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GOLD(I) EFFLUX FROM AURANOFIN-TREATED RED BLOOD CELLS

EVIDENCE FOR A GLUTATHIONE–GOLD–ALBUMIN METABOLITE

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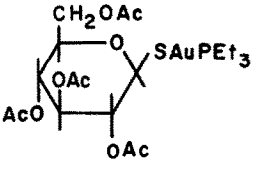
Abstract—The efflux of gold from red blood cells (RBCs) exposed to 10–100 μM auranofin [the second generation chrysotherapy agent, triethylphosphine-(2,3,4,6-tetra-*O*-acetyl-1-β-D-glucopyranosato-*S*-)gold(I)] was studied. RBCs in whole blood were allowed to accumulate gold, and then were placed in fresh plasma or buffered saline solution. In plasma, the kinetics of efflux were first order in gold with an apparent rate constant of $0.81 \pm 0.18\ hr^{-1}$. Serum albumin, in plasma or added to a buffered solution, shifted the equilibrium between intra- and extracellular gold in favor of the latter (compared to saline solution). [^{14}C]Glutathione, generated by *in situ* labeling, also effluxed and associated with the albumin and gold, providing the first direct evidence that the albumin–gold–glutathione complex (AlbSAuSG) may be a circulating metabolite of auranofin formed after both of the original ligands of auranofin are displaced.

The recently-licensed, oral chrysotherapy agent, auranofin (AF¶ or $Et_3PAuSATg$), is an important addition to the rheumatologists' arsenal of treatments for rheumatoid arthritis (RA). AF is a monomeric complex containing a gold(I) ion coordinated to a triethylphosphine ligand (Et_3P) and a thiolate ligand derived from tetraacetylthioglucose (AtgSH) [1, 2] and is represented as $Et_3PAuSAtg$ in this manuscript. The presence of the phosphine causes significant differences in the pharmacological profiles of auranofin and the long-used parenteral drugs [3, 4].



$Et_3PAuSAtg$
AURANOFIN

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¶ Abbreviations: AF, auranofin; AlbSH, mercaptalbumin (reduced Cys-34 group); AuSTm, gold sodium thiomalate (myochrysine); AuSAtg, gold tetraacetylthioglucose; BSA, bovine serum albumin; Et_3P, triethylphosphine; EXAFS, extended X-ray absorption fine structure; FAAS, flame atomic absorption spectroscopy; GSH, glutathione; HSA, human serum albumin; MOPS, morpholinopropane sulfonate; RA, rheumatoid arthritis; and RBC, red blood cell.

The tetraacetylthioglucose ligand of auranofin [5, 6], as well as the thiomalate of myochrysin [7] and the thioglucose of solganol [8] are displaced rapidly *in vivo* [5, 6]. Numerous *in vitro* studies demonstrate that auranofin reacts with serum albumin (AlbSH), initially losing the tetraacetylthioglucose ligand in a reaction at Cys-34 of albumin [2, 9–13]:

$$AlbSH + Et_3PAuSAtg \longrightarrow AlbSAuPEt_3 + AtgSH.$$

Albumin has a high affinity for gold(I) species including the Et_3PAu^+ fragment derived from auranofin [2, 14, 15]. Et_3PO, a metabolite of auranofin identified in human and animal studies [5, 6], can be generated *in vitro* after further incubation in the presence of thiols (RSH), such as tetraacetylthioglucose, thioglucose or glutathione [15]. Disulfide bonds of albumin are the principal oxidants *in vitro*, and the oxygen is provided by water in that case [16]:

$$\begin{matrix} S \\ | \\ S \end{matrix}\!\!>\!AlbSAuPEt_3 + RSH \xrightarrow{H_2O} (HS)_2AlbSAuSR + Et_3PO.$$

