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GROWTH HORMONE MODULATION OF LIVER DRUG METABOLIC ENZYME ACTIVITY IN THE RAT—I

EFFECT OF THE HORMONE ON THE CONTENT AND RATE OF REDUCTION OF MICROSOMAL CYTOCHROME P-450*

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Abstract—The liver metabolism of hexobarbital and aniline was decreased 48 hr after the first injection of growth hormone (GH) in adult male rats. The content and rate of reduction of hepatic microsomal cytochrome P-450 were lowered in these rats as compared with control animals. Liver NADPH-cytochrome *c* reductase showed a similar decrease in activity after GH treatment. The decrease in hexobarbital metabolism paralleled the change in cytochrome P-450 reductase activity as measured with or without addition of this drug substrate to a suspension of liver microsomes from GH-treated rats. The change in aniline metabolism approximated the extent and rate of cytochrome P-450 reduction after GH treatment only when cytochrome P-450 reductase activity was measured without addition of aniline. Injection of GH produced a parallel decrease in the metabolism of both drugs as compared with cytochrome *c* reductase activity. Differences in optimal requirements for drug substrates (hexobarbital or aniline) or NADPH for cytochrome P-450 reductase were not detected. Preincubation studies showed no differences in microsomal drug metabolic enzyme system stability in rats injected with GH. Inhibitors of this system *in vitro* were not demonstrated in liver from GH-treated rats. GH is presumed to affect the level of liver drug metabolism through mechanisms *in vivo* operative at the first stage transfer of reducing equivalents to cytochrome P-450. An additional effect of this hormone on the level or catalytic properties of the hemoprotein cytochrome P-450 may contribute to the decrease in aniline metabolism.

PREVIOUS studies in this laboratory have demonstrated a decrease in drug metabolism with liver from rats injected with growth hormone (GH) or implanted with a GH-secreting pituitary tumor.[1–5] These two animal models have been used to study the specificity of this GH effect in relation to various physiologic or endocrine parameters. For example, when the pituitary tumor was used as a source of rat GH,[6] a low level of drug biotransformation was noted in intact as well as adrenalectomized or castrated male rats, and no inhibitors of the reaction *in vitro* were detected in liver from tumor-bearing animals.[1,2] This microsomal system demonstrated an increase in drug metabolic enzyme activity after phenobarbital treatment even though rats with the GH-producing tumor showed a 60–80 per cent decrease in this enzyme activity.[7] In male rats injected with GH, the decrease in liver drug metabolism was not prevented

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by adrenalectomy, castration, hypophysectomy or starvation,[8,9] and no inhibition of the drug metabolic reaction *in vitro* was observed after addition of a GH-containing mixture to a reaction vessel containing control liver.[10] It was concluded from these studies that GH acts through a mechanism operative *in vivo* and independent of adrenal, testicular or pituitary factors to lower the level of liver microsomal drug metabolism.

The present investigation considers an action of GH at the molecular level of the liver drug metabolic system to: (a) identify possible control points in the system which might serve as a locus for the GH effect; and (b) further characterize functional relations between components of the system and the overall rate of drug metabolism in an animal model which does not require the use of xenobiotic enzyme inducers or inhibitors. The effect of GH on the extent and rate of reduction of liver cytochrome P-450 as related to the observed decrease in the metabolism of hexobarbital or aniline is described. Although the exact mechanism of microsomal drug oxidation remains unclear, evidence from several workers implicates cytochrome P-450 reductase as rate limiting[11–13] and cytochrome P-450 as the terminal oxidase[14,15] in the reaction. The sequence of drug oxidation proposed by Estabrook *et al.*[16] suggests that this process depends on an interaction of at least four factors with liver cytochrome P-450: two electron transfer steps, reaction (binding) with substrate, and formation of an oxygenated P-450–substrate complex. Results from the present study indicate that pretreatment of rats with GH interferes with the first electron step reduction of cytochrome P-450 and decreases the content of this hemoprotein in liver microsomes.

