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Comparative aspects of copper metabolism in silage-fed sheep and deer (*Cervus elaphus*)

By D. O. FREUDENBERGER, A. S. FAMILTON AND A. R. SYKES
Animal Sciences Group, Lincoln College, University of Canterbury, New Zealand

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SUMMARY

Ten yearling red deer stags (*Cervus elaphus*) and ten yearling Coopworth wether sheep were housed in individual pens and offered ryegrass–white clover silage, containing 9–10 mg Cu, 1·4–1·7 mg Mo and 2·4 g S/kg D.M., in amounts close to maintenance energy requirement. For six animals of each species the diet was enhanced with 4·8 mg Mo and 3 g S/kg D.M. Liver biopsy samples were obtained during weeks 1, 6 and 12. The animals were then re-randomized and five of each species offered the basal diet and the remainder the basal diet supplemented with 4 mg Cu/kg D.M. Liver biopsy samples were obtained after a further $4\frac{1}{2}$ weeks. Plasma samples for estimation of total and trichloroacetic acid-soluble Cu were taken weekly.

The mean liver Cu concentration in sheep was 11-fold greater than in deer. Both diets induced liver Cu depletion, though there was a trend for a greater rate of depletion on the Mo- and S-enhanced silage. The rate of depletion (mg Cu/kg liver D.M./day) was 7-fold greater in sheep than in deer, although it was not possible to determine whether this reflected a species-, as opposed to a Cu status-induced effect. In both species highly significant linear relationships were observed between initial liver Cu concentration and rate of liver Cu depletion. This was interpreted to indicate that endogenous loss was directly proportional to liver Cu content in both species. Individual estimates for the minimum rate of endogenous loss of Cu (μg/kg W/day) ranged from 0·2 to 2·71, mean 1·43, and from 2·83 to 14·75, mean 8·35 in deer and sheep, respectively.

During repletion the rate of increase of liver Cu in supplemented groups tended to be greater in sheep than in deer and calculated minimum values for availability of Cu were 0·061 and 0·037, respectively.

Liver Cu concentrations of less than 20 mg/kg D.M. were maintained in deer for several weeks without apparent symptoms of deficiency.

INTRODUCTION

Knowledge of the copper requirements of ruminants is almost entirely based on studies of the absorbability and endogenous losses of copper in animals consuming semi-purified diets (Suttle & McLaughlin, 1976; Agricultural Research Council, 1980). While these provide a framework within which requirements for animals grazing forages can be determined there is some evidence that in absolute terms the values obtaining on semi-purified diets may not hold for herbages (Suttle, 1983*a*).

Sheep have been used as a model for studies of copper metabolism in ruminants because of cost, though it has been recognized that differences in metabolism exist between sheep and cattle (Agricultural Research Council, 1980). The recent moves towards farming the cervidae and virtual complete lack of information on copper metabolism in this species has raised the question of the suitability of data derived from sheep for extrapolation into deer.

This paper describes a pilot study in which the metabolism of Cu in sheep and deer consuming a high quality silage was compared using depletion and repletion techniques and sampling of liver by biopsy.

MATERIALS AND METHODS

Animals

Ten docile red deer stags, aged 8 months and mean live weight (W) 95·7 ± 0·48 kg, from the deer unit on the College Research Farm were brought into indoor pens for a 6-week conditioning period. Ten Coopworth wether sheep, aged 10 months and mean W 48·9 ± 0·77 kg, from a flock on the same property,

were shorn and housed in metabolism crates for a 2-week pre-experimental period. Both species were drenched with 5 mg/kg of Oxfendazole (Synanthic-Syntex Laboratories NZ Ltd) on housing.

Feeding regime and treatments

The trial ran for 112 days and consisted of two periods; an initial 80-day depletion and a subsequent 32-day repletion phase. During both periods silage made from white clover–perennial ryegrass pasture was offered. For the sheep, it was offered at a rate estimated on the basis of individual body weight to be 20 % above maintenance energy requirement (0·48 MJ ME/kgW per day; $\bar{x}$, 1·2 kg D.M./day). It was offered to the stags at the same rate per unit of body weight ($\bar{x}$, 2·0 kg D.M./day). Two silages of similar composition were used, the first during days 0–63 and the second during days 64–112. The silage was changed to maintain consistency of herbage composition. A digestibility trial was conducted in sheep during days 94–100. Water was offered initially but was withdrawn when it became clear that involuntary intake of water in silage was sufficient to meet requirement.

