Some studies on the metabolism and the effects of ^{99}Mo- and ^{35}S-labelled thiomolybdates after intravenous infusion in sheep

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1. Sheep were infused intravenously with ^{99}Mo- and ^{35}S-labelled tri- and tetrathiomolybdates (1–2 mg Mo).
2. Most of the plasma radioactivity was trichloroacetic acid (TCA)-insoluble after infusion, but the stability of this fraction was reduced by pre-infusion or subsequent infusion with unlabelled thiomolybdates. Most of the ^{99}Mo and ^{35}S was shown to be associated with albumin.
3. It was concluded that compounds bound to albumin were relatively stable, but displaced or unbound thiomolybdates were rapidly hydrolysed to molybdate and sulphate.
4. There was no evidence of an irreversible interaction of either ^{35}S or ^{99}Mo with copper in plasma, despite the appearance of a TCA-insoluble Cu fraction. Increased dietary Cu did not increase the retention of ^{35}S in plasma or affect the exchangeability of ^{35}S-labelled thiomolybdates bound to albumin.

Thiomolybdates were first implicated in the pathogenesis of Mo-induced hypocuprosis and molybdenosis in ruminants by Suttle (1974) and Dick *et al.* (1975) and supportive evidence has accumulated since then; see reviews by Suttle (1980) and Mason (1981, 1982). ^{99}Mo-labelled di- and trithiomolybdates ($MoO_2S_2^{2-}$ and $MoOS_3^{2-}$) but not ^{99}Mo-labelled tetrathiomolybdate (MoS_4^{2-}) were detected in the plasma of sheep (Mason *et al.* 1982*a*) and cattle (M. Hynes, D. Poole, P. Rogers and J. Mason, unpublished results) after the infusion of ^{99}Mo-labelled molybdate into the rumen. The compounds could be displaced from their protein carrier(s) in vitro and identified by Sephadex G-25 chromatography.

The duodenal infusion of di- and trithiomolybdate (Mason *et al.* 1982*b*) or tetrathiomolybdate (Mason *et al.* 1980) provokes the appearance of a trichloroacetic acid (TCA)-insoluble copper fraction in plasma. Whether this TCA-insoluble Cu corresponds to the TCA-insoluble Cu which is such a characteristic response to dietary Mo (Smith & Wright, 1975; Bremner & Young, 1978), has not yet been established. There may be two phenomena, a decrease in the TCA-solubility of plasma Cu, associated with high levels of circulating thiomolybdates, and a more persistent TCA-insoluble fraction, less-readily related. Although thiomolybdates will react with Cu in vitro (Clarke & Laurie, 1982), ^{99}Mo-labelled compounds present in plasma can be displaced from their protein carrier, even several days after administration (Mason *et al.* 1982*a*, 1983). TCA-insoluble ^{99}Mo also appears in plasma after the rumen or duodenal infusion of ^{99}Mo-labelled thiomolybdates (Mason *et al.* 1982*b*; Kelleher *et al.* 1983). The fraction corresponds quantitatively to protein-bound ^{99}Mo, although the insolubility in vitro may be a consequence of thiomolybdate breakdown on acidification with TCA. By contrast, after duodenal administration of ^{99}Mo-labelled molybdate, the plasma ^{99}Mo is overwhelmingly TCA-soluble and remains unbound (Mason *et al.* 1982*a*; Kelleher *et al.* 1983).

Little is known of the metabolism of thiomolybdates in vivo. Gooneratne *et al.* (1981) showed that the injection of ammonium tetrathiomolybdate (100 mg twice weekly) prevented the development of Cu toxicity in chronically-Cu-poisoned sheep, despite the recent demonstration (Mason *et al.* 1983) that ^{99}Mo-labelled thiomolybdates injected in this

way, at comparable dose rates, were extensively hydrolysed to $^{99}MoO_4^{2-}$ (di > tri > tetra), particularly over the first few minutes post-injection. As a consequence, more than 95% of the ^{99}Mo was eliminated via the urine mainly over the first 24 h after injection. However, the fate of the sulphur component of the compounds is unknown. This is of interest since the progressive hydrolysis of thiomolybdates would produce sulphides as well as, eventually, molybdate (Mason *et al.* 1983). Thus,

$$MoOS_3^{2-} + H_2O \rightleftharpoons MoO_2S_2^{2-} + H^+ + HS^-.$$

The experiments reported here were designed to examine the metabolism of thiomolybdates in plasma, in particular that of trithiomolybdate, the species which at present seems the one most likely to be of pathological importance (Mason, 1982). The effect of repeated infusions on Cu metabolism and on the fate of the compounds themselves was examined. This is the first reported study employing ^{35}S-labelled compounds.

