The Chemistry of the Gold Drugs Used in the Treatment of Rheumatoid Arthritis

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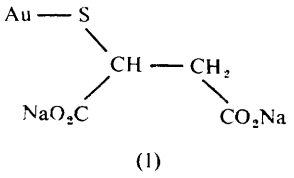
1 Introduction

The biological use of gold[1] can be traced back as far as the Chinese in 2500 B.C., but it was Koch's observation[2] in 1890 that gold cyanide inhibited the growth of tuberculosis bacilli which represented the beginning of systematic gold pharmacology and of attempts to design gold drugs. Over the next 60 years sporadic use of gold drugs in the treatment of tuberculosis led to favourable observations by Forrestier on the use of gold in arthritis which culminated in the favourable report of the Empire Rheumatism Council on the use of gold(I) thiomalate in the treatment of rheumatoid arthritis.[3] These results were confirmed by Sigler,[4] who also reported positive radiological evidence for regression of the disease in the joints of patients with rheumatoid arthritis who were undergoing chrysotherapy (gold treatment). Since then, medical enthusiasm has waxed and waned; the fact that gold drugs can cause remission of the disease being a source of optimism and the high percentage of toxic side-effects causing concern. Nevertheless there is, as yet, no specific drug for the treatment of rheumatoid arthritis. Consequently, gold complexes and drugs originally developed for other diseases, such as penicillamine (Wilson's disease), levamisole (an anthelmintic), and dapsone (leprosy) are standard prescriptions to combat severe rheumatoid arthritis.

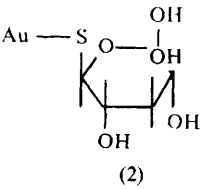
Most gold complexes used medically are thiol complexes (Figure 1) and although the known chemistry of gold has expanded of recent years, the majority of air-stable, water-soluble complexes of gold(I) which do not contain cyanide are still mercapto-gold compounds. Recently,[5,6] successful studies of the use of some phosphine complexes for gold therapy were reported. Unlike standard mercapto-gold drugs which have to be injected, the phosphine complexes are administered orally and, consequently, could have advantages in reducing

[1] G. M. Dyson, *Pharm. J.*, 1929, 123, 249, and 266.
[2] R. Koch, *Deutsche med. Wochenschr.*, 1927, **16**, 756.
[3] Empire Rheumatism Council, *Ann. Rheum. Dis.* 1961, **20**, 315.
[4] J. W. Sigler, B. G. Bluhm, H. Duncan, J. T. Sharp, D. C. Ensign, and W. R. McCrum, *Arthritis Rheum.*, 1972, **15**, 125.
[5] A. E. Finkelstein, D. T. Walz, and V. Batista, *Ann. Rheum. Dis.*, 1976, **35**, 251.
[6] D. T. Walz, M. J. Di Martino, and B. M. Sutton, in 'Anti-inflammatory Agents,' Academic Press, New York, 1974, Vol. 1, p. 217.



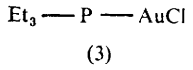
(1)

sodium gold(I) thiomalate (Myocrisin)



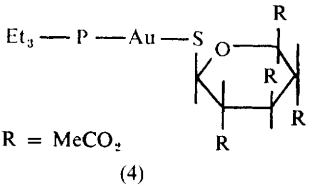
(2)

gold(I) thioglucose (Solganol)



(3)

triethylphosphinegold(I) chloride (SK 36914)



(4)

3-triethylphosphinegold(I)-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (SK and F D-39162)

Figure 1 *Some gold complexes commonly used in the treatment of rheumatoid arthritis. Compounds (1) and (2) are conventional gold drugs which are usually administered by intramuscular injection. Compound (3) is an orally effective form of gold in animal models of inflammation. As a result of further development compound (4) was recently tested as an orally active drug in a clinical trial*[6]

patient discomfort and in ease of administration. Apparently, successful earlier studies on orally absorbed gold complexes were never followed up.[7,8]

One limitation in the development of possible gold drugs as anti-inflammatory or, more specifically, as anti-rheumatic agents has been the lack of suitable animal models for test purposes. Acute models like kaolin rat-paw oedema[9] and chronic models like adjuvant arthritis[10] have given ambiguous or negative results with drugs such as sodium gold(I) thiomalate (myocrisin) and D-penicillamine, both of which are known to be effective to some extent in man.

Another limiting aspect of the development of potential gold drugs has been a lack of basic chemical knowledge of the reactions of gold. The *in vivo* chemistry of gold is likely to be very different from that of transition metals such as copper and iron, which are known to be essential to man. With these elements there are carefully controlled transport, storage, and enzyme functions but there appear to be no similar systems for gold which is distributed more widely in body tissues and fluids. Thus, a general understanding of the chemistry of gold with naturally occurring ligands such as thiols and halides, and the effect of physical properties such as different solvent environments are probably necessary before any chemical understanding of the action of gold drugs can be obtained.

[7] W. Pohl, *Med. Welt.*, 1954, **14**, 659.
[8] B. Makiola, *Med. Welt.*, 1954, **14**, 475.
[9] D. K. Gemmell, J. Cottney, and A. J. Lewis, *Agents and Actions*, 1979, in press.
[10] J. D. Jessop and H. L. F. Currey, *Ann. Rheum. Dis.*, 1968, **27**, 577.

A book has been published recently[11] on the chemistry of gold and there are recent reviews on aspects of its chemistry[12,13] and pharmacology.[6] Consequently, the chemistry is dealt with here only in so far as it is thought to be germane to the *in vivo* reactions of gold.

2 Model Systems for the *in vivo* Reactivity of Gold

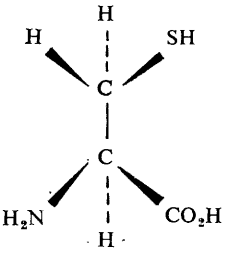
The rationale for the study of model systems is the assumption that the chemistry of gold *in vitro* will differ in detail but not in kind from that *in vivo*. The chemistry of gold will depend on its environment and the closer the model environment is to the *in vivo* system, the better the model will be. As yet, however, no detailed chemical knowledge of the effects of gold complexes are known, so the models examined must therefore be based on general chemistry rather than specific reactions. Thus, typical reactions of gold complexes with the thiols, cysteine, penicillamine, and thiomalic acid; with the dithiol 2,3-dimercaptopropanol; with the tripeptide thiol-glutathione; and with the thiol-containing protein, albumin, are discussed.

A. Reactions of Gold(0), (I), and (III), with L-Cysteine.—*Starting with Gold*(0). Metallic or colloidal gold reacts with cysteine in aqueous or saline solution in the presence of oxygen to give a gold(I)–cysteine complex which[14] can be identified by its circular dichroism (c.d.) spectrum.

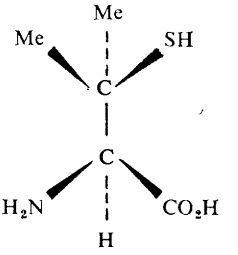
Starting with gold(I). The most common oxidation state of gold with thiol ligands is gold(I) and cysteine forms a 1:1 gold(I) complex. It appears to be polymeric. It is fairly insoluble in dilute acid but soluble in alkali. No structure has yet been published for the complex but i.r. measurements suggest that neither the amine nor carboxylate groups are involved in complexing and that the ligand bonds through the sulphur atom. It can be prepared by adding L-cysteine to almost any water or ethanol soluble gold(I) complex such as gold(I) cyanide, chloride, bromide, iodide, thiomalate, 2,3-dimercaptopropanolate, glutathionate, and triethylphosphinegold(I) chloride. The disulphide cystine in aqueous solution also reacts with gold(I) complexes. Several complexes of the type R_3PAu^I-L-cysteine have also been prepared, starting with R_3PAuCl. They are thought to be linear in structure. Whereas with triphenylphosphine the product is stable, with triethylphosphine the final product is Au^I cysteine.[15] Something of the complexity of these reactions is illustrated in Figure 3 for the reaction of cysteine with triethylphosphinegold chloride. A number of intermediate species are evident from the c.d. spectra.

Starting with gold(III). L-Cysteine reduces most gold(III) compounds in aqueous

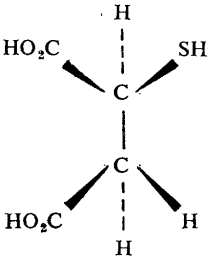
[11] R. J. Puddephat, The chemistry of gold, Elsevier, 1978, p. 274.
[12] P. Sadler, *Structure and Bonding*, 1976, **29**, 171.
[13] H. Schmidbaur, *Angew. Chem.*, 1976, **15**, 728.
[14] G. C. McKinley, 'Some Chemistry of gold complexes', Ph.D. Thesis, Strathclyde University, 1978.
[15] D. H. Brown, G. C. McKinley, and W. E. Smith, *J.C.S. Dalton*, 1977, 1874.



