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N. J. HARPER

ALMA B. SIMMONDS

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Contents

Miscellaneous Antirheumatic Drugs and their Possible Modes of Action

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1 Introduction

Antiinflammatory drugs of the aspirin and corticosteroid types have proved to be very useful agents in the treatment of rheumatoid arthritis. However, in spite of the development of increasingly potent drugs of these types, none have proved to be effective inhibitors of the underlying chronic inflammatory processes which are accompanied by synovial proliferation and degeneration of the organized connective tissues of the rheumatoid joints. There is a great need for the development of a new generation of drugs whose actions may alter fundamentally the disease process and its destructive course, but these are unlikely to be found using existing classical pharmacological screening techniques.

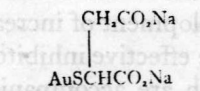
There is an interesting group of miscellaneous nonsteroidal, nonaspirin-like compounds which, though failing to show reproducible activity in most classical screening tests, except perhaps under exceptional conditions, have proved to be efficacious in the treatment of rheumatoid arthritis. The most important of these agents are probably gold compounds, chloroquine

and, more recently, penicillamine. Unfortunately, the use of these compounds is usually restricted to the treatment of more advanced disease because of the associated side effects, which can often be severe and require very careful monitoring of the patient.

The purpose of this chapter is to review actions of these compounds which may be relevant to their antirheumatic activity and possibly provide a basis for seeking new antirheumatic drugs.

2 Gold compounds

Gold compounds are particularly interesting since there is evidence that they can significantly retard joint destruction when given on a long-term basis. In a major study by the Empire Rheumatism Council (1961), treatment with the gold compound sodium aurothiomalate (**1**) was shown to have a beneficial effect on all the disease parameters measured, with the exception of radiological progression where improvement was suggested but was not significant. Further studies by Sigler *et al.* (1972 and 1974), however, did show significant inhibition of the radiologically assessed progression of bone and cartilage destruction in patients treated with sodium aurothiomalate. The most important difference between the studies of the Empire Rheumatism Council and Sigler *et al.* appears to be in the dosing schedules. In the former study, patients received a total dose of 1000 mg of sodium aurothiomalate spread over a five-month period and were assessed radiologically eighteen months after entry to the study without further gold being given. In the latter study, Sigler *et al.* gave "tissue loading" doses of gold in an initial twenty-two-week period and then maintenance doses at increasing intervals up to two years.



(**1**) Sodium aurothiomalate

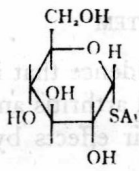
Although the absorption of gold from injection sites appears to be rapid (Mascarenhas *et al.*, 1972) the onset of a clinical response is slow and appears to bear no obvious relationship to the blood levels achieved (Gerber *et al.*, 1972; Mascarenhas *et al.*, 1972; Rubinstein and Dietz, 1973).

The precise mode of action of gold compounds remains unknown but they do have a number of activities which may be relevant to their efficacy in rheumatoid arthritis. These activities are considered below.

2.1 ANTIMICROBIAL ACTIVITY

The role of infective agents in the initiation of rheumatoid arthritis has been a source of argument for many years with evidence having been put forward to suggest that the agent responsible is either bacterial, viral or mycoplasmal (Gardner, 1972). Although different workers have apparently isolated a variety of infective agents from rheumatoid joints, none have been demonstrated reproducibly in a high percentage of joints and by different workers. Perhaps this failure to isolate infective organisms is due to their lack of persistence in viable form but, remaining as antigenic components which cannot be identified (Barland, 1973), they are responsible for a subsequent series of immunological events leading to rheumatoid arthritis. Brostoff *et al.* (1973), for example, reported that leucocyte migration inhibition in the presence of *Mycoplasma fermentans* membranes correlated with the severity of the disease in rheumatoid arthritis, suggesting possible previous exposure to this agent.

The relationship between gold compounds, rheumatoid arthritis and infective agents has an historical basis. Gold compounds appear to have first been used in the treatment of rheumatoid arthritis in the mistaken belief that the disease was related to tuberculosis. As early as 1890 Koch had shown that tubercle bacilli were sensitive to gold cyanide. Landé (1927) reported that aurothioglucose (**2**) relieved joint pains when used to treat bacterial endocarditis and this early work suggested that the effectiveness of gold compounds was due to an antimicrobial activity.