MATERIALS AND METHODS

Animals

The animals used were drawn from a pool of eight male castrated Texel sheep, weighing between 51 and 69 kg, maintained on a basic diet (Mason *et al.* 1978) supplemented with elemental S (3 g S/kg dry matter). The basic diet contained (/kg) 13 mg Cu, 0·35 mg Mo, 1·1 g S, but in Expts 2 and 3, some animals were given additional Cu (20 mg Cu as copper sulphate/kg diet). For the experiments, the sheep were retained in metabolism cages and faeces and urine collected to monitor ^{99}Mo and ^{35}S excretion (Mason *et al.* 1978).

Blood samples and plasma ^{99}Mo and ^{35}S

Plasma samples were obtained and the levels of Cu and TCA-soluble and insoluble ^{99}Mo estimated, as reported by Mason *et al.* (1978). Plasma samples destined for gel-chromatography and Cu determinations were stored at −20°. Samples for ^{35}S counting were prepared by diluting (*A*) 1 ml plasma (total ^{35}S) or (*B*) the supernatant fraction, obtained from precipitation of plasma (1 ml) with TCA (100 g/l) 1:1 (TCA-soluble ^{35}S), with distilled water to 5 ml and shaking with Insta-gel (Packard; 10 ml). TCA-insoluble ^{35}S was taken as the difference between the samples ($A - B$). Counting was delayed by at least 1 month after preparation to eliminate interference by ^{99}Mo (half-life 66 h). The total radioactivity in plasma was estimated by assuming a blood volume of 7% of body-weight and a plasma volume of 60% of blood volume.

Preparation of ^{99}Mo-labelled and ^{35}S-labelled thiomolybdates

^{99}Mo-labelled trithiomolybdate was prepared by the method described by Mason *et al.* (1982*b*), except that the source of ^{99}Mo-labelled molybdate was $^{99}MoO_3$ (Commisariat à l'Energie Atomique, France). The oxide (12 mg Mo) was brought into solution (as molybdate) by the addition of 1 M-sodium hydroxide (2 ml).

[^{35}S]trithiomolybdate was produced by dissolving the contents of an ampoule containing 2–3 mg Na_2 ^{35}S (1 mCi; Amersham International, Amersham, Bucks) in 0·5 M-phosphate buffer, pH 7·3 (200 μl), containing sodium molybdate (350 mM). This was then added to 2 ml phosphate buffer presaturated with hydrogen sulphide (approximate solubility 3·2 mg S/ml). The mixture was then left in a stoppered tube for 10 min after which a further 10 s burst of H_2S gas was passed; the solution was then left for a further 5 min before purification.

[^{35}S]tetrathiomolybdate was produced in a similar manner, except that the reaction in the stoppered tube was allowed to proceed for 4 h and further 10 s bursts of H_2S gas were

given after 25 min, 1·5, 2 and 3 h. Final purification was by Sephadex G-25 chromatography and before injection (immediately after purification), solutions were made isotonic by the addition of appropriate amounts of solid sodium chloride.

Sephadex G-200 gel filtration

Heparinized plasma samples (3 ml) stored at −20° were passed through a column (26 × 900 mm) of Sephadex G-200 superfine grade. Elution at 4° was with 0·01 M-Tris-acetate buffer, pH 7·4, with a flow-rate of 7 ml/h and fractions of 7 ml were collected. The distribution of ^{99m}Technetium was determined after the attainment of isotopic equilibrium (Mason *et al.* 1978) and after one further month, to eliminate ^{99}Mo and ^{99m}Tc, 5 ml of each fraction was taken up in Insta-gel (10 ml) for ^{35}S counting. Cu distribution was determined by atomic absorption spectrophotometry.

Experimental procedures

The labelled (approximately 0·2 mCi per isotope and 1 or 2 mg Mo) and unlabelled thiomolybdates (generally 30 mg Mo) in 20 ml saline (9 g NaCl/l) were infused via a jugular vein over 20 min (labelled), and 2 h (unlabelled), using Gilson six-channel peristaltic pumps. Blood samples were obtained from an indwelling catheter in the other jugular vein at the intervals indicated in the figures, these being intervals from the beginning of infusion, and immediately chilled before centrifugation and processing or storage. Faeces and urine were collected every 8 h and ^{99}Mo counted according to Mason *et al.* (1978). Urine was counted for ^{35}S after at least 1 month delay and samples were diluted in distilled water and centrifuged before suspension in Insta-gel.

