gave the haemin spectrum because of the presence of blood in human bone (figure 1,c); but since both species show roughly the same absorbance values at 700 nm, where haemin absorbs least, it can be assumed that the blank value of human bone, like that of bovine bone, is low and remains approximately constant throughout the bandwidth. Owing to the low absorbance of haemin at 700 nm and the fact that absorption due to the bone substance (blank value) is most readily detectable at this wave-length, 700 nm was selected as the best wave-length for correction of the haemin read-out. The addition of 15 µl of human blood (same blood as in figure 1,b) to the bone homogenate, of which the spectrum is shown in figure 1,c, gave absorbances in the haemin sprectrum at 510 and 700 nm equal to the sum of the 2 individual values (figure 1,d).

The suitability of the method described was demonstrated by analyses of bone homogenates to which blood had been added (table 1). The regression line $y=a+bx$, where y denotes the $A_{510}-A_{700}$ differences for the 3 analyses of mixture 1 and x the blood volumes of these mixtures (10, 15 and 20 µl) (values from table 1), gives an intercept on the y axis at $a=0.0125$, a slope of $b=0.0109$ and a correlation coefficient of 0.999.

$$\text{From the equation } x=\frac{y-a}{b}=\frac{(A_{510}-A_{700})-0.0125}{0.0109}$$

the recovered blood volumes listed in table 1 are obtained for the bone homogenate samples.

Heads of femora removed at surgery from 6 patients requiring prosthetic joints were available to us for analysis. The blood contents of the cancellous bone determined by the haemin method of analysis are shown in table 2.

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Organotin complexes of pyridine-2-carbothioamide

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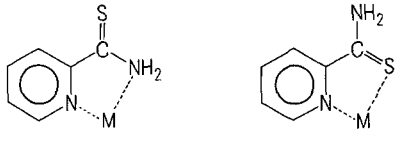
Summary. Complexes of pyridine-2-carbothioamide with diethyltin dichloride, dibenzyltin dichloride and phenyltin trichloride are described. In each case, the chelating agent is bound to the tin(IV) atom by the pyridine nitrogen and the carbothioamide sulphur, giving support to proposals that certain organotin compounds cause enzyme deactivation via the formation of tin-sulphur bonds.

Pyridine-2-carbothioamide could act as a bidentate chelating agent through the pyridine nitrogen and either the nitrogen or the sulphur of the carbothioamide group.

In previous investigations[2–5], the mode of coordination of the carbothioamide group has been inferred from infrared evidence. Bonds in the infrared spectrum of the free base have been variously assigned to N-H stretching and bending modes, to C-N stretching modes and to C=S stretching modes[6,7]. The infrared spectra of the metal complexes reportedly fall into 2 categories, those in which the absorptions due to N-H stretching and bending modes shift to lower energies while the C=S absorption remains unaltered from the values observed for the free base, and those in which the absorptions due to N-H deformations remain unaltered, the C-N absorptions are shifted to higher wavenumbers and the C=S absorptions are shifted to lower wave numbers. The metal complexes with infrared spectra in the 1st category are considered to involve the nitrogen-bonded carbothioamide group, while those in the 2nd category are considered to involve the sulphur-bonded carbothioamide group. The different modes of coordination of the ligand in the various metal complexes appear to correlate with the class A or B character[8,9] of the central metal ion, the class A metals being nitrogen-bonded and the class B metals being sulphur-bonded.

Reaction between pyridine-2-carbothioamide and the mono- and di-alkyltin halides used gave 1:1 complexes in each instance. Infrared data for the free base and the complexes are listed in table 1. For each of the complexes, the N-H stretching and bending modes occur at almost the same frequency as in the free base, the C-N frequency is raised, while the C=S stretching mode has been shifted to lower wave numbers. Thus these complexes fit into the second of the 2 categories described above, and are assigned 6-coordinate structures involving sulphur-bonding to the metal ion by the carbothioamide group.

Tin(IV) is a class A metal ion, and its complexes with pyridine-2-carbothioamide would be expected to be nitrogen-bonded. The unexpected preference by the tin(IV) ion in these complexes for coordination via the sulphur rather than the nitrogen of the carbothioamide group provides some support for the proposed mode of toxic action of dibutyltin diacetate in rats and mice[10], which is considered



Possible modes of coordination of pyridine-2-carbothioamide.

Table 1. Infrared spectral data (cm^{-1})

Compound	ν(NH)	δ(NH)	ν(CN)	ν(CS)
Pyridine-2-carbothioamide (L)	3350	1580	1405	800
$(C_2H_5)_2SnCl_2 \cdot L$	3350	1595	1415	790
$(C_6H_5CH_2)_2SnCl_2 \cdot L$	3340	1590	1420	785
$(C_6H_5)_3SnCl \cdot L$	3350	1590	1430	775

Table 2. Analytical Data

Compound	Found (%)				Calculated (%)			
	C	H	N	Sn	C	H	N	Sn
$(C_2H_5)_2SnCl_2 \cdot L^*$	31.14	4.22	7.37	30.4	31.12	4.18	7.26	30.76
$(C_6H_5CH_2)_2SnCl_2 \cdot L$	47.28	3.88	5.22	23.4	47.10	3.95	5.49	23.27
$(C_6H_5)_3SnCl \cdot L$	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[11]. 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 hepatoprancreas 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[2], and als nerves and serum[3] 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[4], 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[2-4] 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 ecophysiological 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[5] and Saifer[6]. 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.[7].

Result and discussion. Camien et al.[2] 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[4] 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.