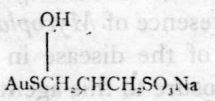


(2) Aurothioglucose ((D-glucosylthio)gold)

The ability of gold compounds to suppress arthritis induced in rodents by infective agents has also been known for many years. Activity was shown against haemolytic streptococci (Rothbard, 1941), mycoplasmas (Findlay *et al.*, 1939; Sabin and Warren, 1940) and other infective agents (Jasmin, 1957). More recently Thomas (1973) discussed several models of arthritis induced in laboratory animals by mycoplasma and suggested that these models might be useful for studying the therapeutic activity of gold compounds. Thomas *et al.* (1966) had found previously that aurothiomalate

was effective therapeutically against *M. gallisepticum* infections in turkeys and chickens.

The studies of Sabin and Warren (1940), however, suggested that gold was not acting directly through an antimicrobial activity. These authors found that neither aurothiomalate, nor the blood of mice treated with it, could prevent the growth of the mycoplasma they had used to induce arthritis. Further support for an indirect effect of gold compounds in mycoplasma-induced arthritis was provided by the study of Wiesinger (1965) using rats. In these experiments allochrysine (**3**) did not inhibit the growth of *M. arthritidis* although it has an apparent antiarthritic effect, as judged by inhibition of the swelling of the rat's paw.



(3) Allochrysine: sodium 3-aurothio-2-hydroxypropanesulphonate

The lack of direct effect of gold compounds on some of the micro-organisms that cause experimental arthritis suggests that they might work through some other mechanism. Further, until active infective organisms can be proven to be responsible for a high percentage of the cases of rheumatoid arthritis, the antimicrobial activity of gold compounds must remain unlikely to explain their therapeutic efficacy.

2.2 ACTIONS ON THE IMMUNE SYSTEM

It is obvious from a weight of evidence that immunological processes are intimately involved in rheumatoid arthritis and it has been suggested that gold compounds could exert their effects by interfering with immunological behaviour.

In the clinical situation, although Mourisden *et al.* (1974) reported that they could not find statistically significant changes in IgG or IgM metabolism in patients treated with sodium aurothiosulphate for three months, Gottlieb *et al.* (1975) did find significant decreases in the serum levels of these immunoglobulins after treatment with sodium aurothiomalate for six and twelve months. Reductions in IgG, IgA, IgM and rheumatoid factor titres appeared to correlate with the clinical response to treatment in the study of Gottlieb *et al.* However, decreases in immunoglobulin levels may reflect either direct effects of gold on the metabolism of immunoglobulins or a beneficial effect on the disease process. Gold was reported to be heavily concentrated in the reticulo-endothelial system, especially

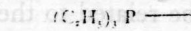
the lymph nodes, of a patient on gold thioglucose (Gottlieb *et al.*, 1972) and this could suggest that lymphocytes are target cells. A direct effect of gold on lymphocytes was suggested by the finding that the lymphocytes of patients receiving gold therapy were unresponsive to the effects of streptolysin S, a blastogenic agent (Fikrig and Smithwick, 1968). However, these authors suggested that this effect might be mediated by an inhibition of lysosomal enzymes and this may be related to the mode of action of gold (section 2.4).

Animal studies also suggest that the reticulo-endothelial system is a site of accumulation of gold (Swartz *et al.*, 1960) though most evidence does not support a direct effect of gold on the lymphocytes. In an attempt to determine the effect of sodium aurothiomalate on immune responses, Persellin *et al.* (1967) measured circulating antibody levels in rabbits following hyperimmunization with bovine serum albumin, typhoid-paratyphoid vaccine and *E. coli*. Gold had no effect on the antibody levels suggesting that it did not have a direct effect on immunoglobulin production by lymphocytes. Further, Persellin *et al.* also demonstrated a lack of effect of sodium aurothiomalate on delayed hypersensitivity to diphtheria toxoid and dinitrochlorobenzene in the guinea pig.

The ability of gold compounds to inhibit the development of rat adjuvant arthritis, a complex disease involving delayed (cell-mediated) hypersensitivity, is debatable. Jessop and Currey (1968) found that sodium aurothiomalate failed to affect the progress of adjuvant arthritis. They also found that primary antibody responses to sheep erythrocytes and delayed skin reaction to tuberculin were not affected in these same animals, even when gold was given prophylactically and in high doses. In contrast, Walz *et al.* (1971), Sofia and Douglas (1973) and Arrigoni-Martelli and Bramm (1975) all reported that sodium aurothiomalate did significantly inhibit adjuvant arthritis. It is possible that the differing results in these studies might be related to differences in absorption from the injection site, since Jessop and Currey gave the gold subcutaneously whilst the other workers gave it intramuscularly. Unfortunately, the blood levels of gold were not measured in the former study so that adequacy of absorption from the subcutaneous injection site cannot be confirmed. Walz *et al.* (1972 and 1974) used rat adjuvant arthritis to evaluate an orally active gold compound, SKF 36914 (4), and found it to be as effective as intramuscularly injected sodium aurothiomalate.

