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Heavy Metal Distribution in Organs and Tissues of the Eastern Great White Egret *Egretta alba modesta*

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Although mercury and other heavy metal contents in birds have been extensively studied from the viewpoint of terrestrial and marine environmental pollution (Bagley and Locke 1967; Martin and Nickerson 1973; Martin and Coughtrey 1975; Munoz et al. 1976; White et al. 1977; Simpson et al. 1979; Hulse et al. 1980), basic methodologies associated with the sampling and detailed distribution characteristics of metals in the various organs have yet to be evolved. In particular, there exists little information on which organ(s) and which part(s) of a tissue should be sampled and analyzed to compare the difference of accumulation levels among birds. Furthermore, consideration of the total body burden of metals in birds is required for understanding the uptake and excretion of metals. While a whole body analysis is possible in small birds, analysis of large birds must be done selectively on a part of a particular organ and/or tissue.

The eastern great white egret (Egretta alba modesta) is relatively a large and long-life bird species, which migrates for breeding to Korea, China and Japan late in March or early in April, and returns to Philippines, Indonesia and Malaysia for wintering late in August or early in October (Min et al. 1984). As the bird is placed in a high trophic level in the food web, it is useful as an "indicator species" for environmental pollution of heavy metals and other chemicals and also for understanding the bioaccumulation processes of pollutants.

The primary purpose of this paper is to present the detailed distribution characteristics of eight metals (Fe, Mn, Zn, Cu, Ni, Pb, Cd and Hg) in different organs and tissues of the eastern great white egret. Based upon these results, the suitability of the organs of an egret for ecological and physiological comparison is also discussed.

MATERIALS AND METHODS

Field examinations of egret were carried out at the breeding site (1.2 ha) on a hill near a small village 2 km of Cheonan City, Chungcheongnando, central Korea. Five adult male egrets were captured every month during the period from April to August

1981, and twenty chicks at different ages during the period from hatching to 70 days. Specimens were immediately frozen at -20°C until autopsy and measurements. The age estimation of adult egrets was made by the color and shape of the beaks.

One chick (female and 12 days of age) and 1 adult egret (male and 2 years of age captured in April) were selected for the detailed study. The brain, liver, kidney, lungs, heart, stomach, stomach content, intestine, skin, bone, muscle, feathers, and the others were dissected and weighed separately. The bone samples were taken from the sternum, humerus, cervical vertebrae, femur and tibia, and then the adhering muscle and ligament were carefully removed from the bones. The surface of the bone samples were gently washed with distilled water, dried by filter paper and weighed before analysis. The muscle samples were taken from the pectoral and femoral regions and then weighed separately. Three types of feathers were collected: the remiges, coverts and abdominals. The primaries were also subdivided into rachis and barbs. The feather samples were rinsed thoroughly in tap water, distilled water and acetone, and were dried at a room temperature. For moisture determination, all the samples were dried at 80°C for 12 hrs, and the lipid content was measured by Soxhlet extraction using ether. All the dry samples were pulverized and stored in polyethylene bags until analysis. The weights of organs and tissues and their moisture and lipid contents in the chick and adult egrets are shown in Table 1, and the biometry results in the other egrets have already been reported by Min et al. (1984).

Table 1. Organ and tissue weights, and contents of moisture and lipid in various organs and tissues of the chick and adult egrets

Organ & tissue	Wet weight (g)		Moisture (%)		Fat (%)	
	Chick	Adult	Chick	Adult	Chick	Adult
Brain	1.50	4.95	84.0	79.5	-	-
Liver	18.9	21.2	76.6	71.4	4.55	3.66
Kidney	4.90	5.86	79.8	71.7	3.20	3.15
Heart	5.70	12.4				10.9
Lungs	4.00	11.2	80.4	74.3	4.20	3.02
Other viscera	27.4	49.5				10.3
Pectoral muscle	2.90	123	82.2	72.0	1.00	7.00
Other muscle	102	246	79.9	68.3	4.25	8.60
Skin	39.3	57.8	79.5	48.9	3.52	21.3
Bone	31.4	263	66.1	31.7	3.20	5.32
Feather 1		38.1		16.8		-
Feather 2	24.2	22.2	79.6	18.6	-	-
Feather 3		41.7		10.0		-
Stomach content	11.6	1.00				
Body weight	268	976				
Content in a body			78.1	52.3		

