STUDIES ON THE METABOLISM OF COPPER

Submitted as a Thesis for the Degree
of Ph.D. of Edinburgh University.

by

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CONTENTS

Section IV /

Introduction

Copper, as a trace element, has received much attention in recent years on account of its great activity as a catalyst involved in reduction-oxidation systems and the important part it plays in plant and animal life. Specific compounds of copper have long been recognised in plants and in the lower animals, notably haemocyanin in the bodies of some marine animals. In the latter, haemocyanin and hence copper diminishes in amount as the biological scale is ascended; and in fishes that are equipped with haemoglobin as their main carrier of oxygen, copper is found only in extremely small quantities.

Most of the early work on the function of copper in the vertebrata showed the essential nature of this element as a supplement to iron in the formation of haemoglobin. It does not influence the assimilation of iron but acts as a catalyst in the incorporation of this element into the haemoglobin molecule. Evidence of this was first seen in the work of Hart et al (1928) who demonstrated the ineffectiveness of highly purified iron salts for haemoglobin formation in anaemic rats and the strikingly beneficial results obtained by the addition of a little copper to the iron taken by the animals.

The/

The sphere of influence of copper is, however, much wider than its role in haemoglobin synthesis and it is quite possible that the effect of copper on haemoglobin formation is entirely secondary to, and dependent upon, its effect on general cell processes. It is intimately associated with both plant and animal tissue oxidases, several of which appear to be copper-protein complexes. In accordance with the view that an enzyme consists of a protein combined with an active prosthetic group, the copper in the enzyme may be looked upon as the prosthetic group, the protein component determining the specificity of the enzyme. The copper-containing proteins, haemocuprein and hepatocuprein, of unknown significance have been isolated by Mann and Keilin (1938) from ox blood and ox liver respectively. Neither the haemocuprein nor the hepatocuprein of Mann and Keilin combined with molecular oxygen or had the catalytic properties of peroxidase, catalase, polyphenol oxidase or cytochrome oxidase. As against this Holmberg and Laurell (1949) claim that a serum copper-protein complex which they have recently isolated, has very specific catalytic properties.

The above work is important but its relationship to the metabolic disorders that occur in

domestic/

domestic animals from copper deficiency is not yet apparent.

The Distribution of Copper in the Organs of Animals.

A general division of the organs of animals may be made into two classes :-

(a) those containing a high proportion of copper;
(b) those containing a low proportion of copper.

In (a) are included liver, kidneys, heart, brain and hair, while (b) includes skin, lungs, pancreas, spleen and muscle. Of all the organs, the liver contains the most copper, either when expressed as total copper in the organs or as a percentage of fresh tissue. However, a very wide range of values for specific organs exists within each species.

In general it is found that a considerable storage of copper occurs in intra-uterine life, (Morrison and Nash, 1930), (McHargue, 1925), Cunningham, 1931) - possibly to enable blood formation and other processes to continue in spite of the low copper content of milk. This accumulation is so considerable that the percentage of copper in the livers of foetal and newly-born animals is many times greater than that of the adult. A notable exception to this is the sheep, in which the adult liver is very much richer in copper than that of the/

the foetal or neo-natal lamb. In all cases, including lambs, there is a steady drop in liver copper concentration throughout early infancy (Cunningham, 1931). This may be attributable to some extent to the relatively rapid increase in size of the liver and other tissues where copper is laid down, as much as to the copper intake not compensating copper wastage. Thereafter, the copper content of the liver in adult life reflects to some extent the copper content of the diet with which the animal has been sustained.

Elvehjem (1935), in commenting on the distribution of normal blood copper between cells and plasma, mentions that he and co-workers (1929) found the major portion in the corpuscular fraction. This was confirmed by Sarata (1933). Schindel (1931) and Guillemet (1932), however, have reported more copper in the plasma than in the cells, whilst Tompsett (1934) found the blood copper to be approximately equally distributed between the two. Eden and Green (1939) confirmed the work of Tompsett. This equal distribution has since been substantiated and it is generally accepted that difficulties with the earlier methods for the estimation of copper, i.e. prior to the work of Tompsett, may have been responsible for lack of decisive information on this point.

"Normal"/

"Normal" values for blood and live copper of sheep.

The copper content of the blood of normal sheep would appear to vary within wide limits. This becomes obvious when large groups of animals are examined (Eden et al, 1945) since a very wide range of blood copper values exists within each group. This reduces the significance of individual values. When the mean values of groups in different areas are compared a wide range is again apparent.

In view of these points, it is not thought possible to accept any single value as a standard for blood copper level.

From a review of the literature it would appear that some confusion exists in defining the term "normal" blood copper level. In some papers this term is loosely used with reference to the blood copper value of sheep in both swayback and non-swayback areas. The term "normal" in these cases is not indicative of a standard "mean" value for an area but implies a blood copper value that is accepted as a standard for differentiating sheep that have given birth to swayback lambs from those that have given birth to "normal" healthy lambs.

As individual values have very little significance, it is intended to use the term "normal" range, throughout this thesis, to indicate the range of/

of means of groups of animals from different areas in which swayback is unknown.

The normal range for blood copper values of sheep in Britain can be taken as 0.060 - 0.120 mg. Cu /100 ml. whole blood. A mean value of $<$ 0.030 mg. Cu /100 ml. (Eden 1945) in a group of animals is taken as indicative of swayback occurring within the group.

Few large groups of sheep have been studied for liver copper values and since such large variations occur it is considered that only some indication of the range of individual values can be given, viz. 0.30 - 10.0 mg. Cu/100 g (fresh weight)(Cunningham, 1931, Eden et al 1945).

Disorders in Sheep and Cattle Associated with Copper Deficiency.

Although various disorders can be produced in laboratory animals, such as the rat, by feeding a copper deficient diet, cattle, sheep and goats, are the only animals known to be affected under natural conditions. These domestic animals have not the varied diet available to man and are largely dependent on the mineral intake from pasture which in turn is dependent upon the mineral uptake from soil over a very limited area. It is not surprising, therefore/

therefore, to find in various countries, syndromes occurring in these animals that are associated with low levels of copper in the blood and organs, especially the liver. These conditions can be prevented, and in some cases cured, by increasing the intake of copper. In other areas, however, disorders are known in which, although the manifestation and preventive treatment are indicative that the animal is suffering from a copper deficiency, the copper content of the soil and herbage may be normal.

Enzootic Ataxia.

Perhaps the most valuable work indicating the importance of copper in the metabolism of ruminants, was carried out by Bennetts (1937) and his colleagues in Western Australia, where they showed that "enzootic ataxia" in lambs, a demyelinating disease developing in the foetus during late gestation, was preventable by the administration of small amounts of copper salts during pregnancy. The occurrence of the disease was shown to be associated with a low copper status of both ewes and lambs and a low copper content of the herbage (< 5 p.p.m.)

The same condition occurs in sheep in other parts of Australia and in limited areas of New Zealand.

Swayback/

Swayback

The condition of "swayback" in lambs is widespread, in varying incidence, throughout Britain and is characterised, clinically, by spastic paralysis and inco-ordination of movement; pathologically, by diffuse symmetrical demyelination of the cerebrum and secondary degeneration of the cord (Innes and Shearer, 1940). In acute cases, gross cavitation of both hemispheres may be found. The clinical signs are usually evident at birth, but where cerebral demyelination is slight a delayed form appears and inco-ordination of movement may not appear for several weeks.

Blood copper values for the ewe are generally low, <0.030 mg/100 ml., but ewes with a blood copper value as low as 0.010 mg/100 ml., may still deliver a healthy lamb (Eden, 1944). Copper reserves in the maternal liver are generally low. This condition corresponds very closely with the "enzootic ataxia" of Western Australia there being the important etiological difference that, whereas in Australia it is associated with pastures low in copper ($<$ 5 p.p.m. dry matter), in Britain it occurs irrespective of the copper status of the pasture, (7 - 15 p.p.m. dry matter or more).

Evidence suggests that in the disease in Britain some unknown factor is involved which either depresses the availability of copper in the plant during/

during digestion in the alimentary tract or in some way disturbs copper metabolism in the animal itself.

Steely Wool in Sheep.

According to Lee and Moule (1947) copper deficiency in the sheep in Australia reveals itself first as a marked deterioration of the fleece. As the copper reserves of the animal are depleted, the crimp becomes progressively less distinct until the fibres emerge entirely devoid of character. The capacity of the follicules to impart crimp returns immediately after the resumption of a normal copper status and the staple that subsequently grows resumes boldly the character of the particular wool-type of the individual.

This condition has also been described by Bennetts (1932) in Western Australia but so far it has not been reported in Britain.

"Falling Disease" in Cattle.

In certain areas in Western Australia where ataxia occurs in lambs, another enzootic disorder is found in dairy cattle. This,"falling disease", is characterised by loss of condition and sudden death (Bennetts et al, 1948). The clinical signs of "falling disease" are evident in adult cattle "especially/

"especially during the flush growth of pasture in spring when the cows are in late pregnancy and producing milk at a high rate. Despite the abundance of food there is a loss of condition, depraved appetite, rough staring coat and often anaemia. This anaemia is of the macrocytic, hypochromic type and is accompanied by haemosiderosis. It spontaneously disappears during the summer months coincidental with the disappearance of the disease. In severe cases the cattle suddenly fall dead when driven or excited in any way. Other symptoms are suppression of oestrus, temporary sterility and the development of abnormally large heads in cows, while calves are stunted but do not succumb." (Bennetts et al, 1948)

Studies of the blood of cows in affected herds show that in addition to the seasonal anaemia, blood copper levels are subnormal throughout the year. Levels of only 0.01 mg.Cu per cent. are common during the spring flush when the anaemia is worst. Liver values are 0.1 - 0.6 mg.Cu per cent. of dry matter as compared with 3.7 - 23.1 mg.Cu per cent. for healthy cows from sound areas.

Optimum response can be obtained by the administration of copper supplements. The seasonal anaemia/

anaemia and clinical signs can be prevented in this way.

Bennetts has found that the essential pathological lesion is atrophy of the myocardium with replacement fibrosis and suggests that the sudden death which characterises the disease is caused by heart failure as a direct result of the cardiac lesion. This myocardial fibrosis is encountered only in copper-deficient cattle and only under conditions of the most acute deficiency.

Copper-Molybdenum Relationship in Disorders of Sheep and Cattle.

"Teart" or Molybdenosis.

Certain pastures, notably in Somerset and adjacent counties in England, give rise to scouring and loss of condition in cattle. Sheep are less affected and horses not at all. Ferguson et al (1943) found that this was due to the presence of relatively large amounts of molybdenum in the herbage, especially in clover. "Teart" pasture may contain 20 to 200 p.p.m. Mo. dry matter whereas pasture on which "teart" does not occur may have from 0.2 - 5 p.p.m. Cows affected with scouring on such pastures can be quickly cured by moving to other grazing low in molybdenum. Alternatively, it is found that the administration of therapeutic doses of copper sulphate/

Sulphate (1 - 2 g. daily) will prevent scouring in animals pastured on "teart" land. "Teart" pastures contain normal quantities of copper (11 - 18 p.p.m. dry matter).

"The levels of copper in the blood of these animals tend to be low, but it is possible to reproduce this scouring within a few days in normal cattle by feeding sodium molybdate, without finding any accompanying depression of blood copper. Sheep grazing within the "teart" area for three years on pastures containing up to 23 p.p.m. Mo. of summer herbage showed normal blood copper (mean of 15 animals 0.07) although with 12 x normal level of blood Mo." (Green, 1949).

"Peat Scour".

Cunningham (1946) has described a condition occurring in cattle, especially calves, on peat land in New Zealand. Calves are difficult to rear because of unthriftiness, debilitating spring scouring and increased susceptibility to internal parasites; some sustain bone fractures. Adult cattle scour profusely and persistently on the flush pastures of spring and autumn, and the production of milk is reduced. This condition is attributed to the presence in the pastures of a moderate excess of molybdenum/

molybdenum varying from 3 - 7p.p.m. in summer to 11p.p.m. in spring (normal 1-3 p.p.m) accompanied by a moderate deficiency of copper, 7p.p.m. (normal 11 p.p.m.). This condition disappears if copper supplies are increased either by top-dressing of the pasture or direct administration of copper salts to the animal.

Allcroft and Parker (1949) have described a condition similar to peat scour occurring in adult cattle on certain peaty farms in Cheshire, and Jamieson and Allcroft (1950) a pining condition, accompanied by scouring, in calves in Caithness. Both these conditions are accompanied by a low blood and liver copper level, and can be cured by the administration of copper.

Discussion.

From these brief cummaries of the diverse conditions arising in sheep and cattle, it would appear that the only common factor is the manner of prevention, viz. increasing the copper intake of the animal. In the minority of cases, occurring chiefly in limited areas of Australia, this therapeutic effect is directly attributable to a deficiency of copper *per se* in the soil, associated with low content of the pasture and low levels of copper in the animal. The/

The deficient animal responds proportionally to the supplement of copper given and the degree of deficiency can be fairly accurately controlled by regulating this supplement.

In the majority of conditions responding to copper therapy, however, it is seldom possible to demonstrate that the prime cause is a deficiency in the soil. Under some conditions, soil copper may be "normal" while the value for the herbage may be low; in other cases, such as occur in Britain, where both soil and herbage copper values come within the "normal" range, apparent copper deficiency in the animal may still arise.

In the latter case the deficiency may arise in two ways :-

(1) by the existence of some factor which suppresses the absorption of copper from the alimentary tract;

(2) by some factor which tends to accelerate the excretion of copper thus maintaining blood and liver values at a low level.

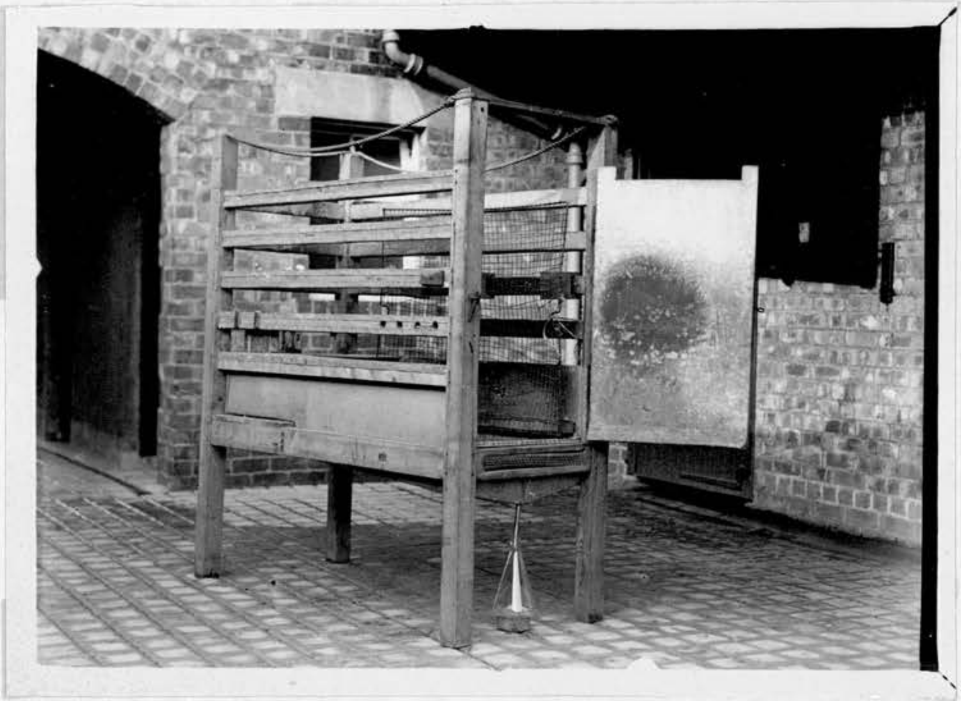
It is highly probable that both these factors exist and that whereas in one county or area the first, in the form of some antagonistic metallic radical or as some sparingly dissosciable copper complex of an organic nature may be involved, in another county there is an upset in the mechanism (hormonal?) controlling/

controlling the level of copper in the animal. The further possibility exists of both acting simultaneously.

With these considerations arising and the nature of the factors still obscure, it is considered that, as yet, there are insufficient grounds on which to base too rigid a comparison between results obtained in different countries (or areas) where the composition of the feeding stuffs may vary widely in fine detail (i.e. trace elements, organic complexes).

The approach to the problem would be considerably simplified if a common diet could be evolved deficient only in copper, but since copper is ubiquitous this is by no means easy.

This thesis presents in four distinct sections work carried out on various aspects of copper metabolism in sheep and which was thought might contribute to the elucidation of the problem of copper deficiency in sheep.



METABOLISM CAGE

General Experimental Procedure.

Metabolism Experiments.

The metabolism experiments to be described in Sections 2 and 3 were all carried out in two identical metabolism cages. These cages, of wooden construction and lined internally with galvanised zinc, permitted free movement of the sheep but were not wide enough to allow the animal to turn round. Food was placed in the front box which, having a fairly high partition proved very efficient in retaining the food. Water was given in a stainless steel trough clipped on to the side of the cage and situated within the food compartment. The animal stood on a strong ½" mesh galvanised grid through which faeces and urine passed. The faeces were collected on a removable small-mesh tray under the grid while the urine passed through on to a solid sloping galvanised zinc bottom and drained through a stainless steel pipe into a two-litre conical flask. The animals were fed regularly at 9.30a.m. and 4.30p.m. with weighed quantities of food. The diet consisted of bruised oats in the morning and hay or dried grass in the afternoon.

