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INACTIVATION OF C1 IN RHEUMATOID SYNOVIAL FLUID, PURIFIED C1 AND C1 ESTERASE, BY GOLD COMPOUNDS*

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SUMMARY

The effects of gold compounds on the functional activities of rheumatoid synovial fluid and normal serum C1, C4, and C2 were investigated *in vitro*. Commercial and purified gold sodium thiomalate in concentrations, as low as 1·25 μg/ml (expressed as elemental gold) inactivated native $C\bar{1}$ and highly purified $C\bar{1}s$, whereas equivalent or higher concentrations of thiomalate had no effect. $C\bar{1}$ inactivation was caused also by other gold compounds such as gold chloride and gold sodium thiosulphate. The $C\bar{1}$ inactivation was not reversible following extensive dialysis. The partial protection of C4 and C2, the two natural substrates for $C\bar{1}$, indirectly verified the $C\bar{1}$ inactivation. This is the first study to show that gold compounds inactivate $C\bar{1}$, one of the reactants in the pathogenesis and/or perpetuation of rheumatoid arthritis.

INTRODUCTION

Chrysotherapy has been shown to be beneficial for patients with rheumatoid arthritis (RA. in several carefully controlled studies (Empire Rheumatism Council, 1960; Freyberg, 1972). Since activation of the classical complement (C)§ pathway by aggregated immunoglobulins or immune complexes is thought to be one of the mechanisms through which the inflammatory mediators of the C system are generated in rheumatoid synovitis (Ruddy & Austen, 1973), an understanding of the effect of gold compounds on this pathway may help to elucidate a mechanism of action in RA. This study shows an inhibitory effect of gold compounds on $C\bar{1}$ in concentrations that are achieved *in vivo*.

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§ The nomenclature used here conforms with that agreed upon by the World Health Organization (*Bull. Wld Hlth Org.*, **39**, 935).

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MATERIALS AND METHODS

Serum

Blood was drawn aseptically from healthy adult donors by venepuncture and allowed to clot at room temperature. Serum was separated by centrifugation, divided into small aliquots, and immediately frozen at −70°C until tested.

Synovial fluid

Synovial fluid from the knee joints of seven patients with definite or classical RA according to the American Rheumatism Association criteria (Ropes *et al.*, 1959) was collected by aseptic arthrocentesis prior to chrysotherapy and handled as above.

Buffers

Glucose gelatin–Veronal buffer containing 0·00015 M Ca^{2+} and 0·0005 M Mg^{2+} ($DGVB^{2+}$), relative NaCl concentration 0·075 M, pH 7·5, was prepared as described previously (Nelson, 1968). A modification of this buffer consisted of glucose gelatin–Veronal buffer without metals ($DGVB^{2-}$).

Gold compounds

The following gold compounds and control chemicals were used: (1) gold sodium thiomalate (GST), 50 mg/ml, (Myochrysine®, Merck, Sharpe and Dohme) purchased commercially; (2) highly purified gold sodium thiomalate (lot number 64708 kindly supplied by Dr Carl Stevenson); (3) ^{195}Au-labelled sodium thiomalate (GST) (New England Nuclear, Boston, Massachusetts); (5) gold sodium thiosulphate ($AuNa_3(S_2O_3)_2.2H_2O$) (G. D. Searle and Company, Chicago, Illinois); (6) gold chloride ($HAuCl_4.3H_2O$) (Mallinckrodt, St Louis, Missouri).

Complement reagents

Functionally purified human C components, whole guinea-pig C, sheep erythrocytes (E) sensitized with rabbit antibody (A) and the stable cellular intermediates (EAC1, EAC4, EAC14) were purchased commercially (Cordis Laboratories, Miami, Florida) or were made by published techniques (Nelson, 1968).

Purified human and guinea-pig C1 were prepared by the technique of Tamura & Nelson (1968) and titrated according to standard methods (Nelson, 1968).

Human $C\bar{1}s$ was purified by the method of Sakai & Stroud (1973), and was homogeneous on immunoelectrophoresis and on analytical acrylamide gel electrophoresis.

Rabbit C1q was purified as previously described (Volanakis & Stroud, 1972). A reagent containing rabbit C1r and C1s was prepared by absorption of functionally pure rabbit $C\bar{1}$ with aggregated gamma-globulin linked to Sepharose in the presence of 0·01 M EDTA.

