EFFECT OF DIET ON BLOOD SELENIUM AND GLUTATHIONE PEROXIDASE ACTIVITY IN THE ALLIGATOR

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(Received 26 April 1983)

Abstract—I. Four groups of immature alligators were fed diets of ground nutria (Myocastor coypus) meat; ground fish (Merluccius spp); nutria plus sodium selenite; and fish plus sodium selenite. Plasma selenium and GSH-Px activity showed a high degree of correlation (r=0.944)

2. Plasma selenium, plasma glutathione peroxidase and erythrocyte glutathione peroxidase (GSH-Px) were measured in blood samples taken 10 days before and at 18, 39 and 60 days after initiating the diets.

3. Selenium concentration and plasma GSH-Px activity remained at low levels in the group fed nutria alone, but increased significantly in the groups fed fish alone, nutria plus selenite and fish plus selenite. Erythrocyte GSH-Px activity was more variable than the plasma enzyme activity, but increased significantly in the groups fed nutria plus selenite and fish plus selenite by 39 days. In the groups fed fish alone enzyme activity was significantly higher than the group fed nutria alone only at 60 days.

INTRODUCTION

The element selenium was first shown to be essential in the prevention of necrotic liver degeneration in rats (Schwartz and Foltz, 1957). Selenium has since been shown to be an essential micronutrient independent of other variables, in a number of domestic animals (Lannek and Lindberg, 1975), rats (McCoy and Weswig, 1969), and chickens (Thompson and Scott, 1969). Recent studies have shown that selenium is also an essential trace element in at least two teleost species (Poston et al., 1976; Hilton et al., 1980). However, the role of selenium in other poikilothermic vertebrates has not been investigated.

The only known function of selenium in vertebrates is as an essential component of the enzyme, glutathione peroxidase (GSH-Px). This enzyme, (E.C. 1.11.1.9; glutathione-peroxide oxidoreductase) contains four gram atoms of selenium per mole covalently linked in the form of seleno cysteine (Flohe et al., 1979). Many diseases caused by a deficiency in dietary selenium can now be explained by a decrease in the activity of this enzyme (Flohe et al., 1979).

A direct relationship between blood selenium levels and GSH-Px activity has been shown for several species, including humans (Lane et al., 1981), horses (Caple et al., 1978), deer (Brady et al., 1978), cattle and sheep (Scholz and Hutchinson, 1979, Thompson et al., 1976; Wilson and Judson, 1976), swine (Silvertsen et al., 1977; Jorgensen et al., 1977) rats (Hafeman et al., 1974; Smith et al., 1974), chickens (Noguchi et al., 1973; Omaye and Tappel, 1974; Gabrielsen and Opstvedt, 1980), quail (Kling and Soares, 1978) and salmon (Poston et al., 1976). Some controversy exists, however, as some authors have been unable to demonstrate a clear relationship between selenium and GSH-Px activity in some species such as pigs (Thompson et al., 1976) and primates (Butler et al., 1982). It has been suggested that in some species such as salmon and trout, selenium requirements may decline as the animal matures, hence the GSH-Px—selenium relationship may not be as marked (Poston et al., 1976; Hilton et al., 1980).

In the present study the effects of dietary selenium on blood GSH-Px activity in juvenile alligators fed two different diets was investigated.

MATERIALS AND METHODS

Animals

Four groups of ten juvenile alligators, which were hatched from artificially incubated eggs nine months prior to the start of the experiment were placed in four separate environmental chambers held at a constant temperature of 29°C. Prior to the experiment the alligators had been fed a diet of ground nutria meat (Myocastor coypus) with a vitamin and mineral supplement lacking selenium. The chambers, egg incubation techniques and alligator rearing practices have been described in detail by Joanne and McNease (1975, 1976, 1979). At the start of the experiment the four groups were fed the following diets (all of which were supplemented with the usual vitamin and mineral supplement): (1) ground nutria meat alone; (2) ground nutria plus selenium; (3) ground fish (Atlantic hake or whiting, Merluccius spp) alone; (4) ground fish plus selenium. Selenium was added in the form of sodium selenite (Nutritional Biochemicals) such that the final mix contained approximately 1 ppm selenium. A blood sample was taken from 20 of the 40 alligators ten days before the experimental diets were initiated, then each animal was bled at 18, 39 and 60 days of the treatment period. At each sampling period the alligators were weighed to the nearest gram and their total length recorded.

In addition to the juvenile alligators described above blood samples were taken from 23 adult wild alligators. 11 adult captive alligators fed fish, and 10 adult captive alligators fed nutria without selenium supplement, and assayed for enzyme activity. Plasma selenium concentration only was measured in over 100 samples collected.
from adult alligators. Blood samples were also taken from alligators immediately after hatching; 12 from eggs collected in the wild and 12 from eggs laid by captive animals, and assayed for enzyme activity only.