Because of the great importance in metabolism experiments of taking representative samples, the greatest care was taken to ensure that the samples kept for analyses were truly representative. The method of sampling the foodstuffs comprising the ration is briefly/

briefly described. The foodstuffs used in the experiment were all ordered in bulk and were generally sufficient for the course of the experiment. The whole amount was mixed and sampled for analysis by quartering. It was found by repeated analyses that the composition of the bulk material varied very slightly. This was checked by taking daily representative samples of food given over a definite period (generally 14 days); these after bulking were sampled and analysed. This method of sampling the foodstuffs comprising the ration, previously used by Stewart(1930), was found to be sufficiently accurate to warrant its use in the metabolism work, so avoiding repeated analysis of the ration.

All samples for analyses were dried to constant weight at 100°C in a stainless steel oven, ground in laboratory mill through a fine steel mesh and stored in air-tight jars.

Faeces and urine were collected and measured each morning at 9.30a.m. In some experiments analyses were carried out on daily samples but generally seven day periods were used. In thelatter cases one-tenth of the daily faeces and urine was collected and kept in cold storage. A period sample was obtaining by mixing the seven daily portions. The period samples of faeces were dried to constant weight and milled in the same way as the feeding stuffs. The moisture content/

content of the faeces, when required was calculated from the total of the seven morning weighings as it was found that the moisture content could alter considerably on standing.

When first put into cages the animals were allowed three to five days to settle down followed by at least one pre-period before commencing the experiment proper.

Chemical Methods of Analysis.

Great care had to be exercised throughout to prevent contamination by copper. The copper in the samples was determined by the wet digestion method of Eden and Green (1940), measuring the colour developed with the reagent, sodium diethyldithio-carbamate, by means of a simple photoelectric cell in conjunction with a standard graph. Estimations on foodstuffs were carried out using from 0.5-1.0g. dry matter; on faeces 0.3-0.5g. dry matter; on urine 25ml. and on blood and liver 5ml. and 0.5-1.0g.(wet weight) respectively. For the blood determination, oxalated whole blood taken from the jugular vein by means of a stainless steel needle was used. Although it was found that the copper in the liver was fairly evenly distributed, liver samples were taken from the same area whenever possible. With these quantities of material the accuracy of the determinations can be taken as ± 2%.

SECTION I.

The Blood and Liver Copper values for sheep in a Scottish hill-sheep area.

The object of this experiment was to establish the range of values for copper in the blood and liver of sheep of all ages grazing on the Southern Uplands of Scotland. The area was selected at random and as far as is known no manifestation of copper deficiency has ever been recorded in this area. By sampling at intervals throughout the year the seasonal variation in both liver and blood was obtained. It was con-:sidered that the data would serve as a basis for assessing the significance of isolated values obtained from sheep in similar hill areas.

Experimental.

A complete flock of 220 Cheviot ewes and lambs was selected on the farm of Gilmanscleuch, situated in the Ettrick Valley. When the experiment was commenced in August 1948 the sheep were of ages ranging by yearly intervals from 4 months to over 6 years old, i.e. year of birth ranging from 1948-1942. They were individually numbered and divided into five groups. Four of these groups (A, B, C and D) each contained 50 animals representing the various ages, while the fifth group contained sheep for use as replacements/

replacements in the event of any deaths occurring in the experimental group.

It was known that with a small number of exceptions, the copper level of the sheep had not been influenced by the therapeutic use of copper salts, as in the anthelmenthic copper sulphate-nicotine mixture, since the spring of 1947. The ex:ceptions are six unidentifiable sheep that were dosed in May 1948. From the fact that they were then suck:ling twin lambs it is known that they were born later than 1946.

Climatic conditions throughout the 10 months of the experiment were normal, no severe winter conditions being encountered.

A group of animals was slaughtered in August 1948, January 1949, April 1949, June 1949, these dates being considered the most likely to reveal any seasonal variation. Blood for copper determination was taken on the day prior to slaughtering. After slaughtering the livers were collected, fresh weight taken, then stored individually in waxed cartons at a temperature of -20 C until required.

The ewes remaining after the first hilling i.e. in Groups B, C, D., were served (in November 1948) to start lambing in the middle of April. In groups B. and C., therefore, all the sheep over 18 months old were/

were pregnant when slaughtered. The killing of Group C. on the 4th of April took place 1-5 weeks before the animal had reached full term. Copper values were obtained for some of the foetal livers from the animals in Group C.

Spectrographic analysis of the soil and pasture for copper content are being undertaken by the Macaulay Institute of Soil Research, Aberdeen, but as yet they have not been completed.

Results.

Determinations of copper content were made on the blood, liver and foetal liver. These analytical values together with the liver weight are contained in Appendix I.

(a) Blood Copper.

Table 1(a) summarises all the blood copper values obtained.

Table 1(a)

	Date of Dampling.	Number of Samples.	Mean Blood copper mg./100ml.	Range. mg.Cu/100 ml.
Group A.	Aug.1948	47	0.059	0.018-0.100
Group B.	Jan. 1949	49	0.055	0.024-0.076
Group C.	Apr.1948	34	0.036	0.014-0.058
Group D.	June 1949	45	0.060	0.028-0.110

From/

Seasonal Variation of Blood Copper.

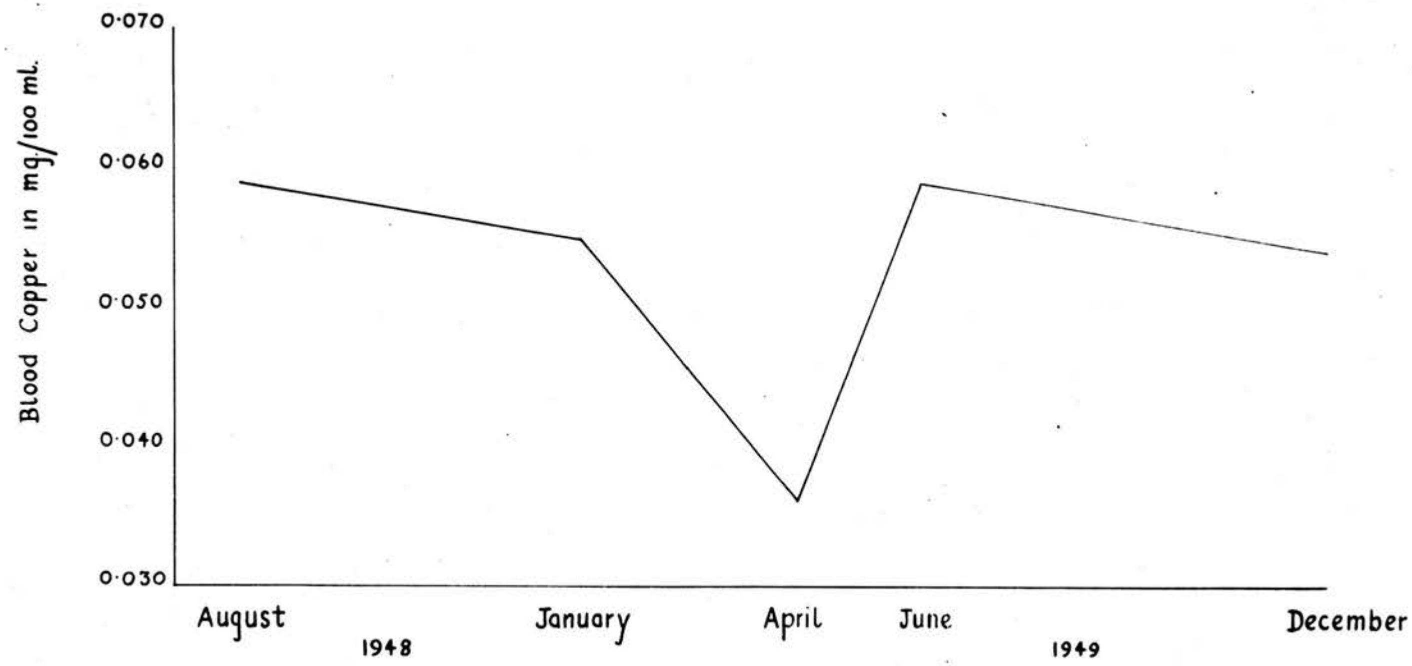


Fig. 1 (a)

From this table it is clear that within each group a very wide range of values exists, the overall range being 0.014-0.110 mg.Cu/100 ml. A comparison of the arithmetic mean of each group indicates that a fall in blood copper level has occurred in April, but has risen again by June. Fig. 1(a) illustrates this trend. Fig. 1(a) includes the arithmetic mean of a small group of 20 animals selected at random from the same area and sampled for blood copper the following December i.e. December 1949. These gave a mean value of 0.054 mg. Cu/100 ml. This group will not be included in the main analysis of the results but seems to show that comparable values were obtain:ed in December 1949 and January 1948.

The above seasonal trend in blood copper level is tested below by statistical analysis. In the analysis the seasonal groups A, B, C, D, are compared for seasonal variation, as also are the overall mean values for the "year of birth" sub:groups.

Table /

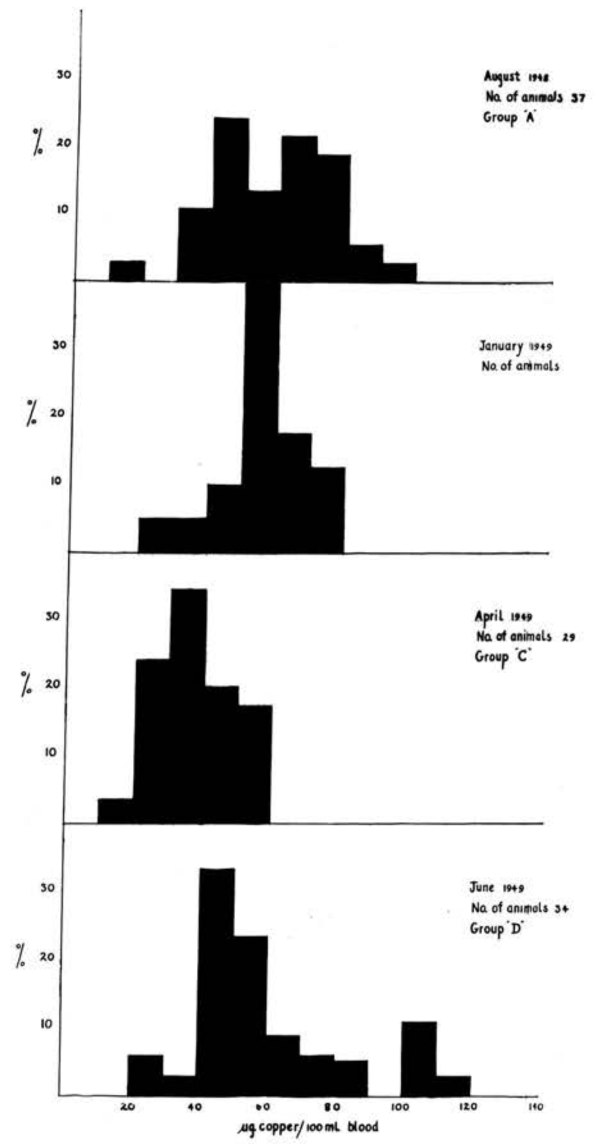
Table 1(b)

Sample Means (X 1000)

Year of Birth.	Group A. Aug.1948	Group B. Jan.1949	Group C. Apr.1949	Group D. Jun.1949	Unweighted Average.
1948	63.8	52.2	33.2	59.0	52.0
1947	63.3	52.0	31.6	49.0	49.0
1946	52.6	50.5	26.8	46.6	44.1
1945	66.3	54.6	42.2	75.0	59.5
1944	62.7	49.7	38.0	51.0	52.3
1943	68.7	60.8	44.4	61.0	58.7
1942	39.5	62.7	37.2	61.0	50.1
Group Mean.	58.5	54.8	36.2	59.5	
Standard Error.	$\pm$3.0	$\pm$3.0	$\pm$3.5	$\pm$3.1	

Animals of group C, i.e. those sampled in April, have clearly a much lower blood copper level than those of the other groups. There is no significant difference between the other three groups, since their means all lie within one standard error of one another.

This/



Seasonal Variation in Blood Copper Level.

Fig.1(b)

This is borne out by an analysis of variance Table 1(c).

Table 1(c)

Analysis of Variance.

Source	Degrees of Freedom	Mean Square	Variance Ratio
Between Seasons	4	3,256	14.3 xx
Between years (unweighted mean)	6	690	3.0 xx
Error (within samples)	147	227	

xx indicates a ratio significant at 1/100 level.

The "Between Seasons" effect is obviously due to the low values in April, and is evident in all age groups including the 1948 lambs (non-pregnant in April). The significant "Between year of birth" effect indicates that some factor associated with the year of birth has had an effect on blood copper. This is clearly not a uniform trend (see last column of Table 1(b), but means that sheep having a particular year of birth have an unusually high or low average compared with the rest.

The frequency distribution of the blood copper values in the four main groups is shown in Fig. 1(b). This histogram can be said to represent the/

the variation occurring in the distribution of the blood copper values of adult sheep (the values for the 1948 age group have been omitted) under the influence of changes in season and changes associated with pregnancy. Here again the lower values for the blood copper in April are evident but it should be noted that from Table 1(b) the values for the 1948 age group or non-pregnant animals exhibit the same trend (Table 1(b) first row).

To summarise these results it would appear that in this hill area a very wide range of blood copper values existed and a significant drop in blood copper level occurred from a mean of 0.055 mg./100 ml. in January to 0.036 mg./100 ml. in April, returning to 0.060 mg/100 ml. in June. This trend is evident in the sheep of all age groups. There is some in-:dication that sheep with a particular year of birth may have a higher or lower average of blood copper values than the remainder.

<u>The relationship between the liver weight, the concentration of copper in the liver and the total copper content of the liver.</u>

In an assessment of any changes occurring in the copper of the liver, either when expressed as concentration of copper in the liver in the usual way,

or/

or as total copper in the organ, it is necessary first to consider changes that occur in the liver weight itself, since the changes in the copper values may be solely dependent upon changes in liver weight.

Liver Weights.

The complete liver weight data (contained in Appendix I) must be considered separately as they are available for many animals on which values for copper concentration and total copper were not obtained.

Fig. 1(e) illustrates the variation in frequency distribution of liver weight with season (1948 age-group omitted).

Table 1(d) gives the average liver weight for each age group (1948-1942) sampled at each season (August 1948, January 1949, April 1949, June 1949), and the unweighted mean for all animals of each age-group and for all in each season-group.

Table/

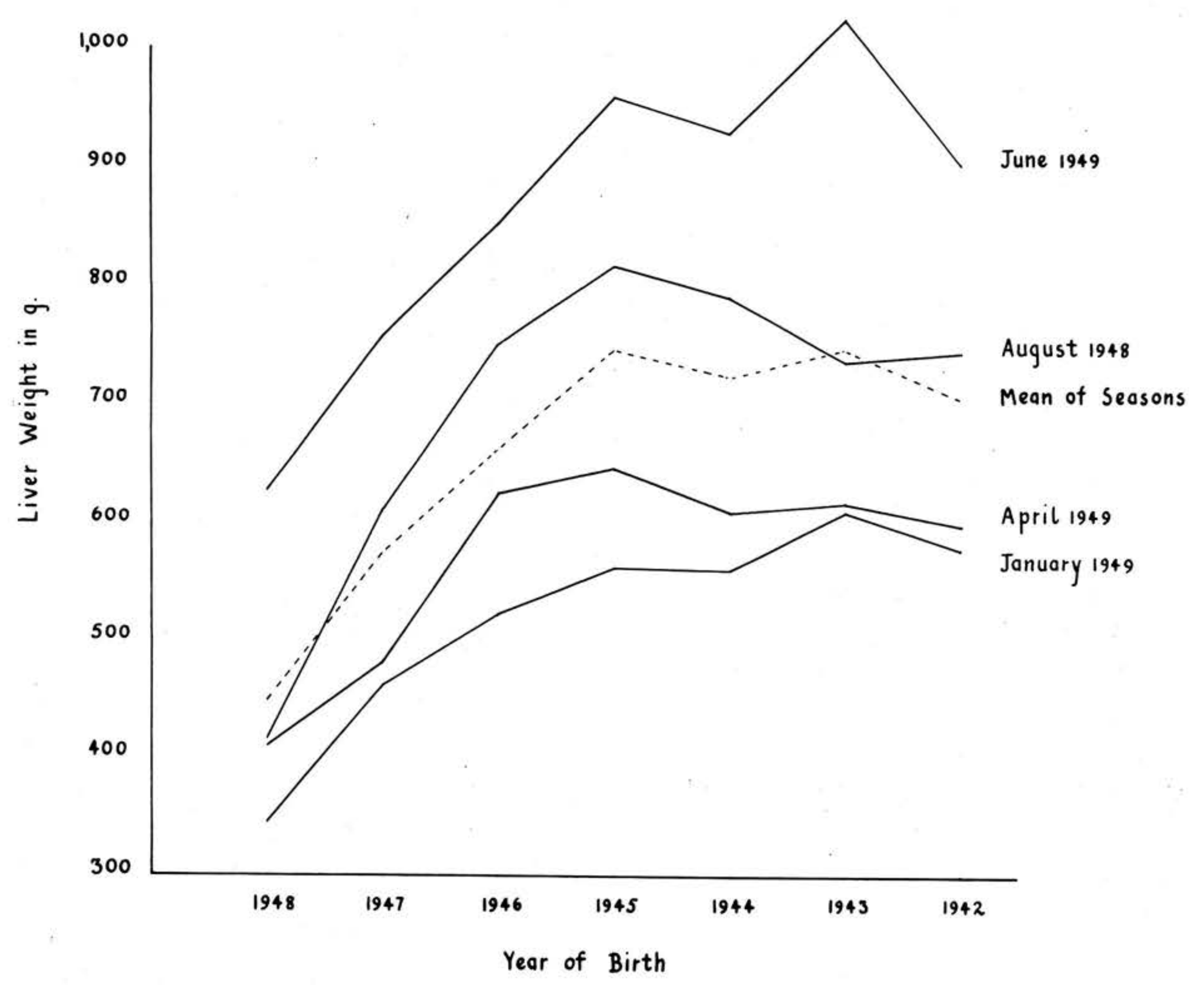

1,000
900
800
700
600
500
400
300
Liver Weight in g.
June 1949
August 1948
Mean of Seasons
April 1949
January 1949
1948
1947
1946
1945
1944
1943
1942
Year of Birth

Fig. 1(c)

Table 1(d)

Liver Weight in.g.

