duces only intragenic changes at this region of chromosome I of *Neurospora*.

The tubes inoculated with the ICR-170–induced mutants alone give the frequency of leakiness among all *ad-3* mutants, and the growth responses with testers (iv) to (vii) give the incidence of allelic complementation and of complementation pattern types among the *ad-3B* strains. Only 5.3 percent (10 out of 187) of the mutants were leaky. Thirty-one out of 130 *ad-3B* mutants complemented at least one of the *ad-3B* testers [testers (iv) to (vii)], giving a frequency of 23.8 percent complementing and 76.2 percent non-complementing *ad-3B* mutants. The complementing *ad-3B* mutants can be further classified as polarized or non-polarized (*6*) on the basis of their response with testers (iv) to (vii). Mutants that do not complement testers (i) and (ii), but do complement testers (iii) and (iv) or only (iv) give a polarized pattern—that is, they map from complon 1 to some distance to the right. The nonpolarized mutants are usually less extensive and can show a variety of patterns (*11*). Among the complementing *ad-3B*'s induced by ICR-170, 64.5 percent (20 out of 31) were polarized and 35.5 percent (11 out of 31) were nonpolarized.

In Table 1 the characteristics of the ICR-170–induced *ad-3* mutants are contrasted with previously described characteristics of *ad-3* mutants isolated in wild-type 74A from four other origins (*6*). It is interesting that the ICR-170–induced mutants are very similar to those induced by x-rays and unlike those induced by 2-aminopurine or nitrous acid in the three characteristics shown. On the other hand, if the incidence of deletion types is considered, the ICR-170–induced mutants are similar to those induced by nitrous acid (*10*) and unlike those induced by x-rays (*7, 9, 15*). Purple adenine mutants induced by ICR-170 are, therefore, unique among the *ad-3* mutants of different mutagenic origins analyzed to date. Furthermore, the low incidences of leakiness, allelic complementation, and of nonpolarized complementation patterns suggest that the enzyme(s) specified by the *ad-3* region is absent or grossly altered in ICR-170–induced *ad-3* mutants. Two explanations of these low incidences can be offered: (i) the mutations are predominantly base-pair additions or deletions rather than base-pair substitutions, and ICR-170 is therefore acting like an acridine (*19*); or (ii), the mutations are predominantly base-pair substitutions giving primarily nonsense codons (*20*) or alternatively mis-sense codons (*20*) at a specific set of sites within the *ad-3* region that specify especially crucial amino acids for determining an active enzyme.

The high mutagenicity of this compound is striking. It would be interesting to know if both components of the molecule are mutagenic in *Neurospora* or if there is a potentiation of the mutagenic activity of the alkylating agent by the acridine nucleus by virtue of its strong affinity for DNA (*21*). Comparative mutation studies of the two components at the *ad-3* region of *Neurospora* should answer this question.

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Immunoassay for Human Chorionic "Growth Hormone-Prolactin" in Serum and Urine

Abstract. *Chorionic "growth hormone-prolactin" in serum and urine from pregnant women, in serum from umbilical cord, and in amniotic fluid was assayed by a sensitive immunological method dependent on the combination of rabbit antiserum to the growth hormone-prolactin with the iodine-131–labeled hormone. The hormone is detectable in serum and urine early in gestation, and with advancing pregnancy its concentration in serum continues to increase to a maximum during the last trimester. It was not found in serum within 8 hours after delivery.*

Human placenta contains a protein which cross-reacts with rabbit antiserum to human pituitary growth hormone (HGH) (*1–6*), and a similar protein has been reported in simian placenta (*3–5*). The placental protein fraction giving an incomplete cross-reaction with antiserum to HGH and exhibiting prolactin-like and luteotropic activity has been called "placental lactogen" by Josimovich and MacLaren (*1*). It was suggested that the placental protein has activity similar to that of growth hormone, the potency of the placental hormone being appreciably less than that of pituitary growth hormone (*3, 4*). We have proposed that this hormone which appears to function as an important metabolic hormone of pregnancy be tentatively designated chorionic "growth hormone-prolactin" (CGP) (*3, 4*). Studies in vivo and in vitro, including production by cultures of human chorionic villi, provide firm evidence that the placenta synthesizes and secretes CGP (*4*). Although exhibiting

