BioMetals **13:** 241–250, 2000.

Arsenic accumulation in three species of sea turtles

Kazutoshi Saeki[1,*], Hiroyuki Sakakibara[2,†], Haruya Sakai[2,‡], Takashi Kunito[3] & Shinsuke Tanabe[3]

[1]*Oita University, Dannoharu 700, Oita, 870–1192, Japan (Fax: +81-975-54-7514; E-mail: ksaeki@cc.oita-u.ac.jp)*
[2]*Department of Environment Conservation, Ehime University, Tarumi 3-5-7, Matsuyama 790 8566, Japan*
[3]*Center for Marine Environmental Studies (CMES), Ehime University, Tarumi 3-5-7, Matsuyama 790 8566, Japan*
Present addresses: †Faculty Agriculture of Kobe University, Kobe, Japan
‡Department of Hygiene, Yokohama-City University, Yokohama, Japan
**Author for correspondence*

Received 19 June 2000; accepted 18 July 2000

Key words: Arsenic, arsenobetaine, dimethylarsinic acid, sea turtles

Abstract

Arsenic in the liver, kidney and muscle of three species of sea turtles, e.g., green turtles (*Chelonia mydas*), loggerhead turtles (*Caretta caretta*) and hawksbill turtles (*Eretmochelys imbricata*), were determined using HG-AAS, followed by arsenic speciation analysis using HPLC-ICP-MS. The order of arsenic concentration in tissues was muscle > kidney > liver. Unexpectedly, the arsenic concentrations in the hawksbill turtles feeding mainly on sponges were higher than the two other turtles primarily eating algae and mollusk which accumulate a large amount of arsenic. Especially, the muscles of the hawksbill turtles contained remarkably high arsenic concentrations averaging 153 mg kg^{-1} dry weight with the range of 23.1–205 mg kg^{-1} ($n = 4$), even in comparison with the data from other organisms. The arsenic concentrations in the tissues of the green turtles were significantly decreased with standard carapace length as an indicator of growth. In arsenic compounds, arsenobetaine was mostly detected in the tissues of all the turtles. Besides arsenobetaine, a small amount of dimethylarsinic acid was also observed in the hawksbill turtles.

Introduction

Sea turtles, classified into two families of six genera and seven species (Limpus *et al.* 1988), are widely distributed in the tropical, sub-tropical and temperate waters of the world's oceans. Recent reports have documented that sea turtles are of great concern due to marine pollutions by plastic debris, tar balls, heavy metals and persistent organochlorine compounds.Although there is no report about arsenic intoxication in sea turtles, arsenic distributions would vary with species and growth. Sea turtles are carnivorous regardless of the species when they are young juvenile soon after ecolosion. For the adult, the species differ from each other in prey items, for instance, green turtles are apparently algicolous, loggerhead turtles tend to be carnivorous and hawksbill turtles prefer sponges (Uchida 1983). High arsenic concentrations are expected in green turtles and loggerhead turtles feeding on algae and mollusk which accumulate high concentrations of arsenic (Phillips 1990; Tamai *et al.* 1992; Francesconi and Edmonds 1993) For the arsenic speciation in sea animals, there are very few reports about reptiles, especially sea turtles. Only one report showed that arsenobetaine dominates along with arsenocholine and arsenate in the leatherback turtle (*Dermochelys coriacea*) feeding on jellyfishes (Edmonds *et al.* 1994). It is anticipated that the differences in prey items of these turtles affect the arsenic speciation in these animals.

Arsenic compounds in sea water are mainly composed of arsenates with some arsenite, methylarsonic

acid (MMA), and dimethylarsinic acid (DMA) (Maher & Butler 1988; Cullen & Reimer 1989; Santosa *et al.* 1996), whereas in biota, organoarsenic compounds predominate. Marine algae contain arsenic at 1000–50000 times the level in their ambient seawater (Francesconi & Edmonds 1993). The majority of algal arsenic is present as arsenosugars (arsenic-containing ribosides) which are believed to be biosynthesized by algae from inorganic arsenic in seawater (Edmonds & Francesconi 1987; Phillips 1990), while arsenobetaine is absent. Dimethylated and trimethylated arsenosugars have been reported in algae, the former being by far the predominant species (Morita & Shibata 1990). Marine animals contain levels of arsenic comparable with algae (Francesconi & Edmonds 1993). Here the major arsenical is arsenobetaine; tetramethylarsonium ion, trimethylarsine oxide and arsenic-containing ribosides also occur as minor constituents in marine animals (Francesconi & Edmonds 1993).

In the present study, arsenic in the liver, kidney and muscle of the three species of sea turtles, e.g., green turtles (*Chelonia mydas*), loggerhead turtles (*Caretta caretta*) and hawksbill turtles (*Eretmochelys imbricata*) were determined using HG-AAS, followed with arsenic speciation analysis using HPLC-ICP-MS, in order to understand the distribution of arsenic species in the turtles.

Materials and methods

Samples

Twenty samples of green turtles (5 males, 13 females and 2 unknown) and 4 samples of hawksbill turtles (3 females and an unknown) were collected at Yaeyama Islands in the East China Sea in March and May 1992, and 4 samples of loggerhead turtles (2 females and 2 unknown) from the north Pacific in May 1990 and March 1992. After dissection, each organ (liver, kidney and muscle) was removed, then stored at 253 K prior to analysis. The biological data are shown in Table 1.

