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CLINICAL AND BIO-INORGANIC STUDIES ON ANTI-ARTHRITIC GOLD COMPLEXES

By

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Thesis submitted for the degree of
Doctor of Medicine

To

The University of Glasgow

From

The Rheumatic Disease Unit and
Bio-inorganic Medicine Laboratories,
McMaster University Health Science Centre.

McMaster University
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To

My Wife Mary, My Son Iain

and My Father Robert

and to the memory of

My Mother Catherine and My Brother Bobby

"We must have clinicians who keep in close touch with physiology, pathology and chemistry, and who are prepared to transfer to the wards through proper channels the knowledge of the laboratory. The organized medical clinic is a clearinghouse for the scientific traders who are doing business in all parts of the body corporate, and the application of new facts to medicine must come through it, or through that small but happily increasing group of men who find time amid the daily cares of practice. One thing is certain; we clinicians must go to the physiologists, the pathologists and the chemists - they no longer come to us."

Sir William Osler (1849-1919)

PREFACE

The work presented in this thesis represents seven years clinical and basic science experience with the anti-arthritic gold complexes. I first became interested in the rheumatic diseases in 1973 during my six months as a senior house officer in pathology with Professor John Anderson at the Western Infirmary, Glasgow. Following the completion of my training in internal medicine, in 1976, I pursued my interest in rheumatology by entering the Specialty Training Programme in the Rheumatic Disease Unit at Queen's University, Kingston, Ontario under the direction of Professor Tassos P. Anastassiades. It was at this time that I became aware of the lack of knowledge regarding the chemistry, the biological mechanisms and the clinical application of the anti-arthritic gold complexes.

In 1980 I was appointed to the Faculty of Health Science, McMaster University, Hamilton, Ontario as an Assistant Professor in the Rheumatic Disease Unit under the chairmanship of Professor W. Watson Buchanan.

It became obvious from my clinical studies with gold sodium thiomalate in the treatment of rheumatoid arthritis, that much could be gained by investigating the true interactions of the gold compounds at a cellular and molecular level. To this end I was fortunate to begin collaborative work with Professor Colin J.L. Lock, Professor

of Chemistry, Institute for Materials Research, McMaster University and to set up my initial laboratory base at McMaster University under the direction of Professor J. Fraser Mustard (Professor of Pathology, Vice-President of Health Sciences).

An outline of the history of gold in medicine and the subsequent serendipitous evolution of the use of gold complexes in the treatment of rheumatoid disease is outlined to illustrate man's interaction with gold through the centuries as a healing agent. The clinical studies in chapters III, IV and V include patients from both Queen's University, Kingston, Ontario and McMaster University, Hamilton, Ontario, Rheumatic Disease Units. These studies were designed to identify the clinical risks and benefits in the use of gold sodium thiomalate in the treatment of rheumatoid arthritis. It is hoped that the information conveyed in these chapters and my corresponding publications in the medical literature will serve as a guide to practising clinicians in the use of the anti-arthritic gold compounds.

The chemical structure of the commonly used anti-arthritic gold compounds, gold sodium thiomalate and gold thioglucose have never been determined: a factor, not related to lack of interest on the part of chemists, but rather to the complexity of gold chemistry. Chapter VI outlines some of these factors. Clearly the mechanism of action of the benefits and toxicities related to the gold compounds could be more easily understood if their chemical structure and subsequently their bio-molecular interactions were known.

The last two chapters contain two completely new observations pertaining to the formulation and mechanism of action of gold sodium thiomalate. Chapter VII illustrates my findings which demonstrate that gold sodium thiomalate as currently marketed is a mixture. Separation of the components of this mixture could potentially result in the production of a compound which is less toxic or perhaps even more effective. In chapter VIII I have described my observations on the inhibitory effect of gold sodium thiomalate on the thrombin enzyme. This finding could have potentially far reaching implications in the management of thrombo-embolic diseases, lupus nephritis and thrombotic vasculitic disorders.

I hope that the content of this thesis will enhance the current knowledge of the clinical use of gold compounds in arthritis and also provide some further insight into understanding the biological action of gold sodium thiomalate.

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"No scientist lives in isolation. What he is, is determined as much by his teachers and all the other influences of his cultural environment as by his innate individuality and his own efforts".

H.S.D. Garven (1959)

This thesis could not have been completed without the invaluable support, direction and guidance I have received from my supervisors Professor W. Watson Buchanan, Professor of Medicine, Regional Co-ordinator of Rheumatology, McMaster University and Professor Colin J.L. Lock, Professor of Chemistry, Institute for Materials Research, McMaster University. I am grateful to Professor Tassos P. Anastassiades (Professor of Medicine and Biochemistry, Director of the Rheumatic Disease Unit, Queen's University, Kingston, Ontario) and Dr. Peter Ford (Associate Professor of Medicine and Pathology, Director of Clinical Immunology, Queen's University) who started me on my initial research studies in biochemistry and immunology and assisted me with my first clinical studies on the use of gold sodium thiomalate in the treatment of rheumatoid arthritis.

The laboratory experience I gained under the direction of Professor J. Fraser Mustard, Professor of Pathology (former Vice-President of Health Sciences) and Professor Raelene Kinlough-Rathbone has been of immeasurable benefit. The many helpful criticisms of

Professor Jack Hirsh, Chairman of the Department of Medicine, Dr. Helen Howard-Lock (Institute for Materials Research) and Dr. Patrick J. Rooney (Associate Professor of Medicine) are gratefully acknowledged.

The work reported in chapters VII and VIII was generously supported by the Arthritis Society Canada (grants No. 14-242-80, 14-242-81, 14-251-82), and the Medical Research Council of Canada (grant No. MA-7836-82). I wish to also acknowledge the Arthritis Society (Canada) for their continued support of my salary as an Associate of the Arthritis Society (Canada). I greatly appreciated the patience, care and quality of effort provided by Mrs. Pearl Davis who typed this thesis. A special mention is also due to the staff of McMaster University Health Science Library for their prompt and expert assistance in the retrieval of manuscripts and books required for this thesis, and also to the members of the Department of Medical Illustration at McMaster University for their help in the production of the many tables and illustrations.

I am grateful to Dr. Peter Sadler, Department of Chemistry, Birkbeck College, London, U.K., for his advise and criticism on aspects of gold chemistry during his visit to McMaster University in 1981.

Two people deserve special mention for their help and guidance with the historical section of this thesis. The first is Dr. Francois Forestier, Aix le Bain, France, who generously provided

photographs of his late grandfather Henri and his late father Jacques and extended kind permission for these to be inserted in this thesis. The second is Dr. Leslie B. Hunt of Johnson, Matthey and Co, Hatton Garden, London, U.K. who kindly provided the historical manuscripts relating to the Figuier family.

Some of the work contained in this thesis has already been published or presented to learned societies.

Presentations to Learned Societies. (Speaker underlined)

1. Kean, W.F., Harvey, D.A., Lock, C.J.L., Singal, D., Kinlough-Rathbone, R.L., Mustard, J.F.

   Chemical and Biological Changes Induced in Myochrisine™ Solution By Heat and Ultra-Violet Light.

   American Rheumatism Association (North Eastern Regional Meeting), October 23, 1981 Montreal, Quebec.

2. Kean, W.F., Lock, C.J.L., Howard-Lock, H., Buchanan, W.W.

   Gold Therapy Does Not Influence the Adverse Effect of D-Penicillamine in Rheumatoid Disease.

   American Rheumatism Association (North Eastern Regional Meeting) October 23, 1981 Montreal, Quebec.

3. Kean, W.F.

   Poly-Pharmacy in the Elderly Arthritic.

   American Rheumatism Association (North Eastern Regional Meeting) October 23, 1981 Montreal, Quebec.

4. Harvey, D.A., Kean, W.F., Lock, C.J.L. and Singal, D.
Chemical and Biological Changes Induced in Sodium Gold Thiomalate (Myochrisine$_{TM}$) by Heat and Light.
14th Inorganic Discussion Weekend (Ontario). Nov. 1st, 1981
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5. Hagar, M., Harvey, D.A., Kean, W.F. and Lock, C.J.L.
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11. Bellamy, N., Kean, W.F. and Brooks, P.

    Gold Therapy in the Elderly Rheumatoid Patient.

    American Rheumatism Association (North Eastern Regional Meeting) October 28, 1982 Boston, Mass, U.S.A.

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    Electron Microscopy (EM) and Energy Dispersive Spectroscopy (EDS) of Platelets in Rheumatoid Patients Treated with Gold Sodium Thiomalate.

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Annals of the Royal College of Physicians and Surgeons (Canada) 15: No. 4. 289, 1982.

8. Kean, W.F., Bellamy, N. and Brooks, P.

Gold Therapy in the Elderly Rheumatoid Patient

Accepted for Publication Arthritis and Rheumatism 1983.

9. Kean, W.F. and Lock, C.J.L.

D-penicillamine Does Not Chelate Gold(I).

Guest Editorial to be published in The Journal of Rheumatology 1983.

The following article has been sent to the Lancet for consideration as a publication.

Harvey, D.A., Kean, W.F., Lock, C.J.L. and Singal, D.

Myochrisine (Sodium Gold Thiomalate) is a Mixture.

SUMMARY

The discovery that gold compounds are beneficial in the treatment of rheumatoid arthritis is possibly the greatest breakthrough in the management of this devastating crippling disease which we will witness in this century. This thesis describes my clinical and research experience with gold compounds over the past seven years and also contains my attempts to understand some of the clinical, chemical and biological actions of the anti-arthritic gold complexes, in the hope that my observations may prove beneficial to the management of patients with rheumatoid arthritis.

Chapter I and II describe the history of gold in medicine and its subsequent use as an agent in the treatment of rheumatoid disease. Gold's presumed magical qualities and its resemblance to the "essence" of the sun made it a natural choice as a healing agent by priests and shamen. The "science" of alchemy revolved around the search for the "quinta essence" or philosopher's stone, which would turn base metals into gold or divulge the secret for the elixir of life. Moses burning of the Golden Calf and forcing the Children of Israel to drink a potion made of the ashes is probably one of the earliest accounts of the practice of alchemy involving gold. Significant milestones in the chemistry of gold were probably Yabir's description in the 8th century of aqua regia

and Roger Bacon's description in the 13th century of gold chloride and its use in leprosy. The first attempt in modern times to relate the medical use of a gold compound to its chemistry was the description in 1811 by two French Professors, Pierre Figuier and Andre-Jean Chrestien, of the use of gold sodium chloride in syphilis and tuberculosis. Koch's observation in 1890 that gold cyanide inhibited the growth of tubercle bacilli in vitro heralded the use of gold compounds in the treatment of tuberculosis over the next 40 years. It was Jacque Forestier's erroneous assumption that tuberculosis and rheumatoid disease had similar pathogenesis, that led to the use of gold compounds in the treatment of rheumatoid arthritis. In 1933, Sir Stanley Davidson of Edinburgh, Chairman of the Scientific Advisory Committee of the Empire Rheumatism Council, proposed a multi-centre controlled double blind trial to investigate the use of gold sodium thiomalate in rheumatoid arthritis. World War II disrupted the success of this initial proposal but the late Dr. Thomas N. Fraser of Glasgow reported his section of the multi-centre tial. Dr. Fraser's trial which was the first published double-blind controlled trial of any anti-rheumatic drug, demonstrated that gold sodium thiomalate was efficacious in 82% of his patients. The Empire Rheumatism Council Trial was eventually published in 1960 and confirmed Dr. Fraser's findings.

Unfortunately gold therapy results in toxicity in at least 30% of patients. It was to identify some of these risk that I engaged in the clinical studies reported in chapters III, IV and V.

My findings indicated that side-effects such as rash, mouth ulcer and proteinuria had predictable patterns of development, all predominantly occurring within the first year, whereas thrombocytopenia and low white blood cell count could appear at any time. No predictive correlates could be established for patients who had gone into sustained remission, but the data strongly suggested that patients who improve within six months may continue gold therapy for up to three years with an increasing margin of safety for mucocutaneous and renal toxicity. In chapter IV my studies indicate that the elderly rheumatoid patient (>60 years) responds just as well to gold sodium thiomalate as his or her younger counterpart and that the toxicity rate is similar in the two age groups. A note of caution is required however, as it was demonstrated that serious haematological abnormality occurred only in patients over 47 years, and nephrotic syndrome occurred only in patients older than 52 years.

In chapter V the clinical study discusses the question, does prior gold therapy influence the subsequent outcome in patients treated with D-penicillamine. The results indicated that there was no significant difference in outcome of patients treated with D-penicillamine, with respect to toxicity, whether they had previous gold toxicity, previous gold but no toxicity or no previous gold therapy. Total gold compound received had no effect on the eventual outcome of D-penicillamine treatment. The discussion in chapter V explains that D-penicillamine is not a chelating agent for the Au(I) contained in the gold complexes and the chemical interaction of

D-penicillamine and Au(I) in vivo should not be referred to as such.

Perhaps one major reason as to why the mechanism of action of the gold complexes have not been identified is the fact that the structure of the two most commonly used gold compounds, gold sodium thiomalate and gold thioglucose are unknown. Both of these compounds have gold in the Au(I) oxidation state and because of the known chemistry of gold, both are probably polymers arranged through recurring gold-sulphur bonds.

My early research with gold sodium thiomalate led to the observation that gold sodium thiomalate could exist as both a colourless and a yellow solution. If gold sodium thiomalate (solid) is added to water, a colourless solution is produced; however, if this solution is heated at 100°C for 30 minutes as in the standard marketing procedure, a yellow solution appears. It is this yellow solution which is given to patients with rheumatoid arthritis. Hydrogen-1 nuclear magnetic resonance spectra show no difference between the two solutions. Both the colourless and yellow solution inhibited the mixed lymphocyte culture reaction and also the action of thrombin on human platelets. However, ultra-violet-visible spectroscopy revealed that there was a difference in the two solutions with an absorbance in the 300-450 nm range accounting for the yellow colour. In their direct interaction with human platelets the yellow solution induced an ADP dependent platelet aggregation. The colourless solution did not affect the platelets. Electron

microscopy and energy dispersive analysis also recorded a difference in that platelets treated with the yellow solution contained particulate material 100-700 nm in length which was phagocytosed within the lysosomes. Platelets treated with the colourless solution also contained a particulate component but these were < 40 nm. The particles from platelets treated with both the colourless and yellow solution contained gold and sulphur. The above findings indicate that gold sodium thiomalate (yellow solution) as currently marketed for human use, is a mixture. If the beneficial and toxic properties of this compound can be identified and separated this could result in a major advance in the therapeutics of rheumatoid disease. In collaboration with my colleagues a separation process for the components of gold sodium thiomalate has been established and initial biological experiments have been completed. Details of this information is not conveyed in this thesis as it is the subject of a patent application submitted in Canada and the U.S.A. by McMaster University on behalf of Professor Colin J.L. Lock (Professor of Chemistry), Miss Debra Harvey (Ph.D. student - gold chemistry) and myself.

Finally, in Chapter VIII my observations that gold sodium thiomalate inhibits the serine esterase thrombin <u>in vitro</u> and <u>in vivo</u> is described. Gold sodium thiomalate inhibits the action of thrombin on washed human platelets and the thrombin clotting time of platelet rich plasma and platelet poor plasma. Using an experimental model of experimentally induced thrombosis it was shown that rabbits treated with pharmacological concentrations of gold sodium thiomalate had significantly reduced thrombus weight compared to

controls. Gold sodium thiomalate had no effect on fibrinolysis nor platelet survival, therefore the reduction in thrombus weight was most likely due to the direct inhibition of the thrombin as was observed *in vitro*. Clearly such a finding could have far reaching consequences in the treatment of vasculitis, lupus glomerulonephritis, coronary artery disease and cerebro-vascular disease.

This thesis is not an end to the work described herein but the smallest of beginnings.

CHAPTER I

THE HISTORY OF GOLD IN MEDICINE

"The history of science is
science itself ... "

Johann Wolfgang Von Goethe (1749-1832)

... "But gold shines like fire
blazing in the night, supreme of
lordly wealth."

Pindar (circa 518-438 B.C.)
Olympian Odes I line i.

Man's worship has made "Gold" the greatest monotheistic deity in the history of civilisation. The dazzle and allure of its brilliance has enthralled men since the dawn of time. Gold is the lodestone of the eyes and the heart, granting peace, waging war, enhancing beauty and perpetuating slavery. Man's lust for gold as a source of wealth and beauty also inflamed his savagery demonstrated by the cruelty of the sweat pits in the ancient Nubian, Egyptian, Arabian and European gold mines.

Gold's rarity and unique stability is the basis of its value. Solar spectral analysis indicates that while gold is present at 0.04 parts per million in the sun, in the Earth's crust it is only approximately 0.004 parts per million (1). It exists as the metal or in mineral form, either as Tellurides, such as calaverite or krennerite or to a lesser extent as auriferous sulphides (1,2). The gold known to ancient man, however, was obviously the familiar alluvial or placer deposits of gold nuggets or granules (Figure 1). We can only surmise the awe and wonder beheld by the discoverer when the first hairy humanoid hand grasped that heavy glittering treasure from the sandy stream.

"Gold! Gold! Gold! Gold!
Bright and yellow, hard and cold."

Thomas Hood (1799-1845)

FIGURE 1: Electrum: A naturally occurring alloy of 80% gold and 20% silver.

Gold: Gold nugget which occurs almost in pure form in nature.

Who the first goldminers and goldsmiths were, we shall never know (Figure 2). Gold was certainly known to the ancient people of Bulgaria. The oldest gold treasure known at present is the Varna treasure of approximately 2,000 gold pieces weighing 5.5 kilograms. According to Dr. T.P. Mohide (2), this treasure is traceable to the Transylvanian gold mines of Romania and probably Mount Pangion in Thrace (now Bulgaria).

The earliest forms of organized retrieval of gold was from alluvial beds by gleaning, washing or strip mining. In the alluvial streams, the silt and sand was washed through animal hides such as sheepskin. The hide was allowed to dry and the gold particles separated. These ancient crude methods were probably the basis of the legend of the Golden Fleece. In the legend, Jason of Thessaly and his Argonauts set sail to Colchis (now in the Soviet Republic of Georgia) to steal the Golden Fleece (3) (Figure 3). The myth was probably based on a Greek expedition to steal some of the gold being retrieved from the shores of the Black Sea.

As with many other aspects of civilisation, the early peoples of the early Mesopotamion cultures developed skills in metal working. Legend suggests that when Atlantis was being engulfed by the waves (now thought to be the destruction of the Minoan civilisation on Crete by the tsunami created by the volcanic destruction of Thera or Santorini) the people set sail in great boats in search of land. Were these tall dark haired Sumerians (Figure 4) who arrived on the shores

FIGURE 2: Metalsmith using blowpipe to intensify the heat in his forge. In his right hand he uses forceps to hold metal pieces over the glowing coals. His trade and tools have not appreciably changed for 5,000 years.

FIGURE 3 : Medea sarcophagus. The sorceress Medea, daughter of the King of Colchis, is depicted using a sleeping draught to drug the serpent guarding the golden fleece (her father's treasure). On the left, Jason is about to steal the fleece. Jason and Medea fled with the treasure but on return to Greece, Jason forsook Medea on behalf of Glauce.
(Antikenmuseum, Basle, Switzerland).

FIGURE 4: Frieze (circa 8th century B.C.) from the palace of King Sargon II, in Khorsabad. It depicts two Sumerian priests. The first is carrying a gazelle and holding a mandrake flower in his hand; the second is holding poppy seeds.

(Musée du Louvre, Paris, Antiquités Orientales).

of the "Land of the Two Rivers" really the skilled metalsmiths of Atlantis (4)? Over 4000 years ago the bustling cities of Lagash, Urak, Sumer and Ur (Figure 5,6) were renowned for their metal-working. These ancestors of the Assyro-Babylonians invented the wheel and produced the exquisitely fine jewellry and ceremonial weaponry found in the diggings of Ur (2,4,5). With the rise of the Pharoanic civilisation in lower Egypt, many of the skills of the other areas of the Middle Eastern cultures were imported into the Nile Delta. Gold was certainly known to the ancient Egyptians from as early as 4000 B.C. Despite the spectacular treasures of Tutankhamun's grave (Figure 7,8,9) which depict the unbelievable wealth and magnificent metal craftsmanship of ancient Egypt (Figure 10), it is of interest that Gordon (5) in his treatise on Medicine Throughout Antiquity makes no mention of the medicinal use of gold by the Egyptians.

Although the span of the years between known events in the dawn of civilisation is minimised by distance, it is perhaps not surprising that by the time 2000 years had elapsed, metal working skills and knowledge of gold mining techniques had spread from the Middle East to the most westerly shores of Europe and to the Shang civilisation in the Yangtse valley in Eastern China. The ancient people of Ireland or Proto-Celts, were goldminers and goldsmiths as early as 2000 B.C. and numerous treasures exist in the National Museum of Ireland, the Royal Irish Academy and Trinity College in Dublin (2).

Early man probably believed that gold possessed magical and even divine properties. It is natural to imagine how man's fanatical worship led to the search for the secret of life itself through the

FIGURE 5 : THE ZIGGARUT OF UR. This is the ruins of the huge Ziggarut, or temple at Ur, first constructed in the 23rd century B.C. According to the Old Testament, Ur was the Patriarchs' place of origin; from here, Terah, with his son Abraham, migrated to Haran. It was from Haran that Abraham, having received the divine mandate, set off for Canaan.

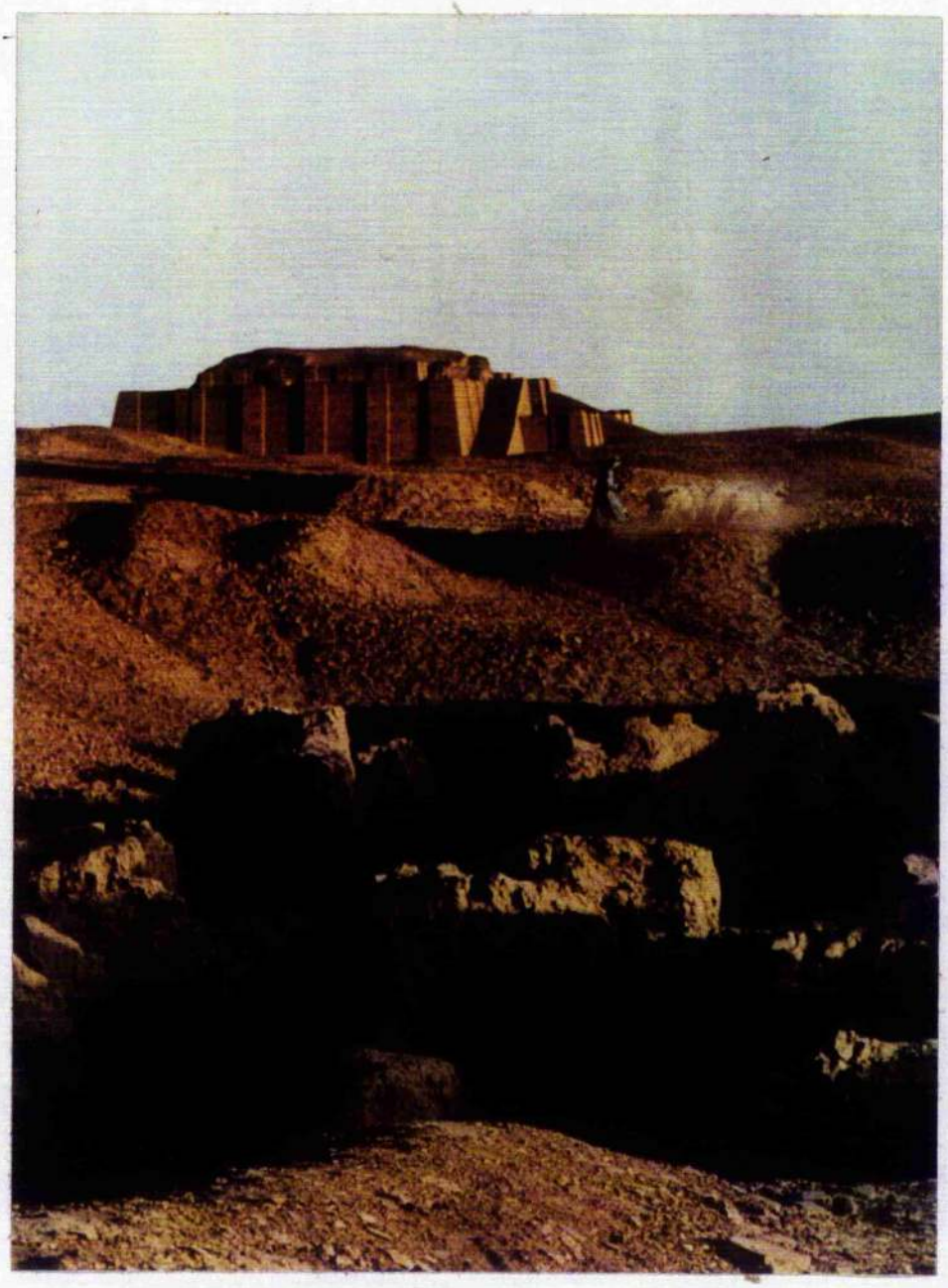


FIGURE 6: CITY OF UR.

A shepherd and his flock pass the ruins of the City of Ur (foreground) with the Ziggarut in the background (see Figure 5). The diggings of Ur have revealed the city's foundations, including residential and commercial centres composed around a network of lanes, which linked schools, shops, dwellings and places of worship.

FIGURE 7 : Gold plated mask of the famous Tutankhamun sarcophagus.

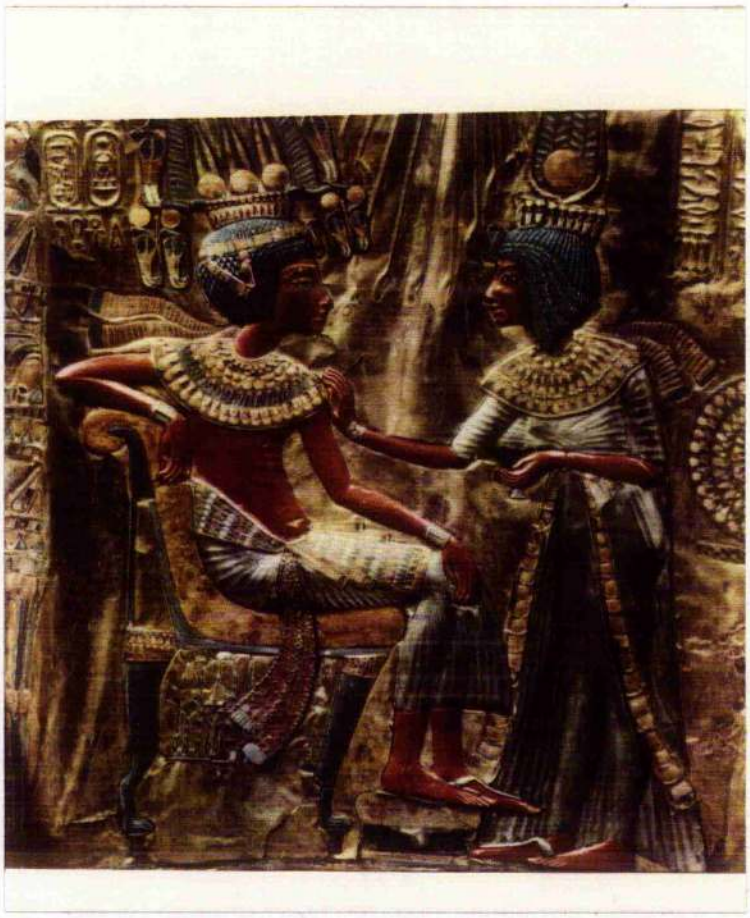


FIGURE 8 : This splendid scene is carved on the back of King Tutankhamun's golden throne. It probably depicts his coronation, with Ankhesenamun, his Queen, anointing him with perfume.

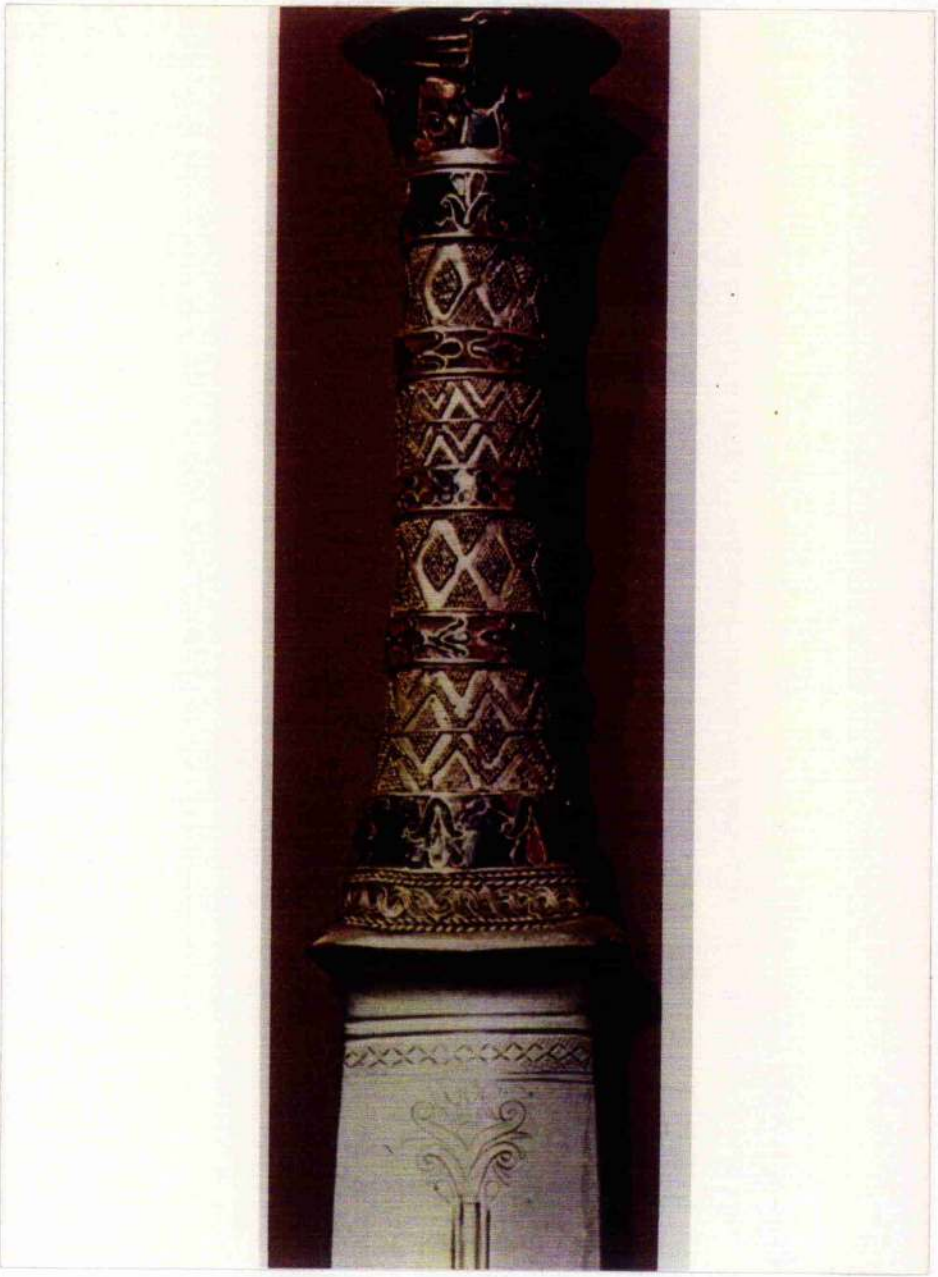


FIGURE 9 : Dagger hilt from the treasure of Tutankhamun's tomb. The artistry in gold is known as granulation. The diamond and zigzag patterns are made of minute gold spheres the size of poppy seeds.

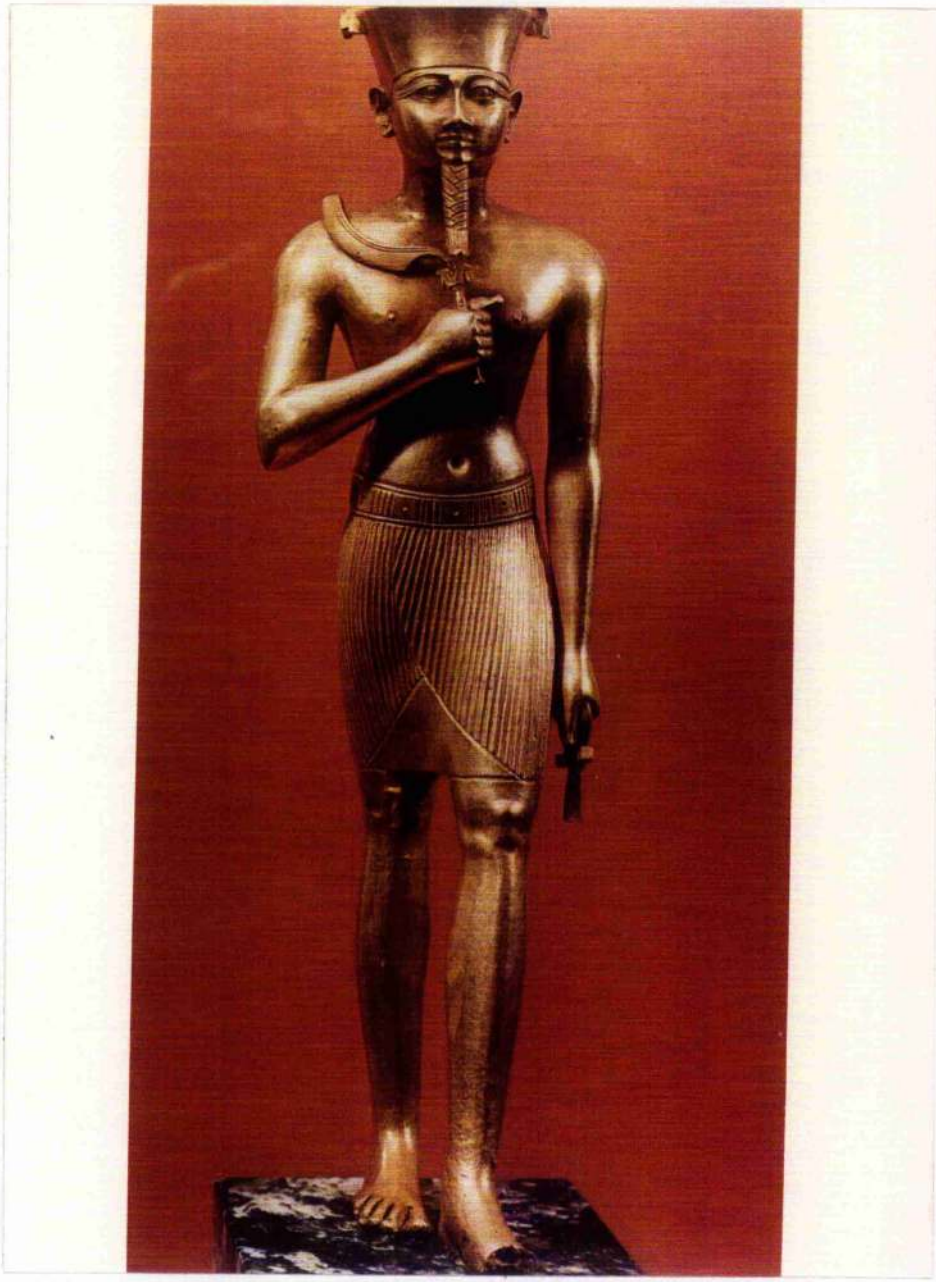


FIGURE 10: Gold statue of Amen, local deity of Thebes elevated to ruling god of Egyptian pharoahs, whose temple priests included healers. (Metropolitan Museum of Art, New York)

"essence" or "spirituality" of this shining metal. The ancient cultures usually ascribed corporeality to natural events such as floods, earthquakes, death and disease. Illness was thought to result from sin and could only be relieved by worship or the incantations of a priest or shaman (Figure 11). It will never be known which culture first used gold as a healing agent, but Weigleb records that the earliest attributable use of gold as a medicinal agent was by the Chinese (6). In Historisch-Kritische Untersuchang Der Alchemie he states that in the writings of the ninth province and the tenth city of the same name, a large block of gold existed which had been used since 2500 B.C. to cure sickness and ills.

To ancient man, gold was the sun on earth. With the rise of the Greek civilisation and the teachings of Aristotle, that all substances are only different forms of the same fundamental substance, came the alchemist's hypothesis that base metals could be turned into gold if one could command the magistery over the four elements or essences, air, earth, fire and water. Manipulation of this principle required the knowledge of the Quinta essence or philosopher's stone. It is easy to imagine how incantations of the ancient priests in search of the quintessence led to the birth of alchemy and thus also to the use of gold as a healing agent.

The aura of reverence bequeathed on the metal gold by the ancient cultures and religions is exemplified in The Holy Bible, Exodus, Chapter 25 where God gives Moses instructions for the construction of the Ark of the Covenant, now beheld as the most sacred symbol

FIGURE 11: Formula on a medico-magical papyrus.
Magic incantation or formula used by an Egyptian Priest.

FIGURE 11 :

Formula on a medico-magical papyrus. Magic incantation or formula used by an Egyptian priest. The text dates from the time of Rameses II (circa 1250 B.C.). The patient who has received a head wound, is identified with Horus 'the avenger of his father' (Osiris). 'He is Horus, o gods, the Lord of Life, who rightfully approaches the house of his father! That no god or goddess, neither male nor female spirit, neither dead man nor dead woman, nor any evil being, male or female, shall be able to take possession of the limbs of the son of any woman, whomsoever he may be, to perpetrate anything evil or bad.'

Then written in red ink: "To be uttered over the talons of a falcon, over the shell of a tortoise. Boil it and put it in oil. Anoint a wounded man (with it) on the site of his wounds. No evil or bad will befall him. A reliable remedy, proven a million times over."

(Rijks Museum Van Oudheden, Leyden, Holland).

of God's presence among the Hebrew people.

> "And they shall make an ark of shittim wood: two cubits and a half shall be the length thereof, and a cubit and a half the breadth thereof, and a cubit and a half the height thereof.
>
> And thou shalt overlay it with pure gold, within and without shalt though overlay it, and shalt make upon it a crown of gold round about." (7) (Figure 12)

The frequent references to the use of gold in Exodus, may not be merely associated with enhancing the beauty of divine objects. Doberer (4) suggests that the Hebrew prophet Moses (circa 1500 B.C.) was probably an accomplished magician and had a knowledge of alchemy. In the Bible, Moses' demonstration of these talents is recorded in the dramatic account of the destruction of the Golden Calf (Figure 13).