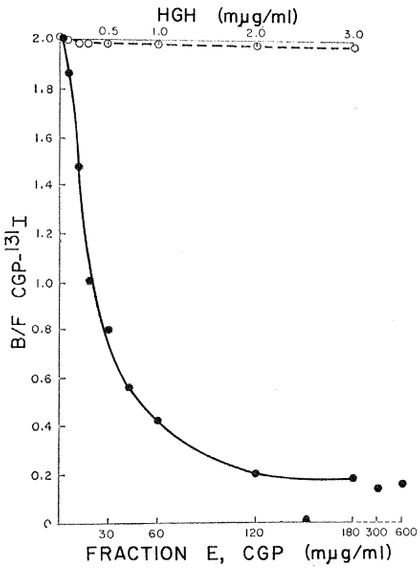

Fig. 1. Standard curve obtained by chromatoelectrophoretic separation of free and antibody-bound CGP-^{131}I from solutions incubated with unlabeled CGP. A sharp decline in the B/F ratio of CGP-^{131}I occurs as the concentration of unlabeled CGP increases. The addition of CGP-^{131}I and antiserum to CGP at 1:20,000 dilution to increasing amounts of human growth hormone did not produce a change in the B/F ratio, as indicated by the dashed line in the upper portion of the graph.

certain biologic characteristics of human growth hormone and sharing common antigenic determinants with the pituitary hormone, CGP is distinguishable from HGH by its physicochemical and immunologic properties (*1–4, 7*). Purified preparations of CGP exhibit little heterogeneity (*2, 7, 8*). However, analogous to the unresolved question of the unimolecular or multiple molecular nature of so-called HGH is the uncertainty whether all of the biologic activities of CGP are attributable to a single molecule (*3, 4*).

Even though CGP has been detected in serum and urine from pregnant women by gel-diffusion methods (*1–4*) a sensitive assay has not been available. We now describe an immunoassay with ^{131}I-labeled CGP and rabbit antiserum to human CGP; the assay was developed for the direct measurement of CGP in the serum and urine of women during pregnancy.

Antiserum to CGP was produced by the intradermal injection of rabbits with a purified preparation of CGP (*3, 9*).

The CGP-^{131}I (specific activities ~ 90 to 145 μc/μg) was prepared by a modification of the method of Greenwood *et al.* (*10*). The following solutions were rapidly mixed in the sequence indicated: (i) 0.025 ml of 0.5*M* phosphate buffer, *p*H 7.5; (ii) 2.0 to 2.5 mc of ^{131}I (IsoServ. Inc.); (iii) 0.015 ml of CGP (1 mg/ml); (iv) 0.020 ml of chloramine T (3.5 mg/ml); (v) 0.080 ml sodium metabisulfite (2.4 mg/ml); and (vi) 0.050 ml of bovine serum albumin (5 mg/ml). The separation of CGP-^{131}I from unreacted ^{131}I, damaged hormone, and traces of other iodinated proteins was carried out by vertical starch-gel electrophoresis with the modified discontinuous buffer system of Ferguson and Wallace (5 volt/cm for 16 hours at 4°C) (*9*). The CGP-^{131}I was localized in the gel by radioautography at a position about 1 to 2 cm more anodal than the albumin marker; it was eluted by syneresis. This procedure yielded a highly purified preparation of CGP-^{131}I.

The assay is based on the method of Berson and Yalow for the measurement of insulin, subsequently adapted for the determination of human growth hormone, glucagon, parathormone, and ACTH (*11, 12*). Unlabeled hormone quantitatively inhibits the binding of labeled hormone to antibody; thus the bound to free (B/F) ratio of labeled hormone decreases as the concentration of unlabeled hormone is increased. An antibody titer is chosen which will bind 60 to 70 percent of a tracer amount of CGP-^{131}I after 4 days incubation at 4°C. The resultant B/F ratio of 1.5 to 2.5 diminishes progressively with increments in the concentration of standard solutions of CGP. The ^{131}I-labeled hormone is separated from the unbound labeled hormone by chromatoelectrophoresis on Whatman 3MM filter paper ("not selected for chromatography") in a barbital buffer system (*p*H 8.6, 0.05 ionic strength) at 4°C as described by Glick *et al.* for HGH (see *12*).

