STUDIES OF COPPER METABOLISM IN SHEEP

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by

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Contents

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The/

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Preface.

The experimental work in this thesis is largely concerned with the quantitative aspects of copper uptake by plants and with the storage of copper in sheep. Sheep were particularly suitable for the experimental work in that they are the smallest commercial ruminant animals and as such are less costly both to obtain and maintain, thus enabling larger numbers to be employed in each experiment and so enhancing the statistical value of the results. Particular attention has been paid to copper absorption and storage by housed sheep as it was considered that future developments in sheep husbandry would lead to greater intensification of production techniques with a consequent increase in the numbers of housed sheep.

The thesis has been divided into five sections. The first comprises an extensive review of the literature pertaining to copper uptake by plants and animals, particularly sheep. The remaining four sections comprise the experimental work undertaken and are concerned respectively with factors affecting copper uptake by herbage; copper absorption by sheep at pasture; copper absorption by housed sheep and finally copper excretion. The effects of an excessive intake of copper by sheep have also been studied. The discussions of and the conclusions drawn from the experimental work described in each section are included at the end of the relevant section.

SECTION I

i) A review of the literature concerning copper metabolism in plants and animals with special reference to previous work pertaining to factors affecting copper absorption.

(a) Copper concentration in herbage.

(b) Copper metabolism in animals.

(c) Copper absorption and utilization.

(d) Copper excretion.

(e) Control of copper deficiency in animals.

(f) Copper poisoning in sheep.

(g) Copper status of housed sheep.

ii) Chemical Methods.

Copper Concentration in Herbage.

A number of factors, other than the basic one of soil copper status, influence the concentration of copper in herbage. The principal features involved are plant species, stage of maturity and the effects of lime and fertilizers. The effect of these factors on the copper content of herbage will be discussed individually.

a) Effect of Plant Species.

Thomas and Thompson (1948) sampled a variety of herbs and grasses from mixed swards grown on a number of long established plots at Cockle Park which had received different manurial treatments. The copper concentrations (in p.p.m. in D.M.) for samples obtained in early June and averaged over the different treatments were:-

Herbs & Weeds		Grasses	
Ribgrass	15.9	Crested Dogs Tail	5.6
Buttercup	16.6	Yorkshire Fog	8.2
Sheep Sorrel	10.8	Red Fescue	4.0
Yellow Rattle	13.0		

The herbs and weeds were much richer in copper than these poorer agricultural grasses.

Thomas, Thompson, Oyenuga and Armstrong (1952) grew a variety of herbage plants under identical conditions and sampled them individually at five growth stages ranging from the young leafy stage to full maturity. Their findings ar summarised in Table 1.

Table 1. Concentration of copper (p.p.m.Cu) in grasses, legumes and herbs (Thomas et al., 1952).

Grasses	Mean of 5 Growth Stages	Early Growth	Late Growth
Perennial Ryegrass	8.5	15.2	9.1
Cocksfoot	10.0	7.0	9.1
Timothy	7.5	8.2	7.6
Crested Dogs Tail	8.0	9.6	6.8
Meadow Fescue	9.5	5.6	6.6
Tall Fescue	4.1	5.4	5.5
Red Fescue	10.3	11.1	8.7
Chewings Fescue	6.3	3.9	7.2
Mean	8.2	8.3	7.6
Legumes			
Trefoil	7.3	9.6	5.3
Alsike	10.5	12.4	12.0
Lucerne	9.1	12.1	6.8
Sainfoin	8.1	10.4	5.0
Mean	8.7	11.1	7.3
Herbs			
Yarrow	10.6	13.4	9.2
Burnet	8.0	10.3	7.1
Plantain	10.5	15.6	6.9
Chicory	12.5	15.4	9.9
Mean	10.2	13.7	8.3

Herbs are found to contain more copper than either grasses or legumes. An interesting feature of the grasses was the wide variation in the copper concentration of the four types of fescue.

Kirchgessner (1965) has also confirmed that grasses generally contain less copper than cloverswhich in turn are appreciably lower in copper than herbs. The mean values recorded for 50 different species were:- Grasses,7.3; clovers,8.5 and herbs, 11.7 p.p.m.Cu.

In order to gain further information on the value of herbs for raising the copper concentration of pasture Thomas, Rogerson and Armstrong (1956) laid down a number of plots containing a combination of chicory, plantain, yarrow, sainfo and burnet at four different seed rates along with Cockle Park type seed mixtur The herbs formed 0, 7, 14 and 21% of the total sward. The mean copper concentrations of five separate cuts during the growing season were 8.1, 8.7, 10.7 and 10.6 p.p.m. respectively. Some practical increase in the copper content of the sward as a whole was, therefore, obtained but this was only achieved at the expense of pasture productivity.

Adams and Elphick (1956) in New Zealand recorded higher Cu concentrations, (18 - 20 p.p.m.) in such weeds as plantain, dandelion and catseer than in clover (7 - 14 p.p.m.) depending on seasonal changes.

Hemingway (1962) in a long term experiment where four samples of cut herbs were obtained in each of three growing seasons, found that clover (about 12 p.p contained more copper than grass (about 8 p.p.m.). Fleming (1965) reported tha red and white clover (8 - 10 p.p.m.) generally contained more copper than grass such/

such as cocksfoot, timothy, rough stalked meadow grass and perennial ryegrass (4 - 8 p.p.m.). This, however, depended on the stage of growth of the grasses and the copper content of the soil.

Mitchell, Reith and Johnston (1957a) obtained many samples of ryegrass and clover from a wide variety of soil types during the month of June of three successive years - 1953, 54 and 55. The copper concentration of the ryegrass ranged from 2.0 - 4.3 p.p.m. (mean, 3.2 p.p.m.) while the Cu concentrations of the clover samples ranged from 1.9 - 12.3 p.p.m. (mean, 7.7 p.p.m.). The mean copper concentration of the clover was consistently higher than that of the ryegrass in each of the three years. In another experiment, however, they found that where soil copper levels were low, grasses contained more copper than clover; the reverse was the case when soil copper was abundant.

b) Effect of Stage of Growth

Thomas, Escritt and Trinder (1945) found little seasonal change in the copper concentration of heather. Samples obtained between June and October ranged from 11.9 - 14.4 p.p.m. There was also no correlation between increasing age (2 - 10 years) and copper concentration. Thomas and Trinder (1947) recorded that the copper concentration in blaeberry remained within the range of 12.9 to 14.4 p.p.m. from June to October. It is noteworthy that where heather is prevalent copper and other minor element deficiencies in livestock are rare. This is a reflection of their higher copper concentration relative to grasses and clovers but it could also be related to soil type (Wilson 1962).

In/

In this same experiment, Thomas et al. investigated the copper concentratio of a range of other moorland plants. They obtained samples at six growth stages from each species ranging from late February/early March to mid-September. They also examined the calcium and phosphorus concentrations of these species. A summary of their findings is presented in Table 2.

Table 2 The copper, phosphorus and calcium content of moorland plants (Thomas & Trinder, 1947).

	Flying Bent	Deer Hair	White Bent	Stool Bent	Draw Moss	Mean
Copper (p.p.m.)						
Late Feb./early March	6.6	5.1	3.5	5.2	5.9	5.3
Late Apr./early May	19.6	9.9	7.7	8.6	9.6	11.1
Mid. Sept.	5.2	4.2	3.8	7.2	8.2	5.7
Phosphorus (%)						
Late Feb./early March	0.07	0.17	0.11	0.20	0.14	0.14
Late Apr./early May	0.41	0.26	0.25	0.19	0.37	0.30
Mid. Sept.	0.12	0.10	0.10	0.13	0.15	0.12
Calcium (%)						
Late Feb./early March	0.10	0.14	0.06	0.07	0.14	0.10
Late Apr./early May	0.12	0.14	0.09	0.07	0.14	0.11
Mid. Sept.	0.20	0.23	0.07	0.07	0.13	0.14

Before spring growth commenced the range of copper concentrations was from 3.5 - 6.6 p.p.m. These rose markedly with the onset of growth in late April/ early May to a mean level of 11.1 p.p.m. and thereafter declined progressively to 3.8 - 8.2 p.p.m. (mean, 5.7 p.p.m.) by mid September. There was no increase in calcium concentration during the spring growth period but phosphorus concentrations/

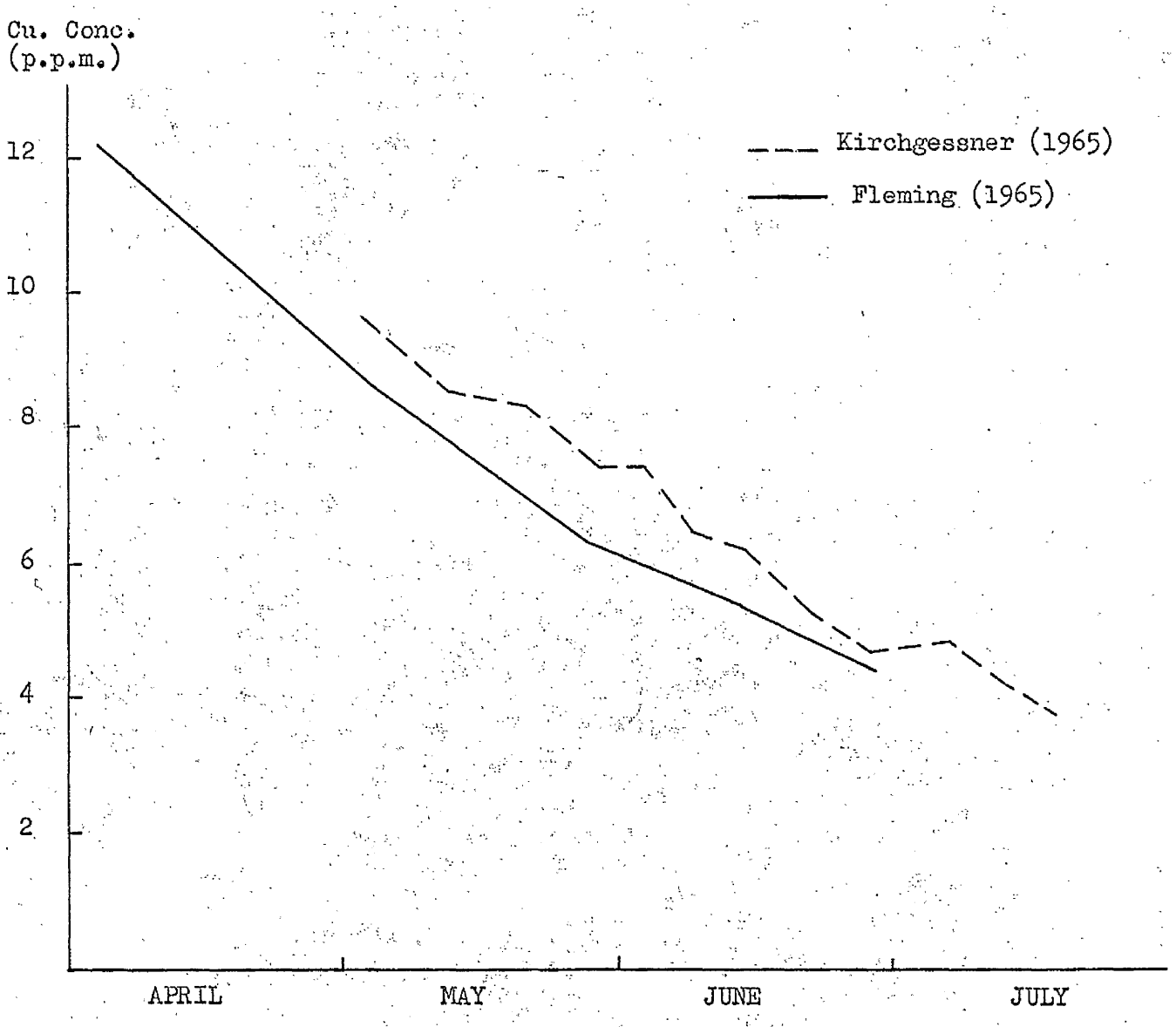
concentrations rose at that time from 0.14 to 0.30% and subsequently fell to 0.12% as the plants matured. This decline in phosphorus concentration with maturity parallels that found for copper concentration. The same trend in phosphorus and copper concentrations was confirmed by Thomas et al (1952).

This general increase in the copper concentration of plants during the spring growth period (Thomas and Trinder, 1947 and Thomas et al, 1952) indicates that the copper concentration of samples of herbage obtained during lambing time may not be a good guide to the copper intake of the ewe during pregnancy. Such herbage samples may, therefore, give misleading information regarding the association between herbage copper concentration and the incidence of swayback.

Figure 1 presents the results of work carried out by Fleming (1965) and Kirchgessner (1965) concerning the changes in the concentration of copper in herbage growing to maturity. Fleming grew cocksfoot, perennial ryegrass, rough stalked meadow grass and timothy in small field plots and obtained five samples from each at different stages of growth. Kirchgessner presented data concerning the mean seasonal changes in copper concentration for three unspecified grasses during the growing season. Their results clearly demonstrate that copper concentration declines with maturity.

Fleming (1965) also recorded that the leaves of grasses at the silage/hay stage contain about twice as much copper as the stems. The increase in the proportion of stem material with advancing maturity may, therefore, explain these overall seasonal changes.

Fig.1 Changes in the copper concentration of grasses growing to maturity.



Hemingway (1962) cut herbage at the silage stage four times in each of three years. He found a progressive increase in the mean copper content at each cut in each of the three years and also that application of nitrogen always increased the copper concentrations. His findings are summarised in Table 3.

Table 3. Copper concentrations (p.p.m.) in herbage cut repeatedly at the silage stage. (Hemingway 1962).

Cut	No N. applied				N. applied			
	A	B	C	D	A	B	C	D
1957	3.6	5.4	9.4	8.9	4.6	7.3	11.5	9.6
1958	5.3	6.4	6.6	8.3	7.6	7.6	9.3	9.6
1959	4.1	5.0	6.8	6.8	6.5	7.1	8.8	11.1
Mean	4.3	5.6	7.6	8.o	6.2	7.3	9.9	10.1

The results published in respect of the effect of stage of growth of clovers on their copper content are rather more contradictory. Piper and Beckwith (1949) in Australia recorded a small but progressive increase in the copper concentration of subterranean clover during the growing season. At the 3 - 4 leaf stage the copper concentration was 9.5 p.p.m. and this increased to 12.0 p.p.m. at flowering. In contrast, Fleming (1965) reported a steady fall in the copper concentration of red clover from 10.8 to 8.4 p.p.m. between early May and late June. Over this period the copper concentration of white clover remained constant at 7.8 - 8.0 p.p.m. Hemingway (1962) found that the mean copper concentrations of four cuts of wild white clover, separated from mixed herbage, increased throughout the season (10.9, 11.0, 13.5 and 13.8 p.p.m.).

c)/

c) Effects of Organic Manures and Fertilizers.

i) Organic Manures. Hemingway (1961) analysed 50 samples of farmyard manure (F.Y.M.) and found the mean concentration of copper in the dry matter to be 19.8 p.p.m. Thus, a dressing of 10 tons F.Y.M. per acre would supply about 40 gms. of copper. This amount of additional copper in unlikely to have any direct effect on the copper concentration of the herbage. Thomas, Holmes, and Clapperton (1955) suggest that applications of organic matter probably increase the copper content of plants by improving the soil texture and water relationships.

Thomas et al.(1955) found that when three grass species (crested dogstail, Yorkshire fog and red fescue) were treated with 8 tons of F.Y.M. that their copper concentration was increased by over 2.0 p.p.m. compared with similar untreated species. Williams, Stojkovska, Cooke and Widdowson (1960) reported that 15 tons of F.Y.M. per acre supplied as much copper as was taken from the soil by cropping with wheat, barley, potatoes, clover and hale. However, they stated that the application of this amount of F.Y.M. depressed the copper concentration in these crops as frequently as it increased copper levels.

ii) Fertilizers. The copper concentrations of some of the common fertilizers were determined by Stojkovska and Cooke (1958). The approximate amounts of copper supplied by three typical dressings were:-

Nitro Chalk	(500 lbs./acre)	4.5 gms.Cu.
Superphosphate	(400 lbs./acre)	9.0 gms.Cu.
Potassium Sulphate	(200 lbs./acre)	0.9 gms.Cu.

Obviously/

Obviously these amounts of copper supplied in the common fertilizers will h little effect in restoring soil reserves of copper.

Fleming (1965) found that the concentration of copper was always higher in the leaf than in the stem of grasses so it would be reasonable to assume that nitrogen applications - which increase leaf growth - would increase the amount of copper in the plant. This effect was indeed found by Hemingway (1962). Pasture treated with ammonium sulphate had a mean copper concentration of 8.4 p.p.m. compared with a mean of 6.4 p.p.m. for untreated pasture. (See Table 3). Stewart and Holmes (1953) reported a 50 per cent increase in copper concentratio after nitrogen applications to herbage.

In contrast to these findings Reith and Mitchell (1964) found only occasional increases in the copper concentration of a mixed herbage of grasses and clovers after nitrogen treatment. They reported no consistent effect of nitrogen application on copper content.

Thomas and Thompson (1948) found no effect on the copper concentration of three different grass species due to an application of 150 lbs. ammonium sulphat per acre. Knabe, Knabe and Kreil (1964) reported a decrease in the copper content of pasture after applications of between 480 - 720 kg/hectare of nitrogen to a sandy soil. This fall in herbage copper content did not occur on fen soils. They suggested that the cause of the decrease was a dilution effect.

Herbage/

Herbage copper concentrations appear to be unaffected by applications of superphosphate and potassium fertilizers (Hemingway 1962 and Reith et al., 1964).

d) Effect of Lime.

Thomas et al.(1945) recorded a slightly lower copper concentration in heather (11.1 p.p.m.) which had been treated with 5 tons of ground limestone per acre compared with untreated heather (12.5 p.p.m.).

Stewart (1951) reported that swayback occurred more frequently on pastures which had been limed. He was, however, unable to show that an alteration in the soil pH significantly influenced the uptake of copper by the herbage to the extent that a pasture which was deemed adequate for copper had become copper deficient.

Mitchell et al.(1957a) found that liming slightly depressed (by about 0.5 p.p.m.) the copper concentration of herbage which had not been top dressed with $CuSO_4$ $5H_2O$. The copper concentration of pasture which had been top dressed with copper was unaffected by liming. Mitchell et al.(1957b) found that liming had no effect on the copper concentration of ryegrass but that it effected some slight increase (about 1.5 p.p.m.) in the copper concentration of red clover.

Reith et al (1954) recorded that the copper concentration of commercially used liming materials is very low (1 - 10 p.p.m.). They suggested that this amount would be insufficient to remedy a deficiency but that it might help to maintain/

maintain adequate levels. In the same publication they reported the effect of annual treatment of 3 tons of lime/acre on the copper content of normal herbage and herbage which had been treated with 20 lb. $CuSO_4$ $5H_2O$ in the spring of each of the years 1954, 55, 56, 57 and 58. Herbage samples were taken in June of each of the years 1958, 59 and 60. On the pasture not treated with copper sulphate liming had a depressive effect reducing the mean herbage copper concentration from 5.0 p.p.m. (unlimed plots) to 3.8 p.p.m. (limed plots). Where the herbage had received supplementary copper there was no such depressive effec due to liming. They suggest that liming can sometimes, but not always, reduce the uptake of naturally occurring copper, especially on copper deficient soils.

Barlow, Purves, Butler and MacIntyre (1960) recorded soil pH levels and herbage copper concentrations during a study of swayback in south-east Scotland. An examination of these results showed that there was no correlation between soil pH and herbage copper content. No significant differences were found betwe the soil pH values from swayback farms (mean 5.79; range 5.0 - 6.8) and control farms (mean 5.96; range 4.9 - 6.7). This was also true for the copper concentration of the herbage which had a mean level of 5.95 p.p.m. (1.8 - 14.8 p.p.m.) on affected farms compared with a mean of 5.65 p.p.m. (3.5tp 7.5 p.p.m.) on the control farms. Andrew and Bryan (1958) found no calcium-copper interactions when growing white clover either in field experiments or in pot culture. An increase in clover yield was found by adding copper and this was unaffected by additions of $CaCO_3$.

e)/

e) Effect of Top Dressing with Copper

Mitchell et al (1957a) laid down a series of plots in 1951 which they treat with 0, 20 or 60 lbs. $CuSO_4$ $5H_2O$ per acre. Two crops of oats were taken and the mixed pasture herbage was sampled in 1953, 54 and 55. The main feature to emerg was the persistent effect of this one application of $CuSO_4$ $5H_2O$ given 3 - 5 years previously. Both the treated herbages had copper concentrations which were 2.0 p.p.m. higher than that of the control herbage for each of the three years. There was no appreciable difference between the copper concentration of the herbages treated with 20 and 60 lbs. $CuSO_4 5H_2O$ per acre.

In another experiment Mitchell et al (1957a) obtained herbage samples in June 1955 from a series of plots which had been treated with 20 lbs. $CuSO_4 5H_2O$ per acre in April 1954 and again in April 1955. Copper analyses of th constituent species of these plots showed a marked response by red clover to applications of copper but only a very small response by ryegrass, cocksfoot, crested dogstail and timothy. The mean increase in the copper concentration of red clover was in the order of 500% while that for the various grass species was only 30%.

These results were confirmed by Reith and Mitchell (1964) when they reported only a small response to applications of 20 lbs. $CuSO_4 5H_2O$ per acre by mixed herbage. The greatest response was again found for clover which increased from 1.4 - 5.7 p.p.m. The copper contents of three grasses - ryegrass, cocksfoot and crested/

crested dogstail - also increased but not nearly so noticeably. Before treatment their copper concentrations had varied from 1.8 - 2.5 p.p.m. while after treatment the range in copper concentration was from 2.6 - 3.1 p.p.m. The effec of the treatment was found to persist for three years.

Morgan and Clegg (1958) compared the effect of spraying a solution of 5 lbs. $CuSO_4 5H_2O$ in 100 gallons water per acre to a peaty soil with spreading a copperised fertilizer which supplied the same amount of copper. Spraying increased the copper content of the herbage to 19.8 p.p.m. but for the first cut only; by the second cut, a month later, it had returned to the same level as the control pasture (8.4 p.p.m.). The copper concentration of pasture treated with the copperised fertilizer was not as high as that of the sprayed pasture at the first cut (14.2 p.p.m.) but it was higher at the second cut (10.7 p.p.m.). However, by the next cut it had also returned to the level of the control pasture (8.5 p.p.m.). A further trial was carried out in the following year when two levels of $CuSO_4 5H_2O$ (5 and 10 lbs./acre) were applied as sprays. The results were similar to those found in the previous year with an increase in copper concentration at the first cut but then returning to the level of the control pasture during the next four weeks.

Morgan and Clegg (1961) in further trials on the effectiveness of supplementary copper applied as sprays for increasing herbage copper concentrations used four different treatments. These were a) control, b) 6 lbs. $CuSO_4$/acre, c) 12 lbs. $CuSO_4$/acre and d) 10 lbs. copper versenate/acre which were all appli in 100 gallons water. The copper concentration of the control herbage was 8.4 p.p.m. whereas treatments b), c) and d) produced levels of 60.0, 80.0 and 20.0 p.p.m. respectively at the first cut. When a second cut was taken a month late the copper concentration of the treated herbage was the same as that of the control pasture. The possibility of surface contamination of the herbage being

responsible for the high copper concentrations was discounted as there was a period of very wet weather between application of the dressing and sampling of t herbage.

Lee (1950) described the results from an experiment where 7 lbs. $CuSO_4 5H_2O$ was spread over an area of soil which had recently been sown with lucerne. The lucerne quickly dominated the copper treated pasture but on untreated pasture grasses remained as the dominant species.

Lee also reported that elsewhere in South Australia copper sulphate has bee applied to calcareous sandy soils at rates varying from 7 - 112 lbs. per acre. The mean legume copper concentration recorded was in the region of 8 - 9 p.p.m. and the highest level obtained was 13.0 p.p.m. The mean copper concentration of grasses in the same areas was about 4.0 p.p.m. Lee stated that he found no exceptionally high herbage copper concentrations after treatment with 7 lbs. $CuSO_4$/ acre. Indeed the highest value he recorded was 10.0 p.p.m.

From the preceding review it can be seen that of all the factors discussed which affect the copper concentrations of plants only the effect of species has led to reasonably consistent results. These have shown that herbs and weeds generally have the highest copper concentration. These are followed by the legumes, except where the soil copper concentration is low, and finally the grasses which have the lowest copper content.

The/

The reports published with regard to the effect of nitrogen, lime and copper sulphate applications to the soil on herbage copper concentrations are, however, much more inconsistent. Applications of nitrogen fertilizers have been reported to have decreased, to have had no consistent effect on, and, more frequently, to have increased herbage copper concentrations. The same range of effect has been attributed to applications of lime while top dressing with copper sulphate has produced reports ranging from little or noincrease or only a transitory increase to a persistent effect lasting over five years.

In view of this disparity among the published work it was thought desirable to obtain further information regarding the effect of soil applications of lime, nitrogen and copper sulphate on herbage copper concentration. This work is described in experiments 1 - 3.

COPPER METABOLISM

More than a century ago copper was shown to occur in living tissues. Harless (1847) found that copper was present, in combination with protein, in the blood of marine bluebloods. Bert (1867) suggested that the blue colour of the blood was related to oxygen transport and in 1878 Fredericq (1878) isolate the pigment from octopus blood and named it haemocyanin. This contains about 0.2% of copper.

Another pigment containing copper, turacin, was isolated in 1869 from the feathers of a species of birds called Turacus found in South Africa (Church 1869). This was shown to contain about 7.0% copper and it was later identified as a copper-porphyrin complex which had a configuration similar to that of haematin, the porphyrin moiety of haemoglobin.

Copper is known to be involved in cell respiration but its precise function has not so far been elucidated. A number of respiratory enzymes are copper-protein complexes; these include ascorbic acid oxidase, polyphenoloxidases, and laccase. Copper is also present in two inactive complexes, hepatocuprein and haemocuprein, which have been isolated from the liver and erythrocytes respectively of mammals. These all contain copper in concentrations similar to that found in haemocyanin. Ceruloplasmin is another Cu - protein complex which is found in the blood. It exhibits weak peroxidase activity. Ninety per cent of plasma copper is held in this form. Ceruloplasmin acts by releasing and binding copper at the appropriate sites in the body thus aiding the regulati of copper absorption and transport.

Perhaps/

Perhaps the most important function of copper so far deduced is the part it plays in iron metabolism. Milk anaemia in rats was found to respond to iron therapy only if a small amount of copper was supplied as well. 5 ug Cu/rat/day was found to be sufficient to initiate and maintain a steady formation of haemoglobin and erythrocytes. Copper is not a constituent of haemoglobin, nor does it appear to be a necessary component of the circulating erythrocyte, but it does facilitate the utilization of iron by the blood-forming organs and also the mobilisation of iron from the tissues. Copper deficiency, however, does not affect the absorption and storage of iron; in fact haemosiderosis is frequently a consequence of it. The mode of action of copper in haemoglobin formation is unknown but it is presumed to be a catalytic one. Schultze (1941) found that the marrow in the bones of copper deficient rats was markedly decreased in cytochrome oxidase and that there was an increase in the concentration of the enzyme upon the administration of copper.

Copper is also concerned in the formation of melanins which are the dark pigments in skin and hair. Their formation from tyrosine is due to the act of the copper proteins - polyphenol oxidases. Copper acts as a catalyst in the oxidation reactions which occur. A copper deficient animal can become seriously depigmented and achromatrichia has been observed in a number of animal including rats, sheep and cattle. Mills (1955) made use of this fact in an experiment with copper deficient rats. He drew up a pigmentation scale relating normal pigmentation to an adequate copper intake and progressively more/

more serious depigmentation to an increasing deficiency of copper. Pantothenic acid also appears to be involved with copper in melanin formation. The skin of rats which become depigmented through a deficiency of pantothenic acid have five times their normal concentration of copper (Hundley and Ing, 1951). Singer and Davis (1950) reported that the depigmentation which occurs in rats being fed on a dried milk diet can be corrected by supplying either pantothenic acid or copper.

The physical nature of hair is also affected by a copper deficiency. In sheep the wool loses its crimp and becomes straight and stringy; the tensile strength is affected and the elastic properties are abnormal. The change is due to a breakdown in the production of disulphide (-S-S-) bonds, which provide the cross linkages in keratin, from the free thiol (-SH) groups of pre-keratin. Under normal conditions the oxidation process proceeds rapidly enough for the keratin fibrillae to be fixed in an orderly pattern as they are forced out through the neck of the follicle. This formation of the disulphide bridges normally takes 8 - 12 hours but in copper deficient sheep it takes at least 3 days and in the meanwhile the fibres remain plastic so that the fibrillae can become disorientated before they are finally fixed by oxidation. Increasing the copper supply to the animal by means of copper sulphate immediately allows for the production of normal crimped wool by the follicle.

Thus, copper is involved both in the pigmentation and keratinisation of hair/

hair but it is not yet known whether both processes are dependent on the same enzyme system. It is suggested that they are not. The evidence for this is that polyphenoloxidase is present only in pigmented hair and feathers where it is involved in the pigmentation process but not in white ones. It has been recorded that the feathers of White Leghorn cockerels benefitted greatly from the addition of copper to their rations thus indicating that keratinisation was aided purely by supplementing with copper in the absence of polyphenoloxidas enzymes (White, Hegsted and Mayer,1951).

Copper was first proved to be an essential nutrient for the rat during a study of milk anaemia by Hart, Steenbock, Waddell and Elvehjem (1928). Since then it has been found to be necessary for all animals and also for all higher plants. Discussion of its effects on animals will, however, be restricted here to sheep and to a lesser degree, cattle.

Sjollema (1933) found that dosing with copper cured the wasting disease - Lechsucht - prevalent among cattle grazing in the North East of the Netherlands. Pasture in the areas where the disease occurredhad a copper concentration in the range 1 - 5 p.p.m. (mean, 2.5 p.p.m.), while that of normal healthy pasture ranged from 6 - 12 p.p.m. (mean, 7.5 p.p.m.). Since then copper deficiency in cattle has been reported from all parts of the world particularly e.g. Wester Australia (Bennetts and Hall, 1939); Florida (Davis, Kidder and Comar, 1946); in New Zealand (Cunningham, 1945); England (Allcroft, 1946); Ireland (O'Donova 1949) and Scotland (Jamieson and Russell,1946). The disease is characterised by/

by the coat becoming harsh and dishevelled as the hair loses its capacity to produce pigment. This is accompanied by a loss of appetite and a gradual loss in condition. Anaemia develops progressively as the copper concentration in the blood falls from a normal level of 0.8 - 1.4 p.p.m. to less than 0.2 p.p.m. At this concentration of copper in the blood the anaemia is so severe that the oxygen carrying capacity of the blood is reduced to less than 30% of the normal range.

The disease in cattle can either be a specific deficiency of copper or else it can be complicated by deficiencies of some other elements such as cobalt. Then, in addition to the symptoms already described, there is a severe and persistent scouring and the animals condition deteriorates more rapidly. In Western Australia, the disease is known as "Falling Disease" and is characterise by sudden death. It usually manifests itself during the period of flush growth of pasture from August to October when there is a loss in condition and some evidence of anaemia. It causes a suppression of oestrus and temporary sterility in the cows while the calves might appear stunted and have limb abnormalities. Diarrhoea may occur but it is not an essential feature.

In South Australia there is a condition prevalent in young lambs born of ewes which have a very low liver copper concentration. This has been termed enzootic ataxia and was first related to a deficiency of copper by Bennetts and Chapman (1937) when they found that treatment of ewes with copper sulphate prevented/

prevented the appearance of the syndrome in their lambs. The central nervous system of the affected lamb is extensively demyelinated and it is likely to die within a few days of birth. The first sign of copper deficiency in the adult sheep is the appearance of "steely" wool due to defective keratinisation.

A similar nervous disorder is found in lambs in the United Kingdom where it is known under the descriptive term "swayback". In less acute cases it is characterised by an inco-ordination of gait while in the more severe forms there may be complete paralysis. Similar symptoms have been described in young lambs in many other parts of the world, e.g. New Zealand (Cunningham, 1944), Australia (Bennetts and Beck, 1942), India (Krishnabba, 1936), South Africa (Dunning, 1933 and Perold, 1949) and Sweden (Magnusson, 1920).

The pathology of the disease in all these cases is essentially the same. There is a diffuse symmetrical demyelination of the central nervous system. The degenerative change varies from small foci in the cerebral white matter to a gross demyelination of both hemispheres with the resulting formation of porencephalic cavities. Secondary demyelination of the motor tracts of the cord generally follows.

The disease usually originates in the foetus but not always since the same symptoms can occur in young sheep more than a year after birth. Swayback is enzootic in areas where the herbage copper content is low but it does not automatically occur in the lambs born to ewes which have a low level of copper in/

in their livers. The aetiology is not clearly understood, however, when it occurs on pastures which are apparently normal in copper concentration and which should provide more than sufficient copper for the needs of the ewes. In such cases the disease is generally termed a conditioned or induced copper deficiency.

Copper deficiency in sheep, or swayback in lambs, has been reported on pastures which ranged in their copper concentrations from 1.8 - 29.0 p.p.m. Values quoted by individual authors include 14.0 - 27.0 p.p.m. for Derbyshire pastures (Innes and Shearer, 1940); 7 - 10 p.p.m. for Northumberland pasture and 13 - 29 p.p.m. for Welsh pasture (Eden, 1944); 4 - 9 p.p.m. for Scottish pasture (Stewart, Farmer and Mitchell, 1946); 6 - 13 p.p.m. for Derbyshire pasture (Mills, 1954) and 7 - 20 p.p.m. for unspecified pasture (Allcroft, 1952, and Allcroft and Lewis, 1957). All these authors suggest that the swayback was due to a conditioned rather than to an actual deficiency of copper in the herbage.

On the other hand Barlow et al. (1960) report a range of copper content from swayback pastures in East Scotland of 1.8 - 25.5 p.p.m. with a mean value of 5.84 p.p.m. Sixty two per cent of the samples analysed by these workers had less than 5.0 p.p.m. Cu. This figure of less than 5.0 p.p.m. copper in the herbage from swayback farms corresponds very well with the copper content of herbage from areas where enzootic ataxia occurs in Australia. Australian herbage which has a copper content of 1 - 3 p.p.m. is regarded as deficient; 3 - 5 p.p.m Cu is regarded as marginal and above 5.0 p.p.m. copper is regarded as being quite safe or normal (Beck, 1962).

Barlow/

Barlow and his colleagues suggest, on the basis of their own work, that swayback may also be a true deficiency disease as in enzootic ataxia. They suggest that the copper content of the herbage can be incorrectly assessed unless great care is taken to eliminate soil from the sample or to allow for some contamination and to correct for it. Soil contamination may have been the cause of some of the high herbage copper concentrations previously reported and particularly those of Innes and Shearer (1940) who suggested that as the sheep would eat herbage contaminated with soil that this is what should be analysed. This viewpoint makes no allowance for the fact that soil copper will likely be much less readily utilized by the animal as it is much more tightly held than the organically bound copper of plants.

It is also noteworthy that most of the herbage samples obtained by previous workers were taken in the late spring and early summer. As already mentioned the copper concentration of grass frequently rises sharply in the spring so that the copper concentration of herbage samples obtained after the lambing may give misleading information concerning the copper concentration of the herbage consumed by the ewe during pregnancy.

Blood Copper Concentration.

The blood copper level of normal sheep ranges from 0.60 - 1.30 p.p.m. (Barlow et al., 1960). Where copper deficiency symptoms or swayback occur the blood copper concentration is generally much lower. Barlow et al.(1960) quote a mean/

mean blood copper concentration of 0.13 p.p.m. (range 0.05 - 0.50 p.p.m.) for th mothers of swayback lambs. Eden, Hunter and Green (1945) report a mean value of 0.30 p.p.m. (range 0.10 - 0.80 p.p.m.) for ewes which gave birth to lambs with swayback. On the other hand Innes and Sheerer (1940) and Shearer and McDougall (1944) quote a mean blood copper concentration of 0.60 p.p.m. (range 0.30 - 1.0 p.p.m.) for similar ewes.

The blood copper concentration of lambs suffering from swayback which exhibit symptoms of the disease at birth or during the first few weeks of life was found to be within the range 0.08 - 0.49 p.p.m. with a mean value of 0.26 p.p.m. (Barlow et al,1960). Swayback can occur in another "delayed" form where symptoms might not appear perhaps for several months after birth. In such cases the blood copper concentrations of the lambs are normally much higher than those found in lambs affected from birth. Barlow et al. (1960) quoted a range of 0.09 - 0.87 p.p.m. with a mean of 0.45 p.p.m. for swayback lambs ranging in age from 10 weeks to 6 months. Thus blood copper analyses may be of no value in diagnosir the delayed form of swayback. The cause of the rise in the blood copper levels is probably due to the lambs commencing to graze from about 4 weeks old and thus being no longer completely dependent on the ewe's milk for all their copper requirements. A whole milk diet is virtually deficient in copper; Elvehjem, Steenbock and Hart (1929) reported that the copper concentration of cows milk was 0.15 p.p.m.

Australian/

Australian work shows very comparable results to those quoted by Barlow et al.(1960) for the blood copper concentration of sheep with lambs suffering from enzootic ataxia. Bennetts and Beck (1942) found that seven out of nine ewes whic had affected lambs had blood copper concentrations of less than 0.10 p.p.m. The highest blood copper concentration was 0.40 p.p.m. Marston (1952) reported signs of copper deficiency when the blood copper level of ewes fell below 0.30 p.p.m.

Liver Copper Concentration.

The normal concentration of copper in the liver of sheep covers a wide range from 100 - 300 p.p.m. However, liver copper concentrations can vary quite considerably in either direction from this range without any risk of either deficiency or toxicity symptoms.

In Australia lambs suffering from enzootic ataxia have liver copper concentrations below 10.0 p.p.m. (Bennetts and Beck,1942; and Bull, Marston, Murnane and Lines,1938). Spais (1956) reported levels of 5 - 10 p.p.m. in the livers of copper deficient ewes and lambs in Greece.

Barlow et al.(1960) reported a mean level of 5.6 p.p.m. Cu for swayback lambs under six weeks old. This compares very well with a mean liver copper concentration of 6.3 p.p.m. for eleven swayback lambs recorded by Allcroft, Clegg and Uvarov (1959). Innes and Shearer (1940) and Shearer and McDougall (19 reported that the mean liver copper concentration of swayback lambs examined by them was 12.0 p.p.m. This is double that reported by Barlow et al. and Allcroft et al.

It/

It is interesting to compare the results of Barlow et al.(1960) and those of Innes and Shearer (1940) and Shearer and McDougall (1944) for the blood copp concentrations of the mothers of swayback lambs and the liver copper concentrations of the lambs. Their results are presented in Table 4.

Table 4. Blood Cu concentration in ewes with swayback lambs and liver Cu concentration in swayback lambs (Barlow et al.1960).

Blood Cu Conc. in Ewes			Liver Cu Conc. in Lambs		
p.p.m.Cu.	Shearer et al.	Barlow et al.	p.p.m.(D.M.)	Shearer et al.	Barlow et al.
0 - 0.10	0	49	0 - 2	0	7
0.10 - 0.20	0	31	2 - 4	0	27
0.20 - 0.30	0	14	4 - 6	7	17
0.30 - 0.40	11	4	6 - 8	7	40
0.40 - 0.50	16	2	8 - 10	18	2
0.50 - 0.60	17	0	10 - 12	22	7
0.60 - 0.70	24	0	12 - 14	14	0
0.70 - 0.80	21	0	14 - 16	18	0
0.80 - 0.90	8	0	16 - 18	14	0
0.90 - 1.00	3	0			

Barlow et al.(1960) found that 94% of ewes which gave birth to swayback lambs had blood copper concentrations below 0.30 p.p.m. whereas Shearer et al. found no blood copper concentrations below this level in similar sheep. Similarly for the livers of swayback lambs 91% of those examined by Barlow et al. had copper concentrations below 8.0 p.p.m. compared with only 14% of those reported by Shearer and his colleagues.

The/

The values reported by Shearer and his co-workers for the blood and liver copper concentrations of ewes and of their swayback lambs are much higher than any which have been recorded since. The improved sampling and analytical techni of the later workers might account for their lower results. Interference from iron in the estimation of copper using sodium diethyldithiocarbamate might also have been a contributory factor in the higher values reported by Shearer et al. The only values which may justify the "conditioned" deficiency idea are those recorded by Shearer and his colleagues, although other workers have recorded normal copper levels in pasture on which swayback has occurred. These pasture samples, however, were generally obtained after lambing rather than during pregnancy.

The Effect of oral dosage with copper sulphate on the concentration of copper in the blood and liver of sheep.

Eden & Green (1939) studied the short term effect of an oral dose of copper sulphate on ovine blood copper concentrations. Two sheep were dosed with a 1% solution of $CuSO_4 5H_2O$ in water; one was given 0.25 g Cu and the other 0.5 g Cu. Blood samples were obtained from both sheep at intervals during several hours after treatment.

The copper concentrations (p.p.m.) of those samples were:

Time(mins.)	0	40	60	75	120	180	300	420
0.25 g Cu	1.38	1.49	-	1.40	1.32	1.32	1.34	-
0.50 g Cu	1.22	-	1.50	-	1.49	1.36	1.36	1.29

There/

There was only a short term increase in blood copper concentration due to the oral dose of copper sulphate administered. It should be noted, however, that the sheep had initially quite high levels of copper in their blood.

Eden (1939 and 1940 - 43) reported that the mean blood copper concentration of sheep which had been regularly dosed with an antihelmintic containing 300 mg $CuSO_4$ was appreciably higher than that of control sheep. Eight dosed sheep had a mean blood copper concentration of 1.12 p.p.m. compared with a mean of 0.72 p.p.m. for the control sheep. The mean liver copper concentration (305 p.p.m.) of the dosed sheep was also significantly higher than that of the control sheep (52 p.p.m.). Eden (1944) working with lambs born in March/April gave regular drenches of 0.17 g $CuSO_4.5H_2O$ (42 mg Cu) from the end of May to mid September. This was on a farm in North Wales where there was a swayback problem, despite a concentration of copper in the pasture ranging from 13 - 21 p.p.m. Before drenching commenced the mean blood copper concentration of all the lambs was 0.47 p.p.m. After seven drenches the mean copper concentration of the dosed lambs was 0.74 p.p.m. while that of the control lambs remained almost unchanged at 0.50 p.p.m.

Hemingway, Brown & Inglis (1962) found significant increases in the liver copper concentration of sheep which had been dosed with either 2.0 or 3.5 g $CuSO_4.5H_2O$ (given as 4 or 7 separate doses of 0.5 g) compared to control animals. The mean liver copper concentration of the control sheep was 64 p.p.m. compared with 140 p.p.m. for those sheep given 4 doses and 162 p.p.m. for those given 7 doses of 0.5 g $CuSO_4.5H_2O$. They reported no differences in blood copper concentration/

concentration between the control and the copper treated sheep. The concentrations had been in the normal range at the commencement of the experiment.

Andrews and Isaacs (1964) in New Zealand reported a significantly higher mean liver copper concentration (933 p.p.m.) in lambs which had been dosed at weekly intervals for 4 months with 70 mgCu (as $CuSO_4.5H_2O$) than in untreated lambs whose mean liver copper concentration was 574 p.p.m. They suggested that this rate of treatment is probably too high as several of the lambs had liver copper concentrations in excess of 1000 p.p.m. Although none of these showed any symptoms of copper toxicity there would have been a definite risk of this occurring if treatment had been continued for a longer period.

Factors Affecting Copper Absorption and Utilization.

The mechanism of copper absorption has not so far been elucidated but it seems probable that it occurs in the higher regions of the small intestine. It is unlikely that copper is absorbed as the free ion but rather in the form of stable organic complexes. Mills (1956) found that three times as much copper was stored in the rat liver when the element was fed in the form of the stable complexes of an aqueous extract of herbage than when fed as the free ion. If it is the case that copper is preferentially absorbed when in the form of soluble complexes then abnormally high concentrations of other metals in the digestive tract might decrease the amount of copper absorbed by competing with it for the metal-binding ligands. Metals which have been reported to reduce copper absorption and storage in the liver include molybdenum, lead, zinc, silver, antimony, barium, calcium and iron.

Under/

Under normal conditions only about 3% of the dietary copper is absorbed and stored in the livers of sheep (Edgar, 1942; Dick 1954, and Hemingway et al., 1962). Under copper deficient conditions, however, absorption may be much more efficient. Marston (1952) suggested that this might explain the remarkable response of copper deficient rats to additions of very minute amounts of copper to their diets. Sheep, under housed conditions, have also been reported to absorb and store large quantities of copper even when being fed on rations which supply a normal intake. This aspect of copper absorption will be reviewed more fully under the section entitled "Chronic Copper Poisoning". The effects of various elements on copper absorption will be discussed here either individually or where appropriate in combination.

1) Molybdenum and Sulphate.

Ferguson, Lewis and Watson (1943) found that the severity of the scouring that occurred in cattle on "teart" pastures in Somerset was directly proportional to the amount of water soluble molybdenum present in the herbage. This condition is an extreme form of induced copper deficiency caused by the excessively high level of molybdenum (50 - 200 p.p.m.) in these pastures. Scouring could be averted by the oral administration of 1 - 2 g $CuSO_4.5H_2O$ per day.

Dick and Bull (1945) reported that liver reserves of copper in both cattle and sheep could be depleted by dosing the animal with molyldate. This finding was later confirmed in New Zealand by Cunningham (1950) and in Australia by Marston/

Marston (1950) who reported markedly reduced liver copper concentrations but no appearance of any deficiency symptoms.

Dick (1954) has since shown that molybdenum effects the liver copper storage of sheep only when the diet contains an adequate supply of inorganic sulphate. Swayback has been produced in lambs by treating the mothers during pregnancy with ammonium molybdate and sodium sulphate(Fell, Williams and Mills, 1961). However, Butler and Barlow (1963) failed to produce swayback in Blackface lambs by similar treatment of their mothers and in fact this treatment raised the blood copper levels of the ewe.

Dick (1954) also found that the addition of ferrous sulphide to the diet lowered copper accumulation in sheep by 75%. He suggested that this might be due to the formation of insoluble cupric sulphide in the rumen. Other sources of sulphur (elemental sulphur and sodium thiosulphate) were ineffective in reducing copper accumulation. Spais (1956) reported a conditioned copper deficiency in sheep grazing salt marshes which had a normal herbage copper content (5 - 15 p.p. Cu) but which had a high level of SO_4 (1 - 4%).

Cunningham (1955) recorded harmful effects in cattle grazing pastures which had 10 p.p.m. Cu if the molybdenum content was over 15 p.p.m. and in pastures with less than 5 p.p.m. Cu if the molybdenum content was in the range 3 - 7 p.p.m. Beck (1962) found normal levels of molybdenum and sulphate in pastures where enzootic ataxia occurs in Western Australia. He concluded that the sub-normal/

normal copper content of the herbage was the constant and most significant factor associated with enzootic ataxia and also with falling disease in cattle. The mean molybdenum content of pastures where swayback is known to occur in Britain is generally not more than 1.5 p.p.m. Only one rather higher value than this (4.1 p.p.m. Mo) has been quoted by Allcroft (1952). This is still only slightly above normal and would not affect copper accumulation unless the copper level of the pasture was very low i.e. < 5 p.p.m. which in this case it was not.

2) Calcium.

There is a widely held belief among farmers and veterinary surgeons that there is a higher incidence of swayback on pastures which have recently been limed or reseeded. Stewart (1951) stated that swayback usually had an enhanced prevalence after liming and Russell and Duncan (1956) reported a high incidence of swayback on limestone rocks in the Scottish border counties. Barlow et al. (1960), however, found no increased incidence of swayback in limestone areas in the Scottish border counties nor did they find any correlation between soil pH and the incidence of swayback. Their results, however, supported the premise that swayback was more prevalent on recently limed or reseeded areas.

Copper deficiency in lambs is known to occur on the calcareous sandy soils of Southern Australia but in this case there is a real deficiency of copper as some plants cannot complete their life cycle unless copper is added to the soils which are virtually copper deficient.

Liming/

Liming a pasture does not generally appear to affect the herbage copper concentration but it might have an indirect effect on the copper status of the grazing animal due to the production of a higher yielding and better type of pasture. This means that either a greater density of stock can be carried or that a given area may support the original livestock for a longer period thus necessitating the supply of less supplementary feeding. Heather, which may have been dominant prior to liming, will give way to a better type of herbage which will generally contain less copper. On the other hand liming may encourage the growth of clover which is normally fairly rich in copper. Liming, to improve hill ground, may, however, be only one of several concurrent factors. Others include improved drainage, reseeding and possibly basic slag applications and these will influence soil type and texture and plant species. A combination of all these factors could be sufficient to cause swayback especially in areas where the copper status of the animals was marginal before pasture improvements were carried out.

In housed sheep Dick (1954) found that 90 g $CaCO_3$ per sheep per day significantly reduced the accumulation of copper in their livers. However, in a repeat experiment, in which he supplied the same amount of calcium, but this time as dicalcium phosphate he found that it had no limiting effect on copper accumulation. Hemingway et al.(1962) reported no reduction in liver copper concentrations in young sheep at grass which had been dosed with 35 g $CaCO_3$ daily for up to 13 weeks.

3) Lead/

3) Lead.

The fact that swayback occurred widely on farms in Derbyshire which had high levels of lead in the herbage led to the belief that this element might be involv in the aetiology of swayback (Innes and Shearer, 1940). Shearer, Innes and McDougall (1940) and Shearer and McDougall (1944) later concluded that lead was not a major factor but they thought that it might play a secondary role.

Blood, liver and kidney copper concentrations in sheep have been reduced by regular daily doses of lead acetate in quantities supplying about 100 - 250 mg.Pb/day (e.g. Ferguson 1948; Hemingway et al, 1962 and Hemingway et al, 1964). However, none of these workers was able to produce swayback by this treatment. Allcroft (1952) also failed to increase the incidence of ataxia in lambs of hypocupraemic ewes with regular doses of lead to the ewes during pregnancy.

4) Zinc.

Ingestion of zinc has been found to reduce the uptake and utilization of copper by the rat (e.g. Smith and Larson, 1946 and Gray and Ellis, 1950). McCall and Davis (1961) reported that zinc reduced copper absorption by the rat only when the protein level of the diet was very low (about 10%). It had no significant effect when the dietary protein level was 17.5 or 25.0%.

Dick (1954) found that the storage of copper in the livers of sheep was unaffected by the addition of 20 mg Zn to the diet but that it was reduced when 100 mg Zn were added. Hemingway et al. (1964) reported no depletion in the liver copper content of ewes given 2 g Zn (as the sulphate) daily during pregnanc

Dynna/

Dynna and Havre (1963) reported a conditioned copper deficiency in cattle which worsened if supplementary Zn was given and only improved slightly when additional copper was given. A complete recovery was effected by the addition of both copper and zinc.

Other elements which have been reputed to reduce blood copper concentration include fluoride, antimony, silver and barium (Ferguson, 1948). Dick (1954) found that up to 50 mg Ni added to the diet of sheep did not affect liver copper storage.

The level of protein in the diet has also been implicated in copper absorption. High dietary protein levels depress the uptake of copper by the rat (e.g. Schultze, Elvehjem and Hart, 1936, and Coulson, Remington and Lynch, 1934). This has recently been confirmed by McCall and Davis (1961) who fed a diet containing 25% protein to rats and found that a supplement of 1000 p.p.m. Cu produced no significant increase in liver-copper concentration. Wallace, McCall, Bass and Combs (1960) found that the toxicity of 750 p.p.m. Cu fed to growing pigs decreased as the protein level was increased from 15 - 25%.

The antagonistic effect of high levels of molydenum and sulphate on copper absorption has been clearly established. However, the concentration of these radicles in the majority of pastures where swayback is known to occur, is generally well within the limits of normality. Thus any increased incidence of swayback due to high levels of molybdenum and sulphate will be of slight/

slight and restricted importance only. High levels of lead in pasture are also found in restricted areas only and even there it is doubtful if they exert any great influence on the incidence of swayback.

Calcium has been reported to increase the incidence of swayback and to decrease copper absorption. No publications have appeared in this country, however, which have reported on the effect of liming on the blood and liver copper status of the grazing animals. It was felt, therefore, that this aspect should be examined and this forms the basis of Experiment 5. The effects of high levels of calcium in the diets of housed sheep were also examined and this work is reported as Experiment 8.

Dietary protein has been shown to have a marked influence on copper absorption and storage in rats and pigs. It was decided to see how it would affect copper absorption in sheep on low, normal and high copper intakes. The results of this investigation are reported in Experiment 7.

Factors affecting copper excretion.

Only a small proportion of the dietary copper intake is absorbed - approximately 3% in the liver of sheep - most of the remainder being excreted in the faeces. Urinary excretion of copper is, under normal circumstances, very slight amounting to only some 100 - 200 ug/day of a total intake of perhaps 15 mg. Bile always contains copper and is probably the main vehicle for its excretion. Part of intravenously injected labelled copper appears in the bile and is then excreted in the faeces.

A/

A number of organic substances are known which affect copper excretion, particularly by increasing the loss in the urine. Their effectiveness in copper excretion was discovered during the treatment of patients suffering from Wilson's Disease. Wilson's Disease, or hepatolenticular degeneration, is an illness in humans which Cumings (1948) showed to be associated with elevated copper concentrations in the tissues, particularly the brain and liver. Cumings advocated the use of British Anti Lewisite (B.A.L. or dimercaptopropanol) for the treatment of patients suffering from this disease. Cumings (1951) and Denny-Brown and Porter (1951) reported marked clinical improvement in a large proportion of patients treated with B.A.L. B.A.L. is a chelating agent and was used to mobilise copper from the tissues and excrete it in the urine. It was found to have unpleasant side reactions, however, and its cupuretic action did not persist if patients were maintained on it for any considerable period of time. Response to it, therefore, was quite promising initially but was not very well maintained.

Bearn and Kunkel (1954) showed that "Versene", calcium disodium ethylenediaminetetra-acetate, was a powerful chelating agent for copper. It had, however, to be given parenterally as it was not very well absorbed from the gut. Despite its powerful chelating ability it has given somewhat disappointing therapeutic results. Bickel, Neale and Hall (1957) suggested that this might be due to the fact that such a highly charged molecule might be unable to cross the blood-brain barrier and remove copper from the main sites of toxic action in the brain.

Walshe/

Walshe (1956) obtained a much larger copper excretion with a new chelating agent, penicillamine (B-B dimethylcysteine), than with either B.A.L. or versene. Penicillamine can be given orally and has been proved very successful in improving the clinical condition of patients suffering from Wilson's Disease over long periods. If it is given as the D-form no undesirable side effects are produced.

Recently a fourth chelating agent has been used successfully in the treatment of Wilson's Disease. This is sodium diethyldithiocarbamate and Sunderman, White and Sunderman (1963) reported that intravenous administration produced a slight increase in urinary copper output and a distinct clinical improvement. Oral administrations did not affect urinary copper output but markedly increased the faecal copper output. Administration by this route however, effected no clinical improvement in the patient.

McDonald (1946) increased the urinary copper output of sheep by giving intramuscular injections of B.A.L. but he found that the effect of the injection persisted for 5 - 8 hours only. Cunningham (1950) stated that large doses of B.A.L. administered to sheep over a period of 3 weeks did not reduce liver copper levels. Todd, Gracey and Thompson (1962) found that intramuscular injections of B.A.L., given to sheep which had been on a high copper intake, did not materially affect the average daily output of urinary copper but that during the two days of treatment the volume of urine excreted was nearly doubled.

Howell/

Howell (1964) reported a reduction in the blood copper concentration of rabbits which had been injected with sodium diethyldithiocarbamate.

Apart from the above little or no work has been published on copper excretion in sheep and the effect on it of dietary changes. It was decided, therefore, to obtain values for the urinary and faecal copper excretion of sheep on normal and high copper intakes and to examine the effect on those of intravenous injections of various chelating agents. It was hoped to show that some, or one, of these chelating agents might be useful in the treatment of sheep suffering from chronic copper toxicosis by reducing the liver copper stores from potentially dangerous to more normal levels. This work is reported as Experiments 13, 14 and 15.

METHODS FOR THE PRACTICAL CONTROL OF COPPER DEFICIENCY IN ANIMALS

Since the initial discovery that copper was an essential nutrient several methods of counteracting a dietary deficiency have been tried. These include top dressing the pasture with copper sulphate, oral dosage with copper sulphate or other salts, the provision of copper-containing licks for voluntary consumption and the injection of copper compounds either intravenously, subcutaneously or intramuscularly. Each of these methods will be discussed separately.

1) The Effectiveness of Pasture Treatment with $CuSO_4$ in Controlling Copper Deficiency in the Animal.

Lee (1950), in Australia, found that a single application of 7 lbs. $CuSO_4 5H$ per acre, drilled in at seeding, maintained flocks free from all symptoms of deficiency for seven years. Comparable sheep in similar adjacent pastures produced steely wool during most of these years. Sheep, introduced to pastures five years after treatment with copper, maintained higher liver copper levels than sheep on similar untreated herbage. Initially the liver copper concentration of all the sheep was 270 p.p.m. After two years on the untreated pasture the liver copper level of the control sheep had fallen to 21 p.p.m. while that of the sheep on the copper treated herbage had only fallen to 190 p.p.m. This illustrates the lasting effect of one treatment of seven pounds of copper sulphate per acre. However, this rate of treatment has not always proved to be successful in Australia. On a different soil type it did not completely prevent the incidence of steely wool and a number of low liver copper concentrations were also recorded.

Munday/

Munday (1963) in Tasmania, considered that 7 lbs. $CuSO_4.5H_2O$ per acre on a fine sandy loam soil increased the liver copper levels of sheep for up to five years. He supported this statement with clinical findings on enzootic ataxia and steely wool together with liver copper values of less than 25 p.p.m. for sheep grazing non top dressed pasture. Where top dressing was practised liver copper concentrations were normal at 100 - 250 p.p.m. On the same sandy soil this treatment increased liver copper concentrations of cattle for several years but only prevented severe scouring for one year. In contrast this same rate of treatment applied on swamp or peaty soils was effective for only one year in increasing liver copper concentrations in cattle. Cunningham and Perrin (1946) in New Zealand found that applications of copper sulphate to peaty soils were of no value in increasing liver copper concentrations of grazing ruminants. Bennetts and Beck (1942) in Australia and Cunningham (1949) in New Zealand have reported that pasture top dressing with copper sulphate is an effective method of controlling ataxia in lambs.

In the United Kingdom, Field (1957) prevented copper deficiency in cattle in East Anglia by top dressing the pasture with a spray of a solution of 10 lbs. $CuSO_4.5H_2O$ per acre. Twenty seven per cent of calves grazing untreated pasture developed symptoms of copper deficiency - in 8 cases the symptoms were severe, - whil e no such symptoms were developed by calves grazing the treated herbage. Herbage copper concentrations were not quoted. Morgan and Clegg (1958) prevented deficiency symptoms in calves grazing in a peat soil area in Lancashire by top dressing the pasture with copper sulphate.

2)/

2) The Effectiveness of Voluntary Consumption of mineral licks containing coppe

Supplying copper-containing mineral licks for voluntary consumption by ewes was one of the earliest methods employed in this country for the control of swayback. The idea was that the ewe would consume sufficient of the lick to correct any copper deficiency that might be present.

Dunlop, Innes, Shearer and Wells (1939) reported an experiment where they collected more than 200 ewes from 40 farms in Derbyshire. These were divided into three groups and put on to 300 acres of land where swayback was known to occur. One group of 50 sheep was used as a control; a second group of 55 sheep had access to licks containing 1% of copper sulphate for periods ranging from 12 weeks to 4 weeks before lambing. The third group of 104 ewes was given acces to a lick containing 0.3% copper sulphate for periods similar to those given to the second group. Twenty five per cent of the ewes in the control group gave birth to swayback lambs. There was a total absence of swayback where copper was fed for 12 weeks prior to lambing and where 1% copper sulphate was fed for the last 4 weeks of pregnancy only one lamb out of seventeen had swayback. However, where 0.3% copper sulphate was fed for this period the incidence of swayback was as high as for the control group. From the information presented it is impossible to assess how much copper each ewe consumed. Also no account was taken of the copper supplied by the 1½ lbs. of supplementary feeding per ewe per day given from December to April. This would account for a large proportion of the ewes' food requirements so that the amount of copper supplied by the "swayback" herbage would be negligible.

Eden/

Eden, Hunter and Green (1945) reported on the use of copperised briquettes of several commercial brands sold in Derbyshire, on eighteen farms in that area. The overall incidence of swayback found was 6.3%. On fifteen of the farms the incidence was only 2.0% while on the remaining three farms it was of the order of 27.0%. Thus on most of the farms a voluntary consumption of copper was effective in reducing swayback. The offered amount of lick per ewe varied from 7 - 39 ounces with a mean of 24 oz. per ewe. This would be equivalent to 14 mg Cu/day during pregnancy if the briquettes contained 0.3% Cu. On the three farms where there was the high incidence of swayback the offered amount per ewe ranged from 18 - 31 oz. The licks were not analysed for their copper concentration so it is possible that these three were low in copper or else an incorrect consumption rate was assumed. On the basis of these results it would seem desirable that copper licks should contain at least 1% copper sulphate when fed for the second half of pregnancy.

3) The Effectiveness of Oral dosing with Copper Sulphate.

Hunter, Eden and Green (1945) and Eden et al.(1945) concluded experiments to investigate the effectiveness of oral dosing with copper sulphate to reduce the high incidence of swayback which occurred on five different farms in Derbyshire. They had control groups totalling 185 ewes and other groups of the same number which were treated fortnightly with 0.5 g. $CuSO_4.5H_2O$ (= 125 mg Cu) throughout pregnancy. Blood samples were obtained from the ewes a) before treatment in October; b) after 5 doses in January; c) after a further 4 doses in March and d) sometime after lambing in May. These results are summarised in Table 5.

Table 5. Concentration of copper (p.p.m.) in the blood of ewes given 0.5 g $CuSO_4.5H_2O$ each fortnight throughout pregnancy and the incidence of swayback in their lambs (Hunter et al., 1945, and Eden et al, 1945)

Sampling Date		Oct.	Jan.	Mar.	May	% Incidence of swayback (30 - 70 lambs per group)
Farm A	With copper	0.34	0.46	0.76	0.56	0
	No copper	0.37	0.27	0.34	0.39	34
Farm B	With copper	0.72	0.62	0.77	0.65	0
	No copper	0.80	0.44	0.51	0.56	3
Farm C	With copper	0.29	0.36	0.52	0.34	8
	No copper	0.31	0.26	0.30	0.30	27
Farm D	With copper	0.71	0.56	0.57	0.71	5
	No copper	0.69	0.42	0.36	0.54	25
Farm E	With copper	0.28	0.34	0.68	0.50	0
	No copper	0.29	0.24	0.35	0.40	10

The copper concentration in the blood of the control ewes generally fell from October to January but there was some recovery by lambing time in March and a further increase by May. The cumulative effect of 5 drenches of 0.5 g $CuSO_4.5H_2O$ given by mid January was to maintain the blood copper level of the treated ewes at their October values. Further administration of copper between January and March, however, generally increased levels to about 0.5 - 0.7 p.p.m. It is interesting to note that in none of the five experiments did the mean blood copper concentration rise above 0.77 p.p.m.

On/

On farms A,C,D and E the mean blood copper concentrations of the untreated ewes at lambing time were in the range 0.30 - 0.36 p.p.m. and the incidence of swayback was 34, 27, 25 and 10% respectively. Treatment of the ewes with a tota of 1.125 g Cu as copper sulphate completely controlled swayback at farms A and E where the mean blood copper concentrations were raised to 0.76 and 0.68 p.p.m. respectively. In contrast on farms C and D where the mean blood copper concentrations at lambing were 0.52 and 0.57 p.p.m. the incidence of swayback was 8 and 5% respectively. Blood copper concentrations were higher, and the incidence of swayback negligible, on farm B where the ewes were better fed.

In these experiments 46 ewes - 40 control and 6 treated - gave birth to swa back lambs. The blood copper concentrations of those 6 treated ewes did not rise as a result of regular drenching and were never above 0.30 p.p.m. The mean blood copper levels (p.p.m.) of these 46 ewes were 0.35 (in October), 0.23 (in January) 0.25 (in March) and 0.30 (in May). In the last blood sample obtained before parturition only 5 of these 46 ewes had more than 0.40 p.p.m. Cu Twenty two of the ewes had blood copper concentrations below 0.20 p.p.m.

4) The Effectiveness of Copper Injections.

The administration of copper by injection was first recorded by Green (1949). He indicated that 20 mg Cu as the sulphate given 6 weeks before parturition controlled demyelination of the foetus of the ewe. The copper was/

was only slowly eliminated and sufficient was stored in the tissues to maintain the ewe and lamb over an intervening period of copper inadequacy. Green gave no supporting evidence in this reference for this statement but was probably quoting the work of Allcroft, Hunter and Salt (1946) which was not reported until 1959 by Allcroft, Clegg and Uvarov (1959).

Apart from this early experiment, publication of which was delayed for 13 years, most of the work in the development of copper injections has been undertaken in Australia and New Zealand. The impetus for the work was largely concerned with the inhibition of growth, and particularly the poor quality of the wool, in sheep in areas where copper sulphate treatment of wide areas of grazing was not feasible.

Harvey (1952) working in Queensland, investigated the potential value of a wide range of copper compounds given by subcutaneous injection. Each injection supplied 50 mg Cu. Highly ionised copper salts such as the sulphate, acetate and borogluconate were highly inflammatory producing severe necrosis and were unacceptable. Weakly ionised salts such as the naphthenate, citrate, oleate, asparaginate and caseinate were generally unsatisfactory when given in aqueous suspension with tragacanth. Severe abscesses formed and much of the copper was exuded from these on bursting. Even when administered in peanut oil there were marked reactions at the site of injection. In contrast copper-8 - hydroxyquinolate, injected in peanut oil, produced little reaction and was largely transported from the site within 24 hours. In each case Harvey recorded at 90% retention of the administered copper in the animal basing this on urine/

urine and faecal analyses of sheep maintained on a constant diet in metabolism cages.

Harvey (1953) recorded a further series of trials where the copper retention was again based on balance trials but where some liver biopsy samples were also obtained. In contrast to his previous findings he reported that only 10% of the copper from copper-8-hydroxyquinolate reached the liver and that the remainder was either exuded from or retained in a walled-off cold abscess at the site of injection. However he found that copper ethylenediamine tetra-acetate was well retained in the liver. Doses supplying 80 - 225 mg Cu, however caused marked increases in urinary copper excretion, induced severe haemoglobin-urea and caused death in periods which ranged from 4 hours to 8 days depending on the dose administered. He also reported that 100 mg Cu, as copper glycine, given in a single dose at one site, produced severe tissue damage and was potentially very toxic. 50 mg Cu, as copper glycine, given at each of two sites produced only small lesions and was considered a safe quantity for practical administration.

The use of subcutaneous injections of copper glycine in an oil base, was further investigated by Sutherland, Moule and Harvey (1955). 80 mg Cu in this form (2.5 mg - 4.0 mg/Kg) was fatally toxic and amounts ranging from 5 - 60 mg injected inside the rear leg produced marked lameness within 24 hours. They concluded that copper glycine supplying 40 mg Cu (1.25 mg/Kg) was a safe dose to give behind the shoulder but that an increase in dosage to 2 - 3 mg/Kg gave marked/

marked reactions at the injection site. This size of injection (40 mg Cu) increased the mean liver copper concentration of six sheep from 157 to 488 p.p.m in the dry matter.

Cunningham (1957) has indicated that although 5 lbs of copper sulphate per acre as a pasture top dressing is useful under New Zealand conditions, its use is impracticable in hilly areas. He recorded that 50 mg Cu, as the sulphate, given intravenously was inadequate for copper deficient animals where there was an excessive molybdenum intake. Larger doses were, however, quite unsafe. He investigated the use of copper glycine , supplying 30, 60 and 120 mg Cu administered to sheep either as an aqueous solution or in marrow fat. Liver copper storage of the aqueous solution when injected subcutaneously was only 42% compared to a 72% storage when given intramuscularly. There was little differen in the storage ratesbetween the two routes when the copper glycine was mixed with marrow fat. The mean increases in liver copper concentration 30 days after the administration of 30, 60 and 120 mg Cu were 81, 191 and 314 p.p.m. respectiv This represented a mean storage of 70% of the injected copper.

Cunningham (1959) investigated the usefulness of subcutaneous and intramuscular injections of copper citrate and the cupric-bis-8-hydroxyquinoline 5 - disulphonic acid salt of tetradiethylamine (Dicuprene) in the hope that they might produce less tissue reaction than copper glycine in an oil or fat base. Copper citrate (152 mg = 52 mg Cu) given in either a beeswax/peanut oil mixture or with beef fat/neats foot oil produced massive reactions at the injection site where/

where up to 50 ml. of pus was recovered. Some 24% (18 - 30%) of the injected copper was recovered in the liver on slaughter after 18 days.

Dicuprene which contained 6.05% Cu was administered either as a) a 10% solution in saline or b) as the cerate formed when mixed with beef fat/neat's foot oil. There were no untoward reactions at the injection site but 3 sheep given 60 mg copper and 1 sheep given 45 mg copper in this form died. No trouble was experienced when 84 hoggets were injected with 30 mg Cu as Dicuprene. The mean liver copper concentration of 4 sheep injected with 45 mg Cu increased by 203 p.p.m. within 14 days which represented an almost complete transfer of the injected copper to the liver.

Fearn and Habel (1961) have investigated the respective values of injections of copper sulphate and copper glycine for improving fleece quality in Australian sheep. Two of 75 sheep given injections of 30 mg Cu, as the sulpha in 15 ml. saline died shortly after treatment. In the remainder liver copper concentrations increased from 18 p.p.m. (pre-treatment) to 127 p.p.m. some 6 weeks after injection but thereafter decreased rapidly to 65 p.p.m. and 28 p.p.m. by 3 and 6 months after injection. Copper glycine injections (≡ 45 mg Cu) given in an oil base increased the mean liver copper concentration to 212 p.p.m. six weeks after treatment but by six months after injection the mean level had fallen to 47 p.p.m. In a number of trials the incidence of steely wool found under copper deficiency conditions ranged from 80 - 100%. In contrast where copper glycine treatment was given every six months almost 90% of the sheep carried normal fleeces.

Hemingway/

Hemingway et al.(1962) found that 8 lambs injected with 45 mg Cu given as copper glycine, had a significantly ($P<0.01$) higher mean liver copper concentration (197 p.p.m.) than that (64 p.p.m.) of 8 similar untreated lambs. The mean liver copper concentration of the injected lambs was also significantly ($P<0.05$) higher than that (151 p.p.m.) of 8 further lambs, 4 of which had been drenched with 2.0 g $CuSO_4.5H_2O$. / Fifty eight per cent of the copper injected and the remaining 4 with 3-5 g. $CuSO_45H_2O$ in separate drenches of 0.5g.$CuSO_45H_2$

as copper glycine was stored in the liver compared with only 2.4% of the copper given as the copper sulphate drench. Five of the eight injected sheep had absce about one inch in diameter, at the injection site. These persisted until slaughter 13 weeks after treatment. Blood copper concentrations were temporaril increased in the injected sheep but they were not maintained at elevated levels.

Copper injections are now being used in Britain for the control of swayback in lambs. The general recommendation is that they be administered subcutaneousl behind the shoulder and not intramuscularly in the leg where they may cause lameness. Only one publication has appeared in Britain, however, concerning their efficacy with ewes which may potentially give birth to swayback lambs.

This was by Allcroft, Clegg and Uvarov (1959) who reported an experiment where they treated 64 ewes with 45 mg Cu, as copper glycine, and had a further 64 ewes as untreated controls. There was a previous history of swayback on the farm. The ewes could be divided into 4 groups; 2 groups of 54 which had been more than a year on the farm and 2 groups of 10 ewes which had been recently introduced to the farm. The mean blood copper concentrations (p.p.m.) recorded were:-/

were:-

	December	January*	February	May**
54 treated ewes	0.22	0.08	0.69	0.40
54 untreated ewes	0.26	0.15	0.20	0.20
10 treated ewes	0.75	0.50	0.70	0.50
10 untreated ewes	0.88	0.35	0.51	0.48

*Immediately prior to injection ** 3 - 4 weeks after lambing.

The injection of copper glycine substantially increased the copper concentration in the blood of the 54 ewes of low copper status but by a few weeks after lambing the level was starting to fall. There were smaller increases for the 10 ewes which were initially of rather higher copper status but in both groups mean blood copper concentrations in late pregnancy were in the order of 0.70 p.p.m.

Allcroft et al. detailed the liver copper concentrations of eleven lambs born to control ewes which had blood copper concentrations at lambing below 0.10 p.p.m. Ten of these lambs, recovered from 8 ewes, exhibited pathological evidenc of swayback, the onset of which varied from 0 to 39 days after birth. The mean liver copper concentration of those eleven lambs was 7.1 p.p.m. (range 3.2 - 8.9 p.p.m.). Whilst these concentrations are very low it should be noted that only those lambs with clinical swayback were examined. The total incidence of swayback in this group was 15.6%.

Lambs were obtained from six of the injected ewes, five of which had blood copper concentrations in the range 0.5 - 0.8 p.p.m. The blood copper concentration of the sixth ewe never rose above 0.1 p.p.m. and it gave birth to a swayback lamb with a liver copper concentration of 5.6 p.p.m. Of the remaining five lambs one was unable to rise after birth and it had a liver copper concentration of/

of 7.6 p.p.m. The remaining four were either stillborn or were found dead and had 32, 46, 52 and 250 p.p.m. of copper in their liver dry matter. The mean liver copper concentration of these six lambs was 67.4 p.p.m. but the standard deviation was as high as ± 96.4. These six lambs, however, cannot properly be regarded as a fair sample of the lambs born to the injected ewes.

