

FREE AMINO ACIDS IN THE HAEMOCYTES AND PLASMA OF THE LARVA OF *CALLIPHORA VICINA*

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SUMMARY

Free amino acid concentrations have been measured in haemolymph samples taken from 3rd instar larvae of the blowfly, *Calliphora vicina*, at various stages prior to pupariation. The amino acids found in the haemocyte fraction only accounted for 6% of the total free amino acid concentration of the haemolymph. However, a high percentage of the dicarboxylic amino acids, glutamate and aspartate, 62% and 69% respectively, appeared to be sequestered in the haemocyte fraction at 72 h prior to pupariation. The percentage of the other individual amino acids found in the haemocyte fraction represented less than 10% of their amount in whole haemolymph. It is proposed that these results, together with the increase in the haemocyte levels of glutamate observed after injecting larvae with saline containing glutamate, are one of the first indications of a homeostatic function of insect haemocytes with respect to haemolymph amino acids.

INTRODUCTION

The presence of relatively high concentrations of free amino acids in the haemolymph of insects has been established as a biochemical characteristic of this class (Tsuji, 1909 - cited by Wyatt, 1961; Florkin, 1954; Price, 1961; Schoffeniels & Gilles, 1970). The apparently high concentrations of glutamate are of particular interest in view of the proposed transmitter action of this amino acid at the insect neuromuscular junction (Kerkut, Shapira & Walker, 1965; Usherwood & Machili, 1968; Kravitz *et al.* 1970; Pitman, 1971). It has been suggested that the measured levels of glutamate in the haemolymph of the locust would activate the excitatory synapses and rapidly desensitize the receptors on the post-synaptic membrane to the action of the transmitter released from the motor nerve terminals (Usherwood & Machili, 1968; Miller, Leaf & Usherwood, 1973). Therefore it has been postulated that the neuromuscular junctions of insects must be protected in some way from exposure to these high levels of free glutamate.

It is possible that the neuromuscular junctions of insects *in vivo* possess some kind of structural barrier, such as a glial cell sheath (Faeder & Salpeter, 1970), which

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could restrict the free diffusion of glutamate from the haemolymph into the synaptic cleft. Alternatively, perhaps not all the measured glutamate is in free solution in the plasma. A large percentage of it could be associated with a blood protein fraction or could perhaps be sequestered in a haemocyte compartment. The latter possibility is interesting in view of the demonstration that 58% of the total free amino acid concentration of crab blood is localized in a haemocyte fraction (Evans, 1972). The latter finding in crab haemolymph was confirmed by Miller *et al.* (1973), but they were unable to find an analogous, amino acid enriched, haemocyte fraction in locust blood. Similarly, Holden (1973), working on cockroach haemolymph, reported that only about 12% of the total free amino acid pool was bound to a haemocyte fraction.

A negative result does not, however, rule out this solution to the apparent paradox, as insect haemocytes are notoriously unstable (Gregoire & Florkin, 1950; Crossley, 1964; Brady, 1967). Also, as Miller *et al.* (1973) point out, it is possible that the cells are so fragile that their contents are released even during mild centrifugation. There are indications that the blood of certain insect species may be less likely to clot than others (Yeager & Knight, 1933; Gregoire, 1971; Crossley, 1974*a*), and that certain inhibitors may be used to prevent haemolymph clotting in some insect species (Post, 1972; Crossley, 1974*b*). Thus it seemed desirable to examine the possible role of haemocytes in concentrating amino acids in further insect species.

In the present investigation we have examined the amino acid distribution between the plasma and haemocyte fractions at various stages prior to pupariation of the 3rd instar larvae of the blowfly, *Calliphora vicina*. The clotting reaction of *Calliphora* consists of cell agglutination and fragmentation, and does not appear to involve plasma gelation. Pharmacological inhibition of the agglutination phase of clotting is possible and has been utilized to investigate the effect of clotting on haemolymph amino acid levels. We have also investigated the effect of elevating the haemolymph levels of glutamate on the activity of the larvae and on the amino acid distribution between the plasma and haemocyte compartments of the haemolymph.

