Rhodanese (Thiosulfate: Cyanide Sulfurtransferase) in the Digestive Tract of Chicken at Different Stages of Development

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ABSTRACT This study was undertaken to investigate the relationships between stage of embryonic development and early posthatch growth and the level of rhodanese (thiosulfate: cyanide sulfurtransferase) activity in different regions of the digestive tract and liver of chickens. The embryos were studied at 14, 17, and 20 d and chickens were 1, 2, and 3 wk old. All tissues studied contained rhodanese. The highest specific activity of rhodanese was present in the liver followed by the proventriculus (P < 0.05). The lowest level was in the esophagus. The level of rhodanese was found to increase with age in the proventriculus and duodenum. The highest rhodanese activity in 3-wk-old chickens was in the proventriculus followed by the liver. These results are discussed in terms of the role of different sections of the digestive tract of the chicken in cyanide metabolism.

(Key words: rhodanase, gastrointestinal tract, embryonic development, cyanide metabolism)

INTRODUCTION

Cyanide is a highly toxic compound that is readily absorbed and causes death by preventing the use of oxygen by tissues (Egekeza et al., 1980). This toxicant is widespread in the environment. Many naturally occurring substances as well as industrial products contain cyanide (Egekeza et al., 1980). More than 2,000 species of plants are known to contain cyanogenic glycosides (Vennesland et al., 1982). It has been reported that ingestion of cyanogenic glycosides in forage crops can result in the death of grazing animals (Keeler et al., 1978). Many studies report the death of birds from cyanide poisoning through several routes, including exposure to cyanide salts or ingestion of cyanogenic plants (Wiemeyer et al., 1986).

The enzyme rhodanese (EC. 2.8.1.1., thiosulfate: cyanide sulfurtransferase) is a ubiquitous enzyme that is known to be responsible for the biotransformation of cyanide to thiocyanate (Westley, 1973). This enzyme is believed to be involved in cyanide detoxification in living organisms (Way, 1984; Aminlari and Gilanpour, 1991). The liver has always been considered to be the major source of rhodanese and is believed to be the major site of cyanide detoxification (Drawbaugh and Marrs, 1987). However, we have recently shown that different parts of the stomach in sheep and cattle (Aminlari and Gilanpour, 1991) and the proventriculus in chickens (Aminlari and Shahbazi, 1994) contain greater rhodanese activity than liver. We report here on the pattern of distribution of rhodanese in different sections of the digestive tract of chickens at different embryonic stages as well as different ages posthatch and compare these with rhodanese distribution in the liver. The results might indicate the parts of the digestive system of chicken that are significantly involved in cyanide metabolism.

MATERIALS AND METHODS

Preparation of Tissue Extract

All tissues were obtained from commercial Lohman strain chicken embryos and chickens after hatching. Five embryos and five chickens were used for each age group. Embryos were at 14, 17, and 20 d of development and chickens were chosen 1, 2, and 3 wk after hatching. All samples, kept on ice, were transferred within 45 min to the laboratory; tissues were separated, stripped from fat, washed a few times with physiological saline, and then blotted. Tissue extracts were prepared by freezing the sample in liquid nitrogen, homogenizing with a hand homogenizer, and suspending the homogenate in 0.025 mL/L sodium phosphate buffer, pH 7.2. The suspensions were centrifuged for 15 min at 4,000 × g in a MSE high speed refrigerated centrifuge. The supernatants were used as the source of enzyme. Samples with too high a rhodanese activity or that were too concentrated in...
protein were appropriately diluted with phosphate buffer and the final results were multiplied by the dilution factor.

**Determination of Rhodanese**

Rhodanese was assayed by the modified method of Sorbo (1953). The reaction mixture contained 16.8 mmol/L sodium thiosulfate, 40 mmol/L glycine buffer pH 9.2, 6.7 mmol/L KCN and 30 μL of enzyme solution in a final volume of 4.0 mL. The reaction was carried out for 20 min at 37°C and stopped by adding 0.5 mL 38% formaldehyde. In control tubes, formaldehyde was added prior to the addition of enzyme solution. The concentration of thiocyanate was measured by the method of Sorbo (1953). The reaction mixture contained 16.8 mmol/L sodium thiosulfate, 40 mmol/L glycine buffer pH 9.2, 6.7 mmol/L KCN and 30 μL of enzyme solution in a final volume of 4.0 mL. The reaction was carried out for 20 min at 37°C and stopped by adding 0.5 mL 38% formaldehyde.

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**Statistical Analysis**

Tissue rhodanese activity data were analyzed by one-way ANOVA using SPSS/PC® software. Duncan’s multiple range test was used to detect significant differences among rhodanese activity of various tissues.

