EXPERIMENTAL SELENOSIS IN CATTLE

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "EXPERIMENTAL SELENOSIS IN CATTLE" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

K.M. Jayak

Mannuthy,
April, 1989.

K.M. JAYAKUMAR

CERTIFICATE

Certified that this thesis entitled "EXPERIMENTAL SELENOSIS IN CATTLE" is a record of research work done independently by Shri. K.M. JAYAKUMAR under my guidance and supervision and it has not previously formed the basis for the award of any degree, fellowship or associate-ship to him.

Dr. N.M. ALEYAS,
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CONTENTS

Introduction

INTRODUCTION

Selenium, a non-metalic element closely related to sulphur was first isolated in 1817 by Berzelius, the Swedish chemist (Robertson, 1966). It is widely distributed in the crust of the earth to about 7×10^{-5} % by weight, as selenides of heavy elements and to a limited extent as free form in association with elementary sulphur. Higher concentration of this element in the soil was found to be associated with volcanic activity.

Selenium was recognized as an essential element in animal nutrition few decades ago, when it was shown that most of the myopathies including 'white-muscle disease' in lambs, calves and foals and hepatosis dietetica of pigs could be prevented by its supplementation in the diet (Underwood, 1971). Rotruck and co-workers in 1973 identified it as a component of glutathione peroxidase, the enzyme that catalyses the removal of hydrogen peroxide from tissues (Lassiter and Edwards, 1982).

The identification of selenium as the causative factor for 'blind staggers' and 'alkali disease' in the great plains of North America stimulated investigations on the distribution of this element in rocks, soils, plants and animal tissues at toxic levels with a view to determine the maximum admissible concentration in feeds and fodder and for developing the means of prevention and control of its toxicity.

Numerous experiments were conducted during the past few decades to determine the toxic effects of seleniferous plants and organic and inorganic compounds both in livestock and laboratory animals. Chronic toxicity with selenium compounds in livestock is manifested by dullness, emaciation, rough coat, loss of vitality, hoof abnormalities including swelling of the coronary band, sloughing of the hooves and severe lameness.

Widespread incidence of gangrenous syndrome simulating chronic selenosis was found to occur seasonally in cattle and buffaloes in Kerala causing serious economic loss to farmers due to high mortality and morbidity rates.

Hence this investigation was taken up with the following objectives:

a) experimental induction of selenosis and analyse the typical manifestations to aid clinical diagnosis by comparison, and

b) study the toxic effects of selenium on body tissues.

Review of Literature

REVIEW OF LITERATURE

Incidence

A fatal disease of horses known as 'Nebraska', characterised by loss of hairs from mane and tail with soreness of feet was described by Madison (1857). Franke (1929) studied alkali disease in detail and coined the hypothesis that this syndrome was caused by the presence of certain toxic elements in feedstuffs. Robinson (1933) discovered selenium as the toxic principle in alkali disease and this finding was later confirmed by Franke and Potter (1935). Beath et al. (1935) identified two diseases of livestock, viz., 'blind staggers' and 'alkali disease' as the respective manifestations of acute and chronic selenosis in the great plains of North America. Fleming and Walsh (1957) reported selenium poisoning in animals from the Republic of Ireland where the selenium content was as high as 1200 ppm against the maximum tolerable level of 5 ppm. Brown and deWet (1962) reviewed the epizootology, symptomatology and pathology of two disease conditions 'tribulosis' and 'enzootic icterus' prevalent in South Africa and postulated the role of selenium as the possible etiological factor.

George et al. (1970) reported the incidence of a gangrenous syndrome involving the extremities in bovine animals particularly buffaloes in Kerala. A similar disease named 'Degnala disease' was subsequently identified from certain provinces of Pakistan by Irfan (1971) and from North India

by Kalra et al. (1974) and Arora et al. (1975). The suggested etiological factors were toxicities due to Fusarium species of fungi (Kalra et al., 1974) and selenium (Arora et al., 1975). Rajan et al. (1977) studied the pathological aspects of gangrenous syndrome of cattle and buffaloes in Kerala and suggested the possible involvement of fungal toxins contained in spoiled paddy straw as the etiological agent. Twomey et al. (1977) reported selenium toxicity in cattle in co-Meath where the average selenium concentrations of soil and herbage were 42 ppm and 37 ppm respectively. Gupta et al. (1982) recorded deformities of hooves and horns in buffaloes, cattle and goats from Hoshiarpur district of Punjab where the selenium content of fodder was estimated as between 3.3 and 5.4 mg per kg. As 'Degnala disease' was not reported from that district they ruled out the possible role of selenium toxicity as the cause of that condition.

Experimental studies

Anderson and Moxon (1942) studied the effect of subcutaneous injection of sodium selenite in dogs and reported the fatal dose of selenite to be from 1.0 to 2.0 mg per kg body weight as a single dose. The organic selenium compounds naturally occurring in plants and grains are more toxic than selenite or selenides (Rhian and Moxon, 1943; Fitzhugh et al., 1944). Magg et al. (1960) observed mortality within a period of 23 weeks in six out of eight steers which received sodium selenite orally three times a week at a dose rate of 0.25 to 0.5 mg per pound body weight. Lenets and Tutushin (1964) studied

selenium toxicity in sheep by oral dosing with sodium selenite at different dose levels. The animals became very weak and thin and developed intermittent haematuria on oral administration of sodium selenite @ 0.5 mg/kg body weight for 2 months. On increasing the dose to 1 mg per kg body weight, the toxic manifestations recorded were gradual depression, anorexia, slight decrease in body weight, pulse and respiratory rates followed by death in 25 to 27 days. The manifestations of sodium selenite toxicosis in pigs were mostly cardiovascular disturbances, depression and paresis (Ogryzkov, 1965). Gardner (1966) observed mortality in sheep maintained on low-protein and low-mineral ration fed with 5 mg sodium selenite every fortnight over a period of 52 weeks. Caravaggi et al. (1970) found the LD_{50} of sodium selenite as 1.9 mg per kg body weight as a single oral dose for lambs. Shortridge et al. (1971) reported mortality in 376 out of 557 calves which received subcutaneous injection of aqueous solution of sodium selenite at a total dose of 100 mg per calf. The toxic effects observed were depression, salivation and respiratory distress prior to death. Herigstad et al. (1973) reported that the toxic manifestations on oral feeding of sodium selenite in swine included anorexia, cachexia, subnormal body temperature and terminally coma and death. Severe convulsions and subsequent death in a two year old dog was reported by Turk (1980) on indiscriminate intramuscular administration of selenium preparation designed for cattle. Bhatia and Kalra (1981) successfully induced 'Degnala disease' in buffalo calves by feeding paddy straw

containing toxic levels of selenium and recorded the toxic manifestations and haematological values during the course of illness. Prasad et al. (1982) could induce a similar disease condition in buffalo calves by feeding rice husk containing 6 to 23 ppm selenium collected from seleniferous areas. Krieger et al. (1986) reported mortality in a herd of cattle after dosing with injectable oil emulsion of vitamin A, vitamin D and sodium selenite due to improper mixing of the preparation.

Selenium detoxification

The toxicity of selenium is believed to be due to replacement of sulphur in essential body proteins and thereby inhibiting the enzymes concerned with tissue respiration (Brown and deWet, 1962). Selenites react with sulfhydyl groups of cysteine and co-enzyme-A, forming stable seleno-sulfides rendering these co-factors unavailable for biological processes in which they are involved in the body (Ganther, 1968). Frost (1972) postulated that the toxicity of selenium is due to excessive accumulation of selenite ion, an oxidant which may interfere with glutathione metabolism. In animal tissues, selenites are converted into dimethyl selenides. The major pathway of selenium detoxification is methylation (Venugopal and Luckey, 1978).