Auranofin metabolites accumulate in the red blood cells (RBCs) of laboratory animals and patients [3–6], whereas gold administered as parenteral drugs enters RBCs only under the influence of cyanide from cigarette smoke [17]. Following a single dose of auranofin, the gold levels in RBCs maximize after 3 days, then decrease, so that for the 3 days after administration, RBC gold content is greater than the serum gold, but subsequently the situation reverses [6]. At present, the literature contains a paucity of information on the biochemical processes that are

responsible for the uptake and efflux of gold from RBCs. Important, cellular studies using RAW 264.7 macrophages examined the uptake and efflux of auranofin metabolites, leading to development of the sulfhydryl shuttle model for transport of auranofin metabolites across the cell membrane [18, 19]. According to this model, protein sulfhydryl groups act as the carrier or shuttle for mediated, but passive, transport of Et_3PAu^+ [18, 19]. While nuclear magnetic resonance (NMR) studies of intact red cells have identified the binding of Et_3PAu^+ to hemoglobin and intracellular glutathione, the concentrations of gold that were employed (1–8 mM) greatly exceeded the concentrations achieved in chrysotherapy [12, 13]. Hemoglobin and albumin, the principal thiol-containing proteins of blood, are compartmentalized in RBCs and serum respectively. Recently, it has been shown that the transfer of Et_3PAu^+ from hemoglobin to serum albumin is thermodynamically favorable [14]. Thus, the accumulation of gold by RBCs is not driven by the binding of Et_3PAu^+ to hemoglobin [14].

It has been suggested that the ultimate metabolites of AF and the parenteral drugs are similar and that their formation is driven by the thermodynamics of rapid ligand exchange reactions, the metabolic alteration of the ligands used to administer the gold, and the large pool of available protein and nonprotein thiols which greatly exceeds the gold concentrations during therapy [20]. Glutathione, the predominant low-molecular-weight thiol in many cells and extracellular fluids [21], is a likely candidate as an *in vivo* ligand to gold(I).

The concentrations of gold that accumulate in and then efflux from RBCs during therapy are too low to allow facile monitoring of the relevant ligands by NMR methods. Therefore, we have used the proven combination of classical biochemical separation methods with flame atomic absorption spectroscopy (FAAS) and radiolabeling to examine (1) the kinetics of gold efflux from RBCs, (2) the role of albumin as an extracellular binding site, and (3) a possible role of glutathione (GSH) as a ligand of gold. Using the γ-glutamyl cycle [21], glutathione can be labeled with [^{2}H]glycine [22] or [^{14}C]glycine [23, 24]. During this cycle, which transports amino acids into RBCs [20], the labeled glycine is added to γ-glutamylcysteine to resynthesize intracellular glutathione [21]. Thus, ^{14}C-labeled glycine introduced into a RBC suspension will be incorporated into the glutathione pool, allowing one to follow reactions of the *in situ* labeled [^{14}C]glutathione.

MATERIALS AND METHODS

Reagents. Sephadexes G-50, G-100 and DEAE-A-50, and 5,5′-dithiobis(2-nitrobenzoic acid) were obtained from the Sigma Chemical Co. Fresh blood from a healthy donor was collected in Vacutainers with sodium heparin (Bectron Dickinson). Auranofin was prepared at Smith Kline & French Laboratories by published procedures [25]. Morpholinopropane sulfonic acid (MOPS) was obtained from the U.S. Biochemical Corp.; EDTA and glucose were obtained from the Aldrich Chemical Co. ^{14}C-Labeled glycine ($H_2NCH_2^*COOH$), Lot No. 2315-079, sp. act. 53.0 μCi/mmol, was obtained from New England Nuclear, Du Pont Chemical Co.

Gold, albumin and ^{14}C-analyses. Gold concentrations were measured by FAAS on an Instrumentation Laboratory model 357, using Spex $Au(CN)_2^-$ standards. The relative detection limit was 0.14 μM, and the sensitivity was 0.84 μM. Measurements were reproducible to ±10% at lower concentration ranges and ±5% or less at higher concentrations. Albumin concentrations were measured by UV absorption (ε_{278} = 39,600 L·mol^{-1}·cm^{-1}. ^{14}C-Activity was measured on a Beckman Scintillation Counter. Aliquots of the chromatographic fractions (0.40 mL) were suspended in 3.0 mL Optifluor and 1.0 mL distilled water and counted for 5 min each.

