Action of "Chorionic Growth Hormone-Prolactin" on Growth and Carcass Composition of Hypophysectomized Rat*† (34063)

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(Introduced by H. A. Waisman)

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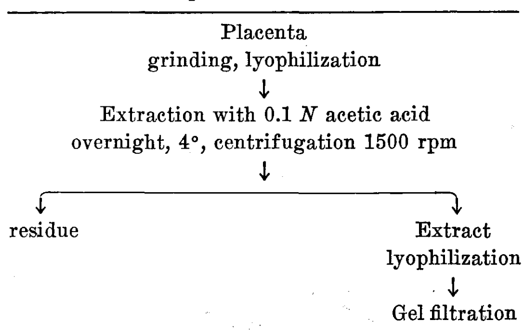
Human placenta may contain a substance similar to pituitary growth hormone. In 1960 Josimovich and Mac Laren (1) described the "placenta lactogen" obtained from human placenta which had the immunological characteristics of growth hormone (GH) and showed prolactin activity. In 1964 Kaplan and Grumbach (2) confirmed these findings and suggested the name "chorionic growth-hormone-prolactin" (CGP) and postulated that the growth hormone—like metabolic changes, such as nitrogen storage, fat mobilization, and glucose utilization, observed in pregnant women could be caused by CGP. Scow and Haven (3) demonstrated that GH increased significantly the concentration of nitrogen and fat in the carcass of hypophysectomized rats, so this could be a good test for the demonstration of GH-like effects of CGP.

We prepared CGP by gel filtration and studied the action of the protein thus obtained on the body composition of hypophysectomized rats.

Materials and Methods. A standard experiment is summarized as follows: (Table I). A recently eliminated placenta free of membranes was homogenized in a Waring Blendor; lyophilized, ground, and extracted with 450 ml of 0.1 *N* acetic acid overnight at 4°; the extract was then centrifuged at 1/500 rpm for 15 min. An abundant brown-red residue was obtained along with a supernatant fraction which had the color of hemolyzed serum.

TABLE I. Preparation of Placental Extract.

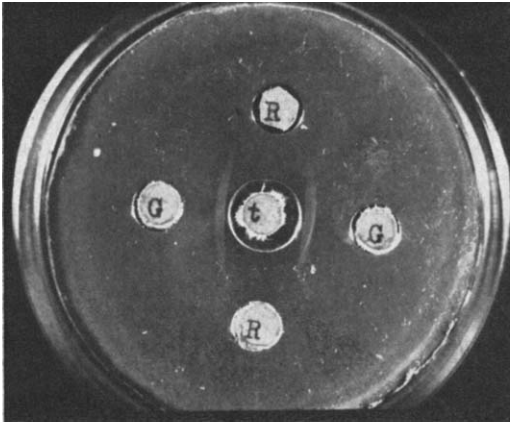


Experimental. Immunological studies. The residue and supernatant fraction were tested for immunological reactions in an Ouchterlony plate (4). The supernatant fluid was lyophilized and subjected to semiquantitative immunological reaction in an agar plate in which a standard solution of GH was distributed in concentrations from 0 to 1000 μg. GH antibody was placed among these standard samples. (See below for preparation of HGH antibody). Successive dilutions of 0–500 mg/ml of placental extract were placed in the same plate beside the antibody (Fig. 5).

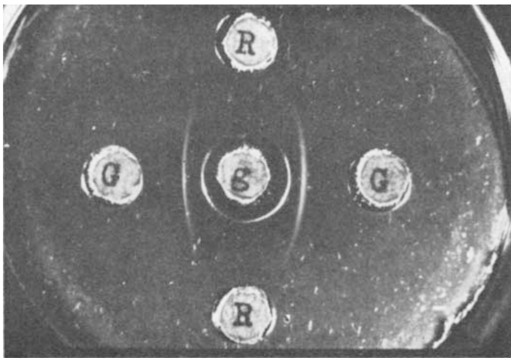
Preparation of antisera. Anti-GH serum was obtained from white rabbits by injecting solution of 2 mg of GH prepared by the method of Raben (5) in 1 ml of saline. The development of the antiserum in the rabbits took about 4 weeks. The first week the GH solution was injected three times intravenously and once subcutaneously, this time being

* This work has been supported by Research Grant A-4226-05 NIH, U.S.

† Read by title at the Thirty-seventh Annual Meeting Society for Pediatric Research, Atlantic City, New Jersey, 1967.