Reagents

All the chemicals and solvents were reagent grade or special grade commercial products. The certified standard reference material, NIES No. 6 (mussel tissue, *Mytilus edulis*), was provided by the National Institute for Environmental Studies, Japan.

Total arsenic concentration

It is generally recognized that certain types of acid digestions (especially those using only nitric acid) do not quantitatively release all the arsenic present in the tissues of marine biota, because some organoarsenic compounds cannot be completely decomposed in the procedure (Phillips 1990; Francesconi *et al.* 1994). The temperature of acid digestion process is likely to play an important role in the total arsenic determination using a Hydride Generation-Atomic Absorption Spectrometer (HG AAS). Anderson & Isaacs (1995) digested the samples at a maximum temperature of 583 K with a temperature controller. Otherwise, the samples were digested by a heating plate at 573 to 653 K (Yasui 1985). These reports suggested that acid digestion for As determination requires a temperature higher than 573 K. Thus, we used the Kjeldahl digestion method with direct gas burner heating to obtain temperatures higher than 573 K. Samples (about 0.2 g dry weight) were exactly weighed into a Kjeldahl flask, and then 3 ml of conc. nitric acid was added. The mixture was gradually heated on the gas burner. After a vigorous reaction, the mixture was cooled, then 2 ml of nitric acid, 2 ml of sulfuric acid and 4 ml of perchloric acid were added, and heated again on the burner. The transparent mixture was concentrated to about 1 ml, cooled, and water added for analyzis by HG-AAS. The procedure prior to a HG-AAS analysis was the modified method of Kubota *et al.* (1990). Ten ml of the digested solution was pre-reduced by addition of 4 ml hydrogen chloride and 2.5 ml of 10% ascorbic acid, then made to 25 ml with water. The mixture solution was analyzed for arsenic by HG-AAS.

The accuracy of the procedure was examined using the certified reference material NIES No. 6 Mussel. The recovery of As in arsenobetaine and the NIES No. 6 sample through the entire procedure was greater than 90%. The detection limit of this method was 0.1 mg g^{-1} dry weight.

Speciation analysis of arsenic

The extraction used in this study was the modified procedure of Shibata & Morita(1992). Fresh samples (about 0.2 g dry weight) were weighed into the centrifuge tube, then 5 ml of methanol/water (1:1 v/v) was added. The mixtures were homogenized, sonicated for 10 min and centrifuged (samples, 2500 rpm for 20 min; standard materials, 2000 rpm for 10 min) to obtain the supernatants. The extraction process was repeated five times, and the extracts were combined,

Table 1. Samples in three species of sea turtles.

	Sampling location	Sex	Carapace length (cm)	Width (cm)
Green turtles	*Chelonia mydas*			
$n = 5$	Yaeyama Is	male	43 to 80	39 to 63
$n = 13$	Yaeyama Is	female	40 to 71	34 to 59
$n = 2$	Yaeyama Is	unknown	42, 55	36, 41
Loggerhead turtles	*Caretta caretta*			
$n = 2$	Tosasimizu	female	86, 92	67, 69
$n = 2$	North pacific	unknown	18, 21	17, 19
Hawksbill turtles	*Eretmochelys imbricata*			
$n = 1$	Yaeyama Is.	unknown	39	32
$n = 3$	Yaeyama Is.	female	38 to 58	37 to 47

then evaporated to dryness. The dried residue was dissolved in 2 ml of water, and filtrated for later analysis.

Arsenic compounds in the extractants were analyzed by high performance liquid chromatography (HPLC, Perkin-Elmer, Integral LC System 100) - inductively coupled argon plasma mass spectrometer (ICP-MS, Perkin-Elmer, ELAN5000) (HPLC-ICP-MS) , with an ODS column GL Science Inc.,Inertsil ODS, 4.6 $\times$ 250). Table 2 indicates the measuring conditions of each instruments.

Figure 1 shows the chromatograms of arsenobetaine, DMA, and the extract from the certified reference material NIES No. 6. Based on the retention time, two peaks, (I) and (II), in the NIES No. 6 chromatogram were identified as arsenobetaine and DMA, respectively. This chromatogram was compared to that obtained by Shibata & Morita (1992), thus expecting that the two rest peaks were identified as arsenosugars (arsenic-containing ribofuranosides).

The data were analyzed using the Statistics Software SPSS/PC+Ver.3.0J.

Results

Total arsenic concentrations in the tissues

Table 3 shows the average concentrations of arsenic in the tissues of the green turtles (Chelonia mydas), loggerhead turtles (Caretta caretta), and hawksbill turtles (Eretmochelys imbricata). There were significant differences in the total arsenic concentrations of the green turtles between the muscle and liver, between the muscle and kidney and between the liver and kidney ($P <$ 0.001 for all cases, U-test). For the three species of turtles, the general order of arsenic concentration in the tissues was muscle > kidney > liver.

