Changes in the distribution of copper and molybdenum after Mo administration and subsequent additional oral or intraperitoneal Cu administration to rats

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1. Male WAG/Cpb inbred rats fed on rations containing 1·5 mg copper/kg (deficient) and 6·0 mg Cu/kg (adequate) were supplemented with molybdenum (500 mg/kg diet). Starting at week 0 rats were killed weekly for up to 6 weeks and the caeruloplasmin activity of plasma, the Cu concentration of plasma, liver and kidney and the Mo concentration of liver and kidney were determined. The experiment was repeated with rats fed on diets of the same composition but given additional Cu for periods of 2 weeks. Cu was given orally by increasing dietary Cu to 6·0 mg/kg and 25·0 mg/kg for Cu-deficient and Cu-adequate rats respectively or intraperitoneally by injecting 75 μg and 250 μg every second day to Cu-deficient and Cu-adequate rats respectively.

2. After Mo administration to Cu-deficient rats plasma and kidney Cu and liver and kidney Mo increased but caeruloplasmin activity and liver Cu decreased. In Cu-adequate rats plasma, liver and kidney Cu and liver and kidney Mo increased to much higher levels than in Cu-deficient rats. Caeruloplasmin activity was not affected. Fluctuations in plasma Cu and kidney Mo were correlated closely.

3. No qualitative difference between the effect of oral or intraperitoneal Cu administered to Mo-treated Cu-deficient or Cu-adequate rats was found. In Cu-deficient Mo-supplemented rats additional Cu increased plasma Cu, caeruloplasmin activity and liver and kidney Cu and Mo. In Cu-adequate Mo-supplemented rats additional Cu decreased plasma Cu and liver and kidney Mo and increased caeruloplasmin activity and kidney Cu and, to a minor extent, liver Cu.

4. In view of the assumption that in rats a Cu, Mo and S containing compound, related to Cu-thiomolybdate, may be formed in vivo the results suggest that Cu binds to the Mo-S part of the compound; when this compound is formed in the gastro-intestinal tract it can not be absorbed and when it is formed at systemic sites it changes the Cu distribution.

Investigations on the copper–molybdenum–sulphur interaction has largely been concentrated on ruminants. This is due to several causes. First, sheep and cattle are the only species susceptible to Mo-induced Cu deficiency under field conditions; secondly, suggestions that the rumen is the main site of the interaction (Suttle, 1974) seem to be confirmed by various investigations; thirdly, single-stomached animals (non-ruminants) have not been considered as very useful experimental models.

This latter view is based on the difference between ruminants and non-ruminants in their reaction to dietary Mo and sulphate. In ruminants liver Cu concentrations are usually reduced after oral Mo and SO_4 supplementation (Marcilese *et al.* 1969) whereas in non-ruminants (rats) liver Cu concentrations are increased after oral Mo supplementation, an effect that is alleviated by SO_4 (Miller *et al.* 1956).

Nevertheless, Mo may have a similar effect on systemic Cu in both ruminants and non-ruminants. This is illustrated by the finding that in both sheep and guinea pigs after Mo supplementation, a Cu fraction can be demonstrated in the plasma that is insoluble in trichloroacetic acid (Smith & Wright, 1975) whereas in sheep (Marcilese *et al.* 1969) as well as in rats (Compère *et al.* 1965; Nederbragt & Van den Hamer, 1981*a*) and rabbits (Gaballah *et al.* 1965) a reduced transport of injected ^{64}Cu from plasma to tissues could be observed in Mo-supplemented animals.

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It has been suggested that the Cu–Mo antagonism in ruminants can be explained by the formation of unavailable Cu-thiomolybdate complexes in the rumen and at systemic sites (Suttle, 1974; Dick *et al.* 1975). Furthermore it was shown in rats (Mills *et al.* 1978) that in vitro synthesized thiomolybdate, when administered orally, inhibited Cu absorption and affected systemic Cu distribution whereas additional dietary Cu, but not intraperitoneal Cu, inhibited the absorption of ^{99}Mo-labelled thiomolybdate. Recently we showed (Nederbragt & Van den Hamer, 1981*b*) that the 'tightly' bound, non-caeruloplasmin Cu fraction in plasma of Mo-supplemented rats is part of an albumin-bound Cu–Mo–S complex that has properties similar to those of in vitro synthesized Cu-thiomolybdate described by Mills *et al.* (1978), thereby confirming the suggestion that the effect of Mo on systemic Cu in non-ruminants may be similar to that in ruminants.

