Influence of different sources of injected selenium on certain enzymes, glutathione and adenosylmethionine concentration in buffalo (*Bubalus bubalis*) calves

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Sodium selenite and selenomethionine were investigated as possible causative factors for the induction of Degnala disease syndrome in twelve buffalo (*Bubalus bubalis*) calves divided into three groups of four. Group 1 was the control group and received no additional selenium. Sodium selenite and selenomethionine were given daily as intramuscular injections on a selenium-equivalent basis, with a weekly increment in the dose of 0·05 mg Se/kg live weight from 0·05 to 0·20 mg Se/kg live weight per day, in groups 2 and 3 respectively. Only one animal from group 3 manifested the lesions of Degnala disease. The blood Se concentration and erythrocyte glutathione peroxidase (*EC* 1.11.1.9; GSH-Px) activity were both greater in groups 2 and 3 than in control group 1. The overall blood Se concentration was 0·22 (SE 0·01), 0·38 (SE 0·12) and 0·77 (SE 0·20) μg Se/ml in groups 1 to 3 respectively with corresponding GSH-Px activities of 63·84 (SE 7·38), 88·37 (SE 12·38) and 165·32 (SE 40·62) enzyme units/mg protein. Erythrocyte glutathione reductase (NAD(P)H) (*EC* 1.6.4.2) activity was not affected by treatment but reduced glutathione content was lower in groups 2 and 3. Liver adenosylmethionine, estimated at autopsy, was lowest (22·87 (SE 6·17) μmol/g) in group 3, and greatest (102·63 (SE 9·39) μmol/g) in group 1 ($P < 0·01$). Organic Se sources seemed to accumulate in tissues more than inorganic sources, and might be the causative toxic factors of Degnala disease.

Glutathione peroxidase: Adenosylmethionine: Selenium

Chronic selenosis is known to occur in some areas of certain geographical zones. A high selenium content in certain straws and fodders, as high as 6·7 mg/kg, has been reported to cause Degnala disease in buffaloes and cattle in India (Arora *et al.* 1975; Prasad & Arora, 1984). Rice straw accumulates Se to the extent of 8·06–11·56 times the Se content of soil (Prasad & Arora, 1980). Certain inorganic forms of Se, such as sodium selenite and sodium selenate in the diet, affect liver cells causing a serious and often fatal disorder (Khirwar & Arora, 1975), but Se which is available in the organic form from certain plants seems to affect liver more severely (Rosenfeld & Beath, 1964; Mathias *et al.* 1967). The exact way in which the inorganic and organic forms of Se at toxic levels interfere with tissue structure and function is unclear. It is likely that certain detoxifying mechanisms may become operative in enhancing the process of Se excretion in the form of its methyl derivatives, such as the trimethyl selenonium ion in urine or dimethyl selenide in exhaled air (Ganther & Hsieh, 1974).

In the present study an attempt has been made to obtain information on the detoxification process occurring with systemic loads of organic and inorganic forms of Se by estimating the activity of enzymes glutathione peroxidase (*EC* 1.11.1.9; GSH-Px) and glutathione reductase (NAD(P)H) (*EC* 1.6.4.2; GSSG-R) and concentrations of related metabolites adenosylmethionine (SAM) and reduced glutathione (GSH).

Table 1. *Feeding regimen used in the experiment* (*Sen* et al. *1978*)

Group	Animal no.	Body-wt (kg)	Feed offered: Concentrate (kg/d)	Feed offered: Green maize (kg/d)
1	2262	94·5	1·2	5·0
	2251	119·0	1·4	5·0
	2237	121·0	1·4	5·0
	2268	79·0	1·2	4·0
	Mean	103·38		
2	2286	93·5	1·2	5·0
	E-2	113·5	1·4	5·0
	2202	92·0	1·2	5·0
	2232	82·0	1·2	4·0
	Mean	95·25		
3	2271	118·5	1·4	5·0
	2291	107·0	1·4	5·0
	2218	130·0	1·5	5·0
	2289	88·0	1·2	4·0
	Mean	110·88		

