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## A Study of the Free Amino Acids from the Haemolymph of Crustacea

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With 4 Figures

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DELAUNAY (1927) reported that the haemolymph of *Astacus astacus* contains 22.2 mg of free amino acids per 100 ml. But CAMIEN et al (1951) reported that the free amino acid concentration of the haemolymph in this species is 80.3 mg/100 ml. Similar differences in results on the content of free amino acids in the haemolymph are found also in respect of *Homarus* sp.; while CAMIEN et al (1951) reported that haemolymph of *Homarus gammarus* contains 64.1 mg of free amino acids per 100 ml, STEVENS et al (1961) observe that the same in *Homarus americanus* is only 21.92 mg/100 ml. The

significance of such differences in the reports by previous workers on one and same species or closely related ones, has not so far been commented

GILBERT (1959) reported that the constituents of haemolymph vary much in relation to size and sex in the crab *Carcinus maenas*. The works of NAIDU (1966) SUNDARA RAJULU (1970) and SUNDARA RAJULU and SANTHANAKRISHNAN (1971) indicate more specifically that the concentrations of free amino acids differ markedly in relation to size and sex in arachnids and myriapods. Therefore it is of interest to know if the variations in the results of previous workers on the free amino acid content of the haemolymph of crustaceans is due to the effect of sex and size. The present investigation has been undertaken to throw some light on this problem.

## Materials and methods

The species investigated were the marine stomatopod *Squilla holoschista*, brackish water prawn *Metapenaeus monoceros*, fresh water crab *Paratelphusa hydrodromous* and the terrestrial sand burrowing crab *Ocypoda ceratophthalma*. The stomatopod is commonly available along the coast of Madras and the ocypods were obtained from the Marina beach. The prawns were collected from Adayar estuary in the vicinity of Madras and the fresh water crabs from the paddy fields in Tambaram.

All the crustaceans were brought to the laboratory within a few hours of collection and kept in separate tanks under conditions similar to their natural habitats. They were starved for a day prior to investigations, to eliminate the variations, if any, in the free amino acids of the haemolymph due to differential feeding. All the animals used were of stage C (DRACH, 1939).

## Collection of Haemolymph

Animals removed from the tanks, were blotted and cleaned using brush to remove the surplus adhering water and sand or mud, and weighed in grams to the nearest first decimal. The haemolymph from *Metapenaeus monoceros* and *Squilla holoschista* was drawn by introducing a syringe needle under the median frontal margin of the carapace into the region adjacent to the posterior gastric mill (DALL, 1964). From the crabs the same was obtained through a small incision in the arthrodistal membrane at the base of the large chela. Samples of haemolymph collected from males and females were pooled separately and centrifuged at 5°C for 15 minutes at 2000 rev/min to separate the haemocytes and the coagulum. The clear supernatant was collected and deproteinized following the method of STEIN and MOORE (1954).

## Quantitative estimation of total free amino acids

Folin method as given in HAWK et al (1954) was adopted for quantitative estimation of total free amino acid concentration of the haemolymph.

## Quantitative estimation of individual free amino acids

Samples of 1 to 2 ml of deproteinized haemolymph were analysed in an automatic amino acid analyzer of the type similar to the one described by SPACKMAN et al (1958). Quantitation was based on the ninhydrin colouring intensity of the effluent from ion exchange columns. The absorbancy of the colour, developed, was measured at 570  $\mu$  and 440  $\mu$  in spectrophotometer. The peaks on the recorded curve were integrated for load varying from 0.1 to 3.0  $\mu$  moles for each amino acid.

## Results

### Total concentration of free amino acids

#### 1. *Squilla holoschista*

The results of estimations of total content of free amino acids from the haemolymph of *Squilla holoschista* of ten different sizes, as indicated by weight, and both the sexes are presented in table 1. It may be noted that males have always higher concentration

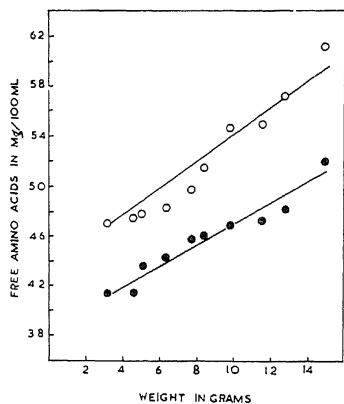


Fig. 1. Figure showing the influence of size on the free amino acid content of the haemolymph of *Squilla holoschista*. Open circles stand for males and closed circles for females.