Statistical differences in the hepatic arsenic concentrations were observed between the green turtles and loggerhead turtles and between the green turtles and hawksbill turtles (U-test, $P < 0.05$ for each case). In the muscle and kidney, the hawksbill turtles had a significantly greater arsenic level than the green turtles and loggerhead turtles (U-test, $P < 0.05$ for each case). These results indicated a remarkably higher accumulation in muscle of the hawksbill turtles.

The arsenic concentrations in the green turtles in this study were slightly higher or similar to those of the same species (Aguirre *et al.* 1994; Gordon *et al.* 1998). The green turtles stranded in south eastern Queensland, Australia, had average concentrations $\pm$ SD (mg kg^{-1} wet weight basis) of 0.26 ± 0.04 in the liver and 0.19 ± 0.05 in the kidney ($n = 23$) (Gordon *et al.* 1998). Arsenic in the species collected in the Hawaiian Islands were less than 0.6 mg kg^{-1} (wet weight basis) ($n = 11$), except for one sample (Aguirre *et al.* 1994). For the loggerhead turtles stranded on southeastern Queensland, the arsenic concentrations were 0.46 ± 0.24 mg kg^{-1} (wet weight basis) ($n = 6$) in the liver and 0.71 ± 0.26 mg kg^{-1} ($n = 3$) in the kidney. As shown in Table 4, arsenic accumulation in leatherback turtles other than green turtles and loggerhead turtles has been reported (Davenport & Wrench 1990; Edmonds *et al.* 1994; Godley *et al.* 1998). The arsenic concentrations in the muscle of hawksbill turtles in the present study were apparently higher than those in the other turtles.

Table 2. Operation and measurement condition of HPLC and ICP-MS

HPLC	perkin-elmer integral LC System 100
Column	GL Science Inc., Inertsil ODS, 4.6 × 250 mm
Buffer	10 mM tetraetylammonium 4.5 mM malonicacid
Flow rate	0.75 ml/s
pH	6.8
Injection	5 μl
ICP-MS	Perkin-Elmer ELAN 5000
RF power	1000w
Scan mode	Graphics
Measured mas	75 m/z
Total integration time	800

Table 3. Total arsenic concentrations, standard diviation and ranges (Min-Max) in three species of sea turtles

		Arsenic concentration (μg/g dry wt.)		
		Liver	Muscle	Kidney
Green turtles				
Chelonia mydas	$n = 19$	1.76 ± 0.95 (0.44–5.34)	24.1 ± 13.1 (2.58–74.9)	5.72 ± 2.99 (0.15–9.99)
Loggerhead turtles				
Caretta caretta	$n = 4$	6.32 ± 1.56 (4.24–9.43)	20.6 ± 13.1 (5.19–45.5)	9.47 ± 5.37 (4.01–20.2)
Hawksbill turtles				
Eretmochelys imbricata	$n = 4$	15.3 ± 8.77 (4.94–32.8)	153 ± 65.1 (23.1–205)	28.3 ± 9.82 (8.62–36.6)

Variation of arsenic concentrations with carapace length in green turtles

To date no one has reported the detailed life cycle of sea turtles and an appropriate age determination method. Thus, the present study used standard carapace length (SCL) as an indicator of the growth stages in sea turtles. Figure 2 showed the relationships between the SCL and total arsenic concentrations in the tissues of green turtles. Arsenic in the muscle was significantly decreased with SCL ($P < 0.005$). The arsenic concentrations widely varied in the young or small body, while the range of fluctuation decreased with growth. The relationship between arsenic and SCL can not be determined in the loggerhead turtles and hawksbill turtles due to the small sample size.

Speciation of arsenic

Figures 3a, 3b, and 3c show the chromatograms of the arsenical compounds extracted from the tissues of the three species of turtles. In all cases, a large peak was detected at the retention time of 300 s. Judging from the chromatograms of the certified materials in Figure 1, this peak was identified as arsenobetaine. The same phenomena were observed even in other samples of each species. In the three species of turtles, arsenobetaine was the most dominant among arsenic compounds which can be experimently detected in the present study. Another small peak other than arsenobetaine was detected in the liver and kidney of the hawksbill turtles, and identified as DMA judging from the retention time in the chromatograms of Figure 1. This slight DMA peak was also present in the liver and kidney of the green turtles, while those tissues of the hawksbill turtles had no DMA peak. The peak was never detected in any of the loggerhead turtle tissues as well as in the muscles of the hawksbill turtles. No other arsenic compounds such as arsenocholine was detected in any of the samples. If arsenocholine and arsenites were to exist in the samples, the peaks should have appeared at retention times of about 270

Table 4. Mean arsenic concentrations in muscle and whole body of marine organisms.

