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INTERACTIONS OF INSULIN, THYROXINE AND DEXAMETHASONE WITH GROWTH HORMONE IN THE CONTROL OF STEROID METABOLISM BY ISOLATED RAT HEPATOCYTES

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Abstract—*In vivo* studies have established that continuous infusion of male rats with growth hormone (GH) results in the feminisation of hepatic steroid metabolism. In this study, however, no feminising effects were observed in response to GH treatment alone in isolated hepatocytes. Combined incubations with insulin and GH or with dexamethasone and GH showed that GH antagonised the stimulatory effects of insulin and dexamethasone respectively. Pretreatment of hepatocytes over 30 min with thyroxine and GH also resulted in a decrease in enzyme activity as compared to the response with thyroxine alone. Thus no feminisation effects were seen with any of the combinations of hormones noted above. Preincubation of hepatocytes with dexamethasone, thyroxine and GH, however, resulted in the expression of the feminisation effect noted *in vivo*. It therefore appears from this study that dexamethasone and thyroxine may play a permissive role in mediating the feminising effects of GH in isolated hepatocytes, and that GH may be interacting with these hormones *in vivo* to produce such effects.

The hepatic drug- and steroid-metabolising system catalyses the oxidative and reductive metabolism of a wide variety of xenobiotics and endogenous steroids [1, 2]. The cytochrome P-450 enzymes play a central role in this metabolism, and at least twenty distinct P-450 forms have been isolated from rat liver [2] and characterised biochemically in terms of substrate specificity, spectral analysis, structural and immunochemical properties [3–5].

The adult rat liver shows marked quantitative and qualitative sex-related differences in the metabolism of drugs and steroids [6]. In adult male rats, steroid metabolism is primarily oxidative, as compared to adult females where steroid metabolism is mainly reductive [7]. Steroid metabolism is low in neonates, and the characteristic enzyme pattern develops at puberty [8]. It has been postulated that the sex differences observed in liver steroid metabolism are secondary to sexual differentiation of the brain at birth [9], and that the adult pattern of steroid metabolism is "imprinted" depending on the presence or absence of neonatal androgens [9]. It has also been proposed that the gonadal hormones do not act on the liver directly, but rather affect the pattern of secretion of growth hormone (GH) by the pituitary, which in turn regulates the hepatic steroid-metabolising enzymes [10]. In adult male rats, GH exhibits regular large peaks (every 3–4 hr) with intervening low or undetectable levels of GH, whereas plasma GH concentrations are more continuous in adult females [11]. Further, continuous infusion of GH into adult male rats has resulted in the "feminisation" of steroid metabolism [12]. Some workers [13] have also indicated a requirement for thyroid and adrenal hormones as permissive factors in the action of GH but this is disputed [12]. Since GH appears to exhibit such a dominant role in the regulation of hepatic steroid metabolism it was of interest to see if this "feminisation" could be achieved *in vitro*, in order to determine whether "feminisation" was a direct effect of GH on the liver cells and if GH required the presence of thyroid and adrenal hormones for action as suggested *in vivo*. The use of isolated hepatocytes is an important technique to study the effects of single hormones, without the ambiguity of the hormonal interactions that may be encountered *in vivo*. This preparation also allows the study of precise combinations of different hormones to attempt to mimic the situation *in vivo* under clearly defined conditions.

We report here the effects of GH on steroid metabolism in isolated rat hepatocytes, and its interactions with insulin, glucocorticoids and thyroid hormones.

MATERIALS AND METHODS

Chemicals. Collagenase and bovine serum albumin (BSA) were obtained from BCL Ltd (Lewes, U.K.). Androstenedione, dexamethasone-21-acetate and L-thyroxine were purchased from Sigma Chemical Co. (Poole, U.K.), and Ham's F-10 medium from Gibco Ltd (Paisley, U.K.). Porcine insulin was obtained from Novo Research Institute (Copenhagen, Denmark), Ultroser G from LKB (Bromma, Sweden) and recombinant Somatonorm(R) (genetically engineered human GH) from KabiVitrum AB (Stockholm, Sweden). [4-^{14}C]Androst-4-ene-3,17-dione was supplied by Amersham International (Aylesbury, U.K.). All other chemicals were of the highest purity available commercially.

