Testosterone conversion to androstenedione by liver homogenates of testicular feminized mice

Genotype	No. of incubations	Recovery of T (%)	Initial T converted to A (%)
BALB/C ♂	6	11.4±0.8	50.5±2.5
Tfm+ (o^+)+/*Y* ♀	4	6.1±0.5	47.5±1.0
Tfm + (o^{hv}) *Blo*/*Y* ♀	4	18.5±1.0	25.7±1.0

All differences are significant $p<0.005$ (Student's t-test). Duplicate incubations were done on liver homogenates of individual mice. *Tfm*$^+$ (o^+), usual testicular feminized mouse; *Tfm*$^+$ (o^{hv}), testicular feminized mouse with protective gene.
Results are expressed as percent per 10 mg tissue per 2 h (Mean+SE).

although these averaged about 30%. The total radioactivity derived from the specific activity of the final crystals and amount of carrier added, expressed as a percentage of radioactivity recovered from the incubations are shown in the Table. Metabolites other than A were not isolated in radiochemically pure form except for small amounts of 5α-androstane-3α,17β-diol (<1%) by livers of BALB/C mice. No estrogens were identified in any of these incubations.

The relationship between the differences in metabolism of T in the liver of testicular feminized mice and the primary genetic defect of *Tfm* remains to be established. However, the results of the present investigation suggest that the presence of the mutant gene (o^{hv}) may have some effect on steroid metabolism in liver homogenates. Liver slices of *Tfm* mice were also found to convert T to A[8]. The results quoted[8] are similar to ours in that no difference could be detected between normal males and *Tfm* animals. In the rat it has been demonstrated that neonatal 'imprinting' of hepatic steroid metabolism is sex-dependent and under hypothalamo-hypophyseal and testicular androgen control[9]. Neonatal androgen insensitivity in the mouse may therefore irreversibly alter adult hepatic metabolism. Further studies in this area are in progress.

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In vitro Effect of Dexamethasone in Conversion of 4-^{14}C Progesterone in the Fetal Rat Adrenal Glands

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Summary. It was found that dexamethasone in vitro inhibited the synthesis of corticosterone and aldosterone.

Fetal rat adrenals synthetise corticosteroids during the last 5 days of intra-uterine development[2–9]. Steroid production in the fetal adrenals is regulated by the fetal pituitary ACTH[7–12]. Administration of cortisone[9,14], of corticosterone[6–8] or dexamethasone[12,13] to pregnant rats produces fetal adrenal atrophy. This effect is mediated by the suppression of fetal pituitary adrenocorticotrophic activity[6–10]. However, the previous experiments have not excluded the possibility that glucocorticosteroids, particularly dexamethasone, directly affects fetal adrenal steroidogenesis. Therefore, in the present study, the conversion of 4-^{14}C progesterone by the fetal rat adrenals in vitro incubated with or without dexamethasone was analysed.

It was found that dexamethasone inhibited 11β-hydroxylation and conversion of progesterone to corticosterone in the fetal adrenal glands when it was added in the incubation medium in vitro.

Materials and methods. Pregnant rats of the Fischer strain were used. On the 22nd day of pregnancy the females were laporotomized, their fetuses were removed, the fetal adrenals dissected and cleaned of adhering tissue under the 10fold magnification of a stereomicroscope and weighed on an analytical balance. Each pair of fetal adrenal glands was incubated in 2 ml of Krebs-Ringer bicarbonate buffer supplemented with glucose (200 mg/100 ml) in the presence of 0.031 μCi of 4-^{14}C progesterone (s.a. 61.0 mCi/mmol; Amersham, Great Britain) without and with various concentrations of dexamethasone. Dexamethasone was dissolved in 0.1 ml of Krebs-Ringer bicarbonate buffer in concentration of 0.1–2.0 μg/ml incubation medium. The samples were incubated in a Dubnoff-type metabolic shaker in an 95% O_2–5% CO_2 atmosphere for 1 h at 37 °C. The media were extracted with 15 ml of a mixture of chloroform and methanol (2:1), evaporated in a stream of N_2. The extracted steroids were separated by two-dimensional chromatography on a thin layer of silica gel GF_{254} (Merck, Darmstadt, BRD) using dichlormethane-*n*-heptane-methanol (15:4:1 v/v/v) and benzene-acetone-*n*-heptane-dichlormethane (4:4:2:1 v/v/v/v) solvent systems. Corticosteroids were identified following acetylation and recrystallization to a constant specific activity, as described previously[15]. Radioactivity was measured in a Mark II Nuclear Chicago liquid scintillation counter. All the values have been corrected for

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Corticosteroid synthesis by the adrenal glands of 22-day-old fetuses incubated with 4-^{14}C progesterone and dexamethasone for 1 h (dpm/mg)