	Species	Parts	As conc. (μg/g dry wt.)	References
Reptiles	*Eretmochelys imbricata*	muscle	153.00	This study
	Chelonia mydas	"	24.1	"
	Caretta caretta	"	20.6	"
	Dermochelys coriacea	"	14.0	Godley et al. (1998)
		" (*)	0.7	Edmonds et al. (1994)
		" (*)	0.21	Davendport & Wrench (1990)
Fishes	*Pleuronectes herzensteini*	"	36.00	Shinagawa et al. (1983)
	Seriola quinqueradiata	"	5.0	"
	Trachurus trachurus	"	25.6	"
	Scomber japonicus	"	5.4	"
	Cololabis saira	"	5.5	"
	Sardinops melanosticta	"	17.3	"
Protochordata	*Halocynthia roretzi*	"	25.0	"
Mollusca	*Batillus cornutus*	Muscle	15.0	
	Tapes philippinarum	soft tissue	17.5	"
	Octopus vulgaris	muscle	49.0	"
Chlorophycaae	*Codium fragile*	whole	3.2	Jin (1983)
	Chaetomorpha moniligera	"	19.0	"
Phaeophyceae	*Gracilaria verrucosa*	"	16.3	"
	Grateloupia filicina	"	14.3	"
Rhodophyta	*Hizikia fusiforme*	"	61.3	Shinagawa et al. (1983)
	"	"	110.0	Jin (1983)
	Sargassum piluliferum	"	110.00	"
	Cystophyllum hakodatense	"	230.0	"

(*): wet weight basis.

and 285 s, respectively, based on the chromatogram of Edmonds *et al.* (1994).

Discussion

Organic arsenic compounds

Commonly in the three species of turtles, of all the arsenic compounds, arsenobetaine was predominantly detected and small amount of DMA existed in the liver and kidney of the hawksbill turtles and green turtles. In the leatherback turtles (*Dermochelys coriacea*), arsenobetaine mostly existed in the muscle and liver together with arsenocholine and arsenate, but DMA was not detected (Edmonds *et al.* 1994). Hanaoka *et al.* (1999) reported that jellyfish, a primary prey for leatherhead turtles, had arsenobetaine as the dominant organic form of arsenic, together with tetramethylarsonium ion and arsenocholine as minor constituents. Arsenobetaine was the dominant form in other prey items (mollusk and marine algae) for the turtles (Francesconi & Edmonds 1993). The occurrence of the DMA according to the species of turtles were not explained by the arsenical speciation in their prey items, implying a different capacity in the arsenic metabolisms in the species of turtles. It is expected that the metabolism for organic arsenic varied with species even in several experimental animals (Vahter 1994). The present results may indicate another assumption that DMA binding proteins exist in the liver and kidney of the hawksbill turtles and green turtles. Styblo & Thomas (1997) found that a rat's hepatic cytosolic proteins combined with DMA. Generally, in mammals, arsenobetaine and DMA are quickly excluded through the urine from their bodies (Shiomi 1994). However in rats, the rmoval of DMA through the urine was slow (Lerman & Clarkson 1983; Vahter *et al.* 1984), due to DMA retention by SH groups on the red blood cells (Cullen & Reimer 1989).

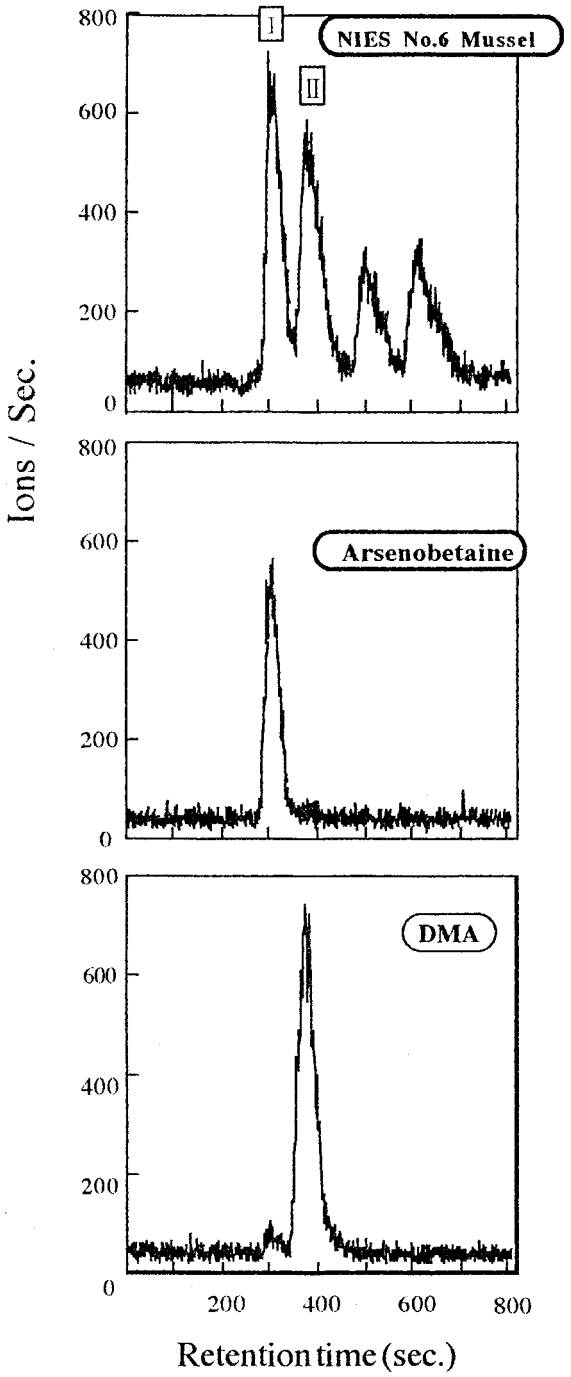

Figure 1. Chromatograms of the extracts of a certified reference of a certified reference material; NIES No. 6 Mussel, arsenobetaine and DMA. Column, GL Sciences Inc., Inertsil ODS, 4.6 × 250 mm: buffer, 10 mM tetraethylammonium 4.5 mM malonicacid (pH 6.8): flow rate, 0.75 ml/min: 5 μl was injected.

