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# DISCRIMINATORY EFFECTS OF GOLD COMPOUNDS AND CARRIERS ON MITOCHONDRIA ISOLATED FROM DIFFERENT TISSUES

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**Abstract**—Four different gold compounds and three gold carriers were used to study the toxicity of gold salts on mitochondria isolated from different tissues. Each of the following compounds—aurothiomalate (ATM), thiomalate (TM), aurothiosulfate (ATS), thiosulfate (TS), aurothioglucose (ATG), thioglucose (TG), and gold chloride (GC)—was tested with mitochondria extracted from rabbit (RbLM) or rat liver (RLM), and from rabbit bone marrow (RbMM) or chloroma tumor (CM). The toxicity of these compounds and carriers on mitochondria was evaluated by determining the inhibition of oxidative phosphorylation as measured by oxypolarography. With glutamate as substrate, mitochondria from hematopoietic tissues (e.g. RbMM and CM) showed a very high sensitivity to low concentrations of ATM, while liver mitochondria (RbLM and RLM) were slightly or not at all affected by those concentrations. Such difference of sensitivity was not observed when ATS, ATG or GC was used. Thus, identical concentrations of ATS or GC were needed to inhibit the oxidative phosphorylation in all mitochondria, and concentrations up to 2500 $\mu$M ATG were without effect on any of the mitochondria studied. While with glutamate ATM and TM primarily inhibited transition state 4/state 3, suggesting the involvement of site 1 in the respiratory chain, ATS, ATG and GC appeared to have the same effect on mitochondria oxidizing either glutamate or succinate. Furthermore, whereas the inhibitory effect of ATS and GC could be prevented or released by cysteine, inhibition by ATM could not. The interaction of the gold compounds and carriers with the highly reactive thiol groups involved in the energy conservation mechanism is discussed.

The administration of gold compounds in the treatment of rheumatoid arthritis has been known for over 50 years [1]. Although accumulating evidence supports their beneficial effects [2–4], disagreements as to their efficacy and incidence of toxicity exist. Hematologic manifestations of toxicity, though relatively rare, can be most serious in the form of aplastic anemia [5–7]. The gold compound aurothiomalate (ATM)‡ has been reported to inhibit *in vitro* myeloid colony formation from human bone marrow [8], *in vitro* lymphocyte responses to mitogens [9], and mononuclear cell responses [10]. Moreover, clinical evidence favors the concept of individual sensitivity to gold compounds [5, 11, 12], but a possible additional factor in their toxicity is that the gold is significantly retained in reticuloendothelial cells, liver, spleen, kidney, and bone marrow [13–17].

The manner in which gold compounds exert their therapeutic effects is poorly understood. However, the prevailing hypothesis suggests that these drugs neutralize and inactivate some lysosomal acid hydrolases which are found in high levels in rheumatoid synovial fluid and presumably perpetuate the inflammatory process [18–20]. Likewise, the harmful side effect of gold compounds at the subcellular level is not clear. Only a few reports have suggested that mitochondria are the target organelles since they appeared to concentrate the gold [21, 22]; uncoupling of oxidative phosphorylation in liver mitochondria oxidizing succinate has also been reported [23].

In the present study we compared the effects of a number of gold compounds and some of their carriers on the metabolism of mitochondria isolated from hematopoietic tissues, liver and kidney. The results indicate that mitochondria of hematopoietic cells are distinctly sensitive to low concentrations of the gold compound ATM. In addition, our studies demonstrate diversity in the interaction of the various gold compounds with mitochondria from different tissues. The reactivity of gold compounds with mitochondrial-SH is discussed.