MATERIALS AND METHODS

Larvae of *Calliphora vicina* R.D. (C. = *erythrocephala* Meigen) were raised on lean meat at a temperature of 25 °C. Samples of haemolymph were taken from 3rd instar larvae at various times prior to puparium formation. Before sampling, the larvae were washed several times in distilled water, blotted dry and then chilled to 4 °C. Haemolymph was released from larvae by puncturing them with a clean s.s. needle and was drawn directly into a prepared microcap (Drummond Scientific, U.S.A.). The microcaps (20 and 50 µl) were prepared by chilling to 4 °C and were stored in a horizontal rack before use. Haemolymph samples were pooled from at least five animals to give a total volume of 100 µl for each sample analysed. The results were corrected for the loss of haemolymph upon delivery from the microcap.

The sample was expelled into 100 µl of unbuffered saline, which included an internal standard of 0.25 µmoles DL-norleucine, and was contained in a 1 ml polythylene centrifuge tube held at 4 °C. The tube was capped and spun at 8800 *g* for 3 min at 4 °C in a Beckman Microfuge to separate the haemolymph sample into plasma and cell fractions. The supernatant plasma fraction was decanted off from the

pellet using a hypodermic syringe chilled to 4 °C, 200 μ l of 0.4 N perchloric acid was added to each tube, and the samples were spun for 2 min as described above. The supernatants were decanted off from the precipitated protein, and the precipitate was washed with two further 200 μ l aliquots of 0.2 N perchloric acid. The supernatants of the washes were pooled with the originals.

The samples were neutralized with KOH and then stored at 0 °C for 30 min to facilitate the precipitation of the insoluble potassium perchlorate, which was then centrifuged off. The samples were dried down under vacuum and taken up in a 4:1 mixture of 0.1 N-HCl:62.5 % sucrose for application to the column of a Technicon Automatic Amino Acid Analyser. The identification and quantification of each amino acid present was made by comparison with a standard mixture of amino acids run under the same conditions (Evans, 1973). The samples were corrected for losses during the extraction procedure by reference to the recovery of the norleucine internal standard.

Third instar larvae were sampled at 96, 72 and 24 h prior to puparium formation and changes in the amino acid concentrations of the various fractions noted. Samples of haemolymph were also obtained from larvae which had been injected 30 min earlier with 5 μ l of an unbuffered saline solution containing 10^{-5} M vinblastine sulphate (Eli Lilly & Co., Indianapolis, U.S.A.). The above results were compared with those from a plasma sample obtained 15 min after the haemolymph had been allowed to clot.

A haemolymph sample was also taken 72 h prior to pupariation and prepared as above. The cell and plasma fractions were split into two halves one of which was hydrolysed in 2 N-HCl for 1 h prior to analysis. The hydrolysed sample was then compared to the unhydrolysed control.

In a separate series of experiments larvae were injected with 5 μ l of buffered saline containing glutamate at various concentrations (0.01 M, 0.025 M, 0.05 M). These concentrations were calculated to give final glutamate concentrations in larval blood of 2 mM, 5 mM, 10 mM, respectively. The calculations were based on a blood volume of 25 μ l per larva. This volume was estimated by the dye dilution technique of Wheeler (1963) and confirmed in a series of bleeding experiments. In the latter case a series of weighed larvae were bled on to absorptive paper and then reweighed. The estimates of the blood volume obtained by the two methods agreed well. The amino acid levels in haemolymph samples taken immediately after injection of 5 μ l of 0.02 M glutamate saline were compared with those from samples taken 15 min after a similar injection, when the activity of experimental animals had regained that of the controls (see Fig. 1). Activity of larvae was assessed by measurement of movement away from a light by klino-taxis. The larvae used were at the 'wandering' stage, and pupated 48–72 h later. They were kept in darkness before each experiment (Mast, 1911). The klino-taxis stimulus was a horizontal beam of tungsten light which, after passing through a heat filter, gave an intensity of illumination of 550 lux at the starting-point of each migration. Distances covered over a paper surface in 1 min were measured from this starting-point (Fig. 1).