Year of birth.	Group A. Aug.1949	Group B. Jan.1949	Group C. Apr.1949	Group D. Jun.1949	Unweighted Mean.
1948	415	344	409	628	449
1947	610	460	470	755	574
1946	747	520	624	850	660
1945	814	560	644	957	744
1944	787	558	607	928	720
1943	732	607	615	1023	744
1942	740	575	596	900	703
Average	692	518	566	856	

From the last column of this Table it is clear that the liver weight increased steadily up to the age of 4 years (1945 age-group) and this trend is shown coincidentally by the average of the liver values for the animals killed in each season.

This is represented graphically in fig. 1(c) where the unweighted mean values for all the liver weights are plotted against year of birth, and likewise the mean seasonal values for liver weight. The in-:crease is almost linear between 1948-1945, then flattens/

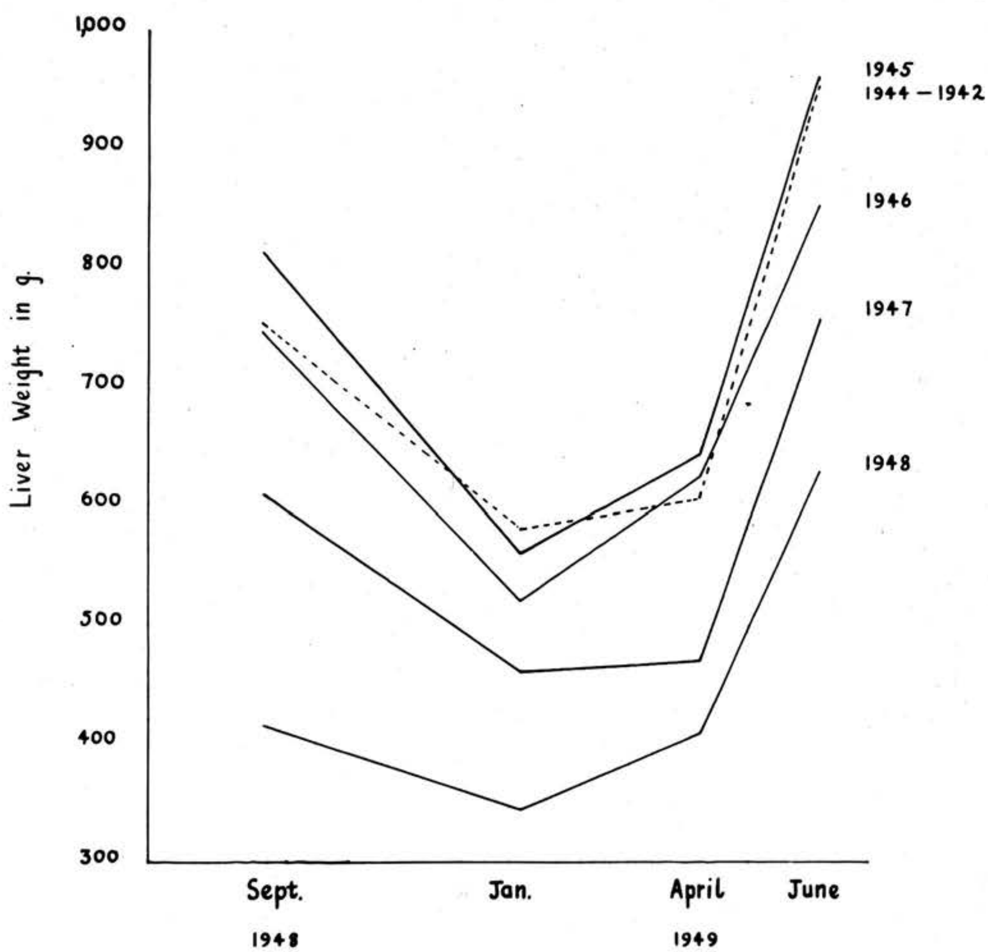


Fig.1(d)

Seasonal changes in liver weight.

flattens out over the years 1944-42.

There is also a striking change in the average liver weight from one season to another (see bottom row of table 1(d)) and is evident in all the age groups. All these changes are apparent in Fig. 1(e) but they are more strikingly represented in Fig. 1(d) where liver weight of individual age groups are plotted against season, the years 1944-42 are grouped together and the mean values taken. This figure shows how there was apparently a decrease in liver weight between August and January followed by a slight increase to April, after which the increased was more marked and in June the liver weight attained a value higher than that of the previous August. It is interesting that in spite of these marked seasonal changes the steady increase in liver weight from year to year is apparent and that the young non-pregnant animals, i.e. the 1948 age-group, exhibit a trend similar to that of the pregnant animals.

The question arises whether there are any significant differences in the way that the various age groups re-act to the seasonal trend. This has been tested by means of an analysis of variance which has been confined to age groups 1946-42 as the two younger age groups have a considerably lower liver weight/

weight. In this analysis one or two individuals have been omitted at random from certain age groups so that each age group is equally represented at all seasons, this leaves 7 animals sampled per season of the 1946 and 1945 age group, 6 of the 1944 group, 4 of the 1943 group and 8 of the 1942 group. The analysis is shown in Table 1(e).

Table 1(e)

Analysis of Variance of liver weight, between seasons and age-groups.

(Age-groups 1946-1942)

Source of Variance	Mean Square.	Degrees of Freedom.	Variance Ratio
Between Seasons	31,200	3	157 x x
Between Years.	765	4	3.84 x x
Interaction	286	12	1.44
Error.	199	134	

x x Indicates a ratio significant at 1 in 100 level of probability.

The Error mean square is based on the variance between animals of the same age-group sampled together and includes all the age-groups (1948-42). The Interaction mean square is a measure of the differences between the responses of the various age-groups to the seasonal trend and is not significant; these differences/

differences are small enough to be accounted for by sampling effects.

There are significant differences between the mean liver weights of the age-groups even when the 1948 and 1947 age-groups are omitted, and, of course, the seasonal trend is highly significant. In view of the very strong seasonal trend and the variations between years, it is remarkable to find such a uni-:formity of reaction of the different age-groups to the seasonal effects as is indicated by the non-significant Interaction mean square.

To summarise, both age and season have an effect on the weight of the liver. With age the liver increases steadily in weight until the animal is 4 years old, after which there is no overall increase in weight. Throughout this period of increase and even when it is stabilised the weight of liver is subjected to considerable seasonal fluctuations, decreasing from August to January, slightly increasing to April, and rapidly increasing from April to June, attaining in June a value much higher than that of the previous August.

The Copper concentration and the Total Copper in the Liver.

Copper concentration in the liver was estimated on a wet, i.e. fresh weight, basis, as with the large numbers involved it was not possible to reduce all the/

the livers to dry matter. However, of 80 samples reduced to dryness and covering the seasonal group August, January, April, all but 7 gave a dry weight value lying between 30-36%. The value of $33\frac{1}{3}$% may therefore be taken as a factor for expressing the concentration of copper in the fresh liver on a dry weight basis.

All the individual results for the copper concentration and the total copper in the liver are tabulated in Appendix I. A wide range of values exists.

The frequency distribution of the copper concentration in the liver for the sheep in the 1947-1942 age-groups sampled at the different seasons (Aug. Jan, April, June) is shown in Table 1(f). The 1948 age-group is omitted.

Table 1(f)

Copper Conc. mg/100 g.liver (fresh weight)	Group A. Aug.48.	Group B. Jan.49.	Group C. Apr.49	Group D. Jun.49
o-0.9	1	2	13	5
1-1.9	4	9	5	3
2-2.9	2	4	5	7
3-3.9	1	2	3	2
4-4.9	4	4	1	1
5-5.9	1	3	1	2
6-6.9	1	1		1
7-7.9				
8-8.9		4		
9-9.9				
10-10.9				
11-11.9				
12-12.9		1		
13-13.9				
14-14.9				
15-15.9		1		
16-16.9				
17-17.9		1		
Total.	14	32	28	21

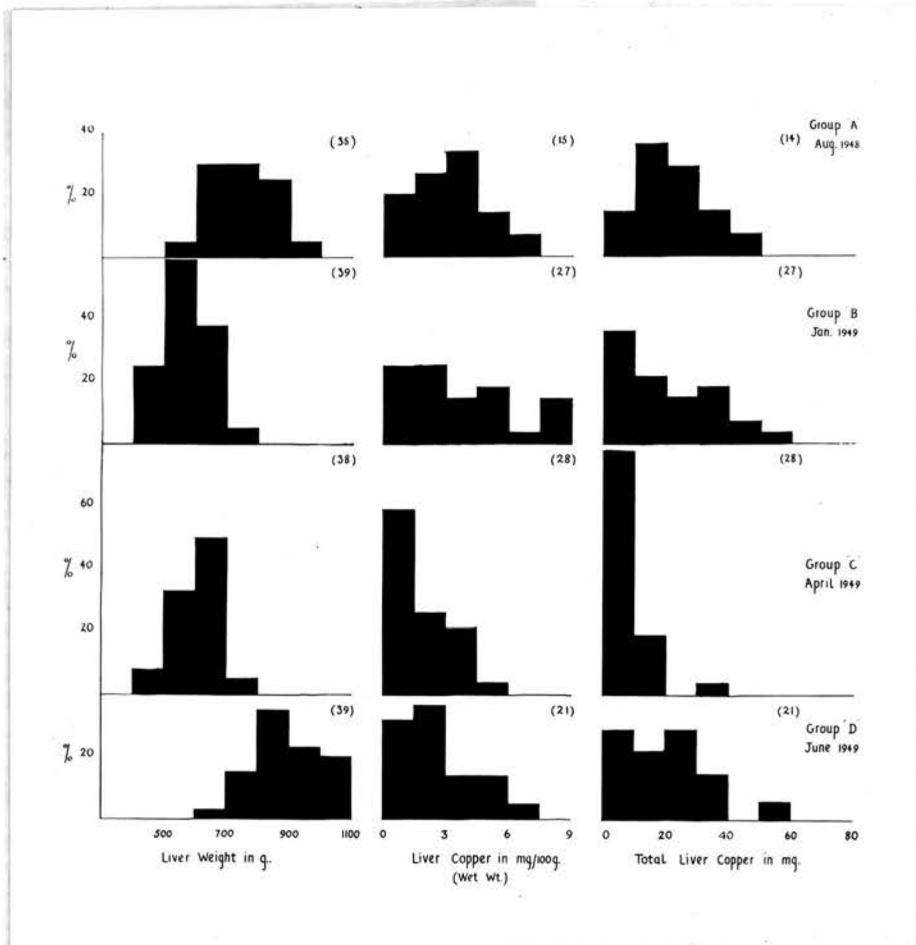


Fig.1(e)

Seasonal distribution of Liver Weight, Liver Copper, Total Liver Copper.

Group B. i.e. the sampling in January is obviously heterogeneous containing seven individuals with copper concentrations well above the range of the remainder and this makes an estimation of the seasonal changes in copper concentration very difficult. There is clearly an association between the season and these excessively high values amounting to about 20% of the sample, since the seven excessive values are confined to the January period, and the probability of this being a sampling effect is very small. Three of the individuals occur in the 1942 age-group, and one in each of the 1943, 1945, 1946 and 1947 age-group. It may be suggested that some factor peculiar to Group B. is responsible for the occurrence of these high values in about 20% of the sample.

The frequency distribution of the copper concentration for all the mature animals i.e. age-group 1947-42, is shown in the histogram fig. 1(e) The three highest values in Group B. had to be omitted.

The frequency distributions of the total copper in the liver show essentially the same picture (Table 1(g)) but here the changes are very much more marked since changes in liver weight are introduced. The 1948 age-group is omitted.

Table/

Table 1(g)

Total Copper. in mg.	Group A. Aug. 48	Group B. Jan.49	Group C. Apr.49.	Group D. June 49
0-4.9		2	14	2
5-9.9	2	7	6	3
10-14.9	4	3	7	2
15-19.9	3	5	1	3
20-24.9	2		1	2
25-29.9		4		4
30-34.9	1	4	1	1
35-39.9	1	1		2
40-44.9				
45-49.9	1	2		
50-54.9				1
55-59.9		1		1
75-79.9		1		
80-84.9		1		
95-99.9		1		
Total.	14	34	33	21

Fig. 1(e) shows graphically the variation of the frequency distributions of the liver copper con:centrations with season for the adult animal i.e. age-groups 1947-42. Because of the skew nature of the graphs a logarthmic scale would probably have been better, but it is thought that the general trend is more obvious as shown.

The question arises whether age affects the copper concentration and total copper in the same way as they affect liver weight. Table 1(h) and Table 1(i) gives the mean copper concentration and the total copper of the livers for age groups 1948, 1947, 1946 and 1945-1942 (grouped together) sampled at each season.

Table/

Table 1(h)

Mean Copper Concentration of Liver.
mg./100 g. (fresh weight)

			Season.	
Age Group.	Aug. 48.	Jan. 49.	Apr.49.	June 49.
1948	-	2.8	1.8	2.9
1947	3.3	2.9	0.8	2.4
1946	-	5.2	1.3	3.1
1945-42	2.9	5.1	1.9	2.2

Table 1(i)

Mean Total Copper in Liver.
mg.

Age Group.	Aug. 48.	Jan. 49	Apr.49.	June 49.
1948	-	10	7	18
1947	19	14	4	18
1946	-	28	6	26
1945-42	21	29	9	22

As some of the averages for single age groups are based on 4-5 individuals, they are not very re:liable, but, omitting the January group for reasons already given, there appears to be no evidence of any trend in copper concentration with increasing age. Total copper shows a slight tendency to increase with age, as might be expected in view of the increase in liver weight.

Comparing/

Comparing the values for the different seasons, it is clear that the copper concentration fell from August to April and then rose again by June. Taking into account the strong seasonal change in liver weight, the result is that total copper dropped from a level of about 20 mg. in August to well below 10 mg. in April, but rose steeply to over 20 mg. in June.

The few high values in January make it im-:possible to determine the nature of the changes from August to January but judging by the frequency dis-:tribution of copper concentration in the liver with season fig. 1(e) any decline from August-January was very slight.

A final question of interest is whether there is any correlation within seasons between liver weight and copper concentration. The correlation coefficient for the season are shown in Table 1(j).

Table 1(j)

Correlation between copper conc. and Liver weight.

Season.	No. of Individual.	Correlation Coeff.
Aug. 1948	14	-0.110
April 1949	28	-0.059
June 1949	21	+ 0.121

None/

None of these values differ signifcantly from zero and as two are slightly -ve and one slightly +ve there is no evidence that the copper concentra:tion is influenced by liver weight.

To summarise the concentration of copper in the liver fell from August to April and then rose again by June. This variation is independent of the seasonal change in liver weight, which did, however, markedly affect the total copper in the liver. This fell from 20 mg. to well below 10 mg. in April, but rose steeply to over 20 mg. in June. The appearance of certain unaccountably high values for liver copper concentration in the January group makes any inter:pretation of the values in this group problematical.

Foetal Livers.

Appendix I contains the individual values for the copper concentration of 29 foetal livers taken from 25 sheep almost all of which were within 1-3 weeks of term. Of the 29, 21 were single and 8 were twins i.e. 4 pairs of twins. The mean value for the copper concentration of the 21 single foetal livers was 0.82 mg./100g. (fresh weight) and for the groups of twins 0.83 mg./100g., it is apparent therefore that no difference exists between the liver copper con:centration of single and of twin foetal lambs. The corresponding/

corresponding mean value for the maternal liver copper concentration was 1.80 mg./100g. (fresh weight). Thus the copper concentration of the maternal liver was very much higher than that of the foetal liver, this finding is in agreement with that of Cunningham (1931).

Discussion.

A review of the literature reveals that in Britain there is little data relating to the levels of blood copper and its seasonal variation that is not complicated, either from having been taken from known swayback areas or from having been taken from farms or experiments where the type of husbandry included supplementary feeding. Although in themselves, these complications are of little significance in relation to the diagnosis and treatment of swayback in these areas, they nevertheless may tend to obscure changes in the blood copper level which may normally occur over large tracts of sheep country, including Scottish hill grazing through subjection to physiological changes associated with pregnancy and to nutritional changes associated with seasonal changes in the pasture.

Shearer et al (1940) showed that the blood copper level of sheep in Derbyshire (swayback area) having the initially low level of between 0.02 and 0.03 mg./

mg./100 ml. in November rose throughout January to April when figures of about 0.07 mg./100ml. were reached. This they considered was a rise associated with pregnancy as reported by Tompsett and Anderson (1935) for the human subject, although they failed to find any indication of a pregnancy rise in sheep with initially higher level of blood copper. Absence of variation during pregnancy was found by Eden (1941) during investigations on park-fed sheep at Weybridge. Eden et al. (1945) again working in a swayback area (Derbyshire) found (considering only the control group not receiving a copper supplement)that there was a slight fall in blood copper value between October and January on five farms, and on one of the farms this decrease continued to the March sampling after which it commenced to increase till sampled in May. The other four farms showed a slight increase in blood copper from January-March continuing until May.

