Veterinary Research Communications, 11 (1987) 467-477
Geo Abstracts Ltd, Norwich - printed in England

MILK AND BLOOD LEVELS OF SILICON AND SELENIUM STATUS IN BOVINE MASTITIS

J. PARANTAINEN[1], E. TENHUNEN[2], R. KANGASNIEMI[3], S. SANKARI[4] & F. ATROSHI[5*]
1. Research laboratories, Medica Pharmaceutical Co. Ltd, P.O.Box 325, 00101 Helsinki 10, 2. Research Center, Neste Oy, 06850 Kullo, 3. Institute of Veterinary Medicine, University of Helsinki, Helsinki 71, 4. Department of Biochemistry, College of Veterinary Medicine, Helsinki 55, 5. Department of Pharmacology and Toxicology, College of Veterinary Medicine, P.O.Box 6, 00551, Helsinki, Finland

*To whom all correspondence should be addressed.

(Accepted: 11 February 1987)

ABSTRACT

Parantainen J., Tenhunen E., Kangasniemi R., Sankari S. & Atroshi F. Milk and blood levels of silicon and selenium status in bovine mastitis. Veterinary Research Communications 11(5), 467-477

Milk and blood levels of silicon, selenium and the selenoenzyme glutathione peroxidase (GSH-Px) were measured in 20 healthy and 21 mastitic cows. In milk samples from healthy quarters the mean silicon concentration was 0.81 and in affected ones 0.39 ppm. In serum the mean silicon values were 1.63 and 1.02 ppm respectively. The selenium status was not altered but the level of erythrocyte GSH-Px was lowered in mastitic animals. Silicon is known to have marked effects on free radical formation, lipid peroxidation and macrophage activity. Its possible role in infection and inflammation is evaluated. Some of the functions of silicon may resemble those of selenium. The possibility of lowered levels of silicon and of the selenoenzyme in mastitis calls for experimentation with dietary or pharmaceutical supplementation of these trace elements.

INTRODUCTION

Mastitis is the most economically devastating disease of dairy cows, causing serious problems of diagnosis and treatment. Mastitis has been defined as inflammation of the mammary gland usually resulting from infectious processes in the udder. Milk stagnating in a blocked duct provides an ideal medium for proliferation of bacteria. However, there are cases of mastitis in which no pathogens are found. Pathogens may be missed in routine bacteriological tests (Schulze et al., 1978) but there is also the possibility that the disease presents itself as a 'sterile inflammation' without involvement of bacteria. Gunther (1965) suggested that the stagnated milk may leak into the surrounding tissue, provoking inflammation as a defence reaction. It is thus obvious that, in addition to pathogens, other factors predisposing the udder to inflammation are important.

0165-7380/87/$03.50

Trace elements may be interesting ... this respect. It has been reported that diets deficient in selenium and vitamin E result in an increased incidence of various infectious and immunological reactions in rodents and dairy cows and the signs may be relieved with a dietary substitution (Maas, 1983, Parnham et al., 1983). Recently Smith and coworkers (1984) demonstrated that selenium supplementation, together with vitamin E, affects the clinical course of bovine mastitis. The target in using selenium is glutathione peroxidase (GSH-Px), a selenoenzyme which represents the biological function of selenium in the organism. In our previous work we demonstrated that GSH-Px may be lowered in the erythrocytes of mastitic animals (Atroshi et al., 1986). The enzyme levels are regulated by the selenium status of the organism, although an adaptation to low selenium intake is possible (Atroshi et al., 1985, Flohe et al., 1973, Sankari & Atroshi, 1983). Any causal association between selenium and the pathophysiology of mastitis has yet to be shown.