Preparation of blood samples

For the juvenile alligators, one ml of blood was drawn from the supravertebral branch of the internal jugular vein as described by Olson et al. (1975), using a heparinized syringe with a 26 gauge needle. Adult alligators and hatchlings were bled from the same vessel, with 50 ml of blood being taken from adults with an 18 gauge needle, and one ml from the hatchlings with a 27 gauge needle. Plasma and erythrocytes were separated by centrifugation for 5 min in a clinical desk-top centrifuge and the plasma stored on dry ice until taken back to the laboratory for assay. Red blood cells were washed twice with a 0.16 M sodium chloride solution then lysed by adding distilled water and drawing the cells up into a pasteur pipette several times. The hemolysate was stored on dry ice. Immediately before assaying, the hemolysate was thawed and then centrifuged at 2000 g for 10 min in a refrigerated centrifuge to remove red cell fragments.

Analytical techniques

Glutathione peroxidase activity in blood plasma and hemolysate was measured using a Beckman 35 spectrophotometer at 340 nanometers by the method of Paglia and Valentine (1967) with minor modifications as described in detail elsewhere (Lance and Elsey, 1983). GSH-Px activity was expressed in units/ml plasma or units/g hemoglobin where one enzyme unit is equal to one μmol NADPH oxidized/min. To avoid inter assay variation, samples from each treatment group were run in each enzyme assay. Selenium was measured in pooled plasma samples (3 to 4 pools per treatment group) from the juvenile alligators, in individual 1 to 2 ml plasma samples from adult alligators, and in three replicates each of ground nutria meat and whole fish, using the spectrophotofluorometric method of Olson (1969).

Statistics

Data were subjected to one way analysis of variance, followed by Duncan’s multiple comparison to obtain P values, with statistical significance given at P < 0.05.

RESULTS

In the initial experiments involving four groups of juvenile alligators fed either nutria alone, nutria plus selenium, fish alone, or fish plus selenium, there were no significant differences in weight gain or growth rate among the different groups (Tables 1 and 2).

Plasma GSH-Px activity

The plasma values for GSH-Px activity in the four experimental groups are presented in Fig. 1. In the group fed nutria alone, no differences in plasma GSH-Px activity were detected throughout the 9 weeks of the experiment. However, when selenium was added to the diet a dramatic increase in plasma GSH-Px activity was evident by 18 days, and this activity remained significantly elevated for the duration of the experiment. In the animals fed fish alone GSH-Px activity also increased to levels not significantly different from the nutria-fed group given selenium or the fish-fed group given selenium. Both fish-fed groups had GSH-Px activity signifi-antly higher than the group fed nutria alone at 18, 39 and 60 days. The fish-fed group which received selenium had slightly, but significantly higher enzyme activity at 39 days than the group fed fish alone. There were no significant differences between the two groups at any of the other sampling periods.

Red cell GSH-Px activity

Enzyme activity in red blood cells of the four treatment groups are given in Fig. 2. There was more variability in red cell than in plasma enzyme activity, and levels in the control group fed nutria alone actually declined during the experiment. Despite this variability, addition of selenium to the diet caused a significant increase in GSH-Px activity by 39 and 60 days, but only a slight but non-significant difference at 18 days. Enzyme activity in red cells at 39 days was significantly higher than activity at 60 days in the nutria-fed group receiving selenium. In the group fed fish alone there were no significant differences in red cell GSH-Px activity throughout the experiment, in contrast to plasma enzyme activity in this group which showed a highly significant increase by 18 days of receiving the diet. Although enzyme activity did not change in the group fed fish alone, the levels were significantly higher at days 39 and 60 than in the group fed nutria. In the fish-fed group receiving selenium however, red cell GSH-Px activity showed a significant increase over red cell activity in the group fed fish alone by 39 days, but not at 60 days.

Selenium

Plasma selenium concentrations in the four treatment groups is shown in Fig. 3. There were no significant changes with time in the nutria-fed control group, whereas addition of selenium to the diet, or a diet of fish alone caused a highly significant increase in plasma selenium. In the group fed fish

<table>
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<th>Days of treatment</th>
<th>Body weight (gm)*</th>
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<tbody>
<tr>
<td></td>
<td>−10</td>
</tr>
<tr>
<td>Group 1</td>
<td>516±23</td>
</tr>
<tr>
<td>Group 2</td>
<td>649±43</td>
</tr>
<tr>
<td>Group 3</td>
<td>607±53</td>
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<tr>
<td>Group 4</td>
<td>627±38</td>
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*n=10 for each group. Means±SEM.