## MATERIALS AND METHODS

Aurothiomalate was purchased from the Aldrich Chemical Co., Atlanta, GA; thiomalate, aurothioglucose, thioglucose and thiosulfate were from the Sigma Chemical Co., St. Louis, MO; aurothiosulfate and gold potassium chloride were from Pfaltz & Bauer, Stamford, CT. All gold compounds and their

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‡ Abbreviations: ATM, aurothiomalate; TM, thiomalate; ATS, aurothiosulfate; TS, thiosulfate; ATG, aurothioglucose; TG, thioglucose; GC, gold chloride; RbMM, rabbit bone marrow mitochondria; CM, chloroma mitochondria; RbLM, rabbit liver mitochondria; RLM, rat liver mitochondria; DNP, 2,4-dinitrophenol; Cys, cysteine; and C, control.

carriers were freshly prepared prior to each experiment. Other reagents were from the Sigma Chemical Co. or were of analytical grade.

*Preparation of mitochondria.* Liver mitochondria from Sprague–Dawley rats (RLM) were isolated as previously described [24]; bone marrow (RbMM) and liver (RbLM) mitochondria from adult New Zealand white rabbits were prepared as described in Ref. 25; and mitochondria from chloroma (CM) tumors grown in Long Evans rats were isolated as described in Ref. 26.

*Measurement of oxidative phosphorylation.* Mitochondrial respiration was determined by oxypolarography in a double-jacketed cell with a chamber of 2 ml capacity at 30°. A Clark oxygen electrode and an oxygen monitor (Yellow Springs Instruments Co., Yellow Springs, OH) attached to a "Linear 255" recorder were used to determine oxygen consumption. The incubations were carried out in a 2 ml reaction medium consisting of either 25 mM glycylglycine, 40 mM KCl, 150 mM sucrose, and 1 mg/ml of bovine serum albumin (for rabbit liver and bone marrow, and also chloroma mitochondria), or 24 mM glycylglycine, 60 mM KCl, and 87 mM sucrose (for rat liver mitochondria), adjusted to pH 7.4 with KOH. Metabolic states were determined according to Chance and Williams [27]. Uncoupled state is the state of mitochondria in the presence of an uncoupler such as 2,4-dinitrophenol. The solubility of oxygen in the air-saturated reaction medium was considered to be 445 ng atoms/ml at 30°.

*Protein determination.* Mitochondrial protein was determined by the biuret method [28], using bovine serum albumin (fraction V) as a standard.

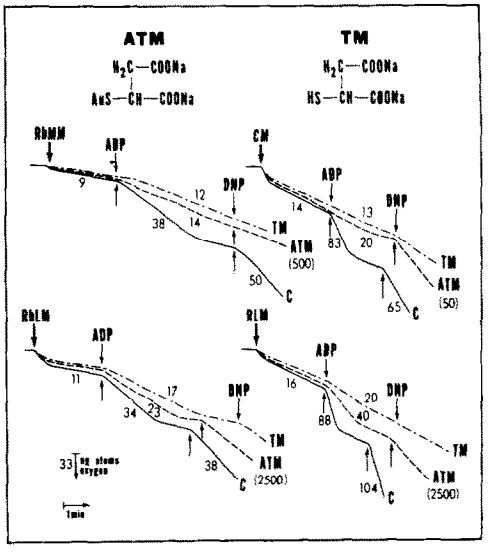


Fig 1. Effects of aurothiomalate (ATM) and thiomalate (TM) on the oxidative phosphorylation of rabbit bone marrow (RbMM), chloroma (CM), rabbit liver (RbLM), and rat liver (RLM) mitochondria, as determined by oxypolarography. Freshly isolated mitochondria of each tissue (1 mg protein) were incubated at 30° in 2 ml of the reaction medium described in Materials and Methods, with 10 mM glutamate as substrate, 5 mM phosphate (control C), and with either ATM or TM added to the medium prior to the mitochondria. ADP (0.1 mM) and DNP (0.18 mM) were added as indicated. Numbers in parentheses indicate ATM concentration in μM; TM concentration was equal to that of ATM used with the same mitochondria. The numbers along the traces give the initial rates of respiration in ng atoms $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$. The formula of ATM and TM is shown.

## RESULTS

*Effect of gold compounds and carriers on mitochondria oxidizing glutamate.* All isolated mitochondria used in this study had well coupled oxidative phosphorylation activity demonstrated by their ability to increase their respiration in response to either ADP or DNP (see control tracings in Fig. 1).