All serums were obtained after subjects had fasted overnight with the exception of those serums collected at the time of delivery. Serums from pregnant women and samples of amniotic fluid were assayed at dilutions of 1:1000 to 1:10,000. Serums from nonpregnant subjects, umbilical cord serums, and aliquot portions of 24-hour urine specimens were assayed at dilutions of 1:10 to 1:20. The diluent used for the serum and urine samples and for the solutions of standards and antibody was a barbital buffer (0.05 ionic strength, *p*H 8.6) containing 3 percent of normal rabbit serum and 0.5 percent human serum albumin (*11*).

No appreciable loss of immunologic activity was noted after repeated freezing and thawing of the serum samples. However, the use of stock solutions of CGP (1 mg/ml barbital buffer, *p*H 8.6) stored at −20°C for more than 1 month resulted in CGP-^{131}I preparations of lower specific activity as well as loss of some immunologic activity in the standard solutions.

Initially, a preparation of CGP obtained after chromatography on diethylaminoethyl (DEAE)-cellulose (fraction E) was used as the reference material. Further purification of CGP has been achieved by filtering fraction E through a column of Sephadex G-75 equilibrated with 0.01*M* tris, *p*H 9.0 (*7*); this has yielded a preparation exhibiting a high degree of electrophoretic homogeneity. Serum and urine values are relative to the more highly purified preparation of CGP, there being no official reference preparation.

The curve obtained by plotting the

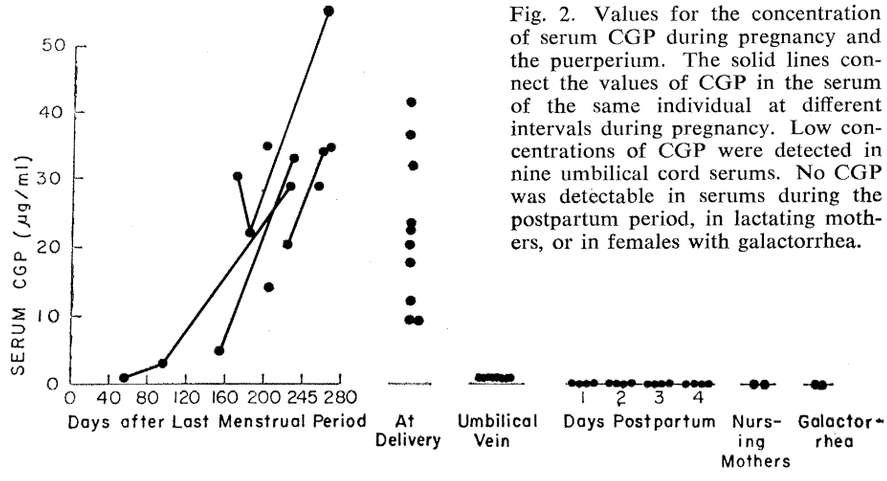

Fig. 2. Values for the concentration of serum CGP during pregnancy and the puerperium. The solid lines connect the values of CGP in the serum of the same individual at different intervals during pregnancy. Low concentrations of CGP were detected in nine umbilical cord serums. No CGP was detectable in serums during the postpartum period, in lactating mothers, or in females with galactorrhea.

Table 1. Excretion of urinary CGP.

Length of gestation (days)	Urine (μg/day)	Serum (μg/ml)
56	10.0	0.5
98	13.1	2.8
101	17.7	
135	56.9	
170	59.5	
178	31.1	
198	100.4	
203	69.2	13.9
222	76.4	
223	36.5	20.6
223	121.5	28.9
239	93.2	
265	105.0	

B/F ratio of CGP-^{131}I against the concentration of unlabeled CGP at a dilution of rabbit antiserum to CGP of 1:20,000 (Fig. 1) illustrates competitive inhibition of the binding of labeled hormone to antibody by unlabeled hormone. The upper dotted curve demonstrates the ineffectiveness of Wilhelmi HGH at concentrations of 0.05 to 10.0 mμg/ml to inhibit the reaction of CGP-^{131}I with antiserum to CGP. In addition, serum from normal adults and children and from patients with active acromegaly did not react in this assay system. The observation that CGP-^{131}I was not bound to rabbit antiserum to human serum at dilutions as low as 1:100 provided further evidence for the specificity of the assay.