METHODS

Animals. Mature (80- to 90-day-old) male Fischer (F344) rats were obtained from an animal vendor* at least 10 days prior to onset of the study. Rats were placed in wire mesh cages suspended over a refuse pan containing Sani-cel. Purina rat chow and water were offered *ad lib.* The animal room environment was controlled at 21 ± 1° and light–dark cycles were alternated every 12 hr beginning at 7.00 a.m. Gentle handling was used at all times and preceding decapitation.

Injection technique. Rats were placed in a restraining device as previously described.[17] The solution of GH was prepared by dissolving 2–4 mg of GH/ml of 0·1 N $NaHCO_3$.† Each rat received a s.c. injection of GH or vehicle ($NaHCO_3$) as 1 ml/200 g body wt. The hormone was administered at 48 and 40 hr before assays *in vitro* were performed.

Tissue preparation. Livers were removed from exsanguinated rats and placed on ice. Two volumes of 1·15% KCl in 0·1 M pH 7·35 Tris buffer were added per g of wet wt liver, and the preparation was homogenized in a glass homogenizer with two passes of a Teflon pestle. The 9000 *g* supernatant fraction was prepared by centrifuging the homogenate for 20 min at 10,000 rev/min in an IEC-B-20 refrigerated centrifuge. An aliquot of the 9000 *g* supernatant fraction was used to prepare the 100,000 *g* pellet containing microsomes. This pellet was washed once prior to dilution with potassium

* Charles River Breeding Laboratories, North Wilmington, Mass. or Simonsen Laboratories, Gilroy, Calif.

† Growth hormone was obtained from Nutritional Biochemicals Company (porcine GH) or as a gift from the NIH Endocrine Study Section (bovine GH). Preparations of GH contained 0·8 to 1·0 i.u./mg dry wt. For the sake of consistency, all results in this paper were obtained from rats injected with porcine GH. Injection of rats with bovine GH produced similar results.

phosphate buffer (0·1 M, pH 7·35) for the assays described below. In some studies the pellet of microsomes from control and experimental rat liver was stored at 4° overnight with minimal loss of enzyme activity.

Assay techniques. Hexobarbital and aniline, drugs which give a respective type I or type II binding spectrum with hepatic microsomes,[18] were used as model compounds for metabolism studies *in vitro*. The reaction mixture contained either hexobarbital (0·6 mM) or aniline (2 mM), and glucose 6-phosphate (3·6 mM), $MgSO_4$ (9·68 mM), nicotinamide adenine dinucleotide phosphate (NADP, 0·83 mM), 0·25 ml of 9000 *g* supernatant fraction equivalent to 1/12 g of liver and 1·45 ml of potassium phosphate buffer (0·1 M, pH 7·35) to adjust final volume of the mixture to 2·5 ml. Incubations were performed at 37° under an atmosphere of oxygen in a Dubnoff-type water bath with shaking speed of 100–110 rev/min.[19] Under these conditions, the reaction rate showed minimal deviation from linearity after 20 min of incubation. The amount of hexobarbital metabolized or of *para*-aminophenol formed from aniline was determined by spectrophotometric methods previously described.[20,21] Hepatic 9000 *g* supernatant fraction and microsomal protein were measured with bovine serum albumin as the standard.[22] Results are expressed per g wet wt liver.

