NUTRITIONAL FACTORS INVOLVED IN WOOL PRODUCTION BY MERINO SHEEP

II. THE INFLUENCE OF COPPER DEFICIENCY ON THE RATE OF WOOL GROWTH AND ON THE NATURE OF THE FLEECE

By Hedley R. Marston and Hector J. Lee*

(Plate 1)

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Summary

A range of nutritional states which varied in relatively small degrees from a normal physiological condition to one of acute copper deficiency was induced in a series of evenly matched groups of Merino sheep depastured on deficient terrain by providing them with supplements of copper which extended through sub-optimum amounts to quantities in excess of their full requirements.

The copper and molybdenum intakes from the pastures were assessed and possible interactions are discussed.

The rate at which the syndrome of copper deficiency developed was governed by the quantity of copper provided in the supplement.

The amount of wool produced by the unsupplemented animals was materially less than that produced by those which received the copper supplements, the increments of increase in wool production above that of the unsupplemented level being related to the additional copper in the supplements by a curve of diminishing returns which reached the asymptote of wool production when the equivalent of between 7.5 and 10 mg. Cu/day was provided.

The capacity to impart crimp to the fibres began to be influenced when the concentration of copper in the systemic blood stream fell below approximately 0.4 mg. Cu/l. and this function failed completely at blood-copper levels <0.2 mg./l. A normal concentration of copper in the bloods of all animals which received supplements $\geqslant 10$ mg. Cu/day was maintained and no untoward effects were suffered by these animals.

I. Introduction

Previous experimental observations (Marston 1946; Marston and Lee 1948; Marston, Lee, and McDonald 1948*a*, 1948*b*) have revealed that the first discernible symptom of an abnormally low copper status in the sheep is the failure of the follicles to impart crimp to the wool fibres.

As these earlier experiments were designed primarily to study symptoms other than the impairment of wool growth, the groups of experimental animals were made up solely on the basis of body weight, and no special precautions were taken either to select them from a flock of particularly even wool type or to distribute the individuals among the groups according to their wool producing capacities. Although the lesions always appeared in the fleeces of the copper-

* Division of Biochemistry and General Nutrition, C.S.I.R., University of Adelaide, South Australia.

deficient animals the individual variation in the fleece weights within the groups was large, and so discussion of the effects of copper deficiency on the overall wool producing capacity of the sheep was withheld until more conclusive data were available.

In the experiment described below, the approach was analogous to a chemical titration. Supplements of copper ranging in increments from nil to an amount that previous experiments (ibid.) had indicated would more than fulfil the physiological requirements of sheep depastured on the deficient terrain where the experiment was conducted, were administered to a series of evenly matched groups of sheep. In this way a graded series of deficiency states was realized. The "end point" after which there was no further response to additional copper was indicated quantitatively by the mean weight of clean scoured wool produced and qualitatively by the complete absence of copper-deficient lesions in the fleeces of the individuals of the particular group.

II. The Experimental Animals

The experimental animals were chosen from a flock of over 5,000 evenly bred (Mutooroo) Merino ewe hoggets by drafting off 140 of desirable size and conformation, and then by individual examination selecting 70 of them for similarity of fleece characteristics. These were tagged and transported by rail to the field station where they were received on to the deficient pastures early in August 1944. A fortnight later a staple of wool was taken from the skin region above the acromion of the right scapula of each individual and the mean fibre diameter of the base of this staple was determined by direct measurement. The animals were ranged seriatim according to the fibre diameters of their fleeces and, after rejection of the extremes, the remaining 64 were bracketed consecutively into 8 lots each of 8 animals. The individuals of each lot were then distributed at random (Tippett) through the 8 experimental groups which thus were as evenly matched as was feasible. The mean age of the animals when they were transferred to the deficient pastures was approximately 10 months; they were about one year old when treatments began. The observations were continued for 4 years; the behaviour of the animals during the first 2 years is reported in this paper.