Silicon is another essential trace element. Not much is known about the roles of silicon in health and disease. Silicon is needed for growth and bone formation (Carlisle, 1974, Schwarz, 1974), and in the synthesis of mucopolysaccharides and collagen (Schwarz, 1974). The arterial content of silicon may decrease with degeneration of tissues, as in atherosclerosis (Loeper et al., 1979), and in advancing age (Akiya et al., 1960). There are also some indications that silicon might be important in some forms of infection and inflammation (Allison, 1976, Craggs et al., 1984, Ghadirian & Kongshavn, 1984, Voronkov, 1975). Silicon is present in normal bovine milk (Archibald and Fenner, 1957, Schwarz, 1978, Varo 1979, Voronkov, 1975). Silicon is known in medicine mainly for its toxicity, as in silicosis caused by exposure of the lungs to silica-containing dust or particles. Interestingly, the toxic actions of silicon include human 'silicon mastitis' but this is a reaction of the breasts to massive injections of silicone oils with possible additives (Chaplin, 1969, Perry et al., 1985, Symmers, 1968). The effects of such exogenous polymerized silicone may not be compared with the role of an endogenous silicon, present only in trace amounts in the tissues.

In the present work we studied milk and blood concentrations of silicon in bovine mastitis.

MATERIALS AND METHODS

Animals: Twenty healthy and twenty one clinically mastitic Finnish Friesian cows of different ages, weights, and stage of lactation were used. The cows were kept indoors in similar husbandry conditions and fed twice daily (6 a.m. and 2 p.m.). The influence of circadian variation was eliminated by always sampling the blood at the same time of the day.

Milk samples (pre-milking) from inflamed and non-inflamed quarters were taken under maximum asepsis into sterile plastic tubes free of silicon. Washed and dried teats were cleansed with a swab dipped in 70% alcohol. The first three streams were rejected before the samples were collected. The samples were kept cool and immediately examined for bacteriology.

Blood samples were taken from the animals at the same time as the milk. Blood was drawn from the jugular vein into silicon-free plastic tubes. EDTA k_3 was added to the tubes used for the selenium, GSH-Px and other hematological determinations, which were performed on the same day. After sampling the serum was separated by centrifugation and stored at -18^{0}C until analyzed.

Mastitis was defined according to the following criteria.

(a) Pathogenic bacteria demonstrable in one or more quarters.

(b) Somatic cell counts greater than 500 x 10^3 cells/ml milk.

In order to show any effects caused by the pathological reaction it was necessary to avoid regarding samples from subclinical cases of mastitis as though they were from healthy controls. Therefore only samples with cell counts below 120 x 10^3 were used as normal controls. Routine milk bacteriology was carried out by the standard Scandinavian method of Klastrup and Madsen (1974). The samples were tested for Staphylococcus aureus, Staphylococcus uberis, Str. dysgalactiae and Micrococci.

For counting the cells we used a Fossamatic Fluorometric Automatic Cell Counter. Before statistical analysis, the values were transformed into a natural logarithmic scale and the cell counts were corrected for milk yield and stage of lactation.

Electrical conductivity was measured at room temperature (22°C) in units of milli Siemens/cm (mS/cm) with an Orion Research Conductivity Meter 101, USA.

Silicon concentrations were measured using an ICP 35000 emission spectrometer (Bausch & Lomb). The calibration standards were Na-silicate water solutions and the silicon concentration was determined directly from the samples. Difficulties were encountered with acidic milk samples (pH < 6), some of which may have given too low values.

Selenium determinations were performed fluorometrically as described earlier (Sankari & Atroshi, 1983).

The glutathione peroxidase (GSH-Px) activity of the packed erythrocytes was determined using tertiary butyl hydroperoxide as the substrate and coupling the peroxidase system with glutathione reductase. (Atroshi et al., 1981).

Determination of fat and protein was done as a routine analysis by VALIO, Finnish Cooperative Dairy Laboratories by the infrared method (Milko-SCAN 300, Fosselectric, Denmark) used in the official milk recording procedures.

Statistical analysis. The mean values were compared by the 'Student's' t-test. Correlation analysis was used according to Snedecor and Cochran (1967). (For details of the statistical analysis see Atroshi et al., 1982).

RESULTS

The results are summarized in Figure 1 and Table I.

Table I. MEAN (+S.E.M.) PATHOPHYSIOLOGICAL PARAMETERS IN MASTITIS

		Healthy	Mastitis	
Milk	conductivity (mS/cm)	4.56±0.05	5.32±0.15	**
	SCC $100x10^3$/ml	39.6±19.2	2149.0±697.7	**
	fat (%)	2.36±0.23	2.90±0.26	N.S.
	protein (%)	3.17±0.06	3.35±0.10	N.S.
Blood	hematocrit (%)	37.2±0.8	32.3±1.3	**
	selenium ng/ml	73.0±4.4	70.6±6.5	N.S.
	GSH-Px uKat/l	1956.0±374	1672.5±248	*

*p < 0.05, **p < 0.01, N.S. not significant
GSH-Px was measured from erythrocytes.