In a recent publication (Nederbragt, 1980) experiments were described in which the effects of Mo supplementation on Cu and Mo distributions in rats were investigated at different dietary Cu levels. The results showed that the effect of Mo was largely dependent on the dietary Cu supply; it was suggested that Cu may somehow affect the uptake and retention of Mo in gastrointestinal tract and tissues. Those experiments were further extended with an investigation on the effect of Mo on Cu and Mo distributions in rats as a function of time and on the influence of additional Cu, given orally or intraperitoneally, on the Mo-induced changes. The results of these studies are presented here and are discussed with respect to the possible formation of Cu-thiomolybdate.

MATERIALS AND METHODS

Male WAG/Cpb rats were purchased from the Centraal Proefdierenbedrijf TNO, Zeist, The Netherlands. Their weight varied from 150–200 g at the start of the experiment. Management, housing conditions and composition of diet have been described before (Nederbragt, 1980). All experimental groups contained six rats. In both experiments Cu-deficient and Cu-adequate diets were given to the rats 2 weeks prior to the start of the Mo supplementation.

In the first experiment rats were maintained either on a Cu-deficient diet, containing 1·5 mg Cu/kg or on a Cu-adequate diet, containing 6·0 mg Cu/kg; both diets were supplemented with Mo to a final concentration of 500 mg/kg. Groups of six rats were killed weekly for up to six weeks of Mo supplementation. In the second experiment rats were maintained on diets similar to those of the first experiment. Either 2 or 4 weeks after the start of Mo supplementation to rats on the Cu-deficient diet (1·5 mg Cu/kg) additional Cu was given to two groups while maintaining the Mo concentration in their diets. One group of rats was supplemented with the same basal diet in which the Cu concentration was elevated to 6·0 mg/kg. In the second group of rats, Cu was administered intraperitoneally by injecting 75 μg Cu as copper sulphate in 1 ml saline (9 g sodium chloride/l) every second day; Cu injections started on day 0 and were given seven times. Both groups of rats were killed 2 weeks after the start of the additional Cu supply and simultaneously a group of Mo-supplemented Cu-deficient rats with unchanged Cu supply was sacrificed. Similar experiments were performed with Cu-adequate rats maintained on a diet containing 6·0 mg/kg. In these experiments additional Cu was given 1 or 3 weeks after the start of Mo-supplementation. One group of rats was given a diet in which the Cu concentration was elevated to 25·0 mg/kg; to the second group of rats 250 μg Cu was administered intraperitoneally in 1 ml saline every second day; Cu injections started on day 0 and were given seven times. Both groups of rats were killed 2 weeks after the start of the additional Cu-supply and simultaneously a group of Mo-supplemented Cu-adequate rats with unchanged Cu supply was killed.

After killing the rats, samples of blood and organs were collected for the

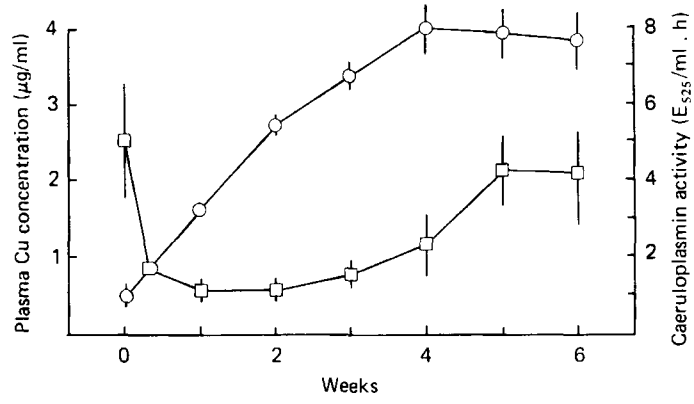

Fig. 1. Changes in the plasma copper concentration (μg/ml; ○—○) and caeruloplasmin activity (extinction units (E_{525})/ml per h; □—□) of Cu-deficient rats following dietary molybdenum supplementation. Cu level of the diet was 1·5 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. Each group consisted of six rats. The points are mean values with their standard errors indicated by vertical bars.

