

Table 2. Analytical Data

Compound	Found (%)				Calculated (%)			
	C	H	N	Sn	C	H	N	Sn
(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> SnCl <sub>2</sub> · L*	31.14	4.22	7.37	30.4	31.12	4.18	7.26	30.76
(C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> ) <sub>2</sub> SnCl <sub>2</sub> · L	47.28	3.88	5.22	23.4	47.10	3.95	5.49	23.27
(C <sub>6</sub> H <sub>5</sub> ) <sub>3</sub> SnCl · L	32.52	2.46	6.51	27.4	32.73	2.52	6.36	26.95

\* L = pyridine-2-carbothioamide.

to involve combination with dithiol groupings in enzymes necessary for mitochondrial oxidative phosphorylation.

**Materials and methods.** Diethyltin dichloride, dibenzyltin dichloride and phenyltin trichloride were obtained from alpha inorganics and used without further purification. Pyridine-2-carbothioamide was prepared from 2-cyanopyridine by the method of Karrer and Schukri<sup>11</sup>. The complexes were prepared by mixing hot benzene solutions

containing stoichiometric quantities of pyridine-2-carbothioamide and the appropriate organotin chloride. On cooling the solutions, the products were obtained as yellow crystals, which were recrystallized from benzene. Analytical data for the complexes are presented in table 2. Infrared spectra were recorded on a Beckman 4250 instrument with samples as KBr discs. Microanalyses were carried out by the Australian Microanalytical Service CSIRO, Melbourne.

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## Free amino acids in some tissues of marine crustacea

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**Summary.** Free amino acids contained in extracts of crustaceans were separated and determined quantitatively by ion exchange chromatography. In the hepatopancreas of the brachyuran crustacean *Carcinus maenas*, glycine, arginine, proline and alanine constituted 78.7% of the total amino acid pool. In *Eriphia spinifrons* and *Maja verrucosa*, glycine, alanine, lysine and leucine comprised 54.9–66.9% of the total content. In the muscle tissue of the macruran crustacea *Palaemon*, glycine, arginine, proline and alanine were the most common free amino acids and comprised 70–95% of the total content. The high concentrations of some amino acids in tissues of crustaceans may play a role in intracellular osmotic regulation and also in the maintenance of ionic hydrogen concentration.

Several authors have shown that crustacean muscles<sup>2</sup>, and also nerves and serum<sup>3</sup> contain high concentrations of free amino acids, particularly glycine. Furthermore, as the absolute free amino acid content appears to be higher in species exposed to higher salinities<sup>4</sup>, it has been suggested that this variation is linked to intracellular osmotic regulation.

The purpose of this study was to determine whether mediterranean crustacea of the section Brachyura contain high concentrations of free amino acids, and also to study the constitution of their free amino acid pools in the hepatopancreas. In addition, as all previous data<sup>2–4</sup> are for benthic crustacea, two sub-planktonic crustacea of the section Macrura were selected for study. Owing to the much reduced hepatopancreas of these latter species their muscle tissue was analyzed.

The data obtained are discussed with regard to their eco-physiological significance.

**Material and methods.** Specimens were caught in the Bay of Naples and kept in tanks with circulating sea water. Dissected organs were stored at –20 °C until analysis. The preparation of amino acid extracts was a modification of the procedure of Tallan<sup>5</sup> and Saifer<sup>6</sup>. For each chromatographic analysis the wet muscle tissue from 3 animals was homogenized in an ice bath for 5 min with a Potter

homogenizer; 10 ml of 6% perchloric acid per g wet weight of tissue was routinely used. The homogenate was centrifuged at 30,000 × g for 30 min at 0–5 °C and the clear supernatant after being neutralized with 2N KOH was left overnight at 4 °C. The potassium perchlorate was removed by centrifugation with 4 ml distilled water and the resulting supernatant fraction was added to the previous one. The supernatant was transferred to a rotatory evaporator and evaporated at 40–50 °C until dry. The residue was dissolved in 4 ml of 0.1 M HCl and centrifuged at 30,000 × g for 30 min. A suitable aliquot of the supernatant was subjected to amino acid-analyses on a Beckman/Spinco mod. 120 according to a modified procedure of Spackman et al.<sup>7</sup>.

**Result and discussion.** Camien et al.<sup>2</sup> determined the non-proteic amino-acid content in muscle of a marine crustacean (*Maja squinado*) and a fresh water one (*Astacus fluviatilis*). They discovered a high concentration of the amino acids glycine, proline, arginine, glutamic acid and alanine. In addition, the absolute concentrations were higher in marine species than in fresh water forms. Others<sup>4</sup> determined the free amino acids in muscle of several crustaceans (*Eriocheir sinensis*, *Carcinus maenas*, *Leander serratus*, *Leander squilla* and *Astacus astacus*) adapted to various salinities. These studies also indicated that the amount of free amino acids increases with salinity.