The suspension of microsomes was diluted with potassium phosphate buffer (0·1 M, pH 7·35) to contain 2–3 mg of protein/ml for determination of the CO-complex of cytochrome P-450 (dithionite reduced),[23,24] 3–4 mg of protein/ml for assay of cytochrome P-450 reductase and extent of NADPH reduced cytochrome P-450,[12,25,26] or 0·2 mg of protein/ml for estimation of cytochrome *c* reductase activity.[27,28] Carbon monoxide was bubbled through a dithionite-reduced microsomal suspension in the sample cuvette and the absorbance at 450 nm relative to 490 nm was determined with a Coleman double beam recording spectrophotometer. No significant absorption differences at 450 nm from cytochrome b_5[14,24,29] were noted with liver microsomes from control and GH-treated rats. The rate and extent of liver cytochrome P-450 reduction were determined at 450 nm in a Gilford 2400 spectrophotometer after rapid addition of NADPH (0·05 ml of 0·05 M solution) to a microsomal suspension adjusted to 37° and equilibrated with the ligand carbon monoxide. The effect of drug substrates on the activity of cytochrome P-450 reductase was examined by adding hexobarbital (1 μmole) or aniline (5 μmoles) to a 3-ml suspension of microsomes 15 sec prior to bubbling of carbon monoxide and 5 min prior to addition of NADPH. The rate of liver microsomal reduction of cytochrome *c* was measured with the Coleman double beam spectrophotometer at 550 nm. For this assay the sample cuvette contained 0·1 ml of the diluted microsomal suspension, 2·6 ml of potassium phosphate buffer (0·1 M, pH 7·35), 0·1 ml of 10 mM NaCN solution and 0·1 ml of a 1 mM cytochrome *c* preparation (from horse heart). A 0·1-ml aliquot of NADPH (2·5 mM) was added to start the reaction. NADPH was omitted from the reference cuvette. A linear reaction rate was observed at room temperature for a minimum of 6 min under these conditions. An extinction coefficient of 19·7 mM^{-1} cm^{-1} was used to express cytochrome *c* results as nmoles reduced per min per g of liver.[30] For the sake of consistency and to facilitate comparison of data, an extinction coefficient of 91 mM^{-1} cm^{-1} was used for cytochrome P-450[24]. An extinction coefficient of 112 cm^{-1} mM^{-1} was used for cytochrome b_5.[31] Inorganic phosphorous formed by the action of glucose 6-phosphatase was measured in a preparation of hepatic microsomes.[32]

Student's *t* test was used to calculate statistical significance ($P < 0.05$) between paired means.[33]

RESULTS

Effect of GH on recovery of microsomes. As an initial step, the effect of GH on recovery of microsomes was assessed with markers such as protein, cytochrome b_5 or P-450 concentration and glucose 6-phosphatase activity. As seen in Table 1, pretreatment with GH did not alter the content of liver microsomal protein cytochrome b_5 or

TABLE 1. ASSESSMENT OF RECOVERY OF MICROSOMES FROM CONTROL AND GROWTH HORMONE (GH)-TREATED RATS*

Parameter	Control	GH	% Change
Protein (mg/g liver)			
200 *g* supernatant fraction	167	160	−4
Microsomal	46	46	0
Cytochrome P-450 (nmoles/g liver)			
200 *g* supernatant fraction	33	18	−46
Microsomal	23	18	−21
Cytochrome b_5 (nmoles/g liver)			
Microsomal	26	27	+4
Glucose 6-phosphatase (μmoles P_i/min/g liver)			
Microsomal	8·4	7·7	−8

* Male Fischer rats were injected s.c. with porcine GH, 4 mg/rat, at 48 and 40 hr before sacrifice. The liver homogenate was centrifuged at 200 *g* to prepare the respective supernatant fraction which was then centrifuged at 9000 *g*. The liver 9000 *g* supernatant fraction was centrifuged at 104,000 *g* to prepare the pellet of microsomes. The mean liver weight was 3·3 and 3·2 g/100 g body weight for control GH-treated rats respectively.

glucose 6-phosphatase activity, but cytochrome P-450 content was decreased. A unidirectional change in content of both cytochromes would have been expected if recovery of microsomes were altered by GH treatment. Cytochrome P-450 content in the 200 *g* supernatant fraction also showed a decrease with liver from GH-treated as compared with control rats. This finding and data from microsomal preparations as well as drug metabolic results with the 9000 *g* supernatant fraction (*vide* infra) indicate that differential recovery of microsomes is not a factor primarily responsible for the the observed changes in mixed function oxidase activity after GH treatment.