III. The Procedure

The procedure adopted was similar to that described in previous papers. The 8 groups were depastured as one flock on the deficient terrain at Robe where it had been demonstrated that sheep suffer a dual deficiency of cobalt and of copper and that appropriate treatment with either element would reveal the uncomplicated syndrome of a profound deficiency of the other. Cobalt deficiency was prevented by providing all animals with an ample supplement of cobalt. The amounts of copper administered to the groups were chosen to range in relatively small increments about the quantity previously proven adequate to provide indefinitely for all physiological functions of sheep depastured on this terrain. The copper and cobalt were administered as 5 ml. of an aqueous solution

of cupric sulphate and cobaltous sulphate in concentrations such as to supply amounts equivalent to those set out below:

Group	Treatment
1-Mu-0	1 mg. Co/day
2-Mu-1	1 mg. Co/day + 1 mg. Cu/day
3-Mu-2.5	1 mg. Co/day + 2.5 mg. Cu/day
4-Mu-5	1 mg. Co/day + 5 mg. Cu/day
5-Mu-7.5	1 mg. Co/day + 7.5 mg. Cu/day
6-Mu-10	1 mg. Co/day + 10 mg. Cu/day
7-Mu-15	1 mg. Co/day + 15 mg. Cu/day
8-Mu-20	1 mg. Co/day + 20 mg. Cu/day

The flock was mustered thrice weekly (Sun., Wed., Fri.) and the doses administered *per os* by means of a syringe in a manner which ensured that the whole of the solution would be swallowed. At monthly intervals the concentrations of copper and of haemoglobin in the blood of each individual were determined in 20 ml. samples withdrawn with special precautions from the jugular. Pasture clips were collected at these intervals from the paddocks on which the animals had grazed and from composite samples the concentrations of copper and of other trace elements were determined.

IV. The Experimental Findings

(*a*) *The Copper and Molybdenum Content of the Pastures*

The concentration of copper in the composite pasture samples fluctuated erratically about a mean of approximately 3 μg. Cu/g. dry wt. (Fig. 1). This vacillation, however, was the consequence of changes in botanical composition of the pastures rather than of transient alterations in the chemical composition of individual plants—the sheep were grazed in rotation over several paddocks and the samples were taken from the pastures on which they were feeding at the time when the blood samples were collected.

The fact that plant species differ widely in their ability to assimilate minor elements from the soils on which the experimental animals were grazed became obvious early in these investigations. The actual and relative amounts of the trace elements (Co, Cu, Fe, Zn, Mo, etc.) that are concentrated within the tissues of a plant were found to be influenced by three main factors – the species, the stage of development of the plant, and the prevailing nutritional environment of the soil in which it is grown.

Although the pastures on the dunes where the experimental flocks were confined consist essentially of a very simple association of the indigenous grasses, *Lagurus ovatus* and *Bromus madritensis*, a sparse growth of herbaceous plants appears during the winter and spring, and some of these undoubtedly are consumed by the grazing sheep. This herbage contained considerably more copper than the grasses in which the concentration varied only within the rather restricted range of from 2 μg. to 3 μg. Cu/g. dry wt. While it is not possible to predict with confidence either the amount of copper in the fodder selected by the sheep from pastures of mixed composition or to assess the extent to which copper retention is complicated by the presence of other elements in this fodder, it may be assumed

that for most of the year by far the greater bulk of the pasture ingested by the experimental animals consisted of grasses, and so the average intake of copper was between 2.5 and 4 mg. Cu/day, i.e. between one-third and one-half of the amount of copper ingested by sheep of similar weight from pastures on areas where copper deficiency symptoms are never experienced.

There is evidence that the retention of copper by the ruminant is influenced by the ingestion of molybdenum – the malady, suffered by cattle on "teart" pastures, which has been claimed to result from chronic ingestion of relatively large quantities of molybdenum, responds to therapy with copper (Ferguson, Lewis, and Watson 1940, 1943), and Dick and Bull (1945) have demonstrated unequivocally that the copper reserves in the liver of a sheep may be depleted by adding ammonium molybdate to the fodder. The quantity of molybdenum involved in "teart" pastures, however, greatly exceeds that present in the pastures on which the following experiments were conducted.

In the alkaline *milieu* of the calcareous littoral, the enhanced availability of molybdenum tends strongly to favour its concentration within the tissues of certain species of plants, and there was certainly more molybdenum in the fodder plants from the dunes on which the experimental flocks were grazed than in similar plants grown elsewhere on more acid soils. Its concentration in the indigenous grasses which grew there varied, according to the season, between the approximate limits of 1.2 and 4.0 μg. Mo/g. dry wt.* It became concentrated to a greater extent in the herbaceous plants and for a period during winter and spring the composite pasture samples contained approximately 8 μg. Mo/g. dry wt. The molecular ratios of Mo/Cu in these samples rarely exceeded unity, but in the spring of both 1945 and 1946 this ratio increased to approximately 1.6.

