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## Selenium Deficiency in Cattle Associated with Heinz Bodies and Anemia

Abstract. *Cattle grazing St. Augustine grass growing on peaty muck soils in the Florida Everglades developed anemia associated with the presence of Heinz bodies and suboptimal concentrations of selenium in blood. Selenium supplementation corrected the anemia, prevented Heinz body formation, increased the body weight of cows and calves, and elevated blood selenium. This may be the first recorded example of widespread anemia in a population due to selenium deficiency.*

Glutathione peroxidase (E.C.1.11.1.9) protects red blood cells from oxidative damage due to hydrogen peroxide and other peroxides (*1*). The enzyme catalyzes the reduction of hydrogen peroxide to water and a large range of lipid peroxides to hydroxy acids (*2*). Reduced glutathione, the unique hydrogen donor in this process, is regenerated by reduced nicotinamide adenine dinucleotide phosphate and glutathione reductase. Glutathione peroxidase is a selenium-containing enzyme (*3*); in bovines it contains 4 g-atoms of selenium per mole (*4*).

Rotruck *et al.* (*5*) found that dietary selenium in the presence of glucose reduced ascorbic acid–induced hemolysis of rat erythrocytes, oxidation of hemoglobin, and the proportion of cells with Heinz bodies. Dietary α-tocopherol had no effect on hemoglobin oxidation or Heinz body formation.

Some cases of hemolytic anemia in humans appear to be associated with reduced levels of glutathione peroxidase in erythrocytes. Investigators have attributed the reduced enzyme activity to genetic causes (*5a*); however, it is possible that dietary selenium deficiency has a significant role in some instances. We report here an investigation of selenium deficiency–associated anemia in grazing beef cattle.

We studied a herd of Santa Gertrudis cattle grazing St. Augustine grass growing on peaty muck soils in the Everglades region of Florida. When subjected to standard ranch practice [96 cows per 40-ha paddock with access to one water trough and one mineral box containing a mixture of dicalcium phosphate, sodium chloride, vitamin A, and trace elements (*6*)], most cattle thrived, but a few developed a wasting condition and anemia. A multifactorial experimental design revealed that, in the absence of mineral supplementation, the cattle developed severe cobalt and copper deficiencies, anorexia, wasting, and anemia. These conditions were, in the main, reversed by the addition of cobalt and copper to the drinking water. Clearly, provision of a trace element mixture in a single box for a herd of cattle is an inadequate method of supplementation for a significant proportion of animals. While provision of cobalt in the drinking water substantially reduced the incidence of anemic animals to about one quarter of those previously shown to be anemic, a residual number with low hemoglobin persisted, particularly through the summer.

In investigating this residual anemia, we established that the grass had a very low selenium content (< 0.02 to 0.055 ppm dry matter, $N = 20$; detection limit, 0.02 ppm). Furthermore, examination of blood smears revealed a high incidence of Heinz bodies in anemic animals. Because of the previous rat studies (*5*) showing that oxidative damage of red cells could be prevented by selenium, the following field trial was undertaken.

Santa Gertrudis heifers ($N = 144$) pregnant with their first calves were selected for uniformity and randomly divided into four groups (A to D) of 36 animals each. Each group was randomly assigned to one of four 22.7-ha pastures, each with a separate water tank of approximately 10,000 liters, the sole source of free water available. Beginning on 11 July 1980 a concentrated solution of sodium selenate was added to the water of groups B, C, and D once weekly such

Table 1. Effect of dietary selenium on hematological parameters and concentration of selenium in whole blood of Santa Gertrudis heifers. Values are means ± standard errors. For each sampling, means in the same column with different superscripts are significantly different ($P < 0.01$, analysis of variance).

