, respectively. We also had noted that, after incubation of JAR cells with tunicamycin for 16 h, a M, 12,000 polypeptide and a M, 15,000 partially glycosylated a polypeptide were present intracellularly (17).

In order to further differentiate the a- and beta-specific polypeptides synthesized in JAR cells, we prepared and characterized a rabbit antiserum directed against the 15 COOH-terminal amino acids of hCG-beta. This antiserum has a high degree of specificity for hCG-beta with minimal cross-reactivity with hCG-alpha (7). Taken together, these data suggested that the M, 18,000 band immunoprecipitated by various anti-beta sera was a partially glycosylated beta precursor, whereas M, 15,000 band was the beta apoprotein. This apparent molecular weight is consistent with that reported for the beta apoprotein by SDS-PAGE (7).

Although intracellular forms of a subunit could be identified in JAR cells, very little accumulation of fully processed, mature a was detected in these cells during a 4-h chase of cells pulsed for 15 min with [35S]methionine (Fig. 2); however, a subunit secreted into the medium (Lanes 7 to 9) migrated identical to that of mature placental hCG-a, and immunoprecipitation of the secreted form of a was blocked by addition of excess placental hCG-a subunit (data not shown).

Fig. 1. Identification of putative hCG-a and hCG-beta precursors in JAR cells by competition with excess unlabeled placental hCG-a and hCG-beta. JAR cells were pulsed for 1 h with [35S]methionine (100 μCi/ml). Immediately before immunoprecipitation, excess unlabeled placental hCG-a (290 μmol/ml) or hCG-beta (140 μmol/ml) was added to the clarified cell lysates (this was approximately a 100-fold excess based on radioimmunoassay of cell lysates for hCG-a and hCG-beta). Immunoprecipitates were separated on the 24,000, 18,000, 15,000, and 10,000 precursors. Lanes 1 to 3 (controls): 1, anti-hCG; 2, anti-a; 3, anti-COOH-terminal beta. Lanes 4 to 6: same sequence of antisera + excess a. Lanes 7 to 9: same sequence of antisera + excess beta.

Fig. 2. Pulse-chase labeling of hCG-a subunit in JAR cells. Cells were pulsed for 15 min with [35S]methionine (100 μCi/ml), washed three times with complete medium, and chased for 15, 30, 60, 120, or 240 min in the presence of complete medium. Cell lysates and media were immunoprecipitated with anti-a. Arrows indicate the migration of secreted a subunit (which migrates identically to placental hCG-a standard) and the M, 18,000 and 15,000 precursors. Lanes 1 to 6 (cells): 1, 0-min chase; 2, 15-min chase; 3, 30-min chase; 4, 60-min chase; 5, 120-min chase; 6, 240-min chase. Lanes 7 to 9 (media): 7, 60-min chase; 8, 120-min chase; 9, 240-min chase. Lane 10: molecular weight standards.
ditions (6, 20, 22). The CBT cell line was established from a glioblastoma multiforme (23) and has been shown to produce hCG or hCG-β, or both, but no free α subunit (6).

All of the data cited above have been derived by radioimmunoassay. It was of interest to examine these cells by pulse-chase techniques to determine the nature of the subunits synthesized and secreted by these cells and to compare these events between cells that produce hCG subunits ectopically and trophoblastic cell lines that produce hCG subunits eutopically.

Fig. 3 shows the results of a pulse-chase experiment in which ChaGo cells were incubated for 1 h with [35S]methionine and then chased for 4 h in the presence of excess unlabeled amino acids. In the lysates from ChaGo cells pulsed for 1 h, the anti-α serum precipitated bands at $M_r = 18,000$ and 15,000 (Lane 6) as well as a diffuse band that migrated identically to that of the secreted α subunit (Lanes 1 and 2). The secreted α migrated at the same molecular weight as standard placental hCG-α. No β-specific polypeptides were present intracellularly or extracellularly (Lanes 3, 7, and 11). In the ChaGo cells chased for 4 h (Lane 10), there was no evidence of the $M_r = 15,000$ band and relatively less of the $M_r = 18,000$ band compared to the amount of material that migrated like the secreted form of α, suggesting a precursor-product relationship between the low molecular weight and mature forms of the α subunit. The immunoprecipitation of both the intracellular and secreted forms of α subunit from the ChaGo cells was blocked by addition of excess unlabeled placental hCG-α subunit (Fig. 4).