Effect of albumin on efflux. Four aliquots (20 mL) of outdated, concentrated RBCs were placed in centrifuge tubes and resuspended in 20 mM MOPS/150 mM KCl/5 mM EDTA buffer, pH 7.4, to approximately their original concentration in whole blood (30 mL). After exposure to 10 μM AF (added as 1.2 mM EtOH solution) for 10 min at 25 ± 0.5°, they were pelleted by centrifugation, washed once with buffer, then resuspended to 30 mL in buffer either with or without 0.625 mM bovine serum albumin and maintained at 25 ± 0.5°. At 0.25, 0.50, 1.0, 2.0, and 4.0 hr, the samples were centrifuged, and 1.5 mL of supernatant fraction was withdrawn for analysis of the gold content by FAAS. After 4 hr, the remaining albumin supernatant fractions were concentrated 10-fold using an Amicon concentrator; then a 0.5-mL aliquot was separated over Sephadex G-100 to assess the extent of albumin-bound gold.

Efflux into plasma ("reconstituted blood"). Aliquots of outdated or fresh human blood were exposed to auranofin at 10, 50, or 100 μM concentrations, and then the RBCs were isolated as described above and resuspended in untreated plasma obtained from the same blood source and maintained at 25 ± 0.5° in a circulating water bath. Small aliquots were removed periodically, and the gold concentration in the plasma was analyzed by FAAS (vs a sodium blank) to establish the efflux kinetics. The plasma remaining at the end of the 10-hr experiment was analyzed chromatographically (Sephadex G-100 or DEAE-Sephadex A-50) to determine the nature of product(s) formed by the effluxed gold.

Chromatography. The plasma obtained after 4 hr from a 50 μM auranofin incubation was fractionated over Sephadex G-100 eluted with 100 mM NH_4HCO_3, pH 7.9. The fractions (9.1 mL) were analyzed for gold and protein. The protein from a similar incubation was chromatographed over DEAE-Sephadex A-50 eluted with a 0–500 mM NH_4HCO_3 salt gradient containing 20 mM MOPS, pH 7.4, buffer. The fractions (10 mL) were analyzed for gold, protein and conductivity.

In situ [^{14}C]GSH labeled RBC efflux studies. Fresh blood (30.0 mL) was centrifuged and the plasma was saved for later use. According to the method of Isab and Rabenstein [22], ^{14}C-labeled glycine (0.30 mL; 100 μCi/mL) was added to 15 mL of buffer (20 mM MOPS, 3 mM EDTA, 150 mM KCl and 5 mM

glucose), then mixed with the RBCs, and gently stirred for approximately 24 hr at 25 ± 0.5°. The RBCs were centrifuged again, and the supernatant fraction (containing unreacted [^{14}C]glycine) was discarded. The RBCs were washed twice with buffer solution, then exposed to auranofin (75 μL of 10 mM MeOH solution) and sufficient buffer to yield 30 mL total volume, and gently agitated for 10 min. The RBCs were washed again with buffer solution to remove extracellular AF. The blood was then "reconstituted" by adding the original plasma to the packed RBCs. The reconstituted blood, about 30 mL total volume, was stirred gently and then maintained at 25 ± 0.5°. Aliquots (4.5 mL) were withdrawn after 0.15, 0.30, 1, 2, 4 and 10 hr. The RBCs were separated by centrifugation, and 0.80 mL of plasma was chromatographed on a Sephadex G-50 column. The eluent was 100 mM NH_4HCO_3 buffer, pH 7.9. Fractions (2.1 mL) were collected, and 0.40 mL of each was used for scintillation counting and the remainder for gold and albumin analyses. Control experiments, omitting the auranofin treatment but otherwise identical, were carried out. In addition, it was demonstrated that unincorporated [^{14}C]glycine did not bind to the albumin fractions under these conditions.

RESULTS

Albumin and the efflux equilibrium. The ability of albumin in the cell culture medium to reduce the uptake of gold into RAW 264.7 macrophage cells [19] suggested to us that the distribution of gold metabolites of auranofin might be an equilibrium between intra- and extracellular binding sites. Therefore, we examined the effect of albumin on the extent of gold effux from RBCs exposed to micromolar concentrations of auranofin. After the red blood cells were incubated in 10 μM auranofin for 10 min, they were resuspended in MOPS/KCl/EDTA buffer, pH 7.4, with and without 0.63 mM albumin. The extent of efflux into the albumin solution (1.8 ± 0.2 μM at 0.25 hr and 6.5 ± 0.3 μM after 4 hr with no subsequent increase) was greater than that into buffer (0.6 ± 0.1 μM at 0.25 hr and 0.8 ± 0.1 μM at 2 hr with no subsequent increase to 10 hr). The difference, almost an order of magnitude, clearly demonstrates the ability of extracellular albumin to alter the distribution of gold between RBCs and plasma.