Figure 1 depicts representative oxypolarographic tracings of mitochondria from different tissues preincubated for 2.5 min with equivalent concentrations of either gold compound ATM or gold carrier TM. While state 4 of respiration was slightly affected by ATM and TM, state 3 of mitochondria isolated from hematopoietic tissues, e.g. RbMM and CM, appeared to be largely or totally inhibited by relatively low concentrations of both ATM and TM, with CM being the most sensitive. On the contrary, respiratory state 3 of liver mitochondria from two different species, e.g. RbLM and RLM, was more resistant to the inhibitory effect of ATM [rabbit kidney cortex mitochondria behaved like RbLM (data not shown)]. Thus, at higher concentration (up to 50-fold), ATM could only partially inhibit state 3 in RbLM and RLM. At this concentration, TM was shown to be more effective than ATM when used with the same mitochondrial preparation. On the other hand, as shown in Fig. 1, the uncoupled state as triggered by DNP addition was affected differently by ATM and TM, indicating presumably different degrees of damage to the mitochondrial membranes.

Figure 2 shows the effects of another gold compound, ATS, and its carrier, TS, on the same mitochondria cited above. Unlike ATM, ATS in the range of 200–500 μM showed (1) slight uncoupling of state 4 in mitochondria of hematopoietic tissues (RbMM and CM) and in RLM; (2) in all mitochondria tested, ATS inhibited state 3 of respiration and (3) in all cases, ATS did not affect the uncoupled state. Moreover, in contrast to TM, the use of TS at the same concentrations as ATS did not produce any inhibitory effect on the metabolic states of any of the mitochondria studied.

ATG or its gold carrier TG at high concentration (up to 2500 μM) caused slight or no inhibition of the different respiratory states in the tested mitochondria (Fig. 3). However, a concentration of only 50 μM of another gold compound, GC, which does not have any thio-group, caused various degrees of uncoupling in the different mitochondria followed by inhibition of respiration blocking further response of the organelles to ADP or DNP (Fig. 4).

*Differential effects of gold compounds on mitochondria of different tissues.* Figure 5 compares the percent inhibition of state 4/state 3 transition in CM, RbMM, RbLM and RLM by a 50 μM concentration of either ATM or TM. Mitochondria isolated from

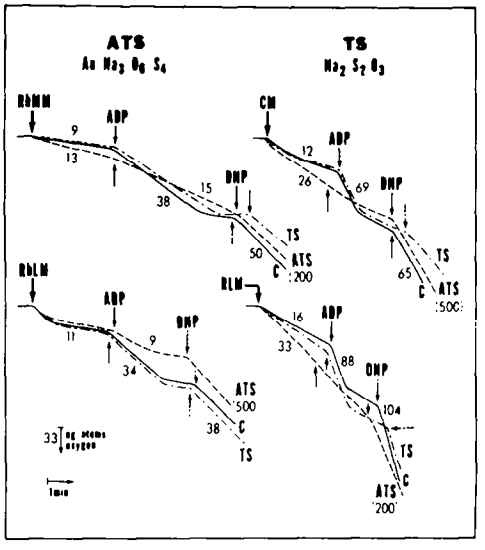


Fig. 2. Effects of aurothiosulfate (ATS) and thiosulfate (TS) on the oxidative phosphorylation of mitochondria isolated from different tissues. Experimental conditions and representations were as in the legend to Fig. 1.

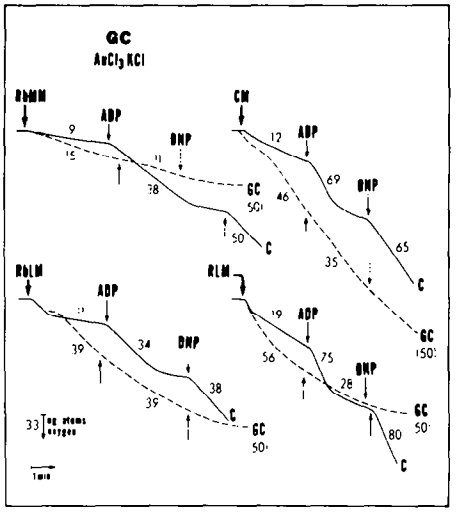


Fig. 4. Effect of gold chloride (GC) on the oxidative phosphorylation of mitochondria isolated from different tissues. Experimental conditions and representations were as in the legend to Fig. 1.

hematopoietic tissues were highly sensitive to ATM and TM as compared to liver mitochondria.

