COMPARISON OF TRACE ELEMENT CONCENTRATIONS IN TISSUES OF THE CHICK AND ADULT ADÉLIE PENGUINS

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Abstract: To improve knowledge of heavy metals accumulated in Adélie penguins (*Pygoscelis adeliae*), concentrations of Cd, Cu, Zn, Hg, Pb, Se, Mn and Fe were measured in livers, kidneys, muscles, skin and feathers collected from 12 chicks and 11 adult penguins which were captured at breeding sites at Cape Hinode and in Hukuro Cove during the chick-rearing period, from 1 January to 9 February, 1991. Antarctic krills (*Euphausia superba*) collected from the stomach content of adult penguins were also analyzed.

Higher concentrations of heavy metals were found in the liver and kidney than in other tissues. The average trace element concentrations of adult penguins were in the order Fe(635 μ g/g wet) > Zn(29.3) > Cu(6.4) > Mn(2.1) > Cd(1.9) > Se(1.4) > Pb(0.31) > Hg(0.18) for the liver, and Fe(280) > Zn(29.8) > Cd (14.7) > Cu(3.5) > Se(2.7) > Mn(1.2) > Hg(0.16) > Pb(0.13) for the kidney. Cadmium and Hg concentrations in the liver, kidney, muscle and skin of Adélie penguins increased with growth; this fact suggests that Cd and Hg were accumulated in the body through food. The Fe concentrations of adults were significantly higher in both pectoral and femoral muscles than those of the chicks (t-test, p<0.01). This might be related to the excellent diving ability of the adult penguin. The conversion ratios of Hg concentrations between feather, liver and pectoral muscle of the adults were calculated at 3:5:1. A comparison of heavy metals of adult Adélie penguins captured in 1981 and those in 1991 indicates that both Hg and Pb concentrations in liver, kidney, muscles and skin of the 1991 samples were obviously higher than those of the 1981 samples.

1. Introduction

As the Antarctic Ocean is far from the major sources of industrial pollution, concentrations of heavy metals accumulated in Antarctic animals like sea birds and marine mammals may be regarded as fairly close to the natural baseline level. However, TANABE *et al.* (1982) reported that organochlorine compounds carried by air and water from polluted areas in the tropical zone and the Northern Hemisphere had polluted sea waters and marine organisms in the Antarctic. In order to evaluate

pollution levels of heavy metals in the Antarctic Ocean, analyses of heavy metals in marine organisms like fishes, sea birds, whales and seals have been done by several scientists (SZEFER et al., 1993; NORHEIM, 1987; HONDA et al., 1986; HONDA et al., 1987a, b; STEINHAGEN-SCHEIDER, 1986; NODA et al., 1993). As Adélie penguins (*Pygoscelis* adeliae) which are endemic species to the Antarctic occupy a higher ecological position and their feeding habits are simple (RIDOUX and OFFREDO, 1989), the species is considered a useful indicator for monitoring environmental pollution in the Antarctic. Long-term monitoring research on heavy metal pollution, however, has not been carried out with regard to Adélie penguins.

In the present study we report on the concentration and distribution of trace elements in the livers, kidneys, muscles, skin and feathers of Adélie penguins in relation to their age groups (chicks/adults), and compare their heavy metal levels between the 1981 and 1991 samples of the adults.

2. Materials and Methods

Adélie penguins were collected during the 32nd Japanese Antarctic Research Expedition (JARE-32). Twelve chick penguins (4 males and 8 females) and 11 adult penguins (5 males and 6 females) were captured with legal permission in the Antarctic at Cape Hinode and Hukuro Cove (Fig. 1) during the breeding season between 1 January and 9 Febraury, 1991. The specimens analyzed in the present study were killed shortly after capture by asphyxiation. These specimens were immediately frozen below -20° C and transported from Syowa Station to the National Science Museum of Tokyo.



Body length, body weight, basic morphometric data and weight of tissues were measured at the National Science Museum (Table 1). Muscle samples were collected from pectoral and femoral regions in each animal, and feather samples were also carefully removed from skin of the breast region. Feather samples were rinsed in tap water, distilled water and acetone, and then dried by air. The samples were stored in polyethylene bags at -30° C until chemical analysis was carried out.