<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>Total length (cm)</th>
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<tr>
<td></td>
<td>−10</td>
</tr>
<tr>
<td>Group 1</td>
<td>57.6±0.6</td>
</tr>
<tr>
<td>Group 2</td>
<td>62.9±1.3</td>
</tr>
<tr>
<td>Group 3</td>
<td>58.2±1.7</td>
</tr>
<tr>
<td>Group 4</td>
<td>61.9±0.7</td>
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</table>
Selenium and glutathione peroxidase in the alligator

Fig. 1. Plasma glutathione peroxidase activity in juvenile alligators fed different diets. Bars represent the mean±SEM of the 10 animals in each treatment group. One enzyme unit (EU) is defined as 1 μmol NADPH oxidized per min.

Plus selenium, plasma selenium concentrations were significantly higher than the group fed fish alone. When plasma selenium concentrations are plotted against plasma GSH-Px activity, a high degree of correlation \( r=0.944 \) is evident. The correlation coefficient for erythrocyte GSH-Px and plasma selenium however was only 0.676.

Plasma selenium in adult wild and captive alligators is given in Table 3. Selenium content of nutria meat was 0.04 μg/g wet wt, whereas fish selenium content was 0.40 μg/g wet wt.

Enzyme activity in plasma of adult and hatchling alligators is shown in Fig. 4. The levels in the

Fig. 2. Erythrocyte glutathione peroxidase activity in juvenile alligators fed different diets. Symbols as in Fig. 1.
juvenile alligators fed nutria alone have been included for comparison. In all groups enzyme activity was significantly higher than the activity in nutria-fed juveniles. Nutria-fed adults had slightly lower plasma GSH-Px activity than wild and fish-fed adults, but the differences were not significant.

In the red blood cells of these same animals, however, GSH-Px activity was significantly higher in wild and fish fed adults than in nutria-fed adults, nutria-fed juveniles, and hatchlings from eggs of wild alligators or eggs from captive alligators (Fig. 5).

**DISCUSSION**

The results of the first part of this study show that alligators have a plasma glutathione peroxidase that is responsive to dietary selenium, and that, as in a number of other species, plasma GSH-Px activity and plasma selenium concentration are strongly correlated. The levels of GSH-Px activity in the plasma of alligators are in the same range as has been reported in a number of mammalian species (Jorgensen et al., 1977; Scholtz and Hutchinson, 1979). On the other hand, levels of GSH-Px activity in the red blood cells of alligators are several orders of magnitude lower than those of rat erythrocytes (Tappel, 1974; Lance and Elsey, 1983). However, in a recent study we have shown that there is considerable species variation in red blood cell GSH-Px activity, with differences of even greater magnitude between closely related mammalian species. Red cell GSH-Px activity in the alligator for example, is more than twice that of the nutria (Myocastor coypus) when measured under identical conditions (Lance and Elsey, 1983). Whether these differences between species are due entirely to differences in dietary selenium intake remains unknown.

Nutria-fed alligators responded to the selenium supplement in the form of sodium selenite with a dramatic increase in plasma and erythrocyte enzyme activity (Figs 1 and 2). Similar responses to selenite in selenium deficient animals have been reported in a number of species (Hafeman et al., 1974; Noguchi et al., 1973). However, the effect of feeding fish alone to alligators previously on a diet of nutria meat was unexpected. The increase in plasma GSH-Px activity by 18 days of initiating the diet of fish alone was identical to that of the nutria-fed group receiving selenium. Selenium concentrations in the two groups were also similar (Fig. 3). The fish (Merluccius spp) used to feed the alligators contains high levels of selenium (0.40 μg/g wet wt), and is obviously a rich source of biologically available selenium, at least with respect to plasma GSH-Px. Adult alligators fed a diet of fish also have plasma selenium levels significantly higher than wild alligators or adult alligators fed nutria (Table 3). However, plasma GSH-Px activity in adult alligators fed fish is not significantly different from wild alligators or adult alligators fed nutria (Fig. 5). In a number of studies in which the ability of sodium selenite to restore plasma GSH-Px activity in selenium deficient animals has been compared to that of selenium from biological sources such as fish

<table>
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<th>Diet</th>
<th>Mean ± SD</th>
<th>n</th>
<th>Range</th>
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<tbody>
<tr>
<td>Wild</td>
<td>0.176±0.055</td>
<td>92</td>
<td>0.07-0.51</td>
</tr>
<tr>
<td>Fish (adult)</td>
<td>0.269±0.064</td>
<td>27</td>
<td>0.11-0.36</td>
</tr>
<tr>
<td>Nutria (adult)</td>
<td>0.191±0.060</td>
<td>56</td>
<td>0.04-0.33</td>
</tr>
<tr>
<td>Nutria (juvenile)</td>
<td>0.035±0.010</td>
<td>18</td>
<td>0.02-0.05</td>
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*Concentration in μg/ml.
meal or vegetable feed, selenite has been shown to be more effective in every case (Gabrielsen and Opstvedt, 1980). In the alligator, fish is equally as effective as sodium selenite in increasing plasma GSH-Px activity, but less effective than selenite in stimulating red cell GSH-Px activity.