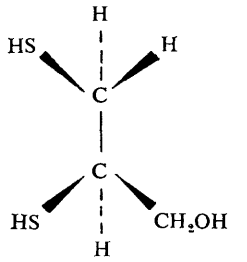
Cysteine



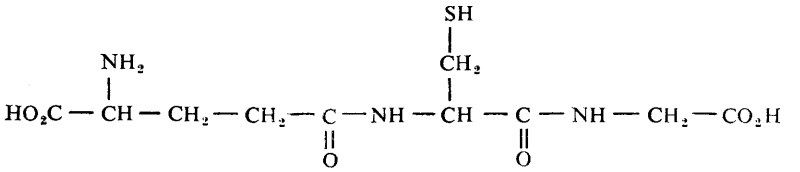
Penicillamine



Thiomalic acid



2,3-Dimercaptopropanol



Glutathione

Figure 2 *Some thiols which are likely to be implicated in the reactions of gold compounds 'in vivo'*

solution to give the gold(I)–L-cysteine complex. Cystine is one major oxidation product.

B. Reactions with D-Penicillamine.—D-Penicillamine (β-mercaptovaline or β-dimethylcysteine) reacts in a subtly different manner from L-cysteine. The difference in complexing behaviour between these ligands has been used in the treatment of Wilson's Disease[16] (D-penicillamine is effective in removing the excess copper present in patients suffering from this disease). Although D-

[16] D. H. Brown, G. C. McKinley, and W. E. Smith, *J.C.S. Dalton*, 1978, 199.

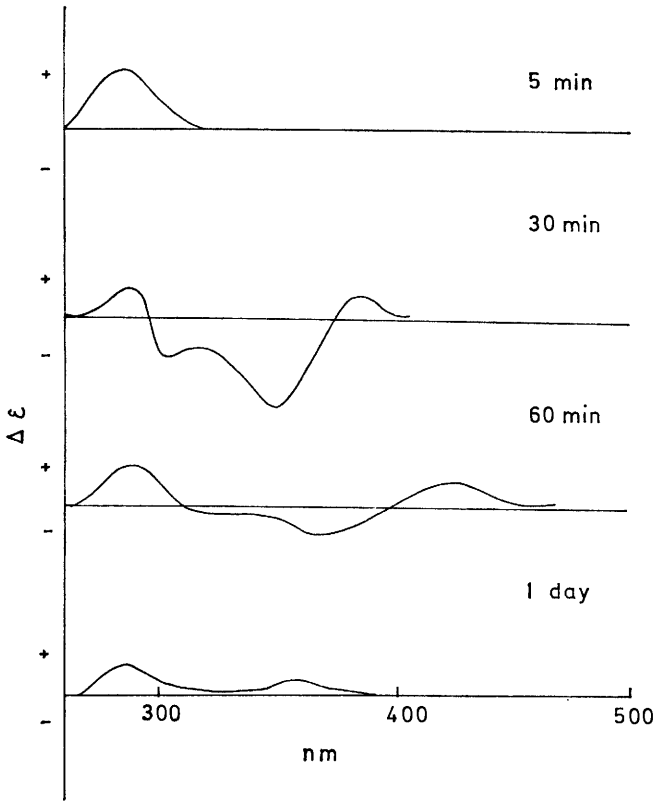


Figure 3 *Circular dichroism measurements after mixing* 5×10^{-4} *solution of triethylphosphinegold*(I) *chloride and cysteine. The reaction proceeds eventually to produce a precipitate of gold*(I)*cysteine* (~ 2 *days*)

penicillamine does not occur naturally *in vivo*, its reactions are of interest both as a model for β-substituted cysteines and because it is used as a drug, sometimes along with and sometimes after, gold therapy.

When gold(0) metal or colloid is mixed with D-penicillamine, solution and complex formation takes place more slowly than with L-cysteine. With gold(I) complexes, D-penicillamine reacts in a somewhat similar manner to L-cysteine, provided that the reactions are carried out under nitrogen. For example, D-penicillamine reacts with phosphine gold(I) chloride producing complexes of formula R_3 PAu penicillamine. In contrast to cysteine, when R = Et, the complex formed appears to be stable to further substitution.[17] Au^I penicillamine is formed on reaction with $AuCl_2$ under nitrogen, but in air, in contrast to

[17] D. G. Palmer and J. V. Dunckley, *Australian and New Zealand J. Med.*, 1973, **3**, 461.

cysteine, oxidation takes place readily and a gold(III)–(D-penicillamine)$_2$ complex can be isolated.[16] Similarly, starting with gold(III) in the form of the tetrachloro anion, substitution takes place, again yielding a bis complex. The oxidation state of this complex was confirmed by its gold Mössbauer spectrum. The shift of 3.01 nm s^{-1} and splitting of 3.74 nm s^{-1} were typical of gold(III)–sulphur complexes. The change in frequency of the NH_2 modes in the i.r. spectra suggested that the amine groups were also complexed.

Thus, comparing the reactions of L-cysteine and D-penicillamine with gold, there appears to be a fine balance in the gold redox system with thiol ligands. *In vivo*, therefore, where there is a variety of thiols in different environments, the possibility of gold(III) complexes being formed with thiols cannot be overlooked.

C. Reactions with Glutathione.—Gold(I) forms a stable 1:1 complex with glutathione[18] in aqueous solution. It is only slightly soluble in water and is stable in excess of ligand. Glutathione, like cysteine, will replace cyanide, chloride, and phosphines from gold(I) complexes. The $AuCl_4^-$ ion oxidizes glutathione to the sulphoxide, the gold being reduced to gold(I) which is stabilized by complexing with unreacted glutathione. If no excess ligand is present the gold is slowly reduced to the metal. Starting with gold(I) glutathione, the ligand can readily be replaced by L-cysteine, D-penicillamine, and 2,3-dimercaptopropanol.

D. Reactions with Thiomalic acid.—Sodium gold(I) thiomalate is a white solid which in aqueous solution is marketed as the drug myocrysin for the treatment of rheumatoid arthritis. In solution it exists in a polymeric form,[19] the structure of this and of the solid is unknown. Aqueous solutions are fairly stable, the thiomalate only being replaced easily by other strong sulphur donor ligands such as L-cysteine.[15,18,19,20] c.d. measurements indicate that immediate substitution of gold(I) thiomalate by cysteine occurs in both acid and neutral conditions. In acid, a stable solution is obtained, whereas at neutral pH the reaction continues more slowly to give a precipitate of gold(I)cysteine and the cysteine–thiomalate disulphide.[18,21] At neutral pH, fast exchange reactions between bound thiols and free SH in solution occur.[19] Alkylation of the thiol also prevents substitution.[20]

E. Reactions with 2,3-Dimercaptopropanol.—2,3-Dimercaptopropanol (BAL, British Anti-Lewisite) has been used in the treatment of gold toxicity with varied success.[22] Its potential use in cases of metal poisoning (it has been used for mercury, cadmium, bismuth, copper, and nickel poisoning) has been restricted by its instability in aqueous solution and by the fact that it is itself slightly toxic.

[18] A. J. Lewis, J. Cottney, D. D. White, P. Fox, A. McNeillie, J. Dunlop, W. E. Smith, and D. H. Brown, *Agents and Actions*, 1980, **10**, 63.
[19] A. A. Isab and P. J. Sadler, *J.C.S. Chem. Comm.*, 1976, 1051.
[20] C. J. Danpure, *Biochem. Pharmacol.*, 1976, **25**, 2343.
[21] C. J. Danpure, *J. Physiology*, 1977, **267**, 25.
[22] H. M. Margolis and P. S. Kaplan, *Ann. Intern. Med.*, 1947, **27**, 353.

If its reactions *in vivo* with gold are varied, so also are its reactions *in vitro*. With the tetrachloroaurate(III) anion, a range of compounds has been described[23] using different ratios of starting materials: $Au_3(BAL)_4Cl_3$, $Au_3(BAL)_5Cl_2$, $Au_2(BAL)_2Cl_3$, $Au(BAL)_2$, $Au_2(BAL)_5$, and $Au_2(BAL)_3$. The Mössbauer spectrum of $Au_3(BAL)_{10}Cl_2$ showed the presence of both gold(I) and gold(III). These compounds are all highly insoluble and presumably polymeric. With gold(I) thiomalate, a precipitate of formula $Au_3(BAL)$(thiomalate) was obtained. With gold(I) glutathionate, the glutathione was replaced by BAL and a precipitate of formula Au_6BAL_9 was obtained.