During the first period (depletion) all animals were offered the basal silage. For six of each species (treated groups) it was enhanced with 4·8 mg Mo/kg D.M. and 3 g S/kg D.M. added as sodium molybdate, in solution, and sodium sulphate, as salt, both being hand mixed, daily, into individual rations. The other four animals from each species remained as controls.

During the second period (repletion), animals were re-randomized and five of each species assigned to each of two treatments: either continued feeding on the basal silage (control groups) or the basal silage enhanced with 4 mg Cu/kg D.M. as copper sulphate in solution hand mixed into the daily ration (treated groups).

Individual silage refusals were weighed daily. Samples of silage offered and refused were collected, weighed, bulked on a weekly basis, dried to constant weight at 75 °C and retained for subsequent analysis.

Tissue samples

Liver biopsy samples were taken from sheep on days 1, 34 and 80 using pentobarbitone-sodium anaesthesia (480–600 mg Nembutal; Ceva) and from deer on days 1, 44, 80 and 112 after immobilization using 1·5–2·5 ml of 2% xylazine hydrochloride (Rompun, Bayer) by the methods of Familton (1985).

The sheep were slaughtered on day 114 and the liver removed, weighed, and D.M. determined after mincing and drying to constant weight at 75 °C. Fresh weight (FW) of the liver of individual deer was predicted from body weight (W) using the following equation derived from data ($n = 68$) supplied by P. F. Fennessy (personal communication):

$$\text{liver FW} = (0{\cdot}298 \pm 0{\cdot}07) + (0{\cdot}0111 \pm 0{\cdot}005)\ \text{W}$$

Liver D.M. was assumed to be 30 % of fresh weight (Long, 1961).

Blood samples were taken each week into heparinized vacutainer tubes. In addition samples were taken from sheep just prior to liver sampling, and from deer soon after each biopsy sampling while the animals were still mildly sedated.

Chemical analyses

Total Cu in plasma and silage was determined by atomic absorption spectrophotometry, the former after dilution (1:5) in 0·1 M-HCl, and the latter on 4 g D.M. after ashing at 550 °C for 12 h followed by extraction in boiling 50 % (v/v) HCl. Tricloro-acetic acid (TCA)-soluble Cu in plasma was determined in the supernatant formed by precipitation of plasma (1 ml) with 5 % w/v TCA (5 ml). Sulphur in silage was determined by the method of Sinclair (1973). Molybdenum in silage and Cu in liver were determined by inductively coupled argon plasma spectrophotometry (Lee, 1983).

Statistical analyses

Differences in liver copper between species were compared by analysis of variance after log transformation of the data. Relationships between initial liver Cu concentration and rate of Cu depletion and effects of diet were examined by linear regression. All mean values are given with standard errors. The sequential measurements of plasma total Cu and the proportion of plasma Cu soluble in TCA were analysed using orthogonal polynomials by the methods of Rowell & Walters (1976). The components of second-order interactions, being homogeneous, were combined to form an error term to test first order interactions.

RESULTS

All the animals remained in apparently good health and no ill effects were observed from the liver sampling procedures. Live-weight change was negligible in deer but sheep gained 6·25 ± 0·54 kg during the trial. Individual weekly mean dry-matter intakes ranged from 1·54 to 1·87 kg D.M./day for deer and from 0·93 to 1·08 kg D.M./day for sheep. Overall, intakes were relatively constant and similar at 18·4 ± 0·54 and 20·1 ± 0·76 g/kg W/day for sheep and deer, respectively.

