Influence of Chelation on Gold Metabolism in Rats. (31725)

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Carefully controlled, double blind clinical study has demonstrated the definite advantage of gold compounds over placebo in treatment of rheumatoid arthritis, thus confirming a long held clinical impression(1,2). There is scant information, however, despite over 40 years of clinical application, of the factors which control the distribution and excretion of the metal. This deficiency is especially noteworthy on those occasions when it is essential to cope with the clinical manifestations of gold toxicity. The unpredictable nature of the hazards of gold therapy also complicates any rational application of this modality. More important even than these unanswered pragmatic questions is the more fundamental query concerning the mechanism by which gold therapy may favorably alter the clinical manifestations of rheumatic disease.

The availability of the facile new analytical technique of thermal neutron activation(3) and an emerging understanding of the factors by which the metabolism and excretion of minerals may be directed and controlled *in vivo*(4), led us to apply this new knowledge to problems of the pharmacology of gold. In this paper we describe our experience with the method of thermal neutron activation analysis for the measurement of gold in biological samples and the influence of some gold binding compounds on the distribution and excretion of the metal administered to rats.

Material and methods. Compounds utilized: *Gold Thioglucose, (Au TG)* (Solganal). The gold content of commercially available ampuled material was established by analysis and then diluted quantitatively with sesame oil to provide a gold concentration of 10 mg/ml.

Gold Mercaptoethyliminodiacetic acid (AuMAIDA) and Gold 1,8-diamino-3,6-dimercaptooctane-N,N-tetraacetic acid (Au BATA): Weighed quantities of the crystalline acids* sufficient to provide a one molar excess of compound were suspended in stock standard gold chloride solutions. The mixture was gradually neutralized to pH 6.5 by the dropwise addition of 10% sodium carbonate solution with continuous stirring. Final volumes were adjusted to provide a concentration of 2 mg/gold/ml.

2,3-Dimercaptopropanol (BAL), Thiomalic Acid† and D,L-Penicillamine were prepared in neutral aqueous solution just prior to use at concentrations of 0.8 mg/ml.

Standard Gold Solutions: Weighed electrolytic gold was dissolved in aqua regia and brought to volume with dilute nitric acid to serve as a stock standard for analysis. For preparation of the experimental gold compounds, the crystalline chloride was dissolved in water shortly before use at an approximate concentration of 10 mg of gold/ml and standardized by analysis.

Neutron Activation Analysis: Duplicate aliquots of 0.10 ml samples were pipetted into polyethylene micro test tubes‡ and sealed with a hot soldering iron. Comparable samples of the standard stock gold solution were prepared for simultaneous analysis. Randomly distributed samples and standards were introduced into an atomic reactor where they were exposed to a thermal neutron flux of about 1×10^{12} n/sec/cm^2 for 6 hours.§. Following irradiation, the resulting radioactivity was allowed to decay for approximately 5 days. In this time the radioactive sodium

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‡ Beckman Instrument Co., California.

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24 decayed but sufficient gold 198 activity remained for convenient counting. A sample and standard which had been activated in the reactor at the same time and location were counted simultaneously in matched, twin 5 inch sodium iodide well crystals with a 400 channel RIDL pulse height analyzer using 200 channels of the memory for each crystal. The results were plotted by an x-y plotter. The area under the 0.41 mev peak was measured. The unknown gold was calculated from the ratio of these areas and the known amount of gold in the standard. It was found that the method was reproducible with a S. D. of ± 5% over a range of 0.05 to 40.0 μg/ml following activation and decay. The standard solution contained essentially only one radionuclide with a gamma energy of 0.41 mev and a half-life of 2.7 days. The major energy peak, and the one used for analysis in the samples was also 0.41 mev with a half-life of 2.7 days.