Systemic and biochemical changes due to selenium

Draize and Beath (1935) reported damage to gastric mucosa and liver due to chronic selenosis in cattle. Rosenfeld and

Beath (1946) observed fall in the level of vitamin A, ascorbic acid and protein in the blood of sheep died of selenosis. Progressive anaemia with decrease in haemoglobin level was observed in mammals due to chronic selenosis (Rhian and Moxon, 1943; Mahalanobis and Roy, 1954 and Bhatia and Kalra, 1981). Fimiani (1949) observed decreased tissue levels of ascorbic acid and glutathione in acute and chronic selenosis. Bien et al. (1957) recorded decreased vitamin A level in the liver of rats due to selenosis.

Magg et al. (1960) observed lesions suggestive of poliomalacia in steers due to acute sodium selenite toxicosis. Gleum et al. (1964) reported focal to diffuse degeneration, necrosis and early replacement fibrosis of myocardium, congestion of lungs and degenerative lesions in liver, kidney and gastro-intestinal tract of sheep due to selenosis. In experimentally induced 'Degnala disease' in buffalo calves, Bhatia and Kalra (1981) observed slight fall in total leucocyte count and haemoglobin level. A decline in eosinophil count was a marked feature during the latter phase of toxicity. Harrison et al. (1983) reported symmetrical poliomalacia leading to paralysis in swine on feeding 19 to 24 mg of selenium/kg of the ration. Dewes and Lowe (1987) reported selenosis in a horse by feeding 35 μ mol selenium (as sodium selenite) daily for 5 consecutive days and observed loss of hairs from mane and tail, disintegration of skin of the anus,

lips, prepuse and scrotum and separation of hooves as the toxic manifestations.

Prophylaxis and treatment of selenosis

a) Effect of arsenic and its salts

Moxon (1941) found that supplementation of arsenic @ 5 ppm through drinking water in pigs counteracted the toxicity of selenium fed at 9 ppm level. Rhian and Moxon (1943) observed that sodium arsenate at 5 ppm level in drinking water prevented chronic selenosis in dogs maintained on a ration added with 13 ppm selenium. Excellent protection has also been reported on supplementing arsanilic acid (0.02 %) and 3-nitro-4-hydroxy phenyl arsonic acid (0.005%) in pigs maintained on a ration containing 10 ppm sodium selenite (Wahlstrom et al., 1955). Arsenic is believed to block transportation of selenite ion from liver (Palmer and Bonhost, 1957). Minyard et al. (1960) claimed the efficacy of daily oral administration of 0.01% arsanilic acid for preventing selenosis in steers maintained on a ration containing 12 ppm selenium. Levander and Baumman (1966) suggested that arsenic helped to stimulate excretion of selenium through gastrointestinal tract and thereby correspondingly decreased the level of selenium in the liver.

b) Effect of sulphates and thiosulphates

Administration of sodium sulphate and sodium thiosulphate was found effective in protecting rats against experimental

selenosis (Halverson et al., 1962). Their protective effect was suggested to be due to enhanced urinary excretion of selenium. Pratley and McFarlane (1974) recorded 50% reduction of selenium content in fodder grown on seleniferous soils by top dressing with sulphate fertilizer. Sulphates are believed to reduce the absorption of selenium from the soils by the plants.

c) Effect of protein diet

Protective effect of protein on selenium toxicity in ewes was studied by Rosenfeld and Beath (1946) and found that high and medium-protein diets afford certain degree of protection when compared to low-protein diet.

d) Effect of miscellaneous agents

Effect of ascorbic acid and potassium iodide on chronic selenium intoxication in rats was studied by Rosenfeld and Beath (1947). Potassium iodide was found to increase the toxicity, while ascorbic acid had no protective effect.

'Degnala disease' experimentally induced in buffalo calves by feeding rice husk collected from seleniferous areas, was successfully treated with a medicinal compound, 'Deg cure' comprising of magnesium sulphate 1 kg, ferrous sulphate 166 g, copper sulphate 24 g, Zinc sulphate 75 g and cobalt sulphate 15 g (Prasad et al., 1982).

Materials and Methods

MATERIALS AND METHODS

Eight apparently healthy cross-bred male calves of the age group of 10-14 months selected at random from the University Livestock Research Station, Thiruvazhamkunnu were used as experimental animals for this study. All the animals were maintained under identical conditions of feeding and management. They were kept under observation for two weeks during which period, samples of dung, urine and blood were examined on 3 occasions to eliminate the possibility of any internal diseases. These animals were divided at random into two groups; six experimental and two control.

Each experimental animal was given sodium selenite at the rate of 1.0 mg per kg body weight orally using an aqueous solution containing 1 mg sodium selenite per ml, three times a week continuously for 24 weeks. Toxic manifestations were recorded during the course of study. Blood samples were collected at weekly intervals and estimated the levels of plasma protein, vitamin A, vitamin C, volume of packed red cells, total erythrocytes, total leucocytes and haemoglobin.

Collection and analysis of clinical samples

Twenty millilitres of blood was drawn from each calf by puncutre of jugular vein in suitably labelled amber coloured vials containing 20 mg sodium EDTA as the anticoagulant. Ten millilitres of anticoagulant treated blood was centrifuged to separate plasma for the estimation of total protein and vitamin A. Estimation of vitamin A was carried out as per the method of Kimble (1939). For estimation of plasma protein,

the biuret method of Inchiosa (1964) was followed. Ascorbic acid level in blood was estimated adopting the method described by Roe and Kuether (1964). Volume of packed red cells, total erythrocyte and leucocyte counts and haemoglobin concentration were determined by the procedures described by Wintrobe et al. (1976). The biochemical and haematological values obtained at different stages of study were divided into 2 sets, first half (week 1-12) and second half (week 13-24) for statistical evaluation.

Post-mortem and histopathological studies

Post-mortem examination was conducted on two experimental animals died on 126th and 132nd day of commencement of study. Animals survived at the end of 24 weeks were sacrificed and the gross lesions in the various internal organs were noted. Samples of liver, kidney, brain and heart muscle were collected and preserved in 10% neutral formalin for histopathologic studies. After fixation, they were processed by routine paraffin embedding technique as detailed in the Armed Forces Institute of Pathology Manual (1968). Paraffin sections were prepared at four to five micron thickness, deparaffinised and stained with Haematoxylin and Eosin method of Haris as described by Disbrey and Rack (1970) and studied the microscopic pathology. The data were analysed using student's 't' test and analysis of variance technique as described by Cochran and Cox (1957) and Snedecor and Cochran (1967).

Results

RESULT

Induction of selenosis

Sodium selenite administered orally @ 1.0 mg/kg body weight three times in a week continuously for 24 weeks resulted in development of toxic manifestations of varying degree in all the experimental animals. Signs of toxicity were noticed from 13th week onwards. Out of the two severely affected animals, one died on the 126th day and the other on the 132nd day of commencement of the experiment.

Toxic manifestations

The salient clinical findings observed in severely affected animals were anorexia, cessation of rumination, cachexia, unsteady gait, rotation of hock during progression, cold and clammy skin, generalised oedema, reduced urine flow, rough starry coat and lacrimation followed by recumbency and death. At the peak of the toxicity, though the rates of respiration and pulse were increased, the body temperature was subnormal. The character of the pulse was soft and weak. The visible mucous membranes were pale.

Four animals have shown less severe signs of toxicity with manifestations evident from 13th week of the experiment. In them, toxic manifestations shown were mild degree of anorexia, general weakness, rough starry coat, resentment or disinclination to move and increased tendency to lie down. The body temperature remained within normal range. The pulse

and respiratory rates were increased. Visible mucous membranes appeared pale. Urine was scanty and the dung was pasty and mixed with mucus.

