The Effect of Copper Excess on Iron Metabolism in Sheep

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Sheep were treated with large amounts of copper (20 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ /kg body wt. per day) for 9 weeks to examine the effect of copper excess on iron metabolism. In addition to confirming that massive haemolysis and accumulation of copper occurs in the liver, kidney and plasma after 7 weeks of exposure to excess copper, it was observed that excess copper produced an increased plasma iron concentration and transferrin saturation within 1 week. Further, iron preferentially accumulated in the spleen between 4 and 6 weeks of copper treatment, producing 3-fold increases in the iron content of both the ferritin and non-ferritin fractions. A 3-4-fold increase was also observed in the amount of ferritin that could be isolated from the spleen. The copper treatment had little or no effect on the concentration of iron in the liver and bone marrow. The following properties of erythrocytes were also unaffected by copper treatment: size, haemoglobin content and pyruvate kinase activity, although the erythrocyte concentration of copper increased after 6 weeks. Copper accumulated in the spleen between 6 and 9 weeks, probably owing to the phagocytosis of erythrocytes containing high concentrations of copper. The data suggest that copper excess influences iron metabolism, initially by causing a compensated haemolytic anaemia, and later by interfering with the re-utilization of iron from ferritin in the reticuloendothelial cells of the spleen.

Copper is required for normal erythrocyte formation and iron utilization. However, only a narrow range of copper concentration is effective, and copper deficiency as well as copper excess have deleterious effects. A deficiency of copper leads to anaemia, which appears to result from a deficit of cytochrome oxidase, a deficiency in the synthesis of haem from iron and protoporphyrin, a decrease in the mobilization of iron from reticuloendothelial cells and hepatic parenchymal cells and impaired absorption of iron (Williams *et al.*, 1976). The decrease in iron transport and absorption has been attributed to a caeruloplasmin deficiency (Roeser *et al.*, 1970). In general, a copper excess in humans and other animals causes hepatic and kidney damage, haemolytic anaemia (Evans, 1973) and methaemoglobinaemia in humans (Chugh *et al.*, 1975) and sheep (Todd & Thompson, 1961). In pigs, copper excess can cause anaemia because of impaired iron absorption (Gipp *et al.*, 1974). Copper poisoning is usually caused by accidental ingestion of large amounts of copper, but in some instances has resulted from the excessive use of copper-rich foods in humans or copper-rich supplements in farm animals. The genetically caused maldistribution of copper in Wilson's disease mimics some of the symptoms of excessive copper ingestion (Scheinberg & Sternlieb, 1965). In spite of the recognized importance of copper in iron metabolism, the specific effects of copper excess on iron metabolism have not been well characterized.

Among animals, sheep are the most sensitive to copper toxicity, and as such should respond to copper excess with the most marked metabolic effects. Previous studies of the effect of copper excess in sheep have been mainly concerned with the distribution of copper and the pathology of copper poisoning, and little is known about the effect of copper excess on iron metabolism, beyond the observed increase in the concentration of iron in the kidney and its decrease in the spinal cord (Ishmael *et al.*, 1971, 1972). Copper-induced changes have been observed in the liver, blood, kidney and central nervous system of sheep. When sheep are exposed to copper excess, copper accumulates in the liver, leading to liver necrosis, which subsequently produces an increase in arginase, glutamate-oxaloacetate transaminase and lactate dehydrogenase activities in the plasma (Todd *et al.*, 1963; Ross, 1966). The liver lesions caused by copper excess are similar to those observed in Wilson's disease. After prolonged exposure, copper is thought to leak from the damaged liver, producing increased plasma and erythrocyte copper concentrations, methaemoglobin formation, massive haemolysis (15-20% of the erythrocytes) and haemoglobinaemia; often the haemolytic crisis is fatal (Todd & Thompson, 1961; Todd *et al.*, 1963; Ishmael *et al.*, 1972). The changes that occur in the kidneys of sheep treated with copper excess (increased iron, copper and haemoglobin) have been attributed to the effects of haemolysis, but apparently some of

the changes (increased copper and iron) are independent of haemolysis and do not affect kidney function (Gopinath *et al.*, 1974). Changes in the white matter of the central nervous system also occur in copper poisoning, even though little copper accumulates there (Ishmael *et al.*, 1971).

The studies reported below show that the early effects of copper excess on iron metabolism led to an increased plasma iron concentration. Subsequently, the copper treatment caused a preferential increase in iron storage and in the ferritin content of the spleen.

