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THE UPTAKE AND SUBCELLULAR DISTRIBUTION OF GOLD IN RAT LIVER CELLS AFTER *IN VIVO* ADMINISTRATION OF SODIUM AUROTHIOMALATE

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Abstract—Using the techniques of differential centrifugation, sucrose sedimentation-gradient and isopycnic-gradient centrifugation and gel chromatography through Sephadex G 75 and G 200, the uptake and subcellular distribution of gold in rat liver has been studied over a period of 1 hr to 36 days after intraperitoneal administration of 0.7 mg kg^{-1} to 80 mg kg^{-1} sodium aurothiomalate ("Myocrisin"). After 1 hr, gold concentrated in the lysosomes of liver cells and, under certain conditions, it was estimated that probably more than 90 per cent of the cytoplasmic gold was associated with these organelles. After longer time intervals partial redistribution of the gold took place, possibly due to sequestration into telolysosomes. The majority of the lysosomal gold appeared to be membrane-bound and increased with increasing aurothiomalate dose and time after injection.

The distribution of gold among the proteins of the cytosol and the lysed granule-fraction supernatant has been investigated and compared with that in the plasma. Whereas in the latter more than 87 per cent of the gold was bound to albumin, the gold in the lysosomal supernatant was bound to molecules over a wide range of molecular weights, and that in the cytosol appeared to be bound to at least three macromolecules, one ~300,000, one ~40,000 (possibly ligandin) and one ~10,000. In addition 10–15 per cent of the cytosol gold appeared to be a much lower molecular weight species (less than 3,000), which was not found in the lysosomal supernatant or the plasma.

Although gold drugs, especially sodium aurothiomalate ("Myocrysin"), have been used with success in the treatment of rheumatoid arthritis for many years [1, 2], little is known about the mechanisms involved in the production of their therapeutic and toxic effects.

In patients undergoing chrysotherapy [3] and in rats administered sodium aurothiomalate [4], the gold was found to accumulate especially in organs containing components of the reticulo–endothelial system, such as the liver. Various qualitative investigations into the subcellular distribution of gold have been made using electron-microscopic and electron-microprobe techniques. After the administration of sodium aurothiomalate, gold was found to accumulate into the lysosomes or lysosome-like organelles of human synovial macrophages [5], rabbit synovial intimal cells, subsynovial macrophages and sometimes articular cartilage chondrocytes [6, 7], rat and rabbit macrophages, hepatocytes and renal epithelial cells [8], rat renal glomerular capillary wall [9] and possibly human dermis [10]. On the other hand when sodium aurothiopropanol sulphonate was injected into rats, the gold in the renal proximal tubule cells appeared to be concentrated in the mitochondria [11]. Very few quantitative investigations into the subcellular distribution of gold have been performed. The preliminary communication of the present work [12] showed that gold accumulated in the lysosomes of rat cells after administration of sodium aurothiomalate. Some of the results in that paper have been recently confirmed [13].

The present studies extend the work of the preliminary communication [12] and investigate in detail the quantitative subcellular distribution of gold in rat liver after intraperitoneal administration of sodium aurothiomalate.

MATERIALS AND METHODS

Materials. Sodium aurothiomalate was obtained from May & Baker Ltd., Dagenham, Essex, U.K. and sodium [^{198}Au or ^{195}Au] aurothiomalate from Radiochemical Centre Ltd., Amersham, Bucks, U.K. Triton WR-1339 was purchased from Winthrop Laboratories, Newcastle, Northumberland, U.K. Sephadex G200 and G75 were brought from Pharmacia (G.B.) Ltd., London, U.K. All the enzyme substrates were obtained from Sigma London Chemical Co. Ltd., Kingston, Surrey, U.K. All other chemicals were bought from BDH Chemicals Ltd., Poole, Dorset, U.K. and were of "Analar" grade except for Triton X-100.

Animal experiments. Male Sprague–Dawley rats were injected intraperitoneally with sodium aurothiomalate containing sodium [^{198}Au or ^{195}Au] aurothiomalate at doses of between 0.7 mg kg^{-1} and 80 mg kg^{-1} (^{195}Au = 10^{-6} Ci to 10^{-5} Ci, ^{198}Au = 10^{-4} Ci to 10^{-3} Ci). The rats were allowed food and water *ad lib.* and for a few of the experiments involving excretion studies were kept periodically in metabolic cages. When plasma was required the blood was obtained by cardiac puncture. The rats were killed after various time intervals between 1 hr and 36 days by dislocation of the cervical vertebrae under light ether anaesthesia. The livers were perfused with

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0.15 M NaCl, excised and washed in ice-cold 0.25 M sucrose. All subsequent fractionation procedures were performed at 4°.

The washed livers were minced, weighed and homogenised in a Potter-Elvehjem apparatus using three up and down strokes of the Teflon pestle. The resulting homogenate was diluted with 0.25 M sucrose to give a concentration of 10% relative to the liver wet weight.

Differential centrifugation. The homogenate was successively centrifuged at 600 *g* for 10 min to provide a cell debris fraction mainly containing nuclei and unbroken cells, 3,000 *g* for 12 min to give a fraction containing mitochondria and heavy lysosomes, 8,000 *g* for 20 min to provide light lysosomes, a few mitochondria and a small amount of endoplasmic reticulum, 30,000 *g* for 30 min to yield endoplasmic reticulum and very light lysosomes, and 105,000 *g* for 120 min to give a fraction containing endoplasmic reticulum and a supernatant essentially free of subcellular organelles.