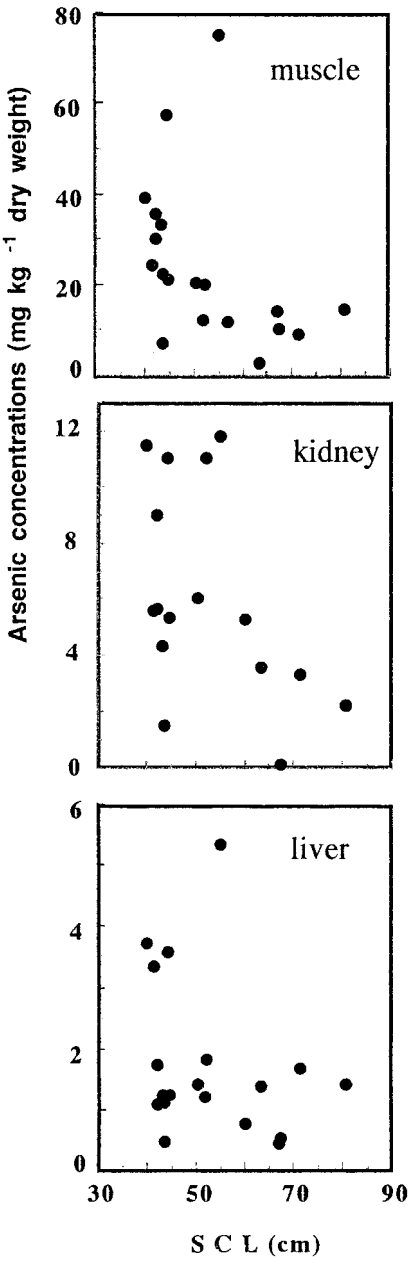

Figure 2. Relationship between the Standared Carapace Length (SCL and arsenic concentrations (μg g^{-1} dry wt.) in the muscle, liver and kidney of green turtle *Chelonia mydas*.

Relationship between arsenic and growth in green turtles

As seen in Figure 2, arsenic in the tissues decreased with SCL in the green turtles. The negative correlation between the hepatic arsenic concentrations and body length was also observed in pilot whales (*Glo-*

bicephala melaena) (Meador *et al.* 1993). On the contrary, no age-dependent accumulations of arsenic are found in the green turtles stranded in Australia (Gordon *et al.* 1998), in pilot whales around the Faroe Islands (Julshamn *et al.* 1987; Caurant *et al.* 1994), and in bowhead whales in Alaska (Krone *et al.* 1999). The main factors for the arsenical decrease with growth in the green turtles are anticipated to be the difference in the metabolic or feeding rates between old and young individuals, and the shift in preferred foods from the young to old individuals. Young green turtles primarily feed on zooplankton, while the mature animals preferably eat sea algae. Arsenic dominant compounds were arsenobetaine in zooplankton and arsenosugars in algae (Francesconi & Edmonds 1993), although the total arsenic level of zooplankton is similar to that of algae, regardless of the variations in location and species. The absorption rate of arsenobetaine is quite high (Vahter *et al.* 1983; Yamauchi *et al.* 1986), whereas arsenosugars are less absorbable through the gastrointestinal tract (Shiomi *et al.* 1990). Thus, a shift in the prey items with growth may decrease the total arsenic absorption in the body of turtle.

Arsenic distribution in the tissues

In general, both organic and inorganic arsenic forms were accumulated in the liver, kidney, lung and spleen of mammals (Yamauchi & Fowler 1994). In all three species of turtles, the order of arsenic concentration in the tissues was muscle > kidney > liver (Table 3). Godley *et al.* (1998) similarly reported that arsenic in the muscle (14 mg kg^{-1} dry weight) was greater than that in the liver (8.2 mg kg^{-1}) in a leatherback turtle from British waters ($n = 1$). In contrast, the hepatic arsenic concentration (0.56 mg kg^{-1} dry weight) was higher than that (0.21 mg kg^{-1}) in the muscle for the same species ($n = 1$) from the Irish Sea (Davenport & Wrench 1990). The high arsenic accumulation in the liver or kidney rather than in the muscle was also observed in other marine animals, pilot whales (Julshamn *et al.* 1987; Muir *et al.* 1988) and narwhale (Wagemann *et al.* 1983). Therefore, the high concentrations in the muscle of the present turtles might indicate that the species have a specific arsenic metabolism mechanism. In birds and terrestrial animals, the metabolism for inorganic arsenic significantly varied with species (Aposhian 1997; Vahter 1999), as well as the organic forms (Vahter 1994). For example, Vahter *et al.* (1983) found that the tissue with the long retention for arsenobetaine included the muscle in rabbits, but other tissues in rats and mice, although the mechanism of arsenobetaine retention has not yet been determined in rabbits.