MATERIALS AND METHODS

Animals and management

Twelve male Murrah buffalo (*Bubalus bubalis*) calves, with body-weights ranging from 79 to 130 kg, were divided randomly into three equal groups (Table 1). The concentrate fed to the calves comprised (g/kg): ground nut cake 430, crushed maize 270, wheat bran 270 and mineral mixture with sodium chloride 30. The resultant mixture was found to contain 0·24 μg Se/kg on a dry matter (DM) basis. The fodder source was green maize which contained 0·14 μg Se/kg DM. The feeding regimen (Table 1) was computed according to Sen *et al.* (1978). After 1 month on this regimen, blood samples were taken and erythrocyte GSH-Px activity was determined. This value was taken as the level of the enzyme activity at week 0.

Se administration and blood collection

The animals of control group 1 received no extra Se but those of group 2 were injected intramuscularly with sodium selenite, and those of group 3 with selenomethionine, each day on an Se-equivalent basis. The weekly increase in dose meant that at weeks 1, 2, 3 and 4, the doses were 0·05, 0·10, 0·15 and 0·20 mg Se/kg body-weight per d respectively. Blood samples were collected in heparinized tubes in ice every week before the injection of the next-higher dose, and brought to the laboratory for analysis.

Measurement of enzyme activities in erythrocytes

Whole blood (0·5 ml) was pipetted into a centrifuge tube and the volume made up to 3 ml with cold 0·4 M-phosphate buffer, pH 7·0. The contents were mixed and centrifuged at 9000 ***g*** for 14 min at 4°. The supernatant fraction was syphoned off. The erythrocyte layer remaining at the bottom of the centrifuge tube was resuspended in buffer to a volume 3·0 ml, mixed and recentrifuged. After the second wash, the erythrocyte layer was haemolysed by addition of cold distilled water to a volume of 3·0 ml, and then mixed again. The GSH-Px and GSSG-R activities in the haemolysate were estimated by following the procedures of Hafeman *et al.* (1974) and King (1965) respectively. GSH concentration was

Table 2. *Whole-blood selenium concentration (μg/ml) of buffalo* (Bubalus bubalis) *injected daily with weekly incremental doses of sodium selenite (group 2) or selenomethionine (group 3)*

Group*	Animal no.	W_0	W_1	W_2	W_3	W_4	Slope
1 (Control)	1	0·24	0·26	0·26	0·21	0·25	−0·003
	2	0·18	0·12	0·16	0·29	0·20	−0·021
	3	0·24	0·22	0·32	0·22	0·20	−0·008
	4	0·10	0·26	0·24	0·27	0·20	0·021
	Mean	0·19	0·21	0·24	0·25	0·21	0·008[a]
2	1	0·18	0·24	0·38	0·60	0·84	0·168
	2	0·04	0·14	0·26	0·42	1·00	0·220
	3	0·12	0·12	0·26	0·50	0·58	0·130
	4	0·12	0·19	0·44	0·68	0·64	0·153
	Mean	0·11	0·17	0·33	0·55	0·75	0·166[b]
3	1	0·16	0·34	0·70	1·40	0·82	0·238
	2	0·20	0·38	0·40	1·40	0·75	0·212
	3	0·22	0·27	1·00	1·18	0·80	0·207
	4	0·30	1·04	0·82	1·70	1·60	0·326
	Mean	0·22	0·51	0·73	1·42	0·99	0·245[c]
						SEM (df 9)	0·020

[a, b, c] Mean values with different superscript letters were significantly different ($P < 0·05$).
W_0-W_4, weeks 0–4 of the experiment.
* For details of treatments, see p. 262 and Table 1.

measured by the procedure of Prins & Loos (1969) and protein concentration was determined by the procedure of Lowry *et al.* (1951). For Se concentration, 1·0 ml whole blood was pipetted into a digestion tube and the determination was carried out using the fluorimetric procedure of Olson *et al.* (1975). Separate samples of whole blood were also taken in Wintrobe haemotocrit tubes for determination of packed cell volume.

