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TIME-DEPENDENT UPTAKE AND METALLOTHIONEIN-BINDING OF GOLD, COPPER AND ZINC IN THE RAT KIDNEY

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Abstract—In rats, in which the whole body burden of Au decreases rapidly, but biphasically, maximum kidney concns are not attained until 10–15 days after a single intraperitoneal dose of either Au(I) or Au(III). The concn of metallothionein-bound Au and of total kidney Cu, which also increases after the administration of the Au compounds, however, reach maxima at 5 days. Between at least 6 and 24 hr after Au treatment, the increases in the concn of Au and Cu in the metallothionein fraction are highly correlated. Measurements on the kidneys of animals at early times (15 min–6 hr) after dosing with Au(III) indicate that the Zn content of the (endogenous) metallothionein is depressed during the first hour, shows a transient increase at 2 hr and then falls to a minimum at 6 hr. Subsequent (6–24 hr) changes in metallothionein-bound Zn parallel those of metallothionein-bound Au and Cu. It seems, therefore, that Au and Cu are incorporated simultaneously into rat kidney metallothionein and this incorporation may be mediated by an initial displacement of Zn. In rats exposed to five doses of Au(III) the half-times of total and metallothionein-bound Au in the kidneys are appreciably longer than those in animals given a single dose. In both groups, the concns of Cu and Zn in the renal metallothionein do not decrease in parallel with that of Au, but change roughly in proportion to their whole kidney concns. In consequence, the metal composition of the metallothionein fraction, which remains above the endogenous concn in the normal kidney throughout an experimental period of 90–140 days, alters considerably with time.

In rats, the renal accumulation of Au, which follows the administration of either Au(III) as sodium aurichloride, or Au(I) as sodium aurothiomalate, is accompanied by an appreciable increase in the Cu concn of the kidneys [1–4]. The renal Cu content is also increased after treatment of the guinea-pig with Au(III) and, in this species, as in the rat, most of this additional Cu is bound, together with Au and a small amount of Zn, in the soluble fraction of the tissue as a metallothionein [3]. In rabbits, hamsters and mice, however, the renal uptake of Au neither affects the Cu concn nor results in metallothionein synthesis [3]. Whereas these species differences indicate that the synthesis of metallothionein is not a direct response to the renal Au concn, there is no proof that, in those species in which this synthesis occurs, the binding of Au to the metalloprotein is secondary to the induction of thionein in consequence of the elevated Cu content of the kidneys. Indeed, experimentally-induced increases in the renal concn of metallothionein-bound Cu in the rat either do not affect or decrease both the kidney uptake and metallothionein-binding of Au [5]. In contrast, administration of Cu and Au, but not of Cu alone, to the hamster leads to the synthesis of small amounts of metallothionein. This metalloprotein also contains Zn in addition to Au and Cu [5]. Furthermore, Au(III) treatment of the Cd-pretreated hamster [which contains a (Cd, Zn) metallothionein in its kidneys] results in further thionein synthesis, the retention of the metallothionein-bound Cd and the incorporation of Au, Cu and additional Zn into this metalloprotein fraction of the renal cytosol [5]. The possibility has been considered, therefore, that the binding of both Au and Cu to kidney metallothionein may occur after induction of the protein in response to changes in renal Zn concn [5].

The inability to answer unequivocally the question whether Cu or Zn is the primary inducer of renal metallothionein synthesis in Au-dosed animals seems to be due, at least in part, to insufficient information about the changes in the kidney concns and distributions of the three metals with time after treatment. The work summarized herein was done, therefore, to investigate these changes in the kidneys of rats after a single dose of either Au(III) or Au(I) and to determine whether an alteration in the content of either metallothionein-bound Zn or metallothionein-bound Cu precedes the incorporation of Au into this metalloprotein. Additional studies, which were made to follow the alterations in the concns and distributions of these metals with time after exposure to multiple doses of Au(III), also are reported.

MATERIALS AND METHODS

Solutions of Au(III) and Au(I) for injection were prepared from ^{195}Au-labelled chloroauric acid and

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sodium aurothiomalate, respectively, as described previously [3, 4].