RESULTS

Expt 1

The effects of repeated infusions of trithiomolybdate were comparable to those seen after a single injection (Mason *et al.* 1983), that is, immediate but transient depressions of the TCA-soluble plasma Cu in vitro on infusion and the appearance of a more persistent TCA-insoluble Cu fraction. There was no evidence of a long-term impairment of caeruloplasmin (TCA-soluble Cu) synthesis, despite the quantities of trithiomolybdate administered (135 mg Mo over 100 h). The results for one of the sheep are shown in Fig. 1. Figs. 2 and 3 show the plasma profiles for ^{99}Mo and ^{35}S respectively of four animals administered double-labelled trithiomolybdate (2 mg Mo) intravenously over 20 min, at zero time in Fig. 1. Two of the animals receiving repeated further infusions of unlabelled trithiomolybdate are compared to two animals infused only once with labelled trithiomolybdate. In all four animals, both the ^{99}Mo and the ^{35}S were overwhelmingly TCA-insoluble, but in the two animals which had been pre-infused with unlabelled trithiomolybdate this fraction was less stable. The difference is apparent after 2 h and obvious after 20 h (Figs. 2 and 3). Further infusions accelerated the decline of both isotopes and for both the disappearance of label from the TCA-insoluble fraction was marked by a transient increase in the TCA-soluble fraction. The urinary excretion patterns were also very different; the animals receiving only labelled trithiomolybdate (2 mg Mo) excreted 37·4 and 24·5% of the ^{99}Mo respectively compared with 36·8 and 25·4% of the ^{35}S over 76 h, in contrast to 65·7 and 66·8% of the ^{99}Mo and 58·7 and 58·3% of the ^{35}S for the two animals repeatedly-infused with unlabelled trithiomolybdate. Faecal output of ^{99}Mo was very low, about 1–2% for all four animals.

The infusion, even of the relatively-small amounts of double-labelled trithiomolybdate (2 mg Mo), was sufficient to produce a transient depression of the TCA-solubility of Cu and the appearance of a persistent TCA-insoluble fraction similar to those shown in Fig. 1.

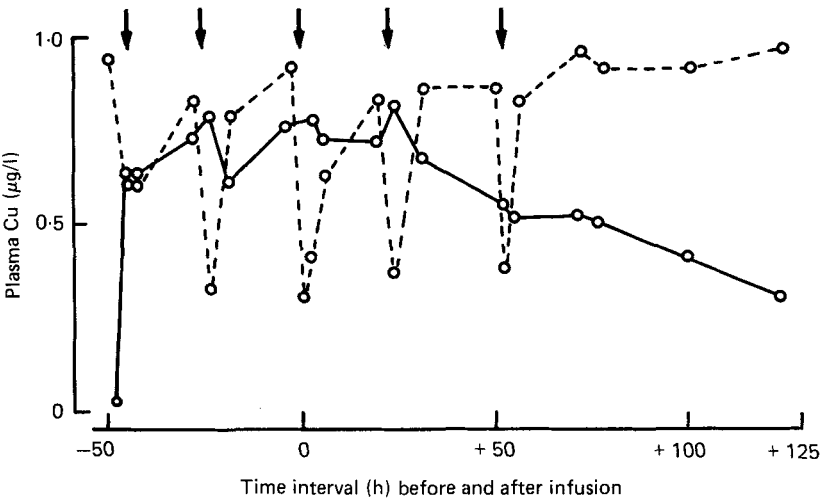

Fig. 1. Expt 1. The changes in trichloroacetic acid (TCA)-soluble (○---○) and TCA-insoluble (○——○) plasma copper of a sheep infused with trithiomolybdate at the time intervals indicated (↓); 15 mg molybdenum at −48 h and 30 mg molybdenum for the four subsequent infusions.