Monospecific antisera to rabbit C1q and C1s were prepared in goats by published methods (Volanakis & Stroud, 1972).

The functional haemolytic activities of C1, C1s, C4 and C2 in rheumatoid synovial fluid and normal serum, in the presence or absence of gold compounds, were determined by previously published techniques (Nelson, 1968; Vroon, Schultz & Zarco, 1971).

RESULTS

Effects of GST on rheumatoid synovial fluid C4

Fig. 1a shows the results of a kinetic study of the effect of GST (final concentration, 5 μg/ml as gold) on C4 haemolytic activity in synovial fluid diluted 1:100 with $DGVB^{2+}$. The percentage haemolysis of EAC1 caused by C4 is plotted versus time in minutes at 37°C. The haemolytic activity in the control mixture without gold progessively diminished until only 16% haemolysis occurred after 120 min. In the GST–synovial fluid mixture, C4 caused 61% haemolysis after 120 min. Thus, the gold compound protected the C4 from inactivation.

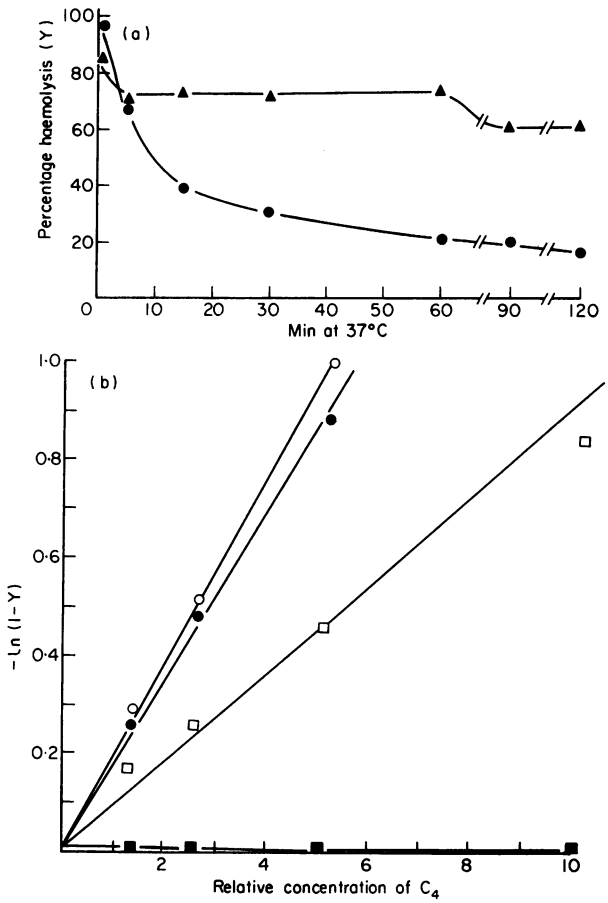

FIG. 1. (a) The effect of gold sodium thiomalate (5 μg/ml as gold) in glucose gelatin–Veronal buffer (▲) or glucose gelatin–Veronal buffer (●) on the functional haemolytic activity of C4 in rheumatoid synovial fluid (diluted 1:100 in buffer) as a function of time at 37°C. (b) The effect of 10 μg/ml of gold sodium thiomalate or buffer on the functional haemolytic titre of C4 in synovial fluid after incubation for 1 hr at 37°C. (○) Zero time, Au. (□) 1 hr, Au. (●) Zero time, buffer. (■) 1 hr, buffer.

The results of a second experiment with C4 are shown in Fig. 1b. Equal volumes of synovial fluid diluted 1:100 in buffer were incubated with GST (10 μg/ml as gold) at 37°C. Buffer was substituted for the GST as a control. Samples were removed immediately after mixing (zero time) and after 60 min at 37°C, quick-frozen in an acetone dry-ice bath, and titrated simultaneously. The C4 titre (CH_{50} units/ml) at zero time was 5000 in the GST–synovial fluid and 4850 in the buffer control. After 60 min, the C4 titre dropped to 2290 in the mixture with gold, but was completely destroyed in the buffer control. Therefore, the gold compound partially protected the C4 from inactivation.

