Biochem. J. (1976) **154**, 433–438
Printed in Great Britain

Growth Hormone and Drug Metabolism

ACUTE EFFECTS ON MICROSOMAL MIXED-FUNCTION OXIDASE ACTIVITIES IN RAT LIVER

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(*Received* 30 *June* 1975)

1. Adult male rats were subjected either to sham operation or to hypophysectomy and adrenalectomy and maintained for a total of 10 days before treatment with growth hormone. Results of the early effects of growth hormone on the activities of the mixed-function oxidases in rat liver over a 96h period after growth-hormone treatment are presented. 2. Hypophysectomy and adrenalectomy result in decreased body and liver weight and decreased drug metabolism (mixed-function oxidases). Concentrations of electron-transport-system components are also decreased. 3. In the hypophysectomized/adrenalectomized rats, growth hormone decreases the activities of the liver mixed-function oxidases and the cytochrome *P*-450 and cytochrome *c* reductases, as well as decreasing the concentration of cytochrome *P*-450 compared with that of control rats. Similar but less dramatic results are obtained with sham-operated rats. 4. It is concluded that whereas growth hormone enhances liver growth, including induction of many enzyme activities, it results in a decrease in mixed-function oxidase activity. Apparently, mixed-function oxidase activity decreases in liver when growth (mitogenesis) increases.

Hepatic mixed-function oxidase activity is low in rats exposed to a high concentration of growth hormone in the blood. This activity for hexobarbital and ethylmorphine metabolism was decreased 48h after injection of male Fischer rats with growth hormone (Wilson, 1969*a*). Implantation of a growth-hormone-producing tumour (the pituitary mammotropnic tumour) (Bates *et al.*, 1962) in Fischer rats also produced a fall in mixed-function oxidase activity (Wilson, 1968*a*,*b*). A decrease in activity was noted after injection of tumour homogenate or a preparation which contained growth hormone in amounts similar to the tumour homogenate (Wilson, 1968*c*, 1969*b*). The effect of the tumour or hormone preparation on this microsomal system was dose-dependent (Wilson, 1969*b*,*c*). Time-course studies with the growth-hormone-producing tumour revealed a small decrease in hexobarbital metabolism at 48h and an 80% decrease in this metabolism by 16 days after tumour implantation (Wilson, 1969*b*, 1971). Injection of a growth-hormone mixture produced a maximum decrease in mixed-function oxidase activity at 48h (Wilson, 1968*c*). Changes in this activity were not related to alterations in content of liver microsomal protein or to secondary effects via adrenals, testes, or pituitary (Wilson, 1968*a*; Wei & Wilson, 1971).

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Failure to demonstrate an effect *in vitro* of growth hormone on liver drug metabolism suggested that the hormone acted through mechanisms operative only *in vivo* (Wilson, 1968*c*). The present study was designed to further examine the effects on the mixed-function oxidase activity of early growth-hormone action in this animal system and to compare it with the early effects of growth hormone on nuclear transcription described in the following paper (Spelsberg & Wilson, 1976).

Materials and Methods

Animals and treatment

Male Sprague–Dawley rats were obtained from a local supplier (Zivic–Miller Inc., Allison Park, Pa. 15101, U.S.A.). Hypophysectomized/adrenalectomized rats were also prepared by this supplier and sent 1 week after surgery. Sham operations of rats (surgery without tissue removal) were performed to assess the effects of the surgery on the growth-hormone response. Completeness of the surgical procedure was ascertained by gross inspection *post mortem*. All animals were maintained in suspended wire-mesh cages and given Purina chow, oranges and 0.9% saline *ad libitum*. The animals were kept under insecticide-free and temperature-controlled conditions, and light/dark cycles alternated every 12h, beginning at 7:00a.m. Rats were placed