Table 1

Results of quantitative estimations of free amino acids of the haemolymph of *Squilla holoschista*

S No.	Wt of animal (g)	Free amino acid content (mg/100 ml)	
		Male	Female
1	3.2	47.12	41.47
2	4.6	47.50	41.52
3	5.1	47.84	43.68
4	6.4	48.32	44.34
5	7.7	49.76	45.75
6	8.3	51.45	46.13
7	9.8	54.65	46.89
8	11.6	55.00	47.25
9	12.8	57.19	48.13
10	14.9	61.20	52.00
Average		52.00	45.72

of free amino acids than females; a smaller male weighing 3.2 grams has 47.12 mg of free amino acids per 100 ml of haemolymph but among females only in larger ones of weight 11.6 grams the free amino acid concentration is 47.25 mg/100 ml.

It may also be seen that size has influence on the haemolymph free amino acid concentration in both females and males; the larger individuals have always higher concentration than the smaller ones (Fig. 1).

#### 2. *Metapenaeus monoceros*

From the results given in table 2, it may be evident that *Metapenaeus monoceros* has a lesser free amino acid concentration in the haemolymph than *Squilla holoschista*; the average value for males being 39.88 mg/100 ml and in females the same is 30.66 mg/100 ml. However, the effect of sex and size on the quantity of free amino acids in this species is the same as in *Squilla holoschista* (Fig. 2).

#### 3. *Paratelphusa hydrodromous*

The content of free amino acids in the haemolymph of *Paratelphusa hydrodromous* is lower than those in *Squilla holoschista* and *Metapenaeus monoceros*. The males of

this species have 28.26 mg/100 ml in average and for females the average value is 25.58 mg/100 ml (Table 3). The effect of sex and size on the concentration of free amino acids in the haemolymph in this species, however, is similar to that in the other two species (Fig. 3).

#### 4. *Ocypoda ceratophthalma*

The least amount of free amino acids in the haemolymph is found in *Ocypoda ceratophthalma*; the females and males of this species have, respectively, 21.45 mg and 25.16 mg per 100 ml in average (Table 4). The influence of size and sex is nevertheless the same as in other species (Fig. 4).

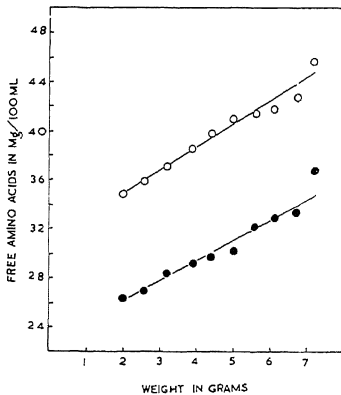


Fig. 2. Figure showing the effect of size on the free amino acid concentration of the haemolymph of *Metapenaeus monoceros*.

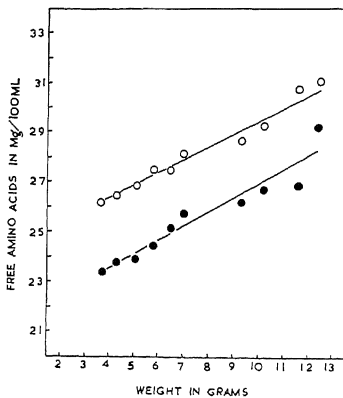


Fig. 3. Figure showing the influence of size on the free amino acid content of the haemolymph of *Paratellphusa hydrodromous*.

