

In both explants, the values for the potential per spike rose during the period that the permanent temperature damage was being inflicted. The frequency of the potentials fell more rapidly than their magnitudes for the same period of time. The difference in the general response of these two aspects of the behavior of the spontaneous potentials and in the effect of the final temperature damage suggests that they may depend upon different underlying mechanisms.

As soon as the experiment was over, both explants were fixed in buffered formalin, processed and stained for histological examination. They both showed the presence of histologically healthy examples of the various normal cellular elements of the telencephalon.

**Zusammenfassung.** Bei zwei verschiedenen Explantaten von Telencephalongewebe 14tägiger Hühnchenembryonen ergab sich als Temperaturabhängigkeit der Aktionspotentiale: Frequenz und Amplitudenabnahme bei sinkender, Zunahme bei steigender Temperatur bis zwischen 37 und 42,5°C. Weitere Temperatursteigerung bringt die Potentiale nach stetiger Verkleinerung bei 47°C schliesslich zum Verschwinden.

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### The Antimicrobial Activity of some Insect Extracts Possessing Juvenile Hormone Activity<sup>1</sup>

The juvenile hormone of insects acts to promote larval syntheses and thus deters adult differentiation. During a general study of the distribution of this molecule, substances possessing juvenile hormone activity have been extracted from vertebrate material (GILBERT and SCHNEIDERMAN<sup>2</sup>; WILLIAMS, MOORHEAD, and PULIS<sup>3</sup> from invertebrates other than insects (SCHNEIDERMAN and GILBERT<sup>4</sup>) and from plants and microorganisms (SCHNEIDERMAN, GILBERT and WEINSTEIN<sup>5</sup>). As part of a broad program to investigate the physiological significance of the ubiquity of this biologically active substance, juvenile hormone extracts were tested on bacteria. The data to be presented show that some of these extracts possess antimicrobial activity against a variety of microorganisms and that such activity is associated with the fatty acid component.

**Methods.** Unless stated otherwise, ether extracts of adult giant silkmoth abdomens, (*H. cecropia*, *S. cynthia*, and *R. orizaba*) were used. The isolation procedure consisted of repeated extractions of finely homogenized abdomens with peroxide-free ether, followed by washing and evaporating to dryness *in vacuo*. The residual oil contains nearly all the ether-soluble material of the abdomen and is rich in juvenile hormone activity (WILLIAMS<sup>6</sup>).

Due to the lipid nature of the test extracts it was not possible to assay the extracts by tube culture or by

impregnation of paper discs. A more suitable assay was developed by placing known quantities of extract into holes cut into seeded agar with a sterile No. 4 cork borer. Plates were seeded by adding 0.3 ml of 24 h trypticase soy broth culture of the test microorganism to trypticase soy or nutrient agar. The assay dishes were incubated at 30° C or 37° C depending on the optimum temperature for growth of the microorganism and read at 24 h by measuring the diameter of the inhibition zone in millimeters. Diameter of the cork borer and agar cut-out was 9 mm. Results are expressed as the radius of inhibition or one-half of the total zone diameter minus the diameter of the agar cut out.

**Results.** Table I reveals the extent of inhibition of microbial growth by extracts of male *cecropia* and male *crizaba* on a wide variety of microorganisms. Royal jelly containing the known antimicrobial agent 10-hydroxy- $\delta$ -2-decenoic acid (BLUM, NOVAK and TABER<sup>7</sup>; McCLESKEY and MELAMPY<sup>8</sup>) was also assayed. While the antimicrobial

<sup>1</sup> Supported by Grants G-7597 from NSF and A-2818 from U.S.P.H.S.

<sup>2</sup> L. I. GILBERT and H. A. SCHNEIDERMAN, *Science* 128, 844 (1958).

<sup>3</sup> C. M. WILLIAMS, L. V. MOORHEAD, and J. F. PULIS, *Nature* 183, 405 (1959).

<sup>4</sup> H. A. SCHNEIDERMAN and L. I. GILBERT, *Biol. Bull.* 115, 530 (1958).