Species-specific accumulation

In Table 4, the arsenic levels in the muscle of loggerhead turtles and green turtles were similar to those in the other marine creatures, whereas that of the hawksbill turtles was extremely high, similar to the red algae. Generally, in marine food webs, arsenic is bioconcentrated but not biomagnified, and the accumulations were large in the lower creatures (Lindsay & Sanders 1990; Francesconi & Edmonds 1993; Eisler 1994). It is interesting that a relatively higher animal, the hawksbill turtles had a high arsenic concentration like the lower creatures. To date, the apparent reason has not been grasped for the high arsenic accumulations in this species. If any, reasons for the phenomeon could be differences in the arsenic concentration and speciation among the prey items. As shown in Table 4, arsenic concentration of mollusk, a prey of loggerhead turtles, was similar to that of the macroalgae for green turtles. Arsenic of two porifera of a prey item for hawksbill turtles were 3.2 and 6.8 mg kg^{-1} wet weight (Shiomi *et al.* 1988), and not extremely higher than the other species. The differences in the element concentration among the species can not be explained by the prey's specific concentrations. Another reason for the phenomeon is not the arsenic speciation in the prey items, because only 15 % of the arsenic compounds in porifera exist as arsenobetaine (Shiomi *et al.*, 1988), thus not being much absorbed more than those for the others. Alternatively, two assumptions for the large accumulation in the hawksbill turtles were considered. One is that the species may feed on prey which contained an abnormal amount of arsenic compounds, and the hawksbill turtles may have a different in specific metabolism for this element compared to the other turtles.

Toxic influences

The present study found a remarkably high accumulation of arsenic in the hawksbill turtles, and predominant arsenic species were arsenobetaine and DMA. The turtles are not likely to be affected by these As compounds, because the toxicities of arsenobetaine and DMA are very low (Kaise *et al.* 1985; Kaise *et al.* 1989). However, a recent study showed that DNA

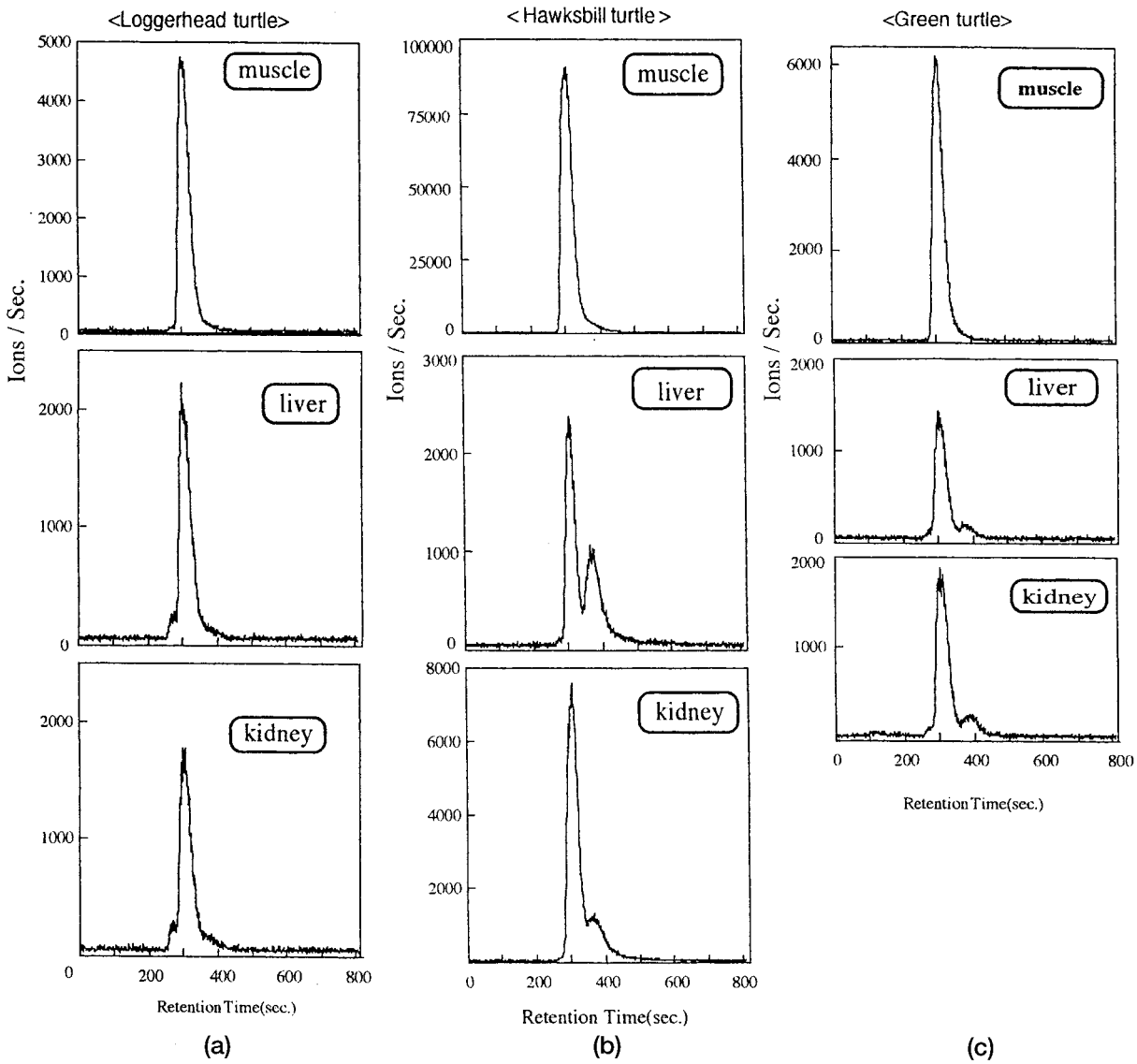

Figure 3. (a) Chromatograms of the extracts from muscle, liver, and kidney in green turtle *Chelonia mydas*. (b) Chromatograms of loggerhead turtle *Caretta caretta*. (c) Chromatograms of hawksbill turtle *Eretmochelys imbricata*.