The lowest silicon values were recorded in those samples that were most inflamed and acidic (pH around 6). In these the silicon values were below the range of our method, i.e. below 0.05 ppm.

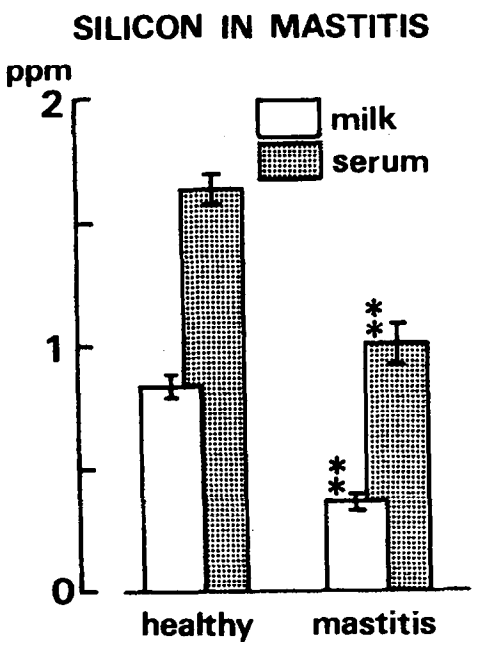

Figure 1. In healthy cows the silicon levels in milk were 0.84 ± 0.05 ppm (x±S.E.M), and in serum 1.63 ± 0.05 ppm. In mastitis the corresponding values were 0.36 ± 0.02 ppm and 1.02 ± 0.08 ppm, respectively.

Negative correlations with silicon concentrations were obtained with both the somatic cell counts ($r = -0.47$, $p < 0.05$) and electrical conductivity ($r = -0.54$, $p < 0.01$). There were low but significant correlations between the erythrocyte GSH-Px and both the serum silicon ($r = 0.36$, $p < 0.05$) and serum selenium ($r = 0.37$, $p < 0.05$) concentrations.

The selenoenzyme GSH-Px, however, was significantly ($p < 0.05$) lowered in mastitis.

DISCUSSION

At 0.8 ppm the silicon content in quarter milk samples from healthy cows was somewhat less than that reported by Archibald and Fenner in 1957 (1.4µg/ml), by Voronkov (1975) referring to studies in the USSR (3.1µg/ml) and by Vavo (1979) at 2µg/ml. The use of silicon-free plastic tubes eliminates the possibility of contamination, a serious problem in the early studies. Up to 400 times higher values have been found by Schwarz (1978) in the literature in extreme cases.

The serum silicon concentrations found in healthy cows were strikingly similar to those reported by Dobbie and Smith (1982). These similar values strongly suggest a strict physiological control over the blood silicon concentration - somewhat like the way that blood electrolytes are regulated. The small variation in silicon concentrations in both studies also supports this suggestion. There seem to be species differences in serum silicon concentrations, herbivores having consistently higher values than some other animals (Dobbie and Smith, 1982).

The most important result in our study was the finding that in mastitic cows both the milk and the serum concentrations of silicon were lowered. In milk the values were less than half the normal. Samples from severely inflamed quarters were the most acidic (pH 6 or less) and had silicon values below 0.05 ppm.

In contrast the blood selenium levels were not significantly lowered but the marked decline in the selenoenzyme GSH-Px, also observed in our previous work (Atroshi et al., 1986), supports the possibility of a relative deficiency in selenium-related biochemical processes. There may thus be a need for selenium supplementation in mastitis (Smith et al., 1984). The blood values may not represent adequately the selenium content in other tissues. For example breakdown of GSH-Px could add to the blood selenium value so giving an erroneous picture of the selenium status.