in a restraining apparatus (Wilson, 1969*d*) to facilitate the injections that were begun at 4:45a.m. Animals were injected once subcutaneously with 5mg of pig growth hormone (Sigma Chemical Co., St. Louis, Mo., U.S.A.; 0.8–1i.u./mg) dissolved in 0.1M-$NaHCO_3$. Control rats received only $NaHCO_3$. Previous studies (Wilson, 1968*a*,*b*,*c*) used 5mg of bovine albumin for control injections and showed that the albumin had no effect on drug metabolism; consequently only the vehicle ($NaHCO_3$) was injected into the control rats. Wilson (1968*c*) also showed that 4–5mg of growth hormone was required for sustained effects (>2 days) of the hormone on drug metabolism. This very high dose (5.0mg/rat) was used in these experiments for the purpose of monitoring growth-hormone effects over long periods (~4 days). Further, the response of drug metabolism to growth hormone was dose-dependent up to 4–5mg/rat. Consequently, a 5mg dose was selected to give a great response. At specific assay times rats were chosen at random from control or growth-hormone-injected groups. Animals were killed by decapitation, and livers were removed after exsanguination. In general, livers from four to five rats were washed with saline, and pooled to prepare one sample for metabolic studies in each control or growth-hormone-treated group.

Isolation of a microsomal fraction containing mixed-function oxidases

The minced liver was rinsed in ice-cold 1.15% (w/v) KCl and blotted dry on a paper towel. Liver (1g) was mixed with 2ml of 1.15% KCl and homogenized in a glass homogenizer with two passes of a Teflon pestle. The homogenate was centrifuged at 9000*g* for 20min to obtain the liver '9000*g*-supernatant' fraction. A portion of this supernatant was centrifuged at 104000*g* and washed once to obtain the microsomal pellet. This pellet was resuspended in 0.1M-potassium phosphate buffer (pH7.35) to contain 2–3mg of protein/ml for analysis of dithionite-reduced cytochrome *P*-450, 3–4mg of protein/ml for assay of cytochrome *P*-450 reductase activity, and 0.20–0.30mg/ml for determination of cytochrome *c* reductase. In general the microsomal pellet was prepared on the day the animals were killed and the microsomal assays were performed on the same day, or on the following morning after resuspension of the pellet, which was stored at 4°C in phosphate buffer.

Assay for mixed-function oxidases

Hexobarbital and aniline, drugs which give respectively type I or type II spectral changes with hepatic microsomal fractions (Schenkman *et al.*, 1967), were used as model substrates to determine mixed-function oxidase activity in the liver 9000*g*-supernatant fraction. A 0.25ml portion (or 0.5ml for hypophysectomized/adrenalectomized rats) was added to a reaction mixture containing 3–6mM-glucose 6-phosphate, 9.7mM-$MgSO_4$, 0.83mM-$NADP^+$ and either 0.6mM-hexobarbital or 2mM-aniline as the substrate. The volume of the mixture was adjusted to 2.5ml with 0.1M-potassium phosphate buffer, pH7.35. Mixtures were incubated at 37°C under O_2 in a shaking water bath (100–110rev./min; Fouts, 1970) for 5–30min. Time-course incubations were performed for each drug, and metabolic data after 20min of incubation were used to express results. Hexobarbital metabolism was determined by the substrate-disappearance method described by Cooper & Brodie (1955). *p*-Aminophenol, formed by the liver hydroxylation of aniline, was determined by the method of Kato & Gillette (1965). Results were calculated as μmol of hexobarbital metabolized or μmol of *p*-aminophenol formed/20min per g of liver.

The extent of dithionite-reduced cytochrome *P*-450

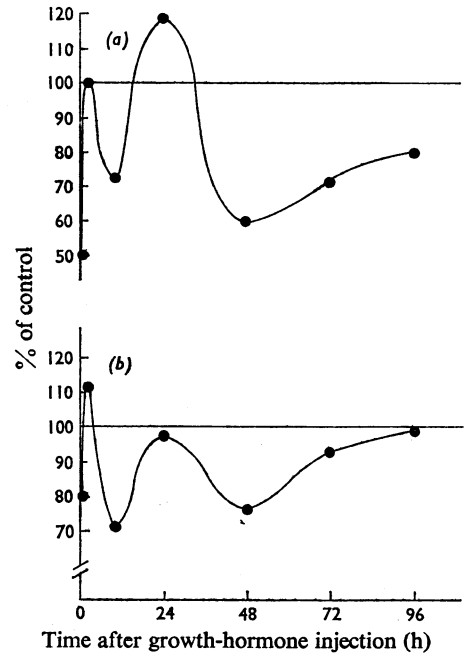

Fig. 1. *Time-course changes in liver metabolism of* (*a*) *hexobarbital and* (*b*) *aniline after injection of growth hormone in sham-operated rats*

