that of the female, probably due to an antagonism to the estrogens secreted by the graft. Progesterone had the ability to facilitate ovulation in the adult rat[8] and in the androgeinized rat when it was submitted to electrical stimulation of the hypothalamus[9,10]. However, in the androgeinized rat, the sole administration of progesterone did not evoke an ovulating response[11]. This difference in response may derive from the possibility that the effect of its own androgens on the hypothalamus in the newborn male rat, does not perform a 'whole or none' result, which apparently occurs in the androgeinized rat. This is supported by the fact that ovulation of ovarian graft in male castrated rat, may be elicited by direct stimulation of the preoptic area without progesterone pretreatment[12].

The ovulatory action of estrogens could thus provide an explanation either by a 'rebound' mechanism of the sudden fall and further increase of LH, or by a facilitating effect on the output of follicullotrophines by estrogens performed at certain levels of the central nervous system[13] or directly at the level of the pituitaries[14,15,16].

Résumé. Des greffons d'ovaire de rat impubère placés dans la chambre antérieure de l'œil du mâle castré, après traitement avec 0,5 mg de dipropionate d'œstradiol ou avec deux doses de 1 mg de progestérone, ont montré une évidente ovulation. On interprète ce fait comme dû à l'action des hormones injectées dans le système nerveux central et qui favorisent le mécanisme de l'ovulation.

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[8] J. W. EVERETT, Physiol. Rev. *44*, 373 (1964).
[9] C. A. BARRACLOUGH and R. A. GORSKI, Endocrinology *68*, 68 (1961).
[10] R. A. GORSKI and C. A. BARRACLOUGH, Endocrinology *73*, 210 (1963).
[11] C. A. BARRACLOUGH, in *Neuroendocrinology* (Eds. L. MARTINI and J. GANONG; Academic Press, New York 1966), chap. 19, p. 61.
[12] J. MOLL and G. ZEILMAKER, Acta endocr. (Copenh.) suppl. *100*, 146 (1965).
[13] S. KANEMATSU and C. A. SAWYER, Endocrinology *72*, 243 (1963).
[14] E. M. BOGDANOVE, Endocrinology *73*, 696 (1963).
[15] Acknowledgment. Estradiol dipropionate was kindly supplied by the CIBA laboratories Montevideo and Progesterone by Schering, Co. A.G., Berlin (Germany).
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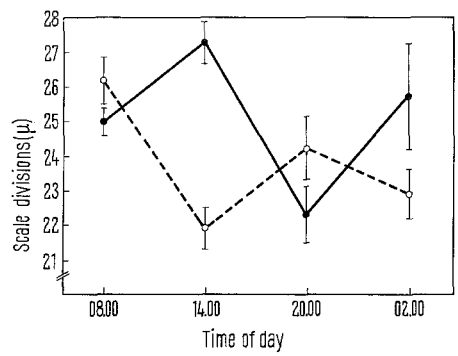
Circadian Fluctuations in Tibia Cartilage — Assayable Pituitary Homogenates of Fed and Starved Weanling Female Rats[1]

Circadian fluctuations of several metabolites in weanling rats have been reported previously[2]. Each one of these has been at some time reported to be related to the metabolic activity of growth hormone (GH). The present communication reports on fluctuations observed in the activity of pituitary homogenates as measured by the tibia test[3] and presumably reflecting GH activity.

Female weanling Holtzman rats were accomodated in single cages in a temperature-controlled room (22.5 °C) with 06.00–18.00 h light and 18.00–06.00 h dark cycles. Lab chow and tap water were given and libitum. On the 4th day the rats were divided into 8 groups of 8 rats each. Groups 1, 3, 5 and 7 were starved 24 h prior to sacrifice (08.00, 14.00, 20.00 and 02.00 h, respectively). Groups 2, 4, 6 and 8 were allowed to eat ad libitum. This was done because of the well-known relation between food intake, fasting and GH levels[4]. At the indicated sacrifice time the rats were quickly killed by decapitation and the pituitaries of a given group were weighed and pooled in a tissue homogenizer and stored at −5 °C. After removal from storage the tissue was disrupted by alternate thawing (45 °C) and freezing and after addition of saline the glands were homogenized. Before final dilution (0.7 mg of fresh pituitary in 0.2 ml of extract) the pH was adjusted to 10. The dosage of 0.2 ml/rat was injected s.c. into young hypophysectomized rats once daily for 4 consecutive days. The assay rats were killed on the 5th day and the tibia cartilage-widening activity of the extracts was determined[3]. Standards of GH were not used since the objective was the comparison of the groups sacrificed at different intervals. Significance of difference in epiphyseal cartilage width (one scale divison = 1 μ) was done using Student's *t*-test.

Fed rats: The pituitary content of tibia-active material rose from the late morning levels to a peak at 14.00 h ($p < 0.01$, Figure). There was a significant depletion by



Circadian fluctuations in the pituitary content of epiphyseal cartilage width assayable substance. Solid black line, fed rats; broken line, animals fasted 24 h prior to sacrifice. Ordinate shows epiphyseal cartilage width in micra, abscissa indicates time of sacrifice. Vertical bars depict standard error of the mean.

[1] This investigation was supported by U.S.P.H.S. Grant No. HD 03331, National Institute of Child Health and Human Development.
[2] M. S. BAHORSKY and L. L. BERNARDIS, Experientia *23*, 634 (1967).
[3] F. S. GREENSPAN, C. H. LI, M. E. SIMPSON and H. M. EVANS, Endocrinology *45*, 455 (1949).
[4] J. ROTH, S. M. GLICK, R. S. YALOW and S. A. BERSON, Science *140*, 987 (1963).