Thus BAL seems to form a range of polymeric species with gold(I) and (III).

F. Reactions with Albumin.—*Gold*(I). Most *in vitro* work on the reactions of albumin with gold complexes has been carried out with sodium gold(I) thiomalate. The evidence suggests[24,25,26] that the latter compound reacts with the free thiol on albumin with the release of thiomalate ion. The reaction, however, is not simple, as the equilibrium appears to be reached slowly and can be affected by the presence of other drugs such as indomethacin and phenylbutazone. As well as the free thiol, there is evidence for several gold-binding sites on albumin and a weak affinity for the gold thiomalate molecule.

Gold(III). In high enough concentration, the tetrachloroaurate ion can precipitate albumin. At lower concentrations reaction can take place with reduction of gold(III) but, as yet, no details of these reactions have been published.

G. Solvent Effects.—In a protein or within membranes or cells, there are hydrophobic as well as hydrophilic regions and, consequently, the solubility and reactions in non-aqueous solvents of the gold species implicated in the treatment of rheumatoid arthritis may be important variables *in vivo*. Sodium aurothiomalate, for example, is soluble in water and reactive to cysteine, whereas in ethanol it is insoluble and unreactive. On the other hand, both the orally active drug auranofin (Figure 1) and triethylphosphinegold chloride, which is orally active in the rat-paw kaolin model, are soluble and reactive to cysteine in ethanol but are insoluble and much less reactive in water.[18] Further, triethylphosphinegold chloride, but not auranofin, can be made soluble and reactive to cysteine in water by the addition of excess sodium thiosulphate. In 2M-HCl, auranofin does react, possibly due to an exchange reaction with the thiol. A similar reaction has been reported for other thiols.[19]

Attempts to prepare a gold(III)–thiomalate complex in aqueous solution have, as yet, proved unsuccessful since the gold is easily reduced to gold(I) thiomalate. In absolute ethanol, however, evidence for a gold(III)–thiomalate complex has been obtained, anhydrous conditions being critical.[27]

[23] D. H. Brown, G. C. McKinley, and W. E. Smith, *J. Inorg. Biochem.*, 1979, **10**, 275.
[24] R. W. Mason, *Pharmacology*, 1977, **15**, 536.
[25] C. J. Danpure, *Biochem. Trans.*, 1976, **4**, 141; *Biochem. Pharmacology*, 1976, **25**, 2343.
[26] E. Jellum and E. Munthe, *Proc. Roy. Soc. Med.*, 1977, **70**, Supp. 3, 136.
[27] D. H. Brown, M. Paton, and W. E. Smith, submitted for publication.

Reactions of gold with amino-acids in non-aqueous solvents have been little studied, partly because of the difficulty of dissolving the amino-acids. One solvent which has been used is glacial acetic acid and it would appear that the chemistry is quite complex. For example, with cysteine a complex of formula Au_4(L-Cys)$_{12}Cl_{10}$ has been isolated and with D-penicillamine, other complex species which contain gold chloride and D-penicillamine can be obtained. These latter compounds have a peak at about 520 nm in the c.d. spectra, suggesting the presence of gold(III).[14]

3 Separation and Methods of Analysis of Gold Complexes *in vivo*

Prime requirements for an understanding of the biological effects of gold *in vivo* would seem to be a method of analysis capable of detecting and determining the concentration of gold in specific proteins and tissues, and methods of determining chemical properties such as reactivity, structure, and oxidation state of molecules and of separated fractions. There has been considerable progress in developing the former of these requirements, with the consequence that distribution and pharmaco-kinetic experiments with gold can now be performed more simply but there has, as yet, been little application of the available techniques to the latter objective. In the following section the most commonly employed biochemical separation techniques are outlined.

A. Biochemical Separation.—As a consequence of the wide distribution of gold in animals and humans, most biological fluids and tissues removed for analysis contain some gold. Since animals are regularly sacrificed during pharmacological studies of gold compounds, a wide range of animal tissues and fluids is available for analysis. In some cases where whole organs have been removed, these have been examined further either by preparing sections which can be scanned by electron microscopy to obtain a map of the gold distribution in the organ, or by mascerating and separating components of the organ. In the latter case, most interest has centred on the separation of the soluble protein fraction which contains melallothionen, a protein with a molecular weight of about 10 000 containing 66 % of cysteine residues.

In human and in animal studies the most extensively studied fraction is blood. A standard method of separating blood is shown in Figure 4. The blood is first split into cells, and extracellular protein and fluid. The extracellular proteins are referred to as plasma if they contain the clotting proteins, fibrin and fibrinogen, otherwise they are referred to as serum. There are about 97 plasma proteins, of which about 25 can be separated with relative ease. Single-stage separations by chromatography or electrophoresis, however, usually produce a less resolved separation. Each fraction contains a range of proteins, although the most common protein, albumin, accounting for about half of the plasma proteins by weight, is predominant in the fraction where it appears.

Rheumatoid arthritis is often stated to be a disease of the immune system and as a consequence the gold level in cells, particularly white cells, is of potential interest. White cells can be subdivided into various types: lymphocytes, poly-

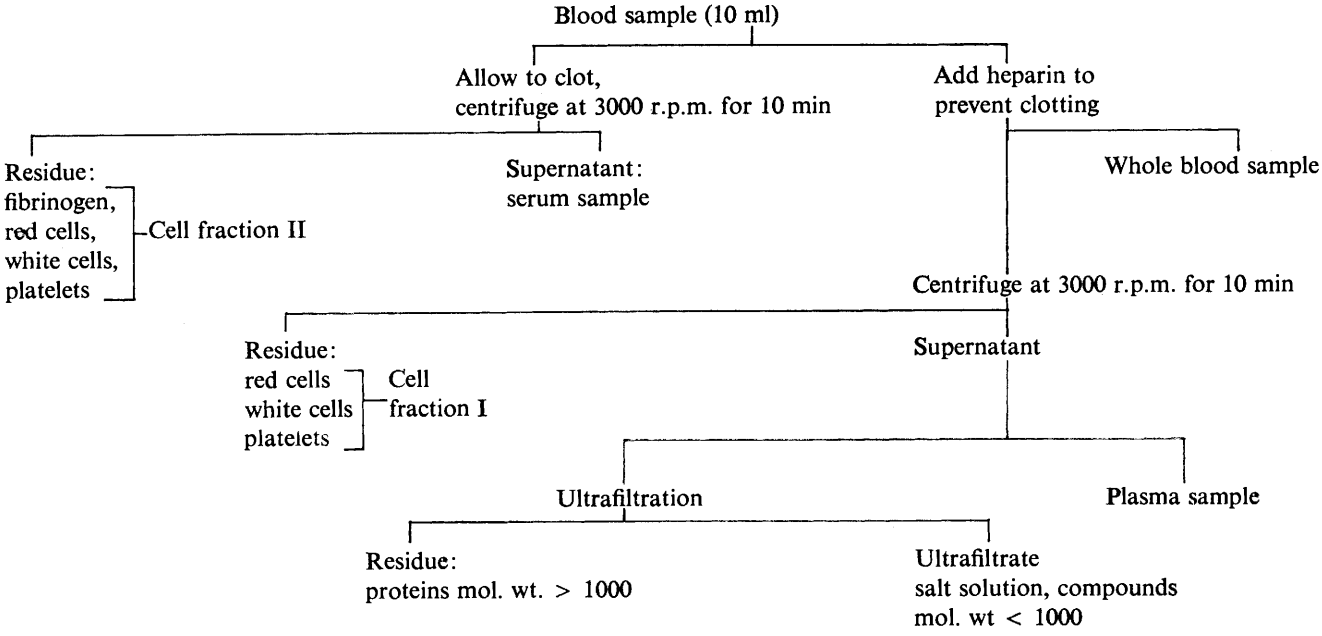


Figure 4 *Procedure for the separation of a blood sample into its components*

morphs, platelets, *etc.* One particular type of cell which has been separated in a number of studies is the macrophage.[18] Macrophages ingest foreign material in the body and can contain gold. Cells have been investigated by electron microscopy and particular attention has been paid to the lysosomes,[28,29,30] small bodies in the cell which are bounded by a single layer of membrane and which contain enzymes implicated in the mechanism of production of pain.[31] The administration of soluble gold salts produces extra lysosomal bodies containing gold called aurosomes and these, too, have been studied by electron microscopy.[32]

In a few cases, tissues and fluids from joints have been studied. The area around the joint is enclosed by the synovial membrane. It encloses a thixotropic fluid, synovial fluid, in which a large number of molecules have been identified,[33] but which is mainly a saline solution of hyalauric acid. The synovial tissue extends across the joint and encompasses important protein-synthesis sites.

