Protective effect of zinc supplementation against copper toxicosis in sheep

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1. A study has been made of the effects of dietary zinc supplementation on the development of copper toxicosis in three groups each of eight 12-week-old lambs.

2. None of the lambs receiving 420 mg Zn/kg diet developed Cu toxicosis in the 24-week experimental period, compared with three in the control group receiving 43 mg Zn/kg and possibly one in the group receiving 220 mg Zn/kg.

3. Liver Cu concentrations were reduced by up to 40% in the Zn-supplemented animals, with concomitant reductions, especially in the early stages of the experiment, in the extent of liver damage, as assessed by measurement of plasma aspartate aminotransferase (*EC* 2.6.1.1) and arginase (*EC* 3.5.3.1) activities.

4. Plasma and liver Zn concentrations were increased only slightly in the lambs receiving the Zn-supplemented diets, and the only indication of possible toxic effects of the Zn supplements was the development of a slight anaemia in those animals receiving 420 mg Zn/kg diet.

5. The results suggest that the incidence of Cu toxicosis in sheep may be controlled by increasing their dietary Zn intake.

The occurrence of chronic copper poisoning has long been recognized as a major hazard in the intensive rearing of sheep. Although this syndrome is associated with extensive hepatic accumulation of the metal, it is not possible to relate its incidence to any particular concentrations of Cu in the liver (Todd, 1969). Problems may arise with diets containing about 30 mg Cu/kg, but dietary components other than Cu also influence the development of Cu toxicosis (Bremner, 1974). Attempts have been made to prevent the occurrence of the disease by dietary supplementation with molybdenum and sulphate (Ross, 1966; Hogan, Money & Blayney, 1968) but, despite the frequent success of this measure, there has been some reluctance to adopt it in commercial practice for fear of inducing a Cu deficiency state.

In an alternative approach, we have examined the possibility of protecting sheep against Cu toxicosis by increasing their dietary zinc intake. It has been found that the absorption of Cu by the rat is reduced by Zn administration (Van Campen & Scaife, 1967). Increase in liver Zn content in the sheep causes a redistribution of hepatic Cu, with an increase in the amount bound to metallothionein, which is thought to be involved in the storage and detoxication of Cu and other heavy metals (Bremner & Marshall, 1974*b*). Furthermore, the liver damage observed in chronic Cu toxicosis (Bremner, 1974) may be associated with Cu-induced lipid peroxidation in lysosomes (Lindquist, 1968) which accumulate Cu within the hepatocyte (Lindquist, 1967; Goldfischer & Sternlieb, 1968). This weakening of the lysosomal membrane by Cu can be reversed in vitro by Zn (Chvapil, Ryan & Zukoski, 1972). Finally, Zn is relatively non-toxic to sheep (Ott, Smith, Harrington & Beeson, 1966)

and has been successfully used in the control of Cu toxicosis in swine (Suttle & Mills, 1966).

The results of preliminary studies have suggested that increasing the Zn intake of sheep to 100 mg/kg diet causes a reduction in their liver Cu content (Mills, 1974). In this paper, the effects of further increasing the dietary Zn concentration to 220 and 420 mg/kg are described. The development of Cu toxicosis was prevented, with concomitant reductions in liver Cu content and liver damage, and with no serious side-effects.

EXPERIMENTAL

Experimental design

Twenty-four Finn-Dorset × Suffolk ewe lambs, aged about 12 weeks and weighing about 24 kg, were allocated at random to three treatment groups, each of eight animals. They were individually penned indoors and were fed *ad lib.* on a diet containing (g/kg): 780 barley, 120 fishmeal, 50 distillers dark grains, 30 distillers solubles and 20 dried yeast. The Cu, Zn and iron concentrations of the basal diet, as fed, were 29, 43 and 206 mg/kg respectively. Lambs in treatment group A were given the basal ration with no additional supplements, whereas those in treatment groups B and C received in addition a supplement of 175 or 375 mg Zn (as $ZnSO_4.7H_2O$)/kg respectively. The animals were weighed and blood samples collected weekly. Liver biopsies were made after 8 and 12 weeks. The experiment was terminated after 24 weeks.

