Zusammenfassung. Der Durchmesser der Zellkerne von Neuronen im Nucleus Medialis Amygdalae (NMA) der normalen männlichen Totenkopfaffen ist grösser als derjenige der ovarektomierten Weibchen mit Östrogen/Progesteron-Substitution. Keine Unterschiede wurden jedoch im Nucleus suprachiasmaticus and cerebralen Cortex festgestellt. Diese morphologischen Unterschiede können auf eine unterschiedliche funktionelle Bedeutung der Amygdala bei Männchen und Weibchen bezogen werden.

G. A. Bubenik and G. M. Brown

Neuroendocrine Research Section, Clarke Institute of Psychiatry, 250 College Street, Toronto M5T JR8 2b (Ontario, Canada), 30 October 1972.

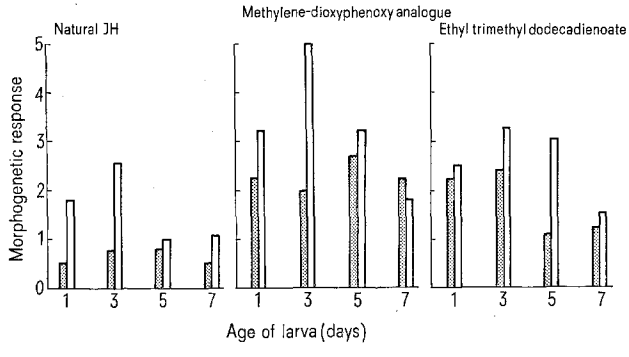
Effect of Diet on Response to Juvenile Hormone in *Galleria mellonella* Larvae

In the course of a study of the morphogenetic activity and metabolism of juvenile hormone (JH) and its analogues in last instar larvae of *Galleria mellonella*, we found that the morphogenetic response of wax moth larvae to JH varied with the diet. Larvae raised on a diet containing beeswax, honey, glycerin and Gerber's mixed cereal[1] showed a higher morphogenetic response to a standard dose of JH than larvae raised on a similar diet lacking beeswax.

Galleria larvae were raised on a diet consisting of Gerber's mixed cereal, glycerin, honey and beeswax in the proportion 12 :1 :1 : 0.5 by volume or on a similar diet but without beeswax. The larvae raised on the diet containing beeswax will be referred to as wax-fed larvae while the other larvae will be referred to as wax-deprived larvae. The larvae were raised from the first instar in plastic containers with unlimited food supply and were maintained at 29 ± 1°C and 70% r.h. Freshly molted last instar larvae can be recognized by their weight, the size of the head capsule[2] and, in this strain of *Galleria*, by pigmentation of the larval integument. The term larva in this communication refers to the last instar larva. The last larval stadium in *Galleria* under these conditions spans a period of 9 to 10 days, of which the last 2 days represents the pharate pupal stage.

Natural juvenile hormone[3] (methyl 12,14 dihomojuvenate), and methylenedioxyphenoxy 6-epoxy 3-ethyl 7-methyl 2-nonene[4] (Hoffmann La Roche, Nutley, N.J.) and ethyl 3,7,11, trimethyl 2,4 dodecadienoate[5] (Zoecon Corp. Palo Alto, California) were used in this study. These compounds are mixed isomers and possess potent juvenile hormone activity in *Tenebrio* bioassay[6]. Morphogenetic response of the larvae to JH was determined according to Sehnal and Meyer[7] modification of a procedure described by Piepho[8]. For this purpose, 25 µg of natural JH, 5 µg of methylenedioxyphenoxy analogue or 2 µg of ethyl trimethyl dodecadienoate were injected into the larvae in 1 µl of peanut oil. Control larvae were injected with 1 µl of peanut oil. Morphogenetic response of the larvae to JH was estimated by determining the extent of retention of larval characters at the succeeding ecdysis which normally occurred within 10 to 12 days. Some of the additional treated larvae pupated later. However, several larvae treated with ethyl trimethyl dodecadienoate failed to pupate, even 35 days after treatment with this analogue. A score of 5 was assigned for larvae which underwent an additional larval molt, and a score of 0 was assigned if the larvae metamorphosed into perfect pupae. At least 30 larvae were used for each determination and thus the score presented in the graph represents an average response of 30 to 50 insects.

