BLOOD GLUTATHIONE PEROXIDASE ACTIVITY IN HORSES IN RELATION TO MUSCULAR DYSTROPHY AND SELENIUM NUTRITION

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SUMMARY: The activity of glutathione peroxidase, a selenium containing enzyme, was measured in the blood of horses to determine its usefulness as an indicator of selenium status. In 15 horses the enzyme activity was positively related to the blood selenium concentration (P < .001, r = 0.98) over the range of enzyme activities of 8.2 to 140 units (µmoles NADP-oxidised/min/gHb) and selenium concentrations of 0.24 to 2.74 µmol/l.

In a group of 8 horses which 2 foals had died with lesions of muscular dystrophy the enzyme activity increased from a mean of 11.8 units before treatment with selenium to 34.5 units after 2 intravenous injections of sodium selenite given one month apart. Another group of 8 horses grazing paddocks adjacent to this affected group did not receive any selenium treatment and had a mean enzyme activity of 11.9 units.

Blood glutathione peroxidase activity was measured in 50 pasture-fed horses and 180 stall-fed horses. The range of activities found (7 to 158 units) indicated that selenium intake in horses varied widely between localities. All pasture-fed horses grazing areas where muscular dystrophy had occurred in foals had low activities (less than 20 units). In stall-fed horses the enzyme activity was influenced by selenium treatment, and horses which had been treated usually had higher activities than horses in the same stable with no history of selenium treatment. It was concluded that blood glutathione peroxidase is a suitable indicator of selenium status in horses.

Introduction

Generalized muscular dystrophy in foals has been associated with deficiencies of selenium and Vitamin E (Schougaard et al 1972; Wilson et al 1976), and is considered to be similar to nutritional muscular dystrophy in ruminants (Hartley and Dodd 1957). As foals with the condition usually die, diagnosis has been based on pathological lesions and confirmed by estimations of selenium and Vitamin E content of feed, serum, blood and animal tissues (Gabbedy and Richards 1970; Wilson et al 1976). There are few studies of selenium levels in horses, and it is difficult to compare values reported by different laboratories (Schougaard et al 1972). Increased activities of serum enzymes such as creatinine phosphokinase and aspartate aminotransferase indicate acute muscular damage (Wilson et al 1976), but may not always be due to deficiency of selenium and/or Vitamin E.

Recently, the enzyme glutathione peroxidase was shown to contain selenium (Rotruck et al 1973). The activity of the enzyme in blood appears to be related to blood selenium concentration and dietary selenium intake, and is considered a sensitive indicator of selenium status in rats, chickens, sheep and cattle (Hoekstra 1974; Ganthier et al 1976; Thompson et al 1976; Wilson and Judson 1976), but there is little information available for horses.

The object of this study was to determine the usefulness of blood glutathione peroxidase activity as an indicator of selenium status in horses. The activity of the enzyme was measured in horses before and after selenium treatment on a property where foals had died with lesions of muscular dystrophy. The enzyme was also measured in stall-fed thoroughbred horses, and in horses grazing pasture where there was no history of muscular dystrophy. The relationship between blood glutathione peroxidase activity and selenium concentration in horses was examined.

Materials and Methods

Animals

A group of 10 horses on a property in Gippsland was examined in November 1976 when 2 foals died. Autopsy examination of the two foals, a 2-week-old palamino foal and a 5-week-old chestnut foal, revealed extensive pale yellow areas in the gluteal and other muscles of the hindlimbs, and in the diaphragm and intercostal muscles. Samples of these muscles from the 5-week-old foal were fixed in 10% formol saline and examined for histopathological lesions.

The mares had grazed in the paddocks without any supplementary feed for up to 5 years and there was no history of deaths of foals in previous years. The pasture had been poor during the autumn and winter of 1976, but growth increased after good rains in late winter and spring pasture consisted mainly of clover.

In December, blood samples were collected from the mares of the affected foals, and from 6 other horses which had been in the same paddock, before they were treated with a selenium.
and Vitamin E preparation*. The preparation contained 5.48 mg sodium selenite/ml and 50 mg Vitamin E/ml, and 10 ml was given intravenously to adult and yearling horses and 5 ml to foals. The horses were treated again in January. Further blood samples were collected from the horses in January and February. In February blood samples were collected from another 4 mares, 3 foals and a yearling which had grazed similar pasture on adjacent paddocks to the affected group. This group of mares had been supplemented during pregnancy with hay cut on the property during the previous summer. Blood samples were also collected from a mare and foal which had just returned to the property after being stall-fed for 9 weeks on another property.

Blood samples were collected from 32 horses which grazed pasture in other areas of Victoria, and from 180 thoroughbred racehorses which were being stall-fed while in work.

Analyses

Blood samples were collected from the jugular vein into plastic tubes containing dried di-potassium diamino-ethane-tetra-acetate (EDTA) and stored at -20°C. Glutathione peroxidase activity was measured by the method of Paglia and Valentine (1967). Preliminary experiments on samples from 4 horses showed that most activity was in the erythrocytes, and only low activities were present in plasma or serum. For this reason the enzyme activity in whole blood was measured in this study. The enzyme appeared to be stable when stored at -20°C as assays on 9 samples after 3 months showed there was no significant loss of activity. Activity was expressed as units, where one unit represented the oxidation of 1 μmole NADPH/min/gHb at 25°C.

Selenium concentration was determined in 15 blood samples by Dr. G. J. Judson, Institute of Medical and Veterinary Science, Adelaide, by the fluorimetric procedure of Watkinson (1966) as described by Wilson and Judson (1976).