Handling of Animals and Preparation of Samples for Analysis: Sprague Dawley male rats, 200-300 g were used. Animals were housed individually and fed Purina chow and water *ad libitum*. Fecal and urinary collections were made daily and pooled for animals in the same study group. Urines were acidified with nitric acid upon collection. Pooled fecal samples were homogenized in a Waring Blender with enough distilled water to provide a smooth paste. Aliquots of 1-3 g were then transferred to 50 ml flasks to which were added 20-25 ml of concentrated nitric acid. After storage at 30-40° for several days, the fecal material was usually sufficiently digested to provide a clear solution. Occasional samples required heating to 60° for clarification. The digests were transferred to volumetric flasks of 25 or 50 ml capacity, brought to volume and aliquots withdrawn for analysis. At completion of the study period the animals were anesthetized with ether, exsanguinated from the abdominal aorta and the organs removed. Following dissection, the tissues of a given experimental group were pooled, weighed and homogenized with distilled water. Weighed aliquots for analysis were digested in nitric acid as described for the fecal samples.

Experimental Design: A. *Distribution and Excretion of Injected Gold:* The first part of the study consisted of giving gold complexed to thioglucose, BATA and MAIDA to 3 groups of rats. Each group had 4 rats who each received 1 mg of gold as the organic compound intramuscularly into the hind limbs daily for 14 days.

B. *Effect of Complexing Agents on Preinjected Gold:* Rats in 7 groups of 6 each were given 1 mg of gold thioglucose intramuscularly daily for 14 days. Following a 4 day rest period, 5 groups were given 4 mg/kg daily of BAL, D,L-penicillamine, thiomalic acid, MAIDA or BATA for 5 days by intramuscular injection. Two control groups were given isotonic saline by the same route on the same schedule.

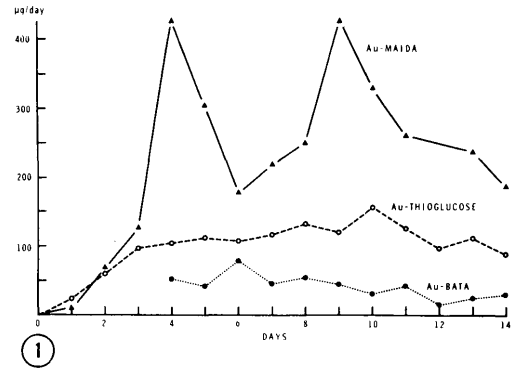
Results: Distribution and Excretion of Gold Compounds: Urinary gold output following daily injections of various gold compounds rose continuously during the first 4 days of administration (Fig. 1). The pattern of fecal gold excretion (Fig. 2) was similar. Noteworthy points in the tissue distribution of gold (Fig. 3) include the relatively high level deposited in kidneys following administration of all these gold compounds and the unusual spleen deposition after Au MAIDA.

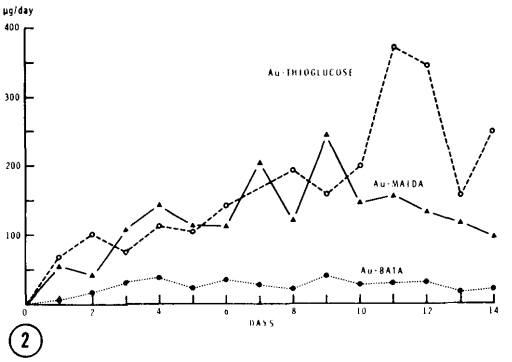
Effect of Treatment: Urinary Excretion: The effect of treatment with various gold binding agents on predeposited gold administered as gold thioglucose is illustrated in Fig. 4 and 5. For ready comparison the data is plotted as a percentage of the initial average gold excretion of 20μg/day/rat in the control groups.

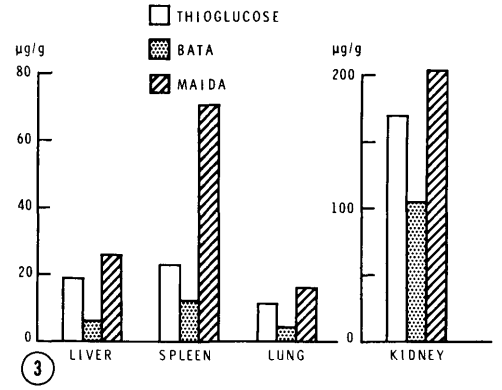
Fecal Excretion: Because of some collection losses, complete fecal excretion data are available only for the results of treatment with MAIDA and BATA (Fig. 6).