At this juncture the influence exerted by these quantities of molybdenum on the capacity of the sheep to retain copper is not clear. But, whether the reduction of the copper status of the experimental sheep depastured on these areas was the result of a dietary deficiency *per se*, or whether it was in part imposed by the interaction of molybdenum or of other constituents of the fodder, there can be no doubt that the *syndrome which developed was the result of copper deficiency.*

(*b*) *Influence of Copper Deficiency on the Body Weight*

It was not feasible to draw definite conclusions from the relatively small differences which became evident between the mean body weights of the groups that received copper supplements. As the groups had been matched originally according to their wool characteristics, the mean body weights at the beginning of the treatments varied over a wider range than in previous experiments, and from their subsequent behaviour it was apparent that in so far as the propensity for further growth was concerned some groups had inadvertently been biased by this method of selection. However, with the exception of the aberrant behaviour of two groups (4-Mu-5 and 6-Mu-10), the mean body weights after two years of treatment ranged in order of the amounts of copper provided in the supplements.

* Molybdenum was determined by the method of Dick and Bingley (1947).

Three months after the beginning of the treatments, the mean body weight of the group which received no additional copper was significantly lower than that of any of the copper-supplemented groups, and subsequently this difference became progressively greater (Fig. 1).

(*c*) *The Influence of Copper Deficiency on the Copper Content of the Blood*

The animals had been depastured on the deficient terrain for approximately 3 months before treatments began, and during this period the concentration of copper in their bloods had fallen to a relatively low level. They had received their first supplementary drenches the day before the first blood samples were taken. The relatively rapid reinstatement of a normal blood-copper level in the groups which were given an adequate supply of copper and the further steady fall in the level in those which received less copper than would provide the full requirements of sheep on this terrain are obvious from Figure 1. The blood-copper levels of all groups fluctuated according to the seasonal changes in the composition of the available fodder but never sufficiently to confuse the order in which they reflected the concentrations of copper provided in the supplements.

Analysis of variance of the concentrations of copper in the bloods of all groups (Table 1) reveals clearly the fact that the supplements containing less

TABLE 1

EFFECT OF COPPER SUPPLEMENTS ON THE COPPER CONCENTRATION OF THE BLOOD

Date of Collection	Mean mg. Cu/l. Blood							
	Treatment, mg. Cu/day							
	0	1.0	2.5	5.0	7.5	10.0	15.0	20.0
18.xi.44	0.29	0.40	0.45	0.39	0.29	0.51	0.34	0.43
19.xii.44	0.32	0.45	0.40	0.61	0.65	0.79	0.79	0.58
15.i.45	0.26	0.39	0.43	0.51	0.70	0.59	0.65	0.69
20.ii.45	0.23	0.31	0.38	0.48	0.51	0.51	0.62	0.63
20.iii.45	0.23	0.33	0.47	0.62	0.67	0.81	0.98	0.82
17.iv.45	0.16	0.27	0.43	0.55	0.50	0.73	0.78	0.70
15.v.45	0.24	0.36	0.49	0.62	0.72	0.81	0.83	0.82
19.vi.45	0.16	0.26	0.39	0.51	0.65	0.80	0.89	0.82
17.vii.45	0.16	0.22	0.32	0.36	0.63	0.73	0.87	0.80
21.viii.45	0.14	0.15	0.28	0.34	0.48	0.62	0.74	0.68
18.ix.45	0.11	0.14	0.17	0.29	0.37	0.69	0.82	0.81
24.x.45	0.12	0.15	0.21	0.38	0.57	0.89	1.12	1.13
22.xi.45	0.08	0.09	0.15	0.23	0.45	0.57	0.62	0.60
20.xii.45	0.15	0.18	0.24	0.52	0.75	0.81	0.89	0.83
22.i.46	0.16	0.22	0.33	0.53	0.67	0.80	0.73	0.80
21.ii.46	0.13	0.16	0.32	0.50	0.73	0.73	0.72	0.77
24.iii.46	0.11	0.13	0.18	0.33	0.46	0.55	0.63	0.72
25.iv.46	0.12	0.13	0.17	0.32	0.45	0.57	0.82	0.77
11.vi.46	0.12	0.15	0.19	0.22	0.44	0.67	0.83	0.93
28.vii.46	0.14	0.11	0.13	0.16	0.45	0.68	0.84	0.72
3.ix.46	0.08	0.20	0.10	0.21	0.41	0.63	0.74	0.79
19.ix.46	0.14	0.25	0.16	0.32	0.49	0.77	0.86	0.99

	Probability		
Significant differences between means	0.05	0.01	0.001
within the table:	0.12	0.16	0.20 mg. Cu/l.

than the optimum concentration of copper merely delayed the depletion of the copper reserves of the animals and that after approximately 2 years had elapsed the blood-copper levels, even of those groups which received supplements which approximated the quantity necessary to fulfil their requirements, fell steadily and approached those of the unsupplemented group. At the end of the first year there was no significant difference ($P < 0.01$) in this respect between the unsupplemented animals and those of the groups that received an additional 1 mg. and 2.5 mg. Cu/day. After 2 years, the mean blood-copper concentrations of the group that received 5 mg. Cu/day had fallen to a level which was not significantly different from those of the three groups which received less copper. The animals that received the equivalent of 10 mg. Cu/day showed no tendency to become depleted in this way.