In order to ascertain whether the glycosylated intermediates in the processing pathway for α subunit were similar between JAR and ChaGo cells, cultures of ChaGo were incubated with 1 or 5 μg/ml of tunicamycin (the latter concentration had been previously shown to inhibit glycosylation but not synthesis of α subunit in JAR cells (17)) for 16 h prior to pulsing for 1 h with [35S]methionine. Cells were then lysed and immunoprecipitated with anti-α serum (Fig. 5). It can be seen in Lane 1 thatChaGo cells were incubated for 1 h with [35S]methionine and then chased for 4 h. Immediately before immunoprecipitation with anti-α, a 100-fold excess of placental hCG-α was added to the clarified cell lysate and medium obtained after the 4-h chase. Lane 1, cells, without excess hCG-α; Lane 2, cells, with excess hCG-α; Lane 3, medium, without excess hCG-α; Lane 4, medium, with excess hCG-α; Lane 5, molecular weight standards. Note: the gels used in this experiment were non-gradient 12% polyacrylamide gels rather than 5 to 20% linear gradient gels used in the other experiments depicted here; hence, the bands appear more diffuse.
seen that treatment with 1 or 5 μg/ml of tunicamycin increased the amount of the $M_r = 15,000$ band relative to the $M_r = 18,000$ band and produced a distinct band at $M_r = 12,000$, which was not seen in the untreated cells. This was in agreement with what was observed in JAR cells (17), suggesting that the glycosylated intermediates of α were similar in JAR and ChaGo.

The secretion of a subunit in HeLa cells treated with sodium butyrate for 72 h prior to pulsing for 1 h with [35S]methionine appeared to be similar to that of ChaGo cells (Fig. 6). Untreated cells pulsed for 1 h contained primarily the $M_r = 18,000$ and 15,000 bands (Lane 2); no β-specific polypeptides were evident (Lane 3). Cells pretreated for 72 h with butyrate contained more of the $M_r = 18,000$ and 15,000 polypeptides as well as mature α subunit (Lane 6). The medium from control and butyrate-treated HeLa cells chased for 4 h contained only free α subunit, and much more α was secreted by butyrate-induced than by noninduced cells (Lanes 10 and 14). Thus, both synthesis and secretion of α subunit appeared to be stimulated by treatment with butyrate.

The pattern of hCG subunit synthesis and secretion by CBT glioblastoma cells was the opposite of ChaGo and HeLa in that only the β subunit appeared to be synthesized and secreted. From the lysates of CBT cells pulsed for 1 h with [35S]methionine, anti-hCG and anti-COOH-terminal β precipitated the $M_r = 18,000$ and 24,000 bands that were also seen with anti-β in JAR cells (Fig. 7, Lanes 1 and 2). Immunoprecipitation of both the $M_r = 18,000$ and 24,000 forms was blocked by addition of excess placental hCG-β subunit (data not shown). Neither free α subunit nor complete hCG appeared to be synthesized in CBT cells since the only specifically immunoprecipitated band that appeared in the 4-h chase medium was a $M_r = 34,000$ band that migrated identically to that of placental hCG-β subunit (Lanes 7 and 8). If complete hCG were being secreted by these cells in significant amounts, the anti-hCG serum should have immunoprecipitated it, and both the α ($M_r = 22,000$) and β bands should have been apparent on the SDS gels under the reducing conditions used in these experiments. Furthermore, anti-α did not immunoprecipitate α precursors in pulse-labeled CBT cells (data not shown).

**DISCUSSION**

The data obtained in this study indicate that although the "kinetics" of secretion of hCG-α subunit is somewhat different between cells that produce α eutopically and those that produce it ectopically, the intermediates in the processing pathway for a subunit appear to be similar. Intracellular $M_r = 18,000$ and 15,000 forms of a subunit occur in JAR, ChaGo, and HeLa cells (Figs. 1, 3, and 6). In JAR cells the intracellular forms are "chased" from the cells without significant accumulation of fully mature α, but mature α does appear in the chase medium (Fig. 2), in ChaGo and butyrate-induced HeLa cells, on the other hand, there is evidence for the accumulation of mature α subunit intracellularly prior to secretion into the medium (Figs. 3 and 6).