The average mineral content of the two basal silages (Table 1) was similar, except that the mean of the weekly estimates for Cu and D.M. concentrations were significantly greater in the second silage

Table 1. *Mean* (±S.E.) *of weekly mineral and* D.M. *analyses for unsupplemented silages and effects of additives during depletion phases on total mineral content*

Silage	Copper (mg/kg D.M.)	Molybdenum (mg/kg D.M.)	Sulphur (g/kg D.M.)	Dry matter (%)
		Basal silage		
1	8·72 (0·16)	1·70 (0·16)	2.45 (0·20)	17·5 (0·8)
2	10·03 (0·16)	1·35 (0·17)	2·35 (0·13)	21·3 (0·4)
		Treated silage during depletion		
1	As above	6·5	5·4	As above
2	As above	6·2	5·4	As above
		Treated silage during repletion		
2	14·03	1·35	2·35	As above

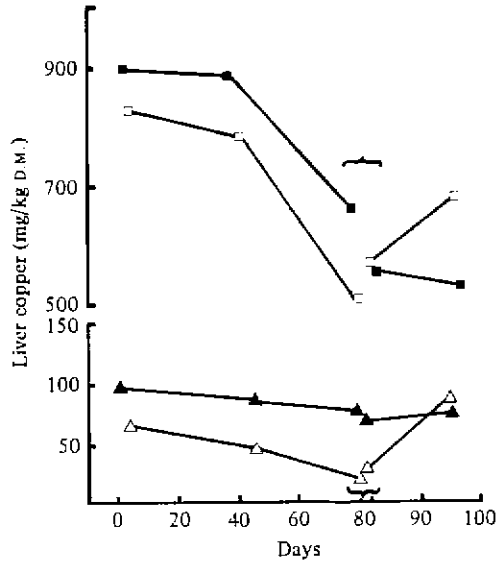

Fig. 1. Changes in mean liver copper concentration during depletion and repletion of deer and sheep. □, ■ and △, ▲ represent sheep and deer. and ■, ▲ and □, △ the basal and supplemented silages, respectively.

($P < 0·01$ in both cases). The mean D.M. digestibility of silage 2 was 0·79±0·004.

Depletion phase

The changes in liver Cu concentration (mg Cu/kg D.M.) in sheep and deer are given in Fig. 1 and Table 2. Values in sheep were initially greater than in deer by a factor of 11 ($P < 0·001$) with three sheep having liver Cu concentrations in excess of 1000 mg/kg D.M.

There were highly significant relationships between initial liver Cu concentration (mg Cu/kg D.M.) and rate of depletion of liver Cu (mg/kg D.M. per day) in all groups (Fig. 2). The coefficients for the relationships were significantly different between treatment groups in both sheep and deer ($P < 0·01$ in both cases). At the termination of the depletion phase, hepatic Cu concentrations in deer, particularly those on the Mo- and S-enhanced silage, Fig. 1, were very uniform and low ($\bar{x} = 20·4 \pm 0·27$ mg Cu/kg D.M.).

Repletion phase

The 4 mg Cu/kg D.M. dietary supplement caused a significant increase in hepatic Cu concentration in both species ($P < 0·05$, in both cases; Table 2, Fig. 1). The increase in concentration tended to be greater in sheep than in deer, though the difference was not significant. On the control silage, on the other hand, deer tended to show a slight gain in liver Cu, whereas sheep suffered further depletion.

There was no apparent relationship between liver Cu concentration and rate of repletion for either species ($r = -0·06$ and 0·142 for sheep and deer, respectively).

The mean dry weight of the livers of sheep removed at slaughter was 0·140±0·0127 kg.

Plasma Cu

The changes in total Cu concentration and the proportion soluble in TCA in plasma from deer and sheep during the two phases of the trial are given in Fig. 3. During depletion there was a significant ($P < 0·05$) species × time interaction for total Cu, mainly as a result of increases in concentration in sheep and decreases in concentration in deer on the Mo- and S-supplemented silage. There was no change in concentration in either species offered the basal silage, but the species × diet component was not significant. There was a large ($P < 0·001$) main effect of species. There was a significant ($P < 0·01$) species × time effect for the proportion of total plasma Cu which was soluble in TCA. Whereas Cu in plasma of deer was 100% soluble, reductions in solubility occurred in sheep on both the basal and supplemented diets during the last 3 weeks of the depletion phase.