Although sodium aurothiomalate appeared to suppress both the non-immune (primary lesion) and immune (secondary lesion) stages of adjuvant arthritis in some experiments, it did not appear to inhibit antibody production to sheep erythrocytes or cutaneous hypersensitivity to purified protein derivative in these same animals (Walz, 1974). Both Walz (1974) and Gerber

(1972) showed that sodium aurothiomalate delayed but did not inhibit rat allergic encephalomyelitis, suggesting that it did not inhibit cell-mediated hypersensitivity. Aspirin-like drugs have also been shown to delay rat allergic encephalomyelitis whilst immunosuppressive drugs, such as cyclophosphamide and methotrexate, can inhibit it completely.



(4) Triethylphosphino-gold chloride (SKF 36914)

On the basis of the evidence available, it must be concluded that gold compounds probably do not have a direct immunosuppressive action on either immediate or delayed (cellular) responses and that other actions are responsible for their activity in immunologically based diseases.

2.3 INTERACTIONS WITH PROTEINS

The apparent ability of gold to inhibit the heat-induced aggregation of human gamma-globulin *in vitro* (Gerber, 1971) could be of relevance to its action *in vivo*.

Complexes of IgG and IgG + rheumatoid factors (IgG or IgM) have been found in rheumatoid synovial fluids and synovial tissues. Their presence was associated with a marked decrease in synovial fluid complement levels (Zvaifler, 1974) following activation of the complement system and complement activation products could contribute to persistent articular inflammation. Further, indigestible immune complexes can be taken up by phagocytically active cells within the inflamed joint (polymorphs, macrophages and synovial lining cells) and this could result in the release of lysosomal enzymes which may cause further inflammatory changes and connective tissue destruction (Allison and Davies, 1975). Recent experiments with cultured cell lines derived from rabbit synovium showed that stimulation of phagocytosis by these cells could also lead to the secretion of nonlysosomal enzymes which can digest connective tissue (Reynolds and Werb, 1975).

It can be seen that if gold compounds did in fact slow down or inhibit the formation of immune complexes or aggregates in the joints (or other sites) they could modify both inflammatory changes and connective tissue degradation. However, an *in vivo* effect of gold on immune complex formation at synovial sites remains to be demonstrated.

The antirheumatic activity of gold may be associated with its interaction with collagen. Adam *et al.* (1965, 1968) studied the uptake of gold by collagen fibres of rat tail tendon following regular administration of sodium

aurothiosulphate and found that the collagen fibres showed distinct staining bands of gold. These authors suggested that, initially, negatively charged gold thiosulphate complexes were bound to collagen molecules by electrostatic forces but these subsequently decomposed forming new coordinate cross-links through the gold resulting in cross striations. It was also suggested that the course of rheumatoid arthritis might be influenced by an increase in the stability of the collagen structure and by the occupation of immunologically active sites on collagen molecules or their breakdown products. However, the relationship of these animal studies to the binding of gold to collagen in the rheumatoid joint remains to be clarified.

2.4 INHIBITION OF ENZYMES

Gold compounds have been shown to inhibit a number of enzymes of both lysosomal and nonlysosomal origin *in vitro*. As already mentioned in section 2.3, the release of lysosomal and non-lysosomal enzymes in the inflamed joint can promote further inflammatory changes and degrade the connective tissue matrix components which are mainly proteoglycans and collagen.

In rheumatoid arthritis, inflamed swollen granulation tissue (pannus) covered with proliferating lining cells grows over the cartilage and into the subchondral bone (Fig. 1).

The major destruction of articular cartilage takes place where the pannus and cartilage are in intimate contact (Ball, 1968) with little breakdown occurring randomly over the cartilage surface except for some loss of proteoglycans (Hamerman, 1969). This suggests that the enzymes necessary for degrading cartilage come either from the pannus, probably the lining cells, or that the pannus releases substances which stimulate the chondrocytes of the articular cartilage to degrade their own matrix. Although the polymorphs which migrate into the synovial fluid and the macrophages which line the synovial cavity can release into the synovial fluid a number of proteolytic enzymes which are active at neutral pH, these enzymes are probably prevented from random attack on the articular cartilage surface by the naturally occurring plasma protein enzyme inhibitors α_2-macroglobulin and α_1-antitrypsin (Ohlsson and Delshammar, 1975).