Table 1 depicts the concentrations of different gold compounds required to cause 50 per cent inhibition of the state 4/state 3 transition. Again, ATM at low concentrations was shown to be a potent ibhibitor of ATP formation in mitochondria of hematopoietic tissues, whereas 50–250 $\mu$M ATS produced a similar degree of inhibition in mitochondria from both liver and hematopoietic tissues. ATG in concentrations up to 2500 $\mu$M had no effect. In contrast, GC in concentrations lower than 25 $\mu$M was inhibitory in all mitochondria.

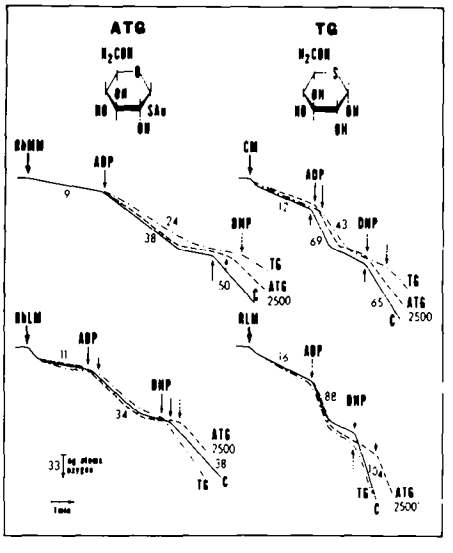


Fig. 3. Effects of aurothioglucose (ATG) and thioglucose (TG) on the oxidative phosphorylation of mitochondria isolated from different tissues. Experimental conditions and representations were as in the legend to Fig. 1.

*Effects of gold compounds and carriers on mitochondria oxidizing succinate.* In sharp contrast to what was observed in mitochondria oxidizing glutamate (Fig. 1), when succinate was used as substrate, CM were more resistant to ATM (Fig. 6) e.g. a 50-fold increase in concentration of ATM failed to block state 4/state 3 transition, although uncoupling of state 4 was observed. Furthermore, up to 1200 $\mu$M TM did not affect CM (data not shown), but at the high concentration of 2500 $\mu$M it became inhibitory to all metabolic states. Since inihibitory concentrations of ATM and TM do not block glutamate transporter system as measured by mitochondrial swelling in isotonic ammonium glutamate (data not shown), these results suggest that the inhibitory effect of the gold compound ATM and its carrier TM may be affecting site 1 of the mitochondrial respiratory chain. On the other hand, the use

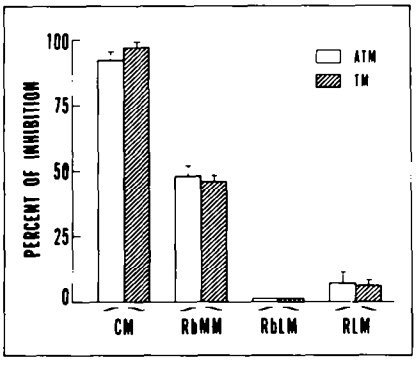


Fig. 5. Influence of the same concentration of ATM or TM on mitochondria from different tissues as determined by the inhibition of state 4/state 3 transition. Conditions were as in the legend to Fig. 1. ATM or TM (50 $\mu$M of either) was added to the reaction medium prior to the mitochondria. Values are means ± S.E.