In addition a mixed granule fraction was obtained by centrifuging the 600 *g* supernatant at 30,000 *g* for 30 min.

Sedimentation gradient centrifugation. Five ml of the cytoplasmic fraction (post cell-debris supernatant) were layered onto a continuous sucrose gradient (0.25 M to 1.17 M) with a 2.40 M sucrose layer at the bottom of the centrifuge tube, to give a total volume of 45 ml. The gradient was centrifuged at 1350 *g* for 2 hr in a swing-out rotor at 4°. Approximately 15 × 3 ml fractions were collected from the bottom of the tube.

Isopycnic gradient centrifugation. A mixed granule fraction obtained from rats, with or without intraperitoneal administration of 0.85 g kg^{-1} Triton WR-1339 4 days previously, was washed in 0.78 M sucrose, resuspended in about 10 ml 0.78 M sucrose and 3 ml or 5 ml layered onto a continuous sucrose gradient (0.78 M to 1.97 M) to give a total volume of 19.5 ml or 60 ml respectively. These isopycnic gradients were centrifuged at 75,000 *g* for 4 hr in swing-out rotors at 4°. 13 × 1.5 ml or 20 × 3 ml fractions, respectively, were collected from the bottom of the tubes.

Nuclear purification. Nuclei were purified by centrifugation of the cell-debris fraction (CD) in 2.4 M sucrose at 41,000 *g* for 1 hr. The pellet was washed once (NP1) or twice (NP2) with 2.4 M sucrose [14]. The specific activities of DNA, relative to those in the homogenates, were 15.8 ± 5.6 (NP1) and 24.6 ± 4.9 (NP2), whereas the relative specific activities for cytochrome oxidase, arylsulphatase, glucose-6-phosphatase and lactate dehydrogenase were all less than 1.

Lysosomal lysis. Mixed granule fractions in 0.78 M sucrose were lysed either by freezing and thawing five times or by incubation for 1 hr at 37° with 0.1% or 1.0% Triton X-100. The membranes (MF) were separated from the lumenal contents (SF) by centrifugation at 105,000 *g* for 60 min.

Gel chromatography. Occasionally 1 ml samples of plasma, high-speed supernatant (S) and lysed-granule-fraction supernatant (SF) were layered onto columns (1.5 cm × 80 cm) of Sephadex G200 or G75 and eluted with 0.01 M Tris-HCl buffer pH 7.4, containing 0.15 M NaCl and 0.005% NaN_3 and sometimes 0.002 M sodium citrate (for plasma samples only). The absorbance of the elute at 280 nm was continuously monitored using a Uvicord II (LKB Instruments Ltd., Croydon, Surrey, U.K.).

Assays. The fractions obtained above were variously assayed for gold, enzymes, protein and DNA activity.

Gold was measured by counting the gamma emission from ^{198}Au or ^{195}Au using a Wallac G.T.L. 300–1000 scintillation spectrometer.

Protein was measured according to Lowry *et al.* [15], with bovine serum albumin as standard.

Inorganic phosphate was determined using a modification [16] of the method of Fiske and Subbarow [17].

Prior to the assay of the subcellular markers, the samples were frozen and thawed once, except for the lysosomal enzymes which were frozen and thawed five times, and the assay methods are set out below:

Mitochondria. Cytochrome oxidase (EC 1.9.3.1) was estimated using 3.3×10^{-5} M reduced cytochrome *c* (from horse heart) in 0.03 M phosphate buffer pH 7.4. The rate of oxidation was monitored by measuring the change in absorbance at 550 nm on fractions diluted 10–100 times, so that a linear oxidation rate was observed [18].

Table 1. The gold content of plasma and plasma fractions after various doses of sodium aurothiomalate

Aurothiomalate dose (mg/kg)	Time after injection (hr)	Total plasma gold		% Total plasma gold eluting with:		
		(μg atom/l)	(% injected dose ml)	Globulins	Albumin	Free
30–80	1	308	1.41	[illegible]	[illegible]	1.2
		413	1.61	[illegible]	[illegible]	0.2
	24	175	0.80	[illegible]	[illegible]	0
		101	1.13	[illegible]	[illegible]	0.3
		226	0.88	[illegible]	[illegible]	0.1
	168	8.7	0.04	[illegible]	[illegible]	0
		15.0	0.06			
	360	13.0	0.05			
	864	0.4	0.002	[illegible]	[illegible]	0
14–17	1	250	1.29			
	24	146	0.75			
		146	0.64			
	168	11.4	0.05			
0.7	168	1.0	0.11	[illegible]	[illegible]	0

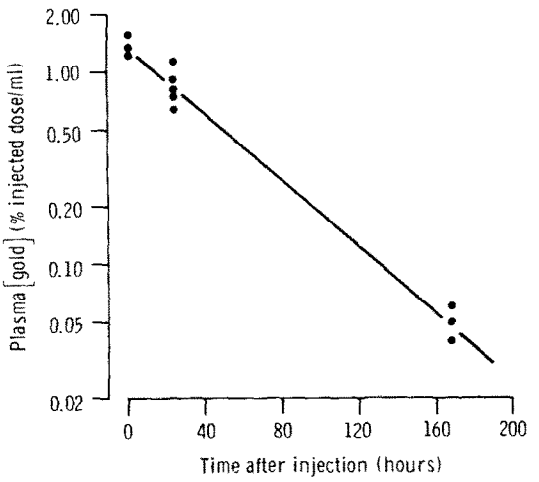

Fig. 1. Plasma clearance of gold after administration of various doses of sodium aurothiomalate.