Since the response of *Galleria* larvae to juvenile hormone varies with the age of the larva[7], larvae of diverse ages, 1, 3, 5 and 7-days-old were used in this investigation. All control larvae (wax-fed as well as those raised on wax-free diet) except some 1-day-old larvae, metamorphosed into perfect pupae. The larval molting of 10 to 14% of the peanut oil injected 1-day-old control larvae is attributed to injury which induces molting in these larvae[9]. The results recorded in the Figure show that wax-fed larvae are more sensitive to JH than the larvae on wax-free diet. This difference in sensitivity to JH is most conspicuous in 3-day-old larvae when compared to the other ages. Except for 7-day-old larvae treated with methylenedioxyphenoxy analogue, the wax-fed larvae always showed a greater morphogenetic response than the larvae raised on wax-free diet. In addition, the data also show that the response to JH in wax-fed larvae varied with age; 3-day-old larvae being most sensitive, confirming the earlier observations of Sehnal and Meyer[7]. Larvae raised on wax-free diet did not show conspicuous age-related changes in their response to JH although there are some minor fluctuations. The morphogenetic response of 3-day-old wax-fed larvae to



Effect of presence of beeswax in the diet on the morphogenetic response of last instar *Galleria* larvae to JH and analogues.
▒ wax-free diet; □ diet with beeswax.
Dose applied: Natural JH, 25 µg per larva; Methylenedioxyphenoxy analogue, 5 µg; Ethyl trimethyl dodecadienoate, 2 µg.

[1] Gerber mixed cereal, Gerber Products Co. Freemont, Michigan Composition: Protein, 11.7%; Fats, 4.5%; Carbohydrates, 73%; Crude fibre, 1.1%; Ash, 2.7%; Moisture 0.7% and vitamins.
[2] S. D. Beck, Wis. Acad. Sci. Arts Lett. *49*, 137 (1960).
[3] H. Roller, K. H. Dahm, C. C. Sweely and B. M. Trost, Angew. Chem. *6*, 179 (1967).
[4] W. S. Bowers, Science, N.Y. *164*, 323 (1969).
[5] G. B. Staal, C. A. Henrick and J. B. Siddall, Abst. ent. Soc. Am. No. 106 (1971).
[6] G. Reddy and A. Krishnakumaran, Life Sci. *11*, part II, 781 (1972).
[7] F. Sehnal and A. S. Meyer, Science, N.Y. *159*, 981 (1968).
[8] H. Piepho, Wilhelm Roux Arch. EntwMech. Org. *141*, 500 (1942).
[9] A. Krishnakumaran, Biol. Bull. mar. biol. Lab. Woods Hole *142*, 281 (1972).

natural JH and methylenedioxyphenoxy analogue is more than 2-fold greater than that shown by larvae raised on wax-free diet in which such high response is obtained (at the most JH-sensitive stages) only on application of approximately a 5-fold higher dose of hormone. Even with ethyl trimethyl dodecadienoate the wax-fed larvae show a significantly higher morphogenetic response. Thus, the difference in the morphogenetic response between wax-fed and wax-deprived larvae appears to reflect differences in sensitivity to JH rather than being the result of individual variations in the experimental animals. Moreover, this difference in morphogenetic response was repeatable on each of the 3 occasions when it was tested. Preliminary studies using another JH analogue, chlorophenoxy 6-epoxy-3-ethyl nonene showed similar differences between wax-fed and wax-free diet-fed larvae. From the above results, we conclude that the presence of beeswax in diet of *Galleria mellonella* influences its morphogenetic response to JH in the last larval stadium.

Reasons for the observed differences in morphogenetic response correlated with the presence or absence of beeswax in the diet of *Galleria* larvae are not clear. Although the presence of beeswax in the diet is inessential for the normal growth of *Galleria*[10], its presence in the diet improved their rate of growth[11,12]. Assuming that observed differences were due to variations in the weights of the larvae raised on the 2 diets which resulted in de facto differences in the dose of applied hormone, we determined the weight of the larvae raised under the 2 diet conditions. These data show that under these conditions of humidity and temperature the weight of the larvae raised on both diets was more or less identical. Furthermore, to avoid any effect of weight we repeated these experiments with larvae of uniform weight and obtained identical results. Thus, the differences noted in morphogenetic response are not the result of variations in the rate of growth of the larvae on the 2 diets. The C^{17}-fatty acid and phospholipid content of *Galleria* maintained for short term on beeswax-containing-diet differs from those deprived of beeswax[11,12]. Whether these differences in fatty acid and phospholipid content or some other hypothetical growth factor in the beeswax or possible differences in lipolytic enzymes in the larvae can account for the differences in their morphogenetic response remains to be proven. If specific substances can be found in the wax-diet which enhance the morphogenetic responses of *Galleria* larvae to JH, they may prove useful in increasing the insecticidal property of juvenile hormones[13].