Clearly, many further separations can be considered but those referred to are the ones most widely discussed at present. This section contains a definition of all the biological terms necessary to understand what follows.

B. Measurement of Gold Concentrations in Biological Fluids and Tissues.— Various methods have been used for the determination of the distribution of gold in biological fluids and tissues. Colorimetric methods[34] lack sensitivity; polarographic methods[35,36] require very careful control of the solution to be analysed, and neutron activation,[37,38] techniques are sensitive and reliable but cumbersome. Possibly the most convenient method, in that it is sensitive enough and relatively rapid, is atomic absorption spectrometry using carbon furnace atomization.[39,40,41]

The major problem with this technique is its sensitivity to the matrix in which the gold is present and it is a technique which requires a skilled operator to ensure reliable answers. With these provisos, however, it is fast, accurate, reliable, and sensitive. A comparison of gold concentrations in human serum protein fractions separated by electrophoresis between atomic absorption and neutron activation (Table 1) illustrates the reliability for one particular separation.[40]

These results and related experiments give some confidence that the more

[28] H. Nakamura and M. Igorishi, *Ann. Rheum. Dis.*, 1977, **36**, 209.
[29] W. L. Norton and M. Ziff, *Arthritis Rheum.*, 1966, **9**, 589.
[30] P. E. Lipsky, K. Ugai, and M. Ziff, *J. Rheumatol.* Supp. 5, 1979, **6**, 131.
[31] J. Chayen and L. Bitensky, *Ann. Rheum. Dis*, 1971, **30**, 522.
[32] F. N. Ghadially, *J. Rheumatology*, Supp 5, 1979, **6**, 45.
[33] J. Heininger, E. Munthe, and J. Pahle, *J. Chromatography*, 1978, **158**, 297.
[34] W. Block and M. S. Buchanan, *J. Lab. Clin. Chem.*, 1947, 118.
[35] G. D. Christian, *Clin. Chem.*, 1965, **11**, 459.
[36] E. S. Jacobs, *Analyt. Chem.*, 1963, **35**, 2112.
[37] S. Solvsten, *Scan. J. Clin. Lab. Invest.*, 1964, **16**, 39.
[38] D. Brune, K. Samshal, and P. O. Webster, *Clin. Chim. Acta*, 1946, **13**, 285.
[39] H. Kamel, D. H. Brown, J. M. Ottaway, and W. E. Smith, *Analyst*, 1976, **101**, 790.
[40] H. Kamel, D. H. Brown, J. M. Ottaway, and W. E. Smith, *Analyst*, 1977, **102**, 645.
[41] R. J. Ward C. J. Danpure, and D. A. Fyfe, *Clin. Chim. Acta*, 1977, **81**, 87.

Table 1 *Gold concentrations in protein fractions separated by electrophoresis, of the serum of patients undergoing chrysotherapy*

Patient	I		II		III		IV	
Serum gold/μg ml^{-1}	3.84		2.6		1.81		1.16	
Gold in plasma fractions	/μg ml^{-1}	% of total	/μg ml^{-1}	% of total	/μg ml^{-1}	% of total	/μg ml^{-1}	% of total
Albumin	1.52	45	1.41	60	1.12	69	0.76	76
α_1	0.13	4	0.09	4	0.03	2	0.03	3
α_2	0.84	25	0.42	18	0.25	15	0.13	12
β	0.47	14	0.23	10	0.09	5.5	0.05	5
γ	0.41	12	0.19	8	0.13	8.5	0.04	4

extensive results described in Section 4 are of sufficient accuracy and reliability. The most serious problems arise during the biochemical separation procedures. Examples of these are:

(i) Gold complexes may react, redistribute, or be removed during separation. An example of this for serum proteins after separation by electrophoresis is shown in Table 2. Both atomic absorption and neutron activation techniques

Table 2 *Comparison of results obtained by carbon furnace atomic absorption spectrometry and neutron activation analysis for gold in different protein fractions separated by the electrophoresis procedure. A and B are different serum samples from rheumatoid patients undergoing chrysotherapy*

Protein fraction	*Neutron activation analysis* /ng Au		*Carbon furnace atomic spectrometry technique*/ng Au	
	A	B	A	B
Albumin	4.2 ± 0.2	3.76 ± 0.14	4.01	3.5
α_1	0.32 ± 0.046	0.36 ± 0.05	0.35	0.38
α_2	0.75 ± 0.097	0.45 ± 0.03	0.65	0.50
β	0.70 ± 0.06	0.41 ± 0.05	0.75	0.39
γ	0.54 ± 0.04	0.24 ± 0.03	0.53	0.26

of analysis were used and both indicate a loss of about 15 % from the original serum gold levels. However, under the experimental conditions used, no gold would be expected to be present in a non-protein-bound form.[40] It is believed that the gold lost is in the form of unreacted drug absorbed on albumin.

(ii) The gold complexes may react with the support media or chemicals used. For example, if the cellulose acetate strips used in electrophoresis are stained with Acid Red dye in the conventional manner to indicate the position of the protein bands for analysis, some of the gold is extracted from the strip by the acetic acid used.[39]

(iii) The matrix containing the gold may itself create problems during analysis. Smoke, for example, is produced by the cellulose acetate strips and the proteins and must be ashed before analysis. A more serious and specific problem arises with halide-rich environments such as are found after using saline solutions to chromatograph gold-containing proteins or are found in ultrafiltrate. Signal depressions of 70 and 90 % have been reported in these cases due to the formation of a volatile gold chloride during the atomization step.[40,41] Solutions to these problems have been proposed, but they could be improved and the size of the depression illustrates the need for caution in this type of experiment.

The above methods involve the isolation and analysis of bulk fractions of biological samples. An alternative approach is to study the gold distribution by electron microscopy. Early studies[30] did not identify gold exactly but referred to

'extra electron dense' deposits in particular parts of cells. More recent studies[29,30,32,42,43] have used *X*-ray absorption methods to analyse for gold and other elements directly. Clearly such methods have great potential although there are difficulties associated with sample preparation. Probably a combination of *X*-ray and bulk analysis will eventually be used for gold analysis in biological samples.

C. Potential for the Measurement of Chemical Properties of Gold *in vivo*.— Techniques used for the determination of the valency and geometry of gold *in vitro* are as yet insufficiently developed for *in vivo* use. The usual techniques of valency identification are by Mössbauer spectroscopy and by ESCA (electron spectroscopy for chemical analysis). Recent Mössbauer and nuclear quadrupole resonance (n.q.r.) studies[44,45,46] have established the nature of the gold signals for a wide range of relevant complexes *in vitro*. As well as identifying the valence state, something of the bonding and geometry of the complexes can be established. The ESCA technique offers mainly a method of establishing valency.[47,48,49,50] Its principal merit is that it does not require the use of a liquid helium cooled detector and for that reason is more readily applicable to biological samples for which storage times may be critical. Both techniques are too insensitive for ready use at physiological gold concentrations.

Since almost all gold complexes are diamagnetic, it is possible to use the n.m.r. spectra of the ligands to determine their geometry and in view of advances made on other biological systems, it may be possible to use this technique as a probe for gold structures *in vivo*. *X*-ray methods would also be helpful in specific cases. Another method which has been applied to gold complexes recently is resonance Raman spectroscopy.[51] Although it has the required sensitivity for halide complexes the Raman spectra of thiol complexes are less satisfactory and there may well be fluorescence problems.

