EFFECTS OF COPPER AND COBALT SUPPLEMENTATION ON THE GROWTH RATE AND FERTILITY OF SHORTHORN HEIFERS IN NORTHERN COASTAL QUEENSLAND

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SUMMARY

A condition of ill-thrift was studied in weaner cattle in an area south of Townsville consisting of plains of swampy ground interspersed with light sandy ridges. A response was obtained to copper but not to cobalt therapy.

Parenteral copper therapy at 3-monthly intervals resulted in a highly significant growth response in winter and spring. Supplemented heifers showed continued superiority for the duration of the experiment from April 1960 to September 1962. Following mating in March 1961 there was a significantly greater number of pregnancies in copper-treated groups as determined by rectal examination in July 1961. Heifers pregnant at this time had grown more rapidly since the commencement of the experiment, but there was no evidence of delayed onset of oestrus in non-pregnant heifers. Differences between copper and non-copper treated groups in the number of pregnancies decreased at subsequent examinations.

Some initial reduction in fertility was attributable to a high incidence of *Vibrio fetus* **but there were no differences between treatments in the incidence of this disorder.**

Liver copper levels were consistently low in untreated animals. Parenteral copper therapy at 3-monthly intervals maintained satisfactory copper reserves except in a period of maximum stress as shown by minimum weight gains from September to December. Calves born to copper-treated cows showed higher initial liver copper reserves. An adequate copper status was maintained in calves treated at 3-monthly intervals.

Analyses of the predominant pasture species showed some seasonal variation in copper content. All findings suggest that copper deficiency in cattle in this locality is due to an interference with copper metabolism in the animal rather than a low copper status in the diet. This interference factor does not appear to be molybdenum in the presence of inorganic sulphate.

Scoring for coat colour and felting established a shorter, smoother coat in copper-treated animals.

I. INTRODUCTION

Copper deficiency has been extensively reported in cattle in Queensland (Sutherland 1952; Harvey 1952; Sutherland 1956; Alexander and Harvey 1957; Chester, Marriott, and Harvey 1957; Donaldson 1960; Harvey *et al.* 1961).

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Underwood (1956) reviewed the wide variety of clinical symptoms which have been associated with copper deficiency in cattle. None of these are specific. Major symptoms are progressive loss of condition, the development of a harsh, dull, discoloured coat, and under some conditions only, the onset of diarrhoea. Adult as well as growing cattle are affected but young stock usually show the most severe symptoms. Frequently, in beef herds, calves are affected while their mothers appear clinically normal. In severely affected animals the response to the administration of small amounts of copper is usually spectacular.

The suppression of oestrus with temporary sterility was reported in copper-deficient cattle by Russell (1944) and Bennetts (1959). Cunningham (1950) also considered that the reproductive capacity of cattle is reduced in copper deficiency. Hignett (1959) stated that hypocuprosis was sometimes but not always associated with reproductive disorders in female cattle. When fertility was thus affected, he found that administration of copper either orally or by injection usually resulted in a rapid and dramatic improvement in breeding performance. This is in agreement with a report by van Rensburg (1961) but is not supported by the observations of Russell (1944), Cunningham (1950) and Bennetts (1959). Dutt and Mills (1960) showed that copper deficiency in rats causes foetal death and resorption in early pregnancy; oestrus cycles were normal.

Cunningham (1950) divided the syndrome of copper deficiency into two types, simple and complicated, the symptoms in each being similar though scouring may be more profuse in the complicated deficiency. He defined a simple deficiency as caused by an inadequate copper intake and a complicated deficiency as due to an excess of molybdenum. The influence of a variety of factors which interfere with copper metabolism in the ruminant has been discussed by a number of workers (Dick 1953, 1956; Allcroft and Lewis 1957; Harvey *et al.* 1961).

Hartmaas (1960) and Deijs and Bosman (1961) have shown that copper deficiency in cattle in Holland occurs in the low-lying, wet areas in which pastures have a high porphyrin content and that at the pH of the rumen porphyrins make copper unavailable to the ruminant.

Cobalt deficiency in cattle in Australia has been reported by Underwood (1956), Bennetts (1959) and Skerman, O'Halloran, and Munday (1961). As with copper, a dietary deficiency of cobalt does not result in any one symptom which can be regarded as specific for this element. A restricted growth rate is characteristic and this is more evident in younger animals. Under severely deficient conditions cattle become debilitated, coats are rough and lack bloom, the shedding of winter coat is delayed and there may be some ocular discharge.