The effect of pregnancy per se in bringing about the fall in blood copper level found in this experiment must be of little significance, apart from the small quantity of copper transferred to foetal tissue, since from Table 1(a) the same trend is evident in the sub-group born in 1948, none of which were pregnant. However, this does not rule out the possibility of there being a rise in blood copper level of sheep throughout pregnancy when they are maintained/

maintained on a level plane of nutrition, since any rise may be masked by other factors in this experiment.

The above argument may equally well be applied to the seasonal changes evident in the liver weight, total liver copper and liver copper concentration, since here again the non pregnant sheep exhibitedthe same trend as the pregnant animals.

As the trends of the seasonal variation in all the items considered in this experiment i.e. blood copper, liver weight, liver copper concentra-:tion, total liver copper, are similar, tending to minimal values between January and April, it may be argued that some common factor exists.

The main factors remaining/to account for this seasonal trend are, the climatic changes and change in the nutritional plane of the animal. Until more is known regarding the mechanism controlling the meta-:bolism of copper, the importance of any direct effect that the former factor may have cannot be assessed. Changes in the plane of nutrition of the animal is the factor most likely to account for all the changes.

Fraser (1939) has shown how "on hill pasture in the West Highlands a Blackface ewe weighing 90 lbs in the Autumn will have commenced to fall back in condition during late November when it may weight 80 lbs. While in lamb between November and April it progressively/

progressively loses weight and may only weigh 60 lbs just before lambing, then throughout the spring and summer the ewe will increase in condition and weight". Although the conditions described by Fraser in the West Highlands are somewhat more extreme than occurs in the Southern Uplands, the same general picture holds. Thus changes in liver weight may be fairly proportional to the overall changes in weight and condition of the animal. According to Fraser, however, it would appear that this decrease in weight was pro:gressive through January to April, whereas in the seasonal changes in the liver weight found in this experiment the minimal value was apparently reached in January with a slight increase by April. It may be that this lowest value is actually reached sometime later, at the end of February and that by the middle of March the early grasses are beginning to reappear and the animal thus regain some of its lost condition and weight, or it may well be that there is a slight increase in liver weight associated with pregnancy and that the growth rate of the still young sheep in the 1948 age-group competing with any nutritional change may produce a final change in liver weight, at the April sampling, similar to that of the pregnant adult in April.

The/

The continual overall increase in liver weight up to the age of 3-4 years as shown by figs. 1(b) and 1(c) is of considerable interest in as much as this might indicate that the Cheviot ewe is not fully mature under its normal environmental conditions until it is 4 years of age. This agreeing very closely with its dentition, the ewe not getting its full complement of permanent teeth until it is from 3-4 years old, although this age may vary slightly with nutritional conditions.

However, it has been shown that besides a decrease in total liver copper associated with a drop in liver weight there is independently a change in liver concentration, with a minimal value at the time of the April sampling, this coinciding with the minimal value for blood copper. These low values may be be associated with a decreased uptake of copper from the herbage either because the copper concentra:tion in the winter pasture is below that of its summer value or because the copper in the winter herbage is present in a form not readily dissosciable and hence not readily absorbed from the alimentary tract. In addition, during the winter period the reserves of the animal are seriously depleted and it may well be that in the utilisation of the animal's glycogen, fat /

fat and protein, there is a greater call on copper as an essential component of the enzymatic systems involved the metabolism of these sources of energy, thus leading to a considerable wastage of copper and hence an ultimate lowering of the copper levels of the blood and organs of the animal.

In the prophylactic treatment of swayback it is well known that supplementary feeding of copper salts during the final six weeks of pregnancy is time enough to prevent the occurrence of the condition. No explanation has been given why it should be effective during the period of late pregnancy but under the conditions of this experiment, this period of dosing would coincide with the time during which the animal has the lowest reserves of copper at its disposal.

<u>Summary</u>.

(a) An experiment in a hill-sheep area is described in which values were obtained for blood copper concentration, total liver copper and liver weight, from sheep ranging from 4 months to 6½ years old, sampled in groups of 50 during August 1948 and January, April and June 1949.

(b) Seasonal variationsand in some cases variations with age, in these values is evident, the minimal values occurring between January and April.

(c) The significance of the seasonal variations are discussed.

(d) Values for the foetal liver copper concentration of 29 foetal lambs, are given.

Acknowledgement

The author wishes to thank Dr. Reeve, (Animal Breeding and Genetics Research Organisation), for carrying out a very full statistical analysis on the results obtained from this experiment.

<u>The effect of increasing the intake of Molybdenum on the copper balance of sheep</u>.

Pastures may be regarded as containing a normal of 1 p.p.m. - 3 p.p.m. Molybdenum expressed on the dry matter and this level is considered as beneficial. Normal traces are certainly important for fixation of atmospheric nitrogen by the free-living Azotobacter in soils and the nodule bacteria of legumes (Arnon and Stout 1939), but when taken up by plants in excessive amounts the element may be harmful to grazing ruminants.

In 1945 Dick and Bull in Australia put forward evidence to show that increased Molybdenum intake has the effect of suppressing the assimilation and storage of copper in the sheep and that the ratio Cu/Mo might have an important bearing in this respect. They showed that when the intake of Mo by a sheep on a normal diet is increased to 10 mg and 100 mg of Mo per day over a period of 6 months, the concentra-:tion of Cu in the liver is significantly reduced.

Evidence in support of this finding has since been put forward by Cunningham (1946) in New Zealand, and recently Marston (1949) in Australia showed that over a period of 9 months, animals grazing/

grazing on pasture moderately deficient in copper, developed symptoms of copper deficiency more rapidly when given supplements of Mo. Using the technique of liver biopsy (Dick 1944) Marston showed that whereas the mean liver copper value for the untreated fell from the initial level of 18.0 - 9.2 mg./100 g. dry matter, those dosed with Mo. showed a fall of from 18.0 - 4.5 mg./100 g. dry matter. This in:creased depletion of stored copper was independent of the quantity of Mo provided (5 - 50 mg. Mo/day). No significant change in blood copper level was found.

In England, Green (1949) has administered Mo as sodium molybdate, to stall fed sheep, "in daily doses raised each quarter on the scale 14, 56, 112, 224 mg. Mo. These sheep had been transferred from a swayback area and were known to have an initial low value for blood and liver copper. Blood copper values rose rapidly from the low average of 0.025 mg. Cu/100 ml. to a normal of 0.085 mg. Cu/100 ml. during the first quarter and reached a value of 0.12 mg. Cu/100 ml. in the latter stages. At the conclusion of the experiment, liver tissue had reached a mean value of 52.8 mg./100 g. dry matter as against

51.2/

51.2 mg./100 g. from controls and a calculated probable initial value of 1.5 mg./100 g. (similar sheep on a swayback farm)".

As these experiments stand it is difficult to draw any conclusions based on a comparison between them, since the design of the experiments, in Australia and New Zealand on the one hand and those in England on the other, is completely different.

The copper balance studies to be described in this section were carried out to see whether the dosing of sheep with Mo could produce any noticeable change in the relationship between the copper intake and the copper excreted.

Experimental.

The copper balance experiments were carried out in two metabolism cages using the procedure previously outlined. The duration of the experiment being from 7 - 15 weeks. For animals No. 800 and No. 632 the diet consisted of bruised oats and hay while for sheep No. 269 and No. 281 dried grass was substituted for hay as it was considered that a more uniform diet could be given and hence more accurate samples for analysis obtained. In general the level of copper in the diet was low (3 - 4 p.p.m.).

The/

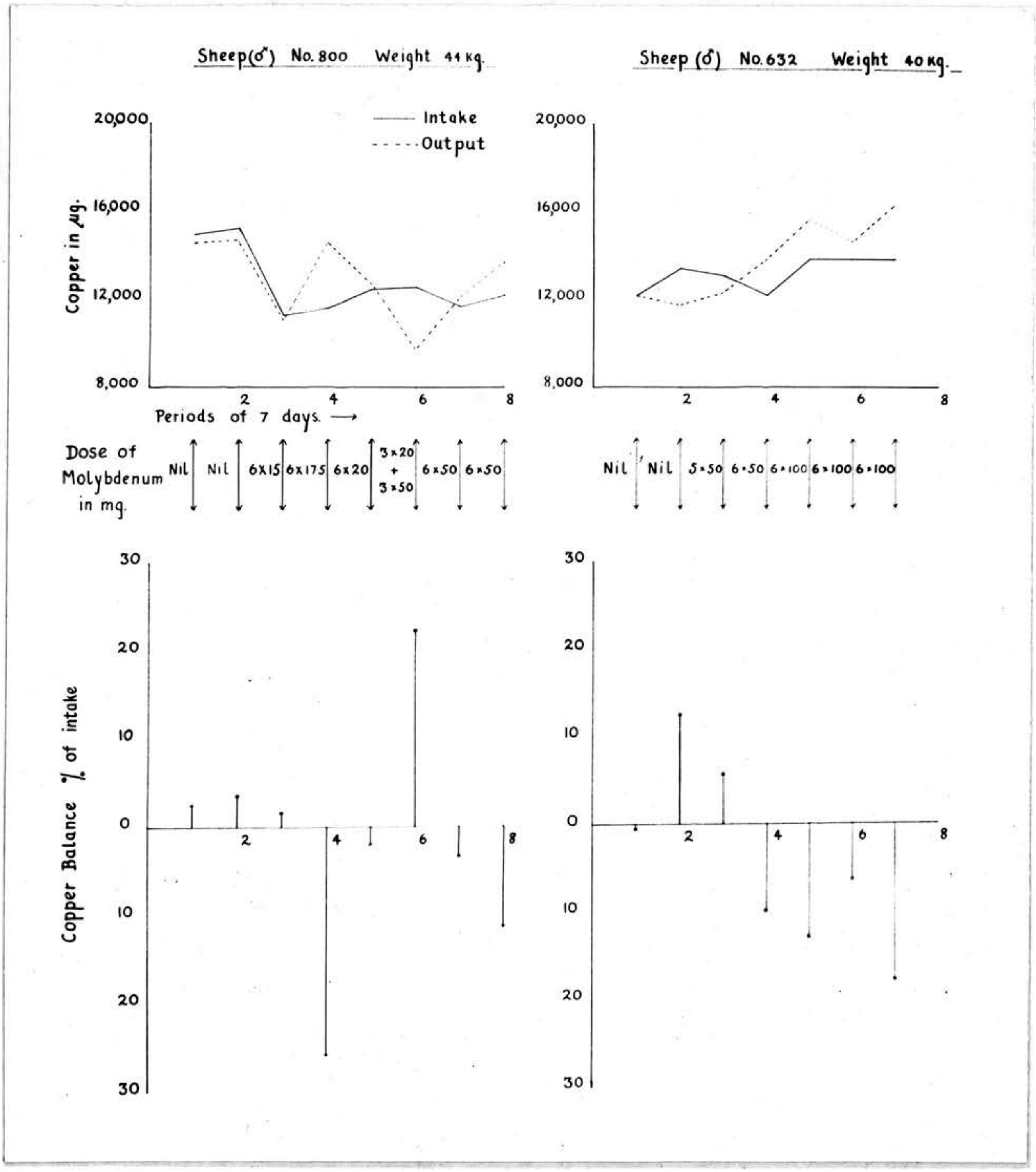


Fig.2(a).

Effect of increasing the molybdenum intake on the copper balance.

The Mo was given orally, as a solution of either sodium or ammonium molybdate, twice daily just before feeding.

Group 1. The results obtained from dosing sheep No. 800 and No. 632 are tabulated in Appendix II, and are represented graphically in fig. 2(a). The copper balance for each period being expressed as a percentage of the copper intake. A negative balance implies that more copper is being excreted than ingested.

The experiments on these two sheep were carried out independently and did not run concurrently.

No. 632 was dosed at a considerably higher level than No.800 and it appeared that the first dose given to No. 632, 100 mg. Mo as sodium molybdate had a considerable diuretic effect, the urine increasing from a normal level of 400 ml. /24 hrs. to over 2,000 ml. The urine volume returned to a low level on the 3rd 24 hour period following this dose. This effect has not been observed again.

From fig. 2(a) it would appear that with the exception of No. 800 during period 6 the balances obtained are suggestive of a swing from a slightly positive balance to a slightly negative balance following dosing with Mo salts and is more pronounced in the case of No. 632 which was dosed at a higher level/

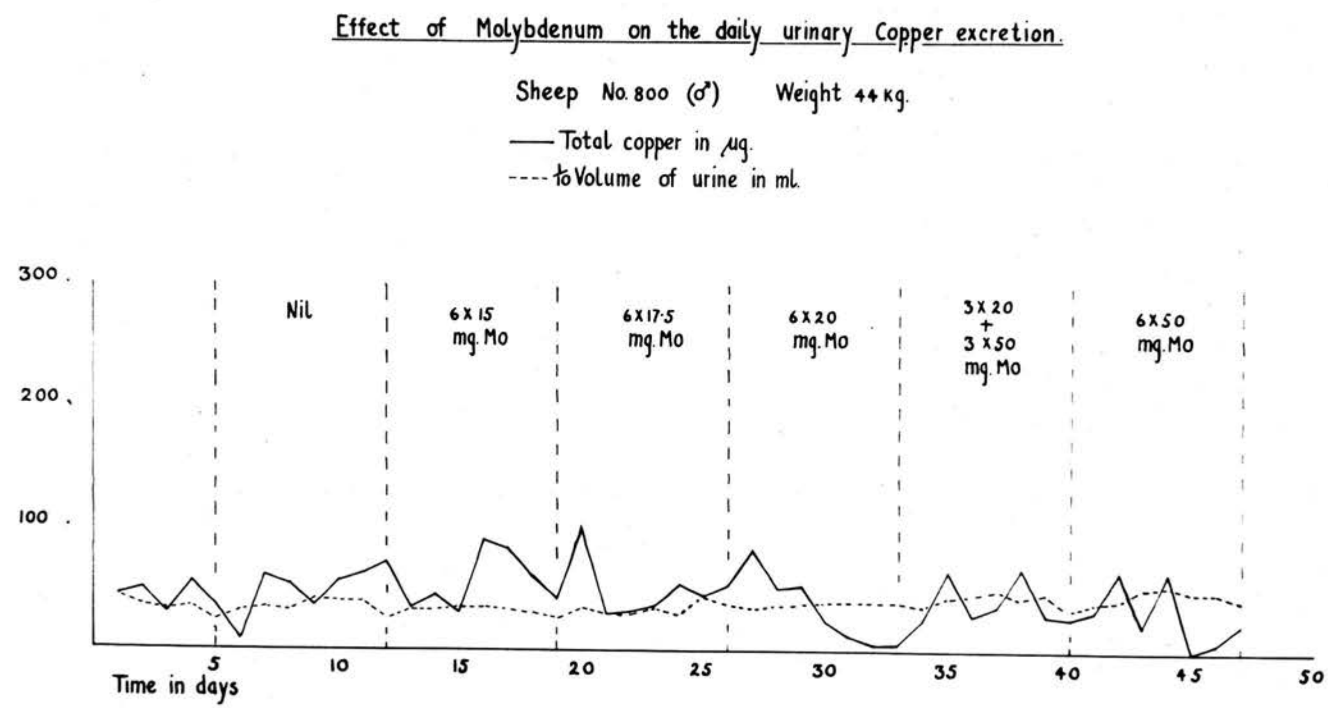

Effect of Molybdenum on the daily urinary Copper excretion.
Sheep No. 800 (♂) Weight 44 kg.
— Total copper in µg.
---- to Volume of urine in ml.
300
200
100
Nil
6 X 15 mg. Mo
6 X 17·5 mg. Mo
6 X 20 mg. Mo
3 X 20 + 3 X 50 mg. Mo
6 X 50 mg. Mo
5
10
15
20
25
30
35
40
45
50
Time in days

Fig. 2(b)

level.

Fig. 2(b) shows the daily excretion of copper in the urine of sheep No. 800 during the first 6 periods. These estimations were made in order to ascertain if any daily variation not evident in the period samples occurred. The daily excretion level of urinary copper appears to be fairly constant, averaging 30-60 μg Cu/24 hours and represents a very small fraction of the total copper ingested (approx-:imately 2,000 μg Cu/day.

At the conclusion of the experiments No. 800 had lost 1.0 Kg. in weight, 44 Kg. - 43 Kg., whereas No. 632 had gained 1.0 Kg. from 40 Kg. - 41 Kg. On post mortem examination No. 800, a male sheep, was found to have considerable quantities of small calculi in both kidneys. No. 632 was quite normal.

Estimation of copper in the liver gave values within the normal range, 2.5 mg. Cu/100 g. (wet weight) for No. 800 and 3.5 mg. Cu/100 g. (wet weight) for No. 632.

The average urinary excretion of copper was 30-70 μg /24 hours.