One animal in treatment group A, and two in treatment C, died after 12 weeks as a result of internal haemorrhage following the biopsies. Another animal in treatment group A was killed after 21 weeks at the point of death, having suffered from anorexia and weight loss for several weeks.

Analytical methods

Concentrations of Cu and Zn in blood and plasma were determined by atomic absorption spectrometry after precipitation of proteins with trichloroacetic acid (50 g/l). Tissue samples were digested with conc. HNO_3–conc. $HClO_4$–conc. H_2SO_4 (4:2:1, by vol.). The activities of plasma ferroxidase (*EC* 1.16.3.1), arginase (*EC* 3.5.3.1) and aspartate aminotransferase (*EC* 2.6.1.1) (AAT) were measured by the methods of Smith & Wright (1975), Cornelius, Douglas, Gronwald & Freedland (1963) and Dickie, Gibson & Albert-Recht (1970) respectively.

Bromosulphthalein (BSP) clearance tests to assess liver function were based on the method of Varley (1962), except that the dose level of BSP was 5 mg/kg body-weight and blood samples were collected from 2 to 12 min after injection. Clearance rates were expressed as a function of the plasma BSP concentrations at 2 min. The distribution of Cu and Zn amongst proteins in the liver cytosol was determined by gel filtration on Sephadex G75 (Pharmacia Ltd, Uppsala, Sweden) as described previously (Bremner & Marshall, 1974*a*), except that liver biopsy samples taken at 8 weeks were homogenized with 9·5 vol. (v/w) 0·01 M-Tris–acetate buffer (pH 7·4).

Statistical analysis of results was by analysis of variance and by the Mann–Whitney U test.

Table 1. *Effect of zinc supplementation on body-weight and plasma copper and Zn concentrations of sheep given a basal diet (treatment group A) or diets with two levels of added Zn (treatment groups B and C)*†

(Mean values for eight sheep/treatment)

Treatment group ...	A	B	C	Approximate SE of differences between means	Statistical significance of effect
Dietary Zn concentration (mg/kg) ...	43	220	420		
Body-wt (kg)					
At start	23·7	24·0	24·2	1·7	NS
After 16 weeks	50·3‡	53·7	49·1‡	4·1	NS
After 24 weeks	57·9§	59·7	51·9‡	5·6	NS
Plasma Cu concentration (μg/ml)					
At start	1·10	1·01	0·99	0·11	NS
After 9 weeks	1·65	1·52	1·50	0·10	NS
After 16 weeks	1·89‡	1·60	1·57‡	0·17	NS
After 24 weeks	1·54§	1·41	1·30‡	0·14	NS
Plasma Zn concentration (μg/ml)					
At start	1·22	1·04	1·05	0·06	NS
After 9 weeks	1·11	1·13	1·49	0·12	**
After 16 weeks	1·13‡	1·29	1·48‡	0·14	NS
After 24 weeks	1·24§	1·13	1·29‡	0·17	NS

NS, not significant.
** $P < 0.01$.
† For details of diets, see p. 552.
‡ Only six sheep/treatment.
§ Only three sheep/treatment.

RESULTS

Body-weight gain. The growth of the sheep was not significantly affected by the dietary Zn supplementation, although body-weights did tend to be greatest in treatment group B (Table 1). The average daily rate of gain was 0·24 kg/d over the first 16 weeks, but only 0·1 kg/d thereafter. This reduction in growth rate was associated with a reduction in food intake from about 1·5 kg/d between 8 and 16 weeks to only 1·0 kg/d from week 17 onwards.

