Research Note

Distribution of selenium binding proteins in liver from two species of penguins from Bouvetøya

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Selenium is an essential trace element and in many animal species and in humans the hepatic level of this element gives an estimate of the nutritional status. At the same time selenium is a toxic element and there seems to be an optimum intake (Moksnes & Norheim 1983). However, in some animal species with very high levels of selenium in the liver, there is no relationship with the intake. On the other hand, the selenium level may be related to the level of some heavy metals, especially mercury (Kari & Kauranen 1978; Norheim et al. 1984; Norheim 1987).

Selenium functions as an integrated part of the enzyme glutathione peroxidase (GSH-Px) and it is assumed that this is its most important role. However, selenium may also have other functions in the body (Magos & Webb 1980).

Little is known about how selenium is bound in animals with high hepatic levels, but it is assumed that it is involved in the detoxification of heavy metals such as mercury and cadmium. The aim of the present study was to make a preliminary investigation of the selenium binding components in species of birds with naturally high hepatic levels of selenium. To this end we have studied two species of penguins.

Materials and methods

Sampling

During the Norwegian Antarctic Research Expedition 1984/85, specimens of chinstrap Pygoscelis antarctica and macaroni penguin Eudyptes chrysolophus were collected at Bouvetøya (54°26'S, 3°21'W) in the south Atlantic. The birds were chloroformed, and samples of liver were removed and stored in liquid nitrogen.

Analytical methods

Selenium was determined by a hydride generator-atomic absorption method after digestion in a mixture of perchloric and nitric acid (Norheim & Haugen 1986).

The glutathione peroxidase (GSH-Px) activity in liver was measured at 37°C after homogenization in 0.15 mol/l of potassium chloride (Paglia & Valentine 1967; Moksnes & Norheim 1983). The total GSH-Px activity was measured with cumene hydroperoxide as substrate (Prohaska & Ganther 1977), while the selenium dependent GSH-Px activity was measured with hydrogen peroxide as substrate.

The gel filtration was carried out at 4°C on a Sephadex G-75 superfine (Pharmacia) column (2.6 x 40 cm). The ascending flow rate was 12.5 ml/h and 5 ml fractions were collected. One part of the liver sample was homogenized in two parts of a...
Table 1: Mean hepatic concentrations and ranges of selenium (µg/g wet weight), total and selenium dependent GSH-Px (µkat/kg wet weight) in chinstrap Pygoscelis antarctica and macaroni penguin Eudyptes chrysolophus from Bouvetøya.

<table>
<thead>
<tr>
<th></th>
<th>Chinstrap penguin</th>
<th>Macaroni penguin</th>
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<tbody>
<tr>
<td>µg Se/g</td>
<td>6.6 ± 2.9</td>
<td>23.6 ± 3.4</td>
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<tr>
<td>n = 10</td>
<td>(4.7–11.4)</td>
<td>(19.7–28.2)</td>
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<tr>
<td>µkat GSH-Px/kg</td>
<td>629 ± 58</td>
<td>838 ± 88</td>
</tr>
<tr>
<td>n = 4</td>
<td>(577–711)</td>
<td>(723–929)</td>
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<tr>
<td>Cumene hydroperoxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>µkat GSH-Px/kg</td>
<td>603 ± 54</td>
<td>802 ± 91</td>
</tr>
<tr>
<td>n = 4</td>
<td>(561–677)</td>
<td>(722–923)</td>
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<tr>
<td>Hydrogen peroxide</td>
<td></td>
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Results

The results of the analysis are given in Table 1. The average selenium levels in the liver of 10 chinstrap and 10 macaroni penguins were 6.6 ± 2.9 and 23.6 ± 3.4 µg/g wet weight (P < 0.001), respectively. In four chinstrap penguins, the average hepatic GSH-Px activity was 629 ± 58 µkat/kg wet weight using cumene hydroperoxide as substrate and 603 ± 54 µkat/kg using hydrogen peroxide as substrate. The corresponding GSH-Px activities in four macaroni penguins were 838 ± 88 and 802 ± 91 µkat/kg, respectively. The difference in enzyme activity between the two species was statistically significant (P < 0.01). On an average, the selenium dependent activity measured with hydrogen peroxide as substrate was 96 ± 3.2% (n = 8) of the total GSH-Px activity measured with cumene hydroperoxide as substrate. There was no difference between the two species with respect to this percentage.