> "And he took the calf which they had made, and burnt it in the fire, and ground it to powder, and strawed it upon the water, and made the children of Israel drink of it." (8)

Whether Moses intended the gold solution as a punishment or a therapeutic purge is not readily interpretable but he did convince the "cleansed" to engage in wholesale slaughter of those Israelite brethren who did not adopt the new Commandments (8,9). In Observationes Chymico-Physico-Medical, Stahl (10) (Figure 14) suggests that the gold solution made by Moses was probably a gold-sulphur compound made from alkaline wood ash, sulphur and gold. This act performed by Moses supports the concept that he was an accomplished alchemist (4) and perhaps one of the earliest of that "faculty" of science.

FIGURE 12: "And they shall make an ark of shittim wood ..... And thou shalt overlay it with pure gold."

Exodus 25 verses 10,11.

Even in very dry places, such as the Sinai desert and the lands around the Dead Sea, acacias (or Shittim wood) still thrive and bear delicate yellow blossoms. Because the wood of this thorny tree is close grained and hard, it has always been prized by cabinet-makers - hence its selection for the Ark of the Covenant.

FIGURE 13: GOLDEN CALF
Heretical idol fashioned by Aaron for the Children of Israel. This gold coated bronze bull, 15 inches long, comes from the City of Byblos (circa 2000 B.C.).
(Musée Du Louvré, Paris)

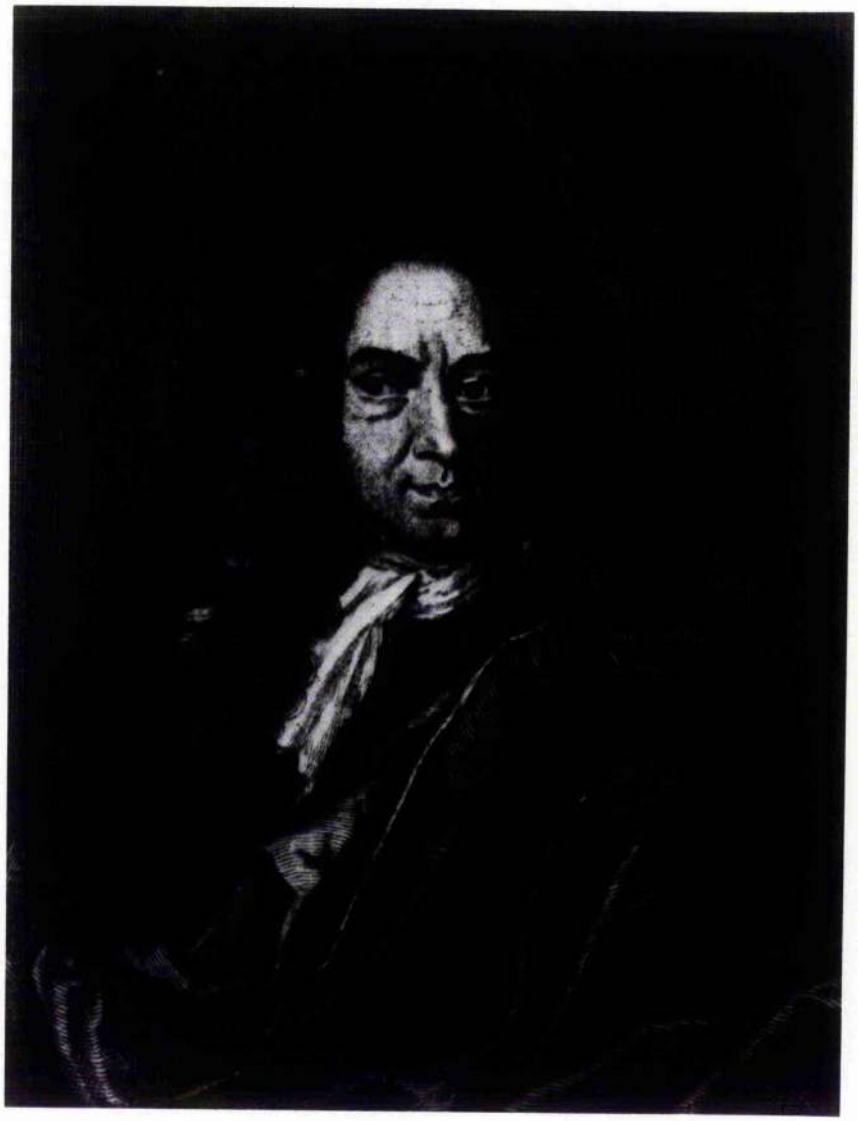


FIGURE 14: Dr. GEORG ERNST STAHL (1660-1734)

In Obervationes Chymico-Physico-Medical Stahl suggests that the gold solution made by Moses from the burnt remains of the golden calf, was probably a gold-sulphur compound, made from the alkaline wood ash, sulphur and gold.

Alchemy, religion, healing and philosophy were entwined in an inseparable mesh. Almost every ancient religion encompasses each of these four "sciences". Circa 600 B.C. there arose in China the religion-philosophy of Taoism (literally - The Way), based on the teachings of Lao-tse (circa 604-531 B.C.). Lao-tse was a philosopher who advocated the principles of simple honest existence coupled with non-interference with the course of life (11,12). However, one would consider the latter principle contradicted by the Taoist physicians who maintained that the medicinal properties of gold used in potions and as coatings for pills could prolong life, cure illness and confer immortality. Like the gold chemistry of the European Middle Ages, Chinese alchemy of the Taoist philosophy fell into a disarray of mystical and allegorical thinking in the search for the elixir of life (13).

Mention of the medicinal use of gold appeared in the Roman and Greek literature by the first century Anno Domini. Plinius Secundus (Pliny the Elder, 23 or 24-79 A.D.), who died at Stabiae (Castellmare in the Bay of Naples) from sulphur fumes during the eruption of Vesuvius, described the use of gold potions as a cure for all ills from skin rash to haemorrhoids in book 33 of his Historia Naturalis (14). The Greek physician, Pedonius Dioscorides (circa 50 A.D.) was a scientist and pharmacologist (Figure 15) who served in Nero's army. During army expeditions to Spain, North Africa and Syria, Dioscorides probably acquired the extensive knowledge to compile De Materia Medica which became a leading pharmacological text for sixteen centuries. In this work he suggests the use of gold as a medicinal agent (15).

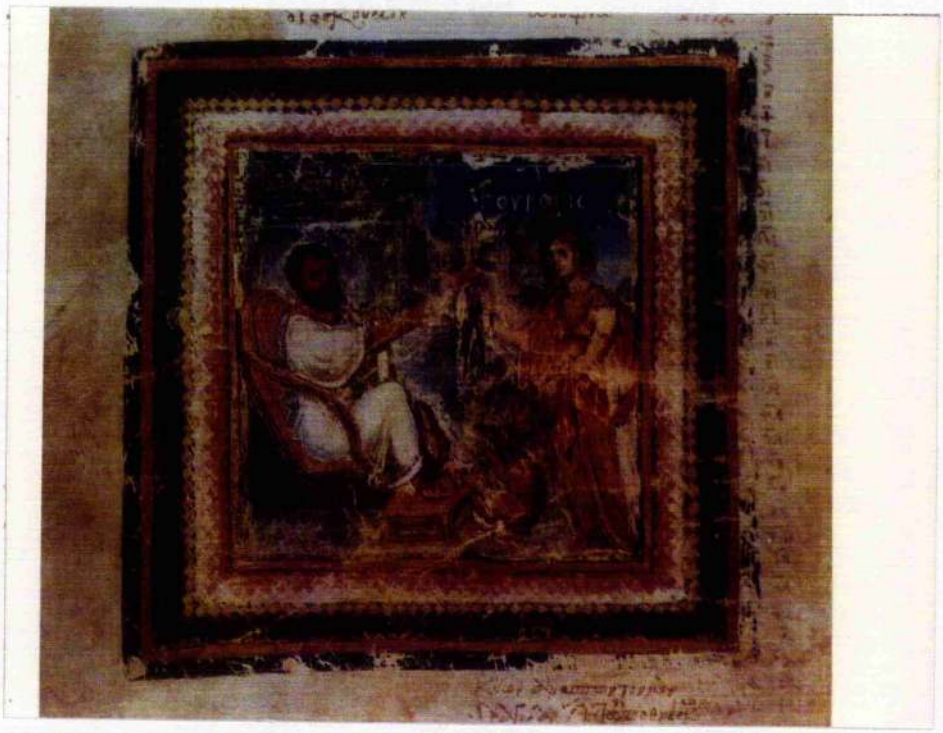


FIGURE 15: Heuresis hands over the Mandragora to the Greek physician, Dioscorides.
(Osterreichische Nationalbibliothek, Vienna).

Prior to modern times, scientific research was rarely used to study medical problems. The first biological experiment in the use of gold can probably be ascribed to Wei Po-yang (circa 150 A.D.) who was a Tao alchemist-physician. He was reported to have had 10,000 formulae for the prolongation of life, some of which contained gold. He realised that his gold solutions did not always provide biologically reproducible results, therefore he would test his potions on dogs before administering them to his patients (13).

Following the decay of the Roman civilisation, the seat of power gradually returned to the Middle East. By the 7th and 8th centuries Anno Domini the Arabs had established their rule in the Near East, Persia, part of the Middle East and North Africa, Spain, Sicily and India as far as the Malay Archipelago. By this time a sophisticated medical and pharmaceutical education system had been established in Persia by the Nestorian Christians. Three centuries earlier, because of religious persecution from rival Christians, they had emigrated from Syria and established hospitals and medical schools in the Persian cities of Nisibis and Gondeschapur (16). It was in these institutions that Arabian pharmacy and medicine were born. One of the most notable alchemists of the period was Abu Musa, Ibn Hayyan, Yabir (circa 722-815 A.D.) also known as Geber. His father Hayyan, was a pharmacist in the town of Kufa, now a small town in Iraq. Hayyan was a follower of the Abbasides and thus politically opposed to the Caliph, Yazid II. For this he lost his life but not before fathering a son who was to become known as the father of modern pharmacy (17). Sometimes referred to as Abu Musa the Wise, Yabir was a scientist rather than a scorcerer. Doberer attributes the following philosophical statement to Yabir:-

> "He who makes no experiments will attain nothing. The researcher does not delight in quantitative experiments; what he is concerned for is the quality of the result attained!" (17)

In his search for aurum potabile Yabir discovered aqua regia - the solvent for the king of metals. Doberer's interpretation of Yabir's recipe for aqua regia is as follows:-

> "Mix a pound of vitriol with a pound of saltpetre and a quarter of a pound of alum from Yemen and distil ..... if thou dissolvest therin the fourth part of salammoniac, for then it dissolveth, gold sulphur and silver."

Despite his father's political antagonism to Umayyad Caliph Yazid II, Yabir became the favoured court scientist and physician to the old Caliph's son, the great Haroun al Rashid, Caliph of Bagdad, city of the "Thousand and One Nights". At this time, the Arab world was the centre of world power, culture, religion and science (18). Over the next two hundred years there followed a succession of great Arabian scientists and physicians: Ali ibn Abbasi (died 994 A.D.); Ibn Sahl al-tabari, Sinon bin subit qurrah (died 942 A.D.); Al-Rhazi or Rhazes (850-932 A.D.); Bar Judens and one of the fathers of modern medicine Abu Ali, Al-Husain, Ibn Abdullah, Ibn Sinna or Avicenna (980-1037 A.D.) (19,20). Rhazes, born in Rayy near Teheran was one of the greatest Arab physicians. He worked in the Baghdad hospitals and taught clinical medicine based on traditional knowledge and his own observations. His major work was Al-hawi - the storehouse which was published in Latin in the 12th century

under the title Liber Contienens (16). Avicenna studied under the Nestorians, Abu Sahel Mosichi and Ab Nasr Alfarabi in Baghdad. He not only became a great physician but a philosopher and an author. His major work Al-Qanum fi'l-Tibb (Canon Medicinal) (Figure 16) was a synopsis of Greek medicine probably acquired through the translations of the Nestorian Christians (16). He believed gold to be purifier of the blood and prescribed pills coated with silver and gold. Al-Qanum became the basis of medical teaching for the next five hundred years(16,20). Moorish scholars of the 10th century spread their knowledge of medicines and alchemy through Spain and into Europe. An example of this perpetual chain of knowledge was the text Al-Tasrif by Al-Zahrawi also known as Albucasis of Cordoba (circa 940-1000 A.D.). This was a comprehensive encyclopaedia of medicine, surgery and pharmacy. The surgical section was translated into Latin by Gerhard of Cremona in the famous Toledo school of translators. Such schools were responsible for transmitting the culture and philosophy of the Arab world to Europe and the British Isles in the early Middle Ages (21).

The Franciscan scholar Roger Bacon (circa 1214-1292 A.D.) (Figure 17) is probably the first known gold researcher in Britain. He suffered for his scientific calling, and was twice persecuted by his own religious Order for his "heresy". He served two separate prison terms, one of 10 years (circa 1255-1265 A.D.) until released by Pope Clement IV, and a second of 14 years (circa 1278-1292), although it is stated that the last few years of his life were spent

FIGURE 16: A page from Al-Qanum fi'l-Tibb of Avicenna, depicting a pharmacy. Insets show sunbathing; three bathing scenes; cupping; bleeding and thoracic puncture.

(Hebrew Biblioteca, Universitaria Di Bologna)

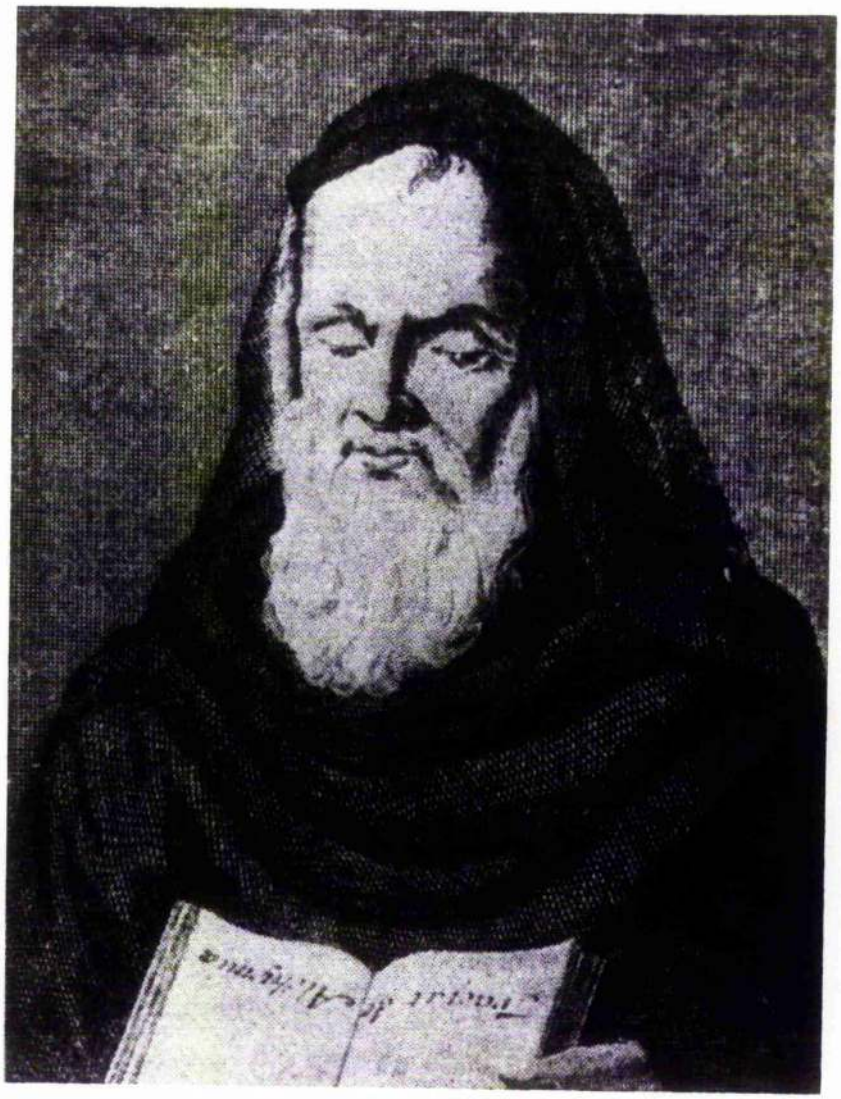


FIGURE 17 : The Franciscan scholar ROGER BACON (1214-1292 A.D.) is probably the first known gold researcher in the British Isles. He is said to have first used gold chloride in the treatment of leprosy.

"in freedom at Oxford in a little room in the squat tower of the gateway at Folly Bridge" (22,23). Bacon's contribution to gold therapy in medicine was his use of auric chloride for the treatment of leprosy (24). This aurum potabile of gold chloride was prepared by dissolving gold in aqua regia and neutralising the solution with calcium carbonate (chalk) (23).

> "Make a diligent purification of the Calx with the water of Alkali, and other acute waters, grind it by several contritions with the salts, and burn it with many assations, that the earth may be perfectly separated from other elements ..." (23)

Exploitation of the people by unscrupulous physicians advocating the value of gold drugs was widespread throughout Europe. Chaucer (Figure 18) captures this ancient and modern vice of doctors in his satire, the Canterbury Tales:-

> "And yet he was but esy of dispence,
> He kept that he wan in pestilence,
> For gold is phisik in a cordial;
> therefore he lovede gold in special." (25)

As with his other characters, Chaucer is able to capture the professional and prescribing habits of the physician of his day, many of whom were no more than charlatans and quacks.

Witchcraft, sorcery and cheap trickery were never far removed from the practice of alchemy. The dogmatic belief in the quintessence and the"Philosopher's Stone" prevented alchemy from ever becoming a true science based on step logic. Sorcerers such as

FIGURE 18: GEOFFREY CHAUCER (1340-1400)

Celebrated English poet noted for his narrative skills, humour and insight. His awareness of the unscrupulous practices of physicians and their love for gold is captured in the Prologue to Canterbury Tales:

"For gold is phisik in a cordial;
therefore he lovede gold in special."

Ficinus (1433-1499 A.D.) not only recommended gold as an elixir of life but advised that pills containing gold be made at the conjunction of Jupiter and Venus to secure their potency. To Ficinus is also ascribed the bizarre logic that eternal youth can be achieved by drinking the blood of children - a diabolical concept adopted by Constantine and Louis XI to recover new vital energy (26). Charlatans and sorcerers such as Ficinus destroyed any hope of establishing scientific fact. The Middle Ages was a period of repressive dogmatism. All new religious, philosophical and scientific thought was met with suspicion and fear. The Church regarded the writings and teachings of the alchemists as the works of the Devil and the herald of the Antichrist. While one can condemn this dogmatic restraint on the advancement of science as it was applied to Roger Bacon (23), the justification of such tenet was perhaps necessary to suppress the witchcraft and sorcery of charlatans such as Ficinus (26). Like Bacon, not all academics of the Middle Ages were quacks. Raymond Lull (1235-1315) (27) the physician-monk, and his friend Arnold of Villanova (1235-1311) (28) in their search for the philosopher's stone, laid the foundations for the embryo of the Renaissance which was to appear 200 years later. Arnold of Villanova was a prolific writer and one of the most famed physicians of his day (28). He was an accomplished alchemist and was reputed to have introduced brandy into the pharmacopeia as an aurum potabile. Arnold, a doctor of theology, law, philosophy and medicine, was consultant to

Peter III of Aragon and also to Boniface VIII whom he had treated for renal calculus (29). The Inquisition was suspicious of Arnold's practice of alchemy and his "heretical" writings. He sought the protection of Boniface VIII which resulted in the latter also being suspected of heresy. Boniface's "soul" was only restored to the Church after his death by Clement V (29). However, Arnold's search for the elixir of life and other theological heresies resulted in his excommunication after death (28).

Search for the philosopher's stone, aurum potabile and the elixir of life intrigued the royal families of Europe throughout the Middle Ages. James IV of Scotland (1473-1513) (Figure 19) was fascinated by medicine and alchemy and was lauded as an astute physician and surgeon (30).

> ... "In the meane tyme this nobill
> King James the fourt was weill
> leirnit in the art of mediecein
> and also one cuning sorugenar
> that nane in his realme that
> wsst that craft bot wald tak his
> counsall in all proceidingis." (31)

James IV not only practised surgery but encouraged patients, doctors and alchemists at his court. He was the patron of John Damian the French (or Italian) alchemist and provided for his laboratory at Stirling Castle (32) Damian's futile search for the quintessence and the production of gold from base metals is overshadowed by his spectacular experiment where he attempted to fly with the aid of artificial wings from the walls of Stirling Castle: an experiment which resulted in him fracturing his femur (33).

FIGURE 19: KING JAMES IV OF SCOTLAND (1473-1513)

"King James the fourt was weill leirnit in the art of mediecein and also one cuning sorugenar."

A Scottish alchemist who suffered for his knowledge at the hands of the nobility was Alexander Seton (32, 34) (Alexander Setonius or Scotus, circa 1550-1604 A.D.). Scotus was reputed to have demonstrated the transmutation of lead into gold at the home of Jakob Hanssen in Enkhuizen, Holland on March 13th, 1602 (34). He revealed this experiment to Dr. Johann Wolfgang Dienheim and Dr. Jakob Zwinger and later in 1603 to Andreas Bletz. He also performed his experiment for Philip Jakob Gustenhöfer, a Strasbourg goldsmith and Hans Leondorp a goldsmith. Scotus told the Scottish Surgeon in Cologne "Meister Georg", that he had a remedy (his gold making powder) "that eats away rotten flesh and leaves healthy sinews alone" (34). This marvellous secret did not go unnoticed. Scotus was arrested and tortured by the Elector Christian II but was rescued by his pupil and friend Michael Sendivogius (born 1556 Cracow) before he revealed the secret. Unfortunately Scotus died of his injuries (32,34). Even then his persecution was not over. Not knowing Scotus was dead, Duke Frederick of Wurttenberg sent a letter to James VI of Scotland (James I of England) on March 10th, 1605 demanding the extradition of Scotus on the grounds that Scotus had promised to reveal all his secrets but had absconded (34).

The Emperor Rudolph II of Germany (1576-1612) was the arch patron of alchemy in the 16th century. In his quest for the philosopher's stone he filled the Hradschin palace in Prague with every sorcerer, alchemist, physician and cheat who claimed a knowledge of

the quintessence. Thus ambitious alchemists seeking court favours and riches would flock to the "Gold Alley". Failure to fulfil expected promises, however, usually resulted in death by execution (35).

The Swiss physician Theophrastus Bombastus von Hohenheim (1493-1541), also known as Philippus Aureolus Paracelsus (Figure 20) believed gold was a universal panacea. He is said to have first used gold in the treatment of tuberculosis and was considered by many as the founder of experimental therapeutics (36,37). Unfortunately his recondite theories of physiology and pathology portrayed in his extensive writings (Figure 21) have cast doubt on his true ability as a scientist, leading to subsequent allegations that he was no more than a charlatan and a quack.

A similar social fate befell the Englishman Francis Anthony (1550-1623 A.D.) (38). Anthony was a Master of Arts from Cambridge (1574) and studied chemistry and physics at that university for 20 years. Although he did not possess a medical license he practised as a general physician and came under the wrath of the Royal College of Physicians when he published Panacea Aurea Seu De Auro Potabili in 1598. Anthony believed vehemently in the properties of his gold potion and claimed that it was an arcanum capable of curing all diseases. The Royal College of Physicians duly compelled him to undergo an examination which he failed so miserably that he was interdicted from practice. He disobeyed this ruling, was fined five pounds and sent to prison. However, he was soon released, but again re-imprisoned, this time for eight

FIGURE 20 : THEOPHRASTUS BOMBASTUS VON HOHENHEIM PHILIPPUS AUREOLUS PARACELSUS (1493-1541)

He is reputed to have first used a gold in the treatment of tuberculosis and was considered by many as the founder of experimental therapeutics.

FIGURE 21 : Woodcut from the title page of Paracelsus, Opus Chirurgicum 1565, showing the interior of a Renaissance hospital. The contrast of medical practice of the time is depicted by the alchemist in the centre, the surgeon amputating a leg on the left and Jesus administering to the praying patient, top right.

months before being released in 1602 on a plea of poverty by his wife. Undaunted he immediately resumed practice and is said to have amassed a considerable fortune from the prescribing and dispensing of his gold cures. In 1610 he published a treatise Medicinae Chymical Et Veri Auri Potabilis Assertio. Anthony's work was severely criticised by many notable scientists and physicians of his day such as Dr. John Cotton of Northampton, and Matthew Gwinne, first professor of Gresham College. In reply to these "attacks" Anthony published Apologie Or Defense Of A Verity Heretofore Published Concerning A Medicine Called Aurum Potabile, in 1616, which according to Evans is in the library of the Royal College of Physicians in London (38). Anthony recommended his aurum potabile "for the strengthening and comforting of the heart and vital spirits". This spiritual belief by Anthony - an unlicensed physician - of the all encompassing qualities of aurum potabile probably served as succour during the periods of attempted pogrom on his panacea by the medical profession.

Not all uses of gold in medicine was by potions and pills. The indestructable properties of gold led the ingenious French surgeon Ambrose Paré to use gold wire or Punctus Aureus as a ligature for inguinal hernia.

> "If the Rupture will not be cured by all these meanes, by reason of the great folution of the continuity of the relaxt or broken Peritonaeum, and the Patient by the confent of his friends there prefent, is ready to undergo the danger in hope of recovery; the cure fhall be attempted by that which they call the Punctus Aureus or Golden tie". (39)

Dwindling health budgets in the last 400 years have no doubt made extensive use of this invention obsolescent!

The use of gold fillings in dental surgery has been widely used for the past 100 years. However, tooth mutilation for decorative purposes was widely practised by the ancient Mayan civilisation (circa 300-800 A.D.) and by other South American peoples (40). In 1913 M.H. Saville (41) described a most fascinating example of "dental art" with gold in a specimen of mandible from La Piedra in Ecuador (Figure 22). The central incisors were inlaid with circular fillings of gold. This specimen is now in the Museum of the American Indian, Heye Foundation, New York. The horrific stories of the ancient South American religious sacrificial practices (Figure 23) is sharply contrasted by their magnificant gold artistry (Figure 24, 25, 26) and the advanced medical and surgical capabilities of these peoples.

The association of gold with medicine has not always been of therapeutic interest, at least not to the patient. Between the late 17th and early 19th century a handsome gold-headed cane became the symbol of prosperity for its six masters (42, 43, 44, 45). Dr. William MacMichael who was a Radcliffe travelling scholar, wrote a light hearted account of the cane's masters told from the point of view of the cane (45). The cane was originally owned by Dr. John Radcliffe, whose estate funded the Radcliffe Library, Radcliffe Infirmary, the Radcliffe Observatory and the Radcliffe Travelling Fellowship (42). The second owner of the gold-headed cane was equally successful - Dr. Richard Mead who attended Queen Anne on her deathbed. He became the most propserous practitioner of his time, earning as much as £7,000 in one year. The gold-headed cane, now a symbol of wealth and success in the medical profession, was passed onto Drs.

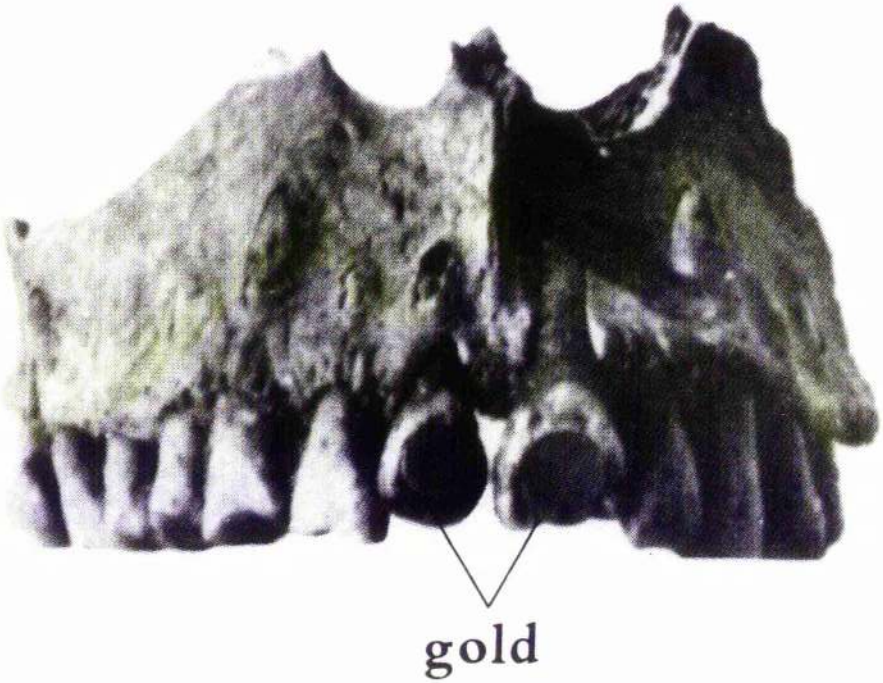


FIGURE 22: Fragment of ancient skull with gold-inlaid teeth excavated at Atacumes site, Esmeraldes Province, Ecuador. (Museum of the American Indian, New York).

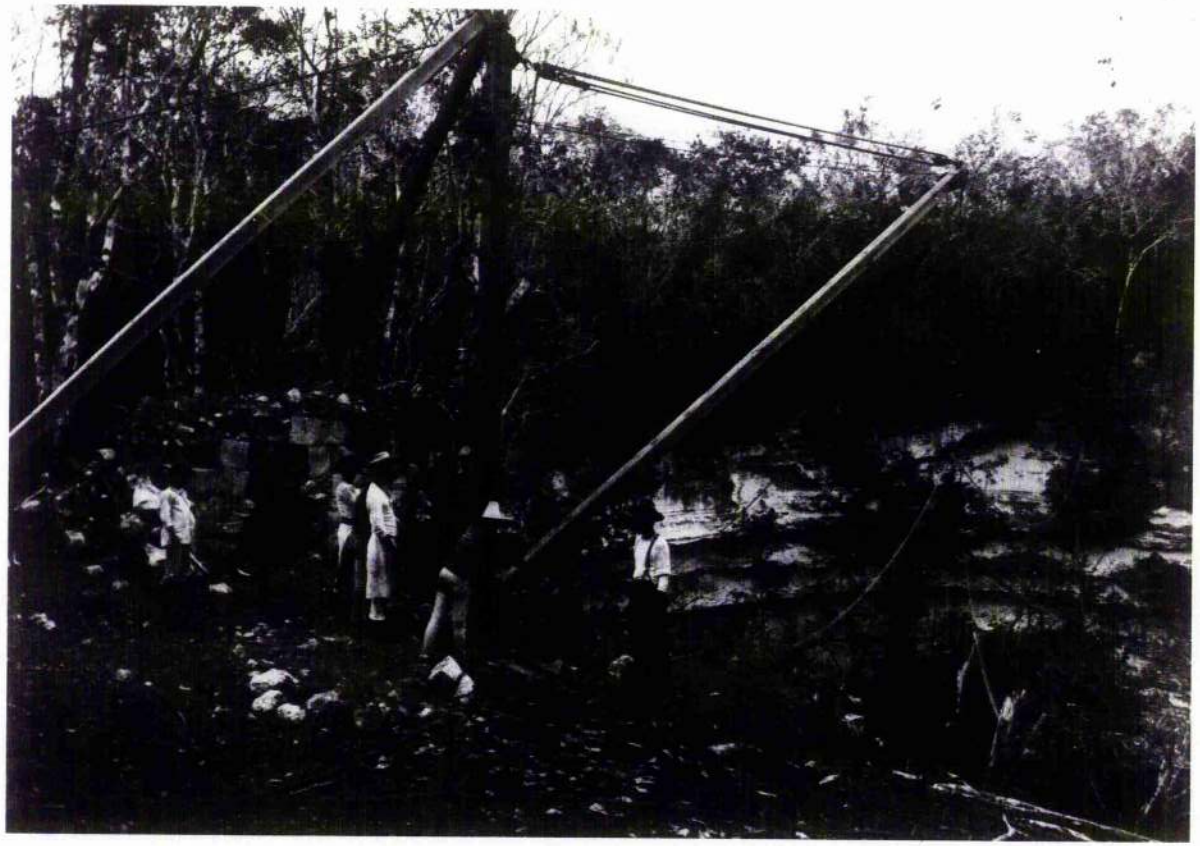


FIGURE 23: Sacred well used by Mayan Indians. Treasure and sacrificial victims were thrown into the well to appease the gods. This well was dredged by Edward H. Thompson, The American Consul General in the Yucatan in the early part of the century and revealed numerous priceless treasures.

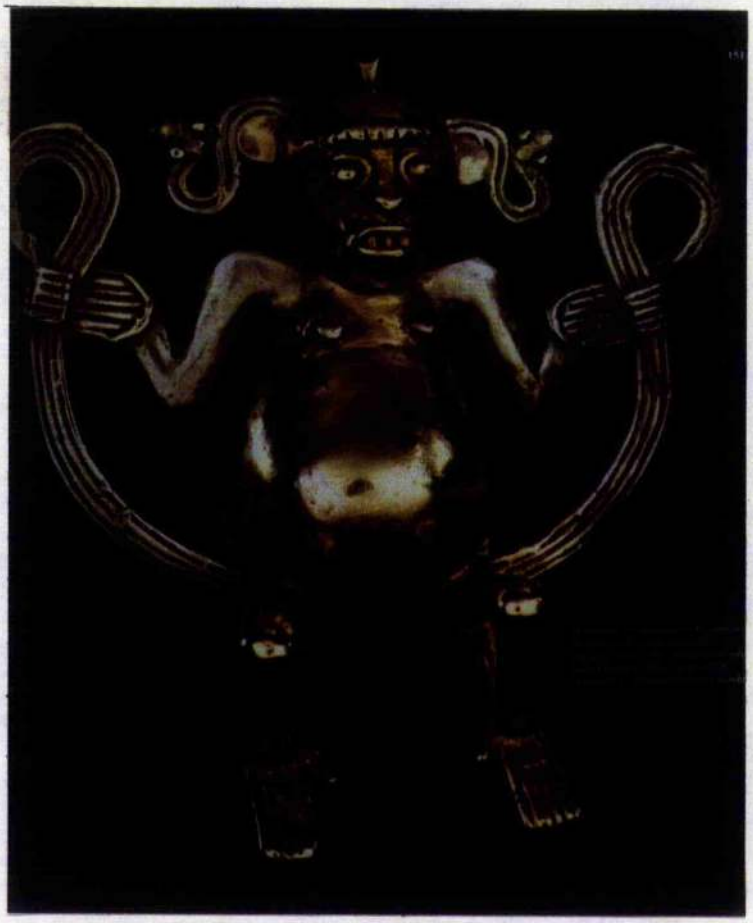


FIGURE 24: Panamanian gold artifact of Veraguas design, of a monkey pendant. The piece is hollow cast and is three inches high (University Museum, University of Pennsylvania).

FIGURE 25 : Peruvian gold work of Chavin period (1200–400 B.C.) Nine and a half inch high columnar crown of beaten gold, embossed with cat-like figures. (Museum of American Indian, New York City).

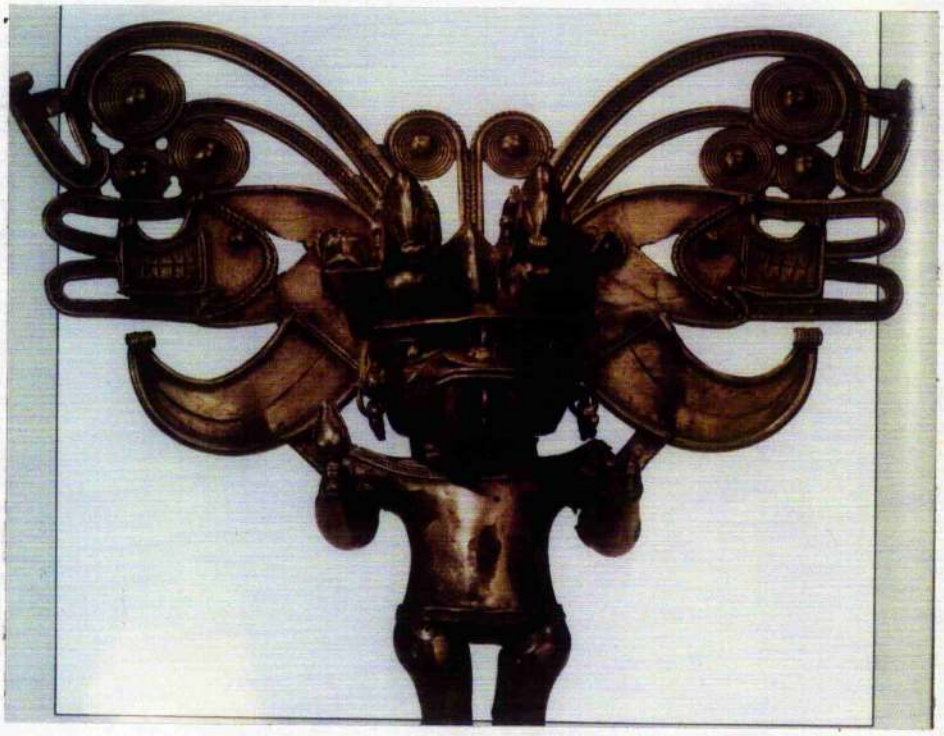
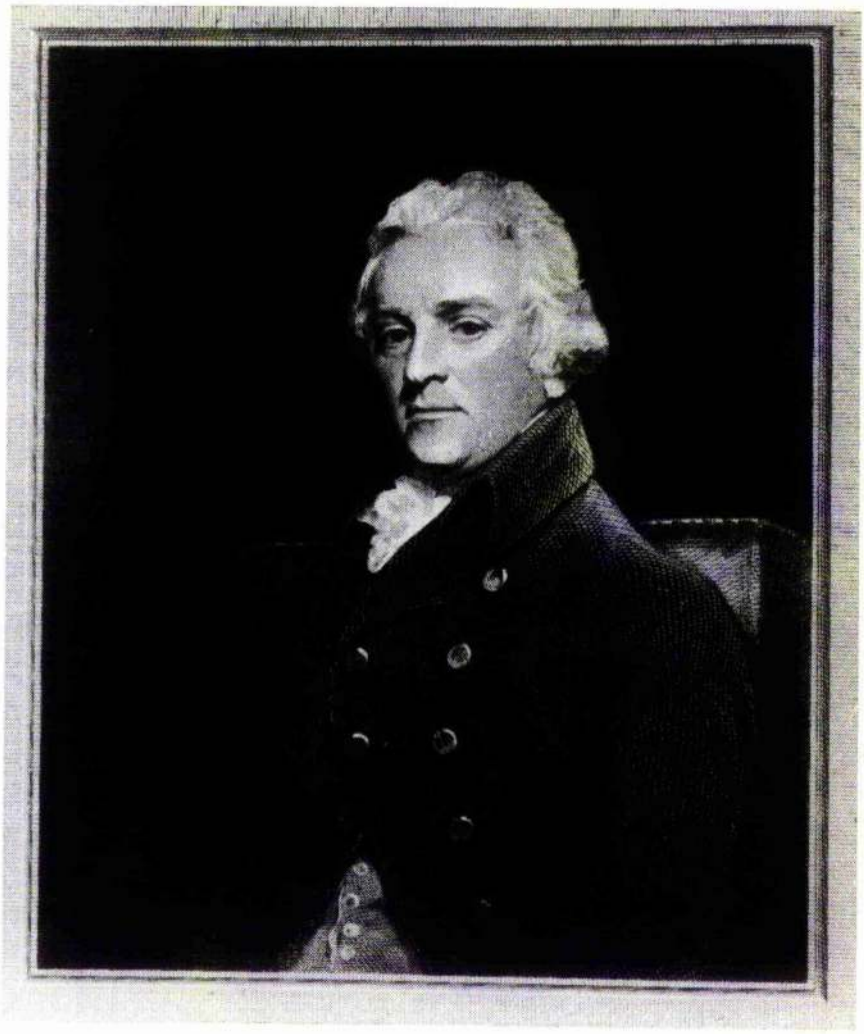


FIGURE 26 : A Fantasy in Gold. This cast figure of a God is a Taironia Indian Specimen which dates from the 13th century A.D. Despite the elaborate work, the figure measures only 5¼" high.