One method which possesses the required sensitivity is u.v.–visible spectroscopy. However, the reliable interpretation of these results in terms of structures and bonding is difficult and the bands tend to be broad and overlapping. Gold(I)[15] complexes generally have strong charge transfer bands tailing towards the

[42] F. N. Ghadially, W. E. DeCoteau, S. Huong, and I. Thomas, *J. Path.*, 1979, **124**, 77; 1979, **125**, 219.
[43] F. N. Ghadially, A. F. Ortschak, and D. M. Mitchell, *Ann. Rheum. Dis.*, 1976, **35**, 67.
[44] P. G. Jones, A. G. Maddock, M. J. Mays, M. N. Muir, and A. F. Williams, *J.C.S. Dalton*, 1977, 1434, 1440.
[45] C. A. McAuliffe, R. V. Parish, and P. D. Randall, *J.C.S. Dalton*, 1977, 1426.
[46] T. P. A. Viegers, J. M. Trooster, P. Bouten, and T. P. Rit, *J.C.S. Dalton*, 1977, 2074.
[47] C. Battistoni, G. Mattogno, F. Cariatti, L. Naldini, and A. Sgamellotti, *Inorg. Chim. Acta*, 1977, **24**, 207.
[48] H. Schmidbaur, J. R. Mandl, F. E. Wagner, D. F. Van de Vondel, and G. P. Van der Kelen, *J.C.S. Chem. Comm.*, 1976, 170.
[49] D. F. Van de Vondel, G. P. Van der Kelen, H. Schmidbaur, A Wooleben, and F. E. Wagner, *Physica Scripta*, 1977, **16**, 364.
[50] D. H. Brown, A. McNeillie, W. E. Smith, M. Gibson, and L. Watson, *J.C.S. Dalton*, 1980, 767.
[51] Y. M. Bosworth and R. J. H. Clark, *Inorg. Chem.*, 1975, **14**, 170.

visible region where weaker bands affect the shape of the tail and produce yellow or colourless species. Gold(III) complexes[52] containing halide ligands have a characteristic peak on the low energy side (300—400 nm) of the charge transfer band. Changes in the energy position of this band with different ligands can be quite small and the much larger changes in molar absorptivity may be a more realistic guide to substitution.

The inclusion of c.d. measurements strengthens the interpretation of electronic spectra, since the broad overlapping bands have different intensities and signs in c.d. and the two together can give a characteristic pattern[14] (Figure 3). For example, for the gold–sulphur bond, a series of peaks has been defined for both gold(I) and gold(III) complexes. Those for gold(III) occur at significantly longer wavelengths and some indication of valence state even at low (10^{-4} molar) concentrations of gold can be obtained.[14] Much remains to be done before any of these methods is an effective *in vivo* probe. However, if the variety of gold oxidation states and geometries evident with model systems is repeated *in vivo*, it is likely that such a probe will be required before a full understanding of the *in vivo* chemistry can be obtained.

4 Gold Studies in Animals and Man

A. Gold in Animal Models of Inflammation.—Although gold therapy has played an important role in the treatment of rheumatoid arthritis for many years, no animal-model studies have shown the same responses to gold complexes as do human rheumatoid arthritis patients. The models generally used are adjuvant arthritis in the rat,[10] kaolin paw oedema in the rat,[6] and u.v. erythema in the guinea-pig[53]. These have all produced conflicting results. In a recent review, Walz *et al.*[6] have compared many of the reported activities of gold injected intramuscularly with the more recently identified orally active forms of gold.

Drugs such as myocrisin and D-penicillamine take six weeks to three months to produce measurable improvements in rheumatoid arthritis in man and consequently the most acceptable animal model would be one in which a response to 'chronic' inflammation rather than 'acute' inflammation is assessed. For this reason, the adjuvant arthritis model is generally regarded as the most acceptable since the effect is measured over a period of three weeks, as opposed to 24 hours for the other models mentioned. However, there are serious deficiencies, for example, penicillamine is ineffective in the adjuvant model.[54] Further, the strain of rat appears to be important. Normal gold drugs partially suppress the physical symptoms of adjuvant arthritis in Lewis[18] or Sprague-Dawley rats[55] but not in the Wistar strain.[56,18] There does not appear to be any correlation in the amount of gold absorbed and the activity in either the Lewis or Wistar strains.

[52] D. H. Brown, G. C. McKinley, and W. E. Smith, *Inorganica Chim. Acta*, 1979, **32**, 117.
[53] E. Law and A. J. Lewis, *Brit. J. Pharmacol.*, 1977, **59**, 591.
[54] E. Arrigoni-Martelli and E. Bramm, *Rheumatol. Rehabil.*, 1976, **15**, 207.
[55] R. D. Sofia and J. F. Douglas, *Agents and Actions*, 1973, **3**, 335.
[56] J. D. Jessop and H. L. F. Currey, *Ann. Rheum. Dis.*, 1968, **27**, 577.

Adjuvant arthritis is produced by injection into one fore paw of the rat. It produces a systemic disease and inflammation of the non-injected paws as well as the injected one occurs. Indomethacin is equally effective in suppressing the injected and hind paw swelling in both strains, which suggests that the Wistar strain is not generally insensitive to inflammatory drugs.[18] Thus, the empirical rationale for originally using gold complexes in the treatment of rheumatoid arthritis has not been convincingly confirmed by animal studies, although some activity has been obtained.

In animal studies, as in human studies, gold is found widely distributed in tissues with major gold accumulations in kidney, liver, spleen, skin, lymph, and bone marrow.[54] Significant gold levels were evident in most other tissue samples examined, including brain tissues. Examination of the uptake of gold [from sodium gold(I) thiomalate] in rat-liver cells showed that, whereas in blood plasma most of the gold was complexed to albumin, in the lysosomes the bulk of the gold was membrane-bound, possibly to thiols.[57] Separation of cytosol and lysosomal supernatant showed that gold was complexed to a wide range of molecular weight species.[58] Recent studies[6,59] have shown that, in the guinea-pig, different gold distributions are achieved by giving gold orally in the form of triethylphosphinegold(I) chloride as compared to intramuscularly administered gold(I) thiomalate. The stomach level five hours after administration of triethylphosphinegold(I) chloride was particularly high, suggesting that a stomach rather than an intestinal absorption mechanism could predominate with this compound. Time course studies of the gold levels found in kidney and liver following the administration to guinea-pigs of 30 mg kg^{-1} of triethylphosphinegold(I) chloride orally and sodium gold(I) thiomalate intramuscularly showed much higher levels from the latter[6,59] (Figure 5). This suggests that with further research, a better balance between therapeutic and toxic effects than that achieved with present drugs and present clinical regimes may be possible. This conclusion is based on the amount of gold present and does not take into account any differences in reactivity of the gold stored in each case. It is notable in this respect that in one experiment the gold accumulated in kidney was not removed during the time of the experiment with myocrisin but was reduced to half with triethylphosphinegold chloride, suggesting that the storage forms of gold were, at least in part, different in the two methods of administration.[59]

It is perhaps a little surprising that very few studies of the gold levels in stomach and intestinal tissues have been made and, in particular, no attempt seems to have been made to check the effect of repeated dosage of the orally active forms of gold on these tissues. The levels achieved one week after a single dose of triethylphosphinegold chloride was administered to guinea-pigs are not markedly different from those produced by intramuscular injection of

[57] D. E. Furst, S. Levine, R. Srinivasan, A. Metzger, R. Bangert, and H. E. Paulus, *Arth. and Rheum.*, 1477, **20**, 1473.
[58] A. Lorber, *Clin. Pharmokin*, 1977, **2**, 127.
[59] H. Kamel, D. H. Brown, J. M. Ottaway, W. E. Smith, J. Cottney, and A. J. Lewis, *Agents and Actions*, 1978, **8**, 546.

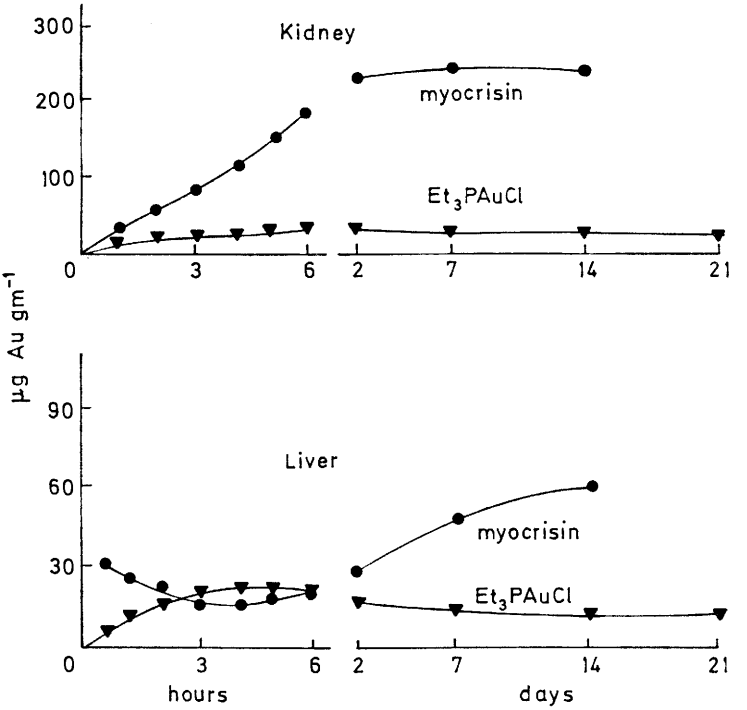


Figure 5 *Gold levels in kidney and liver following the administration of* ½ *dose of* 30 mg kg^{-1} *body weight to guinea-pigs of triethylphosphinegold*(I) *chloride* (*oral*) *and myocrisin* (*intramuscularly*)

myocrisin.[59] However, the reactions discussed in Section 3 of this review make it obvious that different forms of gold could be deposited during oral therapy. In view of the slow clearance rates of gold compounds, it would seem to be necessary to obtain this information before commencing prolonged dosage of humans with these compounds.