Results

Histopathological examination of the skeletal muscle lesions of the 5-week-old foal revealed a progression of changes from hyaline and granular degeneration through to a coagulative necrosis of muscle fibres. Calcification was observed in some degenerate fibres. The endomysial connective tissue showed fibrous proliferation, and macrophages and mononuclear cells, chiefly lymphocytes, had infiltrated the tissue.

The activity of glutathione peroxidase in blood samples collected from the mares of these foals and other horses which grazed in the same paddock is shown in Table 1. The enzyme activity increased from a mean of 11.8 units in December 1976 to 34.5 units in February 1977. In this period the horses had received 2 injections of selenium (Table 1). Blood samples collected in February 1977 from the group of untreated horses grazing adjacent paddocks had a mean enzyme activity of 11.9 units which was significantly lower than the treated group (P<0.001 Student’s ‘t’ test).

Blood glutathione peroxidase activities in a mare and foal which had just returned from another property where they had been stall-fed were 38.5 and 60 units respectively.

TABLE 1

<table>
<thead>
<tr>
<th>Horse</th>
<th>Glutathione Peroxidase Activity (μmoles NADPH oxidised/min/gHb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>December</td>
</tr>
<tr>
<td>1. Mare of foal that died at 2 weeks of age</td>
<td>11.6</td>
</tr>
<tr>
<td>2. Mare of foal that died at 5 weeks of age</td>
<td>9.4</td>
</tr>
<tr>
<td>3. Yearling from Mare No. 2</td>
<td>10.9</td>
</tr>
<tr>
<td>4. Mare</td>
<td>11.1</td>
</tr>
<tr>
<td>5. Foal of Mare No. 4*</td>
<td>17.3</td>
</tr>
<tr>
<td>6. Mare</td>
<td>8.8</td>
</tr>
<tr>
<td>7. Foal of Mare No. 6*</td>
<td>13.7</td>
</tr>
<tr>
<td>8. Stallion</td>
<td>11.7</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>11.8±0.9</td>
</tr>
</tbody>
</table>

*Foals 5 and 7 were 10 and 7 days old respectively when samples were collected in December.
†Not determined.
Although the time course of blood glutathione peroxidase activity after a single treatment of selenium was not followed closely, in one stall-fed horse given 40 mg selenium intravenously the activity increased from 30 units to 79 units after 2 months and decreased to 59 units 5 months after treatment.

**Discussion**

Glutathione peroxidase activity in blood would appear to be a useful indicator of selenium status in horses. Mares of foals which had died with lesions of muscular dystrophy had low activities of glutathione peroxidase, and the enzyme activity increased after treatment with selenium. The glutathione peroxidase activity and selenium concentration in blood were significantly correlated over a wide range. Similar correlations have been reported in cattle and sheep (Thompson et al. 1976; Wilson and Judson 1976). In rats, chickens and sheep it has been shown that the glutathione peroxidase activity in blood and tissues is a function of dietary selenium (Chow and Tappel 1974; Hafeman et al. 1974; Oh et al. 1974), and it appears a similar situation exists in the horse.

Blood selenium concentration estimated in 15 horses ranged from 0.24 to 2.74 μmol/l and this range included nearly all of the horses in this study. Gabbedy and Richards (1970) reported a range of blood selenium concentrations of 0.24 to 0.34 μmol/l in a study of 11 horses and found that stall-fed horses had higher concentrations than pasture-fed horses. In our study the range of blood selenium concentrations and glutathione peroxidase activities found in pasture-fed horses was similar to that found in stall-fed horses (Figure 2). However, some pasture-fed horses in an area where muscular dystrophy occurred in foals had much lower levels than those found in stall-fed horses. The mean blood selenium concentration in the mares of the affected foals was 0.24 μmol/l was not as low as that found by Gabbedy and Richards (1970) in a mare of a foal which developed muscular dystrophy (0.1 μmol/l).

It is interesting to note that not all foals with low blood glutathione peroxidase activity developed muscular dystrophy. The aetiology of the syndrome is considered complex and, in addition to selenium deficiency, a number of factors such as Vitamin E deficiency, stress, and muscular activity may contribute to its development (Wilson et al. 1976).

The time taken for the enzyme activity in blood to reach a maximum level in horses after selenium treatment may be expected to be related to the time taken for synthesis of new erythrocytes. The

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**Figure 2** Histogram showing the frequency distribution of blood glutathione peroxidase activity in 50 pasture-fed horses, 115 stall-fed horses, and 65 stall-fed horses which had a history of selenium treatment.
activity in foal 7 increased more rapidly than in older horses following selenium treatment (Table 1). In sheep and cattle, younger animals have shorter erythrocyte life spans than older animals (Schalm et al 1975).

Muscular disorders of adult horses such as paralytic myoglobinuria and "tying up" are considered not to be associated with a deficiency of Vitamin E or selenium (Lannek and Lindberg, 1975). However, preparations of selenium and Vitamin E are often administered to racehorses with muscular disorders. In some horses this treatment appears to have a beneficial therapeutic effect, but there have been few critical studies undertaken. The clinical response is obtained before there would be any marked increase in blood glutathione peroxidase activity. It would be interesting to determine if horses which showed a clinical response to selenium therapy had low blood glutathione peroxidase activity before treatment.

The range of blood glutathione peroxidase activities found in horses indicate that dietary selenium intake varies widely throughout Victoria. The measurement of activity of the enzyme enables an easy assessment of selenium status in horses, and, in mares, may indicate where preventative measures need to be taken to ensure that foals will not be predisposed to nutritional muscular dystrophy. Low selenium values of suckling foals may indicate the occurrence of subclinical selenium-responsive myopathy (Stowe 1967).

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References

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