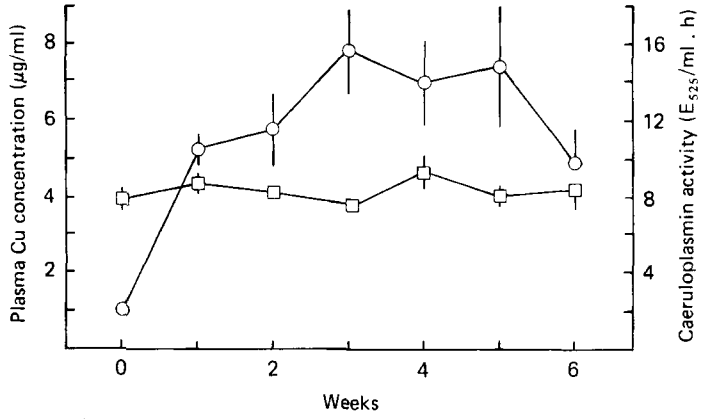

Fig. 2. Changes in the plasma copper concentration (μg/ml; ○—○) and caeruloplasmin activity (extinction units (E_{525})/ml per h; □—□) of Cu-adequate rats following dietary Mo supplementation. Cu level of the diet was 6·0 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

determination of the Cu concentration and caeruloplasmin activity of plasma and the Cu and Mo concentrations of liver and kidney. The methods for these determinations have been described previously (Nederbragt, 1980). Caeruloplasmin activity is expressed as extinction at 525 nm/ml plasma per h (E_{525}/ml per h), plasma Cu as μg/ml and Cu and Mo concentrations of liver and kidney as μg/g dry matter (DM).

RESULTS

Expt 1. Effect of Mo on Cu and Mo distributions as functions of time

Mo supplementation of the diet changed the distributions of Cu and Mo in plasma, liver and kidney of rats. However, a pronounced difference could be observed between the effects on Cu-deficient and Cu-adequate rats when the rate and extent of Cu and Mo accumulations

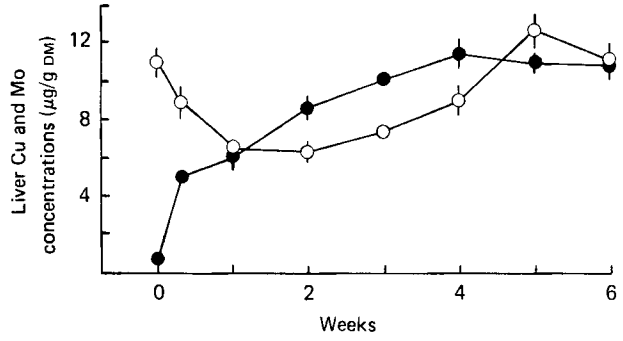

Fig. 3. Changes in the liver concentrations of copper (μg/g dry matter (DM); ○—○) and molybdenum (μg/g DM; ●—●) of Cu-deficient rats following dietary Mo supplementation. Cu level of the diet was 1·5 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

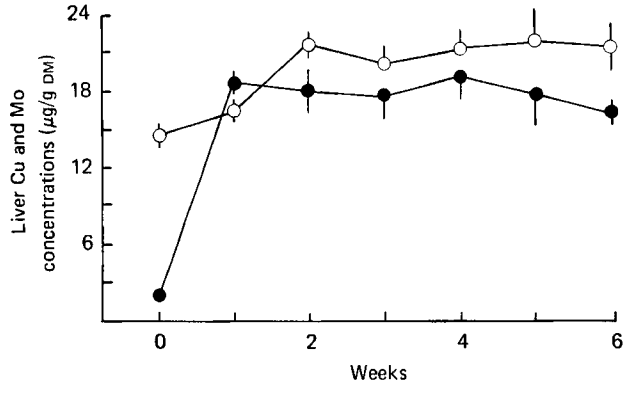

Fig. 4. Changes in the liver concentrations of copper (μg/g dry matter (DM); ○—○) and molybdenum (μg/g DM; ●—●) of Cu-adequate rats following dietary Mo supplementation. Cu level of the diet was 6·0 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

are concerned. This difference was obtained with a diet that contained a similar Mo concentration (500 mg/kg) for both groups of rats.

In the plasma of Cu-deficient rats (Fig. 1) the total Cu concentration increased to 4·0 μg/ml whereas in Cu-adequate rats a level of 7–8 μg/ml was reached (Fig. 2). In the Cu-adequate rats plasma Cu decreased again after 5 weeks. This decrease was the start of a series of fluctuations in which plasma Cu was falling and rising on alternate weeks; they could be observed for up to 12 weeks after Mo administration in rats from which blood samples were taken weekly (details not shown). Although plasma Cu increased in Cu-deficient rats, their caeruloplasmin activity fell rapidly after Mo administration; this was followed by a slow increase towards the end of the experiment (Fig. 1). In Cu-adequate rats the caeruloplasmin activity remained unchanged (Fig. 2).