Lysosomes. Arysulphatase (EC 3.1.6.1) was estimated using 0.02 M *p*-nitrocatechol sulphate as substrate in 0.5 M acetate buffer pH 6.0. The reaction was stopped with 1 M NaOH after an incubation of 1 hr at 37°. The absorbance of the liberated 4-nitrocatechol was measured at 515 nm [19]. β-glucuronidase (EC 3.2.1.31) was estimated using 9×10^{-3} M phenolphthalein β-glucuronide as substrate in 0.1 M acetate buffer pH 5.0. The reaction was stopped with 0.4 M glycine/sodium hydroxide buffer pH 10.0 after an incubation of 1 hr at 37°. The absorbance of the liberated phenolphthalein was measured at 545 nm [20]. Acid phosphatase (EC 3.1.3.2) was estimated using 0.5 M disodium β-glycerophosphate as substrate in 0.1 M acetate buffer pH 5.0. The reaction was stopped with 8% trichloroacetic acid (TCA) after an incubation of 1 hr at 37° [21]. The liberated phosphate was then estimated [17].

Endoplasmic reticulum. Glucose-6-phosphatase (EC 3.1.3.9) was estimated using 4.3×10^{-3} M sodium glucose-6-phosphate as substrate in 0.033 M cacodylate buffer pH 6.5 containing 0.001 M EDTA. The reaction was stopped with 8% TCA after an incubation of 30 min at 37° [22]. The released inorganic phosphate was then estimated [17].

Cytosol. Lactate dehydrogenase (EC 1.1.2.3) was estimated using 10^{-4} M NADH and 7×10^{-4} M sodium pyruvate as substrates in 0.05 M Tris–HCl buffer pH 7.4. The rate of reaction was measured at 340 nm on fractions diluted 100–1000 times [23].

Nuclei. DNA content was estimated using an adaptation [24] of Burton's [25] method.

RESULTS

Plasma. The plasma clearance of gold, after the intraperitoneal administration of various doses of sodium aurothiomalate, is shown in Table 1. Up to 1 week, the plasma clearance corresponded approximately to an exponential with a half time of about 36 hr (Fig. 1).

The distribution of gold in the plasma fractions, as separated by chromatography through Sephadex G200, is shown in Table 1. Over the aurothiomalate dose range 0.7 mg kg^{-1} to 80 mg kg^{-1} and the time interval 1 hr to 36 days, the overwhelming majority of the gold (more than 87.7 per cent) in the plasma co-eluted with the albumin fractions. The gold in the globulin fractions varied from 1.7 to 12.3 per cent and showed a distinct inverse relationship with the total plasma gold concentration. At all time intervals and aurothiomalate doses studied, the concentration of "free" gold was virtually undetectable (less than 1.2%).

Liver. Although a large proportion of the gold was cleared rapidly by renal excretion (10–30 per cent after a dose of 0.7 mg kg^{-1} or 50–70 per cent after 80 mg kg^{-1} in the first 24 hr), some was taken up by the tissues, which still contained about 15 per cent of the injected dose after 36 days (after a dose of 80 mg kg^{-1}).

The liver took up gold to a level of 2.5–4.5 per cent of the injected dose within 24 hr. This remained fairly constant over the subsequent 36 days, even though the whole body retention had dropped to 15 per cent. Therefore the proportion of gold in the body that was located in the liver rose from about 5.4 per cent after 24 hr to 21 per cent after 36 days. Although the initial uptake of gold by the liver varied by a factor of two, no clear relationship was observed between liver uptake and injected dose or liver size.

Intracellular distribution. The intracellular distribution of gold in rat liver was studied 1 hr to 36 days after 0.7 mg kg^{-1} to 80 mg kg^{-1} of sodium aurothiomalate by differential centrifugation, sedimentation-gradient centrifugation, isopycnic-gradient centrifugation with and without prior administration of Triton WR-1339, and Sephadex G200 and G75 chromatography of selected fractions.

Differential centrifugation. The results obtained by differential centrifugation of rat liver homogenates, obtained 1 hr, 24 hr and 7 days after administration of 14 mg kg^{-1} to 17 mg kg^{-1} of sodium aurothiomalate are shown in Fig. 2, together with the profile obtained when sodium aurothiomalate was added to a liver homogenate *in vitro*. The results obtained after a variety of doses between 0.7 mg kg^{-1} and 80 mg kg^{-1} were similar.

One hr after injection, 50.4–65.9 per cent of the cytoplasmic gold (i.e. not including the cell-debris fraction) was located in the high-speed supernatant fraction (S). This had decreased to 7.9–12.3 per cent after 24 hr and remained at about 2–3 per cent between 7 days and 36 days. The decrease of gold in this fraction was paralleled by an increase in gold associated with the granule fractions (Figs 2 and 3). At all time intervals and aurothiomalate doses studied, the highest specific activity of gold (activity/mg protein) was found in the fraction containing the highest specific activity of the lysosomal marker enzyme arylsulphatase, and from 24 hr onwards the highest percentage of gold activity was found in the fraction containing the highest percentage of arylsulphatase.