A statistical evaluation of the data given by Allcroft et al. shows that the injections, while having a noticeable effect on lamb liver copper concentrations did not have a statistically valid effect ($t = 1.52$). Nevertheles the injection reduced the incidence of swayback from 15.6 to 1.5%. It was perhap unfortunate that so few lambs were examined from the injected ewes and that such a high proportion of them were either stillborn or found dead. Hemingway, Inglis and Brown (1964) have shown that the concentrations of copper in the liver and kidney of stillborn or foetal lambs may frequently be exceptionally higher than those in normal lambs at birth. They recorded values of 70 - 440 p.p.m. copper in the kidney of stillborn lambs compared to a normal mean copper concentration of only 11 p.p.m. Similarly where the mean value for the liver of normal lambs was about 10 p.p.m. those found for stillborn lambs could sometimes be as high as 53 - 90 p.p.m.

Allcroft et al.(1959) also reported some work undertaken by Alloroft, Hunter and Salt (1946) which had not been previously published. It is of interest in that it was conducted on the same farm as the 1959 experiment. Alloroft

et/

et al (1946) administered 20 mg Cu, as the sulphate, in physiological saline to 34 ewes of low copper status in mid pregnancy. Ewe blood copper concentrations increased from a pretreatment level of 0.25 p.p.m. in January to 0.55 p.p.m. in February. A mean value of 0.50 p.p.m. was recorded after lambing in May. None of these 34 ewes gave birth to swayback lambs. In contrast the mean blood copper concentration of 34 untreated ewes remained within the range 0.22 - 0.32 p.p.m. from January to May. These ewes produced four swayback lambs.

A further possible use of copper injections is in the early treatment of lambs born in flocks where swayback is prevalent and where no prophylactic treatment has been given to the ewes during pregnancy. Allcroft, Buntain and Rowlands, (1965) have reported the results of observations made at three centres where copper (as the copper calcium ethylenediaminetetra-acetate) was injected subcutaneously into lambs ranging in age from 2 days to 7 weeks. Their results are presented below.

Dose (mg Cu)	Age	No. of Lambs	No. of Deaths
10	4 days - 5 weeks	159	9
20 - 25	4 days - 5 weeks	154	20
30 - 50	2 days - 7 weeks	89	17

It is not clear why such large doses were employed when it is considered that only 45 mg Cu is the recommended safe quantity for an adult ewe. The efficacy/

efficacy of the treatment in swayback control was not reported. Deaths occurred at from 24 - 72 hours after treatment but not all of them were associat with copper poisoning or even with high copper levels in the tissues. Oedema of the lung and a great concentration of pleural fluid were frequent symptoms.

Allcroft et al.(1965) did, however, report that copper calcium EDTA was to be preferred to copper glycine for use both in adult sheep and cattle because of the smaller risk of reaction at the injection site and the better absorption of copper which occurred. They indicated that it had been administered to ewes in mid pregnancy for three consecutive years on a farm where swayback was endemic and that good protection had been obtained. They did not, however, quot any analytical or supporting data.

All these four methods for controlling swayback have been successful in reducing losses to quite low levels in some cases. None have been entirely successful. The least certain of the four is the supply of mineral licks for voluntary consumption since there is no means of ensuring that the ewes consume sufficient copper to supply their own needs and those of the developing foetuses This method has now, to a large extent, been superceded by oral dosage or injection of a controlled amount of copper.

Pasture treatment with copper sulphate has proved quite successful in Australia but it has not really been tried in this country due to the difficulty of adequately covering large tracts of hill ground and thus ensuring that the herbage/

herbage was getting a uniformly adequate supply of copper. Also the fear that sheep might consume excessively large amounts of copper over a long period has discouraged the use of this form of treatment. Experiment 4 was designed to discover the effect of pasture treatment with copper sulphate on the liver copper content of sheep and to see whether this was affected by different rates of application of nitrogen fertilizer since nitrogen applications have been reported to increase herbage copper concentration. It was also hoped to discover whether there was any risk of chronic copper toxicity due to the long term ingestion of herbage which, by Australian standards, had been given a heavy application of copper sulphate.

As the administration of copper by injection is now the favoured means of controlling swayback it was considered useful to obtain further information concerning the value of injectable copper preparations on farms where swayback is a problem. These investigations covered the effects of injection on ewe and lamb blood copper concentrations and on the concentration of copper in the livers of a representative number of lambs shortly after birth. This work is reported in Experiment 6.

Copper Poisoning in Sheep

The occurrence of acute copper poisoning in animals has been known for a long time. Reimers (1908) reported death, due to acute copper poisoning, in a foal which had been grazing in a field of wheat recently sprayed with copper sulphate. Theiler (1912) experimentally produced acute copper poisoning in sheep. He found that the single toxic dose was about 20 mg Cu/kg body weight.

Confusion existed for a long time over the existence of chronic copper poisoning and even as recently as 1920 Wynter Blyth held the opinion that it did not occur. True chronic copper poisoning was first produced experimentally by Mallory (1925) in sheep treated with copper compounds. Since then it has been reported in sheep grazing in orchards which had been sprayed with a copper containing fungicide (Beijers, 1932) and in sheep in Texas which had been given free access to a lick containing copper sulphate for the control of internal parasites (Boughton and Hardy, 1934). These latter workers gave a detailed report of the clinical aspects of the disease.

The first instance of accidental chronic copper poisoning in Britain occurred in sheep grazing near a smelting works in Wales (Bisset, 1934). The literature has since carried a large number of reports of accidental and experimental cases of chronic copper poisoning. These have been reviewed by Eden/

Eden (1940 - 43) and Todd (1962).

There is also a type of chronic copper poisoning which occurs in sheep in Eastern Australia grazing a predominantly subterranean clover pasture which has never been treated with supplementary copper. The occurrence has been attributed to the very low molybdenum content of the herbage. Chronic copper poisoning exists in yet another form in Australia in areas where the weed, Heliotropum europaeum, flourishes. This weed contains an alkaloid - lasiocarpine - which causes atrophy of the liver cells and this in itself can be fatal. The consumption of this alkaloid apparently causes an increased avidity of the liver cells for copper and if the animal then has access to grazing which is high in copper it will rapidly store toxic amounts of copper in the liver. Bull, Albisto Edgar and Dick (1956) termed this type as "hepatogenous chronic copper poisoning"

Occurrence of Copper Poisoning.

Most of the early cases of chronic copper poisoning reported resulted from the grazing of orchards where copper sulphate had been extensively used as a fungicide (e.g. Beijers, 1932; Fincham, 1945; Lafenetre, Monteil and Galtier, 1935; Muth, 1952 and Ogilvie, 1954). Other outbreaks have resulted from treatment of pasture with copper sulphate to correct a deficiency (Pryor, 1959) or to kill snails as a measure in controlling liver fluke (Gracey and Todd, 1960).

Deaths/

Deaths have also occurred where copper-containing mineral licks have been used as antihelmintics (Boughton and Hardy, 1934 and Guilbride, 1963).

High levels of copper in feeding stuffs, some of which were intended for growth promotion in bacon pigs and were fed either inadvisedly or inadvertently to sheep, have caused deaths due to chronic accumulation of copper (Clegg, 1956; Pearson, 1956; Berwyn-Jones, 1960; Kowalczyk, Pope and Sorensen, 1964, and Ross, 1964). One similar case has been reported in an adult Jersey cow fed for 6 months on a bacon production concentrate containing 200 p.p.m. Cu (Todd and Gribben, 1965).

Chronic copper poisoning in sheep has been produced experimentally by Eden (1940 - 43), Sutter, Rawson, McKeown and Haskell (1958), Todd, Gracey and Thompson (1962) and Barden and Robertson (1962).

Housed sheep have been reported to be particularly susceptible to chronic copper poisoning even when the diet contains only a slightly elevated copper content (Bracewell, 1958; Todd, 1962, and Hill and Williams, 1965). Senior (1959) reported death in a housed sheep which received no supplementary copper with its diet. However, this sheep apparently obtained extra copper by chewing at a copper pipe connected to its water bowl.

Course of the Disease.

Eden (1940 - 43) described the development of the disease in four distinct stages. Firstly a relatively limited absorption of copper from the intestine takes/

takes place even when the intake of copper is high. Secondly there follows an adequate elimination of absorbed copper in the bile and urine and possibly through the intestinal wall with limited copper storage in the liver so that blood copper concentrations do not rise materially in the early stages. Thirdly there is gradual functional damage particularly to the liver with slowly rising blood copper. Finally there ensues a sudden breakdown of elimination mechanisms with pronounced lesions in the liver and kidneys associated with icterus and with a sharp premortal rise in blood copper concentration.

Todd (1962) describes the outstanding feature as a sudden and extensive haemolysis which results in haemoglobinurea and jaundice. This haemolysis can be so severe that the blood packed cell volume (P.C.V.) falls from around 40% to about 10% within 48 hours. The blood becomes chocolate-coloured due to the presence of methaemoglobin. Elevated temperatures and inappetance are other features and depression ensues with marked dyspnoea in the terminal stages.

At post mortem the carcase is generally jaundiced and the cirrhotic liver varies in colour from yellow to orange to a muddy brown. The kidneys are dark coloured with a metallic sheen due to the deposition of haemoglobin products and they can be on occasion quite enlarged (Kowalczyk et al., 1962). The gall bladder is usually distended and the bladder can contain dark red-coloured urine The spleen is enlarged and congested with a jelly-like consistency. Excess pericardial/

pericardial fluid can be found and also epicardial haemorrhages.

Degeneration of the cells and central necrosis of the lobules of the liver are the main histological features. In the kidney there is engorgement of the uriniferous tubules with broken down erythrocytes and haemoglobin derivatives. Tubular necrosis has also been reported.

Tissue Copper Distribution.

Blood and Plasma Copper Concentrations.

Eden and Green (1939) examined the whole blood and plasma copper concentrations of sheep and found them to be virtually identical. They then gave intravenous injections of copper (30 - 80 mg Cu) and followed the changes in the whole blood and plasma copper concentrations. During the first hour following injection the plasma copper concentration increased more rapidly and reached higher levels than did the whole blood copper. In one sheep, which died five days after injection, the plasma copper concentration reached a level of 36.8 p.p.m. one hour after injection compared to 23.8 p.p.m. for the whole blood. Both levels equilibrated again some six hours after injection.

Normal blood copper concentrations range from 0.6 - 1.3 p.p.m. (Eden, 1939; Barlow et al.,1960; Beck,1955). It has been generally accepted that the blood copper concentrations of sheep remain within this range during the period of copper accumulation in the liver until about 48 hours before the appearance of clinical symptoms when they suddenly and markedly increase. Only Sutter et al. (1958) have disagreed with this viewpoint. In experimentally induced copper poisoning/

poisoning in sheep they have reported increased serum copper concentrations from 5 to 35 days before death. The increases reported, however, were not very great - the highest value quoted was 2.56 p.p.m. Cu. Once the haemolytic crisis takes place death is reported to occur within a matter of a few days in British breeds of sheep. Australian merino sheep, however, have been reported by Marston (1952) to be able to survive a number of crises before they succumb.

Liver and Kidney Copper Concentrations.

The normal liver copper concentrations in sheep ranges from 100 - 300 p.p.m. In chronic copper poisoning the liver stores large amounts of copper and when death occurs concentrations are usually in excess of 1000 p.p.m. and can exceed 6000 p.p.m. in the dry matter.

The kidney copper concentration in normal sheep is generally less than 20.0 p.p.m. In copper poisoned sheep the concentration can rise to more than 1000 p.p.m. (Eden, 1940 - 43) and is almost always more than 50 p.p.m. However, if the sheep survives for a few days following the initial crisis the kidney copper level can decrease until it is only slightly above normal.

The copper content of the other tissues does not vary very much between normal and copper poisoned sheep although the spleen can sometimes show increased values. Table 6 presents the copper content of the tissues in a normal sheep, a healthy sheep on a high copper intake and a copper poisoned sheep.

Table 6. The copper concentration (p.p.m. D.M.) in the tissues of normal and copper poisoned sheep.

	Normal Sheep. (Underwood, 1956)	Sheep on a High Cu Intake. (Dick, 1954)	Sheep died from Copper Poisoning. (Eden, 1940 - 43)
Liver	237	1250	3090
Kidney	18	14	1365
Spleen	5	5	6
Lungs	10	13	42
Heart	18	16	20
Brain	—	30	14
Muscle	6	3	5
Skeleton	—	1	-

Because of the high levels of copper that can be found in the livers of clinically normal sheep it is unwise to base a diagnosis of chronic copper poisoning purely on a chemical analysis of the tissues. The results of these analyses must be taken in conjunction with clinical findings such as jaundice and haemoglobinurea and must also be related to the diet of the sheep with particular reference to its copper content.

Haemoglobin Content and Packed Cell Volume.

Eden (1939) examined the haemoglobin (Hb) content of the blood of sheep being fed or dosed daily with 1.5 g $CuSO_4.5H_2O$ and which eventually died from chronic copper poisoning. The Hb content of normal sheep ranged from 12 - 14 gms/100 ml. and during the initial dosage period it generally remained within this/

this range. Eden reported a trend for lower values towards the fatal termination of the illness and in one case the level was consistently below 10 gm./100 ml. for the last 3 weeks and it fell as low as 6.1 gm./100 ml. during this period. In contrast, Todd and Thompson (1963) found a marked rise in the Hb content of the blood of sheep during the 10 days prior to the onset of the haemolytic crisis and levels of over 16 gms./100 ml. were recorded. There was a rapid reduction in Hb content with the onset of the crisis and levels fell generally to below 6 gms./100 ml. with one recorded as low as 3 gm./100 ml.

Todd and Thompson (1963) reported a marked increase in packed cell volumes during the period immediately preceding the onset of the haemolytic crisis. Normal values range from 35 - 40% whereas during this period they recorded levels in the range 50 - 55%. Barden and Robertson (1962) also reported an elevated P.C.V. during this period in one sheep but the increase on this occasion was not nearly so much as that reported by Todd and Thompson. As haemolysis occurred the P.C.V. values fell rapidly and were often less than 10 per cent when death occurred (Todd and Thompson, 1963; Barden and Robertson, 1962).

Biochemical Findings.

Boyd (1962) carried out a survey on the distribution of some transaminase and dehydrogenase enzymes in the tissues of normal sheep. He then induced liver necrosis by dosing with carbon tetrachloride and found that the level of activity in the serum of enzymes which were most active in the liver increased most. He reported a relationship between the amount of the increase and the extent of hepatic necrosis.

Todd/

Todd and Thompson (1963) performed various liver function tests to try and determine whether any damage was occurring to the liver cells during the copper accumulation.stage. They found elevated serum levels for glutamic oxalacetic transaminase (GOT) and lactic dehydrogenase (LD) in sheep from 6 - 8 weeks before the onset of the haemolytic crisis. Ross (1964) reported elevated GOT values for up to 4 weeks before death due to chronic copper poisoning. These results indicate that there is severe dysfunction of the liver for some weeks before the haemolytic crisis. Todd and Thompson (1963) reported no increase in serum glutamic-pyruvic transaminase (GPT) levels until after the onset of the crisis. Although these enzyme tests are a measure of liver function they also measure kidney function and disturbances in other tissues. However as copper is not deposited in the kidneys during the accumulation stage of the illness and as there have been no reports of pathological changes in the kidney before the appearance of clinical symptoms elevated values for these tests can be taken as being indicative of liver and not kidney dysfunction.

Todd and Thompson (1963) also examined blood glutathione levels. This estimation includes all non-protein thiol compounds. All the glutathione present in blood is in the red cells and its presence is necessary to protect these from haemolysis (Jocelyn, 1958). It is also involved in the reduction of methaemoglobin so that in the absence of glutathione methaemoglobin accumulates.

Todd and Thompson (1963) found that blood glutathione fell to very low levels/

levels with the onset of the haemolytic crisis. They suggested that the reduction in glutathione levels in the red cells takes place before haemolysis occurs and that it might be involved in the haemolytic process. They also suggested that the methaemoglobinaemia, which occurs as one of the main features of chronic copper poisoning in sheep, results from the reduction in blood glutathione content.

Housed Sheep

The housing of various categories of sheep has now become an accepted standard practice with farmers in this country. The three main classes of sheep involved are 1) ewe hoggs from hill ground which must be overwintered off the hill; 2) pregnant ewes, from about 6 weeks prior to lambing until 3 - 4 weeks post parturition; 3) young Down Cross lambs or 6 month old small hill lambs for fattening.

Apart from the major nutritional and housing problems which arise when dealing with these three different classes of stock another point that has to be borne in mind is the possibility of the accumulation of toxic amounts of copper. As already mentioned Bracewell (1958) and Hill and Williams (1965) have reported chronic copper toxicity in housed sheep on a diet which contained only slightly elevated copper contents.

The first report in this country of the housing of sheep affecting their blood copper concentration was by Eden (1944). During November he housed 8 poor lambs from a hill farm which had not grown since August. At this time their mean blood copper concentration was under 0.8 p.p.m. Three weeks later their mean blood copper concentration had risen to 1.1 p.p.m. despite the fact that their diet of hay and concentrates contained no more copper than the pasture they had been grazing previously. It is possible, however, that they consumed more food indoors than when at grass.

Hunter/

Hunter et al. (1945) and Eden et al. (1945) housed 40 ewes which had previously produced swayback lambs on farms in Derbyshire. Their ration of 1 lb. oats and 1 lb. oat straw provided them with an intake of 5 mg/day Cu compared with an estimated intake of 15 mg Cu/day when at grass. Blood samples were obtained from 31 ewes throughout pregnancy and after lambing in March. Fourteen of these 31 ewes were dosed fortnightly with 0.5 g. $CuSO_4.5H_2O$. The blood coppe concentrations of the dosed and the untreated sheep are presented in Table 7.

Table 7. The mean blood copper concentrations (p.p.m.) of housed sheep (Hunter et al., 1945; Eden et al., 1945).

	27 Nov.	7.Dec.	28 Dec.	18 Jan.	14 Feb.	Post Part.
Dosed copper given(g)	-	0.375	0.5	0.625	2.875	0.875
14 treated ewes	0.32	0.33	0.96	0.17	0.93	1.28
17 untreated ewes	0.27	0.35	0.71	1.12	0.80	1.18

There were no significant differences between the mean blood copper concentrations of the treated and untreated groups. There was, however, a marked rise in the blood copper concentrations of both groups within 1 month of housing. The mean blood copper concentration of comparable ewes which had been left on the farm and which had initially slightly higher blood copper concentrations was 0.46 p.p.m. for treated ewes and 0.34 p.p.m. for untreated ewes in mid-January. The housed ewes produced no swayback lambs.

Apart/

Apart from these reports there has been no work done on the effect of housing of sheep on their absorption and storage of dietary copper. As it was felt that housing would play an increasingly important part in the sheep industry it was thought desirable that this aspect should be thoroughly investigated. To this end a number of experiments were carried out to demonstra the effect of a variety of typical diets on the copper status of housed sheep. The effect of variations in the diet on the copper absorption of sheep on normal and high copper intakes was also investigated. In some instances these absorpti rates were contrasted with those found for similar sheep outdoors (Section IV).

With the possibility of the occurrence of chronic copper poisoning a variet of liver function and haematological tests were performed routinely to find out whether it would be possible to obtain a prior warning of the approach of the haemolytic crisis. These determinations along with whole blood copper estimatio were carried out on sheep which were receiving high copper supplements to see how far in advance of clinical symptoms it was possible to detect elevated or depressed values for each of these measurements.

Chemical Methods

The procedures used for the estimations carried out in this work are fully detailed in Appendix I. Apart from a discussion of the choice of method used for the determination of copper the others will be mentioned briefly except where any modifications to the published method were made and these will be detailed.

Determination of Copper

Elimination of Sources of Contamination.

Copper is present only in trace amounts in biological materials so that any contamination from extraneous sources may lead to grossly inaccurate results. It is, therefore, of paramount importance to eliminate all possible sources of contamination both during the process of obtaining the samples and of analysing them. To this end all glassware used for copper determinations was rinsed immediately after use and left to soak overnight in chromic acid. It was then rinsed thoroughly with tap water and finally washed twice with deionised water.

Blood samples were taken into cleaned glass McCartney bottles which were fitted with rubber stoppers rather than with metal ones. The needles used were made of stainless steel and they were initially boiled in nitric acid. Blood was allowed to flow through the needle for a few moments before collection was commenced/

commenced. Heparin (checked for the absence of copper) was used as the anticoagulant.

Whole livers were obtained and these were subsampled after cutting the liver into a large number of small pieces in order to get a completely representative sample. The liver subsample (100 g. or the whole liver if it was less than this) was put in a pyrex dish and dried at 100°C after which it was ground in a plastic coffee mill fitted with a stainless steel blade. The ground liver was stored in airtight polystyrene containers.

Grass samples were cut 1 - 2 inches above ground level to avoid soil contamination. They were cut either with an Allen scythe or with stainless steel scissors. The grass was dried overnight in a forced draught oven and then ground in a Christy and Norris laboratory mill which was free from any brass parts. The samples were finally stored in airtight polystyrene containers.

Destruction of the organic matter in all samples for copper analyses was performed by wet digestion using a mixture of nitric, perchloric and sulphuric acids. The acids used were specially prepared lead free reagents (Hopkins and Williams Ltd.) which were also satisfactorily low in copper. Adequate blank determinations were made to check all reagents and glassware used.

Choice of method for copper estimations.

During the course of this work many thousands of samples of blood, liver, kidney, urine, faeces and plant material were analysed for copper. It was therefore/

therefore, essential to have a quick but also fairly accurate and reliable method for copper determination. A comparison is made here of a standard method used for copper determination and the one which was eventually chosen for the copper analyses required for this work.

Previously, the most widely used method for the determination of copper in biological material was that devised by Eden and Green (1940). This was a modification of the methods of Thompsett (1934) and MacFarlane (1932). The method depends on the formation of a yellow-coloured complex between copper and the diethyldithiocarbamate reagent used. The optical density of the colour is then measured in a spectrophotometer.

The reagent used by Eden and Green was sodium diethyldithiocarbamate but thi also forms a yellow coloured complex with iron which therefore has to be removed before the complex formation takes place. After the destruction of the organic matter with nitric, perchloric and sulphuric acids they neutralised the solution and then added ammonium citrate in quantity to reduce or eliminate interference from iron. This method, therefore, has the disadvantage that the initial acid solution has to be neutralised and that large amounts of reagents have to be added to eliminate interference.

Marston (1952) and Barlow et al.(1960) have indicated that it is unwise to rely absolutely on ammonium citrate to mask interference from iron in the final copper diethyldithiocarbamate colour. For this reason Butler and Newman/

Newman (1956) considered that for very sensitive work a dithizone method was to be preferred. However, both those analytical methods are rather long and involv many reagent additions and separations. In large scale routine use they are, therefore, perhaps more liable to error.

The aim has been to obtain a method which used as few reagents and pieces of apparatus as possible in this way ensuring that the minimum blank reading was obtained thus enhancing the accuracy of the procedure. Martens and Githens (1952) showed that small amounts of copper could be separated directly in acid solutions from relatively large amounts of most other metals by means of zinc dibenzyldithiocarbamate. Abbot and Polhill (1954) determined the copper concentration in fats and oils by means of the potassium and zinc salts of dibenzyldithiocarbamic acid and found that these methods gave good recovery of added copper and that they were free from interference from other metals, other than bismuth which is unlikely to occur in biological materials.

Andrus (1955) developed a method for the estimation of copper in plant material using zinc dibenzyldithiocarbamate. He also showed a good recovery of added copper and a freedom from interference due to the presence of other ions Brown and Hemingway (1962) in this laboratory developed a method using zinc dibenzyldithiocarbamate for determining copper concentration in blood and liver tissues. This method was found to be free from interference even from massive amounts/

amounts of iron. Copper is estimated directly after wet digestion by means of the zinc dibenzyldithiocarbamate reagent which is dissolved in carbon tetrachloride. No preliminary separation or addition of buffer solution is required. It has the added advantage over sodium diethyldithiocarbamate in that only a single CCl_4 extraction is required. CCl_4 is heavier than water so that it can be drawn off at the base of the separating funnel. Amyl alcohol which was used by Eden and Green is lighter than water so forms the upper layer and is more difficul to separate and requires several extractions.

This, therefore, was a rapid method of analysis which required the minimum amount of apparatus and reagent additions thus reducing the possibility of contamination. It seemed eminently suitable for use in the copper determinations required for this thesis. However, it was felt that before it was definitely accepted that it should be compared with the existing method of copper determination and provided the accuracy and reliability were equally as good then it should be the method of choice.

Ten samples were analysed for copper by both the sodium diethyldithiocarbamate method of Eden and Green (1940) and the zinc dibenzyldithiocarbamate method of Brown and Hemingway (1962). The results of these analyses are presented in Table 8.

Table 8. Copper concentration in whole blood (p.p.m.).

Sample No.	Na diethyldithiocarbamate	Zn dibenzyldithiocarbamate
1	1.29	1.26
2	1.21	1.24
3	1.07	1.16
4	1.09	1.11
5	1.21	1.12
6	1.33	1.32
7	1.10	1.16
8	1.13	1.16
9	0.93	0.95
10	0.93	0.97
Mean	1.13	1.15

The recovery of added copper by both methods was tested by adding various amounts of a standard solution containing 1 ug Cu/ml to whole blood samples. The results are given in Table 9.

Table 9/

Table 9. Recovery of added copper in 5 ml. of whole blood.

Method	Sample No.	5 ml. Blood (ug)	Cu added (ug)	Cu found (ug)	Cu recovered (ug)	Recovery (%)
	1	5.80	4.00	9.75	3.95	98.8
	2	5.60	3.00	8.60	3.00	100.0
Zn dibenzyl.	3	6.60	3.00	9.50	2.90	96.7
	4	6.05	1.00	7.05	1.00	100.0
	5	5.80	1.00	6.85	1.05	105.0
Mean						100.1
	1	6.05	4.00	10.25	4.20	105.0
	2	5.45	3.00	8.75	3.30	110.0
Na diethyl.	3	6.65	3.00	9.75	3.10	103.3
	4	5.50	1.00	6.60	1.10	110.0
	5	5.50	1.00	6.55	1.05	105.0
Mean						106.7

Finally duplicate analyses were carried out on 8 whole blood samples by the zinc dibenzyldithiocarbamate method. The results are presented in Table 10.

Table/

Table 10. Duplicated analyses of 5 ml. portions of 8 whole blood samples for copper.

		Copper Concentration (p.p.m.)	
Sample No.	1	1.11	1.12
	2	1.16	1.12
	3	1.32	1.32
	4	1.16	1.21
	5	1.04	1.05
	6	0.99	1.01
	7	0.97	0.97
	8	0.99	0.95
	Mean	1.09	1.09

From the data presented in Table 8 it can be seen that the results of both methods for whole blood copper estimations compared very closely. Recovery of added copper, however, was more accurate by the zinc dibenzyldithiocarbamate method of Brown and Hemingway. Where the sodium diethyldithiocarbamate method of Eden and Green was used the recovery of the added copper was consistently above 100%, the mean recovery being 106.7%. On the other hand the mean recovery of added copper in 5 samples analysed by the method of Brown and Hemingway was almost precisely 100%, being in fact 100.1%. This slight discrepancy between the two methods is probably due to the larger volume and greater number of reagents used in the copper estimation by the sodium diethyldithiocarbamate method thus causing a slight degree of contamination which/

which resulted in the slightly higher values obtained. As can be seen from Table 10 duplication of analyses by the zinc dibenzyldithiocarbamate gave very good results.

Thus this method compared favourably with an existing method for copper estimation, gave a more accurate recovery of added copper and also gave good duplication of results. It was also much speedier, involved fewer reagent additions and was a much more pleasant method to operate as it did not involve the use of amyl alcohol which is used in copper estimation using sodium diethyldithiocarbamate. This chemical is unpleasant both for the operator performing the copper estimations and also for any other person working in the same laboratory. On the basis of all these factors it was, therefore, finally decided to adopt the zinc dibenzyldithiocarbamate method of Brown and Hemingway for all the copper estimations required for this thesis.

Preparation of Samples for Analyses.

Blood Copper. 5 ml. whole blood used. Wet ashed with a mixture of nitric, perchloric and sulphuric acids. Residue boiled with a small amount of water and the copper concentration determined directly on the solution by addition of zinc dibenzyldithiocarbamate in solution in carbon tetrachloride

Liver, Kidney, Plant and Faecal Copper. 1 gm. of dried and ground sample wet ashed and made up to volume. Aliquot extracted with zinc dibenzyldithiocarbamate and the copper concentration determined.

Urine Copper/

Urine Copper. 25 ml. of sample taken and wet ashed with the acid mixture; additional nitric acid was used if required. Solution made up to volume and copper determined as before.

Plasma Iron. This was estimated by means of the method devised by Bothwell and Mallett (1955). It depends on the formation of a stable coloured complex between ferrous iron and 2 - 2 dipyridyl.

Liver, Kidney, Plant, Faeces and Urine Iron. All these estimations were carried out by an adaptation of Bothwell and Mallett's method for plasma iron determination. The samples were wet ashed and made up to volume as for the copper estimation. A suitable aliquot, generally 5 ml, was then taken and iron was determined on this in precisely the same way as for the plasma after the removal of protein by precipitation with trichloracetic acid (T.C.

Plant Calcium. 1 gm. of dried and ground plant material was dry ashed and made up to volume. The calcium concentration was then analysed by a flame photometric technique described by Hemingway (1956).

Plant Molybdenum. This was determined by means of the method of Purvis and Peterson (1956). This is based on the formation of an orange coloured molybdenum - thiocyanate complex which is produced in hydrochloric acid solution in the presence of a reducing agent.

Plant Sulphur/

Plant Sulphur. This was measured by the indirect flame photometric technique of Cunningham (1962). Sulphur is precipitated as barium sulphate and the barium is then measured by means of a flame photometer.

Blood Packed Cell Volume (PCV). This was measured by the micro-haematocrit method of Fisher (1962) where the blood is centrifuged for 6 minutes at 12000 g.

Haemoglobin. The method of Bell, Chambers and Waddell (1945) for the estimation of oxyhaemoglobin was used. The blood was diluted in 0.04% NH_4OH and the colour was read in an E.E.L. colorimeter using a 625 filter. The concentration of haemoglobin in gms/100 ml. was then read off a prepared standard graph.

Transaminase Enzymes. The activities of serum glutamic-oxalacetic transaminase and serum glutamic-pyruvic transaminase were estimated by the method of Reitman and Frankel (1957). The procedure followed and the reagents used were as detailed in the Sigma Chemical Company's Technical Bulletin No. 505 which was revised in 1963.

Bromosulphthalein (B.S.P.) in Plasma. B.S.P. gives a violet colour in alkaline solution. 1 ml. plasma was diluted with 4 ml. of a 10% NaOH solution and the intensity of the colour determined by reading in a spectrophotometer at 580 mu. The concentration of B.S.P. present was then found by comparison with a standard graph.

Crude Protein/

Crude Protein and Crude Fibre. These were determined by the standard methods of analysis using 2 g. samples of dried plant material.

Section II Experimental

Copper Concentration in Herbage

as affected by

a) Applications of copper sulphate.

b) Applications of nitrogenous fertilizers.

c) Liming.

d) Maturity and protein content.

Experiment 1. The Effects of Nitrogenous Fertilizer and Copper Sulphate on the Copper Concentration of Herbage.

Object. This experiment was designed to assess the effects of varying rates of application of a) Nitro-chalk (ammonium nitrate/calcium carbonate) and b) copper sulphate to the soil on the copper concentration of herbage cut several times each year over two growing seasons.

Field Layout and Herbage Treatment.

A randomised block layout which involved four replicates of each of the various herbage treatments was employed.

The following amounts of copper sulphate were applied on 21st March 1963:-

a) Nil
b) 5 lbs/acre
c) 10 lb/acre
d) 20 lb/acre
e) 5 lbs/acre followed by further applications of 5 lb/acre applied immediately after each cut of herbage.(This treatment is designated as 5* in the subsequent Tables and discussion).

There were two levels of application of Nitro-chalk (21%N), viz. 1 (Low N) and 3 (High N) cwt/acre applied in combination with each of the copper sulphate treatments. These applications of nitrogen were repeated immediately after each cut/

cut of herbage. There were thus a total of 40 small plots arranged in 4 randomised blocks each containing 10 separate treatments. The detailed layout is given in Fig. 2.

Fig. 2. Layout of Plots of Experiment 1

Plot No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
$CuSO_4$(Lb/ac)	10	20	5*	5*	20	10	5	5	0	0	5	20	10	0	0	5*	10	5	20	5*
Nitro Chalk (cwt/ac)	1	1	1	3	3	3	3	1	1	3	1	3	3	1	3	1	1	3	1	3

Plot No.	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
$CuSO_4$(lb/ac)	20	0	5	5	10	20	10	5*	5*	0	10	0	5	5*	0	20	5*	20	10	5
Nitro Chalk (cwt/ac)	3	3	3	1	3	1	1	1	3	1	1	3	1	3	1	3	1	1	3	3

The area of each individual plot was 1/300th of an acre, the dimensions bein 3.5 x 41 ft. The herbage was cut by means of a 3 ft. wide Allen auto scythe thus minimising "edge effects" of adjacent plots. Herbage samples (collected in polythene bags) were obtained by taking about 20 small sub samples along the length of each plot,

The whole area received a basal dressing of 2 cwt. of muriate of potash/acre on 21 March 1963 and again in the spring of 1964. The sward consisted mainly of cocksfoot/

cocksfoot and perennial ryegrass with some crested dogstail and a small amount of wild white clover.

1963 Results.

Four cuts of herbage were obtained in 1963, these being taken at the time of ear emergence - i.e. "the silage stage". The dates were 4 June, 11 July, 23 August and 11 October 1963.

Table 11 presents the data concerning the copper concentration in the herbage obtained from each of the 40 plots on the 4 sampling occasions in 1963.

Table 12 summarises the mean values obtained for the copper concentration of the variously treated herbage at each of the 4 cuts. It also shows the effect of the 2 different levels of nitrogen application on the copper concentration of the herbage.

Table 11. Copper concentration (p.p.m. Dry Matter) of herbage sampled 4 times in 1963.

lbs.$CuSO_4.5H_2O$/ac.		0		5		10		20		5*	
cwt. Nitro chalk/ac.		1	3	1	3	1	3	1	3	1	3
1st Cut; 4/6/63	1	6.6	6.8	7.6	7.5	7.1	8.4	7.2	6.9	6.8	8.1
	2	7.1	6.5	6.9	7.5	6.9	8.2	6.8	9.8	7.0	7.4
	3	5.4	8.1	8.2	7.6	7.0	7.1	6.4	9.0	6.4	7.7
	4	6.8	8.0	6.9	6.6	6.9	7.9	6.1	9.1	7.0	9.1
	Mean	6.5	7.4	7.4	7.3	7.0	7.9	6.6	8.7	6.8	8.1
2nd Cut; 11/7/63	1	7.0	8.6	8.8	7.8	8.2	8.8	10.0	8.3	7.2	8.7
	2	7.3	8.4	9.2	8.2	9.1	11.6	7.5	9.1	9.4	13.0
	3	10.4	7.3	7.2	9.2	10.6	7.8	9.8	9.7	7.4	9.2
	4	8.5	8.0	7.5	11.3	7.5	10.8	9.4	10.4	7.5	10.7
	Mean	8.3	8.1	8.2	9.1	8.9	9.8	9.2	9.4	7.9	10.4
3rd Cut; 23/8/63	1	10.8	12.0	10.2	11.0	10.1	11.7	9.8	11.4	10.9	14.2
	2	12.2	11.3	9.0	11.0	11.9	16.5	15.8	12.7	13.1	12.3
	3	12.7	11.8	9.0	11.9	9.4	11.8	8.8	13.8	11.5	13.1
	4	10.8	13.0	10.7	12.6	10.2	13.4	12.0	14.6	13.1	12.5
	Mean	11.6	12.0	9.7	11.9	10.4	13.4	11.6	13.1	12.2	13.0
4th Cut; 11/10/63	1	12.2	12.2	11.2	14.2	11.6	17.5	10.0	12.8	13.7	15.2
	2	12.1	17.4	13.3	16.7	13.0	14.2	14.9	16.8	16.2	15.1
	3	12.7	12.2	15.1	12.5	12.5	12.7	11.0	12.8	13.0	17.1
	4	12.1	14.4	11.2	14.3	13.9	13.7	13.9	16.8	11.1	16.3
	Mean	12.3	14.1	12.7	14.4	12.8	14.5	12.5	14.8	13.5	15.9

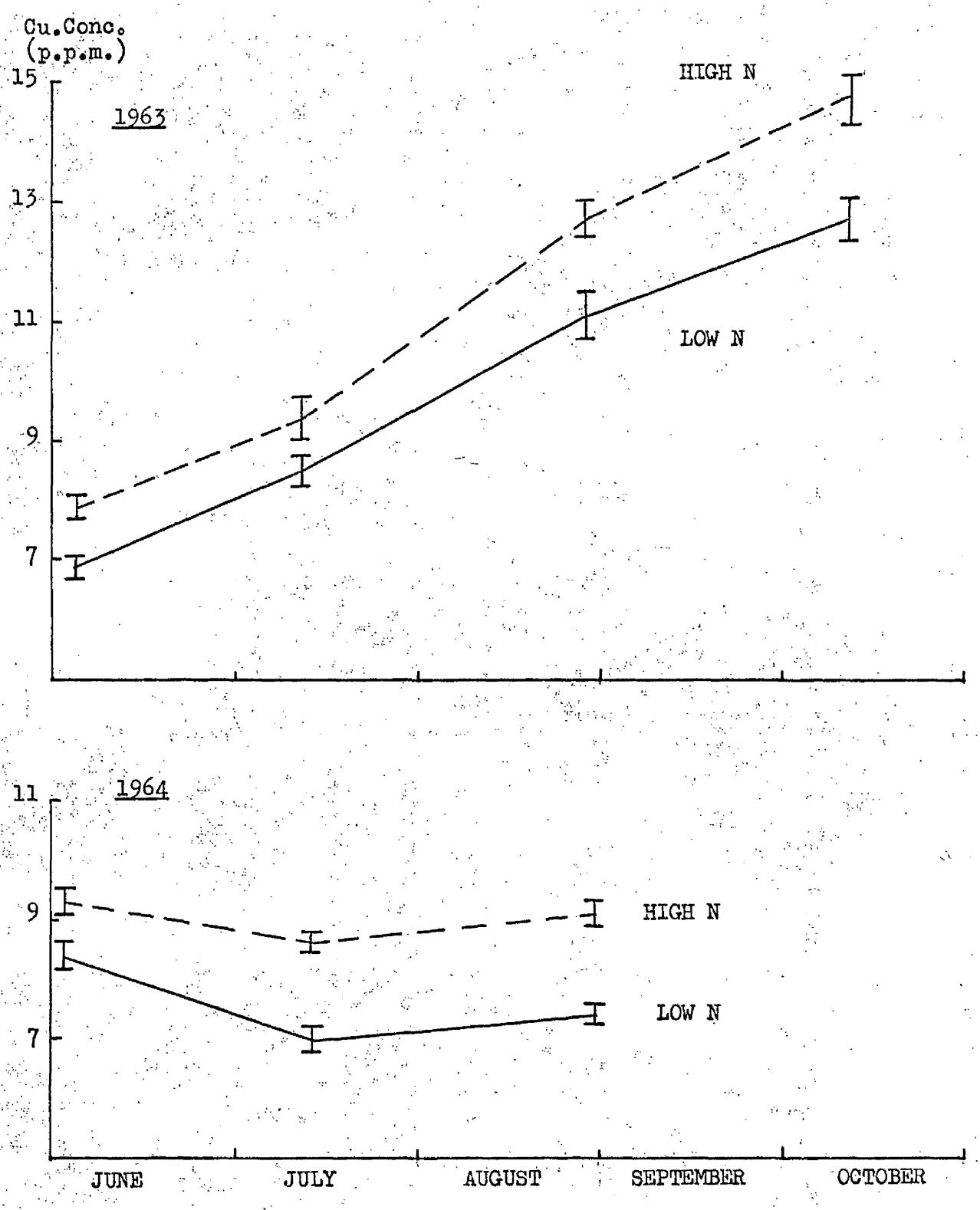
Table 12. Mean copper concentrations (p.p.m.) of 4 cuts of herbage taken in 1963

lb.$CuSO_4$/acre	0		5		10		20		5*		Level of significance
cwt.nitro chalk /acre	1	3	1	3	1	3	1	3	1	3	P =
Cut 1	6.5	7.4	7.4	7.3	7.0	7.9	6.6	8.7	6.8	8.1	0.01
2	8.3	8.1	8.2	9.1	8.9	9.8	9.2	9.4	7.9	10.4	0.05
3	11.6	12.0	9.7	11.9	10.4	13.4	11.6	13.1	12.2	13.0	0.01
4	12.3	14.1	12.7	14.4	12.8	14.5	12.5	14.8	13.5	15.9	0.01
Overall Mean	9.7	10.4	9.5	10.7	9.8	11.4	10.0	11.5	10.1	11.9	
	10.1		10.1		10.6		10.8		11.0		

Nitrogen had a considerable effect on herbage copper concentration. At each of the 4 cuts the herbage which had been treated with 3 cwts. nitro-chalk per acre had significantly higher copper contents than that treated with 1 cwt. per acre (see Fig 3a). This Figure shows the mean copper content and standard error of the herbage, treated with either the low or high nitrogen dressing, at each of the 4 cuts. This shows that there was a tendency for the copper content of both the low and high nitrogen treated herbage to rise with each successive cut. This was in fact borne out by a statistical analysis which showed that the herbage at each cut had a mean copper content which was significantly ($P < 0.01$) higher than that of the herbage at the cut immediately preceeding it.

Copper/

Fig.3. Mean copper concentrations of 7 cuts of herbage taken in 1963 and 1964.



Copper sulphate did not in general have any marked effects on herbage copper concentration. At the first cut the 20 lbs. $CuSO_4$/acre treatment when combined with high N produced a significantly ($P < 0.05$) higher herbage copper concentration than that found for the equivalent plots which had received either 0 or 5 lb. $CuSO_4$ per acre. This effect was not reproduced at the next 3 cuts. At the second and third cuts those plots which had received an initial dressing of 5 lbs. copper sulphate per acre and then further similar dressings immediately after each cut, had significantly ($P < 0.05$) higher copper concentrations than those of the untreated plots and those plots which had received a single application of 5 lb. $CuSO_4$ per acre respectively. There were no other increases in herbage copper concentration which were attributable to the application of top dressings of copper sulphate. It can be concluded, therefore, that applications of copper sulphate in quantities of up to 20 lb/acre had no persistent effect on herbage copper concentration.

1964 Results

In 1964 the plots were again given the appropriate dressing of either 1 or 3 cwt. Nitro-chalk per acre in March. No application of copper sulphate was made in 1964 as it was wished to determine if there would be any "residual" effect from the dressing applied in 1963. The herbage was sampled on 3 occasions (2 June, 13 July and 31 August 1964), each cut again being taken at the silage.stage. Table 13 details the copper concentration in the herbage obtained from each of the 40 plots at the 3 cuts taken in 1964.

Table 13. Copper concentration (p.p.m. D.M.) of herbage sampled 3 times in 1964.

lbs. $CuSO_4 5H_2O$/ac.		0		5		10		20		5*	
cwts. Nitro-chalk/ac.		1	3	1	3	1	3	1	3	1	3
1st Cut; 2/6/64	1	7.7	9.6	7.6	8.8	7.3	10.8	8.7	9.5	8.8	8.8
	2	9.3	8.7	10.0	9.9	8.8	7.8	6.2	10.7	8.2	10.2
	3	8.8	8.8	8.1	8.0	8.7	8.9	8.4	8.6	8.9	8.9
	4	8.3	9.0	7.9	9.5	6.4	8.8	9.6	11.5	9.3	8.4
	Mean	8.5	9.1	8.4	9.1	7.8	9.1	8.2	10.1	8.8	9.1
2nd Cut; 13/7/64	1	6.0	9.2	6.2	8.6	6.7	9.1	9.1	8.4	7.9	7.4
	2	6.3	8.2	6.9	9.2	6.2	8.1	7.9	8.6	7.3	8.2
	3	6.2	7.7	6.6	7.4	6.8	8.5	6.6	9.3	6.6	9.1
	4	6.8	8.5	7.1	8.4	6.7	8.3	6.9	9.2	8.2	9.6
	Mean	6.3	8.4	6.7	8.4	6.6	8.5	7.6	8.9	7.5	8.6
3rd Cut; 31/8/64	1	6.8	9.2	6.7	9.3	7.1	9.4	7.5	9.0	7.7	9.4
	2	8.1	7.4	7.6	9.5	8.4	9.5	8.0	9.5	7.7	10.3
	3	6.5	9.3	7.9	9.2	6.1	7.3	6.1	8.6	7.3	9.7
	4	7.4	8.6	7.4	8.9	8.0	8.6	7.9	10.5	7.0	9.0
	Mean	7.2	8.6	7.4	9.2	7.4	8.7	7.4	9.4	7.4	9.6

Table 14 summarises the mean copper concentrations of the 10 differently treated herbages at each of the 3 cuts. It also shows the overall effect of the 5 separate copper sulphate and the 2 separate nitrogen treatments on the copper concentration of the herbage.

Table 14. Mean Cu concentrations (p.p.m.) of 3 cuts of herbage taken in 1964.

lbs.$CuSO_4 5H_2O$/acre		0		5		10		20		5*		Level of significance		
cwt.Nitro-chalk/ac.		1	3	1	3	1	3	1	3	1	3	1	3	P
Cuts	1	8.5	9.1	8.4	9.1	7.8	9.1	8.2	10.1	8.8	9.1	8.4	9.3	0.0
	2	6.3	8.4	6.7	8.4	6.6	8.5	7.6	8.9	7.5	8.6	7.0	8.6	0.0
	3	7.2	8.6	7.4	9.2	7.4	8.7	7.4	9.4	7.4	9.6	7.4	9.1	0.0
Overall Mean		7.3	8.7	7.5	8.9	7.3	8.8	7.7	9.5	7.9	9.1			
		8.0		8.2		8.1		8.6		8.5				

As in 1963 the overriding effect found was that resulting from the higher rate of nitrogen application. At each of the 3 cuts the mean copper content of the herbage treated with 3 cwts Nitro-chalk per acre was significantly higher than that of the herbage which had been given only 1 cwt. nitro-chalk per acre (see Figure 3b).

As in 1963 there were no marked effects due to the application of up to 20 lb. $CuSO_4$ per acre. At the second cut these plots which had been treated with 20 lbs.$CuSO_4$/acre or with 4 separate dressings of 5 lbs. $CuSO_4$/acre in the previous year had significantly ($P < 0.05$) higher copper contents than the low nitrogen treated control plots.

This, however, was the only residual effect found due to the applications of copper sulphate made in 1963.

There was no general rise in the copper content of the herbage at each successive cut as there had been in the previous year. In fact, the mean copper concentration of the second cut was appreciably lower than that of the initial one (see Fig. 3b). There was a slight rise again with the third cut but the final mean level was still below that of the initial cut.

These results are similar to those reported by Spedding (1965) for trials carried out at Hurley. Raymond and Spedding (1965) and Hemingway (1962) reported that applications of nitrogen fertilizers tend to increase the mineral content of grassland. They found that copper levels were raised appreciably by high nitrogen applications. The results of this present experiment confirm this view as to the effect of high levels of nitrogen application on the copper concentration of herbage. It would appear that this is a fairly general finding and the effect of nitrogen may be of more practical importance than that of copper sulphate application.

Experiment 2. The Effect of Liming on the Copper Concentration of Herbage.

Object This experiment was undertaken to determine the effect of soil applications of various amounts of ground limestone on the copper concentration of herbage over three growing seasons.

Layout/

Layout. The experimental design was a randomised block layout composed of 4 complete replicates of 5 different liming treatments. These were 0,1,2,3 and 4 tons of ground limestone per acre. The plot size, method of sampling and sward composition were all as described for Experiment 1. The site was immediately adjacent to that of Experiment 1 and the layout is illustrated in Figure 4.

Fig. 4. Layout of Plots of Experiment 2.

Plot Nos.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
ton ground limestone per acre.	4	1	2	3	0	2	3	1	4	0	3	0	4	1	2	2	0	4	1	3
Replicate			1					2					3					4		

Lime was applied at the appropriate rates on 22 March 1963 and a basal fertilizer treatment of 3 cwt. Nitro-chalk, 2 cwt. superphosphate and 1 cwt. muriate of potash was given at the same time. This NPK treatment was also given in March 1964 and 1965. In addition 3 cwt. of Nitro-chalk was applied after each cut of grass in 1963 and 1964. Only a single application was given in 1965. No further applications of lime were made after the initial dressing in 1963.

Soil samples (0 - 6 ins. depth) were taken from each plot with a screw type augur; 20 sub samples being combined from each plot. The initial mean soil pH in March 1963 was 5.80. The soil pH values of the samples taken in 1963, 64 and 65 are detailed in Table 15.

Table 15. Soil pH values of samples obtained in 1963, 64 and 65.

Lime tons/acre		0	1	2	3	4
August 1963						
	1	5.75	5.95	6.50	6.60	7.10
	2	5.80	6.05	6.40	6.55	7.05
	3	5.95	6.10	6.35	6.70	6.80
	4	5.70	6.10	6.45	6.80	6.75
September 1964						
	1	5.70	6.15	6.30	6.60	6.85
	2	5.90	6.20	6.50	6.40	7.00
	3	5.85	6.20	6.45	6.90	6.80
	4	5.70	6.10	6.30	6.40	6.60
March 1965						
	1	5.25	5.80	6.05	6.35	6.25
	2	5.70	6.05	6.20	6.30	6.55
	3	5.50	5.95	6.10	6.65	6.80
	4	6.10	6.05	6.10	6.30	6.60
Mean Values						
August 1963		5.80	6.05	6.43	6.66	6.93
September 1964		5.77	6.16	6.39	6.56	6.63
March 1965		5.64	5.96	6.11	6.40	6.55
Overall Mean		5.74	6.06	6.31	6.54	6.70

Over the 3 years the soil pH values ranged from about 5.75 for the control plots to values in the range 6.5 - 7.0 where as much as 4 tons per acre had been given. Successive increases in soil pH were found for each progressive increase in the amount of lime added. The plots, therefore, covered a wide range of soil pH at regularly spaced intervals.

Herbage Copper Concentrations 1963.

Three cuts of herbage were obtained in 1963. Each cut was taken when the grass was at a stage suitable for silage. The copper concentrations of all the herbage samples obtained during 1963 are detailed in Table 16.

During 1963 liming at rates of up to 4 tons per acre had no effect on herbage copper concentration at any of the 3 cuts obtained.

Table 16/

Table 16 Copper concentration (p.p.m. D.M.) of herbage after treatment with lime in the spring.

lime tons/acre		0	1	2	3	4
1st Cut; 4/6/63	1	11.9	10.6	11.7	10.3	10.0
	2	10.8	11.3	10.0	7.8	10.9
	3	11.4	9.5	10.0	9.2	9.5
	4	10.2	11.1	8.9	9.9	8.5
2nd Cut; 26/6/63	1	12.9	9.9	11.9	13.1	10.5
	2	12.5	12.5	11.5	11.9	13.1
	3	10.0	8.1	11.4	12.0	10.2
	4	12.0	11.7	12.2	12.0	11.7
3rd Cut; 2/8/63	1	10.6	10.2	9.9	8.9	9.5
	2	11.0	10.6	9.7	10.2	10.0
	3	11.4	10.6	10.6	10.8	10.8
	4	10.5	11.5	10.8	10.9	10.8
Mean values	1st cut	11.1	10.6	10.2	9.3	9.7
	2nd cut	11.9	10.6	11.8	12.3	11.4
	3rd cut	10.6	10.6	10.3	10.2	10.3
Overall Mean		11.2	10.6	10.8	10.6	10.5

Herbage/

Herbage Copper Concentration, 1964.

Three further cuts at the silage stage were obtained in 1964. In this year, however, only 3 of the replicates were used to obtain samples.

The herbage in the 5 plots in the first complete replicate was allowed to grow to maturity. Samples were obtained from this area at frequent intervals from May to August to determine the seasonal change in the copper content of the herbage. The results obtained will be presented immediately after those obtained for the other 3 replicates.

The copper concentration of the herbage from the 3 replicates at each of the 3 cuts taken during 1964 is presented in Table 17.

As in 1963, liming had no effect on the copper concentration of the herbage. The general level found for the copper concentration of the herbage in 1964 was much lower than that found in 1963. This may have been due in part to a severe frost in the winter of 1963 - 64 which killed some of the ryegrass and left cocksfoot as the dominant species.

Table 17/

Table 17 Copper Concentration (p.p.m. D.M.) of herbage which had been limed in the spring of the previous year.

lime tons/acre		0	1	2	3	4
1st cut; 2/6/64	2	4.9	7.2	7.0	5.2	7.7
	3	7.9	7.7	4.9	9.8	7.9
	4	8.8	7.9	5.3	7.3	4.4
2nd cut; 13/7/64	2	8.4	7.6	6.7	7.6	7.3
	3	8.1	6.9	7.5	6.7	8.2
	4	8.7	7.0	7.1	5.9	7.3
3rd Cut; 25/8/64	2	7.4	6.6	7.0	6.6	6.5
	3	6.3	6.0	7.1	7.4	7.7
	4	7.2	7.0	5.5	7.5	6.3
Mean Values						
First Cut		7.2	7.6	5.7	7.4	6.7
Second Cut		8.4	7.2	7.1	6.7	7.6
Third Cut		7.0	7.2	7.1	7.2	6.8
Overall Mean		7.5	7.3	6.6	7.1	7.0

Changes in copper concentration with advancing herbage maturity.

As already stated in Experiment 2 the first replicate of that experiment was allowed to grow to maturity during 1964. Samples were obtained from the herbage, which was mainly cocksfoot, at regular intervals during the growing season. These samples were analysed for copper, crude protein and crude fibre content. The results of these analyses are presented in Table 18.

Table 18/

Table 18 The copper, crude protein and crude fibre contents of herbage growing to maturity.

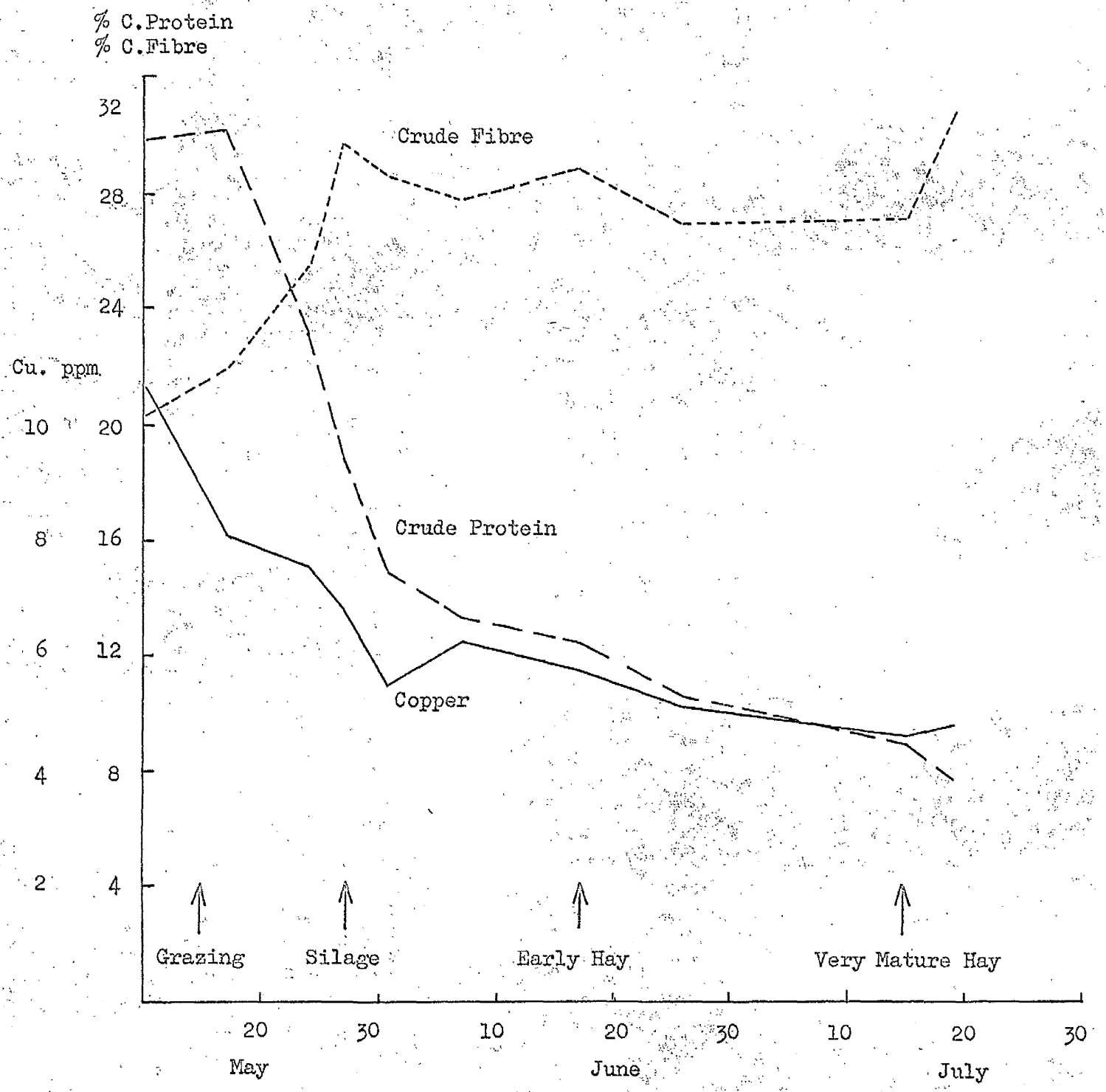
Date	Growth Stage	Cu(p.p.m.)	Crude Protein(%)	Crude Fibre (%)
10 May	4 ins.	10.6	29.9	20.2
17	6 - 9 "	8.1	30.1	21.9
24	12 - 18 "	7.5	23.1	25.4
27	Ear emergence	6.8	18.8	29.7
31	In flower	5.4	14.9	28.6
7 June		6.2	13.3	27.8
17	Progressively	5.7	12.4	28.8
26	More mature	5.1	10.5	26.9
15 July		4.6	9.0	27.1
19		4.8	7.7	30.7

These results are presented graphically in Figure 5. This shows the progressive fall in the copper concentration of the herbage which is paralleled by a fall in the crude protein content as it becomes more mature. There is also an inverse relationship between these two and the increasing crude fibre content.

Herbage Copper Concentration, 1965.

In the investigations carried out in 1964 (reported on page 101) to determine the effect of plant maturity on copper concentration it was found that there was a progressive reduction in herbage copper concentration with advancing maturity. This finding led to a change in cutting technique during 1965. The plots were divided/

Fig.5. Changes in the copper, crude protein and crude fibre contents of herbage growing to maturity.



divided along their length into 3 equal parts; the first part was cut at the grazing stage (12 May), the second at the silage stage at ear emergence (27 May) and the third at the mature hay stage (22 June). As in 1964 only 3 replicates of each of the 5 treatments were available. The copper concentrations of the herbage samples obtained at each of the 3 cuts are detailed in Table 19.

Table 19 Copper concentration (p.p.m. D.M.) of herbage growing to maturity from plots limed 2 years previously.

lime tons/acre		0	1	2	3	4	
1st Cut: 12/5/65		10.6	11.2	9.6	9.0	9.8	
		11.0	10.7	11.1	11.4	8.4	
		12.0	12.0	9.5	10.1	11.8	
2nd Cut: 27/5/65		8.3	7.3	7.7	7.7	6.9	
		7.2	8.0	6.7	6.5	6.8	
		7.6	7.8	6.8	7.5	7.6	
3rd Cut: 22/6/65		5.3	5.2	4.8	5.8	5.5	
		5.8	6.4	5.5	5.2	4.8	
		5.5	6.0	5.3	5.7	4.8	
							Mean
Mean Values	1st Cut	11.2	11.3	10.1	10.2	10.0	10.6
	2nd Cut	7.7	7.7	7.1	7.2	7.1	7.4
	3rd Cut	5.5	5.9	5.2	5.6	5.0	5.5
Overall Mean		8.1	8.3	7.5	7.7	7.4	7.8

Liming again had no effect on the mean herbage copper concentrations. There was, however, a highly significant (P = 0.01) depression in herbage copper/

copper concentration at each growth stage (See Fig 6).

Crude protein was determined on all the grass samples obtained in 1965. The mean results are listed in Table 20.

Table 20. % Crude protein in grass samples taken in 1965 from plots limed in 1963

lime tons/acre	0	1	2	3	4	Mean
Grazing stage	28.6	28.7	29.0	28.9	29.5	28.9
Silage stage	16.9	17.0	17.5	17.1	17.2	17.2
Mature stage	8.5	9.1	8.1	9.0	9.2	8.8

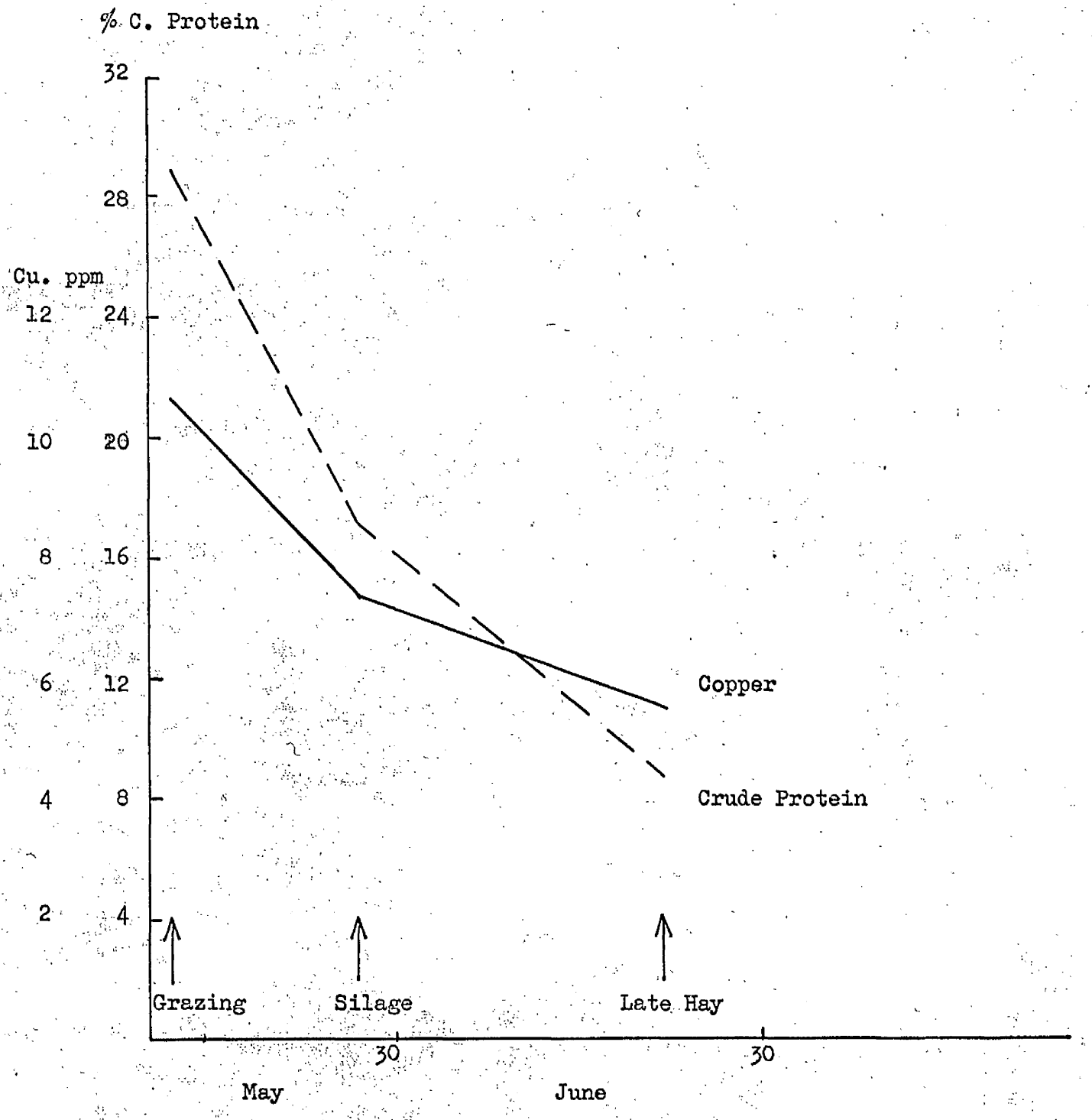
Liming did not influence the mean crude protein content (see Fig 6). There was the normal fall in the crude protein content of the herbage from the grazing to the silage stage and similarly from the silage to the mature hay stage. These falls were highly significant ($P < 0.01$). These results, therefore, confirm the preliminary observation of a reduction in herbage copper with advancing maturity which was made in 1964.

Table 21 summarises the overall mean copper concentrations of the nine separate cuts of herbage, from each treatment, taken during the 3 years. It also lists the mean soil pH values for each treatment over the 3 year period.

Table 21. Mean soil pH and herbage copper concentration, 1963 - 65 (9 cuts of herbage)

lime tons/acre	0	1	2	3	4
Mean soil pH.	5.74	6.06	6.31	6.54	6.70
Mean Cu conc. (p.p.m.)	8.96	8.63	8.22	8.46	8.29

Fig 6. Changes in the mean copper and crude protein contents of herbage growing to maturity on limed plots.



Liming at rates of up to 4 tons per acre effected marked and progressive increases in soil pH so that during the course of the experiment a wide range of soil pH, from 5.5 - 7.0, was covered. Despite this, however, there were no significant differences in herbage copper concentration between any two of the 5 different liming treatments. In this experiment, therefore, liming at rates of up to 4 tons per acre did not influence the herbage copper concentration.

Experiment 3 The Relationship between the Copper and Crude Protein Concentrations in Growing Herbage.

The consistent finding that herbage copper concentrations decreased with advancing plant maturity and also that there was a parallel fall in crude protein content led to an attempt to investigate whether there was a direct relationship between the copper and crude protein contents of herbage. Two experiments were conducted in 1965 and 1966 to investigate this.

1965 Experiment.

A randomised block layout was again used and consisted of 4 complete replicates of 5 different nitrogen treatments. The treatments were 0, 25, 50, 75 and 100 lbs. N per acre applied as Nitro-chalk on 30 March 1965. The first 25 lbs. of N was applied in a compound fertilizer which also supplied a basal dressing of phosphate and potash. Phosphate and potash only were applied to those plots which received no nitrogenous fertilizer.

The individual plots on this occasion were 1/200th acre being 61.5 feet long and 3.5 feet broad. They were divided along their length into 3 equal parts Herbage samples were obtained from the first part at the grazing stage, from the second part at the silage stage and from the third part at the mature hay stage. The fertilizer treatment applied to the individual plots is shown in Fig. 7.

Fig. 7/

Fig. 7 Layout of plots of Experiment 3 (1965)

Plot No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
lb N/ac.	75	50	0	100	25	50	100	75	0	25	100	0	75	25	50	25	100	50	0	75
Replicate	1					2					3					4				

Cut 1 - gra

Cut 2 - sil

Cut 3 - Hay

Unfortunately shortly after the first cut of herbage was obtained a uniform dressing of Nitro-chalk (supplying 25 lbs. N per acre) was applied inadvertently to the whole area covered by the plots. This almost certainly resulted in a levelling out of the crude protein contents of the variously treated plots. The copper and crude protein contents of the variously treated plots are detailed in Table 22.

Despite the inadvertent addition of fertilizer after the initial cut it was still possible to correlate the copper and crude protein contents of the herbage. However, the results of this experiment are not described here but will be deferred to page 113 until the results of a similar trial carried out in 1966 are presented when both sets of results can be examined together.

Table 22/

Table 22. Cu concentration (p.p.m.) and crude protein content (%) of herbage samples from N treated plots.

		Copper Concentration					Crude Protein Content				
lbs. N/acre		0	25	50	75	100	0	25	50	75	100
Cut 1 - grazing stage	1	9.3	9.2	9.7	12.0	10.8	18.5	20.6	23.2	26.5	26.7
	2	7.5	9.8	13.3	9.7	10.5	16.6	22.1	24.0	26.4	26.7
	3	9.4	7.8	9.5	9.9	8.1	19.2	19.3	21.5	25.0	26.2
	4	6.7	7.9	7.4	9.9	11.5	16.3	18.3	22.9	25.8	27.0
Cut 2 - silage stage	1	6.6	6.4	6.4	7.1	7.9	16.3	12.6	14.4	17.9	18.2
	2	5.8	7.2	7.2	6.8	7.6	18.1	16.9	13.7	14.8	14.4
	3	6.0	5.6	5.0	7.0	6.5	17.6	15.3	17.1	15.6	16.4
	4	5.9	5.0	6.2	6.7	6.7	13.1	13.8	12.6	14.4	15.0
Cut 3 - mature hay	1	7.0	7.1	6.3	7.6	8.0	10.9	11.8	11.7	13.4	11.1
	2	8.0	6.6	7.3	6.5	7.5	13.3	11.7	11.3	13.0	11.0
	3	6.7	6.5	7.9	6.5	8.5	13.0	12.6	13.3	14.7	13.9
	4	5.0	5.7	5.3	5.6	5.4	9.4	11.3	8.9	10.6	10.8
Mean Values 1st Cut		8.2	8.7	10.0	10.4	10.2	17.7	20.1	22.9	25.9	26.7
2nd Cut		6.1	6.1	6.2	6.9	7.2	16.3	14.7	14.5	15.7	16.0
3rd Cut		6.7	6.5	6.7	6.6	7.4	11.7	11.9	11.3	12.9	11.7
Overall Mean		7.0	7.1	7.6	7.9	8.3	15.2	15.5	16.2	18.2	18.1

1966 Experiment/

1966 Experiment.

In the 1966 trial only 4 different N treatments were used. These were 0, 25, 50 and 100 lbs. N/acre. The layout of the individual plots, each of 1/200th acre, is shown in Fig. 8.

Fig. 8 Layout of Plots of Experiment 3, 1966.

Plot No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
N lbs/acre	25	50	0	100	50	25	100	0	25	100	0	50	0	25	100	50
Replicate	1				2				3				4			

Cut 1. Grazing.
Cut 2. Silage
Cut 3. Hay.

The plots were again divided along their length into 3 equal parts. Herbage samples were obtained from the first part at the grazing stage (23 May), from the second part at the silage stage (13 June) and from the third part at the mature hay stage (4 July). The copper and crude protein contents of the variously treated plots are detailed in Table 23.

Table 23/

Table 23. Copper concentration and crude protein content of herbage from N treated plots.

		Cu concentration (p.p.m.)				Crude protein content (%)			
Cut 1 - Grazing stage		0	25	50	100	0	25	50	100
	1	7.3	9.2	10.1	10.5	19.4	27.2	31.8	33.4
	2	8.9	9.8	9.6	10.3	21.1	24.6	29.6	33.2
	3	8.3	9.5	10.6	10.5	20.6	26.1	29.7	33.1
	4	8.2	9.0	8.6	10.3	18.5	26.6	30.6	32.5
Cut 2 - Silage stage.									
	1	5.2	9.8	8.1	9.7	13.0	14.0	20.5	24.1
	2	7.9	9.3	7.7	12.6	15.8	14.4	17.6	24.2
	3	7.7	6.8	8.9	6.1	13.9	13.8	18.8	21.7
	4	5.9	7.7	10.6	8.4	12.5	14.5	17.6	20.4
Cut 3 - Mature Hay									
	1	5.8	6.6	6.9	6.5	10.5	11.6	12.4	15.7
	2	5.5	6.0	5.9	6.0	11.8	12.4	13.7	18.4
	3	6.0	7.0	6.8	6.8	12.3	11.4	12.5	18.5
	4	5.4	6.3	6.0	6.4	10.2	11.5	11.1	15.6
Mean Values	1st Cut	8.2	9.4	9.7	10.4	19.9	26.1	30.4	33.1
	2nd Cut	6.7	8.4	8.8	9.2	13.8	14.2	18.6	22.6
	3rd Cut	5.7	6.5	6.4	6.4	11.2	11.7	12.4	17.1
Overall Mean		6.8	8.1	8.3	8.7	15.0	17.3	20.5	24.2

In the 1965 trial those plots which had received a dressing of 50, 75 and 100 lbs. of N per acre had much higher mean copper concentrations (10.0, 10.4 and 10.2 p.p.m. respectively) at the first cut than those of the plots which had received 0 or 25 lbs. N per acre (8.2 and 8.7 p.p.m. respectively). The crude protein contents of the herbage increased progressively with each treatment from 16.3% for the untreated plots to 27.0% for those plots which had been given 100 lbs. N per acre. No comparisons could be made of the effect of the nitrogen treatment at the next 2 cuts due to the inadvertent and indiscriminate addition of nitrogenous fertilizer to all the plots.

At the first 2 cuts taken in 1966 the mean copper concentrations of the variously treated plots increased progressively with each progressive increase in the amount of nitrogen applied. This was also true of the crude protein contents. The results of the 1966 experiment confirmed the finding (already reported in Experiment 2), that the copper concentration of herbage declined progressively as the herbage matured. This was again paralleled by a reduction in the protein content of the herbage with maturity (see Fig. 9).

No such general trend could be deduced from the results of the 1965 trial. Nevertheless the copper concentrations of the second and third cuts in 1965 were appreciably lower than those found for the initial sample taken at the grazing stage (Table 22).

Fig. 10 shows the relationship between the copper concentrations of the 108 herbage/

herbage samples, obtained from the N treated plots in 1965 and 66, and their crude protein contents. A regression coefficient was calculated and this was found to be highly significant ($P < 0.0001$) thus proving that there was a concomitant decline in both the copper concentration and the crude protein content of the herbage samples examined.

Fig 9. Changes in the mean copper and crude protein contents of herbage from nitrogen-treated plots growing to maturity.