Biochemical and haematological values

A. Control group

The level of plasma proteins was in the range of 6.66 to 8.2 g/dl (7.77 ± 0.07) during the first 12 weeks (first half) and 7.2 to 8.4 g/dl (7.29 ± 0.04) in the 13th to 24th week period (second half). The plasma vitamin A level ranged between 30 and 35 µg/dl (32.29 ± 0.48) in the first half and remained unchanged during the second half (30-35 µg/dl (32.29 ± 0.48). The range of ascorbic acid level in the blood was 265-285 µg/dl (274.38 ± 1.41) and 265-285 µg/dl (275.21 ± 1.17) during the first and second halves respectively.

The volume of packed red cells (VPRC) was in the range of 28-34% (30.58 ± 0.30) in the first half and between 28 and 35% (31.88 ± 0.54) in the second half. The total erythrocyte count (TEC) in the first half ranged between 4.92 and 5.96 mill./cmm (5.42 ± 0.09), but during the second half it was in the range of 4.98-5.97 mill./cmm (5.58 ± 0.09). The respective values of total leucocyte count (TLC) were between 5850 and 8100 cells/cmm (7000 ± 168.52) and 6250 and 7850 (7066.67 ± 124.67). The haemoglobin level varied from 9.4-10.6 g/dl (10.08 ± 0.06) and 9.6-11.4 g/dl (10.43 ± 0.09) in the first and second halves respectively.

B. Experimental group

The level of plasma proteins ranged between 6.4 and 8.6 g/dl (7.53 ± 0.09) in the first half while in the second half it was 5.2-7.8 g/dl (6.30 ± 0.13). The vitamin A levels in the plasma were 20 to 35 µg/dl (30.07 ± 0.59) and 20 to 25 µg/dl (22.56 ± 0.38) respectively during the first and second halves. The respective values for ascorbic acid level in blood were between 195 and 240 u/dl (223.12 ± 3.46) and 100 and 210 µg/dl (162.32 ± 4.8).

The VPRC level varied between 26 and 34 % (29.68 ± 0.34) in the first half and between 24 and 30% (25.09 ± 0.49) during the latter half. The values of total erythrocyte count (TEC) in the first half was in the range of 4.68-5.73 mill./cmm (5.16 ± 0.4) and 4.02-5.32 mill./cmm (4.82 ± 0.03) in the subsequent period. The total leucocyte counts were from 5450-8350 cells/cmm (7243.06 ± 66.2) and 4600-6350 cells/cmm (5905.62 ± 94.14) respectively in the first and second halves of study. The haemoglobin level ranged between 7.3 and 12.4 g/dl (10.28 ± 0.11) during the first 12 weeks while in the remaining period it was 6.8-11.4 g/dl (8.31 ± 0.16). The biochemical and haematological values of the experimental and control animals are presented in tables 1 to 8, and the statistically analysed data in tables 9 to 12.

Autopsy findings

a) Gross pathology

There was cardiac dilatation, generalised pallor and

anasarca in two calves died during the course of the experiment due to extreme toxicity. Consistently lesions were seen in the liver in all the experimental calves. The liver was congested and focal areas of haemorrhage were seen. Scattered greyish-white areas of focal necrosis were also seen. The gallbladder was moderately distended with brownish-yellow bile.

Kidneys were enlarged, pale and contained scattered pin-point haemorrhages. Focal greyish-white areas of necrosis were noticed in the myocardium. Petechial haemorrhages of the endocardium were evident in three calves.

Microscopic pathology

Liver of all the experimental animals showed varying degree of hepatitis characterised by focal haemorrhages, fatty degeneration and centrilobular necrosis. The nucleus of the hepatic cells showed varying degree of fragmentation and disintegration. The capillaries of the lobules were congested (Fig. 1 and 2).

The lesions in kidneys consisted of focal areas of degeneration, medullary haemorrhages, tubular necrosis, cloudy swelling and fatty changes. Interstitial tissue showed hyaline casts. The tubules also revealed many hyaline casts (Fig. 3 and 4).

The cardiac muscle showed varying degree of degeneration, oedema, necrosis, lymphocytic infiltration and fibrosis. Petechial haemorrhages were evident in the pericardium (Fig. 5 and 6).

In the brain tissue, there was satellitosis, gliosis and neuronophagia. Oedema of the the brain parenchyma was evident in two calves (Fig. 7 and 8).

In the gastro-intestinal tract, there was oedema, haemorrhage and focal areas of necrosis of the mucosal and sub-mucosal layers.

The liver, kidney, heart, brain and the gastro-intestinal tract from the control animals were subjected to detailed histopathological studies, but no pathological changes were seen in these organs.

Table 1. Biochemical and haematological values - control animal No.I.

Week	Plasma protein (g/dl)	Vitamin A (µg/dl)	Vitamin C (µg/dl	VPRC (%)	Hb (g/dl)	TEC (mill./ cmm)	TLC (cells/ cmm)
1	7.9	35	275	30	9.8	4.92	7250
2	7.9	35	270	32	9.6	5.41	7200
3	7.8	30	280	30	9.6	5.28	7850
4	7.8	30	275	32	9.4	5.31	7650
5	8.0	35	270	32	9.8	5.84	6450
6	8;2	30	285	30	10.2	5.62	6850
7	7.8	30	280	28	10.0	4.98	7150
8	7.6	35	275	30	9.8	5.06	8100
9	7.8	30	270	30	10.2	5.62	6350
10	8.0	30	275	32	10.4	5.72	6200
11	8.2	30	280	30	10.4	5.65	6150
12	8.2	35	265	28	9.8	4.92	6050
13	8.4	30	270	28	10.2	5.15	6650
14	8.2	35	275	32	9.6	5.62	6750
15	8.0	30	280	30	10.2	5.41	6650
16	7.8	35	265	28	9.8	4.98	7250
17	8.2	30	265	32	10.4	6.21	6650
18	8.4	35	270	30	10.2	5.04	7150
19	8.2	30	280	35	10.4	5.82	6250
20	8.0	35	280	30	10.2	5.67	7250
21	7.8	30	270	32	9.8	5.62	6150
22	8.2	35	275	30	10.0	5.82	7250
23	8.2	35	280	30	10.2	5.94	7450
24	8.4	35	280	34	10.0	5.97	6850

Sacrificed after 24 weeks of study

Table 2. Biochemical and haematological values - control animal No.II.

Week	Plasma protein (g/dl)	Vitamin A (µg/dl)	Vitamin C (µg/dl)	VPRC (%)	Hb (g/dl)	TEC (mill./cmm)	TLC (cells/cmm)
1	6.3	35	275	34	10.2	5.92	7450
2	6.8	30	270	32	10.2	5.41	7750
3	6.6	35	270	32	10.4	5.28	7100
4	6.8	30	285	30	10.2	5.18	7600
5	7.0	35	280	32	10.4	5.96	6800
6	7.2	35	270	30	10.6	5.62	6250
7	7.0	30	280	28	10.4	4.98	6850
8	6.8	30	275	30	10.2	5.06	7850
9	7.6	35	265	30	9.8	5.62	7250
10	7.4	30	270	32	10.4	5.78	6850
11	8.2	35	280	28	10.2	5.05	7150
12	7.4	30	265	32	10.0	5.21	5850
13	7.4	30	285	30	10.2	5.05	7150
14	7.2	30	275	32	10.4	5.28	7650
15	7.3	35	270	32	9.8	5.61	6250
16	7.2	35	275	30	9.8	5.25	7450
17	7.2	30	275	30	10.6	5.32	7350
18	7.2	30	275	32	11.2	5.21	7850
19	7.4	30	285	35	10.8	5.91	6650
20	7.2	35	280	30	11.4	5.64	6350
21	7.4	30	270	30	10.8	5.62	7150
22	7.6	30	270	32	11.2	5.82	7350
23	7.4	30	270	32	11.4	5.94	6850
24	7.2	30	275	34	11.0	5.97	7250