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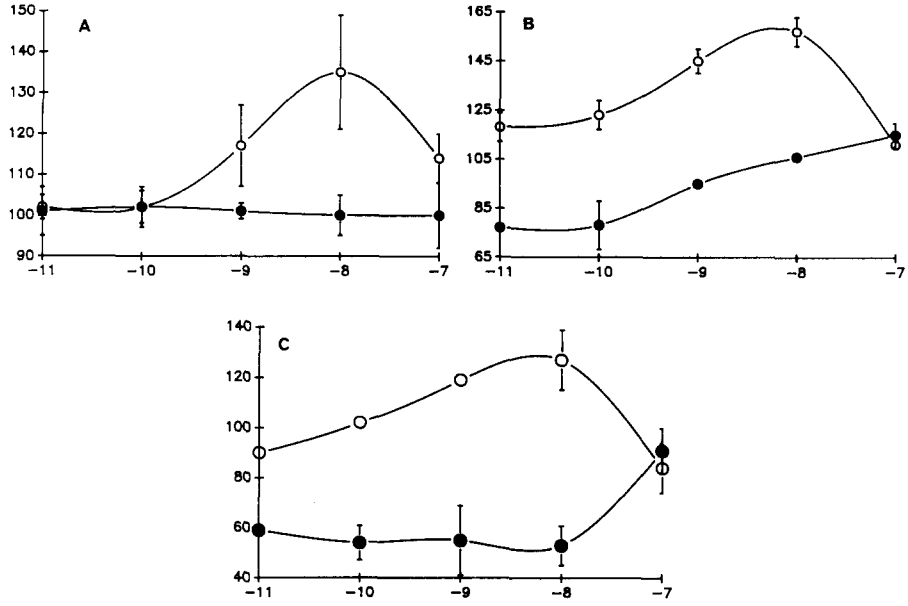

Fig. 1. The effect of 30 min pretreatment with varying concentrations of insulin (10^{-11}–10^{-7} M) in the presence (—●—) and absence (—○—) of GH (10^{-9} M) on the metabolism of androst-4-ene-3,17-dione by 7α-hydroxylase (A), 6β-hydroxylase (B) and 17-oxosteroid oxidoreductase (C).

Animals. Adult male Wistar rats (body weight 350–400 g), bred in the department were used throughout the study. The animals were housed in light- and temperature-controlled conditions (lights on from 07.00 a.m. to 07.00 p.m.; 19 ± 1°) and allowed free access to food (CRM Nuts, Labsure, Croydon, U.K.) and tap water.

Preparation of hepatocytes. Hepatocytes were prepared by the collagenase perfusion technique of Seglen [14] as modified by Hussin and Skett [15]. Briefly, the animal was anaesthetised using halothane/nitrous oxide, and a cannula inserted into the hepatic portal vein. Calcium-free Hank's balanced salt solution (HBSS) was perfused through the liver for 8 min followed by recycled collagenase buffer (HBSS supplemented with 4 mM calcium chloride, 0.5 mg collagenase/ml) for a further 12 min. Following isolation, the cells were washed and resuspended in incubation medium (HBSS + 1 g/l glucose, 100 mg/l magnesium chloride, 100 mg/l magnesium sulphate, 185 mg/l calcium chloride). This method yielded approximately 10^8 cells/g liver with a viability of >90% as assessed by the trypan blue exclusion method and this level of viability was maintained throughout the experiment.

Preincubation of hepatocytes. For the short-term preincubations, the hepatocytes were incubated in Ham's F-10 medium supplemented with 0.1% (w/v) BSA in a final volume of 10 ml (2×10^7 cells/incubation) over a period of 0–60 min and at 37°.