Table 1. Free amino acid pools for the hepatopancreas of *Brachyura* crustacea ( $\mu\text{moles/g}$  wet weight)

Amino acid	<i>Carcinus maenas</i>		<i>Eriphia spinifrons</i>		<i>Maja verrucosa</i>	
	Mean	Range	Mean	Range	Mean	Range
Alanine	14.0	12.50–15.80	15.8	14.90–16.50	9.2	8.40–10.50
Arginine	44.0	41.40–47.50	4.6	4.10– 5.20	2.6	1.90– 3.80
Aspartic acid	1.3	0.80– 1.60	1.3	1.10– 1.60	3.8	3.10– 4.60
Cystine	1.2	0.70– 1.50	4.8	4.40– 5.50	1.8	1.20– 2.50
Glutamic acid	9.2	7.10–10.40	6.3	4.90– 7.50	4.5	3.50– 4.70
Glycine	144.0	135.20–157.40	20.6	18.40–23.50	30.0	28.10–33.70
Histidine	4.4	3.90– 4.90	1.4	0.90– 2.50	0.4	0.30– 0.55
Isoleucine	0.5	0.40– 0.70	3.4	2.70– 4.10	1.3	0.90– 1.80
Leucine	0.6	0.40– 0.90	10.2	8.80–12.10	4.0	2.90– 5.40
Lysine	1.3	1.10– 1.60	10.7	9.20–12.50	5.5	3.20– 6.40
Methionine	trace	–	2.7	1.90– 3.50	1.5	1.20– 1.80
Phenylalanine	–	–	–	–	–	–
Proline	39.0	37.50–41.40	4.9	3.80– 5.50	2.6	2.20– 3.50
Serine	6.3	5.70– 6.60	7.8	6.50– 8.60	3.3	2.90– 4.10
Threonine	trace	–	3.9	3.10– 4.70	1.8	1.20– 2.50
Tyrosine	trace	–	–	–	–	–
Tryptophan	2.3	1.90– 2.50	–	–	–	–
Valine	–	–	6.0	4.10– 9.40	0.4	0.28– 0.57
Total	268.1		104.4		172.7	

The means are calculated from 3 analyses; each analysis is derived from extracts pooled from 3 animals.

Table 2. Free amino acid pools for muscles of *Macrura* crustacea ( $\mu\text{moles/g}$  wet weight)

Amino acid	<i>Palaemon serratus</i>		<i>Palaemon xyphias</i>	
	Mean	Range	Mean	Range
Alanine	15.4	13.10–19.40	12.9	10.10–14.30
Arginine	30.0	26.40–33.03	22.0	19.50–24.10
Aspartic Acid	0.7	0.58– 0.83	0.5	0.39– 0.57
Cystine	0.4	0.31– 0.53	0.7	0.55– 0.84
Glutamic Acid	1.3	1.10– 1.70	2.0	1.60– 3.40
Glycine	49.5	45.20–53.40	80.0	70.50–98.40
Histidine	0.4	0.30– 0.48	0.7	0.40– 0.90
Isoleucine	0.3	0.28– 0.35	1.0	0.80– 1.30
Leucine	0.6	0.51– 0.74	1.2	1.10– 1.50
Lysine	0.8	0.71– 0.91	3.0	2.60– 3.70
Methionine	0.4	0.32– 0.49	1.2	1.02– 1.50
Phenylalanine	–	–	–	–
Proline	44.0	41.50–47.80	76.8	72.40–81.50
Serine	3.0	1.90– 3.90	15.0	13.50–19.40
Threonine	trace	–	trace	–
Tyrosine	–	–	–	–
Tryptophan	0.1	0.09– 1.51	0.2	0.15– 0.29
Valine	–	–	–	–
Total	146.9		217.2	

The means are calculated from 3 analyses, each analysis is derived from extracts pooled from 3 animals.

Tables 1 and 2 show the free amino acid content of several mediterranean species of crustacean (*Brachyura* and *Macrura*). Each value is the mean concentration from three different chromatographic analyses, each analysis is derived from an extract pooled from three animals. The highest and the lowest values are also given.

The hepatopancreas of the *Brachyura* crustacean *Carcinus maenas* (table 1) contains 15 amino acids: glycine, arginine, proline and alanine which constitute 78.7% of the total amino acid pool, whereas in *Eriphia spinifrons* and *Maja verrucosa* glycine, alanine, lysine and leucine comprise 54.9 and 66.9% of the total amino acid content, respectively. A similar situation is found in the muscle tissue of the *Macrura* crustacea (table 2). In *Palaemon serratus* the most abundant amino acids are: glycine, alanine, arginine and proline, representing 95% of the total amount of amino acids. In *Palaemon xyphias*, glycine, arginine, proline and serine comprise 89% of the total amino acid content.

It is interesting to note that in all the species listed in tables

1 and 2 and in those presented in earlier papers<sup>3</sup>, glycine is the most abundant amino acid. In the hepatopancreas of *Carcinus*, *Eriphia* and *Maja* this neutral amino acid represents 42.5, 19.2 and 41.2%, respectively, of the total amino acid content. In the muscles of *Palaemon serratus* and *Palaemon xyphias* the corresponding values are 33.6 and 36.8% respectively.

Owing to the high concentration of several free amino acids in the muscle tissue of crustaceans<sup>3</sup>, and the fact that the absolute concentration increases with increasing salinity of the environment, some authors<sup>4</sup> have suggested that the free amino acids may be linked with intracellular osmotic regulation. Since I found a similar concentration of the amino acids glycine, alanine, arginine, proline and glutamic acid in the hepatopancreas of crustaceans, I consider that these amino acids may also play another physiological role. Firstly, because these 5 amino acids are already present in high concentrations in relation to the other amino acids in the muscle tissue of all fresh water and marine species of crustacean<sup>3</sup> (and present study), and in addition because 3 of these amino acids are neutral while 1 is acidic and the other is basic. Considering that various enzymes are present at different concentrations depending upon the concentration of  $\text{NaCl}$ <sup>8</sup>, I suggest that this additional physiological role may be the maintenance of the ionic hydrogen concentration.

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