| Selenium intake (mg/day) | Selenium in blood (μg/dl) | Packed cell volume (percent) | Hemoglobin (grams per 100 ml) | Number of cows with Heinz bodies | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0 | +1 | +2 | +3 |
| | | *92 days after treatment* | | | | | |
| 0.0* | 0.38 ± 0.02 | 30.7 ± 0.56 | 10.5 ± 0.23 | 5 | 13 | 6 | 11 |
| 0.5 | 0.75 ± 0.03[a] | 40.5 ± 0.43[a] | 14.0 ± 0.90[a] | 36 | 0 | 0 | 0 |
| 1.0 | 1.1 ± 0.03[b] | 40.9 ± 0.38[a] | 14.2 ± 0.10[a] | 36 | 0 | 0 | 0 |
| 2.0 | 2.3 ± 0.10[c] | 40.5 ± 0.44[a] | 14.2 ± 0.12[a] | 36 | 0 | 0 | 0 |
| | | *1 year after treatment* | | | | | |
| 0.0 | 0.8 ± 0.1 | 32.9 ± 0.81 | 12.0 ± 0.26[b] | 0 | 7 | 7 | 22 |
| 1.0 | 5.4 ± 0.2[a] | 38.7 ± 0.43[a] | 12.2 ± 0.20[b] | 31 | 4 | 0 | 0 |
| 2.0 | 6.2 ± 0.3[a] | 39.6 ± 0.53[a] | 14.2 ± 0.17[c] | 35 | 1 | 0 | 0 |
| 0.5 to > 4.0 | 5.0 ± 0.3[a] | 39.8 ± 0.50[a] | 13.5 ± 0.23[c] | 35 | 1 | 0 | 0 |

*$N = 35$. One cow in this group died of undetermined causes before the 92 days after treatment sampling. A replacement cow of similar age was substituted to restore the number to 36.

that individuals received 0.5, 1.0, or 2.0 mg of selenium per day, respectively. Group A received no supplementary selenium. Water was added to the troughs generally on a daily basis but they were not emptied or allowed to overflow during the experiment. Previous measurements on comparable cows at the ranch gave a mean water consumption rate of 46 ± 6 liters per day. This level of intake by the experimental cows would have resulted in the solution in the trough turning over 1.5 times between weekly additions of selenium. The selenium intake of group B was increased from 0.5 to 4.0 mg per animal per day after 7 months. To the water troughs of all four groups aqueous solutions of copper sulfate, cobalt chloride, and phosphoric acid were added once weekly (equivalent to 128 mg, 8 mg, and 10 g of copper, cobalt, and phosphate per cow per day, respectively).

Blood samples were taken from the heifers on 11 October 1980 (at the beginning of calving, and after 92 days of treatment) and again on 25 June 1981 (after about 1 year of treatment). Hemoglobin and packed cell volume were measured by cyanmethemoglobin and microhematocrit methods for all samples. Smears of blood were stained with new methylene blue and examined for Heinz bodies, whose incidence was rated +1 (particles present in ≤ 10 percent of erthyrocytes in the field), +2 (11 to 20 percent), or +3 (> 20 percent). Whole blood was analyzed for selenium by a controlled-temperature acid digestion and an automated fluorometric procedure (*7*).

The effects of selenium treatment on packed cell volume, hemoglobin concentration, incidence of Heinz bodies, and selenium concentration in blood at both samplings are shown in Table 1. At the first sampling period (92 days of treatment), all levels of selenium supplementation completely protected against Heinz body formation. However, untreated cows had a high incidence of Heinz bodies. After 1 year the protective action of selenium was sustained, but an even higher incidence of Heinz bodies was observed in the control cows. At the 1-year sampling the blood from all 36 untreated cows had Heinz bodies, whereas none had been observed in five of these cows at the 92-day sampling.

Cows not receiving selenium had significantly lower packed cell volumes at both samplings. They had lower hemoglobin concentrations in their blood at the 92-day sampling than cows receiving 0.5 mg of selenium or more per day. Also, at the 1-year sampling, unsupplemented cows had significantly lower hemoglobin concentrations than cows receiving 2 or 4 mg of selenium per day. Thus the response of packed cell volume and hemoglobin to selenium is consistent with the observed lower incidence of Heinz bodies and presumably a normal red cell life-span. The presence of Heinz bodies in 4 of 36 cows given 1 mg of selenium per day at the 1-year sampling indicates that the requirement for complete protection exceeds 1 mg of selenium per cow per day. The four cows did not have a significantly lower mean concentration of selenium in blood than the overall group.