To verify that the enhanced efflux was due to an interaction of the gold with the albumin, the albumin solutions remaining at the end of the experiment were isolated, combined, concentrated by reverse osmosis, and then analyzed by gel-exclusion chromatography. The Sephadex G-100 chromatogram of the albumin supernatant fraction showed that the gold was indeed albumin-bound. This result confirms the role of albumin, which contains a high-affinity binding site that shifts the equilibrium between intra- and extracellular gold. (If the efflux were caused by formation of an EDTA–gold complex, the concentrations of gold would be equal in the buffer and albumin solutions, and the gold would be absent from the albumin peak, contrary to our observations here and in Fig. 2, below).

Efflux kinetics. Next, similar experiments were carried out using as the efflux medium additional plasma separated from the same blood sample. That is, RBCs were incubated with auranofin (10–100 μM) in whole blood, then isolated, and resuspended in fresh plasma prepared from the same blood source. This approach was used to study the kinetics of efflux and, as described in the next section, to characterize the resulting extracellular gold species.

As the gold effluxed from the RBCs, aliquots of this "reconstituted blood" were centrifuged periodically to recover the plasma for analysis of the gold present. Figure 1A shows typical plots for the efflux of gold from RBCs pretreated with auranofin (10, 50 or 100 μM). The extracellular gold levels reached a plateau after approximately 4–5 hr, but for the kinetic studies were generally monitored for 10 hr to assure an accurate $[AU]_\infty$ value (Table 1). The final concentrations of the gold in the plasma, $[Au]_\infty$, increased in parallel with the incubation concentrations to which the cells were exposed, $[Au]_i$. This result is consistent with the proposed equilibration of gold between intra- and extracellular binding sites.

Plotting the data according to a standard first-order treatment, $\ln([Au]_\infty - [Au]_t)$ vs time, yielded linear plots (Fig. 1B). The slopes represent apparent rate constants (k_{app}) for the efflux process. The numerical values fall between 0.64 and 1.1 hr^{-1} (Table 1). The differences in the average values obtained for each initial incubation concentration were not statistically significant (within 2 standard deviations). The age of the outdated blood (7–54 days) was examined as a variable, but did not correlate with the changes in k_{app}. The concentrations of albumin, RBCs, glutathione, or other species which might affect the rates of gold efflux may differ among the samples, causing the small variations observed. Since the range of values overlap and cover less than a factor of two, it is most reasonable to average the nine data points, which yielded a value of 0.81 ± 0.18 hr^{-1} for the apparent rate constant. The significance and limitations of this rate constant are addressed in the discussion section (*vide infra*).

Gold binding sites in plasma. To characterize the extracellular species that form as gold effluxes into and equilibrates with the plasma, samples of the plasma were isolated and chromatographically analyzed over gel-exclusion and ion exchange resins. The results of a typical Sephadex G-100 chromatogram are shown in Fig. 2. Gold eluted in the same fractions where authentic (bovine) serum albumin used to calibrate the column eluted. In every case examined, the gold occurred primarily in the albumin fractions and did not appear to any appreciable extent in other high-molecular-weight fractions. Other research allows us to rule out binding to hemopexin, histidine-rich glycoprotein or transferrin, which are other proteins with potential binding sites and similar molecular weights [10, 26]. On some occasions, the FAAS analysis suggested that small amounts of gold might be present in the low-molecular-weight fractions. These peaks were probably artifacts caused by sodium ion interference with the FAAS measurements of the gold concentrations.