B. Clinical Studies of Gold

Gold therapy has been proved effective during many years of clinical experience, and yet the most effective and least toxic dosage schedule for administration of gold has not yet been established. In one double-blind study,[57] patients were selected for treatment using conventional doses (50 mg) or high doses (150 mg) of sodium gold(I) thiomalate given weekly by injection. Neither efficacy nor toxicity correlated with serum gold concentration. Conventional doses were as efficacious as high doses with respect to rapidity and degree of response. However, in another study, the adjustment of gold doses to achieve and maintain a serum level of about 3 $\mu g\ ml^{-1}$ has been recommended,[58] and gold toxicity has

been linked to the dosage regime. Thus, medical studies give little help to the chemist in suggesting a possible mode of action of gold and perhaps exemplify the confusion that still exists medically on the role of gold in the treatment of rheumatoid arthritis. Why do some patients with rheumatoid arthritis respond to gold therapy whilst others do not respond? Can this be due to an inadequate or ineffective dosage regimen[58] or are there different diseases described by the one name? In one study of patients on gold therapy, those with serum positive arthritis responded better to gold than those with serum negative arthritis.[60]

Attempts to relate gold concentration in readily accessible body fluids such as blood serum to therapeutic activities have produced varied results. Some reports have related high gold plasma concentration to therapeutic response,[61] skin rashes,[62] toxic side effects,[63] whereas others have found no correlation.[64,17] Recent work has compared the effects of penicillamine and sodium gold(I)-thiomalate on parameters such as differential agglutination titre in synovial fluid. These results suggest a common mode of action due in part to the reduction of the antigenicity of the IgG complexes.[60]

Although small amounts of gold complex unbound to serum proteins or cells have been found in human serum immediately following injection with fairly high doses of myocrisin,[61] most gold is bound to the serum proteins. In one case, 96 % of the gold was associated with the serum proteins and about 4 % with the white cells, with the level in red cells being beneath detectable limits.[39] The gold in the white cells appears to be distributed between polymorphs and leucocytes.[65] Of the gold in serum, 75–85 % is associated with albumin;[40,41] about 15 % of this gold can be removed during electrophoresis and appears to be a small-molecule, probably unchanged drug.[40] Separations of the serum by chromatography followed by electrophoresis of separated fractions indicated the presence of gold in all fractions which were of sufficient concentration for analysis and suggest, by comparison with the electrophoresis results, that little or no redistribution between the fractions has occurred.[40] Thus, gold would appear to be widely distributed and tightly bound to the proteins, as expected from its chemistry. Penicillamine, for example, does not remove an appreciable amount of protein-bound gold.[66]

Serum-separation studies of this type may be of importance, in that they can provide more specific parameters than the serum gold level for use in monitoring chrysotherapy. However, they can be criticized in that the process of separation and the time between sampling and analysis may lead to a redistribution of the gold in the serum. One study of this type using a chromatographic separation technique has identified small amounts of gold which are not bound to any serum

[60] M. H. Pritchard and G. Nuki, *Ann. Rheum. Dis.*, 1978, **37**, 493.
[61] A. Lorber, G. J. Atkins, G. C. Chang, Y. B. Leeb, J. Starrs, and R. A. Bovy, *Ann. Rheum. Dis.*, 1973, **32**, 133.
[62] J. D. Jessop and R. G. S. Johns, *Ann. Rheum. Dis.*, 1973, **32**, 228.
[63] F. E. Kruzius, A. Markkanen, and P. Peltola, *Ann. Rheum. Dis.*, 1970, **29**, 232.
[64] B. R. Mascarenhas, J. L. Granda, and R. M. Freyberg, *Arth. and Rheum.*, 1972, **15**, 391.
[65] A. Lorber, S. Wilcox, J. Leeb, and T. Simon, *J. Rheumatol.*, 1979, **6**, 270.
[66] D. F. Biggs, D. M. Boland, P. Davis, and J. Wakaruk, *J. Rheumatol.*, Supp 5, 1979, **6**, 68.

protein present in the serum during the first hour after injection.[67] A much larger amount of unbound gold was found in animal studies[68] where a high gold dose in mg kg^{-1} of body weight was used. The amount of gold held by the different protein fractions varies with time during the first few days after injection.[67] It has been shown that during a conventional course of twelve weekly injections of 25 mg of myocrisin, in which the serum level reached approximately the usual level of about 3 $\mu g\ ml^{-1}$, the distribution of gold among the serum proteins did not vary appreciably as the gold level rose.[69] Thus, there appear to be a number of gold-binding sites, and there is no evidence that these become saturated during therapy. There were some differences in the gold distribution between different patients. So far, this information has not proved to be any more successful than the serum gold level in indicating therapeutic or toxic responses.

There are fewer studies of gold in human tissues (largely due to non-availability of samples) than in blood. One study[70] in a patient who died during gold therapy showed relatively high levels of gold in kidney, spleen, liver, skin, lymph, and bone marrow, with smaller concentrations in virtually every tissue sample analysed. Appreciable gold serum levels have been recorded in patients one year to twenty-three years[71] after cessation of chrysotherapy, indicating that the gold store created in tissue is by no means completely inert but gradually releases gold back into the blood-stream. In one attempt to separate kidney fractions, some gold was found in the soluble proteins but the recovery was low, indicating possibly the existence of both a soluble and reactive and an insoluble, less reactive form of gold.[72] Gold has also been shown to accumulate more in inflamed than in normal joints and in synovial tissue: a correlation between gold levels in synovial tissue and the times of gold therapy has been suggested.[71]

A series of papers[73] concerning the use of auranofin in oral therapy imply that this form of treatment may have advantages in ease and comfort of treatment, reduced side-effects[74] and tissue accumulations[75] and possibly even improved therapeutic effect. They suggest that the onset of therapeutic activity is earlier[76] (approximately five weeks after commencing therapy) and confirm that the different enzyme activities and immunological parameters referred to throughout the review do not show a consistent pattern when auranofin and myocrisin are compared. Nor is the distribution of the gold in blood similar, for

[67] C. J. Danpure, D. A. Fyfe, and J. M. Gumpel, *Ann. Rheum. Dis.*, 1979, **38**, 364.
[68] H. Kamel, D. H. Brown, J. M. Ottaway, W. E. Smith, J. Cottney, and A. J. Lewis, *Arth. and Rheum.*, 1978, **21**, 441.
[69] A. Brown, D. H. Brown, J. M. Ottaway, and W. E. Smith, submitted for publication.
[70] N. L. Gottlieb, P. M. Smith, and E. M. Smith, *Arth. and Rheum.*, 1972, **15**, 16.
[71] R. Vernan-Roberts, J. L. Dove, J. D. Jessop, and W. J. Henderson, *Ann. Rheum. Dis.*, 1976, **35**, 477.
[72] H. Kamel, J. R. Auld, N. Cunningham, D. H Brown, J. M. Ottaway, and W. E. Smith, *Arth. and Rheum*, 1976, **19**, 1368.
[73] *J. Rheumatology*, Supp. 5, **6**, 1979.
[74] N. L. Gottlieb, *J. Rheumatology*, Supp. 5, 1979, **6**, 51.
[75] N. L. Gottlieb, *ibid.*, Supp. 5, 1979, **6**, 61.
[76] A. Lorber, T. M. Simon, J. Leeb, A. Peter, and S. A. Wilcox, *ibid.*, Supp. 5, 1979, **6**, 82.

whereas with myocrisin 4 % or so of gold is bound to white cells, 50 % of the gold after auranofin treatment is in the cellular fraction.[77] It has been argued that for auranofin, as for other gold compounds,[6] the action is due to the gold and not to the ligand and it seems most likely that the differences relate more to the reactivities and kinetics of different gold metabolites in the two cases.

C. The Effect of Gold on Enzyme Activities and on the Immune System. Lysosomal enzymes are believed to have an effect on pain and on tissue destruction and it has been postulated that the inhibition of lysosomal glycosidases,[78,79] catheptic proteases,[80] or neutral proteases[81,82] may be involved in the action of gold. However, when a range of enzyme activities was assessed *in vitro* using a variety of gold compounds, a different pattern of activities was obtained for each gold compound tested,[17] a result which is not surprising to the chemist in view of the different reactivities and solubilities of gold compounds (Section 2).