The salines used in this study had the following compositions: (a) unbuffered: NaCl 0.15 M; KCl 0.038 M; glucose 0.054 M. (b) buffered: NaCl 0.15 M; KCl 0.038 M; Tris 0.05 M. These simple salines were isosmotic with *Calliphora* haemolymph.

Table 1. *The levels of free amino acids in haemolymph form 3rd instar larvae of Calliphora vicina 72 h prior to pupariation*

	Whole blood	Plasma	Cells	% in cell fraction	Clotted blood	+inhibitor		
						Whole blood	Plasma	Cells
Taurine	2.67	4.83	0.15	3.0	5.74	2.52	2.43	0.12
Aspartate	0.14	0.04	0.09	69.2	—	0.04	0.03	0.02
Threonine	—	0.91	0.11	10.8	—	—	—	—
Serine	—	2.00	0.19	8.7	—	—	—	—
Glutamine	—	8.20	0.05	0.5	—	—	—	—
Glutamate	1.80	0.50	0.90	61.7	2.97	0.23	0.25	0.15
Proline	7.02	7.10	0.54	7.1	9.50	9.28	7.10	0.24
Glycine	4.62	5.97	0.10	1.6	6.09	5.06	5.97	0.21
Alanine	5.68	6.41	0.69	9.7	9.14	6.59	6.41	0.22
Valine	2.33	1.27	0.08	5.9	1.38	1.96	1.27	0.05
Methionine	0.16	0.13	0.02	13.3	0.53	0.11	0.14	0.01
Isoleucine	0.53	0.51	0.02	3.8	0.38	0.36	0.44	0.03
Leucine	0.82	0.80	0.03	2.0	0.62	0.45	0.61	0.24
Tyrosine	6.66	3.81	0.03	1.3	3.10	7.00	6.68	0.05
Phenylalanine	2.59	2.37	0.06	2.5	1.33	1.78	1.78	—
Lysine	3.51	3.76	0.15	3.8	3.27	2.19	2.30	0.06
Histidine	2.05	2.91	0.06	2.0	2.00	2.59	2.10	0.09
Arginine	2.14	2.81	0.01	3.6	1.17	0.66	0.67	0.07
Total	42.72	54.33	3.28	5.7	47.12	40.82	38.18	1.56

Plasma and whole blood levels are expressed as m-moles/litre of haemolymph. Cell fraction levels are expressed as m-moles of amino acid in cell fraction from one litre of haemolymph. The percentage of the total haemolymph amino acids in the cell fraction is calculated as $\text{cells}/(\text{cells} + \text{plasma}) \times 100$.

RESULTS

Values for the free amino acid levels of whole haemolymph, plasma and haemocyte fractions for samples taken from *Calliphora* larvae at 72 h prior to pupariation are contained in Table 1. The results are expressed as mmoles per litre of haemolymph for the whole haemolymph and plasma fractions, and as m-moles of amino acid in cell fraction from 1 l of haemolymph for the haemocyte fraction. It can be seen that the amino acids in the cell fraction only accounted for about 6% of the total present in the whole haemolymph samples. However, an examination of the distribution of the individual amino acids reveals that a high percentage of the dicarboxylic amino acids, aspartate and glutamate, are localized in the haemocyte fraction. In this species, 69% of the aspartate and 62% of the glutamate appear to be associated with the haemocyte fraction. The other amino acids estimated were present in the haemocyte fraction at levels less than 10% of their respective concentrations in whole haemolymph, at this stage during the development of the larvae.