Table 2. *Change* (±S.E.) *in liver copper concentration* (*mg/kg* D.M.) *in sheep and deer offered basal* (*control*) *or Mo- and S-supplemented* (*treated*) *silage during the depletion phase and the basal* (*control*) *or Cu-supplemented* (*treated*) *silage during the repletion phase*

	Depletion		Repletion	
	Deer	Sheep	Deer	Sheep
Treated	−48·2 (13·0)	−313·0 (65·0)	58·5 (11·0)	96·6 (31·0)
Control	−21·2 (6·40)	−243·0 (139·0)	13·7 (7·1)	−27·8 (25·0)

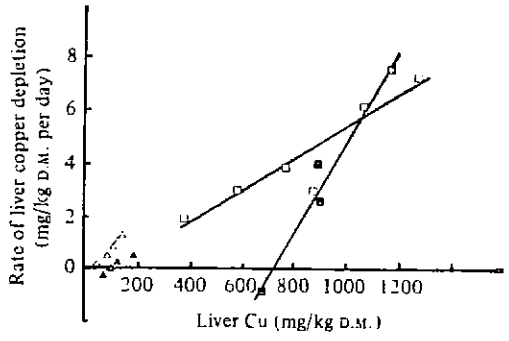

Fig. 2. The relationship between rate of depletion (Y, mg Cu/kg D.M. per day) and initial liver copper concentration (X, mg Cu/kg D.M.). □, ■ and △, ▲ represent sheep and deer and ■, ▲ and □, △ the basal and supplemented silages, respectively. For sheep: $Y = -11{\cdot}7 + 0{\cdot}0163X$ ($r = 0{\cdot}99$); $Y = -0{\cdot}670 + 0{\cdot}0055X$ ($r = 0{\cdot}93$). For deer: $Y = 0{\cdot}1229 + 0{\cdot}0036X$ ($r = 0{\cdot}84$); $Y = -0{\cdot}173 + 0{\cdot}0113X$ ($r = 0{\cdot}99$).

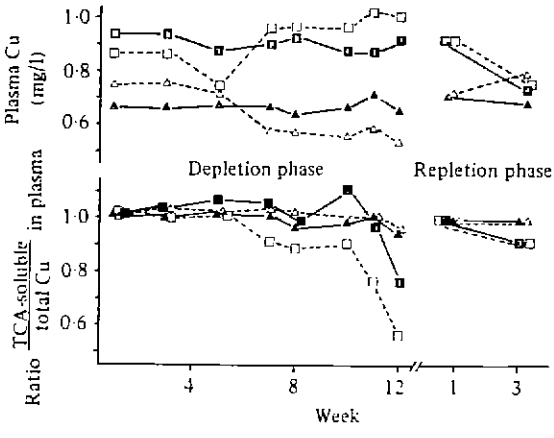

Fig. 3. Plasma total Cu and the proportion of plasma Cu soluble in trichloracetic acid (TCA) in sheep and deer during depletion and repletion phases of the trial. □, ■ and △, ▲ represent sheep and deer and ■, ▲ and □, △ the basal and supplemented silages, respectively.

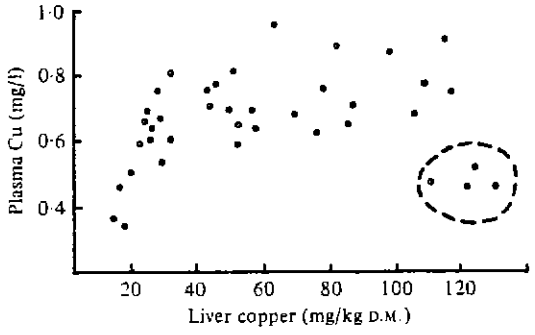

Fig. 4. Relationship between liver Cu and plasma total Cu concentrations at time of liver biopsy sampling in deer.

During repletion there were species × time interactions for both total and TCA-soluble Cu ($P < 0{\cdot}05$ in both cases).

There was no relationship ($r = 0{\cdot}11$) between plasma and liver Cu concentrations in sheep. In deer plasma Cu values below 0·5 mg/l were observed when liver Cu concentrations fell below 20 mg/kg D.M. (Fig. 4), though one individual, indicated by the circle, consistently showed low plasma Cu concentrations in association with high liver Cu concentrations.

DISCUSSION

The much greater hepatic Cu concentrations in the sheep than in the deer probably reflect a true species difference in Cu metabolism since both species grazed similar, though not identical, pasture prior to the trial and there was no history of Cu supplementation of the sheep. There are clinical data accumulating (P. W. Wilson, personal communication) which suggest that hepatic Cu concentrations in the two species may differ by a factor of 6–10. A number of the present sheep had values close to theoretical toxicity levels (> 1000 mg/kg; Grace, 1983), but there was no history of copper poisoning in the flock before or after the trial.

