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Body Size and Organ Accumulation of Mercury and Selenium in Young Harbor Seals (*Phoca vitulina*)

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The seal, being situated at the top of the marine food chain, consumes a considerable amount of fish and accumulates significant concentrations of mercury (Hg) in its tissues. Previous studies on Hg accumulation in seals have so far demonstrated such features as : (1) A positive correlation between Hg and selenium (Se) levels in the liver with a molar ratio of Hg/Se being close to 1.0 (Koeman et al. 1973, 1975; van de Ven et al. 1979) and (2) high ratios of inorganic Hg (I-Hg) to total Hg (T-Hg) in the liver and kidneys (Smith and Armstrong 1978; van de Ven et al. 1979), though most of the Hg in fish consumed by seals exists as methylmercury (Me-Hg), suggesting the presence of an active demethylation process in these tissues.

Elevated percentages of I-Hg/T-Hg in tissues, indicative of Me-Hg demethylating activity, were also reported in other wild animals (Wren et al. 1980) as well as in experimental animals (Norseth and Clarkson 1970; Yamamoto et al. 1986) fed on Me-Hg for a long term.

Recently, using chopped tissue preparations, the authors and coworkers recognized *in vitro* demethylation of Me-Hg in liver and kidney of mouse (Ishihara and Suzuki 1976) and in human fetal liver (Suzuki et al. 1984). In the present study, Hg and Se concentrations were determined in tissue samples of young harbor seals (*Phoca vitulina*) and *in vitro* Me-Hg demethylating activity in seal organs was also examined.

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MATERIALS AND METHODS

Harbor seals were captured in the Sea of Okhotsk off Monbetsu City located in the northeastern part of Hokkaido, Japan. Livers and kidneys were taken from the freshly killed seals and were immediately frozen on the hunting boat. Until the time of measurement, the samples were stored at -20°C for the determination of elements and at -80°C for the *in vitro* demethylation study.

T-Hg and I-Hg were determined by a modified (Yamamoto et al. 1980) Magos' method (Magos 1971) to avoid interference from coexisting Se. For the measurement of Se, portions of the organs (0.3 - 0.5 g, wet weight) were digested at 120°C for 3 hours with 3 ml of nitric acid using a Teflon-lined, high pressure decomposition vessel (Uniseal Decomposition Vessels, Ltd.). Se was determined by a fluorometric analysis with 2,3-diaminonaphthalene (Watkinson 1966). Detection limit of element determination was 1 ng for both Hg and Se. Accuracy of element determination in our laboratory was routinely being proved with reference materials such as NBS SRM No.1577 (bovine liver) for Se and NIES No.5 (human hair) for Hg.

In vitro demethylation of Me-Hg was examined with chopped tissues of the liver and kidneys obtained from seals as well as from rats and mice. Liver or kidney samples were chopped into 0.1 - 0.2 mm pieces and suspended in chilled Eagle Minimum Essential Medium (EMEM, Nissui). A suspension of chopped tissue (200 mg) was incubated with $CH_3{}^{203}HgCl$ (New England Nuclear, Boston, MA) solution and demethylating activity was determined as described previously for human fetal livers (Suzuki et al. 1984).

RESULTS AND DISCUSSION

Table 1. shows the average concentrations of T-Hg, I-Hg and Se, and ratios of I-Hg/T-Hg and T-Hg/Se in the liver and the kidney of 15 seals. Average levels of T-Hg in the liver and the kidney were less than one tenth of those reported in other seals (van de Ven et al. 1979). However, our values were comparable to other data from the studies of young seals such as 1 - 8 year old harbor seals (Sergeant and Armstrong 1973), 1.27 or 5.17 (mean) year old ringed seals (Smith and Armstrong 1978), and juvenile (Ronald et al. 1984) harp seals. Average concentrations of Se in the liver were also in accordance with the values in the literature studying ringed seals of 5.17 year of age (Smith and Armstrong 1978) and juvenile harp seals (Ronald et al. 1984). Though not defined exactly, the