Male Sprague–Dawley rats (250–300g) underwent a sham hypophysectomy/adrenalectomy procedure at least 10 days before the study commenced. Half of the rats were injected once subcutaneously at zero time with 5mg of pig growth hormone/rat. The remaining half (control animals) received an injection of 0.1M-$NaHCO_3$, the vehicle for growth hormone. Results are expressed as a percentage of the control value at each time-period studied. The values are averages of results from two experiments. Liver pooled from four to five rats was used for each study.

was determined in a 3ml solution of hepatic microsomal fraction with a Coleman double-beam spectrophotometer after the sample cuvette was equilibrated with CO (Gillette *et al.*, 1968; Omura & Sato, 1964). The extent and rate of reduction of cytochrome *P*-450 at 37°C was determined in a 3ml solution of hepatic microsomal fraction by first equilibrating with CO and then rapidly adding 0.05ml of 0.05M-NADPH (Gigon *et al.*, 1969; Sesame & Gillette, 1969; Gigon *et al.*, 1968). NADPH reduction of cytochrome *c* was monitored at room temperature (21°C) with a double-beam spectrophotometer for 6min after the addition of hepatic microsomal fraction (0.1ml of a solution containing 0.24mg of protein/ml was added to 2.9ml of reaction mixture in the cuvette) (Phillips & Langdon, 1962; Williams & Kamin, 1962). Linear reaction rates were used to calculate the amount of cytochrome *P*-450 or cytochrome *c* reduced/min per g of liver.

The DNA content of the liver homogenate was measured with diphenylamine reagent (Burton, 1956; Dische, 1930) after extraction with perchloric acid (Schneider & Hogeboom, 1952). The method of Lowry *et al.* (1951) was used to determine protein in the liver homogenate, the 9000*g*-supernatant and microsomal fractions. Bovine serum albumin was used as the standard. Results were subjected to statistical analysis as described by Snedecor & Cochran (1956).

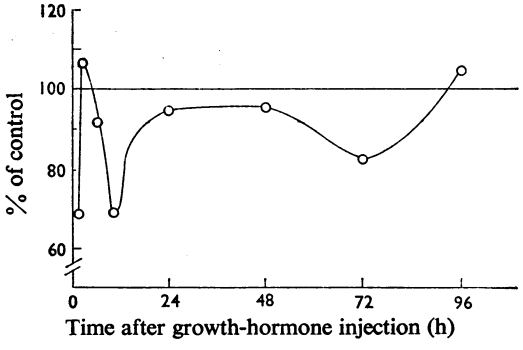

Fig. 2. *Time-course changes in liver content of dithionite-reduced cytochrome P-450 after injection of growth hormone in sham-operated rats*

Animals and treatment conditions were the same as described in the legend of Fig. 1. Values obtained with sham-operated rats injected with the vehicle were used as controls (100%)

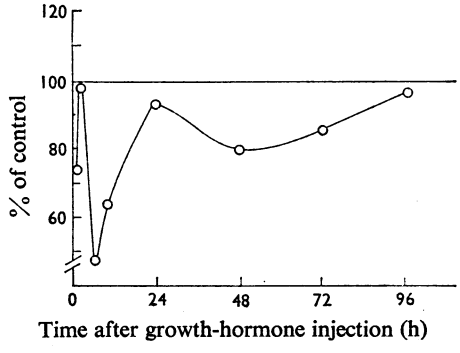

Fig. 3. *Time-course changes in liver microsomal cytochrome c reductase activity after injection of growth hormone in sham-operated rats*

Animals and treatment conditions are the same as those described in the legend of Fig. 1. Values obtained with sham-operated rats injected with the vehicle were used as controls (100%).

