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HEAVY METAL ACCUMULATIONS IN ADÉLIE PENGUIN, *PYGOSCELIS ADELIAE*, AND THEIR VARIATIONS WITH THE REPRODUCTIVE PROCESSES

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Abstract: This paper reports the concentrations and distribution of heavy metals in the organs and tissues of the Adélie penguin collected around Syowa Station, Antarctica, in 1981, and also discusses the changes of heavy metal accumulation with the reproductive activity. The metal concentrations of the whole Adélie penguin were in the order of Fe>Zn>Cu>Mn·Cd>Hg>Pb·Ni. High bioaccumulations of Cd and Hg were observed. Generally, higher concentrations of the metals were found in the liver and kidney. However, the concentrations of Pb, Ni, Hg and Cu were relatively high in feather, and Mn and Zn were higher in the bone. Relatively high concentrations of Mn, Zn and Cd were found in the pancreas. A majority (>60%) of the Fe and Cu burdens in the whole body was in muscle which constituted an average of 50% of the body weight. Relatively high burdens of Mn and Zn were in the bone also, and more than half of the body burden of Cd was found in the kidney. Comparatively high burdens of Cu and Hg were in the feather, especially Hg in the feather was approximately 60% of the body burden. Effect of egg-laying on the body metal contents of adult female was not significant. However, the Fe concentrations in livers of the starved males were higher than those of the females and other males, while the bone Zn showed lower concentrations in males than females. Furthermore, an increase of metal concentrations in the body and a redistribution of body Fe were observed as a result of advanced starvation.

1. Introduction

Some literature concerning heavy metals in the snow, ice, sea water, atmosphere, soil and rock in the Antarctic region (LANDY and PEEL, 1981; YOSHIDA and MUROZUMI, 1977; IWASHIMA *et al.*, 1977; SANO *et al.*, 1977; NRIAGU, 1979), and animals like fish, penguin and seals (MISHIMA *et al.*, 1977; YOSHIYAMA *et al.*, 1979; DENTON *et al.*, 1980; HONDA *et al.*, 1983b; MCCLUNG, 1984) has been published. However, analytical data on the biological materials for understanding the detailed bioaccumulation phenomena of heavy metals are rather scarce.

Birds are useful biological indicators for heavy metal pollution in the environment because of their wide distribution and mostly they occupy higher trophic levels in food chains. Many papers on significance of environmental and biological factors of heavy metal accumulation, as heavy metal levels in water and/or air and their variations with season and location, habitat, growth, sex and migration in birds have been published (BAGLEY and LOCKE, 1967; MARTIN and NICKERSON, 1973; MARTIN and COUGHTRAY, 1975; MUNOZ *et al.*, 1976; WHITE *et al.*, 1977; SIMPSON *et al.*, 1979; HULSE *et al.*, 1980; HONDA *et al.*, 1985).

However, there are some difficulties in elucidating the factors concerning bioaccumulation because of the complexity of the food web as well as the fluctuating anthropogenic load of metals in the environment. On the contrary, in the Antarctic, the anthropogenic pollution of heavy metals is negligible, and also the food web is comparatively simple (KNOX, 1970). So, Antarctic birds are suitable for elucidating the biological factors concerning the bioaccumulation mentioned above. Furthermore, among the Antarctic bird species, Adélie penguin, *Pygoscelis adeliae*, which feed mainly on krill, is an abundant and important component of the upper part of the Antarctic food web (CROXALL, 1983). Since it's life span is more than 10 years (AINLEY and DEMASTER, 1980), the animal is useful as an "indicator species" to understand the complex long-term accumulation characteristics of heavy metals in the Antarctic ecosystem.

This paper reports the concentrations and distribution of heavy metals (Fe, Mn, Zn, Cu, Pb, Ni, Cd and Hg) in organs and tissues of Adélie penguin, *P. adeliae*, and their variations with reproductive processes.

