hemolytic volume. Aloni et al.[8] observed temperature-induced changes in osmotic fragility of rabbit, rat, guinea-pig and human red blood cells. Erythrocytes studied by these authors exhibited a considerably higher stability at 37 °C than at 0 °C.

The decrease in osmotic fragility observed in the present study after erythrocyte irradiation results mainly from the damage to the cell membrane caused by exogenous superoxide anion radicals generated during the water radiolysis process. The destructive action of hydrogen peroxide formed in the dismutation reaction and singlet oxygen may also be of considerable importance.

Gamma radiation causes also peroxidation of membrane lipids. After irradiation of porcine erythrocyte membranes with doses in the range of 0.25–2 Mrad the amount of MDA increases gradually, and above a dose of 2 Mrad it saturates, indicating that at this dose all possible substrates involved in MDA formation have already been utilized.

When membranes were irradiated in the presence of radio-protectors the MDA content was reduced. In the presence of glutathione, catalase and cysteine it was reduced by averages of about 50%, 30% and 20% respectively (figures 2 and 3). In contrast, superoxide dismutase did not protect membrane lipids from MDA formation (figure 3). These results suggest that the main role in radiation-induced damage to membrane lipids should be attributed to hydroxyl radicals and not to superoxide radicals.

Ching-San and Lawrence[9] have shown that OH· radicals generated in liver microsomes initiate NADPH-dependent lipid peroxidation. These radicals are produced mainly according to the classical Fenton reaction. Peroxidative degradation of arachidonic acid proceeds very easily in the presence of OH· radicals and may be stopped by thiourea – a potential scavenger of hydroxyl radicals. Similarly, trapping of OH· radicals by 5,5-dimethyl-1-pyrroline-1-oxide prevents lipid peroxidation. Raleigh et al.[10] have proved that hydroxyl radicals initiate autooxidation of linoleic acid subjected to X-rays.

The results obtained indicate that superoxide radicals and hydroxyl radicals play the main role in radiation-induced damage to porcine erythrocyte membranes.

Table 1. Protective effect of superoxide dismutase and catalase on osmotic fragility of irradiated porcine erythrocytes

Dose	$C_{50\%}$	p
30 krad	0.68±0.03	
+SOD	0.66±0.02	0.01<p<0.02
+Catalase	0.66±0.02	0.01<p<0.02
50 krad	0.72±0.02	
+SOD	0.70±0.01	0.005<p<0.01
+Catalase	0.69±0.02	0.005<p<0.01

Concentration of SOD and catalase, 200 µg/ml erythrocyte suspension; $C_{50\%}$, NaCl concentration at which 50% hemolysis was observed.

Table 2. Protective effect of cysteine and glutathione on osmotic fragility of irradiated porcine erythrocytes. Concentration of cysteine and glutathione: 200 µg/ml

Dose	$C_{50\%}$	p
30 krad	0.70±0.01	
+Cysteine	0.68±0.01	0.01<p<0.02
+Glutathione	0.68±0.01	0.01<p<0.02
50 krad	0.73±0.01	
+Cysteine	0.70±0.02	0.01<p<0.02
+Glutathione	0.71±0.01	0.01<p<0.02

1 This work was performed under the contract R.III.13.
2 D.K. Myers and D.E. Slade, Radiation Res. *30*, 186 (1967).
3 E.D. Wills and A.E. Wilkinson, Radiation Res. *31*, 732 (1967).
4 G. Gerber and K.J. Altman, in: Radiation Biochemistry, vol II, p. 30. Academic Press, New York 1970.
5 J.T. Dodge, C. Mitchell and D.J. Hanahan, Archs Biochem. Biophys. *100*, 119 (1963).
6 K.L. Fong, P.P. Mc Cay, J.L. Poyer, B.B. Keele and H. Misra, J. biol. Chem. *248*, 7792 (1973).
7 J.R. Murphy, J. Lab. clin. Med. *69*, 758 (1967).
8 B. Aloni, A. Eitan and A. Livine, Biochim. biophys. Acta *465*, 46 (1977).
9 L. Ching-San and H.P. Lawrence, Arch. Biochem. Biophys. *190*, 27 (1978).
10 J.A. Raleigh, W. Kremers and B. Gabourx, Int. J. Radiat. Biol. *31*, 203 (1977).

^{13}C NMR-study of osmoregulatory metabolites in the marine mollusc *Tapes watlingi*[1]

R.S. Norton[2] and P. de Rome

Roche Research Institute of Marine Pharmacology, Dee Why (N.S.W. 2099, Australia), 26 July 1979

Summary. Examination of homogenates of tissue from the marine bivalve mollusc *Tapes watlingi* by natural-abundance ^{13}C NMR indicates that taurine, betaine, and some free amino acids play a significant role in osmoregulation.