Group II. It had been intended to repeat the experiment carried out above and to extend the number of control periods, i.e. periods prior to dosing from/

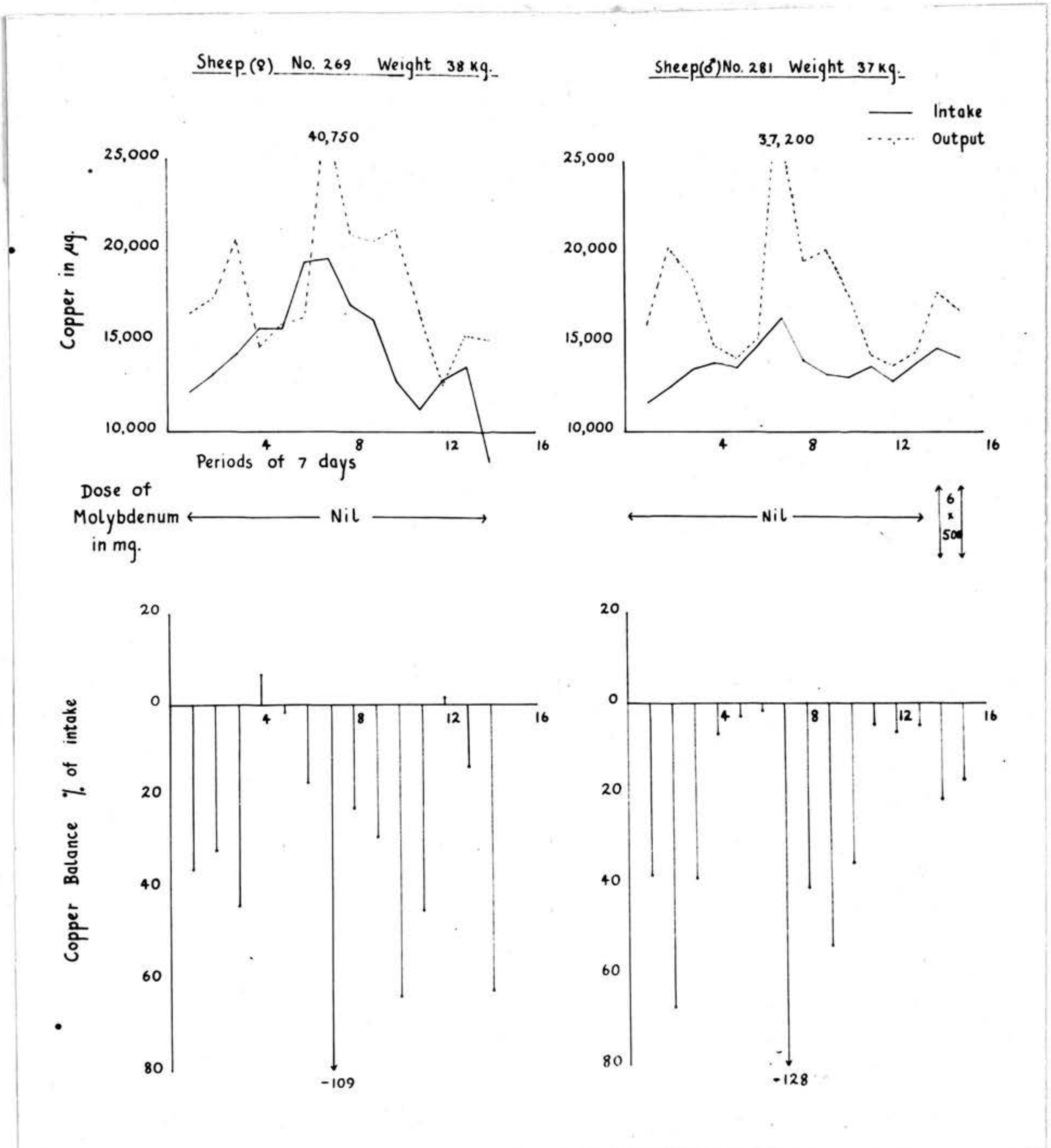


Fig.2(c)

Effect of increasing the molybdenum intake on the copper balance.

from 2 to 5, thus obtaining an extended graph over the period of normal balance. However, the results obtained proved to be so interesting in themselves that the control period was extended to 14 weeks and Mo was only given during the 15th period.

Two sheep, 10 months old, No. 269 weighing 38 kg. and No. 281 weighing 37 Kg. were selected. Their previous history was such that they could be considered comparable animals. They had both been born and reared indoors under the same experimental conditions and on the same diet. This diet was of a similar copper content to the one used in this ex-:periment (3 - 4 p.p.m.) but had been supplemented by 2 ounces per day of a mineral mixture containing 7 p.p.m. copper, equivalent to an additional copper intake of 2,800 μg Cu/week.

The experiments on these two animals ran concurrently under identical conditions.

The results obtained with sheep No. 269 and No. 281 are tabulated in Appendix II and are re-:presented graphically in fig. 2(c). It can be seen that No. 281 maintained a steadier rate of copper ingestion, i.e. a steadier appetite than did No. 269.

Both sheep showed a considerable negative balance over the first three periods coming up to practically/

practically a zero balance over the 4th, 5th and 6th periods. At this point it was thought that dosing with Mo might be commenced but as the animals had been confined to the cages for almost seven weeks (1 pre-period and 6 periods) it was decided to put them out for exercise. They were, therefore, placed in a large stall for three days where they had ample room for movement but were maintained on the experimental diet. On returning the animals to the cages two days were allowed to lapse before the balance experiment was recommenced. The 7th period which followed was a 5 day period but calculated for purposes of comparison as a 7 day period. During this period both sheep were in considerable negative balance (No. 269, -109% and No. 281, -128%), re:presenting a loss over 5 days of 15.1 mg. Cu from No. 269 and 14.9 mg. Cu from No. 281.

Sheep No. 269 continued to maintain a con:siderable negative balance until the 12th week when the balance returned to zero with a slight fall again on the 13th week. During the next two periods the animal did not eat and lost condition rapidly. The result of the 14th period may be discarded, since it can be seen from the graph that the large negative balance, -83%, is due solely to a decreased intake following/

following loss of appetite, and not to an increased output. Molybdenum at a dosage of 500 mg./day. was given during the 15th period but because of the onset of scouring in the animal no results were obtained.

Sheep No. 281 maintained a considerable negative balance until the 11th week when the balance returned to around zero. A slight fall took place on the 14th week. During the 15th period this animal was dosed daily with molybdenum at a level of 500 mg./day. The animal showed no effect from this dosing, maintaining a good appetite. There was no change in the excretion rate of copper.

At the commencement of the experiment the sheep weighed - No. 269, 38.1 Kg., No. 281, 37.4 Kg.; at the end of the 6th period 39 Kg. and 40.3 Kg. respectively and at the termination of the experiment 32.2 Kg. and 42.2 Kg.

Over the 13 weeks (No. 269) and 15 weeks (No. 281) the animals excreted 49.9 mg. and 55.2 mg. more copper than they ingested.

The average uninary excretion of copper was 200-500 µg. Cu per week i.e. 30-70 µg. Cu/24 hours.

Discussion.

Group I. Dick and Bull (1945) in their paper on the effect of dosing with Mo on the copper status/

status of the sheep found that dosing with Mo over a period of 6 months at levels of 10 mg. and 100 mg. Mo/day reduced the copper concentration of the liver of the sheep by an average amount of 23.1 mg. and 28.5 mg./100 g. (dry weight) as compared with an average reduction in the control group of 6.8 mg./100g. Calculating the average nett loss therefore we get ($\frac{23.1 + 28.5}{2}$ - 6.8) or 19 mg./100 g. (dry weight) and with an average fresh liver weight (as quoted) at 350 g. and with moisture content of 70% we get a loss of 19 x $\frac{350}{100}$ x $\frac{30}{100}$ or 20.0 mg. of copper over a period of 6 months, equivalent to 770 μg. copper per week. No figures are given for the copper intake but if based on the average weekly intake of the sheep No. 632 and No. 800 say approximately 13,000 μg of copper per week, this would represent under the conditions of the balance experiment a weekly negative balance of $\frac{770}{13000}$ or approximately 6%. (Since the copper intake of the Australian sheep on free range would be considerably in excess of the 13,000 μg. used in the above calculation, it is probable that a more accurate value would be 4%). This is assuming that all the copper lost from the tissue is ultimately reflected by a loss from the liver. This would appear to be quite a reasonable assumption for livers with/

with such a high copper concentration as quoted by Dick and Bull.

The values for liver copper concentration found in Australia are in general very much higher (Bull 1949) than those found in Britain and in the present series of experiments in particular. In their paper Dick and Bull were dealing with sheep having an initial liver concentration of about 14 mg. Cu/100 g. (wet weight) and a terminal con-:centration of about 6.5 mg. Cu/100 g. It is possible, though it does not necessarily follow, that animals with lower concentration of copper in their liver, viz. No. 632 and No. 800 at concentra-:tions of 3.5 and 2.5 mg. Cu/100 g. (wet weight) respectively, might not excrete their copper at such a high rate. Thus, a comparable value might be less than the 6% negative balance. Negative balances within this range i.e. of at least 6% have been recorded during this experiment, particularly in the case of No. 632 (Fig. 2(a)).

The overall absolute accuracy of the copper estimations for an individual period probably lies within the range of ± 5%, but since a standard technique was used throughout each metabolism ex-:periment, the comparative accuracy between two periods will probably be of the order of ± 2%.

Relative/

Relative changes $>4\%$ in the copper balances of two periods will therefore indicate a significant difference between them.

<u>Group II</u>. The copper balances obtained from sheep No. 281 and No. 632 without any Mo dosing make the value of the results in Group I appear problematical.

The only reasonable explanation would appear to depend upon:-

(a) An initial high concentration of copper in the tissue due to the supplementary intake of 2,500 µg. Cu per week throughout life.

(b) The existence of some factor (hormonal or otherwise) which controls the metabolism and excretion of copper and which is influenced by some physiological or nervous stimuli.

The above two postulates may have operated conjointly, the effects of the stimuli lasting from 20-30 days and being initiated by the sudden change involved in transference from the relative freedom of stalls to the confinement of cages.

The uniformity of the results for the two sheep is very striking. Since the analyses were not always carried out in chronological order it is considered that the technique was not a contributing factor to the similarity.

During the 15th period there was no change in the excretion rate of copper either in urine or faeces by/

by No. 281 with the very high level of dosing, viz. 500 mg. Mo/day (equivalent to a dietry intake con:taining 800 p.p.m. Mo dry matter).

As yet it is not possible in copper balance experiments to differentiate between (a) copper that is ingested and passes through the intestinal tract unabsorbed, (b) total copper that is absorbed, (c) copper that is absorbed and subsequently re-excreted into the intestine. It would appear that unless the quantity of ingested copper actually absorbed is very small, excretion of copper back into the intestine must take place since urinary copper represents such a small fraction of total copper excreted (approx. 500 μg. Cu. per week compared with 13,000 μg. Cu. or 4%).

With the limitation, therefore, in which only total changes in the balance of copper ingested and excreted can be determined and with the further limitations (a) that over a short period of time these changes may be very small, (b) the inadvisibility of keeping the sheep in the cage for a period longer than about 7 weeks without respite; it is doubtful whether balance experiments of this type can give conclusive evidence for or against the view that increasing the intake of Mo over a long period of time brings about a decrease in the copper status of the sheep.

Summary./

Summary.

Experiments are described in which the copper balance of sheep was studied with and without the addition of Molybdenum salts to the diet. The results obtained are discussed and would indicate that the limitations are such that no conclusions can be reached by this method, for or against the view, that increased Molybdenum intake in the sheep either decreases the absorption of copper or increases the excretion of copper, thus producing a lowering of blood and liver copper values.

The possibility of a definite mechanism controlling the metabolism and excretion of copper is discussed.

In these experiments the average urinary copper output was from 30 - 70 μg. for 24 hours.

SECTION III.

The action of B.A.L. (2:3 dimercaptopropanol) on the urinary excretion of copper in the sheep.

McCance and Widdowson (1946) in the human, and McDonald (1946) in the sheep, have shown that the administration of B.A.L. Intrav. (glucoside of dith:ioglycerol) has the effect of increasing urinary copper excretion. The duration of these experiments was only a few hours.

It was decided to study the action of B.A.L. (2:3 dimercaptopropanol) on the urinary excretion of copper in individual non-pregnant sheep with a view to determining whether any appreciable lowering of (a) blood copper level (b) copper reserves in the liver could be brought about by this method.

Experimental.

The B.A.L. (2:3 dimercaptopropanol) was given by intramuscular injection into the thigh. A 5% (w/v) solution of B.A.L., in Arachis Oil containing 10% benzyl benzoate and kept in nitrogen filled ampoules, was used in experiments I, II and III, while stronger solutions for experiments IV and V were obtained by making a solution of pure B.A.L. in 66% (v/v) propylene glycol/water. This stronger solution was such that 1 ml. solution ≡ 200 mgms. B.A.L. and was made up freshly as required. Dosing was/

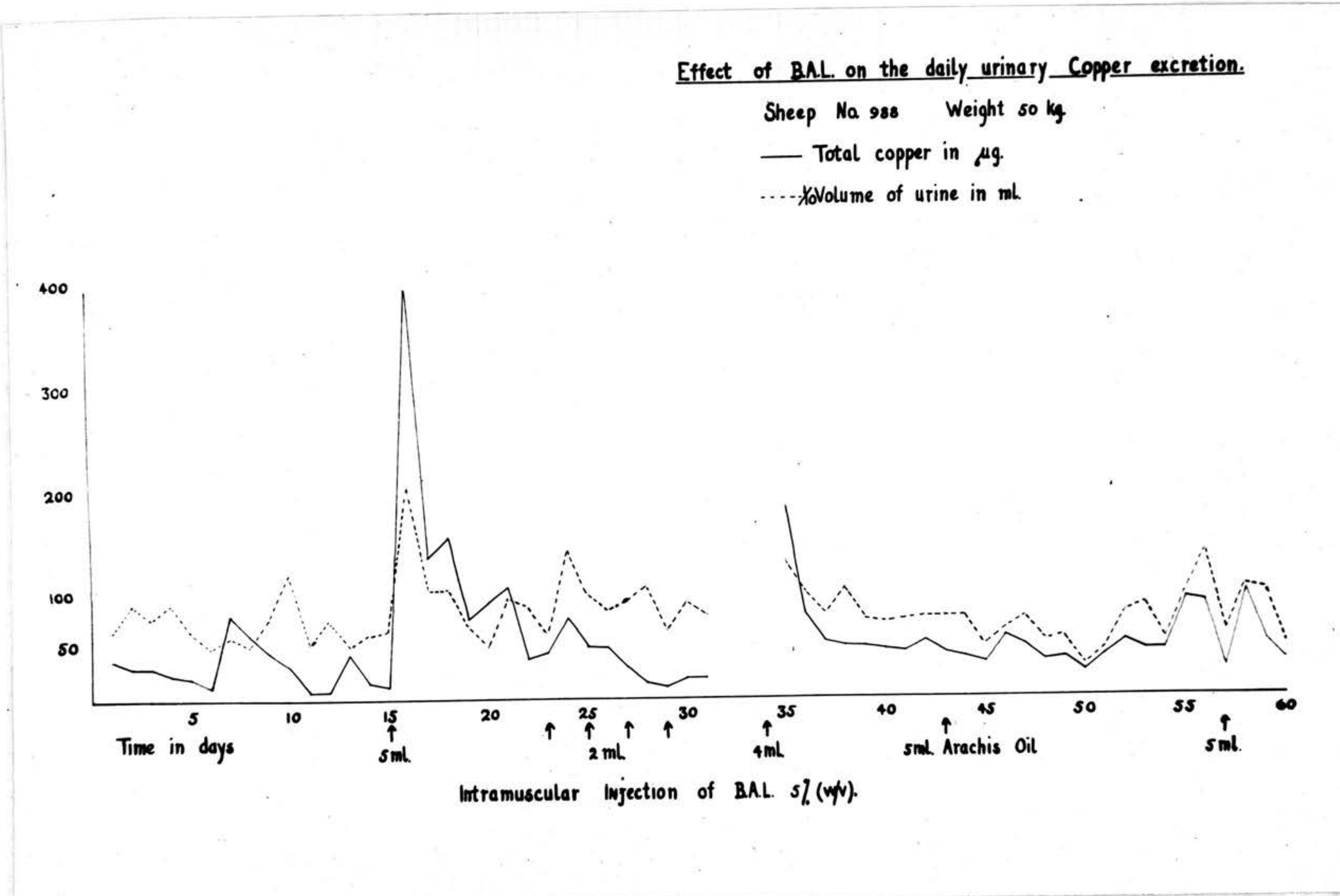

Effect of B.A.L. on the daily urinary Copper excretion.
Sheep No. 988 Weight 50 kg.
—— Total copper in μg.
---- ½₀ Volume of urine in ml.
400
300
200
100
50
5
10
15
20
25
30
35
40
45
50
55
60
Time in days
5 ml.
2 ml.
4 ml.
5 ml. Arachis Oil
5 ml.
Intramuscular Injection of B.A.L. 5% (w/v).

Fig. 3(a)

was carried out at 11.00 a.m. but when two doses were given on the same day, they were given at 11.00 a.m. and 3.00 p.m. Experiments I to V were all carried out in the metabolism cages using the procedure previously described. Since only the urinary copper output was being measured, no record of food intake or faecal excretion was kept.

Experiment I.

Sheep No. 988, weighing 50 Kg., was put in a metabolism cage as previously described and the normal copper output in the urine determined over a period of 15 days. On the 15th day it was given an intramuscular dose of 5 ml. 5% (w/v) B.A.L. (equivalent to a dose of 5 mg./Kg.). Within half an hour the animal was showing mild signs of discomfort, copious salivation and intermittent chewing movements. These symptoms passed off fairly rapidly, i.e. within two hours.

As shown in Fig. 3 (a) the urinary copper output rose sharply following the dose of B.A.L. and although it fell again during the second 24 hour period it did not return to a normal level until six days later. The volume of urine excreted was also considerably increased.