Plasma Cu and Zn concentrations. Plasma Cu concentrations in all groups increased gradually from about 1·0 μg/ml at the start of the experiment to about 1·7 μg/ml at 16 weeks, whereupon they tended to decrease slightly to about 1·4 μg/ml (Table 1). Although no significant differences were found between treatment groups, concentrations were usually greatest in the control group (treatment group A). Cu concentrations were closely related to ferroxidase activities at all times and no values for the enzyme are reported here.

In several sheep there was a transient increase in plasma Cu concentration during the experiment to over 2 μg/ml. These concentrations were either maintained for a few weeks before decreasing to about 1·4 μg/ml or they increased again, very suddenly, to 4–10 μg/ml (Fig. 1). This was associated with the onset of the haemolytic crisis typical of the terminal phase of Cu toxicosis (see below). In calculating mean concentrations in the treatment groups, these very high values have been excluded.

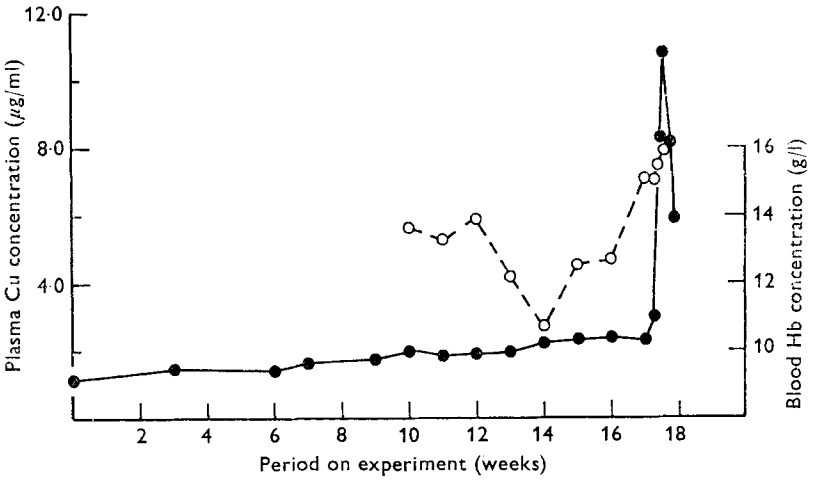

Fig. 1. Changes in plasma copper concentration (μg/ml) (●) and blood haemoglobin (Hb) concentration (g/l) (○) in sheep no. 4 in treatment group A receiving a basal diet with 29 mg Cu/kg and no zinc supplement; for details of diet, see p. 552.

Table 2. *Effect of zinc supplementation on haematological measurements of sheep given a basal diet (treatment A) or diets with two levels of added Zn (treatment groups B and C)*†

(Mean values for eight sheep/treatment)

Treatment group ...	A	B	C	Approximate SE of differences between means	Statistical significance of effect
Dietary Zn concentration (mg/kg) ...	43	220	420		
Haemoglobin concentration (g/l)					
At 10 weeks	132	129	121	4·0	*
At 12 weeks	125	125	100	4·9	***
At 16 weeks	107‡	104	78‡	5·8	**
At 24 weeks	116§	134	105‡	10·1	NS
Packed cell volume					
At 10 weeks	0·344	0·347	0·324	0·011	NS
At 12 weeks	0·357	0·363	0·310	0·010	*
At 16 weeks	0·351	0·349	0·269‡	0·025	**
At 24 weeks	0·318	0·371	0·299‡	0·026	NS
Erythrocyte count ($\times 10^{12}$/l)					
At 10 weeks	10·0	9·6	8·9	0·4	*
At 12 weeks	10·7‡	10·8	9·3	0·5	*
At 24 weeks	8·4§	10·7	8·4‡	0·7	**
Plasma iron concentration (μg/ml)					
At 12 weeks	2·72	2·46	1·97	0·41	NS

NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† For details of diets, see p. 552.
‡ Only six sheep/treatment.
§ Only three sheep/treatment.