When the above results were compared with those obtained from acid hydrolysed samples, it was found that the levels of certain amino acids had increased, in both the plasma and haemocyte fractions. These increases were especially marked for the acidic amino acids, aspartate and glutamate. The increase in the glutamate peak was more than could be accounted for by a quantitative conversion of the glutamine peak alone, and suggests the presence of bound amino acids, presumably as peptides. Similar marked increases in aspartate and glutamate have been reported in the peptide hydrolysates isolated from *Drosophila* (Mitchell & Simmons, 1962), mosquitoes (Chen

Table 2. Amino acid levels in haemolymph samples from larvae of *Calliphora* at various stages of 3rd instar prior to pupariation

Time to pupariation (h)	Whole blood			Plasma			Cells			% in cell fraction		
	96	72	24	96	72	24	96	72	24	96	72	24
Taurine	6.34	2.67	4.82	6.20	4.83	4.73	0.14	0.15	0.09	2.2	3.0	1.9
Aspartate	0.10	0.14	0.09	0.05	0.04	0.03	0.05	0.09	0.06	50.0	69.2	66.7
Glutamate	1.53	1.80	0.95	0.86	0.50	0.49	0.62	0.90	0.46	43.1	61.5	48.4
Proline	10.66	7.02	4.77	10.52	7.10	4.65	0.14	0.54	0.12	1.3	7.1	2.5
Glycine	7.56	4.62	3.12	7.38	5.97	2.97	0.18	0.10	0.15	2.4	1.6	4.8
Alanine	6.70	5.68	11.22	6.58	6.41	10.98	0.14	0.69	0.27	2.1	9.7	2.4
Valine	0.65	2.33	3.46	0.54	1.27	3.38	0.02	0.08	0.08	3.6	5.9	2.3
Methionine	0.24	0.16	0.75	0.24	0.13	0.72	0.01	0.02	0.02	4.2	13.3	2.7
Isoleucine	0.21	0.53	0.77	0.18	0.51	0.74	0.02	0.02	0.03	10.0	3.8	3.9
Leucine	—	0.82	0.91	0.47	0.80	0.84	—	0.03	0.06	—	2.0	6.7
Tyrosine	3.22	6.66	11.00	3.16	3.81	10.67	0.06	0.03	0.33	1.9	1.3	3.0
Phenylalanine	0.86	2.59	3.50	0.83	2.37	3.36	0.03	0.06	0.14	3.5	2.5	4.0
Lysine	2.20	3.51	3.78	2.03	3.76	3.69	0.17	0.15	0.10	7.4	3.8	2.6
Histidine	3.81	2.05	—	3.74	2.91	—	0.08	0.06	—	2.1	2.0	—
Arginine	2.34	2.14	3.91	2.23	2.81	3.87	0.11	0.01	0.04	4.7	3.6	1.0
Total	46.42	42.72	53.05	45.01	43.22	51.12	1.77	2.93	1.95	3.8	6.4	3.7

Values are expressed as in legend to Table 1.

1963), and whole larvae of the blowfly *Phormia regina* (Levenbook & Dinamarca, 1966). The transitory existence of such peptides in the haemolymph of the 3rd instar larvae of *Phormia regina* has been noted by Levenbook (1966).

Also included in Table 1 are values from a sample of whole haemolymph that was allowed to clot before analysis, as well as values for samples taken from larvae injected with 10^{-5} M vinblastine sulphate, which prevents clotting in this species (Crossley, 1974*b*). The values for clotted haemolymph are in close agreement with the analyses of unclotted haemolymph. The results with the inhibitor, however, indicated that this treatment caused specific changes in the levels of certain amino acids such as glutamate and arginine. The latter amino acids were substantially reduced in comparison with their values in the untreated controls. However, in both cases the percentage remaining in the cell fraction was of comparable magnitude to that of the untreated controls.

The variation in the free amino acid levels in haemolymph sampled at various stages of the 3rd instar larva of *Calliphora* prior to pupariation is presented in Table 2. It can be seen that the large percentages of aspartate and glutamate in the cell fraction are maintained at all the sampling times. Other changes observed as pupariation approached were decreases in the levels of proline and glycine, and increases in the levels of alanine in the whole blood and plasma fractions. There were also notable increases in the levels of tyrosine and phenylalanine. The present study indicates that the levels of tyrosine and phenylalanine increase in the haemocyte fraction as well as in the plasma, such that the percentage in the cell fraction remains roughly constant. The slight differences between the sum of the plasma plus cell samples and the whole blood samples are thought to be due to individual variation among the batches of animals sampled.