Table 2

Results of quantitative estimations of free amino acids of the haemolymph of *Metapenaeus monoceros*

S. No.	Wt. of animal (g)	Free amino acid content (mg/100 ml)	
		Male	Female
1	2.0	34.91	26.43
2	2.6	35.85	27.01
3	3.2	37.21	28.45
4	3.9	38.63	29.18
5	4.4	39.75	29.76
6	5.0	41.00	30.28
7	5.6	41.37	32.17
8	6.1	41.80	33.03
9	6.7	42.69	33.49
10	7.2	45.60	36.75
Average		39.88	30.66

Table 3

Results of quantitative estimations of free amino acids of the haemolymph of *Paratellphusa hydrodromous*

S. No.	Wt. of animal (g)	Free amino acid content (mg/100 ml)	
		Male	Female
1	3.7	26.21	23.43
2	4.3	26.52	23.82
3	5.1	26.91	23.97
4	5.8	27.47	24.50
5	6.5	27.50	25.19
6	7.0	28.15	25.80
7	7.9	28.73	26.17
8	10.2	29.25	26.66
9	11.6	30.80	26.93
10	12.4	31.10	29.31
Average		28.26	25.58

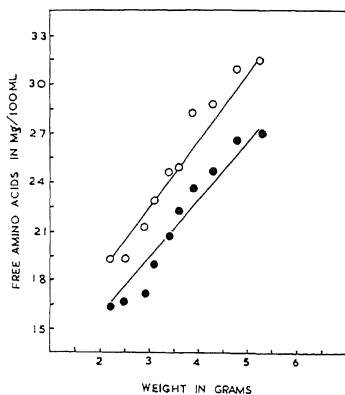


Fig. 4. Figure showing the effect of size on the concentration of free amino acids in the haemolymph of *Ocyroda ceratophthalma*.

Table 4

Results of quantitative estimations of free amino acids of the haemolymph of *Ocyroda ceratophthalma*

S No.	Wt. of animal (g)	Free amino acid content (mg 100 ml)	
		Male	Female
1	2.2	19.25	16.43
2	2.5	19.30	16.70
3	2.9	21.24	17.21
4	3.1	22.82	19.05
5	3.4	24.62	20.80
6	3.6	24.95	22.33
7	3.9	28.21	23.62
8	4.3	28.84	24.73
9	4.8	30.90	26.62
10	5.3	31.50	27.05
Average		25.16	21.45

#### Differential concentration of free amino acids

The sample analysed for estimations of concentration of individual amino acids in every species, is a pool of deproteinized haemolymphs obtained from individuals of different sizes of both the sexes so that the values are applicable to the entire species. It may be evident from the results recorded in table 5, that the stomatopod and the prawn have 16 free amino acids while both the crabs have only 15 of them. The amino acid which is lacking in the crabs is taurine. Otherwise they resemble the other two species in all the respects. The free amino acids common to all the four crustaceans are alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine. A feature of interest is the predominance of glycine over the other amino acids present, in all the forms. The other conspicuous amino acids in all the species are alanine, aspartic acid, proline and tyrosine.

#### Discussion

It may be seen from the results reported in the present investigation that the marine crustacean *Squilla holoschista* has the maximum amount of free amino acids in the haemolymph, i. e. 51.3 mg/100 ml, and the least concentration is found in the terrestrial crab *Ocyroda ceratophthalma*, where it is 24.1 mg/100 ml. Of the other two remaining species, the prawn *Metapenaeus monoceros* from the brackish water has higher free amino acid level, 38.1 mg/100 ml, than the fresh water crab *Paratelphusa hydrodromous* which contains 27.2 mg/100 ml.

Despite these differences, sex and size have similar influence in all the four species; males have always higher concentration than the females and in both the sexes larger ones have higher concentration of free amino acids in the haemolymph than the smaller ones. For instance, a smaller female individual of *Ocyroda ceratophthalma* weighing

2.2 grams has only 16.43 mg/100 ml. But a larger male of this species of 5.3 grams weight contains almost double the concentration, i. e. 31.50 mg/100 ml. GILBERT (1959 a, b) also reports a similar phenomenon with reference to inorganic ions and non-protein nitrogen in a crab *Carcinus maenas*. Therefore when sex and size of individuals studied are not taken into consideration, the values recorded may not be valid for the entire species. In the light of this, it is suggested that the differences in the data on the haemolymph free amino acids in crustaceans by previous workers may be due to these reasons.