Effect of GH on drug metabolism and cytochrome P-450. The liver metabolism of hexobarbital and aniline was decreased 48 hr after the first treatment of male rats with GH (Table 2). This GH effect was associated with a decrease in the content of NADPH-reduced microsomal cytochrome P-450. The extent of the decrease in cytochrome P-450, however, did not approximate the decrease in enzyme activity of the drug metabolic system, especially as measured with hexobarbital. The respective biotransformation of hexobarbital and aniline decreased 32·0 and 26·7 per cent after GH treatment whereas the maximum fall in cytochrome P-450 content was 19·3 per cent. A decrease in the initial rate of reduction of cytochrome P-450 and cytochrome *c* reductase was observed with liver microsomes from GH-treated rats. The per cent

decrease in these two parameters more closely approximated the alteration in hexobarbital and aniline metabolism than did the change in cytochrome P-450 content.

TABLE 2. DRUG METABOLISM AND ELECTRON TRANSPORT SYSTEM IN HEPATIC MICROSOMES FROM GROWTH HORMONE (GH)-TREATED RATS*

Parameter	Control	GH	% Change
Drug metabolism (nmoles/min/g liver)			
Hexobarbital	430 ± 9·5	293 ± 12·0†	−32·0
Aniline	58·0 ± 3·0	42·5 ± 1·0†	−26·7
Electron transport system			
Cytochrome P-450 (nmoles/g liver)			
NADPH reduced	21·1 ± 0·33	17·1 ± 0·33†	−19·3
Cytochrome P-450 reductase (nmoles/min/g liver)	300 ± 11·2	225 ± 8·7†	−24·8
Cytochrome *c* reductase (nmoles/min/g liver)	3490 ± 230	2390 ± 160†	−31·6

* Male Fischer rats (80- to 90-day-old) were injected with porcine growth hormone (4 mg/200 g body wt.) s.c. at 48 and 40 hr before they were used. Controls received an injection of the hormone solution diluent (0·1 M $NaHCO_3$). Drug metabolism results are expressed as nmoles of hexobarbital metabolized or *p*-aminophenol formed per min per g of liver. Hepatic 9000 *g* supernatant fraction was used for drug metabolism studies and liver microsomes for cytochrome studies. Each value represents the mean ± S. E. of five experiments with eight rats per group in each experiment.

† $P < 0·05$.

The effect of GH on the rate and extent of cytochrome P-450 reduction was examined with addition of drug substrates (Table 3). Addition of hexobarbital did not change the extent of cytochrome P-450 reduced by NADPH as compared to microsomes without the drug, but a decrease was observed after addition of aniline in both the control and GH-treated animals. GH treatment decreased the content of cytochrome P-450 as measured in the absence or presence of hexobarbital and aniline. The rate of reduction of cytochrome P-450 by NADPH was increased by addition of hexobarbital and decreased by addition of aniline to the suspension of liver microsomes from control or GH-treated rats. GH pretreatment produced a similar decrease in the rate of cytochrome P-450 reduction with or without hexobarbital. After addition of aniline, however, the decreased rate of reduction was narrowed in control vs GH-treated rats.

Although no change was noted in hepatic 9000 *g* protein in control and GH-treated rats (138 ± 5 and 137 ± 4, respectively), an 8.6 per cent decrease in microsomal protein was noted (37·9 ± 0·6 and 34·6 ± 0·6, respectively) in animals used for work presented in Tables 2 and 3. Results presented in these Tables were recalculated on a per milligram of microsomal protein basis (Table 4). As expected, expression of data on this basis lowered the per cent change of liver drug metabolic parameters in control vs GH-treated rats. It should be noted that no consistent, statistically significant decrease in liver microsomal protein content is found in our laboratory using this GH-pretreatment protocol. It is for this reason that results are expressed per g of liver.