<sup>5</sup> H. A. SCHNEIDERMAN, L. I. GILBERT, and M. WEINSTEIN, *Nature* 188, 1041 (1960).

<sup>6</sup> C. M. WILLIAMS, *Nature* 178, 212 (1956).

<sup>7</sup> M. S. BLUM, A. F. NOVAK, and S. TABER, *Science* 130, 452 (1959).

<sup>8</sup> C. S. McCLESKEY and R. M. MELAMPY, *J. Econ. Entomol.* 32, 581 (1939).

Tab. I. Inhibition of microbial growth by insect extracts

Microorganism	Male <i>H. cecropia</i>				Extract Male <i>R. orizaba</i>				Royal jelly			
	1	2	3	4 <sup>a</sup>	1	2	3	4	1	2	3	4
<i>M. luteus</i>	1.5	2.0	3.0	3.0	0	0	0.5	1.5	7.5	10.5	10.5	13.0
<i>S. aureus</i>	0	0	0	0	0	0	2.0	3.0	0	0	0	0
<i>S. lutea</i>	0	0.5	1.0	1.0	0	0	0	0	3.5	5.5	8.0	8.5
<i>M. smegmatis</i>	2.0	2.5	2.5	3.0	0	0	0	0	3.5	6.5	9.0	11.5
<i>B. megaterium</i>	0	0.5	0.5	1.0	0	0	0	0.5	3.5	5.5	8.5	9.5
<i>B. subtilis</i>	0.5	1.0	1.5	2.5	Not run				4.0	5.0	6.0	6.5
<i>A. fecalis</i>	0	0	0	0	2.5	3.0	4.5	5.5	0	0.5	1.0	1.5
<i>A. aerogenes</i>	0	0	0	0	1.5	4.0	4.5	5.5	0	0	1.0	4.0
<i>E. coli</i> B	0	0	0	0	1.5	4.5	6.5	7.5	0	1.0	1.5	2.5
<i>S. marcescens</i>	0	0	0	0	0	0	1.5	2.0	0	1.0	1.5	2.0
<i>P. vulgaris</i>	0.5	1.0	1.0	1.5	6.0	7.5	8.5	10.5	1.5	3.5	4.5	7.5
<i>S. ellipsoideus</i>	0	0	0	0	0	0	1.5	2.0	0	0	0	0

<sup>a</sup> 1, 0.03 ml; 2, 0.06 ml; 3, 0.09 ml; 4, 0.15 ml.

Tab. II. Assay of agar plates for juvenile hormone activity using chilled pupae of *A. polyphemus* as assay animals

Pupa	Material assayed	Days for assay pupa to molt	Effect	Juvenile hormone activity
1	1/2 of clear inhibitory zone	24	normal adult	—
2	1/4 of clear inhibitory zone	26	normal adult	—
3	1/3 of clear inhibitory zone	25	normal adult	—
4	3/4 material in well	15	pupal-adult intermediate	+++
5	1/2 material in well	14	pupal-adult intermediate	+++
6	1/2 material in well	14	pupal-adult intermediate	+++

spectra are similar, they are not identical, suggesting that the active substance from *cecropia* and *orizaba* differs from that found in royal jelly.

Using *Proteus vulgaris* as the test organism, slight inhibitory activity was found with extracts of adult wax moths (*Galleria mellonella*), male adult *cynthia* and *cecropia* pupae. No activity was found with female *cynthia* or with *cecropia* hemolymph.

Preliminary results indicated that smaller zones of inhibition and a different spectrum of antimicrobial activity were obtained with trypticase soy agar (pH 7.4) than with nutrient agar (pH 6.8). That this is due to the difference in pH of the media is indicated by the finding that the antibacterial activity increases proportionally upon acidification of the nutrient agar from pH 7.5 to pH 6.0. The results suggested that the active principle in these extracts was a fatty acid(s) and that with dissociation of the acid, the antibacterial activity declines. Further evidence supporting this view was obtained by allowing active extract to diffuse into agar containing bromthymolblue indicator. After 2 h an acid reaction occurred in an area approximating the inhibition zone found in a duplicate plate seeded with *P. vulgaris*. To substantiate further the fatty acid nature of the active principle, male *cecropia* extract was gently saponified with methanolic KOH. The ether soluble components were removed, the saponified material brought to a pH of 3 with HCl, and fatty acids extracted with ether. Both fractions were evaporated *in vacuo* and assayed with *P. vulgaris* in nutrient agar. Antibacterial activity was associated only with the saponifiable fraction, supporting the view that a free fatty acid(s) is responsible for the bacterial inhibition. These data exclude the

