The Time Course of the Effect of Hypophysectomy and Oestrogen Treatment on the Hepatic Metabolism of Androst-4-ene-3,17-dione in Male and Female Rats

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(Received 7 April 1978)

Hypophysectomy of male animals has little effect on the hepatic androst-4-ene-3,17-dione (androstenedione) metabolism, except for possible changes in the kinetics of the 16 α - and 7 α -hydroxylases. On the other hand, hypophysectomy of female animals leads to a 'masculinization' of hepatic androstenedione metabolism, following the changes seen in $V_{\max}$ of the enzymes involved, probably due to the removal of the source of 'feminizing factor' thought to maintain the 'female' type of metabolism in the liver. There seems to be a temporal dissociation of the effects on the various enzymes, indicating different cellular control mechanisms for these enzymes. Oestrogen treatment of male rats causes 'feminization' of the hepatic androstenedione metabolism. The time study shows an initial increase in 17-hydroxy steroid oxidoreductase and 6 β - and 16 α -hydroxylase activities, followed by a decrease to the values in females. This biphasic effect is possibly due to an initial direct effect via the hypothalamo-pituitary system.

Sex-dependent hepatic metabolism of steroids and xenobiotics is now well recognized. Studies on the metabolism of testosterone (Denef, 1974), corticosterone (Eriksson & Gustafsson, 1971; Gustafsson & Gustafsson, 1974; Colby *et al.*, 1974; Carlstedt-Duke *et al.*, 1975), 4-dehydroepiandrosterone (Tabei & Heinrichs, 1972), androst-4-ene-3,17-dione and 5 α -androstane-3 α ,17 β -diol (Gustafsson & Stenberg, 1974*a,b*; Gustafsson *et al.*, 1974) and 5 α -androstane-3 α ,17 β -diol 3,17-disulphate (Gustafsson & Ingelman-Sundberg, 1974) all show the existence of sex-related differences. Sexual dimorphism is also shown in the field of drug metabolism: Quinn *et al.* (1958) demonstrated a sex-related difference in the metabolism of aminopyrine, hexobarbitone and antipyrine. Such differences have also been seen by other workers (El Defrawy el Masry *et al.*, 1974; Chung *et al.*, 1975; Gielen *et al.*, 1976) with other drugs.

These sex-specific differences in hepatic metabolism have been shown to be related to the steroid environment in the body during development. The 'masculine'-type steroid metabolism in the rat is related to the presence of perinatal androgen (Denef & DeMoor, 1972; Gustafsson & Stenberg, 1974*a,b*; Tabei & Heinrichs, 1972). Likewise, the higher ethylmorphine metabolism, characteristic of the male rat, is dependent on the presence of testosterone in the neonatal period (Chung *et al.*, 1975). Other steroids, notably the glucocorticoids, are also active in controlling hepatic steroid and drug metabolism (Gielen *et al.*, 1976).

It has been shown that the pituitary gland is involved in the control of hepatic metabolism of steroids and drugs. In fact, in most cases, the presence of the pituitary gland is necessary for the action of steroids on hepatic metabolism, as indicated by studies on androst-4-ene-3,17-dione metabolism (Gustafsson *et al.*, 1974) and on ethylmorphine metabolism (Kramer *et al.*, 1975). Also hypophysectomy of female animals leads to a hepatic metabolism more resembling that of the male (Denef, 1974; Gustafsson & Stenberg, 1974*c*). These data would indicate the existence of (a) pituitary factor(s) released from the female pituitary maintaining the 'female' pattern of metabolism in the liver. This (these) factor(s) have been referred to as the 'feminizing factor' (Gustafsson *et al.*, 1975; Skett *et al.*, 1976).

In a number of instances the sexual differences in drug metabolism have also been examined at the kinetic level. For the *N*-demethylation of ethylmorphine, it was claimed that male animals had a higher $V_{\max}$ and lower K_m value than female (Castro & Gillette, 1967; Davies *et al.*, 1968; Chung *et al.*, 1975). There are indications that the kinetic parameters change during development (Gram *et al.*, 1969; El Defrawy el Masry *et al.*, 1974; Bell & Ecobichon, 1975). No such investigations of hepatic metabolism of steroids have been published.

The present study was performed to answer two questions: (1) what is the time course of changes in hepatic steroid-metabolizing enzymes after hypo-

physectomy or oestrogen treatment; and (2) what are the changes in kinetic characteristics (K_m and V_{max}) after such treatment?