2. Materials and Methods

Field examinations of the Adélie penguin were carried out at the breeding site in Rumpa (island) about 18 km south of Syowa Station (69°00'S, 39°35'E) during the wintering survey of the 22nd Japanese Antarctic Research Expedition (JARE-22). Ten adult penguins were captured by asphyxiation during the reproductive periods from November 6 to December 22, 1981; 3 males and 2 females during the mating period, 1 male and 1 female during the egg-laying period, 1 male and 1 female during the incubation change period and 1 female during the chickrearing period (Fig. 1). The chick was captured within one week after its hatching. Two eggs (133 and 143 g in weight), including the one abandoned during the reproductive periods, were also collected.

The specimens were immediately frozen at −20°C until autopsy and measurements.

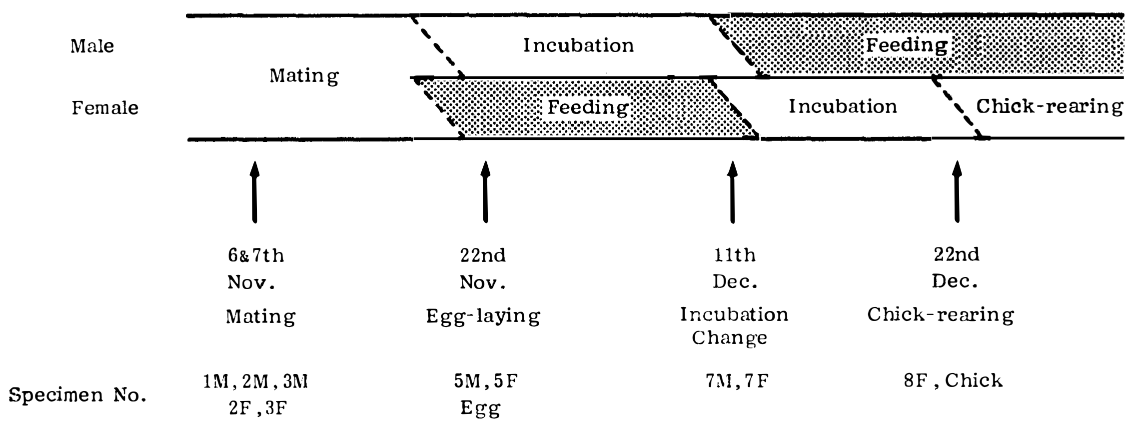

Fig. 1. Schematic representation of the breeding ecology and sampling data of the Adélie penguin, Pygoscelis adeliae.

Table 1. *Biometric data of the Adélie penguin,* Pygoscelis adeliae.

Specimen No.*	1M	2M	2F	3M	3F	5M	5F	7M	7F	8F	Chick
Sampling date	6 Nov. '81	7 Nov. '81	7 Nov. '81	7 Nov. '81	7 Nov. '81	22 Nov. '81	22 Nov. '81	11 Dec. '81	11 Dec. '81	22 Dec. '81	22 Dec. '81
Sex	M	M	F	M	F	M	F	M	F	F	—
Body weight (g)	5020	5500	4710	5860	5420	4110	3850	3740	4540	3790	97.6
Body length (cm)	659	656	629	660	630	684	594	617	652	639	145
Brain	19.2	22.2	17.5	22.0	17.3	18.4	21.6	16.5	16.9	16.4	1.6
Viscera	495.8	552.2	645.2	483.3	596.7	429.5	669.7	382.6	656.2	404.4	15.8
Liver	105.8	107.4	155	109.6	126.8	113.5	113.5	79.5	166.6	100.0	3.2
Pancreas	4.7	4.9	6.5	3.1	4.4	4.2	6.3	2.0	9.0	4.9	0.06
Stomach	47.9	51.8	61.6	58.0	43.2	47.4	70.4	42.2	80.4	49.4	3.2
Intestine	60.8	60.5	79.3	80.1	54.6	41.5	41.5	66.3	93.8	57.8	4.6
Heart	51.6	52.7	54.8	60.1	36.9	42.6	34.4	33.9	47.1	39.4	1.1
Lungs	83.8	97.9	73.9	63.0	82.5	72.4	61.6	83.1	63.7	55.8	1.1
Spleen	1.0	1.1	1.3	—	1.1	0.9	1.2	1.3	2.1	0.7	0.03
Kidney	33.0	43.7	48.1	33.0	31.2	25.5	37.0	21.9	44.8	26.0	2.2
Others	107.2	132.2	164.7	76.4	216	81.5	303.8	52.4	148.7	70.4	0.3
Muscle	2288	2603	2173	2600	2430	1874	1688	1945	1962	1743	44.4**
Fat tissue	1079	1169	719	1162	1118	658	361	286	962	687	—
Skin	234	201	324	327	338	192	205	215	162	151	9.0
Feather	215	218	186	247	210	253	246	226	204	194	1.0
Bone	486	453	426	541	454	509	394	424	375	362	—
Others	89	246	176	191	109	116	156	108	131	160	14.5***