Liver SAM estimation

The animals were killed on day 30 and liver samples were collected on ice and brought to the laboratory for analysis. The Dowex 50 sodium ion-exchange chromatographic procedure of Salvatore *et al.* (1971) was followed to estimate liver SAM concentration. The recovery of SAM was 93%, indicating satisfactory separation of SAM from adenosylhomocysteine (SAH). The isolated fractions were stable at 4° for 24 h.

Statistical analysis

The observations taken at 30 d were subjected to an analysis of variance for a completely randomized design (Snedecor & Cochran, 1968). The observations made at weekly intervals from the start of the experiment (week 0) until week 4 were analysed by linear regression, and the resulting slopes were used in an analysis of variance for a completely randomized design, according to the method of Rowell & Walters (1976). Means were compared using the studentized range test.

RESULTS

Blood Se concentration

The blood concentration remained approximately the same in group 1 at each of the weekly intervals (Table 2). The rate of change of blood Se concentration with time was greater in group 3 animals given selenomethionine than for those in group 2 given the inorganic form of Se on a Se-equivalent basis ($P < 0·05$).

Table 3. *Erythrocyte glutathione peroxidase* (EC *1.11.1.9*) *activity* (*EU/mg protein*) *of buffalo* (Bubalus bubalis) *injected daily with weekly incremental doses of sodium selenite* (*group 2*) *or selenomethionine* (*group 3*)

Group*	Animal no.	W_0	W_1	W_2	W_3	W_4	Slope
1 (Control)	1	55·54	27·47	37·38	28·97	21·38	−6·68
	2	116·63	22·85	59·22	37·36	45·55	−12·77
	3	74·03	109·49	57·71	57·72	49·06	−10·17
	4	106·04	132·88	77·35	75·03	85·14	−9·97
	Mean	88·06	73·17	57·91	49·77	50·28	−9·90[a]
2	1	53·37	104·26	128·90	95·84	55·54	−0·41
	2	87·97	69·88	70·62	75·24	95·56	2·05
	3	43·20	108·16	144·08	123·23	53·36	3·54
	4	54·04	76·79	80·93	166·57	79·95	14·16
	Mean	59·64	89·77	106·13	115·22	71·10	4·84[b]
3	1	57·86	123·09	78·28	129·60	223·28	33·74
	2	42·01	206·04	55·87	208·85	269·18	45·72
	3	54·03	178·03	80·03	231·65	303·93	55·34
	4	74·04	243·05	175·60	213·37	348·57	51·94
	Mean	56·98	187·55	97·45	195·86	288·74	46·68[c]
							SEM (df 9) 3·39

[a, b, c] Mean values with different superscript letters were significantly different ($P < 0·05$).
W_0–W_4, weeks 0–4 of the experiment; EU, enzyme unit.
* For details of treatments, see p. 262 and Table 1.

Erythrocyte GSH-Px activity and liver SAM content

The rates of change of erythrocyte GSH-Px activity differed significantly between treatments (Table 3). In general, it followed a trend similar to that of blood Se concentration and was highest in group 3 animals and lowest in group 1 animals. In group 2, the enzyme activity tended to increase up to week 3, thereafter there was a decrease in activity. In group 3 animals, the enzyme activity increased at each time-interval except at week 2. The higher activity of this enzyme in group 3 also suggested greater tissue Se accumulation from the organic form, which also led to the manifestation of clinical symptoms of Degnala disease in one animal (Plates 1 and 2).

The erythrocyte GSSG-R activity showed no difference with either form of Se (Table 4), indicating similar rates of Se excretion from the tissues. The latter effect was further substantiated by the observed trend of liver SAM concentration (Table 5), which was least in group 3 and greatest in group 1 ($P < 0·01$), indicating a higher Se methylation rate. Metabolic events seemed to occur at a faster rate with the organic form of Se than with the selenite form. GSH concentration did not show any trend with different treatments (Table 6).