juvenile hormone as the active substance since it is associated with the unsaponifiable fraction (SCHNEIDERMAN and GILBERT<sup>9</sup>). Further evidence was obtained for dissociating the antibacterial effect from the juvenile hormone component of the ether extract. The agar containing the inhibitory zone and the residual oil in the wells were separately removed from assay plates and assayed for juvenile hormone activity on chilled *Polyphemus* pupae (GILBERT and SCHNEIDERMAN<sup>10</sup>). Table II reveals that the juvenile hormone remained in the well since this caused the assay pupae to molt into pupal-adult intermediates while the clear agar of the inhibitory zone showed no juvenile hormone activity<sup>11</sup>.

**Zusammenfassung.** Extraktstoffe von *H. cecropia*, *S. cynthia* und *R. orizaba* zeigten antibakterielle Wirkung gegenüber mehreren Mikroorganismen. Ein schwächerer Effekt wurde auch mit den Extraktstoffen von *Galleria mellonella*, adulten Männchen von *Cynthia*- und von *Cecropia*-Puppen beobachtet. Die antimikrobielle Aktivität wurde mit dem Fettsäureanteil der Extraktstoffe in Beziehung gesetzt und von der Wirkung des Juvenil-hormons abgegrenzt.

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<sup>9</sup> H. A. SCHNEIDERMAN and L. I. GILBERT, Anat. Rec. 123, 618 (1957).

<sup>10</sup> L. I. GILBERT and H. A. SCHNEIDERMAN, Trans. Amer. Microsc. Soc. 79, 38 (1960).

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## Hormonal Influences on Leucine Aminopeptidase in the Accessory Reproductive Tracts of the Rat

Effects of various sex hormones on the concentration of numerous enzymes in the seminal vesicles and prostates have been studied, e.g. acid and alkaline phosphatase<sup>1</sup>, carbonic anhydrase<sup>2</sup>, succinate dehydrogenase<sup>3</sup> etc. The behaviour of these enzymes after castration and hormonal treatments is not at all similar.

So far as is known, no corresponding studies concerning the changes observable in leucine aminopeptidase (LAP) in the accessory genital tract have been published. Such a study is described in the present work.

LAP is a proteolytic exopeptidase taking part in protein degradation and possibly also in protein synthesis. A great interest was aroused when it was observed to be increased in the blood during pregnancy and certain malignant tumours<sup>4</sup>. It is a widely distributed enzyme, being present in nearly all tissues so far studied.

In the present study, altogether 121 male rats aged 3–4 months were used. Castration was performed on 12 rats, which were killed 10–14 days later. The following subcutaneous hormone treatments were used: to 13 rats testosterone (Sustanon, N.V. Organon) was injected 1 mg daily, to 11 rats chorion gonadotrophin (Pregnyl, N.V. Organon) 40 IU daily, to 5 rats serum gonadotrophin (Gestyl, N.V. Organon) 46 IU daily, to 15 rats luteotrophic hormone (Prolactin, N.V. Organon) 10 IU daily, to 14 rats cortisone 0.5–2.0 mg (Adreson, N.V. Organon) daily, to 7 rats ACTH (Lääke Oy) 5 UI daily, and in addition to 11 rats reserpin (Lääke Oy) 0.05 mg daily. All these treat-

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<sup>2</sup> T. MIYAKE and G. PINCUS, Endocrinology 63, 64 (1959).

<sup>3</sup> A. TELKKÄ, A. KIVIKOSKI, and V. K. HOPSU, Acta endocr. (Kbh), in press.

<sup>4</sup> J. A. GOLDBERG and A. M. RUTENBURG, Cancer 11, 283 (1958).