Ion-exchange chromatography (DEAE-Sephadex A-50, eluted with a NH_4HCO_3 gradient) was also

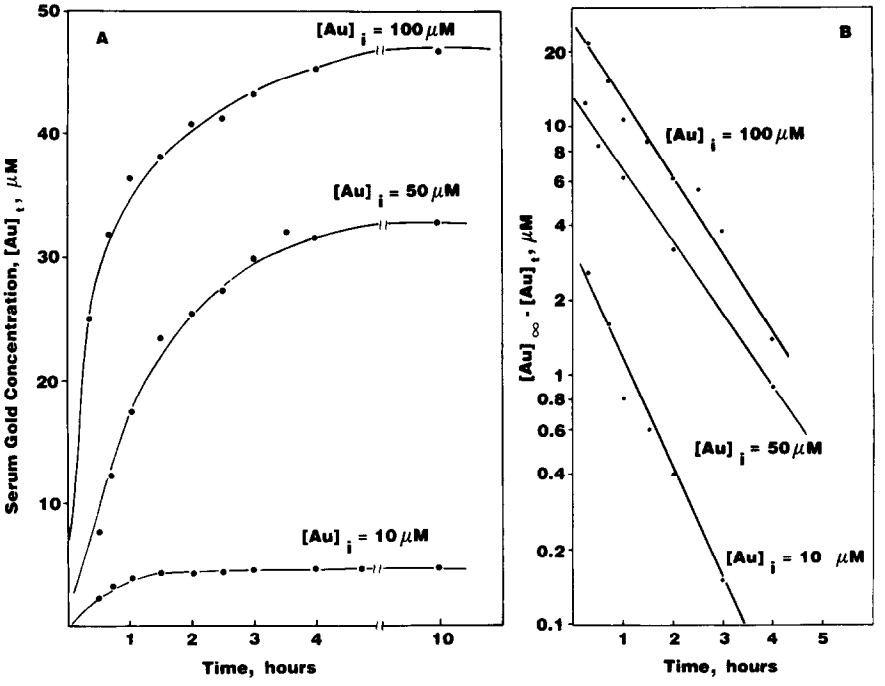

Fig. 1. Efflux of gold from auranofin-treated RBCs resuspended in plasma. (A) Gold concentration in plasma as a function of time. (B) Semi-logarithmic plot of data sets as in panel A. One trial (of three) for each incubation concentration is shown. RBCs in whole blood were incubated with auranofin at the indicated concentration ([Au]$_i$), then resuspended in untreated plasma prepared from the same blood sample and maintained at 25°. The gold efflux was monitored over 10 hr using FAAS to analyze plasma obtained by centrifuging aliquots of the blood.

Table 1. Final gold concentrations and apparent rate constants for gold efflux from RBCs pretreated with auranofin and resuspended in fresh plasma

[Au]$_i$ (μM)	Trials	k_{app}* (hr^{-1})	[Au]$_\infty$* (μM)
10	3	0.78 ± 0.19	4 ± 1
50	4	0.92 ± 0.18	27 ± 8
100	2	0.66 ± 0.04†	47 ± 3†
Average	9	0.81 ± 0.18	

* Mean ± SD, except where otherwise noted.
† Average and range of two data points.

employed to confirm the role of the albumin as acceptor. If other proteins with similar molecular weights, but a different charge, were also binding gold, ion exchange should resolve them from the highly-negatively-charged albumin molecule. In addition, the sodium ion present in the serum is not retained by the resin, thereby eliminating its interference with the FAAS determination of gold. As shown in Fig. 3, the gold was again associated with the albumin component, and not with any of the less-negative protein species which eluted before it.

A role for glutathione. When gold(I) effluxes from the RBC into plasma, it is unlikely to cross the cell membrane as a free ion. The most probable ligands with which it might be transported are the triethylphosphine used to administer the gold and reduced glutathione (GSH) which is prevalent in the red cell. The rate of triethylphosphine oxidation is enhanced in whole blood, compared to the rate in red blood cells or serum alone.* Thus, it is probable that some RBC component accelerates the rate of oxidation. After Et_3P is converted to Et_3PO, which is a poor ligand for gold(I), some endogenous ligand must replace the phosphine in the coordination sphere of the gold before or after it effluxes. We postulated, therefore, that glutathione might be a ligand attached to the effluxed, albumin-bound gold. To test this hypothesis, we labeled GSH *in situ* by exposing RBCs to [^{14}C]glycine which is spontaneously incorporated into GSH by the γ-glutamyl cycle [21–24]. The [^{14}C]GSH-labeled RBCs were then exposed to auranofin to allow gold accumulation under the same conditions described above. The cells were washed and then returned to the original plasma, which had been stored at 4°, thereby forming "reconstituted" blood in which to study the relationship between the efflux of gold and glutathione. Control experiments, omitting the exposure to auranofin, were conducted similarly.