Whole blood concentrations (not given) were generally slightly lower than those in plasma and were also unaffected by Zn intake. However, when animals developed the haemolytic crisis, concentrations of Cu were greater in the whole blood than in plasma and were maintained at a high level after plasma concentrations had decreased.

Plasma Zn concentrations in treatment group C were significantly greater than those in treatment groups A and B for much of the experiment (Table 1). Maximum values of about 1·5 μg/ml were attained after 13 weeks but decreased slowly thereafter.

Haematological measurements. Concentrations of blood haemoglobin (Hb) and packed cell volume (PCV) values were significantly reduced in treatment group C, receiving 420 mg Zn/kg, after week 10, when first measurements were made (Table 2).

The differences between treatment groups were generally small but were most pronounced between weeks 12 and 16, when blood Hb concentrations in treatment group C were about 80 g/l. In the final stages of the experiment, the concentrations were greatest in treatment group B.

The changes in Hb concentration were paralleled by those in PCV and red cell count (Table 2), with the lowest values occurring in treatment group C at about week 16. Similar treatment effects were also found for plasma Fe, but the differences were not statistically significant.

Two animals, in treatment groups A and C, suddenly developed haemolytic anaemia after 16 weeks. Although of unknown aetiology, this was quite distinct from the haemolytic episode normally associated with Cu toxicosis, as there was no change in blood Cu or methaemoglobin concentrations and no haemoglobinuria, and it occurred in animals showing no signs of severe Cu-induced liver damage. In one instance, blood Hb concentrations decreased within 1 week from 94 to 33 g/l, PCV values from 0·30 to 0·15 and erythrocyte counts from 11·6 to $3{\cdot}6 \times 10^{12}$/l. Mean cell volumes were increased, to about 50 fl, there was marked reticulocytosis and 50% of the erythrocytes contained Heinz bodies. Near-normal haematological status was restored after 5 weeks.

Slight increases in blood Hb concentrations and PCV values were noted in the sheep which developed the haemolytic crisis typical of Cu toxicosis (Fig. 1). These occurred at or before the time that blood Cu concentrations suddenly increased.

Plasma AAT and arginase activities. The activities of plasma AAT, which are taken to be a measure of tissue damage, were significantly greater in treatment group A than in the Zn-supplemented groups from week 7, when assays were first done, to week 12 (Table 3). Maximum activities in treatment group A were maintained for several weeks but tended to decrease from week 17 onwards. In contrast, the activities in treatment group C receiving 420 mg Zn/kg, although initially relatively low, tended to increase throughout the experiment. No significant differences were detected between the treatment groups after 13 weeks. There was, however, considerable variation within treatment groups and activities were exceptionally high (> 1000 μmol glutamate produced/l per min) in those animals which developed the haemolytic crisis (Fig. 2). Typical values in normal sheep were about 50 μmol glutamate produced/l per min.

Similar results were found for plasma arginase activities, which more specifically

Table 3. *Effect of zinc supplementation on activities of aspartate aminotransferase* (EC 2.6.1.1) (*AAT*) *and arginase* (EC 3.5.3.1) *in plasma and on liver function* (*bromosulphthalein* (*BSP*) *plasma clearance test*) *of sheep given a basal diet* (*treatment group A*) *or diets with two levels of added Zn* (*treatment groups B and C*)†

(Mean values for eight sheep/treatment)

Treatment group ...	A	B	C	Approximate SE of differences between means	Statistical significance of effect
Dietary Zn concentration (mg/kg) ...	43	220	420		
AAT activity (μmol glutamate produced/l per min)					
At 7 weeks	413	255	153	82	*
At 12 weeks	436	209	176	80	**
At 16 weeks	325‡	212	240‡	96	NS
At 24 weeks	244§	236	323‡	122	NS
Arginase activity (mg urea produced/ml per h)					
At 7 weeks	0·363	0·160	0·156	0·103	NS
At 12 weeks	0·402	0·154	0·137	0·079	**
At 16 weeks	0·709‡	0·437	0·171†	0·179	*
At 24 weeks	0·306§	0·304	0·222†	0·127	NS
Plasma BSP (% dose)					
At 6 min	56·3	43·9	22·5	12·1	*
At 12 min	35·6	24·8	10·8	14·4	NS

NS, not significant.
* $P < 0.05$, ** $P < 0.01$.
† For details of diets, see p. 552.
‡ Only six sheep/treatment.
§ Only three sheep/treatment.