The animal experiments were done with four groups of female Wistar–Porton rats (180 ± 5 g initial body wt) from the laboratory colony. Animals of group 1 were given one dose of Au(III) and those of group 2, one dose of Au(I) (1 mg Au/kg body wt) by intraperitoneal injection and were killed by decapitation at intervals from 15 min to 90 days (group 1) and 2 hr to 30 days (group 2) thereafter. This difference in time scale was due to the limited supply of sodium [^{195}Au]aurothiomalate. Each rat of the third group was given one dose of Au(III) (1 mg Au/kg body wt) every 7 days for 5 weeks. Animals of this group were killed between 1 and 20 weeks after the last dose. Animals of the fourth group remained untreated and were killed at appropriate times as controls to those of groups 1–3. At least three experimental and three control animals were used at each time point. Throughout the experimental periods the rats were maintained on 41B diet (BP Nutrition Ltd, Witham, U.K.) and were given tap water *ad lib.*

Whole body burdens of Au and the concns of Au, Cu and Zn in the kidneys from each animal were determined by the methods that were used in previous studies [3, 4]. The remainder of the kidney tissue (three-quarters of the total) from each animal was homogenized separately in 3 vol. 10 mM Tris–HCl buffer, pH 8, the homogenate being centrifuged

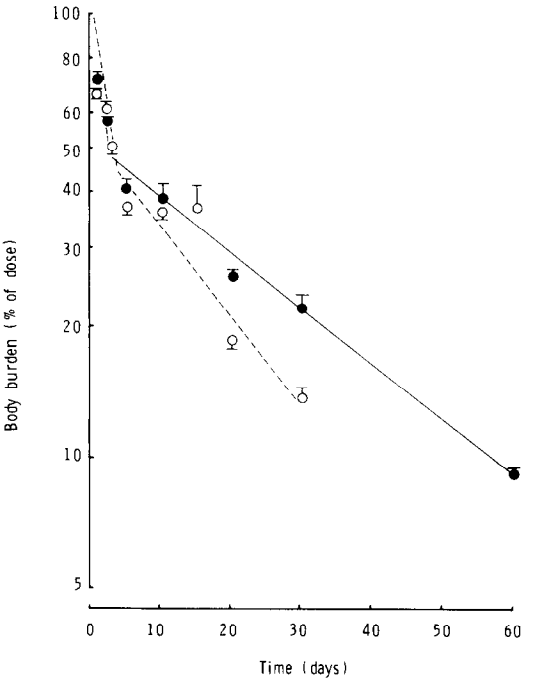

Fig. 1. Decrease in the whole body burden of Au (mean values ± S.E.M.) with time after a single intraperitoneal dose of Au (1 mg/kg) as Au(III) (—●—) or Au(I) (- - ○ - -) in the female rat (N = 3 or 4).

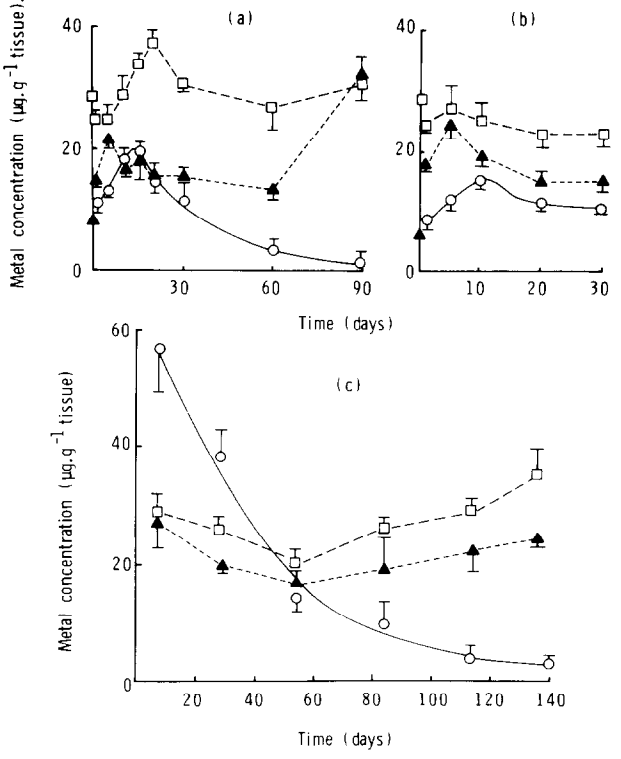

Fig. 2. Time-dependent changes in the concns (mean values ± S.E.M.) of Au (—○—), Cu (- - ▲ - -) and Zn (- - □ - -) in the kidneys of rats (N = 3) after a single intraperitoneal dose (1 mg Au/kg) of: (a) Au(III) or (b) Au(I), and (c) after five doses of Au(III). The points (□, ▲) on the ordinate (a and b) show the endogenous renal Zn and Cu concns in control animals at $t = 0$.

