Methylated arsenic from marine fauna

IT has long been known that certain species of marine animals contain arsenic in their tissues at concentrations up to 100 p.p.m. (ref. 1), and there is little doubt that this arsenic is naturally acquired and does not reflect environmental pollution. The toxicity of arsenic is dependent on its chemical environment and valency state[2,3]. Since the demonstration of the rapid and complete excretion by the human kidney of arsenic consumed in fish and shellfish[1,4], it has been accepted that such arsenic is in an organic and nontoxic form[5]. Clearly full toxicological evaluation of the arsenic in marine animals awaits the elucidation of the precise chemical nature of that arsenic. We have found that mussels (*Mytilus edulis planulatus* Lamarck), western rock lobster (*Panulirus longipes cygnus* George), and stingray (*Dasyatis thetidis Waite*) contain dimethylated and trimethylated arsenic together with a small proportion of inorganic arsenic.

Lobsters were collected off the western shore of Garden Island and mussels and stingray were taken from Cockburn Sound, both in Western Australia. Total soft tissue from the mussels (containing 1 p.p.m. arsenic), tail muscle from the lobster (20 p.p.m. arsenic) and muscle from the disk of the stingray (20 p.p.m. arsenic) were used. Concentrations of arsenic were determined by vapour generation atomic absorption spectrophotometry[6] after digestion of the samples in a mixture of nitric and perchloric acids. Digestion of methanol extracts of the above tissues in aqueous sodium hydroxide led, after neutralisation, to a solution from which arsines were generated on addition of sodium borohydride. Fractional distillation of these arsines from a cold trap into an atomic absorption spectrophotometer[7] led to their identification and has provided information on the alkylation pattern of arsenic contained in fish and shellfish. Sodium borohydride treatment of the methanol extracts before sodium hydroxide digestion produced no volatile arsines.

Preliminary methanol extraction was carried out on macerated tissue of each species. Virtually all arsenic was removed from rock lobster and stingray tissues in this way; 60% of that in the mussel was recovered. The extracts were each treated as follows. Methanol was removed and the residue was digested in sodium hydroxide solution. A small portion of the digest was treated, after neutralisation and addition of buffer, *p*H 4–5, with aqueous sodium borohydride. The arsines generated were trapped in a tube packed with glass beads at the temperature of liquid nitrogen. The trap was linked to an atomic absorption spectrophotometer and allowed to warm, with periodic injection of the volatilised contents of the trap into the flame, as described before[7]. Comparison of the time taken for the arsines from lobster muscle digest to volatilise and enter the flame with those for the standards—arsine, methylarsine and dimethylarsine—revealed 5% unsubstituted arsine, 60% dimethylarsine and 35% of an arsine subsequently identified as trimethylarsine. The percentages represent the constituents of the neutralised base digest when the concentration of sodium hydroxide varied from 10 to 40%: the stronger the base, the greater the release of total arsenic susceptible to reduction. Sodium hydroxide in excess of 40% did not increase the total arsenic released. Digestion was always for 15 min.

Trimethylarsine was identified by use of rock lobster tissue only. The arsines produced by borohydride reduction of the sodium hydroxide digest were trapped by passing all effluent gases through toluene. After concentration, the toluene solution was subjected to gas chromatography and the peak corresponding to the unidentified arsine was located by smell at a sniffing port (strong garlic-like odour). Trimethylarsine was identified by gas chromatography–mass spectrometry[8].

In the sodium hydroxide digest inorganic arsenic is probably present as sodium arsenate or arsenite and dimethylated arsenic as sodium dimethylarsinate. The form of the trimethylated arsenic is more doubtful. Trimethylarsine oxide seems the most likely. The possibility that trimethylated arsenic was demethylating in the alkaline digestion conditions was discounted because the proportions of di- and trimethylated arsenic did not change within the range of sodium hydroxide concentrations that brought about their release. Also, the resistance of dimethyl (trifluoromethyl) arsine to hydrolysis[9] indicated that demethylation would not occur in even the strongest basic conditions used. The close similarities in proportions of the various arsenic compounds from the different species examined may indicate the partial breakdown, in the alkaline conditions used, of a single complex arsenical. That such dissimilar species should all contain arsenic in a single form may seem more likely than that all three should contain the same proportions of three arsenicals. There are, however, no observations on which to base such speculation. Work is in progress to elucidate the chemical structures associated with the methylated arsenicals in both the methanol extract and the sodium hydroxide digest. Preliminary work on the urine of subjects consuming fish containing arsenic suggests that the arsenic remains in a bound state in the body and is excreted in that form. Work is being continued on this aspect of the study.

Although we are still some way from the stage where detailed toxicological assessment of arsenic in marine fauna can be made, it is evident that most arsenic in such situations is organically bound and the general acceptance of the nontoxic nature of this arsenic on such grounds seems to be justified.

JOHN S. EDMONDS
KEVIN A. FRANCESCONI

Western Australian Marine Research Laboratories, PO Box 20, North Beach, Western Australia 6020

Received September 23; accepted December 16, 1976.

1 Chapman, A. C., *Analyst, Lond.*, **51**, 548–563 (1926).
2 Frost, D. V., *Fedn Proc.*, **26**, 194–208 (1967).
3 Schroeder, H. A., and Balassa, J. J., *J. chron. Dis.*, **19**, 85–106 (1966).
4 Coulson, E. V., Remington, R. E., and Lynch K. M., *J. Nutr.*, **10**, 255–270 (1935).
5 *Aust. Fisheries, Canberra* **34**, 23 (1975).
6 Duncan, L., and Parker, C. R., *Varian Techtron, Technical Topics* (Melbourne 1974).
7 Edmonds, J. S., and Francesconi, K. A., *Analyt. Chem.*, **48**, 2019 (1976).
8 Kostyanovskii, R. G., and Yakshin, V. V., *Izv. Akad. Nauk SSSR. ser. Khim.*, **10**, 2363 (1967).
9 Doak, G. O., and Freedman, L. D., *Organometallic Compounds of Arsenic, Antimony and Bismuth*, 151 (Wiley–Interscience, New York, 1970).

Jurassic labyrinthodont

THE discovery of a member of the Labyrinthodontia in the Surat Basin of Queensland, Australia, brings the last known occurrence of this amphibian subclass firmly into the Jurassic.

The specimen was discovered on a property, Kolane, approximately 35 miles ENE of Taroom, within the oolitic member of the upper Evergreen Formation. As marine fossils are largely absent from Jurassic sediments of eastern Australia, the latter have been dated almost entirely by correlation of their microfloras with those of the Jurassic sequence in Western Australia the age of which has been determined by means of its marine faunas. Thus, the first appearance of *Tsugaepollenites segmentatus* and *T. dampieri* in the upper Evergreen clearly dates this formation