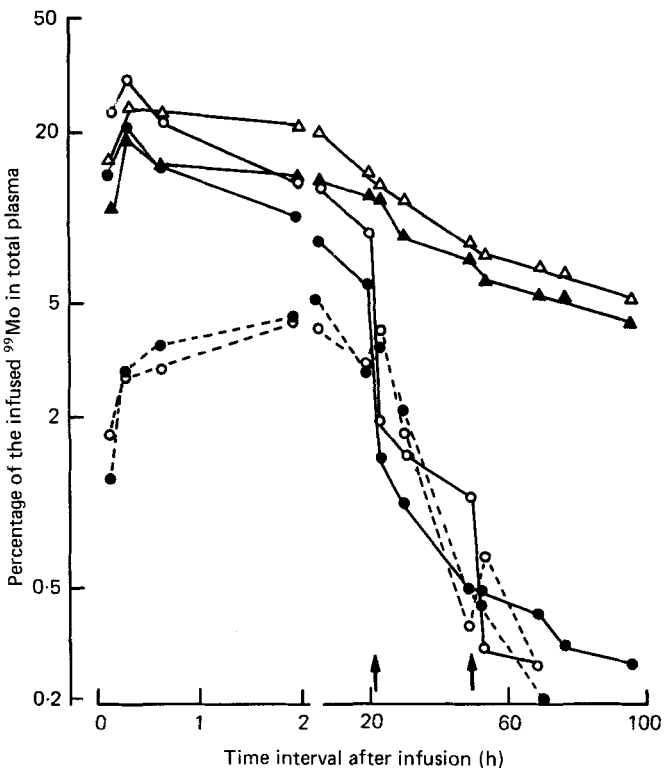

Fig. 2. Expt 1. ^{99}Mo in different fractions in the plasma of four sheep after the intravenous infusion of ^{99}Mo- and ^{35}S-labelled trithiomolybdate (2 mg Mo/animal). Plasma trichloroacetic acid (TCA)-insoluble ^{99}Mo (○——○, ●——●), plasma TCA-soluble ^{99}Mo (○---○, ●---●) of two animals also infused with unlabelled trithiomolybdate at the time intervals indicated (↑). One animal (●——●, ●---●) did not receive the 51 h infusion. TCA-insoluble ^{99}Mo of two animals not infused with unlabelled trithiomolybdate (△——△, ▲——▲).

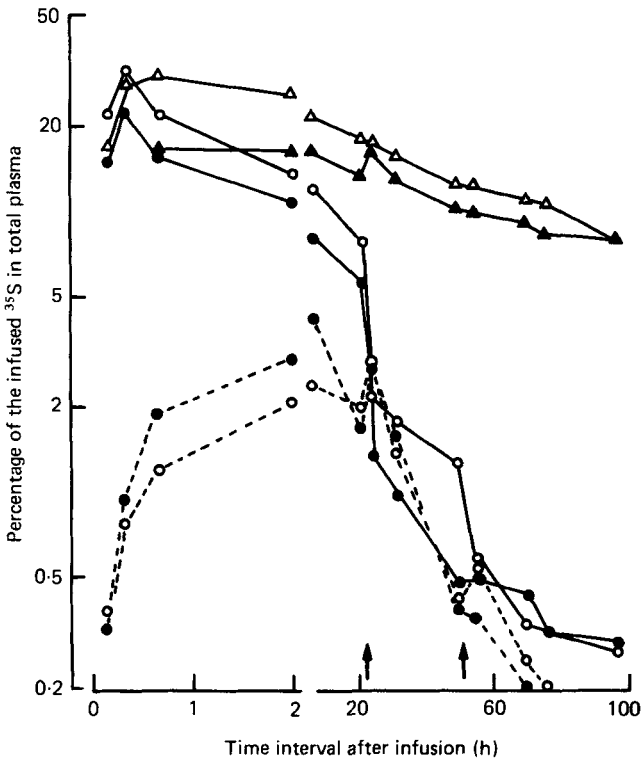

Fig. 3. Expt 1. ^{35}S in different fractions in the plasma of four sheep after the intravenous infusion of ^{99}Mo- and ^{35}S-labelled trithiomolybdate (2 mg Mo/animal). Plasma trichloroacetic acid (TCA)-insoluble ^{35}S (○——○, ●——●), plasma soluble ^{35}S (○---○, ●---●) of two animals also infused with unlabelled trithiomolybdate at the time intervals indicated (↑). One animal (●——●, ●---●) did not receive the 51 h infusion. TCA-insoluble ^{35}S of two animals not infused with unlabelled trithiomolybdate (△——△, ▲——▲).

Sephadex G-200 gel-filtration showed that most of the radioactivity, both ^{99}Mo and ^{35}S, was associated with the albumin fraction of plasma, that is, the protein peak around fraction no. 45 in the samples, shown in Fig. 4*a*. The small-molecular-weight peak eluting after fraction no. 60 was $^{99}MoO_4^{2-}$ and $^{35}SO_4^{2-}$. The effect of the multiple thiomolybdate infusions on plasma Cu was to increase the amount associated with albumin (Fig. 4*b*). Gel-filtration of a pre-infusion plasma sample from the same animal (not shown) showed virtually all the Cu to be associated with caeruloplasmin at around fractions nos. 36–37.

Expt 2

Expt 1 showed that if there was a preferential retention in plasma of ^{35}S over ^{99}Mo (by interaction of S with Cu), the difference must have been quite small (for example, the results in Fig. 3 compared with those in Fig. 2.). To examine the fate of ^{35}S, a second experiment was carried out on four animals without pre-infusion of unlabelled thiomolybdate. The diets of two of the sheep were supplemented with $CuSO_4$ (20 mg Cu/kg diet). The plasma ^{35}S profiles after infusion of [^{35}S]trithiomolybdate (1 mg Mo) are shown in Fig. 5. The increased dietary Cu does not appear to influence the retention of ^{35}S in plasma. An infusion with unlabelled thiomolybdate (30 mg Mo) after 28 h eliminated most of the remaining TCA-insoluble ^{35}S and its disappearance was accompanied by a large transient increase in the levels of TCA-soluble ^{35}S.