The serum concentrations of prolactin and somatotropin were determined to see whether these correlate with any changes in hepatic steroid metabolism.

Experimental

Animals

Sprague-Dawley rats (obtained from a local supplier: Anticimex, Stockholm, Sweden) were used throughout the experiment. The animals were kept in a light- and temperature-controlled room (lights on 06:00–20:00h, $23 \pm 1^\circ\text{C}$) and given free access to food and water or 5% glucose/0.9% NaCl (both w/v) solution for the hypophysectomized animals.

Experimental design and methods

Five groups of animals were used: normal untreated males and females, hypophysectomized males and females and males treated with 1 μg of oestra-1,3,5(10)-triene-3,17 β -diol-3-benzoate (oestradiol benzoate; Sigma Chemical Co., St. Louis, MO, U.S.A.) intramuscularly once daily in 10 μl of propylene glycol. All treatments were started when the animals were 6 weeks old. Hypophysectomy was performed by the parapharyngeal route under light anaesthesia with diethyl ether. The completeness of the operation was ascertained by measuring weight loss caused by the hypophysectomy.

At 1 day after the beginning of the treatment, four animals from each group were randomly selected and killed by decapitation. Trunk blood was collected and the livers excised within 1 min of death and placed in ice-cold Bucher medium (Bergström & Gloor, 1955). The blood was allowed to clot at $+4^\circ\text{C}$ and serum prepared by centrifugation (3600 g for 10 min). The serum was stored at -20°C until assayed for pituitary hormones (prolactin and somatotropin) with the kits kindly supplied by the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism and Digestive Diseases as described previously (Eneroth *et al.*, 1975). The livers were cut into small pieces and homogenized in a Potter-Elvehjem homogenizer. Microsomal fractions were prepared from the resulting homogenates by differential centrifugation (Gustafsson & Stenberg, 1974a,b), and incubated with [4- ^{14}C]androst-4-ene-3,17-dione (500 μg ; 7.5×10^5 d.p.m., from New England Nuclear Corp., Dreieichenhain, West Germany) in an incubation mixture of 3 ml of Bucher medium containing an NADPH-regenerating system (Berg & Gustafsson, 1973). Incubation conditions were 10 min in a shaking

water bath at 37°C . The incubation was terminated by the addition of 10 ml of chloroform/methanol (2:1, v/v), and the steroids present were extracted and analysed (Berg & Gustafsson, 1973; Gustafsson & Stenberg, 1974a,b).

The above procedure was repeated at 2, 4 and 7 days after the start of the treatment for two of the groups (hypophysectomized females and oestradiol benzoate-treated males) to obtain a time curve for the effect of these treatments. At 14 days after the start of the treatment all groups were again sampled and incubations performed as above. In addition to the standard incubations, a series of incubations containing different amounts of steroid (10–500 μg) were performed for each group. In order to perform the 'kinetic' incubations, the microsomal fractions from all the animals in one group were pooled and this was added to the incubation. Duplicate samples at each substrate concentration were taken in all cases. The incubations were terminated and products analysed as above.

Protein determination

The protein concentration in the microsomal fraction was measured by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma) as standard.

Calculation of enzyme activity and statistical methods

Enzyme activities were calculated as nmol of product formed/min per mg of protein, and for the standard incubation were expressed as the mean of the group $\pm$ s.d. Statistical differences were calculated by Student's *t* test (level of significance set at $P < 0.05$) and Duncan's multiple range test. The means were arranged in rank (size) order and values not significantly ($P > 0.05$) different from each other are underlined.

For calculation of the kinetic parameters (apparent K_m and V_{max}) of the various enzymes, the v -versus- $v/[S]$ plot (where v is the initial reaction velocity at substrate concentration $[S]$) was used. Only values of v that could be considered to approximate to the initial reaction velocity were used in subsequent calculations. Apparent V_{max} and K_m values were calculated from the non-weighted linear-regression line of the curve obtained.

Calculation of hormone concentration

Pituitary-hormone concentrations were calculated as μg of NIAMDD-RP-1 standard per litre of serum and expressed as the mean $\pm$ 1 s.d. Similar statistical tests were applied as to the enzyme activities.

