for providing necessary facilities to carry out this work.

- 1. Delamare Deboutteville, C., Biologie des eaux souterraines littorales et continentales, Hermann, Paris, 1960, p. 740.
- 2. Newell, I. M., "A systematic and ecological study of the Halacaridæ of eastern North America," Bull. Bingham Oceanogr. Coll., 1947, 10, 232.
- 3. Chandrasekhara Rao, G. and Ganapati, P. N., "The interstitial fauna inhabiting the beach sands of Waltair coast," Proc. natn. Inst. Sci. India 1968, 34 (2), 82.
- 4. Hartmann, G., "Ostracodes des eaux souterrainee littorales de la Mediterane et de Majorque," Vic et Milieu, 1954, 4 (2), 238.
- 5. Renaud Debyser, J., 'Researches ecologiques sur la faune interrstitielle des sables (Basin d' Arcacoon; ile de Bimini, Bahamas),' Ibid., 1963, Suppl. 15, p. 157.
- 6. Andre, M.. Halacariens marins," Faune de France, 1946, 46, 152.

ON THE FREE AMINO-ACIDS OF THE HAEMOLYMPH AND REGIONS OF THE ALIMENTARY CANAL OF EYPREPOCNEMIS ALACRIS ALACRIS (SERVILLE) (ORTHOPTERA: INSECTA)

M. C. MURALIDHARAN AND T. N. ANANTHAKRISHNAN Entomology Research Unit, Loyola College, Madras-34

ABSTRACT

The distribution and the relative concentrations of the free amino-acids of the hæmo-lymph of the late instars and adult females under diverse physiological conditions as well as the free amino-acids of the various regions of the alimentary canal of the V instar and adult females of Eyprepocnemis alacris alacris (Serville) are discussed.

CONSIDERABLE information on the free amino-acids of the post-embryonic and adult stages of several insects is available⁴ 7 although the free amino-acids of Acridids in particular appear to be confined to the hæmo-lymph of the nymphs of Locusta migratoria.⁶ Schistocerca gregaria,² Anacridium ægyptium and Acrida exaltata.¹¹ Colombo et al.³ have also investigated the free amino-acids of the eggs during embryonic development of Schistocerca gregaria.

Information relating to the free amino-acids of the various regions of the alimentary canal appears to be lacking in the case of Acridids. Data presented here pertains to the qualitative studies on the distribution of the free amino-acids of the fore, mid and hindgut of the V instar and adult females of Eyprepocnemis alacris, alacris, as also the free amino-acids of the hæmolymph of the IV, V instars and adult females under three different physiological conditions, viz., females on the '0' day, i.e, immediately after hatching to adult, mature females (12-15 days), as well as the starved females.

Samples of hæmolymph from the hind-femora and abdomen were collected on a Whatman No. 1 filter-paper, dried, kept for 24 hours in 90% ethanol and was concentrated before spotting. Regions of the fore, mid and hindgut,

removed after dissection in distilled water, were separately ground, centrifuged and stored after adding few drops of isopropyl alcohol. Trichloroacetic acid was added before centrifuging so as to precipitate the minor proteins, peptides, etc.

The extracts were spotted on Whatman No. 1 filter-paper and both two-dimensional and circular methods were employed, using in both cases the same solvent (n-butanol: acetic acid: water)) in the ratio of 4:1:1. After a run of about 10-12 hours at room temperature, the paper was sprayed with 0.2% (W/V) ninhydrin. For the two-dimensional method, phenol: water solvent was also used in the ratio of 3:1. Chromatograms of standard amino-acids were also prepared for comparison and the results tabulated.

The free amino-acid patterns of the hæmo-lymph from the V instar onwards shows that lysine, histidine, serine, glycine, aspartic acid are present in appreciable concentrations, with a fall in concentration in the starved individuals. Serine has also been reported to be present in the hæmolymph of Schistocerca gregaria as in Eyprepoenemis alacris alacris, but has not been reported in Locusta migratoria and Anacridium ægyptium. On the '0' day, a decline in the concentrations of glutamic acid, threonine (absent in Acrida exaltata¹¹),