Feather 1, primaries; Feather 2, secondaries + tails + upper wing-coveries; Feather 3, other feathers (abdominal, dorsal etc.) Body weight does not include stomach content.

For analysis of the metals, one to ten gr of the dry 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 concentrations of Fe, Mn and Zn being directly measured by atomic absorption spectrophotometry (AAS) (Honda et al. 1982). For determination of Cu, Ni, Pb and Cd, extraction with methyl iso-buthyl keton was performed after sodium diethyl-dithiocarbamate chelation, and final measurement by AAS (Honda et al. 1982). The concentration of Hg was determined by flameless AAS (Honda et al. 1983).

RESULTS AND DISCUSSION

The concentrations of Fe, Mn, Zn, Cu, Ni, Pb, Cd and Hg in pectoral and femoral muscles from chick and adult egrets were determined in order to discover their distribution patterns in parts of the muscle (Table 2).

Although the variation in concentrations of the metals between two parts of muscle was not notable in the chick specimen, the adult egret generally contained relatively high concentrations of the metals in the pectoral muscle compared with those in the femoral muscle, in particular with Fe and Cu. However, the concentrations of Zn and Cd were relatively high in the femoral muscle more than twice as in the pectoral muscle. In striped dolphin (Stenella coeruleoalba) the concentrations of Fe in the red-colored muscles, which was relatively abundant in hemoglobin (Hb) and myoglobin (Mb), were higher than those in the white-colored muscles which was relatively rich in fiber and poor in Hb and Mb, and the opposite was observed with Zn and Cd. This difference was recorded in dolphins of 0.5 year and more after birth (Honda et al. 1983). Similar findings were reported for pig and cattle (Underwood 1971). The pectoral muscle of adult egret is abundant in Hb and Mb, which contained most of the muscle Fe. The femoral muscle of adult egret was relatively rich in fiber and poor in Hb and Mb when compared to the pectoral muscle, while in chick the variation of these two components was not significant.

Our results showed that the variation of the metal contents in muscle is dependent on the muscle composition which changes during growth. It has been noted that Zn has several physiological similalities to Cd. However, in contrast to Cd, Zn is essential to animals and counteracts some toxic effects of Cd. In this study, the fact that a similar distribution of Zn and Cd, i.e., higher concentration of both the metals in the femoral muscle of adult egret than in the pectoral one, is interesting for the interaction of these two metals. As the binding forms of Zn and Cd in the muscle of egret are not adequately understood, further information is needed to provide a better understanding of the interaction between these metals, if any, in the muscle.

Table 2. Metal concentrations (μg / wet g) in various parts of muscle, bone and feather for the chick and adult egrets