Zusammenfassung. Mit Juvenilhormon und JH-Derivaten injizierte Larven der Wachsmotte *Galleria mellonella* zeigten stärkere morphogenetische Effekte, wenn ihre Nahrung Bienenwachs enthielt, als wenn diese fehlt.

GUNDA REDDY and A. KRISHNAKUMARAN

Department of Biology, Marquette University, Milwaukee (Wisconsin 53233, USA), 17 November 1972.

[10] M. H. HAYDAK, Ann. ent. Soc. Am. *29*, 581 (1936).
[11] R. G. YOUNG, Ann. ent. Soc. Am. *54*, 657 (1961).
[12] R. G. YOUNG, Ann. ent. Soc. Am. *57*, 321 (1964).
[13] This work was supported by funds from Marquette University and by N. S. F. research grant No. GB 19629. Our thanks are due to Dr. K. TAUTVYDAS for comments on the manuscript and to Mr. R. G. BAGLEY of Hoffmann La Roche Nutley, N. J. and Dr. J. B. SIDDALL of Zoecon Corp. Palo Alto, California for generous supply of the JH analogues used in this study.

A Survey on the Mutagenicity of Various Pesticides

The widespread use of an increasing number of chemicals in agriculture[1,2] and the resulting contamination of food with an increasing variety of pesticide residues imposes several problems. Apart from the dangers of acute and chronic poisoning, the possible mutagenic effects of such contaminations could threaten the genetic health of coming generations[3], and it is therefore of high importance that these effects be investigated. Thus, the present survey should help to establish a priority list of pesticides which would have to be tested thoroughly in mammalian systems to evaluate their mutagenic potential for mankind.

From the array of pesticides registered for use in Swiss agriculture[4] and their derivatives and contaminants, we chose those for testing which fulfilled one or more of the following requirements: a) Structural relationship to known mutagens; b) Structural relationship to natural nucleobases; c) Chemical reactivity towards nucleic acids; d) Pesticidal mode of action through interference with nucleic acid metabolism.

The testing procedure was as described elsewhere[5,6]. Two controls were run on each group, the standard control giving the rate of spontaneous mutations of the bacterial strain used, and a positive control with known mutagens demonstrating the mutagenic responsiveness of this particular strain. The results are summarized in the Table, of which some comments are made below.

It should be made clear that caution has to be exercized in extrapolating these results to higher organisms, a point which is instructively illustrated by the case of captan. Although this fungicide has been shown to act as a mutagenic substance through its alkylating potency[7,8], investigations failed to disclose a similar effect in Drosophila[9], as well as in the 'dominant lethal test' in mice[10]. Moreover it has recently been demonstrated that addition of blood serum destroys its mutagenic activity, apparently by binding the active principle – possibly thiophosgene[11] – to the thiol groups of serum proteins[12].

Another point of interest is the ambiguous response of part of the s-triazines. A mutagenic effect in bacteria could have been expected, since these compounds can be regarded as structurally related to the pyrimidine nucleobases. Supporting evidence for this view was provided by the findings of a small incorporation of s-triazine herbicides into nucleic acids of *Escherichia*

[1] M. MARKKULA, Kemian Teollisuus *28*, 549 (1971).
[2] H.-P. BOSSHARDT, Schweiz. Z. Forstwes. *123*, 650 (1972).
[3] H. V. MALLING, Am. ind. Hyg. Ass. Q. *31*, 657 (1970).
[4] *Pflanzenschutzmittelverzeichnis* (EDMZ, CH–3000 Berne 1972).
[5] B. N. AMES, in *Chemical Mutagens* (Ed. H. HOLLÄNDER; Plenum Press New York-London 1971), vol. 1, p. 267.
[6] J. P. SEILER, Mutation Res. *15*, 273 (1972).
[7] M. S. LEGATOR, F. J. KELLY, S. GREEN and E. J. OSWALD, Ann. N.Y. Acad. Sci. *160*, 344 (1969).
[8] C. H. CLARKE, Mutation Res. *11*, 247 (1971).
[9] P. MOLLET, Diss. ETHZ 1972, in press.
[10] D. ARNOLD, unpublished results (Industrial Bio-test Laboratories) 1967.
[11] R. J. LUKENS, Phytopathology *53*, 881 (1963).
[12] B. BRIDGES, R. P. MOTTERSHEAD, M. A. ROTHWELL and M. H. L. GREEN, Chemico-biol. Interactions *5*, 77 (1972).