These experiments were repeated six times with three different normal human sera, and five times with five different rheumatoid synovial fluids, using gold concentrations ranging from 5 to 20 μg/ml. The C4 activity was always protected by the presence of GST.

Effect of GST on $C\bar{1}$ *in rheumatoid synovial fluid*

To determine whether GST had an effect on $C\bar{1}$, a synovial fluid (J.S.) was diluted 1:100 with buffer and divided into four aliquots of 3·5 ml each. To each was added 3·5 ml of buffer, the first with no GST, the second, third and fourth having final gold concentrations of 1·25, 2·5 and 5·0 μg/ml. The mixtures were incubated at 37°C and sampled at intervals over a period of 120 min. Each timed sample was immediately frozen in an acetone dry-ice bath and kept frozen at −70°C until tested. $C\bar{1}$ was assayed at each time interval, and a plot of percentage haemolysis versus time is shown in Fig. 2. Synovial fluid $C\bar{1}$ haemolytic activity decreased with incubation as the concentration of gold was increased. $C\bar{1}$ caused 83% haemolysis after 120 min at 37°C in the control mixture without gold. By comparison the $C\bar{1}$ in the mixtures containing gold in concentrations of 1·25, 2·5 and 5·0 μg/ml caused 66, 41 and 11 % haemolysis, respectively, after 120 min.

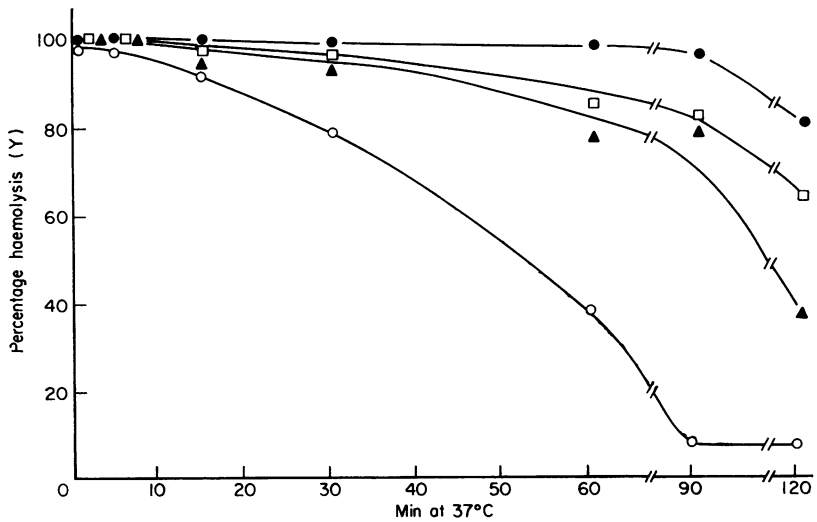

FIG. 2. The effect of increasing concentrations of gold sodium thiomalate (numbers are expressed as elemental gold) on the functional haemolytic activity of rheumatoid synovial fluid $C\bar{1}$ as a function of time at 37°C. The synovial fluid is diluted 1:100 in glucose gelatin–Veronal buffer without metals. (●) Synovial fluid (1:100) + Veronal buffer. (□) Synovial fluid (1:100) + 1·25 μg/ml of gold. (△) Synovial fluid (1:100) + 2·5 μg/ml of gold. (○) Synovial fluid (1:100) + 5·0 μg/ml of gold.

Effects of GST on rheumatoid synovial fluid and normal serum C1, C4 and C2

The effects of GST (3·1 μg/ml) on the haemolytic activities of C1, C4, and C2 in 1:50-diluted rheumatoid joint fluid of patient P.K. as compared to buffer are shown in Fig. 3. In a second experiment, the effects of thiomalic acid (TA) (3·1 μg/ml) and buffer were compared using the same joint fluid. Each test- and buffer-control mixture was incubated at 37°C and stoichiometric haemolytic assays were done after 4 hr. GST caused a loss of $C\bar{1}$ activity compared to the buffer control. A comparison of the C4 and C2 profiles shows significantly higher values in the GST mixture than in the buffer mixture. Since C4 and C2 are the two natural substrates for $C\bar{1}$, the higher titre of these two components in the GST mixture indirectly demonstrates that $C\bar{1}$ was partially inactivated.