DISCUSSION

The highest blood Se levels were observed in group 3 animals with the organic Se source, while there was no increase in group 1 animals, indicating that different Se sources have a different effect (Ehlig *et al.* 1967).

The increase in erythrocyte GSH-Px activity with increasing Se dose from different sources was proportional to the Se dose. Thus, GSH-Px is highly dependent on Se for its activity (Lee *et al.* 1969; Hafeman *et al.* 1974; Ganther *et al.* 1976; Kraus *et al.* 1983). GSH-Px activity increased or decreased in certain groups at certain times which could be explained as a differential pattern of biological response to a given Se dose at one time. The

Table 4. *Erythrocyte glutathione reductase* (EC *1.6.4.2*) *activity* (*mIU/mg protein*) *of buffalo* (Bubalus bubalis) *injected daily with weekly incremental doses of sodium selenite* (*group 2*) *or selenomethionine* (*group 3*)

Group*	Animal no.	W_0	W_1	W_2	W_3	W_4	Slope
1 (Control)	1	2·40	3·44	3·66	1·08	4·18	0·12
	2	4·17	0·86	3·70	2·45	4·88	0·30
	3	4·17	2·85	5·77	5·05	5·19	0·42
	4	3·47	2·79	3·87	4·77	5·05	0·51
	Mean	3·55	2·48	4·25	3·34	4·82	0·34[a]
2	1	2·55	4·89	1·40	2·00	2·78	−0·24
	2	4·69	5·49	1·00	4·03	2·21	−0·64
	3	5·47	6·15	1·65	2·05	2·91	−0·92
	4	3·87	4·11	1·78	1·36	5·00	−0·05
	Mean	4·14	5·16	1·46	2·36	3·22	−0·46[b]
3	1	2·76	2·65	3·26	4·09	4·97	0·59
	2	2·52	3·11	2·15	3·86	5·03	0·58
	3	5·41	2·75	3·00	4·63	5·56	0·22
	4	3·97	2·87	1·59	4·26	5·00	0·35
	Mean	3·66	2·84	2·50	4·21	5·14	0·44[a]
						SEM (df 9)	0·13

[a, b, c] Mean values with different superscript letters were significantly different ($P < 0·05$).
W_0-W_4, weeks 0–4 of the experiment.
* For details of treatments, see p. 262 and Table 1.

Table 5. *Liver adenosylmethionine* (*SAM*) *contents* (*μmol/g fresh liver tissue*) *of buffalo* (Bubalus bubalis) *calves injected daily with weekly incremental doses of sodium selenite* (*group 2*) *or selenomethionine* (*group 3*)

Group*...	1 (Control)		2		3	
	Animal no.	SAM	Animal no.	SAM	Animal no.	SAM
	2262	90·70	2286	69·40	2271	18·30
	2251	99·70	E-2	79·60	2291	16·50
	2237	90·10	2202	35·80	2218	41·30
	2268	130·00	2232	53·50	2289	15·40
	Mean	102·63[a]	—	59·62[b]	—	22·89[c]
	SE	9·39	—	9·56	—	6·17

[a, b, c] Mean values with different superscript letters were significantly different ($P < 0·01$).
* For details of treatments, see p. 262 and Table 1.

enzyme activity was highest in group 3 animals indicating greater Se accumulation, and this might have been the cause of the toxicity symptoms of Degnala disease seen in one animal as a result of selenocysteine or selenomethionine incorporation into certain proteins in the body (Ochoa-Solano & Gitler, 1968; Khirwar & Arora, 1977; Tekchandani & Arora, 1978; Kraus *et al.* 1983). Post-translational incorporation of Se into the GSH-Px enzyme could also have led to the high GSH-Px activity. The erythrocyte GSSG-R activity did not show any trend between treatments, probably because this enzyme was not Se-dependent.