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Materials and Methods

Ferrozine [disodium 3-(2-pyridyl)-5,6-bis-(4-phenylsulphonic acid)-1,2,4-triazine] was purchased from Hach Chemical Co., Ames, IA, U.S.A. Sepharose 6B was obtained from Pharmacia, Piscataway, NJ, U.S.A., and Amberlite IRA-410 from Mallinkrodt Chemicals, St. Louis, MO, U.S.A. Bovine serum albumin and haemoglobin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., and biotin was a product of Calbiochem, La Jolla, CA, U.S.A. Cyanocobalamin, calcium pantothenate, nicotinamide, riboflavin, pyridoxine and thiamin were gifts of Merck, Rahway, NJ, U.S.A. Vitamins A (from fish liver oil) and D (ergocalciferol) were the gift of P. P. Scherer Corp., Detroit, MI, U.S.A., and folic acid was given by Hoffman-La Roche, Nutley, NJ, U.S.A. The A. E. Staley Company, Decatur, IL, U.S.A., kindly provided inositol, and DL-methionine was the gift of the Diamond Shamrock Co., Harrison, NJ, U.S.A. Other growth factors were purchased from ICN Biochemicals, Cleveland, OH, U.S.A. Casein was purchased from the Erie Casein Co., Erie, IL, U.S.A., and sodium selenite from Pfaltz and Bauer, Stamford, CT, U.S.A. All other chemicals were purchased from Fisher Scientific Co., Raleigh, NC, U.S.A., and were of reagent grade.

Ewe lambs (Western Cross-bred; 16–18 kg) were purchased from Mr. Frank White, Abingdon, VA, U.S.A. After adaptation to a purified diet (Diet 17 with cottonseed oil; Matrone *et al.*, 1964) they were given a daily dose of 1% (w/v) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (20 mg/kg) by mouth; an equivalent amount of water was given to the controls. The amount of copper supplement given was 100 times that in the purified diet. Purified diet and drinking water were provided *ad libitum*. Weights of animals were determined, and blood was collected from the jugular vein, weekly. After 6 or 9 weeks, equal numbers of control and copper-treated animals were killed and the organs

were removed, cubed and frozen in solid CO_2 or liquid N_2 . The tissues were stored at -20 or -83°C . Fractionation into components soluble or insoluble after heating was accomplished by heating a 20% (w/v) homogenate in water to 70°C (reaching temperature within 1 min) for 10 min, chilling and separating the fractions by sedimentation at $19000g$ at 4°C for 20 min.

Copper was analysed with a Perkin-Elmer atomic-absorption spectrophotometer (model 305B) with an air/acetylene flame by measuring the A_{325} . Iron was analysed as the ferrozine complex; thioglycolic acid and thiourea were added to complex copper before addition of ferrozine (Yee & Goodwin, 1974). Samples were prepared for copper or iron analysis as follows. Organs and organ fractions were dried and digested with 8.8M HClO_4 /15.6M HNO_3 (3:1, v/v). Plasma was diluted with water (1:1, v/v) for copper analysis or deproteinized by heating with 1.3M HCl and 10% (w/v) trichloroacetic acid at 70°C for 5 min before iron analysis. Erythrocytes were lysed with water and mixed with HCl (final concn. 0.8M) and trichloroacetic acid (final concn. 10%, w/v) before copper analysis (Gubler *et al.*, 1952). Marrow from the sternum was homogenized with 0.85% NaCl (Kerr, 1957). The total iron-binding capacity of plasma was determined by adding iron as ferric ammonium citrate and removing the unbound iron with Amberlite IRA-410 resin (Peters *et al.*, 1956). Erythrocyte pyruvate kinase activity was determined by the procedure of Beutler (1972).

Protein concentrations were determined as described by Lowry *et al.* (1951), except for bone-marrow homogenates, where a modified Folin procedure (Kerr, 1957) was used. Bovine serum albumin was used as a standard throughout. Erythrocyte and plasma haemoglobin were measured in extracts prepared with ethyl acetate/acetic acid (3:1, v/v) and 1M HCl extracts with bovine haemoglobin as a standard (Thunell, 1965).