The concentration of CGP in serums from pregnant women is shown in Fig. 2. At the earliest stage in pregnancy studied, 56 days after the last menstrual period, there was 0.53 μg of CGP per milliliter in the serum. A sharp rise in serum CGP was observed during pregnancy, a maximum being attained by the third trimester. In the few pregnant subjects studied on more than one occasion during gestation, there was a similar rise.

The amount of CGP excreted in 24-hour samples of urine was compared with its concentration in serum on the same day from the same pregnant woman (Table 1). The urinary CGP gradually rose during pregnancy, the pattern differing appreciably from that described for human chorionic gonadotropin. The data suggest a rough correlation of the amount of CGP excreted with the amount in serum. However, the exceptions observed may reflect an incomplete 24-hour collection of urine, variations in the metabolism of CGP, loss of activity with storage, or laboratory error.

At delivery, the amount of CGP in maternal serums was 50 to 200 times higher than that in the umbilical vein, the samples having been obtained simultaneously. In three samples of amniotic fluid, the concentration of CGP was 2.0, 3.3, and 11.1 μg/ml, respectively.

By 8 hours postpartum, CGP has been cleared from the circulation as indicated by assay of serum at dilutions as low as 1:5. The CGP was not detected in the serum of nursing mothers, in the postpartum period, or in individuals not harboring trophoblastic tissue. The serum of two nonpregnant women with galactorrhea did not contain CGP.

That the human placenta is the source of CGP is supported by these studies. The rise in serum CGP during pregnancy, its rapid disappearance postpartum, even in nursing mothers, supports this view and reflects the specificity of the assay. The localization of CGP within the cytoplasm of the syncytiotrophoblast layer of the human placenta (*6*) and the production of CGP by human chorionic tissue grown in vitro (*4*) indicate that, in addition to storing the hormone, the placenta synthesizes it.

The concentration of CGP in the serum of pregnant women is markedly elevated when compared to the concentration of HGH detected in acromegalic patients or in normal individuals after a hypoglycemic stimulus or a prolonged fast (*12, 13*). This substantiates earlier gel-diffusion studies in which precipitin bands were demonstrable when antiserums to HGH were reacted with pregnancy serum but not when reacted with acromegalic serums (*3*). These observations are consistent with data obtained by bioassay showing that the potency of CGP is considerably less than that of pituitary growth hormone, a finding supported by the failure to observe signs of acromegaly in normal pregnancy (*4*).

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Micropuncture Study of Inulin Absorption in the Rat Kidney

Abstract. *By means of a microinjection technique, inulin-carboxyl-C^{14} or inulin-methoxy-H^{3} was injected into single proximal tubules of the rat at various urine flow rates. Urine collected separately from the two kidneys showed negligible amounts of inulin activity on the noninjected side, thus demonstrating directly that there is no significant reabsorption of inulin by the renal tubular epithelium under these conditions.*

The use of inulin as a measure of glomerular filtration rate (*1, 2*) and renal tubular water reabsorption (*3*) is based on the assumption that it is freely filtered at the glomerulus and that no reabsorption or secretion takes place along the nephron. Although the proportional rise in inulin excretion with increasing plasma concentration supports this assumption (*2*), no conclusive evidence for the fate of inulin in the renal tubule has yet been presented. Micropuncture techniques (*4*) provide a possibility of testing this problem directly, and Shehadeh *et al.* have reported that inulin is extensively reabsorbed into the circulation after its infusion into rat proximal tubules blocked proximally by mineral oil (*5*). Their average recovery in the blood of 23 percent of injected inulin may represent an underestimate of inulin reabsorption, since urine was apparently not collected from either kidney during the first 20 minutes after infusion into the