Liver SAM concentration tended to decrease with increasing Se load, particularly with the organic Se source: this may have been because this metabolite was functioning as a

Table 6. *Erythrocyte reduced glutathione concentration (mg/l erythrocyte) of buffalo* (Bubalus bubalis) *injected daily with weekly incremental doses of sodium selenite (group 2) or selenomethionine (group 3)*

Group*	Animal no.	W_0	W_1	W_2	W_3	W_4	Slope
1 (Control)	1	1039·5	763·1	787·5	763·1	688·7	−70·2
	2	730·7	743·9	837·5	744·2	653·0	−15·5
	3	670·0	557·0	725·9	717·6	781·2	38·3
	4	593·2	625·0	560·3	601·8	798·1	38·7
	Mean	758·3	672·2	727·8	706·7	730·2	−2·2[a]
2	1	812·5	762·5	425·0	337·5	267·4	−151·5
	2	664·9	458·3	455·8	486·4	368·4	−56·5
	3	572·9	276·8	459·4	433·9	473·7	−3·9
	4	722·2	750·0	617·6	350·0	458·3	−92·8
	Mean	693·1	561·9	489·4	402·4	391·9	−76·2[ab]
3	1	843·7	810·0	720·0	506·1	325·6	−134·0
	2	662·8	657·6	409·1	439·0	287·5	−96·9
	3	892·8	488·6	652·7	309·5	397·4	−117·0
	4	603·4	480·7	244·2	245·3	144·4	−115·3
	Mean	750·7	609·0	506·5	375·0	288·7	−115·8[b]
							SEM (df 9) 23·8

[a, b, c] Mean values with different superscript letters were significantly different ($P < 0·05$).
W_0–W_4, weeks 0–4 of the experiment.
* For details of treatments, see p. 262 and Table 1.

methyl donor in the detoxification process through methyl transferases in order to form adenosyl homocysteine (Hoffman, 1975) and dimethyl selenide or trimethyl selenonium ion (Hsieh & Ganther, 1975), an action which facilitated the process of its elimination. Additional selenite has been shown to inactivate the methionine-adenosyltransferase (*EC* 2.5.1.6) enzyme system (Hoffman, 1977), and this inhibition might also have prevented the replenishment of SAM. Thus, organic Se sources such as selenocysteine and selenomethionine seem to accumulate in tissues, leading to certain changes and induction of symptoms of Degnala disease.

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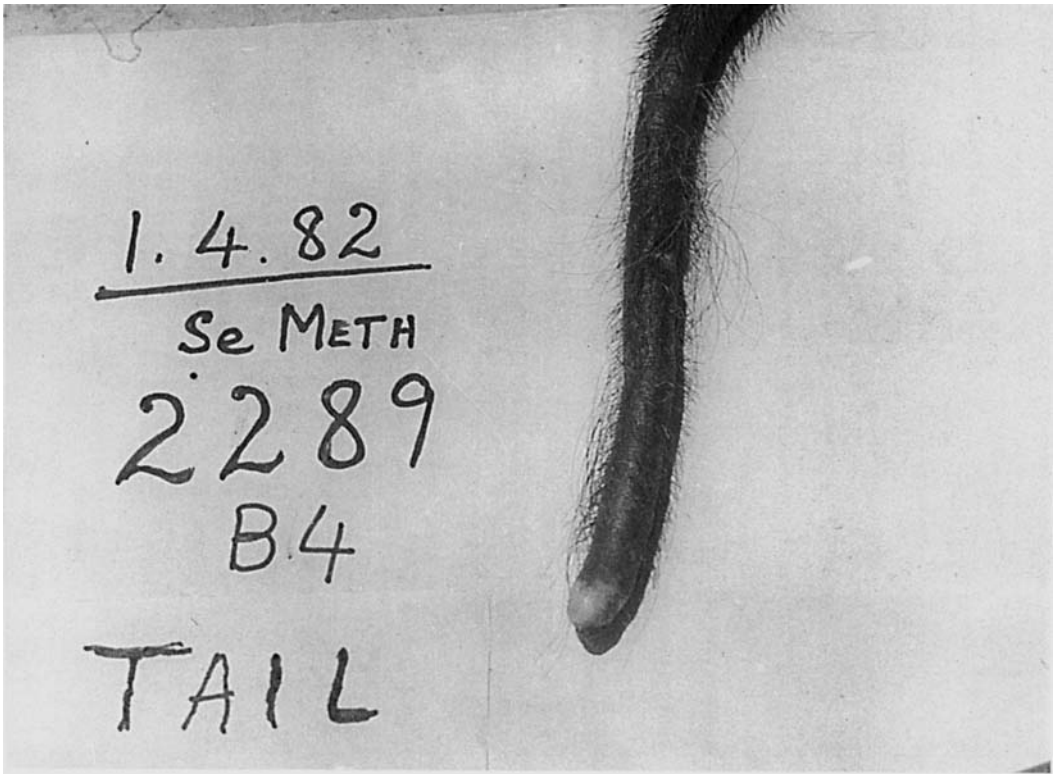