The reasons for the apparent differences between eutopic and ectopic α-producing cells are not yet evident, and they may represent quantitative rather than qualitative differences. We have also observed that another eutopic α-producing cell line, BeWo choriocarcinoma, follows the pattern exemplified by JAR cells. Thus, the secretion of α subunit by malignant trophoblastic cells may not follow the same route reported for other secretory glycoproteins, i.e. incorporation into secretory granules and secretion by exocytosis. This conclusion is consistent with the observations that there is a scarcity of secretory granules in BeWo cells (24) and that agents, such as colchicine, which inhibit hormone secretion by cells in which exocytosis is involved in the secretory process, stimulate secretion of hCG by BeWo cells (25). These authors (24, 25) postulate that secretory granules and microtubules do not play a significant role in hCG secretion by malignant trophoblastic cells.

The differences in α secretion between the various cell lines observed in these experiments are not due to differences in the culture growth phase or the growth rate of the cells because in our hands the doubling time of JAR cells and HeLa cells is similar (±24 h) and that of ChaGo and BeWo is similar (±48 h). Furthermore, cells taken at any point in the growth phase from midlog to early confluency give identical results. It does not appear that differences in intracellular proteases can explain the discrepancies because lysis of cells in the presence of the protease inhibitor phenylmethylsulfonyl fluoride or incubation of clarified cell lysates for 24 h at 37°C before immunoprecipitation do not alter the banding patterns of the immunoprecipitated polypeptides.

Our results suggest the following hypothesis, which can now be tested experimentally, for the processing of the α subunit in cultured malignant cells: the α apoprotein (from which the signal peptide has already been cleaved (17)) is glycosylated during synthesis in a stepwise fashion leading to a $M_r = 15,000$ form containing one asparagine-linked high mannose oligosaccharide; this $M_r = 15,000$ form is then rapidly glycosylated by addition of the second high mannose oligosaccharide to pro-

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**Fig. 6. Pulse-chase labeling of control and butyrate-induced HeLa S1 cells.** Cells were incubated for 72 h without or with sodium butyrate (10 mM) and then pulsed for 1 h with [35S]methionine and chased for 4 h. Cell lysates and media were immunoprecipitated with various antisera. Lanes 1 to 4 (control cells, 1-h pulse): 1, anti-hCG; 2, anti-α; 3, anti-β (R279-V1); 4, nonimmune serum. Lanes 5 to 8 (butyrate-induced cells, 1-h pulse): same sequence of antisera. Lanes 9 to 12 (control medium, 4-h chase): same sequence of antisera. Lanes 13 to 16 (butyrate-induced medium, 4-h chase): same sequence of antisera. Lane 17: molecular weight standards.

**Fig. 7. Pulse-chase labeling of CBT cells.** Cells were pulsed for 1 h with [35S]methionine and chased for 4 h. Cell lysates and media were immunoprecipitated with various antisera. Lanes 1 to 3 (cells, 1-h pulse): 1, anti-hCG; 2, anti-COOH-terminal β; 3, nonimmune serum. Lanes 4 to 6 (cells, 4-h chase): same sequence of antisera. Lanes 7 to 9 (medium, 4-h chase): same sequence of antisera. Lane 10: molecular weight standards.
duce an \( M_s = 18,000 \) form that accumulates intracellularly because one of the next processing steps is a relatively slow one; the \( M_s = 18,000 \) form is further processed by glycosidases and terminal complex carbohydrate synthesis to yield a mature \( M_s = 22,000 \) glycoprotein subunit that is secreted. Although the data for the \( \beta \) subunit are less clear, the following hypothesis is suggested from the data available: a \( M_s = 15,000 \) approtein is glycosylated during synthesis to an \( M_s = 18,000 \) glycoprotein and subsequently to a \( M_s = 24,000 \) glycoprotein containing both high mannose core oligosaccharides and at least one of the serine-linked carbohydrate chains; this moiety is then processed and complex-type and serine-linked carbohydrate chains are added to form a \( M_s = 34,000 \) glycoprotein that is secreted. The processing of the \( \beta \) subunit is complicated by the fact that it contains four serine-linked carbohydrate units in addition to the two asparagine-linked chains (26) and, hence, the shifts in molecular weight are not analogous to those of \( \alpha \). Of course, the molecular weights of all of these putative intermediates are based on SDS-PAGE, a system in which glycoproteins do not migrate strictly according to their molecular weights; thus, the true molecular weights of these intermediates must await more definitive biochemical analysis.