(*d*) *The Influence of Copper Deficiency on the Haemoglobin Content of the Blood*

During the first year of the experiment no significant degree of anaemia developed in the animals of group 1-Mu-0 although over practically the whole of this time the blood-copper level was below 0.2 mg. Cu/l. In the flush of the spring of 1945, however, the individuals of this group became anaemic. The

TABLE 2

THE EFFECT OF COPPER SUPPLEMENTS ON THE OXYGEN CARRYING CAPACITY OF THE BLOOD

Date of Collection	Mean. vol. O_2/100 ml. Blood							
	Treatment, mg. Cu/day							
	0	1.0	2.5	5.0	7.5	10.0	15.0	20.0
18.xi.44	12.51	12.73	12.21	12.24	12.00	11.70	12.16	11.95
19.xii.44	12.35	12.65	12.64	12.60	12.68	12.16	13.21	12.68
15.i.45	12.85	12.94	13.36	12.23	13.49	12.46	13.53	13.23
20.ii.45	13.11	13.01	12.66	12.50	13.34	12.45	13.83	13.68
20.iii.45	11.56	12.29	12.48	12.35	12.69	11.71	12.85	12.83
17.iv.45	11.56	12.86	12.43	11.81	12.70	11.74	12.46	12.39
15.v.45	11.80	12.63	12.34	11.71	13.30	12.26	12.78	12.68
19.vi.45	11.10	11.93	12.59	11.86	13.30	12.29	13.35	13.06
17.vii.45	11.18	11.70	12.15	11.63	12.58	11.88	13.11	13.04
21.viii.45	10.51	10.85	11.96	11.43	12.00	11.41	12.46	11.88
18.ix.45	10.20	11.29	12.15	11.84	12.78	12.00	12.96	12.75
24.x.45	7.36	10.34	10.11	12.17	12.39	12.49	13.55	12.95
22.xi.45	8.28	11.98	13.14	12.96	13.65	13.00	13.91	13.26
20.xii.45	6.91	8.69	10.99	11.60	11.30	12.05	11.63	11.55
22.i.46	8.59	10.93	11.60	11.66	11.94	11.49	12.55	12.01
21.ii.46	8.24	9.93	10.94	10.97	11.45	10.45	11.08	11.34
24.iii.46	8.69	10.73	11.64	11.34	12.13	11.33	11.84	11.35
25.iv.46	8.88	10.48	11.16	11.26	12.89	12.29	13.34	12.94
11.vi.46	10.85	12.21	12.28	12.32	13.81	13.11	14.80	13.74
28.vii.46	9.14	10.08	10.84	11.01	13.84	10.96	11.30	11.10
3.ix.46	9.30	10.73	12.05	11.43	12.91	11.75	11.83	12.61
19.ix.46	8.49	10.71	11.11	11.53	12.55	12.51	10.99	12.20

	Probability		
Significant differences between means	0.05	0.01	0.001
within the table:	0.89	1.16	1.50 vol. O_2/100ml.

mean oxygen-carrying capacities of their bloods fell significantly ($P < 0.01$) and thereafter remained at approximately 75 per cent. of the level maintained throughout by the adequately supplemented groups. It is evident from Figure 1 and Table 2 that a supplement equivalent to 1 mg. Cu/day prevented the relatively rapid fall experienced by the individuals of group 1-Mu-0 and served to maintain the mean oxygen-carrying capacity at a level which rarely was significantly below that of the individuals which received 10 mg. Cu/day.