In Cu-deficient rats the changes in liver Cu (Fig. 3) were well comparable to those of their caeruloplasmin activity (Fig. 2): a decrease followed by a gradual increase. In

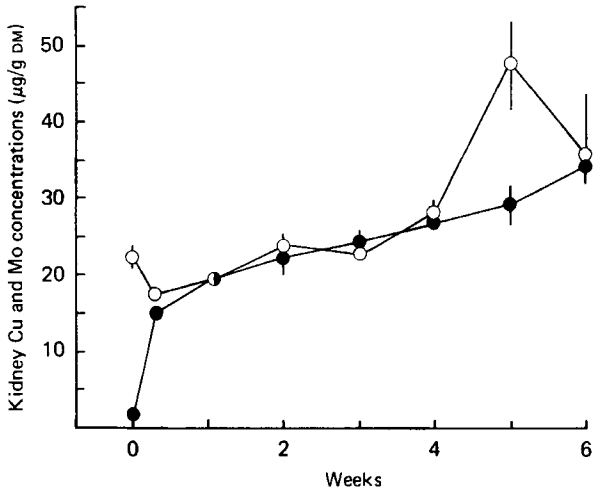

Fig. 5. Changes in the kidney concentrations of copper (μg/g dry matter (DM); ○—○) and molybdenum (μg/g DM; ●—●) of Cu-deficient rats following dietary Mo supplementation. Cu level of the diet was 1·5 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

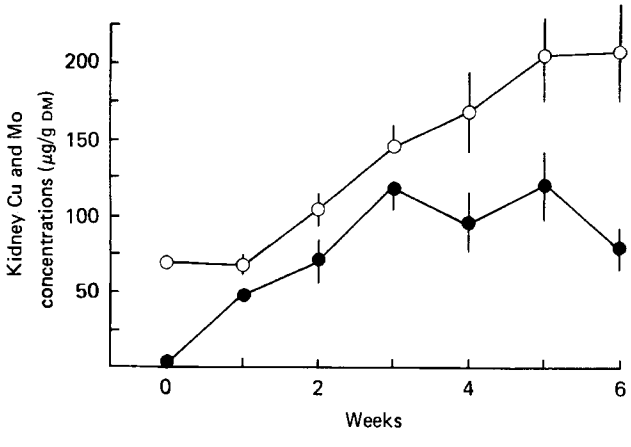

Fig. 6. Changes in the kidney concentrations of copper (μg/g dry matter (DM); ○—○) and molybdenum (μg/g DM; ●—●) of Cu-adequate rats following dietary Mo supplementation. Cu level of the diet was 6·0 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

Cu-adequate rats the liver Cu concentration increased immediately after Mo supplementation and reached a maximum after 2 weeks (Fig. 4). A marked difference was observed in the process of Mo accumulation in the liver. In Cu-deficient rats this accumulation was slow (except for the first 2 d; (Fig. 3)) and reached a maximum that was 0·6 of the Mo level in Cu-adequate rats where the accumulation was very fast (Fig. 4).

The most pronounced differences were found in the kidney. In Cu-adequate rats the net accumulation of Cu amounted to approximately 5 times that in Cu-deficient rats and the Mo accumulation was at least 3 times higher in Cu-adequate rats (Figs. 5 and 6) following

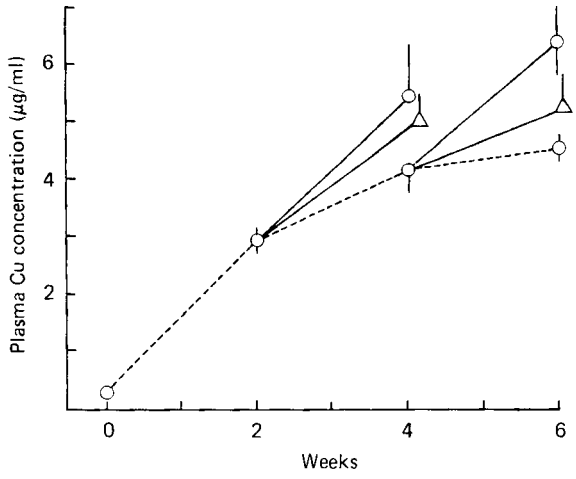

Fig. 7. Changes in the plasma copper concentration (μg/ml) of Cu-deficient rats following dietary molybdenum supplementation and additional Cu supplementation. Cu level of the diet was 1·5 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. (——), Effect of increasing the dietary Cu level to 6·0 mg/kg (○) or of intraperitoneal injections of 75 μg Cu each second day (△). (- - - -), No additional Cu. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

Mo supplementation. Another feature of the changes in kidney Cu and Mo was the small initial decrease of Cu in Cu-deficient rats (Fig. 5) and the lack of increase of Cu during the first week in Cu-adequate rats (Fig. 6). In Cu-deficient rats an abrupt increase at week 5 followed by a decrease was observed (Fig. 5) that can not be explained.