**RESULTS AND DISCUSSION**

A total of 300 samples from different parts of the digestive tract and liver of chicken at different embryonic ages and at ages after hatching were analyzed for rhodanese activity. The results are shown in Table 1. Significant variation exists in the pattern of distribution of rhodanese in different parts of the digestive tract and at different ages. In the liver, no significant differences (P < 0.05) due to age were observed. In the proventriculus, units per gram tissue did not show significant change until chickens reached 2 wk of age, at which time rhodanese starts to increase. In the duodenum similar changes were present but less pronounced. In all other tissues, different values were obtained at different ages; however, these changes do not follow a specific pattern. The lowest level of activity was present in the esophagus. At all ages, liver followed by proventriculus exhibited significantly greater rhodanese activity per gram of tissue than all other parts studied (P < 0.05).

More than 60 yr after the discovery of rhodanese (Lang, 1933), despite extensive biochemical and physiological studies, its true biological function is still an enigma. Although the role of cyanide detoxification has been attributed to this enzyme by many investigators, there is evidence that other functions, such as participation in formation of iron-sulfur centers (Volini et al., 1977) and regulation of energy metabolism (Alexander and Volini, 1987), are also performed by rhodanese. However, compelling evidence provided by various laboratories has re-enforced the long held notion that rhodanese indeed plays a central role in cyanide detoxification (Aminlari and Shahbazi, 1994).

The pattern of distribution of rhodanese in different tissues seems to be highly species specific. In most animals, liver is the richest source of this enzyme. However, the presence of significantly high rhodanese activity in other organs is well documented (Oh et al., 1977; Drawbaugh and Marrs, 1987; Aminlari and Gilanpour, 1991). In the present study, it was shown that...

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**TABLE 1. Mean (± SD) of rhodanese activity (units per gram of tissue) in the extract from tissues of different sections of the digestive tract of chicken at different ages**

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Embryo’s age</th>
<th>Chicken’s age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 d</td>
<td>17 d</td>
</tr>
<tr>
<td>Liver</td>
<td>20.6 (2.8)x</td>
<td>20.9 (4.3)x</td>
</tr>
<tr>
<td>Proventriculus</td>
<td>4.0 (0.6)c,y</td>
<td>4.7 (1.6)c,y</td>
</tr>
<tr>
<td>Esophagus</td>
<td>1.7 (0.2)b,z</td>
<td>0.9 (0.2)c,z</td>
</tr>
<tr>
<td>Crops</td>
<td>1.1 (0.1)b,c,z</td>
<td>1.7 (0.3)d,z</td>
</tr>
<tr>
<td>Gizzard</td>
<td>1.6 (0.4)c,b,z</td>
<td>0.6 (0.2)c,z</td>
</tr>
<tr>
<td>Duodenum</td>
<td>2.3 (0.4)c,y</td>
<td>2.5 (0.8)c,y</td>
</tr>
<tr>
<td>Jejunum</td>
<td>2.2 (0.3)c,b,z</td>
<td>1.2 (0.3)c,z</td>
</tr>
<tr>
<td>Ileum</td>
<td>1.0 (0.3)c,z</td>
<td>1.5 (0.1)c,b,c,z</td>
</tr>
<tr>
<td>Cecum</td>
<td>1.7 (0.2)c,z</td>
<td>2.5 (0.4)c,b,z</td>
</tr>
<tr>
<td>Rectum</td>
<td>2.2 (0.3)c,b,z</td>
<td>1.0 (0.2)c,z</td>
</tr>
</tbody>
</table>

<sup>x</sup>Mean ± SD in each row with no common superscript differ significantly (P < 0.05).

<sup>y</sup>Mean ± SD in each column with no common superscript differ significantly (P < 0.05).

<sup>1</sup>n = 5 for each tissue studied.

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considerable variation exists in rhodanese distribution in different parts of the digestive system of chicken embryos and in posthatch chickens. At all ages, the liver is the richest source of rhodanese. The level of hepatic rhodanese was constant before and after hatching. However, in the proventriculus, the specific activity of rhodanese in the submucosal layer of proventriculus is almost twice of that of the liver (Aminlari and Shahbazi, 1994). This pattern of rhodanese distribution might have significant physiological consequences with respect to cyanide metabolism. It has been suggested that the level of rhodanese in the tissues of animals reflects the efficacy of tissues in cyanide detoxification (Aminlari et al., 1994).

A higher rhodanese activity in the proventriculus than in other parts of the digestive tract is not unexpected because this organ is the first section of the digestive tract in which feed is digested. During a chick’s embryonic life, no contact is made with feeds, hence one might expect low rhodanese activity at this stage of development. After hatching and exposure to feeds which contain cyanogenic compounds, the rhodanese activity of proventriculus starts to increase and at older ages exceeds that of liver. At all ages, part of the absorbed cyanide is metabolized by hepatic rhodanese, giving further protection against the deleterious effect of cyanide. These possibilities can be explored further by experimentally inducing cyanide poisoning in chickens and analyzing rhodanese activity at different stages of development. Furthermore, in view of accumulating evidence that rhodanese is involved in the regulation of mitochondrial electron transport (Ogata and Volini, 1990), high rhodanese activity in the gastrointestinal tract of chicks during development might reflect the energy demands of those tissues that have both high metabolic activity and high turnover rates.

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REFERENCES