* See Fig. 1. ** Sum of muscle and bone weights. *** Sum of yolk and yolk sac weights.

After weighing the whole animal, the muscle, liver, bone, pancreas, heart, spleen, lungs, kidney, brain, testis, ovary, skin, feather, blood, subcutaneous and abdominal fats, and the others were dissected and weighed separately (Table 1). The muscle samples were taken from 2 parts, pectoral and femoral regions. The bone samples were taken from 9 parts, the sternum, humerus, coracoid, femur, tibia, skull, mandible, scapula and clavicle, and then adhering muscle and ligament were carefully removed from the bones. The surfaces of the bone samples were gently washed with distilled water, dried by filter paper and weighed before analysis. Two types of feathers were collected from breast and dorsal regions. The feather samples were rinsed thoroughly in tap water, distilled water and acetone, and were dried at about 25°C. The lipid content was measured by disperser extraction using acetone and hexane. All the wet samples were stored in polyethylene bags until analysis.

For metals analysis, one to ten g of the wet samples were digested in a nitric, perchloric and sulfuric acid mixture, and the bone samples in a nitric and perchloric acid mixture. The resultant solutions were then diluted to a known volume with deionized water, the concentration of Fe, Mn and Zn being directly measured by atomic absorption spectrophotometry (AAS). For determination of Cu, Ni, Pb and Cd, extraction with methyl isobutyl ketone was performed after diethyldithiocarbamate chelation, and final measurement by AAS (HONDA *et al.*, 1982). The concentration of Hg was performed by flameless AAS (HONDA *et al.*, 1983a).

3. Results and Disccussion

3.1. Metal concentrations and their organ and tissue distribution

Table 2 shows the whole body concentrations of metals in the penguin and in their organs and tissues. The whole body concentrations of metals in adult penguins were in the order of Fe > Zn > Cu > Mn · Cd > Hg > Pb · Ni, and this order was higher for Cd and Hg compared with those of Antarctic sea water (HONDA *et al.*, 1985), indicating a high bioaccumulation of these two metals. However, in the Adélie penguin, one order lower concentration of Hg, and a higher concentration of Cd were found as compared with those of pelagic sea birds, such as short-tailed shearwater, fulmar, puffin, *etc.*, from other areas (HAMANAKA, 1984; OSBORN *et al.*, 1979; FURNESS and HUTTON, 1979). It may be explained by the fact that krills which are the main food items of the Adélie penguin, contain a relatively low concentration of Hg (0.0042 μg/wet g) and a relatively high concentration of Cd (0.91 μg/wet g) as reported by HONDA *et al.* (1985).