Spleen ferritin was isolated from 20% (w/v) homogenates in water clarified by sedimentation at $19000g$ for 20 min at 4°C , heated to 70°C for 10 min and fractionated by gel filtration [Sepharose 6B column (2.0 cm $\times$ 47 cm) in 20 mM-potassium phosphate, pH 6.8]. The partially purified ferritin was sedimented at $100000g$ at 4°C for 2 h (Theil, 1973) and redissolved in 20 mM-potassium phosphate, pH 6.8. The preparations appeared to be homogeneous on electrophoresis in 7% (w/v) acrylamide gels (Theil, 1973).

Results

Copper and iron content of organs

The increased concentrations of copper in the liver, kidney and spleen of animals killed after 6 and

Table 1. *Concentration of copper in sheep organs during copper excess*

Ewe lambs (16–18 kg) were fed on a defined diet and were given a daily dose of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (20 mg/kg). After 6 or 9 weeks of treatment, the animals were killed; and the organs were analysed as described in the Materials and Methods section. The results were obtained from three experiments with six control and six treated animals in each. The significance of the differences between control and copper-treated animals was evaluated by a non-parametric rank test (White, 1952); $\dagger P \leq 0.01$ compared with control. The variation among control animals is expressed as the standard deviation.

Organ ...	[Copper] ($\mu\text{g/g}$ of dry tissue)					
	Liver		Kidney		Spleen	
Time (weeks) ...	6	9	6	9	6	9
Control						
Mean	228	229	16.5	18.6	5.7	6.0
S.D.	52	70	0.30	2.04	0.70	1.60
Copper-treated						
Mean	2060 $\dagger$	3890 $\dagger$	85.8 $\dagger$	745 $\dagger$	7.1	39.2 $\dagger$
Range	(905–3410)	(1970–5170)	(33.5–170)	(108–1527)	(4.3–10.3)	(1.7–162)

Table 2. *Concentration of iron in sheep organs during copper excess*

Ewe lambs (16–18 kg) were fed on a defined diet and were given a daily dose of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (20 mg/kg). After 6 or 9 weeks of treatment, the animals were killed; samples of the organs were dried and analysed as described in the Materials and Methods section. Extracts of marrow from the sternum were analysed for non-haem iron (Kerr, 1957). The results were obtained from three experiments with six control and six treated animals in each. The significance of the differences between control and copper-treated animals was evaluated by using a non-parametric rank test (White, 1952); $\dagger P \leq 0.05$ and $\ddagger P \leq 0.01$ compared with control. The variation among control animals is expressed as the standard deviation.

		[Iron] ($\mu\text{g/g}$ of dry tissue)						
Organ	...	Liver		Kidney		Spleen		Marrow
Time (weeks)	...	6	9	6	9	6	9	(μg of non-haem iron/g of protein)
Control								
Mean		264	285	162	192	707	1240	230
s.d.		67	144	26	83	235	708	57
Copper-treated								
Mean		334	469†	409†	4962‡	2710‡	3240‡	278
Range		(225–540)	(167–595)	(145–1800)	(503–10200)	(945–5340)	(1130–6150)	s.d. = 107

9 weeks of copper treatment are shown in Table 1, and agree with the data of Todd & Thompson (1961) and Ishmael *et al.* (1972). For the first time, to our knowledge, a highly significant ($P \leq 0.01$; non-parametric rank test of White, 1952) increase in spleen copper was observed after 9 weeks of treatment. When a single group of animals (three treated and three control) was analysed after 4 weeks of treatment, increased copper concentrations were observed in the liver (1877 $\mu\text{g/g}$ of dry tissue) and kidney (122 $\mu\text{g/g}$) of copper-treated animals, but the copper concentration of the spleen (2.3 $\mu\text{g/g}$) and the iron concentrations of the liver (184 $\mu\text{g/g}$), kidney (138 $\mu\text{g/g}$) and spleen (441 $\mu\text{g/g}$) were similar to those of the control animals.

Analysis of the concentration of iron in the liver and kidneys of copper-treated animals showed little if any change in the iron concentration of the liver after 9 weeks of copper excess, and large increases in

the iron concentration of the kidney after 6 and 9 weeks (Table 2), in agreement with the data of Ishmael *et al.* (1972). In contrast with the liver, we observed that the concentration of iron in the spleen increased 3-fold within 6 weeks of treatment with copper excess (Table 2). By 9 weeks the total amount of iron in the spleen was similar to that in the liver, even though the spleen weight is only 13% that of the liver (Table 3). Increased amounts of iron in the spleen occurred in the absence of detectable haemolysis.