Results and Discussion

The following enzyme activities could be calculated from the results obtained after incubation of androst-4-ene-3,17-dione with rat liver microsomal fractions: 5 α -reductase (giving 5 α -androstane-3,17-dione, 3 α -hydroxy-5 α -androstane-17-one and 3 β -hydroxy-5 α -androstane-17-one), 17-hydroxy steroid oxidoreductase (no attempt was made to separate the 17 α - and 17 β -isomers of the metabolite 17-hydroxy-androst-4-ene-3-one), 16 α -hydroxylase (16 α -hydroxy-androst-4-ene-3,17-dione), 6 β -hydroxylase (6 β -hydroxyandrost-4-ene-3,17-dione) and 7 α -hydroxylase (7 α -hydroxyandrost-4-ene-3,17-dione). Less than 25% of the substrate was metabolized under the conditions used.

During the course of the experiment, both normal male and female animals showed a weight gain of 7–10g, whereas hypophysectomized animals showed a weight loss of 23 ± 8 g. There was no effect on weight of treatment with oestradiol benzoate.

Standard incubations

In previous reports it has been shown that hypophysectomy of female rats leads to a 'male' type of steroid metabolism (Gustafsson & Stenberg, 1974c), but that a similar operation in male animals has little effect (Eneroth *et al.*, 1977). It was also demonstrated that oestrogen treatment of adult male rats could cause 'feminization' of the pattern of hepatic steroid metabolism (Einarsson *et al.*, 1974). All of these effects were confirmed in the present study. Hypophysectomy of the female animals caused an immediate increase in 16 α -hydroxylase activity ($P < 0.01$) without affecting any other enzyme activity (see Figs. 1a–1e). The appearance of the 16 α -hydroxylase (a typical male enzyme not normally seen in the female) in the hypophysectomized female animals 1 day after the operation without simultaneous development of the other masculine characteristics (decreased 5 α -reductase and increased 6 β -hydroxylase activity) indicates a differential control of the various hepatic enzymes, as discussed below. Oestradiol treatment of male animals significantly increased the 17-hydroxy steroid oxidoreductase activity ($P < 0.05$) after 1 day, but was without effect on the other enzyme activities measured (see Figs. 2a–2e). No effect was seen after hypophysectomy in the male animals.

After 14 days, hypophysectomy of the female animals led to a marked decrease in 5 α -reductase activity ($P < 0.01$) and an increase in 6 β -hydroxylase activity ($P < 0.01$). The 16 α -hydroxylase activity noted after 1 day of treatment was still present after 14 days. This combination of changes in the enzyme activities is considered a 'masculinization', as the

overall metabolic pattern moves nearer to that of the male. In contrast with the marked effect seen in the females, hypophysectomy of the male animals had no significant effect on hepatic androstenedione metabolism even 14 days after the operation. Daily oestradiol benzoate treatment (1 μ g) of male animals caused a dramatic change in hepatic steroid metabolism after 14 days of treatment. The 5 α -reductase activity was greatly increased (408% that of the normal male; $P < 0.001$) and the 17-hydroxy steroid oxidoreductase, 6 β - and 16 α -hydroxylase activities decreased ($P < 0.001$ for the 17-hydroxy steroid oxidoreductase and $P < 0.01$ for the hydroxylases). Oestradiol benzoate treatment thus substantially alters the metabolites formed, leading to a more 'female' pattern of metabolism in the male animals.

The time courses of the development of the steroid-metabolizing enzymes in female rat liver after hypophysectomy are shown in Figs. 1(a)–1(e). Statistical analysis by Student's *t* test (indicated by the stars on the curve), comparing the values for each day with those for the untreated female, indicates that the high female 5 α -reductase activity (Fig. 1a) is maintained until between 2 and 4 days after hypophysectomy, after which it rapidly declines, reaching a normal 'male' value 14 days after treatment. The same pattern is also suggested by analysis of the groups by using Duncan's multiple range test (the results of which are shown under each curve). The lines under the symbols show which groups are classed as being not significantly different from each other.

The development curve for the 17-hydroxy steroid oxidoreductase activity after hypophysectomy (Fig. 1b) is more complex. Analysis of the data by *t* test indicates a decrease in activity up to 4 days after operation, followed by an increase to normal values. This interpretation is corroborated by the analysis by the multiple-range test, which also suggests an initial rise in 17-hydroxy steroid oxidoreductase activity after 1 day, then followed by the development pattern described above.