Askew, William and David Pitcairn (Figure 27) and Matthew Baillie (Figure 28). Dr. Askew owned a lavish house in Queen's Square where he housed his enormous library of fine books. These he treasured so much that he did not permit his friends to touch them. The Scotsman, Matthew Baillie, nephew of William and John Hunter (46) was the last owner. Following his death in 1823, his widow presented the cane to the Royal College of Physicians where it is now on display in the library (42).

Although alchemy, sorcery and practice of the occult were rampant in the early 17th century, the emergence of scientists such as Johann Glauber (1604-1679), Thomas Willis (1621-1675), Robert Boyle (1627-1687), John Mayow (1643-1679) and Nicolas Lemery (1645-1715) (47) brought the evolution of analytical chemistry, physiology and anatomy to the fore. Glauber, Boyle and Lemery all dispelled the alchemical belief that aurum potabile had life giving properties. This new era of rational thinking produced the framework for the development of scientific medicine. The Dutch physician Herman Boerhaave (1668-1738) (Figure 29) refuted the medicinal value of aurum potabile in one of his lecturers (48) and in 1778 (49) the Scotsman Dr. Donald Monro published "A Treatise on Medicinal and Pharmaceutical Chymistry and the Materia Medica in which he stated that although gold was long acclaimed to be a panacea, this belief was ill founded and that gold was of no medicinal value.

> "Gold, and some preparations made from it, were long believed to be possessed of great virtues, and to be powerful remedies in many disorders; but this has been found to be a mistake, and they are now never used as medicines." (49)



DAVID PITCAIRN (1749-1809)

FIGURE 27 : DAVID PITCAIRN (1749-1809)
One of the six owners of the Gold Headed Cane.

MATTHEW BAILLIE (1761-1823)

FIGURE 28 : Scottish physician MATTHEW BAILLIE (1761-1823), nephew of William and John Hunter and last owner of the Gold Headed Cane.

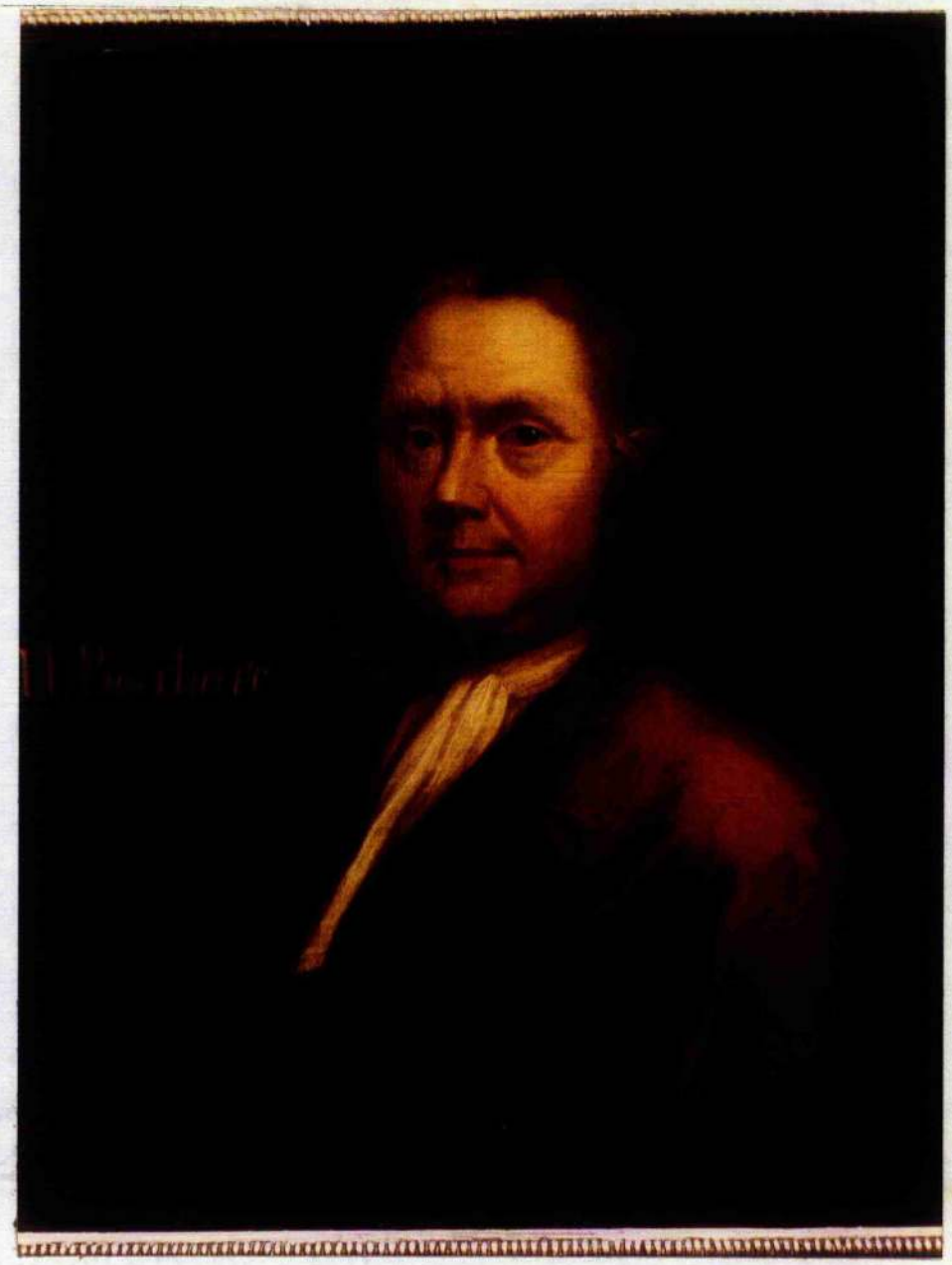


FIGURE 29 : HERMAN BOERHAAVE (1668-1738), famous Dutch physician of the Renaissance Period. In one of his lectures, Boerhaave refuted the medicinal value of aurum potabile.

The revival or re-introduction of the use of gold compounds into medicine can probably be attributed to two French professors at the University of Montpellier, Pierre Figuier (1765-1817), Professor of Chemistry and his friend Andre-Jean Chrestien the Professor of Medicine (50). In 1811 Chrestien published De La Methode Intraleptique (50) in which the last 100 pages were based on the use of gold compounds in several diseases among which were syphilis and tuberculosis. (On this account, both Figuier and Chrestien were 100 years ahead of their time).

In order to enhance the quality of his publication Chrestien asked Figuier to provide a detailed scientific account of the gold compounds he had discussed. This he published as, Sur Les Preparations D'Or, Proposees Par Le Docteur Chrestien, Professeur A L'Universite De Medecine De Montpellier (51). Among the preparations described, Figuier outlined the preparation of sodium gold chloride which was recommended by Chrestien (50). Other members of Figuier's distinguished family contributed to the gold literature. His younger brother Jean Figuier (1776-1824) also a pharmacist, published a paper on gold sodium chloride in 1820 (52). Jean's eldest son Pierre-Oscar Figuier (1805-1879) published a small book entitled, Nouvelles Observations Sur La Preparation Du Cyanure D'Or (Montpellier, 1836). Jean's third son, the famous Guillaume-Louis Figuier (1819-1894) editor of La Presse, was also a pharmacist and like his brother, father and uncle contributed to the literature on gold chemistry with Recherches sur les Combinaisons Oxygénées de l'Or, le Pourpre de Cassius et l'Or Fulminant (53) and Observations Sur La Precipitation De L'Oxyde D'Or (54).

Knowledge of Andre-Jean Chrestien's work quickly spread and according to Higby (48), Dr. Samuel L. Mitchell of New York sent his colleagues at the New York Hospital a copy of Chrestien's book. Researchers at the New York Hospital were investigating the use of sodium gold chloride in the treatment of syphilis. In 1811 John C. Cheesman, a student of Dr. Valentine Seaman at Queen's College in New York, wrote his M.D. thesis on the use of muriate of gold in the treatment of syphilis (55) (Figure 30). Cheesman referred to his drug as gold chloride but according to the method of preparation (55) it was obviously the sodium gold chloride described by Figuier (51). Although Cheesman claimed success with his use of sodium gold chloride in the treatment of syphilis his study was only based on seven patients (55). Further scepticism as to the validity of the effect of sodium gold chloride in the treatment of syphilis is found in the criticism of Chrestien's original work by an anonymous Edinburgh physician, who claimed he was unable to reproduce the beneficial effects of the drug in his own patients (56).

By the early 18th century when medical science began to evolve from the empiricism of alchemy, the time was ripe for revolutionary thought in the treatment of disease. Christian Friedrick Samuel Hahnemann (1755-1843) introduced several unique doctrines in medical thinking which are embodied in his work, Organon Der Rationellum Heilkunde (1810) (57). Some of Hahnemann's doctrines such as Similia Similibus Curantur whereby diseases are cured by drugs which produce similar symptoms were somewhat irrational in thought, but he did advocate the introduction of the "small dose" albeit based on the principle that minute doses enhance the dynamics of a drug. This form

AN

Inaugural Dissertation

ON THE

MEDICAL PROPERTIES OF GOLD,

WHICH UNDER THE AUTHORITY OF
THE REVEREND

JOHN H. LIVINGSTON, SS. T. P.

PRESIDENT, AND THE TRUSTEES OF

Queen's College, in New-Jersey,

AND ALSO BY

AN ORDINANCE OF THE FACULTY OF MEDICINE,
IS SUBMITTED TO EXAMINATION FOR THE DEGREE OF

DOCTOR OF PHYSIC.

By JOHN C. CHEESMAN,

LICENTIATE OF THE MEDICAL SOCIETY OF THE STATE OF NEW-YORK, AND VICE PRESIDENT OF THE PHILO MEDICAL SOCIETY.

On the 28th of September, 1812.

NEW-YORK.

PRINTED BY COLLINS AND CO.

PRINTERS TO THE FACULTY OF MEDICINE OF QUEEN'S COLLEGE,
NEW-JERSEY.

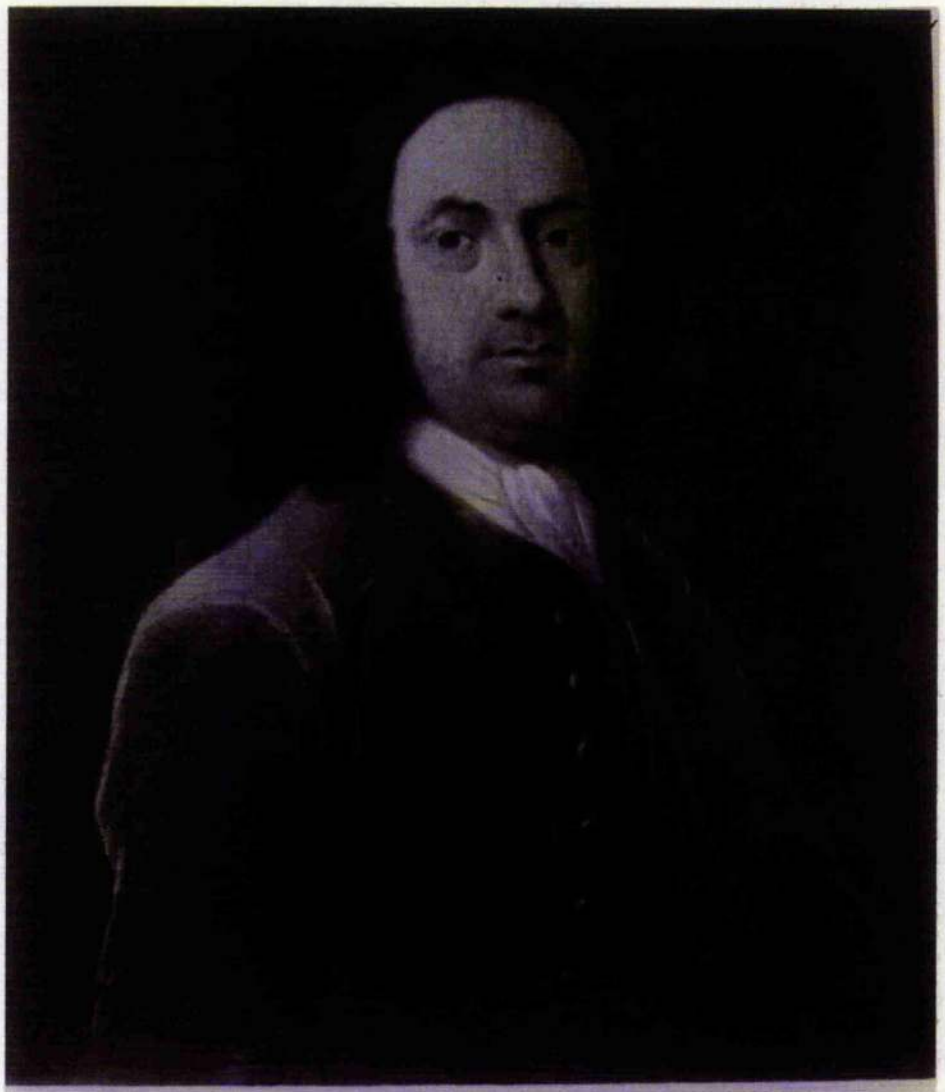
FIGURE 30 : Title page of doctorate thesis by John Cumming Cheesman on the use of gold chloride in the treatment of syphilis.

of logic was scorned and proclaimed as quackery by the majority of the medical profession. In an address at the New Zealand Parliament in 1860, the physician Sir David Monro (1813-1871) said of homeopathy -

> "that most absurd imposition
> on the face of the earth,
> perfectly incredible and monstrous". (58)

Nevertheless, the therapeutic nihilism of miniature dosage of drugs was welcomed by the public who had long been exposed to the iatrogenic diseases induced by the alchemists, charlatans and quacks for centuries. Hahnemann certainly found fame in his practices and when he died in 1843 at age 88 years he was a millionaire (59). Throughout the ninetheenth and early twentieth century, homeopaths advocated the use of gold leaf and aqua regia as almost universal treatments. M. Freligh in his text Homeopathic Practice of Medicine 1868 (60) lists Aurum-Foliat (gold leaf) and Aurum Nitro-Muriat (gold-aqua regia solution) in the chapter on his dispensary. Throughout the text Aurum alone or in combination with other preparations appears as the recommended treatment for numerous unrelated disorders from catarrh, cancer and congestion of the blood to syphilis, scrofula and melancholy of the mind. Unfortunately this almost random application of drugs to symptoms and signs formed very little basis from which to develop a scientific approach to therapeutics.

Hahnemann's era (1755-1843) overlapped with the great period of medical and scientific "revolution" in Britain. Graduates of Glasgow University were among the most notable pioneers - William Smellie (1697-1763) Obstetrician (Figure 31), William Cullen (1711-1790) Professor of Medicine (Figure 32), Joseph Black (1728-1799)



WILLIAM SMELLIE (1697-1763)
Portrait painted by himself in 1719
(Original in the Royal College of Surgeons, Edinburgh)

FIGURE 31 : WILLIAM SMELLIE (1696-1763)
Glasgow graduate and pioneer of modern obstetrical practice.

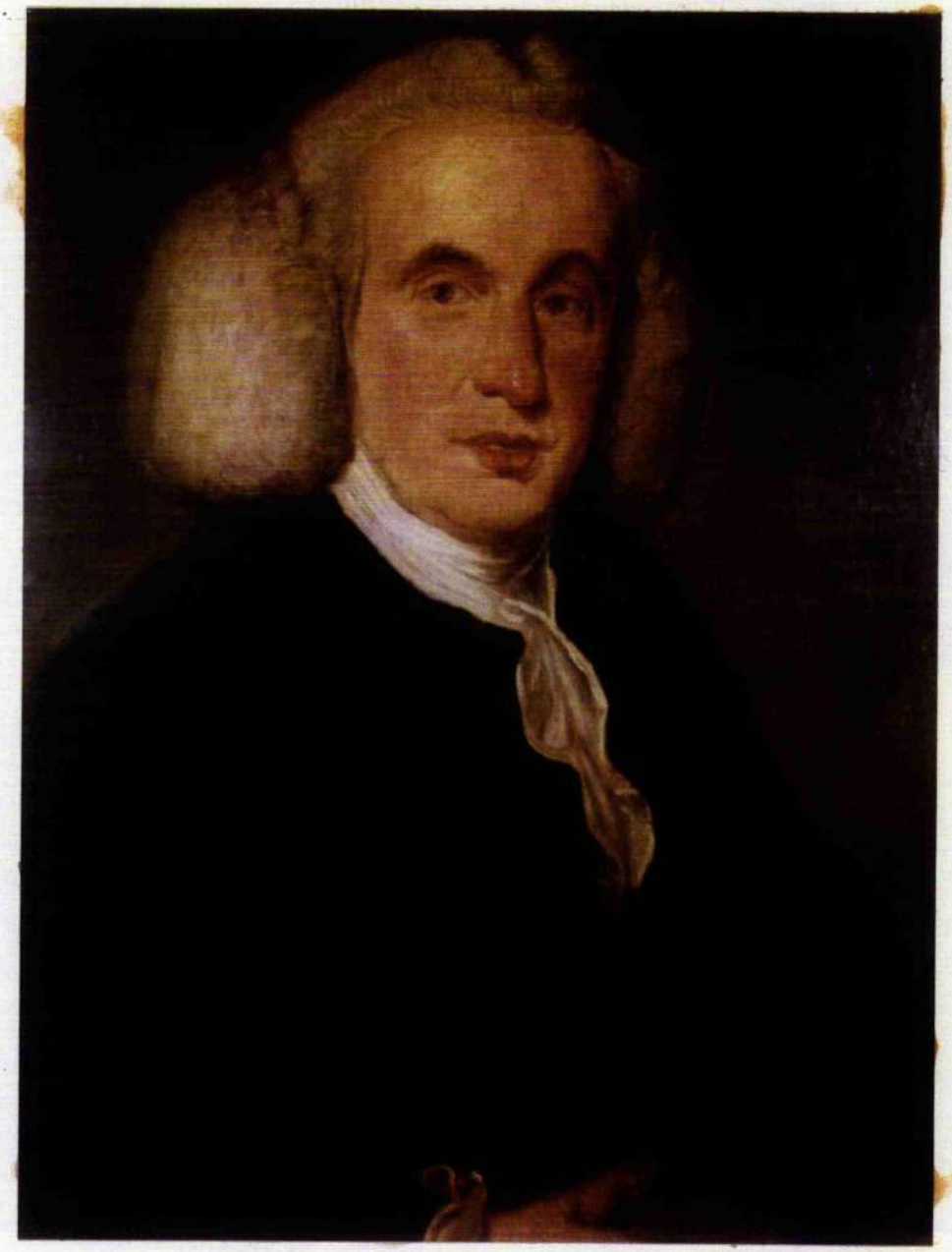


FIGURE 32: WILLIAM CULLEN. 1710-1790. Born at Hamilton.

He was a student at Glasgow University, but did his medical studies in Edinburgh (1734, 1735, 1736). He took his M.D. at Glasgow in 1740 and gave lectures on Clinical Medicine and Chemistry at Glasgow (Joseph Black was one of his pupils). In 1751 he became Professor of Medicine at Glasgow: 1755 he was appointed Professor of Chemistry, University of Edinburgh; in 1766 he became Professor of Physiology at the University of Edinburgh, and in 1733 he was awarded the Chair in Medicine at the University of Edinburgh at the age of 63.

Professor of Medicine and Chemist (Figure 33), William Hunter (1718-1783) Surgeon Anatomist (Figure 34, 35) and John Hunter (1728-1793) Surgeon (Figure 36) (46, 61, 62). Although homeopathy introduced an almost philosophical approach to medical therapy, it did introduce a considerable balance of safety, countering the iatrogenic havoc wreaked among the populace by alchemists and quacks (Figure 37). One contemporary of this transitional era was Robert Burns (1759-1796). Burns' fate at the hands of the medical profession is described by J. Thomson (63).

> "I proclaim that Robert Burns died the doctor's martyr .... The truth stands thus - The Physician of Robert Burns (i.e. Dr. William Maxwell) believed that his liver was diseased, and placed him under a course of mercury. In these days a mercurial course was extremely severe .... Among the last words I ever heard him speak were, 'Well, the doctor has made a finish of it now'."

There is considerable evidence that the much maligned Burns did not die at the hands of an avenging Bacchus or Venus but indeed was a "doctor's martyr" (64). Lenihan and colleagues in Glasgow (65) examined a lock of Burns' hair by neutron activation analysis and found it to contain 8.02 parts per million of mercury - a higher than normal level but not necessarily reflecting an acute dosage. Bearing in mind that hair grows at 20 centimetres per year, the mercurial analysis of the hair sample may not reflect unacceptably high levels in liver, brain and renal tubules (64). Burns' premonition of his fate is aptly expressed in Death and Dr. Hornbrook where he satirizes iatrogenic disease

> "Whare I killed ane, a fair strae death,
> By loss o' blood of want or breath,
> this night I'm free to take my aith,
> That Hornbrook's skill
> Has clad a score i' their last claith,
> By drop an pill." (Figure 38)

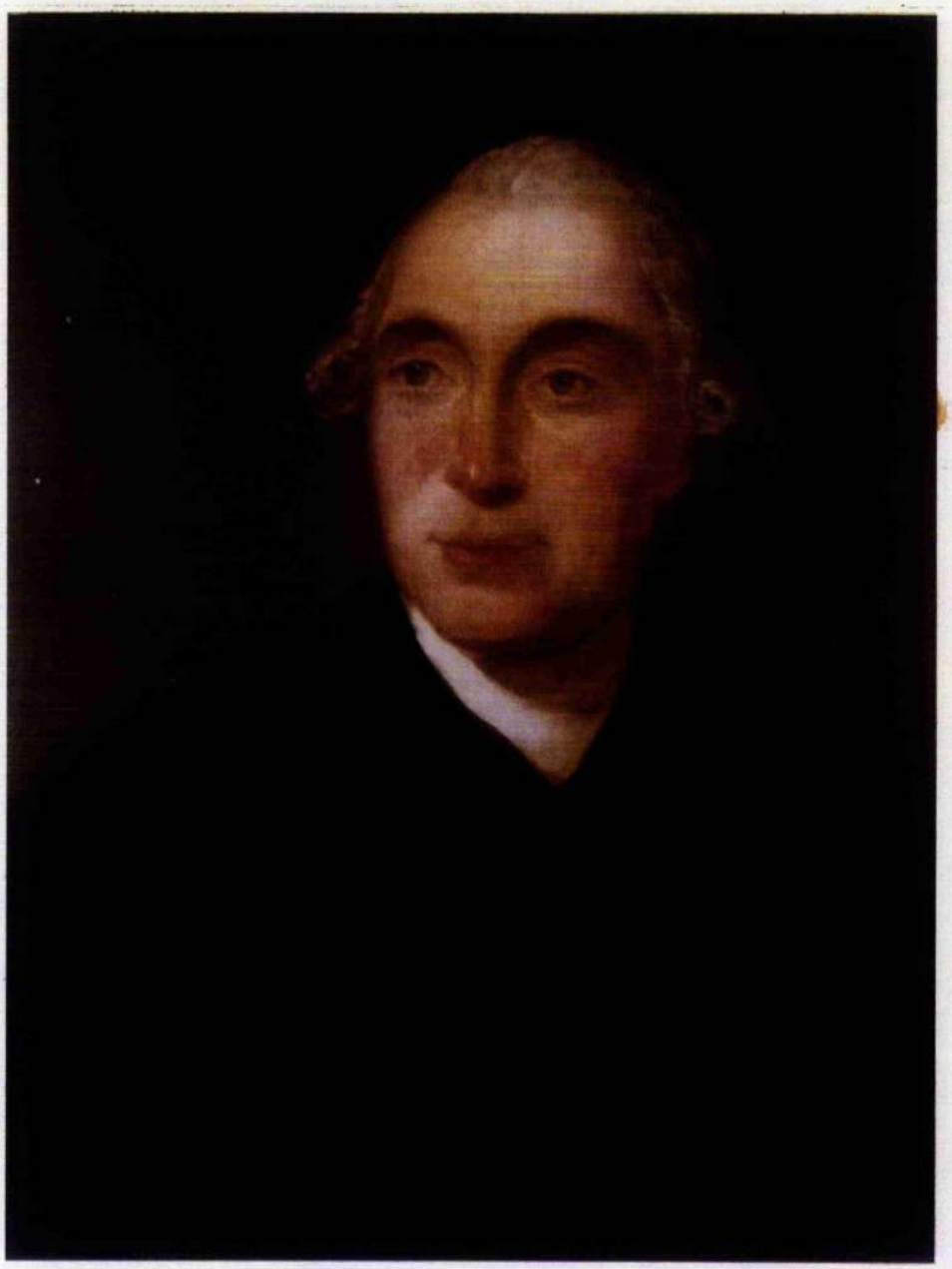


FIGURE 33: JOSEPH BLACK (1728-1799), physician, chemist and physicist. Joseph Black is known for his original definition of "specific heat", "capacity for heat" and "latent heat". He also noted that "fixed air" given off by quickened lime and alkalis is also present in expired air.

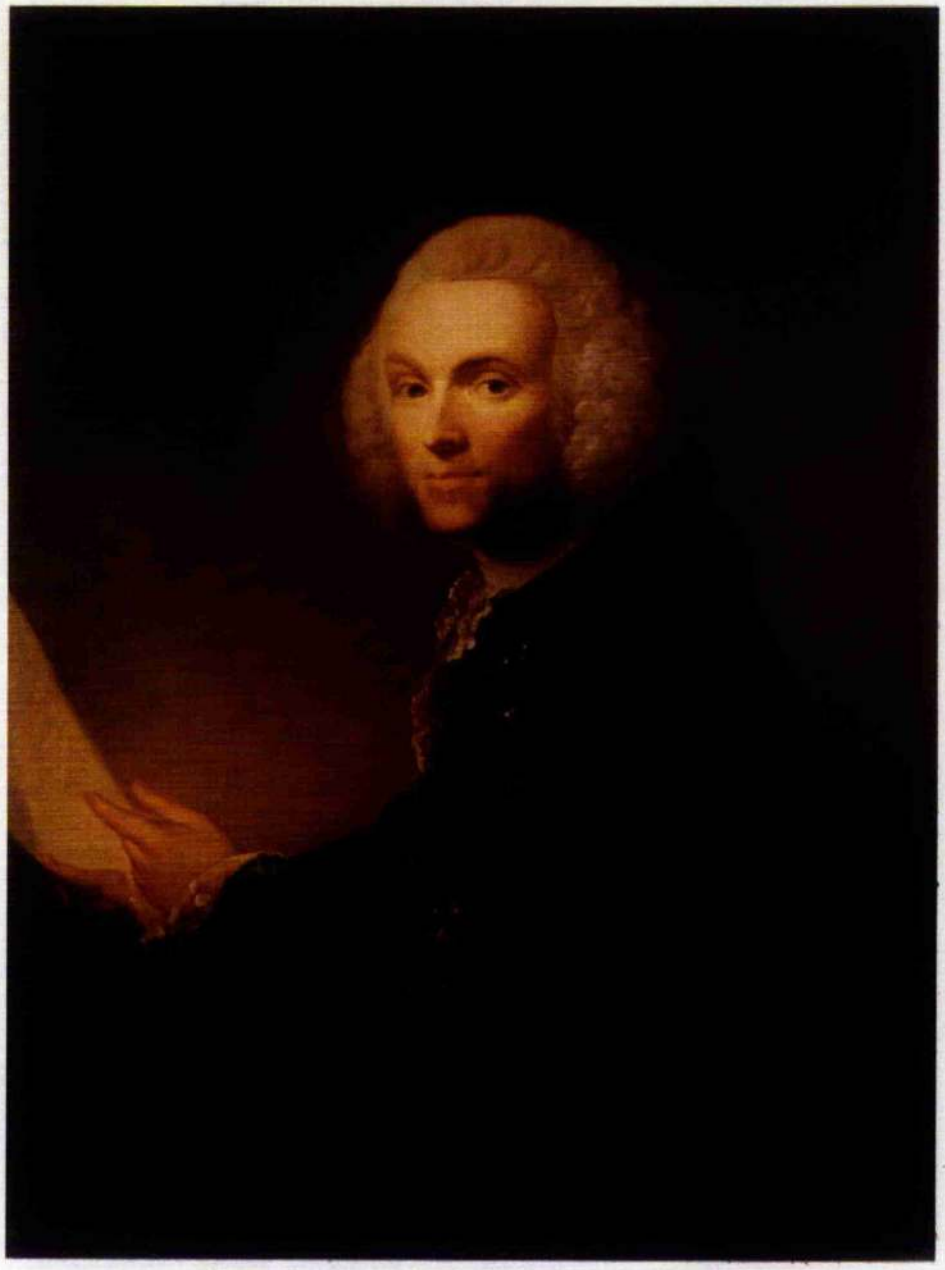
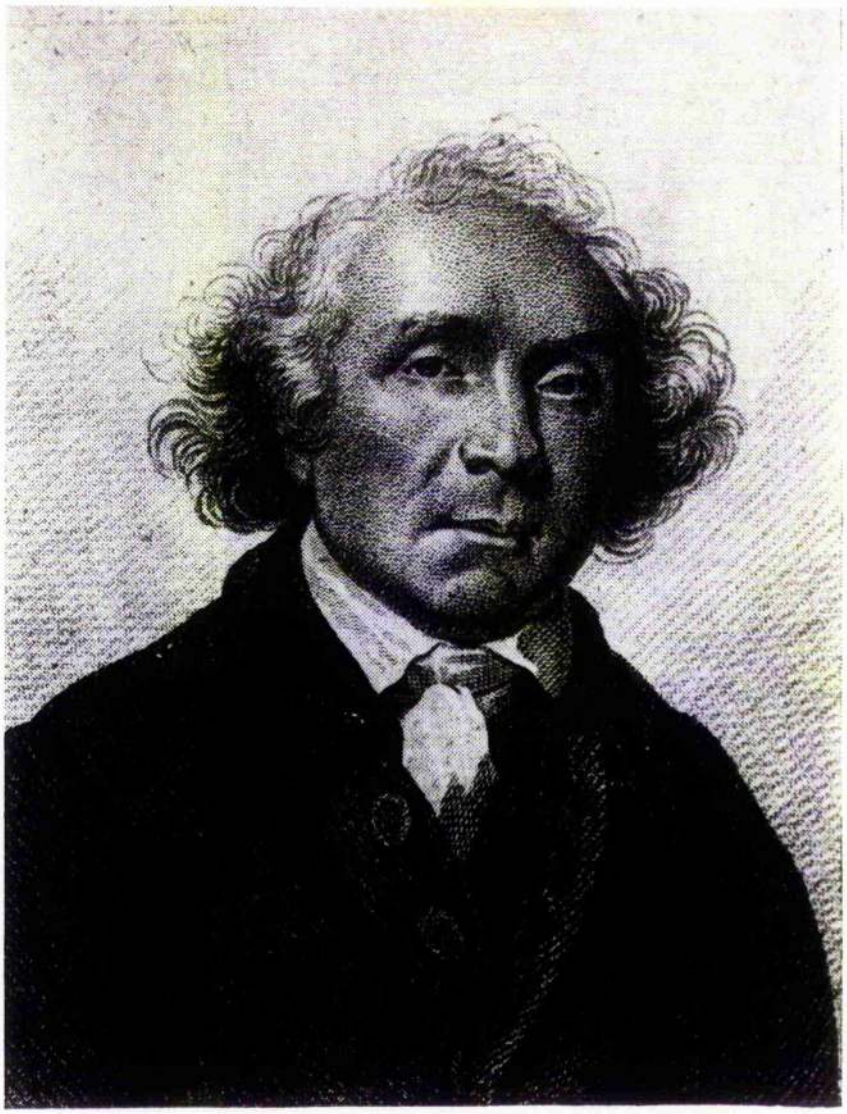


FIGURE 34: WILLIAM HUNTER (1718-1783) surgeon, anatomist and obstetrician. William Hunter followed the examples of his teachers Smellie and Douglass and started, in 1746, a course of private lectures on dissecting, operative surgery, and bandaging. His famous lecture theatre and museum in Great Windmill Street in London was to become the training ground for the best surgeons and anatomists of that era.

FIGURE 35 : Painting of WILLIAM HUNTER by Johan Zoffany.

FIGURE 35 :

Painting by Johann Zoffany of William Hunter demonstrating to members of the Royal Academy in London. Hunter was appointed as Professor of Anatomy to the Royal Academy at its inauguration in 1769-70. The President of the Royal Academy, Sir Joshua Reynolds, can be identified by his long ear trumpet. It is not recorded what Sir Joshua thought of Zoffany's painting, especially as the skeleton has 13 pairs of ribs! In his first Discourse as President to the Royal Academy, Sir Joshua urged students, as beginniners, to learn how to make precise drawings. "He who endeavours to copy nicely the figure before him, not only acquires a habit of exactness and precision, but is continually advancing in his knowledge of the human figure, and though he seems to superficial observers to make a slower progress, he will be found at last capable of adding (without running into capricious wildness) that grace and beauty, which is necessary to be given to his more finished works, and which cannot be got by the moderns, as it was not acquired by the ancients, but by an attentive and well compared study of the human form."



John Hunter.

FIGURE 36 : JOHN HUNTER (1728-1793) Surgeon.

With the advent of John Hunter, surgery ceased to be regarded as a mere technical mode of treatment, and began to take its place as a branch of scientific medicine, firmly grounded in physiology and pathology.

FIGURE 37: The Theriac Vendor or 'Quack Medicine Man', modelled by Simon Feilner 1752. From the Peter Petri Collection, Bahnhof-Apotheke, Offenbach a.m., Germany.

FIGURE 38: Robert Burns converses with the Devil, who complains

"That Hornbrook's skill Has clad a score
i' their last claith, By drop and pill".

In the eighteenth century homeopathy provided a new margin of safety for drug administration, and attempts at disease categorisation by people like Cullen (61) laid the foundation for true scientific diagnostic and therapeutic application.

In the late nineteenth century a unique therapeutic application of a gold compound appeared - the treatment of inebriety by sodium gold chloride. Higby (48) credits Dr. Leslie I. Keeley of Dwight, Illinois as the pioneer of this form of therapy (48). As a young physician serving in the Union Army during the American Civil War, Keeley was appalled to see the social and physical destruction caused by alcohol in the depressed soldiers. Dr. Keeley and a pharmacist colleague, John R. Oughton spent 12 years researching a cure for alcholism and eventually proclaimed sodium gold chloride as a "cure" for drunkenness. Keeley opened his first sanatorium in 1880 and news of his success quickly spread. His life story was recently reviewed by G.A. Barclay in the Journal of the Illinois State Historical Society (66). Satellite branches of Keeley's "Institute: opened in other states and by the 1890's over 30,000 former patients had established the "Bi-Chloride of Gold Club" providing services much like Alcoholics Anonymous do today (66). Dr. Keeley was apparently very secretive with regard to his treatment schedules and died without revealing the dosages he used. Use of sodium gold chloride as a treatment of drunkenness was also referred to in the Canadian literature (67 68). Dr. Oliver Edwards of Ottawa presented his paper on the treatment of inebriety to the Montreal Medico-Chirurgical Society in 1896. He

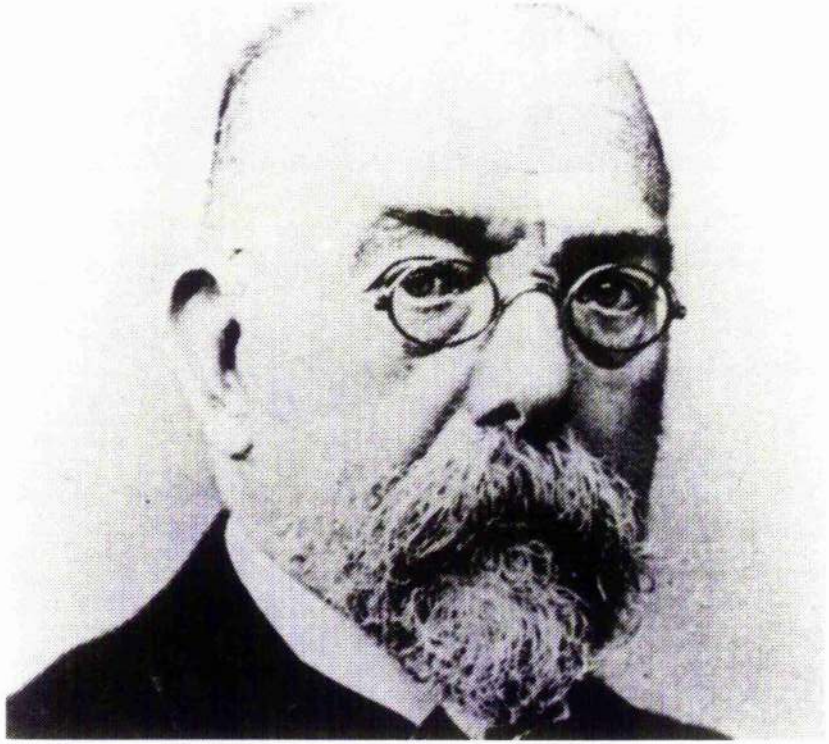
presumably used similar methods to Keeley and detailed instructions for the administration of the sodium gold chloride are given in the text:-

"1st. Wyeth's Hypodermic tablets of chloride of Gold and Sodium, the 1/20th of a grain of 3 or 4 times a day for 2 or 3 days. Then drop to the 1/40th of a grain (that is, one tablet will do for 2 injections), and continue that 3 times a day for at least 3 and sometimes 4 weeks. Use a syringe with a solid piston as sold by Chapman of Montreal, and use always a platinum needle. After a day or two you may have some local hyperaemia - use lanolin or carbolized vaseline, and shower the arm with hot water morning and evening. This does not usually last beyond the second week."