In contrast, TA showed no effect on $C\bar{1}$ in the same synovial fluid. As shown (Fig. 3), the

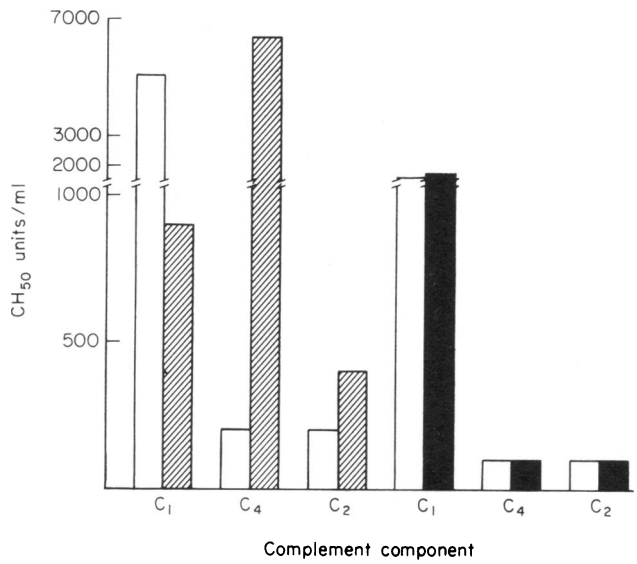

FIG. 3. Effect of gold sodium thiomalate (3·1 μg/ml as gold) (▨), Veronal buffer (□), and thiomalate (3·1 μg/ml) (■) on the functional haemolytic activities of C1, C4, and C2 in diluted rheumatoid synovial fluid P.K. after incubation for 4 hr at 37°C.

component titres decreased in all mixtures after 4 hr at 37°C, regardless of the presence or absence of 3·1 μg/ml of TA, demonstrating that $C\bar{1}$ is not inactivated by this compound. Similar results were obtained with 10 μg/ml of thiomalic acid. The component titres were lower in this experiment than in the test with GST because different lots of the testing reagents were used for the assays.

Similar experiments were done with other rheumatoid synovial fluids and normal human serum. GST inactivated C1 as above, resulting in the partial protection of C4 and C2. Thus, normal serum and rheumatoid synovial fluid gave comparable results.

Non-reversibility of gold inactivation of synovial fluid C1

After completion of the above experiments with synovial fluid, samples of the control and test dilutions (1:50) were extensively dialysed for 12 hr against $DGVB^{2+}$ at 4°C.

The pre-dialysis titres, determined with buffer alone or with GST were not significantly different in equivalent post-dialysis mixtures. Thus, for patient P.K., the pre-dialysis titres for C1, C4 and C2 in the GST mixtures were 900, 6400 and 400 CH_{50} units/ml. The post-dialysis titres were identical. For patient L.C., the pre-dialysis titres for C1, C4 and C2 in the GST mixtures were 600, 6400 and 800 CH_{50} units/ml. The post-dialysis titres were 500, 6400, and 900 CH_{50} units/ml. Therefore, the effects of GST on $C\bar{1}$ were irreversible.

Effects of GST on purified human $C\bar{1}$ and $C\bar{1}s$

Both $C\bar{1}$ and $C\bar{1}s$ were used at a final concentration at which the Z value (-1n(1-Y)) from a haemolytic titration was 1·0. One volume of each component was added to one volume of buffer or to GST having final gold concentrations of 50, 25, 12·5, 6·25 and 3·125 μg/ml. A sample of each mixture was quick-frozen immediately after mixing (zero time) and after incubation for 2 hr at 37°C, a second sample was similarly frozen. Each $C\bar{1}$ or $C\bar{1}s$ preparation was assayed and the percentage inhibition of haemolysis was plotted versus the gold concentration (Fig. 4). The gold compound was markedly inhibitory for both $C\bar{1}$ and

