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Preparation of Human Chorionic "Growth Hormone-Prolactin."* (29602)

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Evidence that the human and simian placenta secretes a hormone with growth hormone-like and prolactin-like activity, which differs in its immuno-chemical, physicochemical and biologic properties from pituitary growth hormone, has been reported from this laboratory(1,2). It was suggested that this hormone, which appears to be similar, if not identical, to human "placental lactogen" isolated by Josimovich and MacLaren (3,4) is an important metabolic hormone of pregnancy(1). In view of uncertainties concerning its major actions, the tentative designation chorionic "growth hormone-prolactin" (CGP) has been applied(1). The human placental hormone shows an incomplete cross reaction when reacted with rabbit antisera to(1-4) human pituitary growth hormone (HGH), and a lesser binding affinity than HGH for rabbit anti-HGH serum(1). Similarities in the relation of the chorionic hormone to pituitary growth hormone and that which exists between human chorionic gonadotropin and human pituitary luteinizing hormone have been pointed out(1). The present report describes a simple method for the partial purification of chorionic "growth hormone-prolactin" from human placentae utilizing immunochemical and zone electrophoretic techniques to detect the hormone and to assess homogeneity.

Materials and methods. Antisera were prepared in rabbits by injection of HGH or of human CGP and rendered specific by absorption with human serum proteins as described elsewhere(1,5). The immunochemical and electrophoretic methods have been reported previously(1,5,6).

In a typical experiment (Table I), 50 g of lyophilized term placental tissue were ground in a Waring blendor and extracted with 1500 ml of 0.3 M KCl at pH 10. All extractions were performed at cold room temperatures. The extract was centrifuged and the dark brown supernatant dialyzed against running tap water for 24 hours. A euglobulin fraction was removed by centrifugation and the clear supernatant lyophilized to yield 12.5 g of reddish powder.

Five grams of the 0.3 M KCl extract were dissolved in 200 ml distilled water, an equal volume of saturated ammonium sulfate added, and the mixture placed in the cold room for one hour. A precipitate was formed which was removed by centrifugation, suspended in distilled water, and dialyzed overnight against distilled water. After centrifugation to remove an inactive precipitate, the solution was lyophilized to yield 370 mg of tan powder, representing 220 g of fresh placenta.

Further purification was obtained by chromatography on a column of diethylaminoethyl (DEAE)-cellulose equilibrated with 0.01 M *tris* buffer, pH 9.0 (Fig. 1). Stepwise elution with 0.05 M NaCl, 0.1 M NaCl, and 0.33 M NaCl in 0.01 M *tris,* pH 9.0, yielded several components, only one of which (Frac. E) formed a strong precipitin band when reacted with rabbit anti-HGH serum or rabbit anti-human CGP serum by

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TABLE I

50 g lyophilized placental tissue extracted for 1 hr at 4°C with 1500 ml 0.3 M KCl pH 10; centrifuged

- solid residue discarded
- soluble extract dialyzed against running tap water overnight
  - precipitate discarded
  - soluble fraction lyophilized Frac. I wt = 12.5 g
    - 5 g dissolved in 150 ml H_2O and equal volume sat. $(NH_4)_2SO_4$ added. Kept at 4°C for 1 hr
      - precipitate suspended in H_2O and dialyzed against distilled water
        - precipitate discarded
        - soluble fraction lyophilized Frac. II wt = 370 mg
          - 75 mg dissolved in 3 ml .01 M Tris buffer pH 9.0 and chromatographed on DEAE cellulose
            - Frac. A-D discarded
            - Frac. E, 14 mg dissolved in 1 ml .01 M Tris buffer pH 9.0, applied to Sephadex G-75 column
              - Excluded fraction (8.5 mg) discarded
              - Retarded fraction (3.5 mg)
      - soluble portion discarded

the double diffusion method of Ouchterlony or by microimmunoelectrophoresis in agar gel(1). The growth hormone-like activity of this preparation was similar to that previously reported(1).

The preparation was purified further by applying 14 mg of Fraction E in 1 ml of 0.01 M *tris* buffer, pH 9.0 to a Sephadex G-75 column (2×25cm) equilibrated with the same buffer (Table I; Fig. 2). The excluded fraction (Tubes 10-15) containing 8.5 mg of protein and the retarded fraction (Tubes 16-20) containing 3.5 mg of protein were dialyzed against distilled water and lyophilized. The retarded fraction, isolated by gel filtration, yielded the major portion of the immunologically reactive CGP.

