the dissection microscope. Inhibition was complete even for tests in the presence of colchicine ($10^{-5}M$), which has been reported[8] to enhance in vitro ovulation in this species. Cytochalasin B thus clearly inhibits amphibian ovulation in vitro.

From the Table it is also evident that although ovulation may be blocked, germinal vesicle breakdown occurs in a significant number of oocytes. (The apparent increase could be due to a greater penetration of the effective hormone in the presence of dimethyl sulfoxide, but this aspect was not pursued). It had been observed earlier that ovulation and germinal vesicle breakdown occur to different and variable extents in a given fragment of hormone-stimulated ovary. A careful analysis of the responses to different hormones[9] indicated that the two responses were independent. This conclusion is confirmed by the present observation, which represents a selective inhibition of the ovulatory response. Germinal vesicle breakdown is clearly not hindered by the prevention of ovulation.

The present study indicates that spawning in an echinoderm, and ovulation in a primitive vertebrate are similar in that each involves a non-muscular contractile system, the formation of functioning of which is initiated by hormones which usually (except in some fish)[10] arise in the ovary.

It should be noted that in this study, actual movements of frog ovarian follicle cells were not observed in control tissue; their occurrence is inferred by analogy with the observed cell movements in the starfish. Cytochalasin B inhibition of spawning in the intact starfish has also not yet been examined (the experiment is impractical at present), but is inferred from the behavior and response of the starfish follicle cells and the fact that 1-methyl adenine stimulates spawining in intact starfish. The fact that the two processes which have actually been observed (cell-movement in the starfish; ovulation in amphibians) are sensitive to cytochalasin B supports the arguement that cell movements in starfish are significant in spawning on the one hand; and that active follicle cell movement is significant in amphibian ovulation on the other.

Ultrastructual studies are required to confirm the analogies drawn above. The basis for the inhibition of intracellular movements by cytochalasin B remains unclear[11], although in many systems cytochalasin inhibition of cell movement is accompanied by a disturbance of the normal morphology of intracellular systems of 50–70 Å microfilaments[4]. It is now apparent that not all microfilaments are sensitive to cytochalasin[12]; but a study of the distribution of cytochalasin-sensitive microfilaments within the ovarian follicles of starfish and frogs should help to identify the amphibian cells whose movements are responsible for ovulation, whatever mode of action may ultimately be demonstrated for the drug.

Zusammenfassung. Cytochalasin B (5 µg/ml) verhindert in vitro vollständig die in *Rana pipiens* und *Hyla regilla* durch Gonadotropinbehandlung stimulierte Ovulation, während die Meiose weiter abläuft. Diese Resultate werden mit früheren Beobachtungen einer ähnlichen Empfindlichkeit der Follikelzellenbewegung bei Seesternen verglichen. Ein für Echinodermen und niedere Wirbeltiere gemeinsamer Follikelmechanismus für die Austreibung des Ovarialeies aus dem Ovar wird vorgeschlagen.

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9α-Fluoro-11β-Hydroxybenzo[d,e]Testosterone 17-Acetate. A Modified Steroid Highly Active on DMBA-Induced Mammary Tumors in Rats

Some years ago we reported the synthesis[1] and the interesting biological properties[2] of benzo[d, e]testosterone acetate (BTA). Now we wish to report on a related new compound, 9α-fluoro-11β, hydroxybenzo[d, e]testosterone 17-acetate[3] (FBTA, IV) endowed with high antitumor activity.

Reaction of 9α-fluoro-11β, 17β-dihydroxy-4-androsten-3-one 17-acetate[4] (I) with triethylorthoformate in the presence of p. toluene-sulfonic acid gave the ethyl enol ether II, m.p. 178–184 °C[5], $[a]_D$ −124°; λ_{max} nm (ε) 239–240 (21,195); ν_{max} 3560, 1736, 1623, 1244, 1172, 1045, 851 cm^{-1}. Anal. Calcd. for $C_{23}H_{33}FO_4$: C, 70.38; H, 8.47; found: C, 70.52; H, 8.41. Pyridine tosylate catalyzed exchange etherification of II with propargyl alcohol in benzene afforded III (m.p. 170–172°, $[\alpha]_D$ −102°; λ_{max} nm (ε) 238 (20,325); ν_{max} 3490, 3300, 1705, 1662, 1631, 1245, 1156, 1024, 852 cm^{-1}. Anal. Calcd. for $C_{24}H_{31}FO_4$: C, 71.76; H, 7.76; found: C, 71.75; H, 7.74) which was converted, by refluxing for 3 h in pyridine and in the presence of 10% Pd/C, into FBTA (IV, m.p. 200–202°, $[\alpha]_D$ −25°; λ_{max} nm (ε) 209–211 (17, 300), 255–256 (10, 300), 300–302 (1,830; ν_{max} 3500, 1722, 1677, 1587, 1555 (w), 1506 (w), 1256, 1047, 781, 752 cm^{-1}. Anal. Calcd. for $C_{24}H_{29}FO_4$; C, 71.97; H, 7.30; found: C, 71.75; H, 7.15).