Table I

Showing the free amino-acids in the hæmolymph and in the alimentary canal of

Eyprepocnemis alacris alacris (Serville)

| | Hæmolymph | | | | | | Alimentary canal | | | | | |
|---------------|-----------|--------------|-------------|----------------------------|----------------------------|-------------------------------|------------------|-----------------|-------------|-------|------------------|-------------|
| Amino-acids | | IV instar | V instar | Adult female '0' day | Adult female matured | Adult female starved for days | Foregut | | MiJgut | | Hindgut | |
| | | | | | | | V instar | Adult | V instar | Adult | V instar | Adult |
| Lysine | • • | + | ++ | ++ | + + | + | + | + | + | + | + | + |
| Histidine | • • | | ++ | ++ | ++ | + | + | - - | + | + | - | 4 |
| Arginine | • • | + | | + | | + | _ | - | + | + | _ | |
| Serine | •• | + | ++ | + + | ++ | + | + | + | + | + | + | + |
| Glycine | • • | + | ++ | ++ | ++ | + | + | + | + | + | + | + |
| Aspartic acid | | + | ++ | ++ | ++ | + | + | + | + | + | + | + |
| Glutamic acid | | + | + + | ⊕ | + | - | + | + | ++ | + | - | + |
| Threonine | •• | + | ++ | Ð | + | _ | + | + | ++ | + | | + |
| Alanine | • • | + | -+ | Ð | + | _ | + | + | ++ | + | - [- | + |
| Proline | | + | + | + | + | + | _ | - | - | - | | - |
| Tyrosine | •• | + | + | () | + | _ | \oplus | _ | ⊕ | ÷ | \oplus | _ |
| Valine | | + | + | $\bar{\oplus}$ | ++ | \oplus | \oplus | () | -+- | + | \oplus | \oplus |
| Methionine | 4. | + | + | $\bar{\oplus}$ | ++ | $\stackrel{\circ}{\oplus}$ | \oplus | Ð | + | + | \oplus | ⊕ |
| Phenylalanine | • • | | - | <u>-</u> | 0 | _ | <u> </u> | - | | - | - | - |
| Leucine | | + | + | \oplus | Ŧ | + | + | + | + | + | + | + |
| Tryptophan | | + | ÷ | Ť | + | ⊕ | _ | - | + | + | _ | _ |

 $^{+ =} Present, - = Absent, + + = Concentrated, \oplus = Traces.$

alanine, tyrosine and leucine is noticed, with a subsequent increase in the normal adult. The meagre presence of tyrosine on the '0' day might appear to be due to its utilisation for the pigmentation process as suggested for Culex sp.⁵ Subsequently, starvation resulted in the absence of some amino-acids, almost as in '0' day condition (Table I). The low concentration of these amino-acids on the '0' day appears to be due to the cessation of feeding for some time before and after ecdysis or possibly be due to the fact that some amino-acids were being used up during ecdysis as in the case of tyrosine. The presence in smaller concentrations of glutamic acid, alanine, proline and tyrosine on the '0' day also confirms the earlier observations that these amino-acids take an active role in the synthesis of cuticle proteins, chitins, etc.,11 and after moulting, in the adult stage these increase in quantity. Phenylalanine is found only in traces in the adult condition while it is absent in the earlier instars.

The free amino-acids of the alimentary canal show clear differences among the three regions. The fore and hindgut present the same amino-acid picture while the midgut differs (Table I). However, no significant changes during transition from V instar to adult were noticed in the alimentary canal, with respect to amino-acid picture except that serine, glycine, aspartic acid, glutamic acid, threonine and alanine were in higher concentration in the midgut region of the

V instar than in the adult. Phenylalanine was absent in both V instar and adult in all the three regions as has been recorded in many insects. The absence of this essential aminoacid may be indirectly explained on the basis, that phenylalanine gets converted readily to tyrosine in the insect body and this conversion has already been recorded by Fukuda. Tyrosine appears to be absent in the extracts of the fore and hindgut and is present only in the midgut, but in the V instar all the three regions have tyrosine but in small concentrations.

Thanks are due to Prof. Dr. A. Srinivasan of the Chemistry Department for help rendered during this investigation.

^{1.} Benassi, C. A. et al., Experientia, 1959, 15(12), 457.

^{2. —} Biochem. J., 1961, 80, 332.

^{3.} Colombo, G. et al., Comp. Biochem. Physiol., 1962, 5, 83,

^{4.} Chen, P. S. Amino-acid Pools, Ed. J. T. Holden, 1962, p. 115.

^{5. —} and Briegel, H., Comp. Biochem. Physiol., 1965, 8.

^{6.} Duchateau, G. H. et al., Arch. int. Physiol., 1952, 60, 539.

^{7.} Marcel Florkin, IV Intl. Cong. Biochem. Vol. XII. Biochemistry of Insects, 1959.

^{8.} Fukuda, T., Proc. IV Intl. Cong. Biochem., Vienna, 1958.

^{9.} Kamlesh Dang and Pant, J. C., Indian J. Ent., 1964, 26, 268.

^{10.} Pant, R. and Agrawal, H., Curr. Sci., 1963, 32, 20,

^{11. -,} J. Insect Physiol., 1964, 10. 443.