Sacrificed after 24 weeks of study

Table 3. Biochemical and haematological values - experimental animal No.I

Week	Plasma protein (g/dl)	Vitamin A (µg/dl)	Vitamin C (µg/dl)	VPRC (%)	Hb (g/dl)	TEC (mill./ cmm)	TLC (cells/ cmm)
1	7.8	35	240	31	11.4	5.21	7150
2	7.8	30	235	30	11.4	5.38	7250
3	7.6	35	235	30	11.6	5.24	7250
4	7.6	30	230	30	11.2	5.28	7200
5	7.3	35	215	32	11.4	5.24	7150
6	7.2	35	215	28	11.4	5.10	7050
7	7.2	30	205	28	11.2	5.06	7250
8	7.2	30	210	30	10.8	5.12	7100
9	7.3	35	205	28	11.4	5.21	7050
10	7.2	35	210	26	10.8	5.01	7050
11	7.2	35	210	26	11.0	5.18	6950
12	7.3	30	205	26	10.8	5.02	6800
13	6.8	25	180	26	10.6	4.84	6650
14	7.0	25	170	26	10.4	4.81	6600
15	6.8	25	160	28	10.2	4.62	6450
16	6.6	20	120	27	9.4	4.60	6200
17	6.2	25	115	25	6.8	4.08	6150
18	5.8	25	100	24	8.2	4.04	6050
19	5.6	25	100	24	7.6	4.02	5950

Died at the end of 18th week

Table 4. Biochemical and haematological values - experimental animal No.II.

Week	Plasma protein (g/dl)	Vitamin A (µg/dl)	Vitamin C (µg/dl)	VPRC (%)	Hb (g/dl)	TEC (mill./ cmm)	TLC (Cells/ cmm)
1	8.6	35	215	33	8.0	5.12	8150
2	8.6	30	215	33	8.0	5.04	8250
3	8.6	30	205	33	8.0	5.42	8100
4	8.6	30	205	33	7.8	5.36	8350
5	8.2	30	220	32	7.8	5.28	8200
6	8.0	25	210	31	7.6	5.41	8050
7	7.8	30	205	32	7.8	5.36	8150
8	7.8	25	200	33	7.6	5.38	8200
9	7.6	25	205	32	7.6	5.32	8050
10	7.6	20	200	33	7.8	5.48	8150
11	7.8	25	205	32	7.6	5.34	7950
12	7.6	20	200	29	7.3	5.21	6850
13	7.2	20	190	28	7.2	5.22	6850
14	6.8	20	185	28	7.4	5.15	6700
15	6.6	20	130	30	7.4	5.14	6450
16	6.8	20	120	30	7.6	5.08	6560
17	6.8	20	115	28	7.2	5.04	6450
18	6.6	20	115	28	7.2	5.01	6350
19	6.8	20	110	28	7.0	5.02	6150
20	6.4	20	110	26	7.0	5.03	6200
21	6.4	20	110	25	6.8	4.98	6250
22	6.2	20	110	24	6.8	4.88	5950
23	6.2	20	105	24	6.8	4.91	4850
24	6.0	20	105	24	6.8	4.89	4600

Sacrificed after 24th weeks of study

Table 5. Biochemical and haematological values - experimental animal No.III.

Week	Plasma protein (g/dl)	Vitamin A (µg/dl)	Vitamin C (µg/dl)	VPRC (%)	Hb (g/dl)	TEC (mill./cmm)	TLC (cells/cmm)
1	8.2	30	230	32	11.8	5.38	7150
2	8.0	30	215	31	10.8	5.24	6850
3	8.0	25	210	31	11.0	5.28	6550
4	8.2	30	230	30	10.8	5.15	6450
5	7.8	30	215	31	11.4	5.42	6500
6	7.6	25	225	32	11.6	5.48	6400
7	7.6	30	220	31	11.2	5.38	6150
8	7.4	30	205	30	11.0	5.27	6200
9	7.6	25	215	32	11.2	5.31	6350
10	7.2	30	200	30	10.6	5.08	5750
11	7.2	25	195	28	10.2	5.04	5450
12	7.0	25	200	28	9.8	5.05	5550
13	6.8	25	185	26	8.2	5.32	5600
14	6.6	25	190	28	8.2	5.29	5450
15	6.8	30	185	26	8.0	5.26	5350
16	6.6	25	190	24	7.4	5.03	4950
17	6.4	25	190	26	7.8	5.08	5050
18	6.2	25	180	24	7.8	5.04	5150
19	6.2	30	165	24	7.8	5.06	5050
20	6.4	25	160	26	7.6	5.01	5200
21	6.0	25	150	24	7.4	4.98	5150
22	6.0	30	150	26	7.6	4.96	4900
23	6.0	25	160	26	7.6	4.97	4800
24	5.8	25	150	24	7.4	4.89	4750

Sacrificed after 24 weeks of study

Table 6. Biochemical and haematological values - experimental animal No.IV.

Week	Plasma Protein (g/dl)	Vitamin A (µg/dl)	Vitamin C (µg/dl)	VPRC (%)	Hb (g/dl)	TEC (mill./ cmm)	TLC (cells/ cmm)
1	8.5	30	215	34	12.4	5.18	7400
2	8.6	35	205	30	12.0	4.98	7650
3	8.4	30	200	30	11.8	4.72	7800
4	8.2	30	210	28	12.2	4.73	7650
5	8.2	30	205	30	12.0	6.21	7350
6	8.3	35	200	32	12.2	5.84	7400
7	8.5	30	205	30	12.0	5.61	7750
8	8.4	30	200	28	11.8	5.73	7450
9	8.2	25	205	28	11.6	4.98	7550
10	8.3	25	205	26	11.8	5.13	7650
11	7.8	20	205	28	11.6	4.78	6850
12	7.6	25	210	26	11.4	5.01	6950
13	7.5	25	210	26	11.4	5.02	6200
14	6.8	20	190	24	11.2	5.01	6250
15	6.6	20	175	26	10.8	4.98	6350
16	6.5	20	180	24	10.6	4.96	6450
17	6.4	20	180	26	10.4	4.96	6750
18	6.3	20	175	24	10.6	4.98	6650
19	6.4	20	170	24	9.8	4.78	6700
20	6.2	20	170	26	9.6	4.76	6550
21	5.8	20	175	26	9.2	4.72	6400
22	5.8	25	170	24	8.2	4.71	6450
23	5.6	20	165	26	8.4	4.78	6550
24	5.8	20	160	24	7.8	4.64	6450

Sacrificed after 24 weeks of study

Table 7. Biochemical and haematological values - experimental animal No. V.

Week	Plasma protein (g/dl)	Vitamin A (µg/dl)	Vitamin C (µg/dl)	VPRC (%)	Hb (g/dl)	TEC (mill./ cmm)	TLC (cells/ cmm)
1	8.5	35	230	30	10.4	5.08	7350
2	8.5	35	225	28	9.8	5.12	7450
3	8.4	35	220	30	10.6	5.05	7550
4	8.3	30	230	30	10.4	5.03	7600
5	8.4	35	225	28	9.6	5.10	7500
6	8.3	30	215	30	9.8	5.26	7450
7	8.3	30	205	30	9.4	5.17	7550
8	8.2	30	200	26	8.8	4.98	7250
9	8.3	30	195	26	8.6	4.97	7150
10	8.3	35	205	28	8.4	4.88	7200
11	8.2	30	205	26	8.0	4.92	7250
12	8.1	30	210	28	8.2	4.68	6950
13	7.8	25	185	28	8.0	4.67	5850
14	7.8	25	170	26	7.8	4.57	5600
15	7.6	25	135	24	7.4	4.52	5750
16	6.4	20	110	26	7.4	4.58	5600
17	6.0	25	105	24	7.6	4.43	5250
18	5.20	20	105	24	7.4	4.65	4950

Died at the end of 19th week

Table 8. Biochemical and haematological values - experimental animal No.VI.