Recently we reported that natural-abundance ^{13}C NMR-spectroscopy provides a convenient means of identifying and quantitating the major organic metabolites in tissue from marine molluscs[3]. As it is likely that the metabolites observed in that study are involved in osmoregulation in those organisms, we decided to investigate this possibility in a representative species. In the work described here, ^{13}C NMR is used to monitor the levels of the major organic ions in tissue from specimens of the marine bivalve mollusc *Tapes watlingi* exposed to a range of external salinities. The results yield information about the osmoregulatory process in *T. watlingi*, while at the same time illustrating the advantages of ^{13}C NMR as a tool with which to study osmoregulation in marine organisms.

Materials and methods. Specimens of *T. watlingi* were collected in Sydney Harbour between March and July 1978, and kept in aerated natural sea water (collected at Dee Why headland) at room temperature (about 22 °C). The animals used in the 2-day experiments (see below) were all from a single collection which was held in natural sea water for 3 weeks before use. Reduced salinities were generated by dilution of natural sea water with deionized water,

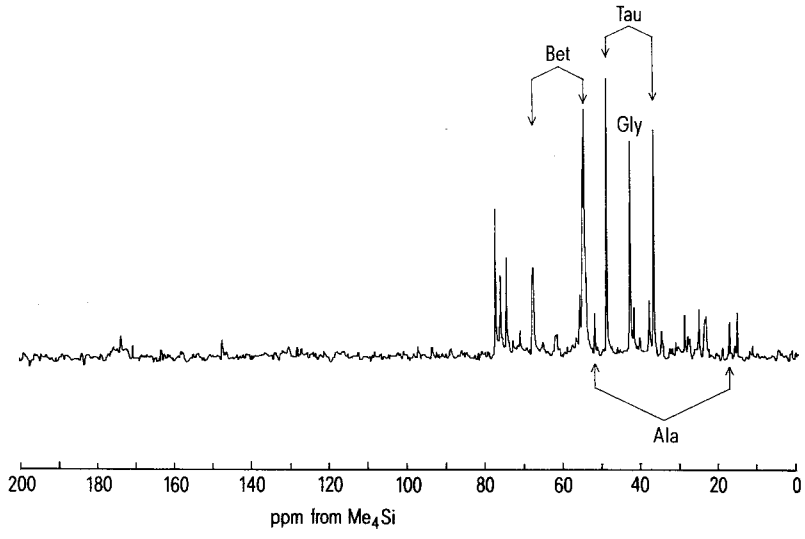

Fig. 1. Natural-abundance ^{13}C NMR-spectrum (recorded at 15.04 MHz, with 10 mm outer diameter spinning sample tubes) of the supernatant of an homogenate of the foot muscle from an animal kept in 1.27× normal sea water for 2 weeks. The freeze-dried supernatant (101 mg) was dissolved in 1 ml D_2O, pH 6.5. 10,000 scans with a recycle time of 5.0 sec were accumulated in 4096 time-domain addresses with a sweep width of 4000 Hz, and processed with 1.1 Hz exponential broadening. Over the whole range of samples, pH varied from 5.8 to 6.6 and probe temperature from 32 to 37 °C (depending on electrolyte concentration).

increased salinities by the addition of artificial sea water salts (Aquasonics, Syndey) to natural sea water. Salinities were checked conductimetrically. The methods of tissue homogenisation and acquisition of ^{13}C NMR-spectra are as described previously[3]. Protein contents were determined by the Lowry method[4].

Results and discussion. Animals were kept at various salinities for periods of 2 days or 2 weeks, then the whole tissue was removed. The foot muscle was separated from the rest of the tissue, blotted and examined independently. The natural-abundance ^{13}C NMR-spectra of homogenates of foot muscle and whole tissue minus foot muscle (hereafter referred to as whole tissue) were then examined. Examples of spectra of intact foot muscle and whole tissue, and of homogenates of these samples, from animals incubated in normal sea water have been given previously[3]. Figure 1 shows the spectrum of an homogenate of the foot muscle from an animal kept for 2 weeks in 1.27× normal sea water. The solution and spectral accumulation conditions given in the caption to figure 1 were used throughout this study.

The major organic ions in *T. watlingi* foot muscle and whole tissue are Tau, Bet and Gly (figure 1)[3]. Resonances from free Ala are also readily identified[3], and are included in our analysis. Polyols and/or sugars give rise to prominent resonances at 70–80 ppm (figure 1), but we have omitted them in this study because they have not been identified as yet, nor are their concentrations as high as those of Tau, Bet or Gly.