at 105,000 *g* for 1 hr (Beckman L5-65 centrifuge) to yield the soluble fraction. This was fractionated by gel filtration on a column (1.5 × 80 cm) of Sephadex G75, the same buffer being used as an eluant at a flow rate of about 20–24 ml/hr. Fractions (3 ml in vol.) were collected and analysed for Au, Zn and Cu (see Ref. 3).

RESULTS

In female rats after a single injection of Au(I) and Au(III) the whole body burden of Au appeared to decrease at about the same rate during the first 5 days and then more rapidly in the Au(I)-dosed animals ($t_{\frac{1}{2}}$ about 17 days) than in those treated with Au(III) ($t_{\frac{1}{2}}$ about 25 days) (Fig. 1). In the latter group of animals the renal Au concn was maximal (approximately 20 μg Au/g tissue) on the 15th day after dosing (Fig. 2a). Although this time point was not included in the studies with Au(I), the results of Fig. 2b suggest that the kidney concn was highest (approximately 16 μg Au/g tissue) about 5 days earlier, i.e. 10 days after the administration of aurothiomalate. In the Au(III)-dosed animals, with which more data were obtained, the renal Au concn decreased slowly from its maximum value to 10 μg/g tissue at about 5 weeks and to 2 μg/g tissue at about 3 months post-injection (Fig. 2a). When these results were expressed as contents to correct for the changes in kidney wt during the course of the experiment, the renal burden of Au appeared to decrease exponentially with a $t_{\frac{1}{2}}$ of 23.9 days ($r = 0.997$) (Fig. 3). A longer biological half-time ($t_{\frac{1}{2}} = 32.0$ days; $r = 0.990$) (Fig. 3) was obtained for renal Au in rats that were given multiple doses of Au(III). In these animals the renal Au concn was maximal (56 μg Au/g tissue) at the first time point (1 week after the injection of the last dose) and then decreased to about 40 and 5 μg/g tissue at 20 and 110 days, respectively (Fig. 2c).

In agreement with previous observations [1–4], the renal uptake of Au was accompanied by an increase in the Cu concn of the kidney. The maximum Cu concn (20–25 μg/g tissue), however, was reached on the fifth day after the injection of a single dose of either Au(I) or Au(III), approximately 5–10 days earlier than the maximum concn of Au (Fig. 2a and b). Thereafter the Cu concn appeared to decrease slowly although, at all time points, it remained between 80 and 30% greater than that (8–13 μg/g tissue) in the kidneys of control animals of the same age. In the prolonged experiment with animals that were given a single dose of Au(III), the renal Cu concn increased again by 150% to 33 μg/g wet wt between 60 and 90 days (Fig. 2a).

In animals that were treated five doses of Au(III) the renal Cu concn was maximal at the time of the first measurement, 1 week after the administration of the last dose. It then declined to a minimum (about 75% above the mean normal concn of 11.5 μg Cu/g tissue in the kidneys of the corresponding controls) at 8 weeks, after which it increased to a second maximum at about 20 weeks (Fig. 2c) (values after 140 days are not shown) and fell slowly thereafter. The pattern of changes in the renal Zn concn in these multiply dosed animals (Fig. 2c) was essentially similar to that of Cu. At 1 day after single doses of Au(I) and Au(III), the Zn concn in the kidney

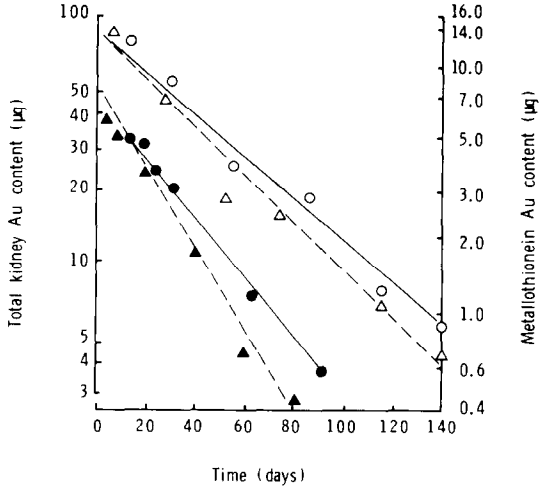

Fig. 3. Semi-log plots of the renal contents of total (——) and metallothionein-bound Au (- - -) in rats at different times after the intraperitoneal injection (1 mg Au/kg) of a single dose (●, ▲) and five doses (○, △) of Au(III). The results are calculated from the mean kidney wts and the mean concns of Au shown in Figs 2a and c, and 5a and c.