The cell-debris fraction contained significant but variable amounts of gold (18–54 per cent of the total liver homogenate). As the time interval from aurothiomalate administration or the dose administered increased, the proportion of gold in the liver that sedimented with the cell debris fraction also increased (Fig. 3). Although the cell debris fraction contained

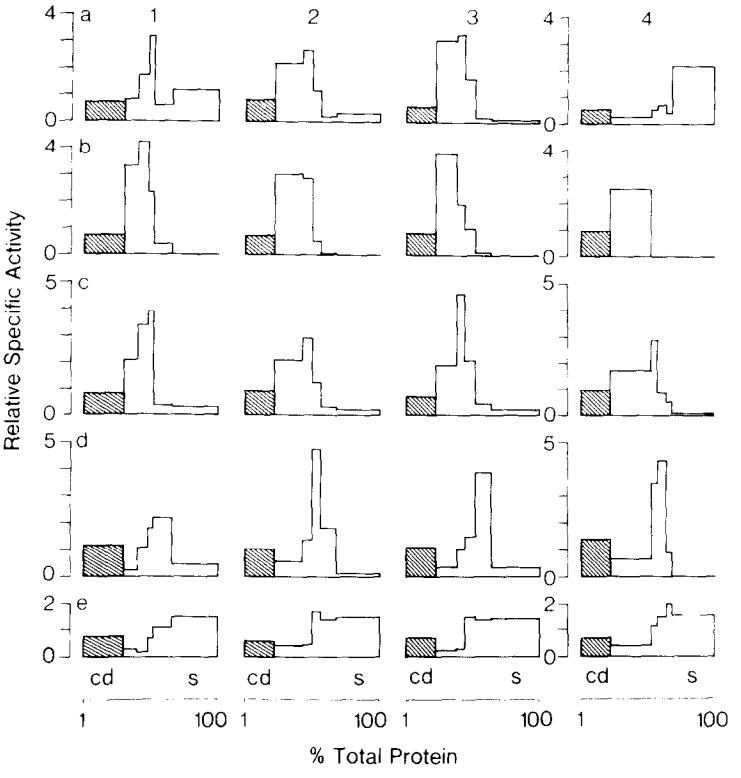

Fig. 2. Differential centrifugation of rat liver homogenates 1 hr (1), 1 day (2) and 7 days (3) after injection of sodium aurothiomalate (14–17 mg/kg). (4) = *in vitro* incubation. a = gold, b = cytochrome oxidase, c = arysulphatase, d = glucose-6-phosphatase, e = lactate dehydrogenase. Fractions CD and S = Cell debris and high-speed supernatant. Fractions in between, from left to right, were obtained by successive centrifugations at 3,000 *g* × 12 min., 8,000 *g* × 20 min., 30,000 *g* × 30 min., and 105,000 *g* × 60 min.

cytoplasmic marker enzymes (Fig. 2), no consistent alteration in cytoplasmic contamination with changes in doses or time intervals were observed.

Addition of aurothiomalate to a liver homogenate prior to differential centrifugation resulted in most of the gold (71.5 per cent) being located in the high-speed supernatant (S) fraction (Fig. 2). When previously incubated with an homogenate for 1 hr at 37°, the gold was more evenly spread across the fractions, but still 50.8 per cent was localised in the high-speed supernatant (S) fraction. With or without prior incubation, the highest specific activity of gold was also in this fraction. This clearly indicates that the *in vivo* distribution of gold was not due to adventitious redistribution during homogenisation.

High-speed supernatant fraction (S). The distribution of gold within the high-speed supernatant fraction (S) was further investigated by gel-permeation chromatography through Sephadex G200 and G75. Figures 4 and 5 show the elution profiles obtained from Sephadex G75 and G200 respectively 7 days after 80 mg aurothiomalate kg^{-1}.

The data obtained from G200 and G75 chromatography show that, when aurothiomalate was administered *in vivo*, the gold in the liver high-speed supernatant (S) could be divided into at least four compartments: a high molecular weight compartment that eluted in the void volume of Sephadex G200, one that co-eluted with ligandin (Y-protein) (as shown by bromosuphthalein binding) [26], one that eluted after Z-protein on Sephadex G75 and a low molecular weight fraction that eluted in the total column volume of Sephadex G75. When aurothiomalate was incubated with rat liver high-speed supernatant (S) *in vitro*, a similar profile was obtained except that the post-Z-protein peak was absent and the missing gold was redistributed evenly among the other three compartments (Fig. 4).

Granule fractions. The separation between the subcellular organelles was improved by subjecting cytoplasmic fractions and mixed-granule fractions to sucrose sedimentation-gradient and isopycnic-gradient centrifugation, respectively.

The overall pattern provided by sedimentation-gradient centrifugation was very similar to that provided by differential centrifugation. Apart from 1 hr after injection, when the gold specific activity and activity profiles most closely resembled those of lactate dehydrogenase, the gold profiles most closely paralleled those of arysulphatase. However with increasing time intervals after the administration of high doses of aurothiomalate (30 mg kg^{-1}), there was a progressive increase in gold associated with the bottom of the gradient, which was not paralleled by any change in enzyme profiles (Table 2).