Table 2. Body weight (kilograms) of cows 1 year after treatment and of their calves at weaning (means ± standard errors).

| Selenium intake (mg/day) | Cows | Calves |
|---|---|---|
| 0 | 447 ± 7 | 261 ± 5 |
| 1.0 | 501 ± 7* | 300 ± 6* |
| 2.0 | 537 ± 7* | 297 ± 7* |
| 0.5 to > 4.0 | 500 ± 7* | 283 ± 6* |

*Signficantly different from control ($P < 0.01$).

Blood selenium concentrations in the four groups at the 92-day sampling were significantly different from each other and were rectilinearly related to the level of selenium supplementation. However, at the 1-year sampling all supplemented groups had similar mean concentrations, which were about six times greater than the concentration in control animals. Body weight, an indicator of the well-being of cattle and progeny, was also affected by selenium supplementation (Table 2). Mean body weight of cows at the 1-year sampling was greater in all the selenium-supplemented groups than in the control group ($P < 0.001$), and body weight of calves at weaning (4 August 1981) was also greater in selenium-supplemented groups ($P < 0.01$ or 0.001).

Supplementation with 1 mg of selenium or more per day for 1 year increased blood selenium to the concentrations reported in healthy cows and sheep (5 to 8 μg/dl), but these levels were still below those measured in unsupplemented cows of the same genetic base on King Ranch in southern Texas (14 to 25 μg/dl).

The importance of selenium in preventing nutritional myopathy (white muscle disease) in calves and paralytic myoglobinuria in yearling cattle is well established (*8, 9*). Selenium deficiency has been implicated in other clinical problems in cattle, such as retained placenta, calf pneumonia, infertility, and poor growth (*9–11*). However, to our knowledge, this is the first recorded example of widespread anemia in a population due to a dietary deficiency of selenium. In humans, selenium deficiency is recognized in Keshan disease (*12*) and during total parenteral nutrition (*13*), and low blood values have been recorded in healthy adult residents of New Zealand (*14, 15*) and Finland (*16*). There is a high correlation between selenium concentrations and glutathione peroxidase activity in whole blood of New Zealand residents, with increasing significance at low selenium concentrations (*17*). However, we know of no reports of Heinz body formation in selenium-deficient humans.

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## Guidance of Peripheral Pioneer Neurons in the Grasshopper: Adhesive Hierarchy of Epithelial and Neuronal Surfaces

Abstract. *An important question in developmental neurobiology is how a neuron finds its way over long distances to its correct target during embryogenesis. Peripheral pioneer neurons in insect embryos have been used for study because of the relative simplicity of the early embryonic appendages, and the accessibility of the identified neurons whose growth cones traverse this terrain. The data presented suggest an adhesive hierarchy of both epithelial and neuronal surfaces that guides the first growth cones from the appendages of the grasshopper embryo.*

Bate first described pioneer neurons in the peripheral (*1*) and central nervous system (CNS) (*2*) of the grasshopper embryo. Since that time, pioneer neurons have emerged as particularly attractive cells with which to study the guidance of neuronal growth cones because of their large size, and the simplicity of the environment through which they navigate. Pathfinding by early differentiating neurons has been extensively studied in the antennae (*1, 3–5*), limb buds (*1, 3, 6–9*), cerci (*10*), and CNS (*2, 7, 11, 12*) of the grasshopper embryo; in the cerci of the cricket embryo (*13*); and in the developing wings of *Drosophila* (*14*) and the moth (*15*). These simple systems may reveal guidance mechanisms common to less accessible growth cones that navigate through more complex environments in these and other organisms.

How do the first growth cones in the peripheral appendages of the grasshopper embryo find their way to the CNS? Bate (*1*) noticed that the first pairs of axons in the periphery became surrounded at particular intervals by other cells, and suggested that if these cells were present early enough, they might serve as "stepping stones" for the first growth cones on their indirect journey to the CNS (*16*). Later, Goodman and co-workers (*11*) noticed that central pioneer neurons turned and grew toward specific neuronal cell bodies in the CNS which they called "landmark cells"; Ho, Goodman, and co-workers (*3, 7*) noticed similar nerve cells, including those described by Bate, in the peripheral appendages appearing to serve a similar landmark guidance role. The idea of the stepping stone–landmark cell was simplified by Bentley and Keshishian (*8*) to the "guidepost cell hypothesis," in which specially placed neurons are the sole source of guidance information. To the exclusion of other sources of guidance, they proposed that the "growth cones from the first pioneers navigate along a chain of cells to the CNS" (*17*).