Difficulties have been encountered when trying to estimate selenium status from determinations in milk (Binnerts et al., 1984). A relative deficiency in form of an increased demand for selenium during inflammation is also possible. Obviously more studies are needed to clarify these discrepancies. In the present study the overall GSH-Px-levels were somewhat lower than in our previous study, in which the mean value for healthy animals was 2394 ukat/L and 1594 ukat/L in mastitis (Atroshi et al., 1986).

Very little is known about the biological roles of silicon. A decline in milk and blood silicon may act as a marker of tissue destruction or other nonspecific events. The relatively high concentrations of silicon in blood vessel intima may decline in atherosclerosis and with advancing age, particularly in acidic conditions (Akiya et al., 1960, Loeper et al., 1979). Silicon has been proposed as a marker of renal damage (Mauras et al., 1980).

An active tissue protective role for silicon has been suggested (Loeper et al., 1979). On the other hand an excessive intake of silicate may cause urinary calculi in cattle (Bailey, 1967). Urolithiasis, like silicosis, represents the toxic action of silicon, possibly contributing to obstruction (fibrosis) and inflammation.

There are some observations which imply that the tissue content of silicon declines during infection. Reviewing the existing data Voronkov (1975) concludes that tissue silicon may protect against tuberculosis. In silicosis and in silica-treated tissues macrophage membranes are disrupted, which leads to an almost total disappearance of these cells (Ghadirian & Kongshavn, 1984, Koike et al., 1982, Zsoldos et al., 1983). In experimental allergic neuritis the causes of the trouble are macrophages and exogenous silicon protects the animals (Craggs et al., 1984, Tansey & Brosnan, 1982). However, in Entamoeba histolytica infection, when macrophages provide the major protecting force, silica makes the infection much worse (Ghadirian & Kongshavn, 1984). The action of silica against macrophages has been decribed as unique and specific (Allison, 1976). The high concentrations of silicon in arterial intima may protect the tissue by inhibiting the attack of macrophages. The role of silicon in neutrophil activity is not known but there is an interesting link that should be checked. Neutrophils from pig and horse blood engulf crystalline particles easily but only those which are charged function as activators and cause the oxidative burst (Higson & Jones, 1984). Silica present in silicotic tissue is known to have a strong negative charge (Weiss, 1978).

Production of the active free-radicals of oxygen, as well as lipid peroxides, may be elevated in hypoxia, inflammatory reactions, infection and tissue destruction (Dormandy, 1983). In silicotic lungs lipid peroxidation is strongly increased and the reaction is accompanied by generation of superoxide (Koike et al., 1982) and/or singlet oxygen (Weiss, 1978, Zsoldos et al., 1983). In these ways silicon could affect several biological phenomena, including tissue destruction and host defences.

The role of selenium and GSH-Px in these reactions is somewhat better known. GSH-Px is considered to have a key role in regulation of lipid peroxidation through its ability to control the generation of oxygen free-radicals (Flohe & Zimmermann, 1970, McCay et al., 1976). A decline in GSH-Px activity, due to a lowered selenium intake, may predispose experimental animals to inflammation (Parnham et al., 1983) and depress the ability of bovine neutrophils to generate the hydroxyl radicals which kill bacteria (Boyne & Arthur, 1979). In previous experiments on mastitis (Atroshi et al., 1986) the low GSH-Px activity was associated with an elevation of plasma and milk prostaglandins, the latter representing a very specific form of lipid peroxidation and mediators of inflammation. In particular, by free radical formation and lipid peroxidation, silicon and selenium may contribute to the reduction of oxygen in a synergistic and complementary way (Figure 2).

The moderate negative correlation between silicon and electrical conductivity may have a theoretical implication as silicon is one of the key physical elements regulating conductivity. Silicon may be compared with the electrolyte's anions, which regulate conductivity by definition. Silicon complexes formed in tissues may have a negative charge (Weiss, 1978). Silicon is present in mucopolysaccharides of collagenous tissues as well as in some other proteins (Schwarz, 1974), i.e. in structures that contain other negatively charged compounds such as amino sugars. In organic material conductivity may be markedly regulated by anionic and cationic components (Greene & Street, 1984), on theoretical grounds possibly also by silicon and selenium (Parantainen, 1985).