The fact that the basal silage supplied inadequate available Cu to maintain body Cu stores in either

species was surprising. The theoretical availability of Cu in this feed, predicted from the equation of Suttle & McLaughlin (1976) for semi-purified diets, was 0·041 and would have provided, in sheep, 0·39 mg Cu/day which is in excess of the theoretical maintenance requirement of 0·22 mg Cu/day (Agricultural Research Council, 1980). Moreover the specific prediction equation for the effect of S on Cu availability in silage (Suttle, 1983*b*) gave a value of 0·048, again suggesting adequacy of supply of Cu. On the other hand the Mo- and S-enhanced silage was predicted (Suttle & McLaughlin, 1976), to provide only 0·009 mg Cu/day (availability 0·0095) and to result in depletion of liver Cu. The present data therefore suggest either that availability of Cu in silages is lower or that endogenous loss is greater than would be predicted from the literature.

The positive association between hepatic Cu concentration and rate of hepatic Cu depletion observed in both species, Fig. 2, has been observed previously in sheep (Suttle & Field, 1983: Woolliams *et al.* 1983) and in cattle (Suttle, 1978: Simpson, Mills & McDonald, 1982). Since intake was offered according to body weight and change in liver mass would not be anticipated at maintenance levels of feeding (Sykes, Coop & Rushton, 1980), a variable rate of endogenous loss, or regulation of rate of Cu absorption according to body Cu status, must be postulated. It is not possible from this study to determine precisely which was responsible. Nevertheless, the closeness of fit of the relationship between rate of depletion and initial liver Cu concentration, in both species on either diet, when the majority of animals were in negative Cu balance and Cu availability clearly very low, does argue for variation in endogenous loss as the major factor. Further support for this argument comes from the lack of association between rate of liver Cu repletion and liver Cu concentration in either species. Moreover, whereas Suttle (1974) observed considerable variation between sheep in rate of Cu repletion when Cu was introduced in the diet much less variation was observed when Cu was infused intravenously, indicating greater likelihood of control within the body.

A minimum estimate of net endogenous loss of Cu can be calculated on the assumption that it was at least equivalent to the product of rate of depletion of liver Cu (mg/kg D.M. per day) and liver D.M. (kg). Actual endogenous loss would be underestimated to the extent of any of Cu absorbed. The calculation also depends on an assumption of zero change in liver weight argued above. Liver weight was measured directly in sheep and predicted (see above) from body weight for deer using accumulated data. Depletion rate for individual animals was calculated from the within-group regression of rate of depletion on liver Cu concentration using individual liver Cu concentrations mid-way through and at the end of the depletion phase (Fig. 2). Estimates for rate of net endogenous loss ranged from 0·20 to 2·71 μg Cu/kg W per day among the deer and from 2·83 to 14·75 μg Cu/kg W per day among the sheep, mean values ($\pm$S.E.) being $1{\cdot}42 \pm 0{\cdot}16$ and $8{\cdot}35 \pm 0{\cdot}70$ μg Cu/kg W per day, respectively. These estimates are much higher than those of 4·0 and 7·1 g Cu/kg W per day for sheep and cattle, respectively, adopted by the Agricultural Research Council (1980). The latter were based on studies in animals with depleted hepatic Cu stores and on diets providing low Cu intake and therefore do not provide for a variable endogenous loss. The rate of increase in calculated net endogenous loss in the Mo- and S-supplemented sheep for each 300 mg Cu/kg D.M. increase in liver Cu content, namely 0·23 mg Cu/day, was, however, very similar to the figure of 0·19 mg Cu/day calculated by Woolliams *et al.* (1983) for the same increment in liver Cu content, though clearly the value for sheep on the control diet was 3-fold greater. The present are the first estimates in deer. The relationship between rate of depletion and liver Cu concentration makes it difficult to compare precisely the endogenous losses of Cu in the two species. Rate of liver Cu depletion (mg/kg D.M. per day) and calculated endogenous loss were six- to eightfold greater in sheep than in deer (Fig. 2), though whether this would be the case at similar liver Cu concentrations is a matter for speculation.

