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.

N. VASANTHA[22], S. A. T. VENKATACHARI, P. MURALI MOHAN[23] and K. SASIRA BABU[23]

Department of Zoology, Yeshwant Mahavidyalaya, Nanded (Maharashtra, India 431601), 12 September 1974.

[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.
[23] Department of Zoology, S.V. University, Tirupati, A.P. India, 517502.

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.

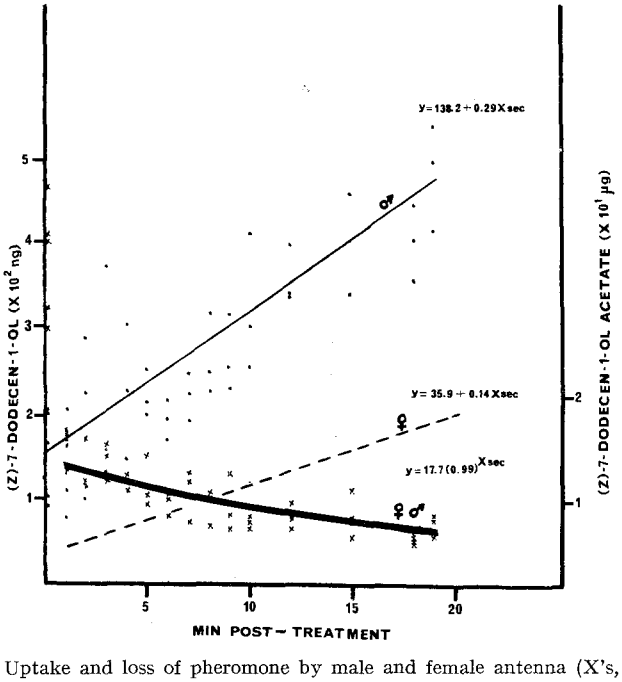
Table I. Comparison of in vivo degradation of pheromone with 6 isomers and analogs on the antenna and legs of male *T. ni* during 4-sec incubations

Chemical	Uptake and degradation by antenna[a]			Uptake and degradation by legs[a]		
	Unchanged chemical recovered/antenna (μg)	Alcohol/antenna (ng)	Ratio	Unchanged chemical recovered/leg (μg)	Alcohol/leg (ng)	Ratio
(Z)-5-dodecen-1-ol acetate	18.4	69.4	0.6	163.1	610.1	0.7
(Z)-7-dodecen-1-ol acetate	25.6	118.1	1.0	40.3	865.8	1.0
(Z)-8-dodecen-1-ol acetate	30.1	36.4	0.3	68.1	183.5	0.2
(Z)-9-dodecen-1-ol acetate	27.3	<12.5	0.1	116.6	168.1	0.2
(E)-7-dodecen-1-ol acetate	16.6	37.8	0.3	48.1	138.4	0.2
Dodecan-1-ol acetate	17.9	60.8	0.5	18.4	107.9	0.1
(Z)-7-dodecen-1-ol butyrate	24.1	29.0	0.2	54.5	161.4	0.2

[a] A total of 30 antennae and legs were used for each test.

were used with any one suspension because contamination of the homogenized pheromone-water interface with scales interfered with pheromonal coating of the antennal surface. The remaining pheromone and its metabolite, (Z)-7-dodecen-1-ol, were removed from the antennae by soaking in diethyl ether (2–3 min except as noted). The volume of the rinse solvents was reduced under nitrogen and quantitatively analyzed by gas-liquid chromatography (5% Carbowax 20 M on chromosorb W 1.8 × 2 mm, 140 °C, 20 ml N_2/min, H_2 flame detector). Standards carried through this procedure revealed an 80% recovery of pheromone and alcohol; therefore, all data in this report have been corrected to 100% recovery. All chemicals were of 94+% purity or better.

The male antenna of *T. ni* degraded pheromone to the corresponding alcohol at 2 × the rate of the female antenna (Figure). These enzymatic rates should be considered maximum because the amount of pheromone coated on the antennae during incubation is far above the physiological range. These data demonstrate a difference between sexes not observed with *B. mori*[2,3]. Even more interesting is the extremely rapid degradation of pheromone during the first 4 sec (the practical zero time in this procedure) of incubation. The data (Figure) show that 138.2 ng of alcohol/male antenna and 35.9 ng of alcohol/female antenna were formed by zero time.



