Growth Hormone Modulation of Liver Drug Metabolic Enzyme Activity in the Rat

II. Specificity of the Hormone Effect[1] (37452)

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(Introduced by A. Bass)

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In a previous study, the hepatic metabolism of hexobarbital, ethylmorphine and *p*-nitrobenzoic acid was demonstrated to be decreased 48 hr after injection of growth hormone (GH) in the male rat (1). A similar effect on liver drug metabolic enzyme activity was noted following injection of a pituitary tumor brei which contained rat GH (2, 3). Preliminary studies indicate that this effect is not mediated via humoral factors from possible target organs (adrenal, gonad) (4), pituitary suppression (5), or by production of inhibitors in rat liver or plasma demonstrable through *in vitro* reaction techniques (4, 6). These studies suggest a specific, direct action of GH on the liver microsomal system responsible for drug biotransformation. The susceptibility of this system to changes associated with factors such as stress (5) or general hormonal environment (7–9), and similarities in structure and action of GH, prolactin and human chorionic somatomammotropin (HSC) (10–14), however, emphasize the need for more detailed examinations on the specificity of the GH effect. This study represents one such examination wherein liver drug metabolism is compared in GH-treated rats vs animals injected with hormonal (prolactin, HCS) or nonhormonal (albumin, globulin) polypeptides. Evidence suggests that, for most drug substrates studied, the action of GH is both specific and unrelated to possible nonspecific effects of polypeptide or amino acid injection.

Methods. Animal and treatments. Male Fischer rats (80–90 days of age, 200–250 g body weight) were obtained at least 2 wk prior to use from either Simonsen Laboratories or Charles River Laboratories, Inc. Temperature (70 ± 2°), humidity (about 50%) and light–dark cycle (12 hourly) were controlled in a room insulated against external noise. Purina rat chow and water were offered *ad libitum.* Injection solutions were prepared by dissolving the polypeptide or amino acid mixture in 0.1 *N* $NaHCO_3$ and adjusting to pH 8–8.5 with drops of 1 *N* NaOH. Solutions prepared 1 day prior to injection were stored at 4° until used. Bovine GH (NIH-B16) and ovine prolactin (NIH, P-S-8) were obtained as a gift from the Endocrine Study Section of the NIH.[3] Porcine GH (Raben) and ovine GH were obtained from Nutritional Biochemicals Co. Lederle Laboratories donated a sample of HCS. Albumin and globulin preparations were obtained from Sigma Chemical Co. An amino acid mixture was prepared such that each gram of the mixture contained amino acids quantitatively and qualitatively similar to those described for human GH (13). Each 100 g of the mixture contained (g): tryptophan, 0.5; histidine, 1.6; methionine, 1.6; half cystine, 2.1; alanine, 3.7; valine, 3.7; asparagine, 4.3; glycine, 4.3; isoleucine, 4.3; proline, 4.3; tyrosine, 4.3; glutamine, 4.8; lysine, 4.8; arginine, 5.3; threonine, 5.3; aspartic acid, 6.4; phenylalanine, 6.9; glutamic acid, 9.0; serine, 9.6; leucine, 13.3. A boiled GH solution was prepared by dis-

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[3] Preparations of GH contained 0.8–1.0 IU/mg dry weight; prolactin contained 28 IU/mg.

solving porcine GH in water, adjusting to pH 7.1 with 0.1 *N* HCl, boiling in waterbath for 20 min, and final adjustment to pH 8–8.5 with a saturated solution of $NaHCO_3$. Concentrations of hormones, polypeptides or amino acid mixtures were adjusted so that rats received 1 ml/200 body weight sc at 48 and 40 hr before performance of *in vitro* assays.