Isopycnic-gradient centrifugation of mixed-granule fractions with or without prior injection of Triton WR-1339 confirmed the association between gold and the lysosomal marker enzyme arylsulphatase. Detergent-induced flotation of gold occurred alongside flo-

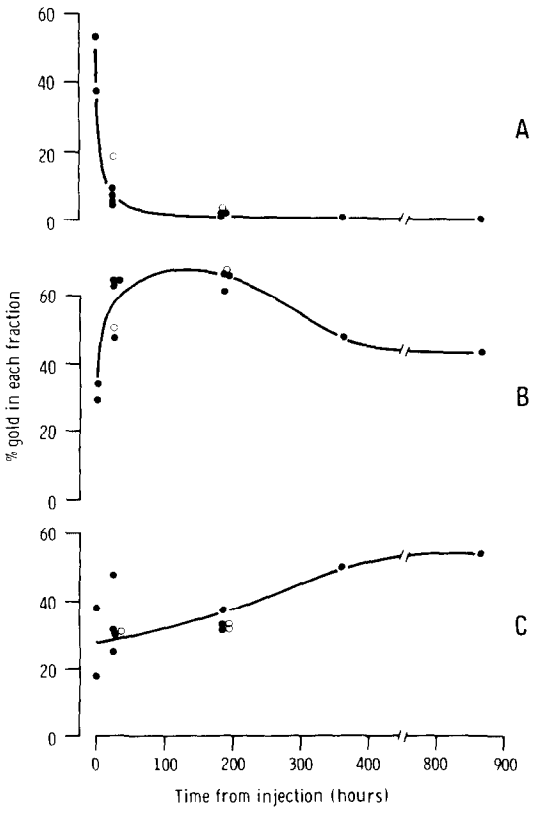

Fig. 3. Distribution of gold in the differential fractions of rat liver at various times after 14–80 mg/kg (●) or 0.7–2.2 mg/kg (○) aurothiomalate. A = High-speed supernatant, B = Mixed-granule fraction, C = Cell debris.

tation of arylsulphatase, the cytochrome oxidase and protein profiles remaining unaltered. However with higher doses of aurothiomalate and at longer time intervals after injection, significantly less gold in the granule fractions could be floated by the detergent than arylsulphatase. For example, 24 hr or less after 30 mg aurothiomalate kg^{-1} or 7 days or less after 0.7 mg kg^{-1}, the increase in the proportion of gold on the isopycnic gradients equilibrating to a specific gravity less than 1.195 after Triton WR-1339 administration was 48.1 ± 4.7 per cent (S.D., $n = 4$) compared to 54.7 ± 13.0 per cent for arylsulphatase. whereas 15 days after 30 mg kg^{-1} only 18.0 per cent of the gold was floated compared with 57.5 per cent for the arylsulphatase.

The distribution of gold within the lysosomes was further investigated by lysing mixed-granule fractions, by freezing and thawing or various concentrations of Triton X-100, and measuring the distribution of gold between the sedimentable membranes and the supernatant. The results (Table 3) showed that the majority of the lysosomal gold was bound to the membrane after freezing and thawing, and increased with increasing time intervals from about 70 per cent after 24 hr to 96 per cent after 36 days (30 mg kg^{-1}). There was also a tendency for the proportion of gold membrane-bound to increase as the aurothiomalate dose increased. Increasing concentration of Triton X-100, as the lytic agent, made little difference to the intralysosomal distribution of gold, when high aurothiomalate doses were administered, but considerably decreased the membrane bound gold when low doses were injected. Prior injection 4 days previously of Triton WR-1339 made little difference to the distribution of gold or the lysosomal enzymes.

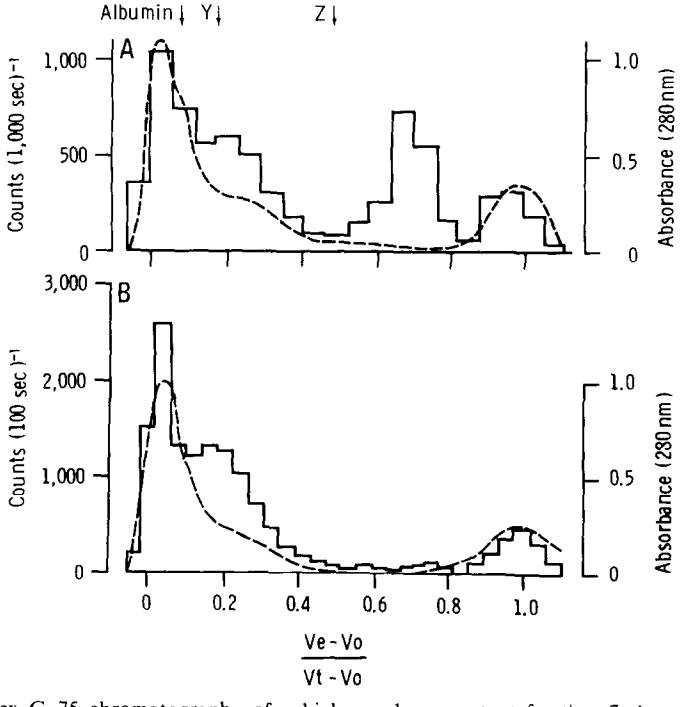

Fig. 4. Sephadex G 75 chromatography of a high-speed-supernatant fraction 7 days after injection of 80 mg/kg aurothiomalate (A) or 1 h after incubation *in vitro* with 1.25×10^{-5} M aurothiomalate (B) —— = ^{195}Au, - - - = A_{280nm}.