Further doses were given as indicated in Fig. 3(a) but at no time did the animal show any signs of/

of discomfort such as it had done following the first dose, and only after the dose of 4 mg./Kg. given on the 34th day was there any marked increase in copper output. On the 43rd day a control dose of 5 ml. arachis oil containing 10% benzyl benzoate was given. This had no effect either on the volume of urine excreted or on its copper content.

Over the period 31st to 34th day the animal was taken out of the cage for exercise. It was con:fined to a stall allowing ample freedom of movement and fed the same diet.

The experiment was concluded on the 62nd day.

This was actually the first experiment carried out and it was not until the conclusion of the experiment that it was noted that the urine drainage pipe leading from the cage, about 5" in length, was made of copper heavily painted on the outside but exposed internally. This was replaced by a stainless steel pipe.

It was decided to try further doses of B.A.L. on the same animal, No. 988, under the new conditions.

Experiment II.

Figs. 3(b) and 3(c) illustrate the effect of increasing doses of B.A.L. on the volume of urine excreted/

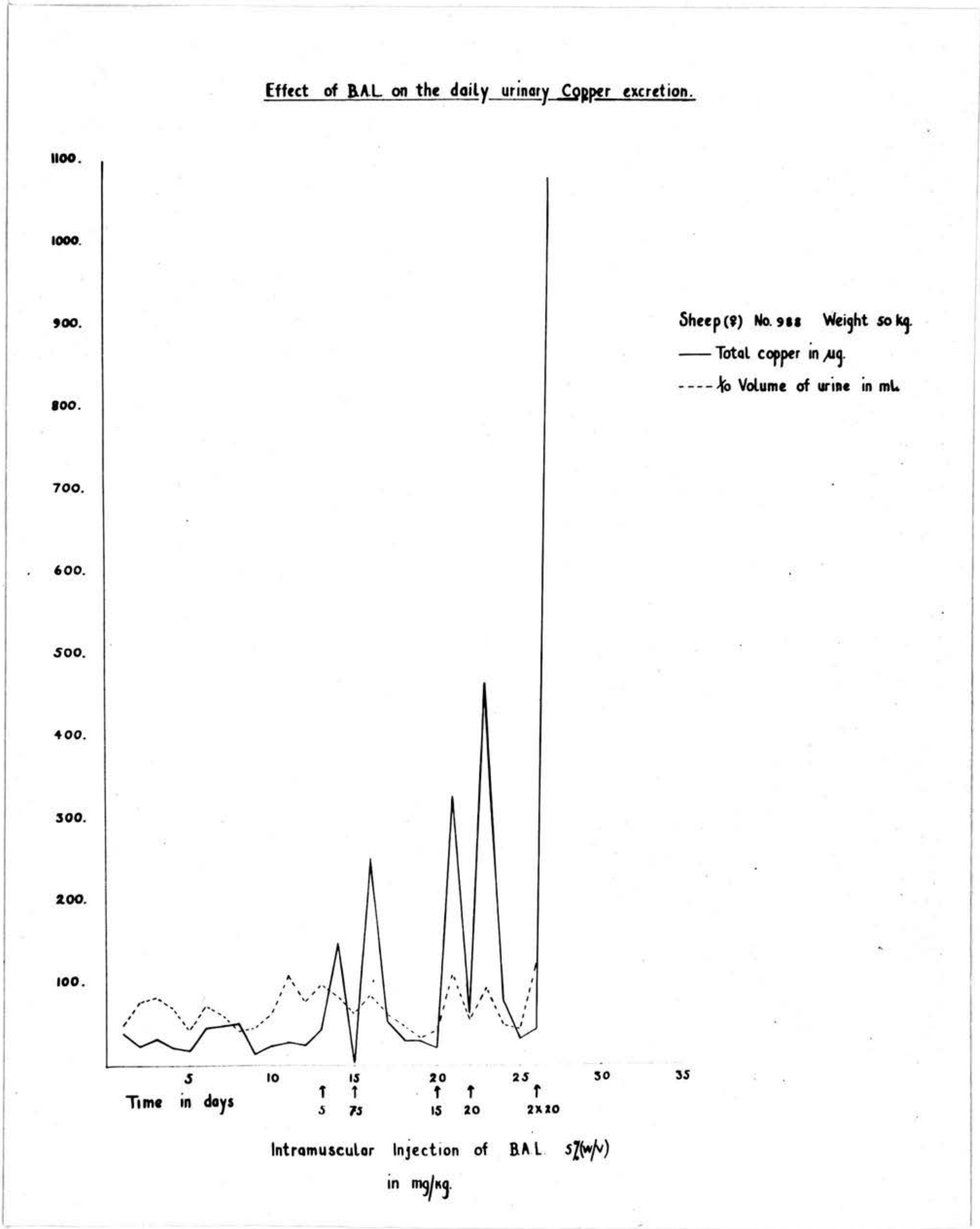

Effect of B.A.L. on the daily urinary Copper excretion.
1100.
1000.
900.
800.
700.
600.
500.
400.
300.
200.
100.
5
10
15
20
25
30
35
Time in days
5
75
15
20
2x20
Intramuscular Injection of B.A.L. 5%(w/v)
in mg/kg.
Sheep (♀) No. 988 Weight 50 kg.
Total copper in µg.
1/10 Volume of urine in ml.

Fig.3(b)

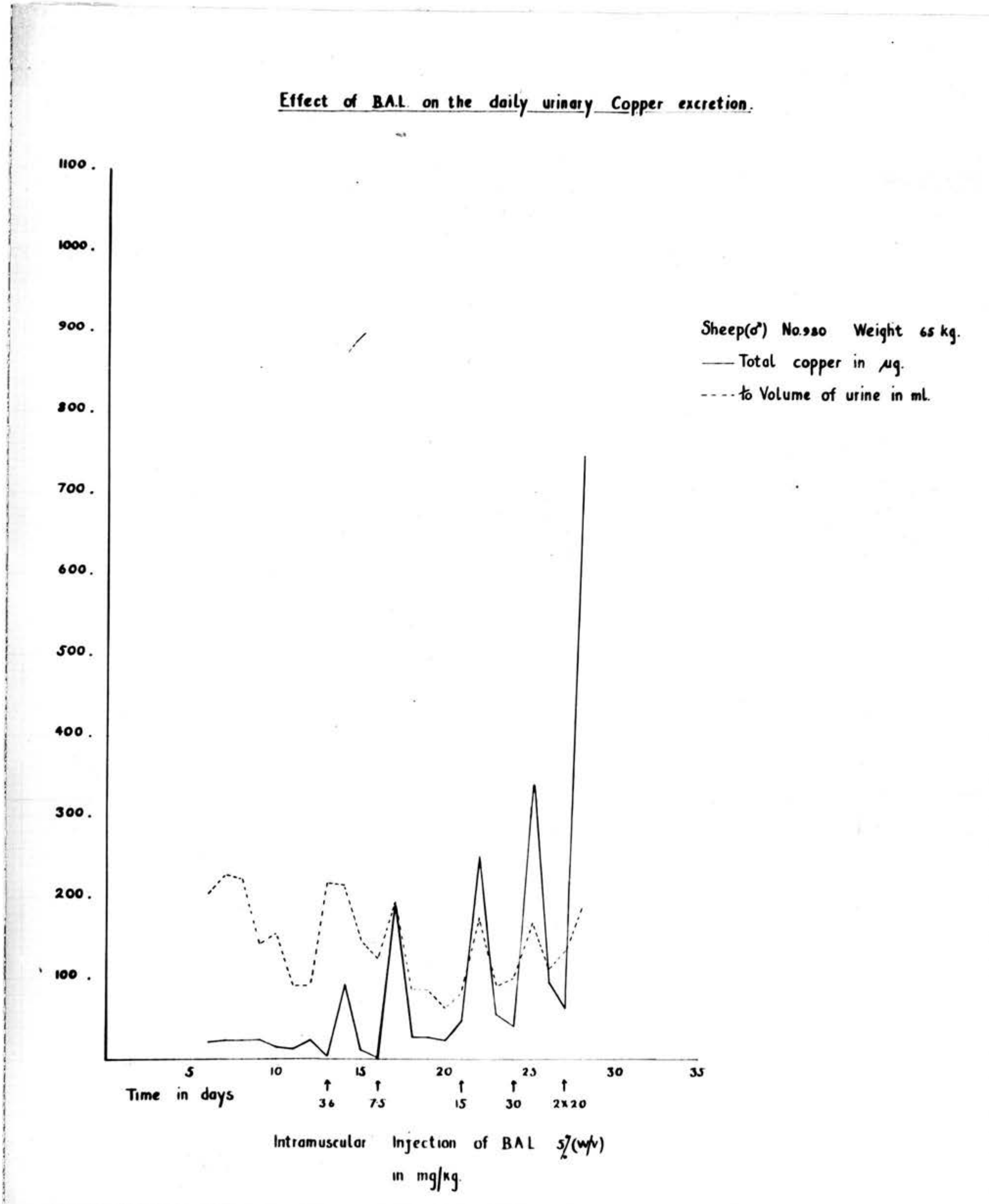

Effect of B.A.L. on the daily urinary Copper excretion.
1100.
1000.
900.
800.
700.
600.
500.
400.
300.
200.
100.
Sheep(♂) No.980 Weight 65 kg.
—— Total copper in µg.
- - - - to Volume of urine in ml.
5
10
15
20
25
30
35
Time in days
36
7.5
15
30
2x20
Intramuscular Injection of BAL 5% (w/v)
in mg/kg.

Fig.3(c)

excreted and on the urinary copper excretion of two sheep, No. 988 as in the previous experiment and No. 980. The dosing of these two animals was carried out concurrently under similar conditions over a period of 26 days.

Blood samples for copper determination were taken at intervals throughout the period and on the morning following the last dose of B.A.L. the animals were slaughtered. Copper determinations were carried out on the liver and the brain. Values obtained for blood, liver and brain copper are given in Table 3(a) below:

SHEEP No. 980.

Copper Values.

Blood.	11th	0.044 mg./100 ml.	at 11.00 a.m.
	12th	0.034 mg./100 ml.	at 2.00 p.m.
	25th	0.024 mg./100 ml.	at 11.00 a.m.
		0.038 mg./100 ml.	at 4,00 p.m.
	26th	0.022 mg./100 ml.	at 11.00 a.m.

Liver. 6.5 mg./100g. (wet weight)

Total copper in liver $6.5 \times \frac{720}{100}$ = 47.2mg.

Brain. 0.166 mg./100 g. (wet weight)

Total copper in brain $0.166 \times \frac{105}{100}$ = 0.170 mg

SHEEP/

SHEEP No. 988.

Copper Values.

Blood.	11th	0.036 mg/100ml.	at 11.00 a.m.
	12th	0.018 mg/100ml.	at 2.00 p.m.
	25th	0.064 mg/100ml.	at 11.00 a.m.
		0.025 mg/100ml.	at 4.00 p.m.
	26th	0.026 mg/100ml.	at 11.00 a.m.

Liver. 5.0mg/100g. (wet weight)

Total copper in liver $5.0 \times \frac{600}{100}$ 30.0mg.

Brain. 0.185 mg/100g.

Total copper in brain $0.185 \times \frac{90}{100}$ 0.163mg.

At the lower levels of dosing, i.e. up to 20 mg./Kg. only mild symptoms associated with the toxic effects of B.A.L. were seen, e.g. slight inter:mittent chewing movements and frequent attempts to urinate with the passage of only small quantities of urine. At the level of 30 mg./Kg. given to sheep No. 980 there was a more marked effect, the animal showing severe body tremors with signs of stiffness and loss of power in the hind quarters. These symptoms gener:ally passed off after 4 - 6 hours.

When the animals were killed and the perito:neal cavity opened the carcases smelled very strongly of B.A.L., apart from this there were no abnormal post mortem findings.

Experiment/

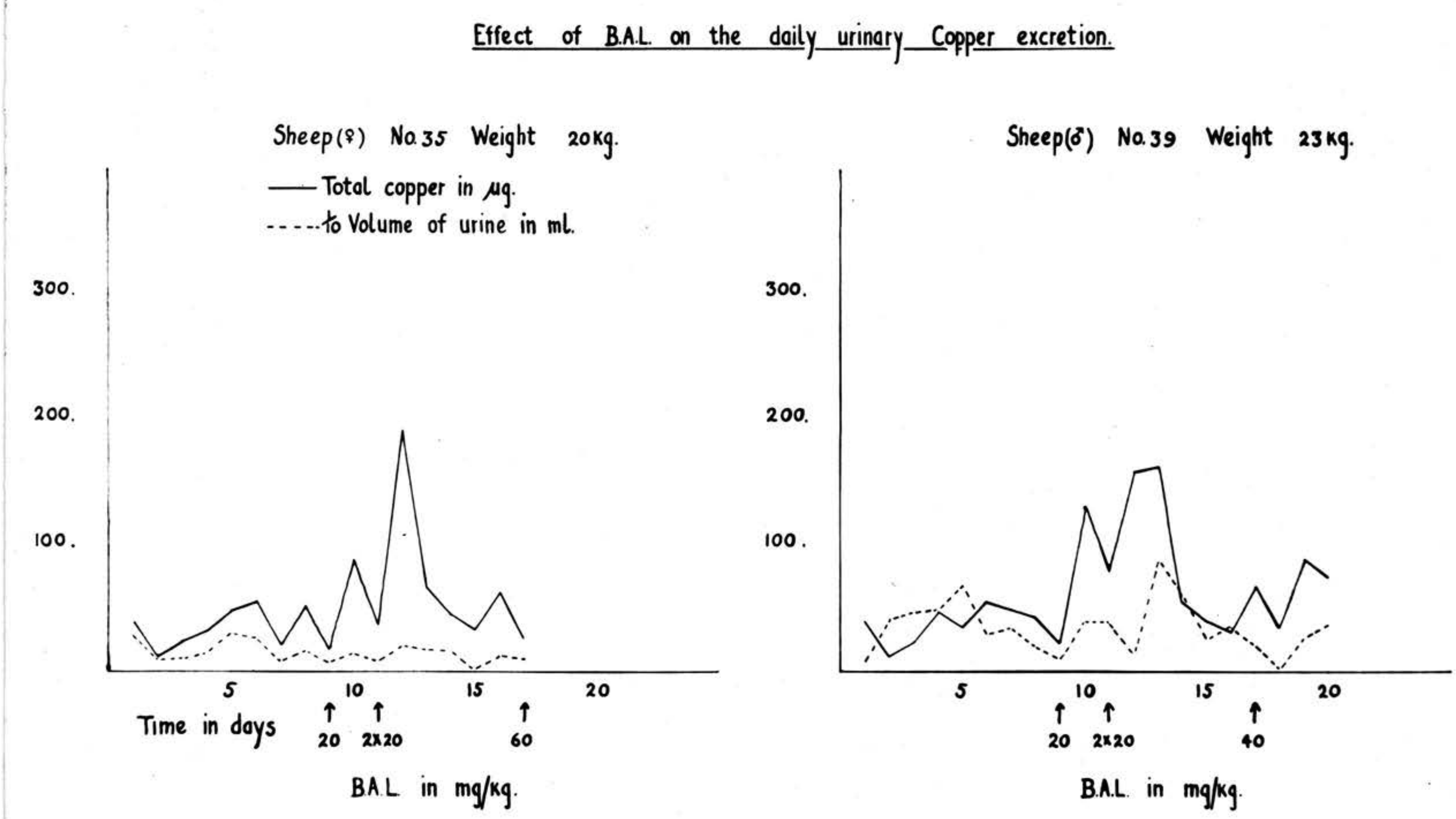

Effect of B.A.L. on the daily urinary Copper excretion.
Sheep(♀) No.35 Weight 20kg.
Total copper in μg.
Volume of urine in ml.
300.
200.
100.
5
10
15
20
Time in days
20
2x20
60
B.A.L in mg/kg.
Sheep(♂) No.39 Weight 23kg.
300.
200.
100.
5
10
15
20
20
2x20
40
B.A.L. in mg/kg.

Fig. 3(d)

Experiment III.

With the size of animal described above and at the high level of dosing, the use of the 5% B.A.L. was very expensive and it was therefore decided to use smaller animals in an attempt to obtain an approx:imate value for the lethal level of dosing. Two lambs, No. 35 and No. 39, weighing 20 and 23 Kg. re:spectively, were selected. In this experiment the B.A.L. was suspended in a 66% (v/v) propylene glycol/ water solution (1 ml. ≡ 200 mg. B.A.L.). Fig. 3(d) illustrates the results obtained.

The symptoms previously produced in sheep No. 980 with a dose of 30 mg./Kg. had indicated that this was perhaps approaching the lethal level of dosing and so animals No. 35 and 39 were dosed with 60 mg./Kg. and 40 mg./Kg. respectively. Lamb No. 35 died within two hours following increased respiration, severe body tremors, general weakness of the hind quarters and eventually complete prostration. At post mortem examination small haemorrhages on the epicardium were evident. Lamb No. 39 after showing symptoms similar to No. 35 was fairly normal again within 24 hours, but appeared weak and listless with a high respiratory rate for the next two or three days. It died three days after the injection, the post/

post mortem examination revealing a parasitic pneumonia. It is very probable that this animal died as a result of the pneumonia.

Throughout this experiment considerable changes in the weather accompanied by sudden fluc-:tuations in atmospheric temperature occurred.

Discussion.

Although the results of the first dosing carried out with sheep No. 988 and given in Fig. 3(a) will have to be neglected owing to the possibility of copper contamination from the drainage pipe, it should be noted that after the first dose of 5 mg./Kg. B.A.L. the animal showed the obvious signs of discomfort usually associated with B.A.L. therapy.

