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INFLUENCE OF GOLD SALTS ON ADJUVANT ARTHRITIS IN THE RAT*

BY

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The Empire Rheumatism Council gold trial (1961) provided reasonably conclusive evidence that gold salts exert a suppressive effect on rheumatoid activity. This being so, the elucidation of their mode of action may be expected to provide information about the pathogenesis of the underlying disease. This prompted us to study the influence of sodium aurothiomalate injections on adjuvant disease in the rat. The polyarthritis of experimental adjuvant disease is generally regarded as being a delayed type of hypersensitivity response to disseminated mycobacterial antigen. It has been widely used for evaluating anti-rheumatic drugs (Newbould, 1963; Winter and Nuss, 1966) and immunosuppressive agents (Ward, Cloud, Krawitt, and Jones, 1964; Currey and Ziff, 1966). We report here a study of the influence of injected gold salts on both adjuvant arthritis and immune responses in the rat.

Material and Methods

Groups of six male Sprague-Dawley rats, weighing approximately 180 g. at the start of each experiment, were given a course of sodium aurothiomalate (Myocrisin) by subcutaneous injection. Different groups received 2·5, 5, and 10 mg. Myocrisin on alternate days, until a total dose of 30, 80, and 160 mg. respectively, had been reached. Control rats were given saline injections.

Fig. 1 shows the design of the experiment. Arthritis was induced by the intradermal injection into the right hind foot-pad of 0·1 ml. Freund's complete adjuvant (containing 0·6 mg. heat-killed *M. tuberculosis* in heavy mineral oil). The injection was given after the animals had been receiving gold injections for 13 days and were thus assumed to be loaded with the metal. Joints were inspected regularly and awarded a score depending on the severity of the arthritis: 0 to 2 for small joints, and 0 to 4 for large joints. The initial swelling in the injected foot (assumed to be a simple inflammatory reaction) and the later generalized arthritis in the non-injected extremities, including the tail, were scored separately.

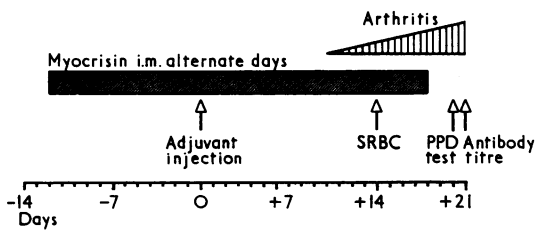

Fig. 1.—Design of the experiments.

All rats were observed for signs of toxicity to gold. They were weighed regularly, and had frequent tests for albuminuria using "Albustix" (Ames). Blood counts were performed at the start, at the time of adjuvant injection, and at the end of each experiment.

The immunological competence of the animals was assessed by measuring the primary antibody response to injected sheep erythrocytes. One ml. of a 1 per cent. suspension of sheep red blood cells (SRBC) was injected intraperitoneally on Day +14. A week later blood was taken to measure the haemagglutination titres. The delayed skin reaction to tuberculin was measured as the diameter of the area of induration produced 24 hours after the intradermal injection of 0·1 ml. 1/100 strength P.P.D. (100 units), given on Day +20.

A separate experiment was designed to study in more detail the influence of gold injections on the time of appearance and magnitude of the primary antibody response. Six rats received subcutaneous injections of 5 mg. Myocrisin daily for 6 days and controls received saline. On the fourth day all rats were given an intraperitoneal injection of 1 ml. of a 1 per cent. suspension of SRBC. During the next 2 weeks, orbital sinus blood was used to carry out daily or less frequent haemagglutination tests using the "Microtitre" method.

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Results

Fig. 2 shows the progressive mean joint scores in the groups of rats which received 160 mg. gold and in a group of controls. No difference between the groups was noted in the severity of the inflammatory reaction in the injected foot, and no suppression of the generalized arthritis occurred in those given gold. In fact, those on Myocrisin had an earlier onset of clinical disease than the controls, and the arthritis tended to be more severe in the earlier stages. The same pattern was evident, but to a less marked extent, in the groups receiving lower doses of gold.

