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Amino Acid Analyses of Haemolymph of *Glossina morsitans morsitans* (Westwood)

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Abstract

Haemolymph from pupae and adult *G. morsitans* was analysed for its content of free amino acids. Of the twenty-one amino acids identified proline occurred in the greatest concentration in the 3 stages tested. Substantial amounts of taurine, glutamine, glutamic acid, alanine, tyrosine and arginine were also measured. There was considerable variation in the content of amino acids in the 3 stages of development of the tsetse fly.

Introduction

Insect haemolymph characteristically contains high levels of free amino acids (LEVENBOOK, 1950; FLORKIN, 1968), but their relative concentrations are subject to variation. Virtually any amino acid may constitute a major component in the haemolymph of a species at some stage of its life history and be almost lacking in another species or in another stage of the same species.

Knowledge of the chemical compositions of haemolymph has served as a valuable guide for the design of several successful culture media which have been used to grow the cells of a variety of insects and other arthropods (WYATT, 1956; SCHNEIDER, 1964; REHACEK & BRZOSTOWSKI, 1969; SCHNEIDER, 1971), but few attempts have been made to cultivate the tissues and cells of tsetse flies. TRAGER (1959) developed a culture medium which supported extensive cell migration from pieces of pupal tissues of *Glossina palpalis,* but NICOLI & VATTIER (1964) were less successful in their attempts to cultivate tissues of *G. fuscipes* in the same medium.

The present study was undertaken in the hope that an analysis of the free amino acids of tsetse haemolymph might therefore provide a guide for the design of a more consistent culture medium for the maintenance and growth of tsetse tissues than that currently in use in which lactalbumin hydrolysate is the main source of amino acids (CUNNINGHAM, 1973).

Materials and Methods

Newly deposited *G. morsitans* pupae supplied by Dr. T. A. M. Nash, Tsetse Research Laboratory, Langford, Bristol, were placed in pots of sterile sand and incubated at 25 °C and at a relative humidity between 65–80 %. Pupae, pre-emerged flies and 2-days old flies were used as sources of haemolymph.

During development of *Glossina* species larval/pupal and pupal/adult apolyses [3] take place and at approximately 16 days after larviposition the dark rigid

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[3] Apolysis = detachment of the epidermal cells from the old cuticle.

puparium contains a pharate [4] fly (SAUNDERS, 1970; HINTON, 1971). In this study the term 10 days old pupae refers to the contents of the puparia 10 days after larviposition.

Collection of haemolymph

Pupal haemolymph – Pupae of 10 days old were washed in sterile distilled water to remove extraneous sand particles. They were allowed to dry and were placed in a refrigerator at 4 °C for 30 minutes. With the aid of watchmakers' forceps, a small fracture was made at the anterior end of the puparium. A capillary tube was inserted through the ruptured puparium into the pupal haemocoel. By capillarity, haemolymph and fat body entered the tube; care was taken to avoid collecting the gut. The contents were expelled onto a few crystals of phenylthiourea in a small Petri dish held over an ice bath. Precooling the pupae and adding phenylthiourea prevented melanization of the haemolymph, which results from oxidation of tyrosine in the presence of an active polyphenol-polyphenoloxidase system (DUBOIS & ERWAY, 1946). The haemolymph of 10 pupae was pooled and centrifuged in a haematocrit tube at 10,000 r.p.m. for 10 minutes. The supernatant fluid, excluding the lipid layer, was transferred to a small tube and stored at –20 °C until used.

Haemolymph of pre-emerged flies – Pupae aged 27 days were washed and precooled at 4 °C for 30 minutes. The anterior end of the puparium was removed with fine forceps and flies at the point of emergence everted the ptilinum. When the ptilinum was fully extended it was cut with iridectomy scissors and the haemolymph was collected in a haematocrit tube. Each tsetse fly yielded approximately 6 μlitres of haemolymph which was treated by the method described above. Haemocytes were removed by centrifugation at 2,000 r.p.m. for 5 minutes and the haemolymph was stored at –20 °C.

Haemolymph of adult flies, 2 days after emergence – Unfed flies were immobilised in the refrigerator at 4 °C for 30 minutes. The haemolymph was collected by amputating the posterior leg at the femur. A capillary tube was held at the stump end and gentle pressure on the thorax of the fly expelled the haemolymph into the tube. A maximum of 2 μlitres could be obtained from one fly. The haemolymph of 15–20 flies was treated as described above and stored at –20 °C.

Amino acid analyses – Immediately before analyses, pooled samples of 10–20 μlitres of haemolymph were placed in small tubes and the volume adjusted to 2.5 ml with 0.1 *N* HCl containing 10 % sucrose and 0.25 μmole norleucine. One ml samples of this solution were analysed for free amino acid content using a Technicon Nc-1 Amino Acid Analyser.

