

sham operated controls only 2 roaches out of 22 (9%) produced ootheca.

Allatectomized female roaches were injected with 50 μ l of a crude cecropia extract prepared according to the method of WILLIAMS⁸, held for 5 days, dissected, and the ovaries removed. The largest oocyte (terminal) in each ovary was then measured and the degree of yolk formation and color noted. The results in the Table indicate that the cecropia extract brought about ovarian growth with the largest oocyte in these insects about 1.6 times that found in the peanut-oil controls. In addition, the terminal oocytes of these insects showed the characteristic yellow color and yolk formation found in normally developing oocytes but absent in peanut-oil injected or noninjected controls. When doses of less than 50 μ l of the cecropia extract were injected, the growth response was found to be less and somewhat erratic. These results were confirmed by injection of 2 other ether extracts from male cecropia abdomens. This was not a nonspecific effect since crude extracts and fractions from several other insects failed to cause growth and yolk deposition. Of the crude extracts tested, only an extract from abdomens of male and female cynthia moths (*Samia cynthia*) showed comparable activity. This insect has also been reported to be a good source of juvenile hormone⁹.

An attempt was made to purify the cecropia extract by chromatographing a 0.5-g aliquot of the crude extract on an 18-g column of silicic acid¹⁰ (2 \times 9 cm) eluted with 300 ml of each of the following solvents: 18% benzene in hexane, 60% benzene in hexane, benzene, diethyl ether, and methanol¹¹. When the fractions were bioassayed as described above, activity was detected only in the diethyl ether fraction (about 60 mg). This fraction was also shown to have juvenile-hormone activity when assayed by a modification of the *Tenebrio* test^{12,13}, kindly performed by R. T. YAMAMOTO, entomologist, of the Entomology Research Division. The active fraction was further enriched by rechromatographing on silicic acid, using combinations of diethyl ether and hexane.

Effect of injected crude cecropia extract on ovarian growth of allatectomized American cockroaches

Treatment injection (50 μ l)	Number of insects injected surviving		Length of * largest oocyte (mm)
Cecropia extract	24	23	2.18 \pm 0.13
Peanut-oil (controls)	24	24	1.33 \pm 0.07

* Mean \pm standard deviation, 3 replicates.

Release of Luteinizing Hormone by Cerebral Cortex Spreading Depression

Recently we have shown¹ that the mere implantation of a needle into the cerebral cortex in rats can induce a release of luteinizing hormone (LH). It was postulated that this effect might be due to a stimulation of specific centers by impulses starting from the injured area or to a phenomenon of cerebral cortex spreading depression. In order to test the second hypothesis, the present experiments were performed.

Farnesol showed little or no activity when injected in peanut-oil solutions or emulsions with a wide range of doses, the highest of which caused some mortality. However, the topical application of farnesol to the cuticle of the abdomen of allatectomized roaches did cause definite ovarian growth and yolk deposition. When farnesol was chromatographed on silicic acid, as described above, it was found to be eluted in a different fraction (60% benzene in hexane) than the active material in the cecropia extract.

These data lend support to the suggestion that the juvenile hormone and the gonadotropic or yolk-forming hormone are either the same compound^{14,15} or are structurally similar compounds, each of which may perform either function.

The above test for gonadotropic activity is currently being used in the examination of crude extracts and fractions from whole insects and insect tissues and the test itself is under study to determine whether it may be used as a quantitative bioassay¹⁶.

Résumé. Les auteurs ont constaté que les extraits abdominaux de Phalènes mâles (*Hyalophora cecropia*) considérés comme une bonne source d'hormone juvénile, stimulent la croissance ovarienne et la formation du vitellus quand ils sont injectés à des femelles allatectomisées de la Blatte américaine (*Periplaneta americana*). Le farnésol, composé qui produit sur certains insectes les mêmes effets que l'hormone juvénile, a également une action gonadotrope définie lorsqu'on l'applique sur l'intégument de la Blatte.

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⁸ C. M. WILLIAMS, *Nature* 178, 212 (1956).

⁹ H. A. SCHNEIDERMAN and L. I. GILBERT, in *Cell, Organism and Milieu* (Ed. D. RUDNICK, Ronald Press Co., New York 1959), p. 157.

¹⁰ Unisil, 100–200 mesh, lot No. M7227, Clarkson Chemical Co., Williamsport, Pa. Mention of a company does not necessarily imply endorsement of its product by the U.S.D.A.

¹¹ M. G. HORNING, E. A. WILLIAMS, and E. C. HORNING, *J. Lipid Res.* 1, 482 (1960).

¹² V. B. WIGGLESWORTH, *J. Insect Physiol.* 2, 73 (1958).

¹³ P. KARLSON and M. NACHTIGALL, *J. Insect Physiol.* 7, 210 (1961).

¹⁴ I. W. PFEIFFER, *Trans. Connecticut Acad. Arts Sci.* 36, 489 (1945).

¹⁵ V. B. WIGGLESWORTH, *J. exp. Biol.* 25, 1 (1948).

¹⁶ The authors gratefully acknowledge the technical assistance of THELMA S. GOLDEN and ETHEL M. JENSEN (Entomology Research Division).