The apparent stepwise addition of high mannose-containing oligosaccharides to asparagine residues of hCG subunits, followed by processing via glycosidases and subsequent addition of complex-type carbohydrate chains, is consistent with what is known about vesicular stomatitis virus G protein (27–30), immunoglobulin G heavy chain (30), and the glycosylated env gene precursor (Pr \( 80^{env} \)) of murine leukemia virus (31). The conversion of lower molecular intracellular forms of hCG-\( \alpha \) subunit to a higher molecular weight form that is secreted is similar to the observation by Weintraub and Stannard (32) that the \( \alpha \) subunit of thyroid-stimulating hormone in mouse thyrotropic tumor cells is lower in molecular weight than the secreted form. Bielinska and Boime (33) have shown that mRNA isolated from first trimester placenta and incubated in a cell-free system containing microsomal membranes directs the synthesis of hCG-\( \alpha \) subunit containing high mannose oligosaccharides and migrating at approximately \( M_s = 18,000 \) by SDS-PAGE. The use of microsomal membranes from tunicamycin-treated cells or incubation of the \( M_s = 18,000 \) form with endoglycosidase H produced lower molecular weight forms of \( \alpha \) (33). These observations are consistent with our data obtained from intact tunicamycin-treated cells (Fig. 5) and from experiments using endoglycosidase H digestion (17).

It is evident that various human cell lines can produce both \( \alpha \) and \( \beta \) subunits (JAR), only \( \alpha \) (ChaGo), or only \( \beta \) (CBT) as well as that some cell lines can be induced to synthesize and secrete much greater amounts of one of the subunits (HeLa). Our studies indicate that these cell lines can be exploited as "biologic reagents" to study further the synthesis, processing, and secretion of hCG subunits by malignant human cells.

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Synthesis and Secretion of Human Chorionic Gonadotropin Subunits by Cultured Human Gestational Cells: Purification of hCG Subunits and Preparation of Antisera

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EXPERIMENTAL PROCEDURES

Isolation of hCG. Crude hCG was purchased from Organon, Inc., Bo, The Netherlands. Purification procedures were performed by modifications of published procedures (1.2). All steps were performed in the cold room at 4°C and particular attention was given to the proper preparation and handling of column matrices. The following were the steps used:

(a) Crude hCG (300 mg) was dissolved in 0.25 M ammonium acetate, pH 5.0, (starting buffer) and dialyzed for 20 h against this solution.

(b) The solution from step a was clarified by centrifugation and applied to a 2.5 × 40 cm column of SP-Sephadex C-25 (previously equilibrated with starting buffer). The column was washed sequentially with starting buffer, 0.1 M ammonium acetate, pH 5.0, and then with a linear salt gradient established by mixing 2.5 liters of starting buffer (containing 0.3 M NaCl) with 2.5 liters of 0.6 M ammonium acetate, pH 5.0, over 6 h at a rate of 60 ml/h. The column eluted between 0.3 M and 0.6 M NaCl.

(c) Partially purified hCG was dialyzed briefly against water and then overnight against 0.04 M Tris, pH 8.6. It was then applied to a 2.5 × 40 cm column of DEAE-Sephadex A-50 previously equilibrated with the same buffer. The column was washed and then eluted using a salt gradient as described in the legend to Fig. 15.

(d) Partially purified hCG obtained by DEAE-Sephadex chromatography was dialyzed briefly against water, lyophilized to dryness, redissolved in 15 mM ammonium bicarbonate, and chromatographed on Mono Q-5/5 (Pharmacia) as described in the legend to Fig. 25.

Isolation of hCG Subunits. Subunits were isolated by modification of published procedures (3,4). Purified hCG was dialyzed against room temperature for 16 h at pH 5.0, and the solution was rechilled. 15 mM ammonium bicarbonate, pH 5.0, and then with a linear salt gradient established by mixing 2.5 liters of 0.15 M ammonium acetate, pH 5.0, with 2.5 liters of 0.6 M ammonium acetate, pH 5.0, over 6 h at a rate of 60 ml/h. The column eluted between 0.3 M and 0.6 M NaCl.