Periodically over 24 hr, aliquots of the plasma were removed and chromatographed over Sephadex G-50. The fractions were collected and analyzed for gold, HSA and ^{14}C-activity. The gold was present

* John Dent, Smith Kline & French Laboratories, personal communication, cited with permission.

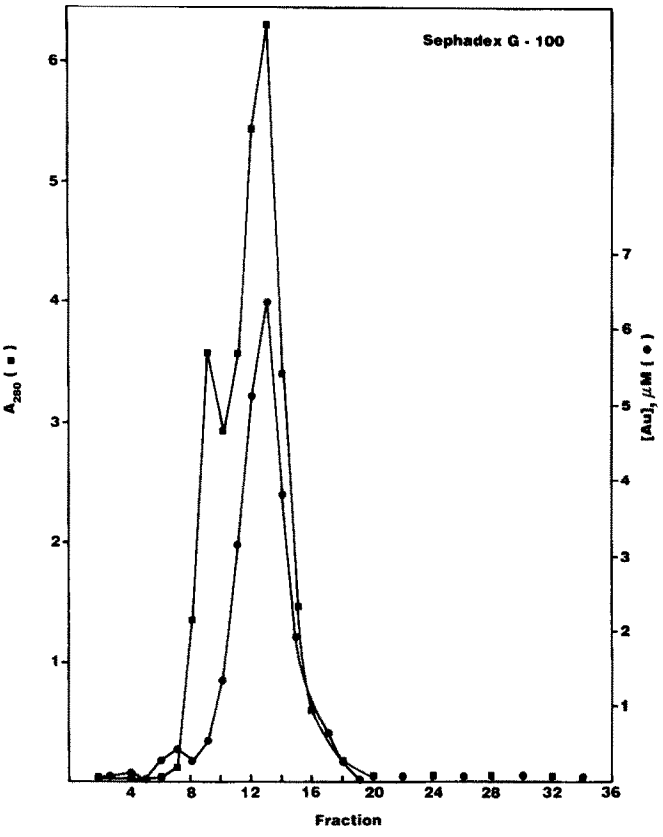

Fig. 2. Gel-exclusion chromatography of plasma after the efflux of gold from auranofin-treated (50 μM, 10 min) RBCs. After 4 hr, the plasma was concentrated approximately 10-fold, and then eluted over a Sephadex G-100 column using 100 mM NH_4HCO_3, pH 7.9. Fractions (9.1 mL) were read for A_{280} to identify protein-containing fractions and for gold by FAAS.