Prostaglandins are likely mediators of inflammation, and gold has been shown to have an effect in the production of prostaglandins.[83,84]

Another approach is to consider the effect of gold on the immune system.[76,85] Standard parameters such as immunoglobulin titres and rheumatoid factor are reduced by chrysotherapy but the results are less consistent if studied in detail. For example, a study[86] comparing myocrisin and auranofin suggested that, whereas both compounds inhibited antibody production *in vitro*, *in vivo* auranofin inhibited antibody production but myocrisin stimulated it. Other effects include inhibition of phagocytic activity of macrophages and polymorphs,[87] inhibition of mast-cell histamine release,[88] and inhibition of complement activity.[89] However, the bulk of evidence seems to focus on the action of sodium gold thiomalate in inhibiting lysosomal enzymes. Work, both in rats[90,26] and humans,[91] on the possible mode of action of orally administered auranofin has also suggested that its therapeutic effect may be due at least in part to inhibition of lysosomal enzyme release and/or decrease in immune-complex formation.

5 Possible Reactions of Gold *in vivo*

Between those mechanisms suggested above and the chemistry of gold discussed

[77] D. T. Walz, D. E. Griswold, M. J. Di Martino, E. E. Bumbier, *ibid.*, Supp. 5, 1979, **6**, 56.
[78] D. Burkhardt, R. W. Stephens, P. Ghosh, and T. K. F. Taylor, *Agents and Actions*, 1978, **8**, 251.
[79] R. Persellin and M. Ziff, *Arth. and Rheum.*, 1966, **9**, 57.
[80] R. S. Ennis, J. L. Granda, and A. S. Posner, *Arth. and Rheum.*, 1968, **11**, 756.
[81] D. Kruze, K. Fehr, H. Menninger, and A. Boni, *Z. Rheumatol.*, 1976, **35**, 337.
[82] A. Janoff, *Biochem. Pharmacol.*, 1970, **19**, 626.
[83] G. A. Higgs, E. A. Harvey, S. H. Ferreira, and J. R. Vane, *Adv. Prostaglandin Thromboxane Res.*, 1976, **1**, 105.
[84] C. Deby, Z. M. Bacq, and D. Simon, *Biochem. Pharmacol.*, 1973, **22**, 3141.
[85] N. Penneys, V. Ziboh, N. L. Gottlieb, and S. Katz, *J. Invest. Dermatol.*, 1974, **63**, 356.
[86] D. T. Walz, M. J. Di Martino, and D. E. Griswold, *J. Rheumatol.*, Supp. 5, 1979, **6**, 74.
[87] J. D. Jessop, B. Vernon-Roberts, and J. Harris, *Ann. Rheum. Dis.*, 1973, **32**, 294.
[88] S.Norn, *Acta pharmacol. toxicol.*, 1965, **22**, 369.
[89] D. R. Schultz, N L. Gottlieb, P. I. Arnold, *Arth. Rheum.*, 1973, **16**, 131.
[90] M. J. Di Martino and D. T. Walz, *Inflammation*, 1977, **2**, 131.
[91] A. E. Finklestein, F. R. Roisman, and D. T. Walz, *Inflammation*, 1977, **2**, 143.

at the beginning of this review, lies a vast gap—the gap in communication between the pharmacologist and the chemist. Little is known of the chemistry of these reactions and so any discussion of the action of gold must, in a sense, be inspired guesswork. Some attempts have been made to prove these mechanisms by comparing the actions of different drugs, *e.g.*, sodium gold(I) thiomalate and D-penicillamine, but as yet little chemical progress has been made. In the following section possible *in vivo* reactions of gold are discussed, based largely on the expected reactions of gold with ligands known to occur naturally and under experimental conditions approximating to natural conditions.

A. Redox Reactions.—Although lysosomal enzyme release is believed to be a key step in tissue destruction, another key step is thought to be the action of superoxide (O_2^-) or hydroxy radicals.[92] The process of tissue damage caused by superoxide release by white cells is believed to be controlled by dismutases containing transition-metal ions such as zinc, copper, and manganese[92] and indeed one such enzyme has been tested for clinical efficacy in the treatment of rheumatoid arthritis. Gold, like other transition-metal ions could act as a superoxide dismutase or peroxidase preventing tissue destruction. The activity of gold(I) could be related to its widespread distribution throughout the body, its facile redox reactions and its relatively low toxicity compared to other transition metal ions. For example, many forms of gold(I) and gold(III) react with peroxide to form gold(0), and gold(III) as sodium tetrachloroaurate reacts with amino-acids such as alanine to form gold(0) and organic by-products including, in the case of alanine, acetaldehyde.[93] Therefore, it is perhaps surprising that little work on the action of gold as a dismutase or as a mediator of dismutase activity has yet to be investigated.

The effect of gold on reactions such as the thiol–disulphide oxidation and ceruloplasmin oxidase activity are discussed later.

B. Effects on Other Trace Metals.—As discussed above, gold(I) will compete *in vivo* mainly with copper(I). It is tempting to pursue this line of enquiry, as copper serum levels can be used as a guide to the severity of several disease states, including rheumatoid arthritis. The rise in serum copper levels during adjuvant arthritis[94] and rheumatoid arthritis[95] is well established and, since the copper is mainly in the form of ceruloplasmin, an acute-phase protein, the elevation in copper levels may reflect the systemic response to inflammation and tissue damage. Gold has been shown to reduce the elevated acute-phase proteins[79] and copper levels[64] in rheumatoid arthritis and to cause a remission of the disease.[96] However, in animal studies, it was found that different gold complexes

[92] Superoxide and Superoxide Dismutases, ed. A. M. Michelson, J. M. McCord, and I. Fridovich, Academic Press, London and New York, 1977.
[93] D. H. Brown and W. E. Smith, unpublished work.
[94] R. Hirschelmann, H. Bekemeier, and A. Stelzner, *Z. Rheumatol.*, 1977, **36**, 305.
[95] L Heilmeyer and G. Stuwe, *Klin. Wochenschr.*, 1938, **17**, 925.
[96] W. Niedermeier, W. W. Prillaman, and J. H. Griggs, *Arth. Rheum.*, 1971, **14**, 533.

exerted different effects on copper homeostasis:[97] triethylphosphinegold(I) chloride given orally suppressed adjuvant arthritis in the Lewis rat and also reduced copper levels, wheras gold(I) thiomalate given s.c. reduced the disease symptoms without affecting serum copper levels, although the latter treatment produced higher serum gold levels.

The role of copper in the inflammatory process has been the subject of much recent discussion[98,99,100] and it is still not clear whether an elevated copper level or a reduced copper level is necessary for anti-rheumatic activity. In particular, the role of 'small' copper complexes (*i.e.* molecular weights of less than 1000) is still not understood. A correlation between low-molecular-weight copper complexes and articular index (a common clinical symptom used to measure the progress of rheumatoid arthritis) has been reported.[101] Whether these complexes involve copper(I) or copper(II) and whether added gold has any role in this chemistry is as yet unresolved.

Therefore, no firm conclusions can be drawn on any direct competition *in vivo* between gold(I) and copper(I).

C. Effects on Thiol Groups.—Gold is generally used medically as a complex with a sulphur donor ligand. This has inevitably led to questioning of the possible role of the ligand in affecting other thiol groups present. However, since there are no satisfactory animal models for testing these drugs, the question is as yet unanswered. Even the activity in animal models of gold compounds like triethylphosphinegold(I) chloride only partly answers the question, as the absorption of gold could involve the formation of a gold–thiol complex and, hence, the alteration of free-thiol concentrations.

Free-thiol concentrations in serum are important in that they are significantly lowered in rheumatoid arthritis and in animal test models like adjuvant arthritis. The reason for this is unknown, although it is presumed to be due to an alteration in the thiol disulphide equilibrium.

$$2RS^- \rightleftharpoons R{-}S{-}S{-}R + 2e$$
$$\text{or } RS^- + RS^- \rightleftharpoons R{-}S{-}S{-}R + 2e$$

Serum thiol concentrations are dominated by albumin, a protein of molecular weight of about 65 000 and containing approximately 0.7 of a free-thiol group per molecule.[91] As mentioned earlier, the relationship of gold to albumin thiol groups is still obscure. It is known that most gold in plasma is bound to albumin and it is thought to be bound to protein thiol. However, as yet no one has convincingly shown *in vivo* that there is a decrease in thiol concentration related to gold(I) uptake in albumin.