The plasma levels of free glutamate from the present study are about 50% lower

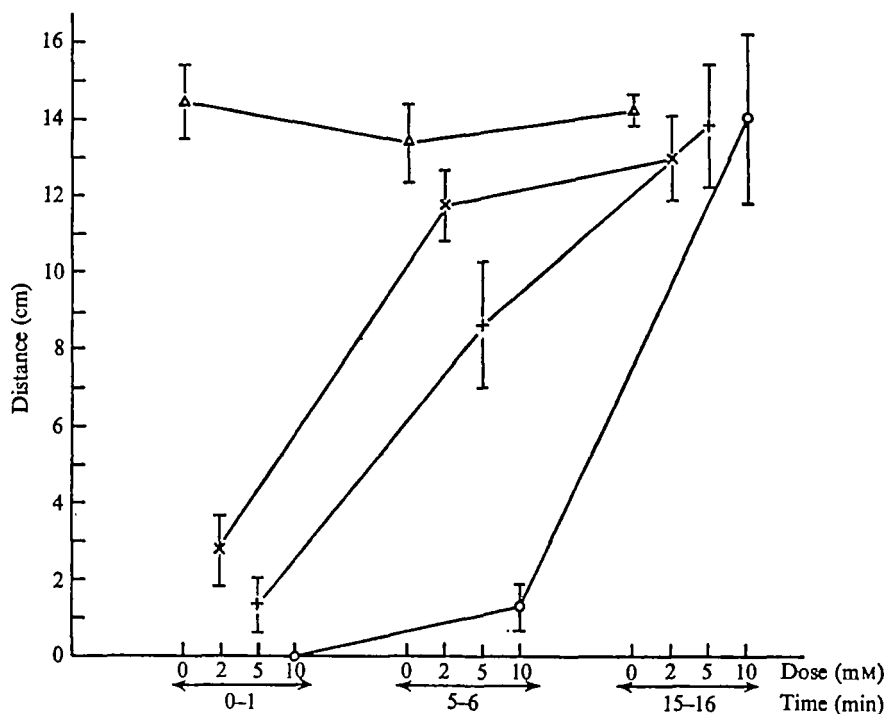


Fig. 1. The distance moved (in cm) by wandering larvae at different times following injection of glutamate. Distances covered in 1 min were measured immediately following injection, and at 5 and 15 min after injection. Injections were adjusted to give final concentrations of 2, 5 and 10 mM glutamate in the haemolymph. Control larvae were injected with buffered saline (0 mM). Bars denote standard errors of the means.

Table 3. *Amino acid levels in haemolymph samples from larvae of Calliphora after injections of 5 μ l of 0.02 M glutamate*

Time after injection (min)	Plasma		Cells	
	0	15	0	15
Taurine	3.21	1.96	0.26	0.21
Aspartate	0.07	0.11	0.03	0.10
Glutamate	1.70	1.86	0.20	0.86
Proline	5.90	7.76	0.37	0.62
Glycine	4.18	5.04	0.29	—
Alanine	—	5.88	0.15	—

The plasma levels are expressed as m-moles/litre of haemolymph and the cell levels as m-moles of amino acid in the cell fraction from 1 l of haemolymph.

than the corresponding whole haemolymph values. It is, however, not known if this level of glutamate in the plasma would interfere with neuromuscular transmission in this species.

A series of experiments was performed in which the blood glutamate levels of larvae were artificially increased by injection of buffered saline containing glutamate. Fig. 1 shows the effect on klino-taxis of larvae at various times after the injection. It can be seen that the activity of larvae injected with saline containing glutamate was

seriously impaired in comparison with the activity of control larvae injected with buffered saline alone. The degree of inactivity was directly related to the concentration of glutamate injected, as was the time taken to return to normal activity levels. Larvae injected with sufficient glutamate to raise the level in the blood to 10 mM were completely paralysed, but were able to recover to normal activity levels within 15 min following the injection. Other kinds of activity measures, such as the number of head movements/unit time, gave results that were similar to the kline-taxis data presented here. The literature on kline-taxis is reviewed by Fraenkel & Gunn (1961).