Biochemical procedures. Livers from decapitated rats were rinsed in 1.15% KCl and homogenized with a Teflon pestle using a smooth-walled glass homogenizer. The homogenate, 1 ml equivalent to 1/3 g liver in 1.15% KCl in 0.5 *M* Tris (pH 7.35), was centrifuged at 9000*g* for 20 min at 4°. Microsomes were obtained by centrifuging the 9000*g* supernatant fraction at 100,000*g* for 60 min and washing in 1.15% KCl–Tris for 45 min. The microsomal pellet was resuspended in 0.1 *M* potassium phosphate buffer (pH 7.35) for use in cytochrome P-450 assays.

A 0.25 ml aliquot of the liver 9000*g* supernatant fraction was added to a reaction vessel which contained (μmoles/2.5 ml final volume): Glucose-6-phosphate, 9; $MgSO_4$, 24.2; nicotinamide adenine dinucleotide phosphate (NADP), 2.08; potassium phosphate buffer (0.1 *M*, pH 7.35), 1.4 ml; and either hexobarbital sodium, 1.5; ethylmorphine, 10; aminopyrine, 8; aniline HCl, 5; or *p*-nitrobenzoic acid, 20. Semicarbazide HCl (25 μmoles/2.5 ml) was used as a trapping agent for formaldehyde produced from ethylmorphine. Incubations were performed in a Dubnoff-type shaking waterbath at 37° for 20 min under oxygen or, for *p*-nitrobenzoic acid, for 30 min under nitrogen. The reaction rate showed minimal deviation from linearity with these incubation conditions. Previously described methods were used for the analysis of hexobarbital metabolized (15), formaldehyde formed from ethylmorphine or aminopyrine (16, 17), *p*-aminophenol from aniline (18), and *p*-aminobenzoic acid from *p*-nitrobenzoic acid (19). In this latter reaction, boiled microsomes were incubated with cofactors, substrate and 100,000*g* supernatant fraction to correct for nonenzymic reduction of *p*-nitrobenzoic acid by hepatic microsomes. Tissue blanks were also used to correct for formaldehyde not formed from ethylmorphine or aminopyrine. Analysis of microsomal protein (20) (with bovine albumin as the standard) revealed no consistent changes in experimental vs control liver. Thus, all results are expressed per gram of wet weight liver.

The content of hepatic cytochrome P-450 was determined after reduction by sodium dithionite (21, 22) or NADPH (23). When dithionite was used, the microsomal suspension was diluted to contain 2–3 mg protein/ml and then divided equally into two cuvettes containing a small amount of solid sodium dithionite. Carbon monoxide was bubbled through the sample cuvette and the absorption peak at 450 vs 490 nm was obtained with a Coleman recording spectrophotometer. No correction was made for content of cytochrome b_5 or hemoglobin because no difference in these hemoproteins was noted at 450 nm with microsomes from GH-treated and control rats. The extent of NADPH-reduced cytochrome P-450 was determined within 3–5 min following the asymptotic phase of P-450 reductase activity. Liver cytochrome P-450 reductase activity was measured in a suspension of microsomes (3–4 mg protein/ml) equilibrated with carbon monoxide for 2 min and reduced by rapid addition of 0.05 ml NADPH (0.5 *M*) delivered by plunger to an Aminco anaerobic cuvette (23). The initial rate of absorbance change at 450 nm was used as recorded with a Gilford 2400 spectrophotometer. Statistical analyses were performed according to methods described by Snedecor (24).

Results. Effect of polypeptide hormones. The effect of bovine, ovine and porcine GH, boiled GH, and prolactin on the metabolism of hexobarbital, ethylmorphine, aniline and *p*-nitrobenzoic acid is depicted in Fig. 1. Rats treated with GH of various origin (porcine, ovine, or bovine) showed a dose-related decrease in hepatic drug metabolism. Injection of boiled porcine GH produced less of an effect on the metabolism of all compounds with the exception of PNBA compared with the effect of nonboiled porcine GH. Since some effect of the boiled preparation on the