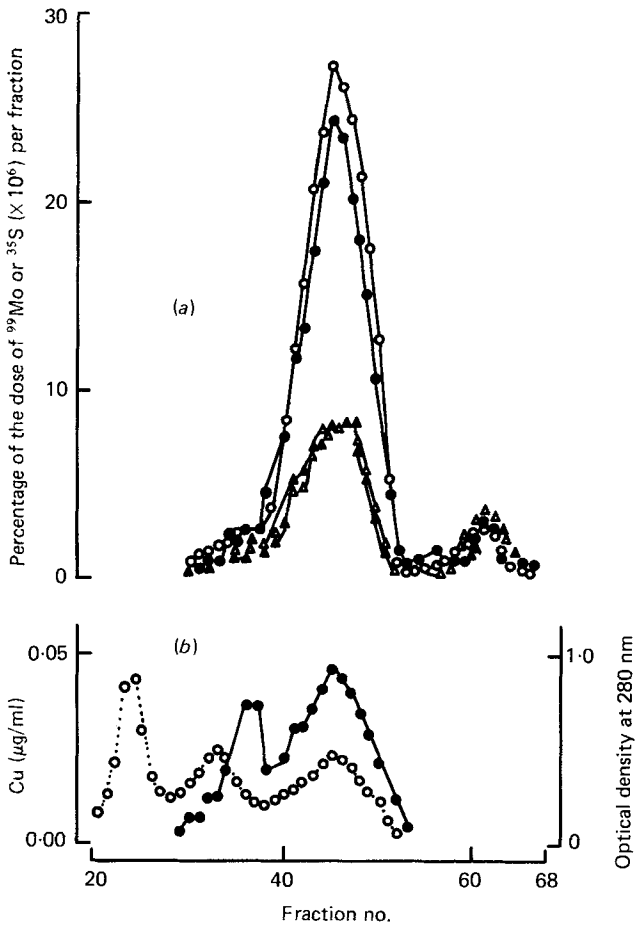

Fig. 4. Expt 1. Sephadex G-200 gel-filtration of 3-ml plasma samples from a sheep after infusion of ^{99}Mo- and ^{35}S-labelled trithiomolybdate. The animal had previously been treated with unlabelled trithiomolybdate (see Figs. 1, 2 and 3). (*a*) ^{99}Mo (●——●) and ^{35}S (○——○) of a sample obtained 10 min after the end of infusion with the labelled trithiomolybdate (2 mg Mo/animal; ^{99}Mo (▲——▲) and ^{35}S (△——△) of a sample taken 6 h post-infusion. (*b*) Copper (●——●) and protein (○---○) profiles of the 10 min sample. Column 26 × 900 mm, fraction volume 7 ml, elution with 10 mM-Tris acetate, pH 7·4, at 4°.

Expt 3

In this experiment, [^{35}S]tetrathiomolybdate (1 mg Mo), a compound more potent biologically than trithiomolybdate (Mills & Bremner, 1980; Bremner *et al.* 1982; Mason, 1982), was used and a longer interval allowed to facilitate any chemical interaction, before reinfusion with unlabelled tetrathiomolybdate. As in Expt 2, two animals were given the Cu-supplemented diet. The results are shown in Fig. 6. Even after 70 h, most of the TCA-insoluble ^{35}S could be displaced and only about 10% of the level of the control animals remained after a second infusion at 95 h. There is, thus, little indication that ^{35}S from tetrathiomolybdate had participated in the formation of a tightly-bound Cu–protein complex in plasma. In this experiment, there was very little TCA-soluble ^{35}S present in plasma, except after the 70 h infusion.