damage was induced by free radical species (dimethylarsenic peroxyl radical and active oxygens) from the DMA metabolites, dimethylarsine and molecular oxygen (Okada & Yamanaka 1994).Therefore, it may be necessary to investigate the influences of DMA in the liver and kidney of the turtles.

Acknowledgements

This study was supported by a Grant-in-Aid for Encouragement of Young Scientists (No. 11780393) and for Scientific Research (No. 12308030) from the Ministry of Education, Science and Culture, Japan.

References

Aguirre AA, Balazs GH, Zimmerman B, Galey FD. 1994 Organic contaminants and trace metals in the tissues of green turtles (Chelonia mydas) afflicted with fibropapillomas in the Hawaiian Islands. *Mar Pollut Bull* **28**, 109–114.

Anderson KM, Isaacs B. 1995 Simultaneous determination of arsenic selenium,and antiony in environmental samples by hydride generation for inductively coupled plasma atomic emission spectrometry. *J AOAC Int* **78**, 1055–1060.

Aposhian HV. 1997 Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. *Annu Rev Pharmacol Toxicol* **37**, 397–419.

Caurant F, Amiard JC, Amiard-Triquet C, Sauriau PG. 1994 Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hg, Se, Zn) in delphinids Globicephala melas from the North Atlantic Ocean. *Mar Ecol Prog Ser* **103**, 207–219.

Cullen WR, Reimer KJ. 1989 Arsenic speciation in the environment. *Chem Rev* **89**, 713–764.

Davenport J, Wrench J. 1990 Metal levels in a leatherback turtle. *Mar Pollut Bull* **21**, 40–41.

Edmonds JS, Francesconi KA. 1987 Transformations of arsenic in the marine environment. *Experimentia* **43**, 553–557.

Edmonds JS, Shibata Y, Prince RIT, Francesconi KA, Morita M. 1994 Arsenic compounds in tissues of the leatherback turtle, Dermochelys coriacea. *J Mar Biol Ass U.K.* **74**, 463–466.

Eisler R. 1994 A review of arsenic hazards to plants and animals with emphasis on fishery and wildlife resources. In: Nriagu JO, eds. *Arsenic in the Environment, Part II: Human Health and Ecosystem Effects*. New York: John Wiley & Sons, Inc.: 185–259.

Francesconi KA, Edmonds JS. 1993 Arsenic in the sea. *Oceanogr Mar Biol Annu Rev* **31**, 111–151.

Francesconi KA, Edmonds JS, Morita M. 1994 Determination of arsenic and arsenic species in marine environmental samples. In: Nriagu JO eds. *Arsenic in the Environment, Part I: Cycling and Characterization*. New York: John Wiley & Sons: 189–219.

Godley BJ, Gaywood MJ, Raw RJ, McCarthy CJ, McKenzie C, Patterson IAP, Penrose RS, Reid RJ, Ross HM. 1998 Patterns of marine turtle mortality in British waters (1992–1996) with reference to tissue contaminant levels. *J Mar Biol Ass U.K.* **78**, 973–984.

Gordon AN, Pople AR, Ng J. 1998 Trace metal concentrations in livers and kidneys of sea turtles from south-eastern Queensland, Australia. *Mar Freshwater Res* **49**, 409–414.

Hanaoka K, Goessler W, Kaise T, Ohno H, Nakatani Y, Ueno S, Kuehnelt D, Schlagenhaufen C, Irgolic KJ. 1999 Occurrence of a few organo-arsenicals in jellyfish. *Appl Organomet Chem* **13**, 95–99.

Julshamn K, Andersen A, Ringdal O, Morkore J. 1987 Trace elements intake in the Faroe Islands I. Element levels in edible parts of pilot whales (Globicephalus meleanus). *Sci Total Environ* **65**, 53–62.

Kaise T, Watanabe S, Itoh K. 1985 The acute toxicity of arsenobetaine. *Chemosphere* **14**, 1327–1332.

Kaise T, Yamauchi H, Horiguchi Y, Tani T, Watanabe S, Hirayama T, Fukui S. 1989 A comparative study on acute toxicity of methylarsonic acid, dimethylarsinic acid and trimethylarsine oxide in mice. *Appl Organomet Chem* **3**, 273–277.

Krone CA, Robisch PA, Tilbury KL, Stein JE, Mackey EA, Becker PR, O'Hara TM, Philo LM. 1999 Elements in liver tissues of bowhead whales (Balaena mysticetus). *Mar Mammal Sci* **15**, 123–142.