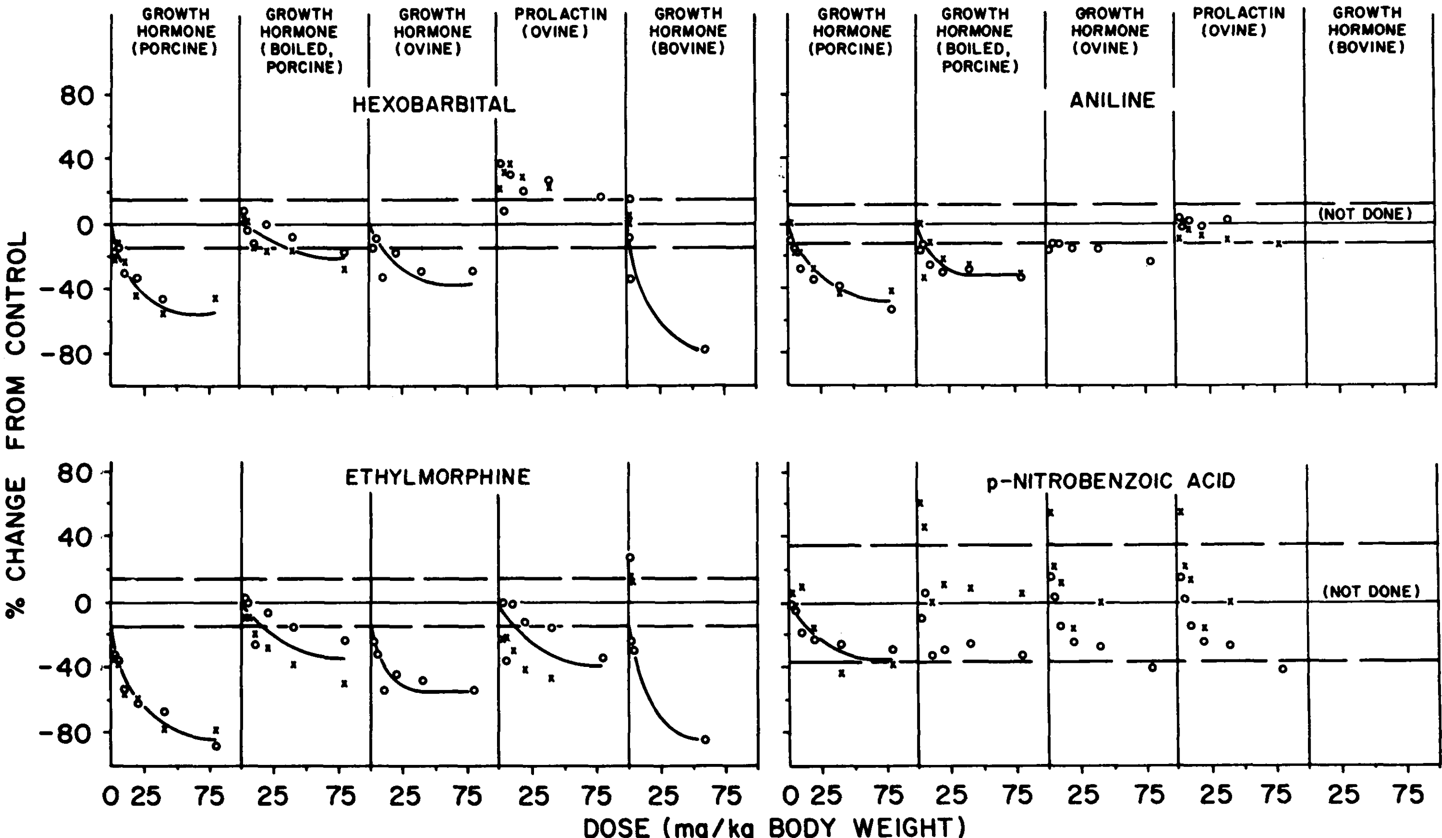

FIG. 1. Effect of polypeptide hormones on liver drug metabolism in the rat. Male Fischer rats were injected with 0.1 *N* $NaHCO_3$ (controls) or a hormone preparation sc at 48 and 40 hr before assays were performed. Each value represents a single determination with liver pooled from 2 to 3 rats. When bovine growth hormone-treated animals were used, the hormone was dissolved in 16% gelatin for sc injection once 48 hr prior to assay; control rats were injected once with bovine albumin (20 mg/ml 16% gelatin). Each value (for bovine GH) represents the mean of 6 determinations for this part of the study. In all other parts of the study each value represents a single determination. The control value for each experiment was used to calculate the percentage change from control. The broken lines on either side of the mean control represent ±3 SE for all control values obtained from different control animals in each part of the study. (O) Expt 1; (X) Expt 2.