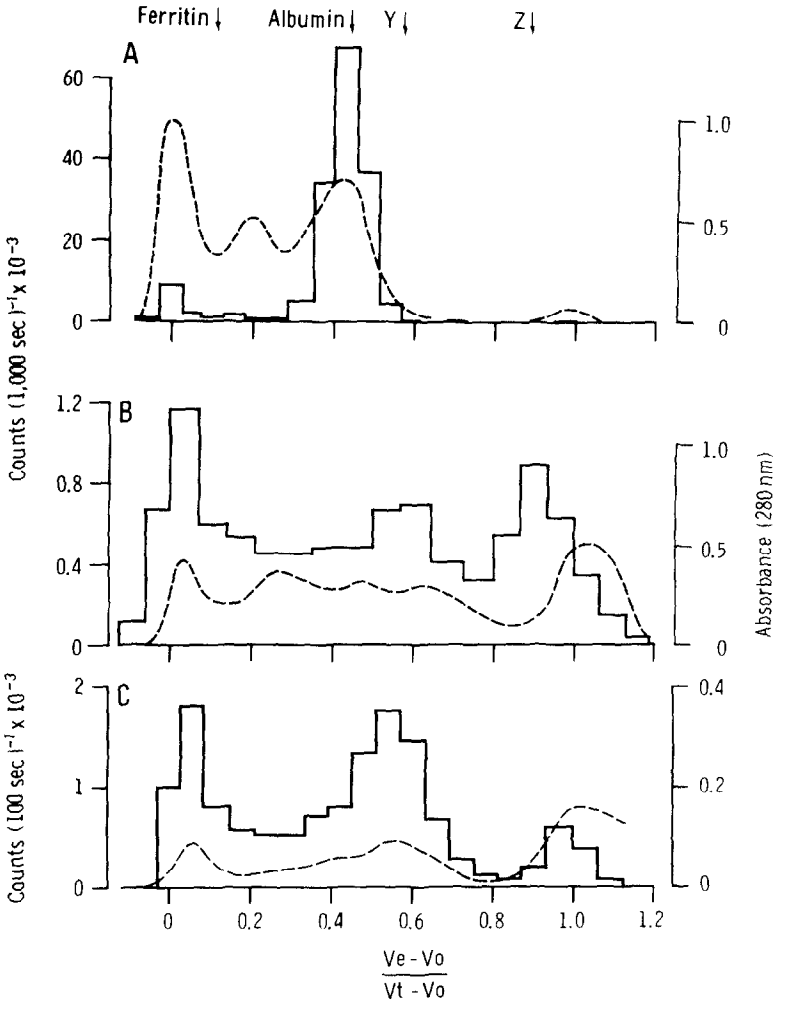

Fig. 5. Sephadex G 200 chromatography of plasma (A) or high-speed liver supernatant (B) 7 days after injection of 80 mg/kg aurothiomalate, or high-speed supernatant after 1 hr incubation *in vitro* with 1.25×10^{-5} M aurothiomalate (C). — = ^{195}Au, - - - = $A_{280\,nm}$.

When a frozen-and-thawed granule fraction from an uninjected rat was incubated with 4.2×10^{-6} M aurothiomalate at 37° for 1 hr *in vitro*, only 42 per cent of the gold was sedimentable, compared with 70–96 per cent in the equivalent *in vivo* experiments (30 mg kg^{-1}). Therefore adventitious binding of gold to the membranes during homogenisation or the fractionation procedure was unlikely to be significant.

The non-membrane bound lysosomal gold (i.e. that released into the supernatant by lysis of the granule fractions) was fractionated by Sephadex G200 chromatography. The elution profiles showed that, after freezing and thawing, the gold was spread over a wide range of eluate fractions, but with very little eluting at the total column volume (i.e. mol. wt less than 5,000). The increase in non-membrane-bound gold, when the lysosomes were lysed with 1% Triton X-100 after administration of 0.7 mg aurothiomalate kg^{-1}, could be entirely accounted for by the appearance of a large peak of gold eluting at the void volume on Sephadex G200. This was accompanied by a large increase in protein ($A_{280\,nm}$) in this fraction.

Cell debris fraction. Attempts to elucidate the subcellular localisation of the gold in the cell-debris fraction were made by purifying the nuclei from this fraction. On assumption that all the gold in the purified nuclear fraction was in fact associated with the nuclei, a maximum level of gold in the nuclei of the total liver could be calculated (Table 4). This value ranged from 0.8 per cent (7 days after 0.7 mg aurothiomalate kg^{-1}) to 32 per cent (36 days after 80 mg kg^{-1}) of the total homogenate gold. The increase of gold in the purified nuclear fraction with increasing aurothiomalate dose and increasing time after injection was not accompanied by any consistent increase in cytoplasmic marker enzyme contamination.

