effect (Figure 3B) but abolished the effect of corticotropin$_{1-24}$ (Figure 3A). [Gly^1]Corticotropin$_{1-18}$amide showed a similar effect on the digested membranes to corticotropin$_{1-24}$.

Discussion. The active site of ACTH was postulared to be located in the NH_2 terminal region (positions 1–13) and the sequence Lys Lys Arg Arg (positions 15–18) was thought to be a likely binding site[9]. The present investigation shows that ACTH analogs bearing binding sequences increased the fluorescence of ANS-membrane complexes, but those without binding sequences did not. This result suggests that the ACTH fragments with binding sequences increased the membrane charge, and therefore enhanced the binding of anionic ANS. The basic amino acids composing binding sequences probably caused the increase of the net positive charge of membranes. The presence of the active site was not requisite for the fluorescent effect. Inability of pig ACTH to increase the ANS fluorescence may be different from that of shorter ACTH fragments which lack binding sequences. A possible explanation is that pig ACTH does not confer enough positive charge on the membranes to increase the bound ANS, owing to the acidic amino acids in the part of the peptide chain over the 25th amino acid.

The augmentation of ANS fluorescence bound to plasma membranes by corticotropin$_{1-24}$ or [Gly^1]corticotropin$_{1-18}$amide was not specific for adrenal cortex, since both corticotropin fragments increased the ANS fluorescence bound to bovine thyroid membranes or human erythrocyte ghosts which do not carry ACTH receptors (unpublished observation). This suggests that the changes of ANS fluorescence by the two ACTH fragments do not show the conformational changes of ACTH receptors, but indicate a rather nonspecific change of the charge of membranes.

Calcium ion is claimed to increase the fluorescence of ANS-membrane complexes through shielding the phospholipid phosphate group[2,8]. The mechanism of ACTH effect seems different, since reduction of phospholipid phosphorus content in the membranes did not affect the fluorescence effect of ACTH fragments, while it decreased the effect of calcium ion. Inability of ACTH fragments to increase the fluorescence of ANS bound to the pronase-treated membranes may suggest that ACTH fragments interact with the pronase-sensitive protein of adrenocortical membranes. On the contrary, membrane protein did not contribute the fluorescence effect of calcium ion so much.

Although the fluorescence effect of ACTH fragments was not related to their binding on ACTH receptors, the present study suggests the possibility that the synthetic, basic ACTH analogs may have a different pharmacological action from that of the natural ACTH by changing the electrostatic environment of the ACTH receptors.

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Conversion of Testosterone to Androstenedione by Liver Homogenates of Testicular Feminized Mice[1]

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Summary. Liver homogenates of testicular feminized (*Tfm*) mice carrying the protective (o^{hv}) gene were found to be less capable of converting testosterone to androstenedione than Tfm without the protective gene.

Testicular feminization in the mouse is genetically determined by the presence of a mutant gene (*Tfm*) on the *X*-chromosome[3]. Recently, a strain of mice has been developed with a further mutant gene (o^{hv}) on the *X*-chromosome, the position of which enables it to modify the expression of *Tfm* so as to restore a measure of androgen sensitivity to some of the target organs[4]. The relationship between the androgen receptor defect[5] and altered steroid metabolism in adult testicular feminized mice[6] is not clear. Differences in the metabolism of androstenedione (A) have been observed in the liver of the Stanley- Gumbreck male pseudohermaphrodite (PS) rat and found to be similar to that of females[7]. Here we report differences in the liver conversion of testosterone (T) to A in two strains of testicular feminized mice.

Mice of genotypes *Tfm*+(o^+)+/*Y*♀, *Tfm*+(o^{hv})*Blo*/*Y*♀, maintained at City of Hope Medical Center, California and normal male BALB/C mice (Health Research Inc.) used in this study ranged in ages from 53 to 190 days. Mice were anesthetized and liver tissue excised, weighed and homogenized in Medium 199 (Gibco). Co-factors were not added in order to simulate in vivo conditions as close as possible. Crude homogenates (1 ml, 11–14 mg wet wt. tissue) were incubated in 5 ml medium 199 with 0.4 μCi [7α-^{3}H] testosterone (25 Ci/m*M*; New England Nuclear) dissolved in propylene glycol, for 2 h in a Dubnoff metabolic shaker at 37 °C with air as the gas phase. Incubations were terminated by quick freezing. Medium was extracted with diethyl ether and the ether extract partitioned between toluene and 1 *N* NaOH. The neutral fraction was subjected to thin layer chromatography in chloroform: methanol (98:2) at 4 °C. Radioactive zones corresponding to T and A were eluted and rechromatographed on Whatman No. 1 paper in heptane:methanol: water (5:4:1) and then crystallized with carrier to constant specific activity. No correction for losses were made

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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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