The concentration of non-haem iron in the marrow was not significantly changed by copper treatment. The values were 278 ± 107 (S.D.) and 230 ± 57 μg of iron/g of protein for copper-treated and control animals respectively (Table 2).

The dramatic increase in the iron content of the spleen of copper-treated animals could represent increased iron stored as ferritin. To test this

Table 3. *Total iron content and iron distribution in the liver and spleen during copper excess*

Ewe lambs (16–18 kg) were fed on a defined diet and were given a daily dose of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (20 mg/kg) for 6 or 9 weeks. After death, organ homogenates were fractionated into heat-soluble and heat-insoluble fractions by heating at 70°C for 10 min (see the Materials and Methods section). The results for animals treated for 6 and 9 weeks were similar and were obtained from two experiments, six control and six treated animals in each; a single experiment showed no difference in organ iron content between control and copper-poisoned animals after 4 weeks of treatment. The significance of the differences between control and copper-treated animals was evaluated by using a non-parametric rank test (White, 1952): * $P \leq 0.01$ compared with control. The range of experimental values is presented in parentheses.

	[Iron] (mg/organ)			
	Liver		Spleen	
	Control	Copper-treated	Control	Copper-treated
Heat-soluble (ferritin)	16.2 (7.8–28.9)	19.0 (3.8–38.9)	3.2 (0.5–9.8)	7.2* (1.7–20.3)
Heat-insoluble	12.6 (8.1–22.5)	13.1 (3.7–21.0)	5.2 (1.7–13.2)	16.7* (4.4–54.0)
Total	28.9 (16.8–46.7)	32.1 (7.5–59.2)	8.3 (2.2–23.0)	23.9* (6.7–74.5)

hypothesis, homogenates of spleen and liver were separated into ferritin (heat-soluble at 70°C, 10 min) and non-ferritin (haemosiderin and haemoglobin) fractions. Copper treatment increased the iron content of both the ferritin and non-ferritin fractions in the spleen about 3-fold (Table 3). Confirmation of the increase in spleen ferritin in copper-treated animals was provided by the observation that the average amount of ferritin that could be purified from 5 g of spleen of copper-treated sheep was 3.2 mg, compared with 0.8 mg from 5 g of spleen of control animals. Thus the increase in the iron content of the heat-soluble (ferritin) fraction of the spleen was approximately equivalent to the increase in the amount of ferritin that was isolated from the spleen of copper-treated animals. Moreover it was found that the iron/protein ratio of purified spleen ferritin was unaffected by copper treatment. Copper treatment did not change the distribution of iron in the liver (Table 3).

Copper excess inhibited the growth of sheep. The average weekly weight gain over the 9-week period of the experiments (for four experiments involving 30 animals each) was 1.03 kg/week (range 0.30–1.91) for control animals and 0.16 kg/week (range –0.38 to +0.49) for copper-treated animals. The difference between the control and copper-treated animals is highly significant ($P \leq 0.001$), by using a non-parametric rank test (White, 1952) to evaluate the data. Copper did not change the ratio of organ weight to body weight or the percentage dry weight of the organs.

Plasma iron and copper content

Massive haemolysis in the second phase of copper toxicity is preceded by a sudden increase in the

copper concentration in the plasma and erythrocytes (Todd *et al.*, 1963; Ishmael *et al.*, 1972). In our experiments, increased plasma and erythrocyte copper concentrations were observed after 6 weeks of exposure to copper excess and were also associated with haemolysis; the mean of all values was higher than the control (Table 4).

In contrast, the iron content of the plasma of animals treated with excess copper increased within 1 week of treatment (Fig. 1), and increases in plasma iron were independent of detectable haemolysis. Transferrin concentrations measured as the total iron-binding capacity of the plasma of copper-treated sheep were normal for 7 weeks and then increased (Fig. 2). The saturation of transferrin was increased to as much as 65.8%, but during weeks 8 and 9 the transferrin saturation decreased as the total iron-binding capacity increased (Table 4).

Haematological analyses

Massive haemolysis is observed after prolonged exposure to copper (Evans, 1973; Scheinberg & Sternlieb, 1965). In the present experiments haemolysis was analysed by measuring the amount of haemoglobin in the plasma. Slight haemolysis was observed as early as 5 weeks after the initiation of copper treatment, but values significantly ($2 \times \text{s.d.}$) greater than the control did not occur until weeks 7, 8 or 9. Anaemia (lowered haematocrit, lowered haemoglobin content) was not observed until plasma haemoglobin content exceeded 300 mg/ml. No effect of copper treatment was observed on erythrocyte pyruvate kinase activity, erythrocyte volume and erythrocyte haemoglobin concentration (Table 4). Pyruvate kinase was assayed because of its sensitivity to inhibition by copper (Boulard *et al.*, 1972).