FIGS. 1 and 2. Gel diffusion by Ouchterlony method in residue of placenta extracted with 0-1 N acetic acid. G: HGH by Raben procedure (1 mg/ml); g:rabbit anti-HGH; R:residue of placenta extracted with 0.1 N acetic acid (1 mg/ml); t:rabbit antiserum to acetic acid or total hypophysis extract.



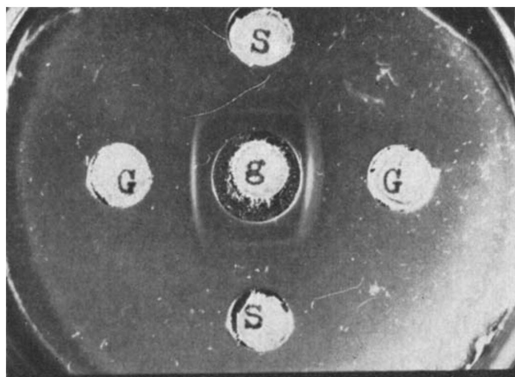
mixed with an equal volume of a mineral oil and lanolin adjuvant (6). During the next 3 weeks the animals received the solution twice intravenously and once subcutaneously, each week. The antibody titer was determined by the method of Wright (7). The antibody solution gave titers between 400 and 300 mg of antigen per ml of antiserum. Total pituitary antiserum was obtained using the 0.1 N acetic extract equivalent to one half of an acetone-dried and ground human gland. The extracts were neutralized with NaOH before injection. The same injection schedule was used.

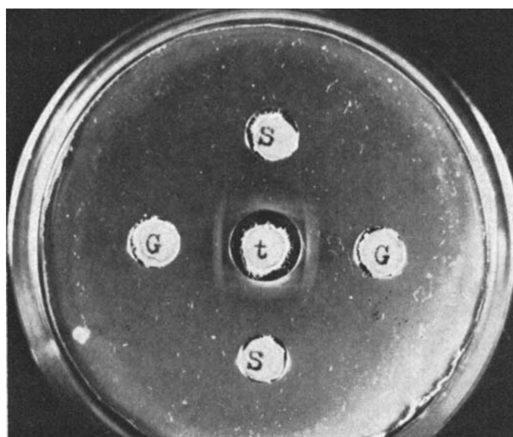
Fractioning in Sephadex G-75 gal°. Two grams of the lyophilized supernatant portion were dissolved in 4 ml of 0.1 N acetic, and placed on a column of Sephadex G-75 (beads) (100 × 1 cm), previously equilibrated with 0.1 N acetic acid. The column was eluted with 0.1 N acetic acid. Three-milliliter fractions were collected with an automatic fraction collector. The protein content was established in a 0.5-ml aliquot of each fraction using the method of Lowry (8), after neutralization with 0.5 ml of 0.1 M NaOH. A graphic representation was obtained plotting the optical density values, against the fraction number (Fig. 6). The immunological reaction was carried out on an Ouchterlony plate (4) using aliquots of each fraction beside the GH antiserum and total pituitary gland antiserum.

Biological assay. The biological activity of the placental extract was studied through bioassay of body weight increment in seven hypophysectomized rats by the method described by Chou *et al.* (9). The animals had been hypophysectomized 10 days earlier and their weights had become stable. The placental extract was injected intraperitoneally for 10 days at a daily dose of 200 μg of the immunological active faction per rat (see second peak in Fig. 6). Hypophysectomized rats were used as controls.

Analysis of the carcass composition of the hypophysectomized rats. After the weight test was completed, the animals were killed in order to study the body composition. Water was determined by the substraction after desiccation to constant weight. Nitrogen was determined by the Kjeldahl method with some modifications (10) on an individual 5-g portion of homogenized carcass; total fat was assayed in an aliquot by extraction in a Soxhlet apparatus; ashes were obtained by calcination and used to determine calcium (11) and phosphorus (12). All assays were performed in duplicate.

Results. Immunological study. Figures 1 and 2 show that a negative immunological reaction was obtained when the residue was placed against GH antiserum and total hypophysis antiserum. Figure 3 shows the results of a qualitative immunological study with the supernatant fluid; only one precipitin line was observed when it was tested with HGH antiserum, while at least two precipitin





FIGS. 3 and 4. Gel diffusion by Ouchterlony method in supernatant fluid of placenta extracted with 0.1 *N* acetic acid. G: HGH, by Raben procedure; g: rabbit antiserum HGH;S: supernatnat of placental extract in 0.1 *N* acetic acid (1 mg/ml); t: rabbit antiserum to total hypophysis acetic extract. Note in Fig. 4 the single band with spur formation.

lines appeared when it was tested against total hypophysis antiserum (Fig. 4). Figure 5 shows results otained with semiquantitative studies of the supernatant fluid. The immunological reaction to the successive dilutions of GH disappears with concentrations of 250 μg/ml and the reaction to the placental supernatant fluid appears to be positive up to concentrations of 31 μg/ml. Because all of the material obtained from the lyophilized supernatant fluid was 23.5 g, we may assume that, under the experimental conditions described, approximately 188 mg of the material prepared (CGP) react with the GH antiserum.