Fig. 3 shows the unexpected pattern of weight loss which occurred in rats on the highest dose of gold. There was an early sharp fall in weight which coincided with a miserable, toxic appearance of the animals, but then, despite continued gold injections, they gained weight and their clinical condition improved strikingly. With the onset of arthritis there was a further loss of weight, appropriate to the severity of the disease and not related to gold treatment.

An increase in the proteinuria which is normal for rats was noted during the first week of gold

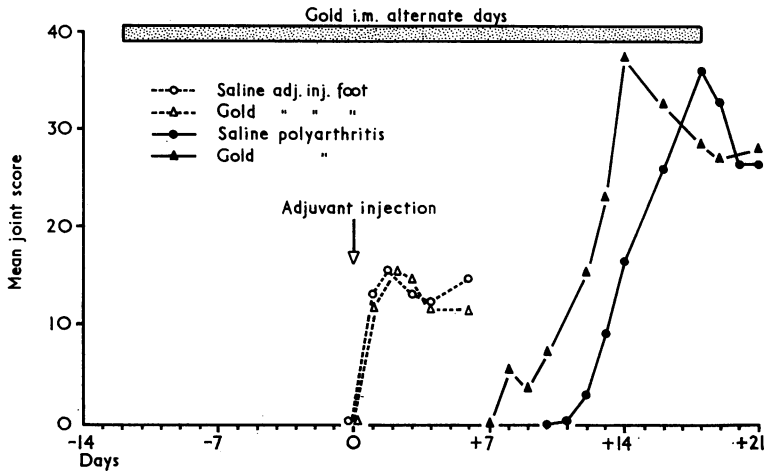

Fig. 2.—Joint scores. The interrupted lines represent early inflammation in the injected foot; the continuous lines represent the later polyarthritis in other limbs.

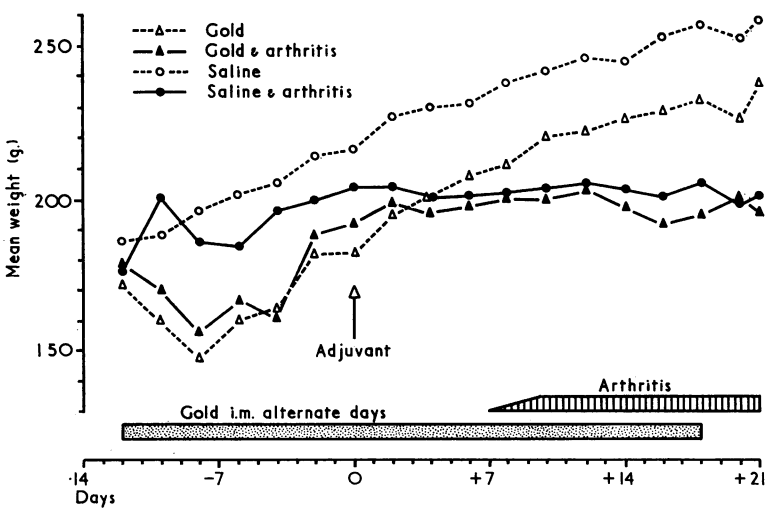

Fig. 3.—Progressive mean weights in four groups of rats. Note the early weight loss in rats given gold, followed by weight gain despite continued medication. The later failure to gain weight by rats developing arthritis is independent of gold treatment.

injections. This also cleared despite continued treatment. This pattern of weight loss followed by apparent tolerance to the metal was reproduced in a group of rats receiving the same course of gold but not given adjuvant.

Rats receiving gold showed no evidence of dermatitis, and their blood counts indicated no bone marrow suppression. No permanent increase in proteinuria resulted from the injections.

Fig. 4 shows the results of the haemagglutination titres of rats in the control group and those which received 80 and 160 mg. gold. No significant differences were noted between the titres in each group. The slightly lower figure in rats on the highest dose of gold was assumed to be a manifestation of general toxicity.