Amongst the enzymes which gold compounds have been shown to inhibit are the lysosomal acid hydrolases, acid phosphatase, β-glucuronidase (Persellin and Ziff, 1966; Ennis *et al.*, 1968), cathepsin D and cathepsin B (Barrett, 1975), the neutral proteases collagenase and elastase from polymorphs (Janoff, 1970) and collagenase from human rheumatoid synovium (Woolley, 1975).

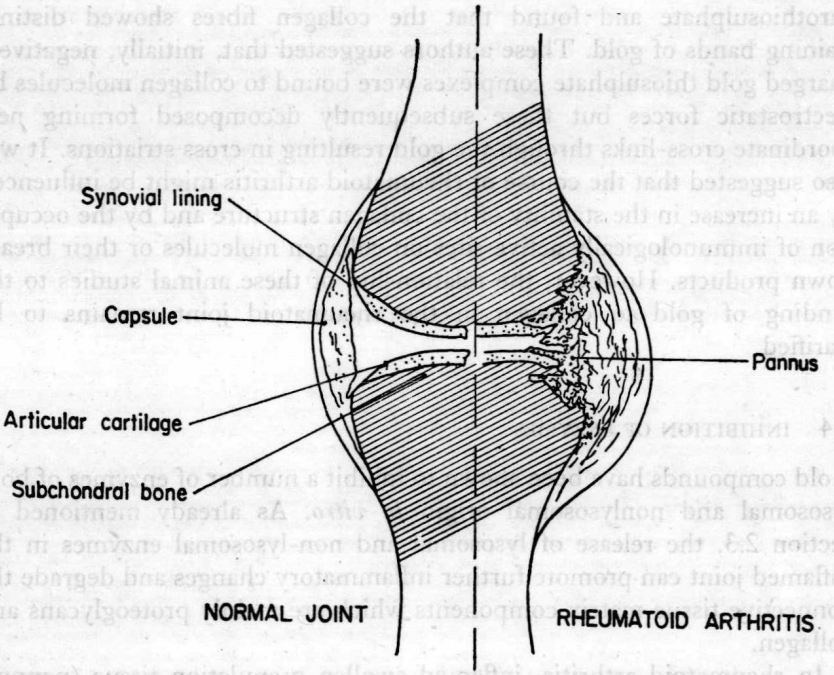


FIG. 1. Changes in the rheumatoid joint. Inflamed swollen granulation tissue (pannus) covered with proliferating lining cells grows over the articular cartilage and into the subchondral bone.

Although the elastase of polymorphs has an exceptionally broad specificity and is the only mammalian enzyme known to degrade all the major structural macromolecules of connective tissues (Barrett, 1975), the relevance of its inhibition by gold to the ability of gold to slow down joint destruction is questionable. As already mentioned, the polymorphs accumulate in synovial fluid which contains natural plasma protein enzyme inhibitors, and very few polymorphs appear in the invasive pannus. The ability of gold to inhibit rheumatoid synovial collagenase may be far more relevant since this enzyme is being produced by cell types which do lie in intimate contact with the articular cartilage and bone where the pannus invades these tissues and natural proteolytic enzyme inhibitors in the synovial fluid are excluded. Further, the free macrophages within the inflamed synovium contain enzymes whose release could be responsible for maintaining the chronicity of inflammation (Allison and Davies, 1975). Inhibition of some of these enzymes, either intracellularly or extracellularly, could account for moderation of inflammatory changes within the synovium by gold.

Although the concentrations of gold compounds required to inhibit some enzymes *in vitro* appear quite high, gold accumulates in endocytically active cells and the concentrations achieved within these cells may eventually be very high. Perhaps this explains why gold compounds take such a long time to exert a demonstrable antiinflammatory and antirheumatic effect.

2.5 INHIBITION OF PROSTAGLANDIN SYNTHESIS

In the past five years it has been shown that prostaglandins are important mediators of inflammation, pain and pyrexia and that aspirin-like non-steroidal antiinflammatory drugs inhibit their synthesis (for a review see Flower, 1974). These discoveries have made a major contribution towards explaining the mode of action of this group of drugs and some workers have considered whether unrelated drugs with apparent antiinflammatory activity such as gold might have similar actions.