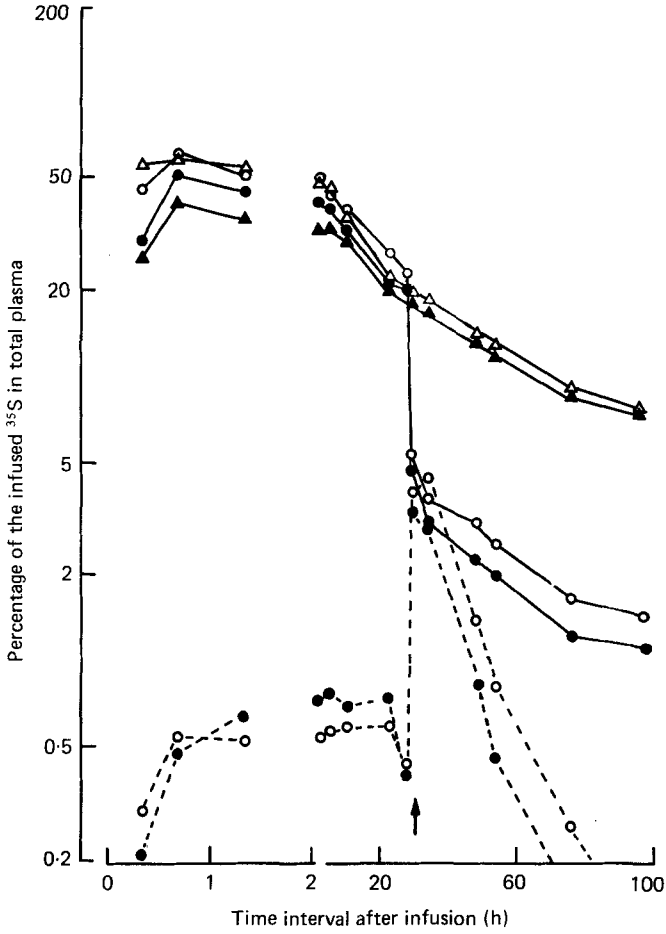

Fig. 5. Expt 2. ^{35}S in different fractions in the plasma of four sheep after the intravenous infusion of [^{35}S]trithiomolybdate (1 mg Mo/animal). Plasma trichloroacetic acid (TCA)-insoluble ^{35}S (○——○, ●——●), plasma TCA-soluble ^{35}S (○---○, ●---●), of two animals also infused with unlabelled trithiomolybdate (30 mg Mo) at the time interval indicated (28 h). TCA-insoluble ^{35}S (△——△, ▲——▲) of two animals not infused with unlabelled thiomolybdate. The diets of two animals (●——●, ●---●, ▲——▲) were supplemented with copper (20 mg Cu/kg diet).

DISCUSSION

The stabilities of the TCA-insoluble ^{99}Mo and ^{35}S fractions in plasma, immediately after infusion of the labelled compounds in these experiments, were higher than those reported by Mason *et al.* (1983) after the rapid intravenous injection of rather larger amounts, that is, 5·4–62·5 mg Mo. In those studies, less than 5% of the dose injected remained in the TCA-insoluble fraction of plasma 2 h post-injection, compared to approximately 50% in Expts 2 and 3 in this series for example. The reduced amount of radioactivity present in the TCA-soluble fraction, that is, $^{99}MoO_4^{2-}$ or $^{35}SO_4^{2-}$, reflects this. Thus, in the previous studies (Mason *et al.* 1983), TCA-soluble ^{99}Mo levels were comparable to the TCA-insoluble ^{99}Mo (tetrathiomolybdate) or even predominant (tri- and dithiomolybdate) over the first few hours, whereas in the present experiments levels were much lower. In Expt 3 in particular, the higher stability of the tetrathiomolybdate ion, coupled with the slow infusion