Blood samples were taken for analysis from larvae injected with 5 μ l of 0.02 M glutamate, immediately after injection and also after 15 min, when the larvae had almost recovered the control activity levels (Table 3). The samples taken immediately after injection showed only a small part of the expected increase in glutamate levels in the plasma fraction. We calculated that the above injection should have raised the total concentration of glutamate by about 3–4 m-moles/l of haemolymph, assuming a haemolymph volume of 25 μ l at 72 h before pupariation. This was probably due to the slow diffusion of the injected amino acid to the sampling site, so that it had not equilibrated throughout the blood space at the time of sampling. After 15 min there was only a slight increase in the plasma levels of glutamate, but the haemocyte fraction, on the other hand, showed a large increase in the glutamate levels between the zero time and 15 min samples.

DISCUSSION

The results of the present study are in close agreement with previous studies on changes in free amino acid levels in the haemolymph of 3rd instar larvae of cyclorhaphous diptera (*Calliphora augur* – Hackman, 1956; *Calliphora erythrocephala* – Price, 1972; *Phormia regina* – Levenbook, 1966; Levenbook & Dinamarca, 1966). Only a small percentage of the total free amino acid content of haemolymph is contained in the haemocyte fraction from larvae of *Calliphora vicina*. This is in agreement with the measurements of Miller *et al.* (1973) on locust blood and of Holden (1973) on cockroach blood. The results on insect haemolymph, however, contrast with the findings on crustacean haemolymph (Evans, 1972; Miller *et al.* 1973), where Evans found that 58% of the total free amino acids of the haemolymph was sequestered in the haemocyte fraction, which only occupied 1% of the total blood volume.

An examination of the free amino acid distribution between the plasma and haemocyte fractions for the individual amino acids in the haemolymph of *Calliphora*, however, revealed that the dicarboxylic amino acids, glutamate and aspartate, were highly localized in the haemocyte fraction. Approximately 69% of the aspartate and 62% of the glutamate found in whole haemolymph samples from larvae of *Calliphora*, 72 h prior to pupariation, were found to be associated with the haemocyte fraction, whilst the haemocyte fraction only occupied about 2% of the total blood volume. In the present study all amino acid values for the haemocyte fraction were corrected for plasma contamination of the cell pellet by reference to the standard aliquot of norleucine added to the haemolymph sample before centrifugation. Although under the conditions of the present estimation little or no clotting of the haemolymph was observed, it is nevertheless possible that a small percentage of the cells, perhaps a selected subpopulation, could have been ruptured during fractionation. The values

presented in this study for the size of the free amino acid pool associated with the haemocyte fraction must therefore be considered as minimal estimates.

Concentrations of dicarboxylic amino acids that were high in comparison to total haemolymph levels have been found in other insect tissues; for example, glutamate concentrations in the gut of *Prodenia* larvae (Levenbook, 1962), and glutamate and aspartate concentrations in the salivary glands of *Drosophila* (Chen, 1966) and in cockroach nerve cord (Evans, 1974). Thus the discovery of relatively high intracellular concentrations of dicarboxylic amino acids in the haemocytes of *Calliphora* could be a general biochemical characteristic shared with many other insect tissues.