(e) The Effect of Copper Deficiency on the Rate of Wool Production

All of the experimental animals were shorn in October of 1945 and of 1946, and the total clean scoured wool produced by each individual was determined. From the findings set out in Table 3 there is no doubt that the amount of copper provided in the supplement was correlated with the amount of wool produced, up to a maximum which appeared to be reached when the animals received the equivalent of between 7.5 and 10 mg. Cu/day in addition to that ingested from the pastures. The asymptote of maximum response and its standard deviation were estimated in each series of observations by relating the annual production of clean scoured wool to the copper intake from the supplement, using for this the expression,

$$Y = k(1 - e^{-B(t+A)}),$$

in which $Y =$ kg. clean scoured wool, $t =$ the supplement in mg. Cu/day, $k =$ the predicted maximum wool production, B and A being constants. The method of least squares was employed to derive the most likely values of k, B, and A and their standard deviations, the latter being based on 55 degrees of freedom. From analysis of variance, $k = 2.92 \pm 0.72$, $B = 0.215 \pm 0.059$, and $A = 5.44 \pm 1.52$ for the 1945 clip, and $k = 3.38 \pm 0.61$, $B = 0.368 \pm 0.073$, and $A = -2.45 \pm 0.565$ for the 1946 clip.

The differential equation of this expression, $dY/dt = B(k - Y)$, specifies that the increase in wool production per unit supplement of copper is proportional to the deficit of wool production below the maximum, k.

The curves obtained in this way are shown in Figure 2 in which the asymptote, k, for each set of data, is indicated with its S.D. plotted on either side. From the closeness of their fit to the observed means there is little doubt that the effect of the copper supplement was governed by the law of diminishing returns and that the maximum response was achieved when the supplement provided the equivalent of between 7.5 and 10 mg. Cu/day.

As the fodder conditions in 1946 were superior to those which prevailed in the previous year, and as the animals were by no means fully grown in 1945, the weight of wool produced in 1946 by all of the supplemented groups was considerably greater than that produced in 1945, notwithstanding the gradual worsening of the state of deficiency of all individuals that received less copper than was necessary to maintain them in copper equilibrium. The relationship between the weight of wool produced and the amount of copper in the supplement was not altered materially in the two clips — at each level of additional copper the proportional deficit from the asymptote remained, within the experimental error of the estimate, practically the same. The rate of wool production

TABLE 3

THE INFLUENCE OF COPPER SUPPLEMENTS ON THE WEIGHT OF CLEAN SCOURED WOOL PRODUCED

Group 1 1-Mu-0		Group 2 2-Mu-1		Group 3 3-Mu-2.5		Group 4 4-Mu-5		Group 5 5-Mu-7.5		Group 6 6-Mu-10		Group 7 7-Mu-15		Group 8 8-Mu-20	
Sheep No.	Total Clean Scoured Wool (kg.)	Sheep No.	Total Clean Scoured Wool (kg.)	Sheep No.	Total Clean Scoured Wool (kg.)	Sheep No.	Total Clean Scoured Wool (kg.)	Sheep No.	Total Clean Scoured Wool (kg.)	Sheep No.	Total Clean Scoured Wool (kg.)	Sheep No.	Total Clean Scoured Wool (kg.)	Sheep No.	Total Clean Scoured Wool (kg.)
1945 Clip															
766	1.92	773	2.16	762	2.45	763	2.29	761	2.75	781	2.78	779	2.88	765	2.72
787	2.22	774	2.65	764	2.84	770	2.71	777	2.83	785	2.62	783	2.75	776	3.52
789	2.07	775	2.23	771	2.47	802	2.62	782	2.88	786	2.91	790	2.95	784	2.88
795	1.82	796	2.10	778	2.52	808	2.48	792	2.75	788	2.70	794	2.58	791	2.88
799	1.85	801	2.21	806	2.15	809	2.46	793	2.59	797	2.45	817	3.00	805	3.23
800	1.66	803	1.80	816	2.34	811	3.19	814	2.76	798	2.90	825	2.68	820	3.15
810	1.87	813	2.35	821	2.38	812	2.47	819	2.80	807	2.53	829	3.33	824	3.04
828	2.28	815	2.39	823	2.37	827	2.73	822	2.57	818	2.75	830	2.59	826	2.68
Mean	*1.96*	Mean	*2.24*	Mean	*2.44*	Mean	*2.62*	Mean	*2.74*	Mean	*2.71*	Mean	*2.85*	Mean	*3.01*
1946 Clip															
766	2.05	773	2.54	762	2.84	763	2.66	761	3.34	781	3.38	779	3.38	765	3.55
787	2.25	774	2.88	764	2.95	770	3.01	777	3.42	785	3.30	783	3.06	776	3.78
789	2.06	775	2.52	771	2.57	802	3.26	782	3.24	786	3.34	790	3.19	784	3.40
795	1.67	796	2.63	778	2.44	808	3.51	792	2.99	788	3.19	794	2.84	791	3.30
799	1.88	801	2.00	806	2.38	809	3.08	793	3.52	797	3.08	817	3.28	805	3.45
800	2.08	803	2.36	816	2.93	811	3.57	814	3.30	798	3.60	825	3.17	820	3.68
810	1.63	813	2.54	821	2.44	812	3.64	819	3.38	807	2.85	829	4.13	824	3.60
828	2.08	815	3.07	823	3.06	827	3.38	822	3.06	818	3.35	830	3.20	826	3.58
Mean	*1.96*	Mean	*2.57*	Mean	*2.70*	Mean	*3.26*	Mean	*3.28*	Mean	*3.26*	Mean	*3.28*	Mean	*3.54*