Another significant feature is the distribution of taurine in the species investigated. This amino acid is present in *Squilla holoschista*, which is marine, and in the brackish

Table 5

Free amino acids of the haemolymph of *Squilla holoschista*, *Metapenaeus monoceros*, *Paratelphusa hydrodromous* and *Ocypoda ceratophthalma* (mg/100ml)

S. No.	Amino acids	<i>Squilla holoschista</i>	<i>Metapenaeus monoceros</i>	<i>Paratelphusa hydrodromous</i>	<i>Ocypoda ceratophthalma</i>
1	Alanine	7.1	3.2	3.1	2.5
2	Arginine	1.7	1.0	0.7	0.6
3	Aspartic acid	5.3	3.6	2.5	2.0
4	Glutamic acid	2.5	1.5	1.8	1.1
5	Glycine	15.1	7.0	6.4	6.2
6	Histidine	3.0	2.2	1.3	0.9
7	Isoleucine	2.4	2.3	0.7	0.4
8	Leucine	1.9	2.5	0.9	0.5
9	Lysine	0.7	0.6	0.6	0.9
10	Phenylalanine	0.6	0.7	1.1	1.2
11	Proline	2.2	4.2	2.3	1.7
12	Serine	0.5	2.2	1.1	1.3
13	Taurine	3.1	1.2	—	—
14	Threonine	0.3	1.1	2.5	1.8
15	Tyrosine	3.1	2.4	1.3	2.3
16	Valine	1.8	2.4	0.9	0.7
	Total	51.3	38.1	27.2	24.1

water inhabitant *Metapenaeus monoceros*; the former has a higher concentration of taurine, 3.1 mg per 100 ml. of haemolymph, than the latter species which contains 1.2 mg/100 ml. The other two species *Paratelphusa hydrodromous* and *Ocypoda ceratophthalma*, which are respectively freshwater and terrestrial forms, do not have this amino acid in the haemolymph. It is of interest to recall in this context that a similar observation has been reported by SIMPSON et al (1959) who found that taurine as free amino acid is absent in the haemolymph of fresh water and terrestrial molluscs but present in brackish water and marine species. This feature has been related to the phenomenon of osmoregulation in molluscs. It is not known if this is the case with Crustacea also. Therefore this feature deserves further study.

A comparison of the results obtained in the present study with those reported from other groups of arthropods may indicate a close similarity among crustaceans and arachnids. SUNDARA RAJULU and SANTHANAKRISHNAN (1971) report that the free amino acid concentration in the haemolymph of arachnids studied by them varies from 22.9 to 51.1 mg/100 ml. A most interesting resemblance is that in Arachnida also males have a higher level of free amino acids in the haemolymph than the females (NAIDU, 1966; SUNDARA RAJULU and SANTHANAKRISHNAN 1971). But the

condition in other groups of arthropods like Diplopoda and Chilopoda is reported to be reverse, in that the females always have a higher quantity of haemolymph free amino acids than males (SUNDARA RAJULU 1970). In addition the values of free amino acids in the haemolymph for Onychophora, Myriapoda and Insecta are much higher than those recorded for Crustacea and Arachnida, the minimum being 236.6 mg/100 ml in a centipede *Ethmostigmus spinosus* (SUNDARA RAJULU 1970; SUNDARA RAJULU and RAMANUJAM 1971) and the maximum value is 2340 mg/100 ml in an insect *Bombyx mori* (FLORKIN 1949; WYATT et al 1956).

This similarity in the free amino acid concentrations of the haemolymph, in Crustacea and Arachnida on one hand and Onychophora, Myriapoda and Insecta on the other seems to have some phylogenetic significance as discussed by SUNDARA RAJULU and SANTHANAKRISHNAN (1971).

### Summary

The free amino acid concentration in the haemolymph has been studied with reference to four species of crustaceans *Squilla holoschista*, *Metapenaeus monoceros*, *Paratellphusa hydrodromus* and *Acyppoda ceratophthalma*. These species contain, respectively, 51.3, 38.1, 27.2 and 24.1 mg of free amino acids per 100 ml of haemolymph. Glycine is predominant over the other amino acids present, in all the species. Taurine is present only in the marine and brackish water forms. The results were compared with those reported by previous workers on other Crustacea and other groups of arthropods. The pattern of distribution of haemolymph free amino acids in different groups of arthropods seems to have phylogenetic significance.