Although electrophysiological data concerning the effects of glutamate on excitatory transmission in larvae of *Calliphora* are not yet available, the present results provide circumstantial evidence that small increases in blood glutamate levels interfere with the functioning of the neuromuscular system. Paralysis resulting from the introduction of glutamate to give a final concentration of 10 m-moles/l of haemolymph is relieved within 15 min. During the same time period, glutamate accumulates in the haemocyte fraction. Haemocytes thus appear to be capable of selectively accumulating and retaining dicarboxylic amino acids against a concentration gradient. The failure of previous workers to obtain a haemocyte fraction with an enrichment of any of the amino acids could be due, as Miller *et al.* (1973) suggest, to the fragility of the blood cells in the species they studied. They further suggested that this possibility was unlikely, since they did not obtain any direct proportionality between simultaneous glutamate estimations and total blood cell counts. However, it has been proposed that the multiplicity of functions, and the diverse morphology and histochemistry of insect haemocytes, are indications of cell differentiation (Crossley, 1974a), or at least of a functional flexibility of one basic cell type (Scharer, 1972). Thus not all the haemocytes counted by previous workers may be involved in the functional accumulation of dicarboxylic amino acids. Indeed, Crossley (1964) presents evidence that the proportions of histochemically different haemocyte types vary at different stages in the development of the larvae of *Calliphora*.

Artificially increasing the haemolymph glutamate levels of *Calliphora* larvae by injections of saline containing glutamate temporarily inhibited the activity of the larvae. The period of recovery depended upon the amount of glutamate injected. The increase in the amount of glutamate in the haemocyte pool 15 min after the injection of 5 μ l of 0.02 M glutamate suggests that one of the factors contributing to the recovery of motility by the larvae could be an uptake of glutamate by the haemocytes. In comparison, it is interesting to note that after injections of large amounts of glutamate (500–5000 μ g) into cockroaches, Holden (1973) noted that the haemolymph glutamate levels were raised during the first hour and then returned to normal. The present findings provide evidence for the protective role of the haemocytes in this species in maintaining plasma glutamate at lowered levels, which will not interfere with normal excitatory neuromuscular transmission during locomotory activity. It also provides the first indications of a homeostatic function of haemocytes with respect to amino acids.

An examination of the variation in haemolymph amino acid levels in *Calliphora* larvae at various stages in the 3rd instar prior to pupariation failed to show any significant changes in the glutamate and aspartate distribution between the plasma and

haemocyte fractions, except for a slight enrichment of the cell fraction at 72 h prior to pupariation. Similar results were observed in haemolymph from the 3rd instar larva of *Phormia regina* (Levenbook, 1966; Levenbook & Dinamarca, 1966). During the sampling period the proline levels in the whole haemolymph and plasma fractions declined and the alanine levels in these samples increased, suggesting that proline could be used as an energy source which is mobilized by the larvae prior to pupariation (Table 2). Proline has also been suggested to be an energy store in the haemolymph and muscles of the cockroach (Holden, 1973).

The present study has also confirmed the findings of Levenbook & Dinamarca (1966), Price (1970, 1972), Post (1972) and Post & DeJong (1973), who found that the levels of free tyrosine in insect haemolymph increase dramatically prior to pupariation. The present study indicates that the amount of free tyrosine in the haemocyte pool also increases in proportion to plasma levels, and that there is also a parallel increase in the levels of phenylalanine in both fractions during this period. However, the percentages of the total free tyrosine and phenylalanine in the haemocyte fraction remains roughly constant. This is probably due to a corresponding increase in the numbers of haemocytes in the haemolymph of *Calliphora* larvae during the same period (Crossley, 1974*a, b*). It seems likely that the increase in tyrosine levels at this stage is due to a release of tyrosine from the fat body at the commencement of the formation of the puparium, which causes a doubling in the levels of tyrosine in the haemolymph (Price, 1972).

The results in this paper provide an indication of the importance of the haemocyte fraction in maintaining low levels of free glutamate in the plasma of *Calliphora* larvae. At present it is difficult to determine whether this is a general phenomenon for all insect species. It would be interesting to have comparative data for insects showing plasma gelation following haemocyte breakdown. However, the difficulty here is to inhibit this type of clotting without grossly interfering with cell function. One possibility might be the use of X-irradiation (Gregoire, 1955). An extension of the preliminary studies on stabilization factors for the haemocytes of various other species is needed before the generality of the above findings can be established.

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