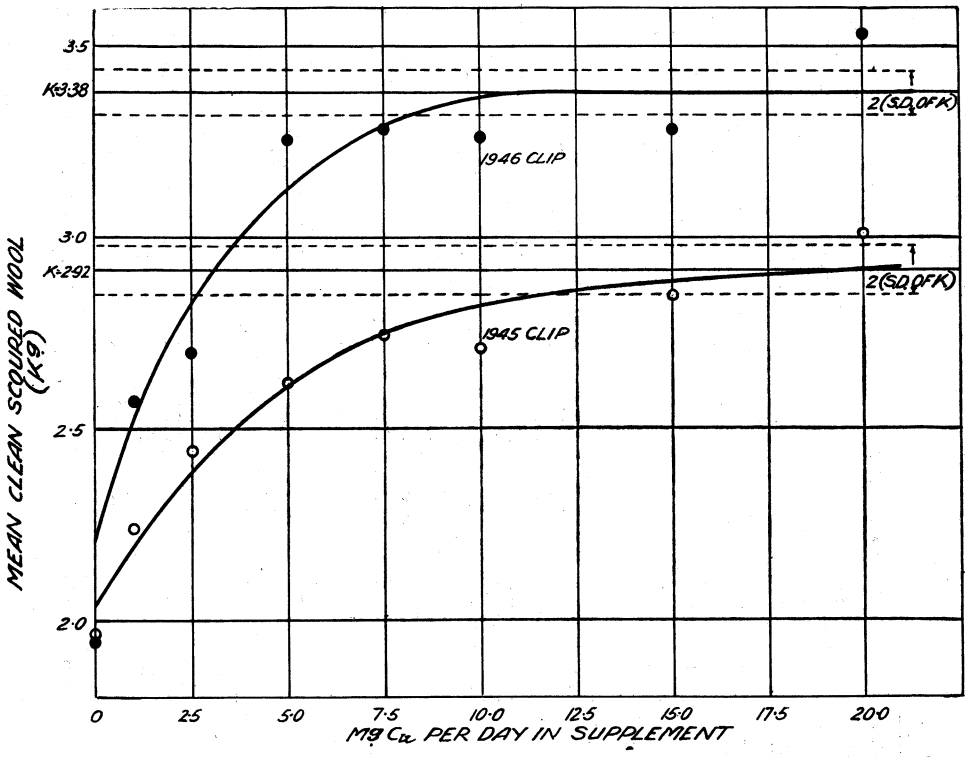


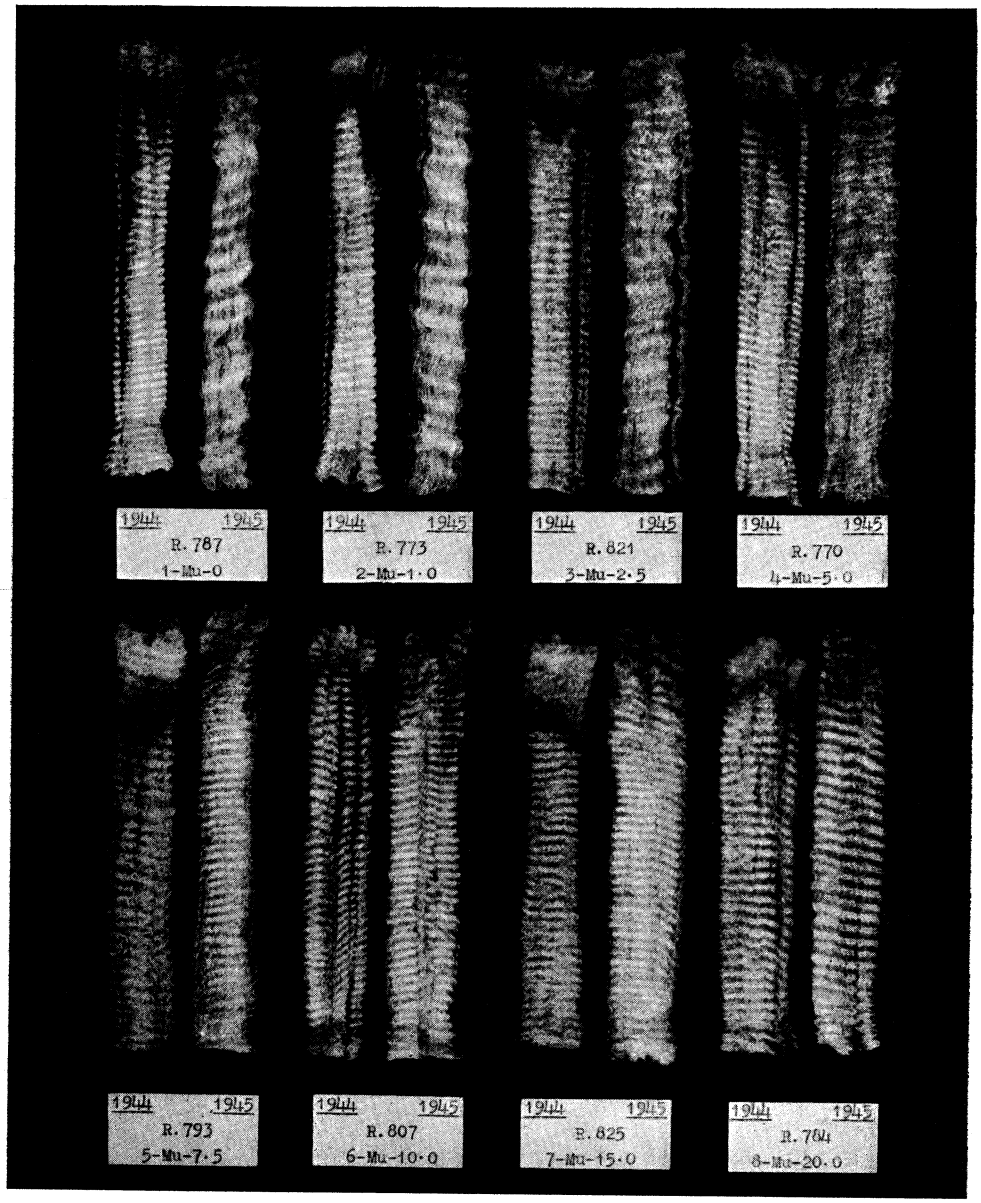
Fig. 2.—The influence of a graded series of degrees of copper deficiency on the annual production of clean scoured wool during the first two years of the experiment is shown. The observed group means are plotted in relation to curves (derived *vide* text) which specify that the increase in wool production per unit supplement of copper is proportional to the deficit of wool production below the maximum, k. The asymptote, k, is indicated with its S.D. plotted on either side. In each year the maximum response was achieved when the supplements provided the equivalent of between 7.5 and 10 mg. Cu/day.

(i.e. the quantity of wool produced per unit time) under these grazing conditions was the resultant of a number of interacting variables — the quantity and quality of the fodder derived from the pastures, the copper intake, the nature and extent of dietary factors which would complicate the absorption or otherwise deplete the copper reserves of the individual etc.— few of which may be evaluated with

Explanation of Plate 1

The series of staples selected at random from each of the experimental groups illustrates changes in the appearance of the wool which supervene on copper deficiency. Both staples of each pair were drawn from the same part of the fleece, the first being grown in 1944 while the animals were depastured on sound country and the second in 1945 during the first year on the deficient terrain.

A range of degrees of deterioration is apparent in the first four pairs of staples which were taken from animals which received supplements (equivalent to 0, 1, 2.5, and 5 mg. Cu/day respectively) insufficient to maintain copper equilibrium. The supplements (equivalent to 7.5, 10, 15, and 20 mg. Cu/day respectively) administered to the sheep that grew the fleeces from which the second four pairs of staples were drawn were adequate to maintain a normal copper status and the process of keratinization was unaffected by the conditions of grazing in 1945.



MARSTON and LEE.—NUTRITIONAL FACTORS INVOLVED IN WOOL PRODUCTION BY MERINO SHEEP

desirable precision in field experiments. A detailed analysis of minor differences in the copper supplement/fleece weight relationships which occurred in the two sets of observations is thus unlikely to yield cogent information.