Adult captive alligators fed nutria have plasma selenium levels and plasma GSH-Px activities considerably higher than the juvenile alligators fed nutria meat (Table 3, Fig. 4). As the adult alligators are kept in ponds in outdoor enclosures under "semi-natural" conditions (Joanen and McNease, 1975), they probably obtain additional selenium from other sources such as accidental ingestion of soil and plant material. Although these adult alligators appear to get additional selenium and show a plasma enzyme activity similar to that of wild alligators or alligators fed fish, the GSH-Px activity in the red cells of these animals is significantly lower than in the red cells of the other two groups of adult alligators (Fig. 5).

Although juvenile fish-fed alligators had plasma selenium concentrations and plasma GSH-Px activities not significantly different from those of nutria-fed alligators receiving sodium selenite, the RBC enzyme activities in these two groups were markedly different. Alligators fed nutria plus selenite had RBC enzyme activity significantly higher than the group fed fish alone at day 39 and day 60. The group fed fish plus selenite moreover, had the highest plasma selenium of all the groups (Fig. 3), but had lower red cell GSH-Px activity than the nutria-fed group receiving selenium. In other words, plasma selenium and plasma GSH-Px activity are
highly correlated (r=0.944), but plasma selenium and red cell GSH-Px activity are only marginally so (r=0.676). These results suggest that while the fish *Merluccius* contains a high concentration of selenium which is as readily available as selenite as a source of selenium for plasma GSH-Px in the alligator, the selenium in the fish is less readily available for incorporation into the enzyme in the erythrocyte.

Hafeman *et al.* (1974) showed that in the rat selenium is incorporated into red cell GSH-Px only during erythropoiesis, and that a period of 60 days (the life span of rat erythrocytes) is required to show maximal stimulation of enzyme activity. The life span of alligator erythrocytes has been estimated to range between 155 and 437 days in animals held at 31°C, and longer by a factor of 3 in animals held at 17°C (Cline and Waldmann, 1962). As alligator RBC enzyme activity obviously increased in animals receiving sodium selenite, it would appear that alligator erythrocytes (which are nucleated) are capable of protein synthesis, and are not dependent on erythropoiesis for incorporation of selenium into GSH-Px. What is not clear however, is why a diet of fish should be equally as effective as sodium selenite in stimulating plasma GSH-Px activity, but relatively ineffective in stimulating erythrocyte activity. What is also difficult to explain is the decline in RBC enzyme activity at day 60 as compared to day 39 in both groups getting sodium selenite, because plasma selenium and plasma GSH-Px activity remained elevated in these animals (Figs 2 and 3). No such decline has been noted in erythrocyte GSH-Px activity in other animal species receiving selenium supplemented diets (Silvertsen *et al.*, 1977; Hafeman *et al.*, 1974).

It is possible that the selenium requirements of alligator red cell GSH-Px are far less than those of plasma GSH-Px, and that the red cell system is only marginally deficient in alligators fed nutria meat. Enzyme activity in red cells of juveniles and adults on this diet are not significantly different (Fig. 6), whereas plasma selenium and plasma enzyme activities differ by a factor of four (Figs 3 and 5, Table 3).

Nutria meat has an extremely low selenium content, close to what has been diagnosed as selenium deficiency in domestic animals (Van Vleet, 1980). It is not surprising therefore, that alligators fed a diet of nutria meat alone would have low plasma selenium and low GSH-Px activity. A blood selenium concentration of less than 0.05 μg/ml has been suggested as diagnostic of selenium deficiency in domestic animals (Van Vleet, 1980). Juvenile alligators on a diet of nutria meat alone have a plasma selenium level of 0.04 μg/ml or less, and a plasma GSH-Px activity lower than all other groups studied (Fig. 5), both measures which would indicate selenium deficiency. However, these juvenile alligators show no obvious symptoms of a trace element deficiency, and in fact grow at a slightly greater rate than fish-fed alligators (Joanen and McNease, 1976). In this instance we appear to have a unique case of a carnivore exhibiting extremely low plasma selenium and GSH-Px activity as a result of being fed on meat from an animal with unusually low tissue selenium.

References—The authors wish to thank Ted Joanen and Larry McNease and the staff at Rockefeller Wildlife Refuge for their kind help and support during this project. We would also like to thank Deborah Wilson for her help with the selenium analysis and Jane Thompson for help with the statistics. This research was funded by the Louisiana Department of Wildlife and Fisheries.

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