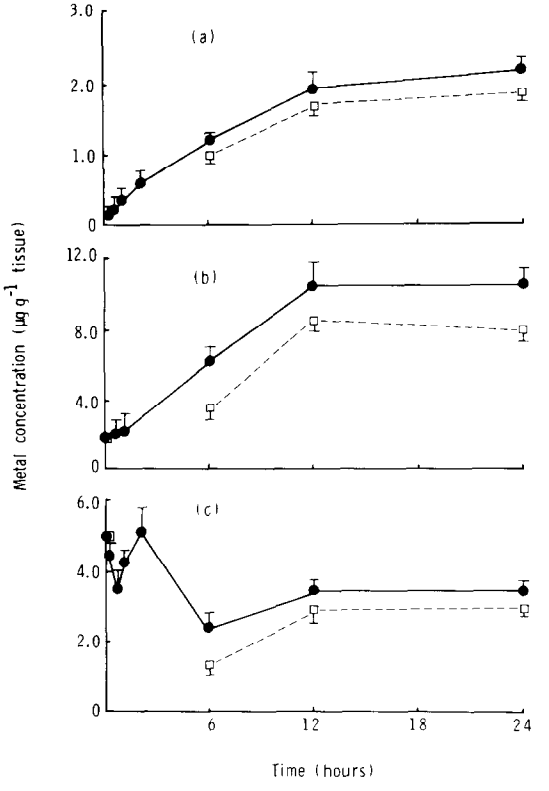

Fig. 4. Concns of: (a) Au, (b) Cu, and (c) Zn in the metallothionein fraction of rat kidney (N = 3) during the first 24 hr after a single intraperitoneal injection (1 mg Au/kg) of Au(III) (—●—) or Au(I) (- - □ - -).

seemed to be decreased slightly (from 28.3 ± 2.3 to about 25 μg/g tissue) and, in the Au(III)-dosed group, remained at this level for 5 days and then increased to a maximum (37.2 ± 2.0 μg/g tissue) at 20 days (Fig. 2a). In Au(I)-dosed animals the maximum of the renal Zn concn was lower and occurred earlier (5 days) than in those dosed with Au(III) (Fig. 2b).

Between 6 and 24 hr after the injection of single doses of Au(III) and Au(I) the increases in the concns of Au and Cu in the renal metallothionein fraction (Fig. 4) were highly correlated [$r = 0.94$ (Student's t-test)]. At earlier times (15 min–6 hr) after injection of Au(III) the metallothionein-bound Zn concn varied appreciably (between 5.1 and 2.3 μg/g tissue), but during the first hour it was depressed below the endogenous level. In the Au(I)-dosed animals, in which measurements were made between 6 and 24 hr only, the Zn in this fraction increased with the increases in Au and Cu concns. Although, in these experiments, binding of Au to metallothionein was found to be most rapid during the first 12 hr after treatment, the concn of Au in this fraction increased for 5 days (Fig. 5a and b). Thus in both Au(I)- and Au(III)-dosed animals the time at which the concn of metallothionein-bound Au was maximal was coincident with that of the maximum concn of Cu in the whole kidneys, but earlier than the maximum concn of Au (Fig. 2a and b). Comparison of Fig. 5a with Fig. 2a, for example, shows clearly that the decrease in the concn of metallothionein-bound Au in animals that were given a single dose of Au(III) began whilst the kidneys were still accumulating Au. At all times about 55% of the Au in the kidneys was located in the soluble fraction but, with the loss of metallothionein-bound Au after 5 days, a larger proportion of this 'cytoplasmic' Au was associated with the high mol. wt proteins that eluted from gel filtration columns at $V_e/V_o = 1$ (data not shown). Nevertheless the half-time of metallothionein-bound Au in these animals [19.6 days ($r = 0.98$)] as calculated from the results of Fig. 5a, was similar to that ($t_{\frac{1}{2}} = 23.9$ days) of total renal Au (Fig. 3).