As the extent of depression of the growth rate of the animals was similarly related to the degree of copper deficiency imposed, the effect on the rate of wool production may be ascribed to the partial failure of appetite rather than to specific effects on the physiological processes involved in wool production *per se*. In the absence of positive evidence to the contrary it may be concluded tentatively that the rate of wool production was limited by the supply of substrate (cf. Marston 1948) rather than by an impairment of the capacity of the cells in the follicles to utilize amino acids and to proliferate normally. The subsequent process of keratinization, however, is materially affected by copper deficiency (Marston 1946).

(*f*) *The Effect of Copper Deficiency on the Nature of the Wool*

Each of the experimental animals had the hereditary propensity to impress a well-defined crimp on its fleece – the flock had been selected, with this function as a criterion, from a strain noted for the character of its wool – and so any impairment of the physiological mechanism involved was readily discernible. The ability to impart crimp was lost very rapidly by the unsupplemented animals and, in all of those that received less copper than would maintain maximum efficiency, the degree of failure of this function was directly related to the extent of depletion of their copper reserves.

The changes in the appearance of the wool that supervened during the first year (1945) of grazing on the affected terrain are shown in Plate 1, in which the type of wool grown while the animals were depastured on sound country (1944) is contrasted with that produced in 1945. The staples were grown on the skin area defined in each animal by the bony protuberance of the acromion process of its right scapula and so are comparable. They were selected at random – the response within each group was remarkably constant.

The lesions that appeared in the fleeces grown by animals which received less than 7.5 mg. Cu/day – which amount was sufficient to maintain normal wool growth over the first year of observation – reflected well-defined stages of the range of deficiency states prevalent among flocks grazing on affected terrain in southern Australia.

The first sign of an impaired copper status is the tendency for the crimps to become less distinct (cf. the wool from R.770 which animal received 5 mg. Cu/day); secondary waves superimposed over the deteriorating crimps then appear (cf. R.821 which received 2.5 mg. Cu/day); and finally the ability to impart crimp is lost altogether (cf. R.787 and R.773 which received no copper and 1 mg. Cu/day respectively). In the second year of observation the condition of the fleeces produced by the animals which received 2.5 and 5 mg. Cu/day deteriorated further, and slight lesions became evident in some of the fleeces from the animals that received 7.5 mg. Cu/day, but the wool from the groups which received supplements containing 10 mg. Cu/day, or more, continued to be normally crimped. Obviously copper balance had been struck by supplements

which provided the equivalent of between 7.5 and 10 mg. Cu/day. This conclusion is identical with that derived from the quantitative considerations discussed in the previous section.

V. Discussion

The rate at which the mean concentration of copper in the bloods of the unsupplemented group fell to a very low level implied a rapid depletion of the copper status under these grazing conditions. Equilibrium was established when between 7.5 and 10 mg. Cu/day were supplied in the supplement: above this level of intake no further improvement was apparent from the criteria employed to measure the physiological state of the animals, and below it the rate of depletion diminished progressively with each additional increment of copper provided in the supplements.

The supplements were administered in relatively concentrated solutions which might tend to favour reflex closure of the oesophageal groove and so lead the drench to by-pass the rumen and flow direct to lower levels of the intestinal tract. The fact that an evenly graded series of deficiency states supervened in the groups that received supplements containing suboptimum quantities of copper suggests, however, that the drenches entered the rumen to be diluted in its voluminous contents before passing to the intestine. It is inconceivable that the small quota of copper absorbed would be so directly related to the amount of copper provided in the drench if the latter flowed intermittently in relatively very high concentration over the absorption areas of the intestine.

The wool fleece was influenced profoundly by the state of copper deficiency induced, and both the rate of wool production and the deterioration of its quality were determined ultimately by the degree of depletion of the copper reserves. The decrease in the weight of wool produced by the copper-deficient animals was more likely the result of an integrated expression of the severity of the deficiency syndrome than of a direct influence of copper deficiency on the rate of cell division in the follicles, but the deterioration of the process of keratinization signified by the failure to impart crimp was certainly a specific effect of copper deficiency. During the course of depletion of the copper reserves this latter function is affected adversely long before there is any sign of an impairment of iron metabolism. The ability to impart normal crimp invariably failed when the blood-copper fell below 0.4 mg. Cu/l., even when the oxygen-carrying capacity was normal, and the extent of this failure was proportional to further decrease in blood-copper. Massive doses of iron exerted no beneficial effect when this function was impaired, but normal keratinization returned immediately when the blood-copper level was reinstated to its normal range by increasing the copper intake.

It is evident that the chemical processes responsible for keratinization depend fundamentally on copper.

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