Gel filtration of the placental acetic acid extract. Figure 6 shows typical curve obtained with aliquots of 0.1 ml of each fraction after Lowry reaction in each fraction. Figure 7 shows the distribution of antigens in the eluate as tested with both HGH antiserum and total hypophysis antiserum; tubes 16 to 22 (second peak) Fig. 6, contained material which reacted immunologically as HGH. The reaction with total hypophysis antiserum was positive in tubes 6 to 13 (first peak) and in tubes 16 to 22 (second peak) (Fig. 7).

Biological assay. Figure 8 shows the results of body weight increment after the administration of the fractions of the placenta with

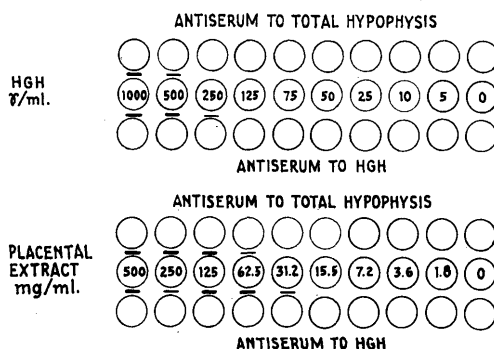


FIG. 5. Immunological study in supernatant fraction of placenta. Semiquantitative reaction.

immunological GH activity (second peak). No significant difference was found between the hypophysectomized control group and the group which received the placental extract.

Analysis of the carcass composition of the hypophysectomized rats. Table II shows the

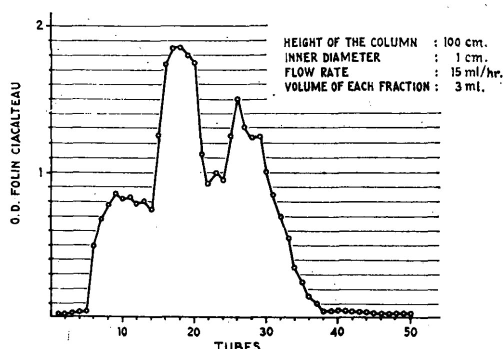


FIG. 6. Gel filtration of a placental extract in Sephadex G-75 beads.

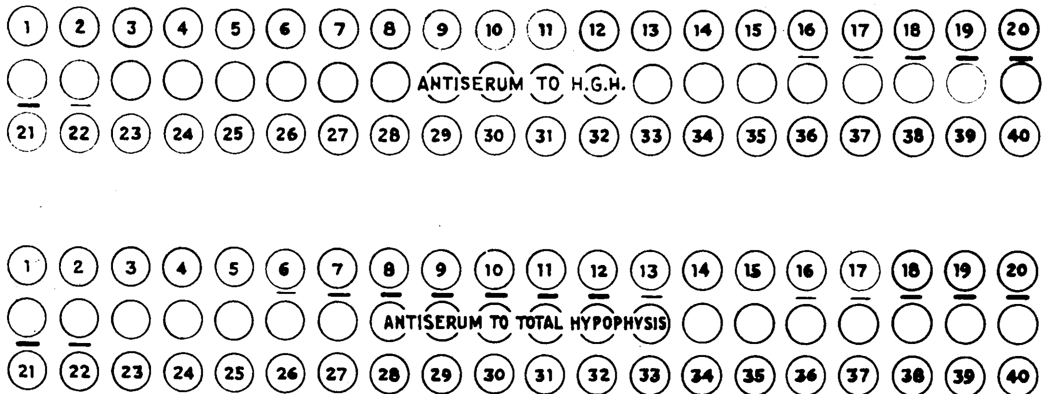


FIG. 7. Immunological reaction of placental fractions obtained by gel filtration. Each number corresponds to each fraction.

carcass composition of the hypophysectomized rats which received the placental protein with immunological GH activity as well as that the hypophysectomized control group. No difference was found.