Fig. 1. The distribution of soluble hepatic selenium binding compounds in a chinstrap penguin (6.1 µg Se/g liver wet weight, dashed line) and a macaroni penguin (19.7 µg/g, solid line) after gel filtration on Sephadex G-75.

Fig. 2. The amount of selenium in Fractions I, II, and III as a function of the hepatic selenium concentration in chinstrap (●) and macaroni (■) penguins.
The average recovery of selenium present in the liver extracted with Tris buffer for five chinstrap and five macaroni penguins was $100 \pm 7.8\%$ (n = 10). All of the selenium was also recovered after gel filtration. The selenium binding compounds present in the cytosol were separated into three main fractions on the Sephadex G-75 column (Fig. 1). Fraction II seemed to coincide with metallothionine. Fraction III, which was eluted near the total volume of the column, contained non-protein selenium containing compounds. The amounts of selenium present in Fractions I, II, and III are shown as a function of the liver concentration in Fig. 2. The difference in selenium amount in Fractions I and III between chinstrap and macaroni penguins is statistically significant (P < 0.01 and P < 0.001, respectively), while there is no difference in Fraction II.

**Discussion**

The hepatic levels of selenium in chinstrap and macaroni penguins found in the present investigation correspond with earlier findings (Norheim 1987). Thus, it is assumed that these levels are representative of the population of the two penguin species at Bouvetøya. It has previously been shown that the selenium concentration may be related to the levels of heavy metals such as cadmium and mercury (Norheim 1987) rather than being representative of the nutritional status.

Studies of GSH-Px in birds which bear relevance to the present study are rare. However, the GSH-Px activities observed seem to be within reasonable limits. If we calculate the amount of selenium present in liver and recovered in Fraction I, the figures for chinstrap and macaroni penguins are 0.64 ± 0.11 and 0.99 ± 0.17 µg Se/g liver weight, respectively. In an experimental study, chickens with a hepatic selenium level of 0.40–0.45 µg/g had a hepatic GSH-Px activity of 285–340 pkat/kg, both on a wet weight basis (Norheim & Moksnes 1985; Moksnes & Norheim 1986). Although the ratio between the amount of selenium bound to GSH-Px and other selenium proteins, that may be present in Fraction I, is not known either in chickens or in penguins, the present results seem to be in agreement with the experimental results. However, the amount of data up till now is very limited. Practically all of the GSH-Px activity recorded was present as selenium dependent activity. In chickens and ducks the selenium dependent activity was about 80%. This percentage was lower in selenium-depleted birds (Schafer et al. 1982; Xu & Diplock 1983; Norheim & Moksnes 1985).

All hepatic selenium seems to be present in the cytosol, but the main part is not bound to proteins. In chinstrap and macaroni penguins, the percentages of hepatic selenium recovered in Fraction III were 83 ± 2.6 and 94 ± 1.1 (P < 0.001), respectively. The distribution of the selenium binding components had a resemblance to the distribution in human liver except for the much lower share of non-protein selenium in this species (Norheim & Steiness 1976). A non-protein fraction seems to be present in rats dosed both with mercury and selenium. However, in these experiments only 16% of the hepatic selenium was present in the soluble fraction (Komsta-Szumsk & Chmielnicka 1977). The first eluted peak in the penguin samples is broad and seems to include several selenium binding proteins. The second peak seems to coincide with metallothionein and contains a mean selenium concentration of 0.32 ± 0.10 µg/g referring to liver weight, with no difference between the species.

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**References**