metabolism of all drugs was observed, it is possible that the boiling procedure did not completely destroy biologic activity of the hormone, or nonspecific stress effects of the hormonally inactive polypeptide were reflected by the small decrease in hepatic drug metabolic enzyme activity. Prolactin pretreatment resulted in a paradoxical response for the metabolism of hexobarbital compared to the response in GH-injected rats. The liver biotransformation of ethylmorphine and PN BA, however, showed an apparent dose-related decrease with prolactin treatment similar to that found with the GH preparations. Little change in aniline metabolism was found after injection of prolactin. The effect of HCS (2 mg/rat injected at 48 and 40 hr prior to sacrifice) on hepatic drug biotransformation was examined. A limited supply of this hormone precluded any dose-response studies. The liver metabolism of hexobarbital was slightly decreased (−9.4%) and that of aminopyrine slightly increased (+10.6%) by HCS compared with $NaHCO_3$-injected control rats. Interpretation of these findings in relation to effects of GH must await availability of HCS in greater quantities to permit more extensive studies.

Changes in the content of liver cytochrome P-450 (dithionite or NADPH reduced) were somewhat variable after injection of the polypeptide hormones (Fig. 2). In general, the extent of dithionite-reduced cytochrome P-450 was decreased by GH and boiling of the hormone produced a slight alteration in its potency for this effect. Chemical reduction of the hemoprotein showed marked inter-experimental variability following injection of prolactin. Porcine GH more effectively lowered the amount of NADPH-reduced cytochrome P-450 than did ovine GH. A dose-related change was not noted in this parameter in prolactin-injected rats. Cytochrome P-450 reductase activity was decreased by injection of porcine or ovine GH and boiling of the porcine preparation decreased its effectiveness. Prolactin affected this enzyme activity only at high doses.

Effect of nonhormonal polypeptides and amino acids. Multiple doses of albumin, globulin, or an amino acid mixture were injected

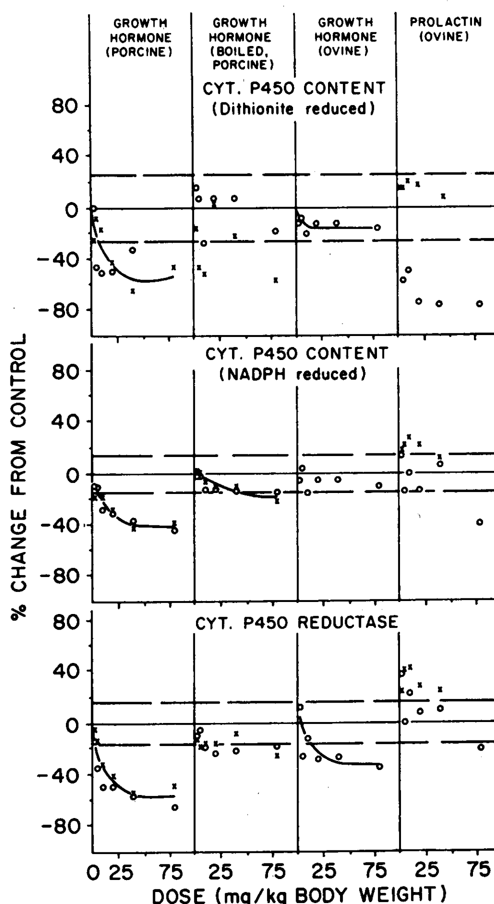

FIG. 2. Effect of polypeptide hormones on liver drug metabolism in the rat. Legend is the same as that described for Fig. 1.