In the case of the 24, 48 and 72 hr incubations, hepatocytes were initially plated in Ham's F-10 culture medium supplemented with 2% Ultroser G, which is a multihormone serum substitute that promotes cell attachment. The medium was replaced with Ham's F-10 medium supplemented with 0.1% BSA after 24 hr. After a further 24 hr, hormones were added to the medium for 24, 48 or 72 hr and incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37°. Additions of hormones were as follows: GH (10^{-9}–10^{-7} M) in 1 M glycine; insulin (10^{-11}–10^{-7} M) in distilled water; dexamethasone (10^{-8} M) in acetone; thyroxine (10^{-8}–10^{-6} M) in 0.05 N NaOH. All additions were made in the minimum volume possible and control incubations received drug vehicle alone. None of the additions significantly altered the pH of the medium.

After preincubation with hormones, the cells were washed, centrifuged and resuspended in incubation medium prior to assaying for steroid-metabolising activity.

Assay of steroid metabolism. Cells were assayed for steroid-metabolising activity in triplicate as follows: to each incubation was added 0.1 μCi, 500 μg of [4-^{14}C]androst-4-ene-3,17-dione in a final volume of 3 ml in incubation medium (7×10^6 cells/incubation) and the mixture was incubated at 37° for 30 min in a shaking water bath. The reaction was stopped by the addition of 5 ml of chloroform/methanol (2:1 v/v). Unchanged substrate and metabolites were separated by thin layer chromatography, located by autoradiography and quantitated by liquid scintillation counting, as previously described [16].

Calculation of results and statistics. Means for each separate experiment were calculated and compared to the test group(s) by means of Student's *t*-test or Duncan's multiple range test as appropriate. The analysis was performed on the actual data (before normalisation) to validate the statistical analysis but results are given as percentage of relevant control at the appropriate time point to simplify the presentation of the data.

RESULTS

The enzyme assay used allows the determination

Table 1. The effect of incubation with 10^{-9} M GH over 0 min to 72 hr on the metabolism of androst-4-ene-3,17-dione by isolated rat hepatocytes

	7α-OHase	6β-OHase	16α-OHase	17-OHSD	5α-red
5 min	112 ± 18	100 ± 12	100 ± 4	90 ± 15	104 ± 3
10 min	94 ± 6	90 ± 10	81 ± 12	63 ± 2*	115 ± 15
15 min	100 ± 14	88 ± 8	87 ± 9	79 ± 4*	108 ± 8
30 min	95 ± 19	100 ± 3	73 ± 18	72 ± 14*	88 ± 10
24 hr	83 ± 7	98 ± 10	104 ± 2	120 ± 6	99 ± 4
48 hr	97 ± 10	110 ± 14	91 ± 10	117 ± 7	87 ± 11
72 hr	109 ± 1	120 ± 15	104 ± 3	101 ± 5	60 ± 3*

Results are expressed as mean percentage of relevant controls ± SD of three experiments (three samples/experiment).

* $P < 0.05$. OHase, hydroxylase; OHSD, oxosteroid oxidoreductase; red, reductase.

Control values (0 min) were: 34 ± 8 (7α-OHase), 36 ± 6 (6β-OHase), 34 ± 8 (16α-OHase), 58 ± 9 (17-OHSD), 106 ± 6 (5α-red) pmol/min/10^6 cells.

Table 2. The effect of 30 min incubation with 10^{-9}–10^{-7} M GH on the metabolism of androst-4-ene-3,17-dione by isolated hepatocytes

	7α-OHase	6β-OHase	16α-OHase	17-OHSD	5α-red
10^{-9} M	112 ± 6	103 ± 14	80 ± 11	83 ± 1*	96 ± 8
10^{-8} M	89 ± 8	92 ± 13	92 ± 9	78 ± 2*	96 ± 6
10^{-7} M	83 ± 9	90 ± 8	89 ± 16	77 ± 4*	114 ± 8

Results are expressed as mean percentage of relevant controls ± SD of three experiments (three samples/experiment).

* $P < 0.05$. Other abbreviations as Table 1.

