Table III. Experiment with E,E-11'-(^{3}H,^{14}C)-farnesol (**2**)

Lytta vesicatoria (couples)		Isotope	Life-time after copulation (h)	Total activity (dpm)	Cantharidin dmp/mg	Cantharidin Total activity (dpm)	^{3}H/^{14}C	Incorporation rate[a] (%)	Transferred total activity (%)	Transferred cantharidin (%)
4	♂	^{3}H	36	3.1×10^7	33 856 ± 224	$(1.69\pm0.011)\times10^6$	9.67 ± 0.07	3.6		
		^{14}C		9.3×10^5	3 500 ± 3	$(1.75\pm0.001)\times10^5$		13.9		
	♀	^{3}H	36	1.5×10^7	28 021 ± 36	$(1.40\pm0.002)\times10^6$	9.75 ± 0.04	3.0	32.8	45.2
		^{14}C		3.2×10^5	2 873 ± 9	$(1.44\pm0.004)\times10^5$		11.4	25.5	45.0

[a] See Table I.

Table IV. Experiment with E,E-2-^{14}C-methylfarnesoate (**3**)

Lytta vesicatoria (couples)		Life-time after copulation (h)	Total activity (dpm)	Cantharidin dpm/mg	Cantharidin Total activity (dpm)	Incorporation rate[a] (%)	Transferred total activity (%)	Transferred cantharidin (%)
7	♂	31	1.0×10^8	2 3507 ± 114	$(1.18\pm0.006)\times10^6$	1.1		
	♀	31	3.0×10^6	9 486 ± 3	$(4.74\pm0.002)\times10^5$	0.5	2.9	28.7

[a] See Table I.

males, 10.3% is transferred into the female sex organs during the short lasting copulation.

The results in Table I show that 93–98% of the cantharidin biosynthesized from 2-(^{3}H,^{14}C)-mevalonate (injection 24–30 h prior to copulation) is transferred from the male *Lytta* to the females during a copulation period of 20–24 h (in particular the ^{3}H/^{14}C-ratio of the isolated cantharidins from the males and females are equal). Of the total activity (100%) present after copulation, only 33.0–52.6% was found in the female *Lytta*. The high rate of incorporation of 18.6–36.7% of 2-^{14}C-mevalonate is remarkable, and it would appear that, in the male, the biosynthesis of cantharidin is stimulated during copulation. Both experiments provide strong evidence against the transfer of an intermediate species, formed from mevalonate during the cantharidin biosynthesis, to the female at copulation.

In further experiments E,E-11'-(^{3}H,^{14}C)-farnesol (**2**)[15] and E,E-2-^{14}C-methylfarnesoate (**3**)[12] were injected into male *L. vesicatoria* from Sicily (1972–1974) during copulation. After an average copulation time of 20 ± 2 h, the couples separated. The males and females were kept alive separately for 30–40 h and then processed. The results of these experiments are presented in Tables III and IV.

Of the total cantharidin biosynthesized from radioactive precursors **2** and **3** by the male *Lytta* during and after copulation, the percentages found in the females were 45.0% and 28.7% respectively. A comparison of these values with those for total activity transferred from male to female during copulation only, show that there is a preferential transfer of cantharidin at copulation. The fact that the value is lower, compared to that in experiment 1, shows that the male insects continue to synthesize cantharidin after copulation from labelled precursors still present in their bodies. Indications suggest that cantharidin is stored in the accessory glands of the male sexual organs. Our observations demonstrate that an almost complete transfer of cantharidin occurs at copulation. Thus it appears very likely that these glands are the site of cantharidin biosynthesis.

[15] S. HAUFFE, Diploma Thesis University Zürich 1975.

[16] M. G. PETER, G. SNATZKE, F. SNATZKE, K. N. NAGARAJAN and H. SCHMID, Helv. chim. Acta *57*, 32 (1974).

Amino Acid Requirements of the Bug *Dysdercus similis* Freeman (Hemiptera: Heteroptera)

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Summary. The amino acid requirements for moulting, growth and development of *Dysdercus similis* have been investigated. The insects could not moult and reach maturity when given 10 essential amino acids only. However, if these were supplemented either with glutamic acid, glycine or aspartic acid growth and moulting was found to be normal. *Dysdercus* has an unusual synthetic mechanism for converting tyrosine into phenylalanine.

A number of papers deal with the amino acid requirements of insects[2–6]. Still we know very little about the amino acid requirements and their role in the growth and moulting physiology of insects[7–9]. Therefore an attempt has been made to elucidate the role of different amino acids during the moulting cycle of *Dysdercus similis*.

Material and methods. Nymphs and the adults of *D. similis* were collected from the fields and reared all the

year round in the laboratory on moist cotton seeds. The experimental insects were sorted out, marked, and kept in separate bottles. Cotton swabs soaked with amino acid solutions were given to the insects daily. The experiments were conducted in 3 sets.

Insects were fed on: 1. a) Essential amino acids (argenine, histidine, lysine, tryptophane, phenylalanine, methionine, threonine, leucine, isoleucine, valine). b) Non-essential amino acids (serine, α-alanine, β-alamine, glutamic acid, glycine, aspartic acid, tyrosine, cystine, proline, hydroxyproline). c) All 20 amino acids. 2. All essential and one non-essential amino acid. 3. All amino acids were given omitting in each experiment a different one of the essential amino acids. The results reported were checked in 3 to 5 series of observations.