DISCUSSION

The fact that most of the gold in rat plasma was bound to albumin (Table 1), after the intraperitoneal administration of sodium aurothiomalate, agrees with the results obtained when rat serum was incubated with sodium aurothiomalate *in vitro* [27], as well as rabbit serum *in vivo* [28] and human serum *in vitro* [27, 29] and *in vivo* (C. J. Danpure, in preparation). The extremely low levels of "free" gold are compatible with the very low dissociation constant

Table 2. Distribution of gold and marker enzymes (as per cent of total gradient activity) on sucrose sedimentation gradients at various times after injection of 30 mg kg^{-1} aurothiomalate*

Parameter	Gradient fractions	Time after injection			
		1 hr	24 hr	7 days	15 days
Gold	1–4	4.8	21.8	38.6	53.4
	5–12	25.8	54.9	46.3	27.9
	13–15	69.4	23.3	15.1	18.7
Cytochrome oxidase	1–4	58.9	57.3	67.0	61.4
	5–12	33.2	32.7	30.6	32.8
	13–15	7.9	10.0	2.4	5.8
Arylsulphatase	1–4	22.4	25.5	22.6	18.6
	5–12	69.4	59.0	66.9	70.6
	13–15	8.2	15.5	10.5	10.8
Lactate dehydrogenase	1–4	10.3	9.7	14.2	11.6
	5–12	24.8	9.6	18.4	21.4
	13–15	64.9	80.7	67.4	67.0
Protein	1–4	21.1	21.1	22.4	21.5
	5–12	30.6	21.1	27.6	26.8
	13–15	48.3	57.8	50.0	51.7

* Fraction 1 = botton of gradient.

for the reaction between serum albumin (human, at least) and aurothiomalate [30].

The strongly negative correlation between the proportion of plasma gold bound to the globulins (except for the extremely long time intervals of 36 days) and total plasma gold concentration or time after injection (Table 1) is unique to the *in vivo* situation, as experiments *in vitro* (C. J. Danpure, unpublished results) showed that there was no such correlation with incubation time or aurothiomalate concentration (over the ranges found in the present experiment). It is interesting to note that a similar correlation has been found in the plasma of patients undergoing aurothiomalate therapy for rheumatoid arthritis (C. J. Danpure, in preparation). On the basis of gold-isotopic exchange, the interaction between aurothiomalate and albumin has been found to be reversible (C. J. Danpure, unpublished results). Therefore, on the assumption that the reaction with the globulins is either irreversible or less reversible, the present results could be explained by the selective removal of gold from the albumin as a result of tissue uptake or excretion.

The rate of clearance of gold from the plasma, found in the present experiment is difficult to compare with the results of other workers. In rats preloaded with aurothiomalate by intramuscular administration, Block *et al.* [31] found an initial rate of removal of gold from the plasma with a half-time of about 7 days, a result that agrees with data obtained from humans [32, 33]. Harth [34] found that the serum half-time of gold in humans was 43 hr over the first 1–2 days with half-time of 6 days subsequently. When rabbits were injected intramuscularly with aurothiomalate, with or without preloading, the plasma half-time was about 40 hr [28]. In the present experiments, the plasma half-time of gold was similar to the latter, although the aurothiomalate was administered intraperitoneally. The effect of the route of administration is uncertain, but Freyberg *et al.* [35] found that, at least for some gold drugs, there was no difference in plasma gold kinetics whether injected intraperitoneally or intramuscularly.

The amount of gold taken up by the liver is similar to the findings of other workers with rats [4, 31] and humans [3], (in the latter case using aurothioglucose). Although it has been implied that liver uptake of gold is a consequence of the activity of the reticuloendothlial system in this organ [13], no unequivocal evidence to that effect is available. Penneys *et al.* [13] found that, whereas the concentration of gold per unit of protein in the Kupffer cells was twice that in the hepatocytes the majority of liver gold was in the hepatocytes. Gold has been detected histologically in hepatocytes as well as Kupffer cells [8] after aurothiomalate administration. In addition, a significant proportion of gold administered is chronically excreted in the faeces [4, 34–36] and if this is indicative of biliary excretion, the role of the hepatocytes in the liver uptake of gold may be important.

Investigations into the intracellular distribution of gold in rat liver cells at various time intervals after a variety of doses of sodium aurothiomalate by a number of different techniques, show unequivocally that gold was concentrated in lysosomes, as demonstrated by the marker enzyme arysulphatase. The results obtained from the differential fractionation, sedimentation-gradient and isoycnic-gradient centrifugation together indicate that, under some conditions, for example 7 days after 14 mg kg^{-1} to 17 mg kg^{-1} aurothiomalate, probably more than 90 per cent of the cytoplasmic gold (more than 70 per cent of the homogenate gold) was associated with lysosomes.

The intracellular distribution of gold became considerably less clear when the time interval and/or the injection dose of aurothiomalate was increased, due to the increased amounts of gold found in the cell debris and partially-purified nuclear fractions, the increased proportion of gold sedimenting to the denser fractions in the sucrose sedimentation gradients, and the decreased ability of prior injection of Triton WR-1339 to decrease the density of gold-containing organelles. These changing characteristics in the distribution of gold were not consistently paralleled by arylsulphatase.

Table 3. Distribution of gold, marker enzymes and protein in mixed granule fractions after varying methods of lysis

Dose (mg kg^{-1})	Time after injection (hr)	±Triton WR1339	Method of lysis: Frozen and thawed					Method of lysis: 0.1% Triton X100					Method of lysis: 1.0% Triton X100				
			Au	Prot	AS	AP	β G	Au	Prot	AS	AP	β G	Au	Prot	AS	AP	β G
30 80	24	−	77	56													
		−	66	85	54	46	24	72	80	47	25	64					
	168	−	85	60									70	37	24	3	9
	360	−	94	79	37			84	42	14							
		+	92	74	23			90	69	17							
	864	−	96	82	63	55	53	94	65	33	32		96	55	35		
0·7	168	−	61	71	70			28	44	23			19	32	12		
		+	51	76	63			34	62	26			15	27	8		

The values represent the per cent sedimented after centrifuging at 105,000 *g* for 60 min. Au = gold, Prot = protein, AS = arysulphatase, AP = acid β-glycerophosphatase, βG = β-glucuronidase.

