LARDY et al.[2], MANN[3], and NEWTON and ROTHSCHILD[5] found 274, 314 and 129 ± 14 (range 79–161) pμ moles of ATP per cell, respectively, in freshly ejaculated bull spermatozoa[5]. NEWTON and ROTHSCHILD[5] also estimated the ADP, AMP, inosine and inosinic acid content of bull spermatozoa; their values for total adenosine and inosine derivatives (AMP + ADP + ATP + inosine + inosinic acid) ranged from 414 to 686 pμmoles/spermatozoon. The content of ATP alone in ram spermatozoa has been reported to be 164 pμ moles/cell[3,5]. A comparison of these values for adenosine (and inosine) derivatives in mammalian spermatozoa, with our values for the total acid-soluble purine and pyrimidine derivatives contained in these cells (Table 1), would suggest that the derivatives of other nucleic acid bases are not present in significant amounts. HULTIN[8], and NEWTON and ROTHSCHILD[5] had attempted a separation of the constituents of the acid-soluble fraction of spermatozoa by ion-exchange chromatography, and did not report the presence of any compounds other than the derivatives of adenine and hypoxanthine in the case of bull spermatozoa, and of adenine and uracil in the case of sea-urchin spermatozoa.

On paper chromatography of the hot PCA hydrolysate of the acid-soluble fraction of buffalo and bull spermatozoa, we, however, always obtained 3 distinct, clearly visible ultraviolet absorbing spots, which were shown to be due to adenine, guanine and cytosine by their Rf value and absorbance ratios at 250/260 and 280/260 mμ. The relative proportions of these 3 bases derived from the acid-soluble fractions of buffalo and bull spermatozoa in typical experiments, are given in Table II. Contrary to the results of earlier investigations[2,3,5,6], the acid-soluble cytosine and guanine derivatives put together were found to be present in these spermatozoa in nearly the same quantity as adenine derivatives.

Thymine and uracil were never detected on the paper chromatogram; their acid soluble derivatives can, therefore, be present in bull and buffalo spermatozoa only in traces. The virtual absence of thymine derivatives in the acid-soluble fraction of spermatozoa is understandable since these cells neither divide nor have any intracellular turnover of deoxyribonucleic acid[9]. The absence of uracil derivatives, is, however, surprising in view of the ability of mammalian spermatozoa to synthesise RNA[9].

Studies are currently in progress on the separation and quantitative estimation of each of the purine and pyrimidine derivatives present in the acid-soluble fraction of mammalian spermatozoa[10].

*Zusammenfassung.* Der Gehalt an säurelöslichen Purin- und Pyrimidinderivaten aus Stier-, Büffel- und Ziegenspermatozoen wurde bestimmt. Es wird gezeigt dass neben Adeninderivaten beträchtliche Mengen von Guanin- und Cytosinderivaten auch in diesen Zellen vorkommen. Uracil- und Thyminderivate waren nicht nachweisbar. Frühere Arbeiten über die freien Nukleotide aus Spermatozoa werden ebenfalls zusammengestellt.

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## The Gonadotropic Action of Cecropia Extracts in Allatectomized American Cockroaches

Ovarian maturation and yolk deposition have been shown to be under the endocrine control of the corpus allatum in a variety of insects[1,2], including the American cockroach (*Periplaneta americana*)[3]. Although most of this work was accomplished by the extirpation and implantation of organs, recently WIGGLESWORTH[4] found that farnesol, a compound that shows juvenile hormone activity in certain insects[5], induces yolk formation when applied to the surface of the cuticle of decapitated adult females of *Rhodnius prolixus*. This latter observation was interpreted as providing further evidence for the probable identity of the yolk-forming (gonadotropic) hormone and the juvenile hormone. However, attempts to initiate ovarian development in allatectomized blow flies (*Calliphora erythrocephala*)[6] and Colorado potato beetles (*Leptinotarsa decemlineata*)[7] with insect extracts containing juvenile hormone have been unsuccessful. This paper reports the use of extracts of abdomens of the male cecropia moth (*Hyalophora cecropia*) to initiate ovarian development and yolk formation in allatectomized American cockroaches.

The cockroaches used in these tests were from a laboratory strain fed commercial dog food. Newly molted adult females were removed from the colony daily and held in containers with adult males. When the females had produced 1 to 3 ootheca, they were allatectomized and held under observation in individual containers for 14–21 days. This was generally found to be sufficient time for the complete regression and resorption of any oocytes undergoing development at the time of allatectomy. Allatectomized females that produced ootheca 7 or more days after the allatectomy were not used as test organisms.

To determine whether these allatectomized female roaches were capable of responding to gonadotropic hormone, 1 to 4 pairs of corpora allata were transplanted into each of 25 allatectomized females. Within 7 to 20 days, 23 of these roaches (92%) produced ootheca. With

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[2] V. J. A. NOVAK, *Insektenhormone* (Ceskoslovenska Akademie ved, Prague 1959).
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[4] V. B. WIGGLESWORTH, J. Insect Physiol. *7*, 73 (1961).
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[6] E. THOMSEN, in *The Ontogeny of Insects* (Ed. I. HRDÝ, the Czechoslovak Academy Sciences, 1960), p. 218.
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sham operated controls only 2 roaches out of 22 (9%) produced ootheca.

Allatectomized female roaches were injected with 50 µl of a crude cecropia extract prepared according to the method of WILLIAMS[8], 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 µl 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 hormone[9].

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 acid[10] (2 × 9 cm) eluted with 300 ml of each of the following solvents: 18% benzene in hexane, 60% benzene in hexane, benzene, diethyl ether, and methanol[11]. 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 µl) | Number of insects injected | surviving | Length of[a] largest oocyte (mm) |
|---|---|---|---|
| Cecropia extract | 24 | 23 | 2.18 ± 0.13 |
| Peanut-oil (controls) | 24 | 24 | 1.33 ± 0.07 |

[a] Mean ± standard deviation, 3 replicates.

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 bioassay[16].

*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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## Release of Luteinizing Hormone by Cerebral Cortex Spreading Depression

Recently we have shown[1] 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.

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 TALEISNIK[2]. 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

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