Results. In the first experiment nymphs kept in 3 separate bottles were provided either with the essential amino acids, the non-essential amino acids or all the 20 amino acids. The nymphs fed on all 20 amino acids grew and moulted to the adult stage, while in the other 2 bottles they died within 3 to 7 days.

In the second set of experiments, the insects were provided with the 10 essential plus 1 of the non-essential amino acids. In these combinations the nymphs died before reaching the maturation stage, except in the presence of glutamic acid, glycine, and aspartic acid.

In the 3rd set of experiments the omission test was applied, i.e. all amino acids were given but for the omission of one of each of the essential amino acid. The omission of each one of the essential amino acids stopped growth and moulting, except when phenylalanine was omitted and tyrosine was given in place of phenylalanine. However, tyrosine could replace phenylalanine only when added in higher quantity; otherwise the insects died before reaching maturity. In the absence of phenylalanine, a few insects survived and reached maturity to some extent.

Discussion. Dysdercus reared only on essential amino acids or on non-essential amino acids could not reach maturity. These observations are in accordance with the results of previous studies on amino acid requirements of insects, which generally grow poorly or not at all with only the essential amino acids provided, even when these are given at higher concentrations, as was noted in the silk worm *Bombyx mori*[10], the aphid *Myzus persicae*[6] and many others. Exceptions to this include the flour beetle *Tribolium confusum*[11], the boll weevil *Anthonomos grandis*[12] and the red banded leaf roller *Argyrotaenia velutinana*[4].

When the essential amino acids were supplemented with aspartic acid, glutamic acid or glycine, growth and moulting of *D. similis* was found to be normal, similar to the situation in *Bombyx mori*, where optimal growth occurred when the essential amino acids were supplemented with either aspartic or glutamic acid[10]. Likewise, cystine was found to be an essential component for *Aedes*[13] and glycine for *Pseudosarcophaga*[14]. In the present study, either glutamic acid, glycine or aspartic acid were found to be indispensable for optimal growth and development in *D. similis*.

It has been observed in many insect species that there exist unusual synthetic mechanisms: for instance, the ability of *Phormia* to use cystine in place of methionine[15], and of *Aedes* to use tyrosine in place of phenylalanine[13], though according to SINGH and BROWN[16], tyrosine cannot replace phenylalanine in *Aedes aegypti*. In the present study, it has been observed that in *D. similis* phenylalanine can be replaced by tyrosine.

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[2] H. L. HOUSE, in *The Physiology of Insecta*, 1st edn. (Ed. M. ROCKSTEIN; Academic Press, New York and London 1965), vol. 2, p. 769.
[3] N. C. PANT, P. GUPTA and J. K. NAYAR, Experientia *16*, 311 (1960).
[4] G. C. ROCK and K. W. KING, J. Insect Physiol. *13*, 59 (1967).
[5] T. ITO and N. ARAI, Bull. seric. Exp. Stn. Japan. *19*, 345 (1965).
[6] R. H. DADD and D. L. KRIEGER, J. Insect Physiol. *14*, 741 (1968).
[7] H. LIPKE and G. FRAENKEL, A. Rev. Entom. *1*, 17 (1954).
[8] D. GILMOUR, *Biochemistry of Insects* (Academic Press, London 1961), p. 6.
[9] H. L. HOUSE, A. Rev. Entom. *6*, 13 (1961).
[10] T. ITO and N. ARAI, J. Insect Physiol. *12*, 861 (1966).
[11] A. F. NAYLOR, Can. J. Zool. *41*, 1127 (1963).
[12] E. S. VANDERZANT, J. Insect. Physiol. *11*, 659 (1965).
[13] L. GOLDBERG and B. DEMEILLON, Biochem. J. *43*, 372 (1948).
[14] H. L. HOUSE, Can. J. Zool. *32*, 351 (1954).
[15] E. HODGSON, V. H. CHELDELIN and R. W. NEWBURGH, Can. J. Zool. *34*, 527 (1956).
[16] K. R. P. SINGH and A. W. A. BROWN, J. Insect Physiol. *1*, 199 (1957).

Production of β-Ergokryptine by a Strain of *Claviceps purpurea* (Fr.) Tul. in Submerged Culture

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Summary. A strain of *Claviceps purpurea*, labelled 231 F.I., produced in submerged culture 1200 μg/ml of a mixture of peptidic alkaloids composed for 30% by β-ergokryptine. Cultural media, conditions of culture and development features of a typic fermentation are reported.

The ergot alkaloid β-ergokryptine was isolated in 1967 from sclerotia[1]. While it is well known that several ergot alkaloids can be produced by strains of *Claviceps* in submerged cultures[2,3], the production of β-ergokryptine in these conditions has never been reported.

In the course of an investigation of strains isolated from sclerotia of *Claviceps purpurea*, a strain able to produce β-ergokryptine in submerged culture was obtained. The strain, labelled 231 F.I., was grown on slants of medium T2 at 28 °C for 8 days and then transferred into 300-ml Erlenmeyer flasks containing 50 ml of the inoculum medium TG. The flasks were incubated 4 days at 24 °C on a rotary shaker operating at 225 rpm with a 3 cm

[1] W. SCHLIENTZ, R. BRUNNER, A. RÜEGGER, B. BERDE, E. STÜRMER and A. HOFMANN, Experientia *23*, 991 (1967).
[2] A. M. AMICI, A. MINGHETTI, T. SCOTTI, C. SPALLA and L. TOGNOLI, Experientia *22*, 415 (1966).
[3] A. M. AMICI, A. MINGHETTI, T. SCOTTI, C. SPALLA and L. TOGNOLI, Appl. Microbiology *18*, 464 (1969).