TABLE 3. EFFECT OF HEXOBARBITAL OR ANILINE ON REDUCTION OF CYTOCHROME P-450 BY HEPATIC MICROSOMES FROM GROWTH HORMONE (GH-)TREATED RATS*

	Control	GH	% Change
Cytochrome P-450-NADPH reducible (nmoles/g liver)			
Without drug	21·1 ± 0·33	17·1 ± 0·33†	−19·3
Hexobarbital	21·6 ± 0·33	16·8 ± 0·33†	−22·3
Aniline	18·0 ± 0·33	14·8 ± 0·44†	−17·6
Cytochrome P-450 reductase (nmoles/min/g liver)			
Without drug	300 ± 11·2	225 ± 8·7†	−24·8
Hexobarbital	386 ± 13·2	284 ± 11†	−26·8
Aniline	171 ± 5·5	163 ± 8·8	−5·7

* See Table 2 for description of animals and treatment. Hexobarbital sodium (1 μmole) or aniline (5 μmoles) was added to a 3-ml suspension of hepatic microsomes equivalent to 1/9 g of liver (3–5 mg protein/ml) prior to bubbling CO. All reactions were performed at 37°.
† $P < 0·05$.

Results of Tables 2 and 3 were used to calculate ratios of hexobarbital or aniline metabolism to each component of the drug metabolic electron transport system. The precise relation of the effect of GH on drug metabolism to changes in one or more components of the mixed function oxidase system was estimated from comparison of these ratios. A positive relationship between two variables is assumed if the per cent change in the ratio of these parameters between control and GH-treated rats

TABLES 4. LIVER DRUG METABOLISM AND ELECTRON TRANSPORT COMPONENTS CORRECTED FOR DIFFERENCES IN MICROSOMAL PROTEIN IN GROWTH HORMONE (GH)-TREATED RATS*

Parameter	Control	GH	% Change
Drug metabolism (nmoles/min/mg protein)			
Hexobarbital	11·2 ± 0·3	8·4 ± 0·4†	−25·2
Aniline	1·5 ± 0·05	1·2 ± 0·04†	−18·9
Electron transport system			
Cytochrome P-450 (nmoles/mg protein)			
NADPH reduced			
Without drug	0·56 ± 0·01	0·49 ± 0·01†	−11·5
With hexobarbital	0·57 ± 0·07	0·49 ± 0·01†	−14·6
With aniline	0·48 ± 0·01	0·43 ± 0·01†	−10·0
Cytochrome P-450 reductase (nmoles/min/mg protein)			
Without drug	7·88 ± 0·24	6·58 ± 0·30†	−16·5
With hexobarbital	10·2 ± 0·36	8·29 ± 0·37†	−19·0
With aniline	4·55 ± 0·15	4·71 ± 0·25	+ 3·5
Cytochrome *c* reduced (nmoles/min/mg protein)	91 ± 6	69 ± 5†	−23·8

* See legend of Table 2 for animals and treatment conditions.
† $P < 0·05$.

approaches zero. As seen in Table 5, hexobarbital metabolism was more closely related to the activity of cytochrome P-450 reductase and cytochrome *c* reductase than it was to the content of cytochrome P-450. The metabolism of aniline was related to the content and rate of reduction of cytochrome P-450 reductase and to activity of cytochrome *c* reductase. It is of interest to note that the overall metabolism of both drugs exceeded the amount of cytochrome P-450 by a factor of 20 (hexobarbital) or 2·8 (aniline) in control rats. The functional relations between hexobarbital metabolized and cytochrome P-450 reductase approached unity whereas the metabolism of hexobarbital and aniline was much less than the amount of cytochrome *c* reduced. Correction of cytochrome P-450 reductase activity for the amount of cytochrome P-450 after GH treatment revealed a smaller decrement than that observed on a per g of liver basis (see Table 2). This suggests that some of the lower activity of cytochrome P-450 reductase after GH pretreatment may be a result of a decrease in the content of hepatic microsomal cytochrome P-450.

TABLE 5. RELATION BETWEEN DRUG METABOLISM AND ELECTRON PARAMETERS IN HEPATIC MICROSOMES FROM GROWTH HORMONE (GH)-TREATED RATS*

	Control	GH	% Change
Hexobarbital metabolized			
(nmoles/min)/nmole P-450	20·4	17·1	−16·0
(nmoles/min)/(nmole P-450 reduced/min)	1·4	1·3	−7·1
(nmoles/min)/(nmole cytochrome *c* reduced/min)	0·12	0·12	0
Aniline (*p*-aminophenol formed)			
(nmoles/min)/nmole P-450	2·75	2·49	−9·5
(nmoles/min)/(nmole P-450 reduced/min)	0·19	0·19	0
(nmoles/min)/(nmole cytochrome *c* reduced/min)	0·017	0·018	+6·0
Cytochrome P-450 reductase			
(nmoles/min)/nmole P-450	14·2	13·1	−7·5

* The mean of each result from Table 2 was used to calculate the metabolism of hexobarbital or aniline.

