Arsenic Accumulation in Great Barrier Reef Invertebrates

Abstract. *Arsenic concentrates in the kidneys of the giant clams of Australia's Great Barrier Reef. The highest concentrations measured were 1004 parts per million, of which most, 2066 parts per million, were in the water-soluble fraction containing trimethylarsoniumlactate and its derivatives. This accumulation is ascribed to a mechanism in which oceanic arsenate is assimilated by symbiotic zooxanthellae and subsequently deposited in host tissues. The gills are the major site of arsenic excretion by these animals. Gill membrane arsenolipids mediate exposure of their trimethylarsonium groups to the sea and its biological oxidative activities.*

Algae of tropical and other waters with extremely low concentrations of phosphate absorb and must metabolize arsenate (*1*, *2*). One end product of arsenate metabolism is a membrane phospholipid, O-phosphatidyltrimethylarsoniumlactate (*3*). The synthesis of this compound and its degradation and excretion proceed simultaneously. The amount of arsenic that accumulates in algae is not excessive, but subsequent transfer through the marine food web commonly gives rise to elevated arsenic contents [10 to 100 parts per million (ppm)] in many marine products (*1*). We report here, however, that massive accumulation of arsenic occurs in kidney tissues of giant clams as well as in other symbiotic invertebrates. This arsenic is primarily in the form of trimethylarsoniumlactate and its derivatives.

In the case of the Australian western rock lobster, the principal organoarsenical was unequivocally characterized (*4*) as arsenobetaine. This could be an oxidation product of the ubiquitous algal arsenical metabolite trimethylarsoniumlactate. Such arsenic compounds of shellfish do not appear to be toxic and do not accumulate in mammals or in fish, being readily excreted into the urine (*5*). Early experiments with synthetic arsenobetaine and arsenocholine (*6*) demonstrated the low toxicity of these compounds in rats and chicks, and it was presumed that they were readily excreted.

Examination of grazing and algae-feeding organisms growing in the low-phosphate waters of the Great Barrier Reef (14°35'S) for arsenic content revealed the surprising results reported in Table 1. Also listed in Table 1 are values for some temperate-water species collected in southern New South Wales (35°S to 36°S) for comparison. Arsenic accumulation was greatest in mollusks and ascidians bearing symbiotic algae (*7*), particularly the large *Tridacna* and *Hippopus* clams, which harbor zooxanthellae, and *Didemnum ternatanum*, which harbors the unique prokaryotic green alga *Prochloron* (*8*). On a total weight basis, the arsenic content of the didemnid exceeded that of the other organisms.

Tridacna maxima kidney tissue (5.0 g, dry weight) was extracted with chloroform-methanol (2:1). The solid residue, 1.9 g, contained 1004 ppm of arsenic as determined by vapor-generation atomic absorption spectrometry. Upon addition of water, the chloroform phase, 0.11 g, contained 191 ppm of arsenic. The aqueous methanol phase, 1.95 g, contained 2066 ppm. This arsenic included 29 percent trimethylarsoniumlactate, 24 percent O-glycerophosphoryltrimethylarsoniumlactate, and 39 percent of a not yet positively identified compound yielding

Table 1. Arsenic concentrations in marine invertebrates from tropical and temperate waters.

Common name	Species	Source	Arsenic (ppm, dry weight)
Oysters, scallops, and mussels	*Ostrea angasi* and *Saccostrea commercialis*; *Pecten fumatus* and *Chlamys asperrima*; *Mytilus* sp.	Jervis Bay, New South Wales (35°5'S)	5–17
Rock oyster	*Saccostrea* sp.	Lizard Island (14°42'S)	58
"Giant clam"			
Kidney	*Hippopus hippopus*	Lizard Island	561, 481
Kidney	*Tridacna maxima*	Lizard Island	1004, 953
Kidney	*Tridacna derasa*	Davies Reef (18°51'S)	784, 893, 1025, 611, 651, 454
Gonad	*Hippopus hippopus*	Lizard Island	21
Gonad	*Tridacna derasa*	Davies Reef	22
Digestive tract	*Hippopus hippopus*	Lizard Island	65
Digestive tract	*Tridacna derasa*	Davies Reef	26
Zooxanthellae	*Hippopus hippopus*	Lizard Island	15, 16
Zooxanthellae	*Tridacna maxima*	Lizard Island	33
Adductor muscle	*Tridacna maxima*	Lizard Island	25, 12
Adductor muscle	*Tridacna derasa*	Davies Reef	12.3, 11.6
Gills	*Tridacna derasa* (1)	Davies Reef	5.2
Gills	*Tridacna derasa* (2)	Davies Reef	24.8
Mother-of-pearl	*Pinctada margaritifera*	Lizard Island	70
Spider strom	*Lambis lambis*	Lizard Island	15
Pink-coated didemnid	*Didemnum ternatanum*	Lizard Island	154, 146, 226
Brown-coated didemnid	*Didemnum ternatanum*	Lizard Island	39
Tunicates	*Pyuva praeputialis*, *Polycitor gelatinosa*, and *Phallusia depressiuscula*	Batemans Bay, New South Wales (35°45'S)	4–18
Foraminifera	*Marginopora vertebralis* (intact organisms)	Lizard Island	3
Foraminifera	*Marginopora vertebralis* (skeleton only)	Lizard Island	0.4
Phytoplankton	Mixed diatoms, and so forth	14 km east from Cape Ferguson, Queensland (19°5'S)	9
Green algae	*Caulerpa* sp., *Halimeda* sp., and *Chlorodesmis fastigiata*	Great Barrier Reef, 14°35'S	4–21