Expt 2. Influence of additional Cu on Mo-supplemented rats

Additional Cu was administered, either orally or intraperitoneally, to Mo-supplemented Cu-deficient rats for a period of 2 weeks. Cu was given during two different periods. The first period, between 2 and 4 weeks, corresponded to that in which plasma Cu in Cu-deficient rats was still increasing; in the second period, between 4 and 6 weeks, the plasma Cu levels of similarly treated rats were maintained at a maximal level (Fig. 1). Dietary Cu was elevated from 1·5 to 6·0 mg/kg and the amount of intraperitoneally-injected Cu was calculated to be equivalent to that of additional dietary Cu, assuming an intestinal Cu absorption in Cu-deficient rats of 0·4 with an average daily food consumption of approximately 22 g/rat. In general, additional Cu caused an increase of plasma Cu (Fig. 7) caeruloplasmin activity (Fig. 8), liver Cu and Mo (Fig. 9) and kidney Cu and Mo (Fig. 10). Qualitative differences could not be observed between the effects of oral and intraperitoneally-injected Cu. The increase of plasma Cu was more pronounced after oral Cu whereas caeruloplasmin activity was increased more after intraperitoneally-injected Cu. The increase of liver Cu was similar when Cu was administered after 2 and 4 weeks of Mo supplementation but the increase in liver Mo was smaller in the second period. Kidney Cu concentrations increased considerably, particularly in the second period and kidney Mo concentrations were approximately doubled by the additional Cu administration.

Additional Cu was also administered, either orally or intraperitoneally, to Mo-supplemented Cu-adequate rats, for a period of 2 weeks. Cu was given during two different periods. The first period, between 1 and 3 weeks, corresponded to that in which plasma Cu in Cu-adequate rats was still increasing; in the second period, between 3 and 5 weeks,

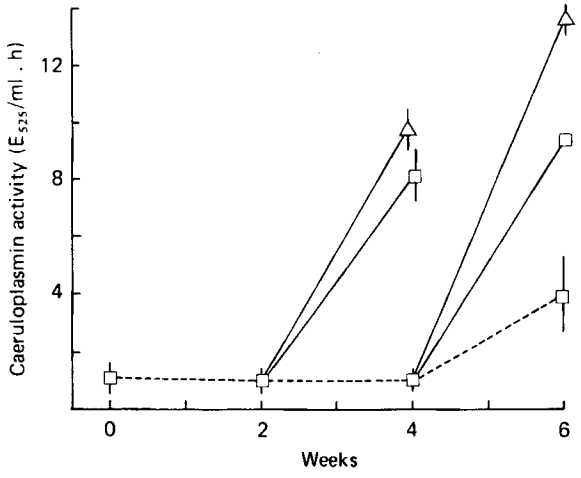

Fig. 8. Changes in the caeruloplasmin activity (extinction units (E_{525})/ml per h) in the plasma of Cu-deficient rats following dietary molybdenum supplementation and additional Cu supplementation. Cu level of the diet was 1·5 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. (——), Effect of increasing the dietary Cu level to 6·0 mg/kg (□) or of intraperitoneal injections of 75 μg Cu each second day (△). (- - - -), No additional Cu. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

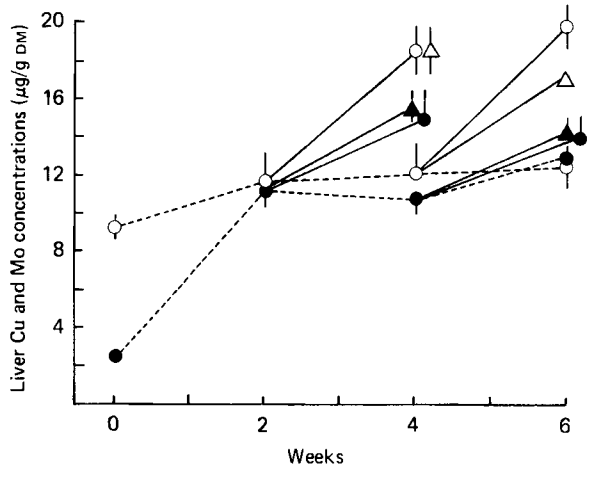

Fig. 9. Changes in the liver concentrations of copper (μg/g dry matter (DM); ○,△) and molybdenum (μg/g DM; ●,▲) of Cu-deficient rats following dietary Mo supplementation and additional Cu supplementation. Cu level of the diet was 1·5 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. (——), Effect of increasing the dietary Cu level to 6·0 mg/kg (○,●) or of intraperitoneal injections of 75 μg Cu each second day (△,▲). (- - -), No additional Cu. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