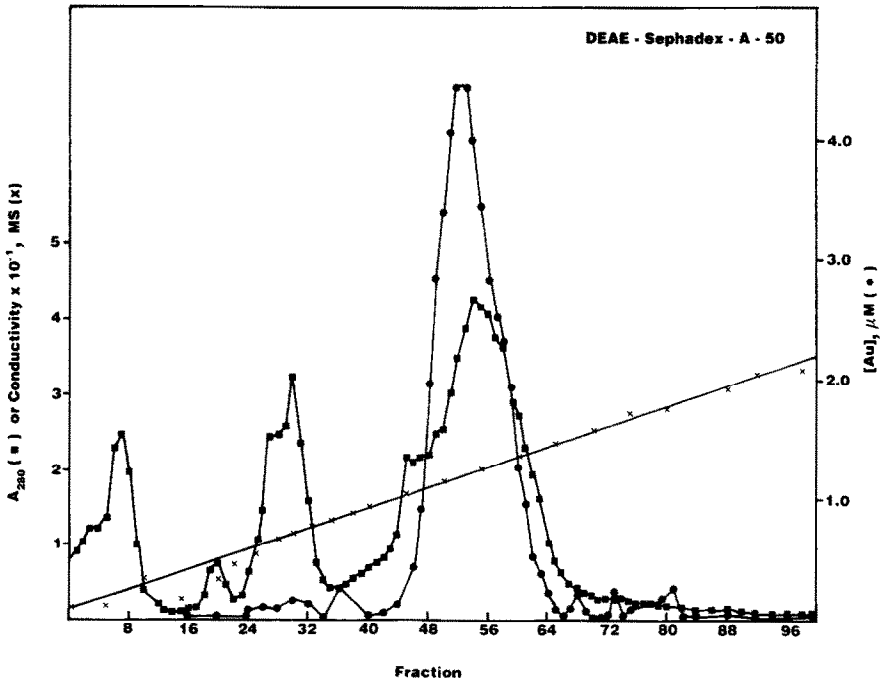

Fig. 3. Ion-exchange chromatography of plasma after the efflux of gold from auranofin-treated (50 μM, 10 min) RBCs. After 4 hr, the plasma was applied to a DEAE-Sephadex-A-50 column and eluted with an NH_4HCO_3 0–500 mM gradient containing 20 mM MOPS buffer, pH 7.4. The fractions (10 mL) were analyzed for gold, protein, and conductivity (milliSiemens).

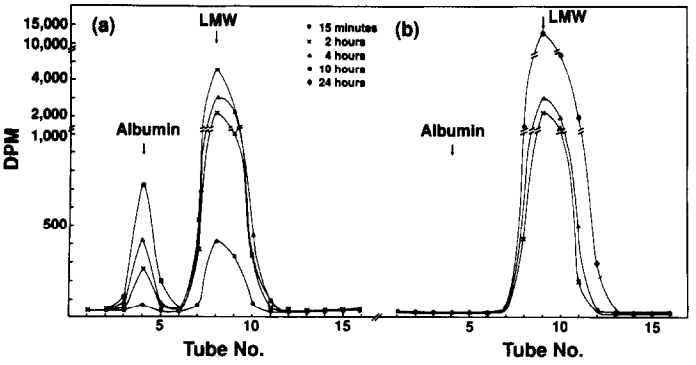

Fig. 4. Chromatograms of plasma from "reconstituted blood" showing the distribution of ^{14}C-activity at successive time intervals after adding fresh plasma to *in situ* [^{14}C]glutathione-labeled RBCs with or without auranofin exposure. Panel a: the labeled RBCs were exposed to auranofin before reconstitution. [^{14}C]Glutathione was associated with albumin (which eluted in fraction 4) and was present in the low-molecular-weight fractions. Panel b: control experiments omitting the auranofin treatment demonstrated that in its absence [^{14}C]GSH effluxed from the RBCs, but did not associate with serum albumin (fraction 4). Conditions: Sephadex G-50 (0.9 × 30 cm column) eluted with 100 mM NH_4HCO_3 buffer, pH 7.9. Aliquots (0.4 mL) of the 2.1-mL fractions were set aside for scintillation counting. The remainder was used to determine the albumin and gold concentrations (not shown).

almost exclusively in the albumin-containing fractions (not shown). The maximum molar ratio of gold bound to albumin (Au_b/BSA) was 0.014. The gold(I) efflux was complete after 10 hr, and the rate of efflux, $k_{app} = 0.66\ hr^{-1}$, was within the range reported above (Table 1), establishing that the [^{14}C]glycine exposure did not alter significantly the gold efflux characteristics.

Figure 4 compares the [^{14}C]GSH distribution in plasma after exposure to auranofin-treated RBCs (a) and control RBCs (b). For the auranofin-treated RBCs, the [^{14}C]glutathione was present in the albumin fractions and low-molecular-weight fractions, while in the control experiment, which omitted the auranofin, the [^{14}C]glutathione appeared only in the low-molecular-weight fractions. Figures 2 and 3 above demonstrated that the effluxed gold was albumin-bound. Thus, the observation that the [^{14}C]glutathione was albumin-bound only when gold was present can be explained as the formation of an albumin–gold–glutathione complex, AlbSAuSG, which is consistent with the high affinity of gold(I) for thiolate ligands. These experiments provide the first direct evidence that glutathione ligates gold(I) in the plasma.