Week	Plasma protein (g/dl)	Vitamin A (µg/dl)	Vitamin C (µg/dl)	VPRC (%)	Hb (g/dl)	TEC (mill./cmm)	TLC (cells/cmm)
1	7.3	30	290	30	11.6	5.08	7350
2	7.3	35	285	28	11.8	5.12	6450
3	7.2	35	280	30	10.8	5.02	7250
4	7.2	30	290	30	10.6	5.04	7300
5	7.6	30	285	28	10.4	5.14	7250
6	7.4	35	270	30	10.2	5.09	7300
7	7.0	35	260	30	10.4	5.10	7150
8	7.0	35	265	28	10.6	5.11	7250
9	6.8	30	255	30	10.2	4.9	7200
10	6.6	30	230	30	10.4	4.87	7150
11	6.8	30	235	28	10.2	4.68	7250
12	6.4	25	225	28	8.8	4.71	6950
13	6.6	25	225	28	9.8	4.78	6900
14	6.4	20	225	26	9.6	4.81	6750
15	6.6	25	215	28	9.6	4.72	6600
16	6.4	20	220	26	9.4	4.74	6500
17	6.2	25	205	26	9.2	4.64	6550
18	6.0	20	210	26	8.8	4.68	6150
19	6.2	25	215	24	8.4	4.67	5900
20	5.8	25	210	26	8.2	4.62	5850
21	5.6	20	195	24	8.4	4.66	5900
22	5.6	25	190	24	8.2	4.59	5650
23	5.8	20	180	24	8.0	4.58	5250
24	5.6	20	185	24	8.0	4.57	5150

Sacrificed after 24 weeks of study

Table 9. Range and mean of biochemical values in calves

Period of study	Group	Plasma protein (g/dl)		Vitamin A (µg/dl)		Vitamin C (µg/dl)	
		Range	Mean	Range	Mean	Range	Mean
0-12 weeks (first half)	Experimental	6.4-8.6	7.53 ±0.09	20-35	30.07 ±0.59	195-240	223.12 ±3.46
	Control	6.6-8.2	7.77 ±0.07	30-35	32.29 ±0.48	265-285	274.38 ±1.41
	t value	2.1201		2.9056		11.65	
13-24 weeks (second half)	Experimental	5.2-7.8	6.30 ±0.13	20-25	22.56 ±0.38	100-210	162.32 ±4.8
	Control	7.2-8.4	7.29 ±0.04	30-35	32.29 ±0.48	265-285	275.21 ±1.17
	t value	10.6559		15.8575		22.8626	

Table 10. Range and mean of haematological values in calves

Period of study	Group	VPRC (%)		TEC (mill./cmm)		TLC (cells/cmm)		Hb (g/dl)	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
0-12 weeks (first half)	Experimental	26-34	29.68 ±0.34	4.68-5.73	5.16 ±0.4	5450-8350	7243.06 ±66.2	7.3-12.4	10.28 ±0.11
	Control	28-34	30.58 ±0.38	4.92-5.96	5.42 ±0.09	5850-8100	7000 ±168.52	9.4-10.6	10.08 ±0.06
	t value	1.7726		2.6563		0.9494		1.5259	
13-24 weeks (second half)	Experimental	24-30	25.09 ±0.49	4.02-5.92	4.82 ±0.03	4600-6850	5905.62 ±94.14	6.8-11.4	8.31 ±0.16
	Control	28-35	31.08 ±0.54	4.98-5.97	5.58 ±0.09	6250-7850	7066.67 ±124.67	9.6-11.4	10.43 ±0.09
	t value	8.2221		7.8758		7.4321		11.4813	

Table 11. Statistical evaluation of mean values - first half (1-12 weeks)

Parameters	Mean value (control group)	Mean value (experimental group)	't' value
Plasma protein (g/dl)	7.77 ± 0.07	7.53 ± 0.09	2.1201 (NS)
Vitamin A (µg/dl)	32.29 ± 0.48	30.07 ± 0.59	2.9056**
Vitamin C (µg/dl)	274.38 ± 1.41	223.12 ± 3.46	11.65**
VPRC (%)	30.58 ± 0.38	29.68 ± 0.34	1.7726 (NS)
TEC (mill./cmm)	5.42 ± 0.09	5.16 ± 0.4	2.6563*
TLC (cells/cmm)	7000 ± 168.52	7243.06±66.2	0.9494 (NS)
Hb (g/dl)	10.08 ± 0.06	10.28 ± 0.11	1.5259 (NS)

DF 10	** P<0.01	* P<0.05
Table value	2.764	2.228

* Significant
** Highly significant
NS Not significant

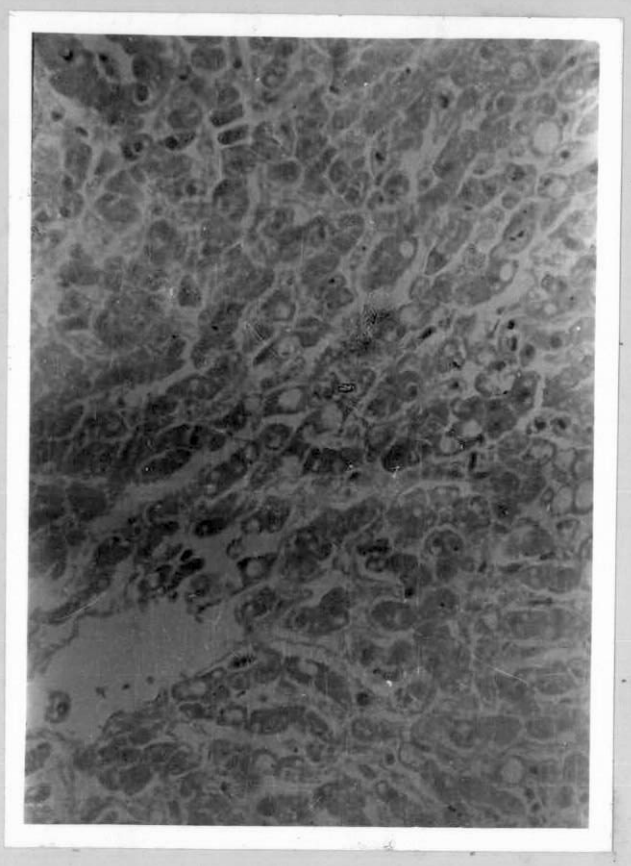
Table 12. Statistical evaluation of mean values - second half (13-24 weeks)