The partially purified hCG was dialyzed briefly against water and then overnight against 0.04 M Tris, pH 8.6. It was applied to a 2.5 × 40 cm column of DEAE-Sephadex A-50 previously equilibrated with the same buffer. The column was washed with the same buffer and then eluted using a salt gradient as described in the legend to Fig. 15.

(e) Partially purified hCG obtained by DEAE-Sephadex chromatography was dialyzed briefly against water, lyophilized to dryness, redissolved in 15 mM ammonium bicarbonate, and chromatographed on Mono Q-5/5 (Pharmacia) as described in the legend to Fig. 25.

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(f) Partially purified hCG obtained by DEAE-Sephadex chromatography was dialyzed briefly against water, lyophilized to dryness, redissolved in 15 mM ammonium bicarbonate, and chromatographed on Mono Q-5/5 (Pharmacia) as described in the legend to Fig. 25.

Preparation of Antisera to hCG and hCG Subunits. HCG (100 µg), alpha or beta subunits (100 µg) were dissolved in 0.1 M phosphate-buffered saline and mixed with an equal volume of complete Freund's adjuvant containing 5% (w/v) heat-inactivated, egg yolk. The mixture was administered subcutaneously over the scapular and the area 20 cm prior to injection of oil or the subunits. Booster injections consisted of 25 µg of subunits emulsified in incomplete Freund's adjuvant and administered subcutaneously in the thigh. Seven samples were each determined at monthly intervals and 3-10 days after each injection.

Preparation of Antisera to hCG Subunits. hCG subunits obtained by the purification procedure shown in Fig. 25 were injected subcutaneously with Freund's complete adjuvant (2.5 µg of beta and 0.5 µg of alpha subunits) and Freund's incomplete adjuvant (7.5 µg of beta and 1.5 µg of alpha subunits), with the following subsequent inoculations of hCG, hCG subunits, or control reagents. Seven samples were each determined at monthly intervals and 3-10 days after each injection.

RESULTS

Isolation of hCG and its Subunits. Polyacrylamide slab gel electrophoresis of samples obtained from each step of the purification procedure for hCG is shown in Fig. 6. It is evident from this gel that a high level of purification was achieved by SP-Sephadex chromatography. The yield from this step (250 µg) was 25%, and 82% of the starting material was removed in the washes before the gradient was begun. Since a major goal of this work was to obtain the subunits, it was necessary to determine the steps following cation-exchange chromatography in order to obtain complete separation of hCG from its subunits. The procedure described here (Fig. 25) was shown to provide this separation and was then tested against the intact hormone as shown in Fig. 6. Additional characterization of the subunits with regard to electrophoretic analysis, which agreed with reports made for these subunits [1] and the demonstration of immunological identity to hCG subunit standards, appear in a separate communication.

Major advantages of the polyacrylamide gel dissociation procedure to obtain hCG subunits are that it is rapid, easy to handle, and requires only 30 µg of purified hCG. This procedure yielded 9 µg of beta and 10 µg of alpha subunits. Polyacrylamide gel electrophoresis of hCG and its subunits obtained by the polyacrylamide gel dissociation procedure shown in Fig. 25. Additional characterization of the subunits with regard to electrophoretic analysis, which agreed with reports made for these subunits [1] and the demonstration of immunological identity to hCG subunit standards, appear in a separate communication.

DEAE-Sephadex chromatography of hCG following cation-exchange chromatography by SP-Sephadex chromatography. The subunits were isolated as follows: 1.25 µg of hCG was applied to a column of DEAE-Sephadex A-50. The column was washed with 0.4 M Tris, pH 8.0, and then with 0.4 M Tris (pH 8.0) containing 0.6 M NaCl, and the hCG was eluted by a linear gradient established by mixing 4.0 liters of 0.4 M Tris (pH 8.0) containing 0.6 M NaCl with 4.0 liters of 0.4 M Tris (pH 8.0) containing 0.8 M NaCl in 20 min. The yield from this purification procedure was 28%. As evidenced in Fig. 9, this preparation was free of impurities, i.e., it did not contain any forms of hCG or its subunits that were not isolated at 98% or more of their expected yield.