Results and Discussion

The results of the analyses of the free amino acids in haemolymph of pupal and adult tsetse flies are illustrated in Fig. 1 and Table 1. The total concentration of amino acids increased from 1,756 mg/100 ml in the pupal haemolymph to 2,021 mg/100 ml in the pre-emerged flies and decreased to 1,486 mg/100 ml in flies which were 2 days old. These values were within the range found in other insect species (100–2,000 mg/100 ml) and were between 30 and 40 times higher than those recorded for vertebrate plasma (40–60 mg/100 ml) (BURSELL, 1970).

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[4] Pharate = that part of a new instar of an arthropod which is enveloped by the cuticle of the previous stage.

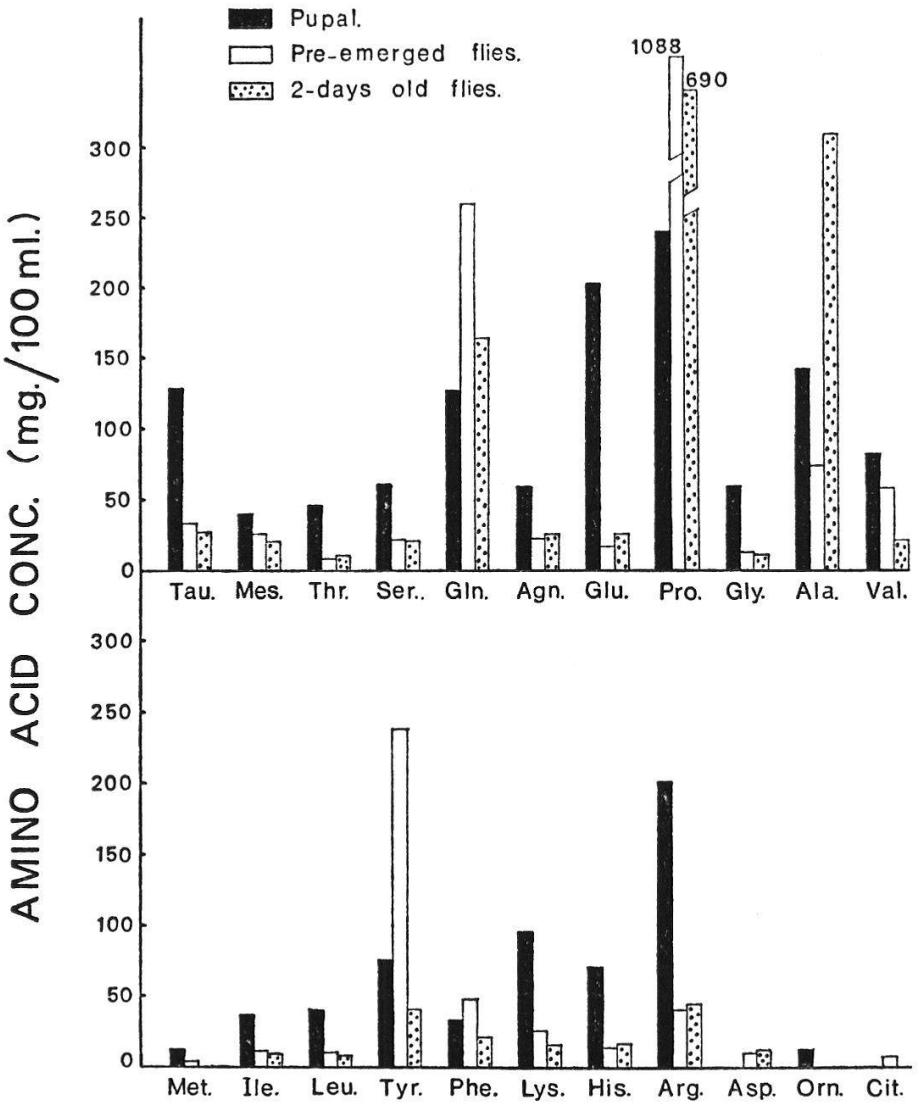

Fig. 1. Amino acid analyses of haemolymph of 10 days old pupae and flies *(G. morsitans).* Tau-taurine; Mes-methionine sulphoxide; Thr-threonine; Ser-serine; Gln-glutamine; Agn-asparagine; Glu-glutamic acid; Pro-proline; Gly-glycine; Ala-alanine; Val-valine; Met-methionine; Ile-Isoleucine; Leu-leucine; Tyr-tyrosine; Phe-phenylalanine; Lys-lysine; His-histidine; Arg-arginine; Asp-aspartic acid; Orn-ornithine; Cit-citrulline.