Specimen No.	Age	Sampling date	Sampling locality	Sex	B.L. (mm)	B.W. (g)	Liver (g)	Kidney (g)
1	Chick	31 Ja n. 91'	Cape Hinode	Female	376	1535	35.2	14.6
2	Chick	31 Jan. 91'	Cape Hinode	Male	386	1687	59.8	16.7
3	Chick	31 Jan. 91'	Cape Hinode	Male	443	2035	73.2	17.3
4	Chick	31 Jan. 91'	Cape Hinode	Female	464	2580	68.5	21.7
5	Chick	9 Feb. 91'	Hukuro Cove	Female	550	2237	56.8	16.3
6	Chick	19 Ja n. 91'	Hukuro Cove	Female	440	2464	117.3	26.9
7	Chick	7 Feb. 91'	Hukuro Cove	Male	546	3752	119.7	30.5
8	Chick	7 Jan. 91'	Hukuro Cove	Male	313	1038	49.0	16.0
9	Chick	9 Feb. 91'	Hukuro Cove	Female	455	3221	95.5	26.4
10	Chick	1 Ja n. 91'	Hukuro Cove	Female	250	593	23.0	9.6
11	Chick	? Jan. 91'	Hukuro Cove	Female	443	2498	108.5	22.9
12	Chick	19 Ja n. 91'	Hukuro Cove	Female	493	3110	112.2	26.3
13	Adult	31 Ja n. 91'	Cape Hinode	Female	651	3858	103.3	30.9
14	Adult	31 Jan. 91'	Cape Hinode	Male	703	4708	118.1	37.0
15	Adult	31 Ja n. 91'	Cape Hinode	Female	701	3870	136.1	38.6
16	Adult	25 Jan. 91'	Cape Hinode	Male	755	5535	141.2	43.4
17	Adult	15 Ja n. 91'	Hukuro Cove	Male	688	4510	91.3	30.4
18	Adult	25 Jan. 91'	Hukuro Cove	Male	686	4487	89.7	31.6
19	Adult	18 Ja n. 91'	Hukuro Cove	Male	708	4590	96.7	39.4
20	Adult	18 Ja n. 91'	Hukuro Cove	Female	674	4259	114.6	33.6
21	Adult	18 Ja n. 91'	Hukuro Cove	Female	657	4400	115.1	34.5
22	Adult	18 Ja n. 91'	Hukuro Cove	Female	683	4191	118.2	37.9
23	Adult	18 Ja n. 91'	Hukuro Cove	Female	684	4642	134.7	40.5

Table 1. Biological data of the Adélie penguins caught in Antarctica.

B.L.: Body length. B.W.: Body weight.

For determination of trace elements, liver, kidney, pectoral and femoral muscles, skin and feather of the penguins as well as Antarctic krills, *Euphausia superba*, collected from the stomachs of several adult penguins, were analyzed. The samples were digested to a transparent solution with a mixture of nitric and perchloric acids. For the analysis of Cd, Cu, Zn, Fe, Mn, Pb and Se, the resultant solutions were then diluted to a known volume with deionized water. The concentrations of Cu, Zn, Fe and Mn were directly measured by flame atomic absorption spectrophotometry. Cadmium and Pb were determined by graphite furnace atomic absorption spectrophotometry. Selenium was analyzed by using a fluorometric spectrophotometer (WATKINSON, 1966). For the analysis of total-mercury (Hg), wet samples were fully digested in a nitric, sulfuric and perchloric acid mixture (KUMAGAI and SAEKI, 1976), and the concentration of Hg was determined by a cold-vapor technique using a fluorest atomic absorption spectrophotometer.

3. Results and Discussion

3.1. Trace element concentrations in tissues

Values of trace elements in the livers, kidneys, pectoral muscles, femoral muscles, skin and feathers of chick and adult Adélie penguins are summarized in Table 2.