Control (0 min) values were: 36 ± 4 (7α-OHase), 38 ± 4 (6β-OHase), 36 ± 4 (16α-OHase), 60 ± 7 (17-OHSD), 100 ± 2 (5α-red) pmol/min/10^6 cells.

of 7α-hydroxylase, 5α-reductase (activities normally higher in female rat), 6β- and 16α-hydroxylases and 17-oxosteroid oxidoreductase (activities normally higher in male rat). Enzyme activities varied over the period of preincubation as previously noted by Hussin and Skett [15] with a marked decline in the activities of the 6β- and 16α-hydroxylases and 17-oxosteroid oxidoreductase but little change in the other enzymes. Control incubations (containing no hormonal additions) are, therefore, performed at each time period and the effects of the hormones assessed against this time-dependent control in each case.

Preincubation of hepatocytes with 10^{-9} M growth hormone (GH) (the approximate average serum GH concentration seen in the control female rat [11]) over both short and long incubation periods (Table 1). show that the hormone has no significant effects on any of the enzyme activities assayed, except for the 17-oxosteroid oxidoreductase activity which showed a significant decrease at 10–30 min and the 5α-reductase activity which was markedly reduced at 72 hr. Table 2 shows that incubation of hepatocytes with supraphysiological GH concentrations ($>10^{-9}$ M) has no marked effects on any of the enzyme activities, except for a marked reduction in 17-oxosteroid oxidoreductase activity at all concentrations of GH. Thus GH at all concentrations and times tested had little effect on steroid metabolism in the isolated cell system used.

Preincubation of hepatocytes with a combination of insulin (10^{-11}–10^{-7} M) and 10^{-9} M GH (Fig. 1) for 30 min showed that GH antagonised the stimulatory effects of insulin on steroid metabolism. As in the previous series of experiments, GH alone had no significant effect on any of the enzyme activities (results not shown). It also appears that the antagonistic action of GH is more marked at lower concentrations of insulin (Fig. 1).

Preincubation of hepatocytes with the synthetic glucocorticoid dexamethasone at a concentration of 10^{-8} M over a period of 24, 48 and 72 hr showed marked stimulatory effects throughout, as is demonstrated in Table 3. Cells incubated with dexamethasone and 10^{-9} GH over this period, however, gave generally lower enzyme activities than with dexamethasone alone, and indeed, showed significant decreases in all enzyme activities at one or more time period. Preincubation with 10^{-8} M dexamethasone over 0 to 30 min (Table 3) show that dexamethasone itself acts as a general stimulatory agent of steroid-metabolising enzymes during this time. Incubation with dexamethasone and 10^{-9} M GH over this period, however, caused a reduction in enzyme activity as compared to the response with dexamethasone alone. Since GH itself exhibited no significant effects (Table 1), but reduced the stimulatory effects of dexamethasone, it appears that the presence of dexamethasone in the medium modifies the response to GH alone.

Preincubation of hepatocytes with 10^{-6} M L-thyroxine (T_4) over 24, 48, 72 hr (Table 4) gave significant decreases in activity, some seen at 24 h (7α-hydroxylase) but others not until 72 hr (16α-hydroxylase). Incubation with 10^{-9} M GH following T_4 treatment resulted in no significant changes in enzyme

Table 3. The effect of incubation with 10^{-8} M dexamethasone (DEX) alone or in combination with GH (10^{-9} M) over 0 min to 72 hr on the metabolism of androst-4-ene-3,17-dione by isolated rat hepatocytes