On the day after hypophysectomy in the female, 16 α -hydroxylase activity (Fig. 1c) had increased from undetectable values (< 0.01) to 0.28 nmol of product/min per mg of protein. This interpretation is supported by analysis by both *t* test and multiple-range-test methods.

6 β -Hydroxylase activity (Fig. 1d) showed a general upward trend leading to a significant increase (by *t* test analysis) 7 days after hypophysectomy. This increase was maintained until at least 14 days after operation.

The development of the 7 α -hydroxylase activity after hypophysectomy (shown in Fig. 1e) indicates a slight initial rise (only significant by multiple range analysis) followed by a marked fall ($P < 0.01$; *t* test

Fig. 1. Time-related development of the hepatic steroid metabolism in female rats after hypophysectomy

Hypophysectomy was performed on day 0 and incubations with androst-4-ene-3,17-dione were carried out 1, 2, 4, 7 and 14 days after the operation. Values for enzyme activities on day 0 are for normal animals. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (according to Student's t test). Analysis of significance by Duncan's multiple range test is indicated on each curve. The underlined and linked values are not significantly different from each other (i.e. $P > 0.05$). (a) 5 α -Reductase; (b) 17-hydroxy steroid oxidoreductase; (c) 16 α -hydroxylase; (d) 6 β -hydroxylase; (e) 7 α -hydroxylase.

analysis) between 2 and 4 days after the operation. The 7 α -hydroxylase activity slowly rises after 4 days, leading to a value slightly under normal after 14 days.

The time courses of the effects (Figs. 1a-1e) indicate that 'masculinization' of the activity of the liver enzymes generally begins between 2 and 4 days after the operation, except for the 16 α -hydroxylase

activity discussed below, despite the fact that the serum concentration of pituitary hormones drops below detectable values by 1 day after operation (see Table 2). The 6 β -hydroxylase activity changes gradually over the period studied, indicating a slow regulation of this enzyme, whereas the 16 α -hydroxylase activity reacts rapidly to hypophysectomy (the full effect is seen after 1 day). The rapid regulation of 16 α -hydroxylase activity indicates the possibility that it is an important regulatory enzyme. For the time course of the development of the 5 α -reductase and 7 α -hydroxylase activities, a lag period of 2 days occurs before any effect is apparent. This lag period could be due to the fact that these enzymes are synthesized using a stable RNA such as has been described for globin mRNA (Schulman, 1968; Hunt, 1974) and ovalbumin mRNA (Palmiter, 1973, 1975), so that these enzymes continue to be synthesized for a time after the stimulus for their synthesis has disappeared. It thus appears that there are different cellular control mechanisms for the various enzymes in the hepatic microsomal fraction which metabolize androstenedione and are under the control of the pituitary gland.

The time curves for the development of hepatic steroid metabolism in male rats during daily exposure to oestradiol are shown in Figs. 2(a)–2(e).

The initial low 5 α -reductase activity (Fig. 2a), characteristic of the male, increases between 2 and 4 days of treatment to a value significantly different (*t*-test analysis, $P < 0.05$) from the control and subsequently continues to rise to approx. 6 times the normal male value ($P < 0.001$) at the start of the treatment. This interpretation agrees in general with that obtained from multiple-range-test analysis, except that the first significant rise was found at 7 days of treatment by the latter test.

17-Hydroxy steroid oxidoreductase activity (Fig. 2b) shows a rather complex development pattern, first increasing above control values 1 day after the start of treatment (significant only in multiple-range-test analysis, $P < 0.05$) and then falling below control values by 2 days after the start of treatment. This lower enzyme activity persists until at least 14 days after the start of treatment, but was only significant by *t*-test analysis 4 and 14 days after the start of the treatment.

The 6 β - and 16 α -hydroxylase activities (Figs. 2c and 2d) show a similar pattern to the 17-hydroxy steroid oxidoreductase activity, a small initial increase in activity after 1 day of treatment (the 6 β -hydroxylase activity not being significant) followed by a sustained decrease in activity down to female values in both cases. The patterns were similar whether analysed by the *t* test or multiple-range test. The 7 α -hydroxylase (Fig. 2e) does not change during the course of the treatment. The gradual change from

'male' to 'female' type metabolism seen in this study contrasts with the sharp change noted in the hypophysectomized females. The biphasic effect of oestrogens seen in this study could be due to an initial direct effect on the liver via an oestrogen receptor (Chamness *et al.*, 1975; Viladui *et al.*, 1975; Eisenfeld *et al.*, 1976), giving 'masculinization' of the hepatic androstenedione-metabolizing enzymes [such an effect has been reported previously (Gustafsson & Stenberg, 1976) when oestrogen, injected into hypophysectomized male animals bearing an ectopic pituitary gland, tended to elevate a number of the hydroxylase activities]. Subsequently, oestrogen affects the hypothalamo-pituitary system, causing release of 'feminizing factor', which changes the androstenedione metabolism to a typically 'female' pattern.

