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Chemotherapy of Chronic Progressive Arthritis of Mice. I. Rôle of Sulfur in Gold-Containing Compounds.

WILLIAM S. PRESTON, WALTER D. BLOCK AND R. H. FREYBERG.
(Introduced by M. H. Soule.)

*From the Rackham Arthritis Research Unit, the Medical School, University of Michigan, Ann Arbor.**

The apparent effectiveness of gold-containing compounds in the treatment of rheumatoid arthritis has stimulated interest in the mechanism by which these substances may act to relieve the symptoms of or arrest the course of this disease. Investigation of this problem is difficult because of the chronicity of the disease, lack of information concerning its etiology, and the inability to reproduce it in experimental animals. The infectious arthritis of mice described by Sabin[1] provides a tool by which certain aspects of the problem may be approached, since this experimental disease resembles rheumatoid arthritis in being definitely responsive to treatment with gold-containing compounds.

It seemed pertinent to determine whether compounds containing gold might be therapeutically effective because of properties other than those conferred by the presence of gold, especially since the undesirable effects which frequently accompany their use are believed to be due to the toxicity of this metal. Since most of the compounds used in therapy have contained sulfur in addition to gold, the question arose concerning the importance of this element, either for its own effect or in combination with gold. One of the more widely used compounds and a typical example of one containing both gold and sulfur is gold sodium thiomalate (Myochrysine). The configuration of the molecule of this compound (Table I) is such that one may conceive that the sulfur present might give rise to a sulfhydryl group on reduction. Because of the known importance of the sulfhydryl linkage in cellular physiology, it seemed possible that the therapeutic effectiveness of this and other gold- and sulfur-containing compounds might be due to the presence of this linkage in the molecule or its formation *in vivo*, rather than to the metallic moiety. The question as to the necessity of sulfur in any form was raised by Sabin[2] in his studies of the use of gold compounds in the therapy

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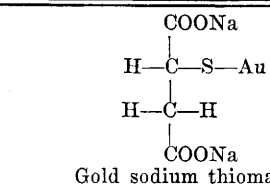
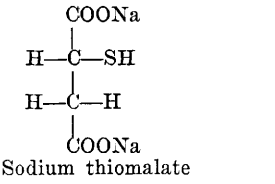
1 Sabin, A. B., *Science*, 1939, **89**, 228.

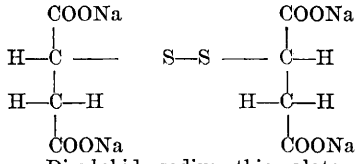
TABLE I.
Structural Formulæ of Compounds Used.



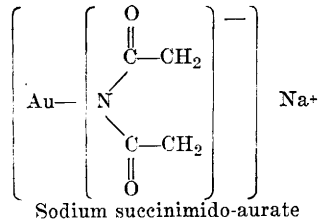
Gold sodium thiomalate



Sodium thiomalate



Disulphide sodium thiomalate



Sodium succinimido-aurate

of the mouse disease; he believed that results with gold chloride ruled out the need of this element. However, the gold chloride was highly toxic for the mice, and it was only on the basis of the cure of the few survivors of the therapy that this conclusion was made.

It is the purpose of this paper to report results of studies of the importance of the non-metallic portion of therapeutically effective gold- and sulfur-containing compounds and the need of sulfur in any form for the action of gold. We have confirmed previous work showing the effectiveness of gold sodium thiomalate in curing the mouse disease, and since this compound is commonly employed in human therapy, the use of sodium thiomalate and its disulfide form appeared to be a satisfactory means of investigating the importance of the sulfhydryl linkage. Additional study of the rôle of sulfur in therapy was possible by the use of sodium succinimido-aurate, a compound which, in contrast to most gold preparations used to treat arthritis, has the gold linked to the rest of the molecule through nitrogen, rather than through sulfur.