Results and discussion. A comparison of the starch gel electrophoretic pattern of human CGP in Fraction E (Channels B) with that of HGH, Raben, Lot No. 16 (Channel C), and of crystalline human serum albumin is given in Fig. 3. Fig. 4 shows a starch gel electrophoretic analysis of the more highly purified preparation of CGP obtained

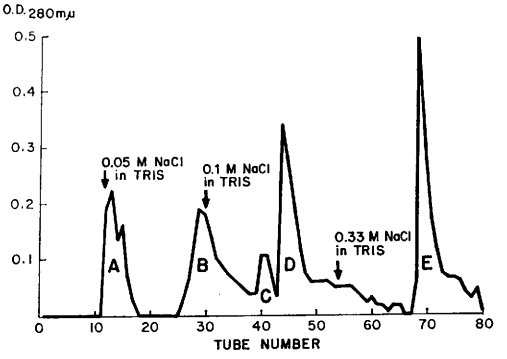

FIG. 1. Chromatography of 75 mg Frac. II. DEAE-cellulose equilibrated with 0.01 M Tris buffer pH 9.0. Column 2 × 38 cm. Five ml aliquots collected. Frac. II dissolved in 3 ml Tris buffer and placed on column, followed by 0.01 M Tris pH 9.0. Discontinuous elution carried out with 0.05 M NaCl, 0.1 M NaCl and 0.33 M NaCl in 0.01 M Tris buffer pH 9.0.

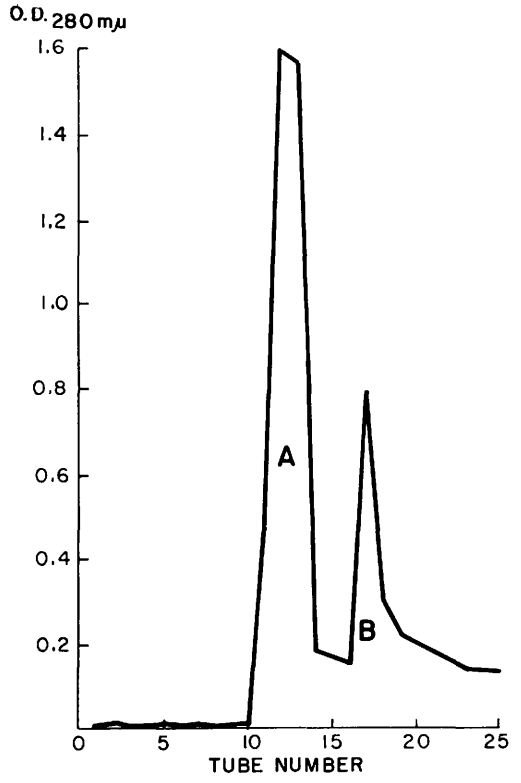

FIG. 2. Effluent pattern obtained by applying 14 mg of Fraction E to a column of Sephadex G-75 (beads) equilibrated with 0.01 M Tris, pH 9.0. Flow rate 60 ml/h; 3 ml fractions. A, excluded fraction; B, retarded fraction.

after Sephadex G-75 filtration. The mobility of the CGP was comparable to that of the component in a freshly prepared saline homogenate of human placental tissue concentrated 10 times by lyophilization (Channels D) which reacted with anti-HGH and which was more mobile than the major component of Raben HGH (Channels C), and of human serum albumin (Channel D). The heterogeneity of the CGP preparation obtained after gel filtration was appreciably less than Fraction E, but a small contamination with serum proteins, mainly albumin (Fig. 4), was still present; the latter were identified by immunoelectrophoresis utilizing horse or rabbit antiserum to human serum(5). However, the homogeneity of CGP seems greater than that of Raben HGH. The CGP in the various preparations was identified by immunoelectrophoresis and by double diffusion in agar according to Ouchterlony using anti-HGH or

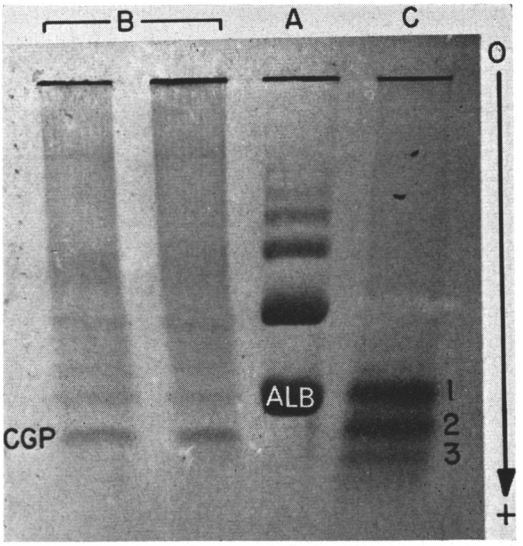

FIG. 3. Vertical starch gel descending electrophoresis(5); discontinuous buffer system of Poulik as modified by Ferguson and Wallace (130 V, 14 hr, 4°C). Channel A contains purified human serum albumin, 8 mg/ml. Channel B contains Fraction E (20 mg/ml). The dark staining anodal band (CGP) cross-reacts with anti-HGH serum. Channel C, HGH Raben Lot No. 16 (10 mg/ml). The latter demonstrates the 3 dark staining bands and several lighter staining bands previously observed with other Raben preparations of HGH.

