hatte LEYDIG (zit. bei WIGGLESWORTH[6]) schon im vorigen Jahrhundert für Käfer vermutet. Die Markierung von Sekretionen hypodermaler oder sonstiger Drüsen würde sehr viel mehr Zeit (mehrere Stunden bis zu einem Tag)[7–10] in Anspruch nehmen, da der «Tracer» zunächst von den Drüsenzellen absorbiert, innerhalb derselben in die Sekrete eingebaut und mit diesen ausgeschieden werden müsste.

Summary. The very short time (5–9 min) between injection of radiophosphorous into the hemolymph of adult fireflies (Lampyridae) and outflow of radioactive droplets after irritation demonstrates with certainty that reflex-bleeding is involved. Labelling the secretions of dermal glands would require much more time (several h or even 1 day.

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18. September 1974.

[6] V. B. WIGGLESWORTH, *The Principles of Insect Physiology*, 7th edn. (Chapman and Hall, London 1972).
[7] W. KLOFT und H. KUNKEL, Proc. XI. Int. Congr. Entomol., Wien 1960, (1961), vol. *1*, p. 746.
[8] W. KLOFT and P. EHRHARDT, Proc. Symposium 'Radioisotopes and Radiation in Entomology', Bombay 1960 (IAEA Wien, 1962), p. 181.
[9] H. NAARMANN, Experientia *19*, 412 (1963).
[10] K. P. LAMB, P. EHRHARDT, V. MOERICKE, Nature, Lond. *124*, 602 (1967).
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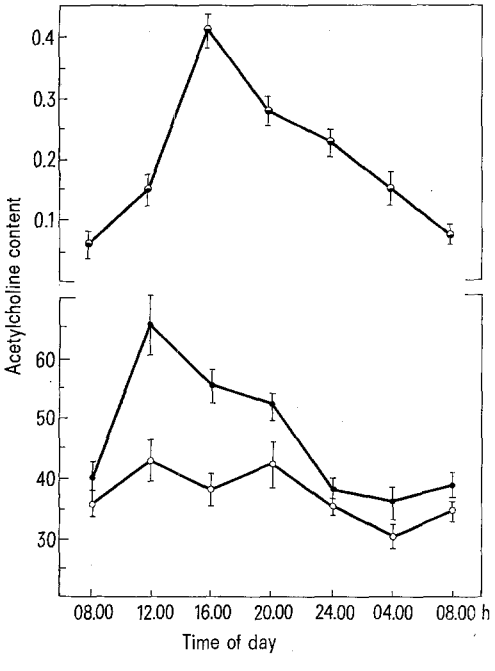
On the Acetylcholine Content in the Scorpion, *Heterometrus fulvipes* C. Koch

Quantitative estimations of acetylcholine (ACh) content in the nervous and non-nervous tissues are carried out in several arthropods[1–5], and there is sufficient evidence to suggest its neurohumoral function[5]. Amongst the arachnids, ACh is detected in the nervous system of *Limulus polyphemus*[6–8] and the ganglia of the spider, *Heteropoda regia* and 2 species of scorpions, *Buthus europaeus* and *Heterometrus maurus*[9].

The central nervous system of the scorpions is shown to produce 2 different neurohormones out of phase with each other, and they seem to control the diurnal rhythmicity of the animal[10–12]. It was also suggested earlier that ACh might act as a neurohumor in the scorpion[13]. If ACh is the neurohumoral substance involved in the diurnal rhythmicity, it would be natural to expect diurnal variations in its content also. The present investigation was carried out to test this hypothesis.

Material and methods. The South Indian scorpion, *Heterometrus fulvipes* C. Koch was used. Cephalothoracic nerve mass (CTNM), ventral nerve cord (VNC) and blood were isolated at various times of the day in cold conditions. The material from 6 animals was pooled to form 1 sample to obtain enough material for the assay. The material was kept in boiling water bath for 5 min to inactivate the enzyme AChE and to release bound ACh. The tissues were cooled and ACh was estimated by the method of Hestrin as given by AUGUSTINSSON[14]. The amount of ACh present in the sample was determined from the standard graph prepared using known amounts of ACh. 6 samples were analyzed at each time to get concordant values.



Acetylcholine content in the CTNM, VNC and blood of the scorpion estimated at various times of the day. The values are the averages of 6 observations ± S.D. and are expressed as µg ACh/g wet weight for CTNM and VNC and as µg/ml in the case of blood. ◒–◒, blood; ●–●, CTNM; ○–○, VNC.