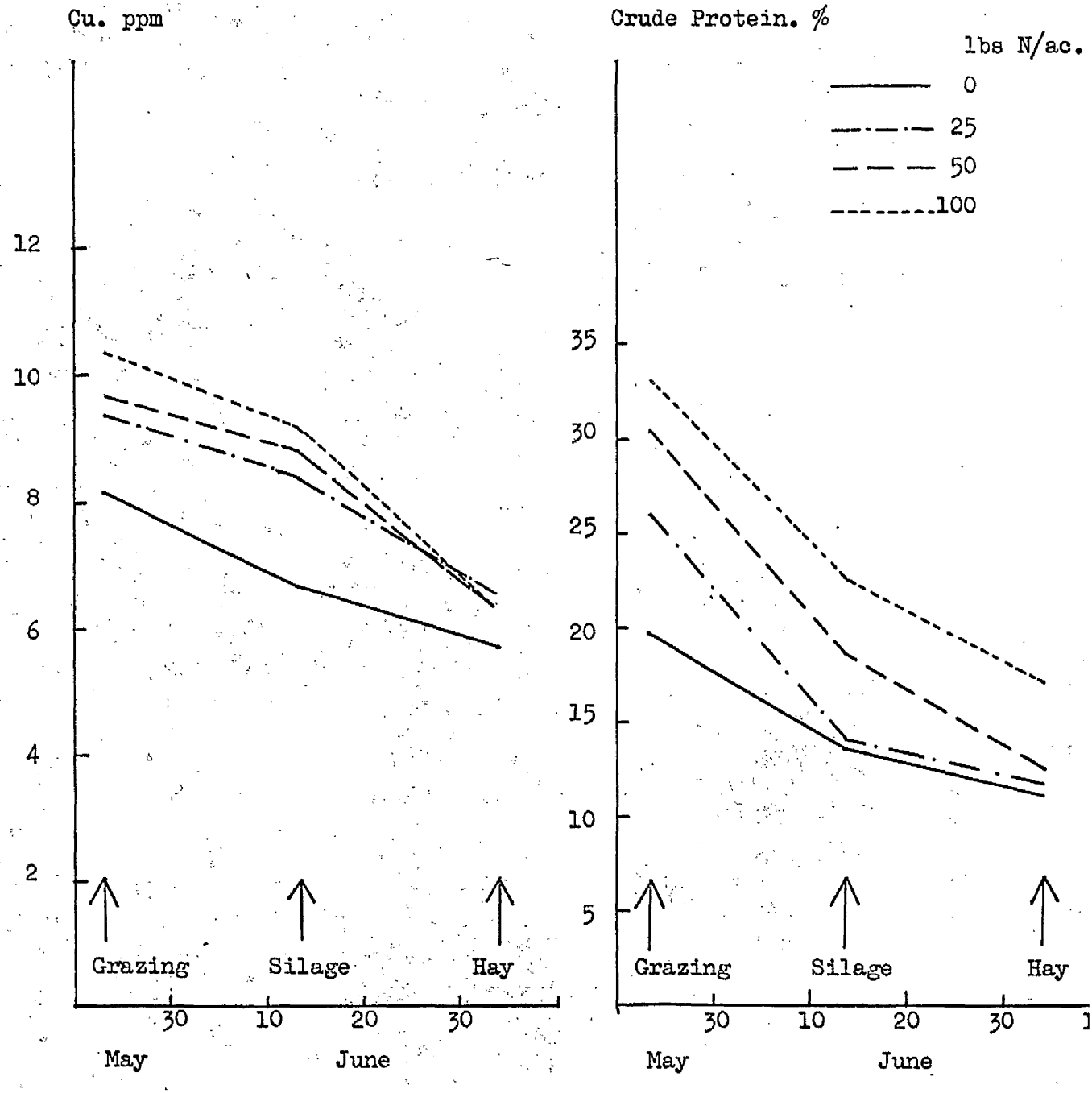
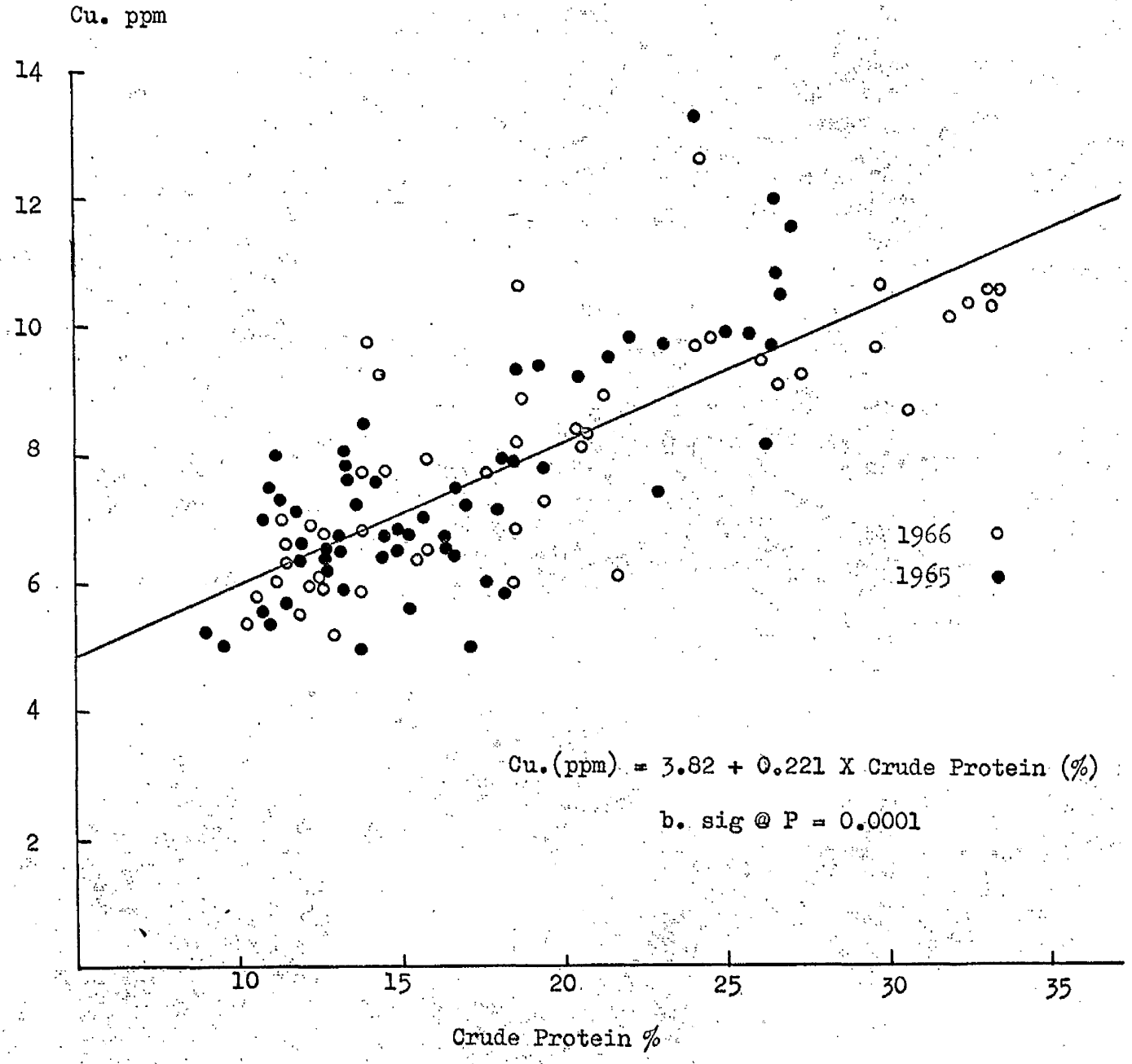


Fig 10. The relationship between the copper and protein contents of 108 herbage samples.



Discussion of Results from Experiments 1 - 3.

Applications of up to 20 lbs. copper sulphate per acre produced no persistent elevation in herbage copper concentrations. Indeed in only a small proportion of the treated plots was there even a temporary increase in herbage copper concentration due to the treatments given. These findings are in agreement with those of Morgan and Clegg (1958) who also found only transitory increases in herbage copper content due to the application of a copperised fertilizer. Morgan and Clegg, however, conducted their trial on a damp, acid, peaty soil typical of those areas where copper deficiency in plants may be found. The preser experiments were undertaken on a heavy loam soil.

These results appear to be in direct contrast to those of trials conducted in the North of Scotland by Mitchell et al. (1957a) and Reith and Mitchell (1964) who reported persistent increases in herbage copper concentrations following soil treatment with copper sulphate. However, the increases recorded by these workers were largely attributable to the marked response by red clover to treatment with copper sulphate. Increases in the order of 500% were recorded. They recorded only very small increases in the copper content of the grass species analysed. There was initially only a very small proportion of clover ($\leq 5\%$) and eventually practically none in the herbage on the plots used for this present experiment. This difference in the composition of the sward between the plots used in the present experiment and those used by Mitchell et al. almost certainly explains the difference in response to the application of copper sulphate.

Applications/

Applications of high levels of nitrogen fertilizers invariably increased herbage copper concentrations at early growth stages. This was a consistent finding in each of the years 1963, 64 and 66 and confirms that of Hemingway (1962). Arnon (1949) has reported that copper is concentrated in the chloroplasts of green leaves and as nitrogen applications increase the proportion of leaf in herbage this probably accounts for the elevation in copper content following nitrogen treatment of the herbage.

Liming at rates of up to 4 tons / acre was found to have no effect on herbage copper concentrations under the conditions of the present experiment covering 3 growing seasons. This finding is in substantial agreement with those of Stewart (1951) and Barlow et al.(1960) who reported no correlation between soil pH and herbage copper concentrations. Mitchell et al.(1957a) and Reith and Mitchell (1964) reported that liming depressed herbage copper concentration but recorded only very small decreases (from 0.5 - 1.2 p.p.m.). These did not occur consistently but were more likely to occur on copper deficient soils.

The copper concentration of herbage was found to decline with plant maturity. This finding which was made initially in 1964 and later confirmed in 1965 and 1966 has also been recorded by Fleming (1965) and Kirchgessner (1965). This decline in herbage copper concentration was found to parallel that for the crude/

crude protein content of herbage and a highly significant relationship was found between herbage copper concentrations and crude protein contents. This relationship is of importance in agricultural practice in that the longer the delay in cutting herbage for hay and silage the lower will be their protein and copper contents. The decrease in the crude protein content of herbage with age is a well known fact but the link between this and the copper concentration of herbage has not previously been identified. An essentially similar relationship between the copper concentration of a wide range of herbage species and their true protein content has been recorded in a very recent publication (Rasheed and Seeley 1966). The importance of this relationship with regard to silage and hay prompted a survey of the protein and copper concentrations in a large number of hay and silage samples grown in Scotland.

A Survey of the Copper, Protein and Calcium Concentrations in Hay and Silage Grown in Scotland.

It is evident from the work reported in Experiments 2 and 3 that with advancing herbage maturity there are depressions in both the copper and protein concentrations in herbage. There is also evidence to suggest that applications of nitrogenous fertilizer may increase copper concentrations. Experiment 2 did not confirm the existence of any relationship between liming and copper concentrations. However, in view of the widespread belief that under practical farming conditions the use of lime increases the incidence of swayback it was thought that a possible relationship between copper and calcium might exist if a wide range of herbage samples were examined. Accordingly, it was decided to determine the copper, protein and calcium concentration in a wide range of hay and/

and silage samples grown in Scotland.

This survey was carried out for two additional reasons. Firstly it was done to fill in a gap in the existing knowledge of the overall picture in connection with the adequacy or otherwise of the amount of copper supplied in the staple diet of a large proportion of the farm livestock in this country. Secondly, to assess whether the recent A.R.C. (1965) recommendation of minimum dietary levels of 5 and 10 p.p.m. Cu for sheep and cattle respectively can be readily achieved on diets consisting largely of hay or silage. This is especially important in self-feed silage systems where little supplementary concentrate feed is offered.

Ashton and Morgan (1952) examined the mineral composition of 45 samples of meadow hay and 55 samples of seeds hay grown in Wales. Their results were as follows:-

	No. of samples	% Crude protein			Cu (p.p.m.)			
		mean		S.D.	mean		S.D.	range.
Meadow Hay	45	9.6	±	1.52	7.4	±	2.2	(4.0 - 1
Seeds Hay	55	9.0	±	2.11	6.3	±	1.7	(2.1 - 1

Although/

Although significant differences were not obtained there was some tendency for seeds hay, which surprisingly had a rather lower protein content than meadow hay, also to contain rather less copper. They recorded that 24% of the samples of meadow hay and 45% of the samples of seeds hay contained less than 6.0 p.p.m. Cu.

Kirchgessner (1965) has recently reported the results of analyses of more th 200 samples of meadow hay grown in Germany. 3% of the samples were found to contain less than 5 p.p.m. Cu and a further 85% contained between 5 and 10 p.p.m. Cu.

The samples obtained in the present work were those submitted to a laboratory which undertakes a wide range of analyses for advisory purposes. 125 hay and 139 silage samples from one winter season and representing farms from all over Scotland were collected. They were, however, more in the nature of a fortuitous selection rather than of a true survey. These samples were analysed for copper, calcium and crude protein.

Tables 24 and 25 present the copper, calcium and crude protein contents of 125 hay and 139 silage samples respectively.

Table 24. The copper, calcium and crude protein contents of 125 hay samples

Cu p.p.m.	Crude protein (%)	Ca(%)	Cu p.p.m.	Crude protein (%)	Ca(%)	Cu p.p.m.	Crude protein (%)	Ca(%)	Cu p.p.m.	Crude protein (%)	Ca (%
8.8	7.4	0.43	6.4	3.8	0.51	6.4	7.0	0.42	7.6	8.6	0.33
7.2	7.8	0.35	4.8	4.2	0.33	9.4	4.6	0.52	8.0	8.0	0.35
7.5	7.2	0.22	5.3	6.6	0.75	10.0	6.0	0.42	6.5	7.5	0.35
5.6	5.2	0.25	5.3	4.6	0.54	11.2	4.9	0.52	5.2	3.6	0.35
8.0	6.8	0.34	6.7	6.4	0.44	5.3	6.4	0.48	7.6	8.2	0.48
11.4	7.2	0.29	6.6	9.2	0.49	3.2	4.0	0.44	7.3	4.1	0.26
14.9	9.4	0.30	6.4	7.2	0.40	6.7	9.8	0.88	9.7	10.2	0.30
10.3	5.4	0.21	5.5	6.2	0.52	3.3	4.7	0.46	4.8	7.2	0.39
9.6	7.8	0.22	4.3	5.8	0.48	6.1	6.4	0.48	9.6	4.1	0.20
9.7	7.0	0.32	7.0	5.4	0.70	8.4	5.0	0.68	15.8	4.4	0.21
7.8	6.6	0.21	5.5	5.6	0.50	6.4	6.0	0.42	6.3	4.1	0.54
6.4	6.0	0.21	4.7	5.0	0.37	8.4	6.6	0.56	6.7	4.2	0.58
9.2	6.2	0.32	6.5	6.6	0.56	7.8	6.4	0.35	6.3	4.6	0.67
14.2	5.6	0.31	5.5	3.8	0.46	4.9	8.2	0.90	6.1	5.4	0.61
3.2	6.8	0.53	3.9	5.0	0.37	5.5	6.6	0.72	11.5	6.6	0.30
8.6	6.2	0.73	4.2	3.6	0.30	7.0	5.6	0.75	11.6	9.6	0.63
9.5	5.4	0.42	6.8	6.0	0.33	6.4	4.4	0.37	4.6	3.3	0.46
7.8	10.9	0.54	8.1	6.6	0.44	5.0	4.6	0.35	10.5	8.5	0.46
6.4	5.2	0.40	5.0	5.8	0.35	3.0	3.0	0.32	9.1	5.6	0.51
6.1	4.8	0.50	7.0	6.0	0.54	5.4	5.2	0.42	8.8	7.5	0.58
5.5	4.6	0.44	7.8	4.6	0.40	3.5	3.6	0.40	8.3	5.6	0.54
6.9	5.0	0.63	4.9	6.2	0.25	5.2	5.9	0.51	6.4	5.4	0.63
4.7	6.4	0.56	6.4	6.0	0.56	5.6	5.0	0.37	5.4	4.1	0.44
5.4	6.6	0.31	5.9	7.9	0.52	6.3	6.6	0.65	8.0	8.6	0.65
5.5	8.0	0.46	7.5	8.4	0.75	6.0	5.7	0.34	6.7	5.6	0.58
4.2	6.6	0.50	4.8	6.6	0.42	5.8	4.2	0.37	7.8	7.2	0.47
3.0	5.6	0.57	7.9	10.2	0.34	5.5	5.8	0.51	10.0	4.6	0.47
6.4	6.0	1.08	4.7	4.2	0.40	7.7	9.5	0.48	9.9	13.3	0.99
9.9	6.6	0.43	6.6	10.8	0.35	4.8	5.5	0.37	16.0	10.6	0.54
5.5	7.4	0.71	7.5	6.0	0.48	4.8	3.8	0.30	9.4	9.1	0.67
3.9	4.0	0.50	12.6	10.6	0.46	7.8	6.2	0.47	6.0	9.1	0.61
7.0	7.0	0.55									

Table 25 The copper, calcium and crude protein contents of 139 silage samples

Cu. p.p.m.	Crude protein (%)	Ca(%)	Cu. p.p.m.	Crude protein (%)	Ca(%)	Cu. p.p.m.	Crude protein (%)	Ca(%)	Cu p.p.m.	Crude protein (%)	Ca(%
8.8	9.0	0.39	10.5	11.4	0.61	14.4	13.8	0.72	7.4	5.2	0.50
4.7	7.2	0.33	6.9	7.8	0.55	9.6	10.8	0.40	12.7	16.0	0.44
8.1	15.2	0.33	11.1	9.2	0.59	10.4	12.2	1.02	5.1	5.5	0.33
10.6	8.0	0.22	14.8	8.4	0.51	9.0	7.0	0.57	7.1	4.5	0.38
9.9	7.6	0.36	15.4	12.6	0.76	11.9	8.6	0.59	7.9	6.0	0.61
10.4	10.2	0.94	8.8	7.6	0.61	10.0	13.4	0.55	5.1	5.0	0.61
18.0	11.2	0.59	7.6	9.8	0.69	8.3	10.6	0.55	19.3	16.1	0.74
14.5	10.2	0.82	10.0	10.4	0.80	5.3	8.6	0.53	8.5	12.0	1.03
8.3	9.2	0.53	7.8	9.6	0.66	7.6	8.8	0.45	9.4	9.5	0.55
17.1	11.8	0.82	9.8	11.0	0.47	9.9	8.2	0.61	8.5	8.0	0.66
6.4	7.8	0.45	11.3	13.2	0.30	9.6	10.2	0.61	9.8	9.0	0.55
5.9	11.0	0.55	8.3	12.2	0.58	9.1	8.6	0.76	14.3	15.1	0.69
9.9	11.4	0.90	6.6	7.4	0.45	5.5	8.8	0.53	11.5	12.0	0.77
8.5	8.0	0.36	9.1	12.6	0.80	8.8	7.6	1.28	14.3	15.1	0.68
8.5	9.2	0.55	9.0	11.2	0.57	8.1	11.6	0.64	6.6	6.4	0.70
9.6	8.8	0.49	9.3	14.2	0.41	8.0	11.8	0.69	10.5	12.0	0.57
11.5	12.6	0.72	9.1	11.8	0.94	6.4	8.6	0.70	6.2	7.1	1.75
11.9	12.4	0.72	9.3	10.0	0.61	11.5	8.8	0.42	11.9	13.5	0.76
7.8	8.4	0.49	4.9	4.4	0.36	5.5	9.4	0.64	6.2	6.8	0.65
6.8	7.8	0.62	12.8	11.6	0.86	7.3	10.0	0.57	10.6	13.0	0.61
10.5	7.8	0.31	6.9	8.4	0.47	7.5	8.8	0.82	11.3	9.0	0.57
10.9	10.8	0.78	8.5	9.4	0.49	6.8	9.2	0.84	6.3	11.6	0.83
10.3	8.8	0.59	10.5	10.0	0.80	6.9	10.6	0.84	17.5	13.0	0.55
10.3	10.2	0.44	7.1	5.5	0.86	6.8	8.0	0.51	7.6	8.0	0.59
5.0	7.8	0.72	8.8	10.0	0.68	9.5	9.2	0.65	10.0	7.1	1.44
18.6	9.6	0.47	7.9	9.8	0.68	16.1	12.2	0.76	7.3	6.5	0.72
13.3	10.2	0.36	8.3	12.4	1.04	10.5	10.5	0.48	13.9	10.1	0.84
7.6	9.6	0.45	10.5	9.0	1.15	10.9	12.0	0.26	9.5	7.5	0.63
10.6	8.2	0.59	10.0	12.4	0.98	8.5	8.2	0.40	11.1	10.3	0.55
8.1	11.6	0.62	11.1	8.3	0.74	7.8	6.5	0.61	10.0	13.0	0.61
12.0	14.0	0.40	11.5	14.5	0.48	6.2	4.5	0.59	10.0	12.0	0.46
9.0	8.1	0.48	10.8	10.2	0.87	7.1	7.5	1.20	7.4	6.1	0.60
4.5	4.7	0.47	8.1	5.75	10.55	16.1	18.10	1.40	9.9	6.79	0.49
9.0	10.8	0.49	7.4	5.0	0.45	12.9	10.5	0.72	14.3	13.2	0.66
9.0	5.1	0.41	12.3	9.8	0.66	8.8	6.5	0.47			

The % crude protein was taken as a measure of maturity. Crude protein content is a rather better indicator of advancing maturity than crude fibre content as the range of values found for the latter quantity is not so wide as for crude protein.

The percentage distribution of hay and silage samples within a range of ol p.p.m. Cu from 3 - 20 p.p.m. is presented in Table 26.

Table 26. Copper concentration (p.p.m.) in 125 samples of hay and 139 samples of silage grown in Scotland.

Copper p.p.m. in D.M.	% of samples 125 Hay Samples		139 Silage Samples		264 Hay & Silage Samples.	
3.0 - 3.9	6.4	17.6	0.0	2.2	3.0	9.5
4.0 - 4.9	11.2		2.2		6.5	
5.0 - 5.9	17.6		5.0		11.0	
6.0 - 6.9	22.4		10.0		16.0	
7.0 - 7.9	14.4	72.0	13.0	59.2	13.6	65.3
8.0 - 8.9	8.8		14.5		11.8	
9.0 - 9.9	8.8		16.6		12.9	
10.0 - 10.9	3.2		15.9		9.9	
11.0 - 11.9	3.2		7.9		5.7	
12.0 - 12.9	0.8	8.8	3.6	33.1	2.3	21.6
13.0 - 13.9	0.0		1.4		0.7	
14.0 - 14.9	1.6		4.3		3.0	
15.0 - 15.9	0.8		0.7		0.7	
16.0 - 16.9	0.8		1.4		1.2	
17.0 - 17.9	0.0	1.6	1.4	5.6	0.7	3.6
18.0 - 18.9	0.0		1.4		0.7	
19.0 - 19.9	0.0		0.7		0.3	
Mean	7.06		9.61		8.40	
S. Dev.	± 2.49		± 2.95		± 2.73	

The mean concentration of copper in the 125 samples of hay was 7.06 ± 2.49 p.p.m. This was significantly ($P < 0.001$) less than the mean copper concentration in the 139 samples of silage (9.61 ± 2.95). 17.6% of the hay samples, but only 2.2% of the silage samples contained less than 5.0 p.p.m. Cu. A further 72.0% of the hay samples and 59.2% of the silage samples contained between 5 and 10 p.p.m. Cu. Only a small proportion of hay (1.6%) and silage (5.6%) samples contained over 15 p.p.m. Cu, and none had more than 20 p.p.m.

Figure 11 shows the relationship between the crude protein and copper concentrations of the hay and silage samples. There were highly significant ($P < 0.001$) correlations between the concentration of copper and the crude protein content in both the hay and silage samples. Regression equations were calculated for the decline in copper content with that in crude protein content. These were:-

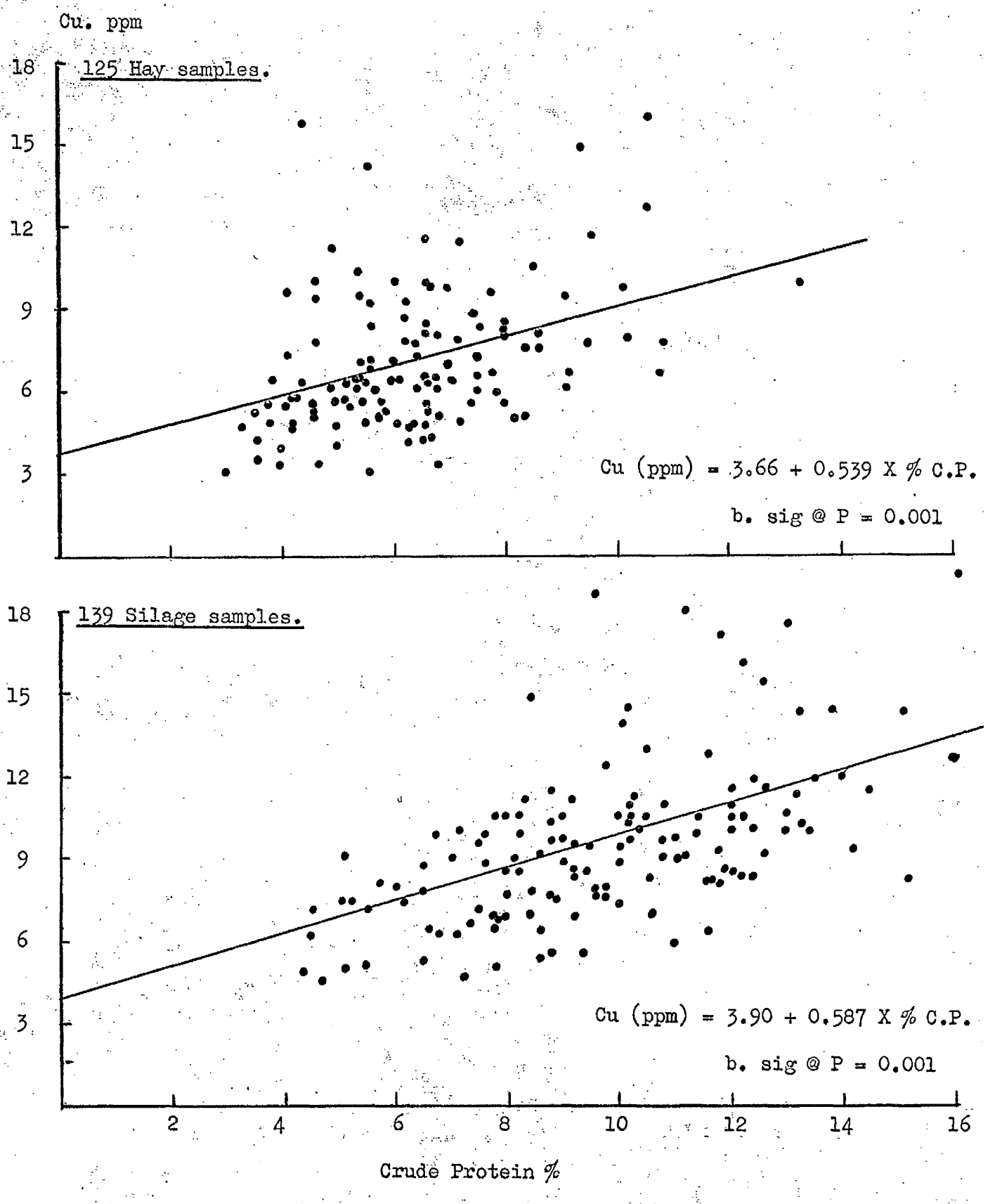
Hay Copper (p.p.m.) = 3.66 + 0.539 x Crude Protein% (t = 4.95)
Mean Crude Protein = 6.31% ± 1.89.

Silage Copper (p.p.m.) = 3.90 + 0.587 x Crude Protein % (t = 7.40)
Mean Crude Protein = 9.73% ± 2.68.

These two relationships are very similar and there was no significant difference between the two regression coefficients. It may, therefore, be considered that as herbage matures from the "early silage" to the "late hay" stage there is a continuous decline in both crude protein and copper concentration. The mean concentration of calcium for the hay samples (0.47% ± 0.166%) was significantly less (P 0.001) than that for the silage samples (0.63% ± 0.19

No/

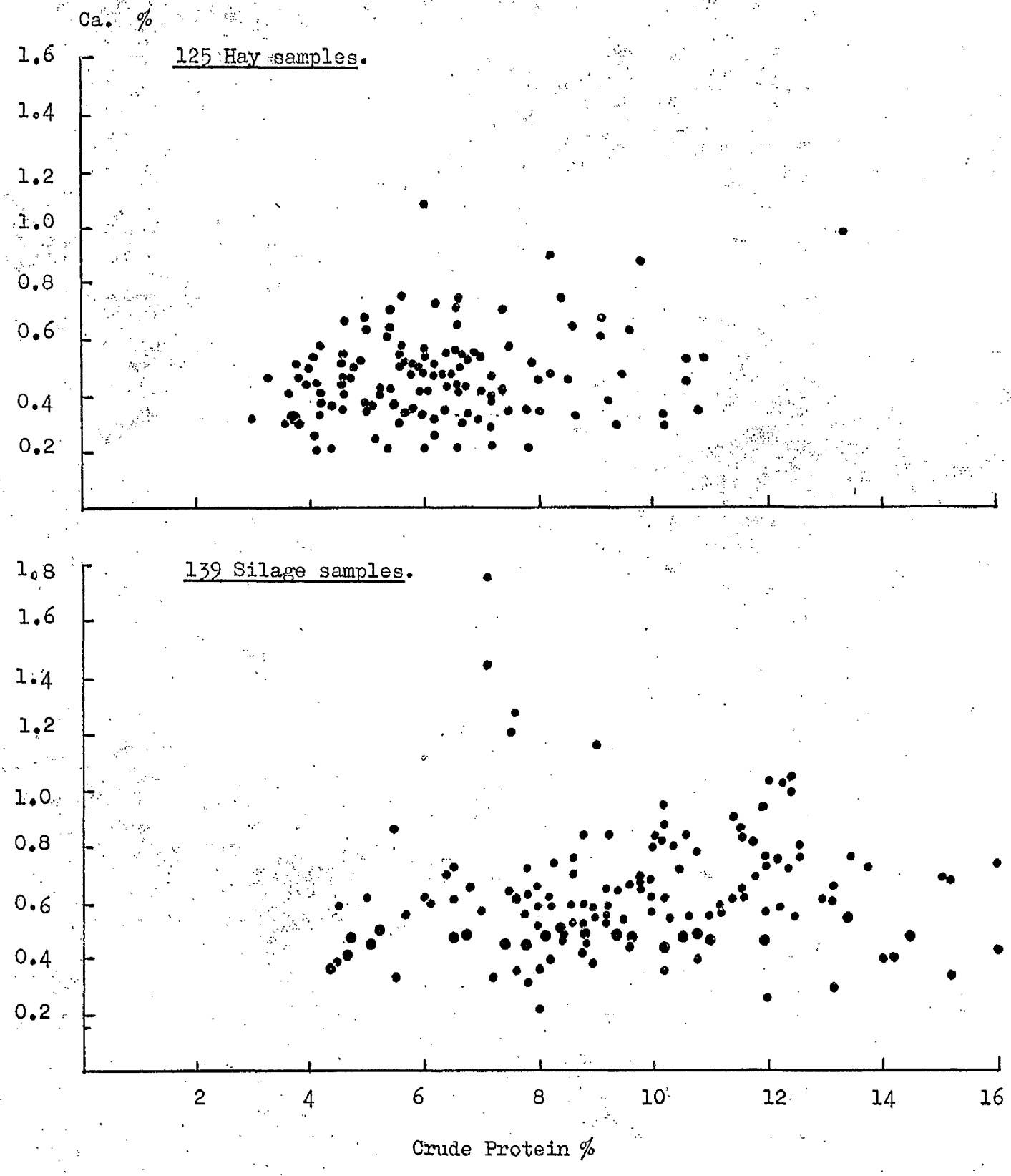
Fig 11. The relationship between the copper and crude protein contents of 125 hay and 139 silage samples.



No correlation was found between the copper and calcium concentrations in either the hay or silage samples (Fig. 12).

On the basis of the results found in this survey, therefore, livestock which are being fed on diets composed largely of hay or silage and particularly when these are made from mature herbage will in a large proportion of cases be consuming a diet which does not meet the recent recommendation of the A.R.C. in respect of its total copper content. Only 25% of all the hay and silage samples examined had more than 10 p.p.m. Cu which is the minimum dietary level recommended for cattle.

Fig 12. The relationship between the calcium and crude protein contents of 125 hay and 139 silage samples.



Section III. Sheep at Pasture

The copper status of sheep as affected by

1) Pasture top dressing with copper sulphate and nitrogenous fertilizer.

2) Liming.

3) Oral administration of various copper compounds.

4) Copper injections.

Experiments (1 - 3) described in Section II were concerned with the effects of lime, nitrogen and copper sulphate on the copper status of herbage cut generally at the silage stage. The effects of these materials on the composition of herbage at the grazing stage may be rather different. There is also the other possible factor of a change in the availability to the animal of the copper of differently treated herbage.

The most useful measurements which can be made in this direction are changes in the concentration of copper in the blood and liver of grazing animals. Sheep have been used in the present work to allow for the use of sufficiently large numbers of animals to enable the results achieved to be examined statistically. A number of experiments (described in this present section) have been designed to measure the storage of copper in sheep grazing herbage treated with varying levels of Nitrogen, lime and copper.

Experiment 4. The Effect on the copper status of grazing sheep of pasture treatment with copper sulphate and N fertilizer.

This experiment was designed to study the effect on the blood and liver copper status of sheep of

a) top dressing a pasture with copper sulphate and

b) the influence on this of different rates of nitrogenous fertilizer application.

Pasture treatment with copper sulphate has not been used to any great extent/

extent in Britain as a means of correcting a deficiency in grazing livestock. This experiment was designed to assess the value of such treatment in increasing liver copper contents in sheep. It was also undertaken to determine whether there would be any increase in the amount of copper stored in the liver as a result of treating the pasture with high levels of nitrogen fertilizer. A further aim was to assess the risk of chronic copper toxicity occuring in sheep grazing pasture for long periods which had been top dressed with fairly high levels of copper sulphate.

Layout. Four different pasture treatments were employed (see Fig. 13). These were:-

a) Low N (1 cwt/acre Nitro-chalk)
b) Low N (" " " " + 20 lbs. $CuSO_4.5H_2O$/acre)
c) High N (3 cwt/acre Nitro chalk)
d) High N (" " " " + 20 lbs. $CuSO_4.5H_2O$/acre).

Each of these treatments was applied twice between early July and early September 1963. The total amount of copper sulphate given to the treated plots was thus 40 lbs/acre. To ensure even distribution the copper sulphate was mixed with sawdust before spreading to increase its bulk.

An area of ground (immediately adjacent to and of the same herbage type as the sites of Experiments 1 - 3) was divided into plots of 0.4 and 0.8 acre (Fig. 13). These areas receiving the Low Nitrogen treatment (1 cwt. Nitro-chalk/acre) were 0.8 acre and these which were given the High Nitrogen application (3 cwt Nitro-chalk/acre) were 0.4 acre. There were two plots available for each of the 4 separate treatments so that the herbage could be grazed and rested/

rested alternately. The layout of the plots and their respective treatments are shown in Figure 13.

Fig. 13. Layout and dates of treatment of plots with Nitrogen and copper.

Low N + Cu 19/7/63 5/9/63	Low N 19/7/63 5/9/63	High N 19/7/63 5/9/63 High N + Cu 19/7/63 5/9/63	Low N + Cu 5/7/63 15/8/63	Low N 5/7/63 15/8/63	High N + Cu 5/7/63 15/8/63 High N 5/7/63 15/8/63

Cu 20 lbs. $CuSO_4 \cdot 5H_2O$/acre (repeated).

High N 3 cwt Nitro-chalk/acre (repeated)

Low N 1 cwt Nitro-chalk/acre (repeated).

Twenty eight Blackface lambs were used in this experiment. These were weaned on 18 July 1963, ear tagged and randomly allocated into 4 groups of 7 lambs for each of the different pasture treatments. The lambs were allowed to graze the plots until they became fairly bare when they were moved to other equivalently treated plots. The vacated plots were then re-fertilized with Nitrogen/

Nitrogen and copper in order to produce sufficient grass to allow the lambs to be moved back into them when the others became bare. In practice the lambs were moved about every 3 weeks. This system was continued until 26 November 1963 by which time growth had practically ceased and grazing was in very short supply. The lambs were then removed from the plots and placed on a permanent pasture where they were to be over-wintered until they could be returned to the plots for another growing season as soon as growth recommenced in the spring

Unfortunately during the course of the winter the sheep were severely savaged by dogs. Some were killed and others had to be slaughtered. Since the numbers in each group were now so drastically reduced it was decided to terminat the experiment and the remainder of the lambs were blood sampled and slaughtered on 24 January 1964.

Results.

Grass samples were obtained at regular intervals throughout the grazing period. Samples were not taken within 2 weeks of the time of application of copper sulphate. These were analysed for their copper content and the results of the analyses are presented in Table 27.

Table 27/

Table 27 Copper concentration (p.p.m. D.M.) of pasture samples taken from July to November 1963.

High N - NoCu	Low N - NoCu	High N - Cu	Low N - Cu
12.7	18.3	49.7	34.2
10.2	11.2	50.8	20.7
13.6	15.8	31.2	17.5
13.4	14.7	68.1	29.4
17.5	8.9	30.3	11.7
11.6	13.8	115.9	54.7
13.6	12.2	84.3	113.0
11.0	13.0	52.9	108.3
13.0	10.2	44.1	65.3
11.5	11.7	70.8	30.0
19.2	14.6	68.9	52.5
17.2	10.8	-	45.7
Mean 13.7	12.9	60.6	48.6

The copper content of these pastures treated with copper sulphate was substantially and significantly ($P<0.01$) higher than for the untreated pastures. The mean copper content, over the grazing season, of the high nitrogen treated plots was higher than that of the plots receiving the low nitrogen treatment. Although/

Although this difference was not significant the effect of nitrogen applications on herbage copper concentration followed the same trend as that reported in Experiment 1. The increase in the copper concentration of the copper treated plots was very much greater than that found in Experiment 1 for similarly treated herbage. This was possibly due to surface contamination although no grass samples were taken within 14 days of copper sulphate applications. Another factor might have been a greater dilution effect in the small plots in Experiment 1 where the herbage was allowed to grow for a month before samples were obtained. Also 40 lbs. $CuSO_4.5H_2O$/acre was applied to the plots in the present experiment compared to a maximum of 20 lbs/acre to the plots in Experiment 1. The copper concentration in the herbage of the plots receiving no supplementary copper remained fairly constant over the period of the experiment.

Blood Copper Concentration.

The mean blood copper concentrations of the 4 groups are shown in Figure 14. The individual values are detailed in Table 28.

Table 28/

Fig 14. Changes in the mean blood copper concentrations of 4 groups of sheep grazing untreated and copper-treated herbage.

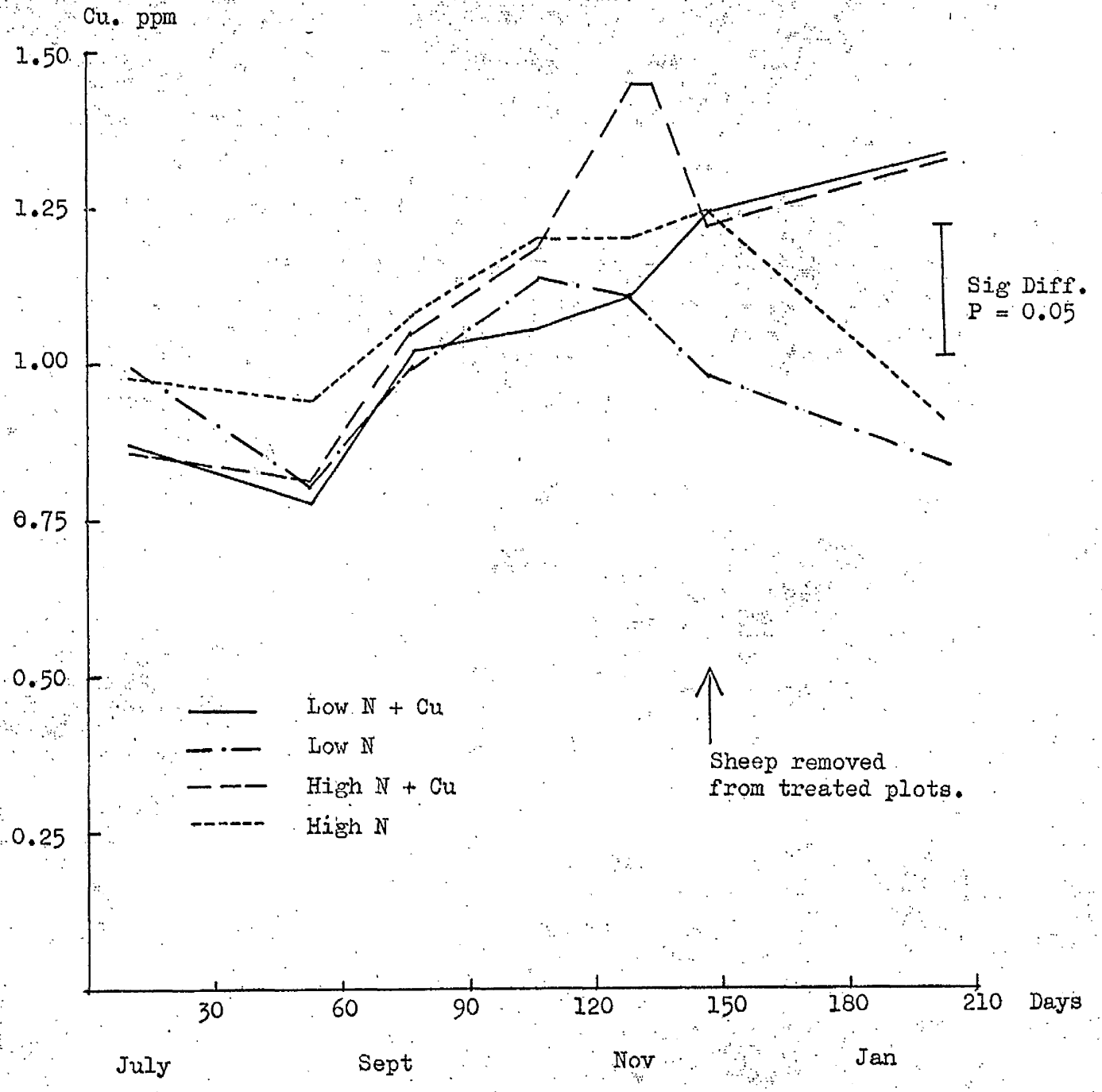


Table 28. Individual and mean blood copper concentrations (p.p.m.) of 4 groups of sheep.

Low N + Cu.							
No.	18/7	22/8	17/9	16/10	7/11	26/11	22/1/64.
864	0.54	0.59	1.03	1.05	1.12	1.76	1.08
874	1.20	0.90	0.80	0.90	0.88	1.04	1.61
875	0.60	0.70	1.16	1.39	1.20	1.09	1.19
882	0.82	0.84	0.92	0.97	1.17	1.16	1.56
883	1.09	0.72	1.10	1.01	1.14	1.18	1.64
891	0.97	0.91	1.07	1.11	1.22	1.60	0.94
892	-	0.81	1.05	0.97	1.03	0.91	-
MEAN	0.87	0.78	1.02	1.06	1.11	1.25	1.34

Low N.							
No.	18/7	22/8	17/9	16/10	7/11	26/11	22/1/64.
865	0.81	0.86	0.91	0.88	1.00	0.81	0.54
866	1.11	0.75	1.11	0.92	1.19	1.14	1.03
876	-	1.02	1.00	1.20	0.84	0.75	0.40
881	1.20	0.77	1.12	1.49	1.38	1.03	1.11
884	1.02	0.71	0.90	1.28	1.27	1.19	0.95
890	0.75	0.77	0.87	1.03	1.03	0.90	-
893	1.06	0.72	1.00	1.17	1.06	1.05	0.99
	0.99	0.80	0.99	1.14	1.11	0.98	0.84

High N + Cu.							
867	091	0.64	1.19	1.14	1.37	1.20	1.41
868	0.67	0.68	0.98	1.01	1.46	1.12	1.08
872	1.22	0.93	1.12	1.36	1.53	1.48	1.58
877	0.75	0.94	1.07	1.60	1.53	1.22	1.39
880	0.76	0.98	1.04	0.99	1.71	1.18	-
886	0.87	0.75	1.05	1.10	0.99	1.09	1.60
889	0.81	0.75	0.91	1.14	1.55	1.26	0.93
MEAN	0.86	0.81	1.05	1.19	1.45	1.22	1.33

High N.							
869	1.14	0.91	1.08	1.12	1.16	1.22	1.16
870	0.80	0.87	0.95	1.00	1.06	1.30	0.92
871	1.32	1.05	0.98	1.09	1.39	1.35	0.83
878	0.65	0.76	1.08	1.23	1.19	1.25	0.91
879	0.90	1.14	1.28	1.39	1.31	1.31	0.74
887	1.11	0.90	1.05	1.16	1.04	1.00	-
888	0.93	0.96	1.15	1.38	1.27	1.32	-
	0.98	0.94	1.08	1.20	1.20	1.25	0.91

The blood copper concentrations of all 4 groups were fairly similar for the first 3 months until 7 November 1963 when the mean blood copper concentration of the sheep grazing the High N - Cu plots rose to 1.45 p.p.m. It remained constant at this level for a further week but by 26 November it had returned to a more normal level of 1.22 p.p.m. Apart from this there were no other differences in the mean blood copper concentrations of the 4 groups until the final sampling just before slaughter. On this date the mean blood copper concentrations of those sheep which had been grazing the copper treated pasture were significantly ($P < 0.01$) higher than those of the sheep which had been grazing untreated pasture.

This difference occurred two months after the sheep had been removed from the plots. This was probably due to the fact that the sheep which had been grazing the treated pasture were releasing some of the copper store which they had built up in their livers to maintain a fairly constant concentration of copper in their blood. The fall in the blood copper concentration of the two groups which had been grazing untreated pasture was probably attributable to a reduced and perhaps insufficient intake of grass rather than to an actual deficiency of copper in the pasture. The copper content of the permanent pasture was 13.5 p.p.m.

Liver/

Liver dry weight, copper concentration and total copper content.

The individual and mean liver dry weights, liver-copper concentrations and total liver-copper contents of the 4 groups are listed in Table 29.

The mean dry weight (162g) of the livers of the High N-Cu treated group was significantly ($P < 0.05$) higher than that of the Low N-Cu group (123 g). There were no other significant differences in liver dry weights between groups.

Both groups of sheep grazing the copper treated plots had mean liver copper concentrations and total liver copper contents which were significantly ($P < 0.01$) higher than those of the two groups grazing the plots which had received no supplementary copper. The total liver copper contents of both groups grazing the high nitrogen treated plots were higher than those of the comparable groups grazing pasture which had been given the low nitrogen treatmen The differences, however, were not significant but they do reflect the same trend as was found for the herbage samples from the plots where those which had been treated with the high level of nitrogen had higher copper concentrations than those receiving low nitrogen applications.

The liver copper concentrations of the sheep which had been grazing the copper treated pasture were, perhaps, not as high as might have been expected in sheep which had been ingesting herbage containing 50 - 60 p.p.m. Cu for several months. As already mentioned in connection with the blood copper concentrations/

Table 29. Individual and mean liver dry weights, liver-copper concentrations and total liver-copper contents of 4 groups of 7 sheep.

Low N + Cu				Low N			
No.	Dry Wt. (g)	Cu Conc. (p.p.m.)	Total Cu (mg)	No.	Dry Wt. (g)	Cu Conc. (p.p.m.)	Total Cu (mg)
864	161	317.4	51.2	865	199	18.7	3.7
874	107	489.4	52.2	866	211	19.3	4.1
875	133	784.5	104.4	876	121	19.0	2.3
882	149	187.9	28.0	881	137	49.4	6.8
883	99	266.7	26.4	884	143	36.8	5.3
891	108	128.6	13.9	890	122	33.2	4.1
892	103	344.5	35.6	893	128	37.3	4.8
Mean	123	359.9	44.5		152	30.5	4.4
High N + Cu				**High N**			
867	205	403.8	82.7	869	123	150.9	18.5
868	154	225.3	34.6	870	162	37.0	6.0
872	179	680.4	121.7	871	147	72.2	10.6
877	180	341.1	61.3	878	150	41.1	6.2
880	143	329.5	47.0	879	125	19.9	2.5
886	129	45.7	5.9	887	118	38.9	4.6
889	149	347.7	51.6	888	113	56.1	6.4
Mean	163	339.1	57.8		134	59.4	7.8
L.S.D. ($P<0.05$)	30.3	161.8	26.1		30.3	161.8	26.1

concentrations it seems probable that the total liver copper content of the sheep had been depleted during the 2 months since they were removed from the copper treated pasture. This supposition is substantiated by the fact that one sheep from the Low N-Cu treated group, which was slaughtered on 28 October 1963 as it had foot-rot, had both a very much higher liver copper concentration (1117 p.p.m.) and total liver copper content (305 mg) than any of the sheep from either of the two copper treated groups which were slaughtered in January 1964. These amounts should be contrasted with a mean liver copper concentration of 350 p.p.m. and a mean total liver copper content of 52 mg. for both groups grazing the copper treated plots.

It would appear, therefore, that liver reserves of copper are maintained only for as long as the animal has an adequate or more than adequate dietary intake of copper. If there is a change to a diet supplying insufficient copper for daily requirements then the existing stores of copper in the liver will be released and the copper concentration will gradually fall. If this were the case there would appear to be little danger in feeding a ration containing excess copper ($>$ 25 p.p.m.) to sheep for a short period provided that they were then allowed to feed on a diet which had a normal or below normal copper content (5 - 10 p.p.m.) as during this period the copper stores, which had been built up in the liver, would be depleted.

Experiment 5. The Effects of 1) Liming of pasture and 2) regular oral dosage over prolonged periods with a) copper sulphate, b) copper glycine and c) copper disodium-ethylene diamine tetra acetic acid, on the blood and liver copper status of sheep.

Liming pasture has been one of the many factors implicated in the aetiology of swayback. However, there has so far been no published evidence which shows that the copper status of sheep has been lowered by applications of lime to pasture. This experiment was designed to determine the effect of liming a pasture on the copper status of sheep grazing thereon over a prolonged period.

The rate of copper absorption and storage in the liver following oral dosage of various copper compounds has also been investigated using similar sheep grazing unlimed pasture. This was undertaken following reports that some copper compounds, particularly copper glycine and copper EDTA, were more readily absorbed than copper sulphate which is the material in general use as a prophylactic treatment for swayback. A comparative study is made here of the degrees of absorption and storage of these three compounds compared to a control group of untreated sheep. A study was also made of the changes in iron concentration in the blood and liver.

Layout. Nine plots each of 0.8 acre were available for this experiment. The herbage was predominantly cocksfoot/perennial ryegrass with some crested dogstail and was considered typical of improved upland pastures.

One/

One of the plots was limed (1½ tons ground limestone/acre) in the spring of 1964 and 2 further plots were similarly treated in January 1965. There were 6 unlimed plots. Nitro-chalk (1 cwt/acre) applications were made at intervals as required to meet the grazing requirements of the sheep.

Thirty orphaned Blackface lambs which had been reared from birth indoors were randomly divided into 5 groups of six lambs per group. One group was allocated to graze the limed plots while the other 4 groups were to graze the unlimed pasture. One of these 4 groups acted as a control group while the remaining 3 groups were dosed each week with a drench containing 70 mg Cu in the form of either a) copper sulphate b) copper glycine or c) copper EDTA.

The experiment was commenced on the 18 August 1964. The system of grazing used was as described in Experiment 4, the lambs being moved to fresh plots when the need arose. By 25 January 1965, however, the amount of herbage available in the plots was insufficient for the maintenance requirements of the sheep and it was decided to feed them some hay (about 1 lb/head per day containing 5.0 p.p.m. Cu) until spring growth produced sufficient grass for their requirements. Hay continued to be fed until 26 April 1965. On 31 May five additional lambs were introduced into the limed plots and six additional lambs were added to the control group. Four of these lambs in each group were Half-breds while the other three were Cross lambs (i.e. Blackface x Cheviot).

Dosing of the lambs in the copper treated groups was carried on weekly

from/

from 24 August 1964 until 19 July 1965 by which time each had received 3.115 g. Cu. The supply of both Cu EDTA and Cu glycine was exhausted on this date and it was decided to terminate this section of the experiment and the sheep in the three copper treated groups were slaughtered on 22 July 1965. There was still a plentiful supply of grass in both the limed and unlimed plots so both groups of sheep grazing these were allowed to continue until the autumn. These two groups were eventually slaughtered on 7 October 1965.

Herbage samples were obtained frequently throughout the grazing period and these were analysed for their copper content. Blood samples were taken on eleven different occasions from the limed and control groups and on nine occasions from the three copper treated groups. The whole livers of all the sheep were obtained at slaughter for the determination of dry matter content, copper concentration and total liver-copper content.

Results

Herbage Copper Concentrations

The copper content of the herbage samples obtained are listed in Table 30.

Table 30. Copper concentration (p.p.m.) of herbage samples (Aug. 1964 - Sept. 1965.

Limed Plots			Unlimed Plots		
7.6	13.5	8.1	8.8	9.9	15.3
6.1	10.5	11.5	8.5	6.1	12.3
7.5	8.9	12.3	8.0	9.9	10.9
7.7	10.7	9.7	7.2	10.1	9.2
7.8	8.5	7.5	9.4	8.1	11.0
9.1	9.1	9.0	6.3	8.8	15.0
8.4	11.0	6.8	9.6	9.3	12.6
9.4	9.5	5.7	9.2	7.0	12.8
6.7		6.9	7.7	13.6	
Mean	8.9 (17 samples)		9.6 (35 samples)		
Standard Error of Mean	± 0.435		± 0.414		

There was no statistically significant difference between the copper concentrations of the limed and unlimed herbages. This confirms the result reported in Experiment 2 where liming was found to have no effect on herbage copper concentration.

Blood Copper Concentration. The individual and mean blood copper concentrations of the 5 groups at each sampling date are presented in Table 31.

Table 31. Individual and mean blood copper concentrations (p.p.m.) of 5 groups of sheep

No.	18.8.64.	16/9.	8/10.	4/11.	3/12.	14.1.65.	12/5.	10/6.	20/7.	14/9.	5/10.
Limed Group											
501	0.96	0.91	0.70	0.70	0.69	0.72	0.40	1.11	1.07	0.90	0.80
502	0.84	0.87	0.93	0.92	0.46	0.38	0.34	0.82	1.05	0.77	0.68
503	1.12	0.83	0.71	0.70	0.41	0.47	0.50	0.97	0.81	0.65	0.49
504	0.98	0.93	0.87	0.68	0.77	0.80	0.50	1.0	1.04	0.86	0.87
524	0.93	0.77	1.09	0.82	0.55	0.60	0.33	0.76	0.75	0.56	0.53
527	1.06	0.85	0.63	0.71	0.55	0.50	0.33	0.54	0.57	0.43	0.48
567								0.92	0.92	0.76	0.60
569								0.76	0.72	0.71	0.58
570								1.06	0.61	0.46	0.37
498										0.87	0.88
499										0.68	0.61
675									0.55	0.44	0.40
Mean	0.98	0.86	0.82	0.76	0.57	0.58	0.40	0.88	0.81	0.67	0.61
Control Group											
513	1.04	0.96	0.92	0.68	0.57	0.67	0.43	0.96	0.75	0.80	0.90
514	1.05	1.23	0.87	0.77	1.05	0.76	0.51	0.97	1.01	1.10	0.85
516	1.16	1.09	0.90	1.01	0.67	0.58	0.44	0.87	1.10	0.82	0.69
528	0.91	0.97	0.87	1.02	0.45	0.85	0.62	0.91	0.83	1.07	1.08
530			1.0	1.20	1.19	0.85	0.66	0.92	0.83	0.96	0.91
495								0.91	1.05	1.10	1.15
565								1.13	1.10	1.07	0.93
566								0.97	0.82	1.02	0.85
574									0.64	0.56	0.74
563								0.69	0.79	-	0.58
674									0.82	0.67	0.69
676									0.69	0.72	0.91
Mean	1.04	1.06	0.91	0.94	0.79	0.74	0.53	0.92	0.87	0.90	0.86
Sig.Diff of means P.	N.S.	0.02	N.S.	N.S.	N.S.	0.1	.05	N.S.	N.S.	.01	.01

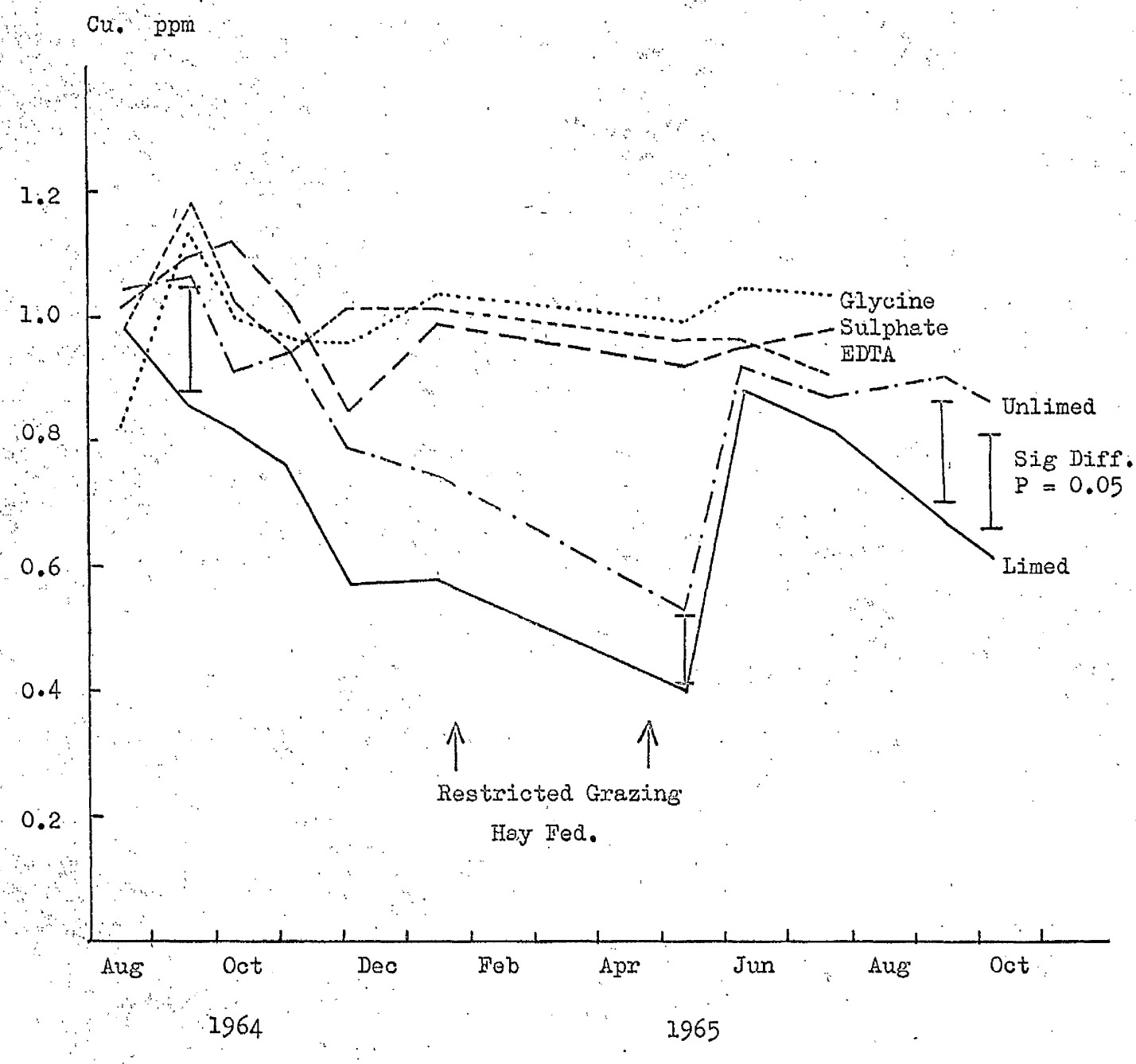
Table 31. Continued.

Group Drenched with $CuSO_4$. (70 mg Cu/week)

No.	18.8.64.	16/9	8/10	4/11	3/12	14/1/65	12/5	10/6	20/7
506	1.18	1.03	1.05	1.17	0.87	1.20	0.93	0.90	0.98
510	0.89	0.96	1.01	0.65	0.55	0.92	0.83	0.93	1.0
511	1.12	1.20	1.31	1.12	0.90	0.75	0.92	0.90	1.01
512	0.94	1.18	1.05	0.96	0.97	0.97	0.98	1.05	0.92
531	0.96	1.12	1.17	1.20	0.96	1.12	-	-	-
Mean	1.02	1.10	1.12	1.02	0.85	0.99	0.92	0.95	0.98
			Cu Glycine Group.						
102	0.73	1.20	1.03	0.87	0.80	0.93	0.96	0.90	0.90
103	1.00	1.19	0.77	1.02	1.09	1.05	0.85	0.85	1.0
104	1.09	1.14	1.10	0.95	1.08	1.19	1.0	1.11	1.13
105	0.62	1.15	1.0	1.12	1.04	1.21	0.92	1.08	1.10
106	0.75	0.87	0.92	0.82	1.02	0.92	1.26	1.06	1.06
107	0.75	1.21	1.10	0.98	0.66	0.87	0.96	1.21	0.97
Mean	0.82	1.13	0.99	0.96	0.95	1.03	0.99	1.04	1.03
			Cu E.D.T.A. Group.						
301	1.15	1.23	0.97	0.97	0.97	0.98	0.86	1.02	0.95
302	0.80	1.20	1.05	0.81	1.08	0.92	1.09	0.92	0.83
303	0.99	1.03	1.04	0.76	0.87	0.93	0.97	0.92	0.88
304	0.98	1.24	0.88	1.09	1.18	1.07	0.90	0.92	0.87
308	-	1.20	1.17	1.09	0.95	1.17	0.98	1.02	0.98
Mean	0.98	1.18	1.02	0.94	1.01	1.01	0.96	0.96	0.90

The changes in mean blood copper concentrations of the five groups are presented graphically in Fig. 15. The mean blood copper concentration of the group grazing the limed pasture was 0.98 p.p.m. at the start of the experiment on 18 August 1964. This level fell steadily over the next nine months until on the 12 May 1965 it had fallen to 0.40 p.p.m. The control group had an initial mean blood copper concentration of 1.04 p.p.m. This rose slightly during the first month but thereafter it followed the same trend as that of the limed group by falling progressively, although not so rapidly, until by 12 May 1965 it had fallen to 0.53 p.p.m. After this date there was a rapid increase in the blood copper concentrations of both groups such that by 6 June the mean blood copper concentration of the limed group was 0.88 p.p.m. and that of the control group was 0.92 p.p.m. This rise occurred some time after the appearance of spring growth. The fall in values in the unsupplemented groups may have been due to the restriction in pasture intake during January to May. There had, however, been a steady fall prior to this period. Thereafter, the mean blood copper concentration of the limed group again decreased fairly rapidly so that at slaughter on 7 October 1965 it was 0.61 p.p.m. During this period the control group maintained a more or less constant concentration of copper with the mean value never falling below 0.86 p.p.m. which was the level on the last sampling date. During the course of the experiment/

Fig 15. Changes in the mean blood copper concentrations of 5 groups of sheep grazing limed or unlimed pasture and in sheep grazing unlimed pasture and receiving regular copper supplementation.



experiment there were four occasions on which the mean blood copper concentration of the limed group was significantly lower than that of the control group and on two other occasions this difference approached significance.

The mean blood copper concentrations of the three groups being given a weekly dose of 70 mg Cu were initially 1.02, 0.82 and 0.98 p.p.m. respectively for the copper sulphate, copper glycine and Cu EDTA groups. These all increased in the first month following the commencement of dosing to between 1.10 and 1.18 p.p.m. but thereafter they fell slightly during the next two months to around 1.0 p.p.m. Mean blood copper concentrations were maintained around this level (range 0.90 - 1.03 p.p.m.) thereafter. None of these three groups exhibited a fall in blood copper concentration comparable to that found for the limed or control groups during the period January to May 1965 when some hay was fed.

Liver dry weight, liver copper concentration and total liver-copper content.

The individual and mean values for liver dry matter, copper concentration and total copper content of the five groups are listed in Table 32.

The mean liver copper concentration of the limed group (19.3 p.p.m.) was significantly ($P < 0.02$) lower than that of the control group (63.1 p.p.m.). This was offset to some extent by the fact that the mean liver dry weight of the control sheep was just significantly ($P < 0.05$) lower than that of the sheep grazing the limed pasture. However if the mean total liver copper content of/

Table 32. Individual and mean liver dry weights, copper concentrations and total liver copper contents of 5 groups of sheep.

Unsupplemented Sheep.

Limed Group				Control Group			
No.	Dry Wt. (g)	Cu Conc. (p.p.m.)	Total Cu (mg)	No.	Dry Wt. (g)	Cu Conc. (p.p.m.)	Total Cu (mg)
501	268	8.3	2.2	513	192	30.8	5.9
502	274	10.8	3.0	514	209	28.2	5.9
503	225	14.6	3.3	516	160	16.6	2.7
504	190	26.6	5.1	528	171	141.4	24.2
524	218	14.9	3.2	530	215	98.4	21.1
527	212	11.8	2.5	495	127	157.2	20.0
567	152	15.2	2.3	565	168	156.6	26.3
569	171	21.9	3.7	566	172	45.2	7.8
570	185	17.0	3.1	574	133	23.1	2.9
498	151	60.0	9.1	563	186	18.4	3.4
499	163	18.8	3.1	674	108	18.4	2.0
675	157	11.3	1.8	676	122	23.5	2.9
Mean	197	19.3	3.5		164	63.1	10.4
L.S.D. (P=0.05)	33.0	35.7	5.8		33.0	35.7	5.8

Sheep receiving 10 mg supplementary Cu/day

$CuSO_4$ Group					Cu Glycine Group					Cu EDTA Group				
No.	Dry Wt(g)	Cu Conc (ppm)	Total Cu(mg)	% Cu Stored	No.	Dry Wt. (g)	Cu Conc (ppm)	Total Cu (mg)	% Cu Stored	No.	Dry Wt (g)	Cu Conc (ppm)	Total Cu (mg)	% Cu Stored
506	178	329.7	58.8	1.55	102	177	325.6	57.6	1.52	301	175	340.3	59.6	1.58
510	179	298.6	53.3	1.38	103	229	326.9	74.8	2.07	302	150	405.5	60.7	1.61
511	230	221.8	50.9	1.30	104	161	361.1	58.1	1.53	303	219	368.2	80.6	2.25
512	241	485.2	116.9	3.42	105	180	302.4	54.4	1.41	304	159	208.6	33.2	0.73
531	87	467.9	40.7	1.31	106	203	214.4	43.5	1.06	308	155	403.7	62.5	1.88
					107	200	550.1	110.0	3.20					
Mean	183	360.6	64.1	1.79		192	346.8	66.4	1.80		172	345.3	59.3	1.61

of the two groups are compared it is seen that there was significantly ($P < 0.02$) less copper in the livers of the limed group (3.5 mg) than in the control group (10.4 mg). This emphasises the fact that there is a real difference between the two groups and not one that is dependent purely on a difference in liver dry matter. The consistently lower blood copper concentrations in the limed compared to the unlimed group support this conclusion.

Both the mean liver copper concentrations and the mean total liver copper contents of the three groups which had been dosed weekly with 70 mg Cu were significantly ($P < 0.001$) higher than those of the control group. There were no differences in mean copper concentration or total liver copper content among the three copper treated groups. All three groups had very similar mean liver copper concentrations (361, 347 and 345 p.p.m.) and mean total liver copper contents (64, 66 and 59 mg) respectively for the $CuSO_4$, Cu glycine and Cu EDTA groups.

The percentage storage rate of dosed copper was also very similar for the three treated groups. The amount of the dosed copper which was stored in the liver by the three groups was 1.79, 1.80 and 1.61% respectively for the $CuSO_4$, Cu glycine and Cu EDTA groups.

Inter-relationship between copper and iron.

An inverse relationship between the concentrations of iron and copper in the livers of pigs was first reported by Cassidy and Eva (1958). This relationship has since been confirmed by Bunch, McCall, Speer and Hays (1962), Ritchie, Luecke, Baltzer, Miller, Ullrey and Hoefer (1963) and Suttle and Mills (1964). O'Donovan, Spillane and O'Grady (1966) reported high liver copper concentrations in pigs which they thought might be due to the low level of iron in their skim milk diet. An unusually high liver copper concentration (1771 p.p.m.) was associated with a very low liver iron concentration (68 p.p.m.)

No reports have been published concerning a similar relationship in sheep. It was considered, therefore, that as the three copper supplements being given to the sheep in this experiment would probably materially increase their liver copper concentrations it would be interesting to investigate the effect of this on liver iron concentrations. Consequently the blood samples obtained from the sheep were analysed for plasma iron concentration and the livers for their iron concentration.

Results.

Plasma Iron Concentration.

The individual and mean plasma iron concentrations of the 5 groups of sheep at each sampling date are listed in Table 33.

Table 33. Individual and mean plasma iron concentrations (p.p.m.) of 5 groups of sheep.

Limed Group

No.	2.9.64.	16/9	8/10	4/11	3/12	14/1/65	12/5	10/6	20/7	14/9	5/10
501	1.26	1.07	1.20	1.69	1.68	2.86	2.05	0.83	1.38	0.89	1.25
502	1.47	1.44	0.70	2.01	2.0	2.43	1.81	1.50	1.38	1.75	1.06
503	1.25	2.12	1.50	2.0	1.93	2.30	1.87	0.51	1.38	1.32	1.69
504	1.63	1.81	1.44	2.06	2.43	2.86	2.0	1.20	1.13	1.38	2.25
524	1.20	1.50	2.20	1.50	1.69	2.55	1.44	1.20	1.63	0.88	1.19
527	1.38	1.50	1.57	1.56	1.63	2.43	1.63	1.38	1.32	-	0.94
567								1.32	1.57	1.26	1.63
569								1.44	1.50	0.95	1.25
570								0.83	1.57	1.32	1.31
498										1.38	1.56
499										1.68	1.75
675									1.69	1.50	1.56
Mean	1.37	1.57	1.44	1.80	1.89	2.57	1.80	1.13	1.46	1.30	1.45

Control Group

513	1.13	1.44	1.13	1.56	-	-	0.83	1.68	1.50	1.32	1.44
514	1.44	1.57	1.38	1.63	1.77	2.61	1.44	1.07	1.01	1.44	1.44
516	0.89	1.57	1.26	1.56	1.87	2.18	1.75	1.44	0.95	1.07	1.06
528		1.38	1.38	1.56	1.75	2.25	1.57	1.50	1.50	1.32	1.0
530	1.57	1.44	1.75	1.75	1.69	2.05	1.44	1.50	1.26	1.32	1.38
495									2.80	1.44	2.31
565								1.57	-	1.40	2.0
566								1.57	1.63	1.76	1.63
574									1.81	1.26	1.0
563								1.20	-	-	1.56
674									1.57	1.20	1.56
676									1.50	1.81	1.75
Mean	1.26	1.48	1.38	1.61	1.77	2.27	1.41	1.44	1.55	1.39	1.51

Table 33. Continued.

$CuSO_4$ Group.

No.	2.9.64.	16/9	8/10	4/11	3/12	14/1/65	12/5	10/6	20/7
506	1.63	1.44	1.38	1.50	1.63	1.07	1.26	1.32	1.32
510	1.07	1.01	1.50	1.25	1.65	1.68	1.57	1.57	1.32
511	1.57	1.57	1.32	1.63	2.24	1.75	1.63	1.93	1.38
512	1.13	1.38	1.75	1.87	1.68	1.87	1.93	1.75	1.38
531	0.95	1.63	1.50	1.63	2.37	2.12	-	-	-
Mean	1.27	1.41	1.49	1.58	1.91	1.70	1.60	1.64	1.35

Cu Glycine Group

102	1.93	1.81	1.63	1.87	2.37	2.30	1.50	1.50	1.87
103	1.45	1.50	1.25	2.12	1.75	1.56	1.75	1.50	1.44
104	1.93	1.57	1.50	1.32	1.87	1.68	0.83	1.07	1.20
105	1.57	1.50	1.63	1.63	2.51	2.18	1.57	1.38	1.38
106	1.75	1.81	1.44	1.44	1.44	1.81	0.63	1.32	1.26
107	2.18	1.57	1.87	1.63	2.06	1.68	2.0	1.63	1.81
Mean	1.80	1.63	1.55	1.67	2.0	1.87	1.38	1.40	1.49

Cu E.D.T.A. Group

301	2.18	2.0	1.69	1.69	2.24	2.05	1.63	1.68	1.69
302	1.47	1.38	1.57	1.57	1.57	-	1.01	1.38	1.13
303	1.57	1.50	1.50	1.57	1.50	1.75	1.32	1.50	1.50
304	1.50	2.0	1.69	1.81	2.0	2.37	1.57	1.63	1.50
308	-	1.57	1.69	1.56	2.0	1.63	1.25	1.01	1.32
Mean	1.68	1.69	1.63	1.64	1.86	1.95	1.36	1.44	1.43

The changes in the mean plasma iron concentrations of the 5 groups are presented graphically in Fig. 16. The most noticeable effect was the seasonal change found in the plasma iron concentrations. Peak levels were found in early winter, in either December or January, with much lower levels recorded in the spring and summer. The increase in plasma iron concentration in the limed and control groups was much greater than that found for the three copper treated groups. The variation in plasma iron concentration in these 3 groups was not so marked as that found for the limed and control groups but all three exhibited the seasonal change with peak values in the early winter. It seems probable that the supplementary copper administered to these 3 groups was instrumental in reducing the variation in plasma iron concentration and for the lower levels recorded in winter compared to those found for the limed and control groups. The fall in plasma iron concentration in the limed group between 12 May and 10 June corresponded with a rapid rise in blood copper concentration during the same period.