Drug metabolism was expressed per electron transfer components of the microsomal system measured in the presence of each drug substrate (Table 6). Hexobarbital metabolism in GH-treated rats approximated the rate of reduction of the cytochrome P-450–hexobarbital complex. Such a relationship was not observed for aniline metabolism which appeared to be associated with the decrease in cytochrome P-450 content and rate of reduction of the cytochrome without aniline (compare values in Tables 5 and 6). The metabolism of hexobarbital in control or GH-treated rats was again found to have a close functional relation to the rate rather than extent of cytochrome P-450 reduction in the presence of this drug substrate. The metabolism of aniline did not show a similar relation since about a 3-fold level of metabolism was noted per mole of cytochrome P-450 and an approximate 3 moles of cytochrome P-450 were reduced for each mole of aniline hydroxylated per min when these microsomal electron transport components were measured in the presence of this drug. Correction of cytochrome P-450 reductase activity for extent of cytochrome P-450 reduction in the presence of each substrate produced paradoxical results. Cytochrome P-450 reductase activity with hexobarbital was slightly decreased whereas with aniline

it was increased after GH treatment. This suggests that the decrease in cytochrome P-450 reductase activity in the presence of hexobarbital is not entirely dependent on a decrease in content of the cytochrome, but a stronger dependent relationship may exist in the presence of aniline. GH may affect both reductase activity and the amount of cytochrome P-450 for aniline metabolism whereas hepatic metabolism of hexobarbital appears to be primarily associated with the effect of GH on cytochrome P-450 reductase activity.

TABLE 6. EFFECT OF GROWTH HORMONE (GH) ON THE RELATION BETWEEN DRUG METABOLISM AND CYTOCHROME P-450 REDUCTASE ACTIVITY IN THE PRESENCE OF HEXOBARBITAL OF ANILINE*

	Control	GH	% Change
Hexobarbital metabolized			
(nmoles/min)/nmole P-450	19·9	17·8	−10·6
(nmoles/min)/(nmole P-450 reduced/min)	1·11	1·03	−6·3
Aniline (*p*-aminophenol formed)			
(nmoles/min)/nmole P-450	3·20	2·87	−10·3
(nmoles/min)/(nmole P-450 reduced/min)	0·34	0·26	−23·5
Cytochrome P-450 reductase (nmoles/min)/nmole P-450			
With hexobarbital	17·8	16·9	−5·3
With aniline	9·5	11·0	+15·4

* The mean of each result from Tables 2 and 3 were used to calculate the metabolism of hexobarbital or aniline. Values for NADPH reduced cytochrome P-450 in the presence of the respective drug were used.

The following kinetic parameters of the liver drug metabolic enzyme system were examined in GH-treated rats to assess possible mechanisms for the observed decrease in the extent and rate of reduction of cytochrome P-450.

Effect of NADPH concentration on cytochrome P-450 reductase activity. Hepatic cytochrome P-450 reductase activity was examined with various concentrations of NADPH. Liver microsomes from GH-treated animals showed a decrease in the rate of reduction of cytochrome P-450 by NADPH as compared with control animals. This decrease was not altered by superoptimal amounts of NADPH when as much as 1·320 mM of this nucleotide was used. This suggests that GH produced a decrease in the amount of flavoprotein for NADPH reduction of cytochrome P-450 rather than a change in its catalytic properties for transfer of reducing equivalents from NADPH. Formation of a tightly bound inhibitor of cytochrome P-450 reductase by GH treatment is an alternative possibility.