Tissue Deposition: The effects of chelate treatment on predeposited gold is evident in Fig. 7 for spleen, Fig. 8 for lung, Fig. 9 for liver and Fig. 10 for kidney.

Discussion. Gold Analysis: The problems inherent in the colorimetric and spectrographic analysis of gold have been critically reviewed by Beamish(5). While we have found it possible to attain satisfactory results in the gold analysis of plasma, urine and wet ashed







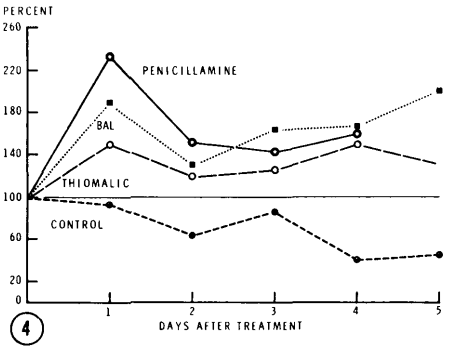
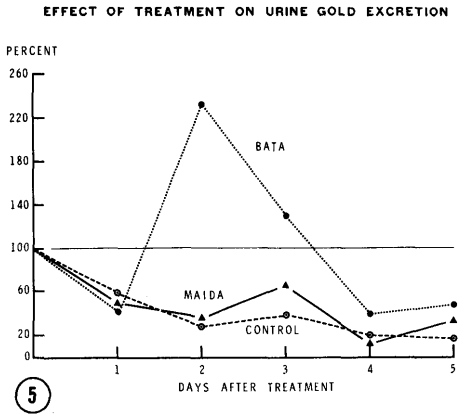
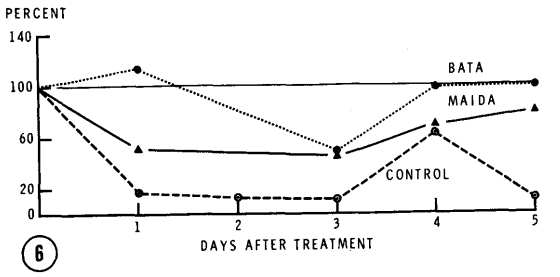


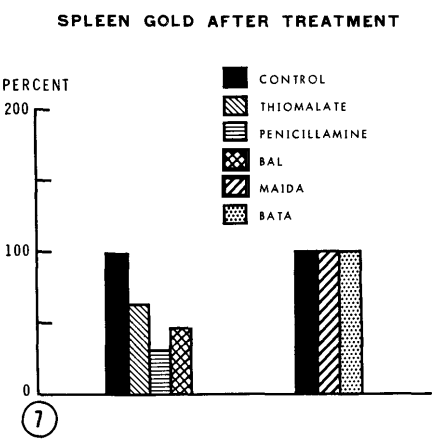
FIG. 1. Urinary gold excretion after gold chelates in rats.
FIG. 2. Fecal gold excretion after gold chelates in rats.
FIG. 3. Tissue distribution of gold after gold chelates in rats.
FIG. 4. Effect of chelate treatment on urinary gold administered as gold thioglucose.

samples of tissue using the o-dianisidine procedure described by Block and Buchanan, we agree with the authors that the method is less useful in analysis of fecal samples(6). In addition, the exacting requirements for control of a lengthy and tedious analytical method mitigates against its routine use. Thermal neutron activation analysis obviates these problems. The sensitivity and accuracy of the procedure readily permits analysis of 0.05 μg/ml of gold in the prepared sample. This is almost two orders of magnitude lower than that attainable by current techniques of atomic absorption spectrophotometry. In addition, thermal neutron activation provides for facile analysis of complex mixtures such as feces. This is possible since the induced radioactivity of the gold in the sample is independent of its chemical form and can be measured directly even in the presence of the activation products of other elements.