In animals that were treated with multiple doses of Au(III) the content of metallothionein-bound Au decreased continually with a half-time [30.3 days ($r = 0.98$)] also similar to that of the total kidney Au [32.0 days ($r = 0.99$) (Fig. 3)]. In these animals, as in those that were given one dose of Au(III), the Cu and Zn concns in the metallothionein fraction (Fig. 5c) did not decrease in parallel with that of Au (Fig. 5c), but each changed roughly in proportion to its whole kidney concn (Fig. 2c). Thus the metal com-

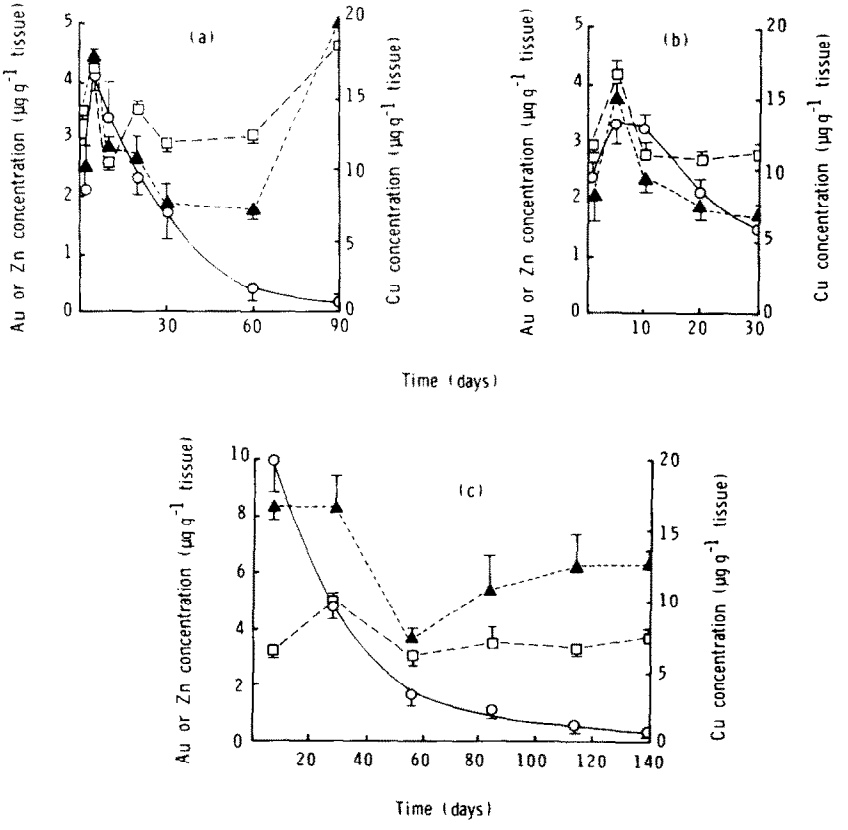

Fig. 5. Time-dependent changes in the concns (mean values ± S.E.M.) of Au (—○—), Cu (- - ▲ - -) and Zn (- - □ - -) in the metallothionein fraction of rat kidney (N = 3) after a single intraperitoneal dose (1 mg Au/kg) of either: (a) Au(III), or (b) Au(I), and (c) after five doses of Au(III).

position of the metallothionein fraction altered with time. After multiple and single doses of Au(III), for example, the molar ratios of Au:Cu:Zn in the metallothionein, as calculated from the data of Fig. 5 were, respectively, 1:9.9:2.4 and 1:13.1:4.2 at 20 days and 1:11.6:4.1 and 1:30.8:12.0 at 50 days. In the renal metallothionein of animals dosed with Au(I) the Au:Cu:Zn ratio was 1:13.8:3.7 at 5 days and 1:11.0:3.9 at 20 days.

