Acta vet. scand. 1991, *32*, 403–404.

Brief Communication

Endotoxin-induced Changes in Plasma Mineral and Vitamin Levels in Calves

Acute infections and endotoxinaemias are associated with alterations in the blood mineral concentrations. The level of calcium, iron and zinc usually decrease, while copper is reported to remain unchanged or to increase. It is well established that hypoferraemia and hypozincaemia are caused by a redistribution of the metals within the body. This redistribution is induced by the endogenous mediators interleukin -1 (Il-1) and tumor necrosis factor (TNF), both of which are produced by activated monocytes and macrophages. *Goldblum et al.* (1987) showed that Il-1 causes a release of granulocyte lactoferrin which chelates iron. The complex is then taken up by the reticuloendothelial system, especially in the liver. Also zinc is taken up by the liver but by a different mechanism.

The redistribution of iron is considered as a non-specific defence mechanism, in that way the necessary iron source is reduced for the invading bacteria. The elevated copper level is generally considered to be the result of increased ceruloplasmin synthesis.

The mechanism behind the fall in calcium is less understood. In humans suffering from the toxic shock syndrome caused by *Staf. aureus* there is evidence that hypocalcaemia is due to a heavy release of calcitonin (*Chesney et al.* 1983, *Sperber et al.* 1990). It is however not known if this mechanism operates also in other types of infections. *Srinivar et al.* (1988) and *Sammalkorpi et al.* (1988) presented results which indicated that also plasma selenium decreases during acute infections.

Plasma or serum levels of minerals and vitamins are sometimes used as nutritional indicators. It seems however quite clear that for correct interpretation of the results, it is necessary that the sampled animals are free from infections.

The primary aim of the present investigation was to study if also the plasma levels of selenium, retinol and α-tocopherol change in calves as a response to endotoxinaemia. Bacterial endotoxins are the most potent stimulus for release of Il-1 and TNF. Lipopolosaccharide (LPS) from *Salmonella typhimurium* was used in the present study. The preparation of LPS was described by *Fredriksson* 1984).

Five calves of the Swedish Red and White Breed, about 4 months of age were injected intravenously with LPS at a dose of 0.5 μg/kg body weight. Blood was sampled at intervals shown in Table 1. Heparin was used as anticoagulant. In order to assure normal blood levels of selenium at the time of the LPS injection the calves were treated intramuscularly with 3.5 mg selenium as sodium selenite about 3 weeks before the LPS injection.

For chemical analysis the separated plasma and erythrocyte samples were digested with nitric acid/perchloric acid by automated wet ashing (*Frank* 1976). The metal concentrations were determined by simultaneous multi element plasma atomic-emission spectrometry (*Frank & Pettersson* 1983). Selenium analysis was performed by continous reduction with sodium borohydride in a flow injection analysis apparatus and atomic ab-

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Table 1. Changes in the plasma levels of minerals, retinol and α-tocopherol in calves after intravenous injection of LPS at a dose of 0.5 μg/kg, $\bar{x} \pm$ SEM, n = 4.

	Hours after injection							
	0	2	4	6	8	10	24	48
Plasma:								
Ca mmol/l	2.73±0.05	2.48±0.17	2.28±0.24	2.23±0.26	2.38±0.27	2.42±0.28	2.36±0.11	2.66±0.13
P "	2.75±0.16	2.82±0.11	2.85±0.26	2.80±0.14	2.70±0.09	2.75±0.09	2.72±0.18	2.78±0.27
Mg "	0.90±0.05	1.00±0.05	1.00±0.07	0.98±0.09	0.98±0.09	0.85±0.05	0.90±0.03	0.88±0.08
Cu μmol/l	6.50±0.43	6.40±0.59	7.30±0.31	7.50±0.86	8.00±0.70	7.80±0.85	7.00±0.77	8.00±0.71
Fe "	48.00±4.50	58.00±5.80	68.00±6.20*	59.30±6.20	50.80±7.60	31.30±5.70	12.30±4.50**	16.80±1.10***
Zn "	14.00±0.41	14.80±1.10	10.30±2.25	5.80±2.50*	3.50±1.50*	3.50±1.89*	7.30±1.44*	13.50±1.50
Se "	0.44±0.01	0.41±0.04	0.41±0.04	0.38±0.03	0.38±0.04	0.42±0.05	0.38±0.04	0.37±0.05
Se "								
(erythrocytes)	2.08±0.03	2.13±0.02	2.05±0.03	2.12±0.04	1.96±0.03	2.00±0.03	1.99±0.03	2.03±0.03
Retinol μmol/l	1.09±0.04	0.98±1.04	1.05±0.11	1.09±0.08	1.05±0.04	0.98±0.04	0.61±0.08*	0.74±0.08
α-tocopherol "	2.32±0.35	2.02±0.26	2.11±0.26	2.08±0.21	1.97±0.21	1.93±0.19	1.81±0.09	1.62±0.20

* Significant difference compared with the preinjection level ($0.01 < p < 0.05$).
** " " " " " " " " ($0.001 < p < 0.01$).
*** " " " " " " " " ($p < 0.001$).

sorption spectrometry using an electrically heated quarts cuvette (*Galgan & Frank* 1988). Retinol and α-tocopherol were analyzed with HPLC and fluorescence detection according to the routine methods of the Department of Chemistry, National Veterinary Institute.

All calves showed typical signs after the LPS injection: anorexia, increased salivation, forced breathing and ruminal stasis. One calf showed very severe clinical signs immediately after the injection and died after 2 h. The results of the plasma analyses are shown in Table 1. Calcium decreased in 3 of the 4 surviving animals. The calf which showed unchanged calcium levels responded however with a profound decrease in iron. from 39 μmol/l to 9 μmol/l after 24 h, which indicates that the fall in iron and calcium is caused by different mechanisms. There were no significant changes in copper, magnesium and inorganic phosphorus. Iron showed a biphasic curve, the cause to the initial rise is not known, but is described earlier (*Luthman et al.* 1989). Zinc decreased more rapidly than iron, minimum levels occurred already after 8 h.

Selenium did not change neither in serum nor in erythrocytes. The preinjection level of selenium was low in spite of the earlier treatment. Retinol showed a significant decrease after 24 h, while there were no significant changes in α-tocopherol.

The changes in calcium, iron and zinc are of the same kind as reported earlier. These changes are considered as typical acute phase reactions. The observation that also selenium decreases was originally made in humans suffering from viral and bacterial infections. The results from the present study are not in accordance with these findings, but indicate that selenium does not change acutely in infections and endotoxinaemias in calves. The number of animals were however small and further studies are needed for more valid conclusions.

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(Received September 30, 1991; accepted October 10, 1991).

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