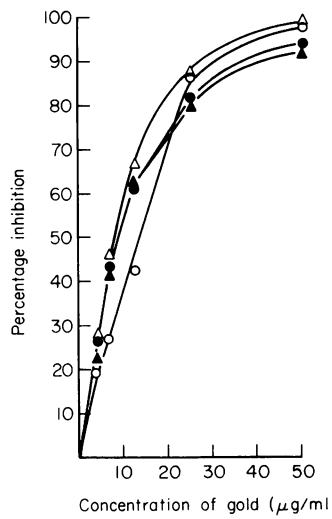

FIG. 4. Effect of increasing concentrations of gold sodium thiomalate (numbers are expressed as elemental gold) on the functional haemolytic activity of $C\bar{1}$ esterase and $C\bar{1}$ after incubation for 2 hr at 37°C. (○) Zero time, $C\bar{1}$ esterase. (●) 2 hr, $C\bar{1}$ esterase. (△) Zero time, $C\bar{1}$. (▲) 2 hr, $C\bar{1}$.

$C\bar{1}s$, even at zero time. For example, 26% of the haemolytic activity of $C\bar{1}$ was inhibited by a gold concentration of 3·125 μg/ml at zero time, and 19% of $C\bar{1}s$ was inhibited. At a gold concentration of 50 μg/ml, 95 and 99% of the haemolytic activity of $C\bar{1}$ and $C\bar{1}s$, respectively, was inhibited at zero time. The inhibitory effect of gold on $C\bar{1}$ and $C\bar{1}s$ was somewhat higher after 2 hr of incubation. Each curve became non-linear at gold concentrations between 12·5 and 25 μg/ml. The inhibitory effect of gold on both $C\bar{1}$ and $C\bar{1}s$ was not reversible following extensive dialysis against $DGVB^{2+}$.

Effect of other gold compounds on $C\bar{1}$

The effect of gold chloride ($HAuCl_4$) and gold sodium thiosulphate ($AuNa_3(S_2O_3)_2$) on functionally pure $C\bar{1}$ was tested using the same methods as those described in the preceding experiments. The results in Fig. 5 show that even at zero time $HAuCl_4$ was markedly inhibitory for $C\bar{1}$, causing 32% inhibition with 3·1 μg/ml of gold, and 100% inhibition with 50 μg/ml. The percentage inhibition for all gold concentrations except 50 μg/ml was greater after 60 min. $C\bar{1}$ was also inhibited at zero time with $AuNa_3(S_2O_3)_2$, having 10% of the haemolytic activity destroyed with 3·1 μg/ml of gold, and 35% after 1 hr. With 50 μg/ml at zero time, 85% was inhibited which increased to 100% after 1 hr.

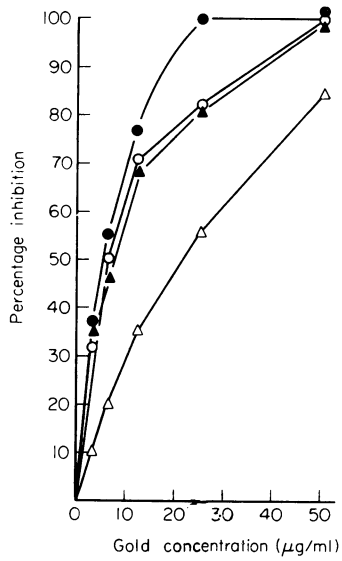

FIG. 5. Effect of increasing concentrations of $HAuCl_4$ and $AuNA_3(S_2O_3)_2$ on the functional haemolytic activity of $C\bar{1}$ after incubation for 1 hr at 37°C. (○) Zero time, $HAuCl_4$. (●) 1 hr, $HAuCl_4$. (△) Zero time, Au Na_3 $(S_2O_3)_2$. (▲) 1 hr, $AuNa_3$ $(S_2O_3)_2$.

Benzyl alcohol, which is used as preservative in commercial GST, was not inhibitory for $C\bar{1}$ at concentrations equivalent to 100 μg/ml of gold. In other tests, commercial GST, highly purified GST without preservative, and thiomalic acid were compared at concentrations ranging from 3·1 to 50 μg/ml. No differences were detected between the commercial and purified preparations at any of the concentrations used, and thiomalic acid caused little or no inhibition. For example, with 50 μg/ml, commercial and highly purified GST caused 48 and 50% inhibition at zero time, respectively, while thiomalic acid caused 8% inhibition.