Although contamination by the copper pipe is the most likely explanation for the high value of copper found after this dose, it is of interest to note that these signs of discomfort have not since been observed in sheep until a much higher dosage level has been reached i.e. 15 - 20 mg./Kg., and, moreover, it was only with this dose and not with subsequent similar doses that both the signs of dis-:comfort occurred and the high value of copper were found.

The second group, comprising sheep No. 980 and No. 988, appears to give a clear cut demonstration of/

of the effect of B.A.L. on urinary copper excretion and its effect at different dosage levels, reaching maximal effect at about 2 x 20 mg./Kg., the interval between dosing being four hours. It is not consider:ed that any interpretation can be made of the varia:tions in blood copper levels as they stand, since subsequent work has indicated that many factors may cause changes in the blood copper level over very short periods and that a better controlled experiment involving larger numbers of animals would require to be carried out. In general, however, the values for copper in the blood of the animals are lower than the accepted normal range, viz. 0.06 - 0.120 mg./100 ml.

From sheep No. 988 one can obtain the value of 2285 µg. of copper excreted during the six 24 hour periods, following the B.A.L. injections, from which, by deducting say 6 x 30 µg. copper as representing normal excretion during this time (from fig.3(b)). It can be concluded that the additional copper ex:creted is about 2,100 µg. It is considered that if a liver biopsy (Dick 1944) had been performed to obtain a value for liver copper prior to the commencement of the experiment, no change in liver copper would have been detected even if all the copper lost had been ultimately reflected by a loss of copper in the liver. The liver of both sheep had "normal values" of copper present/

present and in the case of No. 988, where the total copper in the liver was 30 mg., the loss of copper calculated at 2.3 mg. would not be significant.

The results of the third group, comprising the small animals, No. 35 and No. 39, may be com:plicated by the abnormal temperature fluctuations. This may account for the wide variations in volume of urine excreted, especially by No. 39, (and hence have an effect on the total copper excreted). However, it does appear that a less striking increase in level of copper output is obtained as compared with the animals in Experiment II. It is possible that the age of the animals (7 months as compared with 18 months) might be a factor, although the more likely explanation may be that the increase in copper excretion may depend upon the total quantity of B.A.L. given, as distinct from equivalent doses as reckoned at so much per Kg. weight of animal. At the same dosage level of 2 x 20 mg./Kg. this group of animals was receiving approx:imately 2½ to 3 times less B.A.L. than the group with the larger animals.

It would appear, from the death of No. 35 and the symptoms produced in No. 39, that the lethal level of dosing must lie within 40-60 mg. B.A.L./Kg.

From figs. 3(a), 3(b), 3(c), 3(d), it would appear that in the above experiments all animals had similar/

similar normal levels of urinary copper excretion i.e. 20-40 mg. Cu per 24 hours.

Summary.

(1) In the sheep the normal rate of copper excretion in the urine is 20 - 40 mg. per 24 hours.

(2) Intramuscular injection of B.A.L. (2:3 dimercaptopropanol) increases the urinary copper excretion. At a dosage level of 2 x 20 mg./Kg. the increase may be as high as 30x.

(3) It is unlikely that any permanent change in blood or liver copper level is produced in the sheep by doses of B.A.L.

(4) The toxic dose of B.A.L. for sheep lies between 40 - 60 mg./Kg.

The Effect of Prolonged Dosing of Pregnant Sheep with B.A.L.

Since the main trend of this thesis was directed towards the problem of swayback in lambs, it was decided to try the effect of prolonged dosing with B.A.L. on sheep in the last month of gestation, to determine whether this produced any effect on the developing foetus or on the blood copper level of the ewe.

Experimental.

As the recommended dosage level for humans in the treatment of metallic poisoning is 2 x 2 ml. of 5% w/v B.A.L. and since the sheep were in advanced pregnancy, the dosing was commenced at a level of 2mls. (The work described in the previous section was not completed when this experiment was commenced).

Twelve ewes in the last month of pregnancy and running on free range were divided into two groups. Eight animals in Group A received B.A.L. while the four animals in Group B were controls. Dosing of Group A was carried out between 1st - 25th March, 1948, at levels shown in table 3(b).

B.A.L. INJECTIONS.

Group A.

Date.	Dose. 5% (w/v) B.A.L.
1/3/48	2 ml.
3/3/48	2 ml.
5/3/48	2 ml.
8/3/48	3 ml.
11/3/48	3 ml.
15/3/48	3 ml.
18/3/48	3 ml.
22/3/48	3 ml.
25/3/48	3 ml.

Blood samples taken on 1st March and 15th March gave values for copper, haemoglobin, sugar and chloride as shown in table 3(c). Differential blood counts (carried out by W. Smith) of the animals were also obtained and are given in table 3(d).

In two cases large fibrous lesions were observed on the leg at the site of injection, apart from which no signs of discomfort were observed and all the ewes lambed normally between 15th - 30th March, somewhat earlier than had been expected.

In/

Group B. Controls.

No.	Copper. mg./100 ml.		Haemoglobin. g/100 ml.		Sugar. mg/100 ml.		Chloride. mg/100 ml.	
	1/3/48	15/3/48	1/3/48	15/3/48	1/3/48	15/3/48	1/3/48	15/3/48.
685	.064	.074	7.6	6.5	38.8	41.5	540	540
690	.062	.060	7.8	6.4	54.0	50.5	540	540
691	.042	.042	6.8	5.9	46.1	45.9	550	550
695	.042	.070	6.9	8.6	40.3	45.5	550	540
Mean	.053	.062	7.3	6.9	44.8	45.9	545	540

Table 3(c)

BLOOD VALUES.

Group A. B.A.L. Treated.

No.	Copper. mg./100 ml.		Haemoglobin. g/100 ml.		Sugar. mg/100ml.		Chloride. mg/100ml.	
	1/3/48	15/3/48	1/3/48	15/3/48	1/3/48	15/3/48	1/3/48	15/3/48.
684	.066	.084	7.1	7.7	49.5	46.5	550	520
686	.078	.082	7.3	6.9	44.0	44.9	520	540
687	.062	.062	7.4	6.2	43.1	45.5	550	550
688	.054	.062	7.8	7.1	51.0	47.3	550	540
689	.064	.076	8.5	7.1	51.6	40.0	540	540
692	.040	.050	10.0	10.6	45.7	44.5	540	550
693	.054	.046	6.6	6.9	51.8	49.0	530	540
694	.050	.056	6.6	8.4	47.6	48.3	540	550
Mean	.059	.065	7.7	7.6	48.0	45.8	540	540

DIFFERENTIAL BLOOD COUNT.

1/3/48.

	Group A. B.A.L. Treated.								Group B. Controls.			
No.	684	686	687	688	689	692	693	694	685	690	691	695
Polys.	26	20	20	22	17	26	21	30	52	23	25	34
Lymphs	65	71	66	66	67	63	65	57	35	64	60	52
Monos	5	5	4	4	4	4	5	6	2	4	6	5
Eos.	4	4	10	8	12	7	8	7	11	8	9	9
Basos.	/	/	/	/	/	/	1	1	1	1	/	/

15/3/48

	Group A. B.A.L. Treated.								Group B. Controls.			
No.	684	686	687	688	689	692	693	694	685	690	691	695
Polys.	29	28	27	29	28	39	42	27	33	27	31	29
Lymphs.	58	58	63	61	58	42	40	55	50	56	56	57
Monos	7	6	7	4	7	9	7	10	8	10	5	6
Eos.	6	8	3	6	7	10	11	8	9	7	7	8
Basos.	/	/	/	/	/	/	/	1	/	/	/	/

Table 3(d)

In the following year, 1949, the above ex-:periment was repeated with two pregnant sheep re-:ceiving high doses of B.A.L. (20 mg./Kg. daily) during the period 11th March - 28th March. Table 3(e) gives blood copper values at the beginning and at the end of the experiment.

Table 3(e)

No.	Blood Copper mg./100 ml. 11/3/49	Blood Copper mg./100 ml. 28/3/49
107	0.054	0.053
695	0.070	0.088

Discussion.

No significant change in blood copper levels is evident between the commencement of dosing and 14 days later, nor between the experimental group and the control group.

From the results obtained in the previous section it is now obvious that the first level of dosing would be far too low to produce any appreciable increase in copper excretion, and where an increase was obtained i.e. with 20mg./Kg. the resulting loss would be insignificant. Moreover, changes may occur in blood copper levels as a result of approaching parturition/

parturition which would mask any small change caused by the B.A.L. (cf. Section I).

Conclusion.

Apart from the absence of ill effects on the ewe or the foetus from repeated B.A.L. injections at a low and a high level, no conclusions can be reached from this experiment.

SECTION IV.

Factors influencing the Blood Copper Level - The Effect of an Infection.

During an experiment carried out on a sheep that was simultaneously being used for passaging tick-borne fever, an infection caused by a rickettsia-like organism, it was observed that considerable changes were occurring in blood copper level. The following experiment was carried out to observe in more detail the effect produced by an infection on blood copper levels.

(1) Tick-borne Fevel Infection.

Experimental.

Six sheep were selected and infected separately at intervals of one week with tick-borne fever. The innoculum consisting of 5 ml. of blood taken from an infective animal.

According to Gordon *et al* (1932) - "The disease is characterised by an incubation period of varying length usually varying from 4-6 days (under the conditions of this experiment usually 3-4 days) followed by a febrile phase of approximately 10 days duration, although it is often irregular and prolonged. During the continuance of fever the animal is dull and listless. Uncomplicated cases usually recover. If, during the febrile phase, the animal is destroyed enlargement of the spleen and lymphatic glands constitute the most constant macroscopic lesion present. There/

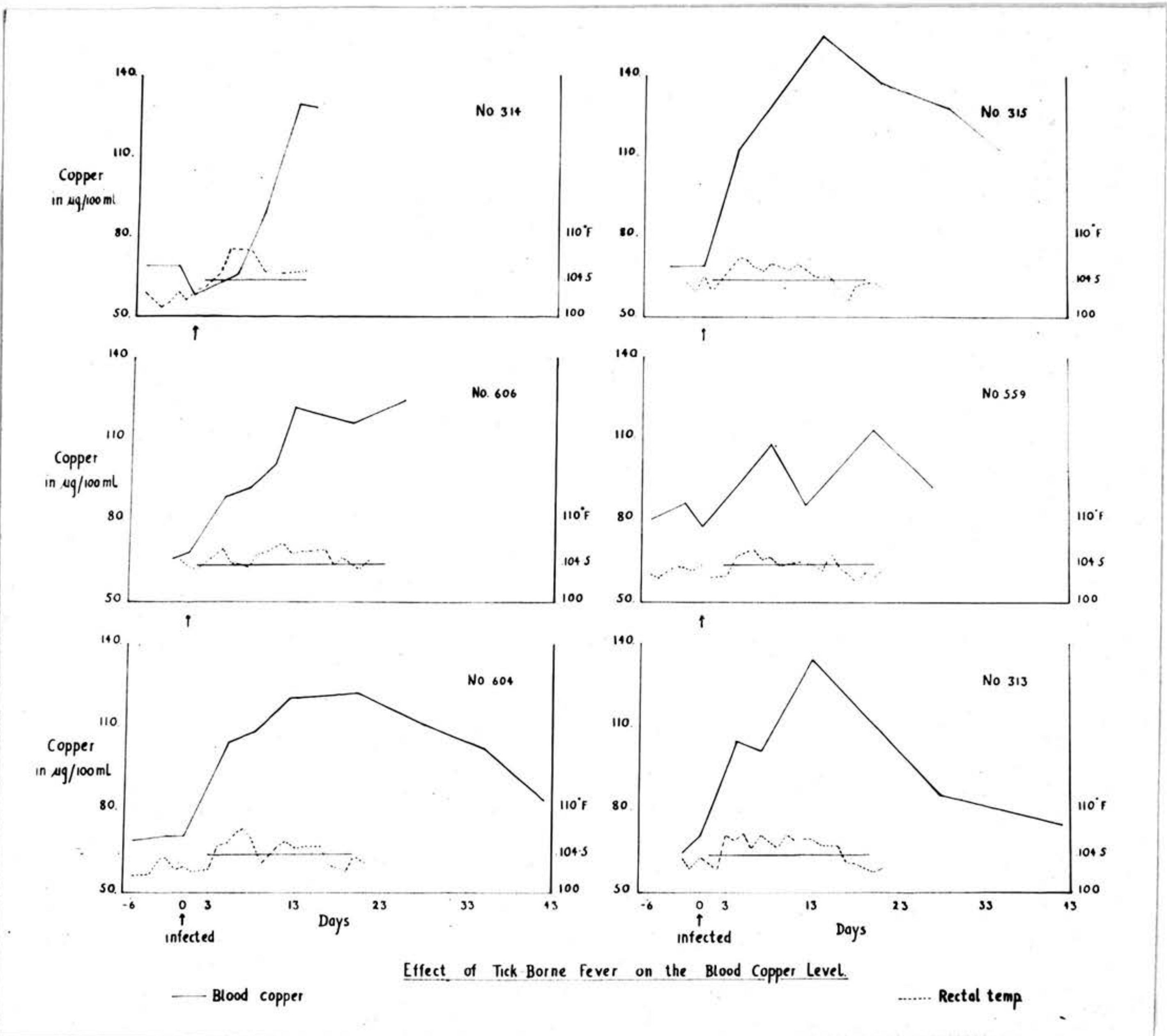

No 314
No 315
No. 606
No 559
No 604
No 313
Copper in µg/100 ml
Days
infected
110°F
104.5
100
Effect of Tick Borne Fever on the Blood Copper Level.
Blood copper
Rectal temp

Fig.4(a)

There is an increase in polymorphonuclear cells."

The rectal temperature of a normal sheep may vary within wide limits but may be taken as between 102-104°F. (Clawson,1928)

All the sheep were running together on free range and there was no complicating factor such as change of diet during the course of the experiment.

Although no sheep were kept as controls, all the sheep had comparable normal commencing values, spread over the 6-weekly periods of infection.

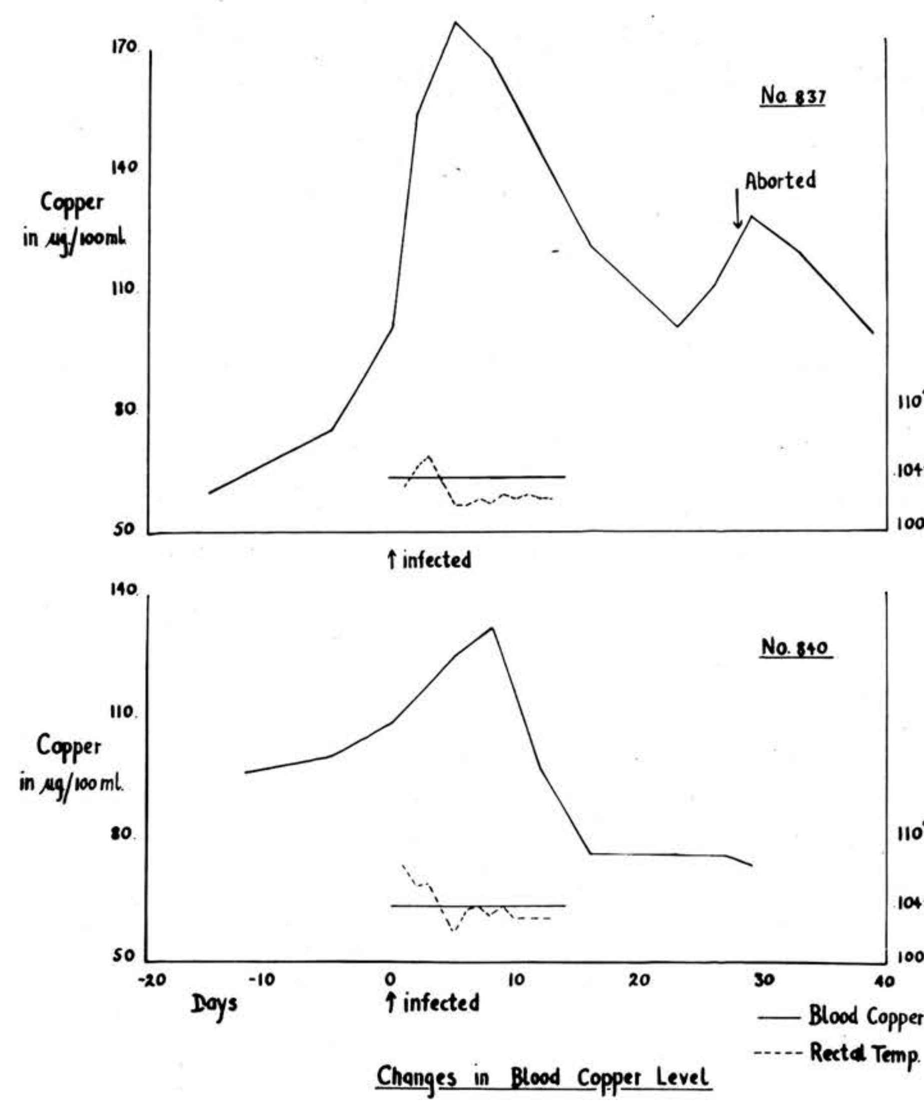
Three blood samples for copper estimation, at intervals of 2-3 days were taken before infecting the sheep and then at frequent intervals thereafter. In some cases the blood copper determination was continued up to 50 days following the infection.

Results.

Fig.4(a) records the changes in the blood copper level in relation to the temperature reaction for the individual sheep Nos. 313, 314, 315, 559 604 and 606.