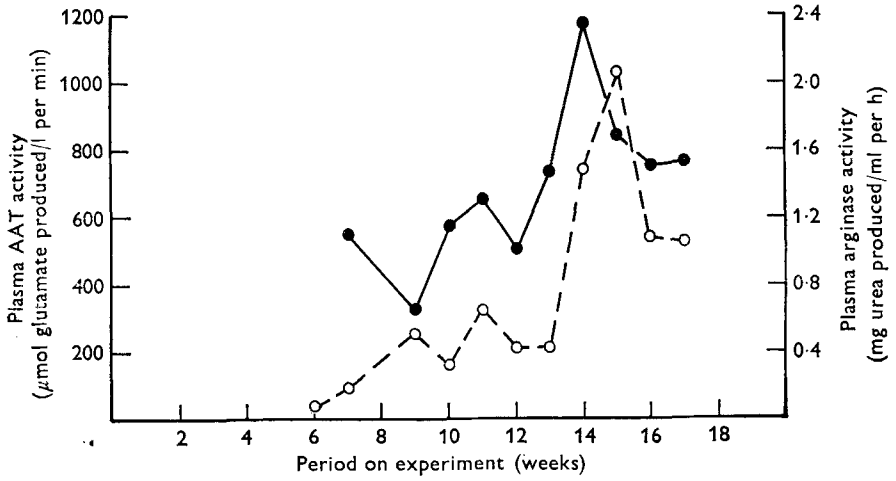

Fig. 2. Changes in plasma aspartate aminotransferase (*EC* 2.6.1.1) (AAT) activity (μmol glutamate produced/l per min) (●) and arginase (*EC* 3.5.3.1) activity (mg urea produced/ml per h) (○) in sheep no. 4 in treatment group A, receiving a basal diet with 29 mg copper/kg and no zinc supplement; for details of diet, see p. 552.

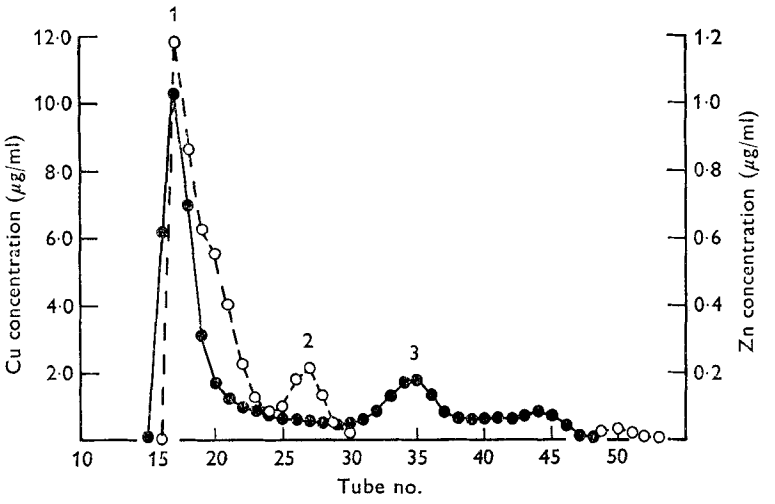

Fig. 3. Fractionation on a Sephadex G75 (Pharmacia Ltd, Uppsala, Sweden) column (900 × 16 mm) of the supernatant fraction from the liver, obtained at the end of the experiment, of one sheep in treatment group A, receiving a basal diet with 29 mg copper/kg and no zinc supplement; for details of diet, see p. 552. The concentrations of Cu (●) and Zn (○) in each tube are shown. Fractions 1 and 3 were contained in tubes 16–23 and 31–39 respectively. Each tube contained 3·5 ml eluate.

reflect liver damage, with values inversely proportional to Zn intake between 7 and 16 weeks (Table 3). As with AAT activities, there was considerable variation within treatment groups and values were extremely high before and during the onset of the haemolytic crisis (Fig. 2). In normal sheep arginase activities were about 0·10 mg urea/ml per h.