Heavy metal concentrations in adult penguins were generally higher in the liver and kidney than in the muscles; this tendency is especially true for Cd and Mn. Concentra-

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Element	Liver (µ	ℓg/g wet)	Kidney (µg/g wet)	Pectoral muscle (μ g/g wet)		
	Chick	Adult	Chick	Adult	Chick	Adult	
Cd	0.089±0.063	1.896±0.656**	0.166±0.114	14.661±2.950**	0.005 ± 0.002	0.066±0.030**	
	(n=12)	(n=11)	(n=7)	(n=11)	(n=11)	(n=11)	
Cu	13.47±6.96	6.40±1.35**	3.10±0.32	3.52±0.86	1.14±0.24	2.41±0.34**	
	(n=11)	(n=11)	(n=7)	(n=11)	(n=10)	(n=11)	
Zn	31.91±8.62	29.34±3.62	23.24±2.17	29.78±7.04*	16.83±3.04	15.38±3.35	
	(n=11)	(n=11)	(n=7)	(n=11)	(n=10)	(n=11)	
Se	1.03 ± 0.15	1.35±0.46	1.47±0.98	2.66±1.11*	0.52±0.23	0.92±0.16**	
	(n=11)	(n=11)	(n=7)	(n=11)	(n=10)	(n=11)	
Fe	565.0±851.0	635.1±215.8	112.9±31.7	279.6±43.9**	68.8±29.4	298.2±41.1**	
	(n=11)	(n=11)	(n=7)	(n=11)	(n=10)	(n=11)	
Mn	2.85±1.07	2.08±0.49*	1.48±0.33	1.21±0.30	0.22±0.14	0.23 ± 0.05	
	(n=11)	(n=11)	(n=7)	$(n=11)^{n}$	(n=10)	(n=10)	
РЪ	0.055 ± 0.029	0.314±0.103**	0.074±0.046	0.134±0.203	0.182±0.130	0.182 ± 0.214	
	(n=12)	(n=11)	(n=12)	(n=10)	(n=11)	(n=10)	
Hg	0.077±0.034	0.175±0.091**	0.047±0.042	0.158±0.086**	0.015 ± 0.005	0.037±0.010**	
	(n=11)	(n=11)	(n=7)	(n=11)	(n=10)	(n=11)	

Table 2. Trace element concentrations in various tissues of Adélie penguins.

Flement	Femoral mus	cle (μ g/g wet)	Skin (µ	g/g wet)	Feather (µg/g wet)		
Liement	Chick	Adult	Chick	Adult	Chick	Adult	
Cd	0.013±0.007	0.450±0.197**	0.0053±0.0056	0.1789±0.0520**	0.1313±0.0817	0.1892±0.0577	
,	(n=12)	(n=10)	(n=12)	(n=10)	(n=9)	(n=10)	
Cu	1.78±0.26	2.53±0.84*	0.908 ± 0.423	0.603 ± 0.302	8.929±3.113	$14.031 \pm 2.132^{**}$	
	(n=12)	(n=10)	(n=12)	(n=10)	(n=12)	(n=10)	
Zn	27.53±5.46	30.89±5.71	19.73±4.64	23.22±4.66	99.07±13.00	75.04±12.48**	
1	(n=12)	(n=10)	(n=12)	(n=10)	(n=12)	(n=10)	
Se	0.84±0.09	1.02±0.13**	0.094±0.031	$0.251 \pm 0.058^{**}$	0.562 ± 0.088	$0.864 \pm 0.239^{**}$	
	(n=12)	(n=10)	(n=11)	(n=11)	(n=12)	(n=10)	
Fe	37.8±9.8	140.4±21.9**	88.2±56.7	107.3±49.4	759.1±1627.7	44.3±19.9	
1997 - 1997 -	(n=12)	(n=10)	$(n=12)^{2}$	(n=10)	(n=12)	(n=10)	
Mn	0.25±0.11	0.19±0.11	N.D.	N.D.	N.D.	N.D.	
	(n=12)	(n=10)	· .	and the second			
Pb	0.215±0.215	0.115±0.072	0.0049 ± 0.0037	0.0521 ± 0.0964	0.3872 ± 0.3841	0.5825 ± 0.4518	
	(n=12)	(n=10)	(n=5)	(n=4)	(n=12)	(n=10)	
Hg	0.036 ± 0.008	0.109±0.016**	$0.0081 \!\pm\! 0.0024$	0.0174±0.0044**	0.0406 ± 0.0188	$0.0850 \pm 0.0461^*$	
• 3	(n=12)	(n=10)	(n=12)	(n=10)	(n=12)	(n=10)	