Binding of ^{195}*Au to rabbit C1q and* $C\bar{1}s$

A 0·1-ml aliquot of ^{195}Au (20 μg/ml) was added to 0·2 ml of fresh rabbit serum containing 0·01 M EDTA. After 2 hr at 37°C, duplicate tubes received 0·3 ml goat antiserum to rabbit C1q (aC1q), to rabbit $C\bar{1}s$, or to rabbit γG. The tubes were incubated for 30 min at 37°C and overnight in the cold. The resulting precipitates were washed twice, suspended in 2·0 ml of buffer, and the supernatant fluids, wash fluids, and precipitates were counted in a gamma

counter. Protein N of each precipitate was measured by a modification of the method of Folin–Ciocalteu in a separate set of duplicate precipitates obtained in a similar way. On a wt/wt basis, the C1q–aC1q immune complex bound approximately three times more ^{195}Au than the $C\bar{1}$s–aC1s complex and sixty-six times more than the γG–aγG complex, whereas the $C\bar{1}$s–aC1s complex bound twenty-three times more ^{195}Au than the γG–aγG complex.

Effect of ^{195}Au on C1q haemolytic activity

The effect of ^{195}Au on the haemolytic function of C1q was examined by incubating rabbit C1q (110 μg N/ml) with an equal volume of either ^{195}Au (20 μg/ml) or buffer for 2 hr at 37°C. Serial two-fold dilutions of C1q were then made in $DGVB^{2+}$ and 0·2 ml of each dilution was incubated with 0·25 ml of EAC4 cells, $1{\cdot}5 \times 10^8$/ml. After 15 min at 30°C, the cells were washed twice with 1·5 ml of $DGVB^{2+}$ and resuspended in 0·25 ml of the same buffer. An excess of functionally pure rabbit C1r, C1s was added and after 10 min at 30°C, the cells were washed twice. EAC14 sites were then measured using guinea-pig C2 and C–EDTA. Fig. 6 shows that incubation with 10 μg ^{195}Au/ml did not influence the ability of

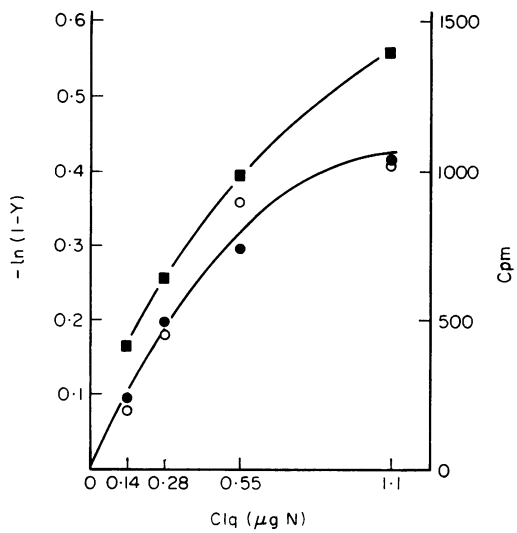

FIG. 6. Functional haemolytic titration of rabbit C1q incubated with ^{195}Au (○) or with buffer (●). Cell-bound radioactivity in counts per minute (cpm) (■). Y = percentage haemolysis.

C1q to bind to EAC4 or its haemolytic activity, as compared to the buffer control. In addition, the amount of cell-bound radioactivity was roughly proportional to the figures obtained in each dilution for the haemolytic assays. This result suggests that the inhibition of $C\bar{1}$s due to bound gold is the mechanism by which $C\bar{1}$ is inhibited. Since C4 and C2 are substrates of $C\bar{1}$s, the protective effect is also explained. C1r was not studied, but C4 and C2 are not known to be substrates for C1r.

Effects of gold on C1q-dependent precipitation of soluble γ-globulin aggregates

One volume of highly purified rabbit C1q (8γN/ml) was incubated with either one volume of GST (25 μg/ml of gold) or buffer at 37° for 2 hr. Both 0 and 2-hr samples were pipetted

into wells punched in agarose plates (Agnello, Winchester & Kunkel, 1970) opposite wells which contained soluble γ-globulin aggregates prepared from 1% Cohn fraction II. The precipitin lines which formed after 24 hr between the GST- or buffer-treated C1q and the γ-globulin aggregates did not differ as judged by visual inspection.