Table 1. Gold compound concentrations required for 50 per cent inhibition of the transition state 4/state 3 of mitochondria from different tissues*

| | ATM | ATS | ATG | GC |
|---|---|---|---|---|
| CM | 25 | 250 | †NI | <25 |
| RbMM | 50 | 50 | NI | <25 |
| RbLM | >2500 | 250 | NI | <25 |
| RLM | 1000 | 50 | NI | <25 |

* Experimental conditions were as described in legend of Fig. 1. Abbreviations: ATM, aurothiomalate; ATS, aurothiosulfate; ATG, aurothioglucose; GC, gold chloride; CM, chloroma mitochondria; RbMM, rabbit bone marrow mitochondria; RbLM, rabbit liver mitochondria; and RLM, rat liver mitochondria. Concentrations are expressed in μM.

† ATG, tested up to 2500 μM, had no inhibitory (NI) effect.

of ATS, ATG, GC, and the respective gold carriers TS and TG (Fig. 5) gave results similar to those observed with glutamate as substrate (cf. Figs. 2, 3 and 4).

In liver mitochondria also (Fig. 7), 2500 μM ATM did not inhibit the respiratory state 3 with succinate oxidation, while the same concentration was over 50 per cent inhibitory with glutamate (cf. Fig. 1). The effects of the other gold compounds and carriers on RLM oxidizing succinate were similar to those with glutamate (cf. Figs. 2, 3 and 4), with the exception that ATS inhibited the uncoupled state in RLM (Fig. 7).

*Prevention and release of the inhibitory effects of gold compounds.* As shown above, 50 μM ATM or TM totally inhibited state 3 of CM. Figure 8 shows that the addition of cysteine (up to 10-fold the inhibitory concentration of ATM) to CM after preincubation with ATM or TM (Fig. 8, B and D), or prior to it (Fig. 8, C and E), neither released nor prevented the inhibitory effect of either compound. Similar results were obtained with RLM (data not shown).

However, cysteine both prevented and released the inhibitory action of ATS on the transition state 4/state 3 of RLM (Fig. 9, B and D). On the other hand, cysteine prevented (Fig. 9C) but did not release (Fig. 9E) the inhibition produced by GC.

## DISCUSSION

Despite some of their serious side effects, gold compounds continue to enjoy wide clinical use. The total dosage employed varies widely among physicians. Retention of gold in body tissues occurs often and may vary from one patient to another. Tissue levels as high as 250 μg gold/ml have been reported [15]. Blood gold levels of 50–350 μg% (μg/100 ml)

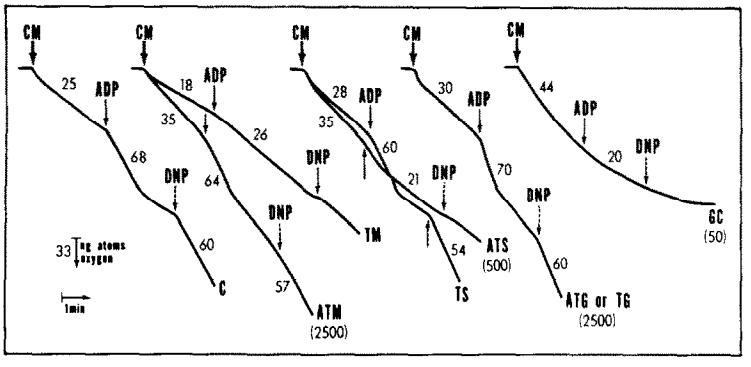


Fig 6. Effects of different gold compounds on chloroma mitochondria when oxidizing succinate. Conditions were as in the legend to Fig 1, with 10 mM succinate replacing glutamate.

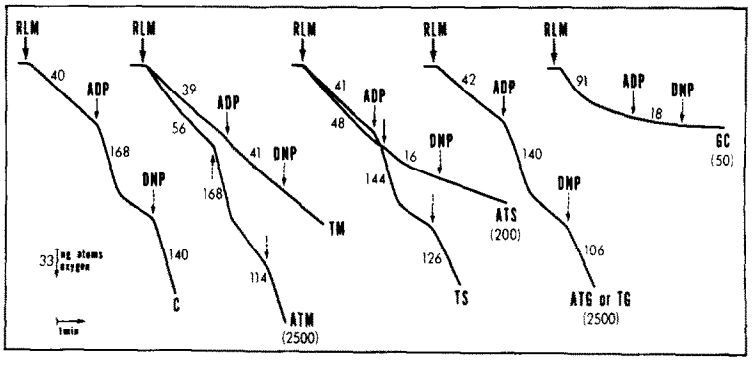


Fig. 7. Effects of different gold compounds on rat liver mitochondria when oxidizing succinate. Conditions were as in the legend to Fig. 6.