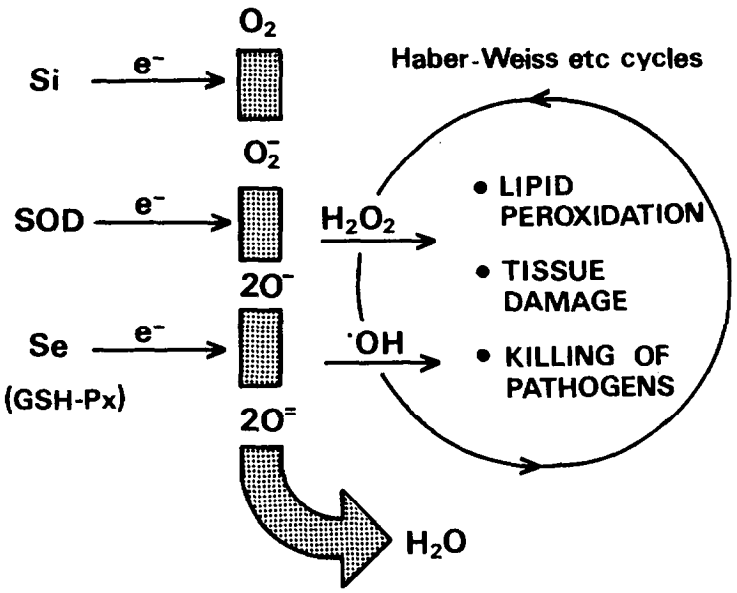

Figure 2. A simplified view of the possible complementary actions of silicon and selenium (with GSH-Px) in free radical formation and lipid peroxidation. The emphasis is on the one-electron steps in the reduction of dioxygen to water. Silicon structures in silicotic lungs having a negative charge may produce superoxide O_2 and/or singlet oxygen. While silicon is able to initiate the process, GSH-Px is needed in the later phases for elimination of hydrogen peroxide and tissue protection. The reduction of the peroxide by GSH-Px may also support an adequate formation of hydroxyl radical for microbial killing. SOD is the free radical scavenger enzyme, superoxide dismutase.

In certain circumstances the nutritional intake of silicon and selenium by cattle may be inadequate. In some geographical areas, as in Finland, the soil is deficient in selenium and this is reflected in a low selenium content in forage (Oksanen & Sandholm, 1970). The existence of selenium-responsive diseases in cattle (Maas, 1983) further points to the possibility of an inadequate intake of this trace element. Selenium supplementation reduced the duration of clinical symptoms of mastitis by 46% and when vitamin E was also given the reduction was as high as 62% (Smith et al., 1984). These results are supported by our finding of a lowered GSH-Px in mastitis. Could silicon be of similar importance? At first glance the idea of a dietary or functional deficiency seems impossible, as grass and animal forage contains plenty of silica. This abundance of silicon may, however, be lost through cultivation, processing and storage of forage, as well as by poor absorption. Bacteria may be important in the natural utilization of silicon (Voronkov, 1975). The bacteria in soil, food, rumen, intestine and inflammatory tissue all have the potential to affect silicon intake and utilization. Studies to clarify the bioavailability of silicon from forage are obviously needed. Serum silicon values do not necessarily change after supplementation (Dobbie & Smith, 1982) but the incidence of urinary calculi may increase when high doses are used (Bailey, 1967). The silicon content in milk may decline towards the end

of the lactation period (Archibald & Fenner, 1957), while the grass silicon may be at its highest during fall and winter (Bailey, 1967). Trials with supplementation of silicon may be worth considering in mastitis once the bioavailability and fate of the trace element are known.

Interestingly, biodynamic (organic) cultivation devotes much of its effort to increasing the silicon uptake from soil in human and animal nutrition. Quartz-containing sand and dust are added as fertilizers, together with bacteria that may utilize the silicon from the stone and glass. The plants are also sprayed with 'silicon preparations'. Some other traditional cultivation habits have also stressed the importance of adding sand or ground stone, consisting mainly of silicate, to improve the quality of the soil.

In conclusion, we have demonstrated that milk and blood levels of silicon may be lowered in bovine mastitis. The reduction was rather large considering the usually narrow range in the milk, particularly, in the serum concentrations. Any inference concerning causality can only be speculative but silicon has been described as an element that may have importance in infection, lipid peroxidation and macrophage activity. The seleno-enzyme GSH-Px was also lowered in mastitis and selenium may be of some help in treating the disease. Both silicon assays and GSH-Px estimations may be useful in diagnosing mastitis and dietary or pharmaceutical supplementation with these trace elements is worth studying.