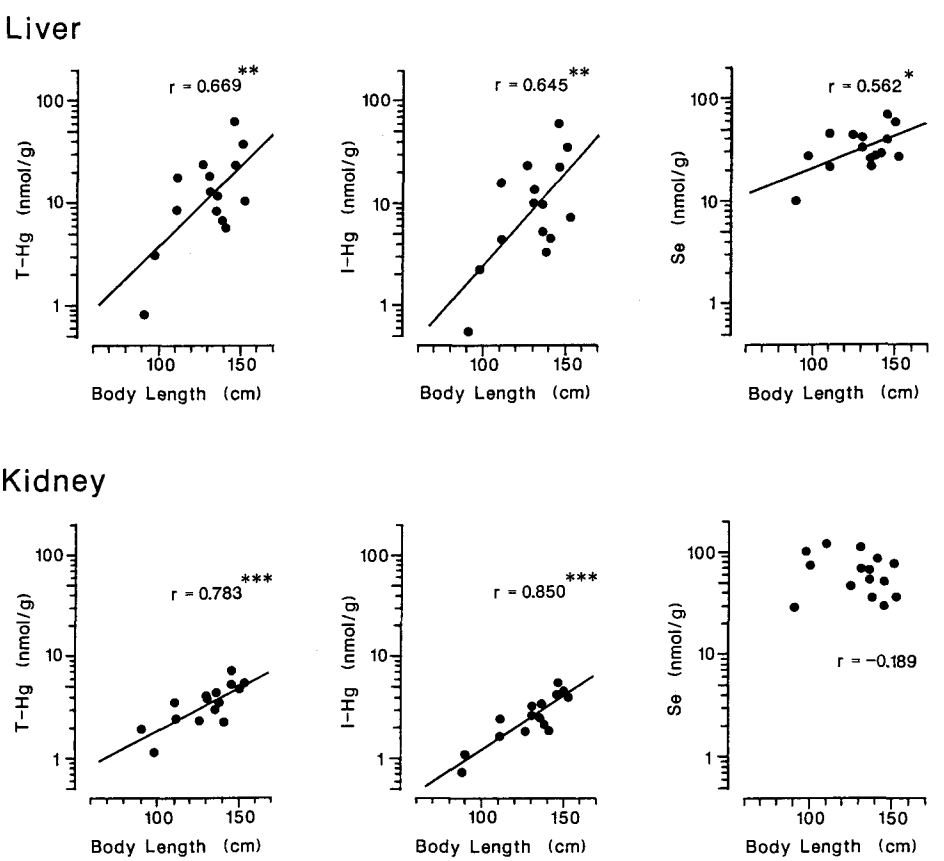

Figure 1. Body length and accumulation of T-Hg, I-Hg and Se in liver and kidney tissues of harbor seals. (Lines in the figures indicate significant regressions of element concentrations on body length. *, **, *** : Statistically significant correlation coefficients; $P<0.05$, $p<0.01$, $p<0.001$)

Table 1. Average concentrations of Hg and Se and molar ratios of I-Hg/T-Hg and T-Hg/Se in liver and kidney tissues of harbor seals[a)]

Elements	Liver	Kidney
T-Hg (nmol/g)	16.7 ± 15.8[b)]	3.60 ± 1.55
I-Hg (nmol/g)	14.3 ± 15.6	2.75 ± 1.30
Se (nmol/g)	34.7 ± 15.3	66.5 ± 29.7
I-Hg/T-Hg	0.77 ± 0.15	0.75 ± 0.09
T-Hg/Se	0.41 ± 0.21	0.071 ± 0.056

a) : 15 seals were used (M,3; F,9; unknown sex,3).
b) : Mean ± S.D.

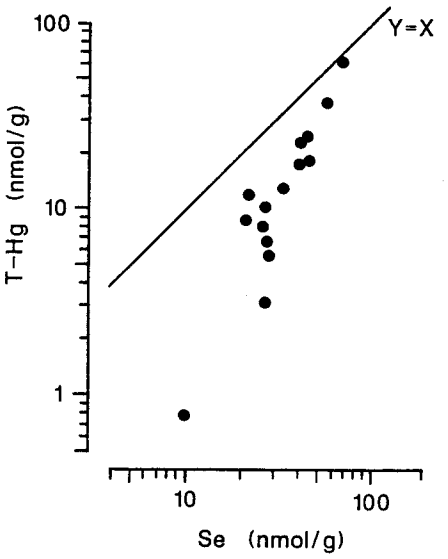

Figure 2. Relationship between T-Hg and Se concentrations in the liver of harbor seals

age of seals in our study was judged by hunters' eyefit to be 2-5 years.