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Synthesis of hCG Subunits

Fig. 30. Gel filtration of hCG released by pronase digestion. hCG was digested by pronase at 37°C in 0.05 M sodium acetate buffer (pH 6.0) and applied to a column (2.5 x 100 cm) of Sephadex G-100. The column was eluted with 0.05 M sodium acetate, the bars on the abscissa mark the fractions pooled for the electrophoresis shown in Fig. 31.

Fig. 31. SDS-polyacrylamide gel electrophoresis of aliquots from the different steps of hCG purification. Electrophoresis was performed with a 35% stacking gel and a 10% running gel according to Kunitz's procedure (12). The gels were impregnated and stained with Coomassie Brilliant Blue R-250. The amount of hCG is expressed as ng/ml and the amount of hCG after dialysis steps Lanes 3 and 4: 50 and 75 ng of hCG after SEP-pak Sephadex, Lanes 5 and 6: 25 and 40 ng of hCG after Blue Dextran. Amounts of hCG were estimated from published kpg values (12) and can only be rough approximations for the smaller bands.

Fig. 32. Dose-response curves for anti-hCG (229-231). The reagents used were: NCS (1:10), RIA, NCS (1:1), hCG (1:1), NCS (1:1), obtained from the National Pituitary Agency. The double antibody radioimmunoassay was based on a 1:10,000 final dilution. Total counts were 2 x 10^6 cpm. Percent specific binding was plotted against each of the various competing antibodies compared to hCG at 50 ng binding. NCS (1:1), NCS, RIA, NCS (1:100), 20% NCS, 2.5% NCS, 1.25%. Bound/Total = 50%; non-specific binding = 1.95% of total counts.

Fig. 33. Dose-response curve for anti-hCG (230-231). The reagents used were; 2nd International Standard NCS, NCS (1:1), NCS, NCS (1:1), prepared as described under Experimental Procedures; hCG (1:1000), NCS, NCS (1:1), NCS (1:10), NCS (1:100), NCS, NCS (1:1000), NCS, NCS (1:10000), obtained from the National Pituitary Agency. There was no reaction with the 2nd International Standard NCS or any of the data samples up to 1000 ng hCG concentration. Only those antibodies which demonstrated competitive inhibition are shown. NCL (1:10000), 20% NCS and 2.5% NCS. Bound/Total = 75%; non-specific binding = 2.5% of total counts.

Fig. 34. SDS-polyacrylamide gel electrophoresis of purified hCG and its subunits. Electrophoresis and gel staining were performed as described in the legend of Fig. 40. Lane 1 contained 20 ng of hCG (fractions 45-50 from Fig. 39), lanes 2 and 3 contained 20 and 40 ng of beta subunit (fractions 60-65 from Fig. 39); lanes 4, 5, and 6 contained 20, 40, and 60 ng of alpha subunit (fractions 71-76 from Fig. 39).
REFERENCES

Fig. 95. Dose response curves for anti-hCG-8 (R359-22). The reagents used were: 2nd International Standard hCG (0-0); hCG-0 (0-0); hCG-a (A-A), prepared as described under Experimental Procedures; and 1st International Reference Preparation hLH (LER960) (A-A); hLH-a (A-A); hTSH (0-0); hTSH-a (0-0); hFSH (0-0); hFSH-a (0-0). Bound/total = 37.02; nonspecific binding = 2.92% of total counts.

Fig. 96. Dose response curves for anti-hCG-8 (carboxy-terminal peptide-hemocyanin conjugate R359-22). The reagents used were: 2nd International Standard hCG (0-0); hCG-0 (0-0); hCG-a (A-A), prepared as described under Experimental Procedures; and 1st International Reference Preparation hLH (LER960) (A-A); hLH-a (A-A); hTSH (0-0); hTSH-a (0-0); hFSH (0-0); hFSH-a (0-0). Bound/total = 39.04; nonspecific binding = 2.92% of total counts.
Synthesis and secretion of human chorionic gonadotropin subunits by cultured human malignant cells.

R W Ruddon, C A Hanson, A H Bryan, G J Puttermann, E L White, F Perini, K S Meade and P H Aldenderfer


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