	7α-OHase	6β-OHase	16α-OHase	17-OHSD	5α-red
5 min (DEX)	121 ± 12*	100 ± 21	80 ± 18	127 ± 2*	120 ± 12*
5 min (DEX/GH)	100 ± 10	110 ± 7	100 ± 18	96 ± 6	94 ± 3
10 min (DEX)	127 ± 20	150 ± 2*	118 ± 3	108 ± 5*	150 ± 16*
10 min (DEX/GH)	95 ± 16	82 ± 9*	77 ± 4*	81 ± 8*	93 ± 4
15 min (DEX)	143 ± 3*	100 ± 14	113 ± 2*	77 ± 2*	114 ± 6*
15 min (DEX/GH)	86 ± 12*	105 ± 18	83 ± 2*	60 ± 4*	93 ± 15
30 min (DEX)	100 ± 6	106 ± 12	115 ± 7*	109 ± 4*	95 ± 14
30 min (DEX/GH)	90 ± 19	111 ± 2*	71 ± 6*	73 ± 2*	102 ± 18
24 hr (DEX)	181 ± 18*	136 ± 9*	144 ± 8*	129 ± 2*	131 ± 4*
24 hr (DEX/GH)	62 ± 7*	88 ± 19	110 ± 10	80 ± 2*	93 ± 1*
48 hr (DEX)	175 ± 4*	149 ± 7*	148 ± 5*	153 ± 6*	163 ± 9*
48 hr (DEX/GH)	80 ± 4*	84 ± 2*	82 ± 11*	100 ± 15	109 ± 3*
72 hr (DEX)	179 ± 13*	188 ± 10*	150 ± 9*	182 ± 14*	193 ± 18*
72 hr (DEX/GH)	91 ± 7*	86 ± 2*	107 ± 2*	90 ± 2*	118 ± 8*

Results are expressed as mean percentage of relevant controls ± SD of three experiments (three samples/experiment).

* $P < 0.05$. Other abbreviations as Table 1.

Control values (0 min) were: 65 ± 5 (7α-OHase), 81 ± 5 (6β-OHase), 74 ± 6 (16α-OHase), 102 ± 3 (17-OHSD), 167 ± 7 (5α-red) pmol/min/10^6 cells.

Table 4. The effect of incubation with 10^{-6} M thyroxine (T_4) alone or in the presence of GH (10^{-9} M) over 0 min to 72 hr on the metabolism of androst-4-ene-3,17-dione in isolated rat hepatocytes

	7α-OHase	6β-OHase	16α-OHase	17-OHSD	5α-red
10 min (T_4)	94 ± 12	87 ± 4	92 ± 8	90 ± 1*	84 ± 8*
10 min (T_4/GH)	60 ± 18*	72 ± 2*	58 ± 14*	54 ± 6*	32 ± 10*
30 min (T_4)	91 ± 11	105 ± 3	96 ± 10	85 ± 1*	76 ± 8*
30 min (T_4/GH)	81 ± 11*	86 ± 4	60 ± 10*	64 ± 3*	32 ± 4*
24 h (T_4)	81 ± 2*	121 ± 5*	100 ± 6	107 ± 9	110 ± 11
24 h (T_4/GH)	125 ± 18	105 ± 13	98 ± 5	96 ± 4	98 ± 6
48 h (T_4)	86 ± 6*	102 ± 8	110 ± 5	108 ± 7	111 ± 8
48 h (T_4/GH)	110 ± 11	86 ± 11	100 ± 5	95 ± 6	98 ± 9
72 h (T_4)	64 ± 6*	64 ± 6*	56 ± 14*	63 ± 2*	63 ± 3*
72 h (T_4/GH)	97 ± 8	93 ± 10	71 ± 14*	97 ± 6	87 ± 8

Results are expressed as mean percentage of relevant controls ± SD of three experiments (three samples/experiment).

* $P < 0.05$. Other abbreviations as Table 1.

Control values (0 min) were: 42 ± 5 (7α-OHase), 58 ± 10 (6β-OHase), 66 ± 9 (16α-OHase), 107 ± 10 (17-OHSD), 112 ± 11 (5α-red) pmol/min/10^6 cells.

activities as compared to control incubations except for a marked decrease in 16α-hydroxylase activity (Table 4).

Exposure of hepatocytes to 10^{-6} M T_4 over a short incubation time course caused no significant changes in any of the cytochrome P-450-dependent enzyme activities assayed but a marked decrease in the 17-oxosteroid oxidoreductase and 5α-reductase activities, as is shown in Table 4. However, incubation with T_4 and 10^{-9} M GH resulted in marked decreases in all enzyme activities by 10 min as compared to activities with either of the hormones alone. Thus, T_4 appears to modify the GH response on hepatic steroid-metabolising enzymes.

Thus, although the effects of GH are altered in the presence of insulin, dexamethasone and T_4, the sex-dependent effects of GH expected from *in vivo* data are not observed, and a general non-specific effect was seen throughout.