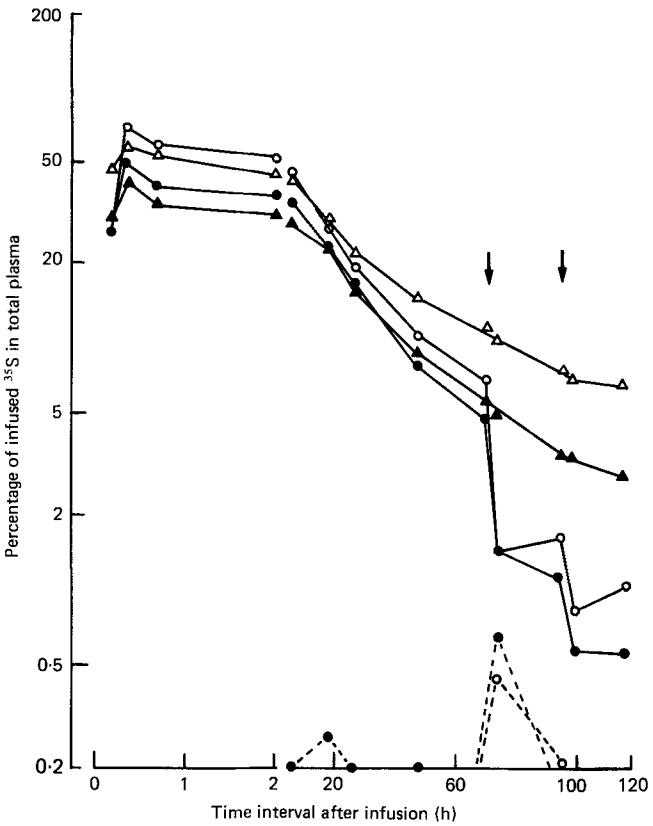

Fig. 6. Expt 3. ^{35}S in different fractions in the plasma of four sheep after the intravenous infusion of [^{35}S]tetrathiomolybdate (1 mg molybdenum/animal). Plasma trichloroacetic acid (TCA)-insoluble ^{35}S (○——○, ●——●), plasma TCA-soluble ^{35}S (○---○, ●---●), of two animals also infused with unlabelled tetrathiomolybdate (30 mg Mo/animal) at the time intervals indicated (↓, 70 and 95 h). TCA-insoluble ^{35}S (△——△, ▲——▲) of two animals not infused with unlabelled tetrathiomolybdate. The diets of two animals (●——●, ●---●, ▲——▲) were supplemented with copper (20 mg/Cu per kg diet).

and the tracer amount employed, reduced the initial hydrolysis so that significant amounts of TCA-soluble ^{35}S were present only after the 70 h infusion with unlabelled tetrathiomolybdate. This pattern is comparable to that observed with compounds absorbed from the rumen (Kelleher *et al.* 1983) and is presumably closer to the situation where animals are exposed to moderate amounts of dietary Mo. The results indicate that the persistence of thiomolybdates in plasma in vivo is dependent on their concentration since the disappearance of label from the TCA-insoluble fraction was accelerated dramatically by pre-infusion or subsequent administration of unlabelled compounds. Experiments carried out with cattle (M. Hynes, D. Poole, P. Rogers and J. Mason, unpublished results) showed that metabolism of trithiomolybdate, in particular that over the first few hours, is indeed dependent on the amount injected.

Overall, these experiments and those of Kelleher *et al.* (1983) and Mason *et al.* (1983) indicate that thiomolybdates bind to albumin in vivo and as such are relatively stable. When the compounds are displaced or where the binding capacity of albumin carrier is saturated, then the free compounds are rapidly hydrolysed to molybdate and sulphate. These

compounds may be recycled, but when the dietary S level is relatively high, as in these experiments, the label tends to be rapidly lost via the urine, presumably since molybdate–sulphate competition for transport processes in the renal tubule blocks reabsorption (Mason, 1981).

While the experiments domonstrated that the amount of Cu bound to albumin increases and that the ^{99}Mo and the ^{35}S were associated with the same protein fraction, this is not necessarily indicative of a chemical interaction. Indeed, most of the ^{99}Mo and ^{35}S remained readily exchangeable in vivo (for example, even after 70 h in Expt 3), and in vitro. This is in common with previous results (Mason *et al.* 1982*a*, 1983) with ^{99}Mo. For example, in Expt 1 even after 97 h, virtually all the residual protein-bound radioactivity from the animals receiving only small amounts of labelled trithiomolybdate (2 mg Mo) could be displaced in vitro and identified as [^{99}Mo]trithiomolybdate (not shown).

The ready exchangeability does not suggest that either Mo or S had formed insoluble, unavailable protein complexes with Cu, at least in plasma. Thiomolybdate infusion did, nevertheless, lead to the appearance of a TCA-insoluble Cu fraction, the characteristic response to Mo first reported by Smith & Wright (1975). In common with other workers, i.e. Bremner & Young (1978) in Mo-fed sheep and Mills *et al.* (1981) using rats, the proportion of plasma Cu associated with albumin increased, but we found no evidence of the 90000-molecular-weight protein reported by Bremner & Young (1978). The protein-bound ^{99}Mo (and ^{35}S) in these experiments is also clearly associated with the albumin fraction and this is analogous to studies with rats (Mills *et al.* 1981), but differs from the studies of Bremner & Young (1978). These authors reported that while some plasma Mo was associated with albumin, there was rather more with the 90000-molecular-weight fraction. There appears to be no association of either ^{99}Mo or ^{35}S with caeruloplasmin. The results indicate, therefore, that Cu and thiomolybdates co-accumulate on albumin, but provide no evidence of any irreversible chemical interaction. However, Smith & Wright (1975) showed that a proportion of the TCA-insoluble Cu could be complexed by diethyldithiocarbamate and Nederbragt & Van den Hamer (1981) have demonstrated that the protein-bound Cu of paramolybdate-fed rats was able to exchange with ^{64}Cu in vitro.