The concentrations of heavy metals in the Adélie penguin were generally high in liver and kidney, and low in brain and fat tissue. However, the concentrations of Pb, Ni, Hg and Cu were relatively higher in the feather also, and those of Mn and Zn were the highest in the bone. Comparatively high concentrations of Mn, Zn and Cd were found in the pancreas also, and such a distribution of metals agreed well with the results in other marine and terrestrial birds (TATSUKAWA *et al.*, 1974; HONDA *et al.*, 1985; OSBORN *et al.*, 1979).

Furthermore, a significant difference of metal concentrations according to the position of the muscle was also found. The observed variation was between the pectoral and the femoral muscle; the former being higher in Fe, lower in Zn and Cd compared

Table 2. *Heavy metal concentrations (mean±SD, range, μg/wet g) in organs and tissues of the adult Adélie penguin,* Pygoscelis adeliae.

	(*n*)	Fe	Mn	Zn	Cu	Cd	Hg**	Pb	Ni
Muscle*	10	143±31.6 (109−204)	0.30±0.05 (0.21−0.35)	21.4 ± 2.8 (18.9 −27.2)	2.72± 0.27 (2.20− 3.05)	0.24± 0.14 (0.04− 0.46)	7± 2 (3−11)	<0.01	<0.01
Pectoral	10	185±51.5 (125−292)	0.31±0.05 (0.21−0.40)	11.2 ± 1.3 (9.40−13.3)	2.70± 0.30 (2.11− 3.00)	0.19± 0.11 (0.03− 0.36)	7± 3 (4−13)	<0.01	<0.01
Femoral	10	82.0 ±15.6 (63.9 −108)	0.29±0.05 (0.20−0.35)	37.5 ± 6.20 (29.6 −48.7)	2.75± 0.32 (2.31− 3.32)	0.33± 0.20 (0.06− 0.64)	8± 2 (3−11)	<0.01	<0.01
Liver	10	733±490 (233−1670)	2.19±0.40 (1.57−2.90)	47.8 ±13.8 (31.9 −73.4)	4.70± 0.81 (3.26− 6.06)	3.85± 1.93 (0.99− 8.46)	60±37 (19−155)	<0.01	<0.01
Pancreas	10	104±43.5 (52.8−210)	2.20±0.52 (1.82−3.67)	109±41.3 (65.5 −203)	1.75± 0.28 (1.20− 2.22)	6.72± 4.30 (1.53−18.0)	25±18 (15−76)	<0.01	<0.01
Spleen	9	321±104 (174−501)	0.38±0.17 (0.06−0.68)	18.2 ± 2.30 (14.9 −22.4)	0.93± 0.21 (0.50− 1.22)	0.55± 0.20 (0.15− 0.85)	58±21 (29±99)	<0.01	<0.01
Blood	10	560±74.0 (471−758)	0.06±0.02 (0.03−0.09)	6.00± 0.60 (4.70− 6.70)	0.52± 0.06 (0.44− 0.62)	0.07± 0.10 (0.01− 0.35)	10± 6 (3−23)	<0.01	<0.01
Kidney	10	220±68.8 (162−360)	1.60±0.31 (0.95−2.18)	48.5 ±13.4 (29.6 −71.4)	3.60± 0.51 (2.89− 4.51)	51.0 ±20.9 (23.8 −93.4)	61±61 (22.6−134)	<0.01	<0.01
Bone	10	23.8 ± 7.53 (12.9 −39.6)	5.60±0.65 (4.33−6.46)	160±26.4 (129−201)	0.63± 0.09 (0.48− 0.80)	0.02± 0.01 (0.01− 0.04)	5± 2 (3−10)	0.07±0.02 (0.03−0.12)	0.11±0.05 (0.07−0.26)
Feather*	10	23.2 ±15.3 (6.75−57.4)	0.69±0.38 (0.17−1.44)	78.2 ± 5.30 (70.6 −87.2)	12.9 ± 1.45 (9.95−15.1)	0.20± 0.09 (0.09− 0.34)	172± 45 (108−271)	0.28±0.07 (0.03−0.42)	0.39±0.47 (0.14−1.78)
White	10	27.0 ±21.4 (5.85−66.2)	0.74±0.53 (0.22−1.66)	70.7 ± 6.30 (64.0 −84.6)	13.1 ± 1.63 (9.42−15.9)	0.22± 0.10 (0.07− 0.40)	150± 49 (81−246)	0.27±0.12 (0.12−0.54)	0.47±0.86 (0.09−3.04)
Black	10	18.7 ±11.7 (5.71−45.9)	0.65±0.36 (0.11−1.30)	87.7 ± 6.70 (79.8 −100)	12.6 ± 1.44 (9.76−14.2)	0.18± 0.11 (0.08− 0.45)	200± 51 (131−304)	0.29±0.07 (0.17−0.37)	0.31±0.07 (0.18−0.43)
Brain	1	0.3	0.02	16.0	0.89	<0.01	3	<0.01	<0.01
Heart	1	182	0.43	24.6	3.58	0.12	13.2	<0.01	<0.01
Lung	1	762	0.11	10.0	0.60	0.71	16.1	<0.01	<0.01
Testis	1	36.6	<0.01	13.4	0.72	0.29	NA	<0.01	<0.01