On the coastal plains at Cromarty, near Townsville in North Queensland, weaner ill-thrift has been evident in beef Shorthorn cattle for a number of years. The symptoms are slow growth, scouring in the most severely affected animals, and a harsh, dull, yellow, discoloured coat. Some deaths are attributed to the general ill-thrift. The stock are most severely affected in the winter and spring following weaning and usually appear clinically normal following the next wet season. Adult cattle do not appear to be affected. In a preliminary investigation, Donaldson (1960) found a clinical improvement and an increase in weight gain following systemic copper therapy. The present investigation was made to provide detailed information on the cause, significance and correction of this condition.

II. DESCRIPTION OF THE PROPERTY

The property is situated approximately 20 miles south of Townsville. It is stocked with Shorthorn cattle and is used for both breeding and fattening. Topographically the area is composed of coastal plains with some associated ridges. The major portion of the property where the experimental cattle grazed consists of plains of swampy ground and light sandy ridges with sparse grass cover. The swampy areas are grassed with para grass (*Brachiaria mutica*), with the transitional area between the swamps and the ridges covered largely by marine couch (*Sporobolus virginicus*) and some *Paspalum vaginatum*. The sandy ridges have some Townsville lucerne (*Stylosanthes humilis*) with blue grasses (species of *Ischaemum*) and wire grass (*Sporobolus poiretii*). The rainfall in the area is reasonably high but quite seasonal, so a seasonal pattern of grazing has developed (Figure 1). The animals graze the ridges during the summer months and work progressively out into the swampy areas in the dry season.

III. MATERIALS AND METHODS

The animals used in this study were 110 Shorthorn heifers which had been weaned between November 1959 and February 1960. They were 10-11 months of age at the commencement of the trial. They were randomized into four groups and allocated to the following treatments:—

(*a*) Control group.

(*b*) Copper-treated group: Each animal was injected subcutaneously in the brisket with 400 mg copper glycinate at intervals of approximately 3 months.

(*c*) Cobalt-treated group: Each animal was given (*per os*) one heavy cobalt pellet containing 5 g of 90 per cent. cobalt oxide (Dewey, Lee, and Marston 1958) at intervals of approximately 6 months.

(*d*) Copper and cobalt-treated group: Each animal was given copper and cobalt treatments as for Groups (*b*) and (*c*).

Mustering for weighing and treatment was carried out as required over the period from April 4, 1960, to September 18, 1962. As the trial progressed the number of experimental animals decreased due to incomplete mustering, escape of some animals into other paddocks, loss of identification ear tags and some deaths from miscellaneous causes. A set number of 10 head from each treatment was used to provide liver samples taken by the biopsy technique of Loosmore and Allcroft (1951), using the instruments described by Dick (1952). Some liver cores were also taken for vitamin B12 assay according to the procedure of H. J. Lee (personal communication). At the same time, blood samples were drawn from the jugular vein to provide serum samples for vitamin B12 assay. Pasture samples were collected using stainless-steel scissors. The samples were packaged in plastic bags and air-freighted to the laboratory for analysis.

Protein in pasture was determined by the official method of the Association of Official Agricultural Chemists (1955) and copper in pasture and liver by the method of Clare, Cunningham, and Perrin (1945). The method of Dick and Bingley (1951) was used in the analysis of molybdenum. Inorganic sulphate was determined by a modification of the benzidene sulphate method of J. B. Bingley and A. T. Dick (personal communication 1953).

In March and April 1961, the experimental heifers along with some others were mated to 10 bulls for a period of five weeks in a small paddock and then run with the general herd, where they had access to herd bulls. Pregnancy status was checked by rectal palpation at each weighing between mating and calving.

In September 1961, serum samples were taken and agglutination tests made for *Brucella abortus, Leptospira pomona* and *Leptospira hyos*. Cervical mucus was subjected to the agglutination test for *Vibrio fetus*. Clinical examinations for the presence of vaginitis were also carried out.