It has been suggested (Mills *et al.* 1981) that TCA-insoluble Cu arises because of the presence of reactive sulphide, possibly persulphide, associated with proteins, and that this reactive sulphide may be protected against hepatic oxidation by thiomolybdates. The authors (Mason *et al.* 1983) have shown that the hydrolysis of the compounds in vivo could be a source of reactive sulphide. The experiments reported here do not appear to support this hypothesis, since the plasma ^{35}S was as exchangeable as the ^{99}Mo and was no more readily related to the persistent TCA-insoluble Cu fraction in plasma. Also the metabolism was not affected by increased dietary Cu which presumably increased the flow of Cu in plasma. The sulphides which must arise as the thiomolybdates are hydrolysed after displacement from albumin thus appear to be very rapidly oxidized. Overall, the results imply that, if there had been an interaction between thiomolybdate-S and plasma Cu, it must have been very small. However, if the reactive S in persulphides, for example, were derived from albumin sulphydryl rather than thiomolybdate, then the fate of the ^{35}S from thiomolybdate would be independent of the bound Cu.

The results also demonstrated why the TCA-insolubility of albumin-bound Cu or plasma Cu may not in itself be meaningful. The acid treatment of thiomolybdates would generate sulphides, so that some entrapment of Cu in TCA precipitates when thiomolybdates are present would be expected. This may explain why there appears to be little relation between changes apparently induced in plasma and the overall potency of the individual compounds in vivo in rats (Bremner *et al.* 1982). However, it is clear from these experiments and from earlier work that Mo does increase the proportion of Cu associated with albumin and its

systemic metabolism is altered. For example, Mills *et al.* (1978) showed that while tetrathiomolybdate dramatically reduced the absorption of ^{64}Cu from the gut, the subsequent tissue distribution was also affected, since much less ^{64}Cu was captured by the liver and a greatly increased percentage was retained in the blood. From the results, it does not appear that the accumulation of Cu on albumin occurs because of direct interaction with thiomolybdates carried on albumin, but is more likely to be a consequence of some modification, induced by thiomolybdates, of the way in which the Cu is bound to the albumin molecule or in the ability of the liver to capture the bound Cu.

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REFERENCES

Bremner. I., Mills, C. F. & Young, B. W. (1982). *Journal of Inorganic Biochemistry* **16**, 109–119.
Bremner, I. & Young, B. W. (1978). *British Journal of Nutrition* **39**, 325–335.
Clarke, N. J. & Laurie, S. H. (1982). *Inorganica Chimica Acta* **66**, 35–38.
Dick, A. T., Dewey, D. W. & Gawthorne, J. M. (1975). *Journal of Agricultural Science, Cambridge* **85**, 567–568.
Gooneratne, S. R., Howell, J. McC. & Gawthorne, J. M. (1981). *British Journal of Nutrition* **46**, 457–480.
Kelleher, C. A., Ivan, M., Lamand, M. & Mason, J. (1983). *Journal of Comparative Pathology* **93**, 83–92.
Mason, J. (1981). *Irish Veterinary Journal* **35**, 221–229.
Mason, J. (1982). *Irish Veterinary Journal* **36**, 164–168.
Mason, J., Kelleher, C. A. & Letters, J. (1982*a*). *British Journal of Nutrition* **48**, 391–397.
Mason, J., Lamand, M. & Hynes, M. (1983). *Journal of Inorganic Biochemistry* **19**, 153–164.
Mason, J., Lamand, M. & Kelleher, C. A. (1980). *British Journal of Nutrition* **43**, 515–523.
Mason, J., Lamand, M. & Kelleher, C. A. (1982*b*). *Journal of Comparative Pathology* **92**, 509–519.
Mason, J., Lamand, M., Tressol, J. C. & Lab, C. (1978). *Annales de Recherche Vétérinaire* **9**, 577–586.
Mills, C. F. & Bremner, I. (1980). In *Molybdenum and Molybdenum-containing Enzymes*, pp. 517–542 [M. P. Coughlan, editor]. Oxford: Pergamon Press.
Mills, C. F., Bremner, I., El-Gallad, T. T., Dalgarno, A. C. & Young, B. W. (1978). In *Trace Element Metabolism in Man and Animals*, pp. 150–158 [M. Kirchgessner, editor]. Weinhensteephan: Arbeitskreis für Tierernahrungsforschung.
Mills, C. F., El-Gallad, T. T. & Bremner, I. (1981). *Journal of Inorganic Biochemistry* **14**, 189–207.
Nederbragt, M. & Van den Hamer, C. J. A. (1981). *Journal of Inorganic Biochemistry* **15**, 293–306.
Smith, B. S. W. & Wright, H. (1975). *Journal of Comparative Pathology* **85**, 299–305.
Suttle, N. F. (1974). *Proceedings of the Nutrition Society* **33**, 299–305.
Suttle, N. F. (1980). *Annals of the New York Academy of Sciences* **355**, 195–207.

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