Effect of Complexing Agents on Gold Metabolism: In recent years the utilization of synthetic chelating agents has provided new possibilities for *in vivo* control of the distribution patterns of some elements. Selective tissue deposition and enhanced urinary and fecal excretion have been achieved by combination of calcium, iron or lead, for example, with various organic compounds(4). That similar results are attainable with gold is suggested by the extensive studies of Freyberg, Block and collaborators(2). These investigators demonstrated that soluble gold compounds, such as thioglucose, differed from colloidal gold preparations by an increased urinary excretion, increased absorption from injection sites, increased kidney deposition and decreased fecal excretion, following intramuscular administration to rats. An insoluble gold compound, gold calcium thiomalate was intermediate in its effect on these parameters. That structural factors related to the nature of the gold complex can result in highly







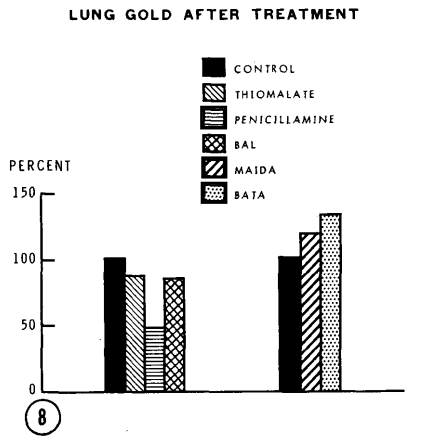


FIG. 5. Effect of chelate treatment on urinary gold administered as gold thioglucose.
FIG. 6. Effect of chelate treatment on fecal gold administered as gold thioglucose.
FIG. 7. Effect of chelate treatment on spleen gold administered as gold thioglucose.
FIG. 8. Effect of chelate treatment on lung gold administered as gold thioglucose.

selective tissue deposition of the metal, is also evident from a consideration of its distribution when administered as gold thioglucose. This soluble gold complex brings about hyperphagia and obesity when injected intramuscularly in mice(8). The response is associated with selective deposition of gold in the hypothalmus(9). Other soluble gold compounds do not produce analagous results(10). The specificity of gold thioglucose has been attributed to the structural similarity of the compound to the presumed glucoreceptor mechanism in the satiety region of the hypothalmus. It is not unexpected that the interaction of administered gold compounds with the many potential gold binding groups *in vivo* should give rise to selective tissue deposition of the metal. Gold, when in the form of auric ion, is able to combine with four coordinating atoms in a planar configuration. Nitrogen, sulfur and oxygen, in addition to the halogens, readily serve as donor atoms in suitably constituted ligand structures which can form gold complexes of varied stability. The widespread occurrence of diverse gold binding ligands, as normal components of tissues, allows for a graded interaction with an exogenous gold compound. These factors when combined with the physical and chemical differences of soluble, colloidal or insoluble gold compounds may be expected to result in characteristic individual patterns of tissue localization. Whether the attainment of a controlled and selective gold deposition by a given carrier can bring about an improved therapeutic response to gold

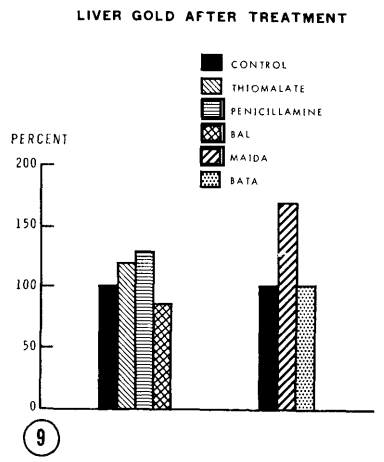
with a simultaneously decreased toxicity remains to be established.