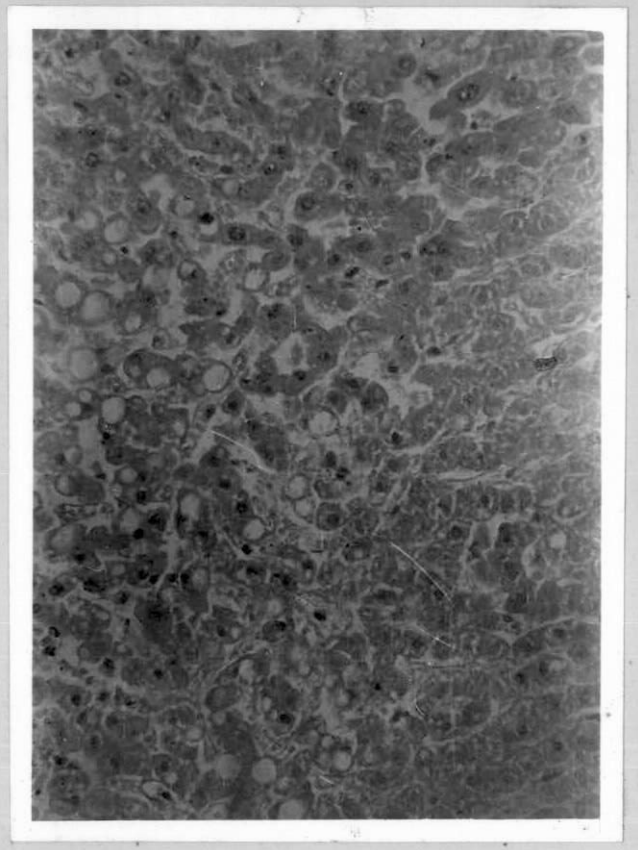
Parameters	Mean value (control group)	Mean value (experimental group)	't' value
Plasma protein (g/dl)	7.29 ± 0.04	6.30 ± 0.13	10.6559**
Vitamin A (µg/dl)	32.29 ± 0.48	22.56 ± 0.38	15.8575**
Vitamin C (µg/dl)	275.21 ± 1.17	162.32 ± 4.8	22.8626**
VPRC (%)	31.08 ± 0.54	25.09 ± 0.49	8.2221**
TEC (mill./cmm)	5.58 ± 0.09	4.82 ± 0.03	7.8758**
TLC (cells/cmm)	7066.69 ±124.67	5905.62±94.14	7.4321**
Hb (g/dl)	10.43 ± 0.09	8.31 ± 0.16	11.4813**

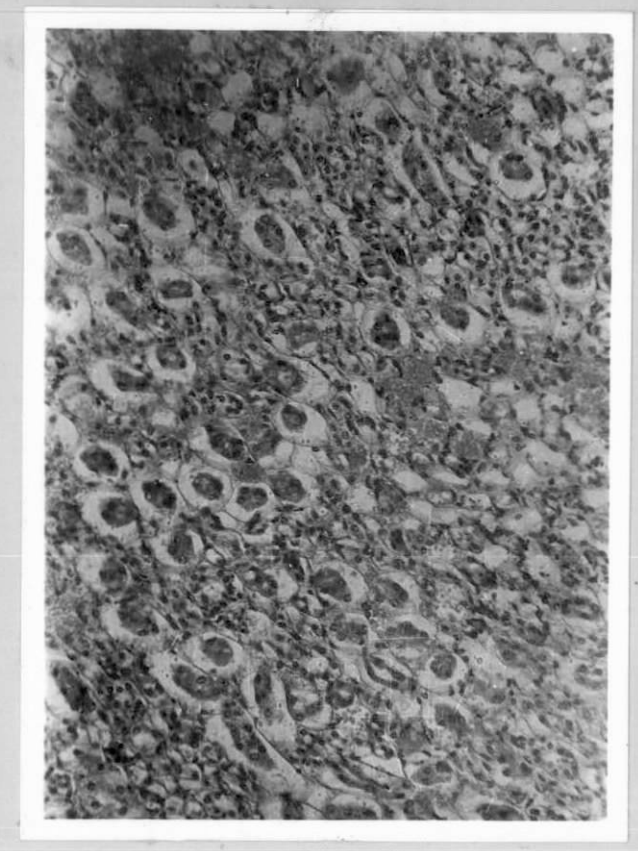
DF 10	** $P < 0.01$	* $P < 0.05$
Table value	2.764	2.228