the plasma Cu levels of similarly treated rats were maintained at a maximal level (Fig. 2). Dietary Cu was increased from 6·0 to 25·0 mg/kg and the amount of intraperitoneally-injected Cu was calculated to be equivalent to that of additional dietary Cu, assuming an intestinal Cu absorption in Cu-adequate rats of 0·3 with an average daily food consumption of approximately 22 g/rat. In general, administration of additional Cu caused a decrease

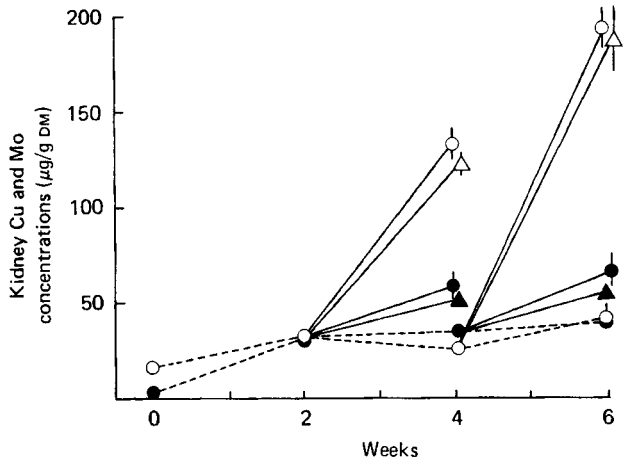

Fig. 10. Changes in the kidney concentrations of copper (μg/g dry matter (DM); ○,△) and molybdenum (μg/g DM; ●,▲) of Cu-deficient rats following dietary Mo supplementation and additional Cu supplementation. Cu level of the diet was 1·5 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. (——), Effect of increasing the dietary Cu level to 6·0 mg/kg (○,●) or of intraperitoneal injections of 75 μg Cu each second day (△,▲). (- - -), No additional Cu. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

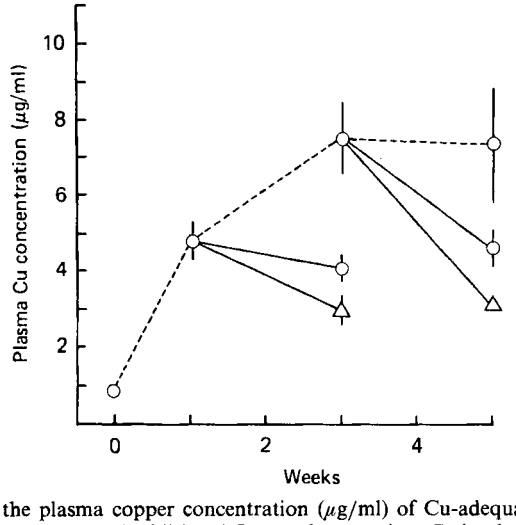

Fig. 11. Changes in the plasma copper concentration (μg/ml) of Cu-adequate rats following dietary molybdenum supplementation and additional Cu supplementation. Cu level of the diet was 6·0 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. (——), Effect of increasing the dietary Cu level to 25·0 mg/kg (○) or of intraperitoneal injections of 250 μg Cu each second day (△). (- - -), No additional Cu. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

of plasma Cu (Fig. 11), liver Mo (Fig. 13) and kidney Mo (Fig. 14), an increase in caeruloplasmin activity (Fig. 12) and a negligible to small increase of liver Cu (Fig. 13) and kidney Cu (Fig. 14). An exception to this was observed in the liver of rats given additional Cu intraperitoneally between 3 and 5 weeks which caused a 2-fold increase of liver Cu. As in Cu-deficient rats no qualitative difference between the effect of orally- and intra-

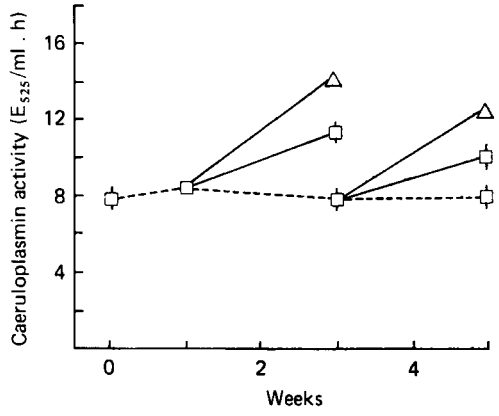

Fig. 12. Changes in the caeruloplasmin activity (extinction units (E_{525}/ml per h) in the plasma of Cu-adequate rats following dietary molybdenum supplementation and additional Cu supplementation. Cu level of the diet was 6·0 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. (——), Effect of increasing the dietary Cu level to 25·0 mg/kg (□) or of intraperitoneal injections of 250 μg Cu each second day (△). (- - -), No additional Cu. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