Liver dry weight, liver iron concentration and liver total iron content.

The individual and mean values for liver-iron concentration and total iron content of the 5 groups are listed in Table 34.

The/

Table 34. Individual and mean liver iron concentrations and total liver iron contents of 5 groups of sheep.

Unsupplemented Sheep.

Limed Group			Control Group		
No.	Liver Fe Conc.(p.p.m.)	Total Fe (mg)	No.	Fe Conc (p.p.m.)	Total Fe (mg)
501	454.3	121.6	513	221.2	42.5
502	324.7	89.1	514	327.5	68.3
503	294.6	66.3	516	231.7	37.1
504	402.7	76.7	528	269.9	46.2
524	310.9	67.7	530	192.7	41.3
527	228.1	48.4	495	174.4	22.1
567	366.6	55.6	565	222.3	37.4
569	174.4	29.8	566	227.9	39.2
570	223.2	41.2	574	373.4	49.8
498	151.4	22.9	563	260.3	48.3
499	206.3	33.5	674	289.9	31.4
675	336.0	52.7	676	210.2	25.6
Mean	289.4	58.8		250.1	40.8

Sheep receiving 10 mg supplementary Cu/day.

$CuSO_4$ Group			Cu Glycine Group			Cu EDTA Group		
No.	Fe Conc. (p.p.m.)	Total Fe (mg)	No.	Fe. Conc. (p.p.m.)	Total Fe (mg)	No.	Fe Conc. (p.p.m.)	Total Fe (mg)
506	480.9	85.7	102	245.5	43.4	301	172.1	30.1
510	325.0	58.0	103	198.7	45.5	302	219.5	32.8
511	204.0	46.8	104	490.3	78.9	303	250.1	54.8
512	197.8	47.5	105	419.0	75.4	304	364.4	57.8
531			106	269.3	54.6	308	344.0	53.3
			107	190.7	38.2			
Mean	301.9	59.5		302.2	56.0		270.2	45.8

The liver iron concentrations and total liver iron contents of all five groups were fairly similar. The mean liver iron concentrations ranged from 250.1 - 302.4 p.p.m. and the mean total liver iron contents were within the range 40.8 - 59.5 mg. It is evident, therefore, that the amount of supplementary copper given to the three treated groups effected no reduction in their liver iron concentration or total iron content.

Discussion. The finding that the liver copper concentration and total liver copper content of the limed group were both significantly lower than those of the control group cannot be attributed to a difference in their copper intake since there was no significant difference in the copper concentration of the limed and unlimed pasture. Liming must, therefore, have had the effect of reducing the availability of the copper present in the herbage or alternatively of reducing the ability of the sheep to absorb the available copper. The blood copper concentration of the limed group was consistently lower than that of the control group, on four occasions significantly so. This indicates that the ability of the sheep to absorb the ingested copper was impaired since similar sheep on a comparable copper intake on unlimed herbage were able to absorb and store the available copper more efficiently.

The percentage of supplementary copper which was stored in the liver was found to be independent of the form in which it was administered. The rate of storage of supplementary copper found in the present experiment, 1.61 - 1.80%, compares/

compares favourably with that reported by previous workers. Edgar (1942) Dick (1954) and Hemingway et al.(1962) have reported that 1.0 - 4.1% of orally administered copper in solution is stored in the liver.

The amount of copper administered to the sheep in this experiment, 70 mg Cu/week, markedly increased liver copper concentrations. These were, however, still only just above the normal upper limit of 300 p.p.m. Cu. and the highest value recorded, 550 p.p.m. was far below the minimum value of about 1000 p.p.m. which has been associated with chronic copper poisoning. There would appear, therefore, to be very little risk associated with the administration of this level of copper supplementation even for protracted periods of time.

High dietary intakes of iron have been reported to decrease copper absorption and an inverse relationship between these two elements has been recorded in pigs. The level of copper supplementation in the present experiment did not influence the liver iron concentration of the sheep in any of the three supplemented groups. However, the total daily intake of copper by these sheep would be only about twice that of normal sheep and thus might not have been high enough to restrict the absorption and storage of iron. There were, however, noticeably lower maximum plasma iron concentrations in the copper supplemented sheep than in either the control or the limed groups.

Experiment 6/

Experiment 6. The effects of various injectable copper compounds in the control of swayback in lambs.

Experiment 6 is divided into three parts (6A, 6B and 6C). In each separate experiment a different proprietary injectable copper compound was given to one half of a flock of sheep in mid-pregnancy; the remaining half of each flock acted as untreated controls. The efficacy of the various compounds was assessed by 1) changes in ewe blood copper concentrations, 2) lamb liver copper contents, 3) lamb blood copper concentrations and 4) the control of clinical swayback.

Experiment 6A.

Fifty Blackface ewes were used in this experiment. There was a past history of swayback on this farm which in some years affected 25% of the lambs born. Previous investigations on this farm indicated that ewe and lamb liver copper concentrations were generally below 20 p.p.m.

Twenty five of the ewes were treated in mid-pregnancy (4 Feb.) with 1 ml. of a copper glycine preparation (R, Young & Co. Ltd. "Swaycop"). This supplied 45 mg Cu and was administered by subcutaneous injection behind the shoulder. The remaining 25 ewes received no treatment. Blood samples were obtained from all the sheep on 4 February immediately prior to injection and again on 8 April. Lambing took place in mid April.

The ewes, which grazed hill herbage throughout, were given about 1 lb./ head/

head/day of a proprietary ewe nut (15 p.p.m. Cu) during the last six weeks of pregnancy.

Results.

The individual and mean blood copper concentrations of the sheep are given in Table 35. The mean blood copper concentrations for both the treated and control groups were 0.44 p.p.m. on 4 February. By 8 April the mean value for the untreated ewes had fallen to 0.34 p.p.m. In contrast the mean value for the injected sheep had increased significantly to 0.77 p.p.m. and the great majority of individual values were between 0.70 and 0.90 p.p.m.

Twelve lambs which were born to the injected ewes were obtained at birth or shortly after and were slaughtered. The majority of these were surplus twin lambs. Ten lambs were obtained from the control group. Six of these had clinical swayback which was confirmed by histological examination of nervous tissue. One further ewe gave birth to a swayback lamb the carcase of which was not available for analysis. In total, 6 of the 25 control ewes produced swayback lambs whereas there were no cases among the treated ewes. The same copper preparation was used in some 600 other ewes on the farm; only one of these gave birth to a swayback lamb.

The liver and, where possible, blood copper concentrations of these lambs are given in Table 36. Some of the lambs from the injected ewes were rather older (up to 2 weeks) than the lambs from the control ewes which were generally obtained at or within a day or two of birth. In consequence the mean liver

dry/

Table 35. Effect of copper glycine (45 mg Cu) given by injection in mid-pregnancy (4 Feb) on blood copper concentrations in late pregnancy (8 April).

Ewe No.	Injected Ewes 4 Feb	Injected Ewes 8 Apr	Ewe No.	Injected Ewes 4 Feb	Injected Ewes 8 Apr	Ewe No.	Control Ewes 4 Feb	Control Ewes 8 Apr	Ewe No.	Control Ewes 4 Feb	Control Ewes 8 Apr.
1	0.48	0.81	694	0.74	0.72	251	0.53	0.25	264	0.37	0.23
2	0.86	0.85	695	0.56	0.86	252	0.70	0.70	265	0.47	0.30
3	0.30	0.74	696	0.45	0.92	253	0.46	0.31	266	0.38	0.28
4	0.47	0.86	697	0.34	0.71	254	0.47	0.36	267	0.33	0.31
5	0.40	0.83	698	0.27	0.77	255	0.38	0.44	268	0.44	0.36
6	0.51	0.71	699	0.35	0.72	256	0.45	0.35	269	0.53	0.40
7	0.53	0.67	700	0.36	0.90	257	0.47	0.46	270	0.33	0.28
8	0.34	0.74	701	0.47	0.86	258	0.34	0.19	271	0.38	0.31
9	0.39	0.68	702	0.61	0.71	259	0.37	0.36	272	0.38	0.30
0	0.30	1.04	703	0.27	0.80	260	0.32	0.22	273	0.69	0.46
1	0.36	0.56	704	0.72	0.86	261	0.65	0.45	274	0.55	0.35
2	0.50	0.70	705	0.31	0.81	262	0.32	0.21	275	0.50	0.32
3	0.31	0.80				263	0.23	0.37			

Mean Concentration	p.p.m. Cu
4 Feb	0.45
8 April	0.79

Mean Concentration	p.p.m. Cu
4Feb	0.44
8 April	0.34

Table 36. Blood and liver copper concentrations in lambs born either to ewes treated with copper glycine (45 mg Cu) or to untreated ewes.

	Injected				Untreated		
	Liver		Blood		Liver		Blood
Dry Wt. (g)	Cu Conc. (p.p.m.)	Total Cu(ug)	Cu Conc. (p.p.m.)	Dry Wt. (g)	Cu Conc. (p.p.m.)	Total Cu(ug)	Cu Conc. (p.p.m.)
14.0	92.5	1295	-	S 18.0	8.3	149	0.44
22.5	163.5	3679	-	S 19.3	8.1	156	0.43
17.6	107.5	1892	0.56	15.0	13.7	205	-
14.0	92.5	1295	-	S 10.0	8.3	83	0.71
22.5	163.5	3678	-	S 14.0	8.4	118	0.36
25.3	57.7	1460	0.86	S 13.5	7.0	95	0.50
36.1	61.4	2217	0.87	15.6	5.8	90	-
34.7	13.1	453	0.51	11.0	7.1	78	-
30.4	20.7	629	0.51	26.7	3.5	93	0.41
35.5	9.8	348	0.33	S 11.5	12.0	138	0.40
31.5	42.2	1329	0.54				
24.8	73.9	1833	0.77				
Mean. 25.7	74.9	1676	0.62	15.5	8.2	120.5	0.49

S = Swayback lamb.

dry weight of the lambs from the injected group was rather higher than that for the control lambs.

The mean liver copper concentration of the lambs from the injected ewes and of those from the control ewes were 74.9 and 8.2 p.p.m. respectively. The mean total liver-copper content of the lambs from the control ewes was 121 u. Treatment of the ewes increased the mean level to 1676 ug. The mean blood copper concentration of the lambs born to the treated ewes (0.62 p.p.m.) was greater than that of the control lambs (0.49 p.p.m.).

Two of the lambs from the injected ewes which had liver copper concentrations of only 9.8 and 13.1 p.p.m. had blood copper levels of only 0.33 and 0.51 p.p.m. respectively. Whilst it is , therefore, clearly evident that this copper glycine preparation effected a striking control of clinical swayback combined with good elevations in ewe and lamb blood copper concentrations it is possible that isolated individuals may not respond to any marked degree. This may be due to inefficiency in the injection or to some tissue reaction at the injection site which seals off the injected copper and prevents full absorption.

Experiment 6B.

The design and conduct of this experiment was similar to that of Experiment 6A. Swayback had occurred spasmodically in the lambs on this farm in previous years but the overall incidence had not exceeded 5 - 10%.

Twenty three/

Twenty three ewes were given a subcutaneous injection of copper calcium ethylene-diamine tetra acetic acid (Glaxo Ltd. "Coprin") which provided 50 mg. Cu on 12 February. Twenty three comparable ewes acted as untreated controls. Blood samples were obtained from all the ewes immediately prior to injection and again on 18 March. Lambing took place in early April. The ewes which grazed hill herbage throughout pregnancy received about 0.5 lb/head/day of a proprietary concentrate food (12 p.p.m. Cu) during the last four weeks before lambing.

Results.

The individual and mean ewe blood copper concentrations are given in Table 37.

The mean concentrations of the treated and control ewes were 0.74 and 0.57 p.p.m. respectively on 12 February. This difference was entirely fortuitous. By 18 March the mean value of the treated ewes had increased to 0.88 p.p.m. whilst that of the control group had fallen to 0.46 p.p.m.

The blood copper level of ewe No. 711 did not apparently respond to the copper treatment and this ewe gave birth to a swayback lamb. There were no other cases of swayback. In all probability the copper injection given to ewe No. 711 was incorrectly administered.

Eight lambs from the control ewes and seven lambs from the treated ewes were obtained for copper analyses. Most of these were surplus twin lambs which were slaughtered at a few days old. Individual and mean blood and liver copper concentrations of the lambs are given in Table 38.

Table 37. Effect of copper calcium EDTA (50 mg Cu) given by injection in mid-pregnancy (12 Feb) on blood copper concentrations in late pregnancy (18 March).

Injected Ewes						Untreated Ewes					
Ewe No.	12 Feb.	18 March	Ewe No.	12 Feb.	18 March	Ewe No.	12 Feb.	18 March	Ewe No.	12 Feb.	18 March
706	0.70	0.84	720	1.09	0.95	331	0.59	0.42	344	0.40	0.50
708	0.97	0.90	721	0.36	0.75	332	1.16	0.70	345	0.32	0.17
709	0.56	0.74	722	0.82	0.85	333	0.33	0.20	346	0.32	0.18
710	0.56	1.20	723	0.45	0.91	334	0.88	0.56	347	0.36	0.43
711	0.35	0.39	724	1.18	0.87	335	0.41	0.33	348	0.54	0.33
712	0.75	0.77	725	0.68	0.74	336	0.81	0.45	349	0.63	0.41
713	1.22	1.15	726	0.46	0.90	338	0.32	0.27	350	0.70	0.53
714	0.72	0.91	727	0.33	0.82	339	0.51	0.42	351	0.36	0.20
715	0.48	0.75	728	0.80	0.76	340	1.20	0.81	352	0.85	0.93
716	1.03	0.86	729	0.97	1.11	341	0.28	0.19	353	0.27	0.38
717	1.06	1.06	730	0.91	1.01	342	0.75	0.86	355	0.74	0.87
718	0.51	0.94				343	0.46	0.38			

Mean concentration	p.p.m. Cu
12 Feb	0.74
18 March	0.88

Mean concentration	p.p.m. Cu
12 Feb.	0.57
18 March	0.46

Table 38. Blood and liver - copper concentrations in lambs born either to ewes treated with copper calcium EDTA (50 mg Cu.) or to untreated ewes.

Injected				Untreated			
	Liver		Blood		Liver		Blood
Dry Wt. (g)	Cu.Conc. (p.p.m.)	Total Cu. (ug.)	Cu.Conc. (p.p.m.)	Dry Wt. (g)	Cu. Conc. (p.p.m.)	Total Cu. (ug.)	Cu.Conc. (p.p.m.)
23.2	177.1	4109	0.90	17.5	23.0	403	-
25.0	74.2	1855	0.87	23.8	7.3	174	0.31
18.0	118.2	2128	0.91	22.5	5.8	131	0.55
25.0	174.9	4373	0.92	26.0	7.4	192	-
16.2	177.9	2882	0.98	15.0	10.4	156	-
15.3	25.7	393	0.76	40.2	7.0	281	0.41
S15.8	7.3	115	0.41	24.5	37.3	913	0.66
				18.8	7.6	143	0.45
Mean							
19.6	107.9	2265	0.82	23.5	13.2	187	0.48

S = Swayback lamb born to Ewe 711.

With the exception of the lamb with swayback born to ewe No. 711, the copper injection markedly increased lamb blood and liver-copper concentrations. The mean blood copper level was increased from 0.48 to 0.82 p.p.m. The mean liver-copper concentration of the lambs born to the treated ewes was 107.9 compared with a mean level of only 13.2 p.p.m. for the control lambs.

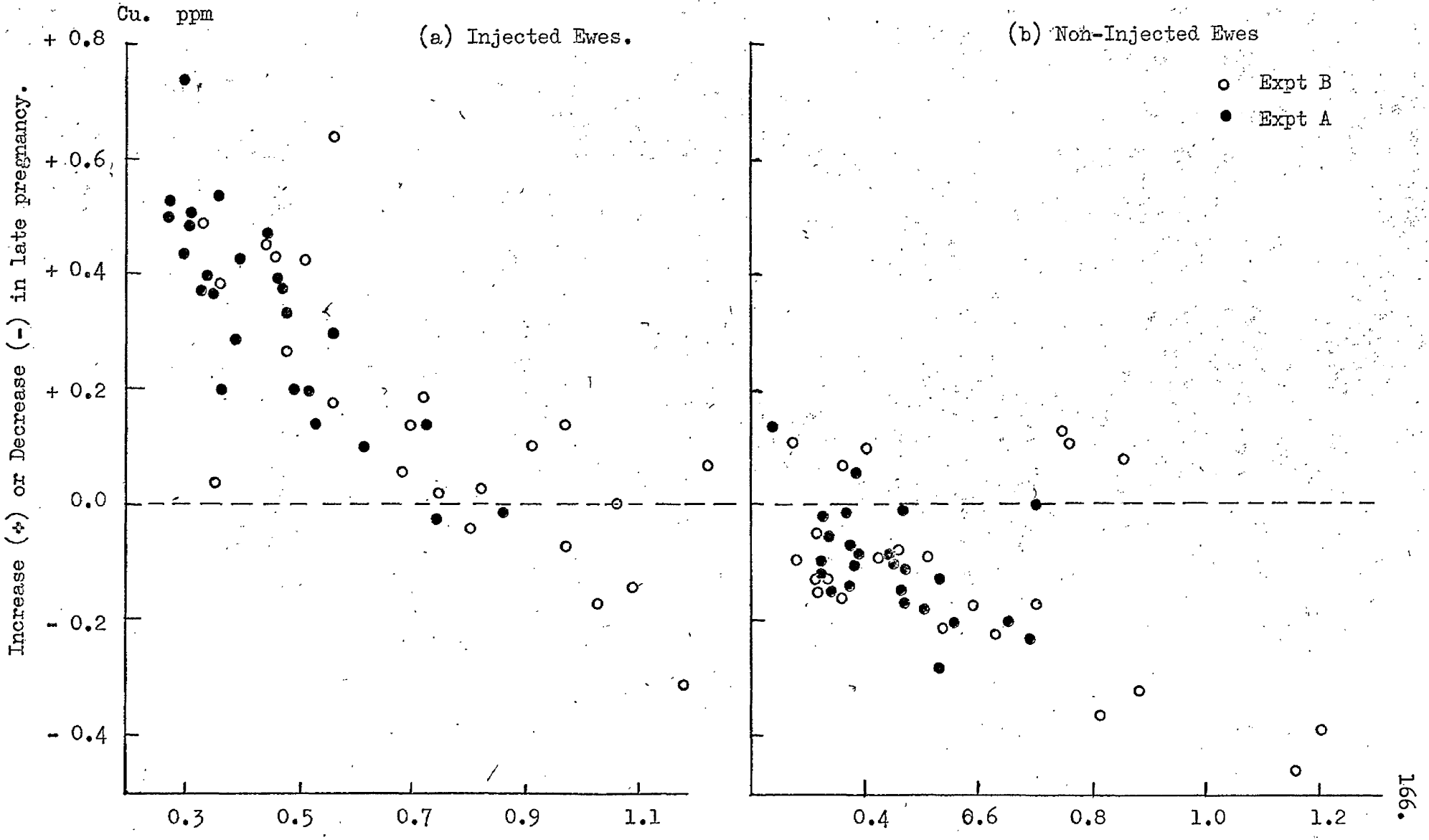
Copper calcium ethylenediamine tetra acetic acid supplying 50 mg. of copper by injection of the ewe is thus an effective agent for increasing lamb copper contents.

It is interesting to note the changes in blood copper concentration of the individual ewes between mid-pregnancy (pre-injection) and late pregnancy for Experiments 6A and 6B. Injection of copper generally increased ewe blood copper concentrations to about 0.8 p.p.m. in late pregnancy.

Fig. 17 illustrates the changes between mid and late pregnancy in ewe blood copper concentration as related to mid pregnancy (pre-injection) values. It illustrates the essentially similar response to both copper glycine (45 mg Cu in Experiment 6A and copper calcium EDTA (50 mg Cu) in Experiment 6B. Injected ewes with pretreatment blood copper levels in excess of 0.8 p.p.m. failed to respond to injection and indeed such values in late pregnancy were generally lower than in mid pregnancy. Ewes with pre-treatment concentrations of about 0.3 p.p.m. increased generally by about 0.5 p.p.m. to around 0.8 p.p.m. in late pregnancy.

In/

Fig 17. Changes in blood copper concentration in late pregnancy in relation to mid-pregnancy values for (a) injected and (b) non-injected ewes.



In contrast for these ewes which were not injected, copper concentrations in late pregnancy were generally lower than in mid-pregnancy. The greatest reductions were shown by these ewes which had the highest concentrations in mid pregnancy.

Experiment 6C.

The copper salt of methionine (Crookes Laboratories Ltd. "Copavet") supplying 40 mg Cu was employed in this experiment. The ewes on the farm on which Experiment 6C was conducted were of low copper status. In previous years ewe blood copper concentrations in the order of 0.3 p.p.m. had been recorded. Dead ewes and lambs in previous years had liver copper concentrations which were generally less than 10 p.p.m. Swayback occurred in most years.

Twenty four Blackface ewes were given copper methionate (40 mg Cu) by subcutaneous injection on 9 February and a further twenty four ewes acted as untreated controls. Lambing took place in mid-April.

Results.

It was not possible to obtain blood samples from these ewes but comparable untreated ewes had mean blood copper concentrations of 0.33, 0.37 and 0.29 p.p.m. in early February, mid March and early April respectively.

Six lambs including one swayback lamb were obtained within a few days of birth from the untreated ewes. The mean liver copper concentration of these lambs was only 8.4 p.p.m. and their mean blood copper concentration was only 0.33 p.p.m. (See Table 39).

Table 39. Blood and liver copper concentrations in lambs born either to ewes treated with copper methionate (40 mg Cu) or to untreated ewes.

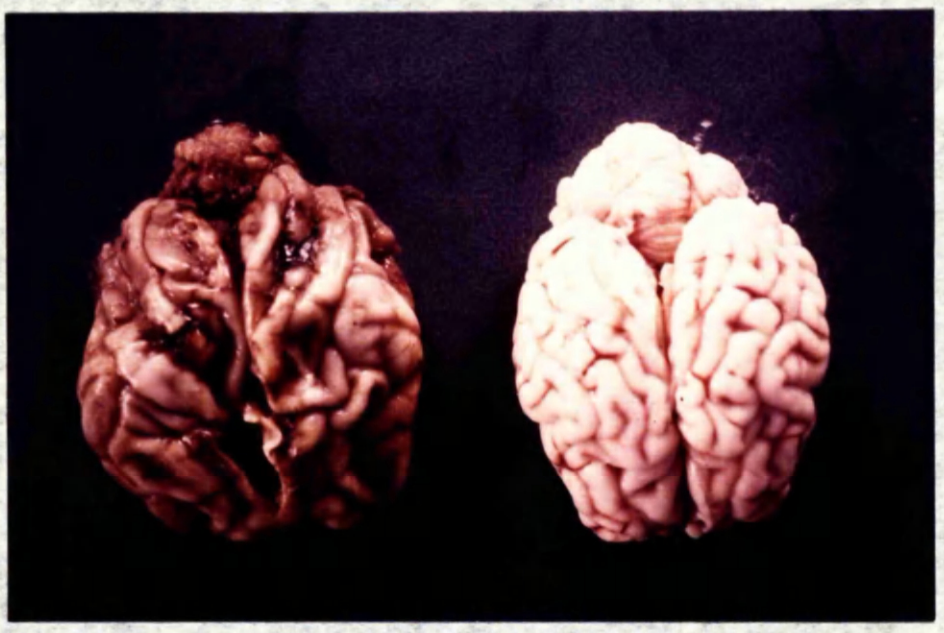
Treated				Untreated			
Liver			Blood	Liver			Blood
Dry Wt. (g)	Cu Conc. (p.p.m.)	Total Cu (ug)	Cu Conc. (p.p.m)	Dry Wt. (g)	Cu Conc. (p.p.m)	Total Cu (ug)	Cu Conc. (p.p.m)
13.5	33.1	447	0.44	18.0	9.8	176	0.40
26.5	36.1	957	-	9.0	13.9	125	-
12.0	51.3	616	-	4.6	9.2	42	0.52
21.0	42.5	893	-	S. 27.0	4.9	132	0.29
17.0	102.8	1748	-	14.0	5.9	83	0.22
10.5	65.8	690	-	25.0	6.6	165	0.23
11.0	13.5	149					
Mean;							
15.9	49.3	786	0.44	16.3	8.4	121	0.33

S = Swayback lamb.

Seven lambs were obtained from the injected ewes. Most of these had died shortly after birth and no blood copper concentrations are available. The mean liver copper concentration was, however, increased significantly to 49.3 p.p.m. compared to a mean of 8.4 for the control lambs. One of the lambs born to an injected ewe had a liver copper concentration of only 13.5 p.p.m.

It can, however, be concluded that 40 - 50 mg. of copper in the form of either copper glycine, copper calcium EDTA or copper methionate given as subcutaneous injections to the ewes in mid-pregnancy is effective in increasing the blood and liver copper status of their lambs at birth. The increases in ewe blood copper concentration and in liver copper levels in the lambs may be substantial. In isolated cases which are probably due to imperfections in the technique of injection or to tissue reaction at the injection site, there may be no response in either the ewe or lamb.

Fig. 17a Brains of a copper deficient and of a normal lamb.



This shows a partial collapse of both cerebral hemispheres in the brain of the copper deficient lamb. This resulted from a diffuse symmetrical gelatinous degeneration and liquefaction of the cerebral white matter.

Section IV

Copper Absorption in Housed Sheep

as affected by

a) The copper content of the diet
b) Copper supplementation
c) The protein content of the diet
d) The calcium content of the diet
e) The fibre content of diet
f) The type of sheep

Housed sheep are reputed to store more copper than similar sheep under more natural conditions outdoors. This has led to the belief that there is a danger of chronic copper toxicity occurring in housed sheep which are being fed diets containing normal or only slightly elevated levels of copper. The recent trend in Britain towards the housing of certain classes of sheepstock is likely to develop much further due to the intensification of farming methods and the comparatively inefficient and uneconomical use of land by the traditional methods of sheep husbandry.

With this possible further development in mind it was decided to make a thorough investigation of copper absorption in housed sheep with special reference to the possibility of the occurrence of chronic copper poisoning. A wide variety of diets have been fed to several different classes of stock and the influence of dietary composition and type of sheep on copper absorption has been examined. In some cases the rate of absorption of dietary copper by housed sheep was compared with that found for similar sheep on a similar diet outdoors. Various levels of copper supplementation were also given in an attempt to determine how quickly symptoms of chronic copper toxicity might appear.

The various experiments which were carried out with housed sheep will be reported in this section. This will be followed by a general integrating discussion on all these experiments paying special attention to any factors found which influence copper absorption and either increase or decrease the likelihood of copper poisoning.

Todd/

Todd et al.(1962) have suggested that in their experience chronic copper poisoning is exceeded only by lead poisoning as a toxic hazard to farm animals. Most of the cases of chronic copper poisoning reported have been in sheep grazing orchards where copper sulphate was used extensively. Housed sheep fed diets either naturally rich in copper or supplemented with copper sulphate may also accumulate copper in potentially toxic amounts.

However, when attempts have been made to produce copper poisoning experimentally, surprisingly large amounts of copper have generally been required. For example, Barden and Robertson (1962) fed housed adult sheep on hay and oats with a daily supplement of 1 g. copper sulphate thus giving an overall dietary copper concentration of about 250 p.p.m. The first death occurred after 15 weeks (104g $CuSO_4.5H_2O$). Todd et al.(1962) fed sheep at grass with 1 g. $CuSO_4.5H_2O$ daily and the first death (of 6 sheep) occurred after 28 weeks. Hemingway (1961) has also fed 1g $CuSO_4 5H_2O$ daily to pregnant ewes at grass for periods of 15 weeks with no obvious signs of distress to the sheep, although liver-copper concentrations increased to very high levels.

Many diverse factors affect the storage of copper in the liver. The dietary protein concentration has recently been shown to influence copper storage in both pigs and rats. Wallace et al.(1960) found that the toxicity of 750 p.p.m. of copper (as the sulphate) fed to growing pigs decreased as the protein level was increased from 15 to 25%. McCall and Davis (1961) found for rats that, when the diet contained 25% of protein, a supplement of 1000 p.p.m. of copper produced/

produced no significant increase in liver-copper concentration. When the diet contained only 10% of protein there was a highly significant increase in liver-copper storage resulting from the additional copper. More recently Hanrahan and O'Grady (1965) found that pigs being fattened on a high protein diet were less susceptible to, but not completely protected against, chronic copper toxicosis than pigs on a lower protein intake.

It was decided to investigate the possible role of protein intake on liver-copper storage by sheep. The effects of two levels of dietary protein intake on copper storage by sheep on low, medium and high copper intakes were investigated. This work is described in Experiment 7.

Experiment 7. The effects of protein intake on the storage of copper in the liver of sheep.

This experiment was designed to study the effect of the dietary protein level on liver copper storage by sheep on low, medium and high copper intakes. The high level of copper supplementation was given in order to determine whether a high dietary protein content would delay the development of chronic copper poisoning.

Fifty four Blackface hoggs, aged about 6 months, were randomly divided into five groups of eight and two groups of seven sheep, in such a way that the separate groups had a similar liveweight distribution. One group of eight sheep was slaughtered at the commencement of the experiment, in order to determine the approximate mean initial liver-copper concentration for the whole group of fifty four.

The remaining six groups of sheep were housed and fed a basal diet of 0.75 lb. of hay per day (5.8 p.p.m. Cu, 9.8% crude protein). Three of the groups received a daily supplement of 0.5 lb. of crushed oats (6.0 p.p.m. Cu, 8.5% crude protein). The other three groups were fed 0.5 lb. per day of a mixture of seven parts of crushed oats and one part of blood meal (6.2 p.p.m. Cu, 18.9% crude protein). Blood meal was chosen as the source of supplementary protein as it had a similar, low copper concentration to that of oats and because only/

only a small proportion needed to be added to the oats to materially increase the protein content of the concentrate supplement. Most other protein rich concentrates contain considerably more copper (from 15 - 25 p.p.m.). These two concentrate diets are subsequently referred to as the low protein and the high protein groups. These rations were chosen to provide comparable amounts of energy to those diets which are commonly fed to Blackface ewe hoggs over-wintered indoors and where the expected liveweight gain over a 6 month period would be 5 - 20 lb.

There were three levels of copper supplementation given as $CuSO_4.5H_2O$; nil, 40 mg (≡ 10 mg Cu), and 1000 mg (≡ 250 mg Cu) per day. One of each of these supplements was given to both a low and high protein group. Table 40 gives the total daily copper intake of the separate groups

Table 40 Total daily copper intake (mg).

Copper supplement (mg Cu)	Low Protein	High Protein
Nil	3.33	3.39
10	13.33	13.39
250	253.33	253.39

Low protein = hay 12 oz, oats 8 oz.
High protein = hay 12 oz, oats 7 oz, blood meal 1 oz.

The/

The sheep were introduced to the indoor diets on 20 November 1962. Administration of copper sulphate (given in solution by mouth), commenced on 10 December 1962. The supplement of 10 mg. per day was given on five days of each week for the period 10 December to 5 April 1963. Dosing was discontinued between 5 and 22 April and resumed during the period 23 April to 2 May. On 2 May these two groups of sheep and the two groups of control sheep receiving no copper supplement were slaughtered. By this date the sheep dosed with the low copper supplement had received a total of 960 mg. of copper given in 96 doses over a period of 163 days.

Administration of 250 mg Cu per day was also commenced on 10 December and continued on 5 days of each week until 5 April when 88 doses (≡ 22 g Cu) had been given. The first death from copper poisoning occurred on 30 January after 49 doses (≡ 12.25 g Cu) and several deaths occurred during the period 30 January - 5 April. Dosing was resumed on 22 April and continued until 12 August for those sheep which did not die earlier, when 183 doses (≡ 45.75 g Cu) had been given. Eleven of the fourteen sheep in these two groups died of copper poisoning and the other three were slaughtered at various stages of the experiment when they were still in good health.

The sheep were weighed monthly. Blood samples were obtained on 6 occasions/

occasions from the group which received either no copper or the low copper supplement. Blood sampling was much more frequent for those sheep which received the high copper supplement and which died of copper poisoning. The whole livers of all the sheep were recovered at slaughter or death for determination of dry matter content and copper concentration.

Results.

1) Control sheep and sheep receiving a daily supplement of 10 mg Cu.

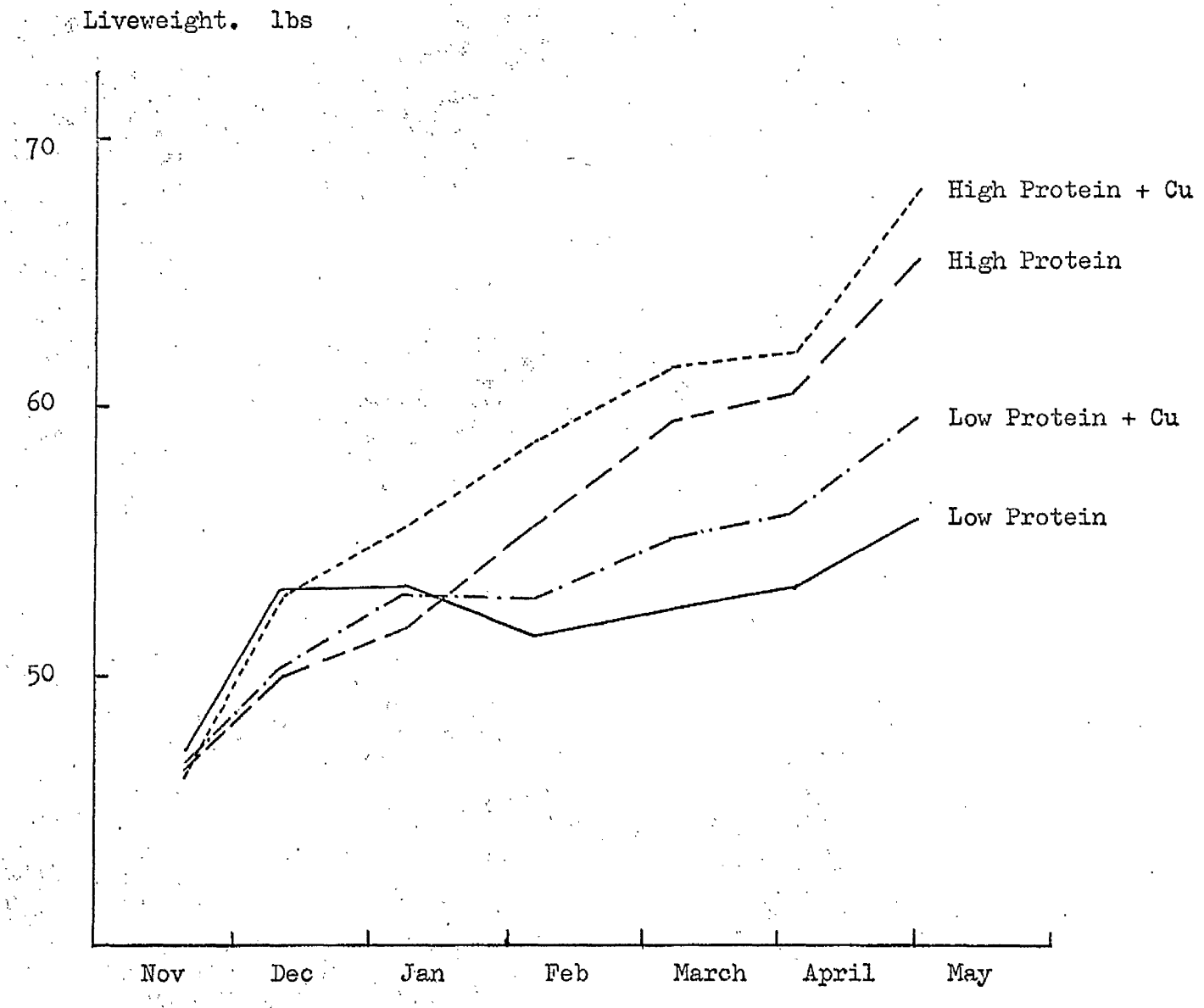
Liveweight Changes.

The mean liveweights of the group of eight sheep slaughtered at the start of the experiment on 20 November was 48.1 lb. and the mean values for the other four groups ranged from 46.7 to 47.3 lb. The progressive changes in mean liveweight of these four groups are shown in Fig. 18.

The two groups which were fed the high protein concentrate had greater increases in liveweight than those fed oats alone. Their combine final mean liveweight (66.95 lb) was significantly ($P<0.05$) greater than that for those fed the low protein diet (57.7 lb). The supplement of 10 mg. of Cu per day effected some increases in liveweight for both the low and high protein groups. Although the increases were not significant there was a constant trend of $2\frac{1}{2}$ - $3\frac{1}{2}$ lb. mean liveweight in favour of the copper supplemented groups. Significant regressions ($P<0.01$) for mean liveweight increases were obtained for all groups except that fed the low protein diet unsupplemented with copper.

Blood/

Fig 18. Changes in mean liveweight.



Blood Copper Concentration.

The individual and mean blood copper concentrations for the four groups of sheep are given in Table 41.

The mean blood copper concentrations for the four groups of sheep are shown in Fig. 19. At the start of the experiment the mean values of the separate groups ranged from 1.02 to 1.12 p.p.m. Cu and the mean value of the slaughtered group was 1.15 p.p.m. Blood copper concentrations of both groups which did not receive supplementary copper decreased steadily over the experimental period, but a significant regression ($P<0.02$) was obtained only for the low protein group. Blood copper concentrations remained constant for both the low and high protein groups which received 10 mg. of additional copper per day.

Liver dry weight, liver copper concentration and total liver copper content.

Table 42 presents the individual and mean dry weights and copper analyses of the livers of those four groups of sheep and of the group killed initially.

Table/

Table 41. Individual and mean blood copper concentrations(p.p.m.) of 4 groups of sheep on 6 sampling occasions.

Low Protein

No Copper Supplement							10 mg. Cu. Supplement						
NO.	20/11/62	9/1/63	7/2	5/3	2/4	1/5	NO.	20/11/62	9/1/63	7/2	5/3	2/4	1/5
2	1.21	1.02	0.84	0.72	0.31	0.44	4	1.21	1.42	1.31	1.21	1.09	1.30
3	0.78	1.00	1.01	0.94	0.98	1.30	19	1.11	1.15	1.09	1.19	1.16	1.05
8	1.06	0.98	0.97	1.00	1.01	0.83	32	1.34	1.13	1.08	1.18	1.16	1.37
9	1.15	0.98	0.85	0.99	0.76	0.99	35	0.96	1.05	1.03	1.02	1.01	1.06
15	0.83	0.83	0.83	0.81	0.82	0.94	37	1.08	1.25	1.23	1.13	0.91	1.28
16	0.90	0.73	0.59	0.50	0.38	0.43	43	0.81	0.93	0.89	1.18	1.05	0.85
31	0.98	0.70	0.78	0.87	0.81	0.82	51	1.36	1.17	1.09	1.02	1.16	0.99
58	1.29	0.99	0.89	1.00	0.77	0.72	62	1.09	0.96	1.03	0.87	0.85	0.90
Mean	1.03	0.90	0.85	0.85	0.73	0.81		1.12	1.13	1.09	1.10	1.05	1.10

High Protein

No Copper Supplement							10 mg. Cu. Supplement						
22	0.98	0.94	0.84	0.81	0.81	0.79	7	1.14	1.17	1.11	1.22	1.28	1.19
27	1.16	1.08	0.98	0.88	0.88	1.06	10	1.14	0.98	0.97	1.10	0.98	1.08
28	0.99	1.00	0.75	0.72	0.72	0.93	13	0.99	0.99	0.86	0.91	0.95	1.16
45	0.74	0.72	0.59	0.40	0.54	0.70	14	1.19	1.24	1.05	1.11	0.97	1.06
50	1.12	0.92	1.11	1.16	1.11	1.19	17	0.97	1.03	1.00	1.26	0.95	1.06
52	1.12	1.12	1.03	1.12	0.97	1.15	24	1.13	1.27	1.16	1.08	0.98	0.97
57	1.06	0.73	1.05	0.97	1.03	1.15	53	1.08	0.97	1.16	1.00	1.27	1.15
59	1.36	0.94	0.90	0.76	0.78	0.78	61	0.80	0.86	1.08	0.90	1.17	1.01
Mean	1.07	0.93	0.91	0.85	0.86	0.97		1.06	1.06	1.05	1.07	1.07	1.09

Fig 19. Changes in mean blood copper concentration.

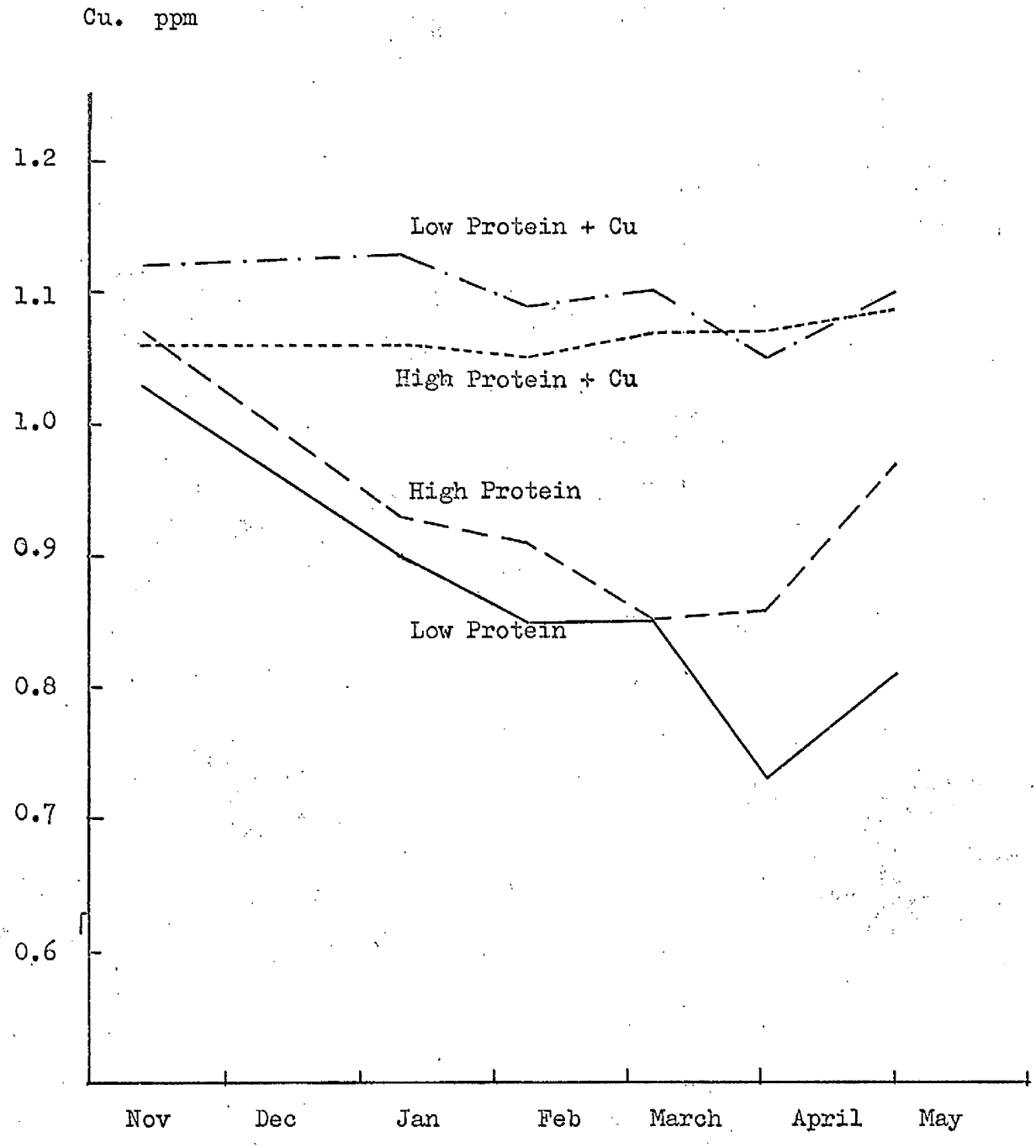


Table 42. Mean and individual liver dry weights (g) liver copper concentrations (p.p.m) and liver total copper contents (mg).

	No Supplementary copper						10 mg supplementary copper					
Killed Initially	Low Protein			High Protein			Low Protein			High Protein		
No.	Dry Wt	Cu Conc.	Total Cu	No.	Dry Wt	Cu Conc.	Total Cu	No.	Dry Wt	Cu Conc.	Total Cu	No.	Dry Wt	Cu Conc.	Total Cu	No.	Dry Wt	Cu Conc.	Total Cu
1	71	327.0	23.1	2	95	16.6	1.6	22	101	137.1	13.8	4	134	272.6	36.6	7	161	179.7	28.9
0	95	48.6	4.6	3	85	145.6	12.3	27	91	47.1	4.3	19	120	115.5	13.8	10	121	248.9	30.1
3	101	54.5	5.5	8	81	31.7	2.6	28	120	36.0	4.3	32	78	527.0	41.1	13	97	275.8	26.6
0	92	83.6	7.7	9	95	49.5	4.7	45	132	20.6	2.7	35	120	230.9	27.7	14	143	486.2	69.3
6	98	78.8	7.7	15	103	64.5	6.7	50	108	220.2	23.8	37	94	746.0	70.1	17	113	505.4	57.2
4	78	147.2	11.5	16	97	18.2	1.8	52	102	207.7	21.3	43	101	369.9	37.5	24	121	424.7	51.3
6	89	82.5	7.3	31	111	203.7	22.7	57	139	149.5	20.8	51	104	117.6	12.2	53	127	152.5	19.4
4	101	131.7	13.3	58	81	29.3	2.4	59	113	34.2	3.9	62	110	401.3	44.0	61	124	368.1	45.6
an	91	119.2	10.1		94	69.9	6.9		113	106.6	11.9		108	347.6	35.4		126	330.2	41.1

S.D. (between means) (P=0.05)

Dry Wt = 15.2g

Cu Conc. = 125.4 p.p.m.

Total Cu = 12.3 mg.

The daily supplement of 10 mg. of copper did not significantly increase the mean liver dry weights. For the low protein group the increase from 93.5 to 107.6 g (13.9 g) and the increase from 113.2 to 125.8 g (12.6 g) for the high protein group should be compared with the least significant difference ($P<0.05$) of 15.2 g. Both high protein groups had significantly greater mean liver dry weights than the corresponding low protein groups. Only the mean liver dry weight of the low protein group fed no supplementary copper failed to increase significantly relative to the group slaughtered initially. These changes in mean liver weight correspond with the changes in mean liveweight of the separate groups.

The mean liver-copper concentration of the unsupplemented low protein group (70.1 p.p.m.) was less than that for the group killed initially (119.5 p.p.m.) and for those fed the high protein concentrate (106.6 p.p.m.), but those differences were not significant. The addition of 10 mg Cu per day markedly (and to a similar degree) raised liver-copper concentrations, irrespective of the high or low protein content of the diet.

The total liver-copper content of the group killed initially was 10.1 mg. and that for the high protein group was 11.9 mg, the mean total liver-copper content of the low protein group being 6.9 mg. Although this reduction was not significant, it shows a similar trend to the significant fall in blood copper concentration for this group, from 1.03 to 0.81 p.p.m. over the period of the experiment (Fig. 19). In the two copper supplemented groups, the mean total liver copper contents of the low and high protein groups were 35.3 and 40.8 mg. Cu respectively; those amounts were significantly ($P<0.01$) greater than for the groups which did not receive additional copper, but the level of dietary protein did not have a significant effect on the total amount of/

of copper stored in the liver. These two groups were given a total of 960 mg Cu (almost 4 g. of $CuSO_4.5H_2O$) more than the other groups. The proportion of this supplementary copper stored in the liver was 2.96% and 3.01% for the low and high protein groups respectively and at the level of copper supplementation used, the difference in protein intake did not affect the proportion of orally dosed copper stored in the liver.

2) Sheep receiving the high copper supplement.

Liveweight changes.

The liveweight gains of both the groups of seven sheep receiving the 250 mg Cu supplement each day were similar to those of the control sheep and those given the small copper supplement when fed the equivalent diets until 6 March when the mean weight of the low protein group was 54.5 lb. and that for the high protein group was 57.2 lb. After this the low protein group started to lose weight. The protein group was not affected at this stage by the additional copper and continued to gain weight at a rate equivalent to both the control group and the low-Cu supplement groups: on 1 May the four surviving sheep in this group had a mean liveweight of 65.3 lb.

In the final stages of copper poisoning loss of weight occurred in most of the sheep as a result of an inappetance which developed a few days before death, and was generally the first sign of distress. One sheep on the low protein diet lost 16 lb. in the 2 weeks before death, but a loss of about 8 lb. was more general. Loss of weight shortly before death was much more evident for the sheep on the low protein diet, and only rarely and to a lesser extent (about 3 lb) did a weight loss occur in sheep on the high protein ration which died of copper poisoning.

Blood/

Blood Copper Concentration

Table 43 shows the blood copper concentrations of the sheep in both the low and high protein groups. Mean values are given up until the beginning of February. After this date individual values were much higher and varied widely. This resulted from the abnormally high values found at or near the time of death of individual sheep. Values of 9.3, 10.6, 12.0 and 12.7 p.p.m. were recorded and as the inclusion of such values would greatly distort the mean for each group, such mean values are not presented. After the beginning of February the blood copper concentrations are related to the time of death of the individual sheep.

The mean blood copper concentrations of both the low and high protein groups remained within the range 0.94 to 1.10 p.p.m. during the first 10 weeks of the experiment. After this some very high values (>9.0 p.p.m.) were recorded for individual sheep as they neared death due to chronic copper poisoning. Such high blood copper concentrations would clearly be most unusual for healthy animals, and would satisfactorily confirm that death from copper poisoning was almost certainly imminent. On the other hand, many blood copper concentrations below 2.0 p.p.m. were encountered during the 2 weeks prior to the onset of obvious clinical symptoms and death. A total of 42 samples, all of which/

Table 43. Blood copper concentrations (p.p.m.) of 2 groups of sheep from November 1962 - February 1963 and thereafter related to their time of death from copper poisoning.

Low Protein

No.	20/11/62	9/1/63	7/2/63	Days before Death 22-28	15-21	11-14	8-10	7	6	5	4	3	2	1	Died
11	1.39	1.15	-	1.15	-	-	-	-	-	-	-	-	-	-	2/2
12	0.81	1.14	1.08	-	1.75	-	2.00	12.00	10.40	5.64	-	4.20	3.60	3.42	21/3
18	1.07	0.92	1.00	1.26	-	1.55) 1.28) 1.26)	1.49	1.31	-	-	-	1.98	-	-	5/4
29	0.99	0.87	1.25	1.25	1.53	1.61	9.30	2.60	2.52	2.56	2.36	2.40	-	-	1/3
42	1.20	1.03	-	1.03	-	-	1.72	-	-	-	-	1.80	-	-	7/2
47	1.00	0.94	0.94	1.85	1.60) 4.56) 1.88)	2.22) 2.44)	2.48) 3.04)	-	-	2.50	-	3.10	-	12.70	14/4
48	1.10	1.29	1.03	-	1.16	-	1.20	-	-	-	-	-	-	2.24	23/3
Mean	1.08	1.05	1.06												

High Protein

5	0.75	0.92	1.16	-	-	1.08	0.87	1.16	0.98	-	-	-	-	1.05	2/5+
33	1.30	1.36	1.00	1.73	-	-	-	-	-	-	-	-	-	-	12/8+
38	0.90	0.96	1.37	-	1.64	1.80	1.45	1.41	1.36	-	-	-	-	1.77	2/5+
39	0.64	0.85	0.78	1.42	1.31	1.77) 1.44)	1.47	1.42	-	-	-	1.55	-	-	5/4
40	0.80	0.92	-	-	0.92	-	-	-	-	-	-	-	-	-	30/1
41	0.90	1.19	1.15	1.26	1.21	-	-	-	-	-	1.47	-	-	-	9/3
55	1.27	1.23	1.13	1.35	-	1.27	-	-	1.75	-	-	-	-	10.60	18/7
Mean	0.94	1.06	1.10												

+ Sheep marked thus were slaughtered while still in good health.

which contained below 2.0 p.p.m. of copper, were obtained from the 11 sheep which died of copper poisoning. The distribution of copper concentration, in the range 1.20 to 1.99 p.p.m. in these samples is detailed in Table 44.

Table 44. Concentrations of copper in the blood of 11 sheep (which died of copper poisoning) during the 2 weeks prior to the onset of clinical symptoms. (Values above 2.0 p.p.m. have been excluded).

Blood Cu Concentration (p.p.m.)	No. of samples
1.20 - 1.29	5
1.30 - 1.39	6
1.40 - 1.49	10
1.50 - 1.59	4
1.60 - 1.69	4
1.70 - 1.79	6
1.80 - 1.89	4
1.90 - 1.99	3

Blood copper varied irregularly in all animals during this period. If 1.30 p.p.m. were taken as the upper limit of copper concentration in the blood of normal sheep, 12 per cent of samples obtained from animals within two weeks of death would have fallen within normal limits; if 1.40 p.p.m. had been chosen, 26 per cent of samples would have appeared normal. Blood copper concentrations/

concentrations within the range 1.20 to 1.29 were found on at least one occasion in 4 sheep and values between 1.30 and 1.39 p.p.m. at least once in 3 more sheep within two weeks of death. Thus, only in 4 of the 11 sheep was the concentration above 1.40 p.p.m. in all samples taken during the two weeks prior to death. A single blood sample may, therefore, sometimes fail to indicate the existence of copper poisoning, even though death is imminent; the usefulness of blood analysis for this purpose is enhanced by increasing the number of samples examined. Blood copper concentrations of individuals during the two weeks prior to death commonly fell within the range 1.3 - 3.0 p.p.m. (with occasional much higher values) for the low protein group whereas a range of 1.3 to 1.7 p.p.m. was more usual for the high protein group.

In some cases, as the illness progressed, the blood packed cell volumes fell as low as 8 compared with values of about 35 for normal sheep. In individuals in which a partial recovery occurred the value rose again to about 20.

Liver dry weight, liver-copper concentration and total liver-copper content.

Table 45 shows the concentrations and total amounts of copper in the livers. It also shows the amount of copper sulphate given to each sheep and the proportion of this stored in the liver.

Table/

189.

Table 45. Quantities of copper sulphate administered and the amounts of copper stored in the liver of sheep which died of copper poisoning or were slaughtered after long-term dosing.

No.	$CuSO_4.5H_2O$ given (g)	No. of days to death	Liver dry wt(g)	Cu Conc.(p.p.m)	Total Cu(mg)	%of dosed Cu in liver
Low Protein.						
11	51	74	60	6530	392	3.00
42	54	79	55	5300	294	2.17
29	65	101	80	3750	300	1.85
12	77	121	103	3674	379	1.97
48	78	123	98	4718	460	2.36
18	88	136	87	4259	370	1.68
47	88	144	72	4689	337	1.53
Mean	84	129	84	4295	337	1.87
High Protein.						
40	49	71	98	3930	386	3.15
41	69	109	109	2370	259	1.50
39	88	136	84	1558	131	0.60
55	158	240	148	1924	287	0.73
5	88	163	101	3313	334	1.52
38	88	163	106	2435	257	1.17
33	183	265	117	2425	284	0.62
Mean	103	164	109	2565	277	1.33
Sign Diff. Bet.Means.	N.S.	0.01	0.05	0.01	0.05	0.1

All the seven sheep on the low protein diet, but only four of the seven on the high protein diet, died of copper poisoning. The other three were slaughtered at various stages of the experiment when they were quite healthy. The first sheep which died (No. 40) was fed on the high protein diet. Death occurred after 49 doses of 1 g $CuSO_4.5H_2O$. The first death in the low protein group occurred after 51 doses. By 5 April (after 88 doses over 144 days) all seven of the sheep fed the low protein diet had died compared with only three from the high protein group. The two sheep (Nos. 5 and 38) slaughtered after 88 doses over 163 days and the other (No. 33) after 183 doses over 265 days, from the high protein group appeared to be in normal health and were not in immediate danger of death from copper poisoning.

If copper sulphate had continued to be administered until a fatal amount of copper had accumulated in those three sheep fed the high protein diet the mean amount given (103 g) and the mean time to death (164 days) would have been increased. Although there was no significant difference in the amounts of copper needed to cause death in these two groups, this largely results from the high standard errors associated with these small groups. The sheep in the high protein group were, however, noticeably better in vigour and condition throughout the experiment. One was given as much as 158 g $CuSO_4.5H_2O$ over 240 days before death and a further one was slaughtered after 183 g had been given over 265 days. In spite of the lack of statistical evidence it seems reasonable/

reasonable to assume that an increased protein intake delayed the time of death of those sheep which were given regular doses of 1 g. $CuSO_4.5H_2O$ over a long period.

The sheep on the high protein diet had greater liver dry weights than those fed oats alone and this was a reflection of their increased live weight. The low protein group had a mean liver copper concentration at death of 4295 p.p.m. This was significantly ($P < 0.01$) higher than both the mean level of 2446 p.p.m. for those four sheep fed the high protein diet which died and for the mean value of 2565 p.p.m. for all the sheep in that group.

The mean total liver copper content of 337 mg. for the low protein group was significantly ($P < 0.05$) greater than the mean of 277 mg. for all the seven sheep in the high protein group. The difference was not quite significant at this level when the mean of 266 mg. for the four sheep which actually died of copper poisoning was considered.

There was a marked tendency for the proportion of dosed copper found in the liver at death to be greater for those sheep which died early than for those which died after a much longer period. Those sheep fed supplementary protein stored a smaller ($P < 0.1$) proportion of the dosed copper than those fed oats alone. The proportion of dosed copper stored in the liver was in the range 1.5 - 3.0% for the sheep fed the low protein diet. Only the first sheep (No. 40) to die in the high protein group stored more than 1.5% of the administered copper in its liver and three of the sheep in this group stored as little as 0.6 - 0.7% of the copper given.

Discussion/

Discussion.

Copper requirement of housed sheep.

Although the differences were not significant there was a tendency for the sheep receiving 10 mg Cu per day to gain rather more weight than those without copper supplement which received a total of only 3.3 mg Cu per day (see Fig. 18). In the absence of supplementary copper both the low and high protein groups showed steadily falling blood copper concentrations compared with the steady values of those given an additional 10 mg Cu/day (Fig. 19). The fall was, however, only significant for the low protein group. The concentration of copper and the total amount of copper in the liver were appreciably lower for the low protein group than for those killed initially (Table 42). Although the differences were not significant reduction of the order of 30 - 40% were obtained. The high protein group however had a similar mean liver-copper concentration to that of the sheep killed initially.

It is generally considered that housed sheep accumulate copper. There is no indication that this occurred in the two groups on diets containing only about 6.0 p.p.m. Cu with no copper supplement, and depletion of liver copper may have occurred in some cases. The results in Table 42 suggest that a supplement of 10 mg Cu allowed some accumulation of copper in the liver, but this was not associated with an increase in blood copper concentration over the five month period.

Proportion/

Proportion of administered copper stored in the liver.

The mean percentages of the total of 960 mg Cu administered in 10 mg doses stored in the liver were 2.96 and 3.01 for the groups fed the low and high protein diets respectively. These figures have been calculated from the total liver-copper contents of these sheep compared with those fed the unsupplemented diets which were slaughtered at the same time.

These results are in good agreement with figures quoted by previous workers where similar amounts of copper have been given. Edgar (1942) gave 25 mg Cu/day (as the sulphate) to 16 sheep at grass for 228 days and found that the mean amount of copper stored in the liver was 2.3% (range 1.0 - 3.9%) of the total given. Dick (1954) found in a large number of experiments with both housed sheep and sheep at grass that when 30 mg. supplementary copper (as the sulphate) was given each day, the proportion stored in the liver ranged from 2.7 - 4.1%. Hemingway et al.(1962) dosed 8 sheep at grass with 0.5 g $CuSO_4.5H_2O$ at fortnightly intervals, the dose averaging 9 mg. Cu/day, and found that 2.4% of this copper was stored in the liver as measured by comparison with untreated sheep.

Of the sheep receiving a supplement of 250 mg Cu ($\equiv$ 1 g $CuSO_4.5H_2O$) the first two which died stored in the liver 3.15 and 3.00% of the total copper administered (Table 45). The mean percentage of dosed copper found in the liver for the eleven sheep which died was 1.77. There was a marked tendency/

tendency for the proportion of copper stored to be less for sheep which survived for a longer period and for those fed supplementary protein.

Amounts of copper required to cause death from copper poisoning.

Table 46 summarises the results of this present investigation and similar experiments conducted by Barden and Robertson (1962) and Todd et al. (1962) where regular daily doses of 1.0 - 2.0 g. of $CuSO_4.5H_2O$ were administered.

Table 46 Amounts of copper sulphate required to cause death in sheep when administered at 1 g. per day.

	Weeks dosed	$CuSO_4.5H_2O$ consumed (g)
Barden & Robertson (1962)	15 - 17	104
Todd et al. (1962) a)	17 - 28	80 - 184
b)	17 - 29	144 - 154
Present experiment		
Low protein diet	11 - 21	52 - 88
High protein diet	10 - 34	48 - 156

The first deaths to occur in the present experiment were recorded after a shorter period of copper supplementation and after the administration of a smaller/

smaller total amount of copper than those found by those previous investigators. In the present experiment, however, the sheep were much smaller (60 lb) than those used by previous workers (about 100 lb). In consequence the daily supplement in terms of unit body weight was proportionately larger in the current experiment.

The amount of copper administered at a rate of about 1 g $CuSO_4.5H_2O$ /day required to kill a sheep ranges from 48 to 156 g as $CuSO_4.5H_2O$ and this wide range may depend on liveweight, liver size and the composition of the diet. It would appear that death of sheep from copper poisoning might occur after 3 - 6 months if the diet contains about 250 p.p.m. Cu. The present investigation suggests that a high protein intake may make sheep less susceptible to copper poisoning resulting from this order of copper supplementation. Where only small amounts of supplementary copper were given (10 mg/day) additional protein had no such effect.

The consistent findings that under 3% of orally administered copper sulphate is stored in the liver of otherwise healthy sheep, allows an estimate to be made of the total copper consumption in a suspected case of poisoning. For this the total liver-copper content is required rather than a simple expression of the copper concentration.

The/

The sheep receiving 10 mg. of supplementary copper per day stored copper in the liver at the same rate (3%) as those which died most rapidly when given the 250 mg Cu/day supplement. The minimum quantity of copper required to kill a sheep under the conditions of the present experiment was 12.5 g Cu (See Table 45).

Copper Concentration in the Wool.

Wool samples were obtained from all the sheep in each of the six groups. All samples were taken from the shoulder and were restricted to the 2" of new growth next to the skin. This was done in order that the copper concentration of the wool might reflect the copper concentration in the diet over the experimental period. All the samples obtained were analysed for their copper concentration and the results are presented in Table 47.

The level of protein in the diet had no effect on the copper concentration in the wool. If, however, the sheep are grouped according to the level of copper supplementation the mean wool copper concentration of those sheep receiving 10 mg. extra copper daily was significantly ($P<0.05$) higher than that of the sheep which received no supplementary copper. Again the mean wool copper concentration of the sheep given 250 mg supplementary copper per day was significantly ($P<0.01$) higher than those of either the unsupplemented sheep or the sheep which received 10 mg. supplementary copper daily.

The wool copper concentrations found in this experiment are rather lower than those reported by Cunningham and Hogan (1958) who recorded values in the range 8.3 - 13.3 p.p.m.

Kidney/

Table 47. Individual and mean wool copper concentrations (p.p.m.) of six groups of sheep.

Copper supplement.	Nil			10 mg				250 mg			
Low Protein.		High Protein.		Low Protein		High Protein.		Low Protein.		High Prot	
No.	Cu.	No.	Cu.	No.	Cu.	No.	Cu.	No.	Cu.	No.	Cu.
2	4.7	22	4.4	4	4.4	7	4.4	11	5.2	5	4.6
3	5.0	27	3.6	19	4.6	10	4.8	12	5.2	33	4.7
8	4.8	28	4.1	32	4.4	13	4.4	18	5.2	38	5.6
9	3.9	45	3.1	35	4.5	14	5.2	29	5.8	39	4.8
15	3.3	50	4.8	37	4.6	17	4.2	42	4.7	40	N.S.
16	2.9	52	4.8	43	3.9	24	5.1	47	5.9	41	4.8
31	4.1	57	5.3	51	4.9	53	4.9	48	5.7	55	5.2
58	4.6	59	4.2	62	4.9	61	5.0				
Mean	4.16		4.29		4.53		4.75		5.39		4.95
Mean		4.225			4.64				5.18		

L.S.D. P = 0.05 = 0.406. L.S.D. P = 0.01 = 0.428

Kidney Copper Concentration

The kidneys were obtained from all the sheep in each of the six groups either at death or at slaughter. These were dried, ground and analysed for their copper concentration. The results are presented in Table 48.

Apart from one sheep the kidney copper concentration of all the sheep in the control and low copper supplemented groups were within the range 12.3 - 23.1 p.p.m. The one exception, sheep No. 8, had a kidney copper concentration of 55.7 p.p.m. There was no difference between the kidney copper concentrations of the unsupplemented sheep and those of the sheep which received a daily supplement of 10 mg Cu. It is evident, therefore, that this level of copper supplementation had no influence on the kidney copper concentrations of the sheep.

Kidney copper levels were much higher for the two groups which received the 250 mg. copper supplement daily. These ranged from 56.1 p.p.m. for sheep No. 5 to 2068.0 p.p.m. for sheep No. 39. The three sheep which were slaughtered (Nos. 5, 33 and 38) had kidney copper concentrations at the lower end of this range (56.1, 249.5 and 185.7 p.p.m. respectively).

A daily copper supplement of 250 mg. per day effected increases in kidney copper levels in all sheep irrespective of whether they died of chronic copper poisoning or whether they were slaughtered while in good health. Sheep No. 33 which survived for 265 days and was eventually slaughtered after receiving 183 g $CuSO_4.5H_2O$ had a kidney copper concentration of 249.5 p.p.m. which is more/

TABLE 48.

Individual and mean kidney copper concentrations (p.p.m.) of six groups of sheep.

Copper Supplement	Nil.		10 mg.				250 mg.			
Low Protein		High Protein		Low Protein		High Protein		Low Protein		High Protein.	
No.	Cu.	No.	Cu.	No	Cu.	No.	Cu.	No.	Cu.	No.	Cu.
2	16.6	22	19.2	4	19.1	7	23.1	11	n.d.	5+	56.1
3	15.9	27	12.3	19	16.6	10	16.6	12	354.9	33+	249.5
8	55.7	28	17.8	32	16.9	13	19.0	18	1148.8	38+	185.7
9	15.0	45	18.9	35	14.3	14	16.8	29	147.8	39	2068.0
15	15.1	50	13.9	37	18.6	17	20.5	42	277.6	40	124.6
16	18.5	52	19.0	43	14.8	24	19.3	47	299.0	41	1304.9
31	15.0	57	15.8	51	17.3	53	15.3	48	60.1	55	1046.3
58	17.9	59	16.8	62	15.5	61	19.7				
MEAN	21.2		16.7		16.6		18.8		381.4		719.3

\+ slaughtered. n.d. = not determined.

more than ten times the normal level. Consequently it would be unwise to diagnose chronic copper poisoning purely on a basis of the kidney copper concentration. Ross (1964) suggests that the copper concentration of the kidney cortex would be better than the liver copper concentration for the diagnosis of poisoning. This, however, is probably too wide a generalisation. For example, sheep No. 29 which survived for two weeks after the initial onset of the haemolytic crisis had a kidney copper concentration of only 147.8 p.p.m. This contrasts with a kidney copper concentration of 249.5 p.p.m. for sheep No. 33 which was slaughtered while still in good health. It appears that if a sheep dies suddenly there is generally a highly elevated kidney copper concentration but if it survives for some length of time after the initial crisis then the kidney copper concentration returns to a nearer normal level.

Experiment 8 (a) The Effect of Varying Calcium Intakes on the Storage of Copper and Iron in the Liver of Housed Sheep.

The experimental design and the day-to-day conduct of the feeding trial on which this experiment was based were under the control of Dr. A.D. Weaver who was conducting the experiment with the primary object of assessing the influence of diet on the incidence of urolithiasis. I am indebted to Dr. Weaver for giving permission for the collection of blood samples and for the provision of the entire livers at the conclusion of the experiment.

Experimental Design.

Four groups of either 10 or 11 Blackface ram lambs (aged about 7 months) were used in this experiment. Table 49 details the mean amounts of hay and concentrates consumed by the separate groups. Groups 1 and 2 received the same concentrate (A containing 0.19% Ca) but Group 1 was offered less hay and considerably more concentrate than Group 2. Group 3 (concentrate B, 0.80% Ca) and Group 4 (concentrate C, 3.83% Ca) were fed the same amount of hay (195 g.) as Group 1 and consumed about the same amount of concentrate (654, 717 and 692 g/day). The three concentrates (A, B and C) were basically of the same composition (20% barley, 20% flaked beans, 30% maize and 30% oats). Calcium carbonate was added as required to increase the calcium concentration in B and C above the level of that in A (unsupplemented). The calcium intakes (Table 49) were only about 2 g/day for groups 1 and 2 (i.e. deficient) 6.4 g/day for Group 3 (i.e. normal) and 26.5 g per day (i.e. grossly excessive) for these sheep in Group 4.

The/

The copper concentration in the various diets ranged from 5.0 to 8.5 p.p.m. (Table 49) and the total daily copper intakes ranged from 3.5 mg (Group 2) to 7.8 mg (Group 3).

The dietary intake of iron for all four groups of sheep was well in excess of the recommended minimum level of 30 p.p.m. suggested by the A.R.C. One unfortunate aspect of the mineral composition of the concentrates used was that the iron level increased in direct proportion to the increase in calcium content. The limestone added to the concentrate must have contained some iron, most probably in the form of Fe_2O_3. Calculation indicates that the limestone might have contained 0.0054% of iron. This is almost certainly of very low availability.

As iron is thought to have an inverse relationship with copper in respect of liver storage the increase in the iron level in the concentrates might have a masking effect on the way in which the calcium content of the diet might alter the absorption and storage of copper. However, the iron content of the diet of Group 4 (412.5 p.p.m.) is only double that of Group 3 (190.0 p.p.m.) compared to a greater than fourfold increase in the calcium concentration. Any effect, therefore due to the additional iron intake will almost certainly be swamped by that due to the massive increase in the amount of calcium present.

The experimental rations were fed from 14 October 1965 until slaughter on 15 January 1965, a total period of 93 days.

Results

The individual and mean blood copper concentrations, liver dry weights, liver copper concentrations and total liver copper contents of the four groups of sheep are detailed in Table 50.

Table 49 Mean Daily Consumption of Hay and Concentrates and Intakes of Calcium, Copper and Iron.

(Groups of either 10 or 11 sheep)

	Group 1	Group 2	Group 3	Group 4
Hay (g)	195	459	195	195
Concentrate (g)	A 654	A 250	B 717	C 692
Calcium Intake (g)				
Hay	0.70	1.65	0.70	0.70
Conc.	1.24	0.48	5.74	25.80
Total	1.94	2.13	6.44	26.50
Ca.% whole diet	0.23	0.30	0.70	3.06
Copper Intake (mg.)				
Hay	0.74	1.74	0.74	0.74
Conc.	4.64	1.78	7.03	6.18
Total	5.38	3.52	7.77	6.92
Cu. p.p.m. whole diet	6.35	5.00	8.50	8.02
Iron Intake (mg.)				
Hay	19.8	46.5	19.8	19.8
Conc.	102.4	39.2	154.0	332.0
Total	122.2	85.7	173.8	351.8
Fe p.p.m. whole diet	144.5	122.0	190.0	412.5

Analyses	Ca %	Cu. p.p.m.	Fe p.p.m.
Hay	0.36	3.8	102.0
Concentrate A	0.19	7.1	157.1
Concentrate B	0.80	9.8	214.7
Concentrate C	3.83	9.2	493.0

Table 50. Individual and mean blood copper concentrations, liver dry weights, liver copper concentrations and total liver copper contents.

Group I						Group 2					
	Blood Cu Conc.(p.p.m.)		Liver				Blood Cu Conc.(p.p.m.)		Liver		
Sheep No.	15/12/64	12/1/65	Dry Wt.(g)	Cu Conc(p.p.m.)	Total Cu(mg.)	Sheep No.	15/12/64	12/1/65	Dry Wt.(g)	Cu Conc(p.p.m.)	Total Cu(mg.)
2	0.88	0.81	146	230.8	33.6	9	1.14	0.90	131	103.4	13.5
3	0.86	0.86	128	151.6	19.3	10	0.72	0.87	90	171.2	15.4
5	1.21	0.86	150	52.3	7.9	11	0.90	0.92	110	86.5	9.5
7	0.79	0.76	151	78.6	11.9	13	1.05	0.94	63	241.6	15.3
8	0.90	0.70	127	110.9	14.0	15	0.75	0.78	97	131.7	12.7
12	0.73	0.76	137	99.9	13.6	17	0.82	0.77	89	170.0	15.1
21	1.10	0.91	126	147.1	18.6	18	0.94	0.70	97	212.4	20.5
26	0.80	1.03	138	173.0	23.9	19	1.00	0.96	101	145.5	14.7
29	1.02	0.87	101	96.0	9.7	20	0.95	0.86	90	115.1	10.3
38	0.88	0.80	160	129.9	20.8	34	0.92	0.85	102	117.6	12.0
41	1.26	1.01	162	167.3	27.1	42	0.97	-	85	108.8	9.3
Mean	0.95	0.85	139	130.7	18.2		0.92	0.86	96	145.8	13.5
			Group 3						Group 4		
1	0.85	0.83	107	98.0	10.5	14	1.04	0.96	100	51.0	5.1
4	1.17	0.86	149	231.8	34.6	25	1.00	0.87	113	90.8	10.3
6	1.03	0.96	75	118.9	8.9	27	1.07	0.78	140	24.6	3.4
16	1.17	0.87	134	184.7	24.7	30	1.26	0.82	134	104.7	14.0
22	0.85	0.99	125	133.9	16.7	31	0.79	0.72	130	42.8	5.5
23	1.14	0.96	161	290.5	46.9	32	0.51	0.86	167	67.8	11.3
24	1.11	1.10	110	206.0	22.6	33	0.93	0.66	119	114.4	13.6
28	1.19	0.93	147	169.5	25.0	36	0.96	0.70	116	77.4	8.9
35	0.62	0.98	156	192.7	30.1	37	0.97	0.92	130	66.1	8.6
39	1.04	0.85	167	115.0	19.2	40	0.77	0.75	130	161.6	21.0
Mean	1.02	0.93	133	174.1	23.9		0.93	0.80	128	80.2	10.2

Blood Copper Concentrations.