Table 5 shows that preincubation of hepatocytes with dexamethasone and T_4 gave a mixed response with no significant effects from 24–72 hr (Table 5). In the presence of dexamethasone, T_4 and GH, however, an interesting response is seen. The 7α-hydroxylase and 5α-reductase activities (normally higher in female rats) are markedly increased, whereas the 6β-hydroxylase, 16α-hydroxylase and 17-oxosteroid oxidoreductase activities (normally

Table 5. The effect of incubation with (10^{-8} M dexamethasone (DEX) + 10^{-6} M T_4) or (10^{-8} M DEX + 10^{-6} M T_4 + 10^{-9} M GH) over 0 min to 72 hr on the metabolism of androst-4-ene-3,17-dione in isolated rat hepatocytes

	7α-OHase	6β-OHase	16α-OHase	17-HSD	5α-red
10 min (DEX/T_4)	80 ± 3*	112 ± 3*	91 ± 3*	100 ± 5	82 ± 5*
10 min (DEX/T_4/GH)	112 ± 5*	92 ± 3*	61 ± 4*	96 ± 6	116 ± 6*
30 min (DEX/T_4)	88 ± 3*	99 ± 4	91 ± 4	113 ± 3*	100 ± 5
30 min (DEX/T_4/GH)	114 ± 6*	88 ± 2*	43 ± 3*	82 ± 3*	134 ± 9*
24 hr (DEX/T_4)	83 ± 15	84 ± 15	112 ± 18	100 ± 5	98 ± 13
24 hr (DEX/T_4)/GH)	122 ± 13*	100 ± 11	79 ± 2*	91 ± 3*	117 ± 5*
48 hr (DEX/T_4)	100 ± 2	110 ± 4	100 ± 8	108 ± 12	97 ± 7
48 hr (DEX/T_4/GH)	150 ± 19*	64 ± 18*	93 ± 8	100 ± 2	121 ± 2*
72 hr (DEX/T_4)	99 ± 4	100 ± 2	110 ± 13	117 ± 12	100 ± 12
72 hr (DEX/T_4/GH)	100 ± 13	78 ± 8*	76 ± 3*	94 ± 15	104 ± 2

Results are expressed as mean percentage of relevant controls ± SD of six values.
* $P < 0.05$. Other abbreviations as Table 1.
Control values (0 min) were: 36 ± 4 (7α-OHase), 36 ± 2 (6β-OHase), 38 ± 2 (16α-OHase), 88 ± 6 (17-OHSD) and 120 ± 6 (5α-Red) pmol/min/10^6 cells.

higher in male rats) are markedly decreased at almost all time periods. This is characteristic of the feminisation effects typical of GH from *in vivo* data.

DISCUSSION

The results presented here indicate that incubation of hepatocytes with GH alone over a range of concentrations and time periods has no significant effects on steroid metabolism in this culture system, except for 17-oxosteroid oxidoreductase activity at short incubations, where reductions in enzyme activity were observed. In contrast to these findings, several groups have reported that exposure to continuous GH *in vivo* results in a feminisation of microsomal steroid metabolism [12, 17, 18]. Human GH is often used in these studies due to its more consistent quality and it has been noted, by comparison to rat GH and ovine prolactin, that it is the somatogenic quality of this preparation rather than its lactogenic quality that gives the effects noted. Recently, Guzelian *et al.* [19] have reported the induction of cytochrome P-450$_i$, (which is associated with the expression of 15α-hydroxylase activity in female rats), in male rat hepatocytes treated with GH. We have, however, found no increases in the female-specific 5α-reductase activity in response to GH alone. Guzelian *et al.* [19] also reported that they were unable to "masculinise" steroid metabolism in female rat hepatocytes, suggesting the need for other hormonal influences. Since GH did not feminise steroid metabolism in our system, it can be postulated that GH maybe acting *in vivo* by interacting with other hormones to achieve its feminising effect.