Effects of gold on the T_{max} of EAC14 cells

Different concentrations of functionally purified guinea-pig $C\bar{1}$ in $DGVB^{2+}$ were added to $EAC4^{hu}$ cells (1×10^9/ml) to form EAC14 containing 260, 65 and 10 effective molecules per cell by published methods (Mayer, 1961). Each of the three sets of EAC14 cells was divided into two parts, one of which was suspended in buffer, the other in buffer containing GST (6·25 μg/ml as gold). After 90 min at 37°C, the cells were washed and adjusted to 1×10^8/ml, and a T_{max} experiment (Mayer, 1961) was done with each set using human C2 and C–EDTA (guinea-pig). There was no significant difference in the T_{max} in the presence or absence of GST. Similarly, no effect on the T_{max} was found when fifty effective molecules of human C1 and 25 μg/ml of GST were used.

Similar T_{max} experiments with $EAC1^{gp}4^{hu}$ cells (1×10^8/ml) containing 250 effective molecules $C\bar{1}$/cell were done with buffer containing 3·1 μg/ml of $HAuCl_4$. There was no difference in the T_{max} time in the presence or absence of gold.

Effects of gold on C2 decay from EAC14 cells

Two and one-half CH_{50} units/ml of human C2 were added to $EAC1^{gp}4^{hu}$ cells (1×10^8/ml), incubated at 30°C for 3 min (T_{max}) and divided into two aliquots of 7 ml each. After centrifugation at 0°C, one aliquot was resuspended in ice-cold $DGVB^{2+}$ and the other in $DGVB^{2+}$ containing GST (3·1 μg/ml of gold). Timed samples were taken at 37°C to measure the C2 decay by published methods (Mayer, 1961). Fig. 7 shows that GST did not influence the decay of C2 from EAC14 cells. Similar results were obtained when C2 decay was done at 30°C.

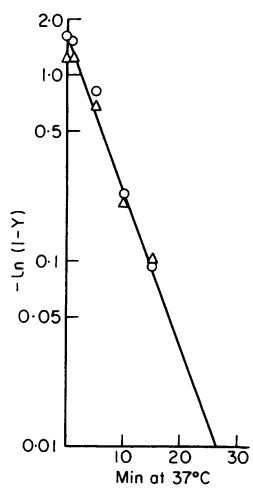

FIG. 7. Effect of gold sodium thiomalate (3·1 μg/ml) (△) or buffer (○) on C2 decay from EAC14 cells. Y = percentage haemolysis.

In other C2 decay experiments, GST (3·1μg/ml), thiomalic acid (6·25 μg/ml), or buffer, was added to three sets of EAC142 cells (1×10^8/ml) after the experiment was in progress for 5 min at 37°C. Neither the GST nor the thiomalic acid altered the rate of decay of C2 when compared to the buffer control.

DISCUSSION

The present study demonstrates that gold compounds *in vitro* are potent non-reversible inactivators of native $C\bar{1}$ in rheumatoid synovial fluid and normal serum. Extension of these studies showed that both partially purified $C\bar{1}$ (C1qrs) and highly purified $C\bar{1}s$ were inactivated by gold. These findings are of particular interest in RA because activation of C1 to $C\bar{1}$ by immune complexes initiates the 'classical' sequence of C reactions (Müller-Eberhard, 1968; Schultz, 1971; Rapp & Borsos, 1970). This pathway has been shown by Ruddy & Austen (1973) to be the predominant pathway for C activation in patients with seropositive RA, although the alternate pathway may also be involved (Götze, Zvaifler & Müller-Eberhard, 1972). Gold in concentrations as small as 1·25 μg/ml inactivated the functional haemolytic activity of $C\bar{1}$.

For the classical C pathway, the loss of haemolytic activity of C4 and C2 is a sensitive index of C1 activation. The haemolytic activities of these two natural substrates for $C\bar{1}$ decreased rapidly following $C\bar{1}$ activation at 37°C in the absence of gold. However, both components were partially protected by the presence of gold because of its inhibitory effect on $C\bar{1}$.