N.D.: Not determined. *Significant level, p < 0.05. **Significant level, p < 0.01.

tions of Cu, Zn and Pb were relatively higher in the feathers. These distribution patterns of heavy metals in Adélie penguins were similar to those of the samples collected in 1981 (HONDA *et al.*, 1986). As expected, the highest concentration of Cd was found in the kidney, followed by the liver. It has been shown that most of the Cd is bound to sulfhydryl groups of metallothionein in the liver and kidney of animal species; biosynthesis of this protein can be induced with Cd and provides a protective mechanism against the toxicity of Cd (FRIBERG *et al.*, 1986). Cadmium concentrations in the liver, kidney and skin of Adélie penguins in the present study were lower than those of the penguins captured in 1981 (HONDA *et al.*, 1986). Though ages of the adults were unknown, the divergence might be caused by the difference of ages, sampling station and/or sampling time relative to reproductive process during the chick-rearing period. Concentrations of Cd and Zn in the femoral muscle were several times higher than those in the pectoral muscle, while Fe concentration in the pectoral muscle was twice as high as that in the femoral muscle. Similar findings were reported for egrets and Adélie penguins (HONDA *et al.*, 1985, 1986). It can be presumed that the variation of heavy metal content in muscle is dependent on the muscle composition, which changes during growth.

In the present study no significant difference was observed in the levels of essential trace elements (Cu, Zn and Se) between Antarctic sea birds (Norheim, 1987) and Adélie penguins. This suggests that the levels of these elements indicate normal physiological levels of Antarctic sea birds. Birds have been widely used as monitors of heavy metal pollution, especially for Hg (THOMPSON et al., 1990; GOCHFELD, 1991), because sampling feathers for Hg analysis minimizes complications that arise from the seasonal variations in soft tissues and eliminates the need to kill birds. The value of Hg concentration in the tissues of Adélie penguins was ten times as low as that of pelagic sea birds (MUIRHEAD and FURNESS, 1988; THOMPSON and FURNESS, 1989). Average Hg concentration in feathers in the present study (0.085 \pm 0.046 μ g/g wet) was lower than the analytical value (0.150 \pm 0.049 μ g/g wet) reported by HONDA et al. (1986). The ratio of Hg concentration(fresh weight) in the feathers, liver and pectoral muscle was about 2:5:1 for chick penguins and 3:5:1 for adult birds. These ratios were different from that for sea birds (THOMPSON et al., 1990). According to HONDA et al. (1986), 60% of the total-mercury burden in adult Adélie penguins was accumulated in the feathers, which constituted only 5% of the body weight. This means that a majority of the Hg in the body is excreted by moulting. Since the moulting process has a marked effect on the Hg concentration in the feathers, the time of sampling is regarded as an important factor for consideration.

As it is controversial to catch Adélie penguins for scientific study, the sampling method should be changed from a lethal method to non-lethal ones. Thus, estimation of metal concentration in each tissue by measuring it in feathers becomes more important. In order to evaluate metal contamination for Adélie penguins, it is necessary to establish a systematic and non-lethal sampling method.

3.2. Comparison between trace element concentrations in the tissues of chicks and adults

In comparing trace element levels among birds, information on their ages and/or growing stage is essential because of the age- and growth-related variations of trace element concentrations.

As shown in Table 2, Cd levels in the liver, kidney, muscles and skin were significantly higher in adults than in chicks (p < 0.01). When Cd concentrations in the tissues were plotted against body weight, significant positive correlations (p < 0.01) were found in the liver, kidney, pectoral muscle, femoral muscle and skin, respectively (Fig. 2). These results suggest that the accumulation of Cd in Adélie penguins is due to their growth stage or ages. However, in the present samples exact ages of adults were not shown, though they are known to reach a mean age of 14 years and breed successfully for 10 consecutive years (CULIK, 1987). Copper concentrations of adults were significantly higher in the pectoral muscles, femoral muscles and feathers, and lower in the



Fig. 2. Relationships between body weight and Cd concentration in the tissues of Adélie penguins. ●, chick; ○, adult.