Effect of substrate on cytochrome P-450 reductase activity. A decrease in the rate of reduction of the cytochrome P-450–substrate complex in GH-treated rats may be a result of: (a) a low level of enzyme; or (b) a qualitative change in the flavoprotein and cytochrome such that interaction with the drug substrate is decreased. To test the second alternative, various amounts of hexobarbital or aniline were added to liver microsomes prior to measurement of cytochrome P-450 reductase activity. The addition of hexobarbital increased the rate of cytochrome P-450 reduction, but increasing amounts of hexobarbital (1·6 μmoles) did not obviate the effect of GH on this system. The addition of aniline (0·13–4 μmoles) narrowed the decrease in

reductase activity observed with control vs GH-treated rats, but no further change was noted with amounts greater than 0·5 μmole of aniline. These results suggest a decrease in the amount of enzyme protein for reduction of the cytochrome P-450–substrate complex in GH-treated rats, although a change in the extent of substrate interaction (binding) with cytochrome P-450 after GH treatment cannot be excluded.

Enzyme stability. A decrease in liver drug metabolism and associated components of the microsomal electron transport system after GH treatment suggests that an alteration of the stability of this system *in vitro* may be produced by GH administration. The metabolism of hexobarbital and aniline by microsomes from GH-treated rats showed no differences in stability as compared to control animals when examined at various times during a 4-hr incubation. Preincubation of hepatic microsomes for periods as long as 3 hr failed to demonstrate enhanced inactivation of cytochrome P-450 reductase activity in GH-treated rats. Addition of hexobarbital or aniline to preincubated microsomes revealed an expected increase and decrease, respectively, in the rate of cytochrome P-450 reduction, but no difference in stability of the control vs GH-treated rat liver preparation was noted. Cytochrome P-450 content with or without substrate (hexobarbital or aniline) was decreased in liver microsomes from GH-treated rats, and preincubation of the microsomal suspension from these rats did not demonstrate an enhanced lability of this cytochrome *in vitro* as compared with preincubated microsomes from control animals. A decrease in drug metabolism in liver from GH-treated rats is not a function of alterations in enzyme or cytochrome P-450 stability as determined by these preincubation techniques *in vitro*.

Presence of inhibitors. Various parts of liver preparations from control or GH-treated rats were combined to note the presence of an inhibitor for drug metabolism or components of the microsomal electron transport system. A decrease in the metabolism of hexobarbital and aniline was found after GH treatment, but no inhibitor of the reaction *in vitro* was detected in the liver 9000 *g* supernatant fraction from GH-treated rats. The observed vs expected extent and rate of cytochrome P-450 reduction and reduction of cytochrome *c* were similar when examined with preparations containing various amounts of control and GH-treated rat liver microsomes.

DISCUSSION

Adult male rats pretreated with growth hormone show a decrease in the liver metabolism of hexobarbital and aniline. The mechanism responsible for this GH effect was the subject of the present study. GH was found to decrease several components of the microsomal electron transport system associated with liver drug metabolism. Cytochrome P-450 content, measured with or without hexobarbital or aniline, was lower in GH-treated as compared with control rats. Cytochrome P-450 reductase activity was decreased in rats pretreated with GH, but this decrease was narrowed when aniline was added to the microsomal suspension. It is of interest to note that the rate of cytochrome *c* reduction by hepatic microsomes was also decreased by prior treatment of rats with GH. The per cent change in activity of cytochrome *c* and cytochrome P-450 reductase activity was similar in these rats (24·8 and 31·6 per cent, respectively, see Table 2). Several workers propose that these two reductases are the same enzyme catalyzing transfer of reducing equivalents to either cytochrome *c* or P-450 as the acceptor.[34,35] Similar changes in the steady state level of cytochrome *c* and P-450 reduction in rats injected with GH support this proposal and implicate

the rate of cytochrome P-450 reduction by NADPH as one possible control point for the effect of GH on this system.