Dr. Edwards also advocated concomitant use of vegetable bitter tonics provided they were nonalcoholic and thirdly induction of a refreshing sleep with "bromide of sodium and chloral" for 1 or 2 nights. Despite the apparent success of these "cures" for drunkenness, the popularity of the therapy did not persist into the 20th century and certainly no mention of this appeared in Osler's Textbook (69).

With the approach of the twentieth century, a new era of gold therapy was about to begin. On March 24, 1882 before the Berlin Physiological Society, Robert Koch (Figure 39,40) astounded the world of science with his announcement that a bacillus was responsible for the dreaded disease, tuberculosis. Eight years later in 1890 at the 10th International Medical Congress in Berlin he announced that

FIGURE 39 : Dr. ROBERT KOCH (1843-1910), Nobel Laureate 1905. Koch astounded the world of science in 1882 with his announcement of the identification of a bacillus as the cause of tuberculosis.



Dr. Robert Koch

FIGURE 40: In 1890 at the 10th International Medical Congress in Berlin Robert Koch reported that gold cyanide inhibited the growth of tubercle bacillus in vitro. The finding led to the use of gold complexes in the treatment of tuberculosis and subsequently by a circuitous route to the use of gold complexes in rheumatoid arthritis.

tuberculin was of benefit for tuberculosis sufferers. This statement was premature as although tuberculin had a marked effect, it was not a cure and many hopes were dashed. However, at the same International Medical Congress he did make an announcement that was to herald the modern management of rheumatoid disease. He reported that gold cyanide was effective against tubercle bacilli in vitro (70). This publication not only led to the use of gold compounds in the treatment of tuberculosis but also by a serendipidous route paved the way for the use of gold compounds in the treatment of rheumatoid arthritis. His results demonstrated that gold cyanide added to culture plates of tubercle bacillus inhibited growth of the organism in vitro but not in vivo in laboratory animals (70). It is not known why Koch elected to use gold cyanide nor did he explain whether it was the gold or the cyanide moiety which produced the biological effects. In view of the findings of Koch, physicians began to use gold compounds in the treatment of tuberculosis. Feldt's observations differed from Koch's, in that, Feldt observed beneficial effects of gold compounds against tubercle bacilli in laboratory animals (71). As well as the reports by Feldt (71,72), Bruck and Glück (73) and Junker (74) published on the effect of gold cyanide compounds on human tuberculosis. In 1925, the Danish veterinarian Holger Møllgard reported on the use of the compound gold sodium thiosulphate in bovine tuberculosis (75. Over the succeeding decade numerous publications reporting the beneficial effect of gold compounds against tuberculosis appeared in the

European literature (76). However, the review by Wells and Long confirms that the effectiveness of the various gold compounds in tuberculosis was if anything minimal (76). In 1927 Dr. K. Landé reported on the use of aurothioglucose in 39 patients with ill-defined conditions, which probably included rheumatic fever and bacterial endocarditis (77). His use of aurothioglucose was based on the premise that gold was a non-specific antiseptic substance. The fortuitous but erroneus hypothesis, that rheumatoid arthritis was a chronic infectious process similar to tuberculosis, led Dr. J. Forestier to start treating his patients with inflammatory rheumatoid disease with gold thiopropanol sodium sulphonate in May 1928 (78). Forestier's application of gold therapy to patients with arthritis thus started a new era of gold therapy in medicine that applied to the treatment of the rheumatic diseases.

SUMMARY:

Elemental gold has been known to man since the dawn of civilisation. The lustre and permanence coupled with its rarity has led to the worship afforded this metal throughout history. Gold's presumed magical qualities and its resemblance to the "essence" of the sun made it a natural choice by priests, healers and shaman. Although reference had been made to the medicinal use of gold by the Chinese circa 2500 B.C., the dramatic account of Moses burning the golden calf, preparing a potion from the ashes, and forcing the Children of Israel to "... drink of it", is probably one of the earliest accounts of the practice of alchemy involving gold. Reference to the medicinal use of gold is also attributed to the Taoist philosophers of ancient China circa 600 B.C. and in later literature, Pliny the Elder and Dioscorides record the use of gold as a medicinal agent. In the great Persian medical schools initiated by the Nestorian Christians, pharmacist-physicians such as Yabir, Avicenna and Rhazes all advocated the use of gold compounds as panaceae. Medical Teaching between the third and ninth centuries spread across North Africa and into Spain with the swoop of the Arab Caliphates. This medical knowledge spread into other parts of Europe and the British Isles by the 11th and 12th centuries. Perhaps the first gold researcher in the British Isles was the Franciscan scholar Roger Bacon (circa 1214-1292 A.D.). According to Stahl, Bacon gave one of the first known descriptions of gold chloride. The repressive religious and scientific attitudes of the Middle Ages prevented the development of any true science but on

the contrary resulted in the flourish of quackery, sorcery and witchcraft. In the search for the "Philosopher's Stone" and the quinta essence, alchemy was patronised by the noble houses of Europe including the court of James IV of Scotland (1473-1513), the Emperor Rudolph II of Germany (157601612) and Duke Frederick of Wurtenberg, all hoping to discover the knowledge for eternal life and the secret of transmutation of base metals into gold. In the Renaissance period the writing and work of Paracelsus was to greatly influence the practice of medicine. He advocated gold as a universal panacea. A similar proclamation that his gold potion was an arcanum came from the Englishman Francis Anthony (1550-1623). Anthony was not a physician but did study chemistry and physics at Cambridge. He was imprisoned on at least two occasions for quackery, and subjected to "examination" by the Royal College of Physicians - an exercise which he failed miserably. In defense of his gold panacea Anthony engaged in a literary interchange with the medical great of his day. Despite their criticism he continued to prescribe his gold potion to his willing patients throughout his lifetime.

Unique association of gold with medical practice have been recorded. Among the more notable is the record by the famous French surgeon, Ambrose Paré, of the use of a gold ligature in the treatment of recurrent inguinal hernia.

Although gold has been used in denfal surgery for almost 100 years, decorative dentistry was practised by the ancient South

American civilisation more than 1000 years ago. A specimen of a mandible with circular gold inlays in the incisors was discovered by the Anthropologist M.H. Saville in Ecuador in 1913 and is now in the Museum of the American Indian in New York City.

The association of gold with medicine has not always been of therapeutic interest. From the end of the 17th century to the beginning of the 19th century a handsome gold headed cane was the prized possession of a series of six notable physicians. The cane was originally owned by Dr. John Radcliffe, benefactor of the now famous Oxford hospital and library. Dr. Radcliffe passed the cane to Dr. Richard Mead, who was an equally eminent and successful physician. The cane was then successively owned by Drs. Askew, William and David Pitcairn, and finally by Dr. Matthew Baillie the nephew of William and John Hunter. The cane is now on display in the library of the Royal College of Physicians in London.

The emergence of medicine and chemistry as true sciences probably began in the early 17th century with such people as John Glauber (1604-1679), Robert Boyle (1627-1687) and Nicolas Lemery (1645-1715). Around this time the famous Dutch physician Herman Boerhaave (1668-1738) and the Scotsman Dr. Donald Monro rejected the idea that aurum potabile was of any medicinal value. The revival of gold compounds in medicine occurred in the early 19th century due to the work of Andre-Jean Chrestien and Pierre Figuier (1765-1817), two professors at the University of Montpellier.

Figuier, a pharmacist, provided the chemical formulation for the gold compounds, in particular gold sodium chloride, which Chrestien advocated as of value in the treatment of tuberculosis and syphilis.

The first record of the use of gold compounds in modern medicine in the United States was the thesis by John C. Cheesman on the use of gold sodium chloride in the treatment of syphilis.

The 18th century was the period of the great pharmacological revolution when homeopaths such as Friedrick Hahnemann rescued the populace from the iatrogenic ravages of the alchemical formulae by introducing the "mini-dose". The homeopath M. Freligh lists gold leaf and a gold-aqua regia solution as a recommended treatment for a wide variety of unrelated disorders, although no rational association of these diseases is apparent. Nevertheless, the advent of homeopathy coincided with the development of structural medical practice as advocated by William Cullen (1711-1790) and Joseph Black (1728-1799), both Glasgow University graduates.

In the past 150 years, perhaps the most unique application of a gold compound was the use of gold sodium chloride by Dr. Leslie I. Keeley (1832-1900) of Dwight, Illinois and Dr. Oliver Edwards of Ottawa in the treatment of inebriety. Dr. Edwards presented his paper to the Montreal Medico-Chirurgical Society in 1896. Despite the lauded remarks of this treatment both by the patients and the physicians, no record of its use exists in current textbooks of medicine and there was no mention in Osler's Textbook.

The herald of the modern use of gold compounds in medicine starts with the observation by Dr. Robert Koch that gold cyanide was bactericidal in vitro to tubercle bacilli. European scientists and physicians over the next 40 years experimented with the use of gold complexes in the treatment of human and bovine tuberculosis. The erroneous but serendipitous assumption by Dr. Jacques Forestier that rheumatoid disease was an infectious disease analogous to tuberculosis, led him to use gold thiopropanol sodium sulphonate on 15 patients with inflammatory rheumatoid disease. The success of this initial experiment was the seed which has led researchers over the past 50 years to investigate both the beneficial and the toxic effects of the anti-arthritic gold complexes.

CHAPTER II

GOLD THERAPY IN RHEUMATOID DISEASE

"We do not what we ought;
What we ought not, we do;
And lean upon the thought
That chance will bring us through."

Matthew Arnold (1822-1888)

> ".... L'analogue de l'evolution clinique de certaines formes graves de rheumatisme chronique avec celle de la maladie tuberculeuse: évolution febrile, avec poussées fluxonnaires au lièvre continue, atteinte de l'etat général, anemie, leucocytose, etc., nous ont fait penser que les sels d'or employes dans la tuberculose pourraient rendre de très grands service aux rheumatisants ...." (78)

The late Dr. Jacques Forestier (Figure 41) thus hypothesised that since the manifestations of rheumatoid arthritis were so similar to those of tuberculosis, gold compounds shown to be of benefit against the dreaded bacillus could be effectively employed against a disease of similar evolution, chronic rheumatoid arthritis. In his original series, Forestier treated 11 women and four men of mean age 42 years, with 250 mgs of gold thiopropanol sodium sulphonate as weekly intramuscular injections. The gold compound suspended in sodium chloride was given every five to seven days depending on how well it was tolerated. A course of gold therapy was defined as 10 or 12 injections. In some cases a second course was commenced after a one month "rest" period. In total 165 injections were given to the 15 patients with a range of between two to 24 per patient. Five patients had an excellent response, five patients were much improved and two were recorded as having a minimal response. In three patients insufficient knowledge was available but according to the author, none of them was worse. Significant improvement was recorded in local and general features of a hithertofore progressive, destructive disease. Patients were recorded as having a decrease in synovial

FIGURE 41 : Dr. JACQUES FORESTIER (1890-1978)
Pioneer in the use of gold compounds in the treatment of rheumatoid arthritis.

swelling and effusion as well as a decrease in joint pain and stiffness. Periarticular tenderness and muscle cramps disappeared, sleep pattern improved and patients required less analgesics especially aspirin.

In his original 15 patients Forestier did record side effects but in general these were not serious. Eight patients had febrile reactions on two or three occasions but none was sustained above 38°C, although in six patients a transient rise to 40°C was recorded. Four of the patients had diarrhoea and vomiting and one of the group also had a generalised morbilliform erythema with desquamation. In one patient who was febrile, the temperature elevation was accompanied by a transient generalised lymphadenopathy. This was not persistent and further therapy was not withheld. Three patients had serious adverse effects. Two had a generalised erythema with pustules and one patient had erythema and mouth ulcers.

This original work by Forestier set in motion a new era in the treatment of rheumatoid arthritis by introducing a drug which markedly improved the disease state. Forestier had shown that gold thiopropanol sodium sulphonate was effective in the treatment of rheumatoid arthritis but he was wise enough to caution that it did induce adverse effects and thus therapy had to be monitored from start to finish.

After more than 50 years of gold therapy in the treatment of rheumatoid disease, with no mechanism of action having yet been determined, it is interesting to note Forestier's speculation as to the mode

of action of the gold thiol compounds in the treatment of rheumatoid arthritis.

> ".... Nous pensons que l'action principle réside dans la combinaison d l'or avec l'atome de soufre et que ce composé doit avoir une action bactericide in vivo sur le ou les agents microbiens qui consent les formes de polyarthrites infectieuses que nous avons traitées." (78)

In February 1930 (79) Jacques Forestier supported his initial findings (78) by reporting the outcome of a further 33 patients with symmetrical inflammatory polyarthritis which he believed to be consistent with rheumatoid arthritis. The outcome of this second study confirmed the efficacy and toxicity data of the first but also showed that the use of 100 mg of gold thiopropanol sodium sulphonate weekly was probably less toxic and equally effective to the original higher dose of 250 mg weekly. Forestier adds a note of caution to potential prescribers of this compound that benefit from the drug is not seen until at least three months of therapy have elapsed. He recommended that therapy be started with three injections of 50 mg intramuscularly 1 week apart followed by 100 mg weekly until a total of 1.0 to 1.5 grams have been given. Repeat courses of the gold compound could be given provided an interval of six weeks had elapsed since the previous course. It is of interest that this second publication on the use of gold compounds by Forestier was the basis for the empirical dosage schedule now used for gold sodium thiomalate.

Although Jacques Forestier is lauded as the pioneer of gold therapy in rheumatoid arthritis, it should be noted that his father, the late Dr. Henri Forestier, founder of La Ligue International Contre

Le Rhumatisme (80) (Figure 42) assisted his son in the original clinical experiments with gold compounds in the treatment of rheumatoid arthritis (78,81).

> "Les première applications therapeutique des sels d'or aux rheumatismes chroniques progressifs d'origine infectieuse faites par le Dr. Jacques Forestier, mon fils et moi-même defins 1928 aut donné des resultats tres satisfaisonts." (82)

Francois Forestier (Figure 43) son of Jacques Forestier is presently a physician in Aix-Les-Bains and is the Vice-President de la Société Française d'hydrologie médicale. Although he is not currently engaged in clinical research with gold, he has published on the subject in collaboration with his father (83, 84).

The first publication on gold therapy in English by Jacques Forestier was published in Lancet in 1932 (85). This was a summary of his original observations published in the French literature (78, 79). In 1934, in the Hunterian Address (86) Forestier presented the results of 500 patients with rheumatoid arthritis, treated with gold compounds. He recorded a 70 to 80% success rate. Fifty per cent of patients treated early in the disease state were permanently improved compared to only 25% of patients with disease of two or more years' duration. In his discussion of gold compounds Forestier stated that gold sodium thiomalate and gold thioglucose were the most useful agents. In this publication, Forestier recorded a fatal case of agranulocytosis, which was his first death due to gold therapy. In a subsequent paper (87) he stated that sedimentation rate was the most

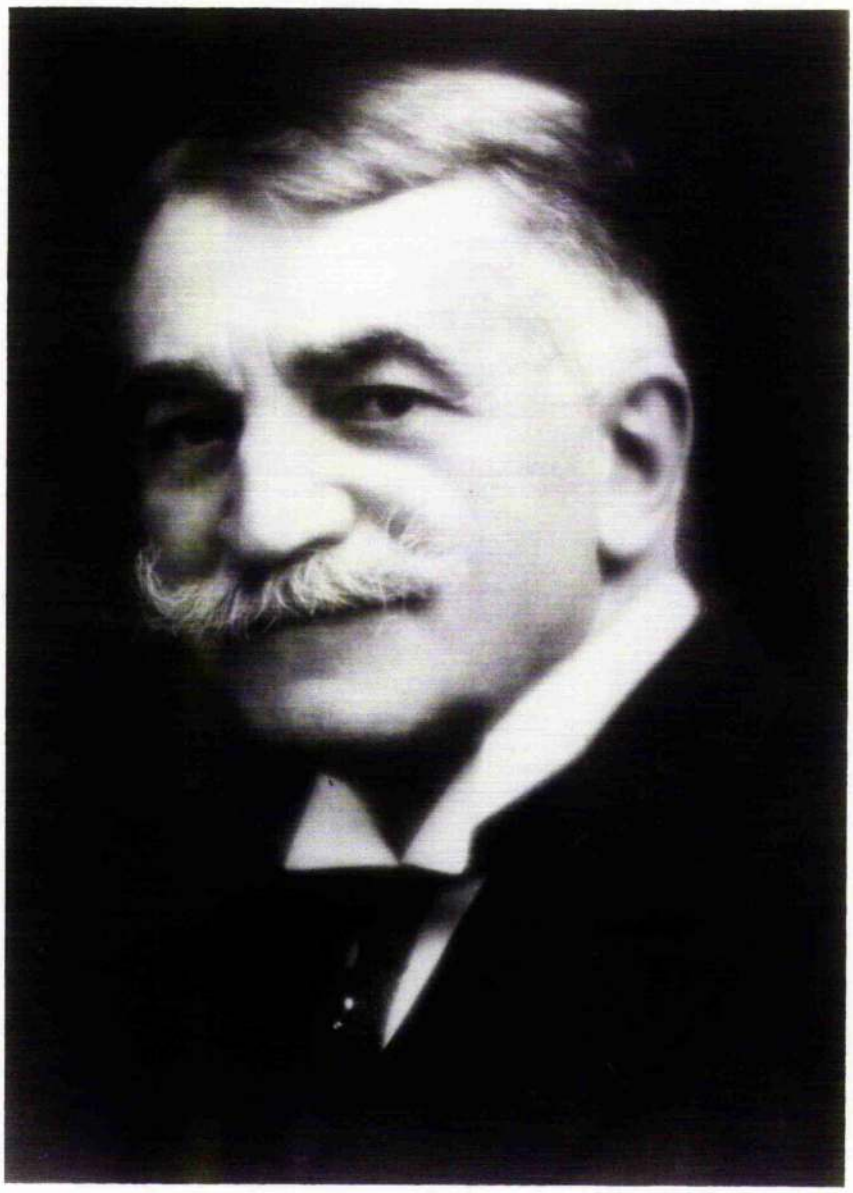


FIGURE 42: Dr. HENRI FORESTIER (1861-1945)
Foundateur de La Ligue Internationale Contre
Le Rhumatisme. Father of Jacques Forestier
and co-pioneer with his son in the use of gold
compounds in the treatment of rheumatoid arthritis.

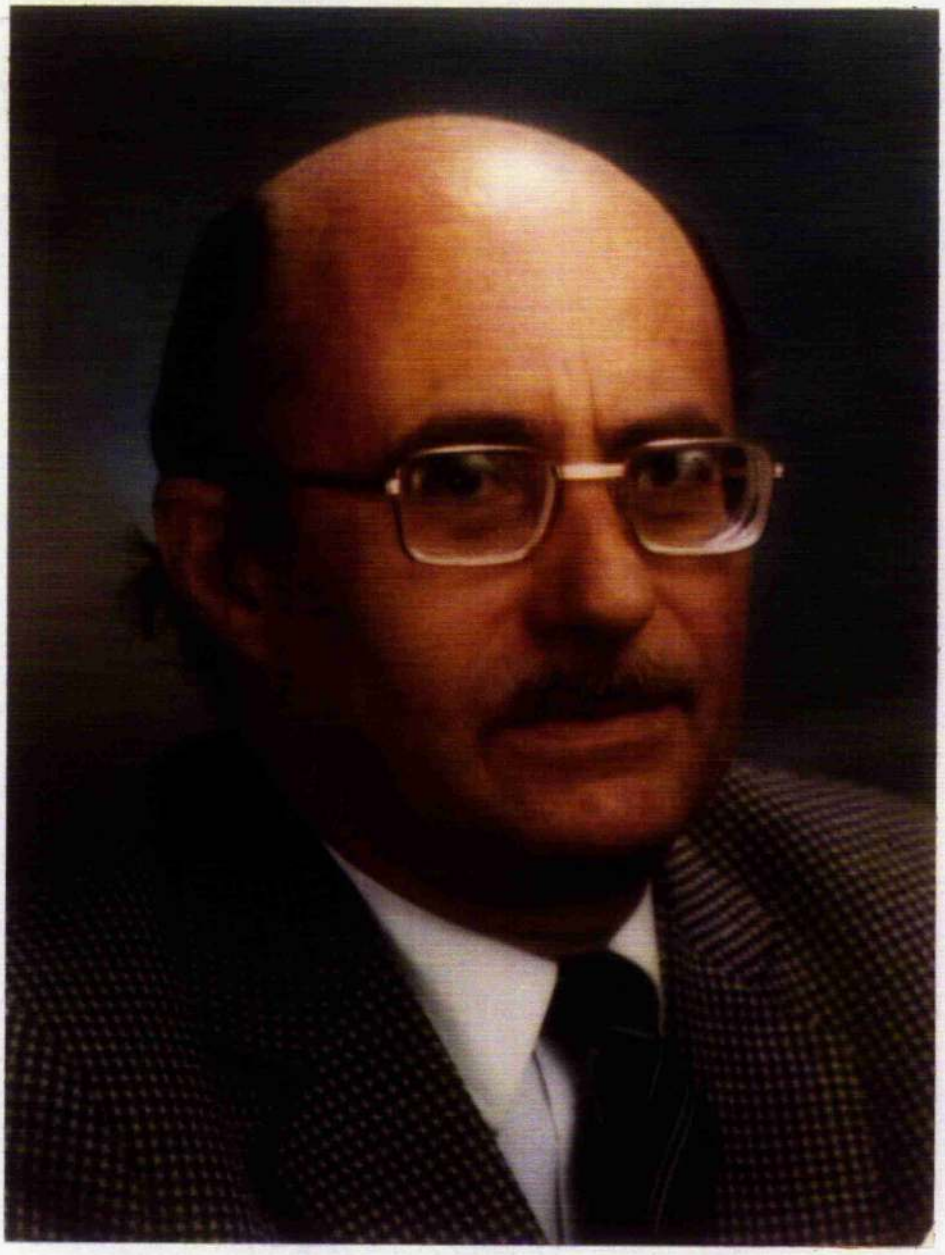


FIGURE 43: Dr. FRANCOIS FORESTIER (Born 1926). Vice-President de la Societe Francaise d'hydrologie medicale. Co-author with his father Jacques Forestier on studies in the use of gold compounds in the treatment of rheumatoid arthritis. Dr. Francois Forestier is a physician in Aix-les-Bains in France.

important check on treatment and that monthly blood counts and urinalysis were an important means of detecting incipient toxic reactions. He also stated that every patient treated with gold therapy should have a coagulation time and a bleeding time before treatment is started in order to identify those patients susceptible to the development of purpura.

Investigators in the United Kingdom (88), Germany (89), the United States (90,91) and Australia (92) confirmed Forestier's findings that injectable gold compounds were effective in rheumatoid arthritis but were not without significant toxicity. Over the next 10 years numerous descriptive analysis of gold therapy in rheumatoid disease appeared in the literature. In a series of 3 publications between 1935 and 1937, Hartfall and colleagues recorded their observations of benefit and toxicity due to gold therapy in patients with rheumatoid arthritis (93,94,95). The final article which described the outcome of 750 patients was the largest series so far recorded (95). Striking improvement was noted in approximately 70% of patients and toxicity occurred in 42% of cases, although only 6% were severe. The relapse rate was 21% and the authors stated that relapse was less common if two courses of gold therapy were given. Seven deaths due to gold therapy were recorded in this series of patients: three cases of haemorrhagic purpura; one case of agranulocytosis; two cases of subacute necrosis of the liver, and one case of exfoliative dermatitis. Hartfall and colleagues also stated that gold therapy was of doubtful value in other forms of arthritis. This statement has never been

challenged by a series of controlled clinical trials although in 1978 Dorwart et al (96) reported a comparative trial of gold therapy with either gold sodium thiomalate or gold thioglucose in patients with psoriatic arthritis compared to patients with rheumatoid arthritis. The authors recorded that the 14 patients with psoriatic arthritis had greater benefit and less toxicity than the 42 patients with rheumatoid arthritis. Except for this study and that of Brewer et al (97) on the use of gold therapy in juvenile rheumatism, no other controlled study of gold therapy has been done to examine efficacy nor toxicity in the other rheumatic diseases.

Uncontrolled studies on the use of gold therapy in rheumatoid disease suggested that these drugs were of benefit in between 50% (98) to 80% (99-101) of patients given these compounds but that a wide range of adverse effects occurred which could be serious (78,79, 85-95. 98-101) and even fatal (86,95,102). There was clearly a need for a strict evaluation of efficacy and toxicity of at least one of the gold compounds with regards to its use in rheumatoid arthritis. In 1939 Sir Stanley Davidson, Chairman of the Scientific Advisory Committee of the Empire Rheumatism Council proposed a multi-centre controlled double blind trial to investigate the compound gold sodium thiomalate in rheumatoid arthritis. World War II disrupted the success of this initial proposal but the late Dr. Thomas N. Fraser of Glasgow (Figure 44) completed his section of the multicentre trial at the Western Infirmary of Glasgow (Figure 45) and published his results independently in the Annals of the Rheumatic Diseases in 1945 (103). Fraser's trial was the first published double-blind controlled

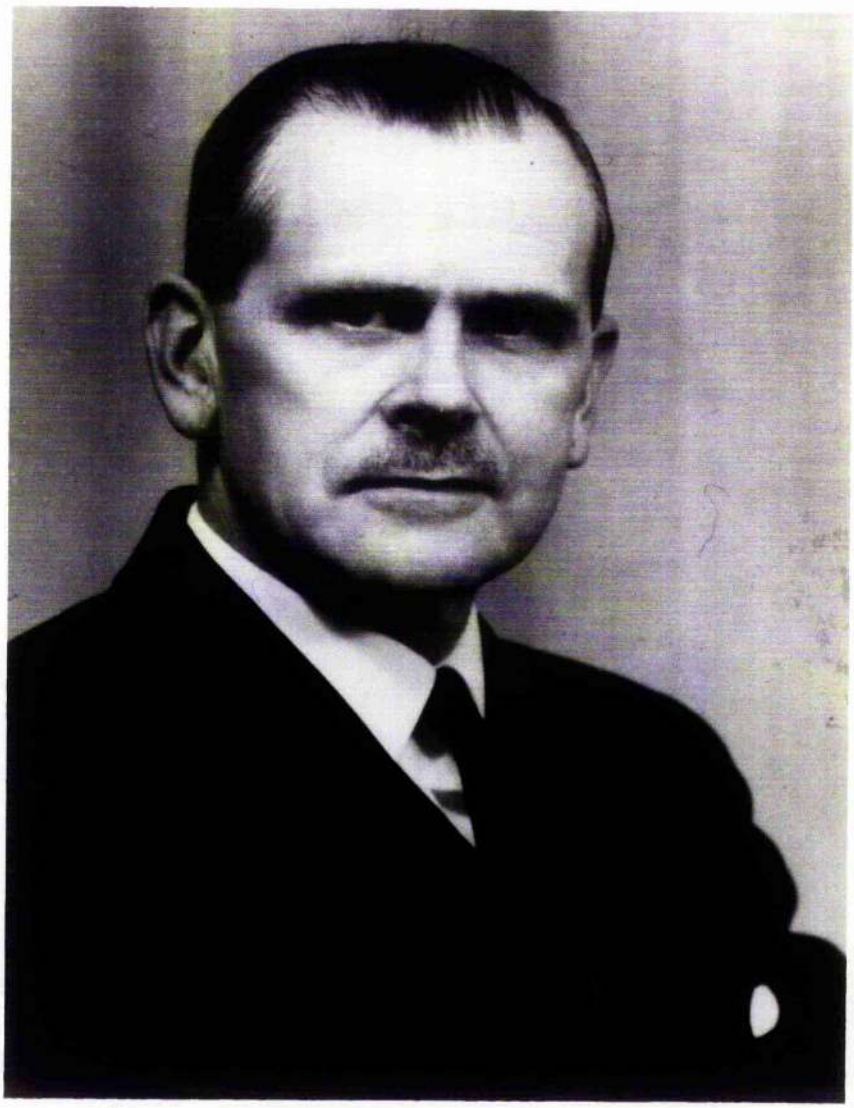


FIGURE 44: Dr. THOMAS N. FRASER (1910-1976)
Author of the first double-blind controlled trial in the use of gold therapy in the treatment of rheumatoid arthritis, for which he graduated M.D. with honours from Glasgow University in 1948. This was the first double-blind controlled trial of any anti-rheumatic drug.

FIGURE 45: One of the wards in the Western Infirmary, Glasgow where Dr. Thomas N. Fraser conducted the first double-blind controlled trial in the use of gold therapy in rheumatoid arthritis, published in the Annals of the Rheumatic Diseases in 1945 (103).

trial of any anti-rheumatic drug. It confirmed Forestier's original findings (78) and demonstrated an efficacy rate of 82% in the patients who received gold sodium thiomalate compared to 45% of the control patients. Fraser did, however, caution that interpretation of the results should only be made within the confines of the study group. His control group had a marked improvement rate which was apparently unexpected. Fraser explained firstly that all study patients received physiotherapy and secondly that some spontaneous remissions might have occurred. If this also accounted for some improvement in the myocrisin group, therefore improvement attributable to gold therapy would be reduced to 42%. It is important to note that 72% of the control group recorded a subjective improvement although for the purpose of the study, improvement was only recorded with 45% who showed objective improvement (103). This suggests that a considerable psychological factor may have been operative. Subsequent to Fraser's work firther trials were reported but these were poorly controlled. (104, 105).

In 1947 Waine and colleagues (104) reported on 58 patients treated with either gold sodium thiomalate or gold thiosulphate. All patients received a minimum of 500 mg with an average total dose of 1600 mg. The authors reported a significant improvement in 57% of the treated group compared to 29% of controls. However, the controls were a group of 62 rheumatoid arthritis patients treated with only "supportive" therapy and did not receive a placebo injection.

In 1950 Adams and Cecil (105) reported on 106 patients with rheumatoid disease who either received gold sodium thiomalate or aurothioglucose during the first year of their disease. The total compound

given was between 1000 mg and 1500 mg. The authors recorded a 66% rate of "remission" by their definition in the gold treated group compared to only 24.1% remission rate in the control patients. However, the control group only received conventional therapy and no placebo injection, thus making these figures non-comparable. Remission occurred on average 10 months later in the control group than in the gold treated group (17 months and seven months respectively). The average time from remission to relapse was 27 months. The authors thus concluded that gold therapy increases the incidence and accelerates the appearance of remission if given during the first year of the disease (105).

In contrast to the beneficial effects of injectable gold therapy over conventional therapy quoted by all authors so far cited, Merliss and colleagues (106) found aurothioglycolanilide (Lauron) given over six months to 27 patients, to be no better than saline or serum injections given to 44 control patients over a similar period of time. Similarly Brown and Currie (107) found that gold sodium thiomalate given as a total dose of 800 mg - 1000 mg was no better than copper, saline nor arsenic injections and no better than physiotherapy or oral aspirin when patients were assessed at the end of the course of gold, and at three months and 12 months later.

Lack of firm statistical evidence as to the efficacy of injectable gold compounds led the Empire Rheumatism Council in 1957 to plan a second multi-centre trial. This was carried out in 24 centres throughout the United Kingdom and the results were published in 1960 and

1961 (108,109). One hundred and ninety-nine patients started the trial. The 99 patients in the treated group received 1000 mg of gold sodium thiomalate as 50 mg weekly injections over 20 weeks. The control group were given 0.01 mg of gold sodium thiomalate as 0.5 µg weekly over a 20 week period (i.e. they received 1×10^{-6} the quantity of gold compound received by the treated group). It was unequivocally demonstrated that in most patients given the 50 mg weekly gold sodium thiomalate, there was progressive improvement in a number of objective variables, including the number of joints clinically inflammed, grip strength and sedimentation rate. Although gold therapy was stopped after 20 weeks (1000 mg of compound), improvement persisted for up to 12 months in many patients and was generally maintained up to 18 months. However, by month 30 (i.e. two years after gold therapy had been discontinued) little if any advantage was recorded in the original gold treated group compared to controls (109). In 1973 the Cooperating Clinics Committee of the American Rheumatism Association (110) reported their double-blind trial of 68 patients with definite or classical rheumatoid arthritis (111). The initial phase of this study compared 36 patients who received gold sodium thiomalate 50 mg weekly for six months and a control group of 32 patients who received sterile water vehicle over the same period. Twelve patients in the gold group dropped out because of adverse effects and eight patients in the placebo group dropped out because of no benefit. The gold treated group showed slight but definite improvement in all parameters

measured, although only the change in sedimentation rate was statistically significant. In the second phase of the study, designed to compare the results of maintenance therapy, patients received six 50 mg doses at two-week intervals, six doses at three-week intervals and then at four-week intervals until a total of two years of treatment had been given. Control patients received the sterile water vehicle in the same fashion. In phase two the gold group showed no increase in the number of involved joints, improved their grip strength and had a fall in sedimentation rate. During the same time period, the control group deteriorated in all of these measurements. The authors commented that the results of the Cooperating Clinics Committee Trial (110) confirmed the results of the Empire Rheumatism Council Trial (108,109) and stated that the larger sample size of the latter allowed the differences recorded for grip strength and number of active joints to reach statistical significance as had been achieved by the sedimentation rate. It should be noted that although the Empire Rheumatism Council Trial patients and Cooperating Clinics Committee Trial patients were comparable in almost all respects, there were marked differences in the category of duration of disease prior to therapy. The majority of Empire Rheumatism Council Trial patients had disease of less than three years duration and had an upper limit of five years, whereas the Cooperating Clinics Committee Trial patients had no disease duration limit and almost one third of the patients had rheumatoid arthritis for longer than five years. If gold works better when given early in the disease process, this would explain the greater demonstrable benefit in the Empire Rheumatism

Council Trial results. However, one must consider that gold therapy may not be as effective as is generally thought and the results of the Cooperating Clinics Committee Trial are a true reflection of its efficacy.

Two subsequent double-blind trials have added further useful knowledge to the management of rheumatoid disease with injectable gold compounds. The first is that of Dr. John W. Sigler and colleagues (112) and the second is that of D.E. Furst and colleagues (113).

Sigler and colleagues (112) reported a two year double-blind study of 13 patients who received gold sodium thiomalate compared to 14 patients who received placebo identical in appearance to the gold compound. Significant improvement in relation to global measurement, ring sizes and grip strength was recorded in the gold-treated group. However, the most striking finding was the claim by the authors that radiological examination showed arrest of bone and cartilage destruction in several patients and that the mean progression rate of destruction was significantly slowed for the treated group. In the Cooperating Clinics Committee Trial (110) posteroanterior radiographs of the hands were taken at the beginning and end of phase one (0-27 weeks). The results obtained by a single observer in blindfold fashion detected deterioration in nine of 19 controls and three of 20 gold treated patients. The difference ($P = 0.06$) was not significant but favoured gold therapy as being possibly beneficial. In the Empire Rheumatism

Council Trial (108,109) no significant radiological differences were detected between the gold treated and the control groups in terms of joint narrowing, development of new erosions or extension of new erosions in any period of the trial. The minimal differences that did occur were in favour of the gold treated group (109). This apparent arrest or even regression of radiological changes recorded by Sigler et al has also been confirmed in a much larger but uncontrolled study by Luukhainen et al (114).

It is difficult to compare the results of the radiological studies in the aforementioned trials (108-110, 112, 114) because of the known interobserver variability in radiographic interpretation (115). However, it does suggest that either gold therapy inhibits the disease process sufficiently well to allow normal tissue repair to take place or that gold therapy enhances tissue repair.

The preceding double-blind trials (108, 109, 110, 112) confirmed that gold therapy was of value in the treatment of rheumatoid disease, but dosage schedule had been achieved by empirical means based on descriptive analyses (79) and poorly controlled comparative studies (101). The question was raised as to whether 50mg of gold sodium thiomalate weekly was equally efficacious and less toxic to higher doses. Furst and colleagues (113) attempted to answer the question by comparing the outcome of 23 patients who were given 50 mg of gold sodium thiomalate weekly, to a group of 24 patients who were given 150 mg weekly. Drug administration and evaluations were carried out double blind. Serum gold concentrations were recorded but did

not correlate with efficacy nor with toxicity. The conventional dose - 50 mg weekly was just as efficacious as the high dose - 150 mg weekly. However, side effects were more frequent and severe in the high dose group (113). These findings are identical to those observed by Forestier in his second publication on the use of gold thiopropanol sodium sulphonate (79).

Since the results of Fraser's trial (103) and subsequent confirmation by the Empire Rheumatism Council Trial, (108,109) gold sodium thiomalate has become the most widely used injectable gold compound in the treatment of rheumatoid arthritis although gold thioglucose is used in the United States and gold thiosulphate is still used in Holland and Denmark. In 1972 Sutton and colleagues (116) reported that orally administered alkylphosphine gold co-ordination complexes exhibited anti-inflammatory properties when administered to adjuvant arthritic rats and in the same year the same group (117) reported that triethylphosphine gold chloride was equipotent to parenterally administered gold sodium thiomalate in suppressing the inflammatory lesions of adjuvant arthritis. Triethylphosphine gold chloride is extremely toxic in man and further studies were not evaluated. However, a related compound 2,3,4,6 - Tetra - o - acetyl - 1 - thio - β - D - glucopyranosato - s - (triethylphosphine) gold has been shown to exhibit potent anti-arthritic properties (118). Subsequent studies have shown that this compound marketed as Auranofin$_{TM}$ (Smith Kline and French, Philadelphia) is effective in human studies of rheumatoid arthritis (119,120,121).

Extensive studies are currently being conducted world wide by Smith Kline and French to investigate the biological, pharmacological and clinical properties of Auranofin$_{TM}$ with a view to marketing the compound as an anti-arthritic agent.