Hair samples over the right ribs were collected in April, July and December 1960 and March 1961 and were scored for colour according to the method of Schleger and Turner (1960). All animals were coat scored in June and December 1961, using the method developed by Turner and Schleger (1960), and the December 1960 hair samples were tested for felting character (Bonsma 1949).

During 1962, the calves were identified with their dams, aged and assigned to the same treatments as their dams. Weighings and treatments were carried out at 3-monthly intervals in March, June and September and liver cores collected for copper analysis in March and September.

IV. RESULTS

There was a highly significant growth response in weaner heifers to copper therapy in the winter and spring of 1960 (Table 1), which resulted in a continued superiority throughout the experiment (Figure 1). Stage of pregnancy did not permit any analysis of weight gains after July 1961, but an examination of Figure 1 indicates that there was some slight compensatory gain in the non-copper groups during the period December 1960 to March 1961 (144, 135, 125 and 121 lb for the control, cobalt, copper, and copper + cobalt groups respectively). However, this was not sufficient to eliminate the overall advantage of the copper-treated groups. Cobalt therapy did not influence growth rate.

TABLE 1

Body-weight Gains of Heifers from April 1960 to June 1961

Treatment	Mean Body-weight Gain	
	4.iv.60–29.ix.60†	29.ix.60–5.vi.61†
Control	1·4 (22)‡	204 (17)
Copper*	44·0 (25)	214 (15)
Cobalt	6·0 (23)	198 (14)
Copper*+cobalt ..	50·9 (26)	205 (16)

* Gain due to copper treatment was very highly significant ($P < 0.001$).

† Standard error of the gains for the first period was ± 6·5 lb and for the second period was ± 7·4 lb.

‡ The figures in parentheses are the number of animals on which the gains were based.

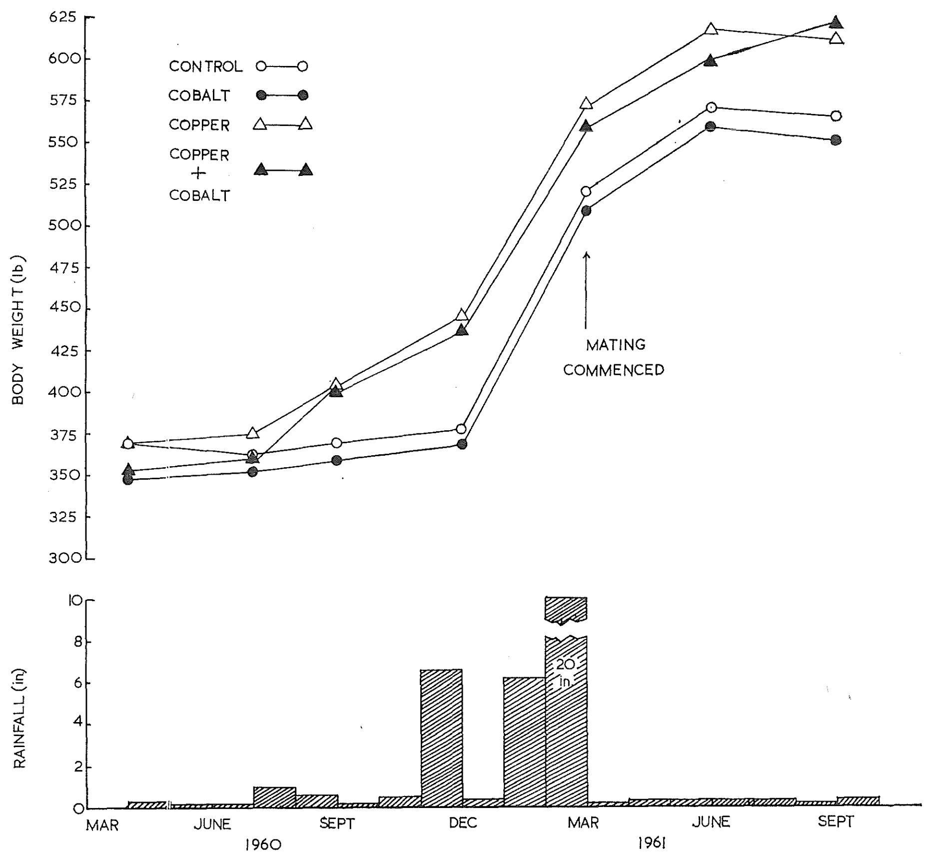

Fig. 1.—Mean body-weight of heifers from each treatment, and monthly rainfall from April 1960 to September 1961.