Table 4. *Haematological properties of sheep during copper excess*

Ewe lambs, fed on a defined diet, were given a daily dose of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (20 mg/kg) for 9 weeks. Blood samples were collected and analysed weekly (see the Materials and Methods section). The mean of all values is presented; three experiments were performed, with six control and six treated animals in each.

	Copper-treated	Control
Plasma iron ($\mu\text{g}/100\text{ml}$ of plasma)	236*	193 (S.D. = 49.8)
Range	(55–1307)	
Transferrin saturation	43.5%*	38.4% (S.D. = 7.3%)
Range	(12.3–65.8%)	
Plasma haemoglobin (mg/100 ml)	27.4†	9.2 (S.D. = 4.7)
Range	(2–742)	
Plasma copper ($\mu\text{g}/100\text{ml}$ of plasma)	123†	88.6 (S.D. = 24.6)
Range	(59–928)	
Erythrocyte copper ($\mu\text{g}/100\text{ml}$ of erythrocytes)	122†	64.0 (S.D. = 19.1)
Range	(47–1045)	
Haematocrit (ml of cells/100 ml of blood)	52.4† (S.D. = 8.9)	52.3 (S.D. = 5.3)
Haemoglobin content (g/100 ml of blood)	15.0† (S.D. = 3.2)	15.4 (S.D. = 2.1)
$10^{-10} \times$ Erythrocyte number (cells/ml of blood)	1.20 (S.D. = 0.03)	1.32 (S.D. = 0.36)
Erythrocyte volume (μm^3)	45.2 (S.D. = 6.7)	43.9 (S.D. = 4.8)
Erythrocyte haemoglobin content (pg/cell)	14.7 (S.D. = 3.6)	14.3 (S.D. = 2.8)
Pyruvate kinase (units/g of haemoglobin)	1.77 (S.D. = 0.49)	1.71 (S.D. = 0.61)

* Significantly different from the controls by the analysis of variance (Bahn, 1972). Plasma iron, $\text{Prob} > F = 0.0001$; transferrin saturation, $\text{Prob} > F = 0.001$. The independent variables considered in the analysis of variance were copper treatment, time, experiment and animal. Only copper treatment affected serum iron concentrations. Both copper treatment and time affected transferrin saturation, the saturation increasing significantly for weeks 1–7 and declining to control values for weeks 8 and 9 when the total iron-binding capacity increased (Fig. 2).

† Values significantly different from the control ($2 \times \text{s.d.}$) were usually observed after 7, 8 and 9 weeks of copper treatment.

Fig. 1. *Effect of copper excess on plasma iron concentrations*

The non-haemoglobin plasma iron concentration was measured as the ferrozine complex (see the Materials and Methods section) by using samples of plasma from blood obtained weekly from the jugular vein of sheep. The data were subjected to an analysis of variance, and the means for each week for control (○) and copper-treated (Δ) sheep were plotted by computer.

Fig. 2. *Effect of copper excess on transferrin content (total iron-binding capacity) of plasma*

The total iron-binding capacity was determined (see the Materials and Methods section) by using samples of plasma from blood obtained weekly from the jugular vein of sheep. The data were subjected to an analysis of variance, and the means for each week for control (○) and copper-treated (Δ) sheep were plotted by computer.

Discussion

The results reported here show that, in sheep, copper excess appeared to affect both iron transport, resulting in increased plasma iron concentration and transferrin saturation (Fig. 1 and Table 4), and iron

storage, causing increases in the amount of iron and ferritin in the spleen (Tables 2 and 3). The spleens of copper-treated animals contained three times as much iron, distributed between the heat-soluble (ferritin) and -insoluble (haemosiderin and haemoglobin) fractions, as the spleens of control animals. Moreover, the amount of ferritin that could be

isolated from spleens of copper-treated animals was also increased 3–4-fold. Liver and bone marrow are the two other major iron-storage sites. In contrast with the spleen, the iron concentration of the liver was only slightly increased by copper treatment (Table 2) and no change in the non-haem iron of the marrow (Table 2) was observed.