Hepatic and renal log-transformed concentrations of T-Hg, I-Hg and Se were plotted against body length (Figure 1). Both T-Hg and I-Hg showed significant correlations with body length in the liver and the kidney. Indeed, we should be careful in regarding body length as an immediate indicator of the age of seals, but the above-mentioned increase of hepatic and renal Hg concentration with body length appears to reflect the age-related accumulation of Hg in the tissues of harbor seals. In accordance with our results, body size or age-related increase of Hg concentrations in seal organs has previously been recognized in several studies (Koeman et al. 1975; van de Ven et al. 1979; Ronald et al. 1984).

Average molar ratio of Hg/Se in the liver of our samples was 0.41 $\pm$ 0.21 (Table 1), being much less than the ratio around 1.0 reported by the earlier studies (Koeman et al. 1973, 1975; van de Ven et al. 1979; Ronald et al. 1984). However, as shown in Figure 2, the ratio increased up to 1.0 with Hg accumulation in the liver. Hg concentrations in the earlier studies included extremely high values reaching several hundreds μg of Hg per g tissue, and clear correlations were usually observed over the Hg levels exceeding 10 μg/g, while most of the Hg concentrations of our samples fell in a range below 10 μg/g. In other words, we have presented an early

phase of Hg accumulation with Se in the liver of younger seals, where the molar ratio of Hg/Se ratios is approaching towards 1.0 with age. Lower ratios of Hg/Se (0.15 - 0.80)have also been reported in pups of common seals (van de Ven et al. 1979).

On the other hand, Hg/Se ratio in the kidney was as low as 0.07 (Table 1.). In addition, no significant correlation was observed between Hg and Se concentrations in the kidneys. In contrast, a positive correlation between I-Hg and metallothionein levels in the kidney, but not in the liver, has been observed in the same samples of ours (Tohyama et al. 1986), suggesting an involvement of metallothionein rather than Se levels in renal Hg accumulation.

As in previous reports (Smith and Armstrong 1978; van de Ven et al. 1979), higher proportions (about 75 % on the average) of Hg was found as I-Hg both in the liver and the kidney (Table 1). The ratio of I-Hg/T-Hg was highest (94-96%) in livers with highest T-Hg concentration (over 20 nmol/g). This suggests that the increased ability for I-Hg formation might have been accompanied with a T-Hg accumulation in the seal liver. An age-related elevation of I-Hg forming ability in the harp seal liver has previously been recognized by Ronald et al. (1977).

Since the harbor seal is a mammal which eats fish principally, Me-Hg degradation may play a role in its survival. In the present study, *in vitro* Me-Hg demethylation in liver and kidney tissues of seals showed about twice the activity of that in rats or mice (Table 2). However, it remains unknown to what extent this species difference in demethylation contributes to the actual I-Hg formation in each species.

Table 2. *In vitro* Me-Hg demethylation activity in liver and kidney tissues of harbor seals, rats and mice

	% I-Hg formed		
	Seal (n=13)	Rat (n=4)	Mouse (n=4)
Liver	0.74 ± 0.23#	0.34 ± 0.05	0.40 ± 0.06
Kidney	0.77 ± 0.15	0.16 ± 0.03	0.37 ± 0.17

: Mean ± S.D.

An enhancement of I-Hg formation caused by long-term feeding of Me-Hg through several generations has been suggested in normal human fetus (Suzuki et al. 1984), and in rats given Me-Hg for three generations (Yamamoto et al. 1986). Although the contribution of intestinal flora to the increase in the I-Hg ratio observed in the experiments described above has not precisely been examined yet, elevated ratios of I-Hg/T-Hg in the tissues and higher activities of *in vitro* Me-Hg demethylation observed in the seals might have resulted from such an adaptive response to the constant intake of Me-Hg with successive generations.

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