DISCUSSION

To our knowledge, these are the first detailed studies of the kinetics of gold efflux from red cells, and the first identification of a gold metabolite formed upon efflux. It is known that Et_3PAu^+ binds to hemoglobin and glutathione, the predominant thiol species in RBCs, after incubation of RBCs with 8 mM Et_3PAuCl [12, 13], but the binding sites populated at therapeutic concentrations have not been identified and may be different [9]. Whatever the actual binding sites may be, it is clear from the data presented here that the uptake of gold is reversible and that a substantial efflux can be observed. The finding that the gold was associated with serum albumin is not surprising, since a large body of evidence points to the fact that cysteine-34 of the albumin sequence is a very high-affinity gold-binding site [14, 15, 27, 28]. Indeed, the recent report that the transfer of gold (as Et_3PAu^+) from hemoglobin to albumin is thermodynamically favorable [14] suggests that albumin may provide a driving force for the efflux.

The simplest interpretation of the apparent rate constant for the efflux, $k_{app} = 0.81 \pm 0.18\ hr^{-1}$, is that it represents a true first-order rate constant for irreversible efflux of gold. Given the effect of albumin on the extent of efflux, however, k_{app} is more likely to be the apparent rate constant for the approach to an equilibrium between intra- and extracellular gold, which must be described by forward (efflux) and reverse (uptake) rate constants. In that case [29], k_{app} is the sum of the rate constants for the efflux and uptake of gold.

$$[Au]_{rbc} \underset{k_{uptk}}{\overset{k_{eflx}}{\rightleftharpoons}} [Au]_{serum} \quad k_{app} = k_{eflx} + k_{uptk}.$$

The thiomalate of myochrysine [7] and the thioglucose of solganol [8], as well as the tetraacetylthioglucose and phosphine ligands of auranofin [5, 6], are displaced from gold after its absorption into the blood. The chemistry of gold(I) clearly requires that other class *b* or soft ligands, of which thiolates are the predominant naturally occurring examples, should replace these medicinal ligands. Identifying the small, endogenous thiolates, or other ligands which complete the coordination sphere of gold(I) protein complexes is necessary for complete characterization of the circulating metabolites of gold drugs. These experiments provide the first direct evidence that glutathione binds to gold after the triethylphosphine of auranofin is oxidized. Since glutathione is the principal thiol in serum as well as

in RBCs and many tissues, it should not be surprising that it plays a role in the chemistry of gold. Indeed, this result confirms a prediction that glutathione and other endogenous thiols would act as ligands in protein–gold metabolites [9, 19, 30].

The metabolite complex AlbSAuSG, identified here, is analogous to the albumin–gold–thiolate adducts of thiomalate and acetylthioglucose, AlbSAuSTm and AlbSAuSAtg, which have been synthesized *in vitro* by reaction of the corresponding oligomers (AuSTm or AuSAtg) with serum albumin [9, 30]. From extended X-ray absorption fine structure (EXAFS) and Mössbauer spectroscopy studies, AlbSAuSTm is known to have a linear two-coordinate structure about the gold(I) ion [30], and a similar structure can be predicted for AlbSAuSG. Thus, it is unlikely that additional ligands render the gold three- or four-coordinate.

The gold and glutathione may efflux concertedly or separately. A concerted efflux mechanism is plausible, since glutathione conjugates of organic electrophiles are transported out of erythrocytes [31], and a bis(glutathione)–gold(I) complex, $Au(SG)_2^{-3}$, would resemble an organic conjugate in many respects.

Significance for chrysotherapy. Auranofin (and its ability to be absorbed orally) represents the second generation of gold-containing complexes for the treatment of rheumatoid arthritis. A rational approach to the design of a third generation drug (e.g. quicker onset of action, fewer side effects) has been blocked by our lack in understanding the precise molecular target(s) responsible for the pharmacologic actions of auranofin and other gold drugs. This situation is in large degree the result of the complex metabolism of gold-containing drugs and the inability to define the immunopharmacologically active gold complex. Our studies, coupled with the documented pharmacokinetics of auranofin in humans [3, 4], clearly suggest that a glutathione–gold–albumin complex is a metabolite of auranofin and, as such, is a precursor to or perhaps the active agent. An obvious corollary of this conclusion is that AlbSAuSG and its precursors $AlbSAuPEt_3$ and AlbSAuSTm (formed by direct reaction of auranofin and myochrysine with albumin) should be used instead of the unmetabolized drugs to test postulated mechanisms of action such as conversion of singlet oxygen to triplet oxygen [32], inhibition of sulfhydryl enzymes [3, 4, 33] and immunoregulatory activity [3, 4, 33].

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