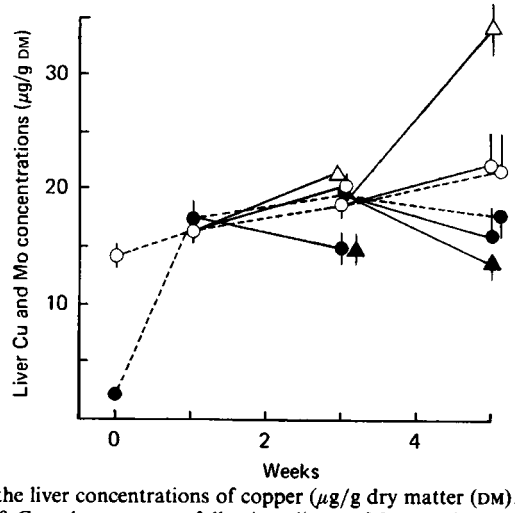

Fig. 13. Changes in the liver concentrations of copper (μg/g dry matter (DM); ○,△) and molybdenum (μg/g DM; ●,▲) of Cu adequate rats following dietary Mo supplementation and additional Cu supplementation. Cu level of the diet was 6·0 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. (——), Effect of increasing the dietary Cu level to 25·0 mg/kg (○,●) or of intraperitoneal injections of 250 μg Cu each second day (△,▲). (- - -), No additional Cu. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

peritoneally-administered Cu was present; furthermore the mode of administration had no influence on the effect of Mo.

DISCUSSION

The studies on both Cu-deficient and Cu-adequate rats offer an opportunity to evaluate more closely the process that takes place after Mo administration. The evaluation presented here is based on the assumption that the formation of a compound containing Cu, Mo and

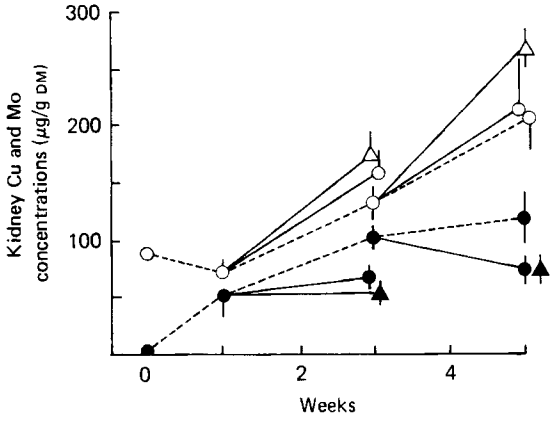

Fig. 14. Changes in the kidney concentrations of copper (μg/g dry matter (DM); ○,△) and of molybdenum (μg/g DM; ●,▲) of Cu-adequate rats following dietary Mo supplementation and additional Cu supplementation. Cu level of the diet was 6·0 mg/kg; Mo administration (500 mg/kg) was started at week 0 and continued throughout the experiment. (——), Effect of increasing the dietary Cu level to 25·0 mg/kg (○,●) or of intraperitoneal injections of 250 μg Cu each second day (△,▲). (- - -), No additional Cu. Each group consisted of six rats. Points are mean values with their standard errors indicated by vertical bars.

a sulphydryl group is responsible for the observed increase in Cu concentrations of plasma and organs of Mo supplemented rats. This compound has been demonstrated in the plasma of those rats and it totally accounted for the increase in plasma Cu (Nederbragt & Van den Hamer, 1981*b*).

The results with Mo administration to Cu-deficient rats (Figs. 1, 3 and 5) suggest that it is the Mo–S part of the compound that is absorbed and subsequently binds systemic Cu. Apart from arguments derived from the experiments with additional Cu supply this can also be seen from the finding that despite their apparent Cu-deficient state (*cf* Cu concentrations and caeruloplasmin activity of Cu-adequate rats in Figs. 2, 4 and 6 at 0 d) Mo caused a considerable increase in plasma Cu but a rapid fall in caeruloplasmin activity; simultaneously liver Cu and, for a short time and to a lesser extent, kidney Cu decreased initially.