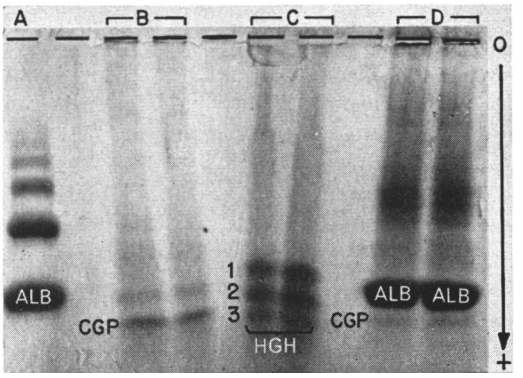

FIG. 4. Vertical starch gel descending electrophoresis. Channel A, purified human serum albumin 8 mg/ml, and in Channel C, HGH (Raben Lot No. 16, 10 mg/ml). Channel B contains the Sephadex G-75 retarded portion, 6 mg/ml and Channel D contains a saline homogenate of human placenta (concentrated 10-fold). The immunologically reactive material which cross-reacted with anti-HGH serum was detected in the band designated CGP. In Channels B the faint staining band more cathodal than the band for CGP and with the mobility of human serum albumin was identified as human albumin by immunoelectrophoresis. This material did not react with anti-HGH or anti-CGP sera.

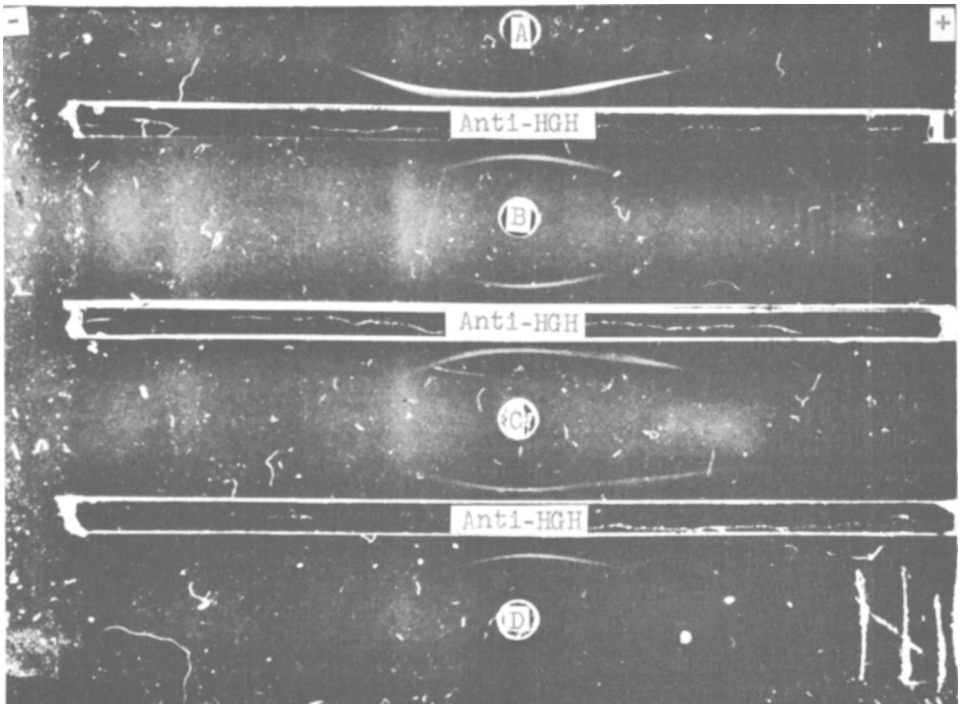

FIG. 5. Immunoelectrophoretogram (barbital buffer, pH 8.2) of the reaction of HGH (Raben Lot No. 16) 250 μg/ml. (Well A), Fraction E, 4 mg/ml. (Well B), Sephadex G-75 retarded fraction 6 mg/ml. (Well C), Sephadex G-75 excluded portion, 16 mg/ml. (Well D), when developed with rabbit antiserum to HGH added to troughs.

anti-CGP as the reactive antisera(2,5). The reactive component was detected only in eluates of the segment of gel(5) corresponding to the dark staining band more anodal than serum albumin. None of the CGP preparations formed a precipitin arc following immunoelectrophoresis and development with rabbit antiserum to human chorionic gonadotropin.

Fig. 5 shows the results of an immunoelectrophoretic analysis obtained by the reaction of Fraction E, of the excluded and retarded fractions following gel filtration, and of HGH with rabbit antiserum to HGH. The crest of the single precipitin arc produced with CGP was slightly more anodal than the arc formed by HGH. Approximately a 4-fold purification, as estimated by immunologic reactivity, was accomplished by gel filtration of Fraction E, prepared by DEAE-cellulose chromatography. Studies of the biologic activity of the purified preparation of CGP are in progress.

Summary. A simple procedure for partial purification of human chorionic "growth hormone-prolactin" is described utilizing DEAE-cellulose chromatography and Sephadex G-75 filtration. The preparation has a high degree of homogeneity. However, small amounts of serum protein contaminants were detected. The electrophoretic mobility in starch gel of the isolated hormone, utilizing a modified discontinuous buffer system was comparable to that of the "native" hormone in a freshly prepared crude placental extract.

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