Kubota M, Asami T, Kubo M. 1990 Determination of As in soil using HG-AAS. *Jpn J Soil Sci & Fertility* **61** (1), 88–91 (in Japanese).

Lerman S, Clarkson TW. 1983 The metabolism of arsenite and arsenate by the rat. *Fundam Appl Toxicol* **3**, 309–314.

Limpus CJ, Gyuris E, Miller JD. 1988. Assessment of the taxonomic status of the sea turtle genus Natator McCulloch, 1908 with a redescription of the genus and species. *Trans Royal Soc S Aus* **112**, 1–9.

Lindsay DM, Sanders JG. 1990 Arsenic uptake and transfer in a simplified estuarine food chain. *Environ Toxicol Chem* **9**, 391–395.

Maher W, Butler E. 1988 Arsenic in the marine environment. *Appl Organomet Chem* **2**, 191–214.

Meador JP, Varanasi U, Robisch PA, Chan S-L. 1993 Toxic metals in pilot whales (Globicephala melaena) from strandings in 1986 and 1990 on Cape Cod, Massachusetts. *Can J Fish Aquat Sci* **50**, 2698–2706.

Morita M, Shibata Y. 1990 Chemical form of arsenic in marine macroalgae. *Appl Organomet Chem* **4**, 181–190.

Muir DCG, Wagemann R, Grift NP, Norstrom RJ, Simon M, Lien J. 1988 Organochlorine chemical and heavy metal contaminants in white-beaked dolphins (Lagenorhynchus albirostris) and pilot whales (Globicephala melaena) from the coast of Newfoundland, Canada. *Arch Environ Contam Toxicol* **17**, 613–629.

Okada S, Yamanaka K. 1994 Induction of lung-specific DNA damage by methylarsenics via the production of free radials. In: Nriagu JO eds. *Arsenic in the Environment, Part II: Human Health and Ecosystem Effects*. New York: John Wiley & Sons, Inc.: 143–157

Phillips DJH. 1980 Quantitative Aquatic Biological Indicators, Applied Science Publishers, Ltd., London.

Phillips DJH. 1990 Arsenic in aquatic organisms: a review, emphasizing chemical speciation. *Aquat Toxicol* **16**, 151–186.

Santosa SJ, Mokudai H, Takahashi M, Tanaka S. 1996 The distribution of arsenic compounds in the ocean: biological activity in the surface zone and removal processes in the deep zone. *Appl Organomet Chem* **10**, 697–705.

Shibata Y, Morita M. 1992 Characterization of organic arsenic compounds in bivales. *Appl Organomet Chem* **6**, 343–349.

Shiomi K. 1994 Arsenic in marine organisms: chemical forms and toxicological aspects. In: Nriagu JO eds. *Arsenic in the Environment, Part II: Human Health and Ecosystem Effects*. New York: John Wiley & Sons, Inc.: 261–282

Shiomi K, Aoyama M, Yamanaka H, Kikuchi T. 1988 Chemical forms of arsenic in sponges, sea anemones and sea hare. *Comp Biochem Physiol* **90C**, 361–365.

Shiomi K, Chino M, Kikuchi T. 1990 Metabolism in mice of arsenic compounds contained in the red alga Porphyra yezoensis. *Appl Organomet Chem* **4**, 281–286.

Styblo M, Thomas DJ. 1997 Binding of arsenicals to proteins in an *in vitro* methylation system. *Toxicol Appl Pharmacol* **147**, 1–8.

Tamaki S, Frankenberger WT Jr. 1992 Environmental biochemistry of arsenic. *Rev Environ Contam Toxicol* **124**, 79–110.

Uchida I. 1983 Principal study of sea turtles. *Kaiyou to Seibutu* **26** (3), 198–204

Vahter M. 1994 Species differences in the metabolisms of arsenic compounds. *Appl Organomet Chem* **8**, 175–182.

Vahter M. 1999 Methylation of inorganic arsenic in different mammalian species and population groups. *Sci Prog* **82**, 69–88.

Vahter M, Marafante E, Dencker L. 1983 Metabolism of arsenobetaine in mice, rats and rabbits. *Sci Total Environ* **30**, 197–211.

Vahter M, Marafante E, Dencker L. 1984 Tissue distribution and retention of 74As-dimethylarsinic acid in mice and rats. *Arch Environ Contam Toxicol* **13**, 259–264.

Wagemann R, Snow NB, Lutz A, Scott DP. 1983 Heavy metals in tissues and organs of the narwhal (Monodon monoceros). *Can J Fish Aquat Sci* **40** (2), 206–214.

Yamauchi H, Fowler BA. 1994 Toxicity and metabolism of inorganic and methylated arsenicals. In: Nriagu JO eds. *Arsenic in the Environment, Part II: Human Health and Ecosystem Effects*. New York: John Wiley & Sons, Inc.: 35–53.

Yamauchi H, Kaise T, Yamamura Y. 1986 Metabolism and excretion of orally administered arsenobetaine in the hamster. *Bull Environ Contam Toxicol* **36**, 350–355.

Yasui A. 1985 Determination method of arsenic. In : Ishinishi S, Okabe S, Kikuchi T eds. *As – chemistry, metabolism, toxicity*. Tokyo: Kouseisyakouseikaku: 157 (in Japanese).