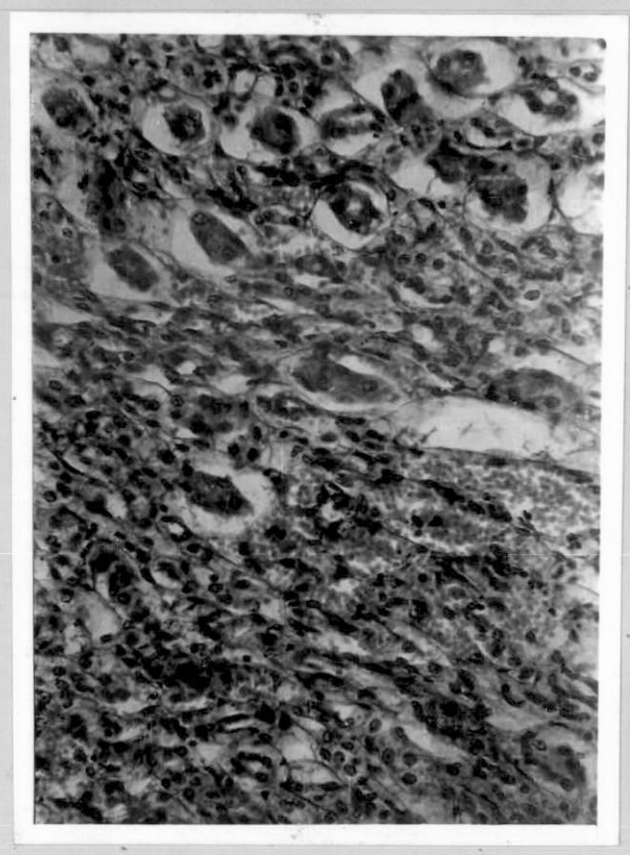
** Highly significant

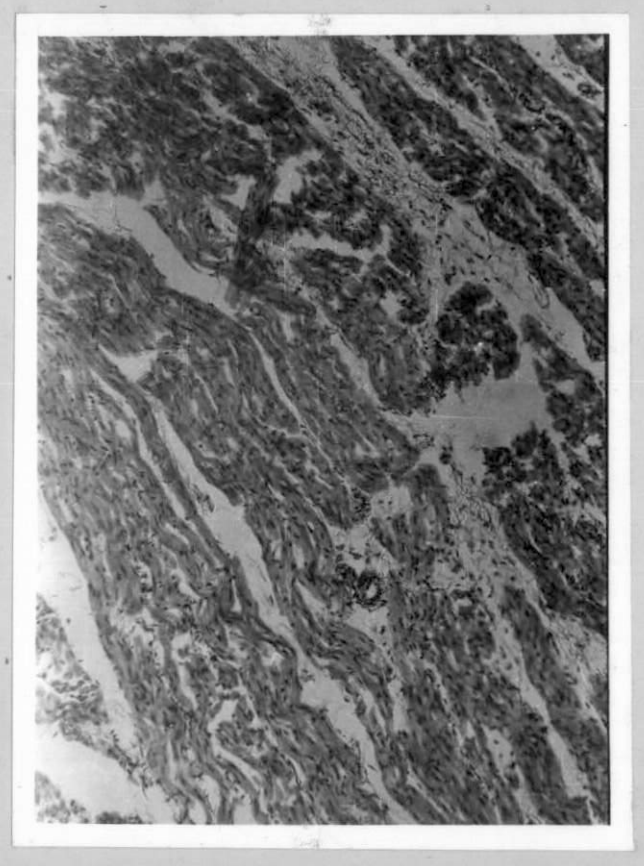
Figures

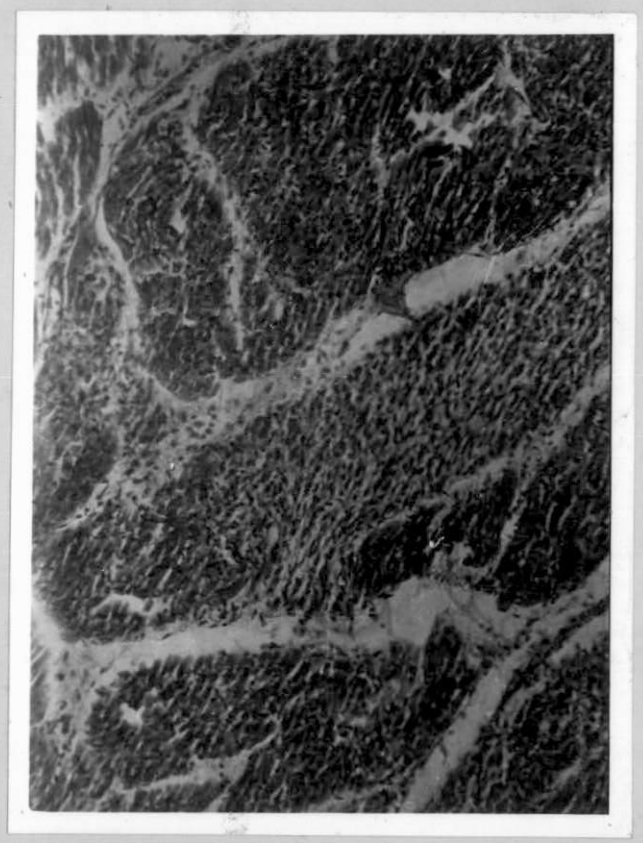


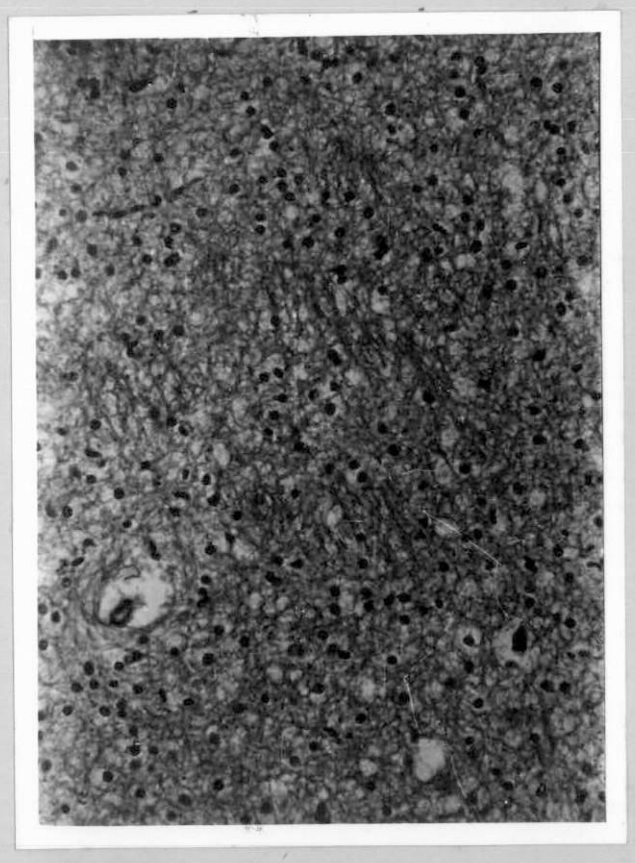












Discussion

DISCUSSION

No signs of toxicity were noticed in any of the experimental animals on oral administration of sodium selenite @ 1.0 mg/kg body weight for the first 12 weeks. But on continuation of administration for 24 weeks, all the animals showed toxic manifestations of varying degree commencing from the 13th week, but death occurred only in two severely affected animals, one on the day 126 and the other on day 132 of the experiment. Magg et al. (1960) also recorded mortality in six out of eight steers within a period of 23 weeks at the above dose level of sodium selenite. In sheep, Lenets and Tutushin (1964) recorded mortality at an early period of 24-27 days, but the dosing of sodium selenite was 1.0 mg/kg body weight, daily. Differences in the duration for development of toxicity and the degree of toxic manifestations might be due to variations in individual susceptibility.

The salient toxic manifestations recorded in the present study were anorexia, cachexia and incoordination of gait followed by recumbency and death. Similar observations in calves were made by Magg et al. (1960) and Shortridge et al. (1971). In the present study subnormal body temperature, cold and clammy skin, rotation of hock during progression, generalised oedema and respiratory distress were noticed as additional signs. Herigstad et al. (1973) also recorded weight loss, subnormal body temperature and respiratory distress prior to death in swine due to selenium toxicity.

Clinical signs such as desquamation of skin of limbs distal to knee or hock joint, muzzle region, gangrenous tail and necrosed eartips as reported by Arora et al. (1975) could not be reproduced in the present investigations.

The gross and microscopic pathology of affected organs in experimental animals revealed haemorrhagic inflammatory or degenerative lesions. Liver was the primary organ affected followed by kidney, heart, gastrointestinal tract and brain. Similar observations were reported by Magg et al. (1960) and Shotridge et al. (1971) in cattle, and Gardner (1966) in sheep; Turk (1980) in dogs and Fitzhugh et al. (1944) in rats. Widespread hyaline degeneration and fibrinoid necrosis of arterioles were stated to be the basis for haemorrhages and degenerative changes in visceral organs (Herigstad et al., 1973). Prothrombin deficiency associated with hepatic damage and capillary fragility caused by ascorbic acid deficiency might be the additional factors responsible for haemorrhages in vital organs. Mortality in two animals may be due to the complications associated with severe damage of vital organs.

There was no significant reduction in the level of plasma protein in experimental animals during the first 12 weeks of study; but it was significantly reduced ($P < 0.01$) in the next 12 weeks. Rosenfeld and Beath (1946) also recorded similar changes in sheep due to selenium toxicosis. Hypoproteinaemia observed in experimental animals might be the sequelae of inanition, damage to the liver, kidney and gastrointestinal tract and reduced biosynthesis of sulphur containing aminoacids

by the microflora of the rumen. The sparing effect of selenium for sulphur for the biosynthesis of sulphur containing aminoacids by the ruminal and intestinal microflora was reported by Rosenfeld and Beath (1946).

The plasma vitamin A level was significantly reduced ($P < 0.01$) in experimental animals both during the first and second halves of the experiment. Eventhough such a finding was not seen recorded in cattle, Rosenfeld and Beath (1946) found significant reduction in the vitamin A level of blood in sheep due to sodium selenite toxicosis. Bien et al. (1957) recorded decreased vitamin A storage in liver of rats due to selenium poisoning. Impaired utilization of carotene by the liver was found to be responsible for this. Reduction in plasma vitamin A level of the experimental animals might also be the sequelae of hepatic damage and inability of inflammed gastrointestinal mucosa to assimilate carotene/vitamin A.

During the first and second halves of the experiment, a significant reduction ($P < 0.01$) in the blood ascorbic acid level of experimental animals was recorded. A supportive finding was reported by Fimiani (1949).

A significant fall ($P < 0.01$) in haemoglobin level was noticed throughout the period of study. Rhian and Moxon (1943), Mahalanobis and Roy (1954) and Bhatia and Kalra (1981) also observed progressive anaemia with decreased haemoglobin levels in dogs, rats and buffalo calves respectively. The reduced haemoglobin levels observed in experimental animals

could be due to bone marrow suppression, hepatic damage and malnutrition. Moreover, selenium in plasma proteins was reported to be linked with globulins, thereby interfering with ready availability of globulins for the synthesis of haemoglobin (Westfall and Smith, 1940). Rigdon et al. (1953) reported bonemarrow depression and decreased production of erythroid cells in selenium toxicity of ducks.

Though the VPRC level was not altered significantly in the first half of the experiment, there was significant fall ($P < 0.01$) in VPRC level during the later half. It could be explained as a sequlae of depression of bone marrow activity, hepatic damage and malnutrition.

In experimental calves the TLC also showed significant reduction ($P < 0.01$), but was evident only during the second half, attributable to bone marrow depression, malnutrition and stress reaction associated with selenium toxicity. Bhatia and Kalra (1981) also recorded similar change in TLC in experimentally induced selenium toxicity.

