The secretion of 20α OH-progesterone diminished progressively from a maximum of 10.7 µg/h between 03.00 h and 05.00 h to a minimum of 3.4 µg/h between 21.00 and 23.00 h. The reductions recorded between 15.00 and 17.00 h (4.0 µg/h) and 21.00 and 23.00 h are significant. Only in the blood samples obtained between 03.00 and 05.00 h was the ratio of progesterone to 20α OH-progesterone less than unity; in all the other samples it was greater.

The weight of the ovary decreased markedly between 05.00 and 09.00 h and remained at the minimum level until 23.00 h (Table).

There appeared to be an increase in the weight of the corpora lutea between 09.00 and 11.00 h, coinciding with the maximum blood levels of progesterone.

Discussion. The basal rates of progesterone and 20α OH-progesterone secretion determined in this study on the 7th day of pseudogestation correspond to those published by FAJER and BARRACLOUGH[1]. Under the same conditions, that is to say in the rat on the same day of pseudogestation (but daily light from 05.00 to 17.00 h), FREEMAN and NEILL[3] demonstrated that the secretion of prolactin is at its greatest between 03.00 and 05.00 h; the release of progesterone by the ovary observed in our experiments (light from 06.00 to 20.00 h) took place 6 h later. It is possible that under our lighting conditions the prolactin peak has been somewhat shifted. It may be assumed that the inverse relation between the rising levels of progesterone and the declining 20α OH-progesterone levels is a result of the liberation of prolactin, since WIEST et al.[6] have shown that prolactin inhibits 20α OH-progesterone dehydrogenase, while according to PUPKIN et al.[7] this enzyme occurs principally in the corpora lutea. Moreover, the variations noted in progesterone: 20α OH-progesterone ratio would seem to be indicative not only of an increase in the de novo synthesis of progesterone but also of a reduction in its rate of conversion to 20α OH-progesterone. These results would then confirm BLENCOE and MOODY's[8] observation that prolactin increases the production of progesterone and causes a reduction in the weight of the ovaries. The variations in the progesterone: 20α OH-progesterone ratio which we noted during the 7th day of pseudogestation could hence be induced by the nocturnal peak of prolactin release. Since this peak has been shown to occur regularly each night throughout pseudogestation[3], it seems reasonable to suppose that peak levels of progesterone also occur daily until pseudogestation ceases.

A circadian rhythm is likewise evident in the weight of the ovaries and the level of 20α OH-progesterone in the ovarian blood. The hormonal conditions governing this phenomenon remain to be elucidated.

Résumé. Par des canulations toutes les 6 heures chez des rattes le 7ème jour de la pseudogestation nous avons pu mettre en évidence un pic de progestérone entre 09.00 et 11.00. Sur la base de nos résultats (progestérone, 20α-OH-progestérone, poids des ovaires et corps jaune) et de la publication de FREEMAN et NEILL[3] nous discutons le rôle de la LTH comme régulation possible de ce phénomène.

P. BISCHOF, C. KRÄHENBÜHL and P. A. DESAULLES[9]

Biological Research Laboratories of the Pharmaceutical Division of CIBA-GEIGY Limited, CH-4002 Basel (Switzerland), 9 November 1972.

[6] W. G. WIEST, W. R. KIDWELL and K. BALOGH JR., Endocrinology *82*, 844 (1968).
[7] M. PUPKIN, H. BRATT, J. WEISZ, C. W. LLOYD and K. BALOGH, Endocrinology *79*, 316 (1966).
[8] BLENCOE and MOODY, cited by W. HANSEL in discussion of J. Reprod. Fertil. Suppl. *7*, 17 (1965).
[9] Acknowledgements. The authors wish to express their appreciation to Dr. L. SCHENKEL-HULLIGER for her helpful advice during this project.

The Role of Nutrition and Endocrine Activity in the Development of Eggs in *Culex fatigans*

A study of the role of nutrition and endocrine activity in the development of eggs of the common Indian mosquito *Culex fatigans* has produced a number of interesting observations. 1. Laboratory-bred female mosquitoes or unmated females obtained from field do not mate in captivity or feed on blood. Mating is essential for the female mosquito to take a blood meal, which is taken only once in the course of a reproductive cycle. Each successive cycle of reproduction must be preceded by ingestion of a blood meal. 2. Both sugar and blood meal cause an increase in the number of the median neurosecretory cells (MNSC). The density of MNSC is at its highest level at 27 h after a blood meal (250% of initial number) when the insect is ready to deposit eggs. The normal MNSC number is restored by 24 h after oviposition. 3. The MNSC of the pars intercerebralis have a major contribution towards the physiological events occurring during the periods of egg maturation and oviposition. However, the MNSC do not show any activity if the mosquito is fed on sugar only, but they show an increase in size in blood-fed mosquitoes with maximum size attained at 72 h after blood meal, at which time fully developed eggs are ready for liberation. 4. The MNSC of the pars intercerebralis are seen to be fully loaded with secretory material up to 48 h after a blood meal. Their maximum size at 72 h after a blood meal and their reduction in size immediately after oviposition clearly indicates that their secretion is released only few hours before oviposition. 5. Starvation and sugar meals fail to induce any activity in the corpora allata (CA), but 24 h after a blood meal, the CA reach their maximal activity, show a sharp decrease at 48 h and thereafter tend to resume the original state of inactivity. 6. Sugar meals do not initiate any development of eggs but ingestion of a blood meal results in an increase in the size of the terminal eggs in a geometrical progression reaching its maximum at 72 h (increase in egg length is 730%). 7. Though the CA activity is very much reduced by 48 h following a blood meal, the eggs continue to grow to their maximum size upto 72 h.

Culex fatigans is an anautogenous species and therefore it does not lay eggs unless allowed to ingest blood, which provides the necessary stimuli for the activation of the endocrine glands and development of oocytes. Several species of mosquitoes of the genera *Aedes*, *Anopheles* and *Culex* have been investigated to establish beyond doubt that egg development is controlled and regulated by hormones. Many earlier workers[1-4] have made very

[1] T. S. DETINOVA, Zool. Zh. *24*, 291 (1945).
[2] A. N. CLEMENTS, J. exp. Biol. *33*, 211 (1956).
[3] D. BODENSTEIN, J. exp. Zool. *140*, 343 (1959).
[4] A. O. LEA, J. Insect Physiol. *9*, 793 (1963).

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.

SHEELA BANERJEE, S. L. SAHNI and D. P. SINHA

Science College, Department of Zoology, Patna–5 (India), 11 November 1971.

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.