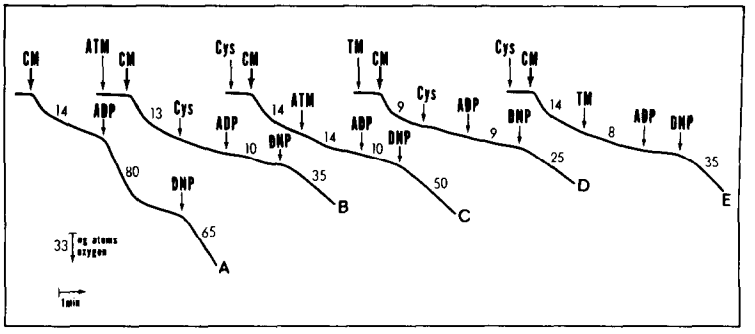


Fig. 8. Irreversible inhibition by ATM and TM in chloroma mitochondria. Conditions were as in the legend to Fig. 1. ATM or TM (50 $\mu$M of either) and cysteine (Cys) (500 $\mu$M) were added as shown.

and plasma levels of up to 450 $\mu$g% have been reported [29, 30]. It is thought that a gold serum level of 300 $\mu$g/100 ml must be maintained to effect a clinical response to chrysotherapy [31].

One of the serious complications of gold therapy is injury to the hematopoietic tissue, resulting in blood dyscrasias ranging from a single cytopenia to complete bone marrow aplasia and pancytopenia. Our studies were designed to address the question of their toxicity, its mechanism, and possible selectivity for hematopoietic tissue. The results have demonstrated clearly that some of the gold preparations are very toxic to oxidative phosphorylation as studied *in vitro*. Among these, GC, ATS, and ATM exerted various degrees of injury to mitochondria even at low concentrations, i.e. less than 10 $\mu$g gold/ml in the case of GC. Other preparations, such as ATG (up to 492 $\mu$g gold/ml), were relatively nontoxic. The difference in the degree of inhibition by ATM or TM (see below) of mitochondria oxidizing glutamate versus succinate, and the ineffectiveness of these compounds to block the glutamate transporter, indicated that ATM may act essentially at the level of site 1 in the respiratory chain. No specific site could be assigned to ATS or GC.

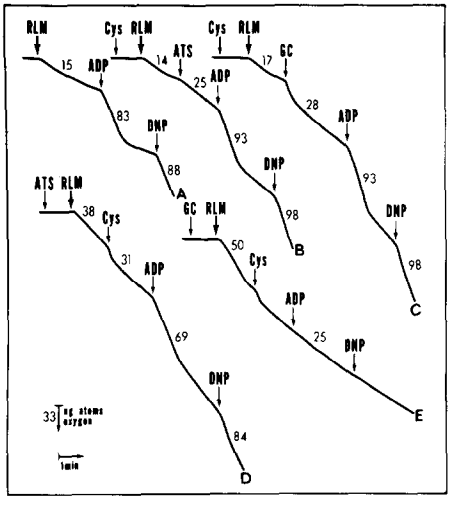


Fig. 9. Prevention and release of the inhibition caused by ATS and GC in rat liver mitochondria. Conditions were as in the legend to Fig. 1. ATS (200 $\mu$M), GC (50 $\mu$M) and cysteine (Cys) (1000 $\mu$M), were added as indicated.