Deby *et al.* (1973) found that Allochrysine (**3**) and gold chloride could inhibit bull seminal vesicle prostaglandin synthetase *in vitro* at 10^{-4} and 10^{-5}M. Similarly, Penneys *et al.* (1974) found that aurothiomalate could inhibit prostaglandin synthesis by a sheep seminal vesicle preparation with an ID_{50} of 5×10^{-5}M. In contrast, Stone *et al.* (1975), also using a sheep seminal vesicle PG synthetase, found that the inhibitory effect of aurothiomalate and aurothioglucose appeared to be specifically on $PGF_{2\alpha}$ synthesis with simultaneous stimulation of PGE_2 synthesis, though the latter results may differ from those of Penneys *et al.* because of addition of copper and glutathione to the *in vitro* system.

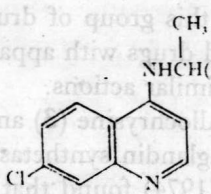
Although these studies do suggest that the inhibition of prostaglandin synthesis by gold compounds is a possible explanation of some of their antiinflammatory activities, inhibition of prostaglandin synthesis *in vivo* in the rheumatoid joint has yet to be demonstrated. Further, since much more potent inhibitors of prostaglandin synthesis such as indomethacin do not appear to have the same antirheumatic actions as gold, it suggests that gold works principally through other mechanisms.

* * *

It is apparent that much remains to be clarified about the relevance of the apparent actions of gold compounds, especially *in vitro*, to their antirheumatic activity. However, further studies are worth while to establish a basis for seeking "gold-like" compounds devoid of many of the side effects of existing drugs.

3 Chloroquine

Although the principal use of chloroquine (**5**) has been in the chemotherapy of malaria, for which the drug was originally developed, it has also been used in the treatment of rheumatoid arthritis for almost twenty-five years. Unfortunately, its use in rheumatoid arthritis remains somewhat limited by its toxic side effects, mainly retinopathy. Chloroquine is similar to gold compounds in that the onset of its antirheumatic effects is slow and seldom noted for at least two months; it appears to slow down joint erosion in long-term treatment (Young, 1959) and its effects continue after drug withdrawal. It does not behave as an antiinflammatory agent in the same way as aspirin-like drugs but appears to affect some of the more basic features of the disease.



(5) Chloroquine

A notable characteristic of chloroquine is its accumulation in tissues and very high concentrations have been found in the liver, spleen, kidney and lungs after administration to many species (Grundmann *et al.*, 1972). Information on the persistence of chloroquine in man varies. Zvaifler and Rubin (1962) found measurable amounts of chloroquine and metabolites in plasma and urine three and a half years after the last administration. Rubin *et al.* (1963) found detectable amounts of chloroquine in urine, erythrocytes and plasma of patients with chloroquine retinopathy five years after the last known ingestion of chloroquine. In contrast, McChesney and Rothfield (1964) reported that significant plasma and urinary levels were not found later than eight weeks after discontinuation of therapy, regardless of the duration of original treatment.

Although chloroquine and related antimalarials have been reported to have effects in a number of biological systems, there is no biological assay agreed upon as being predictive of the utility of this class of agents for the treatment of rheumatoid arthritis (Scherrer, 1974). There is general agreement that chloroquine is not effective in adjuvant arthritis (Newbould, 1963; Graeme *et al.*, 1966; Perrine and Takesue, 1968) or other experimental immunologically based diseases, nor does it appear to affect primary or secondary antibody responses (Kalmanson and Guze, 1965)

and cellular transfer of delayed hypersensitivity or immune complex mediated reversed passive Arthus reactions (Goldlust and Schreiber, 1975).

Some of the activities of chloroquine which do seem of possible relevance to its antirheumatic activity are discussed below.

3.1 EFFECTS ON LEUCOCYTE CHEMOTAXIS

Inflammation within the joint involves both an influx of inflammatory cells from vessels into the local tissues and local cell proliferation. Theoretically, drugs which inhibit the chemotaxis of cells into the inflammatory site, especially macrophages, might have a modifying effect on chronic inflammation in the rheumatoid joint. There have been reports that chloroquine can inhibit rabbit polymorphonuclear leucocyte chemotaxis *in vitro* (Ward, 1966; 1968). However, Perper *et al.* (1974), using a technique involving the adoptive transfer of ^{51}Cr-labelled isologous rat leukocytes and measurement of their accumulation in a carrageenan-induced inflammatory reaction, failed to find any effect of chloroquine at 100 mg kg^{-1} on either polymorphonuclear or mononuclear leucocyte cell chemotaxis. Whether chloroquine has any effect on leucocyte chemotaxis in man is unclear.