Summary:

Based on the assumption that rheumatoid disease was an infectious disease analogous to tuberculosis, Jacques Forestier (78) introduced gold therapy in the treatment of rheumatoid arthritis. This somewhat serendipitous discovery is today perhaps ironically close to the truth, in view of the recent interest in the role of the Epstein-Barr virus (122) and gastro-intestinal commensals (123) as infectious agents which play a role in the pathogenesis of rheumatoid disease. Following Forestier's initial publications (78,79, 83-87) numerous descriptive analyses attested to the efficacy of gold compounds in rheumatoid arthritis but also demonstrated that potentially serious toxicity was a major disadvantage. The double-blind trial by Fraser (103) and the subsequent Empire Rheumatism Council Trial (108,109) and Cooperating Clinics Committee Trial (110) finally established that injectable gold compounds were efficacious in the treatment of rheumatoid arthritis. In an attempt to produce an oral gold compound, Smith Kline and French, Philadelphia investigated the effectiveness of alkylphosphine gold compounds (116-118). 2,3,4,6 - Tetra - o - acetyl - 1 - thio - β - D - glucopyranosato - S - (triethylphosphine) gold has proven to be an effective agent clinically (119-121) and extensive trials are underway on a world wide basis prior to probable marketing.

CHAPTER III

CLINICAL STUDIES ON GOLD THERAPY

LONG TERM INJECTABLE GOLD THERAPY: INCIDENCE OF EFFICACY AND TOXICITY DURING SEQUENTIAL TIME PERIODS

- INTRODUCTION
- PATIENTS AND METHODS
- RESULTS
- DISCUSSION
- SUMMARY

"Man can learn nothing unless he
proceeds from the known to the unknown."

Claude Bernard (1813-1878)

INTRODUCTION:

As already discussed in chapter II, the efficacy of gold therapy with gold sodium thiomalate in the treatment of rheumatoid arthritis is well established (108-110, 112). However, until 1979 (124) there were no clear guidelines on how long gold complexes should continue to be administered to patients who apparently respond to and tolerate the drug. Furthermore, accurate data on the incidence of various forms of toxicity during increasing time intervals of gold treatment were lacking, even though some authors suggested that prolonged administration was usually well tolerated (125, 126).

The following study describes my experience with long term gold sodium thiomalate administration while studying as a rheumatology training fellow at Queen's University, Kingston, Ontario under the directorship of Dr. T.P. Anastassiades (124). The study was conducted at Queen's University, Rheumatic Disease Unit in a special Gold Therapy Clinic (127). It was therefore possible to document virtually all of the toxicity that occurred within one clinic setting. Reliable calculations for both the frequency and incidence of toxicity could therefore be made for periods of continuous treatment ranging from zero to 72 months. Data was also obtained on the outcome of sustained remissions following the discontinuation of long term gold treatment.

PATIENTS AND METHODS:

The methodology of assessment and monitoring were established in 1971 by Dr. T.P. Anastassiades prior to the commencement of the study. I joined his unit on July 1st, 1976 and took charge of the assignment using exactly the same methodology.

The study group consisted of 94 patients who received 100 courses of gold sodium thiomalate (Myochrisine) from April 1971 to June 1978. There were 29 males and 65 females with an average age of 52.3 years (range 10-79) and 49.9 years (range 10-69), respectively. Two males and four females received more than one course of gold therapy. A course is defined as the intramuscular administration of gold compound at weekly to monthly periods for an indefinite period of time. A patient was considered to have received a new course if more than six months had elapsed since the previous injection.

All patients had classic or definite rheumatoid arthritis by the American Rheumatism Association criteria (111) and had objective clinical inflammation of the joints for six months or longer. The decision to start gold therapy was made by a physician specializing in the rheumatic diseases and was based on the presence of active rheumatoid arthritis unresponsive to adequate therapy with nonsteroidal anti-inflammatory drugs. Intramuscular injections of the gold compound were given weekly with starting doses of 10 mg and 25 mg in the first two weeks, followed by 50 mg weekly until a total of 1000 mg had been given. Maintenance doses of 50 mg of gold were then given every two to four weeks. If toxicity developed, the dose was reduced

or stopped at the discretion of the physician. Dosage of gold, toxicity, haemoglobin, white blood cell count, differential white blood cell count, platelet count, erythrocyte sedimentation rate, and urine protein were recorded on each patient's hospital file on a special monitoring sheet. Platelet counts were measured in the hospital routine haematology laboratory on a Technicon Autoanalyzer (model-1A) by using fresh blood collected in EDTA. The normal range was 150,000-450,000/mm^3 and low platelet counts (< 50,000/mm^3) were checked by a Coulter Counter ZBl.

Some patients who lived at considerable distance from the Unit were given gold injections by their family physicians, following the described schedule. Both the family doctor and the patient had duplicate gold monitoring sheets identical to those in the Gold Therapy Clinic, with the information being transcribed from the patient's sheet to a clinic monitoring sheet when the patient was seen at the Gold Therapy Clinic. In addition, family doctors undertaking gold therapy were routinely provided with written information on gold toxicity and efficacy. The 26 patients who were receiving injections from their family doctors were normally seen at the Gold Therapy Clinic approximately every 12 weeks. The remaining 68 patients were followed entirely at the Gold Therapy Clinic.

Patients were asked about the appearance of rash, pruritis, or bruising. Depending on the history and severity of the rash or pruritis, the gold dosage was reduced, temporarily withdrawn, discontinued, or in some instances continued at the same dosage.

If the white blood cell count fell below 4,000 per mm^3 or the platelet count fell below 150,000 per mm^3, gold therapy was immediately discontinued. If there was a downward trend of platelet count, the gold therapy dosage was reevaluated. If the platelet count fell below 200,000/mm^3, the next dosage of gold was omitted. Gold therapy was also withdrawn if the following abnormalities were present: 2+ proteinuria, the persistence of 1+ proteinuria for 3 weeks, or 1000 mg of protein in urine per 24 hours. Following most episodes of toxicity, gold therapy was restarted at reduced dosage (25 mg). Provided there was no recurrence of toxicity, full dosage (50 mg) was resumed on the next or subsequent visit.

The response to the drug was judged by two criteria. The first was the patient's answer to the question, "How do you feel, relative to before you received gold therapy?" (128). The second criterion was the clinical estimation of disease activity based on the total number of active joints. A joint was considered active if it was tender to palpation or painful at the extremes of range of motion, or if it demonstrated soft tissue swelling or effusion. If there was a significant decrease in the number of active joints since the previous assessment (usually an improvement in at least 5 joints), the patient was considered "better"; if the joint count was approximately the same, the patient was considered the "same"; and if there was a significant increase in the number of active joints since the previous visit, the patient was considered "worse". Remission was classified as no clinical evidence of active joints and no evidence of the extra-articular manifestations of rheumatoid

arthritis. Occasionally, where the patient's subjective response differed from the physician's assessment, the latter was taken as the result for inclusion in this study. Response to the drug was recorded at the end of eight periods: 3 months, 6 months, 12 months, 24 months, 36 months, 48 months, 60 months, and 72 months.

RESULTS:

Efficacy and Outcome.

Overall outcome.

The clinical outcome of 94 patients receiving 100 courses of gold therapy is illustrated in Table 1. As described in Patients and Methods, patients were categorized as being in remission, better, same, or worse. For the purpose of the study, outcome was recorded over set intervals of time as shown in Table 1. For the 0-3 month period, 3 patients were withdrawn following immediate toxicity to the drug, and their response is not recorded (see Miscellaneous Toxicity below). The number of patients who showed a favorable response (remission and better) was greater than 70% for each period of treatment analyzed.

Outcome of sustained remissions and reasons for withdrawal of therapy.

Ten patients were discontinued from gold therapy following sustained remission. The outcome after discontinuation of therapy was as follows: Six patients continue in remission and 4 patients have relapsed. The average time of treatment when remission was recorded was similar in both the patients who maintained remission and those who experienced relapses, but the average dose required to gain remission

Table 1. Clinical outcome of 94 patients receiving gold therapy for a total of 100 courses* in 134.4 patient years

Period treatment, months	Number of treatment courses completed per period of study	Clinical response†			
		Remission	Better	Same	Worse
0–3	100	0	71	24	2
3–6	77	9	48	13	7
6–12	60	9	34	9	8
12–24	45	15	17	2	11
24–36	22	10	7	2	3
36–48	13	7	3	1	2
48–72	6	4	1	0	1

* A course is defined as the intramuscular injection of gold salts at weekly or monthly intervals for an indefinite period of time. A patient was considered to have received a new course if more than 6 months had elapsed since the previous injection.

† Numbers are patient responses per period of study. Six patients received two courses of gold therapy.

was higher in the group who have maintained remission. The duration of maintenance therapy after remission was shorter in 6 patients who remain in remission. Two of the 4 who relapsed have been restarted on gold therapy. Both went into remission again after 4 months of therapy, and neither has shown any evidence of toxicity.

The reason for withdrawal from gold therapy during the 134.4 years of treatment is shown in Table 2. Forty-two patients were withdrawn because of toxicity, 19 because of no response, 10 were in sustained remission (Table 2), and 7 had miscellaneous reasons. One patient with Felty's syndrome is in the miscellaneous group, having been stopped because of persistently low white cell count after 135 mg of gold therapy. He has since been restarted and has had a good response in terms of disease activity with disappearance of Felty's syndrome after 1,150 mg of Myochrisine. One patiemt was discontinued because she planned to become pregnant. Eleven patients had more than one reason for withdrawal.

Toxicity.

Overall toxicity (Table 3).

Forty-two patients had to be withdrawn from gold therapy because of toxicity. Eleven patients had more than one toxic episode recorded as a reason for withdrawal. Rash accounted for 45% of the withdrawals in the patients with toxic reactions but only 26% of the reason for withdrawal in the total study group. Mouth ulcers accounted for 20% of withdrawals in the patients with toxic reactions and

Table 2. Reasons for withdrawal from gold therapy during 134.4 patient years of treatment for 94 patients.

Reasons for withdrawal from gold therapy	Number of patients
No response*	19
Remission	10
Toxicity*	42
Miscellaneous	
Defaulted	4
Patient moved	1
Pregnancy	1
Felty's syndrome	1

* Includes 11 patients who had more than one reason for withdrawal.

Table 3. Episodes of toxicity as causes for withdrawal during 134.4 patient years of treatment for 94 patients

Toxic manifestations	Episodes of toxicity leading to withdrawal*	
	Number	%†
Rash	24	45
Mouth ulcers	11	20
Proteinuria	10	19
Low platelets‡	4	8
Immediate allergic reactions	2	4
Low WBC	1	2
Jaundice	1	2
Total	53	100

* Episodes of toxicity occurred in 42 patients (see Table 2).

† Refers to percent of the 42 patients who experienced toxicity.

‡ A platelet count below 150,000 mm^3 was considered a toxic manifestation. See Results section for further discussion.

12% of withdrawals in the study group. Proteinuria accounted for 19% of withdrawal in the patients with toxic reactions and 11% of withdrawal in the study group.

The average time of onset of a toxic reaction necessitating withdrawal was 10 months. The average time of onset of rash, mouth ulcer, and proteinuria necessitating withdrawal was 11 months, 9 months, and 8 months respectively.

Rash, mouth ulcer, and proteinuria (Table 4).

There were 59 episodes of rash in the 134.4 years of treatment of the study group, giving an overall incidence of 3.6 episodes per 10,000 patients per month. Rash occurred most frequently in the 0-3 month period with an incidence of 9.8. The occurrence of rash in patients receiving more than 2 years of therapy was uncommon, and the incidence appears to decrease over progressive time intervals of gold therapy (Table 4).

There were 23 episodes of mouth ulcer in the 134.4 years of treatment of the study group, with an overall incidence of 1.4 episodes per 10,000 patients per month. However, again the highest incidence was observed during the 0-3 month period, with a progressive decrease over subsequent time periods so that the occurrence of mouth ulcers after 12 months of therapy was uncommon.

Sixteen episodes of proteinuria (as defined in Patients and Methods) in 134.4 years of treatment were observed which gives an overall incidence of 0.9 episodes per 10,000 patients per month.

Table 4. The relationship of the length of gold therapy to the frequency and incidence of certain types of toxicity

Period of treatment, months	Number of patients or courses†	Total treatment months	Rash			Mouth ulcer			Proteinuria		
			Number of episodes	Frequency	Incidence	Number of episodes	Frequency	Incidence	Number of episodes‡	Frequency	Incidence
0–3	100	274	27	0.098	9.8	11	0.040	4.0	5(2)	0.018	1.8
3–6	77	450	12	0.027	3.4	4	0.009	1.1	4(2)	0.009	1.1
6–12	60	694	8	0.012	1.9	5	0.007	1.2	3(1)	0.004	0.7
12–24	45	937	9	0.009	2.1	2	0.002	0.4	3(0)	0.003	0.3
24–36	22	745	1	0.001	0.6	1	0.001	0.6	1(0)	0.001	0.1
36–48	13	573	1			0			0		
48–60	6	342	1			0			0		
60–72	2	133	0			0			0		

* Frequency is defined as episodes of toxicity per treatment month during a given period of gold therapy. Incidence is defined as episodes of toxicity per 10,000 patients per treatment month during a given period of gold therapy.

† Ninety-four patients received 100 courses. Incidences of toxicity are calculated on the assumption that 100 patients entered the study.

‡ Numbers in parentheses signify episodes of nephrotic syndrome (proteinuria greater than 3 gm/24 hours).

The incidence of proteinuria also appeared to decrease over progressive time intervals of treatment and was uncommon after 12 months. There were 5 episodes of nephrotic syndrome all occurring within the first 12 months of treatment.

Low Platelets: (Table 5).

Episodes of low platelets did not follow a pattern similar to the incidence of rash, mouth ulcers, and proteinuria with respect to progressive time intervals of gold therapy. Thrombocytopenia as defined in Patients and Methods, occurred when platelet counts were less than 150,000 per mm^3. The average duration of therapy for seven patients who developed thrombocytopenia was 9.7 months (2.5-31.1 month range), and the average dosage was 1,157 mg. Three patients developed platelet counts between 150,000 and 200,000 per mm^3. Their average duration of therapy was 14 months (6-24) and their average dosage was 1,450 mg.

Two patients had severe thrombocytopenia of less than 40,000 and were given prednisone therapy to assist recovery of platelet count. Both patients were given gold injections outside the Gold Therapy Clinic. It is interesting to note that one of the seven patients developed his thrombocytopenia two months after gold therapy had been stopped because of skin rash and proteinuria occurring at six months (625 mg of Myochrisine). All patients except the two with severe thrombocytopenia (less than 40,000 per mm^3) had a recovery of their platelets on withdrawal of gold. In seven patients the gold was restarted after the platelet count rose above 200,000. None of them at any time had a platelet count of less than 125,000 per mm^3.

Table 5. Episodes of low platelets in 134.4 years of gold therapy

Platelet count	Number of patients	Average time low platelets recorded, months	Average dosage when low platelets occurred
$<150{,}000/mm^3$	7*	9.7	1,157 mg
$150{,}000–200{,}000/mm^3$	3	14.0	1,450 mg

* Two patients had severe thrombocytopenia of $<40{,}000/mm^3$; the remaining 5 had counts between $125{,}000–150{,}000/mm^3$ (see Discussion).

Low white blood cell count (WBC)

One patient developed a fall in WBC from 5,000/mm to 2,000/mm^3 after 85 mg of Myochrisine. There was no evidence of Felty's syndrome, and bone marrow appearance was consistent with gold induced marrow toxicity (129).

Miscellaneous toxicity.

There were two episodes of jaundice. One female patient developed cholestatic jaundice after 85 mg of Myochrisine and a male patient developed a cholestatic jaundice after 75 mg of Myochrisine. Liver biopsy in the female patient was consistent with intrahepatic cholestasis. The remission which developed within two weeks of the appearance of jaundice has continued. The male patient's maintenance therapy was being given outside the Gold Therapy Clinic. A liver biopsy was not performed and there were no radiologic investigations. His jaundice resolved after six weeks and gold therapy was restarted 12 weeks later. There has been no recurrence of jaundice and the patient was recorded as better by the criteria outlined in Patients and Methods after 2 years of therapy.

Three patients developed immediate hypersensitivity-like reactions to the Myochrisine therapy. These constituted fever, sweating, headache, and tachycardia. Two patients discontinued the drug after the test dose and one patient was allowed to continue therapy with no further reaction occurring after 135 mg of Myochrisine.

DISCUSSION:

The well controlled British study (108, 109) on the efficacy of treatment with gold sodium thiomalate was carried out for periods of up to 20 weeks, and although the subsequent Co-operating Clinics Committee study (110) had a "phase 2" component for a longer period of time, the numbers of patients that entered that phase were too small for meaningful comment (110). However, subsequent studies have suggested that long term therapy with gold sodium thiomalate is efficacious (130, 131) and there are definite indications that long term treatment with gold sodium thiomalate decreases the rate of progression of erosions (112, 114). Nevertheless, previous studies did not systematically examine the incidence of toxicity with prolonged administration of gold, even though some authors reported that long term therapy was usually well tolerated (125, 126). Until now it has therefore not been possible to make a rational judgement as to the precise risk (and therefore advisability) of continuing treatment with gold sodium thiomalate indefinitely in patients who have responded and who have not developed toxicity.

It will be observed from Table 1 that the clinical response during the first two time periods studied is similar to other studies published (108-110) over similar time periods (i.e. up to 6 months). As suggested by Fraser (103) in his series, there is quite likely an important placebo effect (109, 110, 132) included in the responses of patients (who were seen more frequently than before gold treatment was started), although an accurate estimate of such an effect

is currently not feasible because it would involve withholding potentially remission inducing agents such as the gold complexes, D-penicillamine and chloroquine for prolonged periods of time. It is of interest to note that for progressively longer intervals of time, the "remission" group has increased proportionately to the "better" group (e.g. compare 6-12 and 12-24 months interval Table 1). This is likely explained in part by the policy of the study to continue gold therapy in patients who went into remission before 12 months of gold therapy for past one year, and in part by additional remissions later during treatment. However, also contributing to the decreased proportion of patients doing "better" is an apparent increase in the proportion doing "worse" between 1 and 2 years. After 2 years the number of "remissions" on gold dropped as some patients reverted to an active disease status, and in others, gold was discontinued; the latter patients do not appear in Table 1.

The question of what happens to patients in remission whose gold was discontinued was also explored. It is apparent that while some patients relapsed and others have stayed in remission for prolonged periods of time, the patients who relapsed received on the average, less maintenance gold over a more prolonged period of time. However, these differences in maintenance therapy are not statistically significant for these small subgroups, so that no definite statement can be made about the length of maintenance therapy or frequency of injection in patients who have gone into remission.

Although the total episodes of toxicity as causes of withdrawal (Table 3) are of interest in giving an overview of the types of toxic reactions that are seen in populations treated with injectable gold therapy, they are of little predictive value for patients who continue on the drug. Studies in both the more recent and the older literature (103, 108-110) report toxicity in a fashion similar to Table 3, or as a percentage of the whole population studied during the total period of observation. In view of the lack of predictive value of toxicity reported as in Table 3 certain adverse effects (partially because they constitute frequent occurrences) were subjected to a more rigorous analysis of incidence during sequential time intervals of prolonged therapy with gold sodium thiomalate, as is shown in Table 4. It was deemed useful to calculate incidence data on the basis of 10,000 patients (Table 4) because the numbers derived can be conveniently related to expected toxicity in large populations. The data have also been calculated as a frequency of occurrence (episodes per unit time) since this serves a useful way to express toxicity when one is dealing with relatively small numbers of patients receiving gold for various periods of time (e.g. Gold Therapy Clinics). This approach also has a certain predictive value. For example, it is apparent from Table 4 that there is a progressive decrease in the frequency of occurrence and the incidence of rash, mouth ulcer, and proteinuria during progressive time intervals of injectable gold therapy. This decrease in frequency and incidence is not likely to be due to chance because there is a steady reduction in the frequency

and incidence in all three forms of toxicity. Furthermore, in at least the first five time periods of treatment (0-36 months inclusive) each time period includes a sizeable part of the study population and of treatment months (Table 4).

One explanation for this reduction in the incidence of certain toxicities might be that the authors progressively selected patients who are biologically resistant to these forms of toxicity so that as the duration of gold treatment progresses in time, the proportion of the "resistant" population also progressively increases, since patients who are prone to develop toxicity gradually drop out. However, analysis of episodes of rash occurring in patients who have received gold therapy for prolonged periods of time suggests an alternative explanation. If one examines the group of patients who continued on with gold therapy, for the time interval of two to five years, one finds that the 22 patients who entered that group had experienced 12 episodes of rash during their total period of observation, 11 of which had occurred before two years of treatment; most of these episodes occurred in the 3-6 months period. This gives an overall incidence of 3.5 based on the total period of treatment months for this group of 0-5 years, which is considerably higher than a calculated incidence of 0.2 for rashes occurring during the 2-5 year interval for the group. It can therefore be argued that there is a group of patients who tend to develop rashes early (within 6 months), but if gold treatment continues, they become "resistant" to the development of

further rashes. The possibility that the prolonged gold treatment itself may confer such resistance represents an interesting speculation.

It is evident from Table 5 that the pattern of low platelets correlated to the time of observation during gold treatment is different than that of mucocutaneous and renal toxicity. In fact, no pattern of time of occurrence for low platelets could be identified in this series of patients, as is indicated by the wide range of times when low platelets were observed (see Results). It would therefore appear that the duration of injectable gold therapy bears no correlation to the incidence of thrombocytopenia; thus the useful approach to analysis of data as discussed for the other forms of toxicity in Table 4 has no predictive value for thrombocytopenia. This conclusion is in agreement with previous impressions with respect to the occurrence of gold-associated thrombocytopenia (133, 134). Throughout the study clinically significant thrombocytopenia (or platelet counts less than 125,000/mm^3) were not seen in patients who were followed entirely in the Gold Therapy Clinic (73% of all patients in the study), where the general policy had been to discontinue gold therapy at least temporarily if the platelets dropped below 200,000/mm^3, and to evaluate the situation even if a decreasing trend was noted to levels above 200,000/mm^3 (in the absence of pre-existing thrombocytosis). The validity of this practice, of course, depends on the reliability of the platelet counting method and the method of handling the blood (see Patients

and Methods). Most laboratories specify a value of 150,000/mm^3 as the lower level of normal for their platelet count. A physician monitoring injectable gold therapy, however, should observe a platelet count of less than 200,000/mm^3 as an indication to withhold gold therapy. A falling platelet count even within the normal range, may be equally ominous. Thus a sudden change in a weekly platelet count which has been steady at 400,000/mm^3 to 210,000/mm^3 behoves the physician to withhold gold therapy until a repeat platelet count confirms a stable value above 200,000/mm^3 on at least two occasions one week apart. When a fall in platelet count results in a value which is persistently less than 200,000/mm^3, extreme caution is advised and, where available, blood should be tested for the presence of platelet antibodies (135, 136). Kelton and his colleagues (135) at McMaster University have shown a 100% correlation between the presence of IgG antibodies on the surface of platelets and gold-induced thrombocytopenia. It is not known whether these platelet antibodies are present in the much rarer thrombocytopenia due to marrow suppression. Thrombocytopenia due to gold therapy has been stated as occurring precipitously with no apparent warning. Close observation of changes in platelet count even within the normal range, should result in early identification of some patients who may potentially develop sudden thrombocytopenia. It is of interest, however, that the two cases of severe thrombocytopenia with platelet counts less than 40,000/mm^3 (Table 5) and also the one case of neutropenia, were observed among the 26 patients

whose gold therapy and follow-up were primarily monitored by their family physicians, and who were seen in the Gold Therapy Clinic at less frequent intervals. Thus, while the risk of thrombocytopenia seems to be present at any period of treatment, the risk of clinically important thrombocytopenia is probably significantly decreased by very careful follow-up, preferably at an organised gold therapy clinic. The development of thrombocytopenia due to gold therapy should be recognised as an absolute contraindication to further gold therapy. Other forms of toxicity were observed at too low a frequency to attempt to relate them to duration of injectable gold therapy.

It would therefore appear that patients who have shown significant clinical improvement (or have gone into remission and have tolerated well, six months of gold treatment) should be advised that, depending on their general disease activity and response, they may continue injectable gold therefore for at least three years with an increasing margin of safety for mucocutaneous and renal toxicity. However, close monitoring of haematological parameters remains essential throughout prolonged injectable gold therapy (127).

A detailed account of administration of gold therapy and techniques of monitoring efficacy and toxicity have not been given in this thesis as this information is available in all major rheumatology textbooks. In 1981, I was invited to write a review on the use of gold therapy in the management of rheumatoid arthritis for

the practising physician (127). This review was published in Modern Medicine of Canada in November 1981, and outlines suggested methods of monitoring efficacy and toxicity as well as reviewing various aspects of the individual toxicities and their management.

SUMMARY.

The frequency and incidence of certain forms of toxicity in patients receiving long term gold therapy for rheumatoid arthritis have been reliably calculated for defined, sequential time periods. Ninety-four patients who received 100 courses of gold therapy for a total treatment period of 134.4 patient-years were followed at one gold therapy clinic. For the period of 0-3 months, the incidence of rash, mouth ulcer, and proteinuria were 9.8, 4.0, and 1.8 episodes per 10,000 patients/month, respectively. However, the incidence of these forms of toxicity decreased progressively in subsequent time periods of continued gold therapy. No similar decrease was noted in the incidence of thrombocytopenia, but it would appear that the methodology used in monitoring, significantly affects the incidence of clinically important thrombocytopenia. No predictive correlates could be determined for patients who had gone into sustained remission. However, the data strongly suggest that patients who improve within six months may continue gold therapy for at least up to three years with an increasing margin of safety for mucocutaneous and renal toxicity. No patient should be treated with gold therapy without a strict monitoring system being observed (127) especially for haematological toxicity.

CHAPTER IV

GOLD THERAPY IN THE ELDERLY RHEUMATOID PATIENT

- INTRODUCTION
- PATIENTS AND METHODS
- RESULTS
- DISCUSSION
- SUMMARY

"Let observation with extensive view
Survey mankind, from China to Peru."

Samuel Johnson (1709-1784)

INTRODUCTION

In view of the value of injectable gold therapy in the treatment of rheumatoid arthritis, the next clinical study which I investigated was whether the efficacy and toxicity of gold sodium thiomalate was equally applied throughout the adult population, in particular in the elderly. This study was undertaken using data collected from my years at Queen's University, Kingston, Ontario already referred to in Chapter III, and data collected since starting my appointment as a faculty member at McMaster University in Hamilton, Ontario in March 1980, with Professor W. Watson Buchanan, Professor and Regional Co-ordinator of Rheumatology.

At the present time, the approximate percentage of the Canadian population over the age of 65 is 9%. By the year 2001 the projected estimate by Statistics Canada is that this age group will comprise 12% of 28 million population (137). Similarly figures available for the United States estimate that the population over age 60 years at the present time is 15% of 231 million and will be 14% of 287 million people by the year 2000 (138) If the assumed prevalence of rheumatoid arthritis remains at 1%, there will be 33,600 Canadians over 65 years and 401,800 Americans over 60 years with rheumatoid arthritis. Similar trends are evident in most Western nations.

In the past 20 years gold and penicillamine therapy have received extensive coverage in the rheumatological literature with respect to their value in the treatment of rheumatoid disease.

However, little information is available as a guide to the possible efficacy and toxicity patterns in the elderly rheumatoid patient.

It was originally stated that D-penicillamine therapy resulted in increased toxicity (139) and a decreased efficacy in the elderly compared to the young rheumatoid patient. However, two subsequent studies from our unit failed to confirm these findings (140, 141).

In 1975 Billings et al reported a prospective descriptive analysis of 22 patients receiving injectable gold therapy. The authors stated that the patients with no improvement clinically had a higher mean age (53 years) compared to those with moderate improvement (46 years) and/or major improvement as assessed by their functional indices. These differences, however, were not statistically significant but the authors stated in their "Discussion" that advancing age appeared to be associated with a less favourable outcome (142). Debosset and Bitter reported in abstract form a prospective, descriptive analysis of 24 patients receiving injectable gold therapy for rheumatoid arthritis. Although no basic data is illustrated in the abstract, the authors stated that remission and drug tolerance were less in the 45-65 years age group, and thus questioned the indication for the use of gold in the elderly (143). Thus no good evidence exists at present as to the efficacy or toxicity of injectable gold compounds used in the elderly. In view of our experience with D-penicillamine in the elderly we therefore report a comparative study of the efficacy and toxicity patterns of elderly and young patients receiving injectable gold therapy over prolonged periods of time.

PATIENTS AND METHODS:

One hundred and forty-one patients with classical or definite rheumatoid disease (111) were followed prospectively between April 1971 and April 1982 in order to monitor their toxicity pattern to gold sodium thiomalate therapy (Myochrysine$_{TM}$, Rhone-Poulenc, Quebec). For the purpose of this study, patients were divided into two cohorts, an elderly group of 60 years and greater and a younger group less than 60 years of age.

PATIENTS - Young Group:

In the young group there were 28 males and 73 females with a mean age of 44 (range 10-59) years. All patients were 16 years or greater except 2 females aged 10 years. Both girls were seropositive and since this age group with classical or definite rheumatoid disease (111) respond to gold in a similar manner to the adult population (97), they were included in the analysis. The median functional class was 2, and the duration of disease prior to therapy was 4.4 years. The mean duration of therapy was 15.6 months. The mean total gold sodium thiomalate dosage received was 1392 mg per patient (Table 6).

In order to determine efficacy and toxicity within and between groups, the study population was sub-divided into 4 arbitrary age groups, A, B, C and D. (Table 7)

Group A comprised 14 females and 2 males between 10 and 29 years inclusive with a mean age of 22 yrs ± 1.5 SEM. The duration

TABLE 6

PATIENTS TREATED WITH GOLD THERAPY

	YOUNG	ELDERLY	t	p
Male	101 (28)	40 (17)	–	–
Female	101 (73)	40 (23)	–	–
$\bar{X}$ Age (years)	44 (10-59)	67 (60-83)	–	–
$\bar{X}$ Duration of Therapy ±SEM (months)	15.6 ± 1.4	21 ± 3.7	1.56	>0.05
$\bar{X}$ Duration of Disease Prior to Therapy ±SEM (years)	4.4 ± 0.4	5.7 ± 0.9	1.64	>0.05
$\bar{X}$ Gold Dosage ±SEM (mg)	1392 ± 100	1861 ± 243	2.06	<0.05 >0.02

$\bar{X}$ - mean value
SEM - Standard Error of the Mean
mg - milligrams

Analysis of data by Students t test

TABLE 7

PATIENTS TREATED WITH GOLD THERAPY
(Subdivided into Groups A,B,C and D)

Group	A	B	C	D
N	16	23	62	40
Age Group (years)	<30	30-44	45-59	>60
$\bar{X}$ Age ± SEM (years)	22 ± 1.5	36.7 ± 1.9	53 ± 0.6	67 ± 0.9
$\bar{X}$ Duration of Therapy ± SEM (months)	15.9 ± 3.5	17 ± 3.6	15 ± 1.7	21 ± 3.7
$\bar{X}$ Duration of Disease Prior to Therapy ± SEM (years)	3.9 ± 0.6	3.8 ± 0.6	4.7 ± 0.5	5.7 ± 0.9
$\bar{X}$ Total Gold Dosage ± SEM (mg)	1254 ± 187	1473 ± 234	1459 ± 127	1861 ± 243

N – Number of Patients in each group

$\bar{X}$ – mean duration

SEM – Standard Error of the Mean

of disease prior to therapy was 3.9 years ± 0.6 SEM. Mean duration of therapy was 15.9 months ± 3.5 SEM. Total gold compound received was 1254 mg ± 187 SEM.

Group B comprised 17 females and 6 males in the age group 30-44 years inclusive. The mean age was 36.7 years ± 1.9 SEM. The mean duration of disease prior to therapy was 3.8 years ± 0.6 SEM. The mean duration of therapy was 17 months ± 3.6 SEM. The total gold compound received was 1473 mg ± 234 SEM.

Group C comprised 42 females and 20 males in the age group 45-59 years inclusive. The mean age was 53 years ± 0.6 SEM. The mean duration of disease prior to therapy was 4.7 years ± 0.5 SEM. The mean duration of therapy was 15 months ± 1.7 SEM. The mean total gold compound received was 1459 ± 127 SEM.

PATIENTS - Elderly Group:

The elderly group was designated as group D. In the elderly group there were 17 males and 23 females with a mean age of 67 years ± 0.9 SEM. The median A.R.A. functional class was 2, and the duration of therapy was 21 months. The mean total gold sodium thiomalate administered was 1861 mg per patient. (Table 6 and Table 7)

There was no significant difference among the groups for duration of disease prior to therapy (A versus B $t = 0.11$, $p > 0.05$; A versus C $t = 1.02$, $p > 0.05$; A versus D $t = 1.66$, $p > 0.05$; B versus C $t = 1.41$, $p > 0.05$; B versus D $t = 1.75$, $p > 0.05$; C versus D $t = 0.98$, $p > 0.05$ all not significant). There was no significant difference among the groups for duration of therapy (A versus B $t = 0.217$,

$p > 0.05$; A versus C $t = 0.23$, $p > 0.05$; A versus D $t = 1.00$, $p > 0.05$; B versus C $t = 0.5$, $p > 0.05$; B versus D $t = 0.78$, $p > 0.05$; C versus D $t = 1.47$, $p > 0.05$). There was no significant difference among the groups in terms of mean total gold dosage (A versus B $t = 0.73$, $p > 0.05$; A versus C $t = 0.90$, $p > 0.05$; B versus C $t = 0.05$, $p > 0.05$; B versus D $t = 1.15$, $p > 0.05$; C versus D $t = 1.47$, $p > 0.05$) except for group A (< 30 years) and group D (> 60 years), where the total mean gold dosage was 1254 mg and 1861 mg respectively ($t = 1.979$, $p < 0.05$, > 0.02) (Table 7).

Thus the 4 subgroups were comparable for disease duration prior to therapy, duration of therapy and total gold dosage except group A (< 30 years) and group D (> 60 years) where the elderly group had received significantly more gold compound (Table 7).

METHODS:

All patients with inflammatory rheumatoid disease not responsive to non-steroidal anti-inflammatory drugs were considered eligible to be started on gold sodium thiomalate. A course of treatment was defined as gold sodium thiomalate therapy for 1 month or greater. The drug was given as a 10 mg test dose intramuscularly followed in 1 week by a 25 mg test dose, and then 50 mg per week for 20 weeks and subsequently at 2-4 week intervals for an indefinite period.

Immunosuppressive drugs, chloroquine and D-penicillamine, were not used during gold sodium thiomalate therapy. Equivalent

number of patients (< 10%) in each group were receiving prednisone at less than 10 mg/day.

Clinical response was recorded by rheumatologists at a special gold therapy clinic on a prospective basis using the subjective and objective assessment originally devised by Anastassiades (124). The subjective assessment was based on the patient's answer to the question, "How do you feel now, relative to before starting gold sodium thiomalate treatment, - better, same or worse?" The objective assessment was based on the active joint count. A joint was considered active if it exhibited tenderness to palpation, tenderness on range of motion, effusion, increased temperature or soft tissue swelling. If there was a decrease in joint count of 5 joints or more, the patient was assessed as "better"; if there was an increase in joint count of 5 joints or more the patient was considered "worse". If the subjective assessment differed from the objective analysis, the latter was taken as the result recorded in the study. The response to therapy was recorded at sequential time periods of 3, 6, 12, 24 and 36 months. Remission was classified as no evidence of active joints and no evidence of the extra articular manifestations of rheumatoid arthritis.

Drug toxicity was monitored by a physician at each patient visit. Patients were questioned directly regarding pruritis, rash, bruising, mouth ulcers and joint pain. Laboratory values were assessed at each visit for hemoglobin, white blood cell count, differential white blood cell count, platelet count, erythrocyte sedimentation rate and urinalysis.

For the purpose of this study a skin rash was any eruption which was considered to be related to the gold sodium thiomalate. Mouth ulcers were defined as lesions similar in appearance to aphthous ulcers occurring in the mucous membrane of the mouth. Proteinuria was recorded as the presence of 2+ on dipstick on one occasion, or 1+ on 2 consecutive specimens one week apart. Abormal urinary sediment was considered as any of the following: the presence of red cells >10 per high power field, white cells >10 per high power field, red cell casts, white cell casts or hyaline casts. Thrombocytopenia was recorded as any drop in platelet count below 150,000/cu.mm and leukopenia as any drop in white cell count below 4,000/cu.mm. A fall in polymorph count below 50% and/or a rise in monocyte count above 10% was recorded as a W.B.C. toxicity. A fall in platelet count of greater than 100,000/cu.mm and/or a fall in W.B.C. of 4,000/cu.mm but within the normal range resulted in the drug being withheld until a repeat blood count was obtained, but for the purpose of the study, these events were not recorded as toxicities.

A major toxicity was recorded as an adverse effect which was considered severe enough to require discontinuation of the drug. A minor toxicity was recorded as an adverse effect related to the drug which did notnecessitate total interruption of the course of therapy.

STATISTICAL ANALYSIS:

Statistical analysis of patient data in tables 1 and 2 were performed using the students t test,2 tailed for unpaired data. Values in tables 3, 4, 5 and 6 were calculated using the chi-sequare statistic and/or the Fisher's Exact test where appropriate. A log

Rank chi-square analysis was performed to compare the withdrawal curves of the young (less than 60 years) versus the elderly patients (greater than 60 years) for the summed events of major toxicity and therapeutic failure. An alpha level of 0.05 was chosen for judging statistical significance. The subscript for the chi-square used throughout refers to degrees of freedom.

RESULTS:

Overall Efficacy:

The overall clinical outcome for each age group, group A (< 30 years), group B (30-44 years), group C (45-59 years) and group D (> 60 years) is illustrated in Table 8. There was no significant difference among the groups at any time period examined in terms of "remission", "better", "same" or "worse" (Table 8). The total patients in A, B and C (i.e. all patients < 60 years) were compared to group D (> 60 years). At any time period examined up to 4 years, 58% or greater in the elderly group (> 60 years) and 74% or greater of the young group (< 60 years) were classified as being better or in remission. There was no significant difference in terms of "remission", "better", "same" or "worse" between the young and the elderly group at any time period (χ^2_1 = 1.200-5.874, p = 0.7393-0.1179) except for the first period (0 - < 3 months) when the young patients had significantly greater numbers in the remission and/or better category (χ^2_1 = 7.977, p = 0.0185). However, since the injectable gold compounds are slow acting compounds, this result is of questionable significance.

Reasons for Discontinuation of Therapy.

Remission.