Methods. Two strains of pleuropneumonia-like microörganisms were used to infect mice. One of these was isolated from the lung of a rat showing "bronchiectatic" lesions described by Klieneberger and Steabben.[3] It produces a progressive arthritis in mice indistinguishable from that caused by Sabin's type B. (Serological studies with sera kindly furnished by Dr. Sabin have shown it to be similar to or identical with his type E strains obtained from mice. It resembles the "mouse type" strains of organisms in morphology,

2 Sabin, A. B., *J. Bact.*, 1940, **40**, 823.

3 Klieneberger, E., and Steabben, D. B., *J. Hyg.*, 1940, **40**, 223.

and is apparently different from the L3 type which Klieneberger and Steabben found associated with the lung condition.) In addition, a type B strain obtained from Dr. Sabin was also used. We have found the course of this mouse disease to be essentially as described by Sabin.[2]

Therapy was begun one week after 50% of mice inoculated with 0.5 cc of 48-hour ascitic fluid-broth cultures of the organisms showed the presence of arthritis; this usually occurred within a week following injection. In each experiment, 40 mice were used; 3 groups of 10 mice each received treatment, and one group of 10 served as untreated controls; the mice in each treated group received a constant dose. Each group of treated mice received a different amount of one of the compounds (Table II) intravenously in 0.5 cc volumes on alternate days until 9 or 10 injections had been given, unless obvious cure had been obtained before. A therapeutic effect was considered to have been obtained when evidence of arthritis disappeared from at least 8 of the mice treated with one constant dose of the compound.

The compounds employed are listed in Table II. The sodium thiomalate was prepared by the method of Levene and Mikeska;[4] the disulphide form was made by the oxidation of sodium thiomalate.[5] †

TABLE II.
Results of Chemotherapeutic Experiments.

Compound	Dose		Mice cured,* %
	Compound, mg	Gold content, mg	
Gold sodium thiomalate	1.0	0.5	60
	2.0	1.0	100
	4.0	2.0	100
Sodium thiomalate	1.54	0	0
	7.68	0	0
	15.40	0	0
Disulfide of sodium thiomalate	0.74	0	0
	3.84	0	0
	7.70	0	0
Sodium succinimido-aurate	0.86	0.25	40
	1.73	0.50	60
	8.65	2.50	100

*Average of several experiments with two strains of micro-organisms.

4 Levene, P. A., and Mikeska, L. A., *J. Biol. Chem.*, 1924, **60**, 686.

5 Biilmann, E., *Leibig's Ann.*, 1906, **348**, 120.

† Gold sodium thiomalate (Myochrysine) was obtained from Merck and Company. Sodium succinimido-aurate was obtained from Inventions, Inc., Chicago, Ill.

Results. The results of the experiments, summarized in Table II, emphasize the importance of gold in this type of therapy. Even when the amounts of sodium thiomalate and the disulphide form of sodium thiomalate containing the equivalent of 10 times the maximum amount of thiomalic acid contained in the largest dose of gold sodium thiomalate were administered, no therapeutic effect was observed. It is thus obvious that no part of the therapeutic effect of gold sodium thiomalate can be explained on the basis of the action of sulfur alone or on sulfhydryl formation. In addition, the results with sodium succinimido-aurate rule out the need of sulfur in any form. These results demonstrate that, insofar as the mouse disease is concerned, the therapeutic effects are dependent on the presence of gold in the compounds employed. Further studies are being conducted with other heavy metals.

Conclusions. Arthritis of mice, caused by pleuropneumonia-like microörganisms has been treated with gold sodium thiomalate, sodium thiomalate, the disulphide form of sodium thiomalate and sodium succinimido-aurate. The compounds containing gold, gold sodium thiomalate and sodium succinimido-aurate, were effective in curing this disease, the others had no therapeutic effect. Sulfur alone or in sulfhydryl linkage is not necessary in order that gold-containing compounds have a therapeutic effect.

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Changes in Plasma Amino Acid Nitrogen Concentration Following Nitrous Oxide and Ether Anesthesia and Surgery.

LEE E. FARR, D. A. MACFADYEN, GRANT TAYLOR, A. R. SHANDS, JR., W. R. FERGUSON, E. B. DUNLAP, JR., AND CATHERINE JOHNSON.

From the Alfred I. duPont Institute of the Nemours Foundation, Wilmington, Del.

The present observations were made as part of a general study of factors influencing the concentration of amino acids in the plasma. They are reported at this time because of the growing use of amino acid mixtures intravenously during surgical procedures.

Procedures. Nine patients were observed consecutively. The age, sex and operative procedure are noted in Table I. In each instance a blood specimen was obtained after an overnight fast and immediately prior to induction of anesthesia. Subsequently a second