The TEC level also diminished significantly in both halves of the experiment. During the first half, eventhough the reduction was not much significant ($P < 0.05$), it was highly significant ($P < 0.01$) during the second half. Halverson et al. (1970) reported haemolytic anaemia in rats fed with sodium selenite, but no haemolytic reaction was noticed in the present study. Rigdon et al. (1953) stated that selenites interfere with the enzyme systems concerned with formation of

erythroblasts in ducks. The reduced TEC count in experimental animals could be due to defective erythropoesis on account of malnutrition, bone marrow hypoplasia and hepatic damage.

Summary

SUMMARY

Selenium toxicity was induced experimentally in six calves by thrice weekly oral administration of sodium selenite at the rate of 1.0 mg/kg body weight for a period of 24 weeks while two calves served as control group. All the experimental animals showed signs of toxicity from 13th week onwards. The investigations comprised of recording the toxic manifestations, necropsy studies, estimation of plasma protein, plasma vitamin A, haemoglobin (Hb), volume of packed red cells (VPRC), total erythrocyte count (TEC), total leucocyte count (TLC) and ascorbic acid level in blood.

The salient toxic manifestations observed were anorexia, cachexia, incoordination of gait, cold and clammy skin, lacrimation and respiratory distress followed by recumbency and death. At the peak of the toxicity, there was acceleration of pulse and respiration, subnormal temperature and pale and watery mucous membranes.

There was significant reduction ($P < 0.01$) of plasma vitamin A level throughout the period of study.. Mean values of vitamin A in the first half (1-12 weeks) in the experimental and control groups were 30.07 ± 0.59 µg/dl and 32.29 ± 0.48 µg/dl respectively. The corresponding values in the second half (13-24 weeks) were 22.56 ± 0.38 µg/dl and 32.29 ± 0.48 µg/dl.

The ascorbic acid level in blood also recorded significant reduction ($P < 0.01$) in both the halves. The mean values in

experimental animals were 223.12 ± 3.46 µg/dl and 162.32 ± 4.8 µg/dl respectively in the first and second halves as against 274.38 ± 1.41 µg/dl and 275.21 ± 1.17 µg/dl in the control group. There was no significant change in the plasma protein level between the experimental and control calves in the first half; but it significantly reduced ($P < 0.01$) during the second half. The mean values in the experimental animals were 7.53 ± 0.09 g/dl and 6.30 ± 0.13 g/dl in the first and second halves respectively, while that of control were 7.7 ± 0.07 g/dl and 7.29 ± 0.04 g/dl. Similar changes were observed in VPRC level also. The mean values were 29.68 ± 0.34% and 25.09 ± 0.49% in the first and second halves in the experimental group; while that of control animals were 30.58 ± 0.38% and 31.08 ± 0.54% respectively.

TEC level in experimental animals was slightly reduced (P 0.05) during the first half. But in the second half a significant fall ($P < 0.01$) was observed. The mean values were 5.16 ± 0.4 mill./cmm and 4.82 ± 0.03 mill./cmm respectively in the first and second halves in experimental animals, while that of control group were 5.42 mill./cmm and 5.58 ± 0.09 mill./cmm.

The reduction of TLC level was significant ($P < 0.01$) only during the second half. The mean values in experimental animals were 7243.06 ± 66.2 cells/cmm and 5905.62 ± 94.14 cells/cmm in the first and second halves respectively. The

corresponding values in the control group were 7000 ± 168.52 cells/cmm and 7066.67 ± 124.67 cells/cmm. Comparable changes were observed in the haemoglobin level also. The mean values in experimental group were 10.28 ± 0.11 g/dl and 8.31 ± 0.16 g/dl respectively in the first and second halves whereas that of the control group were 10.08 ± 0.06 g/dl and 10.43 ± 0.09 g/dl.

The gross and microscopic pathology of internal organs in experimental animals revealed that the liver was the primary organ affected followed by kidneys, heart, gastrointestinal tract and the brain. The pathological lesions in the liver were focal, haemorrhages, fatty degeneration and centrilobular necrosis. Kidney showed focal areas of degeneration, medullary haemorrhages, tubular necrosis, cloudy swelling and fatty change. Varying degree of degeneration, necrosis and lymphocytic infiltration with replacement fibrosis were observed in cardiac muscle. Changes noticed in the gastrointestinal tract were oedema, focal haemorrhages, and areas of necrosis of the mucosal and submucosal layers. Oedema of the parenchyma, satellitosis, gliosis and neuronophagia were the changes noticed in the brain.

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EXPERIMENTAL SELENOSIS IN CATTLE

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ABSTRACT OF A THESIS

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ABSTRACT

Experimental studies were conducted on six calves keeping two as control by oral administration of sodium selenite at the rate of 1.0 mg/kg body weight thrice a week over a period of 24 weeks. Visible signs of toxicity were noticed only from week 13 onwards in experimental animals. The clinical toxicologic signs recorded were anorexia, cachexia, incoordination of gait, increased pulse and respiratory rates, pale and watery mucous membrane, subnormal temperature, cold and clammy skin, lacrimation and respiratory distress followed by recumbency and death. Laboratory evaluation of plasma protein, plasma vitamin A, haemoglobin (Hb), volume of packed red cells (VPRC), total erythrocyte count (TEC), total leucocyte count (TLC) and ascorbic acid level in the blood were carried out at weekly intervals. Since visible signs of toxicity were observed from week 13 onwards, the parameters studied were divided into 2 sets; 1st to 12th weeks (first half) and 13th to 24th weeks (second half) for statistical evaluation of data.

In experimental animals, significant reduction was observed only in respect of plasma vitamin A ($P < 0.01$), ascorbic acid level in blood ($P < 0.01$) and TEC ($P < 0.05$) during the first half, whereas all the parameters studied showed significant fall ($P < 0.01$) in the second half. The mean values of plasma protein, plasma vitamin A, haemoglobin, VPRC, TEC, TLC and ascorbic acid level in the blood in

experimental animals were 7.53 ± 0.09 g/dl, 30.07 ± 0.59 µg/dl, 10.28 ± 0.11 g/dl, 29.68 ± 0.34 %, 5.16 ± 0.4 mill./cmm, 7243.06 ± 66.2 cells/cmm and 223.12 ± 3.46 µg/dl respectively. The corresponding values in the control group were 7.77 ± 0.07 g/dl, 32.29 ± 0.48 µg/dl, 10.08 ± 0.06 g/dl, 30.58 ± 0.38 %, 5.42 ± 0.09 mill./cmm, 7000 ± 168.52 cells/cmm and 274.38 ± 1.41 µg/dl. During the second half, the mean values of the above parameters in experimental group were 6.30 ± 0.13 g/dl, 22.56 ± 0.38 µg/dl, 8.31 ± 0.16 g/dl, 25.09 ± 0.49%, 4.82 ± 0.03 mill./cmm, 5905.62 ± 94.14 cells/cmm and 162.32 ± 4.8 µg/dl and in the control group were 7.29 ± 0.04 g/dl, 32.29 ± 0.48 µg/dl, 10.43 ± 0.09 g/dl, 31.08 ± 0.54 %, 5.58 ± 0.09 mill./cmm and 7066.69 ± 124.67 cells/cmm and 275.21 ± 1.17 µg/dl.

The gross and microscopic pathology of internal organs in experimental animals revealed that liver is the primary organ affected followed by kidneys, heart, gastrointestinal tract and brain. The pathological lesions noticed in the liver were focal haemorrhages, fatty degeneration, centrilobular necrosis and varying degree of fragmentation of hepatic cells with congestion and dialatation of capillaries. The lesions in the kidney were focal areas of degeneration, medullary haemorrhages, tubular necrosis, cloudy swelling and fatty changes. Varying degree of degeneration, necrosis and lymphocytic infiltration and replacement fibrosis were observed in the cardiac muscle. Changes noticed in the gastrointestinal tract were oedema, focal areas of haemorrhage and necrosis of the mucosal and submucosal layers.