### Zusammenfassung

#### *Über die freien Aminosäuren in der Haemolymph von Crustacea*

Die Konzentration freier Aminosäuren in der Haemolymph von 4 Arten, nämlich *Squilla holoschista*, *Metapenaeus monoceros*, *Paratellphusa hydrodromus* und *Acyppoda ceratophthalma*, wurde untersucht. Diese Arten enthalten 51.3, 38.1, 27.2 und 24.1 mg freier Aminosäuren in 100 ml Haemolymph. Glycine überwiegt die anderen vorhandenen Aminosäuren bei allen Arten. Taurine kommt nur bei Formen vor, die im Meer oder im Brackwasser leben. Die Ergebnisse werden mit jenen früherer Arbeiten über andere Crustacea- und andere Arthropodengruppen verglichen. Die Zusammensetzung der freien Aminosäuren in der Haemolymph verschiedener Arthropodengruppen scheint von phylogenetischer Bedeutung zu sein.

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### References

- CAMIEN, M. N.; SARLET, H.; DUCHATEAU, G.; FLORKIN, M., 1951: Nonprotein amino acids in muscle and blood of marine and fresh water Crustacea. *J. Biol. Chem.* 193, 881-885.
- DALL, W., 1964: Studies on the physiology of a shrimp, *Metapenaeus mastersii* (Haswell) (Crustacea : Decapoda : Penaeidae). 1. Blood constituents. *Aust. J. mar. Freshw. Res.* 15, 145-161.
- DELAUNAY, H., 1927: Recherches Biochimiques sur l'Excretion Azotee des Invertebres. Siraudan, Bordeaux.
- DRACH, P., 1939: Mue et cycle d'intermue chez les Crustace's decapodes. *Ann. Inst. Oceanogr. Monaco*, 19, 103-391.
- FLORKIN, M., 1949: Biochemical evolution (S. Morgulis, editor and translator) New York: Academic Press.
- GILBERT, A. B., 1959a: The composition of the blood of the shore crab, *Carcinus maenas* Pennant, in relation to sex and size. 2. Blood chloride and sulphate. *J. Exp. Biol.* 36, 356-362.

- 1959b: The composition of the blood of the shore crab, *Carcinus maenas* Pennant, in relation to sex and size. 3. Blood non-protein nitrogen. J. Exp. Biol. 36, 495-500.
- HAWK, P. B.; OSER, B. C.; SUMMERSON, W. H., 1954: Practical Physiological Chemistry New York: Mc Graw-Hill.
- MANTON, S. M., 1964: Mandibular mechanisms and the evolution of arthropods. Phil. Trans. R. Soc. B. 247, 1-183.
- NAIDU, B. P., 1966: Ionic composition of the blood and the blood volume of the scorpion, *Heterometrus fulvipes*. Comp. Biochem. Physiol. 17, 157-166.
- SIMPSON, J. W.; ALLEN, K.; AWAPARA, J., 1959: Free amino acids in some aquatic invertebrates. Biol. Bull. Woods Hole, 117, 371-381.
- SPACKMAN, D. H.; STEIN, W. H.; MOORE, S., 1958: A new method of quantitative estimation of amino acids. Anal. Chem. 30, 1190-1202.
- STEIN, W. H.; MOORE, S., 1954: The free amino acids of human blood plasma. J. Biol. Chem. 211, 915-926.
- STEVENS, T. M.; HOWARD, C. E.; SCHLESINGER, R. W., 1961: Free amino acids in sera of the marine invertebrates, *Cancer irroratus*, *Limulus polyphemus* and *Homarus americanus*. Comp. Biochem. Physiol. 3, 310-314.
- SUNDARA RAJULU, G., 1970: A comparative study of the free amino acids in the haemolymph of a millipede, *Spirostreptus asthenes*, and a centipede *Ethmostigmus spinosus* (Myriapoda). Comp. Biochem. Physiol. 37, 339-344.
- SUNDARA RAJULU, G.; RAMANUJAM, M., 1971: Free amino acids in the haemolymph of *Eoperipatus weldoni* (Onychophora). Experientia (In press).
- SUNDARA RAJULU, G.; SANTHANAKRISHNAN, G., 1971: An investigation of the free amino acids in the haemolymph of Arachnida. Zool. Anz. (In press).
- WYATT, C. R.; LOUGHHEED, T. C.; WYATT, S. S., 1956: The chemistry of insect haemolymph. Organic components of the haemolymph of the silk worm *Bombyx mori* L. and two other species. J. Gen. Physiol. 39, 853-868.