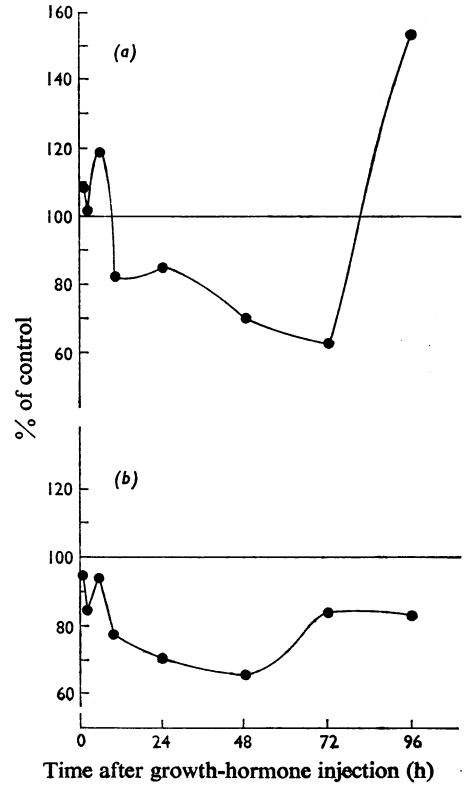

Fig. 4. *Time-course changes in liver metabolism of (a) hexobarbital and (b) aniline after injection of growth hormone in hypophysectomized/adrenalectomized rats*

Animals and treatment conditions are the same as those described in the legend of Fig. 1, except that values obtained with hypophysectomized/adrenalectomized rats injected with the vehicle were used as controls (100%).

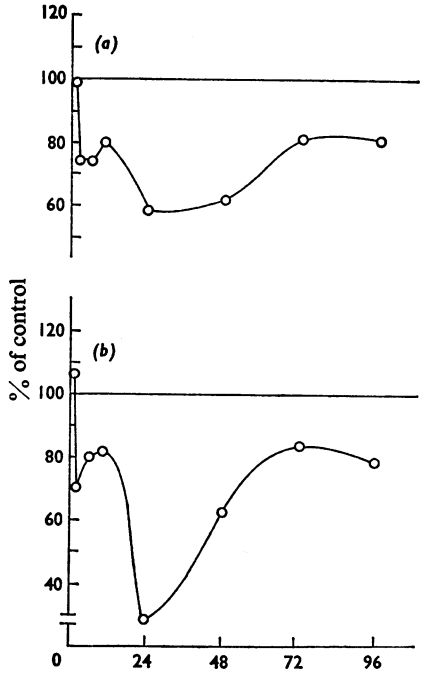

Fig. 5. *Time-course changes in liver content of cytochrome P-450 after injection of growth hormone in hypophysectomized/adrenalectomized rats*

Animals and treatment conditions are the same as those described in the legend of Fig. 4. Values obtained with hypophysectomized/adrenalectomized rats injected the vehicle were used as controls (100%). (*a*) Dithionite-reduced; (*b*) NADPH-reduced.

Results

Overall effects of the operations on rat liver

The effect of hypophysectomy and adrenalectomy on mixed-function oxidase activity and other biological or chemical constituents was noteworthy. Table 1 shows that, compared with control rats, the body and liver weight of the hypophysectomized/adrenalectomized rats are decreased, but the protein concentrations in several liver fractions remain unchanged. The DNA content, on a mg/g of liver basis, is increased in the hypophysectomized/adrenalectomized rats probably owing to losses in cell mass but not cell numbers. Liver drug metabolism (especially that of hexobarbital) shows a decrease in activity. Little change in the extent of cytochrome *P*-450 reduction and aniline (type II) metabolism is observed. Hypophysectomized/adrenalectomized rats were used at 10 days after operation; consequently the above values represent those resulting from a 10-day withdrawal from the growth hormone as well as other hormones.