Table 4. The estimated maximum per cent of liver gold in the nuclei

Aurothiomalate (mg kg^{-1})	Time after injection (days)	RSA		Max % Au in N
		DNA	Au	
30–80	1	23.0	0.9	4.05
	7	16.7	3.3	19.6
	15	7.7	1.7	21.4
	36	30.0	9.6	31.6
0.7	7	20.6	0.16	0.82

N = nuclei, RSA = relative specific activity compared to homogenate.

These results could possibly be explained by the actual redistribution of gold into other subcellular compartments, such as the nuclei. However, as both the sedimentation and isopycnic gradients were essentially free of nuclear contamination, it is more probable that the gold becomes concentrated into telolysosomes, a process that is more noticeable after long time intervals or high doses of aurothiomalate. In the present study, a large proportion of gold was found bound to the lysosomal membrane. It is possible that gold-induced changes in the characteristics of the lysosomal membrane could result in changes of membrane permeability leading to increased lysosome size and/or density and decreased ability to equilibrate with lysosomes containing Triton WR-1339. Many lysosomal enzymes are known to be inhibited by aurothiomalate [37–39], which may explain why the proposed sequestration of gold into telolysosomes was not accompanied by arylsulphatase, especially when it is considered that these telolysosomes are likely to, either contain a higher concentration of gold, or have contained the gold for a longer period of time, than the lysosomes still demonstrating their normal sedimentation and equilibrium characteristics. Support for this concept comes from the work of Yarom *et al.* [8], who showed, histologically, gold-like inclusions in what appeared to by lysosomes but did not stain for lysosomal enzymes.

Over the concentration range used in the present experiments, the majority of gold associated with the lysosomes was probably membrane bound. The dependency of the proportion of gold bound to the membrane on the quantity of aurothiomalate injected and the time after injection could be due to the gold exposing additional binding sites on the membrane, or the exhaustion of interaction sites in the lysosomal lumen. The specific activity of the [^{195}Au] aurothiomalate administered was such as to preclude investigations into the distribution of gold after injection of smaller amounts of drug. However extrapolation of the data obtained after higher doses indicates that the majority of the lysosomal gold would be bound to molecules in the lysosomal lumen.

On the basis of G200 chromatography, the gold complexes in the lysosomal lumen covered a wide range of molecular weights, except that there was virtually no low molecular weight (less than 5000) gold species. This is not surprising if gold is assumed to react with other protein thiols as avidly as it does with that of albumin [30].

The increased solubilisation of gold, when lysis was effected by Triton X-100, was probably a result of solubilisation of high-molecular weight (more than 300,000) membrane protein–gold complexes.

It has been suggested previously [37, 38] that at least part of the anti-inflammatory action of aurothiomalate may be due to the inhibition of lysosomal enzymes. These are known to be partly sequestered on to the lysosomal membrane and partly free in the lysosomal lumen [40]. The distribution of gold in the G200 eluate, found in the present study, compared with the equivalent distributions of some lysosomal hydrolases [41], indicates that gold in the lysosomal lumen, at least, could be bound to lysosomal enzymes.

The significance of the high proportion (50.4–64.9 per cent) of the cytoplasmic gold in the high-speed supernatant fraction (S), 1 hr after injection, is difficult to assess. The absolute amount of gold this represents is very low (0.1–0.4 per cent of the injected dose). Although by 24 hr the proportion of the cytoplasmic gold located in the S fraction had decreased considerably (7.9–12.3 per cent), no consistent changes in the absolute amount of gold in the S fraction (0.2–0.3 per cent of the injected dose) were observed.

This gold is unlikely to represent plasma contamination as the livers were perfused prior to removal. In addition gold in the plasma was almost entirely bound to albumin (Table 1 and Fig. 5) whereas that in the S fraction, at least after 7 days, was bound to proteins other than albumin (Fig. 5). It is also unlikely to be due to adventitious redistribution as a result of lysosomal rupture during homogenisation, because, firstly, only low levels of arylsulphatase were found in the S fraction, and secondly, the distribution of gold among the proteins of the S fraction was quite different to that in the lysosomal lumen. Gold associated with micropinocytic vesicles is also unlikely to be responsible for the gold in the S fraction, as in these the gold would be expected to be bound either to membrane proteins or plasma proteins, neither of which fits the gold distribution actually found.

Although only a small proportion of the total liver gold is found in the S fraction, it may play an important role in the ultimate fate and toxicity of the drug. The proteins to which gold is bound to in this fraction have not been definitively characterised, but it is interesting to note that a significant proportion eluted with the total column volume of Sephadex G200 (mol. wt less than 5000), whereas the gold in both the plasma and the lysosomal lumen appeared to be bound to proteins of much higher molecular weight.

The low molecular weight (less than 5000) gold in the S fraction may be due to the formation of gold complexes with low molecular weight thiols, such as

glutathione and cysteine, which are known to have a relatively high concentration in the cytosol of cells. These mercaptide metabolites may be very complex in nature [42] and may be the forms in which gold is released back into the blood from tissues and organs such as the liver [43].

The possibility that gold in the S fraction is bound to ligandin (Y protein) is interesting with respect to the known role of this protein in the uptake and biliary excretion of a wide variety of drugs and physiological products of catabolism [44].

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