DISCUSSION

The correlation between the binding of Au and the binding of Cu to metallothionein in the kidneys of the rat during the first 24 hr after the administration of either Au(III) or Au(I) (Fig. 4) implies that the two metals are incorporated simultaneously into the metalloprotein. The decrease in the concn of metallothionein-bound Zn at very early times [e.g. 15 and 30 min after injection of Au(III) (Fig. 4)] also suggests that, initially, this binding of Au and Cu occurs by displacement of Zn from the endogenous metalloprotein. At later times, further synthesis of metallothionein must occur, since the contents of total thionein-bound metals (ΣAu, Cu and Zn) in this fraction are greater than the endogenous contents of Zn and Cu. The fluctuations in the metallothionein-bound Zn concns between 0.5 and 6 hr indicate, at least, the possibility that this synthesis is initiated or determined by the Zn that is initially displaced. Nevertheless some of this displaced Zn may be eliminated, since the increase in the whole kidney concn of Zn that follows the administration of Au(III) appears to be preceded by a decrease (Fig. 2a). In contrast the renal concn of Cu begins to increase immediately after the intraperitoneal injection of Au(III) (Fig. 2a). The origin of this Cu, whilst not established unequivocally, may be the liver since, in this organ, the concn of Cu (and Zn) decreases within a short time after the administration of Au(III) (Mogilnicka and Webb, unpublished observations).

In both short- (Fig. 4) and long-term (Fig. 5) experiments, the metallothionein that is induced by Au treatment contains much more Cu than Au. Binding of these metals to metallothionein, although most rapid during the first 12 hr after treatment with a single dose of either Au(I) or Au(III), increases for 5 days (Fig. 5a and b). Thus the concn of metallothionein-bound Au reaches a maximum at the same time as the total kidney concn of Cu (Fig. 2a and b) and then declines with a half-time approximately the same as that of the elimination of Au from the whole organ. The results reported herein, which are in contrast with the observation of maximal concns of total and metallothionein-bound Au in the kidneys of male rats at 2 and 4 days, respectively, after the subcutaneous injection of sodium aurothiomalate [2.5 mg Au/kg (Ref. 2)], suggest that all forms of intracellular Au are in equilibrium. As, however, the concn of metallothionein-bound Au begins to decrease after about 5 days (Fig. 5a and b), whereas the kidney continues to accumulate Au for at least a further 5 days (Fig. 2a and b), the content of some other high-affinity binding component in the kidney must increase slowly with time after treatment and thus alter the intracellular distribution of Au. Since the loss of metallothionein-bound Au between 5 and 15 days after treatment with Au(III) is accompanied by increased binding to the soluble high mol. wt fraction, it is conceivable that the contents of certain cytoplasmic proteins are altered through the effect of Au on lysosomal function [6].

Although the concns of metallothionein-bound Zn and Cu in the kidney are increased in response to the administration of Au, these concns do not decrease in parallel with the subsequent loss of metallothionein-bound Au. Instead, they vary with their concns in the whole kidney, which not only remain above the normal endogenous levels of the untreated controls for at least 150 days after treatment, but also seem to increase disproportionally after about 60 days (Fig. 2a and c). At present these variations cannot be explained. Alterations in dietary Zn and Cu intakes, although possible with an uncontrolled diet, seem unlikely to be a contributory factor, since the treated and control animals received the same food and showed the same pattern of wt gain.

The results of Figs 2 and 3 indicate that the renal burden of Au is higher, but the rate of loss of Au from the kidneys is slower in animals given repeated doses of Au(III) than in those treated with a single dose. The apparent difference between the whole body elimination rates of Au after a single dose of Au(III) and a single dose of Au(I) (Fig. 1), however, cannot be attributed to the different maximal renal concns (20 and 16 μg Au/g tissue, respectively) and remains unexplained. Other estimates of the elimination half-time of Au after a single (subcutaneous) dose of sodium aurothiomalate (1 mg Au/kg body wt) in the rat range from 24 [7] to 26.5 days [8] and are essentially the same as that (25.5 days) reported herein for Au(III)-treated animals. Although differences in the pharmacokinetics of Au have been reported after the administration of auronofin [(2,3,4,6-tetra-*O*-acetyl-l-thio-β-D-glucopyranosato-*S*)-(triethylphosphine)-gold] and sodium aurothiomalate to the rat [9], both the latter and sodium aurichloride are considered to yield Au(I) *in vivo* by metabolic cleavage of the organic ligand and reduction, respectively [10, 11]. Unless, therefore, the reductive process is the more rate-limiting, the elimination half-times of Au in animals, dosed in the same way with these Au(I) and Au(III) compounds, would be expected to be the same.

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