The first rectal examination for pregnancy status was made in July 1961 and indicated a significantly greater number of pregnancies in the copper-treated groups (Table 2). Cobalt did not have any influence on fertility. As shown in Table 2, the difference in number pregnant between the copper and non-copper treated groups decreased at subsequent examinations. Growth rates of pregnant and non-pregnant heifers at the September examination were compared within treatments and results indicated that the pregnant heifers had grown more rapidly from the commencement of the experiment, although at no time did this difference in body-weight attain significance. Rectal palpation indicated normal functioning ovaries in all experimental heifers.

TABLE 2

Effect of Copper Treatment Upon Conception Rate as Judged by Manual Pregnancy Diagnosis

Treatment	No. of Heifers	Pregnancy Diagnosis (%)		
		5.vii.61	11.ix.61	18.xii.61
Copper	42	64*	74	93
Non-copper	52	42	56	83

* Difference due to copper treatment was significant ($P < 0{\cdot}05$).

There was a high incidence of reactors to the agglutination test for *Vibrio fetus,* approximately half the experimental heifers showing a positive reaction (Table 3). There were no differences between groups in the percentage of reactors to this test. As vibriosis is only transmitted venereally, this equal and high incidence suggests no marked differences between groups in the number of

TABLE 3

Pregnancy Status at the Three Examinations Compared with Reaction to the *Vibrio fetus* Test

Reaction	—	Examination of Heifers		
		5.vi.61	11.ix.61	18.xii.61
Positive	No. of heifers	48 (24)*	48	47
	Percentage pregnant	21	42	77
Suspicious	No. of heifers	15 (9)	15	15
	Percentage pregnant	73	80	73
Negative	No. of heifers	34 (16)	34	33
	Percentage pregnant	82	79	85

* Figures in parentheses indicate the number of these heifers that were from the copper-treated groups.

heifers showing oestrus. Initially, reacting heifers showed reduced fertility but as the mating season progressed there was a marked improvement in the fertility status of reactors. At 7 months after the commencement of mating there was no significant difference in the percentage of pregnant animals showing positive and negative agglutination titres. Animals classed as showing suspicious agglutination reactions showed a pregnancy status comparable with that of animals showing a negative titre. There were no positive agglutination titres in tests for *Brucella abortus* nor any association between positive reactors to tests for *Leptospira pomona* or *L. hyos* and the state of pregnancy. Vaginitis was also not associated with the pregnancy status in these animals.

Data from the examination of the hair coat colour are shown in Table 4. Colour score showed a highly significant seasonal trend in animals from both copper-treated groups compared with those not receiving copper. Considering this quadratic component of the seasonal lowering of score, there is a significant difference between groups as evidenced by a smaller trough in treated animals in December. Repeatability of colour score was 0·32, with 90 per cent. fiducial limits between 0·17 and 0·48.

At the single tests for felting character in December 1960, samples showed a difference in score significant at the 5 per cent. level of probability. Within groups, the correlation between colour and felting scores was positive ($r = +0{\cdot}28$) and there was an indication that the relationship was stronger in the non-copper group. The correlation coefficient for the non-copper group was +0·46, as opposed to +0·16 for the treated group.

TABLE 4

Coat Colour Scores of Experimental Animals at the Four Samplings

Treatment	No. of Animals	April 1960	July 1960	December 1960	March 1961
Copper	18	4·4	3·4	2·7	5·0
Non-copper	22	4·2	3·0	1·5	4·8

Standard error of mean scores was +0·2.

Seasonal differences highly significant ($P < 0{\cdot}001$).

Between group differences significant ($P < 0{\cdot}01$).

At the two examinations for coat type score carried out in March and December 1961, no significant differences could be found between treated groups. There was, however, a decrease in score in all groups of about 4 units, indicating a marked change in coat type with season.