[1] J. H. WELSH, *Physiology of Crustacea* (Ed. T. H. WATERMAN; Academic Press, New York, USA 1961), vol. 2, p. 281.
[2] F. HUBER, *Physiology of Insecta* (Ed. M. ROCKSTEIN; Academic Press, New York, USA 1965), vol. 2, p. 333.
[3] J. BOISTEL, *Advances in Insect Physiology* (Eds. J. W. L. BEAMENT, J. E. TREHERNE and V. B. WIGGLESWORTH; Academic Press, New York, USA 1968), vol. 5, p. 1.
[4] B. N. SMALLMAN and A. MANSINGH, A. Rev. Ent. *14*, 387 (1969).
[5] R. M. PITMAN, Comp. gen. Pharmac. *2*, 347 (1971).
[6] C. C. SMITH and D. GLICK, Biol. Bull. *77*, 321 (1939).
[7] W. SCHALLEK, J. cell. comp. Physiol. *26*, 15 (1945).
[8] E. FLOREY, *Neurochemistry* (Eds. K. A. C. ELLIOT, I. H. PAGE and J. H. QUASTEL; Springfield, Illinois, USA 1962), p. 673.
[9] E. CORTEGGIANI and A. SERFATY, C. r. Soc. Biol., Paris *131*, 1124 (1939).
[10] K. P. RAO, J. Anim. Morph. Physiol. *11*, 133 (1964).
[11] K. P. RAO and T. GOPALAKRISHNA REDDY, Nature, Lond. *213*, 1047 (1967).
[12] S. A. T. VENKATACHARI, Ind. J. exp. Biol. *9*, 338 (1971).
[13] S. A. T. VENKATACHARI and V. DEVARAJULU NAIDU, Experientia. *25*, 821 (1969).
[14] K. B. AUGUSTINSSON, *Methods of Biochemical Analysis* (Ed. D. GLICK; Interscience Publishers Inc., New York, USA 1957), vol. 5, p. 1.

Results and discussion. The ACh content of CTNM, VNC and blood and its diurnal variations in the 3 tissues are shown in the Figure. It is evident that ACh content is less in the blood while it is more in the central nervous tissues; there even the CTNM had a higher content than the VNC. ACh content of the blood was less in the early morning hours and gradually rose during daytime and reached a maximum at 16.00 h. Thereafter the content decreased again and came to minimum at 08.00 h the next day. A similar increase in ACh content was also shown by CTNM but the maximum was found at an earlier time i.e. at 12.00 h. The content decreased at 16.00 and 20.00 h and reached a minimum by midnight. The ACh content of the VNC, however, showed no such diurnal variations, though it was seen to fluctuate at various times of the day.

The ACh content of arthropod nervous tissues is generally high and the ACh content of the nervous tissue of the insects is 5–50 times more than that in the vertebrate nervous tissue[3]. The ACh content in the scorpion nervous system is also high. But compared to the ACh content in the nervous tissues of crustaceans[1] and insects[2] it is evident that ACh content in scorpion is less than that in insects and more than that in crustaceans. The content in scorpion is higher than that in *Limulus* where only 15.2 μg/g wet weight are reported in the ventral nerve cord[6]. It is of interest to note that blood maintains a lower level of ACh than the nervous tissue, and such a situation was reported in crustaceans earlier[1].

Variations in ACh content of the nervous tissues of arthropods are shown earlier in relation to development[4,15], temperature acclimation[16] CO_2 and DDT treatment[16,17] and seasons[18]. The present study shows that it undergoes diurnal variations also. It is of interest to note that, while such variations are shown in CTNM and blood, no such changes are seen in VNC and there is a time lag of 4 h in the occurrence of maximal quantities between CTMN and blood. Perhaps ACh is synthesized in CTNM and released into the blood at a later time, and the ACh synthetic processes in VNC may not vary diurnally.

Diurnal variations in electrical activity in the central nervous system of scorpion are shown earlier[12,19]. Interestingly, the period of maximal electrical activity in the cord is the same as the period of maximal ACh content in the blood. This suggests the possibility that higher amounts of ACh in the blood might be responsible for greater electrical activity in the VNC. Such a correlation between the amount of ACh released and the level of nervous activity was also shown earlier in the cockroach[20,21].