Kinetics

The changes in enzyme activity noted after various treatments, as discussed above, have their roots in changes in the kinetic parameters of the enzymes involved. Since, however, the enzymes under investigation are membrane-bound, the enzyme-kinetic data obtained measure the overall effects of the treatment on the interaction *in vitro* between androstenedione and the microsomal fraction, and not necessarily any effect on the enzyme itself. With these reservations in mind, however, a number of tentative proposals can be made from the results obtained (see Table 1). For instance, the 5 α -reductase, with an apparent K_m (30–110 μM) well below the substrate concentration used in the standard incubations (580 μM), shows V_{max} changes similar to the changes seen in the standard incubations: low V_{max} in the normal males and hypophysectomized males and females and high in normal females and oestrogen-treated males. The 16 α -hydroxylase shows a similar apparent V_{max} in both normal and hypophysectomized males (2.0–2.2 nmol of product/min per mg of protein). The apparent K_m and V_{max} of the 6 β -hydroxylase are similar in normal and hypophysectomized male animals. Oestrogen treatment of intact male animals, however, lowers the apparent K_m and V_{max} markedly, to typical female values. Hypophysectomy of female animals increases the apparent K_m and V_{max} , but not to male values, indicating that the pituitary gland is involved in the regulation of the 6 β -hydroxylase but is not the sole regulator. Although the 7 α -hydroxylase activity does not change markedly after hypophysectomy or oestrogen treatment (indicating no change in the V_{max} of the enzyme), some changes in apparent K_m are indicated. All groups, except the normal male, have an apparent K_m between 5 and 10 μM , whereas the male has a much lower apparent K_m of 1.3 μM .

Fig. 2. Time-related development of the hepatic steroid metabolism in male rats during oestrogen treatment. Treatment was begun on day 0; values for enzyme activities on day 0 are for normal animals. For further explanation, see legend to Fig. 1. The equivalent enzyme activities for the hypophysectomized male animals 1 and 14 days after the operation were as follows: after 1 day, (a) 4.5 ± 0.5 ; (b) 1.42 ± 0.20 ; (c) 1.51 ± 0.30 ; (d) 1.65 ± 0.22 ; (e) 0.38 ± 0.08 ; after 14 days, (a) 3.6 ± 1.0 ; (b) 1.59 ± 0.20 ; (c) 1.53 ± 0.40 ; (d) 1.55 ± 0.20 ; (e) 0.43 ± 0.07 [none of the values were significantly different ($P < 0.05$) from the male controls].

This could indicate the requirement for androgen or a pituitary factor to maintain the low apparent K_m of this enzyme. These kinetic parameters are, however, uncertain, owing to the difficulties discussed

above, and cannot be used as the basis for any conclusions about the control of hepatic steroid metabolism.

The apparent K_m values noted in this study are in

Table 1. Measured kinetic parameters (apparent K_m and V_{max}) of the steroid-metabolizing enzymes in rat liver microsomal preparation after various treatments

Apparent K_m is expressed as μM and the V_{max} as nmol of product formed/min per mg of protein. —, Not detectable. The correlation coefficient of the curves was between 0.8 and 0.98 in all cases.

Enzyme	Group parameter	Normal		Hypophysectomized		Oestradiol benzoate-treated male
		Male	Female	Male	Female	
5 α -Reductase	V_{max}	4.2	33.3	5.3	1.3	21.0
	K_m	81	51	110	30	48
16 α -Hydroxylase	V_{max}	2.2	—	2.0	0.26	—
	K_m	100	—	30	28	—
6 β -Hydroxylase	V_{max}	2.5	0.41	4.9	0.93	0.45
	K_m	150	21	140	50	5.2
7 α -Hydroxylase	V_{max}	0.49	0.58	0.9	0.27	0.52
	K_m	1.3	10	7.5	5.2	6.1

Table 2. Serum concentrations of pituitary hormones

Concentrations are given in μg of NIAMDD-RP-1 standard per litre of serum (mean $\pm$ 1 S.D.; $n = 4$). * $P < 0.05$, ** $P < 0.001$ (compared with respective control values).