[97] D. H. Brown, J. Bruin, A. J. Lewis, A. McNeillie, and W. E. Smith, *Brit. J. Pharmacol.*, 1978, **64**, 462.
[98] B. McConkey, R. A. Crockson, A. P. Crockson, and A. R. Wilkinson, *Quart. J. Med.*, 1973, **42**, N.S., 785.
[99] J. R. J. Sorenson, *J. Med. Chem.*, 1976, **19**, 135; *Prog. Med. Chem.*, 1978, **15**, 211.
[100] M. W. Whitehouse, *Agents and Actions*, 1976, 201.
[101] D. H. Brown, W. Buchanan, A. Elghobary, W. E. Smith, and J. Teape, *Ann. Rheum. Dis.*, 1979, **38**, 174.

An associated reaction involves the formation of rheumatoid factor, a macroglobulin thought probably to be formed by thiol oxidation to a disulphide. Treatment with gold is known to decrease the titre of rheumatoid factor in patients[102] and also to increase the free-thiol concentration.[103] Whether this is due to the remission of the disease, to the direct action of gold, or solely to the release of thiomalate is not known.

Using wavelength dispersive *X*-ray microanalysis, gold was detected[77] in the lysosomes of synovial lining type A cells and subsynovial mononuclear cells. Of the two forms of gold deposit detected, sulphur was present in one of them and the S:Au *X*-ray signal of 1:1.5 was higher than that of sodium gold(I) thiomalate. This suggests that an appreciable amount of the gold accumulated in the lysosomes is present as sulphur compounds.

The thermal denaturation of human γ-globulin is mediated in part by a thiol-dependent reaction. This reaction is probably initiated by the small number of free thiol groups (0.2 per molecule) in IgG. It has been suggested that gold compounds complex with this thiol group and, hence, suppress the aggregation of synovial fluid γ-globulin. The evidence for this is not conclusive but the chemistry of gold(I) outlined earlier in this review does suggest that gold(I) will be involved in the thiol equilibria *in vivo*.

Further circumstantial evidence on the thiol involvement of gold arises from the frequent comparisons in medical literature between myocrisin and the thiol-containing drug D-penicillamine (dimethylcysteine) in the treatment of rheumatoid arthritis.[103] Both take many weeks to be effective; both produce a similar decrease in rheumatoid factor and other clinical factors; both have similar side effects, and both affect the differential agglutination titre in synovial fluid, due to their effect on active thiol groups associated with the antigenicity of the IgG complexes. However, such is the wide distribution of gold in the body and also the slow beneficial activity of gold that the specific biochemistry of any therapeutic reaction could involve only a small fraction of the gold present, making the task of isolating this reaction very difficult.

D. Competitive Displacement of Small Biologically Active Molecules.—It has been suggested[104] that the primary action of the anti-inflammatory drugs (including gold) is the competitive displacement of a small biologically active molecule from its binding sites on both circulating and tissue proteins. It is thought that salicylate, for example, occupies protein sites normally occupied by species such as amino-acids, long-chain fatty acids, and other anions. Most clinically active anti-rheumatic drugs, for example, have been reported to displace L-tryptophan from serum protein.[105,106] It is suggested that human serum protein contains protective peptide-like structures in a protein-bound ⇌

[102] E. C. Huskisson, J. Wojtulewski, J. Scott, H. Balme, H. C. Burry, and R. Grahame, XIV International Congress of Rheumatology, San Francisco 1977 (Abstract No. 269).
[103] E. Jellum, E. Munthe, G. Guldal, and J. Aaseth, *Scand. J. Reumatol.*, 1979, Supp. 28, 28.
[104] J. McArthur and P. D. Dawkins, *J. Pharm. Pharmacol.*, 1969, **21**, 744.
[105] J. McArthur, P. D. Dawkins, and M. J. H. Smith, *J. Pharm. Pharmacol.*, 1971, **23**, 393.
[106] M. J. H. Smith, P. D. Dawkins, and J. McArthur, *J. Pharm. Pharmacol.*, 1971, **23**, 451.

protein free equilibrium and in patients with arthritis these anti-inflammatory compounds are bound to an abnormal extent. Anti-inflammatory drugs then act by increasing the proportion of these compounds which are free. If this were the case, a correlation between total serum gold and anti-inflammatory response would be expected. The equilibrium in blood, however, is so complex, *e.g.* free thiol and protein concentration can vary with disease response, that any correlation could be masked.

E. Membrane Stabilization.—An *in vitro* study of the effect of auranofin on human cells demonstrated that the cell membrane was pitted and 'blebbed' by the action of the compound.[107] Further, the effect of myocrisin on lysosomal membranes has often been suggested. The affinity of gold for thiol groups which are involved in the transport of essential substances such as cation and sugars across membranes suggests that at the least, less dramatic effects of the type observed for auranofin *in vitro* would be expected *in vivo*. For example, H-thiamine uptake, essential for DNA synthesis and cell replication, is known to be reduced after auranofin treatment. However, any change in membrane properties caused by binding with thiols might well affect the production of a wide range of enzymes and hormones, the synthesis of which takes place at or in the cell membrane and which are believed to depend on the sulphydril group.

6 Conclusions

Although some plants are known to absorb gold, and gold fossils of plants have been found, in most human and animal studies, the normal gold concentration is beneath detectable limits and it is not generally regarded as an essential element for living systems. In this regard, the administration of gold is more similar to the administration of toxic elements such as mercury than to that of biologically utilized transition elements such as copper and iron. Gold distributes widely, and the number of possible reactions and reaction sites could be very large.

It seems probable that most of the *in vivo* chemistry is concerned with the reaction of gold species with thiols. It is clear that it is well within the compass of mammalian systems to produce gold(0), gold(I), and gold(III) during the metabolic process and that both monomeric and polymeric gold species are to be expected. The nature of gold–sulphur bonding is not yet sufficiently well understood *in vitro*. For example, gold thiomalate in aqueous solution is a polymer, but what form of Au—S—Au link is present? Is it similar to that in the water-insoluble goldcysteine? This type of problem and results such as the formation of a stable gold(III) complex with penicillamine indicate that this is an area of chemistry which could repay further study.

Most gold complexes administered orally are absorbed to some extent, but some are absorbed much more effectively (Et_3PAuCl) than others (sodium aurothiomalate). The very high gold level in stomach tissue five hours after

[107] T. M. Simon, D. H. Kunishima, G. J. Vibert, and A. Lorber, *J. Rheumatol.* Supp. 5, 1979, **6**, 91.

oral administration of triethylphosphinegold chloride would seem to indicate that part at least of the extra absorption efficiency is due to stomach rather than gut absorption. Gold is circulated in blood mainly by the serum proteins, amongst which it is divided, with the largest amount being carried on albumin. It is deposited in many tissues. The amount found in circulating white cells depends on the drug administered. From the chemistry of gold and from analysis of gold aurosomes, it is clear that as well as colloidal gold(0), deposits, of insoluble gold(I) and possibly gold(III) polymers are likely storage forms. Accumulated gold in a form containing both gold and sulphur has been found.

It is an implicit assumption of much of the medical literature, that 'gold' is a single drug. In fact, the reactions of compounds such as triethylphosphinegold chloride, auranofin, and sodium aurothiomalate are likely to be quite different and it is perhaps some confirmation that gold is the active moiety in these complexes that quite a number of compounds have been shown to be effective. They do not, however, produce an immediate common metabolite on adminstration, and from the point of view of reactivity, clearance rates of different compounds from particular tissues vary as do *in vivo* enzyme activities. *In vitro* enzyme studies further illustrate this point, producing quite a different pattern of activities with each compound.

Thus, gold complexes are effective drugs in a widespread and serious disease for which there is at present no effective cure. Sales of myocrisin have increased every year for the past five years although at present gold treatment is a rather toxic form of therapy. There is no reason to believe that the therapeutic and toxic effects are closely linked and the *in vivo* distribution and reactivity can be altered by changing the conditions of administration (compound used, route of administration, dose regime *etc.*). Consequently, it is possible that further research may lead to a more effective form of gold therapy. The major problem which will have to be overcome is that of finding a suitable animal testing-system for rheumatoid arthritis. In this respect an understanding of the mode of action of these compounds would be helpful.

In other respects, however, the problem is more a chemical and analytical one. It is likely that for the next five or ten years, the existing gold drugs with the probable addition of auranofin will remain the normal forms of gold administered. It would clearly be helpful if specific biochemical tests could be devised to give early warning of the onset of toxicity or to evaluate therapeutic benefit more precisely. Here the role of the chemist in developing new specific techniques of analysis and in providing a better understanding of the likely reaction of gold compounds in mammalian systems will be of considerable importance.