Growth-hormone effects on mixed-function oxidase activity in sham-operated rats

The liver metabolism of hexobarbital and aniline was studied for 1–96h after growth-hormone treatment (Fig. 1). Both drugs showed an early (at 12h) decrease in the amount of hepatic biotransformation. This is followed by a 22h period of variability in the directional change of liver mixed-function oxidase activity for hexobarbital and aniline. A consistent decrease in the metabolism of these drugs is observed at 48 and 72h after injection of growth hormone, with a subsequent trend toward control values by 168h (not shown). In this and the following paper, time-course changes emphasize directional trends

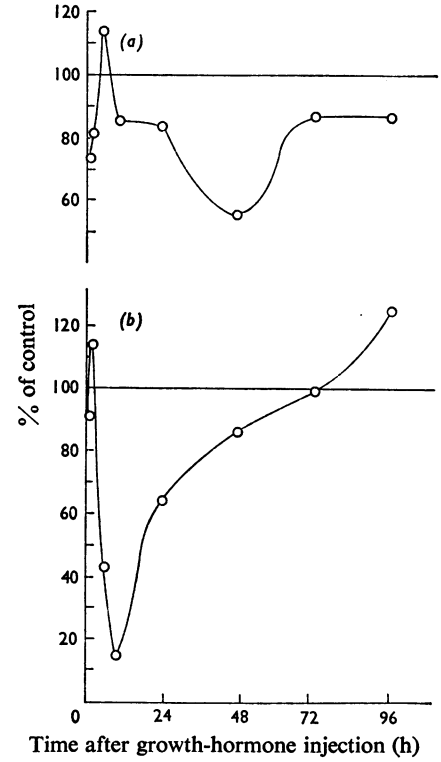

Fig. 6. *Time-course changes in liver microsomal-fraction reductase activity after injection of growth hormone in hypophysectomized/adrenalectomized rats*

Animals and treatment conditions are the same as those described in the legend of Fig. 4. Values obtained with hypophysectomized/adrenalectomized rats injected with the vehicle were used as controls (100%). (*a*), Cytochrome *P*-450 reductase; (*b*), cytochrome *c* reductase.

Table 1. *Effect of hypophysectomy and adrenalectomy on metabolic parameters in the rat*

See the legends to Figs. 3 and 6, and the Materials and Methods section for details of the animals and their treatment. The hypophysectomized/adrenalectomized and sham-operated rats were used 10 days after operation. Only sham control or hypophysectomized/adrenalectomized control rats (treated with vehicle at zero time) were used. Results are expressed as means±S.E. (five observations) from samples obtained between 1 and 24 h after treatment with the vehicle. Thus the S.E. value includes the diurnal variations for each of the parameters studied. *$P<0.05$.

Parameter studied	Control rat	Hypophysectomized/ adrenalectomized rat	Change (%)
Biological			
Body weight (g)	239 ± 6	186 ±3*	−22
Liver weight			
(g)	9.3 ± 0.4	5.7 ±0.2*	−39
(g%)	3.89± 0.09	3.02±0.11*	−22
Protein (mg/g of liver)			
Homogenate	189 ±10	185 ±3	−2
9000*g*-supernatant fraction	122 ± 4	123 ±3	+1
Microsomal fraction	29 ± 1	28 ±0.7	−4
DNA (mg/g of liver)	1.9 ± 0.07	2.8 ±0.04	+49
Drug metabolism (μmol/g of liver)			
Hexobarbital	5.7 ± 0.7	1.9 ±0.07*	−67
Aniline	1.0 ± 0.1	0.9 ±0.07	−16
Electron transport system			
Cytochrome *P*-450 (*E*/g of liver)			
Dithionite-reduced	1.9 ± 0.2	1.9 ±0.2	0
NADPH-reduced	1.8 ± 0.1	1.6 ±0.07	−8
Cytochrome *P*-450 reductase (*E*/min per g of liver)	21 ± 1.8	17 ±1.0	−21
Cytochrome *c* reductase (*E*/min per g of liver)	2.5 ± 1.1	0.9 ±0.1	−63

rather than the absolute magnitude of the change between control and experimental animals. In general, the results for liver metabolism of hexobarbital and aniline after injection of growth hormone in sham-operated rats show an early decrease, a period of variable response, and, beginning at 48h, a low metabolic activity which slowly returns to control values.

Hepatic microsomal electron-transport parameters were examined at various times after growth-hormone treatment of sham-operated rats (Fig. 2). Dithionite-reduced cytochrome *P*-450 shows a decrease and then an increase at 1 and 2h after growth-hormone injection. A consistent decrease in this component was noted at 6 and 10h, with control values being approached by 96h.