Time after start of treatment (days)	Group	Prolactin	Somatotropin
1	Male	22 $\pm$ 8	7 $\pm$ 3
	Female	6 $\pm$ 4	4 $\pm$ 2
	Hypophysectomized male	<2.5	<2.5
	Hypophysectomized female	<2.5	<2.5
	Oestradiol benzoate-treated male	3 $\pm$ 2	34 $\pm$ 14*
2	Hypophysectomized female	<2.5	<2.5
	Oestradiol benzoate-treated male	13 $\pm$ 11	250 $\pm$ 51**
4	Hypophysectomized female	<2.5	<2.5
	Oestradiol benzoate-treated male	22 $\pm$ 2	96 $\pm$ 8**
7	Hypophysectomized female	<2.5	<2.5
	Oestradiol benzoate-treated male	21 $\pm$ 5	72 $\pm$ 30*
14	Hypophysectomized female	<2.5	<2.5
	Hypophysectomized male	<2.5	<2.5
	Oestradiol benzoate-treated male	31 $\pm$ 17	125 $\pm$ 52*

general lower than the corresponding values for drug-metabolizing enzymes reported previously [300–1400 μM for ethylmorphine *N*-demethylase (Castro & Gillette, 1967); 250–950 μM for ethylmorphine *N*-demethylase (Chung *et al.*, 1975)]. This would indicate that the steroids are the natural substrates for these enzymes, rather than the drugs tested above.

Serum concentration of pituitary hormones

The serum concentrations of immunologically active prolactin and somatotropin measured in the various groups after different times of treatment are shown in Table 2. Somatotropin concentrations in normal male and female animals were similar, whereas males exhibited a significantly ($P < 0.05$) higher prolactin concentration than females. Hypophysectomy of male and female animals decreased the concentration of pituitary hormones below the

limit of detection within 1 day of the operation, whereas oestrogen treatment of male animals decreased prolactin ($P < 0.05$) and increased somatotropin ($P < 0.05$) in serum. Continued treatment of the males with oestrogen increased prolactin steadily over the 14-day period, whereas somatotropin increased dramatically over the first 2 days (>200 μg /litre) and subsequently decreased to approx. 60–100 μg /litre for the rest of the period studied.

The marked increase in concentration of immunoreactive somatotropin in serum and the unchanged concentrations of prolactin (except after 1 day of treatment) after oestrogen treatment of male rats contrasts with earlier studies (e.g. Ajika *et al.*, 1972). Different experimental design and time of sampling could, however, explain the apparent discrepancies.

Many pituitary hormones, including somatotropin, have been demonstrated to have an effect on hepatic metabolism of steroids and drugs (Shirasu *et al.*, 1967; Wilson, 1970; Kramer & Colby, 1976),

corticotropin (Colby *et al.*, 1974), prolactin (Lax *et al.*, 1976) and the gonadotropins (Gustafsson & Stenberg, 1975; Kramer *et al.*, 1977). None of the hormones, however, show the same effect as transplantation of a pituitary under the kidney capsule (Eneroth *et al.*, 1977) or oestrogen treatment of male rats (Einarsson *et al.*, 1974).

In the present study, a marked increase in somatotropin secretion seems to be correlated with the effects seen on hepatic steroid metabolism, but there is a temporal dissociation; somatotropin concentrations are high in serum after 1 day of treatment with oestradiol in the male and undetectable in females hypophysectomized 1 day previously, whereas effects on steroid metabolism take up to 14 days to appear. It is possible that the correlation between somatotropin and steroid metabolism is more coincidental than actual.

Further work is necessary to ascertain the mechanism of the differential control of the hepatic steroid-metabolizing enzymes and the postulated active 'masculinization' of the pattern of metabolism in hypophysectomized female animals.

I am grateful to the CIBA-GEIGY Fellowship Trust for financial support in the form of a fellowship. I thank Professor Jan-Åke Gustafsson and Professor Peter Eneroth for helpful discussions during this work. This study was supported by grants from the Swedish Medical Research Council (no. 03X-2819).

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