Unfortunately no blood samples were obtained at the start of the experiment but by mid-period the mean blood copper concentrations of the four groups were within the range 0.92 - 1.02 p.p.m. At the end of the experiment the mean blood copper concentrations of all four groups had fallen from their original level and were in the range 0.80 - 0.93 p.p.m. The mean blood copper concentration of Group 4 receiving the highest calcium intake (0.80 p.p.m.) was lower than those of the other three groups but not significantly so. The fall in blood copper concentration exhibited by all four groups was similar to that found for the low protein group in the previous Experiment (No. 7). The level of protein in the concentrates in the present experiment was also quite low being 11.0% of crude protein.

Liver dry weight, copper concentration and total copper content.

The mean liver copper concentration (80.2 p.p.m.) of group 4 (very high calcium) was significantly ($P < 0.02$; 0.01 and 0.01) lower than those of Group 1, (130.7 p.p.m.); Group 2 (145.8 p.p.m.) and Group 3 (174.1 p.p.m.) respectively. However, this was probably only a real difference for Groups1 and 3 as the mean liver dry weight of Group 2 (low calcium and low concentrates) was significantly ($P < 0.01$) less than that of Group 4. There were no significant differences in liver-copper concentrations among Groups 1, 2 and 3.

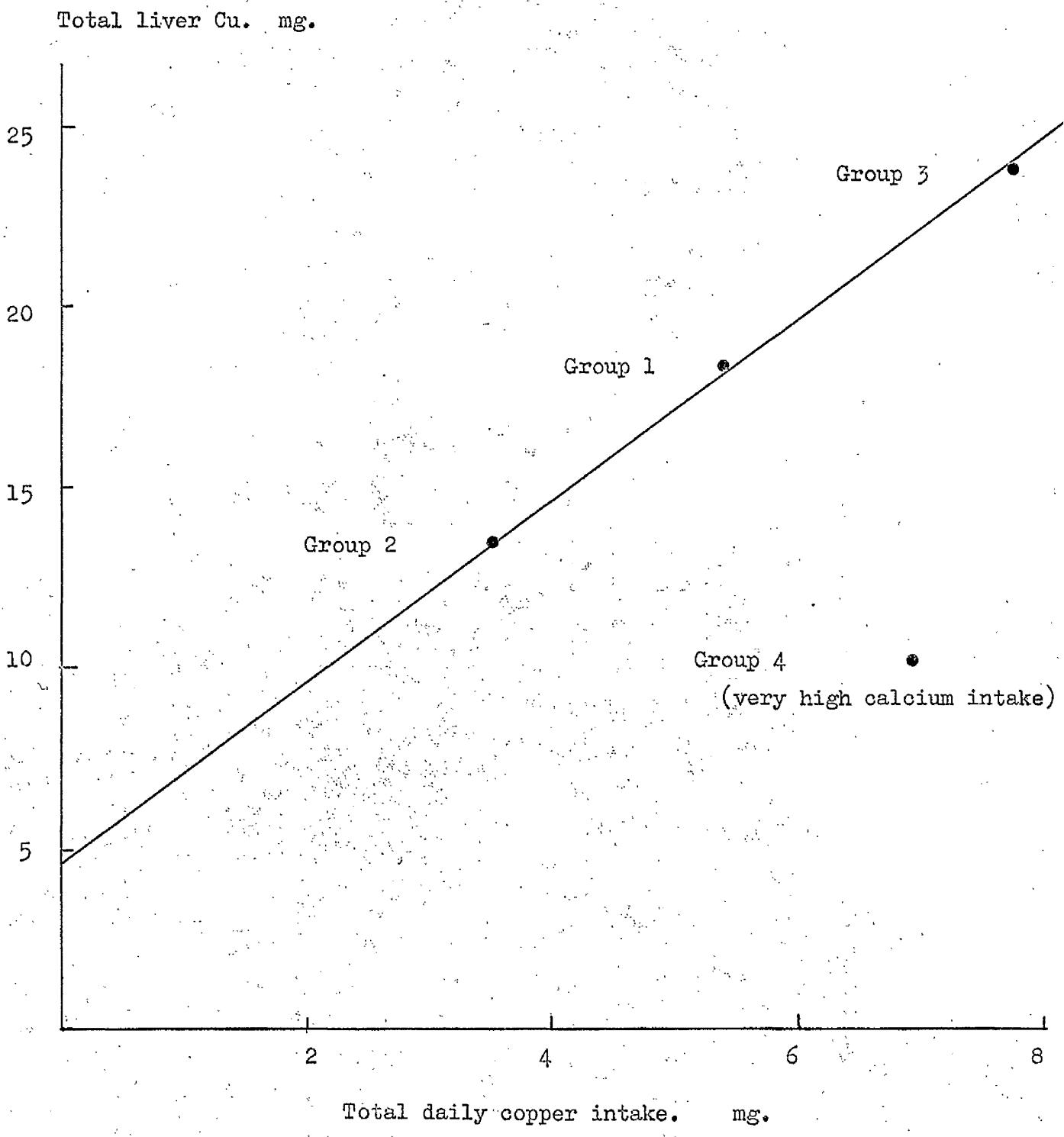
The mean total liver-copper content of Group 4 (very high calcium)(10.2 mg) was/

was also significantly ($P<0.02$ and 0.01) lower than those of Group 1 (18.2 mg) and Group 3 (23.9 mg) respectively but it was not significantly lower than that of Group 2 (13.5 mg). On the other hand, however, the total daily intake of copper by Group 4 (6.92 mg) was almost double that by Group 2 (3.52 mg). When this factor is examined in conjunction with the fact that the mean total liver-copper content of Group 4 (10.2 mg) is appreciably lower than that of Group 2 (13.5 mg) it indicates that there has been a marked reduction in the efficiency of the absorption and storage of dietary copper by Group 4. This is borne out by the fact that where the total daily intakes of copper by Groups 1 and 3 (low and normal calcium respectively) (5.38 and 7.77 mg. respectively) more closely resemble that of Group 4 (6.92 mg) there is a statistically significant difference between the amounts of copper stored in the liver.

The relationship between the daily intake of copper by the four groups and the amount of copper stored in their livers is shown in Fig. 20. This shows that the total liver-copper contents of Groups 1 - 3 are in direct proportion to their daily copper intake. Group 4 (very high calcium) is quite different. If it had conformed to this pattern then it would have had a total liver copper content of about 22 mg. instead of the mean level of 10.2 mg. which the livers did actually contain.

The poor rate of absorption and storage of dietary copper by Group 4 would appear/

Fig 20. Relationship between the total daily copper intake and the total ampunt of copper in the liver of sheep.



appear to be due to the high calcium content of its diet. The mean total liver copper content (13.5 mg) of Group 2 (low calcium and low concentrates) was significantly less than that (23.9 mg) of Group 3 (normal calcium). This, however, is purely a reflection of the difference in their daily intakes of copper (3.52 mg Cu/day by Group 2 and 7.7 mg Cu/day by Group 3) rather than due to any difference in the calcium contents of the two diets.

By taking the dietary copper intake and total liver copper content of Group 2 as control levels it was possible to calculate the proportion of the additional dietary copper fed to Groups 1 and 3 which was stored in their livers. The proportion of the additional copper stored by Group 1 was 2.72% and by Group 3 was 2.63%. This, therefore, provides further evidence of the fact that the low and normal calcium levels had no effect on copper storage.

Liver Iron Concentration and Total Iron Content.

The individual and mean liver iron concentrations and total iron contents of the four groups are presented in Table 51.

The mean liver iron concentration and the mean total liver iron content of group 2 (low calcium, low concentrates) were significantly ($P<0.01$) higher than those of the other three groups. This appears to be a rather paradoxical result at first sight since the iron concentration in the diet of the sheep in Group/

Table 51. Individual and mean liver iron concentrations and total iron contents of 4 groups of sheep.

Group 1			Group 2			Group 3			Group 4		
No.	Fe Conc. (p.p.m)	Total Fe (mg)	No.	Fe Conc. (p.p.m)	Total Fe (mg)	No.	Fe Conc. (p.p.m)	Total Fe (mg)	No.	Fe Conc. (p.p.m)	Total Fe. (mg)
2	193.5	28.2	9	152.1	19.8	1	262.8	28.2	14	184.5	18.5
3	143.7	18.3	10	674.2	60.6	4	232.6	34.7	25	169.4	19.2
5	263.6	39.6	11	420.3	46.2	6	533.6	40.0	27	244.3	34.1
7	185.8	28.0	13	616.6	39.0	16	236.4	31.7	30	149.0	20.0
8	220.6	27.9	15	569.6	55.1	22	247.3	30.9	31	181.2	23.5
12	113.5	15.5	17	592.4	52.6	23	155.2	25.0	32	168.1	28.0
21	163.7	20.7	18	484.7	46.9	24	253.8	27.9	33	395.1	46.8
26	188.2	26.0	19	254.4	25.7	28	160.7	23.7	36	211.0	24.4
29	175.1	17.7	20	359.4	32.2	35	161.5	25.2	37	228.9	29.7
38	190.0	30.4	34	331.4	33.8	39	243.7	40.8	40	163.2	21.2
41	205.8	33.3	42	378.2	32.3						
Mean	185.8	26.0		441.8	40.4		248.8	30.8		209.5	26.5
L.S.D. (bet. means)	96.5	8.3		96.5	8.3		96.5	8.3		96.5	8.3

Group 2 is lower than that in the diets fed to the other three groups. The mean total daily intake of iron by the sheep in Group 2 was 86 mg. compared with intakes of 122, 174 and 353 mg. respectively for Groups 1 - 3. Group 2 was also fed twice as much hay as was fed to any of the other groups. However, the major factor causing the difference in liver iron concentration between Group 2 and the other three groups is likely to be the daily intake of dietary copper. The sheep in Group 2 had a very low daily intake of copper (3.52 mg). Marston (1950) has reported that sheep on copper-deficient diets lay down extensive deposits of iron particularly in the liver. Although the diet consumed by the sheep in Group 2 was not copper deficient it was still below normal and it would be this factor that allowed for the comparative build up of iron in their livers compared to the normal amount of iron in the livers of the other three groups which were being fed a diet containing an adequate supply of copper.

Experiment 8 (b). The Effect of Varying Hay and Concentrate Intakes on the Storage of Copper in the Liver of Sheep.

As was the case for Experiment 8 (a) this present trial had also been designed by Dr. Weaver to assess the effects of various feeding regimes on the incidence of urolithiasis in housed Blackface ram lambs. Dr. Weaver kindly provided the entire livers at the termination of the experiment so that the effect of varying hay and concentrate intakes (which greatly altered the amounts of copper consumed by the different groups of sheep) on copper storage in the liver could be assessed.

There were three basic diets offered to groups of 12 or 14 Blackface ram lambs aged about 6 months at the commencement of the experiment. The rations fed are listed in Table 52. The concentrate mixture was composed of 30% maize, 30% oats, 20% beans and 20% linseed. Group 1 received 300 g (low), Group 2 received 600 g (medium) and Group 3 received 1000 g (high) of concentrates per day. The hay intakes were 675, 312 and 125 g. per day respectively and all groups were fed 750 g. of swedes per day.

The mixed concentrate contained 17.1 p.p.m. Cu and the hay and roots (D.M.) contained 4.6 and 4.1 p.p.m. Cu respectively. As the concentrate intake increased from 300 to 1000 g. per day the copper intake increased from 8.34 - 17.99 mg. Cu per day (Table 52).

Feeding/

Table 52.

Mean Daily Consumption of Hay and Concentrates and Copper Intakes.

	Group 1. Low Concentrates.	Group 2. Medium. Concentrates.	Group 3. High Concentrates.
Hay. (g)	675	312	125
Concentrates (g)	300	600	1000
Swedes (g)	750	750	750
Copper Intake (mg)			
Hay.	2.90	1.44	0.58
Concentrates.	5.13	10.26	17.10
Roots.	0.31	0.31	0.31
Total.	8.34	12.01	17.99
Cu. p.p.m. in whole diet.	7.95	12.12	15.00

Feeding of the experimental diets commenced on 1 December 1963 and continued until slaughter on 24 March 1964, a period of 115 days.

Liver dry weight, copper concentration and total copper content.

The individual and mean liver dry weights, copper concentrations and total copper contents of the 3 groups of sheep are detailed in Table 53. The relationship between the mean daily copper intake and the mean total liver copper content is shown in Fig. 21.

The mean liver dry weight (195 g.) of Group 3 (high concentrates) was significantly ($P<0.01$) higher than that (143 g.) of Group 1 (low concentrates) and that (164 g.) of Group 2 (medium concentrates). The mean liver dry weight of Group 2 was also significantly ($P<0.05$) higher than that of Group 1.

The mean liver copper concentrations of Group 2 (medium concentrates) and Group 3 (high concentrates) 208.8 and 240.7 p.p.m. respectively) were significantly ($P<0.01$) higher than the mean liver copper concentration (125.7 p.p.m.) of Group 1 (low concentrates). The mean total liver copper contents of Group 2 (34.7 mg) and Group 3 (47.4 mg) were also significantly ($P < 0.01$) greater than the mean total liver copper content of Group 1 (18.0 mg). However, despite the fact that Group 3 (high concentrates) had a much higher daily intake of copper (17.99 mg) than Group 2 (12.01 mg) its mean liver copper/

Fig 21. Relationship between the total daily intake of copper and the total amount of copper in the liver of sheep.

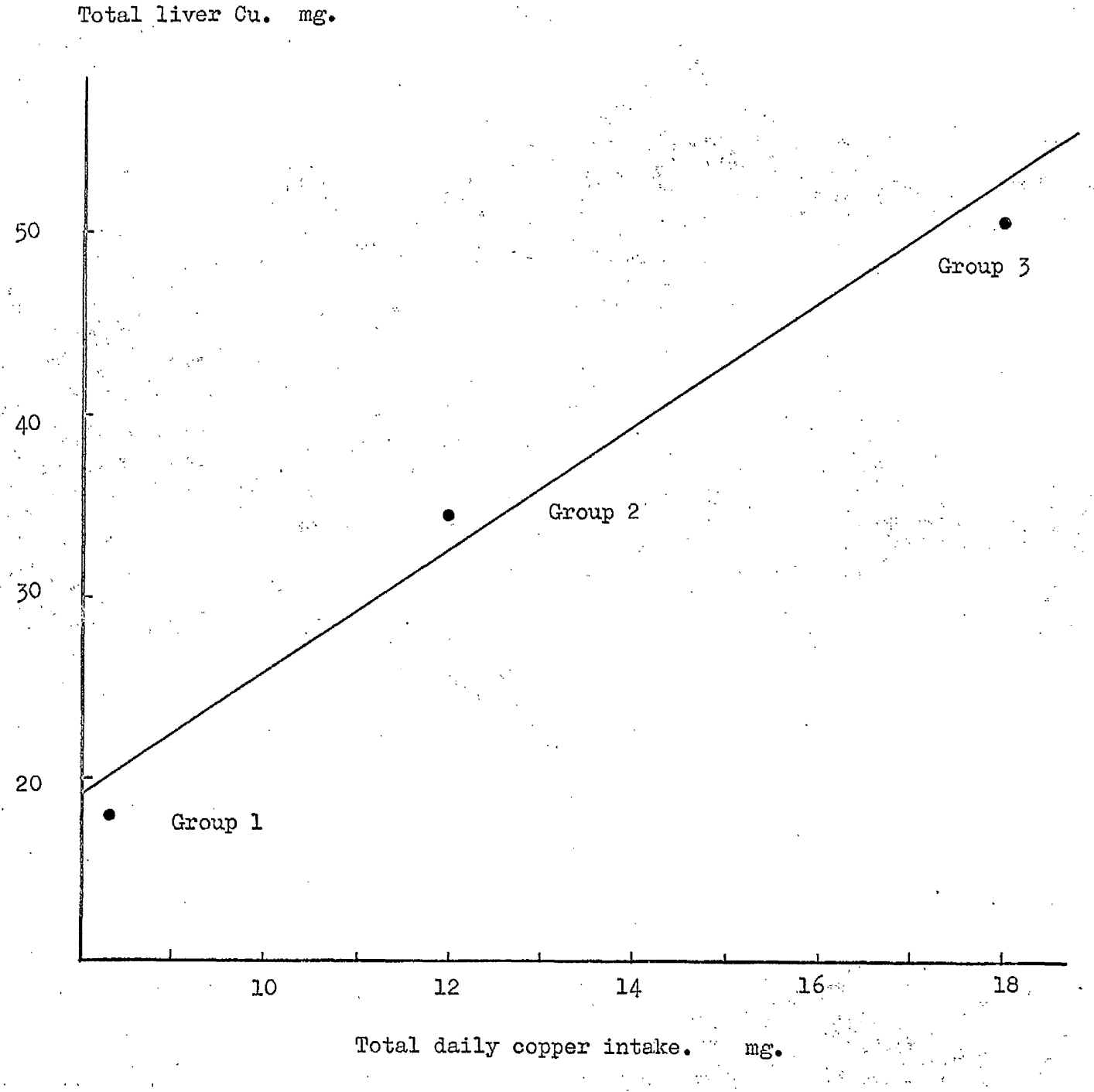


Table 53. Liver dry weights, copper concentrations and total copper contents of 3 groups of sheep.

Group 1 - Cu Intake 8.4 mg.				Group 2 - Cu Intake 12.01 mg.				Group 3 - Cu Intake 17.99 mg.			
No.	Dry Wt. (g)	Cu Conc. (p.p.m.)	Total Cu. (mg)	No.	Dry Wt. (g)	Cu Conc. (p.p.m.)	Total Cu. (mg.)	No.	Dry Wt. (g)	Cu. Conc. (p.p.m.)	Total Cu. (mg).
6	121	138.3	16.7	2	171	232.4	39.7	1	178	56.8	10.1
8	158	145.0	22.9	4	163	199.8	32.6	3	150	243.5	36.5
12	147	78.8	11.6	5	202	304.9	61.6	7	181	153.6	27.8
13	180	192.3	34.6	10	145	291.0	42.2	9	206	220.2	45.4
15	165	76.8	12.7	11	213	246.7	52.5	21	175	115.2	20.2
16	115	113.5	13.1	14	175	225.7	39.5	22	173	356.2	61.6
17	132	193.2	25.5	19	162	207.1	33.6	33	206	328.6	67.7
18	104	117.6	12.2	23	136	140.2	19.1	35	252	259.2	65.3
20	161	80.8	13.0	25	171	205.4	35.1	37	211	267.9	56.5
26	142	170.3	24.2	27	152	156.6	23.8	38	205	433.0	88.8
28	134	118.3	15.9	29	120	205.0	24.6	41	225	204.9	46.1
30	159	83.2	13.2	36	156	239.9	37.4	42	173	249.9	43.2
				39	152	128.1	19.5				
				40	181	133.0	24.1				
MEAN	143	125.7	18.0		164	208.3	34.7		195	240.7	47.4

copper concentration (240.7 p.p.m.) and mean total liver copper content (47.4 mg) were not significantly higher than those (208.8 p.p.m. and 34.7 mg. respectively) of Group 2 (medium concentrates). Since the mean total liver copper content of Group 2 was significantly higher than that of Group 1 but not significantly less than that of Group 3 this implies that Group 2 stored the small amount of additional copper (3.67 mg) in their ration more efficiently than did Group 3 with a much larger amount of additional copper (9.65 mg). This was borne out when the proportion of the additional copper fed to Groups 2 and 3 which was stored in the liver was calculated by taking the dietary copper intake and the mean liver copper content of Group 1 as control levels. The storage rate of the additional dietary copper was 3.95% for Group 2 compared with 2.67% for Group 3.

The highest liver copper concentration found in this experiment was 433.0 p.p.m. for sheep No. 38 in Group 3. This can be regarded as a "high normal" level but one which is certainly well below that at which chronic copper toxicity becomes a possibility. Thus although there was a progressive increase in liver copper concentration with progressive rises in dietary copper intake there was a falling off in the efficiency of copper absorption and storage as the dietary level of copper increased. This means, therefore, that the build up of toxic amounts of copper in the liver was certainly not accelerated in this present experiment by feeding diets containing slightly elevated copper concentrations to housed sheep.

Experiment 9 (a). Liver Copper Storage in Young Lambs being Fattened on Various Feeding Regimes.

This experiment was designed to study the storage of copper in the liver of young lambs which were being fattened under three different systems. These were a) grass alone, b) grass + concentrates ad lib and c) various cereal-based diets fed indoors. The rations covered a wide range of copper concentrations and crude protein contents. One hundred and ten Half-bred lambs running initially with their respective ewes were used in this experiment.

A total of sixty seven lambs were fattened at grass. Of these, 28 (Group A) received grass only and 39 (Group B) were at grass but had additionally an ad lib supply of a proprietory concentrate (Lambwena) offered by means of a self-feeder. The grass contained 10.6 p.p.m. of copper assessed as the mean of three samples obtained during the growing season.

The remaining 43 sheep were fattened indoors on slatted floors in four groups (C, D, E and F) and received the diets enumerated in Table 54. The diets were freely available ad lib and in addition all four groups received about 4 oz. of hay per day. This amount was small in relation to the consumption of the various concentrates. Group C received only the proprietary Lambwena concentrate, Groups D and E were fed on barley or oats/barley dominant rations supplemented with a protein/vitamin/mineral concentrate (Quicklamb) and Group F had a similar diet but including some sugar beet pulp to increase the fibre content. The copper concentrations of these four diets ranged from

9.9/

Table 54. % Composition of Diets fed to the four groups of housed lambs in Experiment.9a

% of Diet	Group C	D	E	F	Analysis of Foods + Cu.p.p.m.	C. Protein %
Oats	-	-	40	26.6	4.0	8.0
Barley	-	80	40	26.6	5.3	9.0
Sugar Beet Pulp	-	-	-	26.6	19.3	11.0
Lambwena *	100	-	-	-	21.0	18.0
Quicklamb **	-	20	20	20	31.0	30.0
Whole diet						
Cu. p.p.m.	21.0	10.4	9.9	13.8		
C. Protein %	18.0	13.2	12.8	13.4		

* Lambwena 5.5% Groundnut, 2.5% decorticated Cotton Cake, 7.5% Copra, 20.5% Barley, 5% Oats, 15% Maize, 10% Wheatfeed, 10% Distillers Grains, 2.5% Malt Culms, 3% Sugar, 5% Molasses, 3.75 % Minerals + Vits. A.D. and E.

**Quicklamb 36.75% Groundnut, 13.5% Barley, 12.25% Soya, 10% Wheatfeed, 5% Maize Gluten, 2½% Fish Meal, 2½% Herring Meal, 5% Molasses, 12.5% Minerals + Vits. A and D.

\+ Mean of values obtained on 3 sampling occasions.

9.9 to 21.0 p.p.m. (Table 54). The ration fed to Group C was appreciably higher in protein (18.0%) than were those fed to the other three groups of housed lambs (12.8 - 13.4%).

The lambs were slaughtered on four different dates ranging from 27 June to 4 September 1964 as they reached about 75 lbs. live weight. The whole livers of all the lambs were obtained at slaughter for copper determination.

Results.

The individual liver dry weights, copper concentrations and total copper contents of the six groups of sheep are detailed in Table 55 and the mean values are summarised in Table 56.

For those sheep which were at grass, the feeding of a supplementary concentrate (Lambwena) to Group B resulted in a mean liver-copper concentration (303.4 p.p.m.) and a mean total liver-copper content (64.6 mg) which were significantly higher ($P = 0.01$) than for those sheep (Group A) which received grass only (199.4 p.p.m. and 44.8 mg. respectively). The mean amount of Lambwena concentrate fed to the lambs in Group B was 56 lbs. per head. Calculation indicates that 3.6% of the additional copper supplied in this way was stored in the liver of these sheep at grass.

The mean liver copper concentrations and total liver copper contents of all the four groups of housed lambs were significantly higher ($P = 0.01$) than for the two groups at grass (Table 56).

Table 55. Individual and mean liver dry weights, copper concentrations and total liver copper contents of 6 groups of sheep.

Group A.

No.	Dry Wt. (g)	Cu.Conc. (p.p.m.)	Total Cu. (mg.)	No.	Dry Wt.	Cu.Conc.	Total Cu.
1	185	184.0	34.0	13	254	262.6	66.7
2	168	176.6	29.7	14	226	271.1	61.3
3	189	185.6	35.1	15	221	334.3	73.9
4	179	100.1	17.9	16	209	262.2	54.8
5	187	111.3	20.8	17	210	99.8	21.0
6	174	172.6	30.0	225	230	227.0	52.2
7	194	137.9	26.8	223	217	193.5	42.0
8	217	82.3	17.9	207	230	133.8	30.8
29	231	257.9	59.6	121	257	223.4	57.4
219	269	295.2	79.4	52	231	171.1	39.5
230	203	123.3	25.0	21	227	168.8	38.3
9	253	338.3	85.6	204	243	163.5	39.7
10	183	277.6	50.8	43	270	239.5	64.7
11	261	236.5	61.7				
12	239	152.5	36.4	Mean	220	199.4	44.8

Group B.

1	203	378.6	76.9	217	231	303.9	70.2
2	170	211.8	36.0	209	182	161.5	30.1
3	237	204.4	48.4	218	221	260.0	57.5
4	210	265.1	55.7	174	172	323.1	55.6
5	186	323.7	60.2	117	254	241.4	61.3
167	231	287.2	66.3	106	207	287.1	59.4
192	239	327.2	78.2	147	283	200.5	56.7
189	239	344.2	82.3	99	200	437.4	87.5
187	220	314.8	69.3	135	204	370.4	75.6
88	223	198.4	44.2	213	203	342.4	69.5
95	215	145.1	31.2	92	245	420.2	102.9
98	224	393.2	88.1	172	130	363.9	47.3
115	203	337.9	68.6	171	231	298.0	68.8
118	190	419.0	79.6	228	165	280.1	46.2
124	213	324.5	69.1	229	170	278.6	47.4
143	243	215.3	52.3	170	198	349.8	69.3
145	208	393.4	81.8	202	256	273.7	70.1
158	193	214.9	41.5	201	270	366.4	98.9
194	226	311.5	70.4				
199	219	321.2	70.3				
200	217	341.5	74.1	Mean	214	303.4	64.6

Table 55 Continued.

Group C.

No.	Dry Wt. (g)	Cu.Conc. (p.p.m.)	Total Cu. (mg.)	No.	Dry Wt.	Cu.Conc.	Total Cu.
1	201	1220.3	245.3	67	204	1092.4	222.8
2	254	749.5	190.4	13	193	951.1	183.6
3	225	980.4	220.6	50	188	800.9	150.6
4	200	979.1	195.8	7	133	975.9	129.8
5	230	870.9	200.3	8	228	875.4	199.6
6	251	1132.7	284.3	9	185	577.5	106.8
48	220	622.8	137.0				
41	202	725.3	146.5	Mean	208	896.7	186.7

Group D.

1	240	828.2	198.8	191	198	564.3	111.7
2	188	913.4	171.7	58	151	936.2	141.4
3	193	553.3	106.8	163	171	663.9	113.5
4	227	717.7	162.9	20	186	995.4	185.1
5	174	432.8	75.3	10	193	1426.0	275.2
6	169	1798.9	304.0	55	181	1131.0	204.7
7	171	1894.7	324.0	205	236	832.2	196.4
8	204	647.4	132.1	11	129	2184.8	281.8
9	239	1256.1	300.2	Mean	191	1045.7	193.3

Group E.

1	225	837.8	188.5	3	194	1230.5	238.7
2	197	763.7	150.4	4	218	776.7	169.3
18	197	925.6	182.3				
36	261	895.3	233.7	Mean	215	904.9	193.8

Group F.

1	208	932.6	194.0	5	221	842.0	186.1
2	192	1246.9	239.4	224	239	1280.7	306.1
3	187	1418.0	265.2				
4	179	1757.4	314.6	Mean	204	1246.3	250.9

Table 56 The mean liver dry weights, copper concentrations and total copper contents of 6 groups of lambs

Group	No. of lambs.	Dry Wt. (g)	Cu conc.	Total Cu content (mg)	Diet Composition Cu ppm.	C. Protein %
Lambs at Grass						
A	28	220	199.4	44.8	-	-
B	39	214	303.4	64.6	-	-
Housed Lambs						
C	14	208	896.7	186.7	21.0	18.0
D	17	191	1045.7	193.3	10.4	13.2
E	6	215	904.9	193.8	9.9	12.8
F	6	204	1246.3	250.9	13.8	13.4

Of the four groups of housed sheep Group F, which was fed a diet consisting of 26.6% oats, 26.6 Barley, 26.6% sugar beet pulp and 20% Quicklamb, had a mean liver copper concentration (1246.3 p.p.m.) which was significantly ($P = 0.05$) higher than both that (896.7 p.p.m.) of Group C - fed on Lambwena only, - and that (904.9 p.p.m.) of Group E which was fed 40% oats, 4)% barley and 20% Quicklamb. The copper concentration in the ration fed to Group F (13.8 p.p.m.) was appreciably higher than that in the ration fed to Group E (9.9 p.p.m.). The protein contents of both rations were essentially the same (13.4 and 12.8% respectively for Groups F and E). It could, therefore, be assumed that the higher liver copper concentration found for Group F was directly related to the higher copper concentration of its diet in comparison to that in the diet of Group E.

On the other hand no such assumption could explain the higher liver copper concentration found for Group F in comparison to Group C (Lambwena only) since the dietary copper concentration of Group F (13.8 p.p.m.) was much lower than that of Group C (21.0 p.p.m.) There must, therefore, have been some factor which either depressed copper absorption in Group C or increased the efficiency of copper absorption in Group F. The difference might almost certainly be explained by the difference in the levels of dietary protein. Group C which did not store the dietary copper as effectively had a higher level of dieta protein (18.0%) than Group F which had a dietary protein content of 13.4%. High dietary protein levels have been reported to depress the rate of copper absorption/

absorption and storage in the livers of sheep (MacPherson and Hemingway, 1965), rats (McCall and Davis, 1961) and pigs (Wallace et al., 1960 and Henrahan and O'Grady, 1965). The difference in the dietary protein levels of the rations fed to Groups C and F would explain their different rates of copper absorption and their different liver copper concentrations. The high protein level in the diet fed to Group C would also account for the fact that the mean liver copper concentration and mean total liver copper content of Group C are not significantly different from those of Groups D and E despite the fact that the copper concentration in the diet fed to the Group C (21.0 p.p.m.) is more than double that fed to either of the other two groups (10.4 and 9.9 p.p.m. respectively). This indicates that Groups D and E must have stored their dietary copper at more than twice the rate of Group C.

The sheep in this experiment belonged to a commercial organisation who were running it as a trial to test the effectiveness of various diets in fattening young lambs. They kindly gave permission to obtain the livers of the lambs from the various groups at slaughter and also to take food samples for analyses. However, it was not possible to get an accurate check on the amount of food consumed by each group and thus to calculate their total copper intakes. In order to get an approximate measure of the storage rate of dietary copper by the 4 groups of housed sheep a food conversion ratio of 6:1 was assumed. On this basis Group C stored 3.9% of the dietary intake of copper compared to more than 8.0% by Groups D, E and F. This confirms what was already postulated that the absorption of dietary copper was being depressed in Group C. The absorption/

absorption rates found for Groups D, E and F are substantially higher than those that have been quoted previously by other workers. This is possibly due to the fact that they are very young lambs being fattened on an intensive system with no herbage. Absorption rates quoted by previous workers were generally for older sheep on more traditional methods of husbandry.

The storage rate of dietary copper by Group B which was being fed lambwena at grass was 3.6%. This is very similar to that found for Group C (3.9%) which was fed on a similar diet indoors and suggests that housing in itself had no effect on increasing the efficiency of absorption of dietary copper.

Despite some very high liver copper concentrations no symptoms of chronic copper toxicity were found in any of the lambs. Fourteen of the 43 housed lambs had liver copper concentrations over 1000 p.p.m.; the remainder, apart from No. 5 in Group D, all had liver copper concentrations between 500 and 1000 p.p.m. Liver copper concentrations of this magnitude are potentially toxic and it is probable that if the lambs had been fed on these diets for much longer than the 12 weeks taken to fatten them some manifestations of chronic copper poisoning would have appeared. However the risk of chronic copper poisoning in this type of young stock, could be reduced either by having the fattening period as short as possible or alternatively by increasing the protein content of the diet.

Experiment/

Experiment 9 (b). Liver copper storage in young sheep being intensively fattened indoors.

This experiment was carried out as a further investigation into copper absorption in lambs being intensively fattened indoors. On this occasion somewhat older lambs were used. Fifty nine small Blackface 7-month old lambs weighing 40 - 50 lbs. were housed on 30 October 1964. These were divided at random into 4 groups of 14 or 15 sheep per group and allocated to one of 4 experimental rations. These rations were basically made up of 50% barley and 20% of one of two different proprietary protein concentrates. In two of them, however, 15% of the barley portion of the ration was replaced by sugar beet pulp in order to increase the fibre content of the diet. The composition of each ration is detailed in Table 57. In addition each lamb received about 4 oz. of hay per day.

Table 57. % Composition of the rations fed to four groups of lambs.

Group	1	2	3	4
Barley	80.0	80.0	65.0	65.0
E.K.94	20.0	-	20.0	-
Quicklamb	-	20.0	-	20.0
Sugar Beet Pulp	-	-	15.0	15.0
Analysis of whole ration				
C. Protein %	13.5	13.2	13.8	13.5
C. Fibre %	4.8	4.8	6.1	6.1
Copper p.p.m.	6.8	9.6	8.3	11.2

E.K.94 and Quicklamb were two proprietary protein concentrates manufactured specifically for intensive lamb production. Their compositions were:

	Quicklamb	E.K. 94.
Ground nut	36.75	36.75
Soya Meal	12.25	18.0
Barley	13.5	17.75
Wheatfeed	10.0	10.0
Maize Gluten	5.0	-
Fish Meal	2.5	-
Herring Meal	2.5	-
Molasses	5.0	5.0
Minerals and Vits. A & D	12.5	12.5
Analysis		
C. Protein %	30.0	28.7
C. Fibre %	4.4	4.5
Copper p.p.m.	29.1	14.8

The difference between the two concentrates lies in the fact that E.K. 94 contains no animal protein while Quicklamb contains 2.5% of Fishmeal and 2.5% Herring/

Herring meal. Quicklamb contained 29.1 p.p.m. Cu whilst E.K.94 contained 14.5 p.p.m. Cu. Feeding of the experimental rations was commenced on 21 November 1964. The lambs were fed these rations until they attained a suitable slaughter weight of between 75 and 85 lbs. liveweight. They were then slaughtered on 4 different dates ranging from 6 January to 10 March 1965. Four of the lambs were killed at the start of the experiment in order to obtain an initial value for the liver copper concentration and total liver copper content of the lambs

Samples of the foods were taken on 5 separate occasions and these were analysed for their copper concentration, crude protein and crude fibre content (Table 57). The barley contained 4.8 p.p.m. Cu and the sugar beet pulp contained 14.9 p.p.m. Cu. Blood samples were obtained from all the sheep on two separate occasions and for a third time from those sheep which took longer to reach an acceptable slaughter weight. All blood samples obtained were analysed for their copper concentration. Whole livers were obtained from all the lambs at slaughter. These were weighed, dried, ground and analysed for their copper concentration.

There was a range in the copper concentration of the rations fed to the 4 groups from 6.8 p.p.m. for Group I to 11.2 p.p.m. for Group IV. The addition of sugar beet pulp increased the copper concentration in each case by 1.5 - 1.6 p.p.m. The rations containing Quicklamb contained 2.8 - 2.9 p.p.m. more Cu than those containing E.K.94. The crude protein content of all 4 diets was essentially the same ranging only from 13.2 - 13.8 %. In this experiment, therefore/

therefore, there should be no effect due to a difference in the level of crude protein in the diet. The four diets contained two different levels of crude fibre content. These were 4.8% for Groups I and II and 6.1% for Groups III and IV which includedsugar beet. This should allow some indication as to whether the crude fibre content of the diet has any effect on copper absorption in sheep.

Results.

Blood Copper Concentration. The individual and mean blood copper concentrations of the 4 groups of lambs are listed in Table 58.

The mean blood copper concentrations of all 4 groups were very similar and varied little throughout the course of the experiment. They were always within the range 0.89 - 1.05 p.p.m. and were unaffected by the different levels of dietary copper concentration in the rations fed. There were no significant differences in mean blood copper concentration between any two groups on any of the three sampling dates.

Liver dry weight, copper concentration and total copper content.

The individual and mean values for liver dry weight, liver-copper concentration and total liver-copper content are listed in Table 59.

Table/

Table 58 The individual and mean blood copper concentrations (p.p.m.) of 4 groups of lambs.

Group I				Group II			
No.	7.12.64	6.1.65	7.2.65	No.	7.12.64	6.1.65	7.2.65
42	0.82	0.96	1.25	68	0.92	0.99	0.95
43	1.10	1.12	1.30	69	1.09	0.91	1.09
44	0.62	0.90	-	70	0.94	0.86	-
45	1.19	1.13	1.24	71	0.69	0.73	0.90
46	1.05	0.87	-	72	1.07	1.01	0.81
49	1.07	0.87	0.91	73	1.14	1.00	-
50	0.87	0.91	-	74	0.95	1.09	0.81
51	0.77	0.95	-	75	0.64	0.85	-
52	0.45	1.01	0.82	76	0.85	1.00	0.81
53	1.15	1.24	1.05	77	1.25	0.90	-
55	0.99	0.92	-	78	1.04	-	0.91
56	0.95	0.86	-	80	1.23	0.97	0.82
57	1.20	1.02	0.81	81	1.34	1.39	-
				82	0.80	0.90	-
Mean	0.94	0.98	1.05	83	1.02	0.86	0.95
				Mean	1.00	0.96	0.89

Group III				Group IV			
58	0.80	1.01	1.01				
59	0.90	1.12	0.82				
60	0.72	0.92	0.80	84	0.96	0.81	1.12
61	1.07	0.87	1.01	85	0.96	0.88	-
62	0.94	0.92	0.95	86	0.77	0.87	0.74
63	1.33	1.15	0.88	88	0.81	0.88	-
64	0.89	0.90	1.09	89	1.31	0.83	1.17
65	0.82	0.82	-	91	0.88	0.87	-
66	1.09	0.77	-	92	0.60	1.02	0.86
67	1.05	1.07	1.09	93	1.18	1.14	-
68	1.00	1.00	-	94	0.93	1.04	-
69	1.10	0.91	-	95	1.17	0.97	-
70	0.77	0.94	0.86	97	0.82	0.92	-
71	1.18	1.14	0.97	98	1.21	0.97	-
72	0.85	0.94	1.20				
Mean	0.97	0.97	0.97	Mean	0.97	0.93	0.97

Table 59 The individual and mean liver dry weights, liver copper concentrations and total liver copper contents of 4 groups of sheep.

Group I				Group II			
No.	D.M.(g)	Cu Conc.(p.p.m.)	Total Cu (mg)	No.	D.M.(g)	Cu Conc(p.p.m.)	Total Cu (mg)
42	132	267.2	35.3	68	188	275.4	51.8
43	186	225.5	41.9	69	191	253.8	48.5
44	213	258.3	55.0	70	233	202.5	47.2
45	163	303.4	49.5	71	174	151.8	26.4
46	151	153.0	23.1	72	124	136.6	16.9
49	166	348.9	57.9	73	201	351.9	70.7
50	115	220.4	25.3	74	156	348.8	54.4
51	133	166.4	22.1	75	206	211.7	43.6
52	190	302.5	57.5	76	149	338.5	50.4
53	157	99.7	15.7	77	158	327.4	51.7
55	177	124.1	22.0	78	169	378.8	64.0
56	197	163.3	32.2	80	182	410.2	74.7
57	137	364.5	49.9	81.	178	269.3	47.9
				82	192	314.9	60.5
Mean	163	230.6	37.5	83	195	321.0	62.6
				Mean	180	286.2	51.4
Group III				Group IV			
58	114	349.7	39.9				
59	184	263.9	48.6				
60	168	316.7	53.2	84	194	509.9	98;9
61	164	281.1	46.1	85	260	314.9	81.9
62	174	241.6	42.0	86	133	221.1	29.4
63	253	270.0	68.3	88	236	309.9	73.1
64	168	252.5	42.4	89	249	504.9	125.7
65	195	222.8	43.4	91	139	185.8	25.8
66	215	137.0	29.5	92	120	467.8	56.1
67	163	263.6	43.0	93	251	151.6	38.1
68	194	301.6	58.5	94	225	380.9	85.7
69	255	213.4	54.4	95	207	267.8	55.4
70	128	316.7	40.5	97	215	218.8	47.0
71	183	297.7	54.5	98	166	192.0	31.9
72	155	178.2	27.6				
Mean	181	260.4	46.1	Mean	200	310.5	62.4

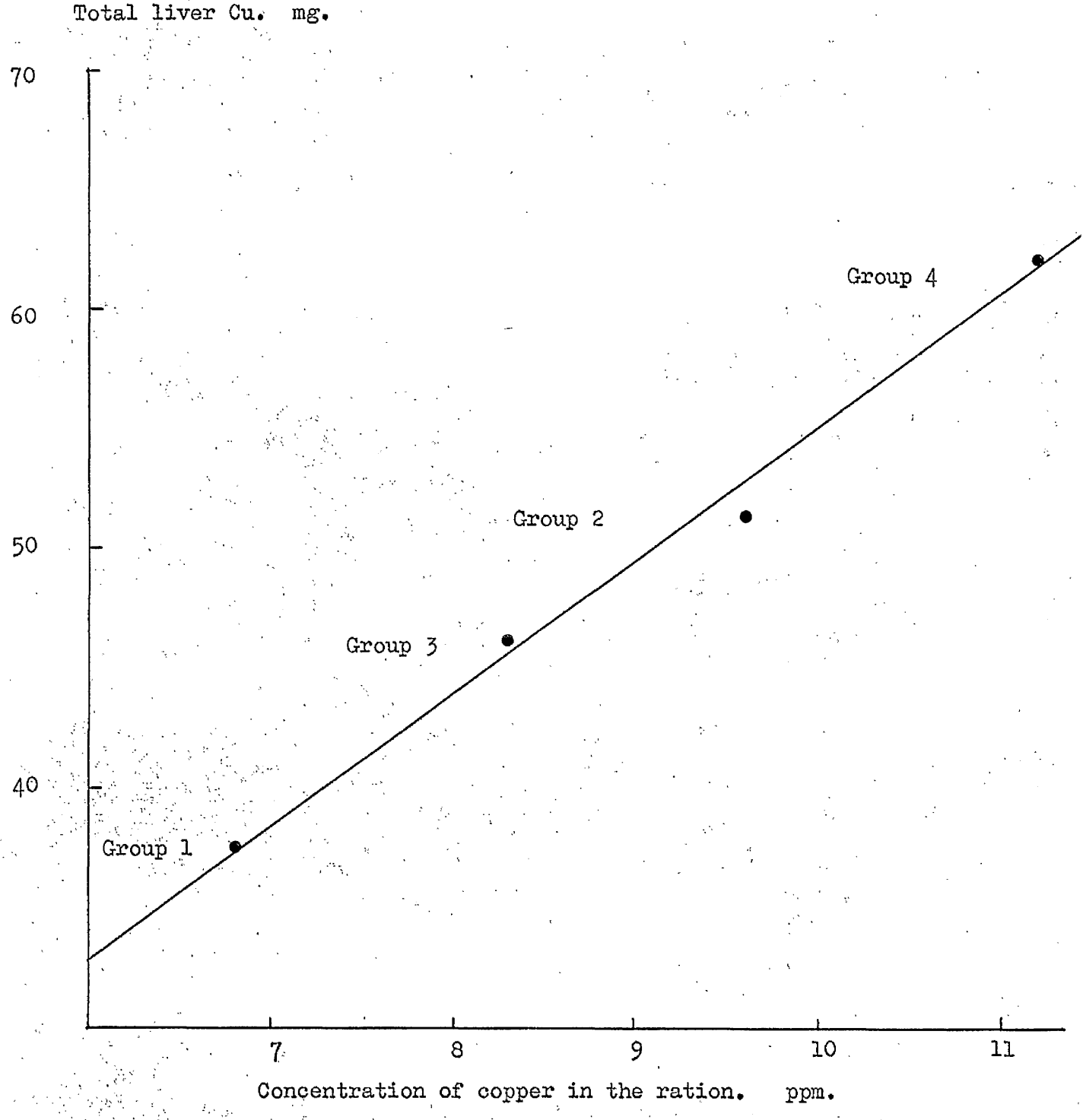
A summary of the mean values for the liver dry matter, liver copper concentration and total liver copper content of the 4 groups and of the 4 sheep killed initially is given in Table 60.

Table 60 Mean liver dry matter, copper concentration and total copper content of 5 groups of sheep.

	Dry Matter (g)	Cu Concentration (p.p.m.)	Total Cu (mg)
Group killed initially	134 (120-142)	206.6 (157.9-245.1)	27.5 (24.5-33.8)
Group I	162	230.6	37.5
Group II	180	286.2	51.4
Group III	181	260.4	46.1
Group IV	200	310.5	62.4
Sign.Diff. (bet.means)	Gp.IV > Gp.I ($P<0.05$)	-	Gp.II > GpI($P<0.05$) Gp.IV > GpI($P<0.02$)

Fig. 22 shows the relationship between the concentration of copper in the food and the mean liver copper concentrations of the four groups. A linear relationship is found to exist between those two factors; the liver copper concentration increases as a consequence of an increased copper concentration in the food. There were no significant differences, however, in liver copper concentration/

Fig 22. Relationship between the concentration of copper in the food and the total amount of copper in the liver of sheep.



concentration among the 4 groups. That the mean liver copper concentration of Group IV (310.5 p.p.m.) was not significantly higher than that of Group I (230.6 p.p.m.) can only be explained by the fact that the mean liver dry matter of Group IV (200 g) was significantly (P = 0.05) higher than that of Group I (162 g). The combined mean liver copper concentration of Group II and IV (297.0 p.p.m.) which were being fed 20% Quicklamb was significantly (P = 0.05) higher than that of Group I and III (246.6 p.p.m.) which were being fed 20% E.K. 94. This is purely a reflection on the difference in copper concentration between the two concentrates. The Quicklamb had a copper concentration of 29.1 p.p.m. compared with one of 14.8 p.p.m. for the E.K. 94.

The mean total liver copper contents of Group II (51.4 mg) and Group IV (62.4 mg) were significantly ($P < 0.05$ and 0.02 respectively) higher than the mean total liver copper content of Group I (37.5 mg). This confirms what was suggested earlier that if the livers had been of equivalent size the mean liver copper concentration of Group IV would have been significantly higher than that of Group I. The combined mean total liver copper content of the sheep fed 20% Quicklamb (56.3 mg) was significantly (P = 0.01) higher than that of the sheep fed 20% E.K. 94 (42.1 mg).

Liver Storage of Dietary Copper. There were no significant differences in the rates of absorption and storage of dietary copper found among the 4 groups. The percentage of dietary copper stored in the liver by the 4 groups (calculated from the total Cu content of the food consumed) was 3.5, 2.7, 3.6 and 2.9% respectively for Groups I to IV.

Effect/

Effect of Fibre (from sugar beet pulp). The addition of 15% sugar beet pulp to the rations was found to have no effect on the proportion of dietary copper stored in the liver. This can be seen from Table 61 where a comparison is made between the difference in copper concentration and total liver copper content of the sheep being fed Quicklamb and those being fed E.K.94 both with and without sugar beet pulp.

Table 61. Comparison between liver copper concentration and total liver copper content of sheep on a low and high fibre intake.

	Cu.Conc in food (p.p.m.)	Liver Cu Conc.(p.p.m.)	Total Liver Cu (mg)
Barley + Quicklamb (Group II)	9.6	286.2	51.4
Barley + E.K.94 (Group I)	6.8	230.6	37.5
Difference.	2.8	55.6	13.9
Barley + Quicklamb + S.B.P. (Group IV)	11.2	310.5	62.4
Barley + E.K.94 + S.B.P. (Group III)	8.3	260.4	46.1
Difference	2.9	50.1	16.3

This demonstrates that the copper was stored equally irrespective of the fibre content of the diet. This is also evident from Fig. 22 which shows a linear relationship between the concentration of copper in the food and in the liver. If the addition of sugar beet pulp had been affecting the copper storage rate a straight line graph would not have been obtained.

In/

In contrast to Experiment 9 (a) where almost all the housed lambs had liver copper concentrations in excess of 500 p.p.m. only two sheep in this present experiment had liver copper concentrations above this level. The copper concentration in the diet of most of the groups in these experiments was within the range 8.3 to 13.5 p.p.m. and the crude protein content was around 13.0%. The difference between the liver copper concentrations of the sheep in the two experiments cannot, therefore, be explained by a difference in the dietary intake of copper or crude protein. The most probable cause of the higher liver-copper concentrations found for the lambs in Experiment 9 (a) was the fact that they were introduced to those intensive rations almost from birth when they are possibly able to absorb the dietary copper much more efficiently. The sheep in the present experiment had been weaned before they were introduced to the intensive fattening rations and were about 6 months older than the lambs in Experiment 9 (a).

There was no imminent danger of copper toxicity occurringin the present experiment and in fact the highest liver copper concentration recorded, 509.9 p.p.m., is only just outside the upper normal limit.

Experiment/

Experiment 10. The storage of copper in the livers of lambs fattened at grass with access to creep feed.

This experiment involved an investigation into the comparative rates of storage of copper in the liver of lambs either (a) born indoors and housed for the first 10 - 12 weeks of life and then fattened off grass alone or with some supplementary concentrate or (b) similar lambs born outside and fattened on a similar dietary regime.

Seventy seven Halfbred lambs were used in the experiment; 25 of the lambs were born indoors while the remaining 52 had been born outside. Lambing took place between 1 February and 13 March 1965. All 77 lambs had access to creep feed - Lambwena - until 6 May after which time the housed lambs were taken outside and 12 of them put to graze with 36 of the lambs which had been born outside. These lambs ran with their mothers at a stocking rate of 7 ewes/acre and were offered no supplementary creep feed. The remaining lambs, 13 born inside and 16 born outdoors, were placed in an adjacent paddock with their mothers at a stocking rate of 10 ewes/acre. These lambs were offered supplementary creep feed ad lib. The creep feed was originally Lambwena alone but this was gradually diluted with barley until by 3 July a mixture of equal parts Lambwena and barley was being fed.

The lambs were slaughtered on either 25 May, 8 June or 13 July 1965 when they reached an acceptable weight of 75 to 80 lbs. liveweight. The whole livers were obtained at slaughter and weighed. The caudal lobe was then separated/

separated, weighed, dried, ground and analysed for its copper concentration. If the copper concentration in the caudal lobe is taken as representative of that in the whole liver the total amount of copper present in the liver could be calculated.

Samples of the pasture being grazed and of the barley and Lambwena fed were obtained on several different occasions and these were then analysed for their copper concentration (Table 62).

Results.

The total amount of creep food consumed by the lambs was recorded. Until 6 May the housed lambs consumed 22.8 lbs. Lambwena/head compared with 20.1 lbs. /head for the lambs born outside; i.e. a mean consumption of 21.5 lbs/head to this date. After 6 May the mean total amount of barley and Lambwena consumed by the group which had access to the creep feed is listed in Table 62.

Table 62 Copper Concentration and creep feed consumption after 6 May 1965.

Sheep killed on	Lambwena (lb/head)	Barley (lb/head)	Grass.
25/5/65	26.82	-	Ad Lib.
8/6/65	48.88	2.65	"
13/7/65	104.35	32.75	"
Copper conc.(p.p.m.)	22.5	6.5	10.5

From/

From this data the mean intake of copper from the creep feed for each group was calculated. By comparing this with the total amount of copper stored in the liver the proportion which was stored in the liver could be calculated.

Liver dry matter, copper concentration and total copper content.

The individual and mean liver dry weights, liver copper concentrations and total liver copper contents of the 4 groups of sheep are listed in Table 63.

A summary of the mean liver dry weights, copper concentrations and total copper contents of the 4 groups of sheep is given in Table 64.

The mean liver-copper concentration of the sheep in Group II (155.0 p.p.m.) which were born outside and given no creep feed was significantly ($P = 0.01$) less than those of the other 3 groups (226.8, 430.1 and 262.9 p.p.m. respectively for Groups I, III and IV). The mean liver-copper concentration of Group III (430.1 p.p.m.) which were born indoors and given creep food at grass was highly significantly ($P < 0.001$) greater than those of the other 3 groups. No significant difference was found between the mean liver-copper concentration of the sheep in Group I (226.8 p.p.m.) which were born indoors and given no creep feed at grass and that of the sheep in Group IV (262.9 p.p.m.) which were born outside and then given access to creep food at grass.

The mean total liver-copper content of Group III (born indoors and given creep feed at grass) (86.1 mg) was highly significantly ($P = 0.001$) greater than the mean total liver-copper contents of the other 3 groups (43.9, 32.6 and 52.0 mg. respectively for Groups I, II and IV). The mean total liver copper/

Table 63. Individual and mean liver dry weights, copper concentrations and total copper contents of 4 groups of sheep.

Group I. Born Indoors. No creep feed at grass.

No.	D.M.(g)	Cu Conc. (p.p.m.)	Total Cu(mg.)	No.	D.M.(g)	Cu Conc. (p.p.m.)	Total Cu(mg.)
1	239	421.2	100.7	36	169	233.8	39.5
2	179	232.7	41.7	64	211	249.0	52.5
4	178	157.4	28.0	66	217	128.5	27.9
8	195	151.8	29.6	82	192	245.4	47.1
9	196	196.3	38.5	140	179	243.7	43.6
11	161	299.6	48.2				
19	185	162.0	30.0	Mean(12)	192	226.8	43.9

Group II. Born Outside. No creep feed at grass.

1	233	110.6	25.8	18	206	119.6	24.6
5	213	144.5	30.8	19	190	108.7	20.7
6	202	215.0	43.4	29	185	148.4	27.5
7	195	155.8	30.4	30	200	101.1	20.2
8	165	185.5	31.1	31	203	149.0	30.2
10	257	174.9	44.9	46	252	170.8	43.0
11	216	138.3	29.9	47	213	134.8	28.7
13	217	280.4	60.8	58	205	80.8	16.6
17	234	178.5	41.8	65	187	517.3	96.7
70	219	94.5	20.7	R5	176	155.5	27.4
71	182	176.3	32.1	6	233	23.3	54.3
84	175	196.3	34.4	10	216	147.9	31.9
90	167	136.5	22.8	12	217	105.3	22.9
106	240	111.9	26.9	15	181	241.4	43.7
120	201	98.9	19.9	17	215	159.7	34.3
124	193	121.9	23.5	22	217	170.6	37.0
173	176	101.5	17.9	204	173	127.8	22.1
185	198	153.6	30.4				
195	175	143.1	25.0	Mean(36)	204	155.0	32.6

Group III. Born Indoors. Creep feed at grass.

14	231	469.8	108.5	80	181	448.0	81.1
15	207	403.1	83.4	102	177	380.8	67.4
26	183	373.8	68.4	104	165	569.5	94.0
32	179	321.4	57.5	125	220	452.0	99.4
50	212	637.7	135.2	R7	232	294.7	68.4
51	210	450.3	94.6				
53	221	489.9	108.3				
67	177	300.8	53.2	Mean(13)	200	430.1	86.1

Group IV. Born Outside. Creep feed at grass.

16	216	269.8	58.3	114	173	289.1	50.0
48	219	28.4	6.2	115	197	138.4	27.3
49	240	118.2	28.4	130	221	218.6	48.3
56	193	376.0	72.6	135	172	347.6	59.8
57	186	183.7	34.2	136	201	180.9	36.4
63	199	349.0	69.5	144	172	592.5	101.9
76	214	200.0	42.8	207	212	336.7	71.4
96	211	349.8	73.8				
98	225	228.1	51.3	Mean(16)	203	262.8	52.0

Table.64. The mean liver dry matter, liver-copper concentrations and total liver copper content of 4 groups of sheep.

Group	No/Group	Dry Matter (g)	Cu Concentration (p.p.m.)	Total Cu (mg.)
I (Born indoors; No creep at grass)	12	192	226.8	43.9
II (Born outside; No creep at grass)	36	204	155.0	32.6
III (Born indoors; Creep fed at grass)	13	200	430.1	86.1
IV (Born outside; Creep fed at grass)	16	203	262.9	52.0

	Cu Concentration	Total Cu
Sign. Diff. (between means)	Gp.II < Gps.I, III & IV ($P<0.01$) Gp.III > Gps.I, II & IV ($P<0.001$)	Gp.IV > Gp.II ($P<0.005$) Gp.III > Gp.I, II & IV ($P<0.001$)

copper content of the sheep in Group IV (52.0 mg) which were born outside and given creep feed was significantly (P 0.005) higher than that (32.6 mg) of the sheep in Group II which were also born outside but given no creep feed. It was not, however, significantly higher than the mean total liver copper content (43.9 mg) of the sheep in Group I which were born indoors but given no creep feed when turned out to grass. Despite the fact that there was a highly significant difference between the mean liver-copper concentrations of Groups I and II there was not quite a significant difference in their mean total liver-copper contents (43.9 and 32.6 mg respectively). This is due to the fact that the mean liver dry weight (204 g) of Group II, although not significantly higher than that (192 g.) of Group I, is sufficiently above that of Group I to reduce the difference in mean total liver-copper content to a non significant level. The actual difference in mean total liver copper contents between the two groups is 11.3 mg. while the least difference required for significance is 12.5 mg.

<u>The proportion of copper in the concentrates fed after 6 May which was stored in the liver.</u>

No lambs were killed at the start of this period so that no control values were available for either the liver copper concentration or total copper content prior to the final fattening stage of the four groups at grass. As this was the case it was possible only to calculate the storage rate of dietary copper in these two groups which had access to creep feed at grass by comparing them with the 2 groups which had been similarly reared up until 6 May but which after/

after this date were fattened solely off grass. The final difference between the total liver-copper contents of the two groups being compared would be due to the additional copper intake from the creep feed and as the mean food consumption of each animal was known the percentage storage in the liver of this supplementary copper could be calculated.

When the sheep in Group III, which were born inside and then given creep feed at grass, are compared with Group I which were also born inside but were given no creep feed at grass it was calculated that 10.4% of the supplementary copper has been stored in the liver by Group III. Similarly when we compare the two groups which were born outside we find that Group IV has stored 4.9% of the additional copper it received in the creep feed.

Discussion

The most significant result to emerge from this experiment was that copper was stored apparently much more effectively by lambs which had been housed than by lambs which had been reared on an equivalent diet outdoors. This is evident if we compare the total liver copper contents of Groups III and IV. Both groups had received almost exactly equal amounts of creep feed but the sheep in Group III which had been housed for the first 10 - 12 weeks of their lives had stored 34.1 mg. more copper than had the sheep in Group IV which had been outdoors all their lives. Also the % storage rate of dietary copper at grass by the sheep which had previously been housed was 10.4% compared with a storage rate of 4.9% for those sheep which had been outdoors continually. This supposition/

supposition that the housed lambs stored their dietary copper more efficiently is emphasised by the fact that the mean total liver copper content of Group IV, which had access to concentrate feeding all their lives was not significantly higher than that of Group I which got no supplementary feeding after the initial 10 - 12 weeks of life during which time they had been housed. The fact that the mean liver-copper concentration of Group I (226.8 p.p.m.) was significantly higher than that of Group II (155.0 p.p.m.) also suggests that housed lambs store dietary copper more readily than do those at grass. Group I had been housed for the first 10 - 12 weeks of life and thereafter both groups had been fattened at grass with no supplementary creep feed. It is probable that there would be more copper at birth in the livers of the lambs born indoors as the ewes were on an indoor diet. However, as the liver size would be small the total amount of extra copper would be unlikely to exceed 2.5 mg. This difference is slight compared to the differences at slaughter between the mean values of the lambs born indoors (130.0 mg) and those born outdoors (84.6 mg).

Free access to concentrates containing about 20.0 p.p.m. of copper increased the mean liver-copper concentrations and total liver-copper contents of sheep grazing pasture containing 10.5 p.p.m. Cu. This can be seen by comparing Groups III and IV which had access to supplementary creep food and had total liver-copper contents of 86.1 and 52.0 mg. respectively with Groups I and II (which were living solely off grass) and had mean total liver-copper contents of 43.9 and 32.6 mg. respectively.

Experiment/

Experiment 11 (a). The storage of copper in the livers of young sheep fattened intensively indoors on cereal based diets.

This experiment was designed as a further investigation into copper absorption in small 6 month old Blackface lambs which were intensively fattened indoors on a largely cereal diet. Maize, barley and oats were used in different combinations as the cereal portion of the diets while the protein requirements were supplied by groundnut and soya bean meal. As groundnut and soya bean contain appreciably more copper than cereals, in one treatment urea was used as a substitute for half the groundnut and soya bean meals. In all six different diets were constructed and these have been detailed in Table 65.

Table 65 % Composition of Diets*

	Group I	Group II	Group III	Group IV	Group V	Group VI
Barley	85.0	-	35.0	-	-	-
Maize	-	85.0	-	35.0	91.3	78.8
Oats	-	-	50.0	50.0	-	-
Soya	7.5	7.5	7.5	7.5	3.75	-
Groundnut	7.5	7.5	7.5	7.5	3.75	-
Urea	-	-	-	-	1.2	1.2
Lucerne (dried)	-	-	-	-	-	20.0
Analysis of Concentrate Diet						
Crude Protein%	14.1	14.2	14.9	14.9	14.8	13.6
Copper (p.p.m.)	5.85	4.33	5.96	5.33	3.29	2.81

* All diets contained additions of 1% $CaCO_3$, 1% NaCl and Vitamins A & D.

The/

The crude protein contents of all six rations were between 13.6 and 14.9%. It is unlikely that such small differences would influence copper storage. The copper levels of the concentrate diets ranged from 2.8 - 5.9 p.p.m. These are low levels of copper concentration and the diets fed to Group V (3.3 p.p.m.) and Group VI (2.8 p.p.m.) could almost be regarded as being copper deficient.

Sixty eight 5 - 6 month old Blackface ewe lambs were used in this experiment. These were brought in from hill grazing in Argyll in September and housed directly. On housing they were put through a footbath, innoculated against pulpy kidney, treated with carbon tetrachloride against liver fluke and Thibenzole against other internal parasites. Eight of the lambs died as a result of acidosis during the initial changeover period on housing and their livers have been used to provide an initial value for the liver copper concentration and total liver copper content of the sheep at the start of the experiment. The remaining sixty lambs were divided at random into 6 groups of ten; each group was assigned to one of the experimental rations.

Five of the lambs in each group received a supplement of 10 mg Cu per day. This was given as a drench of copper sulphate once weekly, each drench containing 70 mg Cu (280 mg $CuSO_4.5H_2O$). All the lambs in each group were ear-tagged before randomisation into the six groups of 10 and it was decided to drench those five lambs in each group which had the five highest tag numbers.

Feeding of the experimental diets commenced on 12 October 1964. The maize

and/

and protein - rich supplements and urea were all fed originally as meals. This, however, was found to be rather wasteful and unsuccessful due to lack of palatability. Consequently from 7 December 1964 kibbled maize was fed in place of maize meal and the groundnut, soya bean meal and urea (as appropriate) were pelleted together with the appropriate amounts of minerals and vitamins. The rations continued to be fed in this form until the sheep were slaughtered 14 weeks later on 16 March 1965 when their individual weights ranged from 70 - 100 lbs.

Hay ($\frac{2}{3}$ lb./head/day) was fed until 7 December. Thereafter a mixture of hay and oat straw was fed before finally changing to chopped ($\frac{1}{2}$ - 1") oat straw. The sheep consumed about 50 g of this per head/day.

Samples of all the foods were obtained frequently and these were then analysed for copper and crude protein (Table 66).

Table 66 Analyses of Foods (Mean of 5 Samples).

	Barley	Maize	Oats	Soya	G.nut.	Lucerne	Hay	Straw
Crude Protein (%)	8.4	8.6	10.1	45.9	46.4	16.8	5.0	2.1
Cu (p.p.m.)	4.1	2.3	4.3	16.6	15.0	5.0	4.4	2.9

Blood samples were taken on four separate occasions during the course of the experiment and were analysed for their whole blood copper concentrations. The whole livers were obtained from all the sheep at slaughter on the conclusion of/

of the experiment. These were weighed and subsampled and the subsamples were dried, weighed, ground and analysed for their copper concentration.

Results

Liveweight Gains

Table 67 presents the mean liveweight changes of the separate groups together with their consumption of the various foods. The column headings are abbreviations of the full ration details given in Table 65.

The sheep in Group VI (maize, lucerne and urea) were always rather more advanced in growth than the other groups. Diets based on maize were rather better than those based on barley. Substitution of one half of the groundnut and soya used in Group II by urea (Group V) did not in the long term have any adverse effects on growth. During the early stages however, (up to mid December before the rations were pelleted) those sheep given urea (Group V) were rather poorer than all the other groups.

Those diets containing a high proportion of maize (Groups II and V) had more efficient food conversion ratios (6.1 and 6.5) than the other groups. Group I based on barley (9.5) and Group III based on barley and oats (8.8) were much the poorest in this respect.

Blood Copper Concentration. The individual and mean blood copper concentrations of the 6 groups of sheep are detailed in Table 68. Two mean values will be given in each case; one for those sheep which got no supplementary copper and the other for those that did. Group I will be used to refer to those 5 sheep on the basal diet while Group I + Cu will refer to the 5 supplemented sheep in Group I.

Table 67. Mean liveweight changes (lbs) food consumption (lbs) and concentrate conversion ratios (10 sheep/group).

Group		I	II	III	IV	V	VI
		Barley	Maize	Barley Oats	Maize Oats	Maize Urea	Maize Lucerne Urea
Weeks	Mean Liveweight						
0	28th Sept.	49.9	50.1	49.9	48.1	48.5	49.6
5	2nd Nov.	52.6	53.8	54.4	56.1	50.9	54.8
10	7th Dec.	57.0	58.2	60.1	57.6	54.0	62.0
15	11th Jan.	59.9	67.0	66.0	66.0	61.9	74.2
20	15th Feb.	72.1	79.8	80.3	81.3	79.6	85.2
22	1st Mar.	78.1	84.8	84.8	83.6	82.7	92.2
24	15th Mar.	79.8	89.3	88.6	89.9	86.9	96.2
Total Concentrate Consumption (lbs/head).		283	233	340	315	255	339
Concentrate conversion ratio (lb/lb L.W.G.).		9.5	6.1	8.8	7.5	6.5	7.2
Roughage Consumption (lbs/head).							
Hay (lbs)		47	47	47	47	47	47
Straw (lbs)		11	13	7	9	11	11

Table 68. Individual and mean blood copper concentrations of 12 groups of 5 sheep.

Group I					Group I + Cu.				
No.	12/10/64	18/11	25/1/65	9/3.	No.	12/10/64	18/11	25/1/65	9/
815	1.05	0.96	1.02	0.96	853	1.14	-	1.07	1.
819	0.79	1.10	0.96	1.00	854	0.62	0.91	1.10	1.
820	0.91	1.01	-	-	857	1.01	0.91	0.90	0.
826	1.30	1.02	0.99	0.91	881	1.14	1.05	1.19	1.
851	1.06	1.30	0.92	1.03	885	0.95	0.85	0.89	1.
Mean	1.02	1.08	0.97	0.98		0.97	0.93	1.03	1.

Group II					Group II + Cu.				
No.	12/10/64	18/11	25/1/65	9/3.	No.	12/10/64	18/11	25/1/65	9/
814	1.39	1.01	1.00	0.86	843	0.69	0.91	0.92	0.
817	0.90	1.21	1.02	1.14	849	1.17	0.96	0.91	0.
830	0.97	0.85	-	1.11	861	1.01	1.26	0.92	0.
831	1.01	1.23	1.09	1.09	877	1.01	0.90	0.96	1.
834	0.90	0.98	-	-	879	0.86	0.97	0.98	1.
Mean	1.03	1.06	1.04	1.05		0.95	1.00	0.94	0.

Group III					Group III + Cu.				
No.	12/10/64	18/11	25/1/65	9/3.	No.	12/10/64	18/11	25/1/65	9/
803	0.93	0.75	0.92	0.76	837	0.59	0.72	0.82	0.
804	1.17	0.87	1.01	0.92	855	1.11	1.13	0.82	1.
805	1.03	0.95	0.81	0.76	864	0.88	1.06	1.01	0.
821	1.31	0.81	0.82	0.83	869	0.70	0.92	0.86	0.
829	1.02	0.84	0.97	1.00	878	0.98	1.25	1.26	
Mean	1.09	0.87	0.91	0.85		0.85	1.02	0.95	0.

Group IV.					Group IV + Cu.				
No.	12/10/64	18/11	25/1/65	9/3.	No.	12/10/64.	18/11	25/1/65	9/
806	1.25	1.10	0.99	1.02	833	0.91	0.77	0.87	0.
809	1.01	1.03	1.01	0.90	845	0.96	0.72	0.96	1.
818	0.96	1.39	0.88	0.94	863	0.95	0.80	0.87	0.
827	0.92	0.97	1.01	0.92	872	0.55	0.64	0.77	0.
832	0.96	0.95	1.09	1.08	882	1.14	1.37	1.07	1.
Mean	1.02	1.09	1.00	0.97		0.90	0.86	0.91	0.

Table 68. (Contd.)

Individual and mean blood copper concentrations of 12 groups of 5 sheep.

Group V					Group V + Cu.				
No.	12/10/64	18/11	25/1/65	9/3.	No.	12/10/64	18/11	25/1 /65	9/
801	1.04	0.92	0.85	0.86	844	0.65	1.03	1.30	0
823	0.82	1.28	0.86	0.86	852	1.14	1.02	0.85	0
825	1.51	1.31	1.02	1.10	860	1.02	1.11	0.91	0
828	0.90	0.76	1.02	0.93	871	0.87	0.65	0.96	0
838	0.91	0.89	0.92	0.87	875	0.86	0.94	1.00	1
Mean	1.04	1.03	0.93	0.92		0.91	0.95	1.06	0

Group VI.					Group VI + Cu.				
No.	12/10/64	18/11	25/1/65	9/3.	No.	12/10/64	18/11	25/ 1/65	9/
824	0.96	0.65	0.82	0.81	858	0.87	1.01	0.94	0
836	0.97	-	0.82	0.92	862	0.93	1.07	1.09	1
839	0.97	1.01	0.85	0.79	865	0.87	1.06	0.91	1
842	0.91	0.78	0.72	0.86	870	0.90	1.06	1.05	0
856	1.01	0.72	0.92	0.96	873	1.19	1.11	0.98	0
Mean	0.96	0.79	0.83	0.87		0.95	1.06	0.99	0

No definite trend was found for the mean blood copper concentrations of either the supplemented or the unsupplemented sheep. The mean blood copper concentrations of all 12 groups were always within the range 0.79 to 1.09 p.p.m. The unsupplemented sheep in Group VI had an initial drop in their mean blood copper concentration from 0.96 - 0.79 p.p.m. but thereafter it increased slowly until by the end of the experiment it had reached a level of 0.87 p.p.m. despite a dietary copper concentration of only 2.8 p.p.m. It was nevertheless generally rather lower than all the other groups. Supplementation with 10 mg Cu per head/day had no effect on mean blood copper concentration. The mean values of all the samples obtained from the unsupplemented and supplemented sheep respectively were 0.98 and 0.96 p.p.m.

Liver dry weight, liver-copper concentration and total liver-copper content.

The individual and mean liver dry matters, liver-copper concentration and total liver-copper contents of the 6 groups of sheep are presented in Table 69, divided into two sub-groupsaccording to whether they received supplementary copper or not.

The mean liver dry weights, copper concentrations and total liver copper contents of the group killed initially and of the other 12 groups are summarised in Table 70.

The six groups which received 10 mg. of supplementary copper per day had mean liver copper concentrations which were significantly (but not vastly) higher than those of the comparable groups which received no supplementary copper. Mean concentrations were about doubled (231 cf. 498 p.p.m.). However, the mean liver copper concentration (398.4 p.p.m.) of Group I + Cu was not significantly/

Table 69. Individual and mean liver dry weights, copper concentrations and total copper contents of 6 groups of sheep.