in male rats to note possible nonspecific effects on liver drug metabolism (Fig. 3). Injection of these protein or amino acid-containing solutions did not produce a consistent change in the hepatic metabolism of hexobarbital or ethylmorphine compared with control rats which received $NaHCO_3$. The metabolism of aniline and PNBA showed similar results except for an increase in PNBA metabolism following administration of high doses of egg globulin. In general, the metabolism of all drugs showed a variable and random response to the injection of these nonhormonal substances. It is assumed that this response is a nonspecific one which follows the injection of a protein-containing solution and thus partially explains the varia-

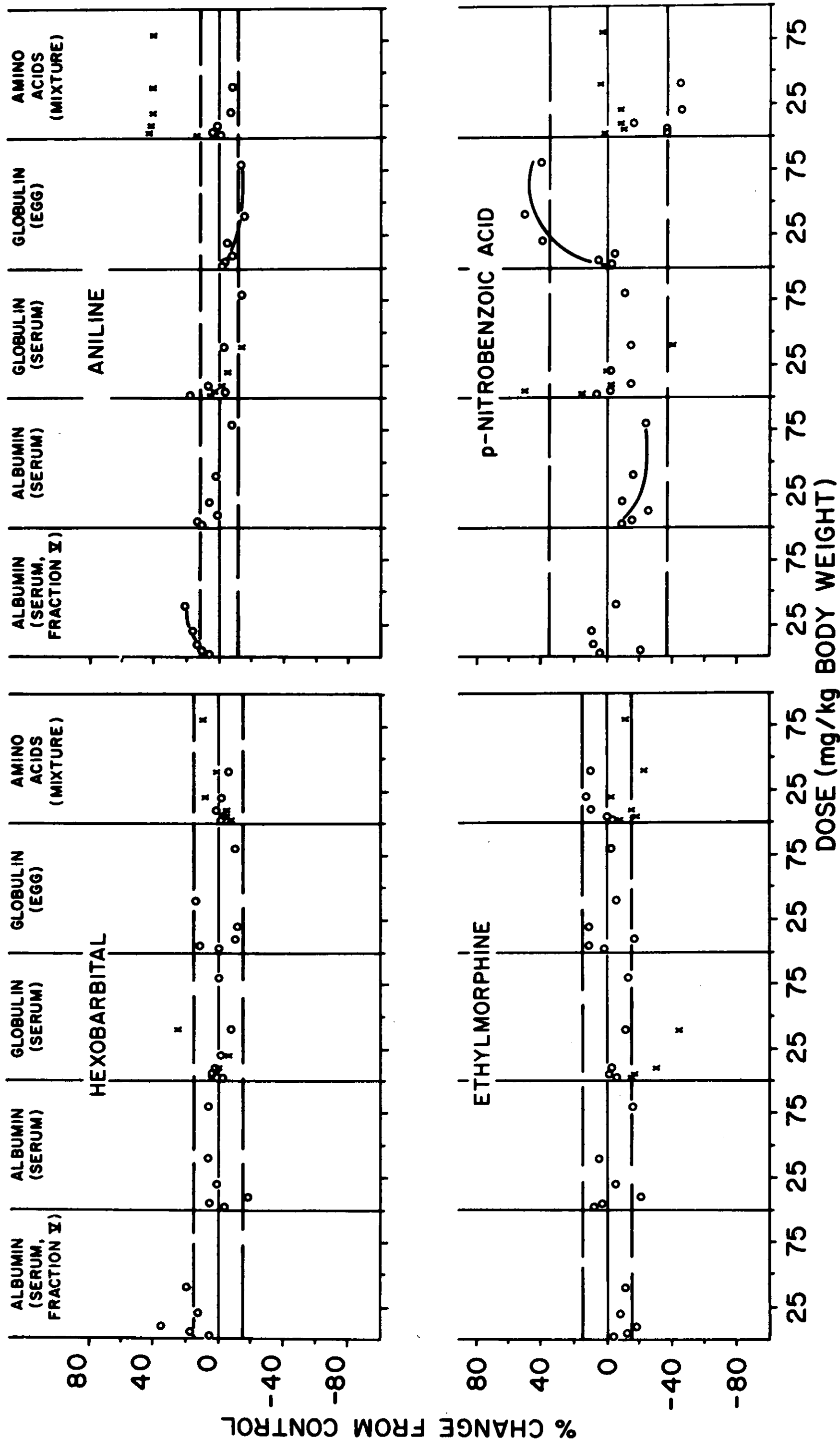

FIG. 3. Effect of polypeptides and amino acids on liver drug metabolism in the rat. Legend is the same as that described for Fig. 1 except for the use of nonhormonal polypeptides or an amino acid mixture in the injection procedure.

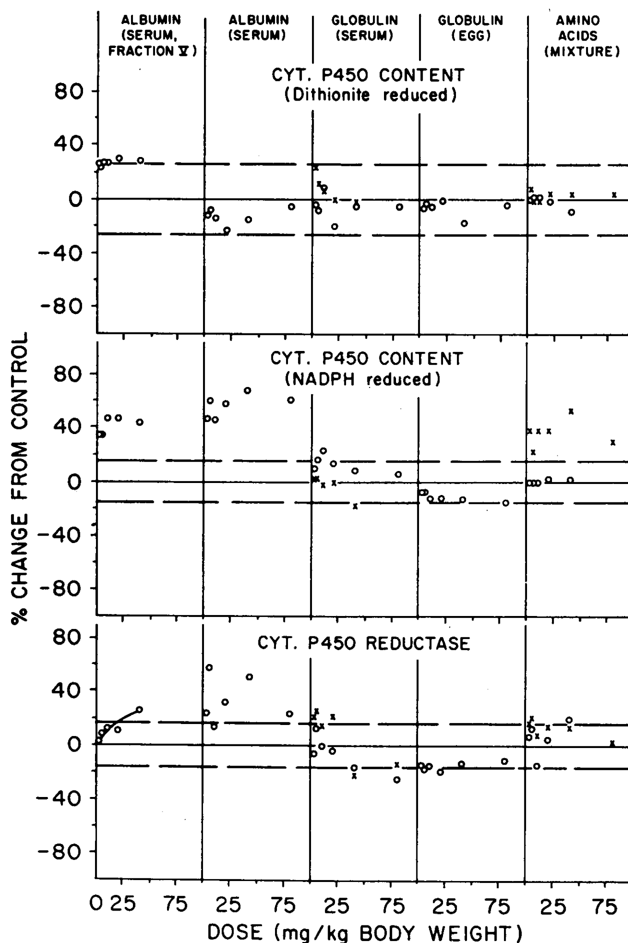

FIG. 4. Effect of polypeptides and amino acids on liver cytochrome P-450 in the rat. Legend is the same as that depicted for Fig. 3.

bility in magnitude of a GH effect on this metabolism observed from day to day in our laboratory.

The extent and rate of reduction of cytochrome P-450 by hepatic microsomes was examined following administration of polypeptide- or amino acid-containing solutions (Fig. 4). In general, the amount of dithionite- or NADPH-reduced cytochrome P-450 showed no dose-dependent change after injection of albumin, globulin, or an amino acid mixture. An apparent increase in NADPH-reduced cytochrome P-450 was noted after treatment with two preparations of serum albumin, but this effect was not dose related. Although a trend toward a dose-related increase and decrease in cytochrome P-450 reductase activity was observed after injection of albumin (fraction V) or serum globulin, respectively, the extent of the changes differed little from the range of control values. The globulin-induced decrease in reductase activity was quantitatively less than that produced by porcine GH (see Fig. 2) and was quite variable with doses less than 25 mg/kg. In general, these nonhormonal polypeptides had little dose-dependent effect on the rate or extent of cytochrome P-450 reduction.