The repletion phase was designed to provide initial estimates of the relative availability of Cu in sheep and deer. Several approaches can be used to provide these estimates. The increment in liver Cu caused by the Cu supplement provides a measure of the availability of the supplement, on the assumption

Table 3. *Calculated minimum rates* ($\pm$S.E.) *of absorption and availability of copper in copper-supplemented sheep and deer during the repletion phase*

	Predicted endogenous loss (mg/day)	Liver retention (mg/day)	Growth (mg/day)	Copper absorbed (mg/day)	Copper intake (mg/day)	Availability of copper
Deer	0·185	0·750	0	0·96	25·7	0·037
	(0·048)	(0·154)		(0·08)	(1·1)	(0·008)
Sheep	0·406	0·450	0·062	0·92	15·0	0·061
	(0·021)	(0·147)		(0·08)	(0·4)	(0·012)

that the additional Cu absorbed does not augment endogenous loss. On this basis estimates of 0·08 and 0·24 were derived for deer and sheep, respectively, which, particularly for the sheep, are high. The second approach, in which Cu absorption (A) is calculated as $A = HR + E + G$, where HR is the hepatic retention of Cu (change in liver Cu concentration × liver D.M.), E the minimum endogenous loss calculated, as above, during the present depletion phase and G the calculated gain in extrahepatic Cu, estimates the overall availability of Cu in the diet and supplement. The present estimate of endogenous loss derived in unsupplemented sheep was based on only four animals and, in predicting an approach to zero at liver Cu concentrations of 700 mg/kg D.M. (Fig. 2), seems unrealistic. The relationships derived in the Mo- and S-supplemented deer and sheep during depletion were therefore used to predict individual endogenous loss from the mean of pre- and post-repletion values for liver Cu concentration. This approach would tend to overestimate availability if Mo supplementation enhances endogenous loss (Woolliams *et al.* 1983; Suttle & Field, 1983). It was assumed that 1·1 mg Cu/kg is deposited in body gain (Agricultural Research Council, 1980) though more recent estimates have been slightly lower, namely 0·8 mg Cu/kg (Grace, 1983). The factorial calculations are given in Table 3. These estimates, though lower than the values derived from the increment in liver Cu induced by the Cu supplement, especially in the case of sheep, further suggest availability of Cu to be much lower in deer than in sheep. Such a situation would be consistent with the current and other findings of considerably lower liver Cu concentrations in deer.

An interative approach would provide higher values for both endogenous loss and availability, but the limited nature of the data preclude such calculations. Nevertheless the data do suggest that the very high rate of depletion of liver Cu in animals on the basal silage was the result of a higher rate of endogenous loss than would be predicted from the literature, rather than a low availability of Cu *per se*. Moreover, the dependence of endogenous loss on copper status suggests that the adoption of the single value of the Agricultural Research Council (1980) will underestimate the dietary requirement to maintain Cu balance in animals with normal Cu status. A reduction in endogenous loss with reducing Cu status will presumably provide a protective mechanism against reduction in Cu intake and result in stabilization of Cu status at a lower value. The nature of this mechanism and the rapidity and effectiveness of response requires more detailed examination. A physiological approach to prediction of Cu requirement would, however, appear to be required.

Whether the dietary requirement for copper in deer should reflect a lower availability will depend on the critical plasma and tissue Cu concentrations for Cu-dependent enzyme activity, and the relative effectiveness of Cu conservation mechanisms at low liver Cu concentrations. We are not aware of systematic work to establish these or deficiency symptoms. The relationship between liver Cu and plasma total Cu concentration in deer (Fig. 4), although based on limited data, does suggest a similar relationship to that observed in other ruminant species (Kellaway, Sitorus & Leibholz, 1978) in that plasma Cu concentration did not appear to be depressed until liver Cu concentration fell below 20–30 mg/kg D.M. One animal, data from which are circled did, however, consistently show low plasma Cu concentrations in association with high liver Cu values.

The appearance of a TCA-insoluble fraction in the plasma of sheep was surprising since this has generally not been seen until dietary Mo exceeds 8 mg/kg D.M. (Smith & Wright, 1975). The fact that this fraction was not seen in deer on the same diet is interesting and supports the conclusion of Mason *et al.* (1984), based on very limited data, that deer may also differ from other ruminants in the metabolism of molybdenum.

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