Tissue	Moisture[a]		Ca[b]		Fe		Mn		Zn		Cu		Pb		Ni		Cd		Hg	
parts analyzed	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad
Muscle																				
Pectoral	81.2	72.0	-	-	36.4	77.6	0.26	0.43	13.6	20.9	0.83	3.72	0.16	0.06	0.05	0.04	0.004	0.002	0.05	0.26
Femoral	79.9	68.3	-	-	37.1	63.7	0.27	0.32	14.7	44.4	0.83	1.33	0.15	0.05	0.07	0.03	0.003	0.006	0.05	0.21
Bone																				
Sternum	73.8	26.2	0.4	109	46.4	95.6	0.35	3.48	18.8	92.9	1.60	3.32	0.26	1.13	0.056	0.19	0.043	0.017	0.033	0.208
Humerus	65.4	15.6	48.1	163	37.7	24.7	1.29	2.90	58.1	123	1.29	0.69	0.20	0.22	0.048	0.03	0.038	0.003	0.028	0.042
Cirvical vert.[c]	65.2	30.9	71.0	123	36.6	81.8	2.18	3.26	87.9	94.9	1.10	1.39	0.23	0.40	0.049	0.08	0.035	0.006	0.029	0.087
Femur	64.5	32.0	67.4	85.2	35.9	91.6	1.68	3.38	89.0	78.1	1.06	1.63	0.19	0.44	0.048	0.09	0.033	0.007	0.027	0.109
Tibia	59.5	25.6	66.4	112	30.0	70.7	1.88	3.41	76.1	91.5	0.65	0.76	0.16	0.31	0.043	0.06	0.033	0.006	0.022	0.072
Feather																				
Remiges		16.8	-	-		87.4		5.62		80.1		7.35		1.48		0.29		0.032		2.43
Coverts	79.6	18.6	-	-	18.2	168	1.00	10.4	40.4	116	1.25	7.50	0.08	1.51	0.10	0.42	0.002	0.034	0.23	2.46
Abdominal		16.0	-	-		286		17.5		165		7.90		2.30		1.59		0.059		3.12
Primaries																				
Rachis	-	-	-	-		18.0	-	0.93	-	97.5	-	12.0	-	0.37	-	0.14	-	0.007	-	2.37
Barbs	-	-	-	-		147	-	15.2	-	125	-	7.50	-	2.34	-	0.22	-	0.050	-	3.26

a, % ; b, mg / wet g ; c, cirvical vertebrae ; Ch, chick ; Ad, adult

A wide variation in the concentrations of metals in bones of different regions of the body was evident (Table 2); the concentrations of Fe, Cu, Pb, Ni, Cd and Hg were highest in the sternum and lowest in the humerus although the lowest concentrations in the chick existed in the tibia. When the concentrations of Fe, Cu, Pb, Ni, Cd and Hg were plotted against the contents of moisture (not shown), their metal concentrations increased with the increase of moisture content. This suggests that the accumulation of these five metals in the bones depends on the blood content. However, relatively high concentrations of the metals were observed in the sternum of adult egret, this possibly suggesting the different forms of the metals between the sternum and the other bones. Bone Fe is present in the blood and in red marrow as storage Fe, where Fe exists as ferric hydroxide (Underwood 1971). A relatively high concentration of Fe in the sternum is due to an accumulation of ferric hydroxide in the red marrow, and also Cu, Ni, Pb, Cd and Hg might be highly accumulated in red marrow of the sternum in the same way as the Fe accumulation.

On the other hand, the distribution patterns of Zn and Mn concentrations in the bones were similar to that of Ca, which implies that both the metals are accumulated in the bone with ossification. However, the relations between the concentrations of Zn or Mn and the Ca concentration were different between the chick and adult. The concentration ratios of Zn/Ca in bones of the chick were higher than those of the adult, and this suggests that the chick has relatively high absorption and accumulation capacity of Zn when compared to the adult. Furthermore, the concentration of Mn in the chick bones increased with the increase of Ca concentration, while for the adult the concentration of Mn did not increase with the Ca concentration. The distribution of Zn and Mn in this study agreed with the results in the bones of striped dolphin (Stenella coeruleoalba) (Honda et al. 1984). Although it is well known that both Zn and Mn are essential for the formation of bone (Underwood 1971), the different accumulation patterns of both metals in the adult bone suggest a different function of two metals on bone calcification.