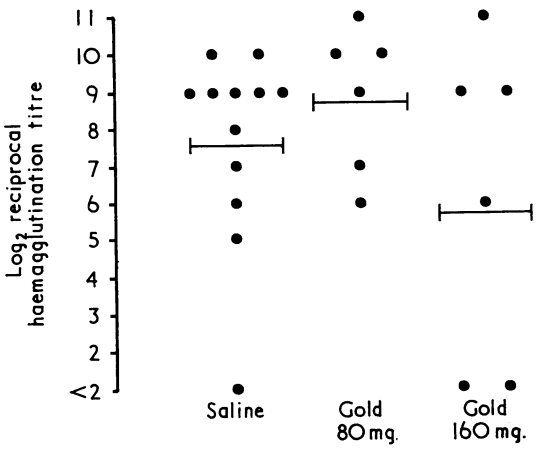

Fig. 4.—Haemagglutination titres. Each dot represents one animal. The horizontal bars indicate the mean.

The Table shows the results of P.P.D. skin tests in rats on moderate and on high doses of gold. Again, no significant differences were noted between those on gold and the controls.

The early appearance and rapid progression of arthritis in gold-treated animals had suggested that the metal might, in this situation, be exerting a (conventional) adjuvant affect. The influence of gold injections on the evolutions of the primary antibody response was therefore studied. Fig. 5 shows the progressive mean haemagglutination titres after the intraperitoneal injection of 1 ml. of a 1 per cent. sheep red cell suspension. Neither the time of onset nor the magnitude of the antibody response showed any evidence of adjuvant effect by the gold. No significant differences emerged when the responses of individual animals were analysed separately, nor when the sera were tested after pre-treatment with mercaptoethanol.

TABLE
DELAYED SKIN RESPONSE TO P.P.D.

Treatment	No. of Rats	Induration: 24 hrs. (Diam. mm.)	
		Mean	Range
Myocrisin 80 mg.	6	5·8	0—13·0
Saline	6	7·5	4·0—11·0
Myocrisin 160 mg.	6	7·1	5·0—10·0
Saline	6	10·1	8·0—13·0

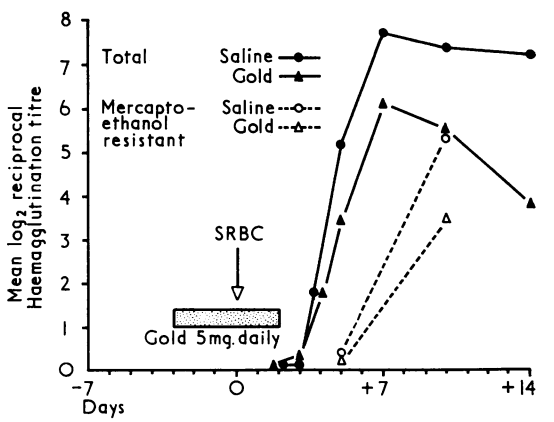

Fig. 5.—Failure of gold treatment to exert an adjuvant effect on the antibody response to injected SRBC.

Discussion

The results of these experiments show that gold salts injected into the rat do not inhibit adjuvant arthritis or suppress non-specific inflammation, and do not materially depress immune responses. This held true even in the high, near lethal, dosage range (equivalent, on a weight basis, to a course of about 40 g. in man).

Although adjuvant arthritis in the rat has been successfully treated with a variety of anti-rheumatic and immunosuppressive agents (Newbould, 1963; Ward and others, 1964; Winter and Nuss, 1966), only Newbould included gold in his experiments. He gave a group of rats a course of 5 mg. Myocrisin daily, starting one day before the injection of adjuvant and continuing for 13 days. He showed some inhibition of the arthritis on this regimen both in the injected foot and in the non-injected extremities. The fact that, in rheumatoid subjects on gold treatment, a considerable period of time may elapse before a therapeutic effect is observed persuaded us

to load our rats with gold before the injection of adjuvant, in an effort to magnify any anti-arthritic action especially in the injected foot. However, even with the near lethal doses employed, no suppression was noted. We are unable to reconcile Newbould's conclusions with our own.