Steroids	Controls	Dexamethasone µg/ml incubation medium 0.1	0.2	0.5	1.0	2.0
Aldosterone	47.4[a] ±5.7	36.0 ±1.0	31.6 ±0.4	18.5[b] ±1.4	17.1[b] ±5.1	9.6[b] ±0.1
18-Hydroxy-11-deoxycorticosterone	403.7 ±15.3	424.8 ±2.7	428.5 ±6.5	466.1 ±17.9	503.0 ±15.0	544.0[c] ±33.0
Corticosterone	334.1 ±8.9	273.7 ±20.5	264.4 ±5.0	261.2[c] ±8.8	233.7[b] ±11.8	231.0[b] ±9.8
11-Deoxycorticosterone	196.0 ±21.0	224.4 ±12.2	269.9 ±37.1	291.5 ±1.4	236.0 ±17.0	233.0 ±45.0
Progesterone (residual substrate)	21,902.1 ±982.1	22,025.5 ±376.0	21,891.2 ±899.4	22,742.1 ±114.4	22,202.3 ±237.8	21,081.8 ±498.3

Values are mean ± S. E. [a] 4 fetuses per sample; [b] $p < 0.01$ (vs control); [c] $p < 0.05$ (vs control).

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procedural losses from the recovery at the last stage of purification of non-radioactive standards added immediately after incubation. The results obtained were statistically evaluated by the analysis of variance. In the case of homogeneity of variances, Student's *t*-test was used, and Kramer's test when the variances were not homogeneous.

Results and discussion. The results indicate that fetal adrenal glands in vitro hidroxylated 4-^{14}C progesterone in 11-deoxycorticosterone, 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) corticosterone and aldosterone. The major steroid produced was 18-OH-DOC. The adrenal glands of fetuses incubated with 0.5–2.0 µg dexamethasone/ml incubation medium synthetized less corticosterone and aldosterone than the adrenal glands of control fetuses. However, with higher concentrations of dexamethasone the conversion of 4-^{14}C progesterone to 18-OH-DOC was increased.

It is known that dexamethasone inhibits the fetal adrenals by decreasing fetal ACTH activity[12,13]. This experiment demonstrated in vitro inhibition of 11β-hydroxylation of progesterone to corticosterone by the high concentrations of dexamethasone. Therefore the results indicate that dexamethasone may affect steroidogenesis in the fetal adrenal gland directly, as well as its already known inhibitory effect mediated by the fetal pituitary[12,13].

RNA Biosynthesis in Isolated Prothoracic Glands of *Tenebrio molitor* in vitro

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Summary. The prothoracic Glands of *Tenebrio molitor* synthesize in vitro mainly rRNA. The rate of RNA synthesis reaches a maximum at day 10 of the last larval instar, which coincides with the event of apolysis.

The prothoracic glands (PGs), the sites of biosynthesis of the moulting hormone α-ecdysone[2–4] in insects, undergo periodic morphological changes during growth and metamorphosis which can be correlated with their secretory activities. Prior to each secretory activity, an increase in nuclear and nucleolar volume is observed[5] which is followed by high cytoplasmic vacuolization, abundant appearance of rough endoplasmic reticulum and Golgi areas[6]. The activation is brought about by neurohormones from the brain[7]; this stimulation can be demonstrated by an increased ^{3}H-uridine incorporation into RNA[8,9], which was also demonstrated after addition of brain hormone extracts to prothoracic glands in vitro[10]. Since the activated glands retain their ability to synthesize α-ecdysone both after transplantation[11] and in vitro[2–4], it seemed reasonable to assume that they would also be able to maintain their RNA biosynthetic

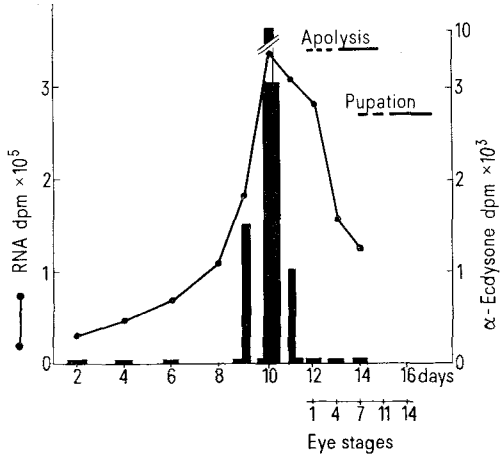

Fig. 1. RNA and α-ecdysone biosynthesis of PGs in vitro: total RNA synthesis of 10 gland pairs in 24 h (– ● – ● –), mean values of 2 independant series; α-ecdysone synthesis: each bar represents a single incubation. Eye stages are given according to [14].

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