The concentrations of Tau, Bet, Gly and Ala were determined as described previously[3], and expressed as mmoles per g soluble protein[4]. The results for the foot muscles from animals incubated at various salinities for periods of 2 days and 2 weeks are shown in figure 2[5]. After 2 days, the levels of all 4 compounds are greater at the highest salinity than at lower salinities, indicating that the animals begin to respond to changes in external salinity within this period. However, the dependence of metabolite levels on salinity after this time is only slightly greater than the natural variation among animals. After 2 weeks' acclimatisation, there are quite large increases in levels of these compounds with increasing external salinity. Thus, it appears that the foot muscle from *T. watlingi* responds to variations in the salinity of its environment at least partly by adjusting the concentrations of its 3 major organic ions Tau, Bet and Gly. The level of Ala exhibits the same trend, although this solute is quantitatively less important in terms of its contribution to the overall osmotic response (figure 2).

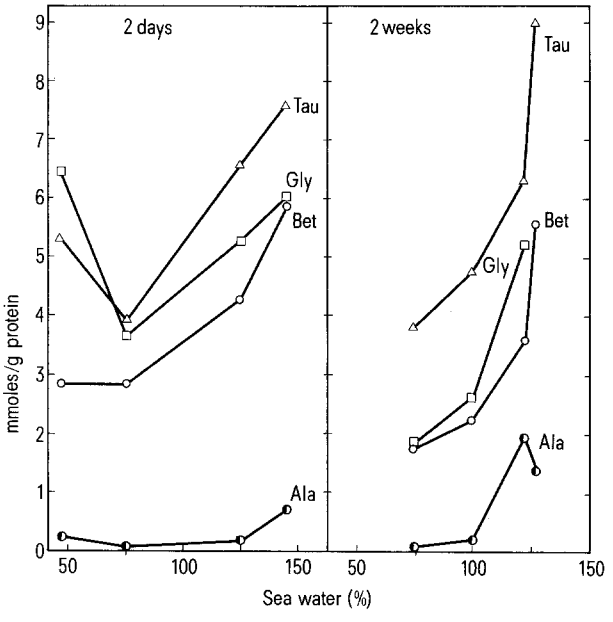

Fig. 2. Levels of Tau (△), Bet (○), Gly (□) and Ala (◐) in the supernatants from homogenates of *T. watlingi* foot muscles removed from animals incubated for periods of 2 days or 2 weeks in natural sea water diluted or supplemented with sea water salts as described under 'materials and methods'. Levels of Gly are slightly low due to some decomposition during assay[5]. At each salinity 2–4 animals were examined. The likely errors in quantitation have been discussed previously[3].

In the rest of the tissue there was no significant dependence of the levels of Tau, Bet or Gly on environmental salinity, even after 2 weeks. After 2-days' exposure the level of Ala showed no marked dependence on salinity, but, after 2 weeks, Ala had increased significantly in 1.22× and 1.27× normal sea water. However, even at the highest salinity its concentration was less than that of Tau, Bet or Gly.

The present study indicates that Tau, Bet, Gly and, less importantly, Ala are involved in osmoregulation in *T. watlingi* foot muscle. Although our work was carried out using tissue homogenates, we believe that the metabolite concentrations so obtained resemble fairly closely those in intact tissue because of the similarity found previously[3] between ^{13}C NMR-spectra of intact tissue and those of tissue

homogenates. No clear trends emerged in the levels of Tau, Bet or Gly in the rest of the tissue, although Ala showed a dependence on salinity after 2 weeks' incubation. This suggests that these metabolites may not be the major osmoregulatory solutes in every organ in *T. watlingi*, although their presence in fairly high concentrations requires that they contribute significantly to the overall osmotic equilibrium. It is also possible that some organs do not achieve osmotic equilibrium with the haemolymph during this period, or that they do not maintain constant volume under conditions of osmotic stress.

In foot muscle the levels of the major organic solutes begin to respond to alterations in environmental salinity within two days. As *T. watlingi* is an estuarine mollusc, and therefore subject to significant salinity fluctuations under natural conditions, this response is of physiological importance. Notwithstanding this, specimens of *T. watlingi* are often found buried in the sand or mud with only part of the shell protruding, and their initial response to environmental stress may be valve closure. It is known that isolated tissue from these bivalves can withstand long periods of anoxia without addition of nutrients[6,7].

Tau, Bet, Gly and Ala have all been found in a wide range of marine organisms[8]. In a number of organisms, including many species of molluscs[9–13], one or more of these compounds has been found to play a role in osmoregulation. Examination of a number of other molluscs by ^{13}C NMR reveals that Tau, Bet and some polyols are present in all species, with Gly being present in some[3]. Thus, it is likely that the major organic solutes observed in ^{13}C (and ^{1}H) NMR-studies of tissue from marine organisms will be those involved, at least to some extent, in osmoregulation. These techniques should, therefore, be particularly useful for the study of osmoregulation in marine organisms.