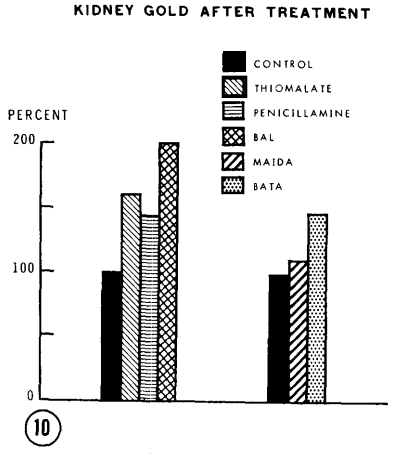
The present study has been patterned on earlier work of Block *et al*(11,12). Our results for gold distribution and excretion following gold thioglucose show excellent agreement with these earlier workers. They reported a daily urinary excretion of between 10-15% of the administered dose. Our data provide a daily average of 10.5%. Block *et al* found a fecal gold excretion of 19.6% of the cumulative dose; we obtained 16.2%. Our results on soft tissue deposition also follow those of the earlier authors. The highest concentration of gold was deposited in kidney, with spleen, liver and lung following in that order. It may be noted that while the gold concentration in spleen is higher than that in liver, the greater weight of the latter results in deposition of a larger percentage of the injected dose in this organ. The carriers MAIDA and BATA brought about a modified gold distribution compared to thioglucose. Possibly because of the increased solubility and diffuseability of gold resulting from binding to MAIDA urinary excretion is enchanced and deposition of the metal in spleen, liver, lung and kidney is increased. The less soluble Au BATA compound showed a uniformly low level of gold in the analyzed tissues and excreta. While the point was not proven by analysis, we suspect that the compound accumulated at the local injection sites. These results and interpretations are in keeping with analogous data reported for gold thioglucose and the less soluble gold calcium thiomalate.

Few agents are available to induce the mobilization and excretion of injected gold. When gold toxicity occurs during therapy the usual regime is to cease further administration of the gold compound and treat with BAL. Since enhancement of urinary gold following treatment with BAL is relatively slight, it may be presumed that part of the observed amelioration of toxic effects is as much a consequence of gold redistribution as enhanced excretion. Results obtained in the present study are in keeping with such a view. Urinary gold excretion is increased by all compounds except MAIDA. Tissue distribution is modified by all. Gold mobilized from spleen (Fig. 7) and lung (Fig. 8) by penicillamine, BAL and thiomalate, may be partially redeposited in liver and kidney during transport into the urine. This could account for the observed increased concentration in these organs. The relation between renal excretion and kidney deposition is more evident in Fig. 11. When treatment resulted in enhancement in urinary gold, there was a concomitant increase in the concentration in kidney tissue. It is clear that the most useful compounds for treatment of gold toxicity are those which induce urinary gold excretion with least deposition of the element in the kidney. By this criteria, penicillamine and BATA appear to be the most effective agents since the ratio of urine/kidney gold is highest for these compounds. However, the absolute amount of gold mobilized is also significant. In this respect penicillamine is far superior to BATA. Likewise the toxicology of the compounds and their other pharmacologic, physical and chemical characteristics need to be considered in their comparative evaluation.

Certain implications of these findings have significance for clinical problems of gold toxicity. The redistribution of gold with deposition in kidney tissue suggests that careful attention to renal function is mandatory in the chelate therapy of gold toxicity. In this respect the situation is analogous to the calcium disodium ethylenediamine tetraacetate therapy of lead poisoning. Deleading with this agent may be accompanied by increased kidney deposition simultaneously with enhancement of urinary lead excretion.

Our studies on the mobilization of predeposited gold are difficult to compare to other reports in the literature since variability in the experimental design markedly affects the results. In a previous evaluation, for example, of the effect of MAIDA and BATA on the excretion of lead and mercury in rats(13) some of these variables became evident. The elapsed time between deposition of the element and initiation of therapy modified the ability of these compounds to mobilize the elements. The longer this interval, the less effective was the therapeutic response. It may





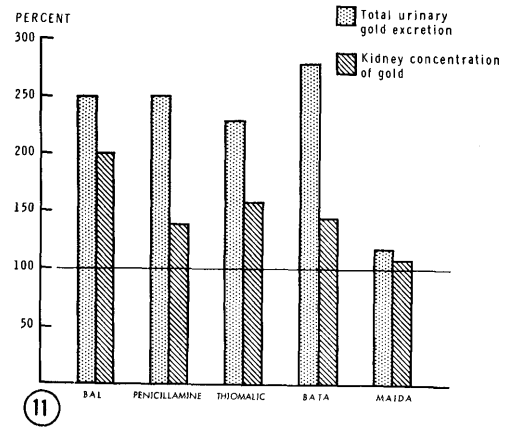


FIG. 9. Effect of chelate treatment on liver gold administered as gold thioglucose.