Three patients in group B (30-44 years), 5 patients in group C (45-59 years) and 4 patients in group D (> 60 years) discontinued therapy because they were judged to be in sustained remission. These differences were not significant among the groups (Table 9). The

TABLE 8

CLINICAL OUTCOME OF GROUPS A, B, C AND D OVER SEQUENTIAL TIME PERIODS

TIME PERIOD (months)	GROUP	N	REMISSION	BETTER	SAME	WORSE
0 - <3	A	16	0	12	4	0
	B	23	0	18	5	0
	C	62	0	45	17	0
	D	40	0	23	15	2
	$\chi^2_6 = 7.031$ p=0.3180 N.S.					
3 - <6	A	15	1	8	2	4
	B	20	1	15	3	1
	C	52	4	39	8	1
	D	38	1	16	10	2
	$\chi^2_9 = 15.276$ p = 0.0636 N.S.					
6 - <12	A	13	1	8	2	2
	B	16	2	11	1	2
	C	43	6	29	7	1
	D	29	1	16	6	6
	$\chi^2_9 = 9.705$ p = 0.3749 N.S.					
12 - <24	A	9	0	8	0	1
	B	13	3	8	1	1
	C	32	6	15	3	8
	D	20	2	12	3	3
	$\chi^2_9 = 8.332$ p = 0.5011 N.S.					

TIME PERIOD (months)	GROUP	N	REMISSION	BETTER	SAME	WORSE
24 - <36	A	3	0	2	0	1
	B	6	2	4	0	0
	C	14	5	5	1	3
	D	11	3	7	1	0
	$\chi^2_9 = 7.520$ p = 0.5832 N.S.					
36 - <48	A	1	0	0	0	1
	B	3	1	2	0	0
	C	7	4	2	0	1
	D	9	3	4	1	1
	$\chi^2_9 = 8.838$ p = 0.4526					
>48	A	1	0	0	0	1
	B	1	0	1	0	0
	C	1	1	0	0	0
	D	4	3	1	0	0
	$\chi^2_9 = 10.063$ p = 0.1221 N.S.					

N = Number of patients per group in each time frame

N.S. = Not Significant

Statistics by Chi-Square Method.

mean time at which remission occurred was recorded as 27 months ± 11 SEM for group B, 31 months ± 4.9 SEM for group C and 52 months ± 4.3 SEM for group D. There was no significant difference in these times between group B and C ($t = 0.35$, $p > 0.05$) but the differences between group B and D ($t = 2.21$, $p < 0.05$, > 0.02) and group C and D ($t = 3.16$, $p < 0.01$) were significant. One female patient in group A (< 30 years) developed a sustained remission after 1 month of gold therapy following the occurrence of cholestatic jaundice.

The mean total gold compound received at the time remission was recorded was 2467 mg ± 8.9 SEM for group B, 2430 mg ± 265 SEM for group C and 2946 mg ± 1405 SEM for group D. There was no significant difference between the groups in terms of total gold compound received (B versus C $t = 0.41$, $p > 0.05$; B versus D $t = 0.29$, $p > 0.05$; C versus D $t = 0.36$, $p > 0.05$).

Default.

Two patients in group A and 4 patients in group C dropped out because of default. This did not represent a significant difference between the groups A, B, C nor D (Table 9). At the time of default, 2 patients were recorded as "worse", 1 recorded as "same" and 3 recorded as "better".

No Response.

Three patients in group A, 3 patients in group B, 9 patients in group C and 9 patients in group D dropped out because of failure to respond to the drug and were recorded as having "No Response". These differences among the groups were not significant (Table 9).

TABLE 9

NUMBER OF PATIENTS WHO STOPPED GOLD TREATMENT (In Each Age Group)

	A	B	C	D	X_3^2	p
N	16	23	62	40	–	–
Age (years)	30	30-44	45-59	60	–	–
Remission	0	3	5	4	2.25	0.5270 N.S.
Default	2	0	4	0	6.203	0.1021 N.S.
No Response	3	3	9	9	1.417	0.7015 N.S.
Toxicity	8	11	21	18	2.542	0.4676 N.S.

N - Number in each group

N.S. - Not Significant

Statistics Analysis by Chi-Square Method

Toxicity.

Eight patients in group A, 11 patients in group B, 21 patients in group C and 18 patients in group D discontinued therapy because of a major toxicity. These differences were not significant (Table 9).

In order to compare the rate of dropout from therapy for the two most significant events, withdrawal curves of the young (group A, B and C) versus the elderly (group D) were calculated for the summed events of major toxicity and therapeutic failure. As shown in Figure 46, there was no significant difference between the young and the elderly group (Log Rank $\chi^2 = 2.419$, $p > 0.1$), in terms of dropout from therapy due to toxicity and drug failure.

Overall Toxicity.

The number of episodes of toxicity, major and minor, and major alone occurring in the young patients less than 60 years (group A, B and C) compared to the number of episodes occurring in the elderly group (group D) are illustrated in Table 10. There were 76 episodes of major plus minor toxicity in 56 patients from the young groups (group A, B and C) and 36 episodes of major plus minor toxicity in 27 patients from the elderly group (group D). The number of patients with major plus minor toxicity was not significantly different between the young and the elderly. ($\chi^2_1 = 1.719$, $p > 0.05$; Fisher's Exact $p = 0.2547$). There were 49 episodes of major toxicity in 39 patients from the young groups (group A, B and C) and 20 episodes of major toxicity in 18 patients from the elderly group. The number of patients developing major toxicity was not significantly different ($\chi^2_1 = 0.485$, $p > 0.05$; Fisher's Exact $p = 0.5688$).

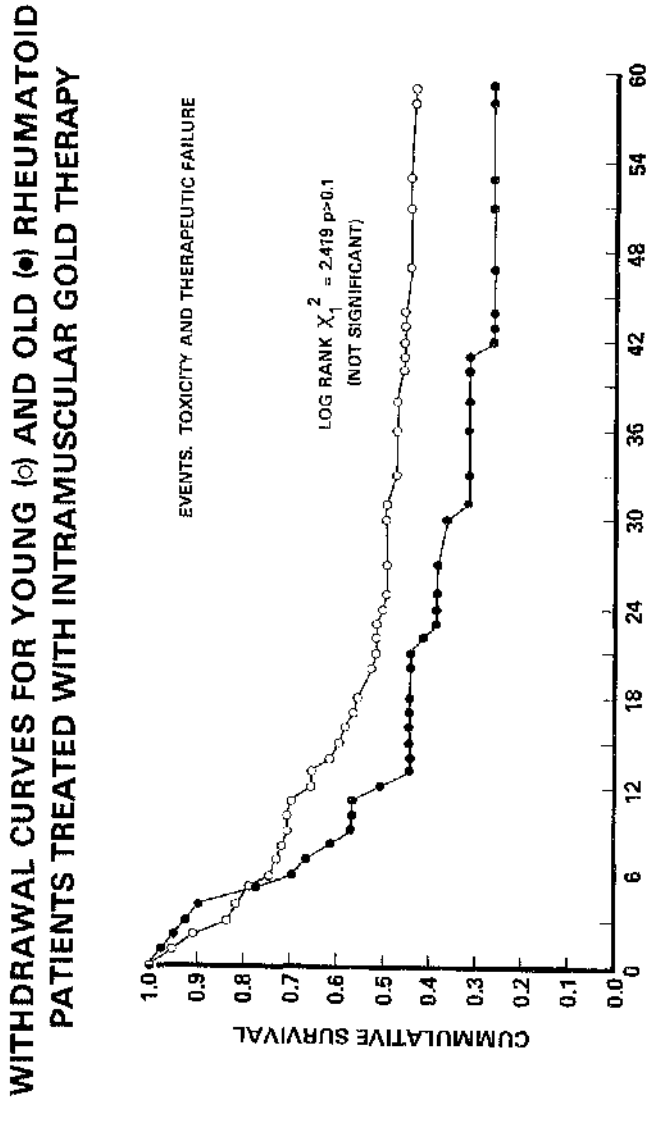


FIGURE 46: Withdrawal curves for young and old rheumatoid patients treated with intramuscular gold therapy.

Skin Rash.

There were 39 episodes of skin rash in the young group and 21 episodes of skin rash in the elderly group. There was no significant difference between these groups ($\chi^2_1 = 2.260$, $p = 0.133$; Fisher's Exact $p = 0.1856$). Twenty-five patients in the young group and 13 patients in the elderly group had a serious skin rash and thus required discontinuation of therapy. These values were not significantly different between the two groups. ($\chi^2_1 = 0.874$, $p = 0.350$; Fisher's Exact $p = 0.4014$). (Table 10)

Eighteen of the 25 major episodes of rash in the young occurred before 12 months and 11 out of 13 major episodes of rash in the elderly occurred before 12 months. Thus the distribution pattern for time of occurrence of major skin rash was similar. This was also true for minor rash.

Proteinuria.

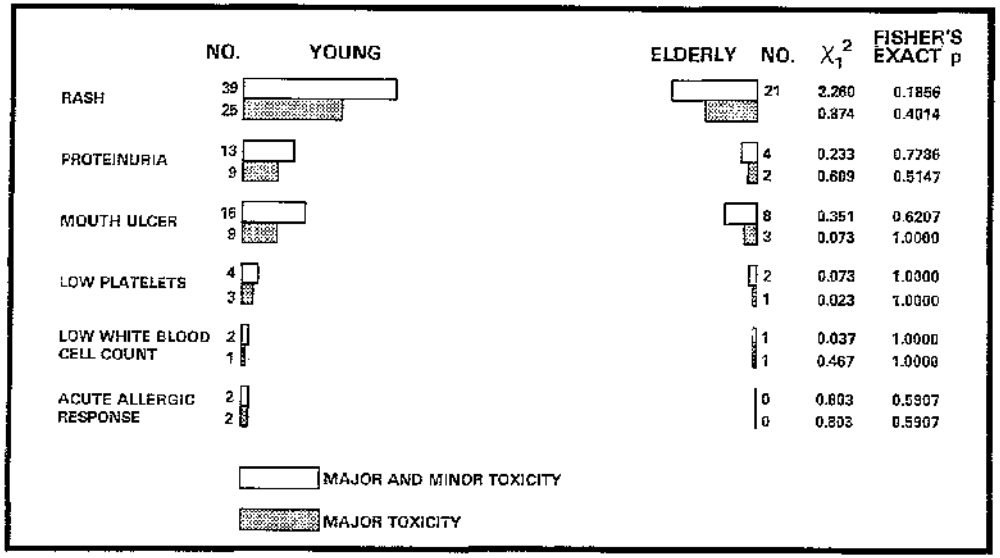
Thirteen episodes of proteinuria occurred in the young group and 4 episodes in the elderly group, but these differences were not significant ($\chi^2_1 = 0.233$, $p = 0.637$; Fisher's Exact $p = 0.7786$). Only 2 elderly patients required discontinuation from therapy due to proteinuria as opposed to 9 of the young patients, but this difference was not significant ($\chi^2_1 = 0.609$, $p = 0.5147$; Fisher's Exact $p = 0.5147$). (Table10)

Mouth Ulcer.

Eight episodes of mouth ulcer occurred in the elderly and 16 episodes of mouth ulcer occurred in the young patients. These differences were not significant ($\chi^2_1 = 0.351$, $p = 0.554$; Fisher's Exact

TABLE 10

EPISODES OF TOXICITY IN YOUNG AND ELDERLY PATIENTS TREATED WITH INJECTABLE GOLD THERAPY



YOUNG — All patients < 60 years
ELDERLY — All patients > 60 years
NO. = Number of episodes of toxicity

Statistical analysis is by X_1^2 and Fisher's Exact p.

The p value for X_1^2 is not given.

$p = 0.6207$). Three elderly patients and 9 young patients had severe mouth ulcers and were discontinued from therapy. There was no difference between the numbers in these groups ($\chi^2_1 = 0.073$, $p = 0.787$; Fisher's Exact $p = 1.0000$). (Table 10)

Low Platelets.

Two episodes of low platelet counts were recorded in the elderly group and 4 episodes of low platelets in the younger group ($\chi^2_1 = 0.073$, $p = 0.783$; Fisher's Exact $p = 1,0000$). Only 1 elderly patient and 3 young patients were recorded as having a major toxicity due to low platelets and had to be discontinued from therapy ($\chi^2_1 = 0.023$, $p = 0.879$; Fisher's Exact $p = 1.0000$). (Table 10)

Low White Blood Cell Count.

There was 1 episode of major toxicity due to low white blood cell count in either group ($\chi^2_1 = 0.467$, $p = 0.494$; Fisher's Exact $p = 1.0000$, not significant). One other patient in the young group had a low white blood cell count but this was not considered serious enough to require discontinuation of therapy. (Table 10)

Acute Allergic Response.

Acute allergic response characterized by onset of generalised aching, fever, malaise was noted in two of the young group. These reactions were severe enough to require discontinuation of the drug. None of the elderly group experienced an allergic response, however, these differences were not significantly different (major and minor, $\chi^2_1 = 0.803$, $p = 0.370$; Fisher's Exact $p = 0.5907$) (Table 10)

Major Toxic Events in Each Group.

In order to exclude differences between the age groups the major adverse effects occurring in group A (< 30 years), group B (30 - < 44 years), group C (45 - < 60 years) and group D (> 60 years) were analysed and are illustrated in Table 11.

Skin Rash

There were 5 episodes of major skin rash in group A, 7 episodes in group B, 13 episodes in group C and 13 in group D. There was no significant difference between the groups ($\chi^2_3 = 2.04$, $p = 0.56$, not significant). (Table 11)

Proteinuria.

There were 3 episodes of major proteinuria in Group A, 2 in group B, 4 in group C and 2 in group D. These differences were not significant ($\chi^2_3 = 3.27$, $p = 0.3496$) (Table 11). The 4 patients in group C and the 2 patients in group D all had nephrotic syndrome (> 3 grams of proteinuria per 24 hours). All patients with nephrotic syndrome were 52 years of age or greater. All cases resolved within 12 months.

Mouth Ulcer.

Four episodes of major mouth ulcer occurred in group B, 5 episodes of major mouth ulcer occurred in group C and 3 episodes of serious mouth ulcer occurred in group D. There was no significant difference among the groups ($\chi^2_3 = 3.88$, $p = 0.274$) (Table 11).

Low Platelets.

Three episodes of major low platelet count occurred in group C and 1 episode occurred in group D. These differences were not significant ($\chi^2_3 = 2.04$, $p = 0.561$) (Table 11). All patients with serious low platelet count were 47 years of age or greater.

Low White Blood Cell Count.

One serious episode of low white blood cell count occurred in a 57 year old male in group C and 1 serious episode of low white blood cell count occurred in a 65 year old male in group D. There was no significant difference between the two groups ($\chi^2_3 = 0.91$, $p = 0.8224$) (Table 11).

Acute Allergic Response.

One episode of serious acute allergic response occurred in a 30 year old female in group B following her second test dose of 25 mg of gold sodium thiomalate. The reaction was characterized by the onset of urticaria, within 24 hours of her injection. One episode of serious acute allergic response occurred in a 57 year old female in group C after her first 50 mg of gold sodium thiomalate (i.e. she had received 10 mg and 25 mg respectively in the two previous weeks). The reaction to the drug was characterised by urticaria, itching and fever within 24 hours of receiving the injection. There was no significant difference in the numbers of acute allergic responses between the groups ($\chi^2_3 = 2.21$, $p = 0.5253$) (Table 11).

TABLE 11

EPISODES OF MAJOR TOXICITY DUE TO GOLD THERAPY (In Patients in Group A,B,C and D)

Group	A	B	C	D	X^2_3	p
Age (years)	<30	30-44	45-59	>60	–	–
N	16	23	62	40	–	–
Rash	5	7	13	13	2.04	0.56 N.S.
Proteinuria	3	2	4	2	3.27	0.3496 N.S.
Mouth Ulcer	0	4	5	3	3.88	0.274 N.S.
Low Platelets	0	0	3	1	2.04	0.561 N.S.
Low White Blood Cell Count	0	0	1	1	0.91	0.8224 N.S.
Acute Allergic Response	0	1	1	0	2.21	0.5253 N.S.

N – Number of Patients in each age group

N.S. – Not Significant

Statistical Analysis was by Chi-Square Method

DISCUSSION:

The potential for increased drug toxicity is well recognised in the elderly population (144, 145, 146) and although attributed to defective metabolic handling (146), patient and physician mal-compliance contribute to the potential development of adverse effects. The use of gold therapy in the elderly rheumatoid patient has received little attention in the rheumatological literature and the few statements to date, state that injectable gold is less efficacious (142) and more toxic (143) in the elderly rheumatoid patient than in the young group. This prospective analysis comparing an elderly to a young population did not confirm these statements but suggest to the contrary that the elderly rheumatoid should not be denied the potential value of injectable gold therapy. This study demonstrates that the elderly are just as likely to benefit from gold therapy as the younger population.

Several authors have reported that the prognosis of rheumatoid arthritis in the elderly is either just as favourable (147, 148, 149) or better (150, 151) than in the younger age groups. A sudden onset severe form of rheumatoid arthritis is found in approximately 20-30% of the elderly (149, 152, 153, 154) but it is usually self limiting, can be readily controlled during the acute phase and has a good prognosis (154). However, some authors do hold the view that rheumatoid disease does follow a more severe course in the elderly than in the young (155, 156, 157). This controversy will

only be resolved by a prospective controlled comparative analysis of functional outcome to assess disease severity in an elderly population compared to a younger population who develop rheumatoid arthritis.

In order to determine efficacy or toxicity within or between a certain age group, the data for clinical outcome and toxicity were analysed for four arbitrary age groups: group A less than 30 years; group B 30-44 years; group C 45-59 years and group D the elderly population greater than 60 years. No differences in terms of efficacy were found among the groups. Remission (see Methods) was just as common in the elderly as in the young groups. It is of interest that the mean time of remission was 27 months, 31 months and 52 months in groups B, C and D respectively, and that the mean gold dosage received was greater than 2400 mg of gold compound. This suggests that the 20 week course of gold therapy or the arbitrary 1 gram of compound administered (158) may be insufficient to afford maximum benefit to the patients, especially the elderly.

Similarly, adverse reactions were not more common in the elderly group compared to the young groups. Analysis of the individual age groups - A, B, C and D did not uncover any hidden susceptibility to toxicity within a group. It should be noted, however, that low platelet count and low white blood cell count, although infrequent, occurred in patients greater than 47 years and 57 years

respectively and the six patients with nephrotic syndrome were older than 52 years of age.

Therefore, although haematological toxicity did not occur with increased frequency statistically in this elderly population (group D), strict criteria for recording haematological adverse effects should be used. The guidelines used in this study of a low platelet count less than 150,000/mm^3 and a low white blood cell count of less than 4000/mm^3 as well as the differential white blood cell count (see Methods) are strict by most standards, but are designed to identify haematological toxicity at its earliest stages.

Caird et al found that the range of leukocyte count in the normal elderly person was 500 - 3,500/mm^3 (159), but indeed I recorded only one major reduction in W.B.C. below 4,000/mm^3 in this elderly population. However, since the prognosis for aplastic anaemia is especially poor in the elderly (160) I strongly recommend a strict monitoring system for all the haematological indices in any patient receiving gold therapy (127).

Skin rash was not increased in this elderly group although skin rash has been reported to be increased in the "normal" elderly population in as many as 50-95% of people (161, 162, 163) and was also increased in the elderly population reported by me who received D-penicillamine therapy (141, 142).

It has been suggested that the elderly are at risk of developing the nitritoid reaction (164). The authors base their conviction on the outcome of three cases (165, 166) which refer to nitritoid reactions occurring in two patients over 60 years of age and recommend the use of gold thioglucose as an alternative. It should be noted, however, that the third patient reported was 49 years of age. In my own study none of the elderly patients developed an allergic-like response of any kind. Thus my study did not uncover any predisposed risk of nitritoid reaction in the elderly group. The predisposed risk, if it is genuine, most likely exists with patients of all ages who have serious cardiovascular disease.

The results reported in this chapter indicate that gold sodium thiomalate is equally efficacious in the elderly rheumatoid patient as in the young rheumatoid patient, and that the incidence of adverse effects does not appear to be increased, although serious toxicity such as nephrotic syndrome and haematological toxicity occurred in patients 47 years of age or older. There are no apparent differences in clinical outcome nor in toxicity due to gold therapy among the age groups: less than 30 years; 30-44 years; 45-59 years and greater than 60 years. Thus gold sodium thiomalate therapy can be administered to the elderly population provided the same strict guidelines for monitoring toxicity is applied to the young population are enforced (127).

SUMMARY:

Forty elderly patients (> 60 years) and 101 young patients (< 60 years) with rheumatoid arthritis receiving injectable gold therapy were followed prospectively between April, 1971 and April, 1982. In the young group, there were 28 males and 73 females with a mean age of 44 years (range 10-59). The median functional class was 2, the duration of disease prior to therapy was 4.4 years, and the duration of therapy with injectable gold was 15.6 months. The mean total gold compound received was 1392 mg. The elderly group comprised 17 males and 23 females with a mean age of 67 years (range 60-83). The median functional class was 2, the duration of disease prior to therapy was 5.7 years and the mean duration of therapy was 21 months. The mean total gold compound administered was 1861 mg. As well as age, the only significant difference between the two groups was the increased gold compound received by the elderly. In order to determine efficacy and toxicity within and between certain groups, the 141 patients were divided into four arbitrary age groups - group A (< 30 years), group B (30-44 years), group C (45-59 years) and group D (> 60 years).

The elderly responded to the gold therapy just as well as the young patients at any time frame examined after 3 months of therapy. There was no difference in terms of clinical benefit between groups A, B, C and D.

Nine patients in the elderly group (> 60 years) and 15 patients in the young group (< 60 years) had therapy discontinued

because of no response. This difference was not significant between the groups A, B, C and D ($\chi^2_3 = 1.417$, $p = 0.7015$).

There was no difference in outcome of individual toxicity between the elderly and the young groups, and there was no difference in frequency of toxicity between the age groups A, B, C and D. Serious haematologic toxicity occurred only in patients over 47 years of age, and nephrotic syndrome occurred only in patients older than 52 years of age.

In this study, clinical benefit from gold therapy was just as good in the elderly as in the young patients, and the toxicity rate and drug failure rate was not significantly different.

CHAPTER V

THE EFFECTS OF PRIOR GOLD THERAPY ON SUBSEQUENT TREATMENT WITH D-PENICILLAMINE IN RHEUMATOID ARTHRITIS

- INTRODUCTION
- PATIENTS AND METHODS
- RESULTS
- DISCUSSION
- SUMMARY

"An' forward, tho' I canna see,
I guess an' fear!

Robert Burns (1759-1796)

INTRODUCTION:

The thiol compound penicillamine currently used in the treatment of rheumatoid disease (167, 168, 169) was first used in medicine because of its properties as a chelating agent. J.M. Walshe (170, 171) first published the beneficial effects of this compound in Wilson's disease in 1956 based on the hypothesis that the in vitro chelating properties of the penicillamine for copper was the mechanism of action of the new drug. New knowledge of the many varied actions of penicillamine suggest that possibly other mechanisms are also active in its beneficial effects in Wilson's disease. These actions have been reviewed in an article by me and my colleagues but their further discussion is outwith the scope of this thesis (141). Many textbooks suggest that if patients receiving gold compounds develop toxicity, a chelating agent such as D-penicillamine should be used (172-175) with the clear implication, often not stated, that the D-penicillamine may act as a chelating agent towards gold. Along with my colleagues Professor C.J.L. Lock and Dr. Helen E. Howard-Lock of the Department of Materials Research and Professor W. Watson Buchanan, Professor of Medicine at McMaster University, I addressed the question - does prior therapy with gold compounds influence the adverse effects of D-penicillamine in rheumatoid arthritis?

The treatment of rheumatoid disease with gold compounds is not effective in all patients (108-110) and the rate of adverse reaction is approximately 30%. Therefore, an increasing number

of patients with rheumatoid disease are treated with D-penicillamine subsequent to gold therapy. Despite their widely dissimilar chemical structures, the thiol compounds gold sodium thiomalate and D-penicillamine have remarkably similar clinical effects. Improved patient well-being and a reduction in erythrocyte sedimentation rate occur after approximately three months of treatment (108-110, 167) with either drug, and there is a marked similarity in incidence and type of adverse effects (108-110, 124, 167, 168).

In view of this similarity of gold sodium thiomalate to D-penicillamine, several authors have commented on the outcome of rheumatoid patients who were treated with D-penicillamine and who previously had received gold therapy (168, 176-183). It is not surprising that these authors have different opinions in regard to the toxicity rate of patients who received D-penicillamine after gold therapy, because there havebeen no standard criteria by which to judge toxicity in the management of rheumatoid disease. The grading and assessment of adverse effects of gold compounds and D-penicillamine have been merely a collective series of personal observations; there have been no common standard definitions among the different groups (108-110, 124, 167-169, 176-183). In parallel controversy in the literature, researchers have asked whether penicillamine effectively binds to gold and could indeed be responsible for the occurrence or recurrence of adverse effects in patients who receive D-penicillamine and who have previously had an adverse reaction to a gold compound.

The objective of my study was to determine whether there is a difference in outcome in patients who receive D-penicillamine therapy and who have had previous gold therapy. I attempted to meet these objectives by comparing: (1) overall outcome, (2) effect of gold dosage, (3) effect of time between end of gold therapy and start of D-penicillamine therapy, (4) effect of time between end of gold therapy and the onset of D-penicillamine toxicity, (5) the time of onset of toxicity to D-penicillamine in those patients having previous gold therapy compared with those having no previous gold therapy, and (6) the toxicity pattern of D-penicillamine with respect to previous gold therapy.

PATIENTS AND METHODS:

One hundred fourteen patients with definite or classic rheumatoid arthritis {diagnosed according to criteria of American Rheumatism Association (111)}were followed prospectively between January 1976 and April 1981, in order to monitor their toxicity pattern to D-penicillamine therapy. There were 34 male patients and 80 female patients; the average ages were 54 years (range 10-79 years) and 50 years (range 11-74 years), respectively. The mean functional class was 2. The duration of disease before therapy was 6 ± 3 years, and the mean duration of therapy was 11 ± 8 months. The average dosage of D-penicillamine was 490 ± 160 mg/day. All patients with inflammatory rheumatoid disease not responsive to nonsteroidal antiinflammatory drugs were considered eligible for D-penicillamine therapy. A course of D-penicillamine is defined as daily or alternate-day administration of the drug for an indefinite period of time. Patients who were taking the drug for the first time were given 250 mg/day for a minimum of 4 weeks, and increments in dosage were made at the discretion of the attending physician and were based on clinical outcome. If D-penicillamine therapy was stopped for 6 months and then started again, we defined that as a second course of the drug for that patient.

Immunosuppressive drugs, gold compounds, and chloroquine were not given during D-penicillamine therapy. A few patients were taking low-dose prednisone at less than 10 mg/day.

Fifty-three patients had previously received gold sodium thiomalate therapy. A standard course of gold sodium thiomalate was a weekly injection of 50 mg intramuscularly for 20 weeks, followed by 50 mg every two to four weeks for an indefinite period of time. The time between discontinuation of gold sodium thiomalate therapy and the start of D-penicillamine therapy ranged from 0-84 months. The physician who was managing the patient's rheumatoid disease decided whether to start D-penicillamine treatment and when to start it for each patient.

For the purpose of the study, the patients were divided into three groups. Group A consisted of 11 men and 19 women, who had a mean age of 55 ± 12 years and who had received previous gold sodium thiomalate therapy that had been discontinued because of an adverse reaction. Group B consisted of 21 women and two men who had a mean age of 51 ± 16 years and who had received prior gold sodium thiomalate therapy that was discontinued because of no response. For one patient in Group B, gold sodium thiomalate therapy was discontinued because of remission. When a relapse occurred, she chose to receive D-penicillamine because of a fear of injections. Group C consisted of 40 women and 21 men who had a mean age of 49 ± 15 years and who had never received previous gold sodium thiomalate.

The methods used to measure efficacy and toxicity in these patients have been described previously (124, 168). A major toxicity was defined as an adverse effect that was severe enough to

necessitate discontinuation of the drug. A minor toxicity was defined as an adverse effect related to the drug that did not necessitate total interruption of the course of therapy.

For the purpose of this study, a skin rash was any skin eruption considered to be related to the treatment drug. Mouth ulcers were defined as lesions similar in appearance to aphthous ulcers occurring in the mucous membrane of the mouth. Dysgeusia was the loss or alteration of taste perception, and proteinuria was the presence of 2+ on dipstick on 1 occasion or 1+ on 2 consecutive urine specimens 1 week apart. Abnormal urinary sediment was defined as any of the following: the presence of red cells > 10 per high power field, white cells > 10 per high power field, red cell casts, white cell casts, or hyaline casts. Thrombocytopenia was defined as any drop in platelet count below 150,000/mm^3. As previously reported (124,168), a fall in polymorpholeukocyte count below 50% and/or a rise in monocyte count above 10% was recorded as a white blood cell (WBC) toxicity. If there was a fall in platelet count of greater than 100,000/mm^3 and/or a fall in WBC of greater than 4,000/mm^3 but within the normal range, the drug was withheld until a repeat blood count was obtained, but, for the purpose of the study, these events were not recorded as toxicities. In one report by Kay (184), the author lends support to the strict measures we have instituted to intercept potential hematologic toxicity, by the observation that bone marrow suppression may be preceded by a gradual fall of platelets and neutrophils, even within the normal range.

RESULTS:

In Table 12, the outcomes of the 114 patients who received or are receiving D-penicillamine are shown. Fifty-three patients (group A and B) previously received gold therapy. Thirteen of group A (43%), 10 of group B (43%), and 25 of group C (41%) had no toxicity to D-penicillamine. Seventeen of group A (57%), 13 of group B (57%), and 36 of group C (59%) had a major or minor toxicity to D-penicillamine. Thirteen of group A (43%), 10 of group B (43%), and 24 of group C (39%) had a major toxicity to D-penicillamine therapy.

There was no significant difference among patients in groups A, B, or C in their eventual response to D-penicillamine with respect to toxicity ($\chi^2_1 \leq 0.08$, $p > 0.9$, not significant).

In Table 13, I have compared the total accumulated gold therapy administered in groups A and B with respect to the development of D-penicillamine toxicity. In group A, those patients with no D-penicillamine toxicity had received a mean total gold dosage of 920 mg, and those patients who developed toxicity to D-penicillamine had received a mean total gold dosage of 777 mg. A t-test for comparison of the mean values showed no statistically significant difference between these values ($t = 0.92$, $p > 0.25$, not significant). In group B, those patients with no D-penicillamine toxicity had received a mean total dose of 1,636 mg of gold, and those patients who developed D-penicillamine toxicity had

Table 12. Outcomes of 114 patients treated with D-pencillamine*

Toxicity to D-penicillamine	Group A (n = 30)†	Group B (n = 23)†	Group C (n = 61)†
None	13 (43%)	10 (43%)	25 (41%)
Major and minor	17 (57%)	13 (57%)	36 (59%)
Major	13 (43%)	10 (43%)	24 (39%)

* n = number of patients; χ_1^2 was calculated for all 2 × 2 combinations, as well as χ_2^2 for the overall table (3 × 2); in all calculations $\chi^2 \leq 0.1$, $P > 0.9$ not significant.

† Group A had toxicity to previous gold therapy; Group B had no toxicity to previous gold therapy; Group C had no previous gold therapy.

Table 13. Mean total gold dosage

Toxicity to D-penicillamine	Total gold dosage, mg*	
	Group A (n = 30)†	Group B (n = 23)†
None		
Mean	920	1,636
Range	60–2,800	490–3,700
Toxicity		
Mean	777	1,778
Range	35–1,850	150–4,800

* n = number of patients. Statistical analysis was by *t*-test. In group A, $t = 0.92$, $P = 0.25$, not significant. In group B, $t = 0.46$, $P = 0.25$, not significant.

† Group A had toxicity to previous gold therapy; Group B had no toxicity to previous gold therapy.

received a mean total dose of 1,778 mg of gold. These values were not significantly different ($t = 0.46$, $p > 0.25$). A Mann-Whitney U test (for stochastic comparison of skewed distributions) was applied to the results of Tables 14-17.

The mean time in months between discontinuation of gold therapy and the start of D-penicillamine therapy with respect to the development of D-penicillamine toxicity is shown in Table 14. In group A, the mean time between discontinuation of gold therapy and the start of D-penicillamine therapy was 25 months in those patients who did not develop D-penicillamine toxicity (subset A1). The mean time between the discontinuation of gold therapy and the start of D-penicillamine therapy, however, was 16 months in those who developed both a major and minor D-penicillamine toxicity (subset A2), and 16 months in those who developed a major D-penicillamine toxicity only (subset A3). The difference between these times approached the level of statistical significance ($U_{A1,2} = 50.5$, $0.02 < p < 0.05$; $U_{A1,3} = 36.5$, $0.02 < p < 0.05$).

In group B, there was no significant difference in time between the discontinuation of gold therapy and the start of D-penicillamine therapy in those patients who did not develop D-penicillamine toxicity (subset B1, mean = 15 months), those who developed both a major toxicity (subset B2, mean = 16 months), and those who developed only a major D-penicillamine toxicity (subset B3, mean = 16 months) ($U_{B1,2} = 62.5$, $p > 0.10$) ($U_{B1,3} = 47$, $p > 0.10$).

Table 14. Time in months between the discontinuation of gold therapy and the start of D-penicillamine therapy*

Subset	Toxicity to D-penicillamine	Group A (n = 30)†	Group B (n = 23)†
1	None		
	Mean	25	15
	Median	26	4
	Range	3–53	0–72
2	Major and minor		
	Mean	16	16
	Median	5	5
	Range	0–84	0–72
3	Major		
	Mean	16	16
	Median	5	4
	Range	0–84	0–72

* n = number of patients; Mann-Whitney U test for comparison of medians of subsets A1, 2, 3 and B1, 2, 3 was used: $U_{A1,2} = 50.5$, $0.02 < P < 0.05$; $U_{A1,3} = 36.5$, $0.02 < P < 0.05$; $U_{B1,2} = 62.5$, $P > 0.10$ not significant; $U_{B1,3} = 47$, $P > 0.10$ not significant; $U_{A1,B1} = 28$, $0.05 < P < 0.10$; $U_{A2,B2} = 105$, $P > 0.10$ not significant; $U_{A3,B3} = 60$, $P > 0.10$ not significant.

† Group A had toxicity to previous gold therapy; group B had no toxicity to previous gold therapy.

In a comparison between groups A and B, there was a significant difference between subsets A1 and A2 but not A2 and B2 or A3 and B3, in regard to the time between discontinuation of gold therapy and the start of D-penicillamine therapy, regardless of outcome on D-penicillamine ($U_{A1,B1} = 28$, $0.05 < p < 0.01$) ($U_{A2,B2} = 105$, $p > 0.10$) ($U_{A3,B3} = 60$, $p > 0.10$).

In Table 15, the mean time in months between discontinuation of gold therapy and the onset of D-penicillamine toxicity in group A (mean = 22 months) and group B (mean = 20 months) is shown. There was no significant difference between these times ($U = 104$, $p > 0.10$).

In group A, 13 patients had a major toxicity to both gold therapy and D-penicillamine (Table 16). Six of the 13 patients had the same major toxicity to both drugs (three rash, three proteinuria). The average time between discontinuation of gold therapy and the development of D-penicillamine toxicity was 17 months in those with the same toxicity and 24 months in those with different toxicities. These times were not significantly different ($U = 17$, $p > 0.60$, 2-tailed).

In Table 17, the mean time of development of toxicity to D-penicillamine is compared between group A + B and group C (i.e. patients with previous gold therapy and patients without previous gold therapy). The mean time at which D-penicillamine toxicity occurred was four months for group A + B and six months for group C. These values are not significantly different ($U = 129$, $p > 0.10$, 2-tailed).

Table 15 Time in months between discontinuation of gold therapy and the onset of D-penicillamine toxicity*

	Group A (n = 30)†	Group B (n = 23)†
Mean	22	20
Median	10	9
Range	2–85	1–73

* n = number of patients; Mann-Whitney U test was applied to compare medians. (U = 104, $P > 0.10$ not significant)
† Group A had toxicity to previous gold therapy; group B had no toxicity to previous gold therapy.

Table 16 Mean time between discontinuation of gold therapy and the onset of major D-penicillamine toxicity in 13 patients with a major reaction to both drugs*

	Patients with the same adverse reaction (n = 6)	Patients with different adverse reaction (n = 7)
Mean	17	24
Median	9	10
Range	2–42	5–85

* n = number of patients; Mann-Whitney U test was applied to compare medians (U = 17, $P > 0.60$, 2-tailed).

The toxicity patterns are also shown in Table 17 for two groups of patients, those with previous gold sodium thiomalate therapy (group A + B, n = 53) and those with no previous gold sodium thiomalate therapy (group C, n = 61). There was no significant difference in the number of episodes of individual toxicity between the two groups. (See Table 17 for χ^2_1 and p values).

Table 17 Toxicity in two groups of patients receiving D-penicillamine*

	Group A + B† (n = 53)	Group C† (n = 61)	χ^2	P	
Time of onset					
Mean	4	6			
Median	3	6			
Range	1–19	1–11			
Type of episodes					
Rash	8 (16%)	8 (13%)	0.092	0.8	NS
Proteinuria	8 (16%)	5 (8%)	1.336	0.2	NS
Thrombocytopenia	4 (8%)	3 (5%)	0.340	0.5	NS
Taste abnormality	2 (4%)	1 (2%)	0.504	0.47	NS
Mouth ulcer	1 (2%)	1 (2%)	0.010	0.92	NS
Leukopenia	0	3 (5%)	2.67	0.10	NS
Gastrointestinal intolerance	0	3 (5%)	2.67	0.10	NS

* n = number of patients; NS = not significant.
† Group A + B had previous gold therapy; group C had no previous therapy.

DISCUSSION:

Several authors have commented on the outcome of rheumatoid patients treated with D-penicillamine who had received previous gold therapy (140, 168, 176-183). The Multicentre Trial Group (176), Tsang et al (177), Webley and Coomes (178), and Weiss et al (179) did not find an increased evidence of toxicity in their D-penicillamine-treated patients who had received previous gold therapy. This has been confirmed in both younger (< 60 years old) and elderly (> 60 years old) patients (140, 141). Our findings are consistent with these reports and suggest that overall development of toxicity is not influenced by previous gold therapy, since the toxicity pattern of group A + B was not significantly different from that of group C and the time of onset of D-penicillamine toxicity was not significantly different between group A + B and group C (Table 17).