ACKNOWLEDGEMENTS

The study was supported by a grant from the Finnish Academy of Sciences. We thank Ms Marjatta Kaukonen for drawing the figures.

REFERENCES

Akiya S., Motohashi N. & Niwase T. 1960. Studies on the silica in animal tissue. Bulletin of the Tokyo Medical and Dental University, 7, 327-350

Allison A.C. 1976. Fluorescence microscopy of lymphocytes and mononuclear phagocytes and the use of silicate to eliminate the latter. In: B.R. Bloom & J.R. David (eds), In vitro methods in cell-mediated and tumor immunity. (Academic Press Inc., New York), 395-404

Archibald J. G. & Fenner H. 1957. Silicon in cow milk. Journal of Dairy Science, 40, 703-706

Atroshi F., Kangasniemi R., Honkanen-Buzalski T. & Sandholm M. 1982. Beta-lactoglobulin phenotypes in Finnish Ayrshire and Friesian cattle, with special reference to mastitis. Acta Veterinaria Scandinavica, 23, 135-143

Atroshi F., Sankari S., Osterberg S. & Sandholm M. 1981. Variation of erythrocyte glutathione peroxidase activity in Finnsheep. Research in Veterinary Science, 31, 267-271

Atroshi F., Sankari S. & Kaipainen P. 1985. Erythrocyte and liver characteristics in low and high glutathione Finnsheep. Pharmacological Research Communications, 17, 23-32

Atroshi F., Parantainen J., Sankari S. & Osterman T. 1986. Prostaglandins and glutathione peroxidase in bovine mastitis. Research in Veterinary Science, 40, 361-366

Bailey C.B. 1967. Siliceous urinary calculi in calves: prevention by addition of sodium chloride in the diet. Science, 155, 696-697

Binnerts W.T., Rijken J. & Viets T.C. 1984. The selenium content in milk as indicator of the selenium status in cows. In: Trace element - analytical chemistry in medicine and biology, Vol. 3, (Walter de Gruyter & Co., Berlin/New York), 129-137

Boyne R. & Arthur J.R. 1979. Alterations of neutrophil function in Se-deficient cattle. Journal of Comparative Pathology, 89, 151-158

Carlisle E.M. 1974. Silicon as an essential element. Federation Proceedings, 33, 1758-1766

Chaplin C.H. 1969. Loss of both breasts from injections of silicone (with additive). Plastic and Reconstructive Surgery, 44, 447-450

Coombs J., Spanis C. & Volcani B.E. 1967. Studies on the biochemistry and fine structure of silica shell formation in diatoms. Photosynthesis and respiration in silicon starvation synchrony of Navicula pelliculosa. Plant Physiology, 42, 1607-1611

Craggs R.I., King R.H.M. & Thomas P.K. 1984. The effect of suppression of macrophage activity on the development of allergic neuritis. Acta Neuropathology, 62, 316-323

Dobbie J.W. & Smith J.B. 1982. The silicon content of body fluids. Scottish Medical Journal, 27, 17-19

Dormandy T.L. 1983. An approach to free radicals. Lancet, 2, 1010-1014

Flohe L. & Zimmermann R. 1970. Role of GSH peroxidase in protecting the membrane of rat liver mitochondria. Biochimica Biophysica Acta, 223, 210-213

Flohe L., Gunzler W.A. & Schock H.H. 1973. Glutathione peroxidase: a selenoenzyme. FEBS Letters, 32, 132-134

Ghadirian E. & Kongshavn P.A.L. 1984. Effect of silica on resistance of mice to entamoeba histolytica infection. Infective Immunity, 45, 399-402

Greene R.L. & Street B.G. 1984. Conducting organic materials. Science, 226, 651-656

Gunther M. 1965. Acute mastitis. Lancet, 1, 175-180

Higson F.K. & Jones O.T.G. 1984. Oxygen radical production by horse and pig neutrophils induced by a range of chrystals. Journal of Rheumatology, 11, 735-741