* Average concentration was calculated by multiplying of each tissue weight and its metal concentration.
** ng/wet g, NA: Not analyzed.

Table 2. (Continued).

	(*n*)	Fe	Mn	Zn	Cu	Cd	Hg**	Pb	Ni
Subcutaneous fat	3	25.1 ±11.4 (12.4 −40.0)	0.07±0.03 (0.04−0.12)	11.3 ± 6.40 (2.90−18.4)	0.44± 0.26 (0.14− 0.77)	0.30± 0.22 (0.10− 0.61)	2 (<2−3)	<0.01	<0.01
Skin	3	31.0 ± 8.60 (23.7 −43.1)	0.41±0.24 (0.17−0.73)	27.2 ± 0.90 (26.0 −28.1)	1.45± 0.44 (0.93− 2.00)	0.70± 0.12 (0.54− 0.84)	4±3 (2−9)	<0.01	<0.01
Whole body* concentration	10	102±28.1 (68.7 −163)	0.80±0.13 (0.60−1.02)	30.8 ± 2.60 (27.1 −35.7)	2.06± 0.09 (1.89− 2.20)	0.60± 0.22 (0.33− 1.07)	13± 4 (8−19)	—	—

* Average concentration was calculated by multiplying of each tissue weight and its metal concentration.
** ng/wet g, NA: Not analyzed.

Table 3. The concentrations (mean±SD, μg/wet g) and burdens (mg) of metals in penguin's egg, chick and adult penguins.

			Fe	Mn	Zn	Cu	Pb	Ni	Cd	Hg
Whole body concentration										
Egg		(*n*=2)	45.9, 40.8	0.36, 0.26	14.9, 7.60	1.51, 1.03	<0.01, <0.01	<0.01, <0.01	0.053, NA	NA, 0.014
Chick		(*n*=1)	47.9	0.29	13.3	1.40	<0.01	<0.01	0.004	0.007
Adult	Male	(*n*=5)	112±31.4	0.85±0.15	30.3±3.27	2.06±0.11	<0.01	<0.01	0.63±0.23	0.014±0.003
	Female	(*n*=5)	91.3±19.6	0.75±0.08	31.4±1.39	2.05±0.06	<0.01	<0.01	0.60±0.25	0.013±0.004
Whole body burden										
Egg		(*n*=2)	5.98, 5.79	0.05, 0.04	1.94, 1.08	0.20, 0.15	—	—	0.007, —	—, 0.002
Chick		(*n*=1)	4.03	0.02	1.12	0.18	—	—	0.004	0.0006
Adult	Male	(*n*=5)	511±105	3.93±0.50	141±11.9	9.71±1.41	—	—	2.69 ±0.48	0.06 ±0.01
	Female	(*n*=5)	399±98.7	3.28±0.58	137±13.4	8.93±1.12	—	—	2.58 ±1.13	0.06 ±0.02