From these graphs it is clear that a considerable rise in the blood copper levels occurs in response to this infection, attaining a maximal value between the 10th-14th day, i.e. from 6-10 days after the onset of the temperature reaction. In the light of findings of Gordon _et al_ (1932) this increase in blood copper appears to bear no relation to change in the blood cytology of the animal.

(2)/



No. 837
Aborted
Copper in µg/100ml.
170
140
110
80
50
110°F
104·5
100
↑ infected
No. 840
Copper in µg/100 ml.
140
110
80
50
-20
-10
0
10
20
30
40
Days
Blood Copper
Rectal Temp.
Changes in Blood Copper Level

Fig.4(b).

(2) Enzootic Abortion Infection.

This disease in sheep is caused by as yet unclassified rickettsia-like bodies which localise themselves on the cotyledons of the placenta causing the sheep to abort generally within 14 days of term (Stamp, McEwen et al, 1950). Very little is known regarding the course of the disease.

Experimental.

Two sheep, No.837 (pregnant) and No.840 (non-pregnant) were put in stalls within four days of each other and the blood copper level followed for 16 and 12 days respectively prior to infection.

The animals were then infected intravenously with egg yolk sac suspension containing the organism and the blood copper followed for a further 30 days.

Sheep No.837 aborted on the 28th day following infection. The abortion was diagnosed as having been caused by the infective agent.

Results.

Fig.4(b) shows the changes obtained in the blood copper levels together with the temperature reactions.

The results are complicated by the effect of bringing the sheep indoors (both sheep were very nervous) and by a change in the feeding stuffs which would probably have a different copper concentration. This/

This may be reflected in the rise in blood copper observed in both sheep during the control period. However, a further rise in blood copper following infection does appear to take place, particularly in the case of No.837 which reached the very high level of 0.175 mg./100 g. blood. The response to this infection would appear to occur much more rapidly, i.e. within 5-8 days, than in the case of tick-borne fever infection.

An interesting point arises in the case of No.837 where a further increase occurred, from 0.100-0.126mg/ 100 g. blood, coinciding with the time this animal aborted.

Discussion.

The results obtained would appear to be in agreement with those of Nielsen (1944) and of van Damme *et al* (1945), who observed increases in the blood copper level of humans during infection of various kinds.

Since tick-borne fever, in particular, is very widely spread throughout Scottish hill sheep and the elevation observed in blood copper level persists over a prolonged period, it would appear that this factor would have to be carefully considered in any experiment designed to follow changes in the level of blood copper.

Further experiments would require to be carried out/

out before the nature of the factor controlling this change can be determined. These would be designed to show whether the rise is determined by :-

(a) Fever involving a breakdown in the heat regulating mechanism accompanied by a corresponding breakdown in the control of blood copper level.

(b) Increase in general metabolic rate during febrile phase causing an increase in the metabolism of copper with subsequent increase in copper blood level. This might be effected through the thyroid.

(c) Increase in antibody formation possibly involving Cu-protein complexes.

The most likely factor probably involved the thyroid activity for it has been shown by Naraska (1937) that in rabbits thyroidectomy has the effect of producing an appreciable lowering of blood copper, while injection of thyroxine produces a considerable rise in the blood copper.

Summary.

(1) A considerable rise in blood copper level took place in six sheep after infection with tick-borne fever, reaching maximal value 10-14 days after infection some of them not returning to normal values till approximately 40 days after infection.

(2) A rise in blood copper level took place in two sheep after infection with enzootic abortion. In the case of one sheep an extremely high level was reached. Other factors may have influenced these changes.

General Conclusions.

So far it has been in the field of veterinary science that almost all the work on copper deficiency syndromes occurring in domestic animals (summarised in the introduction) has been carried out with emphasis on diagnostic methods, pathological changes and therapeutic method of control and prevention. More recently the problem of the relationship between the copper content of the soil and of the pasture has been receiving the attention of workers in the field of soil and plant research with emphasis on the possible existence, in certain areas, of some metallic radical antagonistic to the uptake of copper either by the plant or the animal. This general trend of investigation has been continued in Sections, I, II and III of this thesis using animals, normal at the commencement of the experiment and not animals actually exhibiting or having exhibited syndromes associated with copper deficiency, and from observations made particularly in Sections II and IV it would appear that the level of copper in the animal and its rate of excretion, are controlled or associated with some definite physiological mechanism.

Although the wide approach to the problem,as outlined above, has been of primary economic importance and of practical value in the control of the syndromes, it has contributed little, if anything, to/

to a better understanding of the metabolic processes involving copper in the normal animal and to the elucidation of the changes in biochemical equilibrium that apparently occur in the dynamic systems within the animal as a consequence of a reduction in its copper status. It is considered that any real advance must now come from a more fundamental approach to the problem, involving the isolation and study of the properties of the actual copper complexes present in the soil, plant and in the animal. The properties of the more important complexes in the animal will undoubtedly be enzymic in character and measurements of the activities of these enzyme systems in the normal and copper deficient animal would be the most likely approach to lead to a better understanding of the functions of copper in the normal animal and of the manner in which the clinical signs and pathological picture found in animals suffering from copper deficiency, are produced.

SUMMARY

This thesis, after a review of the various syndromes arising in sheep and in cattle through a reduction in the copper levels of their blood and organs, describes in four sections experiments carried out on certain aspects of copper metabolism in these ruminants.

Section I: Variations associated with the season and in some cases with the age of the animal have been found to occur in the blood copper concentration, liver copper concentration, total liver copper and liver weight of 200 Cheviot sheep of all ages, in a Scottish hill-sheep area.

Section II: The copper balances of sheep have been studied with and without supplementary feeding of Molybdenum salts, to ascertain whether molybdenum has any effect on the copper balance. No conclusive results were obtained.

Section III: Experiments are described showing how dozing with B.A.L. (British-Anti-Lewisite, 2:3 dimercaptopropanol) increases the urinary excretion of copper.

Section IV: Considerable elevations in the blood copper level of sheep have been obtained as a consequence of two types of infection given to the animal.

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APPENDIX I

GROUP "A"
Slaughtered 31st Aug.48

Year of Birth	Number	Blood Copper mg/100 ml.	Liver Copper wet wt. mg/100 g.	Fresh Wt. of Liver g.	Total Liver Copper mg.
	802	0.066		425	
	807	0.062		320	
	808	0.100		380	
	812	0.080		415	
1948	814	0.074		380	
	825	0.052		425	
	828	0.044		395	
	832	0.064		580	
	837	0.044		355	
	841	0.052		475	
	725	0.074	1.82	605	11.0
	727	0.074	4.02	685	27.5
1947	733	0.070		685	
	873	0.042	2.65	515	13.6
	874	0.060	4.64	510	23.7
	875	0.060		660	
	670	0.088		830	
	678	-		665	
	867	0.038	4.16	680	28.3
1946	868	0.048		675	
	869	0.032		680	
	870	0.046		830	
	871	0.054		750	
	872	0.062		870	
	896	0.068	4.00	845	33.8
	702	0.082		915	
	707	0.062		855	
1945	719	0.074		815	
	863	0.070		600	
	864	0.048		915	
	865	0.060	0,804	750	6.03
	745	0.074		735	
	748	0.058		825	
1944	752	0.052		795	
	760	0.056		780	
	762	0.066		745	
	866	0.070		790	
	859	0.096	3.24	690	22.3
1943	860	0.048	1.33	795	10.6
	861	-	1.73	710	12.3
	862	0.062		735	
	781	0.052		860	
	850	0.034		820	
	852	0.044	1.06	670	7.1
1942	853	0.038		815	
	854	0.042	2.17	700	15.2
	855	0.044		670	
	856	0.018	5.84	635	37.1
	857	0.044	6.21	755	46.9
	858	-	4.37	-	-

GROUP "B"
Slaughtered 10 Jan.49.

Year of Birth	Number	Blood Copper mg/100 ml.	Liver Copper Wet Wt. mg/100g.	Fresh Wt. of Liver g.	Total Liver Copper mg.
	801	0.036	3.33	320	10.7
	803	0.054	2.25	395	8.9
	805	0.004		375	
	811	-		285	
1948	813	0.058		340	
	816	0.038		320	
	821	0.066		330	
	833	0.054	3.02	405	12.2
	842	0.054	2.75	330	8.4
	845	0.046		-	
	726	0.060		585	
	732	0.054	1.18	525	6.2
1947	738	0.052	1.46	508	7.4
	739	0.070	8.17	460	37.6
	743	0.024	0.69	400	2.8
	671	0.056	4.52	580	26.2
	675	0.058	17.1	555	95.0
	476	0.076	2.76	545	15.0
1946	679	0.060	6.77	500	33.9
	680	0.038	1.44	420	6.1
	684	0.036		525	
	693	0.052	1.43	535	7.6
	695	0.028	2.38	545	13.0
	697	0.056	4.44	575	25.6
	698	0.048	0.43	580	2.5
1945	701	0.054	1.77	445	7.9
	703	0.056	8.87	660	58.6
	716	0.056	2.88	540	15.5
	717	0.062	1.73	550	9.5
	720	0.050		565	
	744	0.044		615	
	746	0.046	1.37	620	8.5
1944	749	0.042		555	
	759	0.060	3.45	510	17.6
	761	0.052	5.50	540	30.1
	966	0.054	5.68	510	28.0
	654	0.064	4.85	705	34.2
	655	0.056	1.22	545	5.9
1943	657	0.062	2.27	650	14.7
	658	0.062	1.67	635	10.6
	664	0.060	15.97	480	76.5
	769	0.070	4.5	560	25.2
	770	0.066		525	
	771	0.054	8.57	570	49.3
1942	772	0.072	8.93	575	46.9
	786	0.062		550	
	789	0.056	5.33	605	32.2
	790	0.064		710	
	791	0.056	13.9	605	84.1
	794	0.070	3.43	480	16.5

GROUP "C"

Slaughtered 4th April 1949

ear of irth	Number	Blood Copper mg/100 ml.	Liver Copper Wet Wt. mg/100 g.	Fresh Wt. of Liver g.	Total Liver Copper mg.	Foetus LiverCopper Wet Wt. mg/100g.
	809	0.045	2.57	405	10.4	0.53
	815	0.038	0.67	450	3.01	
	817	0.032	3.0	365	16.0	
	820	0.029	1.93	425	8.21	
948	826	0.022	0.74	350	2.59	
	829			390		
	834			405		
	835			475		
	838			425		
	844			400		
	728	0.044		510		
	730	0.030	0.59	470	2.77	1.1
947	734	0.036	0.46	470	2.19	0.73
	736	0.034	1.88	400	7.52	0.82
	740	0.014	0.40	500	2.00	0.41
	668	0.032	2.0	625	12.5	0.31
	674	0.022	0.60	765	4.29	0.60
	677	0.022	1.14	600	6.84	0.24 0.40
946	685	0.030	0.58	640	3.71	0.29
	687	0.028	2.30	530	1.22	
	688			655		
	690			705		
	691			525		
	700	0.052	3.37	840	21.6	0.91
	704	0.044	1.46	550	8.03	2.4
945	705	0.022	0.46	670	3.08	0.48
	708	0.038	1.30	650	8.45	0.75
	711	0.055	1.85	665	12.3	1.1
	715			670		
	722			655		
	750			555		
	756	0.039	0.53	650	3.44	0.13
944	757	0.028	0.48	670	3.21	
	758	0.044	2.1	620	13.0	1.03
	764	0.041	5.6	565	31.6	0.74
	767		0.25	585	1.46	0.49 0.43
	652	0.028	3.45	555	19.1	0.89
	653	0.032	0.68	690	4.69	0.92
943	659	0.052	2.5	570	14.3	1.35 2.03
	661	0.058		690		
	665	0.052	0.84	470	3.95	1.35
	776	0.020	0.69	530	3.66	
	777	0.048	4.2	615	2.58	1.19 0.91
	780	0.036	3.3	555	1.83	0.36
942	782	0.036	0.32	600	4.92	
	783	0.046	2.0	555	11.1	0.86
	792			640		
	793			645		
	797			600		
	798			625		

GROUP "D"
Slaughtered 7 June 1949

ar f	Number	Blood Copper mg/100 ml.	Liver Copper Wet Wt. mg/100 g.	Fresh Wt. of Liver g.	Total Liver Copper mg.
	816	0.074	2.10	585	12.3
	818	0.84	4.30	625	26.9
	819	0.070	3.81	755	28.8
	822	0.068	-	580	
8	831	0.048	3.34	560	18.7
	836	0.052	1.00	625	6.25
	840	0.044	3.06	700	21.4
	843	0.056	2.83	620	17.6
	846	0.050	1.57	630	9.89
	847	0.044	3.75	600	22.5
	724	-	5.16	760	39.2
	731	0.028	3.33	870	29.0
7	735	0.056	0.32	725	2.32
	737	0.056	0.70	735	5.14
	742	0.054	2.35	685	16.1
	669	0.048	6.76	815	55.1
	673	0.044	2.83	890	25.2
	681	0.048	2.43	765	18.6
6	682	0.068	0.66	870	5.74
	686	0.034	-	955	
	689	-	1.80	830	14.9
	692	0.044	5.08		
	694	0.040	4.34	830	36.0
	699	0.054	1.02	1010	10.3
	700	0.110	2.76	1040	28.7
	709	0.084	2.04	1005	21.0
5	710	0.084	0.57	715	4.07
	712	0.100	1.17	895	10.5
	713	0.040		750	
	721	0.050	3.37	970	32.3
	723	0.078	2.46	1070	26.3
	747	0.040	5.15	1050	54.1
	751	0.068	2.43	910	22.1
4	753	-	0.69	935	6.45
	735	0.028		810	
	765	-		850	
	768	0.100		1015	
	651	0.042		1030	
	656	0.104		1055	
3	662	0.054		970	
	663	0.044		980	
	666			1080	
	775	0.044		880	
	778	0.080		820	
	784	0.068		890	
1942	785	0.056		890	
	787	0.108		980	
	788	0.078		950	
	795	0.056		860	
	796	0.048		920	

APPENDIX II

SHEEP No. 632

riod	Copper Excretion		Total Copper Output in µg.	Total Copper Intake in µg.	Copper Balance		Molybdenum Supplement in mg.
	Faeces in µg.	Urine in µg.			in µg.	% of Intake	
1	11700	450	12150	12100	- 50	- 0.41	NIL
2	11550	230	11780	13450	1670	12.4	NIL
3	12090	280	12370	13110	740	5.7	250
4	13100	340	13440	12220	-1220	-10·0	300
5	15500	180	15680	13880	-1800	-13·0	600
6	14540	190	14730	13880	- 850	- 6.2	600
7	16130	240	16370	13880	-2490	-18·0	600

SHEEP No. 800.

Period	Copper Excretion		Total Copper Output in μg	Total Copper Intake in μg.	Copper Balance		Molybdenum Supplement in mg.
	Faeces in μg.	Urine in μg.			in μg.	% of Intake	
1	14200	350	14550	14890	340	2.3	NIL
2	14300	390	14690	15200	510	3.4	NIL
3	10700	360	11060	11220	160	1.5	90
4	14360	240	14600	11580	-3020	-26·0	105
5	12420	280	12700	12450	- 250	- 2·0	120
6	9540	210	9750	12560	2810	22.3	210
7	11570	500	12070	11680	- 390	- 3.3	300
8	13280	410	13690	12200	-1490	-11.4	300

SHEEP No. 269.

Period	Copper Excretion		Total Copper Output in μg	Total Copper Intake in μg	Copper Balance		Molybdenum Supplement in mg.
	Faeces in μg	Urine in μg			in μg.	% of Intake	
1	15300	1200	16500	12140	- 4360	- 36.0	NIL
2	16880	510	17390	13150	- 4240	- 32.0	NIL
3	20240	410	20650	14350	- 6300	- 44.0	NIL
4	14220	410	14630	15640	1010	6.5	NIL
5	15480	450	15930	15650	- 280	-1.8	NIL
6	15420	970	16390	19320	2930	15.2	NIL
7	39800	950	40750	19520	-21230	-109·0	NIL
8	19780	1070	20850	17090	- 3860	- 22.6	NIL
9	19700	820	20520	15920	- 4600	- 28.9	NIL
10	20200	920	21120	12870	- 8250	- 64.1	NIL
11	15000	1530	16530	11380	- 5150	- 45.3	NIL
12	12000	600	12600	12810	210	1.6	NIL
13	14820	570	15390	13560	- 1830	- 13.5	NIL
14	14630	470	15100	8260	- 6840	- 83.0	NIL

SHEEP No. 281

riod	Copper Excretion Faeces in µg	Copper Excretion Urine in µg	Total Copper Output in µg	Total Copper Intake in µg	Copper Balance in µg	Copper Balance % of Intake	Molybdenum Supp. in mg.
1	15400	540	15940	11570	-4370	-37.9	NIL
2	19660	540	20200	12450	-7750	-67.0	NIL
3	18350	290	18640	13450	-5190	-38.6	NIL
4	14700	150	14850	13860	- 990	- 7.1	NIL
5	13800	260	14060	13660	- 400	- 2.9	NIL
6	14300	840	15140	14900	- 240	- 1.6	NIL
7	36810	390	37200	16340	-20860	-128·0	NIL
8	18670	840	19510	13940	-5570	-40.8	NIL
9	19480	680	20160	13220	-6940	-52.5	NIL
10	16900	760	17660	13070	-4590	-35.2	NIL
11	13640	670	14310	13690	620	- 4.6	NIL
12	12940	690	13630	12810	- 820	- 6.4	NIL
13	13900	540	14440	13820	- 620	- 4.5	NIL
14	17020	690	17710	14660	3110	-21.3	NIL
15	16060	670	16730	14260	-2470	-17·0	3,000