The results of tests of liver function after 13 weeks indicated that the plasma clearance of BSP was more rapid in the Zn-supplemented animals. The proportion of the dyestuff remaining in the plasma after 6 min was inversely related to Zn intake (Table 3). Less than 30% of the BSP remains after 6 min and less than 5% after 12 min in normal sheep (W. J. Lawson, unpublished results).

Incidence of haemolytic crises. Three sheep in treatment group A died, or were killed at the point of death, after developing Cu toxicosis at 17, 19 and 22 weeks. In all instances, there was loss of appetite, jaundice and a sudden increase in AAT and arginase activities (Figs. 1 and 2). There was also methaemoglobinaemia and, if the syndrome was allowed to develop, haemoglobinuria. In one animal in treatment group B, blood Cu, AAT and arginase levels had started to increase when the animal was slaughtered at 24 weeks.

Tissue concentrations of Cu and Zn. Liver biopsy samples were collected after 8 and 12 weeks and analysed for Cu and Zn. At both times, the Cu concentrations in treatment groups B and C were significantly less, by about 25 and 40% respectively, than those in treatment group A (Table 4). Although liver Zn concentrations were significantly related to Zn intake, values in the sheep receiving 420 mg/kg were only 1·5 times those in the control group (treatment group A) (Table 4). Similar effects were found at the end of the experiment but the differences between treatment groups were not significant.

Table 4. *Effect of zinc supplementation on concentrations of copper and Zn in liver and kidney cortex of sheep given a basal diet (treatment group A) or diets with two levels of added Zn (treatment groups B and C)*†

(Mean values for eight sheep/treatment)

Treatment group ...	A	B	C	Approximate SE of differences between means	Statistical significance of effect
Dietary Zn concentration (mg/kg) ...	43	220	420		
Liver Cu concentration (μg/g fresh liver)					
At 8 weeks	265	209	174	27	*
At 12 weeks	308	232	180	33	**
At end of experiment	394‡	362	274‡	80	NS
Liver Zn concentration (μg/g fresh liver)					
At 8 weeks	31·4	38·7	52·7	4·8	***
At 12 weeks	35·3	43·4	48·0	3·6	**
At end of experiment	31·3‡	40·4	43·1‡	7·3	NS
Kidney Cu concentration‖ (μg/g kidney cortex)	13·1§	10·9‡	10·1‡	3·5	NS

NS, not significant.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diets, see p. 552.

‡ Only six sheep/treatment.

§ Only four sheep/treatment.

‖ Values from sheep with Cu toxicosis and from single animals in treatment groups A and B (see p. 558) have been omitted.

Kidney cortex Cu concentrations were not affected by Zn intake (Table 4). They were generally about 12 μg/g except in the four Cu-poisoned sheep (122 ± 41 (SE) μg/g) and in one other animal in each of treatment groups A and B, when they were 53·5 and 136·8 μg/g respectively.

Separation on Sephadex G75 (Fig. 3) of the supernatant fractions of each of three liver homogenates from treatment groups A and C revealed effects of Zn supplementation on the distribution of Cu among metal-binding proteins at 8 weeks. In particular the concentration of Cu present as metallothionein (fraction 3) (Bremner & Marshall, 1974*b*) was only 19·6 ± 3·7 (SE) μg/g liver in treatment group A compared with 32·7 ± 10·8 μg/g in treatment group C. This was equivalent to 6·8 ± 1·4 and 18·3 ± 5·8% respectively of the total hepatic Cu, these proportions being significantly different ($P < 0.05$). In all instances most of the soluble Cu occurred in fraction 1, which was excluded by the gel, and there was no Zn in the metallothionein fraction.