Discussion. The present study was designed to define the specificity or otherwise of GH on microsomal drug metabolic and electron transport parameters of rat liver. Rats injected with either of two proteins representing a major class of polypeptides—albumin

and globulin—or a mixture of amino acids quantitatively and qualitatively similar to those contained in human GH (13) were used to test the response of drug metabolic enzyme activity to nonhormonal agents. Little change was found in the liver metabolism of four drug substrates or in the content and rate of cytochrome P-450 reduction. Since intact male rats were used, an opportunity was available for humoral or other mediators of acute stress effects to act upon the drug metabolic system. Absence of a consistent, dose-related effect following injection of nonhormonal polypeptides strongly suggests that a biologically active portion of GH is operative on this system and that the predominant effect is exclusive of a stress effect or other nonspecific factors produced by injection of hormonally inactive proteins.

Growth hormone, prolactin, and human chorionic somatomammotropin (HCS or human placental lactogen) are three protein hormones which demonstrate similarities with regard to primary structure (25), secondary configuration (26, 27), and biologic activity. A recent review summarized evidence that GH and HCS are two distinct hormones (28), and small differences in molecular weight have been noted between GH, HCS, and prolactin (29). Studies on the structure of HCS have permitted further comparisons between this hormone and GH or prolactin (13, 14), but large amounts of HCS are probably necessary to produce biologic effects similar to GH (30). In view of the marked similarities between GH, HCS, and prolactin, it is somewhat surprising that GH showed a relative specificity for its effect on the liver microsomal drug metabolic system. Doses of HCS higher than those used here may be required to mimic the effect of GH on this system, but, in general, high doses of prolactin did not decrease the liver metabolism of hexobarbital or aniline, and no change was observed in cytochrome P-450 content or reductase activity. Species differences in source of prolactin may explain some of these results since ovine prolactin was used and ovine GH was less potent than either porcine or bovine GH with respect to their effect on drug metabolic enzyme activity. HCS and prolactin may show differences in relative potency for an action on this microsomal system, or the specificity of GH may be produced by amino acid sequences or residues of the hormone not common to HCS or prolactin. An alternative argument proposes that GH affects the system indirectly through mediators such as sulfation factor (31). Postulation of an intermediary factor specifically released by GH would help explain some of the variability in GH action encountered by us and others (32) who work with hepatic metabolic systems. Presence of an intermediary factor is not unlikely since the repressive effect of GH on liver tyrosine transaminase activity was not observed with the perfused liver preparation (32). In our study, hormones structurally similar to GH exerted minimal effects on the hepatic system responsible for drug metabolism and thus suggest that (a) this system may be used as a model to study relatively specific *in vivo* effects of GH under conditions of controlled endogenous substrate flux and (b) that GH may have a role in the modulation of this metabolic enzyme activity.

Summary. The specificity of growth hormone (GH) on liver drug metabolic enzyme activity was examined by (a) injecting rats with one of three hormones [GH, prolactin, or human chorionic somatomammotropin (HCS)] known to have similar structural and biological characteristics, and (b) by treating animals with nonhormonal polypeptides (albumin or globulin) or an amino acid mixture similar in composition to the amino acid content of human GH. The liver metabolism of hexobarbital, ethylmorphine, aniline and *p*-nitrobenzoic acid (PNBA) and the rate and extent of cytochrome P-450 reduction showed a dose-dependent decrease following injection of GH. Boiling the GH reduced its effectiveness. With the exception of ethylmorphine and PNBA metabolism, prolactin did not decrease activity of this metabolic system. When one dose of HCS was studied, the metabolism of hexobarbital was slightly decreased and that of aminopyrine was slightly increased. The spectrum of effects of GH on the overall rate of drug metabolism and on components of the microso-

mal cytochrome P-450 system could not be reproduced with the nonhormonal polypeptides or with the amino acid mixture. It is concluded that, within the dose and time periods used for this study, the decreased steady state level of liver drug metabolism is a relatively specific action of GH in the rat.

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