Cytochrome *c* was used as an acceptor of reducing equivalents from NADPH to determine liver microsomal reductase activity in growth-hormone-treated sham-operated rats (Fig. 3). This time-course change with cytochrome *c* reductase shows an early decrease, with subsequent low but fluctuating activity between 24 and 72h. There is a slow return to control values 96h after injection of growth hormone.

Effect of growth hormone on mixed-function oxidase activity in hypophysectomized/adrenalectomized rats

Time-course changes in the overall rate of liver mixed-function oxidase activity were studied in hypophysectomized/adrenalectomized rats to obviate possible indirect or permissive effects mediated via the pituitary or adrenals. Fig. 4 shows that the use of these animals somewhat minimizes the early variation in aniline metabolism induced by growth-hormone treatment found in sham-operated animals. The hydroxylation of aniline by the hepatic microsomal fraction shows a consistent decrease for 96h, whereas that of hexobarbital shows an initial increase followed by a prolonged decrease (to 72h) after growth-hormone treatment. Differences in hexobarbital metabolism after growth-hormone injection between the hypophysectomized/adrenalectomized rats and those found with sham-operated rats were minimal (except at 96h).

Changes in the content of dithionite- or NADPH-reduced liver microsomal cytochrome *P*-450 at variable periods after growth-hormone injection were studied in the hypophysectomized/adrenalectomized rats (Fig. 5). An early decrease in cytochrome *P*-450 is noted, with even more pronounced effects observed between 24 and 48h. The activity of cytochrome *P*-450 reductase shows an early biphasic effect and then a decrease between 10 and 96h after growth-hormone treatment of hypophysectomized/adrenalectomized rats (Fig. 6). The activity of cytochrome *c* reductase by the hepatic microsomal fraction is decreased by 6–10h and slowly returns to control values by 72h. In general both the extent and reduction rate of cyto-

chrome *P*-450 shows an early and prolonged decrease after injection of growth hormone in these hypophysectomized/adrenalectomized rats. As with the mixed-function oxidase activities the concentrations of the components of the electron-transport system in hypophysectomized/adrenalectomized rats are more dramatically effected by growth hormone than they are in the sham-operated rats.

Discussion

It is known that growth hormone enhances the growth rate and body weight of animals (Harris, 1964). Removal of growth hormone from an animal causes a reverse effect. The hypophysectomy and adrenalectomy of our animals substantiated these early reports by causing a decrease in both body and liver weight, in some drug-metabolizing enzymes, as well as in some components of the electron-transport system (Table 1). Both the mixed-function oxidase activities in the early periods (1–10h) after growth-hormone administration show multiphasic patterns. This was the case for both the sham-operated and the hypophysectomized/adrenalectomized rats. Generally the mixed-function oxidase activity of the growth-hormone-treated animals remained below that of the control during the early periods after treatment. These results confirm those reported earlier (for different systems) on the growth hormone-induced decrease in drug-metabolizing-enzyme activity (Wilson 1968*a*,*b*; 1969*a*,*b*). During the later periods (72–96h after growth-hormone treatment), the mixed-function oxidase activity in most instances also returned to, or was approaching, the control values. The hexobarbital metabolism and cytochrome *c* reductase activities in the hypophysectomized/adrenalectomized rats are an exception.

The results described in the present paper are consistent with the hypothesis proposed by Wilson & Frohman (1974) wherein mixed-function oxidase activity (as well as other specialized-function enzymes not associated with growth) is decreased in situations associated with liver growth. Similar to the case of malignant transformation and to mitogenic activity increases in liver cells of hypophysectomized/adrenalectomized animals in response to growth hormone, we propose that the specialized functions not associated with growth are decreased, probably as a result of the diversion of the cellular resources (i.e. energy) to growth requirements. The mechanism by which growth hormone might alter these enzyme activities is the subject of the following paper (Spelsberg & Wilson, 1976).

We are indebted to Mr. Gary Colin, Mr. Kenneth Dill and Dr. Joseph Chrastil for their excellent assistance in the laboratory. This work was sponsored by grants from NIH (CM-15431) and (HD 9140-B) and NCI (CA 14920). J. T. W. is the recipient of a Research Career Development Award from the National Institutes of Health (no. K4 42-539). T. C. S. is a Fellow of the National Genetic Foundation.

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