There were, however, no significant effects of treatment on the Cu distribution in livers taken at the end of the experiment. In contrast to the earlier samples, the tendency was for Zn supplementation to decrease the amount of Cu present in metallothionein, the concentrations being 36·1, 29·8 and 21·9 (SE of difference 4·0) μg/g liver in treatment groups A, B and C respectively and equivalent to about 7% of the total liver Cu. With the exception of the livers from the sheep which developed Cu toxicosis and from one other animal with extremely yellow plasma, the

concentrations (μg/g fresh liver) of Cu in metallothionein (y) were best expressed as a function of liver Zn concentration (x) by the equation:

$$y = 0{\cdot}91x - 15{\cdot}5 \text{ (SE of regression coefficient } 0{\cdot}12).$$

Concentrations in the Cu-intoxicated animals were much greater than that predicted from this equation and usually accounted for 15% of the total liver Cu. No Zn was present as metallothionein in any of these livers.

DISCUSSION

It is clear that substantial protection against the development of Cu toxicosis in sheep was obtained by increasing the dietary Zn concentration to 220 or, more effectively, 420 mg/kg. In this way, the onset of the haemolytic crisis was prevented and, moreover, there was a decrease in the amount of liver damage which occurred even in the animals which did not enter the terminal phase of Cu toxicosis. This protection was achieved without any significant deleterious effect on the Zn-supplemented sheep other than the production of a very mild anaemia.

Although the mechanism whereby Zn exerted this protective effect was not studied in detail, it was probably associated in part with the decrease in liver Cu concentration in the supplemented animals during the first half of the experiment.

Some measure of the effects of Zn on the availability of the dietary Cu can be obtained from the changes in liver concentrations of the metal between weeks 8 and 12, when biopsy samples were taken. If the liver accounts for about 1·6% of the total body mass in sheep of this age (Wallace, 1948) then, for an average daily food intake of 1·5 kg, the proportion of the dietary Cu retained in the liver was calculated to be 4·0, 2·8 and 1·5% in treatment groups A, B and C respectively. Although it cannot be excluded that these differences arose from effects of Zn on Cu excretion, it seems more probable that, as in the rat (Van Campen & Scaife, 1967), Zn affected Cu uptake at the intestinal level.

It is also possible that Zn affects the toxicity of Cu in other ways, especially as the protective effect of Zn in pigs is not associated with a decrease in liver Cu concentrations (Suttle & Mills, 1966). Zn may promote the formation in the liver of relatively non-toxic forms of Cu. These could include metallothionein, whose occurrence in the liver of ruminants (Bremner & Marshall, 1974*b*) and pigs (Bremner, 1976; Bremner & Young, 1976) is dependent on Zn status and which is thought to be involved in the storage and detoxification of Cu and other heavy metals (Bremner, 1974). In accord with previous studies (Bremner & Marshall, 1974*b*) Cu-thionein concentrations were increased by Zn supplementation at 8 weeks and were related to liver Zn content at the end of the experiment. However, this was a relatively minor Cu component in these livers, accounting for only 7% of the total liver, although it did increase to 15% in the sheep which developed Cu toxicosis. Whether this reflected a response to increased levels of circulating Cu or impaired biliary excretion of the metal is not known and must await improved understanding of the physiological role of this protein.

Another possible explanation for the protective effect of Zn against the development

of Cu toxicosis was that it inhibited the Cu-induced lipid peroxidation of membranes (Chvapil *et al.* 1972). However, in preliminary studies on erthrocyte fragilities and on malondialdehyde production by liver homogenates, no significant differences were found between the treatment groups. Furthermore, examination of the livers by electron and light microscopy (T. P. King & I. Bremner, unpublished results) revealed similar effects of Cu on lipid peroxidation of membranes in all groups.