Group I				Group I + Cu.			
No.	D.M.(g)	Cu Conc. (p.p.m.)	Total Cu(mg.)	No.	D.M.(g)	Cu Conc. (p.p.m.)	Total Cu(mg.)
815	154	153.8	23.7	853	178	597.1	106.3
819	149	270.9	40.4	854	129	514.3	66.3
820	116	164.5	19.1	857	177	229.6	40.6
826	179	124.6	22.3	881	172	346.7	59.6
851	219	158.5	34.7	885	144	304.2	43.8
Mean	163	174.5	28.0		160	398.4	63.3
Group II				Group II + Cu.			
814	201	196.9	39.6	843	164	798.5	131.0
817	219	259.0	56.7	849	151	502.2	75.8
830	188	183.0	34.4	861	199	690.5	137.4
831	198	244.3	48.4	877	268	531.4	142.4
834	139	144.0	20.0	879	209	403.1	84.2
Mean	189	205.4	39.8		198	585.1	114.2
Group III				Group III + Cu.			
803	153	134.3	20.5	837	193	575.3	111.0
804	149	349.4	52.1	855	236	367.2	86.7
805	160	284.8	45.6	864	184	374.1	68.8
821	254	255.7	64.9	869	159	623.3	99.1
829	174	286.2	49.8	878	154	533.9	82.2
Mean	178	262.1	46.6		185	494.8	89.6
Group IV				Group IV + Cu.			
806	226	205.2	46.4	833	187	607.9	113.7
809	153	317.3	48.5	845	209	448.9	93.8
818	149	452.5	67.4	863	173	509.6	88.2
827	185	299.2	55.4	872	153	583.5	89.3
832	234	351.7	82.3	882	203	431.0	87.5
Mean	189	325.2	60.0		185	516.2	94.5
Group V				Group V + Cu.			
801	160	247.0	39.5	844	153	446.4	68.3
823	232	240.4	55.8	852	163	681.4	111.1
825	267	141.3	37.7	860	237	396.4	93.9
828	171	318.7	54.5	871	179	496.0	88.8
838	167	303.5	50.7	875	178	466.6	83.1
Mean	199	250.2	47.6		182	497.4	89.0

Table 69 (continued)

Group VI				Group VI + Cu.			
No.	D.M.(g)	Cu Conc. (p.p.m.)	Total Cu(mg.)	No.	D.M.(g)	Cu Conc. (p.p.m.)	Total Cu(mg.)
824	193	130.1	25.1	858	240	563.8	135.3
836	208	171.0	35.6	862	215	447.8	96.3
839	175	186.1	32.6	865	246	600.4	147.7
842	210	147.3	30.9	870	204	379.8	77.5
856	206	211.6	43.6	873	241	503.4	121.3
Mean	198	169.2	33.6		229	499.0	115.6
Group which died initially							
802	99	79.5	7.9				
810	127	82.1	10.4				
835	102	149.0	15.2				
859	92	32.9	3.0				
866	90	97.5	8.8				
867	102	79.5	8.1				
874	81	135.7	11.0				
876	100	103.6	10.4				
Mean	99	95.0	9.4				

Table 70 Mean liver dry weights, copper concentrations and total liver copper contents of 13 groups of sheep

Group Died Initially

Liver Dry Wt. (g)	99
Cu. Conc.(p.p.m.)	95
Total Cu. (mg.)	9.4

Unsupplemented	Barley	Maize	Barley Oats	Maize Oats	Maize Urea	Maize Lucerne Urea
Liver Dry Wt. (g)	163	189	178	189	199	198
Cu. Conc. (p.p.m.)	174.5	205.4	262.1	325.2	250.2	169.2
Total Cu.(mg.)	28.0	39.8	46.6	60.0	47.6	33.6

Supplemented with copper

Liver Dry Wt. (g)	160	198	185	185	182	229
Cu. Conc.(p.p.m.)	398.4	585.1	494.8	516.2	497.4	499.0
Total Cu.(mg.)	63.3	114.2	89.6	94.5	89.0	115.6

significantly higher than those (262.1, 325.2 and 250.2 p.p.m.) of Groups III, IV and V respectively which received no supplementary copper. The remaining five groups which received supplementary copper had mean copper concentrations which were significantly higher than those of all the six unsupplemented groups. Although the mean liver copper concentrations of the supplemented groups ranged from 398.4 p.p.m. for Group I (barley) to 585.1 p.p.m. for Group II (maize) there were no significant differences between any two of the six groups.

In the unsupplemented groups the range in mean liver copper concentration was from 169.2 p.p.m. for Group VI (maize, lucerne, urea) to 325.2 p.p.m. for Group IV (maize and oats). The mean liver copper concentrations of Group III (262.1 p.p.m.) and Group IV (325.2 p.p.m.) which were on a diet containing 50% oats were significantly ($P = 0.01$) higher than those of Group I (174.5 p.p.m.) and Group VI (169.2 p.p.m.) which were on diets composed largely of barley and maize respectively. The mean liver-copper concentration of Group IV (maize and oats) was also significantly higher than that of Group II (205.4 p.p.m.) which was fed on a diet composed mainly of maize.

The mean total liver-copper contents of the six unsupplemented groups ranged from 28.0 mg. for Group I (barley) to 60.0 mg. for Group IV. These were all significantly ($P < 0.001$) higher than the mean total liver copper content (9.4 mg) of those sheep which died at the start of the experiment. The mean total liver copper content of Group IV (60.0 mg) (maize and oats) was significantly ($P = 0.01$) higher than those of Group I (28.0 mg), Group II (39.8 mg)(maize) and Group VI (33.6 mg)(maize, Lucerne & urea). The mean total/

total liver copper contents of Group III (46.6 mg)(barley and oats) and Group V (47.6 mg)(maize and urea) were significantly (P = 0.01) higher than that of Group I (28.0 mg)(barley).

The range in mean total liver-copper content of the copper supplemented groups was from 63.3 mg. for Group I (barley) to 115.6 mg. for Group VI (maize, Lucerne and urea). The mean total liver copper contents of Group II (maize) (114.2 mg.) and Group VI (115.6 mg.) were significantly higher than those of Groups I, III, IV and V (63.3, 89.6, 94.5 and 89.0 mg. respectively). The mean total liver copper contents of Groups II - VI were all significantly higher than that of Group I.

The Proportion of Dietary Copper Stored in the Liver.

The proportion of the dietary copper of the unsupplemented groups and the proportion of the additional dosed copper which was stored in the liver are shown in Table 71.

The proportion of dietary copper which was stored in the liver was calculated by comparing the total liver copper of the six groups with that of the mean of those sheep which died at the start of the experiment. The proportion of the dosed copper (1330 mg. at 10 mg.Cu/day) which was stored was calculated by comparing the supplemented group with the equivalent unsupplemented group.

For/

Table 71. Total copper intake and the proportion of dietary and dosed copper which were stored in the liver (mean of 5 sheep/grou

Group	I	II	III	IV	V	VI
Copper Intake (mg)						
Concentrates	690.9	481.5	919.0	740.1	370.1	419.2
Hay	92.8	92.8	92.8	92.8	92.8	92.8
Straw	14.5	17.1	9.2	11.9	14.5	14.5
Total	798.2	591.4	1021.0	844.8	477.4	526.5
Unsupplemented Groups.						
Additional copper stored in liver (mg)	18.6	30.4	37.2	51.6	38.2	24.2
as % of Total Intake	2.33	5.14	3.64	6.11	8.00	4.59
Supplemented Groups. (Total supplement 1330 mg Cu)						
additional copper stored in liver (mg).	35.3	74.4	43.0	34.5	41.4	82.0
as % of supplementary dosed copper.	2.65	5.59	3.23	2.59	3.11	6.17

For the unsupplemented groups the mean storage rate of dietary copper ranged from 2.33% for Group I (barley) to 8.00% for Group V (maize and urea) while for the supplemented groups the mean storage rate of dosed copper ranged from 2.59% for Group IV to 6.17% for Group VI (maize, lucerne, urea). For the unsupplemented sheep the mean storage rates of dietary copper by Groups I (2.33%) and III (3.64%) which were fed on diets consisting largely of oats or barley were appreciably lower than those of Groups II, V and VI (5.14, 8.00 and 4.59% respectively) which were fed diets containing maize as the main cereal.

The distinction between the storage rates of the additional dosed copper by the groups on a largely maize diet and those on one consisting mainly of barley or oats was not so marked but here also the two groups which stored the dosed copper most efficiently were those on a maize diet. These 2 groups were Group II which stored 5.59% and Group VI which stored 6.17% of the dosed copper. A more detailed comparison of liver copper storage by sheep on maize or barley and oats based diets will be given at the end of the next experiment.

There was no apparent risk of chronic copper toxicity on the unsupplemented diets. The highest individual liver copper concentration recorded was 452.5 p.p.m. for sheep No. 818 in Group IV. The highest liver copper concentration found for the supplemented sheep was 798.5 p.p.m. for sheep No. 814 in Group II. There were no signs or symptoms of chronic copper toxicity in any of the supplemented sheep despite having been given 10 mg. additional copper daily for 19 weeks.

Experiment/

Experiment 11 (b). The storage of copper in the livers of young sheep fattened intensively indoors on cereal based diets and supplemented with a variety of forms and levels of protein.

In this experiment crushed barley and kibbled maize were again compared as cereals sources for the indoor fattening of 6-month old Blackface lambs. Supplementary nitrogen was provided in a variety of forms to achieve a range of crude protein levels in the concentrates from about 9.5% (no extra protein) to about 13.0%. The principal nitrogen sources were fishmeal, groundnut and soya, dried lucerne meal and urea.

The concentrate mixtures employed were as detailed in Table 72 and the composition of the various protein concentrates were as shown in Table 73. Rations 1 - 4 and 7 supplied 12.2 - 13.6% crude protein. Rations 1 and 3 gave a comparison between maize and barley where the protein supplement provided about equal amounts of vegetable protein (soya and groundnut) and non protein nitrogen (urea). Similarly rations 2 and 4 contrasted maize and barley but with a protein concentrate containing a higher content of urea pelleted in dried lucerne. Ration 7 (62% barley, 30% sugar beet pulp and 8% fishmeal) provided about the same total protein and was included as this is a widely advocated diet for this purpose for use in the West of Scotland.

Rations 5 and 6 (based on barley) had lower protein levels of about 11.3% the former relying exclusively on urea as a supplement and the latter containing only groundnut and soya. Ration 8 included no supplementary protein.

Comparison/

Table 72. Compositions of Diets.

Group	1	2	3	4	5	6	7	8
Abbreviated Designation	Maize ½ Veg ½ Urea	Maize Lucerne Urea	Barley ½ Veg ½ Urea	Barley Lucerne Urea	Barley ½ Urea	Barley ½ Veg	Barley Sugar Beet Fish meal	Barley
% Maize	80	82	-	-	-	-	-	-
% Barley	-	-	80	82	80	80	62	92.5
% Sugar Beet Pulp	7.5	7.5	7.5	7.5	7.5	7.5	30	7.5
% Fishmeal	-	-	-	-	-	-	8	-
Type and % of Concentrate Pellet	12.5 A	10.5 D	12.5 A	10.5 D	12.5 B	12.5 C	-	-
Analysis of Mixture								
Crude Protein %	12.6	12.2	13.2	12.8	11.2	11.4	13.6	9.5
True Protein %	10.5	9.1	11.1	9.6	9.2	11.3	13.5	9.4
Non Protein N as % of Total N.	16.8	25.5	15.9	25.0	18.0	-	-	-
Copper p.p.m.	10.5	10.4	13.4	13.3	8.2	8.7	9.9	6.9

Table 73. Composition and Analysis of Pelleted Protein Concentrates.

Pellet Abbreviated Designation	A ½ Veg ½ Urea	B ½ Urea	C ½ Veg	D Lucerne Urea
Soya	23.0	-	23.0	-
Groundnut	23.0	-	23.0	-
Maize	26.5	72.5	32.5	10.0
Molasses	10.0	10.0	10.0	10.0
Minerals & Vits*	11.5	11.5	11.5	15.0
Urea	6.0	6.0	-	12.0
Lucerne	-	-	-	53.0
Analysis				
Crude Protein %	39.0	23.1	25.0	40.6
True Protein %	22.3	7.1	23.7	10.9
Copper p.p.m.	58.0	16.7	20.8	67.3

* Salt, dicalcium phosphate, calcium carbonate and Vitamins A and D.

Comparison between these three rations and Ration 3 should give some assessment of the usefulness of urea as a dietary source of nitrogen.

All the rations (except No. 7) included 7.5% of sugar beet pulp as a possibl safeguard against acidosis. The copper contents of the diets ranged from 6.9 (Group 8) to 13.3 - 13.4 p.p.m. (Groups 3 and 4). Food samples were obtained for analysis at regular intervals during the experiment. The analyses of the concentrate pellets are shown in Table 73.

The mean analyses of the other foods were:-

	Maize	Barley	Sugar Beet	Fishmeal
Crude Protein %	8.8	9.5	9.6	60.8
Copper p.p.m.	2.6	6.2	15.5	17.8

A total of 82 Blackface lambs (aged about 6 months) were housed on 16 September 1965, and allocated at random into eight groups of 10 or 11 each. A further group (No. 9) of seven comparable lambs remained outside and were allowed free grazing (10 p.p.m. Cu) and access to turnips (3.5 p.p.m. Cu). All the lambs were run through a footbath, innoculated against pulpy kidney and dosed against fluke and other endoparasites.

The housed lambs were initially fed on hay alone but after 5 days the appropriate concentrates (but omitting sugar beet from all but Group 7) were introduced to each group. The amount of hay fed was reduced from about $\frac{3}{4}$ lb. to only $\frac{1}{4}$ lb. per head per day. Some oat straw was then offered but by 16 November/

November all roughage was withdrawn apart from a small amount of chopped oat straw, the consumption of which was considerably less than 1/10 lb. per head per day for the sheep in all the groups.

During this change-over period (2 - 3 weeks after housing) one of the maize fed sheep died of acidosis and another was quite ill. A total of six of the barley fed sheep died from a similar cause and another ten were seriously ill but eventually recovered. None of the sheep in Group 7 (62% barley, 30% sugar beet pulp and 8% fishmeal) were affected. Some 7.5% of sugar beet pulp was introduced to all the diets in an attempt to alleviate this severe acidosis. This proved to be quite successful and was retained as a constituent of all the diets throughout the remainder of the feeding period (Table 72). There can however, be no doubt that kibbled maize was a far safer food during the change-over period than crushed barley.

The whole livers of the seven sheep which died were retained and their mean copper content was taken as a measure of the copper status of all the sheep at the start of the experiment.

All the sheep were slaughtered on 2 February 1966. Blood samples and the entire livers were obtained at slaughter from all the sheep for the determination of copper concentrations.

Results/

Results.

Liveweight Changes.

Table 74 presents the changes in mean liveweight of the 8 groups of housed sheep. As was the case in Experiment 11 (a), these sheep fed maize were markedly superior in growth rate and appearance to these groups fed barley. The maize fed sheep (Groups 1 and 2) reached optimal slaughter weights of 75 - 80 lbs. some 4 or 5 weeks in advance of the barley fed sheep. Groups 2 (maize based) and 4 (barley based) where non protein nitrogen formed about 25% of the total nitrogen intake were not inferior to Group 1 (maize based) and Group 3 (barley based) where only about 16% of the total nitrogen was supplied as urea.

Group 7 (barley, sugar beet and fishmeal) had a comparable protein level to Groups 1 - 4. Whilst initially it was rather better than Groups 3 and 4 (barley based) this may have been a reflection of greater palatability and gut fill. By the end of the experiment the lambs in this group were 3 - 4 lbs. lighter than those in Groups 3 and 4. The protection against acidosis given by the inclusion of 30% beet pulp was, however, a most noticeably beneficial feature of the ration fed to this group in which the individuals grew in a uniform manner.

The/

Table 74. Mean Liveweight changes (lbs), concentrate consumption (lbs) and food conversion ratios.

Group	1	2	3	4	5	6	7	8
Abbreviated Designation	Maize ½ Veg ½ Urea	Maize Lucerne Urea	Barley ½ Veg ½ Urea	Barley Lucerne Urea	Barley ½ Urea	Barley ½ Veg	Barley Sugar Beet Fishmeal	Barley
Mean Liveweight								
Weeks Date								
0 16 Sept.	47.6	48.8	47.0	46.7	47.6	48.3	48.0	48.1
5 21 Oct.	54.2	53.2	50.6	48.6	48.8	50.0	53.0	48.6
10 25 Nov.	64.8	65.4	61.6	60.8	55.6	57.6	59.4	54.6
15 30 Dec.	76.4	80.8	70.6	69.2	60.6	65.6	67.8	63.8
20 1 Feb.	89.3	92.2	83.4	82.0	73.3	78.4	79.2	73.5
Concentrate Consumption (lbs/head)	276	279	281	277	215	227	240	208
Concentrate Conversion Ratio (lbs/lb L.W.G.)	6.6	6.4	7.7	7.8	8.4	7.6	7.7	8.2

The performance of the lambs in Group 8 (barley and sugar beet only) was poor. Where this was supplemented with urea (Group 5) to increase the crude protein level from 9.5 to 11.2% growth was not improved. However, both groups were very irregular in that some individuals grew well whilst others gained weight only slowly. In Group 6 where a protein level of 11.4% was achieved by supplementation with groundnut and soya, individual growth rates were more uniform and the final mean liveweight was intermediate between Group 8 (barley and sugar beet) and Groups 3 and 4 (barley supplemented to about 13% crude protein).

The sheep at grass achieved a mean liveweight of 72.0 lbs. at slaughter. This was very comparable to the sheep in Group 8 which had unsupplemented barley.

As in the previous experiment (No. 11(a)) sheep fed maize based diets had much superior food conversion ratios (about 6.5) to those fed barley at comparable protein intakes (about 7.8). Where no protein supplement was given (Group 8) the food conversion ratio increased to 8.2.

Blood and Liver Copper Concentrations.

Table 75 details the individual and mean concentrations of copper in the blood at slaughter and livers of the separate groups of sheep together with the liver dry weights and total liver copper contents. The mean values together with the respective mean total dietary copper intakes and the percentage/

Table 75 — Mean and individual blood copper concentrations (p.p.m.) liver dry weights (g) liver copper concentrations (p.p.m.) and total liver copper contents (mg) of 10 groups of sheep.

roup.	1				2				3				4				7			
	Blood		Liver			Blood		Liver			Blood		Liver			Blood		Liver			Blood		Liver	
.	Cu. Conc.	Dry Wt.	Cu. Conc.	Total Cu.	No.	Cu. Conc.	Dry Wt.	Cu. Conc.	Total Cu.	No.	Cu. Conc.	Dry Wt.	Cu. Conc.	Total Cu.	No.	Cu. Conc.	Dry Wt.	Cu. Conc.	Total Cu.	No.	Cu. Conc.	Dry Wt.	Cu. Conc.	Total Cu.
	0.86	157	483.8	76.0	71	1.20	285	564.3	160.8	8	0.76	131	453.2	59.4	77	1.01	178	804.1	138.3	3	0.87	170	714.6	121.5
	0.72	150	423.2	63.5	72	1.04	171	487.4	83.3	48	0.85	164	413.9	67.9	82	0.90	161	350.1	56.4	7	0.82	171	392.6	67.1
	0.85	181	666.4	120.6	74	1.10	157	497.2	78.0	55	0.85	178	641.7	114.2	89	0.60	148	655.2	97.0	12	0.90	185	410.6	76.0
	1.00	170	357.5	60.8	80	0.98	150	449.8	67.5	67	0.76	180	499.6	89.9	98	1.16	155	474.8	73.6	22	1.00	222	264.6	58.7
	1.01	126	710.6	89.5	86	0.90	236	469.3	110.8	69	0.83	231	849.7	196.3	105	0.89	182	404.3	73.5	33	1.10	151	519.6	78.5
	0.80	156	530.9	82.8	96	0.86	154	407.2	62.7	79	0.80	170	490.0	83.3	106	0.80	169	450.1	75.9	36	0.85	163	692.7	112.9
	0.99	173	346.7	60.0	97	0.86	142	839.6	119.2	101	0.89	157	713.8	112.1	117	0.89	178	408.8	72.8	47	0.75	181	210.5	38.1
	1.24	218	386.8	84.3	103	0.90	219	622.9	136.4	110	1.14	140	319.2	44.7	118	0.80	135	645.3	87.1	50	0.85	128	225.6	28.9
	1.04	143	389.9	55.8	104	0.85	118	345.4	40.8	124	0.85	155	383.0	59.4	119	0.89	149	470.3	70.1	57	0.88	191	413.9	79.1
	0.98	173	519.1	89.8	122	0.77	167	829.8	138.6	129	0.65	164	586.4	96.2	121	0.86	134	713.5	95.6	60	0.96	170	192.1	32.7
1	0.95	165	481.5	78.3		0.95	180	551.3	99.8		0.84	167	535.1	92.3		0.88	158	537.7	84.0		0.90	171	403.7	69.4
roup	5				6				8				9			Died Initially.								
	0.79	219	328.4	71.9	1	1.05	150	228.8	34.3	4	0.89	171	380.5	65.1	18	0.77	168	14.2	2.4	10		70	56.8	4.0
	0.94	142	339.2	48.2	5	0.76	283	320.4	90.7	13	1.09	136	280.2	38.1	88	0.56	184	18.4	3.4	16		92	70.9	6.5
	0.87	169	295.5	49.9	24	1.04	133	363.7	48.4	19	1.05	194	309.2	60.0	94	0.66	127	19.8	2.5	17		127	22.7	2.9
	1.25	177	417.5	73.9	28	0.86	145	220.4	32.0	30	1.08	165	278.5	46.0	100	0.70	153	12.7	1.9	32		95	81.1	7.7
	1.11	152	501.7	76.3	31	0.95	169	222.3	37.6	38	0.91	148	197.6	29.2	107	0.67	134	24.2	3.2	37		109	62.1	6.8
	0.75	194	218.3	42.4	45	0.95	141	240.6	33.9	41	0.97	162	395.4	64.1	120	0.75	146	52.1	7.6	44		103	33.4	3.4
	1.16	150	265.1	39.8	46	0.86	154	245.9	37.9	42	0.95	174	318.7	55.5	130	0.76	113	15.0	1.7	114		83	94.0	7.8
	1.03	127	354.8	45.1	49	0.86	190	414.1	78.7	43	0.95	163	152.3	24.8										
					84	0.75	161	227.3	36.6															
1	0.99	166	340.1	55.9		0.90	170	275.9	47.8		0.99	164	289.1	47.9		0.70	146	22.3	3.2			97	60.1	5.6

percentage of the dietary copper which was stored in the liver (by comparison with the mean value for those which died initially) are further summarised in Table 76.

The mean blood copper concentration (0.70 p.p.m.) of the sheep at grass was much lower than those of the 8 groups of housed sheep. These differences were very highly significant at $P<0.001$ for all the housed groups except Group 3 when it was significant at $P<0.02$. The mean blood copper concentrations of the housed sheep ranged from 0.84 p.p.m. for Group 3 to 0.99 p.p.m. for Groups 5 and 8. The mean blood copper concentration of Group 8 (barley only) was significantly ($P<0.01$) higher than that of Group 3 (barley, $\frac{1}{2}$ veg. $\frac{1}{2}$ urea), which oddly enough had the highest dietary concentration of copper. There were no other significant differences in mean blood copper concentrations among the 8 groups of housed sheep.

The mean liver copper concentrations of all eight groups of housed sheep were highly significantly ($P<0.0001$) greater than the mean liver copper concentrations of the group at grass and of those sheep which died initially. The mean liver copper concentrations of Groups 1 - 4 which were fed either maize or barley with a protein pellet based on either soya, groundnut and urea or lucerne and urea and which had a copper concentration in their diet ranging from 10.4 - 13.4 p.p.m. were all significantly higher than those of Groups 5, 6 and 8 which were fed barley based diets with copper concentrations ranging from/

Table 76. Mean liver dry weights, liver copper concentrations, total liver copper contents and the proportion of dietary copper stored in the liver by 10 groups of sheep.

Group	Mean total Cu intake (mg)	Liver Dry Wt(g)	Liver Cu Conc.(p.p.m.)	Liver Total Cu (mg)	% Cu Intake Stored
1.(Maize, ½ Veg, ½ Urea)	1316	165	481.5	78.3	5.52
2.(Maize, Lucerne Urea)	1318	180	551.3	99.8	7.15
3.(Barley, ½ Veg, ½ Urea)	1709	167	535.1	92.3	5.07
4.(Barley, Lucerne, Urea)	1673	158	537.7	84.0	4.69
5.(Barley, ½ Urea)	800	166	340.1	55.9	6.29
6.(Barley, ½ Veg)	897	170	275.9	47.8	4.70
7.(Barley, Sugar,Beet, Fishmeal)	1079	171	403.7	69.4	5.91
8.(Barley only)	651	164	289.1	47.8	6.50
9.(at grass)	-	146	22.3	3.2	-
10.(Died initially)	-	97	60.1	5.6	-

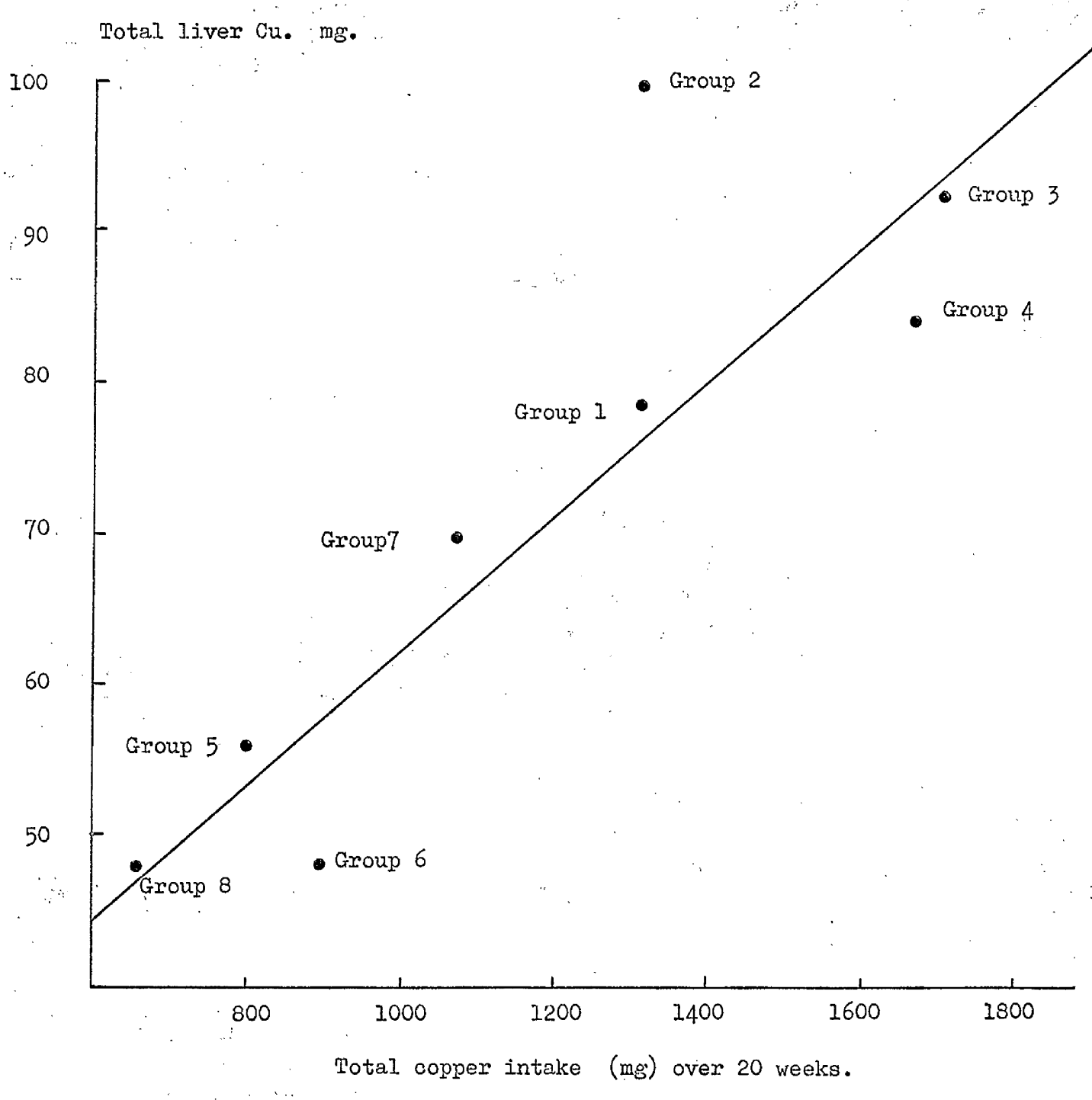
from 6.9 - 8.7 p.p.m. This difference in liver copper concentration was purely a reflection of the difference in the copper concentration of the diets. Group 7 which was fed a diet of barley, sugar beet pulp and fishmeal with a copper concentration of 9.9 p.p.m. had a mean liver copper concentration (403.7 p.p.m.) which was neither significantly higher than those of Groups 5, 6 and 8 nor significantly less than those of Groups 1 - 4.

The mean liver copper concentration of the group at grass (22.3 p.p.m.) was significantly ($P<0.01$) less than that of the mean of those sheep which died initially (60.1 p.p.m.) This was possibly due as much to a growth in liver size as to an actual depletion of liver copper stores. However, it does indicate that there was insufficient copper in the grass and turnips to maintain the initial liver copper concentration.

Fig. 23 shows the relationship between the total intake of copper by the eight groups of housed sheep and their respective total liver-copper contents. The mean total liver copper contents of all the housed groups were significantly ($P < 0.001$) greater than those of the group at grass and of the group killed initially. As for the liver-copper concentration the mean total liver copper contents of Groups 1 - 4 were all significantly ($P<0.02$) greater than those of Groups 5, 6 and 8 but not significantly greater than that of Group 7 which in turn was not significantly higher then the mean liver copper contents of Groups 5, 6 and 8.

The/

Fig 23. Relationship between the total copper intake over 20 weeks and the total amount of copper in the liver of sheep.



The mean total liver-copper content of the group at grass (3.24 mg.) was not quite significantly less than that of the group which died initially (5.59 mg.) despite the fact that there was a significant difference in their liver-copper concentrations. However, the difference (2.35 mg.) was almost significant as the least difference required for significance at P = 0.05 was 2.40 mg.

Proportion of Dietary Copper Stored in the Liver.

The percentages of the dietary copper which were stored in the livers of the eight groups of housed sheep are detailed in Table 76. The mean proportion of the dietary copper which was found in the liver additional to the mean of the group which died initially ranged from 4.70% for Group 6 to 7.15% for Group 2.

Group 2 which was fed a ration of maize, lucerne and urea with a copper concentration of 10.4 p.p.m. stored a higher proportion of its dietary copper intake than any of the other seven groups. This can be seen from Fig. 23 which shows a more or less linear relationship between total copper intake and total liver-copper content for all groups other than Group 2. Group 2 has in fact stored about 25 mg. more copper than would have been anticipated had it shown a similar trend to that exhibited by the other seven groups.

Although the copper concentrations of the diets in this present experiment (6.9 - 13.4 p.p.m.) were very much higher than those (2.8 - 6.0 p.p.m.) in the similar experiment (No. 11 (a)) carried out during the preceding winter more than 70 per cent of the housed sheep still had liver-copper concentrations

below/

below 500 p.p.m. Only eight sheep (out of 75) had liver-copper concentrations over 700 p.p.m. the highest of these being 849.7 p.p.m. for sheep No. 69 in Group 3. These concentrations are approaching the tentative danger level of over 1000 p.p.m. Cu but none of the sheep exhibited any symptoms of chronic copper toxicity. It can be assumed, therefore, that although dietary copper concentrations of up to 13.4 p.p.m. will markedly increase liver copper concentrations they are unlikely to elevate them to potentially dangerous levels within a period of 5 - 6 months which is the maximum length of time during which such stock would be housed.

Discussion.

The results of Experiments 11(a) and 11(b) appeared to indicate that the sheep which were fed diets composed largely of maize stored a greater proportion of the dietary copper than those which were fed on barley-based diets. Over the two experiments the mean amount of the dietary copper which was stored by the maize fed sheep was 5.40%. This compares with a mean storage rate of 4.26% for the barley fed sheep. Although this difference in the amount of dietary copper stored by those two groups of sheep was not significant it does reflect a constant trend.

Fig. 24 shows the relationship between the dietary copper concentration and the total amounts of copper in the livers of the sheep on the maize and barley-based diets. This substantiates the difference in the ability of the sheep on the maize and barley diets to store the available copper as it clearly shows that at any given dietary copper concentration the amount of copper stored in the livers of maize fed sheep is always greater than that stored by sheep on a barley ration.

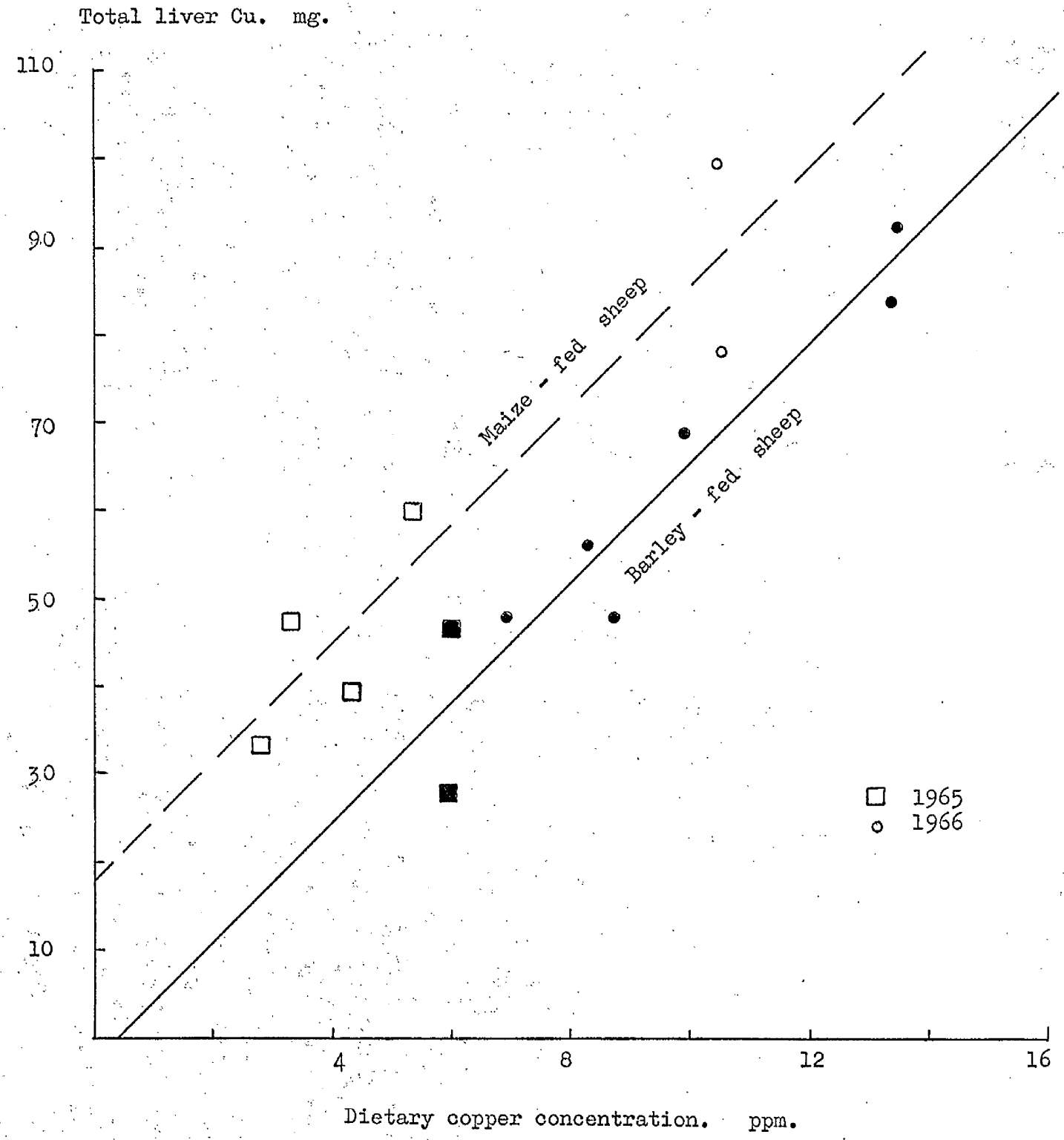
Regression coefficients were calculated for increasing total liver copper content with increasing dietary copper concentration for both the maize and barley fed sheep. These were both found to be highly significant ($P<0.01$) thus indicating that there was a direct relationship between total liver-copper content and dietary copper concentration irrespective of the nature of the diet. The regression equations were:

Maize Fed Sheep

$$X = 17.68 + 6.779\ y \quad (t = 5.30)$$

(X = total liver Cu (mg.); y = dietary copper concentration (p.p.m.)

Fig 24. Relationship between the dietary copper concentration and the total amount of copper in the liver of maize and barley-fed sheep.



Barley Fed Sheep

$$X = -2.66 + 6.819y \quad (t = 7.46)$$

Values for y were substituted in these equations and regression lines have been superimposed on Fig. 24.

Assessment of the risk of chronic copper toxicity developing in housed sheep.

During the course of eight experiments described in this section a total of 340 sheep had been housed and fed rations ranging in copper concentration from 2.8 to 21.0 p.p.m. for periods of 16 - 20 weeks. Table 77 presents the range in copper concentration of the rations, the total number of housed sheep in each experiment and the distribution of the liver-copper concentrations of the sheep.

Thus out of 340 sheep which had been housed for periods of up to 7 months 271 or 79.7% had liver copper concentrations below 500 p.p.m. A further 55 sheep (16.2%) had liver copper concentrations in the range 500 - 1000 p.p.m. and only 14 sheep (4.1%) had copper concentrations in the liver of over 1000 p.p.m.

In only one experiment (No. 9 (A)) did the majority of the housed sheep have liver copper concentrations of over 500 p.p.m. and in this same experiment one third of the housed sheep had liver copper concentrations of over 1000 p.p.m. The sheep in Experiment 9 (a) were much younger than those in the other experiments described in this section and they were introduced to the concentrate/

Table 77. Distribution of liver-copper concentration in housed sheep.

Expt. No.	Range of Cu Conc. (p.p.m.)	Total No. of sheep.	Liver Cu Conc. (p.p.m.)						
			<500	500-600	600-700	700-800	800-900	900-100	>1000
7	3.3-13.4	32	29	2	-	1	-	-	-
8(a)	5.0-8.5	42	42	-	-	-	-	-	-
8(b)	8.0-15.0	38	38	-	-	-	-	-	-
9(a)	9.9-21.0	43	1	3	3	5	8	9	14
9(b)	6.8-11.2	55	53	2	-	-	-	-	-
10	10.5-15.0	25	23	1	1	-	-	-	-
11(a)	2.8-6.0	30	30	-	-	-	-	-	-
11(b)	6.9-13.4	75	55	6	6	4	4	-	-
		340	271	14	10	10	12	9	14

concentrate rations almost from birth. It seems probable that young lambs of this age have an enhanced ability to store copper in their livers and will, therefore, be more susceptible to copper poisoning. Apart from this effect of age the protein content of the ration also influences the proportion of the dietary copper which is stored in the liver in that the higher the protein content the smaller is the proportion of the copper intake stored and also the less susceptible are the sheep to chronic copper poisoning (See Experiments 7 and 9(a)).

It was decided to investigate the relationship between the total daily intake of copper over an 18 week period and the liver-copper concentration of the housed sheep at slaughter. This was undertaken in order to determine the length of time it would take to attain liver-copper concentrations of over 1000 p.p.m. on any given intake of copper. The sheep in Experiment 9(a) have been excluded from this calculation as they were much younger than those in the other experiments and also because they stored a much larger proportion of their dietary copper and were as a consequence atypical. Table 78 details the copper concentrations in the diets and total daily copper intakes of the housed sheep in Experiments 7, 8a, 8b, 9b, 11a and 11b. It also presents their liver-copper concentrations and total copper contents.

Table/

Table 78. Dietary copper intake and liver copper concentrations of housed sheep.

Expt.No.	Group	No.sheep/ group	Dietary Cu.Conc.	Daily Cu. Intake	Liver Cu.Conc.	Total Liver Cu.Content
7	1	8	5.8	3.3	69.9	6.9
	2	8	5.9	3.4	106.6	11.9
	3	8	17.6	13.3	347.6	35.4
	4	8	17.6	13.4	330.2	41.1
8(a)	1	11	6.4	5.4	130.7	18.2
	2	11	5.0	3.5	145.8	13.5
	3	10	8.5	7.8	174.1	23.9
	4	10	8.0	6.9	80.2	10.2
8(b)	1	12	8.0	8.3	125.7	18.0
	2	14	12.1	12.0	208.3	34.7
	3	12	15.0	18.0	240.7	47.4
9(b)	1	13	6.8	6.0	230.6	37.5
	2	15	9.6	8.5	286.2	51.4
	3	15	8.3	7.4	260.4	46.1
	4	12	11.2	9.9	310.5	62.4
11(a)	1	5	5.9	5.0	174.5	28.0
	2	5	4.3	3.7	205.4	39.8
	3	5	6.0	6.4	262.1	46.6
	4	5	5.3	5.3	325.2	60.0
	5	5	3.3	3.0	250.2	47.6
	6	5	2.8	3.3	169.2	33.6
11(a)	1	5	16.5	13.3	398.4	63.3
	2	5	17.2	12.0	585.1	114.2
	3	5	14.9	14.7	494.8	89.6
	4	5	14.9	13.7	516.2	94.5
	5	5	15.1	11.3	497.4	89.0
	6	5	11.7	11.6	499.0	115.6
11(b)	1	10	10.5	9.4	481.5	78.3
	2	10	10.4	9.4	551.3	99.8
	3	10	13.4	12.2	535.1	92.3
	4	10	13.3	12.0	537.7	84.0
	5	8	8.2	5.7	340.1	55.9
	6	9	8.7	6.4	275.9	47.8
	7	10	9.9	7.7	403.7	69.4
	8	8	6.9	4.7	289.1	47.8

A regression equation was calculated for the increase in liver-copper concentration related to the daily intake of copper. This was found to be highly significant ($P < 0.001$) thus indicating that the liver-copper concentration was dependent on the copper intake of the individual sheep. Fig. 25 illustrates the relationship between the daily copper intake and the liver-copper concentration. The regression equation was:

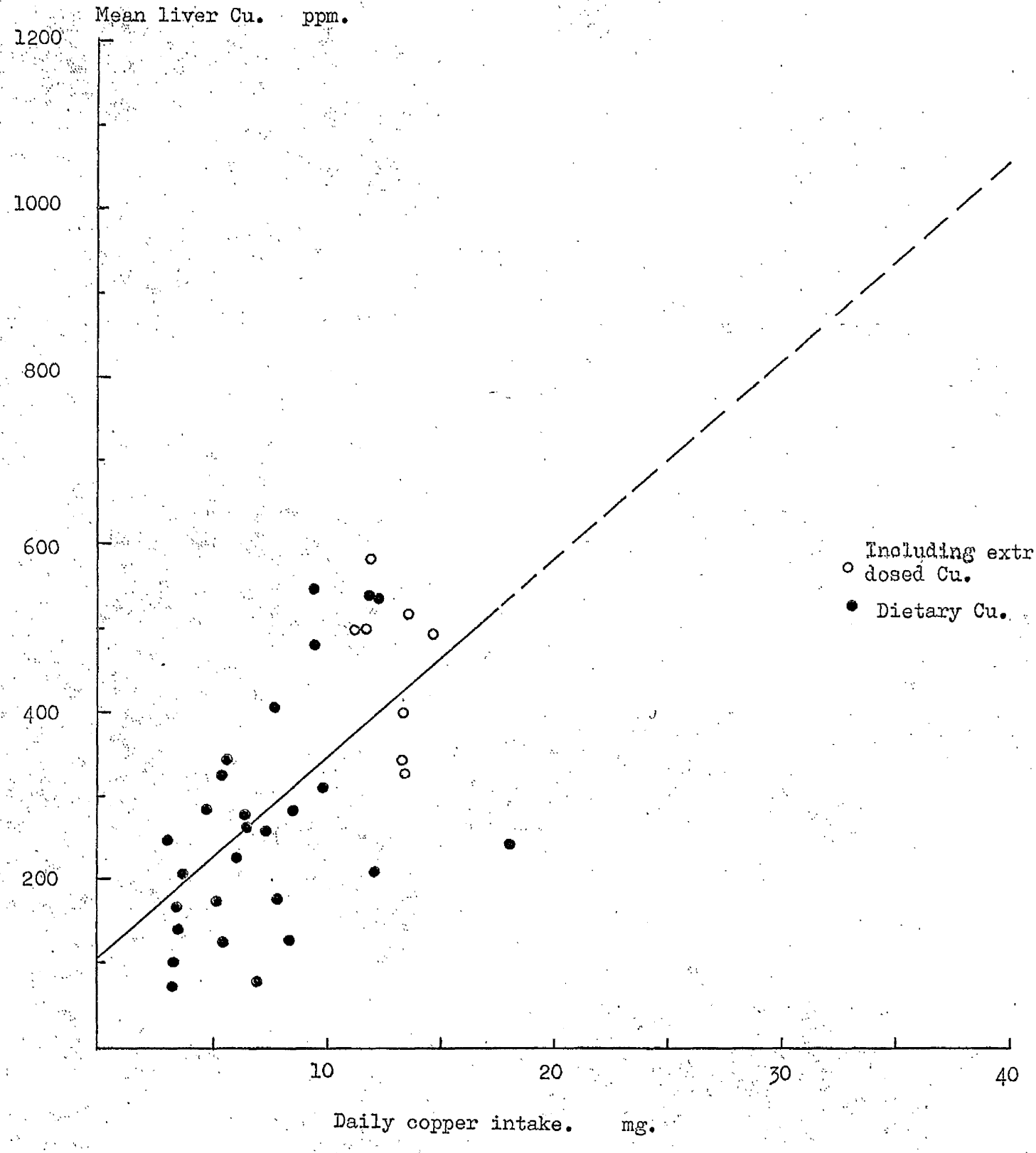
$$X = 109.34 + 23.54\ y \qquad (t = 4.476)$$

Where X = liver copper concentration (p.p.m.); y = mean total daily copper intake (mg.) over an 18 week period.

By substituting values for y in the regression equation a regression line has been superimposed on Fig. 25. The intercept on the y axis (109 p.p.m.) reflects the initial liver copper concentration of the sheep before the commencement of feeding of the experimental rations. This compares very well with the value of 109.6 p.p.m. for the mean of 27 sheep which were killed or died at the commencement of Experiments 7, 9(b), 11 (a) and 11 (b). Extrapolation of this regression line (as illustrated in Fig. 25) indicates that liver-copper concentrations of over 1000 p.p.m. would be attained only by feeding a diet containing at least 37 mg.Cu/day for this period of 18 weeks. Conversely liver-copper concentrations of over 1000 p.p.m. would be reached by feeding a diet providing a daily intake of 16.7 mg. Cu for a period of 36 weeks.

However/

Fig 25. Relationship between the daily copper intake of housed sheep and their mean liver copper concentrations.



However, the fact that the liver-copper concentration of a sheep exceeds 1000 p.p.m. does not mean that it will automatically die of copper poisoning within a short period. A review of the literature on chronic copper poisoning in sheep reveals that only very few sheep die of copper toxicity having liver-copper concentrations which are just slightly in excess of 1000 p.p.m. More frequently values exceeding 1500 p.p.m. have been recorded with occasionally much higher concentrations of from 6000 - 8000 p.p.m. Table 79 summarises the liver-copper concentrations of sheep which died from copper poisoning both during the present work and also those recorded by other workers.

The range in liver-copper concentration found for the sheep which died from copper poisoning during the present work was from 1224 - 6530 p.p.m. This compared with a range of 1000 - 8140 p.p.m. recorded by other workers. Of all the sheep which died of copper poisoning 79.2% had liver copper concentrations in excess of 1500 p.p.m.

In Experiment 9(a) fourteen sheep had liver copper concentrations ranging from 1092 to 2185 p.p.m. None of these, however, exhibited any of the symptoms of chronic copper toxicity and all had acceptable carcases at slaughter. Liver copper levels of this magnitude, although potentially toxic, can, therefore, evidently occur without manifest signs of toxicity. Ten of these fourteen sheep in Experiment 9(a) had liver copper concentrations below 1500 p.p.m.

Despite/

Table 79.

Liver copper concentrations (p.p.m.) of sheep which died from copper poisoning.

Present Work.		Other Workers.		
6530	1078) (Muth, 1952)	1300 - >2600	(6)	(Gracey & Todd, 1960).
5300	1340)	2866	(2)	(Barden & Robertson, 1962)
3750	1900 (Clegg, 1956).	1100 - 1500	(2)	
8674	1400	1500 - 2250	(7)	
4718	1400 (Pearson, 1956)	2250 - 3000	(2)	(Todd et al., 1962).
4259	1900	>3000	(2)	
4689	1700	3300		
3930	1100	1376		
2370	1400 (Bracewell, 1958)	1420		(Guilbride, 1963)
1558	1600	2028		
1924	2100	1144		
3313	1600	1678		
2434	2500	1362		
2425	2160 (Sutter et al., 1958).	2055		
1224	1580	1400		(Ross, 1964).
2965	1985	2020		
3105	1100	8140		(Howard, 1965).
6323	1000 (Berwyn-Jones, 1960)	1670		(Hill & Williams, 1965).
2480	2100			
3288	1600			

Despite the high liver copper concentrations found in Experiment 9(a) it is unlikely that any serious problem would be encountered from chronic copper poisoning in this type of young stock as they are normally fed these intensive rations for only 10 - 12 weeks prior to being slaughtered. Somewhat older lambs which take longer (4 - 6 months) to fatten appear to be less liable to build up toxic amounts of copper in the liver as they store only about 3% of their dietary copper intake. Chronic copper poisoning is unlikely to be a serious problem with this type of stock as long as they are fed diets with normal copper concentrations. In the experiments described in this section only 7.9% of this type of sheep had more than 500 p.p.m. Cu in the liver and none of these exceeded 900 p.p.m. Cu.

Pregnant ewes may be housed for some 6 weeks before lambing and for a further 4 weeks after parturition. Copper stores in the liver are unlikely to approach toxic levels during this period and any stores that are built up will be depleted when the ewes are turned out to grass (See Experiment 4). A large proportion of the diet fed to housed ewes may consist of hay or silage which normally have copper concentrations in the range 5 - 10 p.p.m. This level of dietary copper would not lend itself to the build up of elevated levels in the liver and thus the risk of chronic copper poisoning on such a regime would be minimal.

Ewe/

Ewe hoggs which are being overwintered indoors may be housed for periods of up to six months. They are, however, fed only the minimum amount of food that is consistent with keeping them in good health and giving a liveweight increase of around 2 lbs. per month. The foods fed (e.g. hay and oats with a little protein concentrates) are generally low in copper perhaps ranging from 3 - 8 p.p.m. Thus the development of chronic copper toxicity in sheep under this type of husbandry is highly improbable for two reasons; firstly because the dietary copper concentration is low and secondly because of the small total intake of food. The results of Experiment 7 indicated that there might even be a depletion in liver copper content in such sheep when fed on a diet of hay and oats.

The results of the Experiments (7 - 11 (b)) described in this section demonstrate that the only real risk of chronic copper poisoning in housed sheep arises when very young lambs are being intensively fattened on highly concentrated diets. In such circumstances the risk can be reduced by increasing the protein content of the diet and also by having the fattening period as short as possible. Other classes of sheep might have some increase in liver-copper concentration during the period of housing but these are unlikely to be maintained when they are returned to more natural regimes outdoors.

Section V

1) Copper Excretion

as affected by

a) Dietary intake of copper and copper supplementation.

b) Intravenous injection of various heavy metal chelating agents.

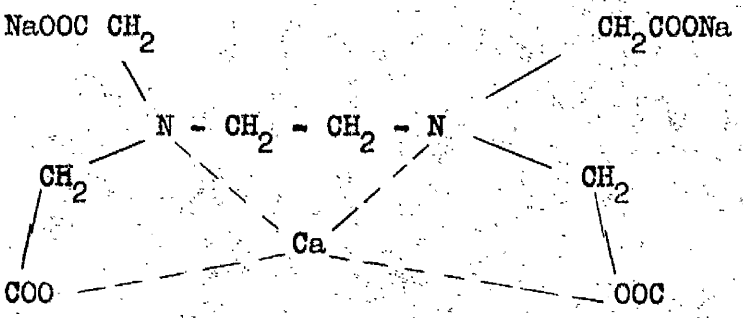
c) Chronic copper toxicity.

II) Biochemical Changes in the Blood of Sheep during the Development of Copper Toxicity.

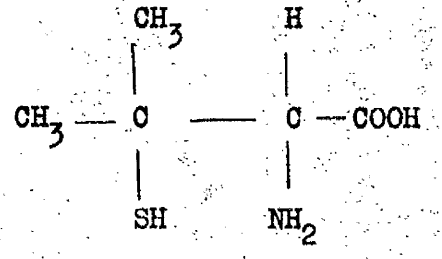
Most of the work which has been published with regard to the excretion of copper has been done in the field of human medicine particularly in connection with Wilson's Disease. Patients suffering from this disease store excessive amounts of copper in the tissues of the brain and liver. These deposit of copper in the liver cause severe hepato-lenticular degeneration. A similar deposition and accumulation of copper with hepato-lenticular degeneration can occur in the livers of sheep which are fed diets with a high copper content.

In human medicine various methods are used in the treatment of this disease. Potassium sulphide may be given orally in an attempt to reduce the absorption of copper from the gut. Low copper diets are fed to reduce the copper intake to the minimum level and the intravenous administration of various drugs which increase urinary excretion of copper is another form of therapy employed. The main drugs used are calcium disodium ethylenediamine tetra acetic acid (EDTA) and penicillamine. These have been used with a fair measure of success. The structural formulae of these two chelating agents are represented heree-

<u>EDTA</u> (Disodium salt)



<u>Penicillamine</u>



Little/

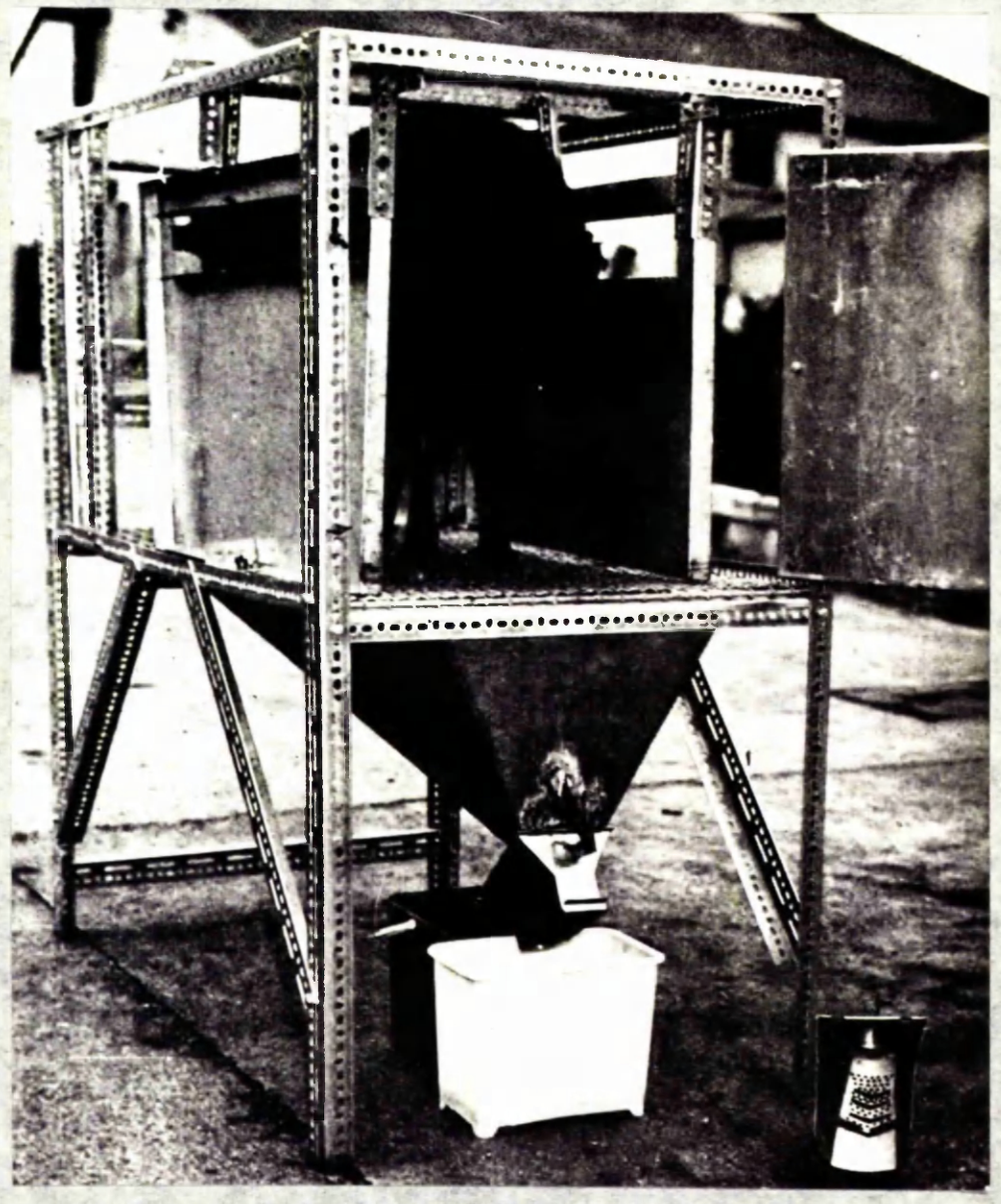
Little or no work has been published with regard to the effect of dietary copper intake on the level of copper excretion in the urine of sheep. It was felt that this should be investigated and also that the effect of intravenous injections of various drugs on urinary copper excretion should be studied. It was hoped that one of these chelating agents might prove useful in the treatment of sheep suffering from chronic copper toxicosis. This forms the basis of the work described in the present section.

However, before any experiments were conducted on urinary copper excretion it was deemed essential to discover whether accidental contamination of urine with faeces or other extraneous substances would materially alter its copper concentration. A small trial was performed to examine this and it is described here before entering into a description of the main work of this section.

The Effect of faecal contamination of urine on its copper concentration.

All the sheep used in the experiments described in this section were kept in metabolism cages for some time prior to and during the period of collection of urinary and faecal samples. One of these cages which were constructed to the design of Duthie (1959) is illustrated in Fig. 26. The urine runs through the seive part of the separator and is collected in a polythene container while the faeces does not pass through the sieve but is collected in a separate container at the forward end of the separator. The shute and separator are made/

Fig. 26. Type of metabolism cage used for sheep in Experiments 12-16.



made of stainless steel as are the mesh flooring and any other parts which may come in contant with the faeces or urine. Welded to the end of the shute is a toothed piece of stainless steel which is set to direct the urine on to the top set of holes and the first V of the separator.

Unless these separators are kept scrupulously clean and free from wool and food particles there is a tendency for the faeces to become stuck to them. Consequently when this occurs any urine excreted has to pass through faeces before it can be collected. Occasionally also a small amount of faeces may fall into the urine container. It was decided, therefore, to determine whether this type of contact with faeces would materially effect the copper concentration of urine.

To this end four sheep were put into metabolism cages and urine samples were collected. Great care was taken to ensure that no contamination of the samples occurred. Faeces samples were also collected from the same sheep. Two of the sheep were then given 5 daily doses of 1 g $CuSO_4.5H_2O$ and further collectionsof urine and faeces were made. This was done to discover if there would be any increase in contamination due to a higher copper concentration in the faeces.

Copper was determined on 1) uncontaminated urine 2) 25 ml. samples of 200 ml. of urine which had been run once over a separator containing 25 g. faeces

3)/

3) urine which had been run 4 times through 25 g. faeces and 4) urine which had been left in contact with either a small or a large amount of faeces for several hours. This level of contamination is much worse than would be normally encountered but it should serve to illustrate the seriousness of any such contamination.

The copper concentrations of uncontaminated and contaminated samples of urine from 2 sheep both prior to and after receiving 5 doses of 1 g $CuSO_4.5H_2O$ are listed in Table 80.

Table 80 Cu concentration (ug/100 ml) of urine before and after contact with faeces.

Sheep No.	Uncontaminated Sample	200 ml. Sample run 1 x over 25 g. faeces	200 ml. Sample run 4 x over 25 g faeces	Sample after long contact with faeces
30	44.5	51.0	57.0	62.0
31	64.0	71.0	75.0	65.0
After receiving 5 g $CuSO_4.5H_2O$.				
30	120.0	126.0	130.0	136.0
31	87.0	92.0	101.2	108.0

The copper concentrations of urine samples from a further 2 sheep are listed in Table 81. One of those, No. 16, had been fed on a diet of hay and concentrates with no supplementary copper whereas the other, No. 34, was fed a similar ration but had been getting 5 g $CuSO_4.5H_2O$ per week for 19 weeks. Samples were taken from both sheep on 2 successive days.

Table/

Table 81 Cu Concentration (ug/100 ml) of urine before and after contact with faeces.

Sheep No.	Uncontaminated Sample	Sample run 1 x through 30 g. faeces.	Sample after long contact with faeces (50 g. in 75 ml)
16	23.0	22.5	72.5
	25.0	26.0	41.5
34	94.0	225.0	320.0
	101.5	157.5	220.0

From the results presented in Tables 80 and 81 it is clear that any form of contact with faeces increases the copper concentration of urine. For sheep Nos. 30 and 31 the increase in copper concentration was in the order of 12 - 14% for samples which had been run once through 25 g. faeces. However, when their urinary copper concentration was increased after having received five 1 g. doses of $CuSO_4.5H_2O$ the percentage increase in concentration due to faecal contamination was in the order of only 5 - 6%. When the urines from these two sheep were run four times through 25 g. faeces the copper concentration was in all cases higher than when it had been run through only once. The increase in concentration was in the range 3 - 11%.

For three of the four sheep the greatest amount of contamination occurred when the urine was left in contact with faeces for several hours. The increases in urinary copper concentration, compared with the uncontaminated samples, ranged/

ranged from 13 - 39%. There was no increase whatsoever in the urinary copper concentration of sheep No. 31 after standing for 6 hours in contact with 2 g. faeces.

There was little appreciable increase (1%) in the urinary copper concentration of sheep No. 16 after 75 ml. had been run once through 30 g. faeces. There was, however, a marked increase (up to 200%) when the urine (75 ml) was left for several hours almost saturated with faeces (50 g). On the other hand there were large increases (50 and 100%) in the urinary copper concentration of sheep No. 34 after it had run once through 30 g. faeces. When left in contact with faeces for several hours the increases in the urinary copper concentration of sheep No. 34 were in the order of 100 and 225%. The difference in the degree of contamination found between the 75 ml. samples of the urines of sheep Nos. 16 and 34 after they had been run once through 30 g. faeces can be explained by the difference between their faecal copper concentrations. The faecal copper concentration of sheep No. 16 was 11.7 p.p.m. while that of sheep No. 34 was 361 p.p.m.

Although these trials were designed to show the situation at its worst they nonetheless demonstrate the necessity of ensuring that any collections of urine, made for copper analyses, be kept free from any form of contact with faeces. This is especially true of any prolonged form of contact so great care must be taken in placing the recepticles for urine collection in such a way that no faeces is allowed to fall into them.

In/

In the light of those observations it was considered desirable to inspect the separators two or three times a day during collection of urine and faeces samples to ensure that no bits of wool, or food or faeces were caught in them which might impede the flow of urine or misdirect the faeces into the wrong receptacle.

During the course of all the experiments carried out on copper excretion a number of urine samples did become inadvertently contaminated with faeces. These were discarded and are not included in the results of the various experiments.

Experiment 12. Influence of dietary copper supplementation on the level of urinary and faecal copper excretion of housed sheep.

This experiment was designed to determine the level of copper excretion in the urine and faeces of Blackface hoggs which were being fed indoors on a diet providing comparable amounts of energy to those commonly fed to this type of stock when they are being over-wintered. The effect of 2 levels of copper supplementation (10 and 250 mg Cu/day) was studied.

Six Blackface hoggs were used in this experiment. These sheep formed part of the control and copper supplemented groups of Experiment 7 already described in Section IV. Sheep Nos. 8 and 50 which came from the control group had a daily copper intake from the basic diet (0.75 lb. hay and 0.5 lb. oats) of 3.4 mg. Sheep Nos. 24 and 32 received an additional 10 mg. of supplementary copper while sheep Nos. 41 and 42 were given 250 mg. supplementary copper.

These 6 sheep were removed from their groups on 8 December 1962 and placed in metabolism cages. They were fed rations identical to those they had been receiving before removal from their groups. Dosing with 10 or 250 mg. Cu (given as the sulphate in solution by mouth each morning) was commenced on 10 December 1962. The cages were washed down and set up for the collection of urine and faeces on the same day. The first collection was made on 11 December and further collections were carried out for another 11 days until 21 December 1962. The sheep were then removed from their cages and put back into their respective groups.

Results/

Results

Urinary Copper Excretion

The total daily urine volume, the urine copper concentration and the total urine copper excreted by the 6 sheep are detailed in Table 82.

The total daily excretion of copper by the 3 pairs of sheep is illustrated in Fig. 27. This shows that dosing with 10 or 250 mg Cu produced no immediate increase in urinary copper excretion as the output of copper in the urine of the 4 sheep receiving the supplementary copper was no higher than that of the control sheep on the day following the administration of the first dose. However, the effect of the supplementary copper was becoming evident 2 days after the commencement of dosing. By this time the total daily excretion by the 2 sheep which were receiving a daily supplement of 10 mg Cu had increased to twice the original level. This trend continued so that by 6 days after the initial dose their daily excretion of copper was 5 times the original level.

The pattern of excretion for the 2 sheep being dosed daily with 250 mg.Cu was similar although one of them, No. 42, did not show any marked increase in urinary output of copper until 3 days after the initial dose. However, by 6 days after the start of dosing both sheep had a daily copper output in the urine which was 10 times that found on the first day after the initial dose.

Over the 11 day collection period the mean total urinary excretion of copper/

Table 82 (a) Total daily volume of urine (ml.) excreted by 6 sheep.

Amount of Supplementary Cu given daily (mg.)	No.	11/12	12/12	13/12	14/12	15/12	Mean of 6 days 16-21/12
0.0	8	210	250	250	275	270	163
	50	n.d.	n.d.	n.d.	225	350	510
10.0	24	315	250	300	275	350	270
	32	180	120	130	130	200	112
250.0	41	250	300	260	260	235	250
	42	170	90	125	70	140	105

(b) Urinary copper concentration (ug/100 ml)

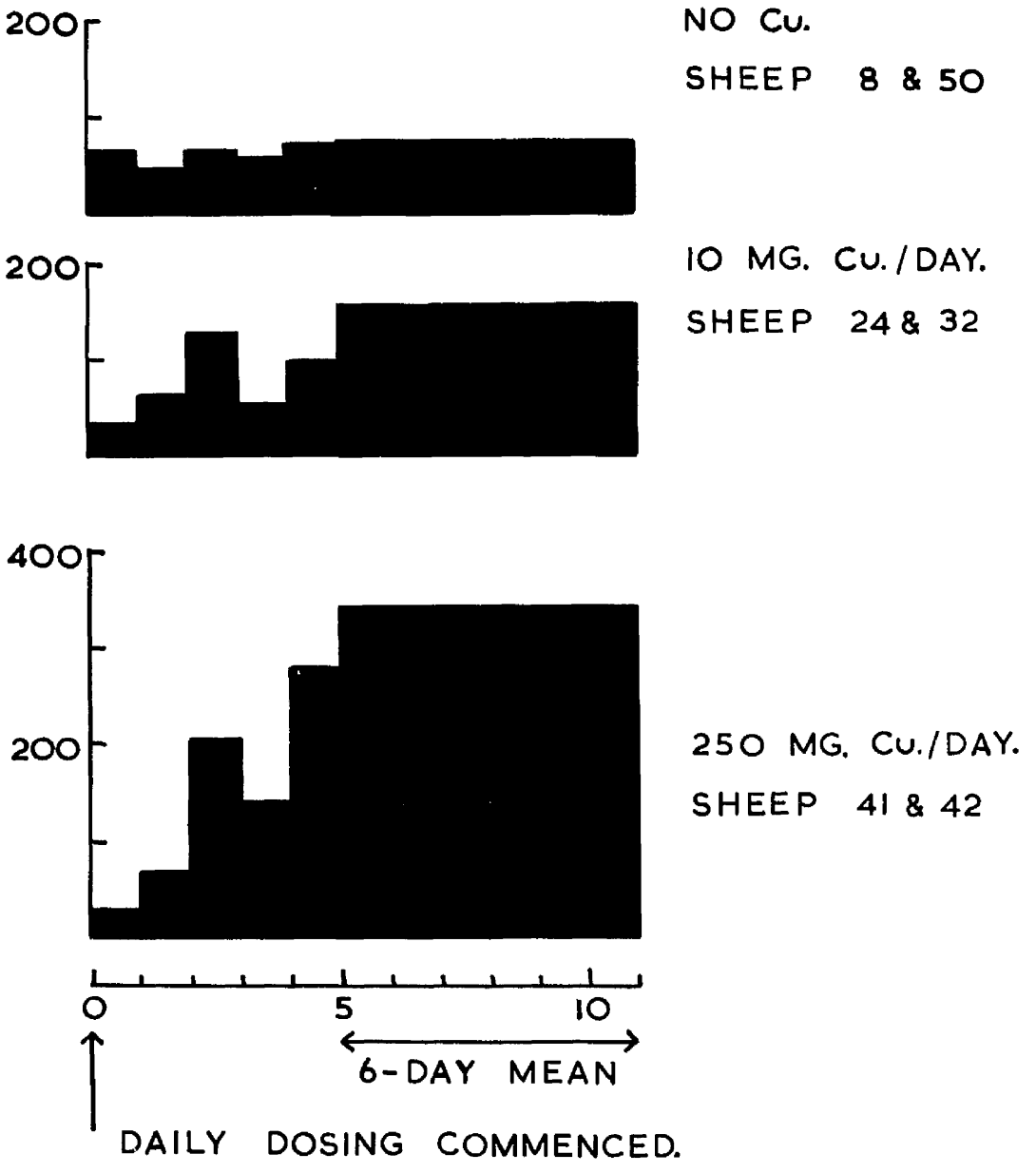
0.0	8	30.4	19.0	25.6	26.8	25.0	34.4
	50	n.d.	n.d.	n.d.	17.4	22.0	17.5
10.0	24	12.7	25.0	49.8	22.0	38.8	59.2
	32	12.8	52.8	81.0	35.0	30.8	134.4
250.0	41	14.9	33.4	94.2	86.4	170.4	146.4
	42	13.5	40.2	136.8	78.2	112.8	304.0

(c) Total Urinary copper output (ug)

0.0	8	63.8	47.5	64.0	73.7	67.5	56.1
	50	n.d.	n.d.	n.d.	39.2	77.0	89.3
10.0	24	40.0	62.5	149.4	60.5	135.8	159.8
	32	23.0	63.4	105.3	45.5	61.6	150.5
250.0	41	37.3	100.2	244.9	224.6	400.4	366.0
	42	23.0	36.2	171.0	54.7	157.9	319.2

(n.d. = not determined)

Fig. 27. Mean daily urinary copper excretion (ug) of (a) two control sheep (b) two sheep receiving 10 mg Cu/day and (c) two sheep receiving 250 mg Cu/day.



copper by the 2 control sheep was 65.3 ug. This compares with 88.1 ug Cu for the 2 sheep receiving 10 mg. supplementary copper and with 178.0 ug. Cu for the 2 sheep receiving 250 mg. supplementary copper daily.

Faecal Copper Excretion The amount of faeces dry matter excreted daily, its copper concentration and the total daily amount of copper excreted by the 6 sheep are listed in Table 83.

Table 83. Faeces dry matter (g), copper concentration (p.p.m.) and total copper output (mg) of 6 sheep.

		11 - 15 Dec.			16 - 20 Dec.		
No.	Extra Cu (mg)/day	Dry Wt.	Cu Conc.	Total Cu	Dry Wt.	Cu Conc.	Total Cu.
8	0.0	135	12.8	1.73	194	12.7	2.46
50	0.0	259	12.8	3.32	163	12.4	202
24	10.0	173	37.9	6.56	177	50.6	8.96
32	10.0	132	35.5	4.69	137	52.3	7.17
41	250.0	147	700.5	102.97	197	777.5	153.17
42	250.0	138	722.3	99.68	172	911.1	156.71

During/

During the first 5 day period the total copper excreted by the 2 sheep receiving 10 mg supplementary copper was more than double that of the 2 control sheep whilst the amount excreted by the 2 sheep receiving 250 mg. supplementary copper had increased 50 fold when compared with the 2 control sheep.

There was a further increase in the total faecal output of copper by the four supplemented sheep during the second 5 day period of collection. The two sheep getting 10 mg. supplementary copper had a mean daily excretion in the faeces (8.08 mg Cu) which was almost 4 times that of the 2 control sheep (2.24 mg.). The 2 sheep being given the 250 mg. copper supplement had increased their mean total output to 154.94 mg Cu. which was 70 times more than that of the 2 control sheep.

Experiment 13 (a). The effect of intravenous injection of EDTA on the output of copper in the urine and faeces of sheep.

This experiment was undertaken to determine the effect of intravenous injections of EDTA on the rate of excretion of copper in the urine and faeces of sheep. A study was also made of its effect on blood copper concentration and it was also hoped to correlate any increase in urinary output of copper with the total copper content of the liver.

Six old ewes were used in this experiment; Five of these were Cheviots, the other (No. 27) being a Blackface. These were placed in metabolism cages where they were left for a week to accustom them to their new surroundings. They were fed to appetite on a ration consisting of hay and concentrates. Water was always available.

After the preliminary settling in period the cages were set up for the collection of urine and faeces. The total volume of urine and the mass of faeces excreted by each sheep was measured daily. Daily collections were carried out for 5 days prior to the injection of EDTA. Control values were thus obtained for the mean total daily excretion of copper in the urine and faeces of the 6 sheep.