Dodd et al (180) suggested that D-penicillamine may react with protein-bound gold and mobilize it, so that the gold can again cause adverse reactions. I found that in group A, those patients who did not develop D-penicillamine toxicity had a larger time interval between the gold toxicity and the start of D-penicillamine therapy than those patients who developed a major and minor toxicity or those who developed a major toxicity alone (Table 14). Although these differences just reached statistical significance, it is difficult to draw a conclusion that D-penicillamine toxicity is less common if the time between gold toxicity and D-penicillamine therapy

is long, because the range of intervals for individual patients overlapped among the 3 subsets. Similarly, the time between discontinuation of gold and the development of D-penicillamine toxicity was no different between group A and group B (Table 15). Dodd et al (180) also suggested that adverse reactions in patients receiving gold therapy and subsequently D-penicillamine are commonly of the same type, if the time interval between administration of the 2 drugs is short. In my series, 13 patients had a major reaction to both drugs, but I did not find any significant difference in the time between toxicity to the 2 drugs in the 6 patients who had the same adverse effect compared with the 7 patients who had a different adverse effect.

Many authors have commented that specific adverse reactions occur more commonly in patients with D-penicillamine toxicity if previous gold has been administered. Day and Golding (181) found an increase in marrow hypoplasia in patients who were receiving D-penicillamine and who had previously received gold therapy. Billingsley and Stevens (182) reported an increased incidence of proteinuria, and Webley and Coomes (183) reported an increased incidence of skin rash and proteinuria. As in Webley and Coomes' original report (178), the Multicentre Trial Group (176), Tsang et al (177), and Weiss et al (179), however, I did not find any significant differences in the incidence of skin rash, proteinuria, low platelets, taste abnormality, mouth ulcers, low white cell count, or gastrointestinal upset when I compared my patients with

previous gold therapy (group A + B) with those who had never received gold therapy (group C) (Table 17).

Total gold dosage received did not influence the outcome of D-penicillamine toxicity, regardless of previous gold toxicity. Dodd et al (180) mentioned that gold is a chelating agent for metals and suggested that D-penicillamine may react with protein-bound gold and mobilize it, thus making it available to cause adverse reaction. While D-penicillamine does chelate Au(III), an oxidation state of gold known to bond to four atoms arranged in a square about it, there is no evidence that D-penicillamine chelates Au(I), the oxidation state of gold used in anti-arthritic drugs and that found *in vivo*. I suggest, therefore, that the terminology *chelation* not be used when referring to the *in vivo* interaction of gold and penicillamine.

Although penicillamine was originally introduced as a drug in the treatment of Wilson's disease because of the compound's capacity to chelate copper (170, 171), this knowledge that penicillamine is a metal chelator has led to three basic problems in the medical literature:-

(i) The assumption that penicillamine acts as a chelator for all metals, including the Au(I) administered in gold thiol complexes, e.g. gold sodium thiomalate (185, 186).

(ii) The assumption that adverse effects occurring in patients receiving D-penicillamine might be related to D-penicillamine "chelating" previously administered gold.

(iii) The perpetuation of assumptions (i) and (ii) in the medical literature (172-175).

The first concept, that D-penicillamine chelates the Au(I) of gold sodium thiomalate has recently been disputed (187). The results of my findings in this chapter have disputed concept (ii) and indicate that there is no clinical correlation between prior gold therapy (adverse effects or not) and eventual outcome on D-penicillamine therapy (188).

It is evident that the term chelation is not completely understood by some members of the medical fraternity. Apart from the confusion mentioned herein, it has been stated that D-penicillamine on oxidation to D,D-2,2,5,5, tetramethylcystine cannot act as a chelate (189), even though this ligand has quite clearly been shown to chelate copper (190) (Figure 47).

In view of this apparent knowledge gap in the medical literature, Professor C.J.L. Lock (Department of Materials Research, McMaster University) and I collaborated to provide a description of the chemical mechanism of chelation, and to illustrate that although D-penicillamine-Au(I) binding may occur, D-penicillamine-Au(I) chelation is an unlikely chemical reaction <u>in vivo</u>. It seems

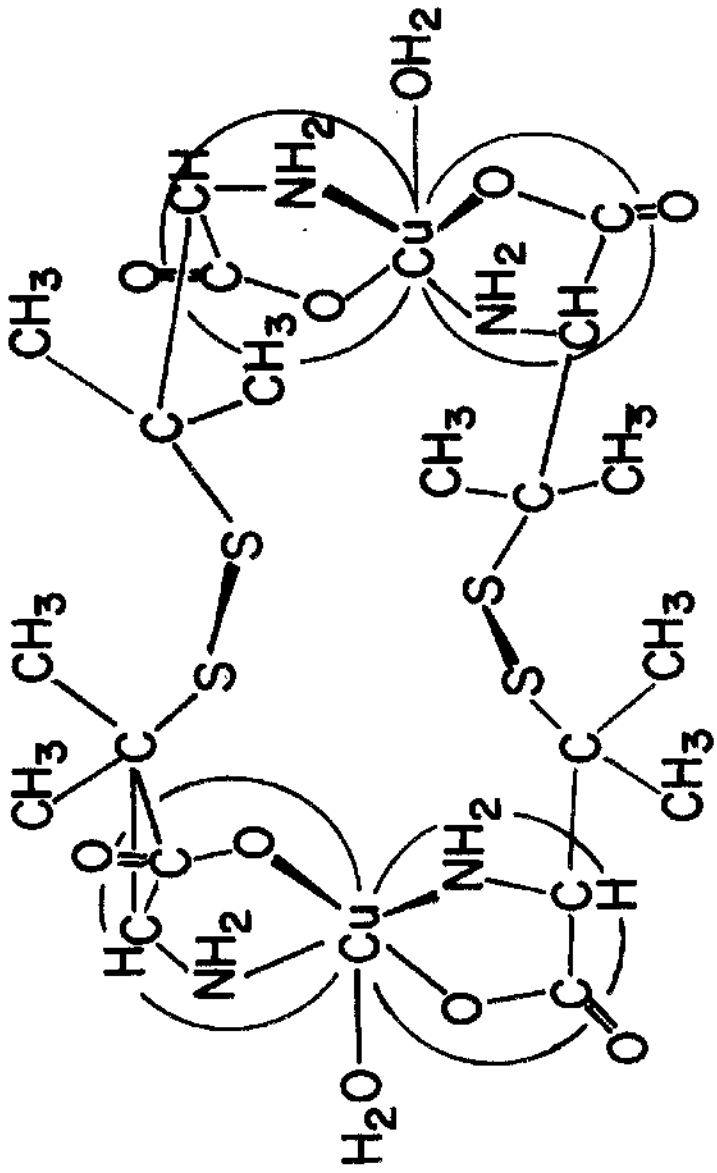


FIGURE 47: The complex of β,β,β′,β′-tetramethylcystine (D-penicillamine disulphide) with Cu(II) showing the ligand chelating through nitrogen and oxygen atoms. The four chelate rings are shown in the circles.

relevant that this description be added to the discussion of the clinical studies in this chapter.

Chelation (Greek: khele, Latin: chele, claw) is said to occur when two or more electron-pair donor sites from a polydentate ligand attach to the same metal ion simultaneously and form a ring structure.

A ligand is any atom, ion or molecule which can form a bond to another atom, usually a metal atom or cation, by donating a pair of electrons (Figure 48a). Polydentate ligands have more than one potential donor site (Figures 48b and 48c). When two or more donor electron sites of a polydentate ligand attack to the same metal simultaneously, a ring structure as illustrated in Figure 48d occurs and a chelate is said to be formed.

D-penicillamine forms at least one complex with Au(I), and although the exact structural nature is unknown, it is assumed to be a linear gold-cysteine compound $(RS\text{-}Au(I)\text{-}SR)^-$ (191) as shown in Figure 49a. This complex, however, is readily oxidised in <u>air</u> to the Au(III)-penicillamine chelate complex Au(III)-$(NH_2CH(CO_2^-)C(CH_3)_2S)_2^{3-}$ as shown in Figure 49b (192).

It was this latter compound which Eyring and Engleman used to determine their chelation constants (185) and it is apparently from this work that the misapprehension arose that D-penicillamine chelates gold in all its oxidation states. It should be noted, however, that gold contained in injectable gold

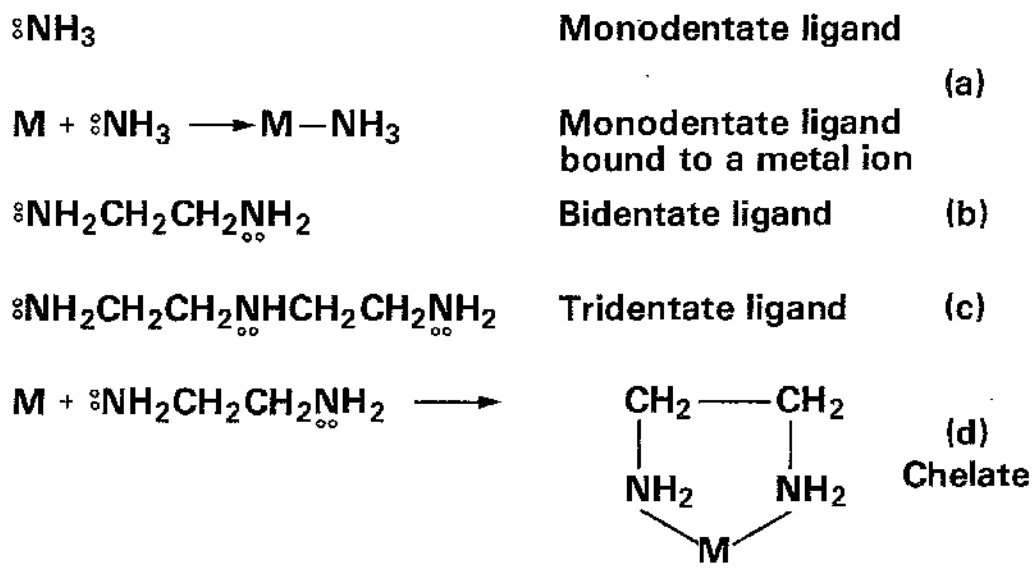


FIGURE 48: (a) Ammonia a monodentate ligand; one donor electron pair.
(b) Ethylenediamine, a bidentate ligand; two donor electron pairs.
(c) Diethylenetriamine, a tridentate ligand; three donor electron pairs.
(d) The formation of a metal chelate by the binding of the two donor electron pairs of ethylenediamine to the same metal ion.

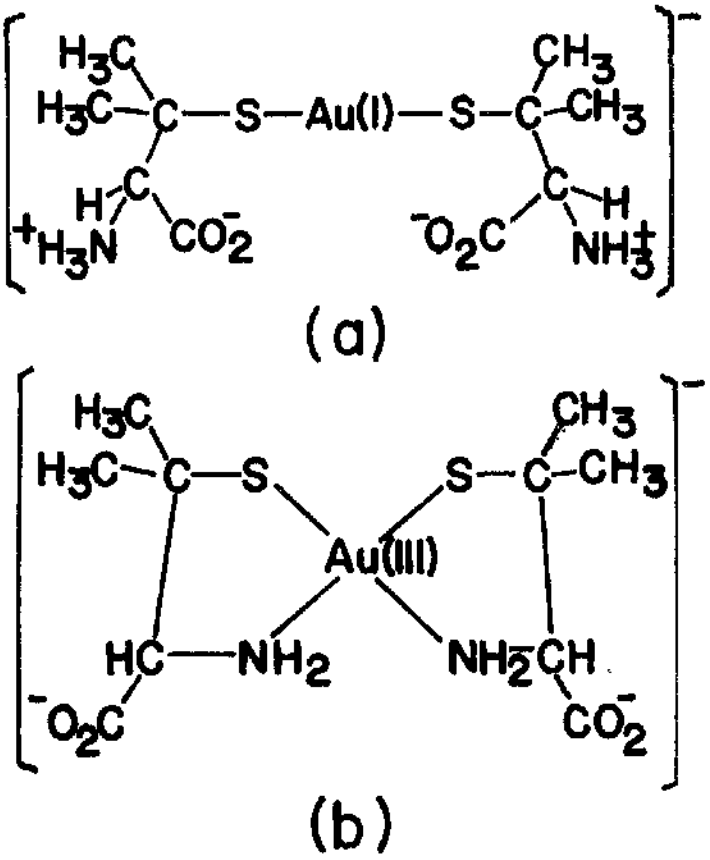


FIGURE 49: (a) Two D-penicillamine molecules bound to Au(I), but not as a chelate.

(b) Two D-penicillamine molecules bound to Au(III) and chelating through the S and N atoms.

compounds is Au(I) (193). If gold sodium thiomalate is administered to laboratory animals, gold recovered in tissue and urine exists only in the Au(I) oxidation state as determined by Extended X-ray Absorption Analysis of Fine Structure techniques (EXAFS) (193). Reduction from Au(III) to Au(I) is most likely caused by the powerful sulphur containing reducing enzymes present in vivo. Thus although penicillamine forms a chelate with Au(III), this oxidation state is not present in the commonly used anti-arthritic compounds, gold sodium thiomalate, gold thioglucose and gold sodium thiosulphate, and there is no evidence that Au(III)-containing molecules occur in vivo following administration of the anti-arthritic gold compounds.

Au(I) compounds normally have a geometry in which the Au(I) atom is attached to only two ligand atoms (X) such that the X-Au(I)-X angle is 180° (191). Less frequently, and only for rather specific ligands, Au(I) binds to three (X-Au(I)-X=120°) (194) or four (X-Au(I)-X=109.5°) (195) ligand atoms. Schaeffer et al (191) have pointed out that chelation of Au(I) by D-penicillamine is impossible if the two ligand bonds are at 180°; the ligand is not large enough to span the distance involved. Chelation can only occur if the X-Au(I)-X angle is less than roughly 120°. Complexes of Au(I) with X-Au(I)-X angles of $< 120^{\circ}$ are known and indeed some may involve chelate ligands. These three and four-coordinate complexes are, with one exception, only found with organic phosphine ligands and α,α´-dipyridyl or O-phenanthroline (196). In one case a sulphur containing ligand forms a chelate

to Au(I). This is an exceptional compound containing the tetrathiotungstate ion as a ligand and again is stabilized by an organophosphine (197).

There have been no descriptions of other sulphur bound ligands forming more than one bond to a Au(I) atom, so the complex contains two ligands with the bonds at 180° to each other, and there have now been many such structures determined. Even when a ligand has two potential sulphur bonding sites, the ligand binds to two different gold atoms rather than form a chelate (Figure 50) (198). There is no apparent reason why D-penicillamine should show exceptional behaviour and thus one would expect D-penicillamine to bind to Au(I) only through the sulphydryl site. (Indeed there is good reason why D-penicillamine should not chelate Au(I)). Au(I) prefers to bind to polarizable (soft) ligand groups such as sulphur or phosphines (199). It does not bind readily to weakly polarizable (hard) ligand groups (199). The amine and carboxylate groups of D-penicillamine are "hard". Higher oxidation states of metals prefer to bind to increasingly harder sites so the chelation observed in Au(III) to the S and NH_2 sites is not surprising.

The ability of penicillamine to compete for Au(I) attached to protein is well established when a large excess of penicillamine is present (192, 200), but the D-penicillamine is probably only bound through a simple Au-S bond. This competition appears not to be important at standard pharmacological doses.

These chemical concepts lend credence to the results of my clinical study in this chapter (188) which indicates that prior

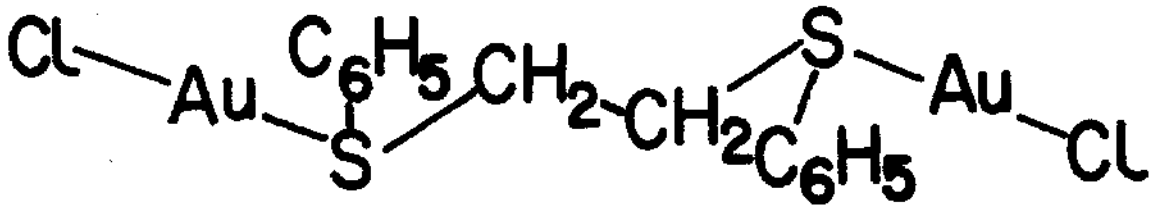


FIGURE 50: The structure of μ-{1,2-bis(phenylthio)ethane}-bis{chlorogold(I)}. Even though the ligand has two potential sulphur bonding sites, the ligand binds to two different gold atoms rather than form a chelate.

gold therapy does not influence the clinical outcome of patients with rheumatoid disease receiving D-penicillamine. In my opinion a more likely hypothesis regarding the similarity of adverse effects noted for gold sodium thiomalate and D-penicillamine is the fact that both react with thiol compounds and will thus have similar chemical interaction with biologically active compounds and structures.

In conclusion, the evidence is against thiol bound ligands such as thiomalate, thioglucose, thiosulphate and D-penicillamine forming chelates to Au(I) unless a phosphine is present, and thus mobilisation of bound gold *in vivo* by D-penicillamine through the mechanism of chelation is unlikely (200).

Therefore, the *in vivo* interaction of D-penicillamine and gold in the Au(I) oxidation state should not be referred to as chelation.

SUMMARY:

One hundred and fourteen patients with definite or classical rheumatoid arthritis were followed prospectively between January 1976 and April 1981 to monitor their toxicity pattern to D-penicillamine. The influence of previous gold sodium thiomalate therapy on the toxicity patterns of D-penicillamine is described. There was no significant difference in overall outcome of the patients treated with D-penicillamine with respect to adverse effects, whether they had previous gold toxicity, previous gold therapy but no toxicity, or no previous gold therapy. The time from gold toxicity to the start of D-penicillamine therapy was greater in those who did not develop D-penicillamine toxicity compared with those who did. This difference just reached statistical significance. Total gold therapy received had no effect on eventual outcome of D-penicillamine treatment, and the toxicity pattern of D-penicillamine in those patients who had previous gold therapy was similar to those patients who had never received gold therapy.

There are references in the medical literature which state that D-penicillamine may be used as a chelating agent for gold administered in the anti-arthritic injectable gold compounds.

The binding of thiol containing polydentate ligands, particularly D-penicillamine, to Au(I) and Au(III), is discussed.

It is concluded that while these ligands bind to both Au(I) and Au(III) through the sulphur atom, chelation occurs only to Au(III). Chelation of thiol ligands to Au(I) does not occur.

CHAPTER VI

GOLD CHEMISTRY OF THE AU(I) ANTI-ARTHRITIC GOLD COMPLEXES

- INTRODUCTION
- GOLD CHEMISTRY
- THE ANTI-ARTHRITIC GOLD COMPLEXES AND RELATED STRUCTURES
- SUMMARY

"By happy alchemy of mind
They turn to pleasure all they find."

Matthew Green (1696-1737)

INTRODUCTION:

Shortly after being appointed to the Faculty of Health Sciences at McMaster University my research interests became increasingly directed towards the mechanism of action of the anti-arthritic gold complexes. This work has involved studies on the chemical nature of the gold complexes (in collaboration with Professor Colin J.L. Lock of the Department of Materials Research), the effects of gold complexes on lymphocytes (in collaboration with Dr. Dharam Singal, Professor of Pathology and Director of Histocompatability Studies) and platelets (supervised by Dr. J. Fraser Mustard, former Vice Principal of Health Sciences, Dr. Raelene Kinlough-Rathbone, Professor of Pathology, Director of Post-Graduate Studies, and Dr. Jack Hirsh, Chairman of the Department of Medicine). In 1981 Professor Colin J.L. Lock and I formed the discipline of Bio-inorganic Medicine at McMaster University, which in addition to ourselves as co-directors has four other faculty members, as well as one Ph. D. student, one research scholar, one B.Sc. student, two laboratory research assistants, and three technicians. The major research thrust of this bio-inorganic medicine team is the study of the anti-arthritic gold complexes and related compounds (e.g. D-penicillamine) in the characterisation of their structure, the structural relationship of the complexes to peptide and protein binding and the interaction of the gold-thiol complexes with cell and enzyme

systems. Some of these studies will be outlined in the succeeding chapters, but the entire scope of the research work is too varied and lengthy to include in one thesis. In the next two chapters, I will address two avenues of my own research made in conjunction with my colleagues over the past two years, which I consider to have been significant observations. Firstly, a brief review of the basic chemistry of gold relevant to the compounds used is given to facilitate the understanding of the concluding chapters. Four excellent extensive reviews of gold chemistry are available which provide a more detailed account of the structural analysis and biological action of the gold complexes as are known to date (1, 201-203).

GOLD CHEMISTRY

Gold is a soft, yellow lustrous metal. Since it is not attacked by either oxygen or sulphur at any temperature, it has a unique stability in its elemental form (1). Solar spectral analysis confirms that gold is present at 0.04 parts per million in the sun; it is, however, only present as approximately 0.004 parts per million in the Earth's crust (1). Gold deposits on Earth exist as the metallic form or in mineral form, either as Tellurides such as calaverite and krennerite (different crystalline forms of $AuTe_2$), montbrayite (Au_2Te_3) and mixed gold-silver tellurides such as sylvonite (Au Ag Te_4) (1). To a lesser extent mineral forms of auriferous sulphides also exist (2). Metallic gold and silver mixtures also exist, perhaps the most well-known being electrum, which contains 20% silver (1). Gold ores also contain small quantities of other valuable metals such as iridium, osmium, platinum, rhodium, ruthenium, palladium, copper and nickel (1).

Gold is extremely malleable and highly ductile, such that one ounce can be beaten to an area of 300 square feet. Pure gold metal has a density of 19.32g cm^{-3} at 20°C; it melts at 1063°C and boils at 2966°C. (1) Gold has an atomic number of 79 and an atomic weight of 196.9665. The stable isotope ^{197}Au contains 79 protons and 119 neutrons. There are 29 other isotopes of gold with mass numbers in the 177 to 204 range. The most important of these is ^{198}Au

which is prepared by neutron capture by the reaction:

$$^{197}_{79}Au + ^{1}_{0}n \rightarrow ^{198}_{79}Au$$

^{198}Au decays by β-emission accompanied by gamma radiation (1). The half-life of 2.7 days makes it a relatively safe and useful tool in medical science, in radiotherapy, radio-diagnostic and radiotracer studies.

Gold is classified as a Group I.B. metal in the periodic table with an electronic configuration of $\{Xe\}\ 4f^{14}5d^{10}6s^{1}$. The most commonly recognised oxidation states are I, II, III and V (referred to in this thesis as Au(I) etc.), although according to Puddephatt (1), metal-metal bonds do exist in complexes in which it is difficult to assign a formal oxidation state to the gold atom. Au(I) has the closed shell electronic configuration $\{Xe\}\ 4f^{14}5d^{10}$ and thus like the isoelectric mercury (II) forms two, three and four co-ordinate complexes. The Au(I) complexes are diamagnetic and adopt regular structures which are most commonly of the linear two co-ordinate type. The true salts of Au(I) such as the halides are unstable in the presence of water and disproportionate to Au^{o} (metallic gold) and Au(III). However, Au(I) can be stabilised by the formation of complexes with "soft" ligands (199) such as the thiolates (204-206) and phosphines (204,207,208). All currently ised anti-arthritic gold complexes exist as Au(I) thiol or phosphine compounds (209). The Au(III) complexes are diamagnetic and have the low-spin $5d^{8}$ electron configuration. The vast majority of

these complexes have a four co-ordinate square planar stero-chemistry (1, 201-203). The high toxicity of the Au(III) complexes such as chloroauric acid ($HAuCl_4$), makes them unsuitable for human use (209). As already referred to in Chapter V, Elder and colleagues have shown that if gold sodium thiomalate is administered to laboratory animals, the gold recovered from the tissue and urine exists in the Au(I) oxidation state (i.e. the same oxidation state as the gold in gold sodium thiomalate) and not the Au(III) oxidation state. Further, if $Au(III)Cl_3$ is administered to laboratory animals, gold recovered in tissues and urine exists only in the Au(I) oxidation state as determined by x-ray absorption near edge spectroscopy (XANES) and extended x-ray absorption fine structure spectroscopy (EXAFS). XANES distinguishes Au(III) from Au(I) and Au^0 due to a $2p \rightarrow 5d$ transition which occurs only for Au(III). Elder and colleagues also showed that EXAFS with appropriate curve fitting techniques could demonstrate that the gold atoms in solution of gold sodium thiomalate (i.e. Au(I) oxidation state) are co-ordinated to two sulphur atoms with a Au-S bond length of 2.29 A^0 (193). This was consistent with their finding that in rats chronically treated with gold sodium thiomalate, the gold within particles obtained from the rat kidney cells was in the Au(I) oxidation state and was bound to two sulphur atoms with a Au-S bond length of 2.30 A^0 (193). The Au(I) oxidation state therefore appears to be the primary oxidation state in a biological milieu: reduction from AU(III) to Au(I) is most likely caused by the powerful sulphydryl containing reducing enzymes present in vivo.

A common misconception among members of the medical profession, is the idea that D-penicillamine may be used as a chelating agent for gold, if a toxic reaction occurs during or following the administration of the anti-arthritic gold complexes (172-175). There is no theoretical (see Chapter V, Discussion) nor biochemical (187) evidence that D-penicillamine chelates Au(I) in vivo. As referred to above and in Chapter V, Au(I) compounds normally have a linear geometry in which the Au(I) atom is attached to only two ligand atoms (X) such that the X-Au(I)-X angle is 180° (191). Less frequently, and only for rather specific ligands, Au(I) binds to three (X-Au(I)-X angle equal to 120°) (194) or four (X-Au(I)-X angle equal to 109.5) (195) ligand atoms. These three andfour-coordinate complexes are, with one exception, only found with organic phosphine ligands and α,α´-dipyridyl or O-phenanthroline (196). The only sulphur containing compound to form a chelate and thus non linear structure to Au(I) is an exceptional compound containing the tetrathiotungstate ion as a ligand. It is stabilised by an organophosphine (197). Thus Au(I)-S bonds are at 180° to each other except in exceptional circumstances. Even when a ligand has two potential sulphur bonding sites, the ligand binds to two different gold atoms rather than form a chelate (198). There is no apparent reason why D-penicillamine should show exceptional behaviour and thus one would expect D-penicillamine to bind to Au(I) only through the sulphydryl site. Furthermore, Au(I) binds more readily to

polarizable (soft) ligand groups such as sulphur or phosphines (199) as opposed to weakly polarizable (hard) ligand groups (199), and the amine and carboxylate groups of D-penicillamine are "hard". Thus, as supported by the studies of Davis and Barraclough (187), there is no theoretical reason why D-penicillamine should chelate Au(I).

THE ANTI-ARTHRITIC GOLD COMPLEXES AND RELATED STRUCTURES.

The current commonly used gold complexes in the treatment of rheumatoid arthritis and in related research work are illustrated in figure

Gold sodium thiomalate (figure 51a) has been used in the treatment of rheumatoid arthritis since the early 1930s (87). It is marketed by May and Baker (U.K.), Rhone-Poulenc (Canada) and Merck, Sharp and Dhome (U.S.A.) as vials of 50 mg/ml of compound suspended in sterile water for use as an intramuscular injection. The compound contains approximately 51% gold and 0.3 mols of glycerol per mol of thiomalate. During the manufacturing process glycerol is used in the final purification step (210). The vials of compound marketed for human use also contain the following preservatives: phenylmercuric nitrate 0.002% (May and Baker preparation); chlorocrescol 0.05% (Rhone-Poulenc preparation); benzyl alcohol 0.5% (Merck Sharp and Dhome preparation). May and Baker (U.K.) have kindly allowed Professor Colin J.L. Lock and me access to the details of their patented manufacturing process. Since a secrecy agreement exists, no details of this process will be given. The currently assumed molecular weight of gold sodium thiomalate is 390.12. This value is based on the empirical formula of the compound (figure 52a) but clearly this structure is incompatible with the known chemical properties of Au(I) (1, 201-203) and a more likely explanation of the structure is a polymer or oligomer as graphically depicted in figure 51 (a). According to Shaw (211) gold sodium thiomalate elutes from Sephadex G-100 as a polymeric

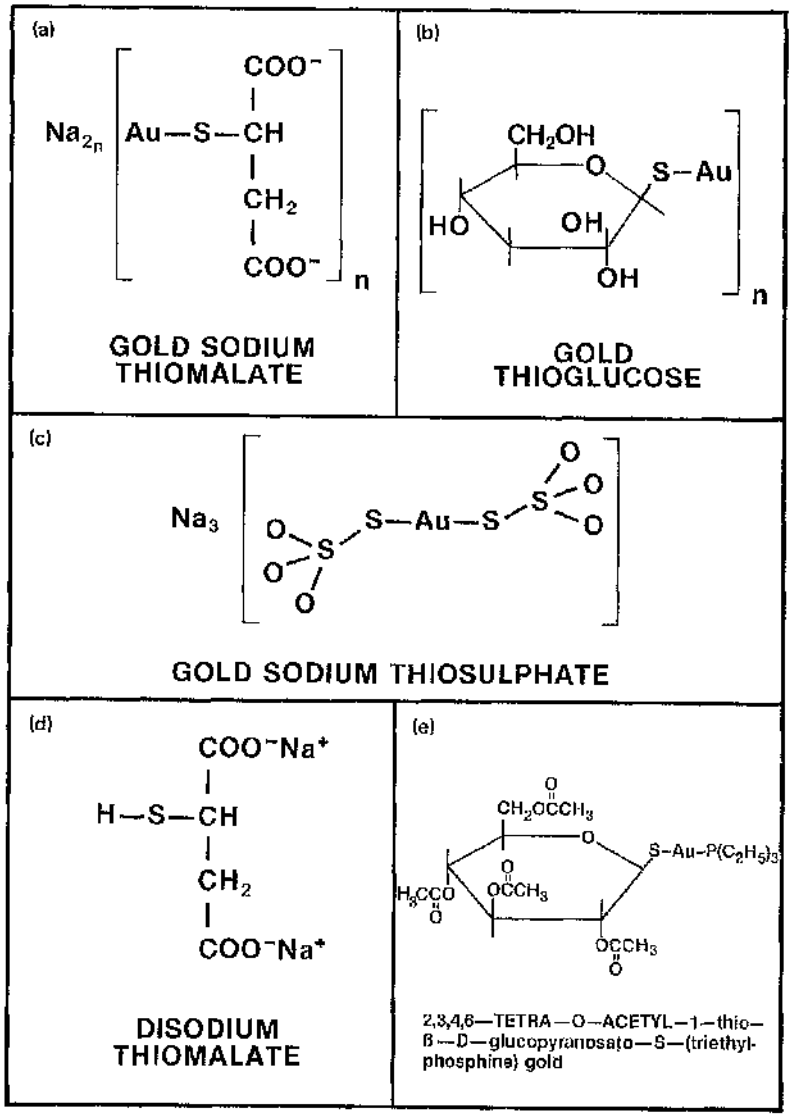


FIGURE 51:

(a) The formula of gold sodium thiomalate depicted as a polymer.

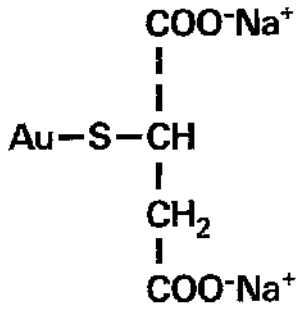
(b) The formula of gold thioglucose depicted as a polymer.

(c) Gold sodium thiosulphate, structural formula. The anion contains two thiosulphate moieties bound to gold through the sulphur atoms.

(d) Disodium thiomalate formula. The disodium thiomalate salt is a monomeric species.

(e) 2,3,4,6-Tetra-o-acetyl-I-thio-β-D-glucopyranosato-S-(triethyl-phosphine)gold is characterised as having a gold-phosphine bond as well as a gold thiol bond, and is a monomeric species.

(a)



(b)

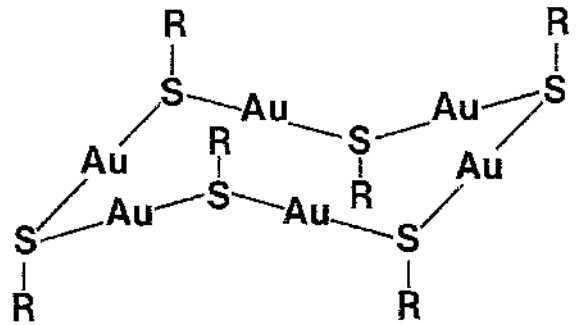


FIGURE 52: (a) Empirical formula of gold sodium thiomalate.

(b) Graphic illustration of a probable hexameric structure for gold sodium thiomalate, based on model building.

structure. Ultracentrifuge and hydrogen-1 and carbon-13 nuclear magnetic resonance studies by Sadler and colleagues (201,210,212) support this concept of the gold sodium thiomalate structure. Based on model building, a likely structural formula is a hexamer as is shown graphically in figure 52b Clearly the dearth of information of the structural formulation of gold sodium thiomalate has contributed considerably to the lack of knowledge available on the biological interactions of this Au(I) anti-arthritic complex.

Gold thioglucose (figure 51b) was manufactured in the United States by Merck Sharp and Dhome in the early 1930s (87) and is currently manufactured under the brand name Solganol by the Schering Corporation of New Jersey and is adiministered as an intramuscular injection. Although it is water soluble, it is marketed as 50 mg of gold thioglucose in sterile sesame oil with 2% aluminium monostearate; 1 mg of Propyl p-Hydroxybenzoate is added as a preservative. Gold content is approximately 50.25% and the molecular weight based on the empirical formula is 392.18, but as with gold sodium thiomalate, the structural formulation of gold thiolgucose based on the known chemistry of gold, is most likely an oligomer (1, 201-203, 209) (Figure 51b).

Gold sodium thiosulphate (figure 51c) is marketed as Sanochrysine in Europe by Ferrosan, Sweden, in vials of 100 mg of compound/ml for use as an intravenous injection. It has been available since 1924 when it was first introduced by Møllgard (75).

The compound has a molecular weight of 490.21 and contains 40.19% gold. The anion contains two thiosulphate moieties bound to gold through the sulphur atoms. Gold sodium thiosulphate is one of the few Au(I) compounds whose structure is known and which has been studied crystallographically (206).

Mercaptosyccinic acid is manufactured in the United States by Sigma Chemicals, St. Louis, Missourri and has a molecular weight of 150.15. The disodium thiomalate salt in equimolar concentrations of thiomalate to gold sodium thiomalate is prepared by the addition of 1 molar sodium hydroxide to the mercaptosuccinic acid solution (19 mg/ml) until a pH of 7.75 is achieved. The disodium salt is a monomeric species (figure 51d).

2,3,4,6-Tetra-O-acetyl-I-thio-β-D-glucopyranosato-S-(triethyl-phosphine) gold (figure 51e) is manufactured as Auranofin$_{TM}$ by Smith Kline and French, Philadelphia, U.S.A. It is characterised as having a gold-phosphine bond as well as a gold thiol bond and is a monomeric species (figure 51e). It is currently being tested in numerous clinical trials as a 3 mg tablet administered orally. The compound is a white, odourless crystalline solid which is insoluble in water. The powder is unstable and must be protected from light and heat. On a weight basis it contains approximately 29% gold and has a molecular weight of 678.5 with a melting point of 112°-115°C (116-121). It is currently only available for research studies.

SUMMARY.

Following my appointment as a faculty member at McMaster University, Hamilton, Ontario, I established the discipline of Bio-inorganic medicine along with Professor C.J.L. Lock of Materials Research. The principle areas of research of this group are the investigation of the structural formulae of the anti-arthritic gold complexes, their structural interaction with peptides and proteins and the mechanism of interaction of the gold complexes with cell and enzyme systems such as occur in lymphocytes and platelets.

Gold is a valuable metal with a unique stability in its elemental form. It is present in the Earth's crust as approximately 0.004 parts per million either in the metallic state or in mineral form usually as a Telluride.

Gold has an atomic number of 79 and is classified as a IB metal in the periodic table. Its stable isotope is ^{197}Au although 29 other isotopes exist with mass numbers in the 177 to 204 range. Au(I) is the oxidation state of the presently used anti-arthritic gold complexes. Au(I) complexes are diamagnetic and adopt regular structures which are most commonly of the linear two co-ordinate type. Extended x-ray absorption fine structure spectroscopy studies have shown that the Au(I) state is the primary oxidation state present in a biological milieu. The linear geometry of Au(I)-S bonds and the presence of gold in the Au(I) state in vivo,

makes the concept that D-penicillamine chelates Au(I) in vivo unlikely. Biochemical studies have also shown that D-penicillamine does not chelate Au(I), therefore D-penicillamine cannot be used as an in vivo chelating agent for gold administered as the anti-arthritic gold complexes.

The current commonly used anti-arthritic gold complexes are gold sodium thiomalate, gold thioglucose, gold thiosulphate and 2,3,4,6-Tetra-o-acetyl-1-thio-β-D-glucopyranosato-S-(triethyl-phosphine) gold. The structures of these complexes differ in that both gold sodium thiomalate and gold thioglucose are oligomer,s gold sodium thiosulphate contains one gold molecule and two thio-sulphate moieties and 2,3,4,6-Tetra-o-acetyl-1-thio-β-D-glucopyro-nosato-S-(triethylphosphine) gold as a monomer. Gold sodium thio-malate and gold thioglucose are administered by the intramuscular routine whereas gold sodium thiosulphate is administered intra-venously. 2,3,4,6-Tetra-o-acetyl-1-thio-β-D-glucopyranosato-S-(triethylphosphine) gold is administered orally.

CHAPTER VII

THE CHEMICAL AND BIOLOGICAL DIFFERENCES IN GOLD SODIUM THIOMALATE FOLLOWING EXPOSURE TO HEAT AND ULTRA-VIOLET IRRADIATION

- INTRODUCTION
- MATERIALS AND METHODS
- RESULTS
- DISCUSSION
- SUMMARY

"What one knows is, in youth,
of little moment;
They know enough who know how
to learn."

Henry Brooks Adams (1838-1918)

INTRODUCTION

Gold sodium thiomalate has been used in the treatment of rheumatoid arthritis for at least 50 years (87), but to date, the definitive in vivo actions resulting in efficacy and toxicity have not been identified. As referred to in Chapter VI the exact chemical structure of gold sodium thiomalate has not been described. The simple formulation given in figure 52a, as $Na_2\{AuSCH(CO_2)CH_2CO_2\}$ is inconsistent with the known chemistry of gold (1, 201-203). Physical and chemical studies suggest that the structure is likely an oligomer (201, 210-212) and model building shows a hexamer is possible (212) (figure 52b). The correct chemical name for gold sodium thiomalate based on the structure given in the Martindale's Extra Pharmacopoeia (213) is sodium (dithiomalato-S)aurate(I), but since the structure (figure 52a) is almost certainly incorrect, I shall use the term gold sodium thiomalate throughout. In collaboration with Professor Colin J.L. Lock (Institute for Materials Research, McMaster University) I have undertaken various chemical and biological experiments in order to establish the exact nature of the compound, gold sodium thiomalate. Like Dr. Frank Shaw and colleagues (202) at Ann Arbor University in Michigan, U.S.A. and Dr. Peter Sadler and colleagues (201), Birkbeck College, University of London, U.K., we have had difficulty in crystallising gold sodium thiomalate and agree that it is most likely an oligomer. One important observation which we have demonstrated for the first time is that gold sodium thiomalate as currently marketed for human use, is a mixture.