In these studies also, marked changes were found in liver ultrastructure after 12 weeks which were consistent with the impaired liver function and the increased levels of AAT and arginase (Ross, 1966) in the plasma, and confirmed that considerable liver damage had occurred by this time. Similar biochemical changes have been reported previously (Todd & Thompson, 1963; Ross, 1966; Ishmael, Gopinath & Howell, 1972) and it has been reported that morphological and histochemical changes in hepatocytes and Kupffer cells occur several weeks before the onset of the haemolytic crisis (Ishmael, Gopinath & Howell, 1971).

Dietary Zn supplementation did not eliminate the effects of Cu on liver ultrastructure or on plasma enzyme activities, which were above normal even in the animals receiving 420 mg Zn/kg diet. However, the Cu-induced changes were delayed sufficiently to allow the sheep to attain a normal slaughter weight before serious damage had occurred. The absence of significant differences in plasma enzyme levels between treatment groups towards the end of the experiment was a consequence mainly of the decreased activities in treatment group A. This could have resulted from the death by this time of the animals most affected by Cu, or alternatively from some adaptation to Cu accumulation similar to that reported in Cu-poisoned pigs (Suttle & Mills, 1966).

The absence of any serious effect of Zn supplementation on the sheep is in accord with the relatively non-toxic nature of this element (Bremner, 1974). Dietary Zn concentrations of about 1000 mg/kg are necessary to impair growth and food conversion efficiency in this species (Ott, Smith, Harrington & Beeson, 1966). A dietary intake of 420 mg Zn/kg did cause a mild anaemia in the sheep, although growth was unaffected and the changes in plasma and tissue Zn concentrations were small compared with those found in calves receiving 600 mg Zn/kg for only a few weeks and showing no clinical signs of Zn toxicosis (Stake, Miller, Gentry & Neathery, 1975). This implies that there was effective homoeostatic control over Zn uptake in the sheep and indeed the decreased plasma and liver Zn concentrations during the experiment indicate a possible adaptation to the high Zn intake.

A mild anaemia has also been reported in growing cattle receiving 200 mg Zn/kg diet (Ott, Smith, Harrington, Parker & Beeson, 1966). The cause of this has not been established, but high Zn intakes in rats increase ferritin turnover in liver (Coleman & Matrone, 1969) and decrease erythrocyte life-span (Settlemire & Matrone, 1967). Similar effects may occur in the sheep, perhaps exacerbated by the tendency of the high Cu intake to reduce blood Hb concentrations in all animals. In view of the high Fe concentration in the diet (206 mg/kg), it is unlikely that Fe supplementation would have prevented the development of this anaemia.

The pattern of change in plasma Cu concentrations was similar to that found

by McCosker (1968), with an increase to over 2 μg/ml occurring a few weeks before the onset of the haemolytic crisis and the associated dramatic increase in Cu concentrations. The initial increase often coincided with the first major changes in AAT and arginase activities. In contrast, Todd & Thompson (1963) and Ishmael *et al.* (1972) reported that plasma Cu concentrations were unaffected in the period before the crisis. In most other regards the pattern of development of Cu toxicosis was similar to that described previously (Todd & Thompson, 1963; Ross, 1966; Ishmael *et al.* 1972), with increases in blood Hb concentrations and PCV values occurring immediately before the final increase in blood Cu concentrations. The formation of methaemoglobin and Heinz bodies was also typical of chronic Cu toxicosis in sheep and indicative of considerable oxidant stress in the erythrocytes at this time (Todd & Thompson, 1963).

These results suggest therefore that substantial protection against the onset of Cu toxicosis in sheep might be obtained by increasing their dietary Zn intake. However, before these findings are applied commercially, further studies with larger numbers of animals are merited to establish the significance of the effect of the higher level of Zn on the haematological status of the sheep. In addition, attention should be paid to the increased susceptibility of pregnant animals to Zn toxicosis (J. K. Campbell, unpublished results).

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