Table 2 shows the concentration of metals in three types of feathers and two parts of a primary feather of adult egret. Their variations both between the types and between the parts are evident. All the metal concentrations were highest in the feathers collected from the abdominal surface, and decreased in the order of coverts and remiges. But the concentration change of Cu among three feathers was not so large. In the primary feather, however, most of the metal concentrations was much higher in the barbs than the rachis, while the opposite was observed with Cu. The abdominal feather of adult egret is mainly composed of down and powder feathers, while the coverts and remiges such as primaries and secondaries are made of plumes and semiplumes (Mcfarland et al. 1979). The abdominal feathers has relatively large percentage in weight of barbs to rachis when compared to the coverts and remiges. Therefore, the different feather

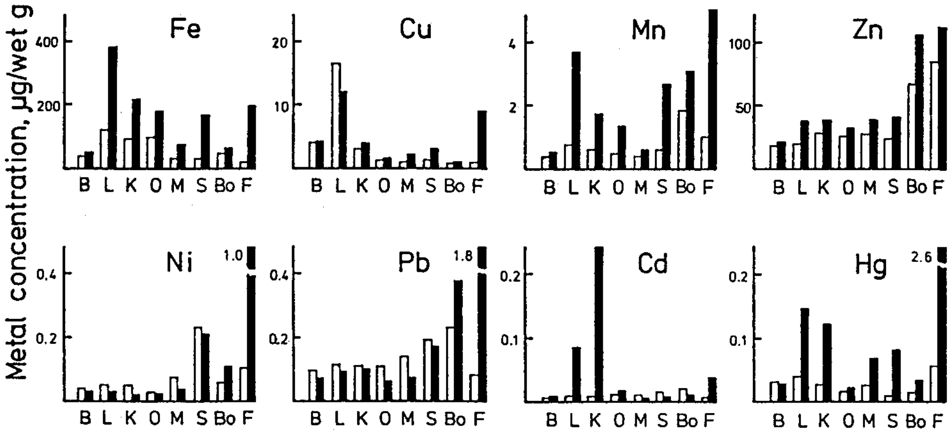

Figure 1. Comparison of metal concentrations in brain (B), liver (L), kidney (K), other viscera (O), muscle (M), skin (S), bone (Bo), and feather (F) of the chick (□) and adult (■) egrets

structure may account for the variations of metal concentrations among the bodily positions of feathers. Furthermore, all the metal concentrations in the adult feathers were higher than those in the chick (Table 2), suggesting that the metals in feather are accumulated with the growth of feather, and the details in growth-related accumulation of the metals will be published elsewhere.

The concentrations of the metals in brain, liver, kidney, muscle, bone, feathers, skin and the other viscera are shown in Figure 1. The metal concentrations in the adult egret were generally high in liver, kidney, skin, bone and feathers, and low in brain and muscle. These distribution patterns agreed with those in many other birds species (Tatsukawa et al. 1974; Finley and Stendell 1978; Furness and Hutton 1979; Osborn et al. 1979; Cheney et al. 1981; Nicholson 1981; Delbeke et al. 1984). In particular, a low concentration of metals in the brain may be explained by the "blood-brain barrier" (Okada 1978), although the mechanism is not clear. But there are some discrepancies and metal-specific accumulation. The concentrations of Fe, Cu, Mn, Cd and Hg were relatively high in liver and kidney, but not noticeably so with Zn, Ni and Pb. A relatively high accumulation of Cu was observed in the brain and feathers and it has been reported that Cu in the brain is associated with Cu-protein as albocuprein-I and -II (Fushimi et al. 1971). Furthermore, high accumulations of Mn, Zn, Ni, Pb and Hg were found in the hard tissues such as bone, skin and feathers. It is well known that high accumulations of these metals have already been reported in the hard tissues of upland and sea birds (Tatsukawa et al 1974; Osborn et al. 1979; Hulse et al. 1980; Cheny et al. 1981). According to Underwood (1971), Zn and Pb are stored in bone with ossification and Mn, Zn and Ni are related to the pigmentation of feathers. However, chemical form of these metals