NA: Not analyzed.

with the latter. Similar findings were reported for several birds and mammals, such as adult egret, fulmar, Manx shearwater, herring gull, pig, cattle and dolphin (HONDA *et al.*, 1982, 1985; UNDERWOOD, 1971; OSBORN *et al.*, 1979). The muscle of the Adélie penguin is rich in myoglobin (Mb) (24.7–44.1 mg Mb/wet g) compared with those of other birds species (PAGES and PLANAS, 1983) also, and its high Mb content is associated with special capability of the animals for diving (WEBER *et al.*, 1974). In this study, the pectoral muscle of adult penguin was also abundant in hemoglobin (Hb) and Mb, which contained most of the muscular Fe. While poor in Hb and Mb, the femoral muscle was relatively rich in fiber, which may contain a relatively high concentration of Zn metalloenzymes. Zn has several physiological similarities to Cd. However, in contrast to Cd, Zn is essential to animals and counteracts some toxic effects of Cd. In this examination, the fact that a similar distribution of Zn and Cd, *i.e.* higher concentration of both the metals in the femoral muscle of adult penguin than in pectoral, is an interesting phenomenon of the interaction of these two metals.

Although many studies on the accumulation of heavy metals in birds concerning the specific accumulation site of metals have been published, the information on the whole body burden of metals and the organ and tissue distribution is scarce so far. This situation makes it rather difficult to understand the detailed bioaccumulation process of metals in birds and also ecosystem transport. Figure 2 shows the metal burdens in organs and tissues of the Adélie penguin which were calculated from the weight of organs and tissues and their metal concentrations, and the results are expressed as percentage in the organ and tissue to total body burden.

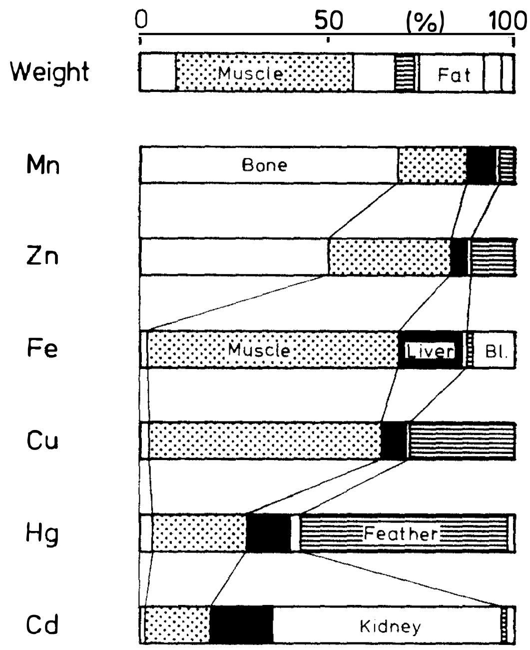

Fig. 2. Distribution of heavy metal burdens in the various organs and tissue of adult penguin, Pygoscelis adeliae.

The organ and tissue distribution of the metal burdens was metal specific. More than 60% of Fe and Cu burdens in the penguin's body was in the muscle, the weight of which constituted an average 50% of the body weight. A relatively high burden of Mn and Zn was in the bone also, and that of Cd, say about 60%, was found in the kidney. Comparatively high burdens of Cu and Hg were present in the feather, which constituted only 5% of the body weight. Especially Hg in the feathers was approximately 60% of the body burden, which means that a majority of Hg in a bird's body is excreted by molting.