TABLE 5

MEAN LIVER COPPER LEVELS OF EXPERIMENTAL ANIMALS

Treatment	Liver Copper (p.p.m. Cu)									
	1960				1961		1962			
							March		September	
	April	July	September	December	March	June	Cows	Calves	Cows	Calves
Control	15 (10)*	10 (10)	5 (10)	9 (9)	9 (8)	10 (6)	13 (3)	14 (3)	15 (7)	9 (3)
Cobalt	14 (10)	8 (10)	6 (8)	9 (8)	11 (8)	16 (9)	15 (4)	12 (4)	13 (4)	10 (2)
Copper	11 (10)	32 (9)	43 (7)	20 (8)	40 (7)	71 (7)	27 (7)	76 (8)	66 (8)	195 (5)
Copper + cobalt	19 (10)	28 (9)	51 (9)	33 (9)	51 (8)	86 (5)	37 (5)	81 (5)	68 (8)	72 (3)

* Figures in parentheses indicate the number of animals sampled from each treatment.

Liver biopsy samples were taken for analysis on each occasion immediately prior to treatment. Although copper levels (Table 5) represent reserves at approximately 3 months after treatment, they still indicate a higher copper status in animals receiving copper therapy. In view of advanced pregnancy, samples were not taken prior to treatment in September and December 1961. Liver samples were also not taken prior to treatment in June 1962.

Levels for vitamin B12 in liver and serum are shown in Table 6. No differences between treatments are evident.

TABLE 6

MEAN LEVELS FOR VITAMIN B12 IN LIVER AND SERUM
(μg/g fresh liver; mμg/ml serum)

Treatment	Vitamin B12		
	September 1960		September 1961
	Liver	Serum	Serum
Control	0·91 (8)*	0·23 (8)	0·32 (9)
Copper	0·93 (8)	0·25 (8)	0·25 (7)
Cobalt	1·14 (7)	0·23 (8)	0·32 (10)
Copper + cobalt ..	1·01 (10)	0·25 (10)	0·34 (9)

* Figures in parentheses indicate the number of animals sampled from each treatment.

Results of analyses of the three pasture species forming the major part of the diet indicate a seasonal fluctuation in copper status, levels tending to be lowest in winter and spring (Table 7). Molybdenum levels are low and show little seasonal variation; they tend to be highest in Townsville lucerne. All pastures show appreciable levels for inorganic sulphate, with maximum concentrations in marine couch. Protein levels are adequate for production but the amount of available feed was limited in winter and early spring.

At the first sampling in March 1962, calves from the copper-treated cows had liver copper reserves greater than those of calves from the cobalt and control groups (Table 5). Thereafter, copper therapy at 3-monthly intervals maintained adequate reserves in calves from the treated groups, whereas liver copper levels tended to decline still further in untreated groups. Due to the earlier onset of pregnancy in copper-treated cows (Table 2), calves in these groups were born earlier; in September 1962 the mean age of calves in the copper-treated groups was 7·8 months, compared with 6·6 months for calves in the control and cobalt-treated groups. No statistical analysis of the growth rate data on calves was attempted but there were no marked differences between treatments at the conclusion of the experiment in September 1962.

TABLE 7

COPPER, MOLYBDENUM, INORGANIC SULPHATE AND PROTEIN IN THE MAJOR PASTURE SPECIES (dry-matter basis)

Pasture Plant	Time of Sampling	Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Sulphate (% SO_4)	Protein (%)
Brachiaria mutica	Apr. 1960	6·9	0·25	0·26	7·5
	July 1960	9·5	0·10	0·83	. .
	Sept. 1960	. .	1·2	0·71	16·8
	Dec. 1960	7·9	0·32	0·55	13·0
	Mar. 1961	6·4	0·4	0·56	10·3
	May 1961	5·9	1·5	1·14	13·7
	Sept. 1961	12·7	0·1	0·59	8·2
	Jan. 1962	8·2	0·93	0·32	13·3
Sporobolus virginicus . .	Apr. 1960	7·1	0·23	0·21	8·4
	July 1960	8·5	0·5	2·4	11·9
	Sept. 1960	7·6	0·65	1·77	9·4
	Dec. 1960	11·8	0·06	1·94	13·0
	Mar. 1961	8·5	0·2	1·4	10·0
	June 1961	4·6	0·5	0·66	7·7
	Sept. 1961	10·8	0·54	1·96	9·4
	Jan. 1962	9·0	0·27	1·66	11·2
Stylosanthes humilis . .	Apr. 1960	10·4	0·12	0·13	19·1
	July 1960	5·6	0·9	0·35	12·4
	Sept. 1960	8·8	1·8	0·35	12·3
	Dec. 1960	. .	. .	. .	. .
	Mar. 1961	10·3	0·5	0·41	22·9
	June 1961	9·2	2·5	0·24	14·7
	Sept. 1961	. .	. .	. .	. .
	Jan. 1962	20·7	1·0	. .	23·3