Insulin and GH are known to interact *in vivo* [1, 6]. Chemically induced or spontaneous diabetes mellitus has been shown to induce sex-dependent effects on both drug [20] and steroid [21] metabolism. Further, the diabetic state is reported to cause marked changes in the GH secretory pattern [22], thereby implying a certain degree of interaction between insulin and GH in this system. We therefore attempted to investigate the link between GH and insulin in isolated hepatocytes.

Insulin itself is known to act as a general stimulator of steroid metabolism in rat hepatocytes [23]. Preincubation of hepatocytes with insulin and GH showed that GH antagonised the stimulatory effects of insulin in a non-specific manner in that both male- and female-specific enzyme activities were affected in a similar way. Hence it may be concluded that although insulin may be one of the hormones that GH is interacting with *in vivo* to mediate the feminisation effects, it is not the sole hormone, as the sex-dependent effects characteristic of exposure to continuous GH are not manifested here.

Several workers have previously reported that the effects of GH are potentiated in the presence of other hormones [13, 17, 24–30]. Colby [13] has reported that the adrenal steroids and thyroid hormones are involved in the regulation of the steroid-metabolising system since both adrenalectomy and thyroidectomy affected drug metabolism markedly.

Earlier studies with glucocorticoids indicated that adrenalectomy caused reductions in drug metabolism in male animals only [24], suggesting a permissive role of these hormones in mediating androgen action. These results were confirmed by Gustafsson and Stenberg [25] who found that adrenalectomy caused reductions in male-specific steroid-metabolising enzymes only. Colby *et al.* [26] also showed that a combination of ACTH and GH resulted in a decrease in reductive metabolism.

Extensive work has also been carried out to determine the effects of thyroid hormones on steroid and drug metabolism [27–30] the general consensus being that thyroxine exhibits marked sex- and substrate-dependent effects.

The results obtained from the present study show that dexamethasone stimulates steroid-metabolising enzymes non-specifically over both short and long incubation periods, and that the GH response is modified in the presence of dexamethasone. Dexamethasone is known to be a potent inducer of the cytochrome P-450 system [31], and so the 24, 48, 72 hr studies may represent induction of P-450$_{PCN}$ [31]. It is not clear, however, whether the

stimulatory effects of dexamethasone on steroid metabolism represent a glucocorticoid function or cytochrome P-450 induction [31] and this confuses the interpretation of the results with dexamethasone and GH.

Thyroxine alone is seen to decrease the activity of all of the enzymes studied over the shorter time periods—an effect which is enhanced by GH. The effects of thyroxine over the longer time periods also tend to be stimulatory except for the 6β-hydroxylase at 24 hr. The effects of long-term thyroxine treatment are inhibited by GH. The inhibitory actions of high thyroxine concentrations are similar to those seen *in vivo* by Skett and Weir [29].

Colby [13] has reported that, although the feminising effects of GH were not demonstrated in hypophysectomised rats, feminisation of steroid metabolism was achieved in the absence of the pituitary gland provided that the correct hormonal environment was established. They found that hypophysectomised animals treated with ACTH and thyroxine manifested the feminising effect typical of continuous GH infusion in normal animals [13]. Thus these studies suggested that the apparent requirement for ACTH and T_4 to exhibit the actions of GH may account for the inactivity of GH *in vitro*. This is substantiated by evidence from numerous groups who have stressed the inclusion of both corticosteroids and thyroid hormones in the culture medium in order to maintain cytochrome P-450 levels equivalent to those found *in vivo* [32–37]. Further, Mode *et al.* [38] have recently reported that the induction of the female-specific P-450$_{15\alpha}$ by continuous administration of GH into hypophysectomised male rats was markedly enhanced by simultaneous treatment with thyroxine and cortisol, a natural glucocorticoid. In agreement with the above reports [13, 38], we have found that the feminisation effects of GH on steroid-metabolising enzymes are only manifested in the presence of *both* dexamethasone and thyroxine in the culture medium at periods ranging from 10 min to 48 hr. It therefore follows that dexamethasone and thyroxine may have a permissive role in mediating the feminisation effects characteristic of GH, and that GH may be interacting with these hormones *in vivo* to produce such effects.

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