An interesting finding in this study was that the gold compound ATM exerted different degrees of inhibition in mitochondria isolated from different tissues. Thus, mitochondria of hematopoietic cells were found to be very highly sensitive to ATM as compared to mitochondria of liver or kidney. The biochemical basis for this difference is not clear. Of equal importance is that, in order to inhibit the oxidative phosphorylation in mitochondria (CM, for example), different amounts of each gold compound were required (cf. Figs. 1–4). Moreover, our data demonstrated that, if mitochondria were oxidizing succinate, all gold compounds tested are capable of various degrees of uncoupling. This is in agreement with previous results [23]. However, with glutamate as oxidizable substrate, the uncoupling activity, assumed to be a general property of gold compounds, was not always evident (cf. Figs. 1–4).

Since gold compounds were first used, abundant evidence has indicated that gold can react with -SH groups of different proteins [32–35]. The implication of thiols in the energy conserving mechanism has been well demonstrated [36–38], and the number of the very reactive thiols involved in that process has been determined [39, 40]. Moreover, cadmium ions were reported to uncouple oxidative phoshorylation by binding a dithiol function in mitochondria [41], and mitochondrial-SH groups were suggested to play an important role in the uncoupling activity of non-steroidal acidic anti-inflammatory drugs [42]. Accordingly, in our experiements the use of gold carriers (with no gold atom) and cysteine provided us with basic information on the mode of action of each gold compound on mitochondrial functions. The inhibition observed with the use of ATS in all mitochondria, whereby this inhibition was either released or reversed by the addition of cysteine on the one hand, and the total ineffectiveness of the carrier TS on oxidative phosphorylation on the other hand, are clear indications that the inhibition caused by ATS is mediated by gold-SH interactions, with gold being rapidly dissociated into its ionic form. This also appears to be the case with GS, since cysteine was able to protect its inhibitory effect. However, the inability of cysteine to reverse the inhibition of GC could be related somehow to the auric form of gold in this compound.

Surprisingly, the mode of action of ATM seems to follow another pathway. In all cases, the gold-free compound TM gave more inihbition than ATM, suggesting that the inhibitory effect of the latter was in fact produced by the thiomalic moiety of the molecule. This suggestion is also favored by the inability of cysteine to protect or release that inhibition. Thus, TM may act as a potent inhibitor of oxidative phosphorylation with glutamate as substrate, while at higher concentrations it becomes inhibitory with succinate (cf. Figs. 1, 6 and 7). Furthermore, ATG, which is known to be less toxic than other gold compounds [30, 43], and the gold-free TG did not affect any of the mitochondria studied. In the light of these data, it is suggested that, unlike the case of ATS and GC, the dissociation of gold from ATM and ATG may not have occurred under the conditions of our experiments. Thus, while ATG did not produce any toxicity at the mitochondrial, level, the toxic effect of ATM was mostly caused by the carrier of gold, which requires detailed study to elucidate its molecular mechanism.