Adult female rats were prepared with pregnant mare's serum and then with human chorionic gonadotrophin in order to induce heavily luteinized ovaries as described by McCANN and TALEISNIK². Seven days after the beginning of the injections, and under ether anaesthesia, the first ovary was removed and 1 h later the second one. The depletion in ascorbic acid concentration in the second

¹ S. TALEISNIK, L. CALIGARIS, and J. DE OLMOS, *Amer. J. Physiol.*, in press.

² S. M. McCANN and S. TALEISNIK, *Amer. J. Physiol.* 199, 847 (1960).

Effect of cerebral cortex spreading depression on LH secretion

	No. of cases	No. of positive stimulations ^a	O.A.A.D. %	P value
(a) Control (uniovariectomy) <i>bilateral</i>	12	—	-1 ± 1.9^b	—
(b) Bone removed	12	1	3.2 ± 2.5	vs. a. n.s.
(c) Bone + dura removed	11	1	6.1 ± 1.2	vs. a. n.s.
(d) (c) + local KCl solution	12	10	18.6 ± 2.0	vs. a. <0.001 vs. c. <0.001
<i>unilateral</i>				
(e) Bone + dura removed	12	2	5.1 ± 2.2	vs. a. n.s.
(f) (e) + local KCl solution	19	5	8.1 ± 1.8	vs. a. <0.01

^a O.A.A.D. higher than mean control + 2 standard deviations. ^b Mean and standard error of mean. n.s. Not significant.

ovary as compared to the first was taken as an index of endogenous liberation of LH. Ovarian ascorbic acid depletion (O.A.A.D.) has been reported^{2,3} as a very sensitive and specific test for LH.

As has been shown⁴, spreading depression (S.D.) can easily be elicited in rats' cerebral cortex by local application of 25% KCl. This technique was used in the present experiment. Cotton soaked with 25% KCl was applied to the cerebral cortex after a window of 0.5 cm diameter was opened in the skull in the occipital area and the dura carefully removed under microscopic control in order to avoid cortical injury. Groups of animals were prepared in which only bone, or bone plus dura, were removed without application of KCl. These operations were performed immediately after the removal of the first ovary.

As can be seen in the Table, removal of one ovary does not induce O.A.A.D. In contrast to this, a highly significant O.A.A.D. takes place when 25% KCl is applied bilaterally to the cortex. Also unilateral application of KCl has induced a significant LH secretion. No effect was obtained by removing the bone alone or together with the dura.

As has been shown⁴, S.D. not only produces a severe impairment of electrical spontaneous activity of the cortex but can also suppress its functional activity. In that way this technique can be used as a means to induce functional ablation of the neocortex.

The results obtained in the present work show that cortical S.D. evoked by local application of KCl induces a liberation of LH. This fact is an evidence that the neo-

cortex activity is involved in the mechanisms of secretion of this hormone, probably through a modification of hypothalamic activity. BUREŠ⁴ has also reported, under the same circumstances, symptoms of hypothalamic involvement such as thermoregulation disturbances, water retention and hypoglycemia. The fact that stressful stimuli do not influence LH liberation² renders this phenomenon more evident.

In order to explain these results, we have to postulate that in normal conditions the cerebral cortex has an inhibitory influence on LH secretion and when its spontaneous activity is depressed either by its injury, as in the case of the needle, or by local application of 25% KCl, the inhibition is removed and release of the hormone takes place.

Résumé. La «spreading depression» du cortex cérébral provoquée chez les rats par l'application locale d'une solution de KCl à 25% déclenche une sécrétion d'hormone lutéinisante mesurée par la déplétion de l'acide ascorbique ovarien chez des animaux pseudogrades.

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Instituto de Investigación Médica "Mercedes y Martín Ferreyra", Córdoba (Argentina), July 11, 1962.

³ A. PARLOW, Fed. Proc. 17, 402 (1958).

⁴ J. BUREŠ, Josiah Macy Jr. Foundation. Second Conference on the Central Nervous System and Behavior, Madison (1959).

Presynaptic Effect of the Neuro-Muscular Transmitter

At the neuro-muscular (n-m) junction a presynaptic effect of acetylcholine (ACh), and of certain compounds related to this drug, has been indicated by the drug conditioned antidromic nerve activity described by MASLAND and WIGTON¹, FENG and LI², RIKER et al. (e.g. FUJIMORI et al.³, RIKER et al.⁴), WERNER⁵ and others, and discussed by KOELLE⁶. Since the postsynaptic transmitter effect may presumably influence the presynaptic events, it is of importance for the study of the latter to establish conditions which exclude the post-synaptic and maintain the presynaptic effect of the transmitter.

The following is a preliminary report on an attempt to exclude the postsynaptic response by cutting the muscle fibres transversally on either side of the endplate region, leaving the muscle fibres to depolarization by the de-

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³ H. FUJIMORI, W. F. RIKER JR., J. ROBERTIS, and F. G. STANDAERT, J. Pharmacol. 121, 286 (1957).

⁴ W. F. RIKER JR., G. WERNER, J. ROBERTIS, and A. S. KUPERMAN, Amer. N. Y. Acad. Sci. 81, 328 (1959).

⁵ G. WERNER, J. Neurophysiol. 23, 453 (1960).

⁶ G. B. KOELLE, Nature 190, 208 (1961); J. Pharm. Pharmacol. 14, 65 (1962).