- (1) $Y = 0.00929 \times 1.0001^{x}$; r = 0.765; p < 0.01. (2) Y = 0.00554X 11.841; r = 0.795; p < 0.01.
- (3) $Y = 0.000836 \times 1.009^{x}$; r = 0.747; p < 0.01. (4) $Y = 0.000163 \times 1.001^{x}$; r = 0.738; p < 0.01.

(5) $Y = 0.000592 \times 1.001^{x}$; r = 0.798; p < 0.01.

kidneys than those of chicks. These patterns correspond with those in the eastern great white egret, *Ergretta alba modesta* (HONDA *et al.*, 1985). A significant difference of Zn concentration in soft tissues between chicks and adults was not found, which seems to suggest that several control mechanisms have operated to maintain the physiologically required level of Zn. On the other hand, Se concentrations in the tissues except for liver were significantly different between them.

As shown in Table 2 and Fig. 3, Fe concentrations in the pectoral and femoral muscles of adults were about four times as high as those of chicks. Iron concentration in pectoral muscles was twice as high as that in femoral muscles in both growth stages. Pectoral muscles of adult penguins are rich in myoglobin, which contains a significant amount of Fe. According to WATANUKI *et al.* (1993), the diving depth of Adélie penguins is more than 20 m and their diving duration is about 2 min. This high myoglobin content in pectoral muscle of Adélie penguins seems to explain their excellent diving ability. Remarkable variation of Fe concentrations in chick feathers might be due to the time of sampling or the contamination of soil owing to inadequate washing before analysis.

The relationships between Hg concentrations in six tissues and body weight are shown in Fig. 4. As shown in Table 2 and Fig. 4, Hg concentrations in the tissues of chicks were almost constant in all stages and significantly lower than those of adults.





 $\begin{array}{l} penguins. \quad \bullet, \ chick; \ \bigcirc, \ adult. \\ (1) \ Y = 0.036 \times 1.0003^{x}; \ r = 0.491; \ p < 0.05. \\ (3) \ Y = 0.0076 \times 1.003^{x}; \ r = 0.652; \ p < 0.01. \\ \end{array}$

(5) $Y = 0.00042 \times 1.0003^{x}$; r = 0.745; p < 0.01. (6) Y = 0.0000173X + 0.0048; r = 0.578; p < 0.01.

Mercury concentrations in the tissues of adult Adélie penguins were significantly lower than those of pelagic sea birds (MUIRHEAD and FURNESS, 1988; THOMPSON and FURNESS, 1989). It is well known that the bioaccumulation process of Hg depends on the food chain, that Hg content of high trophic animals is significantly higher than that of lower trophic ones, and that the Antarctic marine ecosystem is relatively short in the length of the food chain. Moreover, Hg concentration in Antatctic sea water (5.8 ng/l) is lower than that in the western North Pacific, Bering Sea and Japan Sea waters (Honda *et al.*, 1987a). When the bioaccumulation factor of Hg was calculated from the concentrations of Hg in the tissues of adults and Antarctic sea water, it was estimated to be 3.1×10^4 (liver), 2.7×10^4 (kidney), 6.4×10^3 (pectoral muscle), 1.9×10^4 (femoral muscle), 3.0×10^3 (skin), 1.5×10^4 (feathers).

3.3. Correlations between different elements

Correlations between the concentrations of Hg, Se and Cd were calculated in liver, kidney, muscles, skin and feathers, respectively (Table 3). Significant positive correlations (p < 0.05 or p < 0.01) were found between Hg-Se, Cd-Hg and Cd-Se in all tissues except for feathers. No correlations were found between Cd-Hg and Cd-Se in feathers.

Elements	Liver	Kidney	Pectoral muscle	Femoral muscle	Skin	Feather
Hg/Se	0.458*	0.555*	0.657**	0.729**	0.790**	0.577**
	(n=22)	(n=19)	(n=21)	(n=22)	(n=22)	(n=22)
Cd/Hg	0.676**	0.673**	0.794**	0.815**	0.780**	0.306
	(n=22)	(n=18)	(n=21)	(n=22)	(n=22)	(n=19)
Cd/Se	0.616**	0.587*	0.668**	0.660**	0.890**	0.393
	(n=22)	(n=18)	(n=21)	(n=22)	(n=22)	(n=19)

Table 3. Correlation coefficients between two elements concentrations in the tissues.

