important contributions in the field of reproductive physiology of mosquitoes. The observations made in the course of this study suggest that events occurring between the ingestion of a blood meal and oviposition involve the activity of the CA and the MNSC of the pars intercerbralis. There is evidence that the CA are responsible for maturation of eggs and the secretion of the MNSC for oviposition. The two hormones are not present in the body systems in sufficient quantities at the same time. It appears that the presence of one in sufficient quantity antagonizes the elaboration of the other. The details of the evidence and the pathway linking the blood meal and ovulation will be published elsewhere.

Zusammenfassung. Begattete weibliche *Culex fatigans* legen ihre Eier 72 h nach einem Blutmahl, wenn die Aktivität der medianen neurosekretorischen (MNSC) Zellen am höchsten ist. Die Corpora allata (CA) erreichen 24 h nach dieser Mahlzeit ihre höchste Aktivität und es wird daraus geschlossen, dass das Hormon der CA Eireifung und die Sekrete der MNSC die Eiablage bewirken.

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The Inhibition of Amphibian Ovulation in vitro by Cytochalasin B[1]

Follicle cells associated with isolated oocytes of the starfish *Patria* respond to in vitro administration of the starfish ovarian hormone 1-methyl adenine by moving away from the surface of the oocyte[2]. The movement of these cells is inhibited in vitro by the mold metabolite cytochasasin B[3], which has been shown to interfere with a number of non-muscular cellular movements and intracellular shape changes[4,5]. Since 1-methyl adenine is known to stimulate spawning is intact starfish[7] it was suggested[3] that this active follicle cell movement played an important part in the spawning process.

A number of similarities exist between starfish and amphibians with respect to both the endocrine control of female reproduction and the histological structure of the ovary[2,6]. This suggests that if amphibian ovulation were mediated by similar follicle cell movements, the process should also be sensitive to cytochalasin B.

To examine this hypothesis, ovulation was observed in fragments of ovaries from mature female *Rana pipiens* (commercially obtained) or *Hyla regilla* (field collected), each containing 30–50 full-grown oocytes. The ovarian fragments were incubated at room temperature (20–23 °C) on a slowly rotating gyratory shaker in a total volume of 5 ml of amphibian Ringer's solution. Suspensions of homogenized *R. pipiens* pituitary (1/20 of a pituitary/ml) were used to stimulate ovulation. The degree of ovulation was observed after 24 or 48 h of incubation by counting both loose oocytes and constricted oocytes in the process of leaving the ovary. Germinal vesicle breakdown was determined by boiling the eggs at the end of the incubation period and dissecting them induvidually under a dissection microscope. Cytochalasin B (a generous gift of S. B. CARTER, Imperial Chemical Industries, Ltd.) was dissolved in 100 %dimethyl sulfoxide (DMSO) and used at a final concentration of 5 μg/ml in 1% DMSO.

Successful ovulation was obtained in fragments of ovaries from 10 specimens of *Rana* (Table) and 3 of *Hyla*. In each case in which significant ovulation was obtained in controls, inhibition by cytochalasin was complete or nearly so. The carrier (1% DMSO) had no effect on ovulation, and the presence of cytochalasin resulted in no gross morphological changes that could be observed under

[1] Supported by funds provided for biological and medical research by State of Washington Initiative Measure No. 171. The competent assistance of P. PENDERGRASS and M. VANDENBARK is gratefully acknowledged.

[2] H. KANATANI, Gen. comp. Endocrin., Suppl. *2*, 582 (1969).

[3] P. C. SCHROEDER, Naturwissenschaften *58*, 270 (1971).

[4] N. K. WESSELS, B. S. SPOONER, J. F. ASH, M. O. BRADLEY, M. A. LUDUEÑA, E. L. TAYLOR, J. T. WRENN and K. M. YAMADA, Science *171*, 135 (1971).

[5] J. MCGUIRE and G. MOELLMANN, Science *175*, 642 (1972).

[6] J. W. ANDERSON and M. B. YATVIN. J. Cell Biol. *46*, 491 (1970).

Effect of cytochalasin B on frog ovarian responses to gonadotropins in vitro

	Ovulation (%)						Germinal vesicle breakdown (%)		
Animal No.	PIT only	PIT + DMSO + CCB	PIT + DMSO	PIT + COL	PIT + COL + DMSO	PIT + COL + DMSO + CCB	PIT only	PIT + DMSO + CCB	PIT + DMSO
1	97 (79)	4 (47)	—	—	—	—	64 (73)	90 (62)	—
2	25 (40)	0 (41)	39 (62)	—	—	—	—	—	—
3	28 (32)	0 (28)	90 (31)	—	—	—	—	—	—
4	86 (26)	0 (34)	63 (38)	—	—	—	19 (37)	94 (34)	57 (35)
5	85 (20)	0 (27)	90 (40)	—	—	—	67 (15)	67 (27)	97 (34)
6	36 (44)	3 (35)	37 (38)	—	—	—	56 (43)	80 (35)	51 (39)
7	86 (29)	0 (25)	—	13 (25)	18 (31)	0 (29)	—	—	—
8	72 (36)	0 (36)	—	87 (30)	58 (45)	0 (56)	—	—	—
9	73 (44)	0 (45)	—	97 (45)	91 (33)	0 (56)	—	—	—
10	64 (44)	0 (37)	—	41 (59)	28 (39)	0 (43)	—	—	—

Numbers in parentheses are the number of oocytes examined to determine the percentage given; abbreviations: CCB, cytochalasin B, 5μg/ml; DMSO, 1% dimethyl sulfoxide; PIT, 1/20 *Rana pipiens* pituitary/ml; COL, $10^{-5}M$ colchicine.

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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[7] H. KANATANI and H. SHIRAI, Devel Growth, Differ. *12*, 119 (1970).
[8] P. A. WRIGHT, Gen. comp. Endocrinol. *2*, 389 (1962).
[9] S. SUBTELNY, L. D. SMITH and R. E. ECKER, J. exp. Zool. *168*, 1 (1968).
[10] S. V. GOSWAMI and B. I. SUNDARAJAJ, J. exp. Zool. *178*, 467 (1971).
[11] S. B. CARTER, Endeavour *31*, 77 (1972).
[12] R. D. GOLDMAN, J. Cell Biol. *52*, 246 (1972).

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

[1] R. GARDI, R. VITALI and P. P. CASTELLI, Tetrahedron Lett. *27*, 3203 (1966).
[2] G. BRIZIARELLI, Endocrinology *81*, 390 (1967).
[3] Systematic name: 2, 3, 3a, 4, 5, 5a, 5b, 6, 7, 12, 12a, 12b-dodecahydro-5aα-fluoro-3β, 5β-dihydroxy-3aβ, 5bβ-dimethylbenzo[f, g]-cyclopent[a]anthracen-8(1H)-one 3-acetate.
[4] G. H. THOMAS and J. FRIED, U.S. Patent No. 3,001,990 (September 26, 1961); Chem. Abstr. *56*, 2485 (1962).
[5] Melting points are uncorrected. Optical rotations were taken in 1% dioxane solution at 24° ± 1. UV-spectra were determined in EtOH with an Optica CF_4 spectrophotometer. IR-spectra (w, weak) were measured in Nujol with a Perkin-Elmer instrument. We are indebted to Dr. C. PEDRALI for the spectral determinations.
[6] L. G. HERSHBERGER, E. G. SHIPLEY and R. K. MEYER, Proc. Soc. exp. Biol. Med. *83*, 175 (1953).