Increases in plasma iron and transferrin saturation have been observed after increased iron absorption, liver damage and erythrocyte lysis (Finch, 1972). Our data suggest that the increased plasma iron concentrations were caused by erythrocyte destruction rather than by increased absorption of iron or liver damage. First, increased iron absorption leads primarily to deposition of iron in the liver and sometimes in the bone marrow and/or spleen (Brink *et al.*, 1976). Since copper treatment of sheep produced a markedly increased iron concentration in the spleen, with little or no change in the iron concentration of liver and bone marrow, it appears unlikely that copper excess affected iron storage by increasing the absorption of iron; further, iron absorption in pigs is inhibited by copper excess (Gipp *et al.*, 1974). Secondly, liver damage probably could not account for the observed higher concentration of plasma iron, because liver damage, measured as an increase in plasma copper concentration and total iron-binding capacity, occurred much later than the increase in plasma iron concentration. The increase in plasma iron concentration and transferrin saturation occurred after 1 week of copper treatment (Fig. 1 and Table 4), but the increase in plasma copper concentration occurred after 6 weeks and the increase in total iron-binding capacity occurred after 7 weeks of copper treatment (Table 4 and Fig. 2). There was no correlation between plasma iron and copper concentration. Finally, increased erythrocyte destruction can cause small increases in plasma iron concentration, because of the increased rate of haemoglobin catabolism and new erythrocyte formation. Increased erythrocyte destruction can occur in the absence of detectable haemolysis. Therefore the results presented here suggest that copper excess caused a chronic, compensated haemolysis soon after copper treatment began. Later, after liver damage, the commonly observed massive acute haemolysis occurred.

Iron concentrations in the spleen can be increased by impaired re-utilization of iron. The data suggest that copper interfered with the re-utilization of iron from spleen ferritin for the following reasons. Normally old erythrocytes are phagocytosed by the reticuloendothelial cells, mainly in the spleen and to a lesser extent in the liver. The iron from catabolized erythrocytes is released to the plasma in two stages, a rapid phase in which the intracellular form of iron is unknown and a slow phase in which the iron passes through ferritin (Fillet *et al.*, 1974). The release of iron from the ferritin micelle, a micro-

crystalline hydroxy-ferric oxide, can be effected *in vitro* by reducing agents such as thioglycolic acid and sodium dithionite or by chelators such as nitrilotriacetate and dioxogulonic acid (Harrison, 1974). The mechanism of mobilization of iron from the ferritin micelle *in vivo* is unknown, but biological reductants such as FMN H_2 and NADH can reduce the iron micelle *in vitro*. A ferrireductase in the liver has also been observed. In addition to a reducing enzyme and/or a reductant or chelator of low molecular weight, the structure of the iron micelle itself and possibly the ferritin apoprotein are involved in the mobilization process (Harrison, 1974; Sirivech *et al.*, 1974; Dognin & Crichton, 1975). When a block occurs in the rapid phase of re-utilization, as in acute infections, there is a decrease in three properties of the plasma: iron concentration, total iron-binding capacity and transferrin saturation (Lynch *et al.*, 1974). Since copper excess in sheep caused an increase in both the concentration of iron in the plasma and transferrin saturation (Figs. 1 and 2, and Table 4), it appears that the rapid phase of iron re-utilization, accelerated by haemolysis, proceeded without interference. However, the accumulation of iron and ferritin in the spleen (Tables 2 and 3) suggests that copper interfered with the slow phase of iron re-utilization. Copper could interfere with the reduction and/or the chelation of iron or change the iron micelle and/or the apoprotein.

Among the iron-storage sites, the spleen was preferentially affected by copper excess. This observation can be attributed to the major role that the spleen plays in erythrocyte catabolism and iron re-utilization. Moreover, large numbers of phagocytosed erythrocytes have been observed in the spleen of copper-treated sheep, but very few were observed in the liver (Søli & Nafstad, 1976). Phagocytosis of the erythrocytes with a high copper content that appeared after 7 or 8 weeks of copper treatment could account for the increased concentration of copper in the spleen after 9 weeks (Table 1).

In summary, copper excess was observed to influence iron metabolism in sheep, rapidly producing an increase in plasma iron concentration, probably caused by chronic compensated haemolysis. In addition, copper excess caused a dramatic increase in the amount of iron and ferritin in the spleen, possibly by interfering with the re-utilization of iron from ferritin. Analysis of the influence of excess copper on iron metabolism not only provides information about copper toxicity but also provides a means for examining iron re-utilization in the spleen.

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