Initially it was of concern whether gold, thiomalic acid, or an impurity in commercial GST preparations (Myochrysine) was the moiety responsible for $C\bar{1}$ inactivation. It was shown that the inactivation of $C\bar{1}$ was caused by the gold in GST, because thiomalic acid at equivalent or greater concentrations than the gold compound had no direct effect on $C\bar{1}$ and did not protect C4 and C2 from $C\bar{1}$. Furthermore, benzyl alcohol, the preservative used in commercial GST, had no effect on $C\bar{1}$ at the same concentrations in which the GST was used. This was expanded further by showing that highly purified GST without a preservative was equally as effective as the commercial preparation. Two other chemically pure gold compounds that were highly inhibitory for $C\bar{1}$ were $HAuCl_4$ and $AuNa_3(S_2O_3)_2$.

It was of interest that gold as ^{195}Au bound to Clq as well as $C\bar{1}s$. The 'classical' C pathway is initiated by the binding of macromolecular $C\bar{1}$ through the C1q subunit to a site on the Fc portion of immunoglobulins. Bound gold did not appear to impair the binding of C1q to EA, nor was its functional haemolytic activity or ability to precipitate soluble γ-globulin aggregates altered. As mentioned above, the inhibitory effect of gold is more likely to be due to the demonstrated direct effect on highly pure $C\bar{1}s$.

T_{max} experiments with EAC14 cells are very sensitive for demonstrating alterations in cell-bound $C\bar{1}$. Partial inactivation of cell-bound $C\bar{1}$ would increase or eliminate the T_{max} (Stroud, Austen & Mayer, 1965). Thus, the lack of a demonstrable effect of gold on the T_{max} could be interpreted as indicating that gold effects only fluid phase $C\bar{1}$ and not cell bound $C\bar{1}$.

Many theories have been advanced to explain the mechanism(s) of action of gold compounds in RA. Gold was shown to act directly on free sulphydryl groups of proteins (e.g. cysteine), rendering them biologically inactive (Libensen, 1945) and has been reported to inhibit lysosomal enzymes in guinea-pig macrophages (Persellin & Ziff, 1966), cathepsin-

D isolated from rheumatoid synovium (Boyle, Tabachnick & Granda, 1972), phagocytosis and killing of bacteria *in vitro* (Messner, Carlson & Jelinek, 1970), synthesis of antibody to bovine serum albumin in rabbit lymphocytes (Persellin, Smiley & Ziff, 1963), and to prevent the aggregation of human γ-globulin (Gerber, 1971). Most of these studies required higher gold concentrations than that needed to inactive C components. The findings of this *in vitro* study offer yet another mechanism of gold action, but do not exclude other known effects of gold.

The observation that $C\bar{1}$ is inactivated by small quantities of gold may possibly warrant consideration in the treatment of other diseases were C plays a role. For example, patients with hereditary angioneurotic oedema are deficient in a serum inhibitor of C1, the C1 esterase inhibitor (C1I) (Donaldson & Evans, 1963; Donaldson & Rosen, 1964). This functional deficiency is thought to lead to sporadic C1 activation, which in turn acts on its two natural substrates, C4 and C2. A peptide is released from this interaction, the so-called 'C-kinin', which is thought to be responsible for the recurrent episodes of non-inflammatory circumscribed oedema of the skin, respiratory, and gastrointestinal tracts. Inasmuch as GST inactivates $C\bar{1}$ *in vitro*, treatment of such patients with gold compounds may also block $C\bar{1}$ *in vivo* and prevent the peptide-induced oedema. This is especially appealing since gold is retained many months after treatment. The recent demonstration that gold favorably influences the course of pemphigus, allowing for reduction of corticosteroid dosage and associated with a fall in anti-epithelial antibody titre, suggests that gold may be useful in the treatment of other diseases as well (Penneys *et al.*, 1972). Pemphigus has been shown to be associated with activation of C components in the blister fluid (Jordon *et al.*, 1973).

In summary, we have shown that gold compounds have a deleterious effect on the sequential reactions of C via the 'classical' pathway by causing non-reversible destruction of $C\bar{1}$ in serum, synovial fluid, and in functionally pure $C\bar{1}$. The same effect was shown on highly purified $C\bar{1}s$. These *in vitro* results add another dimension to the action of gold compounds on proteins in biological fluids, offer an additional mechanism of action of gold which may explain some of its therapeutic efficacy in RA, and stress the importance of using *functional* haemolytic assays to measure the effects of therapeutic agents on proteins.

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