The 6 sheep were injected with EDTA, at a rate of 1 gm/30 lbs. liveweight. This was given as a 5% solution in normal saline, by means of a slow intravenous drip on 3 December 1962. Blood samples were taken immediately before/

before and again 2 and 5 days after injection. Urine and faeces samples were collected for a further 5 days after injection. Both in this experiment and in those succeeding it each collection of urine represents the total volume excreted in a 24 hour period e.g. from the time of injection to 24 hours post injection.

The sheep were removed from the metabolism cages on 8 December and slaughtered on 11 December 1962. The whole livers were obtained at slaughter, weighed, dried and analysed for their copper concentration. Copper was determined on all samples obtained during the experiment.

Results

Blood Copper Concentration The blood copper concentrations of the 6 sheep on 3 sampling dates are given in Table 84. The liver dry weights, copper concentrations and total copper contents are also presented here.

Table/

Table 84 Blood copper concentrations (p.p.m.), liver dry weights, liver-copper concentrations and total liver-copper contents of 6 sheep.

	Blood			Liver		
No.	3/12	5/12	8/12	Dry Wt(g).	Cu Conc (p.p.m.)	Total Cu(mg)
27	0.87	0.81	1.02	206	925	191
453	1.01	0.72	0.87	180	307	55
468	0.83	0.49	0.66	170	148	25
473	1.22	0.90	0.99	184	714	131
474	0.73	0.43	0.79	217	435	94
481	0.52	0.56	0.77	175	82	14
Mean	0.86	0.65	0.85			

The mean blood copper concentration of the 6 sheep 48 hours after injection was 0.65 p.p.m. This compares with a mean value of 0.86 p.p.m. prior to injection and one of 0.85 p.p.m. 5 days after injection.

The blood copper concentrations of 5 of the sheep 48 hours after injection had fallen from their initial concentration immediately prior to injection. On resampling 3 days later the blood copper concentrations of all 5 sheep had risen to concentrations which were similar to those of the pre-injection samples. The blood copper concentration of the sixth sheep, No. 481, was slightly higher 2 days after injection (0.56 p.p.m.) than it had been at the pre-injection sample (0.52 p.p.m.). It exhibited a further rise in concentration 5 days after injection when the level was 0.77 p.p.m.

Urinary/

Urinary Copper Excretion.

The total volume of urine excreted, the copper concentration in the urine and the total amount of copper excreted in the urine are detailed in Table 85.

The total daily output of copper in the urine of the 6 sheep is presented diagramatically in Fig. 28. For all 6 sheep the total urinary excretion of copper on the day after the injection of EDTA was substantially higher than the corresponding mean excretion rate for the 6 day pre-injection period. The mean total daily excretion of urinary copper for the last 4 days of collection (2 - 5 days after injection) was similar to the corresponding mean values for the initial 6 day period.

Three of the 6 sheep had a significantly ($P<0.05$) higher output of copper in the urine on the day following the injection of EDTA compared to their mean output for the preceeding 6 days (Table 86). These 3 sheep (Nos. 27, 473 and 474) all had fairly high liver-copper concentrations (925, 714 and 435 p.p.m. respectively) whereas the other three sheep (Nos. 453, 468 and 481) which did not have a significant increase in urinary copper output on the day after injection had normal to low liver-copper concentrations (307, 148 and 82 p.p.m. respectively)(See Table 84).

The mean copper excretion over the six day pre-injection period, the copper expretion on the day after injection, the mean copper excretion for the final 4 days of collection and the total liver-copper contents of the 6 sheep are detailed in Table 86.

Table 85. (a) Total daily volume (ml) of urine excreted by 6 sheep.

						Injected					
No.	28/11	29/11	30/11	1/12	2/12	3/12	4/12	5/12	6/12	7/12	8/12
27	900	1060	520	605	640	1000	2150	1500	1000	670	430
453	580	280	700	500	400	450	900	390	350	375	160
468	875	1200	775	1150	-	350	650	395	350	675	400
473	340	450	560	500	395	-	900	480	600	640	400
474	400	400	670	610	1310	375	460	270	370	300	120
481	1050	1100	450	575	510	600	700	600	450	685	560

(b) Urinary Copper Concentration (ug/100 ml)

27	11.6	13.6	20.2	21.0	26.4	11.4	10.2	8.5	13.2	15.3	17.2
453	14.0	11.2	16.8	16.3	7.8	9.8	11.4	14.1	16.5	14.1	21.6
468	8.1	5.8	8.9	7.0	-	12.1	15.6	11.0	12.5	11.6	7.2
473	12.6	13.0	13.8	16.1	10.8	-	25.5	15.0	11.9	15.0	13.4
474	26.6	42.0	18.0	17.4	18.6	15.3	38.1	36.6	17.0	15.3	16.4
481	7.5	6.2	7.8	12.6	18.8	6.2	13.8	9.9	10.9	11.0	14.5

(c) Total Urinary Copper Output (ug).

27	104	144	105	127	169	114	209	128	132	103	74
453	81	43	118	82	31	44	103	55	58	53	35
468	71	70	69	79	-	42	101	43	44	78	29
473	43	59	77	81	43	-	230	72	71	96	54
474	106	168	111	106	144	57	175	99	63	46	20
481	79	68	35	72	96	37	94	59	49	75	81

Fig. 28. The daily output of copper (ug) in the urine of 6 eyes prior to and following intravenous injection of E.D.T.A.

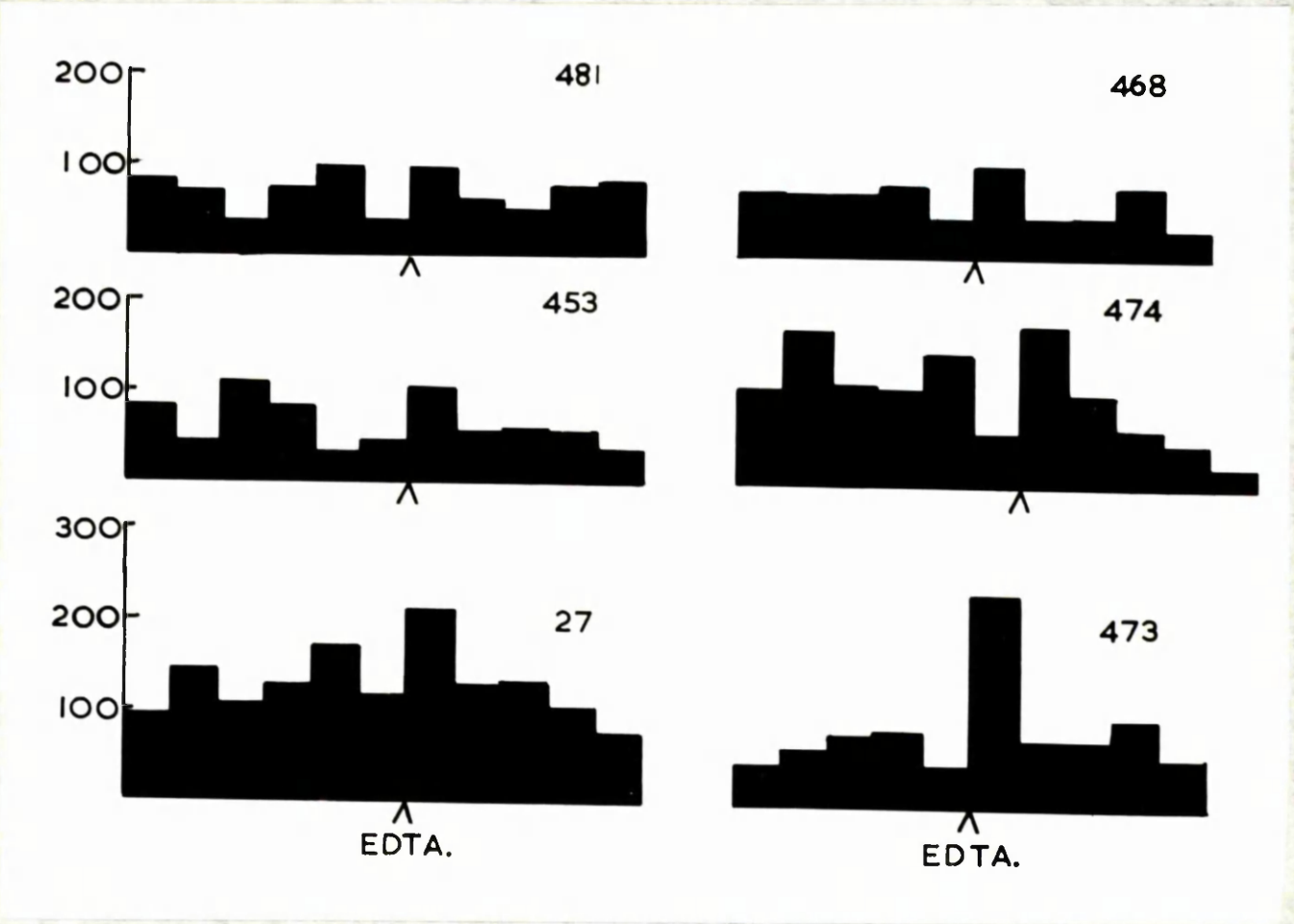


Table 86 Urinary copper excretion (ug) prior to and following injection of EDTA and the total liver-copper content (mg) of 6 sheep.

No.	Total Liver Cu Content (mg)	Mean Cu Output (ug) 6 days pre-inj.	Cu output (ug) day after injection.	Mean Cu Output (ug) 2-5 days after inj.
27	191	127.2 ± 25.4	209*	109
453	55	66.5 ± 32.9	103	50
468	25	66.2 ± 29.8	101	49
473	131	60.6 ± 29.5	230*	73
474	94	115.0 ± 34.6	175**	57
481	14	64.5 ± 24.1	94	66

*Significant increase (P 0.01) ** Significant Increase (P 0.05)

Following the reporting of Experiments 13 (b), 15(a), 15(b) and 15(c) which involved the injecting of further sheep with EDTA an attempt will be made to correlate the increase in urinary copper output on the day following injection with the total liver copper content of the sheep.

One feature of the EDTA injections was the marked increase in volume of urine excreted in the 24 hours following injection. These increases were in the order of 100% for three of the sheep and in the case of sheep No. 27 this increase in the total volume of urine excreted accounted for the significant/

significant increase in copper excretion as there was no rise in the copper concentration of the urine. In the case of the other 2 sheep (Nos. 473 and 474) which had significant increases in urinary copper output following the intravenous injection of EDTA there was also a marked rise in urinary copper concentration.

Faecal Copper Concentration.

The faeces dry matter excreted daily, its copper concentration and the total daily output of copper in the faeces by five of the sheep are detailed in Table 87. The results for sheep No. 474 had to be discounted as the faeces had been unfortunately contaminated with an extraneous source of copper.

The mean daily faecal excretion of copper by these 5 sheep for the 6 day pre-injection period, for the first 48 hours after injection and for the last 3 days of collection is given in Table 88.

Table/

Table 87 Faeces dry matter (g), copper concentration (p.p.m.) and total copper output (mg Cu) of sheep before and after EDTA injection.

(a) Faeces dry matter (g) excreted daily.

No.	28/11	29/11	30/11	1/12	2/12	3/12 ↓ Injected	4/12	5/12	6/12	7/12	8/12
27	105	111	119	92	104	126	102	26	113	117	31
453	217	172	121	146	87	90	120	163	89	159	70
468	179	192	135	85	114	8	90	125	73	181	41
473	137	100	112	85	137	-	110	87	82	79	77
481	95	137	144	53	92	104	130	107	0	122	32

(b) Faecal copper concentration (p.p.m.)

27	83.5	84.0	87.7	84.0	91.0	88.7	88.2	86.2	87.2	103.5	88.0
453	31.7	36.0	59.8	63.9	58.6	64.8	60.9	66.5	63.1	71.5	68.8
468	36.7	42.7	81.5	70.0	70.4	69.9	64.7	95.9	101.5	81.6	85.6
473	50.0	62.4	61.9	77.4	65.0	-	82.3	115.1	92.5	84.0	89.4
481	42.0	47.0	74.6	49.9	85.6	84.4	82.5	99.2	-	104.4	102.4

(c) Total Faecal Copper Output (mg).

27	8.76	9.32	10.44	7.73	9.46	11.18	9.00	2.24	9.85	12.11	2.73
453	6.88	6.19	7.24	9.33	5.10	5.83	7.31	10.84	5.62	11.37	4.82
468	6.57	8.20	11.05	5.95	8.03	0.56	5.82	11.99	7.41	14.77	3.51
473	6.85	6.24	6.93	6.58	8.91	-	9.05	10.01	7.59	6.64	6.88
481	3.99	6.44	10.74	2.64	7.88	8.78	10.73	10.61	-	12.74	3.28

Table 88 Mean daily excretion of copper (mg) in the faeces of sheep before and after intravenous injections of EDTA.

No.	6 days pre-injection	0 - 48 hours after injection	48 - 120 hours after injection
27	9.48	5.62	8.23
453	6.76	9.08	7.27
468	6.73	8.91	8.56
473	7.10	9.53	7.04
481	6.28	10.67	5.34
Mean	7.27	8.76	7.29

Over the 6 day pre-injection period the mean daily output of copper in the faeces of these 5 sheep ranged from 6.28 to 9.48 mg. During the 48 hours immediately after injection there was an increase in the daily output of copper by 4 of the 5 sheep. The fifth sheep, No. 27, showed a decrease in the daily output o f copper during this period from 9.48 to 5.62.mg. During the last 3 day collection period its output increased to 8.23 mg/day. During this period the daily output of copper in the faeces of the other 4 sheep returned to similar levels to those found during the initial 6 day period.

Experiment 13 (b). The effect of intravenous injections of EDTA and penicillamine on the output of copper in the urine and faeces of sheep.

This experiment was designed as a further trial of the usefulness of intravenous injections of EDTA in increasing urinary copper excretion in sheep. The effect of the EDTA injections on urinary copper output was to be compared with that of injections of penicillamine given (at a different time) to the same sheep. The effect of these injections on blood copper concentration was also to be studied and it was further wished to determine whether there was any relationship between increased copper excretion in the urine following these injections and total liver-copper content.

Six Blackface hoggs were used in this experiment. These sheep formed part of the control and copper supplemented groups of Experiment 7 already described in Section IV. The 6 sheep were introduced to the metabolism cages on 8 April 1963 where they were left for 10 days before preliminary collections of urine and faeces were made. The sheep were fed the same diet as they had been receiving previously, i.e. 0.75 lb. hay and 0.5 lb. oats/blood meal mixture. Sheep Nos. 28 and 45 had been receiving the basic diet only with no supplementary copper; sheep Nos. 17 and 53 had received 5 doses of 10 mg. Cu per week while the remaining 2 sheep, Nos. 5 and 38 had received 5 doses of 250 mg Cu per week since 10 December 1962, i.e. totals of 0.90 g Cu and 22.0 g Cu respectively. No further doses of copper sulphate were given to these 4 supplemented sheep for the duration of the present experiment.

The/

The metabolism cages were washed down thoroughly and then set up for the collection of urine and faeces on 18 April 1963. The first collection was made on 19 April and daily collections were taken thereafter until the conclusion of the experiment on 28 April 1963.

2 g. EDTA was administered to each of the 6 sheep on 22 April by means of aslow intravenous drip. The EDTA was given as a 5% solution in N. Saline. The liveweight of each of the six sheep was about 60 lbs. The penicillamine injections, also given by slow intravenous drip, were administered to the 6 sheep on 25 April. Each sheep was injected with a solution made by dissolving 600 mg. D-penicillamine hydrochloride in N. saline and neutralising the resulting solution with 0.2 NKOH.

Blood samples were obtained from the sheep immediately prior to both injections and again on the day after injection. The whole livers were obtained from all 6 sheep at slaughter on 1 May 1963. All samples of blood, liver, urine and faeces were analysed for their copper concentrations.

Results

Blood Copper Concentrations.

The blood copper concentrations of the 6 sheep on 6 separate sampling dates are presented in Table 89. The liver dry weights, copper concentrations and total copper contents are also presented.

Table/

Table 89. Blood copper concentrations (p.p.m.), liver dry weights (g), liver-copper concentrations (p.p.m.) and total liver-copper contents (mg) of 6 sheep.

	Blood						Liver		
		EDTA injected ↓		Pen. injected ↓					
No.	19/4	22/4	23/4	25/4	26/4	1/5	Dry Wt.	Cu. Conc.	Total Copper
28	0.78	0.92	0.56	0.80	0.90	0.93	120	36.0	4.3
45	0.60	0.60	0.54	0.65	0.60	0.70	132	20.6	2.7
17	1.07	1.22	1.05	1.25	1.37	1.06	113	505.4	57.1
53	0.98	1.20	1.06	1.09	1.38	1.15	127	138.4	17.6
5	1.08	1.08	0.87	1.16	0.98	1.05	101	3313.3	334.6
38	1.64	1.80	1.45	1.41	1.36	1.77	106	2434.8	258.1
Mean	1.03	1.14	0.92	1.06	1.10	1.11			

This demonstrates that there was a marked fall in the blood copper concentrations of all 6 sheep on the day following the administration of EDTA. The decreases ranged from 0.06 p.p.m. for sheep No. 45 to 0.36 p.p.m. for sheep No. 28. After a further 2 days the blood copper concentrations of 5 of the 6 sheep had returned to their pre-injection levels. The blood copper concentration of the sixth sheep, No. 38, remained at the level to which it had fallen on the day after injection.

Only/

Only 3 of the 6 sheep exhibited a fall in blood copper concentration 24 hours after the intravenous injection of penicillamine and for 2 of these 3 sheep the decrease was very slight (0.05 p.p.m. or less). The third sheep, No. 5, had a decrease in blood copper concentration of 0.18 p.p.m. The other 3 sheep all had increased blood copper concentrations (from 0.10 to 0.29 p.p.m.) 24 hours after the penicillamine injections. By 5 days after the administration of penicillamine the blood copper concentrations of all 6 sheep had returned to similar concentrations to those found immediately before the previous injection of EDTA.

Urinary Copper Excretion.

The total volume of urine excreted, its copper concentration, and the total daily output of copper in the urine of the 6 sheep are presented in Table 90.

The total daily output of copper in the urine of the 6 sheep is presented diagramatically in Fig. 29. On the day following the administration of the EDTA injections all six sheep exhibited increases, ranging from 50% to more than 200% of the pre-treatment values, in the total amount of urinary copper excreted. The increases were in all cases highly significant ($P<0.01$). During the next 2 days the total daily excretion of copper in the urine of all 6 sheep returned to the pre-treatment levels.

There were marked increases in the total output of copper in the urine of all 6 sheep during the 24 hour period following the administration of penicillamine. The increase in output for all 6 sheep was again highly significant ($P<0.05$). During the last 2 days of collection the daily level of copper excretion returned to that existing during the pre-treatment period.

The/

Table 90 (a) The total volume (ml) of urine excreted daily by 6 sheep.

No.	19/4	20/4	21-22/4	EDTA Inj. ↓ 23/4	24/4	25/4	Pen. Inj. ↓ 26/4	27-28/4
28	480	500	650	1675	1100	750	850	715
45	210	200	228	450	425	1000	1000	413
17	1100	950	665	750	700	450	860	350
53	1300	1150	910	1140	675	700	1175	1075
5	250	200	218	450	175	235	480	250
38	500	415	505	1150	600	430	500	400

(b) Urinary copper concentration (ug/100 ml).

28	36.3	36.3	38.2	18.8	13.5	18.4	45.0	20.9
45	60.0	60.0	28.1	48.8	30.6	11.6	47.5	32.0
17	21.7	21.7	37.5	45.9	35.9	10.4	40.7	27.5
53	24.0	25.0	24.4	71.9	36.3	40.7	87.5	7.2
5	143.0	143.0	143.0	142.5	177.5	118.8	440.0	225.0
38	37.0	37.0	57.5	38.1	55.0	34.3	235.0	45.7

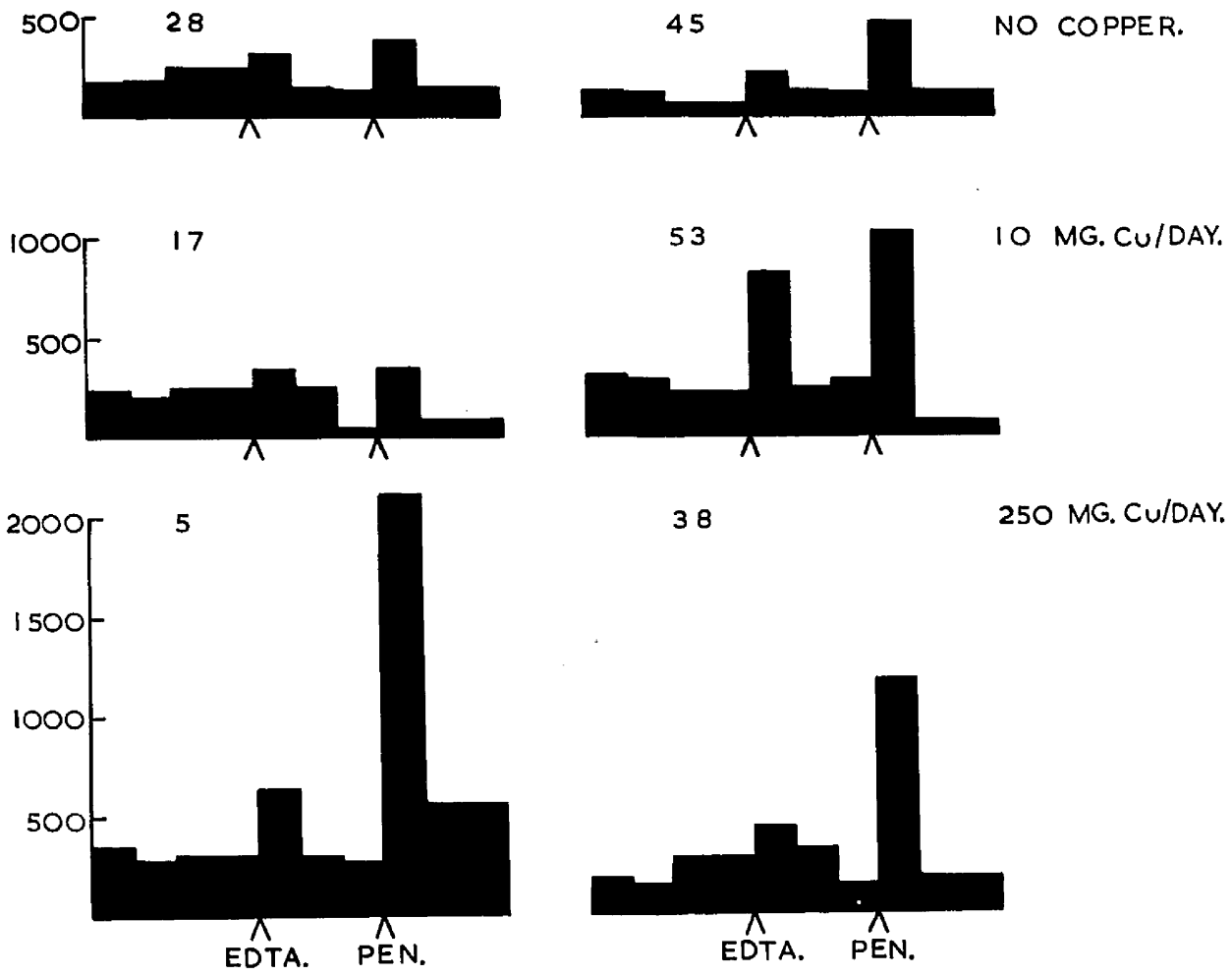
(c) Total urinary output of copper (ug).

28	174	182	248	315*	149	138	383*	149
45	126	120	64	220*	130	116	475*	132
17	239	206	249	344*	251	47	350+	96
53	312	288	222	820*	245	285	1028*	77
5	358	286	312	641*	311	279	2112*	563
38	185	154	290	438*	330	148	1175*	183

* Significant increase over mean pre-injection output ($P < 0.01$)

\+ " " " " " " " ($P < 0.05$)

Fig. 29. The daily urinary copper excretion (ug) of two control sheep, two sheep which received 10 mg Cu/day and 2 sheep which received 250 mg Cu/day prior to and following intravenous injections of E.D.T.A. and penicillamine.



The mean increase in copper output in the urine of the 6 sheep after the EDTA injections was 238 ug. This contrasts with a mean increase of 703 ug Cu following penicillamine administration. This increased output after treatment with penicillamine was found for all 6 sheep and indicates that it was much more effective in increasing the rate of excretion of copper in the urine than was EDTA at the dosage rates used in the present experiment.

The effect of the EDTA injections has largely been to increase the total volume of urine excreted without any concurrent increase in its copper concentration. Only one of the 6 sheep, No. 53, showed a marked increase in urinary copper concentration during the 24 hour period immediately following the EDTA injections. On the other hand all six sheep exhibited increases in their urinary copper concentrations following treatment with penicillamine. This increase in urinary copper concentration was accompanied by an increased volume of urine excreted in only one of the 6 sheep, No. 5.

Relationship between total liver-copper content and increased output of copper in the urine following injection.

Table 91 shows the relationship between the total liver-copper content of the 6 sheep and their increased output of urinary copper following injection with EDTA and penicillamine.

Table/

Table 91. Total liver-copper content (mg) and the urinary copper excretion of 6 sheep treated with chelating agents.

No.	Total Liver Cu	Mean Cu output 4 days pre-inj.	Increased output after EDTA inj.	Increased output after pen. inj.
28	4.3	213	102	193
45	2.7	94	126	372
17	57.1	236	108	143
53	17.6	261	559	766
5	334.6	317	324	1802
38	258.1	230	208	942

There was not such a clear relationship found between the total liver-copper content of the sheep and their increased output of copper following EDTA injection on this occasion as was found in the preceding experiment where the greatest increases in copper excretion were associated with the highest total liver-copper contents.

If the seemingly abnormally high values found for sheep No. 53 after both the injections of EDTA and penicillamine when it excreted inordinately large amounts of copper in relation to its total liver-copper content are excluded then the 2 sheep (Nos. 5 and 38) with the highest total liver-copper contents had by far the greatest increases in the amount of copper excreted in the urine/

urine. Sheep No. 17, however, which had a total liver copper content of 57 mg. did not have a greater increase in urinary copper output than sheep No. 28 which had a total liver copper content of only 4.3 mg. These results will be examined in greater detail at the end of this section when an attempt will be made to establish the existence of a relationship between the increased amounts of urinary copper excreted following treatment with chelating agents and the total liver-copper contents of the sheep.

Faecal Copper Concentration.

The faeces dry matter excreted daily, its copper concentration and the total daily output of copper in the faeces of the 6 sheep are detailed in Table 92.

There was a slight decrease in the total amount of copper excreted in the faeces of all 6 sheep over the 2 day period following the EDTA injections. This, however, corresponded to a decrease in the total faecal dry matter excreted and did not indicate any reduction in the copper concentration of the faeces. There was a small decrease in the amount of faecal copper excreted by 3 of the sheep following the penicillamine injections but this was also related to a drop in the total amount of faeces excreted. Neither treatment exhibited any effect on the concentration of copper in the faeces or on the total amount of copper excreted.

Table 92. Faeces dry matter, copper concentration and total copper output of 6 sheep prior to and after injections of chelating agents.

(a) Faeces dry matter (g) excreted daily.

		EDTA inj. ↓		Pen. Inj. ↓		
No.	19-20/4	21-22/4	23-24/4	25/4	26/4	27-28/4
28	346	305	246	312	222	285
45	300	305	125	84	141	261
17	315	305	265	300	300	295
53	251	235	191	232	212	110
5	185	245	231	242	222	200
38	265	280	240	242	270	225

(b) Faecal copper concentration (p.p.m.)

28	17.8	12.9	13.5	13.2	12.4	10.7
45	9.9	10.3	13.7	15.1	15.9	14.2
17	14.1	10.8	11.7	14.0	14.0	15.9
53	23.3	15.2	14.4	15.3	14.2	8.4
5	12.7	13.0	12.9	15.8	12.7	18.0
38	13.5	16.7	15.6	17.3	17.5	20.0

(c) Total faecal copper output (mg).

28	6.16	3.94	3.33	4.12	2.76	3.04
45	2.97	3.13	1.71	1.27	2.24	3.70
17	4.44	3.30	3.10	4.21	4.20	4.70
53	5.84	3.56	2.75	3.55	3.00	0.92
5	2.35	3.19	3.0	3.81	2.83	3.59
38	3.57	4.68	3.74	4.18	4.73	4.50

Experiment 14. The effect of intravenous injection of sodium diethyldithiocarbamate on the output of copper in the urine and faeces of sheep.

Na diethyldithiocarbamate is a powerful chelating agent for copper (and other elements) and has been extensively used in the quantitative determination of copper. Its ability to chelate copper has prompted its use as a therapeutic agent in the treatment of Wilson's Disease where it has effected marked clinical improvement when administered intravenously. It has been reported to slightly increase urinary output of copper (Sunderman et al., 1963) in humans and to lower blood copper concentrations (Howell, 1964) in rabbits.

This present experiment and the succeeding one were designed to examine the effect of intravenous injection of varying amounts of Na diethyldithiocarbamate on copper excretion in the urine and faeces of sheep and on blood copper concentration in sheep.

Three old Blackface ewes were used in this experiment. These were placed in metabolism cages on 5 December 1963 where they were fed 1 lb. hay and 1½ lbs. of a concentrate mixture daily. The concentrate consisted of flaked maize, bran and a proprietary calf rearing concentrate. This diet provided a daily intake of 15.6 mg Cu. The 3 sheep were left for a week in the metabolism cages before collections of urine and faeces were commenced in order that they might become accustomed to their new surroundings. The first collection of urine and faeces was made on 12 December 1963. Daily collections were taken thereafter until the conclusion of the experiment on 19 December 1963.

On/

On 16 December 1963 two of the sheep were given injections of Na diethyldithiocarbamate by means of a slow intravenous drip. Sheep No. 441 was injected with 75 ml. of a 4% solution of Na diethyldithiocarbamate in an equal volume of N. saline. Sheep No. 961 was given an injection of 150 ml of the 4% solution of Na diethyldithiocarbamate in 100 ml. N. saline. These two injections were equivalent to 3 and 6 gms. Na diethyldithiocarbamate respectively. The third sheep, No. 420, acted as a control and was given an injection of 150 ml. N. saline.

Blood samples were obtained from the sheep prior to injection and then 3, 7, 24 and 72 hours after injection. All samples of blood, urine and faeces obtained were analysed for their copper concentration.

Results.

Blood Copper Concentration.

The blood copper concentrations of the 3 sheep on 5 sampling occasions are listed in Table 93. The time scale used relates to a zero time at injection.

Table 93 Blood copper concentrations (p.p.m.) of 3 sheep prior to and after intravenous injections of Na diethyldithiocarbamate.

No.	Before Injection	Hours after Injection			
		3	7	24	72
961	0.77	0.55	0.66	0.69	0.82
441	0.93	0.76	0.74	0.78	0.84
420 (control)	0.84	0.77	0.73	0.68	0.74

There/

There was a marked fall in the blood copper concentrations of the 2 ewes which had received the injections of Na diethyldithiocarbamate within 3 hours of the time of injection. By this time the blood copper concentration of No. 961 had fallen from the pre-injection level of 0.77 p.p.m. to 0.55 p.p.m. while that of No. 441 had fallen from 0.93 to 0.76 p.p.m. By 24 hours after injection the blood copper concentrations of both sheep had risen again to nearer their original levels and after another 48 hours they had returned to normal.

Sheep No. 420, which had only received an injection of 150 ml. N. saline also exhibited a fall in blood copper concentration during the first 3 hours after injection. The fall in concentration, however, was only slight (0.07 p.p.m.) and was less than half that shown by Sheep No. 441 and less than one third of that shown by sheep No. 961. However, it showed further slight decreases (0.04 and 0.05 p.p.m.) in blood copper concentration in the samples obtained 7 and 24 hours respectively after injection. By 72 hours after injection the blood copper concentration of sheep No. 420 had begun to rise towards its original level.

Urinary Copper Excretion

The total volume of urine excreted, its copper concentration and the total daily excretion of copper in the urine are detailed in Table 94.

Both sheep injected with Na diethyldithiocarbamate exhibited increases in the total urinary excretion of copper on the day following injection compared with their mean total daily copper excretion on the 5 preceding days. Sheep

No/

Table 94 (a) The total volume of urine (ml) excreted daily by 3 sheep.

	Before Injection				After Injection		
Days	4 to 3	2	1	0	1	2	3
No.	12-13/12	14/12	15/12	16/12	17/12	18/12	19/12
961	263	350	850	200	750	625	400
441	550	350	580	400	425	460	750
420 (control)	425	775	825	675	Nil	675	740

(b) Urinary copper concentration (ug./100 ml)

961	16.9	9.0	12.0	9.3	13.0	9.8	8.8
441	11.8	12.0	30.8	9.8	25.2	8.6	12.0
420 (control)	10.4	13.0	16.5	16.7	Nil	13.8	26.0

(c) Total urinary output of copper (ug)

961	44.4	31.5	102.0	18.6	97.5	61.3	35.2
441	64.9	42.0	178.6	39.2	107.1	39.6	90.0
420 (contol)	44.2	100.8	136.1	112.7	Nil	93.2	192.4

Table 95 Faeces dry matter, copper concentration and total copper content of 3 sheep before and after injection of Na diethyldithiocarbamate.

No.	Faeces dry matter(g)			Faecal Cu Conc.(p.p.m.)		
	12-13/12	14-16/12	17-19/12	12-13/12	14-16/12	17-19/12
961	70	234	235	32.6	46.7	36.4
441	177	230	192	44.2	45.3	41.4
420 (control)	125	133	133	44.0	48.6	43.5

Total Faecal Cu Content (mg).

	12-13/12	14-16/12	17 - 19/12
961	2.3	10.9	8.6
441	7.8	10.4	7.9
420 (con.)	5.5	6.5	5.8

No. 961 had an increased output of 50 ug. Cu while sheep No. 441 had an increase in output of 29 ug. Cu. Neither of those increases, however, was statistically significant.

Sheep No. 420 which had received an injection of 150 ml. N. saline excreted absolutely no urine in the 24 hour period following injection. On the next day it exhibited no increase either in urinary copper concentration or in the total amount of copper excreted.

Faecal Excretion of Copper.

The faeces dry matter, copper concentration and total amount of copper excreted by the 3 sheep are presented in Table 95.

There was a slight reduction in both faecal copper concentration and the total amount of copper excreted over the 3 day period following injection compared to the 3 day period preceding it for both sheep injected with Na diethyldithiocarbamate. This occurred for sheep No. 420 which received the control injection of N. saline. No significance can, therefore, be attached to these small changes.

Since the dosage rates of Na diethyldithiocarbamate used in the present experiment had had no significant effect upon the rate of excretion of copper in either the urine or faeces it was decided to conduct a further trial using the same sheep but on this occasion to increase the amounts of Na diethyl-dithiocarbamate administered to 5 times the original quantities.

For/

For this purpose the 3 old Blackface ewes were again introduced to the metabolism cages on 20 January 1964. Collection of urine and faeces samples was commenced on 23 January and daily collections were taken until 31 January 1964. The diet fed to the 3 sheep was precisely the same as that fed to them during the first part of the experiment in December 1963.

On 28 January 1964 sheep No. 441 (control) was given an injection of 150 ml. N. saline; sheep No. 420 was injected with a solution of 15 g. Na diethyldithiocarbamate in 150 ml. N. saline and sheep No. 961 received an injection of a solution of 30 g. Na diethyldithiocarbamate in 150 ml. N. saline.

Very shortly after receiving its injection of 30 g. Na diethyldithiocarbamate sheep No. 961 began to show signs of stress. Its respiratory rate increased rapidly and its head hung forward and rolled from side to side. By 3 hours after injection it had recovered somewhat and appeared normal apart from a rapid heartbeat. After a further 4 hours it had collapsed and was breathing very rapidly. It eventually died about 24 hours after injection. Post mortem examination showed marked pulmonary congestion and oedema. Mild epicardial petechia were also found. Sheep No. 420 which had been given 15 g. Na diethyldithiocarbamate showed similar signs of stress shortly after injection to those exhibited by sheep No. 961. Its respiratory rate increased but not so markedly as that of No. 961. By 3 hours after injection, however, it had completely recovered. Sheep No. 441 (control) showed no obvious signs of stress or discomfiture after receiving the injection of 150 ml. N. saline.

Blood/

Blood samples were again obtained before injection and (where possible) at 3, 7, 24 and 72 hours after injection. All samples of blood, urine and faeces obtained were analysed for their copper concentration.

Results.

Blood Copper Concentration

The blood copper concentrations of the 3 sheep on 5 separate sampling occasions are detailed in Table 96.

Table 96. Blood copper concentrations (p.p.m.) of 3 sheep prior to and after injection of Na diethyldithiocarbamate.

No.	Before Injection	Hours After Injection			
		3	7	24	72
961	1.12	0.88	0.97	Died	Died
420	1.12	0.93	0.82	1.07	1.01
441 (control)	1.30	1.37	1.19	1.36	1.27

As in the previous experiment the blood copper concentrations of both sheep injected with Na diethyldithiocarbamate fell sharply within 3 hours of injection. The blood copper concentration of sheep No. 961 fell from the pre-injection level of 1.12 to 0.88 p.p.m. and that of sheep No. 420 fell from 1.12 to 0.93 p.p.m. After a further 4 hours the blood copper concentration of sheep No. 961 had begun to rise again towards its original level and was at 0.97 p.p.m. In the case of sheep No. 420 the blood copper concentration continued to fall during this period so that by 7 hours after the time of injection it was at 0.82 p.p.m. No further sample was obtained from sheep No. 961 but the

blood/

blood copper concentration of sheep No. 420 had risen to its original level by 24 hours after injection. This level was maintained over the next 2 days.

Sheep No. 441 which had received an injection of 150 ml. N. saline exhibited no reduction in blood copper concentration during the first 3 hours after injection. There was in fact a slight rise in its blood copper concentration during this period from 1.30 to 1.37 p.p.m. However, by 7 hours after injection its blood copper concentration had fallen to 1.19 p.p.m. This fall in blood copper concentration was less than half that exhibited by the 2 sheep which had received the injections of Na diethyldithiocarbamate. By 24 hours after injection of N. saline the blood copper concentration of sheep No. 441 had returned to its pre-injection level at which it remained during the succeeding 2 days.

It is worth noting that at the commencement of this part of the experiment the blood copper concentrations of all 3 sheep were about 0.30 p.p.m. higher than they had been when housed initially at the beginning of December 1963. Prior to having been brought indoors for this experiment in December the sheep had been feeding on pasture where their blood copper concentrations had ranged from 0.77 to 0.93 p.p.m. After 5 weeks indoors during which time they were fed on hay and concentrates their blood copper concentrations ranged from 1.12 to 1.30 p.p.m. Eden (1944) found a similar increase in the blood copper concentration of hill lambs after they had been housed for 3 weeks.

Urinary/

Urinary Copper Concentration.

The total volume of urine excreted, its copper concentration and the total daily excretion of copper are detailed in Table 97.

The injections of Na diethyldithiocarbamate had no effect on the urinary excretion of copper in either sheep. The total output of urinary copper excreted on the day following the injections was no greater than the mean daily excretion during the preceding 6 days. Neither was there any increase in the amount of urinary copper excreted by the control sheep No. 441 on the day after it had been injected with 150 ml. N. saline compared with the mean daily excretion rate for the pre-treatment period.

Faecal Copper Excretion.

The faeces dry matter, copper concentration and total amount of copper excreted by the 3 sheep are presented in Table 98.

There was a marked decrease in the total daily excretion of copper in the faeces by sheep No. 420 over the 3 day period following injection compared to the mean daily output of copper during the 6 day pre-treatment period. This decrease was not due to a drop in the faecal copper concentration but to a drop in the total amount of faeces excreted. The decrease in the amount of faeces excreted is almost certainly attributable to the stress caused to the sheep by the injection of 15 g. Na diethyldithiocarbamate.

Sheep/

Table 97. (a) The total volume of urine (ml) excreted daily by 3 sheep.

No.	23/1	24/1	25/1	26/1	27/1	28/1 ↓ Injected	29/1	30/1	31/1
961	180	400	515	450	600	530	500	-	-
420	15	560	750	675	1170	765	900	770	730
441 (control)	25	800	700	30	900	1250	700	980	625

(b) Urinary Copper concentration (ug/100 ml).

961	38.5	11.7	11.7	12.1	13.1	11.2	10.0	-	-
420	64.4	18.9	9.3	15.7	17.6	8.4	8.8	15.6	10.8
441 (control)	41.5	14.9	10.7	29.3	16.7	11.2	10.8	12.4	8.4

(c) Total urinary output of copper (ug).

961	69.3	46.8	60.3	54.5	78.6	59.4	50.0	-	-
420	9.7	105.8	69.8	106.0	205.9	64.3	79.2	120.1	78.8
441 (control)	10.4	119.2	74.9	8.9	150.3	140.0	75.6	96.7	52.5

Table 98 Faeces dry matter, copper concentration and total copper content of 3 sheep before and after injection of Na diethyldithiocarbamate.

No.	Faeces dry matter (g)				Faecal Cu Conc(ppm)				Total faecal Cu content (mg)			
	23-24	25-26	27-28	29-31/1	23-24	25-26	27-28	29-31	23-24	25-26	27-28	29-31
961	250	300	270	Dead	28.6	37.3	35.5	Dead	7.2	11.2	9.6	Dead
420	240	225	200	116	37.8	41.9	42.6	48.1	9.1	9.4	8.5	5.6
441 (contr)	140	140	115	136	40.6	42.7	43.5	53.1	5.7	6.0	5.0	7.2

Sheep No. 961 excreted no faeces following the injection of 30 g. Na diethyl-dithiocarbamate. There was a slight increase in the faecal copper concentration and the total copper output of sheep No. 441, which had been injected with 150 ml. N. saline, on the day following injection compared with the control period.

Thus the injections of from 3 - 30 gm. Na diethyldithiocarbamate effected little or no increase in urinary copper concentration and no increase in output of copper in the faeces. The most noticeable effect of these injections was the sharp decrease in blood copper concentration found within 3 hours of administration of the injection.

Experiment 15(a). The effect of intravenous injections of EDTA and Penicillamine on the output of Cu and Fe in the urine of sheep being dosed with copper sulphate.

Earlier experiments described in this section (Nos. 13(a) and 13(b)) have shown that intravenous injection of EDTA caused only a slight increase in the urinary output of copper and that this increase in output was due to largely to the increased volume of urine excreted. Penicillamine injections on the other hand effected marked increases in urinary copper concentration and total copper output without producing any great changes in the total volume of urine excreted. Theoretically EDTA should chelate ferric iron (Fe^{3+}) in preference to the cupric ion (Cu^{2+}) since the complex formed between EDTA and Fe^{3+} is less dissociated (or more stable) than that formed between EDTA and Cu^{2+}. The uptake of cupric ions from solution into a complex is governed by the ionisation constant of the metal-binding groups of the complex-forming ligand and the stability constant of the complex formed. Thus the presence of metals other than copper, especially those which form more stable complexes, would lead to competition for binding sites and consequently decrease the amount of copper complexed.

It was thought that competition might be occurring between Fe^{3+} and Cu^{2+} in the bloodstream for suitable organic ligands on the intravenously injected EDTA. Consequently it was decided to investigate the effect of intravenous injections of EDTA on the outputs of both copper and iron in the urine. It was felt that a similar investigation should be undertaken with regard to the effect/

effect of penicillamine injections. This work formed the basis of the present experiment. It was decided to discontinue the collection of faeces since previous experience indicated that the injections used had little or no effect on faecal copper output.

Four Blackface ewe hoggs were used in this experiment. They were placed in metabolism cages on 24 March 1965, and left there for a settling in period of more than a week. The sheep were being fattened on a largely cereal based diet and this was fed to them ad lib. During the acclimatisation period two of the sheep, Nos. 848 and 850, were to receive 7 daily doses of 1 g. $CuSO_4.5H_2O$ while the other two, Nos. 841 and 868, were to act as controls. Unfortunately the last dose of 1 g. $Cu\ SO_4.5H_2O$ was given in error to the two control sheep. Thus sheep Nos. 848 and 850 received 6 g. supplementary copper sulphate and sheep Nos. 841 and 868 received 1 g. supplementary copper sulphate.

The first collection of urine was made on 2 April and Daily collections were taken thereafter until the termination of the experiment on 10 April 1965. The four sheep received their injections of EDTA on 5 April and of penicillamine on 7 April 1965. The individual liveweights of the four sheep and the dosage rate of EDTA and penicillamine administered to each are detailed in Table 99.

Table 99/

Table 99. Liveweight and dosage rates of EDTA and penicillamine administered to 4 sheep.

No.	Amount of $CuSO_4$ given (g)	Livewt(lbs)	EDTA (g)	Penicillamine (mg)
841	1.0	80	2.75	900
868	1.0	110	4.0	1200
848	6.0	75	2.5	750
850	6.0	80	2.75	900

Blood samples were obtained from the four sheep on 6 occasions throughout the course of the experiment. These were analysed for their whole blood copper concentrations. The whole livers of two of the sheep (Nos. 841 and 868) were obtained at the termination of the experiment and these were dried and analysed for their copper and iron concentrations. All urine samples obtained were analysed for their copper concentration.

Results.

Blood copper Concentration.

The blood copper concentrations of the four sheep on six separate sampling occasions are detailed in Table 100.

Table/

Table 100. Blood copper concentrations (p.p.m.) of 4 sheep before and after injections of EDTA and penicillamine.

		EDTA inj. ↓		Pen. inj. ↓		
No.	24/3	5/4	6/4	7/4	8/4	13/4
841	0.98	0.89	1.00	0.87	0.95	0.98
868	1.29	0.96	1.08	0.93	1.00	1.48
848	1.00	1.25	1.54	1.20	1.29	1.14
850	0.94	1.05	1.15	1.04	1.09	1.06

The blood copper concentrations of all four sheep had elevated values on the day following EDTA injection compared to the pre-injection samples. The increases ranged from 0.10 to 0.29 p.p.m. This is contrary to previous findings which showed, almost without exception, that there was a reduction in blood copper concentration on the day following the intravenous administration of EDTA. The blood copper concentrations had all returned to their pre-treatment level by 48 hours after injection.

On the day following the administration of intravenous penicillamine all 4 sheep exhibited slight increases in blood copper concentration ranging from 0.05 p.p.m. to 0.09 p.p.m. This finding is more in accord with that found earlier (Experiment 13(b)) when elevations in blood copper concentration were found for 3 of 6 sheep which had been treated with penicillamine. The other 3 sheep in that experiment had shown only negligible reductions in blood copper concentration.

Urinary/

Urinary Copper Concentration.

The total daily volume of urine excreted, its copper concentration and the total daily output of copper in the urine of the four sheep are detailed in Table 101.

The total daily output of copper (and of iron) in the urine are presented diagramatically in Fig. 30. Only two of the 4 sheep - Nos. 850 and 868 - exhibited any increase in their output of urinary copper following the injection of EDTA. The increase in output for sheep No. 868 (94 ug) which had received 1 g. supplementary $CuSO_4$, was highly significant ($P<0.01$) whereas that for sheep No. 850 (172 ug) which had received 6 g. supplementary $CuSO_4$, was only just significant ($P<0.05$). There was no change in the level of urinary copper output by sheep No. 841 (1 g. additional $CuSO_4$) but there was a distinct decrease in the total amount of urinary copper excreted by sheep No. 848 (6 g. additional $CuSO_4$) on the day following injection compared to the mean output for the previous 4 days.

On the day following the intravenous injection of penicillamine there were marked and highly significant increases in output of urinary copper by all four sheep. These increases ranged from 766 ug. for sheep No. 841 (1 g. additional $CuSO_4$) to 1527 ug for sheep No. 868 which had also received 1 g. supplementary $CuSO_4$. Within 48 hours of having received either injection the urinary output of copper by all 4 sheep had returned to the preinjection level/

Table 101 (a) The total volume of urine (ml) excreted daily.

No.	Extra $CuSO_4$ given (g).	2/4	3/4	4/4	5/4	EDTA inj. ↓ 6/4	7/4	Pen. inj. ↓ 8/4	9/4	10/4
841	1.0	925	775	870	400	460	815	455	250	500
868	1.0	400	425	530	325	380	280	435	340	480
848	6.0	380	390	760	320	300	370	440	410	250
850	6.0	1450	1500	1760	575	1200	950	1150	960	850

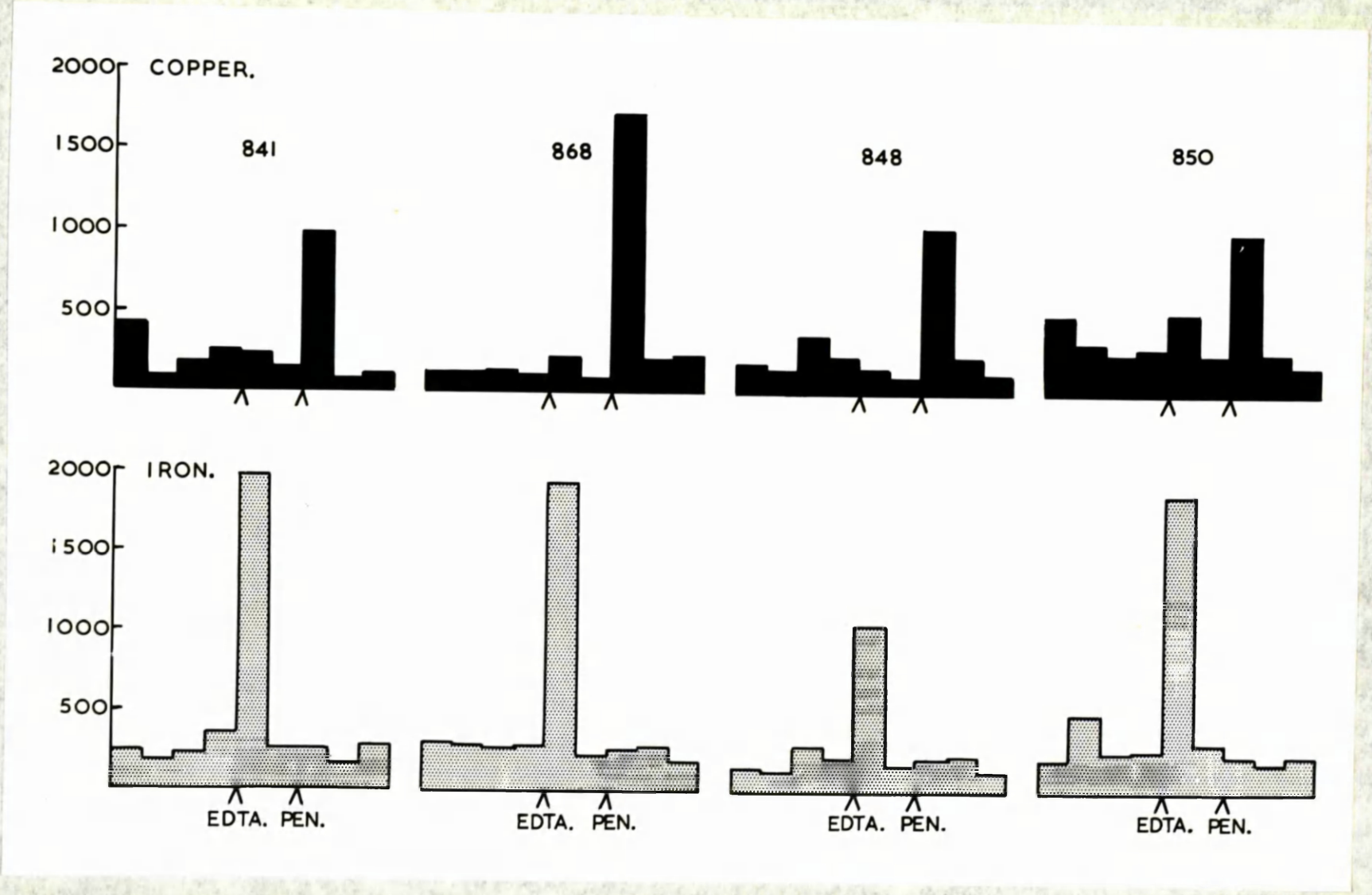
(b) Urinary copper concentrations (ug/100 ml)

841	1.0	45.2	11.6	20.5	62.0	50.4	18.4	216.0	29.0	23.0
868	1.0	32.0	31.2	25.6	35.3	58.4	34.8	397.6	61.0	49.6
848	6.0	48.0	38.4	47.0	71.6	49.6	27.0	234.0	57.6	48.0
850	6.0	34.0	21.2	14.0	50.8	42.4	25.6	87.6	26.8	20.8

(c) Total urinary output of copper (ug)

841	1.0	418	90	178	248	232	150	983	73	115
868	1.0	128	133	136	115	222	97	1730	207	238
848	6.0	182	150	357	229	149	100	1030	236	120
850	6.0	493	318	246	292	509	243	1007	257	177

Fig.30. The daily output of Cu and Fe (ug) in the urine of 4 sheep prior to and following intravenous injection of E.D.T.A. and penicillamine



Additional Cu given to sheep 841 and 868 = 1 g $CuSO_4 \cdot 5H_2O$
" " " " " 848 " 850 = 6 g $CuSO_4 \cdot 5H_2O$

level. This experiment has again demonstrated that penicillamine is very much more effective than EDTA in increasing the urinary output of copper.

The total liver-copper contents of sheep Nos. 841 and 868 and the effect of the intravenous injections of EDTA and penicillamine on their urinary outputs of copper are presented in Table 102.

Table 102. The total liver-copper content of 2 sheep and the increases in output of urinary copper following intravenous injections of chelating agents.

No.	Additional $CuSO_4$ (g.)	Total Liver Cu (mg.)	Increase(+)or decrease(-) in Cu excretion after EDTA	Increase in Cu following pen. (ug.)
841	1.0	27.6	Nil	766
868	1.0	43.7	+ 94	1527

The larger increases were recorded for the sheep (No. 868) with the higher total-liver-copper-content. This is in accord with earlier findings. These results will be discussed at the end of this section in an attempt to discover a relationship between total liver-copper content and increased output of copper in the urine following injection of chelating agents.

Urinary Iron Concentration.

The concentration and total daily excretion of iron in the urine of the four sheep are presented in Table 103.

Table 103 (a) Urinary Iron Concentration (ug/100 ml).

No.	Extra $CuSO_4$ given (g)	2/4	3/4	4/4	5/4 ↓ EDTA inj.	6/4	7/4 ↓ Pen. inj.	8/4	9/4	10/4
841	1.0	26.4	23.2	26.4	88.8	429.2	32.4	57.6	70.0	57.6
868	1.0	76.0	70.0	51.2	70.0	509.6	79.2	60.8	85.6	40.0
848	6.0	38.4	32.4	38.4	67.2	350.4	44.8	48.0	54.4	52.8
850	6.0	14.0	32.4	14.0	44.8	156.4	32.4	20.0	20.0	28.0

(b) Total urinary output of Iron (ug).

841	1.0	244	180	230	355	1974	264	262	175	288
868	1.0	304	290	271	288	1936	222	264	291	192
848	6.0	146	126	292	215	1051	166	211	223	132
850	6.0	203	486	246	258	1877	308	230	192	238

During the 4 day pre-treatment period the level of iron in the urine remained fairly constant within the range of 200 - 300 ug. There was a spectacular rise in the total amount of iron excreted by all 4 sheep during the 24 hour period immediately after the administration of the EDTA injections. These increases in iron excretion ranged from 856 ug. for sheep No. 848 (6g. extra $CuSO_4$) to 1722 ug. for sheep No. 841 (1g. extra $CuSO_4$). Within 48 hours of having received the injections of EDTA the output of urinary iron had returned to the pre-injection level of around 200 ug. for all four sheep. The output of urinary iron was maintained at this level over the following 4 days despite the fact that during this period all 4 sheep received injections of penicillamine. The penicillamine injections, therefore, did not influence the urinary output of iron.

The pattern found for urinary iron excretion in this experiment is, therefore, the converse of that found for copper excretion. The injection of penicillamine caused large increases in urinary copper output but had no effect on iron output whereas the injection of EDTA greatly increased the level of iron excretion and only produced relatively much smaller increases in copper output/in two of the four sheep and had no effect on copper output in the other two sheep. From these results, therefore, it would appear that iron in the bloodstream is being chelated in preference to copper by the EDTA injected.

Experiment 15(b). The effect of intravenous injections of EDTA and Penicillamine on the output of copper, iron and zinc in the urine of sheep being dosed each day with copper sulphate.

The preceding experiment (No. 15(a)) illustrated the differing specificity of the two chelating agents used for copper and iron. It was decided to conduct a further trial with a view to establishing that this was a consistent pattern of mineral excretion following the intravenous administration of these two chelating agents.

The ingestion of zinc has been found to reduce the uptake ond utilization of copper by the rat (Smith and Larson, 1946 and Gray and Ellis, 1950). Mills (1955) has postulated that this might be due to competition between these two elements for suitable binding sites on organic complexes in which form he suggests copper in more readily absorbed. Thus an increased concentration of zinc in the diet might reduce copper absorption by occupying a greater proportion of the suitable organic ligands thus reducing the amount of copper which is complexed. It was, therefore, thought that it would be interesting to study the comparative rates of excretion of copper and zinc in the urine following the intravenous injection of chelating agents which would provide suitable sites for combination with these metals.

Six Blackface ewe hoggs were used in this experiment. These were being fattened on a cereal based ration consisting of 80% barley and 20% of a protein concentrate. This ration was fed ad lib. The 6 sheep were placed in metabolism cages on 12 April 1965 and left for a four day acclimatisation period. Two of the sheep/

sheep, Nos. 848 and 850, were to be used as controls and these were given no supplementary copper. A second pair, Nos. 812 and 887, were to receive 7 daily doses of 1 g. $CuSO_4.5H_2O$ while the remaining 2 sheep, Nos. 808 and 884, were to be given 7 daily doses of 2 g. $CuSO_4.5H_2O$. The supplementary copper sulphate was given in solution by mouth between 12 and 18 April 1965.

Collection of urine was commenced on 16 April and continued daily until the conclusion of the experiment on 25 April 1965. The 6 sheep were given intravenous injections of EDTA (1g./30lbs. liveweight) on 19 April and of penicillamin (150 mg/15 lbs. liveweight) on 22 April 1965.

Blood samples were obtained from all 6 sheep immediately prior to both injections and again on the day after injection. The whole livers were obtained from all 6 sheep at slaughter on 27 April 1965. All samples of blood, liver and urine obtained were analysed for their copper and iron concentrations. In addition all urine samples were analysed for their zinc concentration.

Results.

Blood Copper Concentration

The blood copper concentrations of the 6 sheep on six separate sampling occasions are listed in Table 104. This also details the liver dry weights, copper concentrations and total liver-copper contents of the 6 sheep.

Table 104 Blood copper concentrations (p.p.m.), liver dry wts.(g), liver-copper concentrations (p.p.m.) and total liver-copper contents (mg) of 6 sheep.

No.	Additional $CuSO_4$ (g)	Blood 24/3	19/4	EDTA inj. ↓ 20/4	22/4	Pen. inj. ↓ 25/4	27/4	Liver Dry Wt.	Cu.Conc.	Total Cu Content
848	0.0	1.0	1.12	1.02	1.07	1.08	1.20	184	306.0	56.3
850	0.0	0.94	0.98	1.02	1.19	1.24	1.23	198	440.5	87.2
812	7.0	0.90	0.93	0.97	1.05	1.00	0.99	228	532.2	121.3
887	7.0	1.08	1.24	1.12	1.11	1.20	1.61	178	928.8	165.3
808	14.0	1.17	2.44	1.98	2.95	2.50	1.28	146	1387.8	202.6
884	14.0	1.14	1.59	1.69	1.71	1.73	1.84	98	2131.2	208.9

Table 105 (a) The total volume of urine (ml) excreted daily.

No.	Additional $CuSO_4$ (g)	16/4	17/4	18/4	EDTA inj. ↓ 19/4	20/4	21/4	Pen. inj. ↓ 22/4	23/4	24/4	25/4
848	0.0	250	230	290	300	320	700	825	725	410	300
850	0.0	900	1190	800	840	860	785	370	270	250	530
812	7.0	1150	1270	1160	1360	1380	670	1040	1120	750	975
887	7.0	525	545	565	400	375	320	240	440	265	270
808	14.0	1115	950	1075	700	1325	1650	1575	1130	1475	1780
884	14.0	290	675	700	400	405	440	810	710	430	285

(b)/

Table 105 (b) Urinary copper concentration (ug./100 ml.)

No.	Additional $CuSO_4$ (g)	16/4	17/4	18/4	19/4 ↓ EDTA Inj.	20/4	21/4	22/4 ↓ Pen. Inj.	23/4	24/4	25/4
848	0.0	99.0	42.0	50.0	34.4	62.4	26.0	21.2	128.8	44.0	34.4
850		39.0	34.4	31.2	12.6	22.4	15.5	22.0	41.2	23.2	23.6
812	7.0	83.0	35.0	37.0	35.2	29.0	30.8	21.6	159.6	35.2	30.4
887		139.0	123.2	101.0	138.4	68.8	41.6	42.0	352.0	87.6	60.8
808	14.0	184.0	140.0	154.0	355.0	158.4	96.0	84.0	358.8	80.0	72.0
884		278.0	161.0	308.0	288.0	153.6	115.0	298.0	656.0	216.0	230.0

(c) Total urinary output of copper (ug)

848	0.0	248	97	145	103	200	182	175	934	180	103
850		351	409	250	106	193	122	81	111	58	125
812	7.0	955	445	429	479	400	206	225	1788	264	296
887		730	671	571	554	258	133	101	1549	232	164
808	14.0	2052	1330	1656	2485	2099	1584	1323	4054	1180	1282
884		806	1087	2156	1152	622	506	2414	4658	929	656

Three of the sheep (Nos. 812, 850 and 884) exhibited slight increases (of up to 0.10 p.p.m.) in blood copper concentration on the day following the administration of EDTA. The remaining three sheep (Nos. 848, 808 and 887) all ha reduced blood copper concentrations following EDTA treatment. The reductions ranged from 0.10 p.p.m. for sheep No. 848 to 0.46 p.p.m. for sheep No. 808. The falls in the blood copper concentrations of sheep Nos. 887 and 808 might possibly have been occasioned by the fact that dosing with 1 and 2 g. respectivel of copper sulphate had been discontinued from the day on which they had received their injections of EDTA.

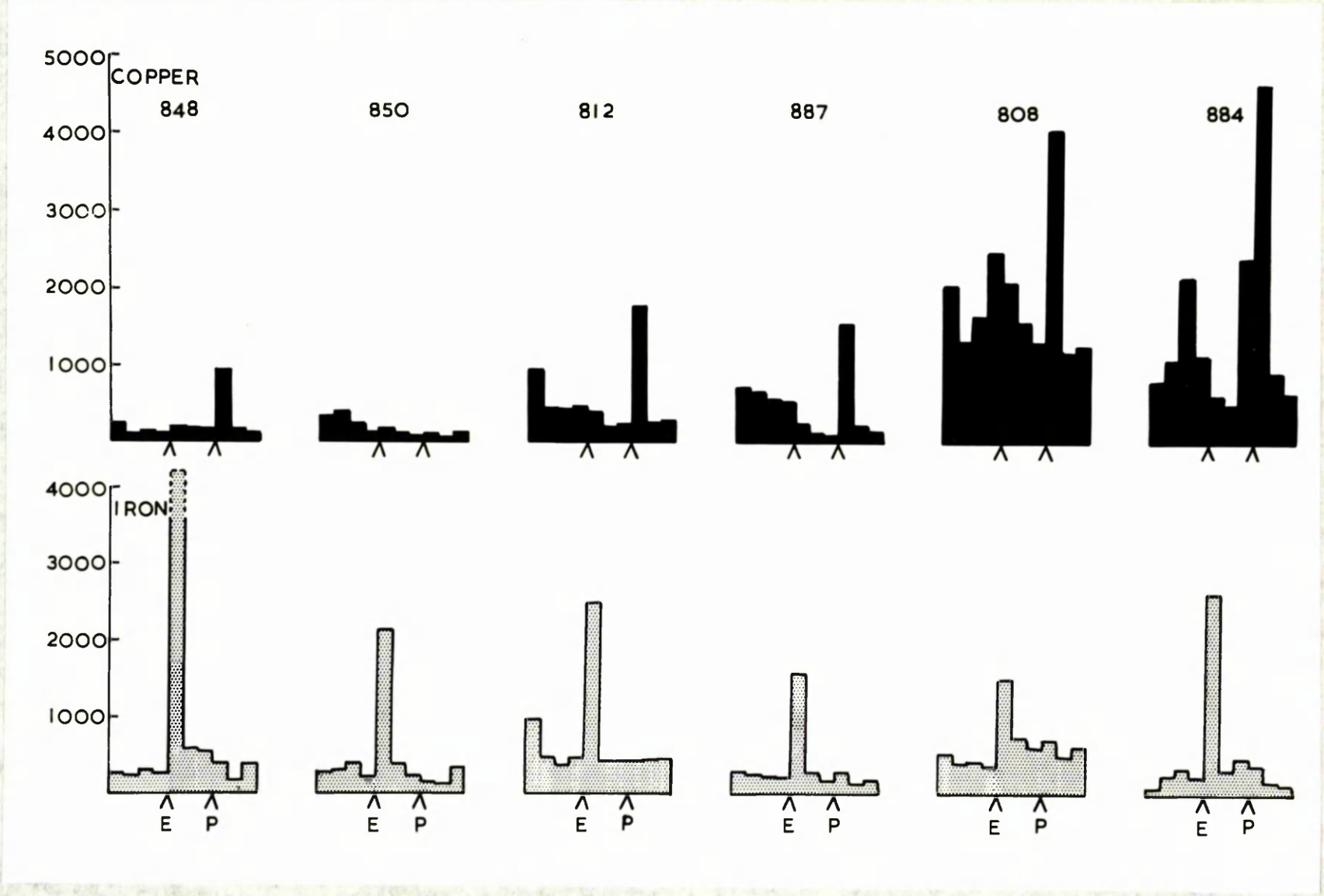
No change was observed in the blood copper concentrations of four of the 6 sheep on the day after the administration of penicillamine. One sheep (No. 887) showed a slight rise (0.09 p.p.m.) in blood copper concentration while the last one (No. 808) exhibited a fall in blood copper concentration of 0.45 p.p.m. Sheep No. 808, however, had an exceptionally high blood copper concentration (2.95 p.p.m.) immediately before injection and consequently the fall during the next 24 hours might have occurred naturally since concentrations of this magnitude are rarely maintained for any length of time even in sheep approaching death due to chronic copper poisoning (MacPherson, Brown & Hemingway, 1964).

Urinary Copper Excretion.

The total daily volume of urine excreted, its copper concentration and the total daily output of copper in the urine of the 6 sheep are detailed in Table 105. The total daily output of copper and iron in the urine of the 6 sheep is presented diagramatically in Fig. 31.

The/

Fig. 31 The total daily output of Cu and Fe (ug) in the urine of 6 sheep prior to and following intravenous injections of E.D.T.A. and penicillamine.



Additional copper given to sheep	848 and 850	Nil
" " " " "	812 " 887	7g $CuSO_4.5H_2O$
" " " " "	808 " 884	14g $CuSO_4.5H_2O$

The pre-injection level of urinary copper excretion varied with the treatment given to the 3 groups of sheep. The two control sheep (Nos. 848 and 850) excreted about 200 ug/day; the two sheep (Nos. 812 and 887) which were receiving 1 g. supplementary copper sulphate per day excreted just over 400 ug/day while the remaining 2 sheep (Nos. 808 and 884) which were being dosed daily with 2 g. $CuSO_4.5H_2O$ had a mean output of over 1400 ug/day.

In previous experiments (Nos. 13(a), 13(b) and 15(a)) intravenous injections of EDTA had always effected an increase in output of urinary copper in at least some of the injected sheep. In contrast in this present experiment there was no increased output of urinary copper by any of the 6 injected sheep.

The injections of penicillamine produced marked and highly significant increases in the amount of copper excreted in the urine by five of the six sheep. These increases ranged from 770 ug Cu for sheep No. 848 to 3409 ug. for sheep No. 884. The urinary output of copper by all five sheep had returned to the pre-injection level within 48 hours. The other sheep (No. 850), however, exhibited no increase in urinary copper output following the administration of penicillamine. It did have an elevated urinary copper concentration but this was offset by a reduction in the volume of urine excreted. In the previous experiment (No. 15(a)) this same sheep had shown a marked increase in urinary copper output after treatment with penicillamine. The relationship between the increase in amount of copper excreted following penicillamine injections and the total liver copper content of the 6 sheep will be discussed at the end of this section.

Urinary/

Table 106 (a) Urinary Iron Concentration (ug/100 ml)

No.	Additional $CuSO_4$ (g)				EDTA inj.			Pen. inj.			
		16/4	17/4	18/4	19/4	20/4	21/4	22/4	23/4	24/4	25/4
848	0.0	113.2	104.0	110.4	94.8	6576.0	82.4	67.2	54.4	44.8	132.0
850		32.4	26.4	51.2	26.4	248.8	51.2	64.0	57.6	54.4	67.2
812	7.0	85.6	38.4	32.4	35.6	181.2	64.0	41.6	38.4	60.8	48.0
887		57.6	48.0	44.8	48.0	422.4	91.6	76.0	67.2	54.4	73.2
808	14.0	48.0	41.6	38.4	51.2	113.2	44.8	38.4	64.0	32.4	35.6
884		35.6	38.4	51.2	57.6	651.6	76.0	60.8	54.4	41.6	48.0

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(b) Total urinary output of iron (ug)

848	0.0	283	239	320	284	21043	577	554	394	184	396
850		292	314	410	222	2140	402	237	156	136	356
812	7.0	984	488	376	484	2501	429	433	430	456	468
887		302	262	253	192	1584	293	182	296	144	198
808	14.0	535	395	413	358	1500	739	605	723	478	634
884		103	259	358	230	2639	334	492	386	179	137

Urinary Iron Excretion.

The concentration and total daily excretion of iron in the urine by the 6 sheep are presented in Table 106.

Over the pre-injection period of 4 days the level of iron excreted in the urine of the 6 sheep varied between 200 to 400 ug. daily. Following the injections of EDTA there was a marked and highly significant increase in urinary Fe excretion by all 6 sheep. In the case of sheep No. 848 in particular this increase was of massive proportions. It excreted 21 mg. Fe compared to a mean pre-injection excretion of 0.28 mg./day. The increases in iron output by the other five sheep were not so great but they ranged from 1075 µg. for sheep No. 808 to 2400 ug. for sheep No. 884. Within 48 hours of injection the output of iron in the urine of all 6 sheep had returned to the pre-injection level. The injections of penicillamine again produced no effect whatsoever on the output of iron in the urine.

Urinary Zinc Concentration.

The concentration and total daily excretion of zinc in the urine are detailed in Table 107.

The total daily output of zinc in the urine of the six sheep during the pre-injection period ranged from 0.18 to 1.77 mg. with a mean daily excretion of 0.69 mg. There were marked and highly significant increases in the output/

Table 107 (a) Urinary zinc concentration (ug/100 ml).

No.	Additional $CuSO_4$ (g)	16/4	17/4	18/4	19/4 ↓ EDTA inj.	20/4	21/4	22/4 ↓ Pen.inj.	23/4	24/4	25/4
848	0.0	369.0	371.0	611.0	402.5	2358.0	299.0	152.0	347.2	196.0	351.0
850		86.0	61.9	212.0	68.8	1830.0	n.d.	102.0	334.8	258.5	72.0
812	7.0	59.0	41.3	39.0	48.0	1080.0	230.3	68.8	317.2	89.4	174.0
887		65.3	41.3	34.4	113.5	3520.0	440.0	172.0	481.3	123.8	96.3
808	14.0	141.0	89.0	58.5	58.5	909.0	105.0	n.d.	158.0	62.0	65.5
884		88.2	44.7	37.9	44.7	3194.0	n.d.	68.7	199.5	152.0	72.2

(b) Total urinary output of zinc (mg).

848	0.0	0.95	0.85	1.77	1.17	7.10	2.10	1.20	2.50	0.80	1.05
850		0.77	0.74	1.70	0.57	15.70	n.d.	1.02	0.91	0.65	1.55
812	7.0	0.67	0.52	0.45	0.66	14.90	1.60	0.72	3.50	0.67	1.70
887		0.34	0.23	0.19	0.45	13.20	1.40	0.41	2.10	0.33	0.26
808	14.0	1.57	0.85	0.63	0.41	12.000	1.70	n.d.	1.80	0.90	1.20
884		0.25	0.30	0.27	0.18	13.00	n.d.	0.48	1.40	0.65	0.21

n.d. = not determined.

output of zinc following the administration of EDTA. These increases ranged from 5.92 mg. for sheep No. 848 to 14.75 mg. for sheep No. 850. The level of zinc excretion was still about twice normal during the period 24 - 48 hours after injection but returned to the pre-injection level on the third day. The penicillamine treatment effected much smaller increases, ranging from 0.93 to 2.75 mg. Zn, in the urinary output of zinc by five of the sheep. The other sheep, No. 850, had an elevated urinary zinc concentration but a decreased volume so that its total zinc output remained at the pre-injection level of 0.91 mg. Normal levels of zinc were being excreted again within 2 days of the administration of the penicillamine injections.

One possible explanation for the large increase in urinary zinc output compared to little or no increase in urinary copper output following the EDTA injections is the difference in the concentrations of zinc and copper in whole blood. Normal blood contains about 1.0 p.p.m. Cu compared to 7.0 - 8.0 p.p.m. Zn. There is thus, a much greater chance of Zn being chelated by the EDTA administered than there is of Cu being chelated.

The increased amounts of copper excreted following the penicillamine treatment ranged from 0.77 to 3.4 mg Cu. This was similar to the increase in the amounts of zinc excreted which ranged from 0.93 to 2.75 mg. following penicillamine. This would indicate that penicillamine chelates copper in preference to zinc since the outputs are virtually identical despite a blood zinc concentration which is seven times greater than the copper concentration.