From the present investigations it may be suggested that the neurohormone produced during daytime, resulting in the enhancement of electrical activity, might be ACh[22].

Zusammenfassung. Beim südindischen Skorpion *Heterometrus fulvipes* zeigt der Gehalt an Acetylcholin folgende Verteilung: Nervenkomplex des Cephalothorax > ventraler Nervenstrang > Blut; er unterliegt tageszeitlichen Schwankungen im Nervenkomplex des Cephalothorax und im Blut. Aus der zeitlichen Verschiebung der Maxima wird abgeleitet, dass der Nervenkomplex des Cephalothorax Acetylcholin produziert und dieses an das Blut abgibt.

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[15] K. GRZELAK, Z. LASSOTA and A. WRONISZEWSKA, J. Insect Physiol. *16*, 1405 (1970).
[16] E. H. COLHOUN, J. Insect Physiol. *2*, 108 (1958).
[17] J. M. TOBIAS, J. J. KOLLROS and J. SAVIT, J. Cell. comp. Physiol. *28*, 159 (1946).
[18] R. I. SMITH, J. cell. comp. Physiol. *13*, 335 (1939).
[19] K. P. RAO, Proc. 16th Int. Congr. Zool. Washington D.C. (1963).
[20] S. J. MIKALONIS and R. H. BROWN, J. cell. comp. Physiol. *18*, 401 (1941).
[21] E. H. COLHOUN, J. Insect Physiol. *2*, 117 (1958).
[22] Acknowledgment. The present investigation was carried out at S. V. University, Tirupati, A.P. India and the authors are highly beholden to Prof. G. KRISHNAN, Head of the Department of Zoology, S.V. University, for the facilities provided. Thanks are also due to the University Grants Commission, New Delhi, India, for selecting one of us (NV) as a National Research Associate which made the present work possible.
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Hydrolysis of Sex Pheromone by the Antennae of *Trichoplusia ni*[1]

Degradation of bombykol, (E)-10, (Z)-12-hexadecadien-1-ol, in and/or on the antenna of *Bombyx mori* (L.) has been demonstrated[2–5]. However, dihydrobombykol and tetrahydrobombykol were similarly degraded by *Bombyx* antennae, indication of a relative lack of enzymatic specificity. A similar lack of specificity was indicated by the enzymatic degradation of both the pheromone of *Porthetria dispar* (L.)[6] (disparlure); (Z)-7,8-epoxy-2-methyloctadecane), and its precursor, ((Z)-2-methyl-6-octadecene), by male antennae. Enzymatic degradation of the pheromone of the cabbage looper (*Trichoplusia ni* Hübner), (Z)-7-dodecen-1-ol acetate) by isolated proteins presumably released from olfactory sensilla has also been demonstrated[7,8], however, the pheromone seemed to be degraded more slowly than other isomers and analogs of the pheromone[9]. In this report, evidence is presented that demonstrates in vivo a specificity for pheromone by degradation of enzymes on the antenna and legs of male *T. ni*.

For most of these tests antennae of live intact moths were briefly dipped in a 1 mg/ml (4.42×10^{-3} *M*) sonicated suspension of pheromone in water. No more than 10 insects

[1] Mention of a pesticide or a proprietary product in this paper does not constitute a recommendation or an endorsement of the product by the U.S. Department of Agriculture.
[2] G. KASANG, in *Gustation and Olfaction* (Eds. G. OHLOFF and A. F. THOMAS; Academic Press, New York 1971), p. 245.
[3] G. KASANG and K.-E. KAISSLING, in *Olfaction and Taste IV* (Ed. D. SCHNEIDER; Wissenschaftliche GmbH, Stuttgart 1972), p. 200.
[4] R. A. STEINBRECHT and G. KASANG in *Olfaction and Taste IV* (Ed. D. SCHNEIDER; Wissenschaftliche GMBH, Stuttgart 1972, p. 193).
[5] G. KASANG, Naturwissenschaften *60*, 95 (1973).
[6] G. KASANG, B. KNAUER and M. BEROZA, Experientia *30*, 148 (1974).
[7] S. M. FERKOVICH, M. S. MAYER and R. R. RUTTER, Nature, Lond. *242*, 53 (1973).
[8] S. M. FERKOVICH, M. S. MAYER and R. R. RUTTER, J. Insect Physiol. *19*, 2231 (1973).
[9] M. S. MAYER, S. M. FERKOVICH and R. R. RUTTER, Unpublished data.