Uptake and loss of pheromone by male and female antenna (X's, bold line right-hand ordinate) and resultant (Z)-7-dodecen-1-ol on male antennae (filled circles, constant line, left-hand ordinate) and female antennae (broken line, left-hand ordinate) after various incubation intervals.

Attempts to demonstrate enzymatic specificity with various isomers and analogs of the pheromone over time intervals of 5–20 min were inconclusive because the data (Figure) indicated first order enzyme kinetics. It seemed that specificity might be demonstrable if observations were confined to the first 4 sec of the reaction when zero-order kinetics would predominate. The results of these studies (Table I) demonstrated that with maximum velocity conditions the enzyme of both the antenna and legs was specific for the natural pheromone. The legs produced 2–10 × more alcohol than the antennae for all chemicals employed though more substrate was available, probably as a reflection of the greater surface area (Table I). It can be concluded from these data that there is associated with the antenna and legs of *T. ni* an enzyme(s) that rapidly and specifically catalyzes the degradation of the sex pheromone to the free alcohol.

The next problem was to determine more precisely the location of this reaction. A serial extraction with 3 lipid solvents of increasing polarity was used to successively remove unbound, lipid bound, and other more strongly (protein?) bound pheromone and alcohol[3]. In these tests the pheromone was compared with its geometric isomer, (E)-7-dodecen-1-ol acetate, because of their chemical similarity, because of their degradative dissimilarity (Table I), and because the (E)-isomer is behaviorally inactive[11,c.f.,12,13]. Almost 96% of the two acetate isomers but only 56–62% of the two alcohol isomers were removed by the short pentane extraction (Table II). Only about half (20%) the alcohol remaining in or on the antenna was extracted by diethyl ether, which confirms previous observations[2–5]. The number of pheromone molecules (3.5×10^{14}) was remarkably similar to the number of alcohol molecules (2.6×10^{14}) remaining in or on the antenna after the ether extraction. These data confirm previous data[3] that show a small but significant amount of pheromone and metabolite remaining in or on the antenna even after relatively vigorous extraction. However, there was only a small difference in the amounts of behaviorally active or inactive isomers.

Table II. Results of successive solvent extractions of male antennae incubated 10 min with (Z)- and (E)-7-dodecen-1-ol acetate

Chemical	No. antennae	Recovery	1st extraction pentane (15 sec)		2nd extraction diethyl ether ether (30 sec)		3rd extraction chloroform-methanol	
			Acetate recovered/ antenna (μg)	Alcohol/ antenna (ng)	Acetate recovered/ antenna (μg)	Alcohol/ antenna (ng)	Acetate recovered/ antenna (μg)	Alcohol/ antenna (ng)
(Z)-7-dodecen-1-ol acetate	80	Amount	6.76	241.41	0.18	70.80	0.13	79.53
		%	95.6	61.6	2.5	18.1	1.9	20.3
(E)-7-dodecen-1-ol acetate	60	Amount	7.15	135.36	0.19	53.78	0.13	51.30
		%	95.7	56.3	2.5	22.4	1.7	21.3

Each extraction was divided as nearly as possible into 2 intervals to obtain a rinse of the antennae and the container.

The simplest interpretation of these data is that the degradative enzyme of males functions to remove excess pheromone from the body surface of males resulting from either chance contact with females or long exposures during orientation to a 'calling' female. The female antenna (and probably the rest of the body) also degrades the pheromone to the alcohol, which would reduce or inhibit male orientation to the pheromone[14,15] and in this context may convey spurious information.

If, on the other hand, we speculate that the enzymatic conversion is an event closely related to the transductory process, then some other considerations must be mentioned. The male perceives the alcohol through a primary receptor cell in the same sensillum with the primary pheromone receptor cell[10]; sensitivity to the alcohol is almost as great as that to the pheromone[10]. The female also perceives the pheromone, though much less sensitively than the male[10,16]. Could this difference between male and female sensitivity to pheromone be related to the slower rate of pheromone degradation by the female (Figure)?

Calculations based on published[17] and unpublished data[10] indicate there is a total of about 6500 pheromone-receptive sensilla on the *T. ni* antenna. If there are approximately 3000 olfactory pores/sensilla[18]; then calculation shows there are approximately 2×10^7 pores/antenna. Because the results (Table I) show that 29.5 ng (9.6×10^{13} molecules) of alcohol are formed/sec per antenna, then 1.5×10^{10} molecules of alcohol were produced per sensilla trichodeum. Because 20% of the alcohol may have been bound to protein (Table II, $CHCl_3$:MEOH extraction) and if this protein were the enzyme, then 9.8×10^5 molecules of pheromone degradative enzyme would be present in or at each pore.