Recent work by Kapusta and Mendelson (1967) has again raised the possibility that a microbial agent is concerned in the pathogenesis of adjuvant arthritis. In the past, gold salts have been found to be curative in certain forms of infective arthritis in rodents. Sabin and Warren (1940) demonstrated the curative effect of certain gold compounds on the arthritis in mice caused by pleuropneumonia-like organisms (PPLO). The mode of action of the gold salts remains unknown, as growth even of minimal inocula was not inhibited or prevented by varying concentrations of myocrisin, and micro-organisms grown for several generations in the presence of gold did not lose the capacity to produce arthritis.

The pattern of toxicity of gold compounds in rats has previously been described (Cortell and Richards, 1942 a, b; Denko and Anderson, 1944). These authors noted the development of transient renal damage, mainly tubular, and raised blood urea, followed by recovery despite continued gold injections. They attributed these findings to the development of "tolerance" to the metal within a certain dose range, and record similar findings with another heavy metal, arsenic. We think that our rats on the high dose schedule of 10 mg. Myocrisin on alternate days, showed this same phenomenon. Sections of the kidney made at the end of our experiment were apparently normal on light microscopy, apart from the presence of gold in the tubules which was demonstrated by the histochemical technique of Elftman and Elftman (1945).

There is evidence that gold accumulates at sites of inflammation both in animals (Bertrand, Waine, and Tobias, 1948; Jeffrey, Freundlich, and Bailey, 1958) and in man (Lawrence, 1961). There is also evidence that lysosomal enzymes may play an important role in the pathogenesis of joint inflammation (Weissmann, Becher, Wiedermann, and Bernheimer, 1965). This has raised the possibility that gold may act locally in inflammatory arthritis by influencing the release and activity of lysosomal enzymes in the synovial tissues. Persellin and Ziff (1966), studying lysosomal enzymes of the peritoneal macrophage, have provided some support for this theory. In a later study (Persellin, Hess, and Ziff, 1967) these authors explored the possibility that gold might exert its therapeutic effect by acting as an immunosuppressive agent. In fact, they found no evidence that gold suppressed any aspect of immune competence in rabbits. Our work in the rat is in agreement with this.

Thus, gold given to the rat, even in near-lethal doses, does not materially suppress immune responses, and will inhibit neither non-specific inflammation nor adjuvant arthritis. It would seem to follow either that adjuvant arthritis is not such a good experimental model for screening anti-rheumatic drugs as previous experience has suggested, or that gold does not, in fact, have anti-rheumatic activity.

Summary

Gold salts injected into the rat neither inhibited adjuvant arthritis nor suppressed non-specific inflammation. They did not materially depress immune responses.

DISCUSSION

DR. W. R. M. ALEXANDER (*Edinburgh*): The tidy series of experiments which you have done gives a fairly unequivocal answer within their design, but there are many factors which could explain differences in response to gold in the human and in the rat with adjuvant arthritis. There are, for example, differences in the nature of the diseases, and in the length of time for which the drug is given. As you have concluded, your results do not explain the action of gold, and I do not think this is altogether unexpected.

DR. JESSOP: I would agree except that some response had been noted by Newbould in 1963, and we were in fact starting off with the idea of repeating that experiment, the chief difference being in the timing of gold administration in relation to adjuvant reaction. Results turned out to be negative on a much larger series of rats, even with double the dose of gold used by Newbould.

DR. F. DUDLEY HART (*London*): We compared our clinical anti-inflammatory effects with drugs with Newbould's series with the same drugs in the adjuvant arthritis, and our list was more closely parallel with his with adjuvant arthritis than with any other of the animal experimental models; but I was surprised by his gold results, particularly by the rapidity with which he obtained his effect.

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Influence des sels d'or sur l'arthrite à adjuvant du rat

RÉSUMÉ

Des sels d'or injectés au rat n'inhibèrent pas l'arthrite à adjuvant et ne supprimèrent pas l'inflammation non spécifique. Ils n'abatirent pas matériellement la réponse immunitaire.

Influencia de sales de oro sobre la artritis experimental en la rata

SUMARIO

Sales de oro injectadas á la rata no inhibieron la artritis provocada por el adyuvante y no suprimieron la inflamación no específica. Tampoco reprimieron materialmente la respuesta inmunitaria.