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## REFERENCES

1. M. J. Forestier, *Bull. Mém. Soc. méd. Hôp.* **8**, 323 (1929).
2. R. H. Freyberg, in *Arthritis and Allied Conditions* (Eds. J. L. Hollander and D. J. McCarty, Jr.), p. 455. Lea & Febiger, Philadelphia (1972).
3. P. Davis, *J. Rheumatol.* (Suppl. 5) **6**, 18 (1979).
4. D. M. Sagransky and R. A. Greenwald, *J. Rheumatol.* **7**, 477 (1980).
5. M. M. Wintrobe, A. Strowell and R. M. Roll, *Am. J. med. Sci.* **197**, 689 (1939).
6. D. J. McCarty, J. M. Brill and D. Harrop, *J. Am. med. Ass.* **179**, 161 (1962).
7. J. L. Baldwin, R. Storb, E. D. Thomas and M. Mannik, *Arthritis Rheum.* **20**, 1043 (1977).
8. A. Howell, J. M. Gumpel and R. W. E. Watts, *Br. med. J.* **1**, 432 (1975).
9. M. Harth and C. R. Stiller, *J. Rheumatol.* (Suppl. 5) **6**, 103 (1979).
10. M. Harth and C. R. Stiller, *J. Rheumatol.* (Suppl. 5) **6**, 112 (1979).
11. N. L. Gottlieb, P. M. Smith and E. M. Smith, *Arthritis Rheum.* **15**, 582 (1972).
12. N. L. Gottlieb, *J. Rheumatol.* (Suppl. 5) **6**, 51 (1979).
13. R. H. Freyberg, W. D. Block and S. Levey, *J. clin. Invest.* **20**, 401 (1941).
14. W. D. Block, D. H. Buchanan and R. H. Freyberg, *J. Pharmac. exp. Ther.* **82**, 391 (1944).
15. N. L. Gottlieb, P. M. Smith and E. M. Smith, *Arthritis Rheum.* **15**, 16 (1972).
16. P. M. Smith, E. M. Smith and N. L. Gottlieb, *J. Lab. clin. Med.* **82**, 930 (1973).
17. N. L. Gottlieb, *J. Rheumatol.* (Suppl. 5) **6**, 2 (1979).
18. R. H. Persellin and M. Ziff, *Arthritis Rheum.* **9**, 57 (1966).
19. R. S. Ennis, J. L. Granda and A. S. Posner, *Arthritis Rheum.* **11**, 756 (1968).
20. F. N. Ghadially, *J. Rheumatol.* (Suppl. 5) **6**, 45 (1979).
21. J. Stuve and P. Galle, *J. Cell Biol.* **44**, 667 (1970).
22. R. Yarom, H. Stein, P. D. Peters, S. Slavin and T. A. Hall, *Archs Path.* **99**, 36 (1975).
23. M. W. Whitehouse, *Biochem. J.* **92**, 36P (1964).
24. S. Abou-Khalil, W. H. Abou-Khalil and A. A. Yunis, *Biochem. Pharmac.* **29**, 2605 (1980).
25. S. Abou-Khalil, Z. Salem and A. A. Yunis, *Am. J. Hemat.* **8**, 71 (1980).
26. S. Abou-Khalil, Z. Salem, W. H. Abou-Khalil and A. A. Yunis, *Archs Biochem. Biophys.* **206**, 242 (1981).
27. B. Chance and G. R. Williams, *Nature, Lond.* **175**, 1120 (1955).
28. G. Allan, A. G. Gornall, C. J. Bardawill and M. M. David, *J. biol. Chem.* **177**, 751 (1949).
29. B. R. Mascarenhas, J. L. Granda and R. H. Freyburg, *Arthritis Rheum.* **15**, 391 (1972).
30. N. O. Rothermich, V. Philips, W. Bergen and M. H. Thomas, *Arthritis Rheum.* **19**, 1321 (1976).
31. A. Lorber, C. J. Atkins, C. C. Chang and Y. B. Lee, *Ann. rheum. Dis.* **32**, 133 (1973).
32. L. Libenson, *Expl. Med. Surg.* **3**, 146 (1945).
33. D. A. Gerber, *J. Pharmac. exp. Ther.* **143**, 137 (1964).
34. C. J. Danpure, *Biochem. Soc. Trans.* **2**, 899 (1974).
35. D. F. Biggs, D. M. Boland, P. Davis and J. Wakaruk, *J. Rheumatol.* (Suppl. 5) **6**, 68 (1979).
36. C. Cooper and A. L. Lehninger, *J. biol. Chem.* **224**, 561 (1957).
37. A. Fluharty and D. R. Sanadi, *Proc. natn. Acad. Sci. U.S.A.* **46**, 608 (1960).
38. W. W. Kielley, in *Intracellular Respiration, Proceedings of the Fifth International Congress of Biochemistry, Moscow* (Ed. E. C. Slater), Vol. 5, p. 378. Pergamon Press, New York (1963).
39. D. C. Gautheron, *Biochimie,* **55**, 727 (1973).
40. S. Abou-Khalil, N. Sabadie-Pialoux and D. Gautheron, *Biochem. Pharmac.* **24**, 49 (1975).
41. A. L. Fluharty and D. R. Sanadi, *Biochemistry* **2**, 519 (1963).
42. J. P. Famaey and J. Mockel, *Biochem. Pharmac.* **22**, 1487 (1973).
43. J. S. Laurence, *Ann. rheum. Dis.* **35**, 171 (1976).