1 Acknowledgments. We thank Dr L.J. Borowitzka and D.D. Jamieson of this Institute for helpful discussions, and the RRIMP marine biology staff for assistance in collecting the animals.
2 Queen Elizabeth II Fellow in Marine Science, 1976–1978. Please address correspondence to this author.
3 R.S. Norton, Comp. Biochem. Physiol. B *63*, 67 (1979).
4 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
5 As noted in reference 3, the height of the Gly methylene resonance decreased during spectral accumulation. This was not corrected for in the present report, so the average heights give a slight underestimate of the true Gly concentrations. The magnitude of this decrease showed no significant dependence on the salinity at which the animal was kept.
6 D.D. Jamieson and P. de Rome, Comp. Biochem. Physiol B *63*, 399 (1979).
7 K.D. Barrow, D.D. Jamieson and R.S. Norton, Eur. J. Biochem., in press (1980).
8 J.T. Baker and V. Murphy, Handbook of Marine Science, vol. 1, Compounds from Marine Organisms. CRC Press, Cleveland, Ohio, 1976.
9 S.K. Pierce and M.J. Greenberg, J. exp. Biol. *57*, 681 (1972).
10 S.K. Pierce and M.J. Greenberg, J. exp. Biol. *59*, 435 (1973).
11 R.M. Baginski and S.K. Pierce, J. exp. Biol. *62*, 589 (1975).
12 R.M. Baginski and S.K. Pierce, Comp. Biochem. Physiol. A *57*, 407 (1977).
13 E. Schoffeniels and R. Gilles, in: Chemical Zoology, vol. 7, p. 393. Ed. M. Florkin and B.T. Scheer. Academic Press, New York 1972.

Purification and partial characterization of two lectins from *Momordica charantia*[1]

S.S.-L. Li

Department of Microbiology, Mount Sinai School of Medicine, New York (New York 10029, USA), and Laboratory of Environmental Mutagenesis, NIEHS, NIH Research Triangle Park (North Carolina 27709, USA), 9 May 1979

Summary. 2 different lectins have been purified from the seeds of *Momordica charantia* by gel-filtration and ion-exchange chromatography. These 2 lectins appear to be composed of 2 subunits of 26,000 daltons. Protein fraction I, but not II, showed agglutinating activity toward human type-O red blood cells. The amino acid compositions and amino-terminal sequences of these two homologous proteins are quite different.

The fruit of *Momordica charantia* is widely used in the orient, although the seeds are not eaten. The D-galactose-binding agglutinin from *Momordica charantia* has been shown to agglutinate human type-O red blood cells, but not Yoshida sarcoma cells[2]. Recently, toxic momordin and non-toxic momordica agglutinin have also been separated by CM-cellulose chromatography, and the momordin inhibits protein biosynthesis of Ehrlich ascites tumor cells[3]. In this report, 2 lectins have been purified from the seeds of *Momordica charantia*, and their molecular weights, amino acid compositions and aminoterminal sequences of 27 residues have been determined.

Materials and methods. The seeds of *Momordica charantia* were obtained from Chan Man Hop Seed Co., Hong Kong. The proteins were isolated as previously described[4]. Hemagglutination assays were performed in microtiter plates with human type-O red blood cells[5]. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed on 12.5% slab gel in Tris-glycine buffer, pH 8.3[6]. The gels were stained for protein with Coomassie brilliant blue and for carbohydrate with periodic acid-Schiff reagent[7].

The proteins were hydrolyzed in 6 N HCl at 110 °C for 24, 48 and 72 h, and the hydrolysates were analyzed with an automatic amino acid analyzer (Beckman 121). Cysteine and/or half-cysteine were determined as cysteic acid after performic acid oxidation[8]. Automatic Edman degradations were performed with the Beckman protein sequencer using N, N′-dimethylallylamine buffer and single acid cleavage[9,10]. Phenylthiohydantoin-amino acids were identified by GLC[11], TLC[12], and/or amino acid analysis after back hydrolysis with 6 N HCl or 56% HI[13]. Phenylthiohydantoin-arginine was also identified by the phenanthrene quinone spot test[14].

Results. The crude protein extract was chromatographed on a column of DEAE-Sephadex (figure 1,a) followed by gel filtration on a Sephadex G-150 column (figure 1,b). The proteins under peak GI were further separated into fractions I and II on CM-cellulose column using a linear gradient of sodium phosphate buffer (figure 1,c). The