20.00 h ($p < 0.001$) and a reattainment of morning levels by 02.00 h. The depletion at 20.00 h could be expected since the period of greatest activity is known to begin at approximately 18.00 h (onset of darkness[5]). Muscular exercise is a known powerful stimulus for GH secretion in man[4].

Fasted rats: The effect of the 24-hour fast appears to influence the GH-activity in a manner opposite to that in fed rats. The highest content of GH-activity in the pituitaries occurred in the late morning (08.00 h) and dropped to its lowest level by 14.00 h ($p < 0.001$).

Diurnal fluctuations of plasma GH have been reported for children[6] and adults[7]. In children, GH levels are low during the day and high during the night, the latter being attributed to the long fact since the previous meal. It has been reported by CLARK[8] that high prolactin levels occur in rats at 16.00 h and low values were found at 22.00 h.

The data suggest circadian fluctuations in the pituitary content of tibia-test-assayable material, presumably GH. The fact that the removal of the exogenous stimulus of food intake did not eliminate significant variations in pituitary GH content suggests that the latter might be subject to the function of the 'Biological Clock'[9].

Zusammenfassung. Mit Tibiatest auf Wachstumshormonaktivität geprüftes Hypophysenmaterial zeigte trotz Fastenperioden zirkadische Gehaltsschwankungen. Die Wahrscheinlichkeit besteht, dass Wachstumshormone und eventuelle andere hypophysäre Hormone in ihrer Aktivität der «Biologischen Uhr» unterworfen sind.

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Buffalo (New York 14214, USA), 17 March 1969.

[5] C. P. RICHTER, Proc. Congr. Endocr. London, Excerpta med. Found. Series *183*, 119 (1964).
[6] W. M. HUNTER, J. Endocr. *34*, 147 (1966).
[7] W. M. HUNTER, J. A. R. FRIEND and J. A. STRONG, J. Endocr. *34*, 139 (1966).
[8] R. H. CLARK and B. L. BAKER, Science *143*, 375 (1964).
[9] E. BÜNNING, *The Physiological Clock* (Springer Verlag, Heidelberg 1964).

Injury and the Axial Organ of Echinoids

When foreign cells or organisms are injected into the perivisceral cavity of certain echinoids, the axial organ responds in a distinctive way[1]. Similar responses occur when coelomic fluid is removed, allowed to clot and re-introduced into the perivisceral coelom of the individual from which it was obtained. This suggested that the organ is responding to conditions likely to be associated with serious injury[1]. The observations to be reported substantiate this suggestion.

Material and methods. Injuries which penetrate the test lead to loss of coelomic fluid and coelomocytes both by escape and by clot formation. It is pertinent to discover whether the axial organ responds to such losses. Healthy individuals of *Arbacia punctulata* were deprived of coelomocytes in 2 ways. First, by withdrawing coelomic fluid into a sterilized syringe inserted through the peristome; second, by repeatedly removing the clot of coelomic fluid which sealed an opening 3–5 mm in diameter made in the test. The effects produced on the axial organ were compared and related to the condition of the organ in an individual having a naturally acquired lesion of similar kind.

Results and discussion. When *Arbacia punctulata* is robbed of about 1.0 ml of coelomic fluid the fluid lost is replaced within 24 h and the axial organ shows well-defined changes. Cells, cell debris and secretion begin to leave the lacunae and move into the contractile vessel, so that the channels in the trabeculae which connect the 2, become swollen with such material (Figures 1 and 2). Some of the cells resemble lymphocytes and fusiform cells[2], others peritoneal cells and those which produce mucus. Cell divison is active in both the migrating cells and those of the peritoneum which covers the axial organ and lines its central cavity. In such areas the peritoneum may lose its integrity, allowing cells and secretion to escape from the underlying lacunae and to pass, together with the loosened and proliferated peritoneal cells, into the lumen of the organ or into the perivisceral coelom. The nature of this secretion and that which passes into the contractile vessel in *Arbacia punctulata* has not yet been determined, but in other species much of it is acid mucopolysaccharide. It could be significant in wound healing[3].

These effects are evident $3^1/_2$ h after a single withdrawal but urchins can survive up to 9, provided that the withdrawals of fluid are separated by intervals of no less than 24 h. Under such conditions the coelomic fluid declines in clotting power, presumably owing to loss of coelomocytes and the lacunae show signs of depletion (Figure 3). Similar effects were clearly observed in all of 15 experiments in only 3 of which were there signs of debility or general tissue disintegration. One such case had suffered 6 withdrawals, the other two 9. The effects in instances such as the last mentioned produced by severe stressing can be accepted only with reserve, but they are useful in so far as they indicate that the depleted lacunae appear to be partially replenished with cells and secretion from the area where the mesentery and its contained 'haemal' vessel are attached to the axial organ. This hints at a possible function for the enigmatical 'haemal' system.

Parallel experiments in which the peristome was penetrated by the syringe, but no fluid withdrawn, produced comparably clear effects on the axial organ in only 2 instances out of 7. This suggests that loss of coelomocytes rather than unavoidable tissue damage is the major cause.

Clear and consistent effects were reproduced in 4 urchins from which healing clots were removed at intervals of 1–6 days. After 4 or 5 such operations clotting and healing were impaired or ceased and the axial organ showed changes precisely similar to those which followed withdrawal of coelomic fluid.

It proved possible to relate these findings to one instance taken from the normal environment. Naturally occurring lesions of the test proved rare in *Arbacia*:

[1] N. MILLOTT, Nature *209*, 594 (1966).
[2] R. ENDEAN, in *Physiology of Echinodermata* (Ed. R. A. BOOLOOTIAN; Interscience, New York 1966), p. 301.
[3] N. MILLOTT and H. G. VEVERS, Phil. Trans. *B 253*, 201 (1968).