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the lactate upon acid hydrolysis. Toxic components included 7.5 percent cacodylate and 0.5 percent methanearsonate as determined by radiochromatographic analysis of kidney extracts of ^{74}As-labeled animals. The ratios of these compounds are similar to those produced from [^{74}As]arsenate by isolated zooxanthellae of the *Tridacna* mantle. As with CO_2 fixation products (*9*), such algae released part of their arsenic compounds to the surrounding seawater medium (7 percent cacodylate, 21 percent trimethylarsoniumlactate, and 72 percent of its unidentified derivative).

Concentric concretions occur in the kidneys of *Tridacna* as well as in the kidneys of other mollusks (*10*); their remarkable heavy metal contents have been reported. Using radioarsenic, we found that these concretions can serve as absorbents for [^{74}As]arsenate and [^{74}As]arsenite, much as claimed for similarly concentric concretions (*11*), the Bezoar stones of early pharmacy (*12*). Reports of arsenic detoxication by Bezoar stone have been published (*13*).

The accumulation of algal arsenicals in the clam kidney suggests that their excretion by way of the urine is slow. The gills appear to be a major avenue for arsenic release to the sea. *Tridacna maxima*, maintained for 24 hours in radioarsenate, incorporated equal amounts of radioactivity into the kidney and the gills (the arsenic content of gill tissue was only 5.2 ppm). The gill radioactivity was distributed among at least four novel membrane arsenolipids. Gill membranes exposed to bacterial oxidative activity, therefore, provide a site for arsenic excretion in *Tridacna* and possibly for other organisms as well.

Arsenate is absorbed and methylated primarily by algae growing in tropical waters where phosphate concentrations may be comparably low. This observation is reflected in the generally low arsenic concentrations measured for temperate-water mollusks (Table 1) and reported for marine fishes and invertebrates (*1*, *14*) from northern waters of much higher phosphate concentrations. The results demand further study of the regulation of arsenate metabolism by environmental phosphate and a comparative study of renal excretion of known arsenical metabolites.

A. A. BENSON
Scripps Institution of Oceanography, University of California, San Diego, La Jolla 92093

R. E. SUMMONS
Research School of Biological Sciences, Australian National University, Canberra 2601, Australia

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Phenytoin-Induced Teratogenesis: A Mouse Model

Abstract. *Malformations associated with the fetal hydantoin syndrome have been reproduced in a mouse model. The occurrence of these defects was correlated with maternal serum concentrations, but not with maternal or fetal genotype or the presence of a seizure disorder.*

The suspected teratogenicity of phenytoin (Dilantin; Parke, Davis) in humans requires investigation, especially since 0.3 to 0.5 percent of pregnant women are epileptic and therefore candidates for anticonvulsant drug therapy (*1*). The association between epilepsy, birth defects, and anticonvulsant drugs was recognized in 1963 (*2*), but recently clinical reports have indicated a specific pattern of abnormalities in the offspring of mothers receiving hydantoin anticonvulsants during pregnancy (*3–7*). This pattern, known as the fetal hydantoin syndrome, includes craniofacial malformations, developmental and psychomotor delay, pre- and postnatal growth deficiency, impaired intellectual performance, and cardiac, genitourinary, and skeletal anomalies (*6*).

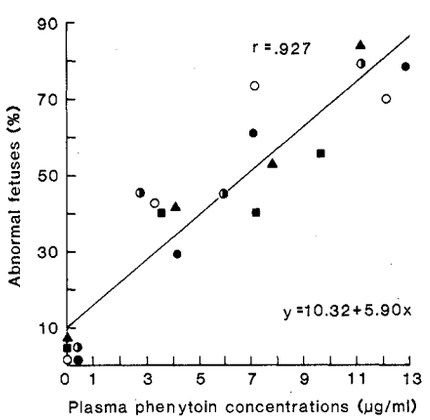

Fig. 1. Relation between the maternal plasma concentration of phenytoin and the frequency of fetuses with malformations. Data points represent averages of all litters: (○) C57 (+/+); (◑) C57 (+/*qk*); (●) C57 (*qk*/*qk*); (■) C_3H; and (▲) SWV.

It has been argued that maternal anticonvulsant therapy is but one variable common to the syndrome (*8*). Shapiro *et al.* (*9*), analyzing data from a collaborative perinatal project, concluded that epilepsy, either maternal or paternal, is the decisive factor in the etiology of the malformations, and not a teratogenic effect of the anticonvulsant drugs. Rather than trying to factor out all of the possible etiologic variables in an epidemiologic approach to the problem, we developed an animal model of the fetal hydantoin syndrome.

To separate the teratogenic effect of epilepsy from that of phenytoin treatment, we required an animal model that would closely approximate the human condition by meeting the following criteria (*10*): (i) The test animal should have spontaneous seizures. (ii) The seizures should be controlled or eliminated by phenytoin treatment. (iii) The drug should be administered orally. (iv) Plasma drug concentrations should fall within the optimal therapeutic range for mice, of 2.5 to 10 μg per milliliter of plasma. (v) The offspring of treated ani-

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