Klastrup O. & Schmidt Madsen P. 1974. Nordiske recommendationer vedrorende mastitisundersogelser af kirtelprover. Nordisk Veterinaermedicin, 26, 197-204

Koike S., Kuno Y. & Morita H. 1982. The effects of silica on lipid peroxidation, and the production of superoxide radicals by phagocytizing rabbit macrophages. Nippon Eiseguku Zasshi, 37, 510-515

Loeper J., Goy-Loeper J., Rozensztajn L. & Fragny M. 1979. The antiatheromatous action of silicon. Atherosclerosis, 33, 397-408

Maas J.P. 1983. Diagnosis and management of selenium responsive diseases in cattle. The Compendium of Continuing Education in Practitioning Veterinary, 5, S393-S399

Mauras Y., Riberi P., Cartier F. & Allain P. 1980. Increase in blood silicon concentrations in patients with renal failure. Biomedicine, 33, 228-230

McCay P.B., Gibson D.D., Fong K.L. & Hornbrook K.R. 1976. Effect of glutathione peroxidase activity on lipid peroxidation in biological membranes. Biochimica Biophysica Acta, 431, 459-468

Oksanen H.E., Sandholm M. 1970. Selenium content of Finnish forage crops. Journal of the Scientific Agricultural Society of Finland, 42, 251-254

Parantainen J. 1985. Trace elements as possible keys to biological semiconduction: pharmacological implications. 4th South-East Asian/Western Pacific Meeting of Pharmacologists. Penang, Malaysia, Abstr. p6

Parnham M.J., Winkelmann J. & Leyck S. 1983. Macrophage, lymphocyte and chronic inflammatory responses in selenium deficient rodents. Association with decreased glutathione peroxidase activity. International Journal of Immunopharmacology, 5, 455-461

Perry R.R., Jaques D.P., Lesar M.S.L., d'Avis J.C. & Peterson H.D. 1985. Mycobacterium avium infection in a silicone-injected breast. Plastic and Reconstructive Surgery, 75, 104-106

Sankari S. & Atroshi F. 1983. Effect of dietary selenium on erythrocyte glutathione peroxidase and blood selenium in two types of Finnsheep genetically selected for high and low glutathione peroxidase activity. Zentralblatt fur Veterinarmedizin A, 30, 452-458

Schulze W.D., Thompson P.D. & Bright S.A. 1978. Inflammatory response of the bovine mammary gland to an irritant in the streak canal. American Journal of Veterinary Research, 39, 785-790

Schwarz K. 1974. Recent dietary trace element research, exemplified by tin, fluorine, and silicon. Federation Proceedings, 33, 1748-1757

Schwarz K. 1978. Significance and functions of silicon in warm-blooded animals. Review and outlook. In: G. Bendz & I. Lindqvist (eds), Nobel symposium 40, 1977, (Plenum Press, New York), 207-230

Smith K.L., Harrison J.H., Hancock D.D., Todhunter D.A. & Conrad H.R. 1984. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. Journal of Dairy Science, 67, 1293-1297

Snedecor G.W. & Cochran W.G. 1967. Statistical methods. 6th edition, (Iowa State University Press of America)

Symmers W. St C. 1968. Silicone mastitis in 'topless' waitresses and some other varieties of foreign-body mastitis. British Medical Journal, 3, 19-22

Tansey F.A. & Brosnan C.F. 1982. Protection against experimental allergic neuritis with silica quartz dust. Journal of Neuroimmunology, 3, 169-179

Varo P. 1979. Kivennaisainetaulukko (Tables of trace elements, in Finnish), Otava.

Voronkov M.G., 1975. Silizium und Leben. K.Rhlmann (ed), (Akademie-Verlag, Berlin)

Weiss A. 1978. Isolation and characterization of a characteristic phosphato-silicate from human lungs with silicosis. In: G. Bends & I. Lindqvist (eds), Biochemistry of silicon and related problems. Nobel Symposium 40, 1977, (Plenum Press, New York)

Zsoldos T., Tigyi A., Montsk T. & Puppi A. 1983. Lipid peroxidation in the membrane damaging effect of silica-containing dust on rat lungs. Experimental Pathology, 23, 73-77