Experiment 15(c). The effect of intravenous injections of EDTA and penicillamine on the output of copper and iron in the urine of sheep.

This was the final experiment conducted to investigate the effects of the two chelating agents, EDTA and penicillamine, on the urinary excretion of copper and iron. It was undertaken principally to increase the overall number of sheep which had been thus treated in order to establish a relationship between increased urinary copper output and total liver copper content. This was thought necessary in order to reduce as far as possible the influence of individual variation on the overall relationship.

Only two sheep, Blackface ewe hoggs, were available for this experiment. These were put into metabolism cages on 25 April 1965, where they were left for 6 days to become accustomed to their new surroundings. They were fed an identical diet to the 6 sheep in the previous experiment i.e. 80% barley and 20% of a protein concentrate. No supplementary copper was given to either of the two sheep.

Collection of the daily volume of urine excreted was commenced on 1 May and continued thereafter until the termination of the experiment on 11 May 1965. The 2 sheep were given intravenous injections of EDTA (1 g./ 30 lbs. liveweight) on 5 May 1965, and of penicillamine (150mg/15 lbs liveweight) on 8 May 1965.

Blood/

Blood samples were obtained from both sheep immediately before both injections and again 24 hours later. The whole livers were obtained from both sheep at slaughter on 13 May. All blood and liver samples obtained were analysed for their copper concentration. Copper and iron determinations were carried out on all urine samples.

Results

Blood Copper Concentration

The blood copper concentrations of both sheep on 5 separate sampling occasion are presented in Table 108. This also details their liver dry weights, copper concentrations and total copper contents.

Table 108. Blood copper concentrations (p.p.m.), liver dry weights (g), liver copper concentrations (p.p.m.) and total liver copper contents (mg) of 2 sheep.

	Blood					Liver		
	EDTA inj. ↓			Pen. inj. ↓				
No.	5/5	6/5	8/5	9/5	11/5	Liver Dry Wt.	Cu Conc.	Total C Content
31	0.90	0.95	0.95	0.92	0.97	251	513.3	129
878	1.18	1.34	1.28	1.32	1.37	154	533.9	82

The blood copper concentration of sheep No. 31 remained within the range 090 to 0.97 p.p.m. throughout the course of the experiment and was not affected by either treatment. Sheep No. 878 exhibited a rise of 0.16 p.p.m. in blood copper concentration following the intravenous injection of EDTA. It was unaffected/

unaffected by the intravenous injection of penicillamine.

Urinary Copper Concentration.

The total daily volume of urine excreted, the urinary copper concentration and the total daily output of copper in the urine are detailed in Table 109.

The urine samples collected from both sheep on 4 May had to be discarded as they were badly contaminated with faeces. The injection of EDTA did not affect the output of urinary copper by sheep No. 31. It did, however, produce a significant ($P<0.05$) increase in output of urinary copper by sheep No. 878. During the 4 day pre-treatment period the mean daily excretion of copper by sheep No. 838 was 138 ug. whereas during the 24 hours following injection it excreted 253 ug. Cu. By 48 hours after injection its copper excretion was back at the pre-treatment level.

There were marked and highly significant increases in copper output by both sheep following the administration of penicillamine. The increase in urinary copper output for sheep No. 31 was 938 ug. while for sheep No. 878 it was 866 ug. The urinary excretion of copper by both sheep returned to normal within 48 hours of injection.

Urinary Iron Concentration.

The urinary iron concentration and the total daily iron excretion are detailed in Table 110. Fig. 32 shows the daily excretion of iron and copper in the urine.

Table 109 (a) Total volume of urine (ml) excreted daily.

					EDTA inj.			Pen. inj.			
No.	1/5	2/5	3/5	4/5	5/5↓	6/5	7/5	8/5↓	9/5	10/5	11/5
31	380	800	850	700	1320	650	490	530	320	355	820
878	340	625	270	300	400	460	570	475	380	515	890

(b) Urinary Copper Concentration (ug/100 ml).

31	41.0	19.6	15.6	-	16.4	29.2	22.8	23.2	340.0	53.6	26.4
878	28.5	36.4	36.0	-	32.0	55.0	21.6	18.2	261.2	53.2	37.6

(c) Total daily output of copper (ug).

31	156	157	133	-	216	190	112	123	1088	190	216
878	97	228	97	-	128	253	123	86	993	274	335

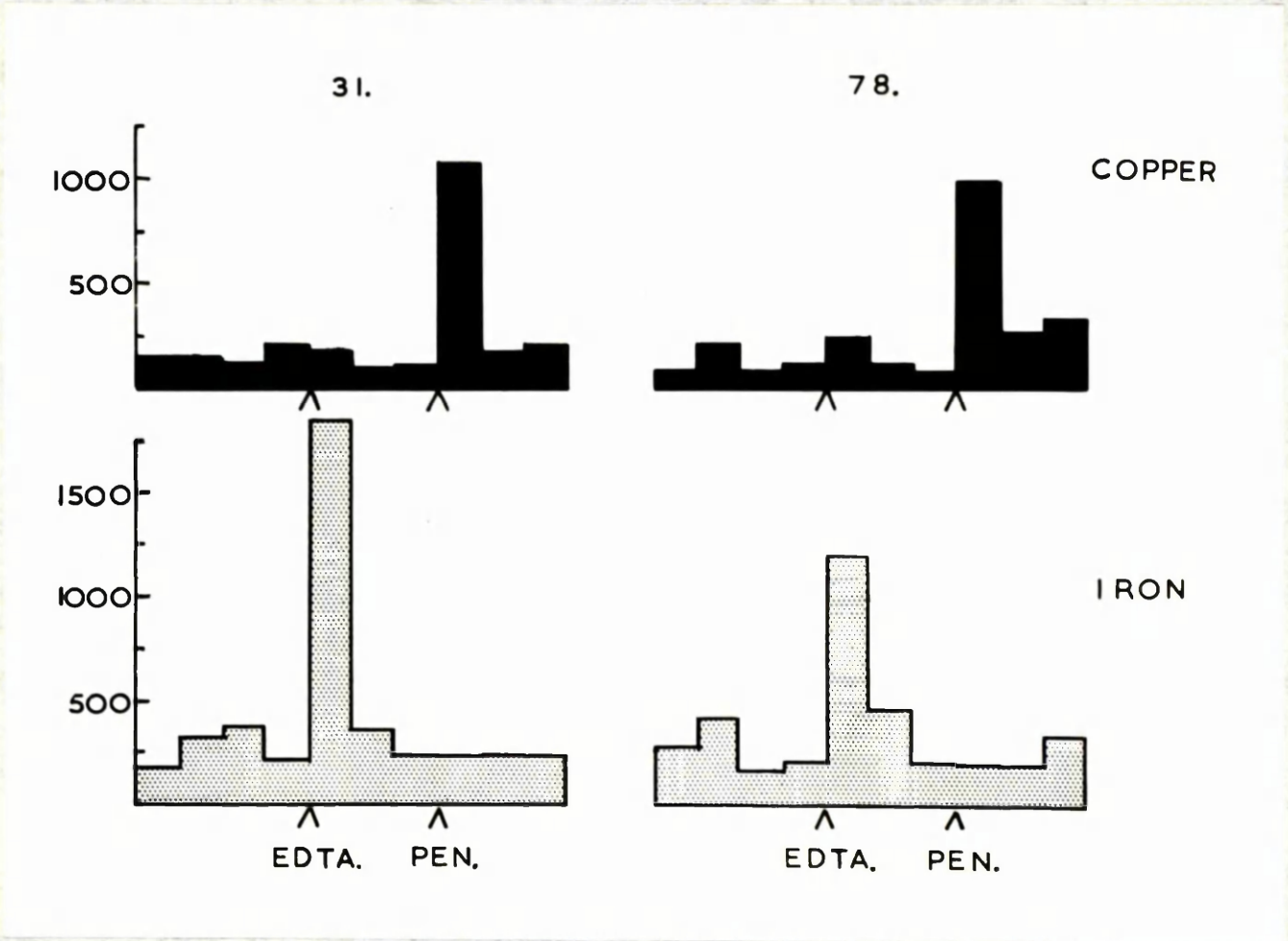
Table 110 (a) Urinary iron concentration (ug/100 ml)

31	51.2	41.6	44.8	-	17.2	286.0	76.0	48.0	76.0	70.0	29.6
878	85.6	67.2	64.0	-	54.4	261.2	82.4	44.8	54.4	38.8	38.4

(b) Total daily output of iron (ug)

31	195	333	381	-	227	1859	372	254	243	249	243
878	291	420	173	-	218	1202	470	213	207	200	342

Fig. 32. The total daily copper and iron excretion in the urine (ug) of 2 sheep prior to and following intravenous injections of E.D.T.A. and penicillamine.



EDTA injection again produced highly significant increases in the total daily excretion of iron in the urine. The increase in urinary iron output by sheep No. 31 was 1575 ug. while for sheep No. 878 it was 928 ug. By 48 hours after injection the output of urinary iron had fallen to the pre-treatment level and it remained at this level (of less than 300 ug/day) for the remainder of the experiment. Urinary iron excretion was unaffected by the administration of intravenous injections of penicillamine.

Summary of Experiments 13 and 15 involving injections of penicillamine and EDTA

A. Penicillamine Effects.

A total of 18 sheep have been injected with penicillamine in the course of the four Experiments 13(b), 15(a), 15(b) and 15(c). The results of these Experiments are summarised in Table 111.

The increase in urinary copper excretion on the day after injection has been plotted against the total liver copper content of the sheep in Fig. 33. An attempt was made to determine whether the increase in copper excretion following injection was related to the total liver-copper content. This was done by calculating a regression coefficient for the increases in urinary copper output and their respective total liver-copper contents. The regression coefficient was found to be highly significant ($P < 0.01$) thus demonstrating that the size of the increase in urinary copper output following the intravenous administration of penicillamine was dependent upon the total liver-copper content of the sheep.

The regression equation was

$$X = 534.4 + 5.198\,y \qquad (t = 2.95)$$

(X = increased copper excretion in ug; y = total liver Cu (mg))

This regression line has been superimposed on Fig. 33.

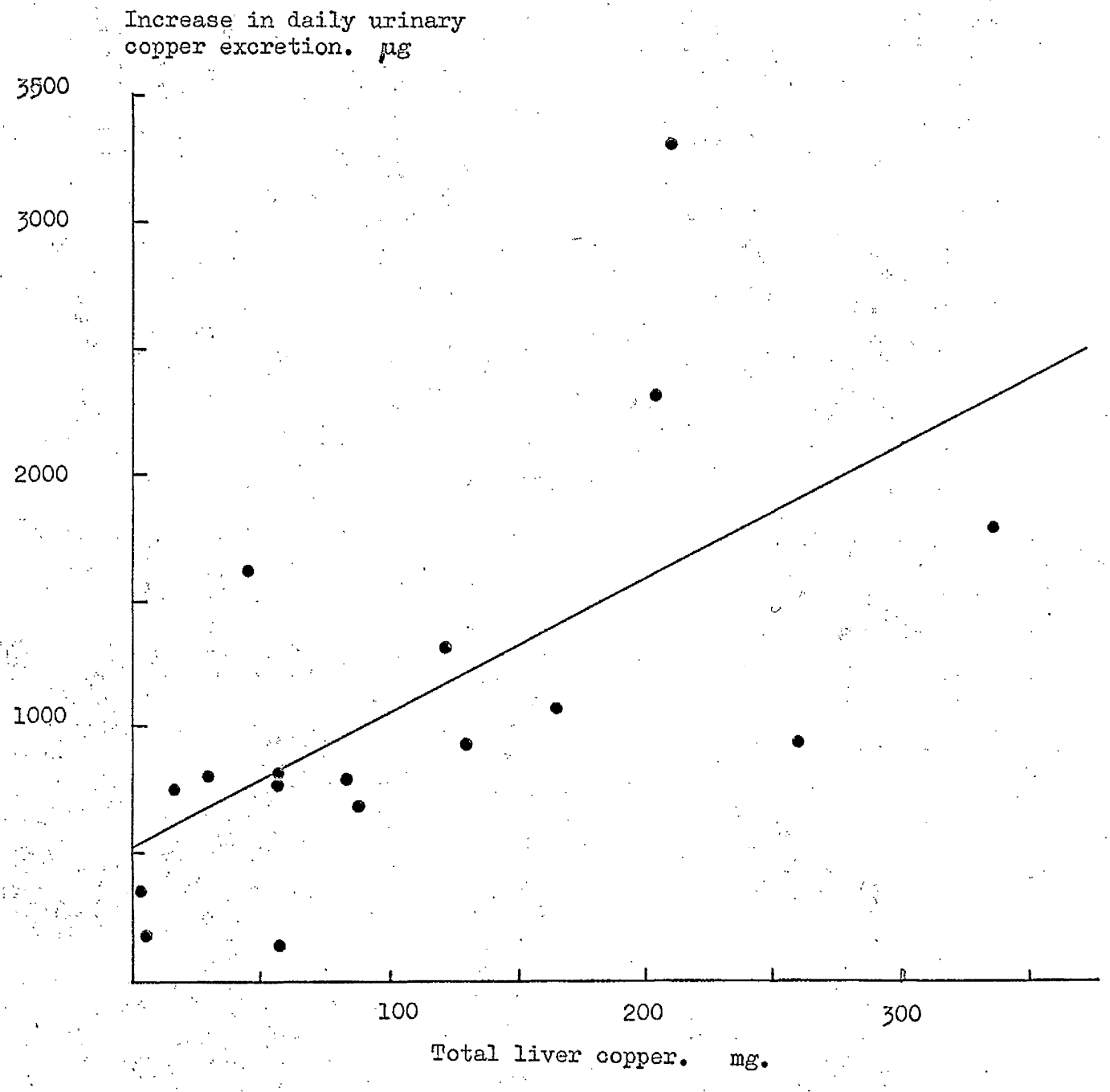
B. EDTA Effects.

During the course of the five Experiments (Nos. 13(a), 13(b), 15(a), 15(b) and/

Table 111. The mean daily urinary excretion of copper over the pre-treatment period, the urinary copper excretion from 0-24 hours after injection, the increase in urinary copper output following injection and the total liver-copper content of the sheep.

No.	Mean daily Cu excretion pre-treatment (ug)	Cu excreted 0-24 hrs. after injection(ug)	Increase in Cu output(ug)	Total liver Cu (mg)
31	150	1088	938	129
878	127	933	806	82
848	158	934	776	56
812	462	1788	1326	121
887	460	1549	1089	165
808	1738	4054	2316	203
884	1354	4658	3304	209
841	217	983	766	28
868	122	1730	1608	44
848	204	1030	826	56
850	318	1007	689	87
28	193	383	190	4
45	103	475	372	3
17	207	350	143	57
53	262	1028	766	18
5	310	2112	1802	335
38	233	1175	942	258

Fig 33. Relationship between the increase in daily urinary copper excretion following injection of penicillamine and the total amount of copper in the liver of sheep.



and 15(c)) in which intravenous injections of EDTA were given a total of 24 sheep were treated. Of these five have been excluded from the present discussion; four of these because they were being dosed with copper sulphate up until the day of injection but not thereafter and the fifth because its urine was very badly contaminated with faeces on the day after injection. The results from these five experiments are summarised in Table 112.

Fig. 34 shows the relationship between the total liver-copper content of the sheep and the increase in urinary copper output following EDTA treatment. A highly significant ($P < 0.02$) correlation was found between the total amount of copper present in the liver and the increase in urinary copper output following the EDTA injection. A regression equation was calculated for the increase in urinary copper output with that in total liver-copper content. It was:

$$X = 19 + 0.693\ y \qquad (t = 2.69)$$

(X = increase in urinary copper output (ug); y = total liver copper content(mg))

This regression line has been superimposed on Fig. 34.

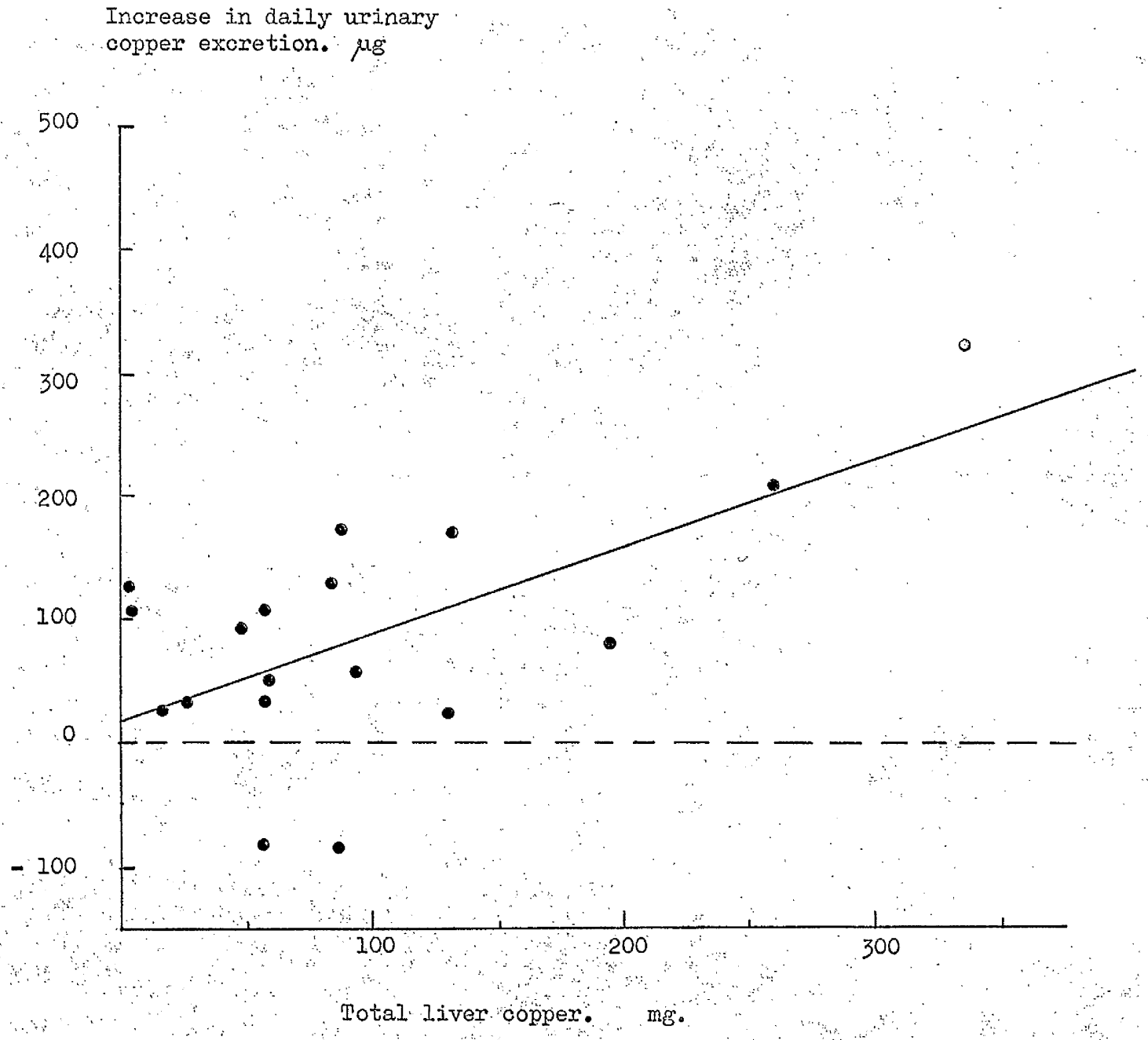
An attempt was also made to correlate the increased amount of iron excreted in the urine on the day after the administration of EDTA with the total amount of iron present in the liver. The coefficient was, however, found to be non-significant having a t value = 1.807 while that required for significance = 2.262 ($P = 0.05$).

A/

Table 112. The mean daily urinary excretion of copper (pre-treatment), the urinary copper output from 0 - 24 hours after injection, the increase or decrease in urinary copper output following injection and the total liver copper content of the sheep.

No.	Mean daily Cu excretion pre-treatment (ug)	Cu excretion 0-24 hrs. after injection(ug)	Increase or decrease in Cu output(ug)	Total liver Cu (mg)
31	166	190	+ 24	129
878	138	253	+115	82
848	148	200	+ 52	56
850	279	193	- 86	87
841	234	232	- 2	28
868	128	222	+ 94	44
848	230	149	- 81	56
850	337	509	+ 172	87
28	213	315	+ 102	4
45	94	220	+ 126	3
17	236	344	+ 108	57
5	317	641	+ 324	335
38	230	438	+ 208	258
27	127	209	+ 82	191
453	67	103	+ 36	55
468	66	101	+ 35	25
473	61	230	+ 169	131
474	115	175	+ 60	94
481	65	94	+ 29	14

Fig 34. Relationship between the increase in daily urinary copper excretion following injection of E.D.T.A. and the total amount of copper in the liver of sheep.



A comparison was made between the regression coefficients for the increases in urinary copper output following penicillamine and EDTA administration related to total liver-copper content. The regression coefficient for the increase in output of urinary copper following the intravenous injection of penicillamine was found to be significantly ($P < 0.02$) higher than that for the increase in copper excretion following EDTA administration. This demonstrates, therefore, that, at the dosage rates used, as the total liver copper content of the sheep increased there was a more rapid rise in output of urinary copper after treatment with penicillamine than there was following EDTA treatment.

The pattern of urinary copper excretion in sheep found in the experiments described in this section is essentially similar to that found for humans who have been similarly treated with chelating agents. Intravenous administration of EDTA produced small increases only in copper excretion of sheep and this corresponds with disappointing therapeutic results in humans reported by Bickel et al. (1957). Treatment with EDTA would, therefore be of little use in effecting an improvement in the condition of sheep suffering from chronic copper poisoning.

Penicillamine injections produced relatively much larger increases in urinary copper output. However, the total daily output after treatment is still extremely small when related to the total amount of copper in the liver. From Fig. 33 it can be seen that an increased excretion of 2.0 mg Cu. per day would correspond to a total liver copper content of 275 mg. If this liver-copper content were to be reduced to a reasonably normal total content of 100 mg Cu then this rate of treatment would have to be maintained over a period of 12 weeks.

Long/

Long term treatment with penicillamine has been used extensively and successfully in the treatment of patients suffering from Wilson's Disease (e.g. Fister, Boulding, and Baker, 1958; Seven, Kliman and Peterson, 1959; Walshe, 1960; Boulding, 1961 and Warren and Broughton, 1962). This is obviously, therefore, a feasable method of treatment but the cost of such treatment over a prolonged period is unlikely to be such as to encourage its widespread adoption. The only circumstances in which its use might be economically justifiable would be in the treatment of pedigree rams suffering from chronic copper poisoning (e.g. Clegg, 1956). Otherwise, however, the monetary value of an individual sheep would not justify the expenditure of a large sum of money in an attempted cure.

If penicillamine were to be used in the treatment of chronic copper poisoning in sheep it would be imperative to have as early a diagnosis as possible in order that the treatment should have the best possible chance of success. It is probable that if treatment was commenced in the final stages of the illness it might only precipitate its course. Additional copper would be released from the liver into the bloodstream with a consequent rupture of the red cells and haemolysis. Eventual recovery after such an haemolytic crisis, particularly in British breeds, is improbable. A reliable method for the early diagnosis of the illness would, therefore, be advantageous.

Despite/

Despite the significant correlation found between the increase in the urina output of copper following the administration of penicillamine and the total liver copper contents of the sheep it is unlikely that this could be employed to detect sheep either with very low liver-copper contents which might subsequently produce swayback lambs or with elevated liver-copper contents which might shortly show symptoms of chronic toxicity. The use of penicillamine for this purpose would be unreliable due to the wide variation in the amounts of copper excreted in the urine by sheep having equivalent total liver-copper contents. Thus a sheep which had a total liver copper content of 4 mg. might excrete as much extra copper as one with a liver copper content of 60 mg. (See Experiment 13(b)). On the basis of such treatment both sheep might be regarded as being normal but it is possible that the former one might give birth to a swayback lamb.

Similarly at the other extreme a sheep which had a total liver copper content of 165 mg. excreted more copper after treatment with penicillamine than did another with a total liver copper content of 260 mg (See table 111). This, therefore, indicates the difficulties that could arise in attempting to diagnose chronic copper toxicity in sheep by comparing the rates of urinary copper excretion following the intravenous administration of penicillamine.

Intravenous penicillamine was found to have no effect on urinary iron excretion in sheep. A similar finding has been reported following the treatment of human patients with penicillamine (e.g. Seven et al., 1956 and Boulding and Baker, 1957). Penicillamine would therefore be of little use in the treatment of haemosiderosis which can sometimes occur as a consequence of copper deficiency.

Experiment 16. The usefulness of various blood analyses in indicating an approaching haemolytic crisis.

Experiment 7 indicated that blood copper analyses of samples obtained from sheep during the two weeks prior to death from chronic copper poisoning would not always confirm that death is imminent. Indeed in only 4 of 11 sheep was the blood copper concentration consistently above 1.40 p.p.m. in all samples taken during the two weeks prior to death. Copper analyses, therefore, may fail to detect the existence of chronic copper poisoning even within a short time of death. Consequently it was considered that an attempt should be made to discover whether any other blood test would give an earlier, more sensitive and more consistent indication of the approach of an haemolytic crisis due to copper poisoning. This was considered desirable in view of the fact that the earlier a treatment can be commenced the greater is the chance of effecting a successful cure.

This present experiment describes the results obtained for 6 different blood tests, including blood copper analysis, performed frequently on samples obtained from sheep which were being dosed with 1 g. $CuSO_4.5H_2O$ every other day, over a long period. The five other tests used were two plasma transaminase concentrations - glutamic-oxalacetic transaminase (GOT) and glutamic - pyruvic transaminase (GPT) - blood packed cell volume (PCV) and haemoglobin content (Hb) and the rate of clearance of a dyestuff - Bromosulphthalein (BSP) - from the bloodstream.

Eight/

Eight Blackface 6-month old hoggs were used in this experiment. These sheep were similar to those used in Experiment 11a in Section IV and they were fed an identical ration which consisted of 82% barley and 18% of a protein concentrate composed principally of soya and groundnut. This was fed ad lib.

The eight sheep were given a dose of 1 g. $CuSO_4.5H_2O$ (given in solution by mouth) every other day. This would be equivalent to a dietary concentration of 125 p.p.m. Cu if each sheep ate 1 Kg. of food per day. Dosing of the eight sheep was commenced on 19 October 1964, and continued until just before death due to copper poisoning.

Blood samples were obtained at regular intervals from the eight sheep and more frequently when symptoms of copper toxicity appeared. These samples were analysed for blood copper concentration, GOT and GPT concentrations, and also for PCV and Hb content. Also on several occasions each sheep was injected intravenously with a solution of BSP in N. saline and blood samples were obtained at regular intervals after injection to determine how quickly the dye was eliminated from the blood-stream.

The whole livers were obtained from each sheep after death and these were dried and analysed for their copper concentrations. The total liver-copper content was then calculated and by comparing this with the mean total liver-copper content of the control group the proportion of the dosed copper which was stored in the liver could be determined.

Results/

Results

Liver dry weights, liver-copper concentration and total liver-copper content.

Table 113 shows the concentrations and total amounts of copper in the livers of the 8 sheep. It also shows the amount of copper sulphate given to each sheep and the proportion of this stored in the liver. The dried liver sample of sheep No. 847 was unfortunately mislaid so no values are available for its liver copper concentration and total liver-copper content. Kidney copper concentrations are also detailed in Table 113.

The first sheep to show symptoms of chronic copper toxicity was No. 813 when on 11 January 1965 a blood sample was found to have the dark chocolate colour associated with copper poisoning. After this it exhibited a progressive inappetance and began to appear jaundiced. By 18 January it could no longer stand so it was slaughtered. Post mortem examination showed a slightly jaundiced carcase and a bronze coloured liver. The kidneys, which were found to be grossly enlarged were a dirty brown colour and completely lacked the metallic sheen considered typical of copper poisoning. A pathological examination of the kidneys revealed severe tubular necrosis and also some slight oedema. The enlarged size of the kidneys was probably caused by a prolonged haemolysis which along with the tubular necrosis caused blockages with a consequent absorption and retention of water. Each kidney weighed 187 g. which is almost twice the normal for a Blackface hogg. By the time it was slaughtered sheep No. 813 had received 46 doses of 1 g. $CuSO_4.5H_2O$ (≡ 11.5 g Cu).

During/

Table 113 Quantities of copper sulphate administered and the amounts of copper stored in the liver of sheep which died of copper poisoning.

No.	No. of doses	Amount Cu given(g)++	Kidney Cu conc.(ppm)	Liver dry wt.(g)	Liver Cu conc.(ppm)	Liver Total Cu (mg)	% of dosed Cu in liver.**
813*	46	11.50	77.0	158	1224.0	193.3	1.44
807	57	14.25	155.5	98	2965.0	291.5	1.85
840	60	15.00	1590.2	108	3105.1	336.3	2.06
811	65	16.25	1181.5	64	6323.3	406.5	2.33
880	66	16.50	79.5	104	2480.0	258.9	1.40
846	86	21.50	570.3	111	3288.8	366.0	1.57
847	108	27.00	n.d.	-	-	-	-
883+	185	46.25	360.6	190	973.0	184.9	0.34
Mean			573.5	[illegible]	2908.5	291.1	1.57

n.d. = not determined

* Slaughtered

** Calculated by reference to mean copper concentration of control sheep (Group I in Experiment IIa which received the same diet but no supplementary copper.

\+ Died from other causes

++ x 4 = g $CuSO_4 \cdot 5H_2O$

During the next 5 weeks a further four sheep died from copper poisoning having been given from 14.25 to 16.5 g. of Cu. A further 6 weeks passed before sheep No. 846 succumbed on 7 April after having received 21.5 g. Cu. Another period of 6 weeks elapsed before sheep No. 847 died after having been dosed with 108 g. $CuSO_4.5H_2O$ (= 27 g. Cu.).

Thus only one sheep (No. 883) survived beyond this time. It was showing no signs of distress due to the large amount of copper and was in fact extremely healthy and eating well. It weighed 118 lb. on 5 May and during the next few months it continued to thrive so that by 24 September 1965 it weighed 153 lb. It showed no outward signs of distress and continued to eat well until it died suddenly on 26 October more than 5 months after the time of death of the longest survivor of the other seven sheep. By this time it had received 185 doses of 1 g $CuSO_4.5H_2O$ (=46.25 g. Cu). Pathological examination of its liver showed no indication of degenerative changes which might be attributed to copper poisoning but did show some sign of damage due to fasciola and indeed some fluke were found in the bile ducts.

Typical liver lesions found on pathological examination of the sheep which died were diffuse micro abscesses throughout the lobules and polymorphonuclear infiltration into the liver sinusoids. Kidney damage found included acute tubular necrosis with haemoglobin deposits in the tubular epithelium and diffuse interstitial/

interstitial fibrosis. Haemoglobinuric nephrosis was also found with heavy deposits of haemoglobin in the distal tubules with numerous haemoglobin casts and slight focal lymphocytic infiltration.

The mean liver copper concentration of the sheep was 2909 p.p.m. with a range from 973 to 6323 p.p.m. This range of liver copper concentration is very similar to that found in Experiment 7 where the range was from 1558 to 6530 p.p.m. The mean copper concentration of the control group was 175 p.p.m. (range 125 - 271 p.p.m.). The lowest liver-copper concentration was found for sheep No. 883 (973 p.p.m.) which survived the longest and received more than twice as much copper sulphate as six of the other seven sheep.

The mean total liver-copper content was 291 mg. (range 185 - 407 mg). This compares with a range of 131 to 470 mg. in Experiment 7 where sheep were similarly dosed with copper sulphate. Sheep No. 883 also had the lowest total liver copper content (185 mg.) despite having received a far greater amount of supplementary copper.

The mean percentage storage rate of dosed copper in the liver was 1.57% (range 0.34 - 2.33%). This compares favourably with results from the previous experiment when the proportion of supplementary copper stored ranged from 0.60 to 3.13%. It is also similar to the results quoted by previous workers for the proportion of dietary or supplementary copper stored in the liver.

Kidney Copper Concentration/

Kidney Copper Concentration.

The range in kidney copper concentrations (from 77.0 to 1590.2 p.p.m., Table 113) in the present experiment compares favourably with that found in Experiment 7 where the range was from 56.1 to 2068.0 p.p.m. Sheep No. 813 which was slaughtered had the lowest kidney copper concentration (77.0 p.p.m.). Sheep No. 883 which was the longest survivor and which had received a total of 46.25 g Cu. had a kidney copper concentration of 360.6 p.p.m. This sheep, therefore, which did not die of chronic copper poisoning had a kidney copper concentration higher than those (77.0, 79.5 and 155.5 p.p.m.) of three sheep (Nos. 813, 880 and 807 respectively) which died from this cause. This further illustrates the difficulty of basing a diagnosis of chronic copper poisoning purely on an analysis of the copper content of the tissues.

Blood Copper Concentration.

Fig. 35 shows the changes that occurred in the blood copper concentrations of the individual sheep during the course of the experiment. The initial blood copper concentrations ranged from 0.75 - 1.44 p.p.m. and they remained within this range for the next 2 months. After this time the blood copper concentrations of individual sheep varied widely due to the abnormally high values found at or near the time of death.

Table 114 (a) details the individual blood copper concentrations of each of the 8 sheep on the separate sampling occasions. Table 115 lists the number of days to death when an elevated blood copper concentration was first found for each of the 8 sheep.

Table 114 (a) Blood copper concentrations (p.p.m.) of 8 sheep which received 1 g. $CuSO_4.5H_2O$ on alternate days related to their time of death.

Sheep No.	Days before death. 150	125	100	75	50	30	20	15	10	6	5	4	3	2	1
807	-	1.30	1.23	-	1.57	1.20	5.64	2.55	1.95	2.04	-	2.44	-	-	-
811	-	0.98	1.49	1.14	1.46	2.23	1.78	2.08	-	8.35	-	-	4.40	-	8.25
813	-	-	1.44	1.33	-	1.09	-	-	-	2.26	2.43	-	-	1.80	1.85
840	-	0.96	1.10	1.08	0.95	1.06	1.03	1.28	-	-	-	-	-	9.10	4.60
846	1.18	1.36	1.01	1.13	0.98	0.88	1.28	1.74	2.30	5.04	11.00	-	4.15	3.20	3.85
847	1.00	0.71	1.01	0.75	1.09	1.43	1.07	1.29	0.97	-	-	1.44	-	-	9.47
880	0.76	1.04	0.97	1.19	1.24	1.51	-	2.20	2.25	2.25	2.50	-	1.95	-	2.20
883	1.39	1.32	1.37	1.25	1.45	1.49	1.43	1.39	-	1.39	-	-	-	-	

(b) SGOT concentrations (S.F./ml)

807	-	145	180	-	640	300	730	1050	555	1100	-	1040	-	-	-
811	-	84	123	420	290	930	540	910	-	1555	-	-	1610	-	1550
813	-	-	123	242	-	260	-	-	-	1860	1810	-	-	650	510
840	-	85	95	119	110	105	155	240	-	-	-	-	-	3000	1760
846	255	950	150	450	112	94	100	580	950	2020	1610	-	1610	1140	1420
847	142	150	146	112	181	330	190	430	270	-	-	710	-	-	1290
880	88	137	137	280	240	630	-	1915	2550	1810	1120	-	1460	-	1100
883	720	430	1000	510	660	1055	510	510	-	450	-	-	-	-	-

(c) SGPT concentrations (S.F./ml)

807	-	8	6	-	35	20	15	64	18	13	-	13	14	-	-
811	-	9	5	26	26	57	21	64	-	14	-	-	42	-	22
813	-	-	5	10	-	11	-	-	-	190	130	-	-	34	19
840	-	7	10	13	15	14	23	20	-	-	-	-	-	125	92
846	14	18	14	16	11	11	10	15	14	15	21	-	125	119	79
847	7	13	14	7	10	17	11	12	13	-	-	11	-	-	122
880	12	13	10	16	15	17	-	80	34	28	37	-	46	-	64
883	14	11	16	11	8	10	11	11	-	11	-	-	-	-	-

(d) Packed Cell Volumes (%).

	Days before Death.														
	150	125	100	75	50	30	20	15	10	6	5	4	3	2	1
807	-	-	-	-	18.5	36.0	43.0	21.0	18.5	22.5	-	22.0	-	-	-
811	-	-	-	27.0	25.0	18.5	21.0	22.5	-	24.0	-	-	24.0	-	24.0
813	-	-	-	-	-	42.0	-	-	-	25.5	-	-	-	-	-
840	-	-	-	-	37.0	36.5	38.0	39.0	-	-	-	-	-	20.0	19.0
846	-	43.5	35.5	37.5	32.5	35.0	35.0	47.5	48.5	42.0	39.0	-	13.5	14.5	15.5
847	34.0	34.5	33.5	32.0	33.0	34.0	33.5	38.0	34.5	-	-	38.0	-	-	-
880	-	-	-	38.0	41.0	41.0	-	29.0	33.5	30.5	-	-	31.0	-	32.0
883	37.0	34.0	36.5	36.0	36.0	39.0	42.0	41.0	-	43.0	-	-	-	-	-

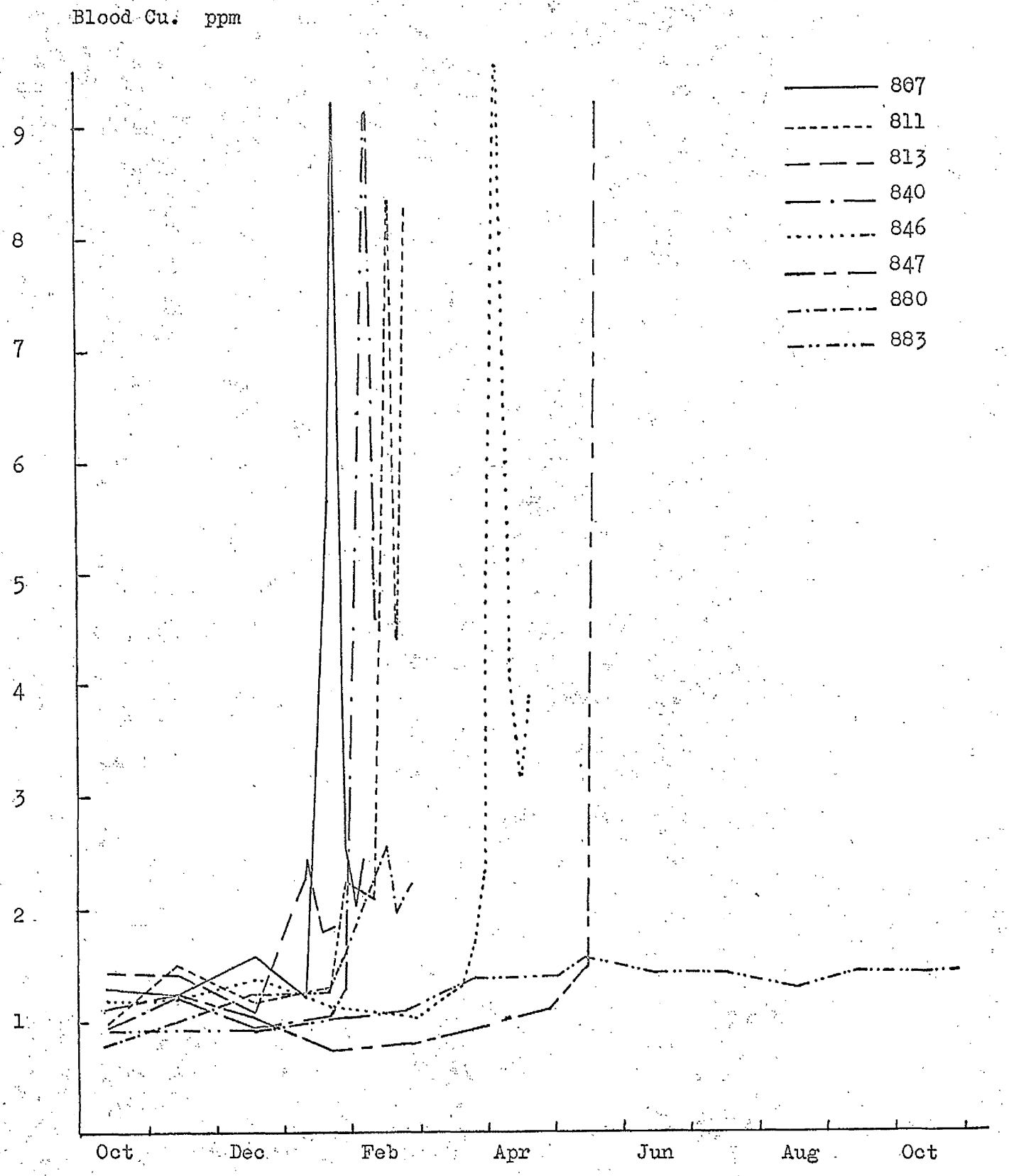
(e) Haemoglobin Contents (gm/100 ml)

807	-	-	-	-	-	-	-	6.3	6.0	7.8	-	7.7	-	-	-
811	-	-	-	-	-	5.6	5.9	7.4	5.2	7.4	-	-	-	-	7.8
813	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
840	-	-	-	-	-	-	-	-	-	-	-	-	-	7.8	5.7
846	-	-	-	13.2	11.9	12.6	11.9	16.3	16.2	14.4	13.7	-	6.8	6.3	6.0
847	-	12.0	11.4	11.2	11.8	13.2	11.9	13.4	12.0	-	-	13.1	-	-	-
880	-	-	-	-	-	-	-	9.6	12.0	10.3	-	-	11.2	-	10.7
883	14.0	11.9	11.3	11.7	12.5	12.6	14.4	15.0	-	15.0	-	-	-	-	-

Table 115. Days to death when first elevated blood copper concentration found; the copper concentration on that day and over the previous range of samples.

No.	Days to Death	Cu conc(p.p.m.)	Previous Range
807	52	1.57	0.60 - 1.23
811	26	2.23	0.98 - 1.49
813	7	2.26	1.09 - 1.44
840	2	9.10	0.95 - 1.28
846	14	1.74	0.88 - 1.36
847	4	1.44	0.71 - 1.29
880	28	1.51	0.76 - 1.26
883	-	-	0.91 - 1.55

Fig 35. Changes in the blood copper concentration of sheep which died of copper poisoning.



An elevated value for each of the following tests is taken as one which was noticeably and generally significantly higher than the previous range of values obtained for any individual sheep.

This shows that for sheep No. 807 there was a significant increase in blood copper concentration some seven weeks before death while for sheep No. 840 this occurred only 2 days before death. In four sheep elevated blood copper concentrations were found only during the last week before death and in fact an elevated blood copper level was never found for sheep No. 883 although samples were obtained until 6 days before death. The mean number of days to death when elevated blood copper concentrations were first found was sixteen.

SGOT concentration

The SGOT concentrations of all the individual blood samples taken from each of the 8 sheep are detailed in Table 114 (b). Table 116 lists the number of days to death when abnormal GOT values first occurred.

Table 116 The first abnormal SGOT concentration found in relation to the time to death and the previous range of concentrations.

No.	No. of days to death	SGOT conc. (S.F.)*	SGOT Previous Range	Cu Conc. on same day
807	52	640	140 - 180	1.57
811	33	880	84 - 420	1.30
813	31	260	121 - 240	1.09
840	16	240	85 - 155	1.28
846	14	580	90 - 450	1.74
847	39	430	59 - 181	1.29
880	35	580	88 - 280	1.26
883	-	-		

* S.F. = Sigma Frankel units/ml. serum.

The normal range of SGOT concentration found prior to the elevated values which occurred during the latter stages of the illness was 80 TO 200 S.F. SGOT concentrations for the control group were always within the range 68 - 186 S.F. throughout the course of the experiment. Abnormally high SGOT concentrations were found over periods varying from 14 days before death for sheep No. 846 to 52 days before death for sheep No. 807. In the case of sheep No. 883 SGOT levels were abnormal and varied widely for 10 months prior to death so it was impossible to relate a high SGOT value on a specific date with an approaching haemolytic crisis. The abnormal SGOT values found for this sheep must have been attributable to some cause other than the toxic effect of copper. Excluding sheep No. 883 the mean number of days to death when elevated SGOT concentrations first occurred for the other seven sheep was thirty-one.

SGPT Concentration

The SGPT concentrations of all the blood samples obtained from each of the 8 sheep are presented in Table 114 (c). Table 117 details the number of days to death when elevated SGPT levels were first found for each of the 8 sheep.

Table 117. The first elevated SGPT concentration found in relation to the time to death and the previous range of concentrations.

No.	Days to death	SGPT conc. (S.F.)	SGPT Previous Range	Cu conc. (p.p.m.) on same day.
807	52	35	6 - 10	1.57
811	33	60	5 - 26	1.30
813	7	130	5 - 11	2.26
840	2	125	7 - 23	9.10
846	3	125	8 - 21	4.15
847	1	122	4 - 17	9.47
880	28	30	10 - 17	1.51
883	5	-	6 - 17	-

The normal range of SGPT concentrations was from 5 - 26 S.F. with the majori of samples having a concentration below 20 S.F. For five of the eight sheep no elevation in SGPT concentrations occurred until one week or less before death due to copper poisoning. Elevated SGPT values were found for the other three sheep (Nos. 807, 811 and 880) from between 4 and 7 weeks before death. The elevated values found ranged from 30 - 130 S.F. Generally, however, no elevated values were recorded until after the onset of the haemolytic crisis. The mean number of days to death when elevated SGPT levels were first obtained was sixteen.

Elevated PCV Levels.

The packed cell volumes of the blood samples obtained from the 8 sheep are detailed in Table 114 (d). Table 118 details the number of days to death when elevated PCV levels were first found for each of the 8 sheep.

Todd and Thompson (1963) reported that an increase occurred in the PCV of sheep during the last 10 days to a week before the haemolytic crisis. They quoted the normal range as being between 35 - 40% which rose during this period in some cases to as much as 50 - 55%. The normal range found in the present experiment was from 34 - 38% while the elevated values recorded ranged from 40 - 49%. In three cases no elevation in PCV was found and in three others the elevated values found were in the range of only 40 - 42%.

Table/

Table 118. The first elevated PCVs found in relation to their previous range and to the time of death of the individual sheep.

No.	Days to Death	PCV (%)	PCV (previous range)	Cu conc. (p.p.m.) on same day
807	19	43.0	18.5 - 36.0	5.64
811	-	-	-	-
813	31	42.0	-	1.09
840	-	-	-	-
846	13	47.5	31.0 - 39.0	1.74
847	58	40.0	30.5 - 35.5	1.37
880	-	-	-	-
883	21	42.0	27.0 - 39.0	1.43

Table 119 The first lowered PCV found in relation to their previous range and to the time of death of the individual sheep.

No.	Days to Death	PCV(%)	PCV (Previous range)	Cu conc. on same day
807	52	18.5	-	1.57
811	33	22.0	25.0 - 27.0	1.30
813	7	25.5	42.0	2.26
840	2	20.0	36.5 - 39.0	9.10
846	2	13.5	31.0 - 48.5	3.20
847	-	-	30.5 - 40.0	-
880	13	29.0	38.0 - 41.0	2.20
883	-	-	27.0 - 43.0	-

Lowered PCV Levels

The number of days to death when lowered PCV levels were first recorded for each sheep is presented in Table 119.

In six of the eight sheep abnormally low PCVs were found only within the last 2 weeks before death and five of these six did not occur until 7 days or less before death. The other two sheep, Nos. 807 and 811, had abnormally low PCVs some seven and five weeks respectively before death. In the case of sheep No. 807 this level returned to normal and even above normal during the following 4 weeks before finally falling again to an abnormally low level (20%) during the last 10 days before death. The PCV of sheep No. 811, however, maintained an unusually low level (14.0 - 24.0%) during the 4 weeks preceding death from copper poisoning.

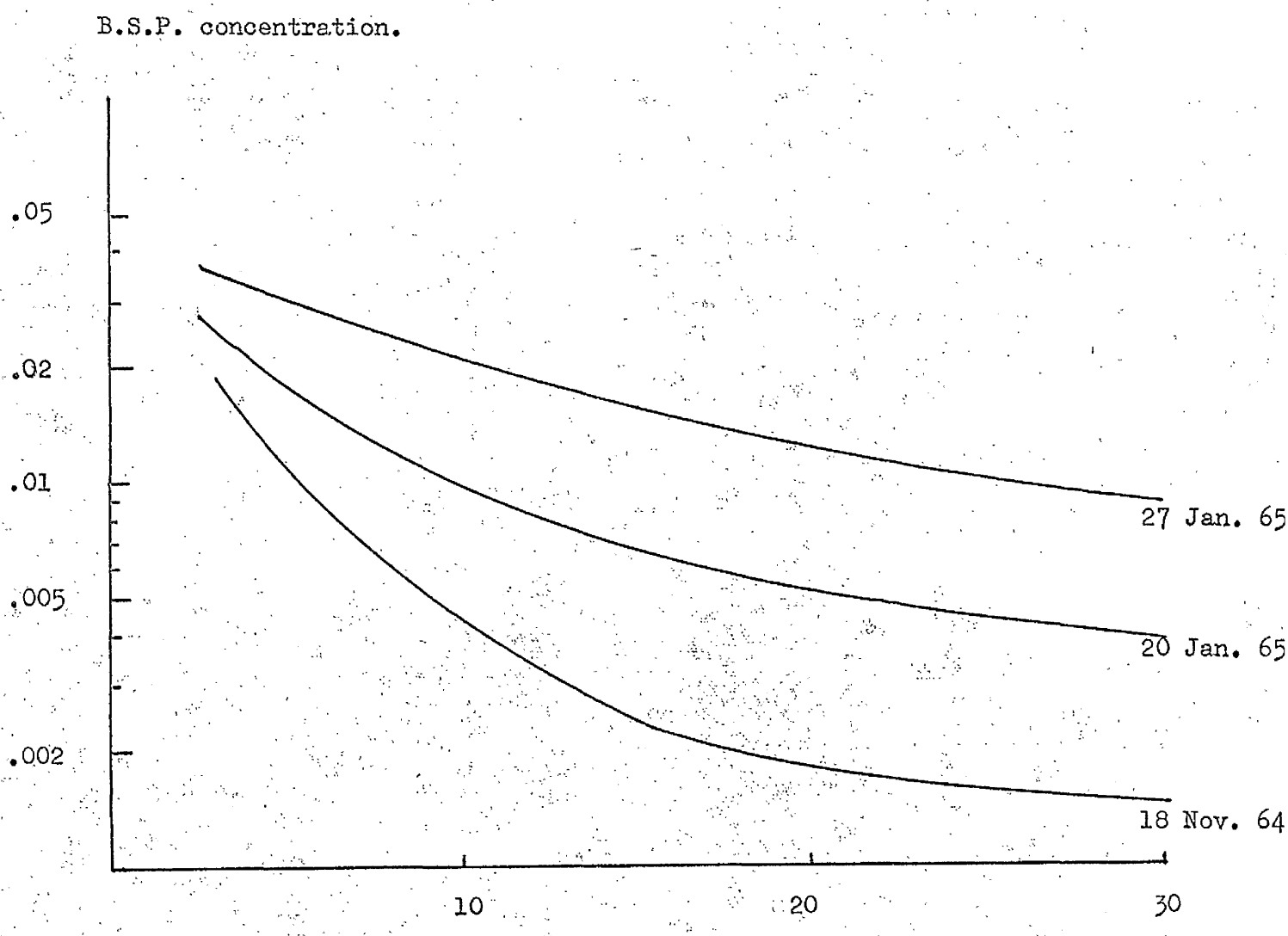
Haemoglobin Contents.

Haemoglobin (Hb) contents of the blood samples obtained from the 8 sheep are detailed in Table 114 (e). These showed a similar trend to the PCVs. Elevated PCVs of over 40% were associated with Hb contents of 13 - 16 gm/100 ml. while lowered PCVs of around 20% were accompanied by Hb contents of 5.0 - 7.5 gm/100 ml.

B.S.P. Clearance.

In normal sheep intravenous injections of bromosulphthalein (B.S.P.) are generally cleared from the bloodstream within 15 - 30 minutes of injection. As the liver becomes progressively more damaged there is a progressive increase in the length of time taken for the dye-stuff to be eliminated. This is illustrated in Fig. 36 which shows the rate of clearance of B.S.P. from the bloodstream/

Fig 36. The rate of clearance of B.S.P. from the blood of sheep 807 which received 1.0 g of copper sulphate each alternate day.



bloodstream of sheep No. 807 on three different occasions. Firstly when the sheep was in good health it cleared the dye stuff within 15 mins; secondly a much slower rate of clearance of the dye stuff with some still remaining after 30 mins. and finally as death due to chronic copper toxicity approached with a consequent degeneration of the liver cells there was a very poor rate of clearance with a high concentration of the dye stuff still present 30 minutes after injection.

This technique was not employed very frequently but on three occasions when it was used during the 14 days before death of sheep Nos. 807, 846 and 880 the dye was not cleared from the bloodstream within 30 minutes of injection. B.S.P. was rapidly cleared from the blood of another three sheep (Nos. 811, 840 and 880) which were injected during the period 16 to 30 days before death.

The Relative Merit of the Various Tests in providing an indication of an Approaching haemolytic crisis.

Abnormal SGOT concentrations were the earliest indicators of an approaching haemolytic crisis for six of the eight sheep. The two exceptions, sheep Nos. 847 and 883, both had elevated PCVs before there was an increase in their SGOT concentrations. Sheep No. 883 however, is perhaps exceptional in that it had some very high SGOT levels (1300 S.F./ml) in the early stages of the experiment long before it exhibited any increase in PCV. These high SGOT levels could not be related to the approach of the terminal phase of chronic copper poisoning and must have been associated with some other cause.

Table/

Table 120 gives an indication of the comparative usefulness of the various tests in diagnosing the approach of the climactic stage of chronic copper toxicit in sheep.

Table 120. The % of sheep found with abnormal values for each of six separate tests in the latter stages of chronic copper poisoning.

Days before Death	Cu	SGOT	SGPT	Elevated PCV	Lowered PCV	B.S.P.
36 - 64	16.7	50.0	14.3	28.6	14.3	0.0
22 - 35	25.0	71.4	25.0	14.3	14.3	0.0
16 - 21	33.3	80.0	20.0	33.3	16.7	33.3
11 - 15	80.0	100.0	60.0	40.0	60.0	100.0
5 - 10	71.4	100.0	28.6	33.3	57.1	100.0
0 - 4	100.0	100.0	85.7	0.0	81.7	100.0

This illustrates that of the tests employed the SGOT concentration was the most sensitive indicator of copper poisoning. All sheep examined during the last 15 days, before death, had elevated SGOT levels; 80% of those examined during the period 16 - 21 days before death had elevated concentrations and 50% still had elevated values during the period 36 - 64 days before death. There was at best only a one in three chance of obtaining elevated values for the other five tests during the 15 - 21 days period before death. Apart from the possibility of an elevated PCV there was very little chance of abnormal values in determinations other than SGOT during the 36 - 64 day period before death.

In/

In all cases there was at least one of the six tests which showed an abnormal value more than two weeks before death. On the basis of the results found in this experiment, therefore, it would appear probable that where blood sampling is carried out fairly regularly some indication of the onset of illness due to copper poisoning would be detected at least two weeks before death. This would enable any treatment prescribed to have a perhaps more reasonable chance of effecting a cure.

However, under normal circumstances regular blood sampling is not practised and a slightly elevated concentration for one of those tests might be discarded as being due to contamination or incorrect analysis and thus the opportunity for early and successful treatment might be lost. Sampling a larger number of sheep than those with existing clinical signs might be useful.

The difficulty of assessing the importance of a slightly elevated SGOT concentration can be simply illustrated by comparing the SGOT concentrations of sheep Nos. 840 and 883 on 28 January 1965. On that date the SGOT concentration of sheep No. 840 was 240 S.F. and of sheep No. 883 was 580 S.F. If these had been isolated samples it would have been reasonable to assume that sheep No. 883 was the more severely affected of the two. However, the previous range of SGOT concentrations for sheep No. 883 was from 94 - 790 S.F. while that for sheep No. 840 was from 85 - 155 S.F. Thus the concentration for sheep No. 840 on 28 January was significantly higher than the mean of the previous samples while that for sheep No. 883 fell within the previous range.

On/

On this basis then the more reasonable assumption would be that the condition of sheep No. 840 was deteriorating while that of sheep No. 883 was remaining static. This was indeed borne out by subsequent events in that sheep No. 840 died within 16 days of 28 January. In contrast sheep No. 883 survived for a further 9 months after this date.

Changes in the output of urinary copper by sheep approaching death due to chronic copper toxicity.

When a sheep was found to have either an elevated blood copper or SGOT concentration and/or lowered PCV and Hb values it was removed from the group and placed in a metabolism cage and daily collections of urine were taken. It was thus possible to gain information on the changes in the pattern of urinary copper excretion in sheep nearing death due to chronic copper toxicity.

Fig. 37 shows the total daily excretion of copper in the urine by five of the sheep related to their time of death. The urinary copper output of a sixth sheep is also shown. This is sheep No. 883 which although it was receiving the same treatment as the other five showed no elevation in urinary copper excretion by the time the remainder had succumbed.

The period during which collections were made varied from only two days before death for sheep No. 840 to more than a month before death for sheep No. 846. During the period when the sheep were being dosed regularly with 1 g. $CuSO_4.5H_2O$ on alternate days the normal level of copper excretion in the urine appeared/

appeared to be around 300 ug per day. This was the level of copper excretion found for sheep No. 883 and also for sheep No. 846 during the 3 weeks collection period prior to the onset of symptoms of copper toxicity.

During the last few days of life, however, the amount of copper excreted in the urine increased markedly and was only rarely less than 1000 ug/day. The mean daily output of copper in the urine by sheep No. 811 over the 12 day period immediately before death was more than 2000 ug. In the case of all five sheep shown in Fig. 37 which died of copper poisoning there was an appreciable decrease in the output of urinary copper on the day before death compared to the mean output over the previous number of collections.

This increased copper excretion in the urine during the terminal stages of the illness is probably caused by the release into the bloodstream of the copper stores which have been built up in the liver. This is thought to cause the extensive haemolysis which is characteristic of chronic copper poisoning. As a result of this free haemoglobin is released into the plasma and is eventually excreted in the urine.

Haemoglobinurea is fairly common in the latter stages of the disease and causes the urine to become a reddish or even purply black colour. This colour varies with the severity of the haemolysis and a typical example is illustrated in Fig. 38.

Fig. 37 Changes in the total daily excretion of copper in the urine (ug) of sheep approaching death due to chronic copper poisoning.

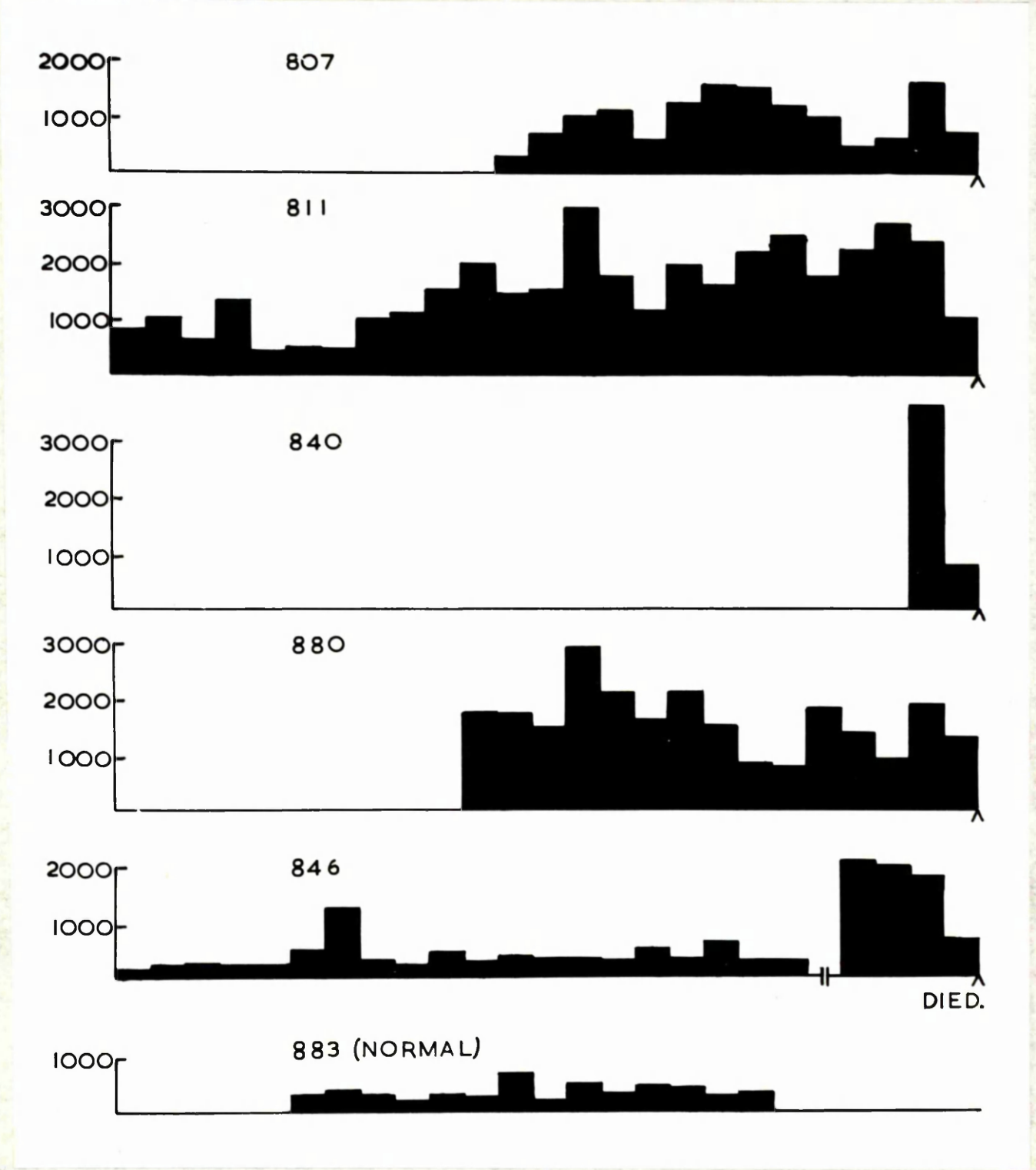
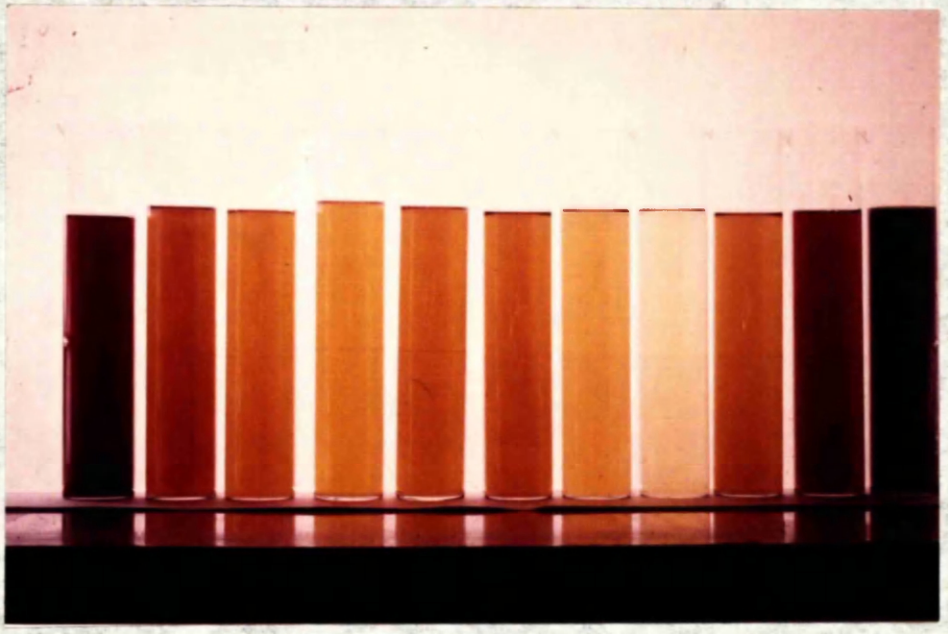


Fig. 38. Sheep No. 811. Changes in urine appearance over 11 days.



Following an initial crisis in the first sample a progressive recovery was made until the onset of a second crisis nine days later.

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APPENDIX

Chemical Analyses - Laboratory Procedures.

Copper. (Brown & Hemingway, 1962)

Reagents. 1. Acid Digestion Mixture. 400 ml. nitric acid, 200 ml. perchloric acid and 100 ml. sulphuric acid are mixed together. All acids should be Hopkins and Williams lead free reagents.

2. Zinc dibenzyldithiocarbamate. Stock solution - 0.5 g. dissolved in 500 ml. carbon tetrachloride A.R. Stock solution diluted 1:1 with carbon tetrachloride to give 0.05% working solution.

Whole Blood.

Procedure. Pipette 5 ml. whole blood into a micro kjeldahl flask. Add 7 ml. acid digestion mixture. Heat gently until brown fumes appear and then allow reaction to subside. Digest slowly over a period of several hours to remove the nitric acid. If any black organic matter remains, cool, add more nitric acid and redigest. Continue heating for 10 minutes after appearance of white fumes of sulphuric acid. The liquid should be colourless. Allow to cool, add 10 ml. de-ionised water and heat to boiling. Cool and transfer to a 50 ml. separating funnel, washing with de-ionised water until volume is about 30 ml. Add 5 ml. 0.05% zinc dibenzyl dithiocarbamate, stopper and shake vigorously for 2 minutes. Withdraw lower layer and filter through cotton wool into optical cuvette. Measure colour intensity in Eel Spectra at 435mu. Set zero with carbon tetrachloride. Carry out blank determination at same time.

Liver/

Liver, Kidney, Grass and Faeces.

Procedure. Weigh into a micro kjeldahl flask, 1 g. sample which has been dried and ground and digest in similar manner to that for blood. Make the resulting acid solution up to a 100 ml. in the case of liver, kidney or faeces and to 50 ml. in case of grass. Pipette a suitable aliquot into a 50 ml. separating funnel, adding de-ionised water to give a total volume of about 30 ml. and proceed as for blood.

Urine.

Procedure. Pipette 25 ml. sample into a 100 ml. beaker and add 10 ml. acid digestion mixture. Cover with watch glass and heat on hotplate, till white fumes of sulphuric acid appear. Solution should be colourless. Allow to cool, add 10 ml. water and heat to boiling. Make up to 100 ml. Pipette suitable aliquot into a separating funnel and proceed as before.

Preparation of Standard Curve.

Standard curve is prepared by digesting a series of copper solutions containing between 0 and 8 ug. copper and following the above procedure.

Iron (Bothwell & Mallett, 1955).

Reagents. 1. Acid Digestion Mixture. Prepared as for copper.

2. Hydrochloric Acid. 2N aqueous solution.

3. Trichloroacetic Acid. 20% aqueous solution.

4. Thioglycollic Acid. Analar.

5. 2:2'-dipyridyl. 0.4% aqueous solution.

6. Sodium Acetate. Saturated solution.

Blood Plasma

Procedure. Add 2 ml. 2N HCl to 4 ml. plasma and stir with glass rod. Add 2 ml. 20% TCA and stir vigorously for 45 seconds. Centrifuge for 15 minutes at 3000 rev./min. Pipette 5 ml. supernatant liquid into a tube containing 2 drops thioglycollic acid 0.5 ml. 0.4% 2:2'-dipyridyl and 2.5 ml. saturated solution of sodium acetate. Stopper tube and shake mixture thoroughly. Measure colour intensity in Eel Spectra at 520 mu. Carry out blank determination at same time.

Liver, Kidney, Grass, Faeces and Urine.

Digest samples and make up to 100 ml. or 50 ml. as for copper. Pipette 5 ml. solution (or less plus de-ionised water to 5 ml.) into tube containing reagents and proceed as for plasma iron.

Preparation of Standard Curve.

Standard curve is prepared by pipetting 5 ml. of a series of solutions containing 0.4 to 4 ug./per $\frac{\text{iron}}{\text{ml.}}$ into previously prepared tube and following above procedure.

Calcium in Plant Material.(Hemingway, 1956.)

Cation Exchange Resin Columns. Resin used is Amberlite IR - 120 (H), ground so that it passes the 60 but is retained by the 120 mesh B.S. sieve. It is packed to depth of 5 cm. in 35 cm. x 7 mm. tapered open glass tubes and held by cotton wool plugs. 10 ml. water should run through in not less than 20 minutes. Resin is prepared for use by washing with 5 N nitric acid and then several times with water.

Procedure. Ash 1 g. of plant material at 500°C, add 1 ml. concentrated HCl and evaporate to dryness. Dissolve residue in hot water and filter the solution into a 100 ml. volumetric flask, dilute to the mark and shake. Pipette 5 ml. into ion exchange column, allow to drain and wash column twice with 5 ml. portions of water, rejecting all the washings. Place 10 ml. calibrated flask beneath the ion exchange column and elute the calcium with 5N nitric acid until 10 ml. of eluate are obtained. With appropriate filter in position set the flame photometer at 0 with 5N nitric acid and at 100 with solution containing 100 p.p.m. calcium in 5 N nitric acid and then take readings for solutions.

Preparation of Standard Curve.

Prepare a series of solutions containing from 0 to 100 p.p.m. calcium and pass 10 ml. of each through the ion exchange columns and then follow the above procedure.

and wait exactly 5 minutes before extracting with ether. Add 10 ml. isopropyl ether. Shake 100 times by hand. Draw off aqueous layer and measure colour intensity of ether layer in Eel Spectra at 475 mu.

Preparation Standard Curve.

This is prepared as above by using aliquots of molybdenum solution to give a range of 0 to 8 ug. 10 ml. concentrated HCl is added to each standard before making up to 100 ml. and adding reagents.

Sulphur in Plant Material. (Cunningham, 1962).

Reagents. 1. Magnesium Nitrate. 125 ml. 8N nitric acid are added to 20 g. magnesium oxide. Magnesium nitrate is dissolved in water, filter and made up to 250 ml.

2. Barium Chloride. 5% aqueous solution.

3. 6N Hydrochloric Acid.

4. 1 N Hydrochloric Acid.

5. Fuming Nitric Acid. A.R.

6. 2% Ammonium EDTA solution. 10 g. ethylene diaminetetracetic acid are dissolved in 100 ml. water containing 50 ml. ammonia solution (s.g. 0.88) and diluted to 500 ml.

Procedure. Weigh 200 - 500 mg. of dried plant material into a 100 ml. beaker. Add 1 ml. water and 2 - 5 ml. fuming nitric acid, cover with a watch glass and leave overnight. Rinse watch glass, add 2 - 4 ml. magnesium nitrate solution and evaporate to dryness on a steam bath. Place in cold muffle furnace and heat for 2 hours at 450°C. Cool add 4 ml. 6N HCl and evaporate to dryness to dehydrat the silica. Add 1 ml. 1N HCl and 5 - 10 ml. water. Warm until all salts are dissolved and filter through no. 52 filter paper into a glass centrifuge tube. Rinse the beaker and wash the filter paper with warm water until the volume in the tube is 25 ml. Add dropwise 1 ml. 5% barium chloride solution to the filtered digest. Mix allow to stand for 15 minutes and centrifuge at/

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at 4000 rev/min. for 3 minutes. Remove the supernatant liquid without disturbing the barium sulphate precipitate. Wash precipitate with 25 ml. water. Centrifuge/the and remove supernatant liquid. The precipitate is dried on a water bath. Pipette 10 ml. ammonium EDTA solution into the tube and measure the barium using Eel flame photometer with Wratten No. 77 filter and air pressure 10 lb/sq. Set zero with re-agent blank and set at 100 with solution prepared with 6 ml. aliquot of sulphate standard containing 3000 ug.S.

Preparation of Standard Curve.

This is prepared by taking 1 to 6 ml. aliquots of standard sulphate solution containing 500 - 3000 ug.S and preparing barium sulphate solutions in ammonium EDTA as previously described.

SUMMARY.

The literature related to the copper concentration of plants and anima has been reviewed and an account has been given of the effects of copper deficiency and toxicity in sheep. Reference has also been made to the meth employed in the correction of copper deficiency in sheep.

Herbage copper concentrations were found to be affected only transient by soil applications of copper sulphate and not at all by liming. Copper concentrations were, however, invariably increased by treatment with nitrog fertilizers. A positive relationship between herbage copper concentration and crude protein content was established. A similar relationship was foun exist between the copper and crude protein contents of hay and silage.

Sheep grazing limed pasture were found to have lower blood and liver c levels than similar sheep grazing unlimed pasture although there was no di in the copper concentration of the grazed herbage. Injections providing 4(mg Cu given to ewes in mid-pregnancy increased ewe blood copper concentrat and the liver copper concentrations of their lambs when compared with untr controls. The incidence of swayback was almost completely prevented by th treatment.

A high level of dietary protein was found to be effective in reducing toxicity/

toxicity of an excessive intake of copper to sheep. It apparently reduced proportion of the copper intake which was stored in the liver. A very high calcium intake (3.8%) markedly reduced the proportion of the dietary intake copper which was stored in the liver. Sheep on barley-based diets stored a smaller proportion of their dietary intake of copper than did similar sheep f maize-based diets. Very young lambs being fattened intensively indoors wer apparently more susceptible to the build up of high levels of copper in the livers than older sheep on similar intensive diets. Chronic copper toxicit was unlikely to develop in sheep housed from 6 months of age unless they we housed for very long periods.

The effect on the urinary and faecal copper excretion of sheep of a) copper supplementation and b) intravenous injection of various chelating ag has been studied. Penicillamine given intravenously markedly increased the urinary copper output of sheep. In contrast, EDTA had only a small effect urinary copper excretion but markedly increased the urinary output of iron.

The usefulness of various blood tests in giving a prior indication of approach of the terminal stages of chronic copper toxicity was examined. increased concentration of glutamic-oxalacetic transaminase in the plasma w a constant feature for at least two weeks (and frequently for period of up seven weeks) before death. The other tests employed gave irregular results until after the occurrence of obvious clinical distress.