These was, however, considerable variation in the levels of individual amino acids between the 3 stages. This was particularly marked for taurine, glutamine, glutamic acid, proline, tyrosine, lysine, histidine and arginine. Most of the amino acids occurred in greater concentrations in the pupal haemolymph than in the haemolymph of flies. Exceptions to this were glutamine, proline, alanine, tyrosine, phenylalanine and aspartic acid. The last of these was not detected in the pupal blood. In the pre-emerged and 2-days old flies, the levels of most of the amino acids were similar but there was a marked decrease in the amount of glutamine, proline, valine and tyrosine in the older flies. Variation in the levels of amino acids between the 3 stages was not unexpected since significant differences in amino acid content are known to occur during the life history of a single insect species (FLORKIN & JEUNIAUX, 1964; CHEN, 1966). Some of the observed variations were probably associated with short-term fluctuations in the concentration of particular amino acids concerned with different metabolic activities.

Table 1. Free amino acids in haemolymph of tsetse *Glossina morsitans*

Amino acids	10 days old pupae		Pre-emerged flies		2 days old flies	
	conc. mg/ 100 ml	conc. mM	conc. mg/ 100 ml	conc. mM	conc. mg/ 100 ml	conc. mM
Taurine	127.2	10.17	33	2.67	27	2.15
Methionine sulphoxide	38	2.31	25	1.53	20	1.22
Threonine	45	3.74	8	0.67	10	0.86
Serine	60	5.74	21	2.03	20	1.93
Glutamine	126	8.61	259	17.65	164	11.25
Asparagine	57	4.31	22	1.66	24	1.79
Glutamic acid	203	13.79	16	1.08	25	1.70
Proline	239	10.74	1,088	94.60	690	60.04
Glycine	59	7.93	13	1.72	12	1.64
Alanine	142	15.91	73	8.15	309	34.68
Valine	86	7.34	57	4.90	21	1.78
Methionine	12	0.79	4	0.28	–	–
Isoleucine	36	2.78	12	0.92	9	0.67
Leucine	40	3.06	10	0.77	9	0.66
Tyrosine	76	4.22	237	13.07	40	2.21
Phenylalanine	33	2.03	47	2.84	20	1.19
Lysine	94	6.45	25	1.68	15	1.04
Histidine	70	4.52	14	0.90	16	1.01
Arginine	201	11.54	40	2.28	44	2.54
Aspartic acid	–	–	10	0.78	11	0.86
Ornithine	12	0.93	–	–	–	–
Citrulline	–	–	7	0.42	–	–
Total	1,756		2,021		1,486	

A striking feature was the high concentration of proline particularly in the haemolymph of the pre-emerged flies in which it reached a level of 1,088 mg/ 100 ml. Proline plays an active part in general protein metabolism and is a major component in the protein of insect cuticle (HACKMAN, 1953). The investigations of BURSELL (1963) on the amino acid content of the musculature of *G. morsitans* during the hunger cycle revealed large amounts of proline in the resting flies but the level diminished during flight. The decrease in the concentration of proline in the 2-days old flies in the present studies might have been associated with either flight metabolism or cuticle formation or both. BALOGUN (1969, 1971) recorded relatively low concentrations of proline in the amino acid analysis of extracts of homogenised whole *G. palpalis* flies using the technique of paper partition chromatography. The notable increase in the level of alanine in the 2-days old flies confirms the findings of BURSELL (1963) in which the alanine content of the musculature of the thorax of tsetse flies increased dramatically after flight. Large amounts of alanine were also identified on chromatograms of haemolymph of adult *G. pallidipes* (KNIGHT, 1961) and in extracts of whole flies *G. palpalis* (BALOGUN, 1971). The fluctuation in the quantity of tyrosine could be explained by the general accumulation of tyrosine in the blood during adult development in preparation for the final stages of sclerotization and tanning of the cuticle which takes place at emergence of adult flies. Biochemical studies of this compound

indicate that it enters important metabolic activities of most insects at metamorphosis.

Certain amino acids such as threonine, glycine, methionine, isoleucine, leucine, aspartic acid, ornithine and citrulline were poorly represented in the haemolymph of the flies. A large proportion of the methionine was detected as the sulphoxide which is produced during storage of the haemolymph at –20 °C. The variations in the other amino acid levels are not fully understood and any attempt at interpretation with our knowledge of tsetse physiology would be speculative at present.

The figures obtained may indicate the range of amino acids desirable for the formulation of media for the cultivation of tsetse fly tissues, and they may also be of value for the design of media for the growth of tsetse-borne parasites *in vitro*.

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