A parallel functional relationship between liver drug metabolism and the rate and extent of cytochrome P-450 reduction would be expected if either were rate limiting for the reaction.[11–15,36] Comparisons of nanomoles of drug metabolized per each of these parameters revealed differences in type I (hexobarbital) vs type II (aniline) spectral binding substrates with regard to the effect of GH. The liver metabolism of hexobarbital paralleled a decrease in the rate of cytochrome P-450 reduction, especially when this rate was studied in the presence of this type I substrate. Little relationship was observed between differences in the amount of hexobarbital metabolized and cytochrome P-450 content with liver from control vs GH-treated rats. This agrees with previous studies in which little relation was found between liver drug metabolism and cytochrome P-450 content.[36,37] The metabolism of aniline after GH injection, however, showed changes which more closely paralleled the decrease in cytochrome P-450 content than the rate of reduction of the cytochrome when measured with aniline. The metabolism of hexobarbital and aniline was closely associated with the change in cytochrome P-450 reductase activity after GH treatment when this activity was estimated without addition of drug substrate. Examination of certain kinetic parameters revealed that the level of drug substrate or NADPH required for an optimum rate of cytochrome P-450 reduction was not altered by pretreatment with GH. Furthermore, liver microsomes from GH-treated rats did not show differences in stability as measured with hexobarbital or aniline metabolism and the rate or extent of cytochrome P-450 reduction. Inhibitors of these reactions *in vitro* were not detected when combinations of liver preparations from control and GH-treated rats were studied. These observations suggest that GH acts *in vivo* to decrease cytochrome P-450 reductase activity and hexobarbital metabolism in parallel irrespective of observed changes in the level of cytochrome P-450. Presumably, the decrease in reductase activity is secondary to a decrease in enzyme protein, but qualitative changes in activation sites,[38] substrate interaction with different amounts of high and low spin cytochrome P-450[39–41] or a decrease in unknown sources of reducing equivalents must be considered. Similar interpretations may apply to the GH effect on aniline metabolism, but a decrease in the extent of cytochrome P-450 after GH treatment may influence the metabolism of this type II compound. This decrease could be produced by variation in the amount of rough and smooth liver microsomes, together with inherent differences in the extent or rate of reduction of cytochrome P-450,[41] in GH-treated rats. The effect of GH on the amount of cytochrome P-450 may also reflect a change in hemoprotein synthesis or degradation, or an alteration in extinction coefficient.[42–44]

Several models have been used to study functional relationships between the overall rate of drug metabolism and components of the microsomal electron transport system. Changes in drug metabolic enzyme activity have been noted with animals of different ages, sex, species and under the influence of xenobiotic inhibitors or inducers of this enzyme activity. In general, a relatively poor association has been found between the change in liver drug metabolism and the content of cytochrome P-450.[36,37,45] Overall metabolic enzyme activity for hexobarbital and aniline was in excess of the amount of cytochrome P-450 in hepatic microsomes of control or growth hormone-treated rats. A better parallel relationship exists between this metabolism and rate

of reduction of cytochrome P-450 or cytochrome *c*. The rate of cytochrome *c* reduction, however, was much greater than the rate of hexobarbital or aniline oxidation. Results of this study support the suggestion that the metabolism of a type I substrate approximates the rate of cytochrome P-450 reduction[41] and demonstrate that another model system—the GH-treated rat—may be used to examine functional relationships between liver microsomal constituents.

GH is known to decrease the activity of some enzymes concerned with intermediary metabolism. The activity of tryptophan pyrrolase,[46,47] tyrosine transmainase[46–48] and serine dehydratase[49] is decreased in GH-treated animals. In contrast to these enzymic reactions which involve endogenous substrates, activity of the liver microsomal drug metabolic system may be examined under conditions of controlled substrate (drug) flux. Thus, this is the first report which: (a) considers a mechanism of GH action in a metabolic system without the use of endogenous substrates: (b) demonstrates alterations in the level of a hemoprotein (cytochrome P-450) after injection of GH; and (c) shows that GH treatment decreases the activity of a flavoprotein involved in the first stage transfer of reducing equivalents to cytochrome P-450. Subsequent studies will examine further the mechanism and hormonal specificity of this GH effect to define its possible role in the postnatal maturation and daily modulation of liver microsomal drug metabolic enzyme activity.

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