3.2. Changes of metal concentrations with reproductive activity

Table 3 shows the concentrations and burdens of metals in penguin's egg, chick and adult. Although most of the metals showed lower concentrations in the eggs than in the adult penguins, the concentration of Hg in the egg was nearly the same as that in the adult female, suggesting that Hg is more transferable from mother to her egg when compared with the other metals. The metal burdens in the eggs were very low compared with those in adult penguins, which indicates that effect of egg-laying on the accumulation of metals in a penguin's body is practically negligible. However, sexual differences of the metal concentrations were observed in the liver and bone. The concentration of Fe in the liver was about two times higher in males (987 ± 530 μg/wet g) than in females (478 ± 264 μg/wet g), while the bone Zn showed lower concentration ($p < 0.05$, Mann-Whitney, U-test) in males (140 ± 7.93 μg/wet g) than in females (179 ± 24.1 μg/wet g).

In the case of this breeding site, female penguins returned to sea for feeding soon after egg-laying, while male penguins continued to starve through the periods from mating to incubation change, resulting in the decrease (approximately 30%) of body weight, which accounted 13% for fat, 12% for muscle and 5% for the others, respectively (Table 1). During this starvation period, the body concentrations of metals in the males gradually increased, and a redistribution of the body Fe was observed between the muscle and liver. Figure 3 shows the changes of Fe burdens in the organs and tissues with the starvation. The hepatic Fe burden increased with advancement of the starvation until egg-laying and afterward slightly decreased at the incubation change, while the opposite trend was found with the muscular Fe. These observations suggest that the muscular Fe was transferred to the liver with the progressing starvation, and then might have been stored mainly as hemosiderin- and ferritin-Fe. Therefore, signifi-

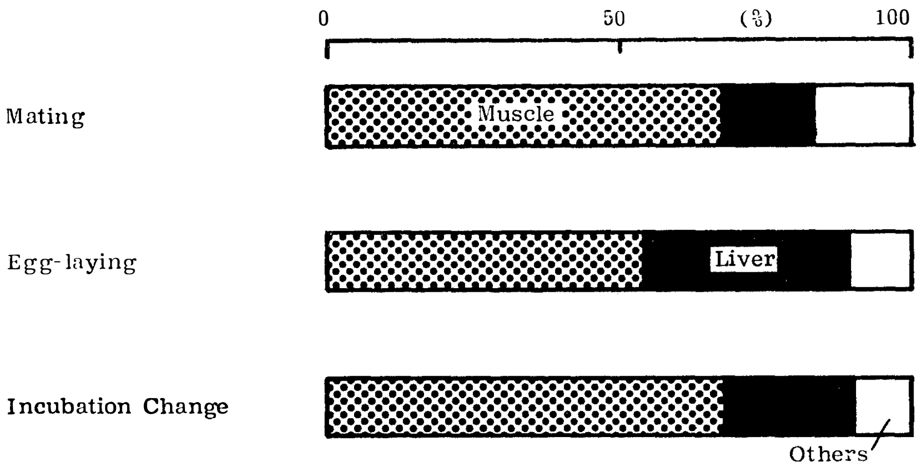

Fig. 3. Starvation-related change of the body Fe distribution in the Adélie penguin, Pygoscelis adeliae.

cantly higher concentrations of Fe in the male livers than the females might be due to the high hepatic accumulation of Fe during the starvation period.

It is known that alkaline phosphatase activity in bone is related to ossification. Some investigators have also speculated that Zn may participate in the synthesis of alkaline phosphatase (IQBAL, 1969; WESTMORELAND and HOEKSTRA, 1969). OJANEN *et al.* (1975), examining the seasonal changes of Zn and alkaline phosphatase activity in the house sparrow, reported that bone Zn content and alkaline phosphatase activity in females were at significantly higher levels during egg-laying than at other times of the year, and also that their levels in females were higher than those in males. They also assume that the high Zn content during the egg-laying period is probably connected with medullary bone formation and other synthetic processes. In the Adélie penguin, significantly higher concentrations of Zn in the female bones than those in the males can also be explained by such physiological differences between sexes.

Such reproduction-related changes of the metal distributions suggest that consideration of the sexual stage and starving process is needed for detailed understanding of the bioaccumulation process and also for the toxicological criteria of heavy metals.

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