3.2 LYSOSOMOTROPIC ACTIVITY AND EFFECTS ON LYSOSOME FUNCTION

Although the precise mode of action of chloroquine is obscure, the relationship between chloroquine and the lysosomal system seems to be central to explaining many of its actions. The possible role of lysosomes and their enzymes in inflammation and connective tissue destruction in the joints has already been referred to in the section on gold compounds and there is evidence that chloroquine might exert its therapeutic effects through inhibition of lysosome function.

Many cells exposed to chloroquine take it up avidly and achieve much higher intracellular concentrations than in the surrounding medium. This rapid uptake is accompanied by a high degree of cytoplasmic vacuolation and there is substantial evidence that the site of accumulation of the drug is in the lysosomes (Allison and Young, 1969; Fedorko *et al.*, 1968). Autophagy, a mechanism whereby cells sequester and digest, within vacuoles, portions of their own cytoplasm, is associated with a high degree of vacuolation and has been shown to be induced by chloroquine in leukocytes of man (Fedorko, 1967), pancreatic cells of rats (Fedorko, 1968), cultured fibroblasts (Gaddioni *et al.*, 1964) and macrophages (Fedorko *et al.*, 1968). The latter authors suggested that these effects could conceivably be the basis, at least in part, for some of the toxic complications of

long-term chloroquine treatment in man. However, similar effects might also explain the drug's antimalarial and possibly other therapeutic activities.

Erythrocytes containing chloroquine-sensitive malaria parasites concentrate the drug to high levels and the drug produces typical vacuolation and autophagy in the parasite indicating a lysosomal localization similar to that found in mammalian cells. Homewood *et al.* (1972) proposed that chloroquine may act by inhibiting intralysosomal haemoglobin digestion in the parasite either by raising the intralysosomal pH or directly by inhibiting proteolytic enzymes. The reduced ability of the parasite to digest haemoglobin, essential for its nutrition, inhibits its growth.

The concentrations of chloroquine achieved within mammalian lysosomes are probably also adequate to inhibit directly a number of hydrolytic enzymes. Evidence of enzyme inhibition was provided by the inability of human fibroblasts exposed to chloroquine *in vitro* to digest proteins and mucopolysaccharides (Lie and Schofield, 1973), an effect similar to that seen on raising the medium pH (Lie *et al.*, 1972). De Duve *et al.* (1974) also showed that chloroquine inhibited the breakdown of exogenous proteins by macrophages *in vitro*. Of direct relevance to the effects of chloroquine on enzymes involved in connective tissue degradation, *in vitro* experiments have shown that chloroquine inhibited a chondromucoprotease of cartilage (Barrett, 1975; Cowey and Whitehouse, 1966), a cathepsin B in cartilage (Ali *et al.*, 1967) and collagenases from rat skin and bovine cartilage (Cowey and Whitehouse, 1966). Since collagenases have not been shown to be lysosomal in origin, it is not known if the concentrations of chloroquine achieved *in vivo* at the site of collagenase action would be high enough for it to have an inhibitory activity.

Although many antiinflammatory drugs and chloroquine stabilize the leakage of enzymes from isolated lysosomes (Filkins, 1969; Hyttel and Jorgensen, 1970; Abraham and Hendy, 1970; Ignarro, 1971), much of this *in vitro* work, especially with liver lysosomes, is of doubtful relevance to the behaviour of lysosomes in intact cells of the types found in the rheumatoid joint. As already described in the section on gold compounds, the *in vivo* stimulus for enzyme release from lysosomes may well involve the uptake of indigestible immunological or other materials and require the combined responses of the intact cell to achieve lysosomal enzyme secretion. Indeed, in contrast to the results with isolated lysosomes, Ringrose *et al.* (1975), using cultured mouse peritoneal macrophages, showed that chloroquine at concentrations in excess of 10^{-4}M tended to stimulate release of lysosomal enzymes induced with zymosan. In another model involving stimulated cells, in this case human leukocytes in contact with aggregated IgG immobilized on cartilage discs, Perper and Oronsky (1974)