V. DISCUSSION

The findings from this experiment confirm the earlier report by Donaldson (1960) that weaner ill-thrift in this locality is associated with a low copper status and is alleviated by copper therapy. There is no evidence of an associated cobalt deficiency. Vitamin B12 levels were adequate in liver and blood of all experimental animals tested (H. J. Lee, personal communication) and no response was obtained to cobalt therapy.

Pasture analyses indicate that the low copper status in cattle in this locality is not associated either with a low dietary copper status or with interference with copper metabolism in the animal due to an excess of molybdenum in the presence of inorganic sulphate. This suggests the presence of some other factor in the pasture which interferes with copper metabolism and is in agreement with the findings of Harvey *et al.* (1961). Hartmaas (1960) and Deijs and Bosman (1961) have shown that in the low-lying, wet areas in Holland porphyrin in pasture is appreciable and interferes with the availability of copper to the ruminant. Although porphyrin was not determined in pastures in our experiment, there is no doubt that in this locality copper deficiency is associated with low-lying, wet areas. The predominant vegetation on the marine plain is green para grass (*Brachiaria mutica*) which becomes increasingly available and sought after by cattle with the seasonal drying out of extensive swamps.

A significant growth response of weaner cattle to copper therapy was obtained only during the dry season of late winter and early spring. In the absence of therapy, cattle tend only to maintain body-weight at this time. They are then grazing almost exclusively on the dried-out swamps. This suggests that the factor which interferes with copper metabolism may occur in para grass and swamp couch to a greater extent than in pasture species which predominate on the ridges. A further limitation would be a restriction in feed intake at this time, due to the amount of available feed. There was a significant fertility response to copper in July 1961 which decreased as the mating season progressed. Two explanations are possible. This could be a direct influence of copper or fertility as described in cattle by Hignett (1959) and in rats by Dutt and Mills (1960). Alternatively, it could be an indirect effect associated with the growth response in heifers which had received copper treatments. Sorensen *et al.* (1959) showed that growth rate influenced only the age at which the first oestrus occurred in heifers. In our experiment early pregnancy tended to be associated, on both a within-group and a between-group basis, with heifers which had grown more rapidly, but there was no evidence of delayed oestrus in lighter animals. Rectal examination and the high incidence of vibriosis indicated regular oestrus cycles. It would therefore seem likely that the significantly greater number of early pregnancies in copper-treated animals may have been due to the direct influences of copper.

Although there was a high incidence of reactors to the mucus agglutination test for *Vibrio fetus,* the distribution of infected animals was random among all groups and did not influence results attributable to treatments. The high incidence of *V. fetus* and the improvement in the level of fertility with time are in agreement with the findings of Newsam (1960).

Colour scoring indicated a deepening of coat colour attributable to copper therapy and a lightening of coat colour during the dry season when animals are under greater stress as evidenced by lower weight gains. Felting score is an index of both coat type and length. Differences in coat type were most marked in December 1960 and felting score at this time established the shorter, smoother coat of copper-treated animals. As animals matured, no differences between treatments could be detected, suggesting that cattle become less susceptible to seasonal stress with age.

Under the seasonal conditions encountered during the course of this study from April 1960 to September 1962, treatments at 3-monthly intervals with subcutaneous injections of copper glycinate, equivalent to 100 mg copper (Cu), were sufficient to maintain liver copper reserves at a fairly satisfactory level.

However, in the critical period of low weight gains from September to December 1960, treatments were barely adequate and some fall in liver copper reserves was evident.

Due to the earlier onset of pregnancy in copper-treated animals, comparisons could not be made on the effect of copper therapy on the growth rate of calves. Analyses of the initial liver biopsy samples taken from the calves in March 1962 indicated higher liver copper reserves in calves from copper-treated dams. Thereafter, copper therapy at 3-monthly intervals maintained an adequate copper status.

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