Fig. 10. Effect of chelate treatment on kidney gold administered as gold thioglucose.

FIG. 11. Relation of urinary and kidney gold concentration after chelate treatment compared to untreated controls (100%) after gold thioglucose administration.

be presumed that metabolic turnover in the interval prior to treatment results in both excretion and redeposition of the metal in less readily accessible tissue receptors. Equally critical was the effect of the quantity of element administered. The frequently observed "threshold effect" by which trace quantities of injected cations have a differential distribution compared to larger amounts is a significant factor influencing later mobilization efforts. Thus the mobilization of predeposited mercury by various-SH containing chelating compounds results in increased liver accumulation at low but not at a high mercury dosage. Redeposition in kidney tissue was variably influenced by the dose. Effects such as these may be the basis of the difference between our results and those recently reported by Davidson and Engleman concerning the effect of penicillamine on kidney gold concentration (15). While the total gold administration in the two studies was the same (14 mg/rat) the latter study involved a 28-day injection period compared to 14 days in the present work. Because of the urinary and fecal excretion during the longer period of gold administration, the body burden of gold at time of initiation of penicillamine therapy was significantly less in the study of Davidson and Engleman. In addition these authors sacrificed their animals after 28 days of treatment compared to 5 days in this study, and penicillamine was administered *per os* in contrast to the intramuscular route we have used. The combination of these variables may account for the fact that whereas we observed an increase in kidney gold after penicillamine therapy, the earlier study noted the opposite result.

Summary. Thermal neutron activation has been applied to the analysis of gold in biological samples. A convenient procedure is described for measurement of gold with a standard deviation of $\pm$ 5% in the range of 0.05 to 40.0 μg/ml. The tissue distribution and excretion of gold injected in rats is dependent upon the nature of the gold carrier. Mobilization of preinjected gold by various agents may enhance excretion but causes simultaneous redistribution of the metal in tissues. D,L-penicillamine, the most effective

of a group of -SH containing gold binding compounds studied, decreased the gold content of spleen and lung but caused an increased gold deposition in liver and kidney during mobilization of predeposited tissue gold.

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A Simple Method for the Production of Anuria in Mice.* (31726)

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Anuric animals are presently prepared by bilateral nepherectomy (BLN), a time-consuming and difficult procedure which involves major surgery. The purpose of this report is to describe a rapid and simple means of preparing mice whose kidneys do not elaborate urine. Anuria is produced without major surgery by simple penile ligation (PL). PL-induced anuria is compared to BLN-induced anuria in respect to completeness of the anuric state, survival time, and digitoxin toxicity.

Methods. Groups of 10-20 male Swiss Webster-type mice weighing 25-40 g were anesthetized with ether and bilaterally nephrectomized using the dorsal approach as described by Farris and Griffith(1). Other mice were anesthetized for equal time periods during which the penis was extruded by gentle pressure on both sides of the mons pubis and ligated with 000 silk suture materials. The penis was the retracted by lifting the skin on both sides of the mons pubis. BLN and PL sham operated animals were prepared as controls. Digitoxin (K & K Laboratories, lot 53375) was administered intraperitoneally in 0.1 ml of 47.5% ethanol in dosages of 1.25 and 2.5 mg/kg to BLN, BLN-sham, PL, and PL-sham mice 2 hours after surgery; lethality was determined 12 hours after digitoxin treatment. Phenolsulfophthalein (PSP), 1 mg/kg, was administered intravenously. Urine collected from the bladder by aspiration 2 hours after BSP administration was treated with 9 volumes of 0.1 N NaOH and the absorbency determined on a Bausch and Lomb Spectronic 20 spectrophotometer at 560 mμ (2). Bladders of mice which succumbed during experiments were examined soon after death; survivors were sacrificed and bladders examined at termination of experiments. Median time to death (LT50) was calculated by the Litchfield method(3). Digitoxin lethality data were treated by the binomial expansion method(4) and the PSP data by Students "t" test. The $P_{0.05}$ level of significance was used.

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