Another model has been suggested to explain the guidance of peripheral neurons in the developing appendages of insects. Nardi and Kafatos (*18*) previously proposed a proximo-distal gradient of epidermal cell adhesiveness in the developing wing of the moth. Nardi (*15*) then used this model to explain the asymmetric response of growing sensory neurons when confronted with epithelial grafts that changed the proximal-distal axis. Nardi suggested that such an adhesive gradient might also direct the initial polarized growth of the pioneer neurons.

Several important questions are raised by these studies. (i) How do the pioneer neurons initiate their growth cones with a particular polarity, that is, proximally toward the CNS? (ii) What guides pioneer growth cones proximally down the

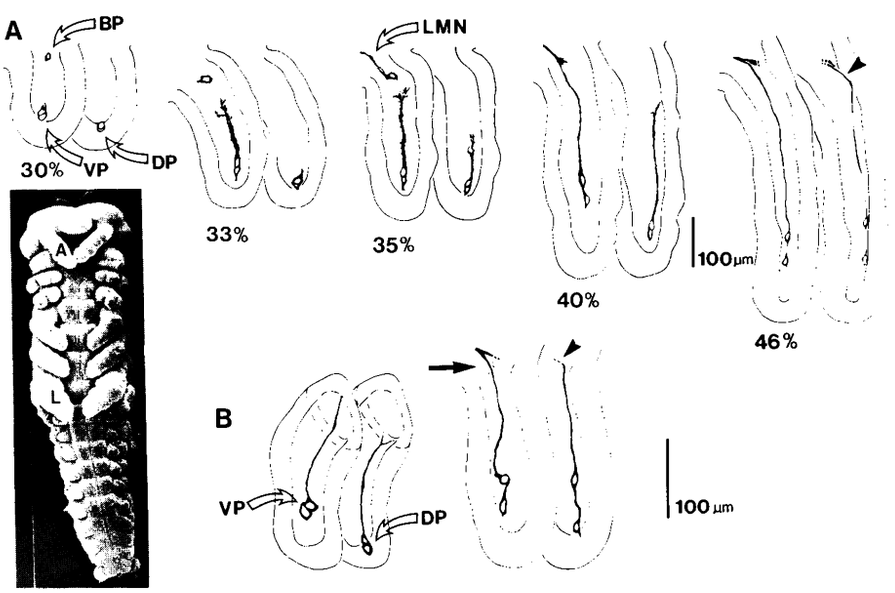


Fig. 1. Camera lucida drawings of antennae stained with the I-5 monoclonal antibody and a biotin-avidin horseradish peroxidase system. (A) Drawings of the antenna spanning the period of 30 to 46 percent of embryonic development reveal the spatio-temporal pattern of neuronal differentiation. In all split-plane drawings, the ventral plane is shown on the left and the dorsal plane on the right. Arrowhead points to where DP axons curve around lateral surfaces to ventral surface of epithelium as they fasciculate with and turn medially along the LMN axons; *VP*, ventral pioneers; *DP*, dorsal pioneers; *BP*, base pioneer; *LMN*, lateral motoneuron growth cone. (B) The left antenna was removed from a 33 percent embryo, and the right antenna was left intact; both were then placed in culture for 43 hours at 29°C and 5 percent $CO_2$ in a culture medium containing 20.8 nmole β-ecdysterone and 1.7 n*M* juvenile hormone I. In the control antenna (right), the VP growth cones reached the CNS, the BP neuron (left arrow) died as normal, and the DP growth cones made their characteristic ventral and medial turns along the LMN axon and entered the CNS (right arrowhead). In the experimental antenna (left), both pairs of pioneer growth cones have grown proximally along their stereotyped circumferential position to the base of the antenna; the DP growth cones did not turn ventrally and medially. (Inset) The inset on the left is a scanning electron micrograph of a 35 percent grasshopper embryo showing the antenna (*A*) and the metathoracic limb bud (*L*) used in these experiments. [Photograph by Robert Ho and Michael Bastiani]