Plate 1. Loss of hair from tail, and tail tip necrosis in buffalo (*Bubalus bubalis*) calves receiving selenomethionine as the selenium source, with the tail switch having dropped off.

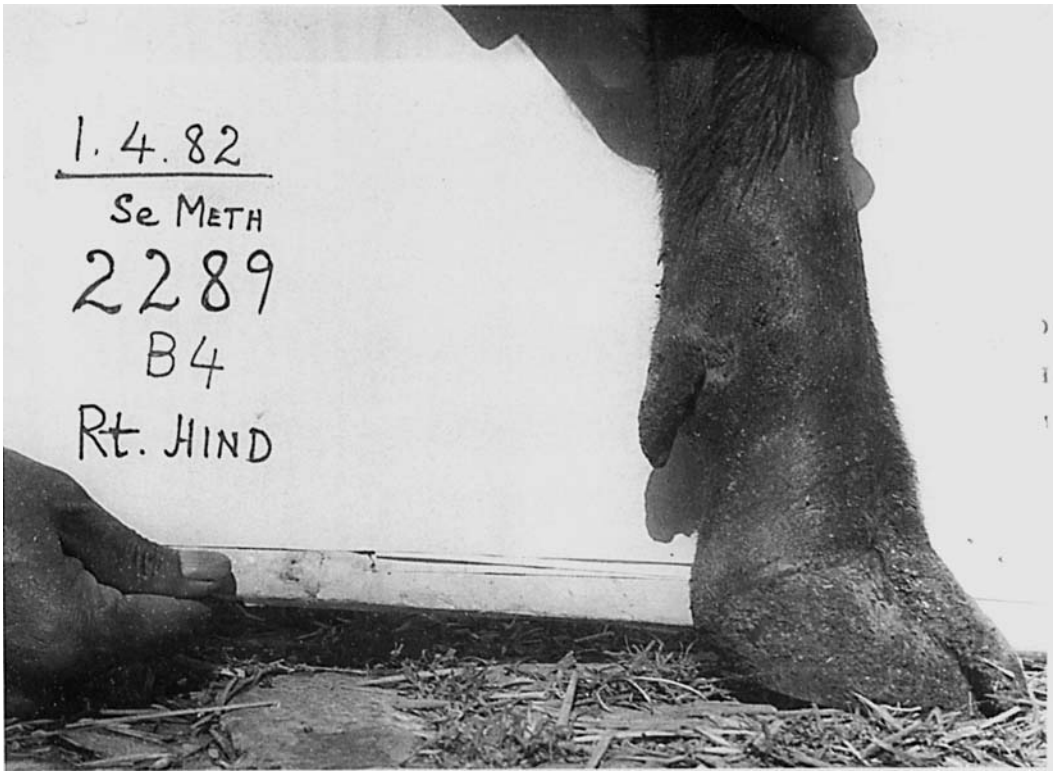

Plate 2. Wounds on the skin of legs near the coronary band of buffalo (*Bubalus bubalis*) calves receiving selenomethionine as the selenium source.

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