KAISSLING[19] estimated the total number of 'acceptors'/primary olfactory receptor cell (A_{TOT}) of *Bombyx* to be 32,300 although later he estimates a larger number to be present[18]. If the enzyme demonstrated here is comparable to such theoretical acceptors, then A_{TOT} can be estimated by multiplying the 9.8×10^5 molecules of enzyme/pore by 3000 pores/sensilla trichodeum (1 primary receptor cell/sensilla trichodeum) to arrive at the value of 2.9×10^9 acceptors/cell in *T. ni*. KAISSLING[19] also estimated a turnover number of 180/min for the queen substance receptor and 100/min for the bombykol receptor. If, for comparison, the 1.5×10^{10} molecules of alcohol produced/sensilla trichodeum/sec is divided by 2.9×10^9 acceptors (enzyme)/cell, the result is 5.2/sec or 310/min. This value is remarkably similar to the values KAISSLING estimated for the queen substance and bombykol receptors.

While none of the isomers or analogs are behaviorally active, they also are degraded, which would be essential if this enzyme serves a role other than in the transductory mechanism. Although these experiments are informative, it should be kept in mind that the levels of pheromone substrate used are far above the physiological range of behavioral effectiveness.

The data tend to confirm the speculation by KAISSLING[20] that there is an early and a late inactivation process associated with pheromone perception. The specificity of the enzymatic activity is observable only with short-duration incubations and becomes more complex with increasing incubation intervals. Enzyme kinetics would aid in this interpretation; however, substrate concentrations and product are not measurable at physiological concentrations. The possibility exists that the kinetics can be examined in vitro[9].

Résumé. Les antennes du mâle *Trichoplusia ni* (Hübner) dégradent la pheromone femelle sexuelle, (Z)-7-dodécen-1-ol acétate en donnant l'alcool correspondant deux fois plus vite que les antennes de la femelle. La pheromone elle-même est hydrolysée plus rapidement que 6 isomères et proches analogues. Les jambes des mâles dégradent aussi la pheromone.

M. S. MAYER

Insect Attractants, Behavior and Basic Biology, Research Laboratory, USDA, Agricultural Research Service. P.O. Box 14565, Gainesville (Florida 32604, USA), 6 November 1974.

[10] M. S. MAYER, unpublished data.
[11] J. R. MCLAUGHLIN, E. R. MITCHELL, M. BEROZA and B. A. BIERL, in press (1975).
[12] H. H. TOBA, N. GREEN, A. N. KISHABA, M. JACOBSON and J. W. DEBOLT, J. econ. Entomol. *63*, 1048 (1970).
[13] M. JACOBSON, H. H. TOBA, J. DEBOLT and A. N. KISHABA, J. econ. Entomol. *61*, 84 (1968).
[14] J. H. TUMLINSON, E. R. MITCHELL, S. M. BROWNER, M. S. MAYER, N. GREEN, R. HINES and D. A. LINDQUIST, Envir. Entomol. *1*, 354 (1972).
[15] J. R. MCLAUGHLIN, E. R. MITCHELL, D. L. CHAMBERS and J. H. TUMLINSON, Envir. Entomol. *3*, 677 (1974).
[16] G. G. GRANT, Ph. D. Dissertation, Virginia Polytechnic Institute and State University (1970).
[17] R. N. JEFFERSON, R. E. RUBIN, S. U. MCFARLAND and H. H. SHOREY, Ann. ent. Soc. Am. *63*, 1227 (1970).
[18] K.-E. KAISSLING, in *Olfaction and Taste III.* (Ed. C. PFAFFMANN; Rockefeller Univ. Press. New York 1969), p. 52.
[19] K.-E. KAISSLING, in *Handbook of Sensory Physiology IV Chemical Senses 1 Olfaction.* (Ed. L. M. BEIDLER; Springer-Verlag, Berlin 1971), p. 351.
[20] K.-E. KAISSLING, in *Olfaction and Taste IV.* (Ed. D. SCHNEIDER; Wissenschaftliche GmbH, Stuttgart 1972), p. 207.