The results with Mo administration to Cu-adequate rats show that there is a striking difference in the response of liver and kidney. Maximal concentrations of Mo and Cu in the liver are reached at 1 and 2 weeks respectively and are maintained for the remaining experimental period (Fig. 4). In the kidney the increase in Mo and Cu concentrations continues for 3 and 5 weeks respectively and the Mo concentration of the kidney starts to fluctuate after it has reached its maximum. Furthermore a high correlation (r 0·94; $P < 0{\cdot}005$) exists between plasma Cu and kidney Mo for all rats in this experiment. This finding seems to confirm the suggestion of Bremner & Young (1978) that an unavailable Cu fraction (probably Cu-thiomolybdate) may be taken up by the kidney before its excretion into the urine. Anyhow, these results show that in handling Cu and Mo in Mo-supplemented rats the role of the kidney markedly differs from that of the liver.

The effect of Mo administration is more dramatic in Cu-adequate rats where Cu and Mo concentrations become several times higher than in Cu-deficient rats (except for liver Cu); this confirms earlier observations on the influence dietary Cu has on the effect of Mo administration in rats (Nederbragt, 1980). Furthermore it is in accordance with the results in the Cu-deficient rats of Expt 2, where additional Cu administration intensified the effect of Mo administration (Figs. 7–10), although some effects (e.g. the rise in caeruloplasmin

activity) may be due to alleviation of the Cu-deficient state. The main effect of additional Cu administration should be caused by an increased storage of Cu and Mo, bound together in a Cu–Mo–S compound as suggested before. Under those conditions an increased retention of Mo should be measurable as a reduced excretion of Mo into the urine because this is the main route of Mo excretion (Bibr & Lener, 1973; Rosoff & Spence, 1973).

Reversed results are obtained when Cu is administered to Mo-supplemented Cu-adequate rats (Figs. 11–14): plasma Cu and liver and kidney Mo are decreased (or are not further increased as in the kidney between 1 and 3 weeks) whereas liver and kidney Cu are mostly slightly increased; the rise in caeruloplasmin activity is more substantial and suggests that the Cu supply of these rats is not optimal. The observed decreases of plasma Cu and liver and kidney Mo provide a second argument for the suggestion that an Mo–S compound is formed in the gastrointestinal tract: the presence of a large amount of dietary Cu may give rise to a higher concentration of a Cu–Mo–S compound in the gastrointestinal tract and, as it cannot be absorbed, less of the Mo-S compound is available at systemic sites for Cu binding. That Cu has no effect on molybdate is shown by Mason *et al.* (1978) who found no influence of additional Cu on the absorption of ^{99}Mo administered as molybdate to sheep via the duodenum and furthermore by the lack of influence of intraperitoneally-injected Cu on the retention of ^{99}Mo-labelled molybdate that was administered intravenously to rats (Nederbragt and Van den Hamer, unpublished results).

In a recent paper (Nederbragt & Van den Hamer, 1981*b*) it was suggested that the Cu–Mo–S complex that was demonstrated in the plasma of Mo-treated rats was related to Cu-thiomolybdate (CuTM). For the suggested formation of an Mo–S compound or thiomolybdate (TM) in the gastrointestinal tract of the rat there is no direct evidence. However, it has been shown that micro-organisms in the intestinal lumen of the rat are involved in the metabolism of S-containing compounds (Huovinen & Gustafsson, 1967) which may be influenced by the presence of molybdate as it is in rumen micro-organisms (Gawthorne & Nader, 1976). Furthermore the finding that oral Cu inhibits the absorption of orally administered ^{99}Mo-labelled TM (Mills *et al.* 1978) confirms the suggested physiological behaviour of the Mo-S compound and its relation to TM.

The amount of Cu to be injected was chosen somewhat arbitrarily because no convincing data could be found concerning the efficiency of Cu absorption under the different conditions used in these experiments. Nevertheless no qualitative and only small quantitative differences were found between the effect of oral and intraperitoneally-injected Cu. This may indicate that in Mo-supplemented Cu-adequate rats Cu excreted into the bile should behave similarly to oral Cu. Although it is assumed that little re-absorption of biliary Cu occurs in rats nothing is known of the binding of Cu to biliary compounds; it cannot be excluded that the Cu-chelating properties of TM in the intestine are equal to those of TM at systemic sites and therefore binding of Cu from biliary proteins may inhibit the absorption of TM.

Although further experimentation is necessary, the results of this study strongly support the hypothesis that the formation of CuTM-related compounds may occur in the gastrointestinal tract and in plasma and tissues of non-ruminants.

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