*Significant level, p < 0.05. **Significant level, p < 0.01.

Animals usually show increased tolerance to Cd toxicity when they have been previously treated with sublethal amounts of heavy metals, such as Cd, Zn, Cu and Hg (KOJIMA, 1988; YAMAMOTO and INOUE, 1985). The mechanism of tolerance is most likely altered intra-cellular distribution of Cd with more Cd-binding to metallothionein in pretreated animals. Selenium is known to have a detoxifying effect on heavy metals, such as Hg and Cd, and affects the distribution of Hg in animal tissues. As a consequence of this redistribution of Hg and Se that forms a Hg-Se protein complex, decreased toxicities of both Hg and Se have been observed. Further investigations concerning biosynthesis of metal-binding proteins and the antagonistic effects between elements are needed to confirm the heavy metal-detoxifying mechanisms. Detailed information about metallothionein in the liver and kidney of Adélie penguins will be published elsewhere.

3.4. Comparison of trace element concentrations between the 1981 and 1991 samples

Adélie penguins feed mainly on Antarctic krill widely distributed in the Antarctic Ocean (VOLKMAN et al., 1980; WATANUKI et al., 1993). Table 4 shows a comparison of trace element concentrations in the tissues of adult Adélie penguins sampled in 1981 and 1991. Compared with the 1981 samples, the 1991 samples showed higher concentrations of Hg in 4 tissues (liver, kidney, muscle and skin), Pb in 5 tissues (liver, kidney, muscle, skin and feathers), and Fe in 4 tissues (kidney, muscle, skin and feathers). YAMAMOTO et al. (1987) reported that the mean concentrations of elements in Antarctic krill were in the order Cu > Zn > Fe > Mn > Cd > Pb > Hg. Although there is a slight difference in the order of elements, their values for Zn, Cu, Mn and Cd were nearly close to those found in the present study. But the values for Fe and Pb were considerably

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Samples	Year	Fe	Mn	Zn	Cu	Cd	Hg	Pb
Liver	1981*	733	2.19	47.8	4.70	3.85	0.060	< 0.01
	1991**	635	2.08	29.3	6.40	1.90	0.175	0.314
Kidney	1981*	220	1.60	48.5	3.60	51.0	0.061	< 0.01
	1991**	280	1.21	29.8	3.52	14.7	0.158	0.134
Muscle								
Pectoral	1981*	185	0.31	11.2	2.70	0.190	0.007	< 0.01
	1991**	298	0.23	15.4	2.41	0.066	0.037	0.182
Femoral	1981*	82	0.29	37.5	2.75	0.330	0.008	< 0.01
	1991**	140	0.19	30.9	2.53	0.450	0.109	0.115
Skin	1981*	31	0.41	27.2	1.45	0.70	0.004	< 0.01
	1991**	107	N. D.	23.2	0.60	0.179	0.017	0.052
Feather	1981*	23	0.69	78.2	12.9	0.200	0.172	0.280
	1991**	44	N. D.	75.0	14.0	0.189	0.085	0.583
Krill	1986***	3.6	0.71	9.6	12.7	0.43	0.008	0.040
	1991**	29	1.30	19.2	13.2	0.26	0.020	0.170

Table 4. Comparison of trace element concentrations (mean, $\mu g/g$ wet) between the 1981 and 1991 samples of adult Adélie penguins and Antarctic krills.

*Honda et al., 1986. **Present study. ***Yamamoto et al., 1987. N.D.: not determined.

lower than those discovered in the present study. Although the reason for the difference is not clear, it has been shown that element concentrations in Antarctic krill vary in relation to sex, growth, maturity stage and sampling season (YAMAMOTO *et al.*, 1990). This fact indicates that bioaccumulations of trace elements in Adélie penguins appear to be influenced by the higher accumulation of Fe and Pb in the Antarctic krill, the main diet of the penguins.

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