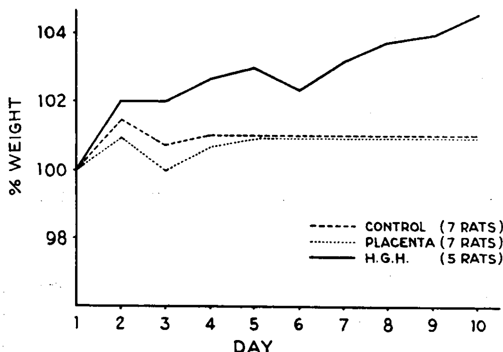


FIG. 8. Body weight increment test in rats treated with placental extract and HGH (Raben)

Discussion. Different methods have been suggested to prepare GH-like placental extracts (1, 2, 13). The method reported here was formerly used to prepare a human urinary fraction with growth hormone activity and was described elsewhere (14).

The use of 0.1 N acetic acid for this purpose seems adequate since, in our experience, the sensitivity of this reaction is about 15–20 μg/ml and after extraction no immunological activity was found in the residue. Extraction with this acid and gel filtration guarantees better preservation of proteins than other methods which produce marked changes in pH and in ionic strength (5–15).

Immunological studies of the supernatant fluid against the total hypophysis antisera showed at least two proteins which are immunologically identical to proteins from human hypophysis. One of them shows immunological identity with HGH (Fig. 4).

In order to separate these two proteins, the supernatant fluid was filtrated. Under the conditions described, three protein concentration peaks were obtained and two of them (the first and the second) correspond with the two lines of immunological activity. The second of these peaks is immunologically similar to the HGH of Raben (5). Kaplan and Gumbach (2) described the physicochemical properties of this protein and the spur described by them as an indicator of partial

TABLE II. Carcass Composition of Hypophysectomized Rats Treated with Placental Extract.

	Weight (g)	Water (%)	Fat (%)	Ash (%)	N (%)	P (%)	Ca (%)
Placenta	103.57	60	36.20	9.97	8.39	1.81	2.96
SD	±8.66	±5.19	±4.12	±0.78	±0.40	±0.36	±0.33
Controls	104.0	64.3	36.40	10.14	8.43	1.76	3.12
SD	±10.7	1.78	4.47	±1.22	±0.61	±0.72	±0.50

immunological identity was found. Each one of these peaks gave only one precipitin line when placed separately against total-pituitary antiserum (Fig. 7). Studies on the nature and properties of the first and third peaks are being carried out in our laboratory.

Studies for weight increment with placental extract in hypophysectomized rats show that in the doses used there is no significant differences between the treated rats and the controls. It seems that, under the experimental conditions described, the extract has no growth-promoting activity. It is important to point out that the doses given are quite high. These results are in agreement with those of Kaplan and Gumbach (2) who obtained a positive response on the growth of the tibial epiphysis and on the incorporation of radioactive sulfate by costal cartilage in hypophysectomized rats only when they gave doses around 1 mg of placental material. This response is lower than that produced by 6 μg of bovine GH under the same experimental conditions. A similar and equivocal response was found by Frish (13).

Young (16) has shown that GH administered to normal rats produces a change in body composition as far as fat, nitrogen, and water content are concerned. Scow and Haven have demonstrated that HGH and testosterone increase significantly that amount of protein and fat in the carcass and pelt of hypophysectomized rats (3). It appears that this could be a good test for demonstrating a similar effect by CGP, although under these conditions the hormonal environment of pregnancy is not present.

On the basis of the findings of Hummel *et al.* (17) and of Chesley (18) concerning utilization of fat and nitrogen stores in pregnancy, Kaplan and Gumbach (2) have suggested that the CGP may be responsible for these changes. Results obtained in the present experiments seem to indicate that the placental extract in the doses tested does not have this kind of biological activity, and the absence of changes in body composition observed in hypophysectomized rats after the injection of placental extract contradicts this hypthesis.

Summary. It has been suggested (1) that CG-P may serve as the metabolic hormone of pregnancy ensuring a maternal store of nitrogen and minerals and mobilizing fat.

CG-P (immunologically active fraction, after gel filtration) was injected intraperitoneally to hypophysectomized rats at a daily dose of 200 μg per rat during 10 days in order to demonstrate these actions.

The carcass composition of the animals was analyzed (water, fat, nitrogen, protein, and calcium).

A group of hypophysectomized rats which did not receive placental extract was used as control. The absence of changes in body composition in hypophysectomized rats treated with CG-P seems to indicate that under described experimental conditions, the placental protein does not have the postulated metabolic activities.

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Received April 2, 1969. P.S.E.B.M., 1969, Vol. 131.