FBTA was compared with testosterone propionate (TP) and with 19-nortestosterone phenylpropionate (NTPh), a weak androgen, in the androgenic and myotrophic test performed on castrated male rats[6]. With 2 mg (5 µMoles) of FBTA, the weigths of the prostate, seminal vesicles and levator ani plateaued at values which were remarkably less than those seen at equimolar doses of NTPh and comparable with those obtained with doses of TP lower than 0.01 mg. The antigonadotrophic test in

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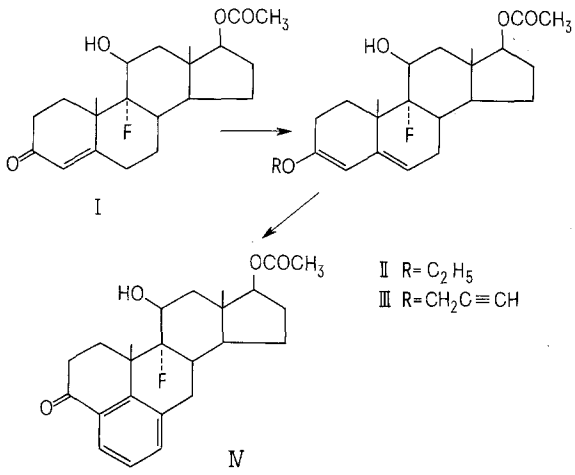
parabiotic rats[7] revealed that FBTA produces the same effect as TP with doses 20 times greater, yet scarcely androgenic. Also, 10 mg of FBTA proved to be less uterotrophic than 0.4 μg of estrone and as anti-uterotrophic as 20 mg of TP in the mouse test[8].

Tested in rats bearing hormone-dependent mammary tumors induced by 7,12-dimethylbenz[a]anthracene (DMBA)[9], FBTA after 30 days of s.c. treatment at daily doses of 2 and 4 mg decreased the death rate from 50% of the controls to 11 and 25% respectively. Tumor regression, evaluated as the ratio between the average areas of treated and untreated control tumors, was 0.05 and 0.13, respectively. The lack of a dose-related effect is not explainable; this observation has been frequently reported with other compounds used in anti-tumor screening[10,11]. The anti-tumor effect was still evident 30 days after discontinuing treatment.

FBTA is biologically very similar to its analog, BTA, except for a higher antigonadotrophic and anti-tumor potency at low doses. The hypothesis is proposed that the antigonadotrophic and anti-estrogenic activities make FBTA a very effective inhibitor of experimental hormone-dependent mammary tumors of rats.



Riassunto. È stato preparato un nuovo analogo pentaciclico del testosterone e ne sono state saggiate le proprietà ormonali e l'attività nel test del tumore mammario indotto dal DMBA nel ratto. Il conposto ha dimostrato un'elevata attività antitumorale e una scarsa attività androgena.

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Morphologic Sex Differences in Primate Brain Areas Involved in Regulation of Reproductive Activity

The major region regulating sexual behavior and secretion of gonadotrophins in mammals is the hypothalamus[1]. However, an equally important area in this control is the 'limbic system', an area mediating the connections between the old an new brain structures. The amygdala plays a particularly important role in the regulation or modification of both sexual behavior[2] and gonadal function[3]. RNA metabolism in both the hypothalamus and amygdala shows marked alterations in response to neonatal androgen administration as compared to other parts of the brain[4]. To determine whether morphological sex differences could be found in these areas in the primate brain, nuclear size measurements were performed on neurons in the nucleus medialis amygdalae (NMA), the suprachiasmatic nucleus (SCH), medial preoptic area (MPOA), arcuate nucleus (ARN) and cerebral cortex (CC) in normal male and ovariectomized female squirrel monkeys on replacement estrogen/progesterone therapy.

Material and methods. Five mature females were treated for 10 days (beginning 1 week after ovariectomy) with an i.m. dose of 2.5 mg of estradiol benzoate and 12.5 mg of progesterone[5]. These females and 5 normal males were left undisturbed for 17 h prior to sacrifice. To avoid an alteration in nuclear size due to a stress response[6], the animals were quickly heparinized and then decapitated in less than 1 min after the room was entered. Brain perfusion with saline through the carotid artery and later fixation with Bouin's solution was finished in 5 to 6 min after death in order to protect against nuclear changes due to hypoxia[7]. The brains were embedded in paraffin and for measurement of the cell nucleus diameter in Hematoxylin-Eosin slides a modified Szenthagothai et al method was used[8]. In each described area, 200 cell nuclei were measured.

Results. A marked sex difference was found in the nuclear diameter of neurons in the NMA. The mean diameter of the cell nuclei in the NMA in the female group was 8.74 μm ± S.E. 0.028 and was significantly smaller ($P \leq 0.01$) than in the male group (Figure 2) which averaged 9.57 μm ± 0.054. The SCH area together with the neighboring MPOA are known to be involved in the

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