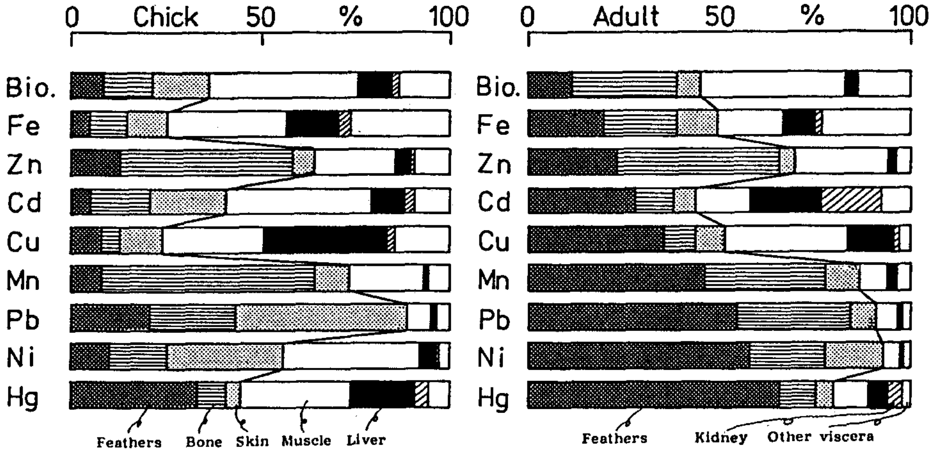

Figure 2. Distribution of metal burdens in the various organs and tissues of chick and adult egrets. Bio: organ and tissue weight %

in the hard tissues is not adequately understood.

Similar distribution patterns of the metals in organ and tissue were found in the chick egret also, but there are some differences between the chick and adult. Cu in liver, and Pb and Ni in soft tissues such as brain, liver and kidney show a comparatively high accumulation when compared to the adults.

The metal burdens in organs and tissues were calculated from the weight of organs and tissues and their metal concentrations, and the results are expressed as percentage of organ and tissue burden to total body burden (Figure 2).

In adult egret, relatively high percentages of the metal burdens in the whole body were presented generally in the feathers, which comprised only 10 % of the body weight, being 50 % or more with Hg, Ni, Pb and Mn. This means that a majority of these metals in a bird body is excreted by moulting. Cd and Cu burdens in liver and Cd in kidney were relatively high.

In contrast to the adult, chick egret showed relatively high burdens of the metals in the soft tissues such as muscle and liver compared with those in the hard tissues such as feathers, bone and skin, and very low burdens of Mn and Ni in the chick feathers. Furthermore, among the soft tissues of the chick, hepatic Cu consisted relatively high burden, while hepatic and renal Cd showed relatively low burdens. These observations suggest that accumulation of metals vary with the development processes of organs and tissues.

Higher accumulations of Fe and Cd were observed in soft tissues such as liver and kidney than in hard tissues. Cu, Hg and Mn showed high accumulation in feathers, with relatively high accumulations in liver and kidney. Whilst higher accumulations of

Zn, Pb and Ni were found in hard tissues such as feathers and bone than soft tissues. Consequently, when the accumulation processes of these metals in egret are discussed ecologically, analyses of liver, kidney, bone and feather are essential. If the tissue of the highest accumulation is needed, liver and kidney for Fe and Cd, liver and feather for Cu, feather for Hg, and feather and bone for Mn, Zn, Pb and Ni are to be selected and analyzed. The accumulation of toxic metals such as Hg, Pb and Cd in muscle can be used for a comparative study in residue levels between birds species, and also for hygienic metal pollution, in some cases. However, because the concentrations of all the metals vary with the growth stages, the parts of an egret to be analyzed must be determined by considering like stages and development process of the organ and tissue. Furthermore, when the heavy metal accumulation is considered with muscle, bone and feathers, the significant regional variation in concentration within the organs and tissues should be kept in mind. Usually, the pectoral muscle, femur and primary feather are recommended for a comparison. Detailed variation of the metals distribution according to biological conditions such as growth, reproductive processes and moulting, will be published elsewhere.

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