This important finding was the result of a simple observation. In 1980 the pharmaceutical company Rhône-Poulenc, Quebec, Canada kindly agreed to supply gratis samples of gold sodium thiomalate for my experimental use. I noted that solutions of the compound supplied for human use were yellow, whereas a batch of compound supplied for research purposes was a colourless solution. I made enquiries to the company in Montreal as to the difference in the two solutions and was informed by their chief pharmacist that although both samples were prepared in exactly the same way, by dissolving the solid form (white powder) in sterile water, the samples for human use were further sterilized by heating at 100°C for 30 minutes. This procedure resulted in the familiar yellow solution which is marketed by their company, whereas the solution which was not exposed to 100°C for 30 minutes was colourless.

$$\text{White Powder (Gold Sodium Thiomalate)} \xrightarrow{H_2O} \text{Colourless Solution} \xrightarrow[30\text{ mins}]{100^{\circ}C} \text{Yellow Solution}$$

This simple experiment was confirmed in my laboratory. Since both the colourless and yellow solution of gold sodium thiomalate were of exactly the same concentration, clearly the heat sterilization process had resulted in some change in order to produce the yellow colour. In conjunction with my colleagues in the discipline of Bio-inorganic medicine, we undertook physical, chemical and biological investigations in order to compare and contrast the properties of the colourless and yellow solutions of gold sodium thiomalate.

The physical and chemical studies referred to in the chapter were done by Professor C.J.L. Lock, Dr. Helen Howard-Lock and Miss Debra Harvey, a Ph.D. student. The biological experiments involving lymphocytes were done in collaboration with Dr. D. Singal, Director of Histocompatability Studies and the experiments on platelets, electron microscopy and energy dispersive analysis were done solely by myself. Lymphocytes were chosen for study, as the effect of gold sodium thiomalate (yellow solution) on lymphocyte function is well established (214-217). Platelets were chosen as a study cell because several aspects of the interaction of the yellow solution of gold sodium thiomalate with platelets were already known to me from my research studies on the interaction of gold complexes with thrombin and platelets. (These will be outlined in Chapter VIII).

The experimental question addressed in this chapter was: What is the physical, chemical and biological significance of the change from the colourless solution of gold sodium thiomalate to the yellow solution of gold sodium thiomalate, especially since the latter is marketed for human use?

MATERIALS AND METHODS

Gold Complexes and Related Compounds

Gold sodium thiomalate was supplied gratis by Rhône-Poulenc, Quebec as: (a) the yellow solution in vials as currently marketed for human use; and as (b) a sterile white powder which, when mixed with sterile water, resulted in a colourless solution (figure 53). Both the yellow and the colourless solution contained equal quantities of glycerol (0.3 mols per mol of thiomalate) and chlorocresol (0.05%) as stated in Chapter VI. Gold thioglucose and thiomalic acid were obtained from the Sigma Chemical Co., St. Louis, Missouri, U.S.A. Gold sodium thiosulphate was prepared in the Bio-inorganic medicine laboratories at McMaster University after the method of Brown (204). 2,3,4,6-tetra-ortho-acetyl-1-thio-β-D-glucopyranosato-S-(triethyl-phosphine) gold (Auranofin$_{TM}$) was supplied gratis by Smith, Kline and French, Philadelphia, U.S.A. Disodium thiomalate in equimolar concentration to the thiomalate in gold sodium thiomalate was prepared by the addition of 1 molar sodium hydroxide (NaOH) to thiomalic acid 19 mg/ml until a pH of 7.75 was obtained. Auranofin is insoluble in water. For the platelet studies a stock solution was prepared by dissolving 17 mg of compound in 0.5 ml of absolute alcohol (C_2H_5OH) followed by the addition of 0.5 ml of water to achieve a concentration of $5.1x10^{-4}$M as elemental gold. Suitable alcohol control concentrations were used for each dilution of Auranofin added to the platelet suspensions.

All gold compound concentrations listed in this chapter are expressed as molar quantities of elemental gold.

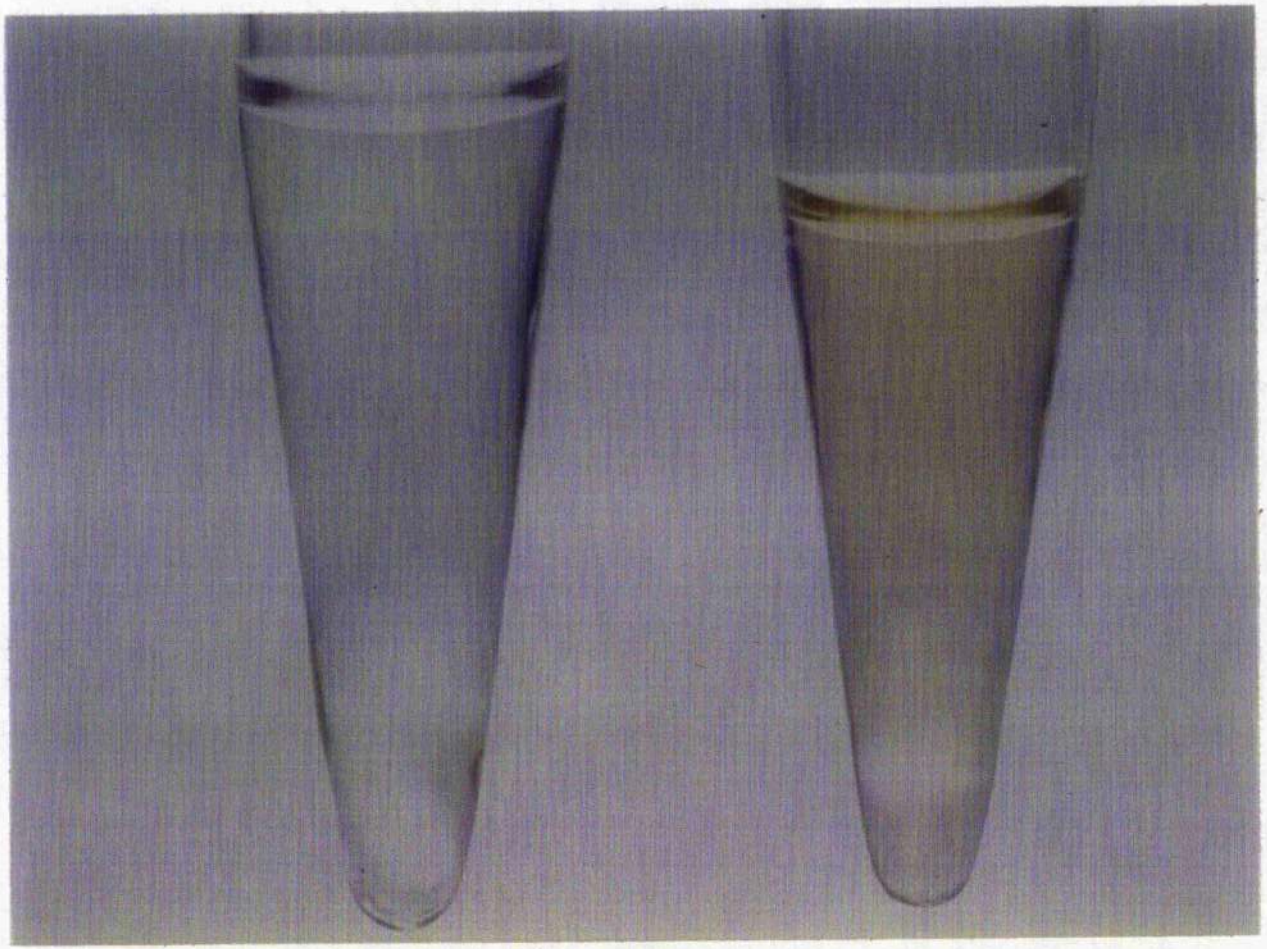


FIGURE 53: Test-tubes containing the colourless solution of gold sodium thiomalate (0.13M) on the left and the yellow solution of gold sodium thiomalate (0.13M) on the right. The yellow solution is the compound currently marketed for human use and is available world-wide.

Physical and Chemical Studies

Nuclear Magnetic resonance spectroscopy (N.M.R.) studies were carried out on a Varian EM-390 spectrometer. The colourless solution of gold sodium thiomalate was prepared from solid in heavy water (D_2O) to a concentration of 3.2×10^{-2}M. N.M.R. studies were also carried out on the colourless solution of gold sodium thiomalate following long term ultra-violet (U.V.) irradiation over 750 minutes at 300 nanometer (nm) wavelength in a Rayonet$_{TM}$ Photochemical Reactor.

Ultra-violet-visible spectra were performed on a Pye Unicam SP8-100 ultra-violet spectrophotometer. Spectroscopy of the visible light range (350-450 nm) was carried out using concentrations of colourless and yellow gold sodium thiomalate at 3.2×10^{-2}M. Spectra in the ultra-violet range (180-350 nm) were examined using concentrations of 3.2×10^{-4}M for the colourless and yellow solution of gold sodium thiomalate.

In order to follow the colour change from colourless to yellow by ultra-violet irradiation, 3.2×10^{-2}M of the colourless solution of gold sodium thiomalate in distilled water was irradiated with 350 nm wavelength in a Rayonet$_{TM}$ Photochemical Reactor. The visible spectrum of the sample was observed at specific time intervals over 500 minutes at 425, 450 and 475 nm wavelength and the optical density (absorption) plotted.

Biological Studies

(a) Mixed Lymphocyte Culture (MLC)

Lymphocytes were separated from freshly drawn blood as described by Boyum (218) and suspended in minimum essential media, supplemented with heat-inactivated (56^{o} for 30 min.) pooled AB serum from normal healthy donors. The one way mixed lymphocyte culture (MLC) test was performed as described by Singal et al (219) using 100 x 10^3 responder lymphocytes and 100 x 10^3 X-irradiated stimulator cells in 0.1 ml volume in flat-bottom microtitre plates (linbro 1S MRC96). Heat-inactivated pooled AB serum was added in 0.05 ml volume per culture. The cultures were incubated at $37^{o}C$ in water-saturated 5% CO_2-in-air atmosphere. Cultures were labeled with 0.5 µCi of ^{3}H-thymidine (6.7 Ci/mM, New England Nuclear, Boston, Mass.) for 18 hours. Total culture time, including thymidine pulse, was 120 hours. The cultures were harvested onto glass filter papers by a multiple sample harvester.

MLC inhibition tests were carried out by adding a range of concentrations of the colourless or yellow solutions of gold sodium thiomalate (0 - 60 µg of Au /ml of culture) at the initiation of culture. Percent inhibition in MLC in gold treated cultures compared to controls was calculated for the concentrations 3.8×10^{-5}M, 7.6×10^{-5}M, 1.5×10^{-4}M and 3.0×10^{-4}M (expressed as elemental gold) per milliliter of culture.

(b) Platelet Studies

Materials

Adenosine diphosphate (ADP) and adenosine 5'-monophosphate (AMP) were obtained from the Sigma Chemical Co., St. Louis, Missourri. Creatine phosphate and creatine phosphokinase were obtained from the Sigma Chemical Co., St. Louis, Missourri. Creatine phosphate/creatine phosphokinase is prepared by the addition of 5×10^{-2}M creatine phosphate to 0.85% sodium chloride and mixing this solution with 100 units/ml of creatine phosphokinase. Bovine thrombin was obtained from Miles Laboratories, Kankakee, Illinois, U.S.A. Radioactive serotonin (^{14}C-serotonin) was obtained from Amersham/Searle, Arlington Heights, Illinois, U.S.A. as 5-hydroxytryptamine-3'-^{14}C-creatine sulphate, 57 mCi/mmol. Radioactive chromium (^{51}Cr) as $Na_2{}^{51}CrO_4$, 100-400 mCi per mg of Cr was also obtained from Amersham/Searle. Radioactive counting was performed on a Philips Liquid Scintillation Analyser (P.W. 4510).

PREPARATION OF WASHED HUMAN PLATELETS

Washed human platelet suspensions were prepared by the method of Mustard et al (220). Briefly, 129 ml of blood was obtained by antecubital vein puncture from volunteers. Volunteers were selected who were on no medications and had taken no medication for two weeks. The blood was immediately transferred in equal volumes to 3 plastic centrifuge tubes (5 ml), each containing 7 ml of acid-citrate-dextrose (ACD) anticoagulant solution, as described by Aster and Jandl (221). The whole blood-ACD mixture was gently but rapidly mixed to prevent

clotting. The suspension was then centrifuged at 37°C in an R.C.3 Sorval centrifuge at 1200g for 3 minutes. The platelet-poor plasma was discarded and the platelets were suspended in Tyrodes solution containing 0.35% albumin (220,222). Apyrase prepared according to Molnar and Lorand (223), with a nucleotidase activity of 5.3 units of adenosine diphosphatase/mg and 4.2 units of adenosine triphosphatase/mg was included in the Tyrodes albumin solution at a concentration of 10 µl/ml (220, 224). Platelets were incubated in this first washing solution of Tyrodes albumin with 2 µCi of ^{14}C-serotonin and 200 µCi of disodium chromate/mg for 30 minutes. This first washing solution was then centrifuged at 1200g for 10 minutes, the supernatant discarded and the platelets resuspended in Tyrodes albumin solution (second washing solution) for 10 minutes. The second washing solution of platelets was centrifuged at 1200g for 10 minutes and the supernatant discarded. The platelets were suspended in a final suspension of Tyrodes albumin and the platelet count adjusted to 500,000/mm^3. The platelet suspensions were stored at 37°C in a water bath prior to use.

Platelet Aggregation Studies

Platelet aggregation was studied by a modification of a turbidimetric method as previously described (220). Briefly, light transmission of 1 ml suspensions of washed platelets was measured on a Payton Aggregation Module (Payton Associates, Scarborough, Ontario, Canada) and recorded on a Rikadenki Pen Recorder (Kogyo, Japan). Platelet shape change and aggregation were recorded following the interaction with test compounds (e.g. gold complexes) and aggregating agents (e.g. ADP).

Electron Microscopy

Specimens of platelets treated with the gold compounds were studied by electron microscopy. 0.5 ml samples of the platelet suspensions were added to 0.5ml of 4% gluteraldehyde in 0.2M sodium cacodylate buffer (pH 7.3). The specimens were fixed for one hour at 4°C and then centrifuged at 3200 r.p.m. to obtain a pellet of platelets. These platelets were post-fixed in 1% osmium tetroxide in 0.1M sodium cacodylate buffer. The platelets were block stained with 2% aqueous uranyl acetate and dehydrated in graded ethyl alcohol solutions and embedded in Spurr's resin. Ultrathin sections were stained with Reynold's lead citrate. Electron microscopy was performed on a Philips 301 microscope at McMaster University Health Science Centre.

Energy Dispersive Spectroscopic Analysis

Platelet specimens were prepared as described for conventional electron microscopy except for the post-fixation in osmium tetroxide and block staining, which were omitted. The unstained, ultrathin sections were coated with carbon and analysed in a Philips 400 transmission electron microscope equipped with an energy dispersive x-ray detector and analyser. Energy dispersive spectroscopic analysis was carried out at the Ontario Research Foundation, Mississauga, Ontario, Canada.

RESULTS

Physical and Chemical Studies

^{1}H-N.M.R. spectra of freshly prepared colourless solution of gold sodium thiomalate made from solid (3.2×10^{-2}M) are shown in figure 54a. There is no significant difference in the pattern of this spectrum when compared to the yellow solution of gold sodium thiomalate (3.2×10^{-2}M) (heat sterilised for human use) as shown in figure 54b. The extra peak in figure 54a as marked by an arrow is a marker. 13C N.M.R. were not measured. The difference in intensity of the ^{1}H N.M.R. spectra is because they were measured at different gains. The spectrum of the yellow was well known, but the colourless was measured at the higher gain in order to determine whether there were any different weak features.

The ultra-violet-visible spectra of the colourless and yellow solutions are shown in figures 55(i) and 55(ii). They illustrate that although there is a reduction in the peaks at 230 and 270 nm {see arrows figure 55(i)} there is much greater absorption for the yellow solution in the 350-450 nm range {figure 55(ii)}, consistent with the colour difference of the two solutions.

In order to follow the colour change from colourless to yellow, the colourless solution (3.2×10^{-2}M) was irradiated with U.V. light at 350 nm over a prolonged time period, and optical observations were made over 3 wavelengths in the 400-500 nm regions, as shown in figure 56. The curves are consistent with a peak growing in the 450-475 nm range. After 500 minutes of irradiation the colourless solution had become dark brown. Thus, the yellow colour of the commercially available gold sodium thiomalate (myochrisine) is caused by an absorption at 450 nm.

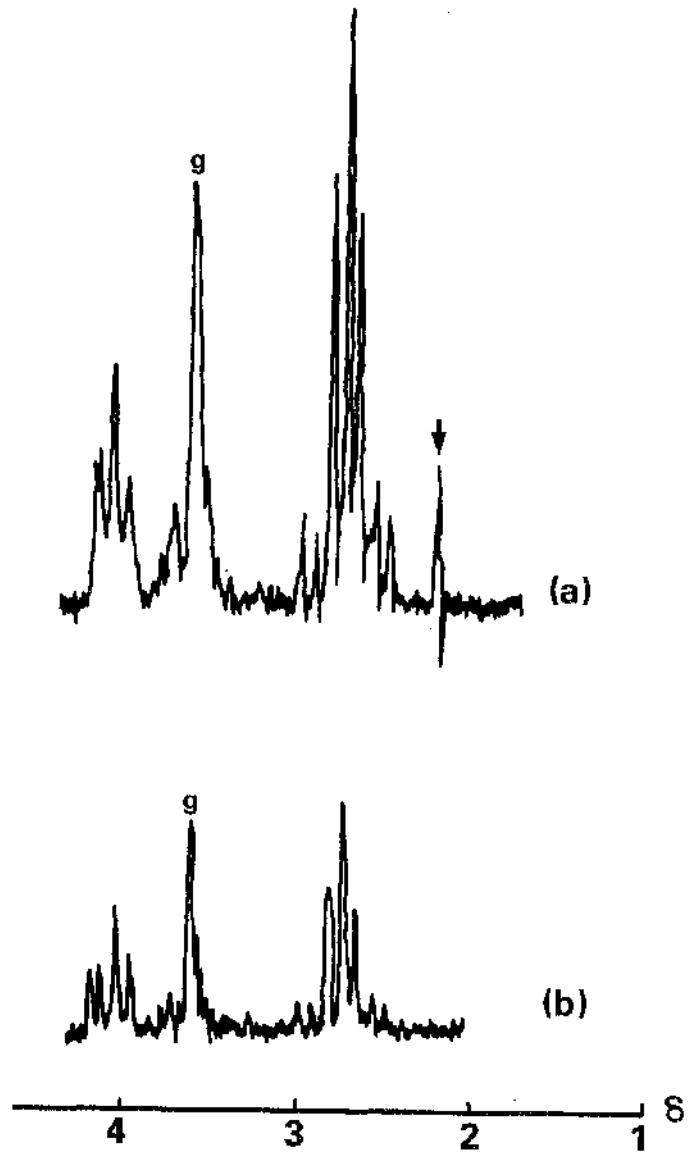


FIGURE 54: Hydrogen I nuclear magnetic resonance spectra of (a) a freshly prepared colourless solution of gold sodium thiomalate (3.2×10^{-2} M) and (b) a sample of the yellow solution of gold sodium thiomalate (3.2×10^{-2} M). There is no significant difference in the patterns of these spectra.
g = glycerol peak. arrow - is a marker
abscissa - δ is the shift downfield from tetramethylsilane in p.p.m.
ordinate - intensity

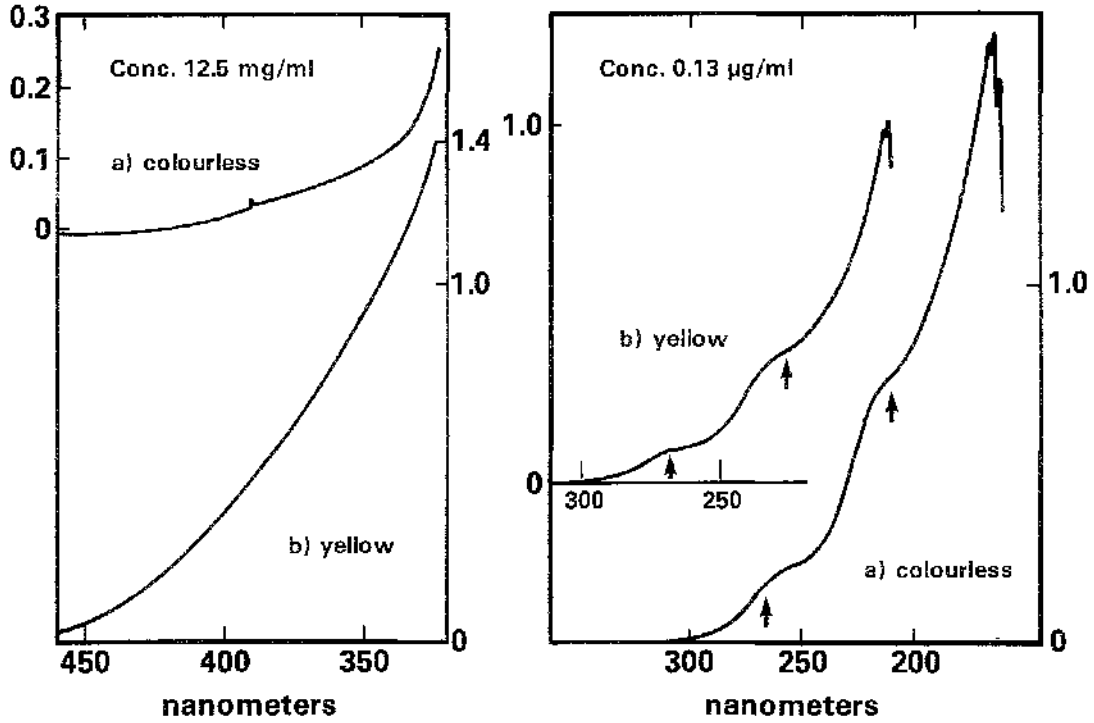


FIGURE 55: Ultra-violet-visible spectroscopy of (a) colourless and (b) yellow sodium thiomalate. Concentrations of 3.2×10^{-4}M were observed in the ultra-violet range (180-350 nm) and 3.2×10^{-2}M were observed in the visible light range (350-450 nm). Light wavelengths in nanometers (nm) is displayed on the abscissa and optical density is displayed on the ordinate.

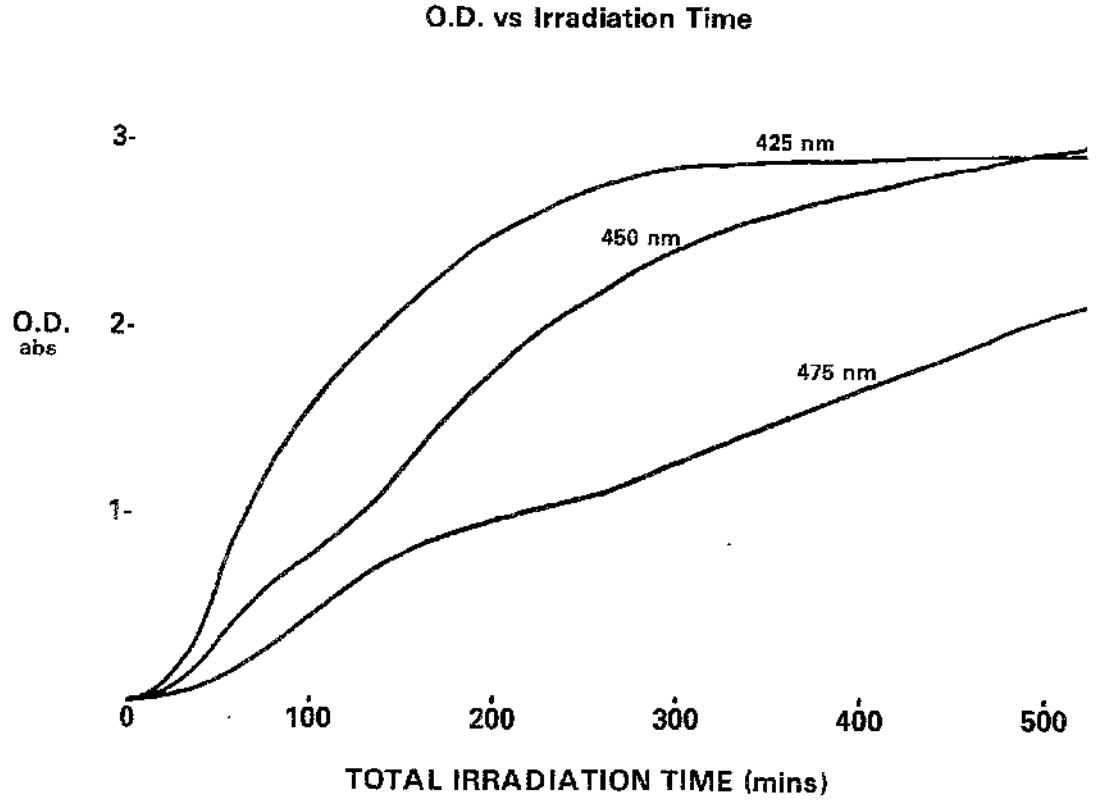


FIGURE 56: Ultra-violet irradiation (350 nm) of colourless gold sodium thiomalate over 500 minutes Observations were made at 425, 450 and 475 nm. Irradiation time is displayed on the abscissa and absorption or optical density (O.D.) is displayed on the ordinate. The appearance of these curves is consistent with a peak growing in the 450-475 nm range.

Biological Studies

(a) Mixed Lymphocyte Culture

The addition of the colourless and yellow solutions of gold sodium thiomalate to the M.L.C. cultures resulted in significant ($p < .005$) decreases in response. The data with colourless gold sodium thiomalate solution are given in figure 57 and with the yellow solution of gold sodium thiomalate are given in figure 58. The average percent inhibition in stimulation ratios (SR) with the colourless solution were 29% and 58% with 3.8×10^{-5}M and with 7.6×10^{-5}M respectively. The percent inhibition with the yellow solution were 18% and 44% at these concentrations.

The percent inhibition in M.L.C. with varying concentrations of the colourless and yellow solution of gold sodium thiomalate (3.8×10^{-5} - 3.0×10^{-4}M) are given in figure 59. It is evident that the percent inhibition increased with increase in amount of gold in cultures (except for colourless gold sodium thiomalate at 3.0×10^{-4}M, which produced a lower percentage inhibition than 1.5×10^{-4}M of the colourless solution (figure 59)).

(b) Platelet Studies

Thrombin Inhibition

The serine esterase thrombin which converts fibrinogen to fibrin will cause platelet aggregation and release of ^{14}C-serotonin when added to washed human platelets. Both the colourless and yellow solutions of gold sodium thiomalate in a concentration range of

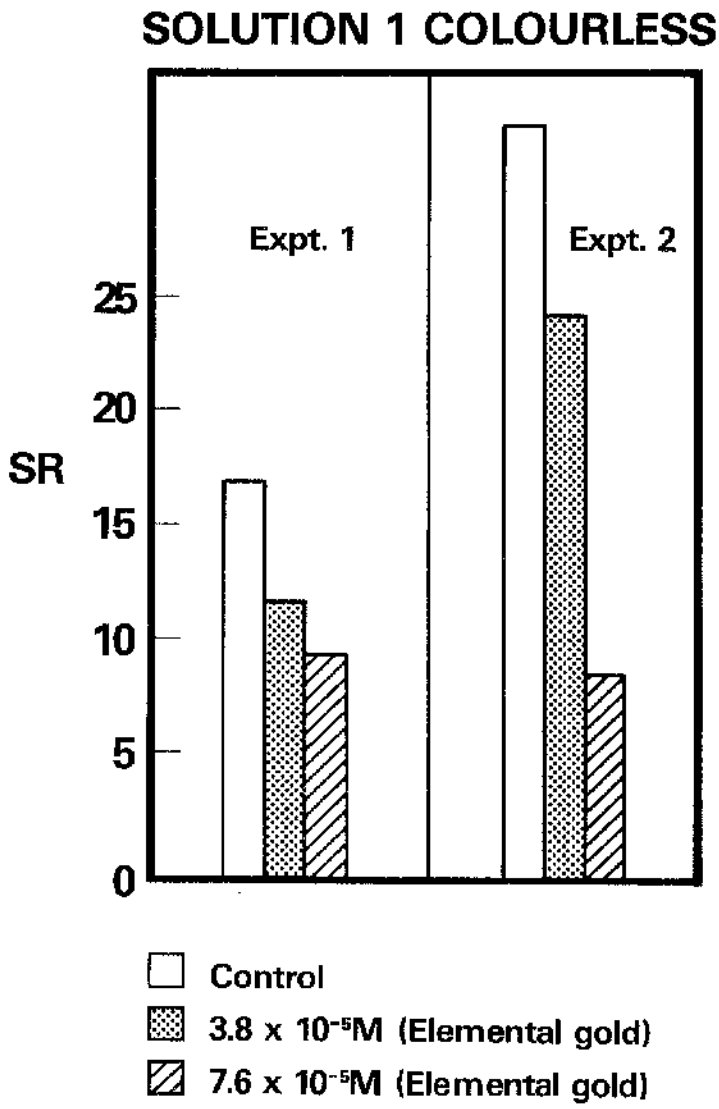


FIGURE 52: The effect of colourless gold sodium thiomalate on the mixed lymphocyte culture (M.L.C.) reaction. The data from two experiments are given in this figure. The results are expressed as stimulation ratio (S.R.) on the ordinate. The concentrations are expressed as elemental gold.

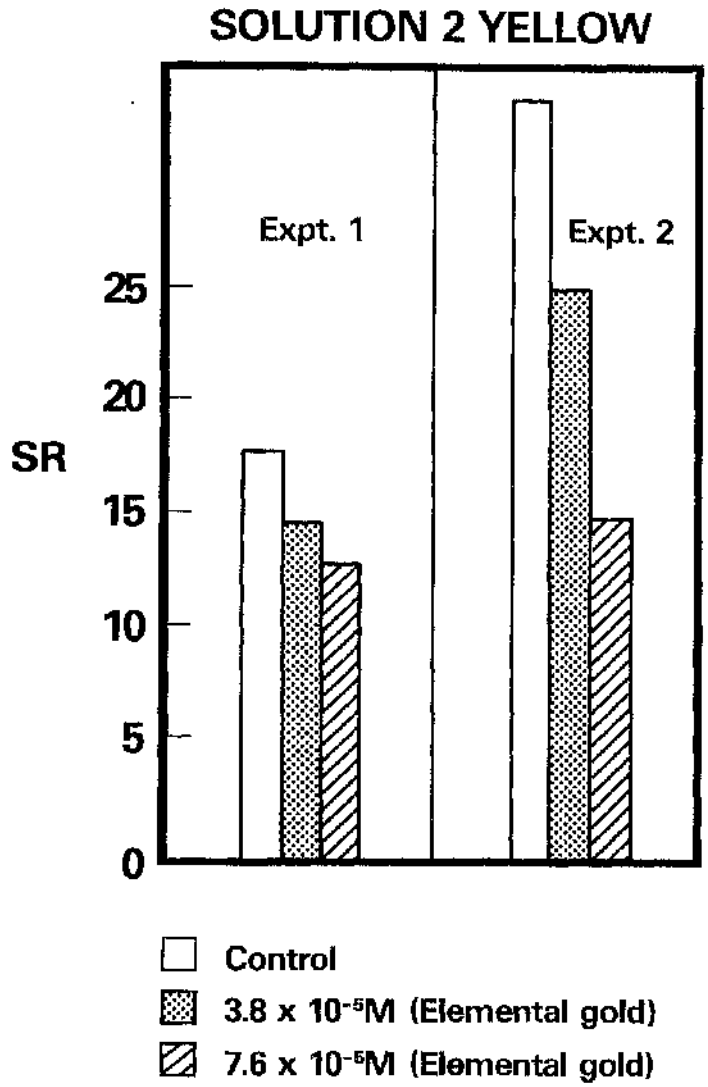


FIGURE 58: The effect of yellow gold sodium thiomalate on the mixed lymphocyte culture reaction. The data from two experiments are given in this figure. The results are expressed as stimulation ratios (S.R.) on the ordinate. The concentrations are expressed as elemental gold.

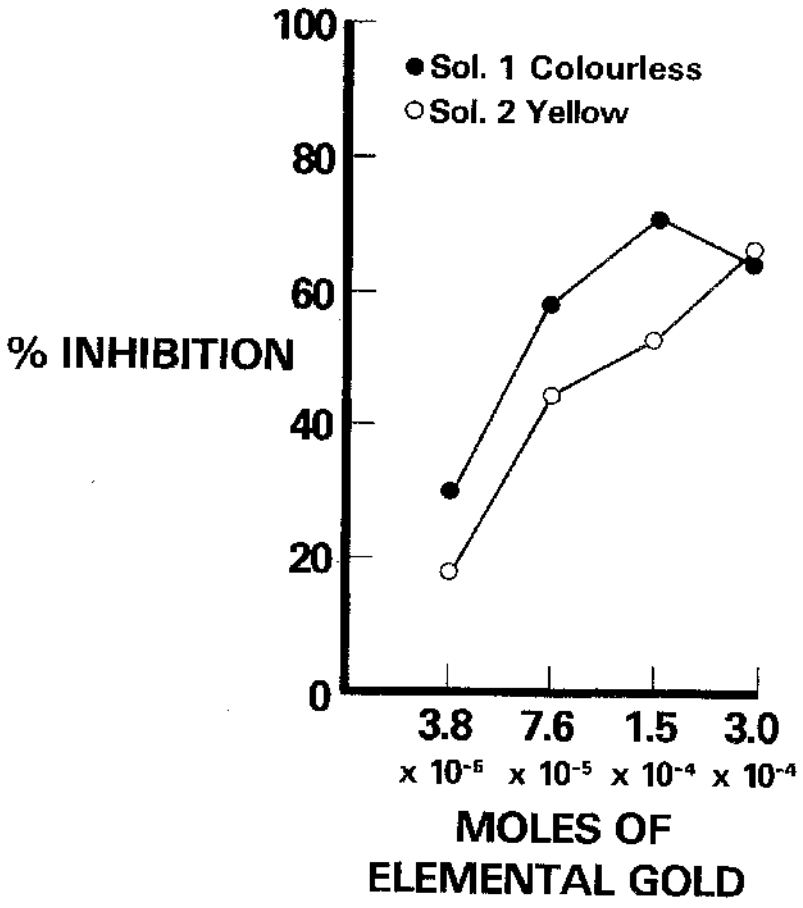


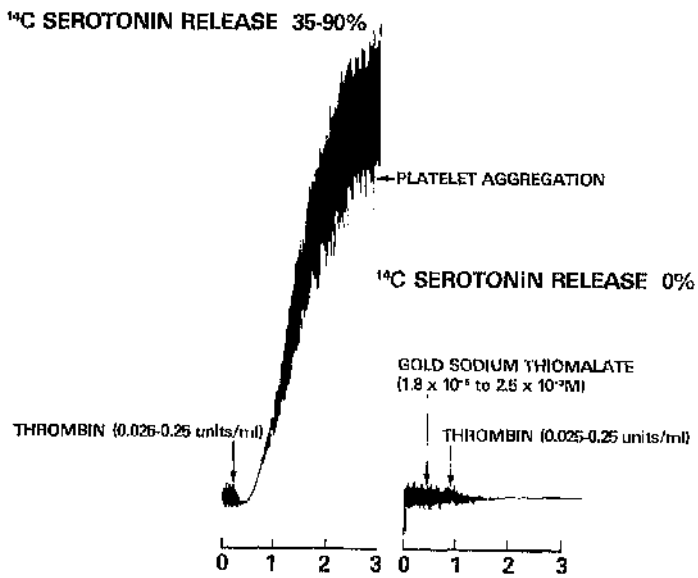
FIGURE 59: The effect of varying concentrations of gold sodium thiomalate (colourless and yellow solutions) on the mixed lymphocyte culture reaction. The data are expressed as percent inhibition on the ordinate and as the concentration of elemental gold per millilitre of culture on the abscissa.

$1.8x10^{-5}$ - $2.5x10^{-3}$M inhibited the action of bovine thrombin (0.025-0.25 units/ml) on 1 ml of washed human platelets if added to the platelet suspension prior to the addition of the thrombin (figure 60) or simultaneously with the thrombin. (These effects will be further discussed in Chapter VIII).

Platelet Aggregation Studies

The yellow solution of gold sodium thiomalate ($1.3x10^{-3}$ - $6.4x10^{-3}$M as elemental gold) added to washed human platelets resulted in shape change, within 30 seconds followed by platelet aggregation and release of internal granule contents as measured by ^{14}C-serotonin radioactivity (figure 61). De-aggregation of platelets usually occurred within 2 to 4 minutes (figure 61). Due to the wide biological variability demonstrated by the platelets, ^{14}C-serotonin release values could not be compared between individual samples obtained from different volunteers. However, within samples, ^{14}C-serotonin release values, paralleled the increase in concentration of the yellow solution of gold thiomalate. The usual values for ^{14}C-serotonin release were between 5% and 20% for the gold concentrations, $1.3x10^{-3}$ - $6.4x10^{-3}$M (figure 61). Platelet lysis as measured by ^{51}Cr release did not occur within this concentration range of gold. Platelet aggregation was not observed for gold concentrations of the yellow solution of gold sodium thiomalate less than $1.3x10^{-3}$M.

When a range of concentration of the colourless solution of gold sodium thiomalate ($1.8x10^{-5}$ - $6.4x10^{-3}$M) was added to washed human platelets absolutely no reactivity of the platelets was observed (figure 61).



Thrombin (0.025-0.25 units/ml) added to washed human platelets causes platelet aggregation and release of ^{14}C-serotonin. Prior addition of Gold Sodium Thiomalate (1.8 x 10^{-5} to 2.5 x 10^{-3}M)* inhibits the action of thrombin (0.025-0.25 units/ml) on washed human platelets.

* GOLD SODIUM THIOMALATE values are expressed as moles of elemental gold.

FIGURE 60: Thrombin (0.025-0.25 units/ml) induced platelet aggregation, with 35%-90% release of ^{14}C-serotonin. Prior addition of either colourless or yellow gold sodium thiomalate (1.8×10^{-5} - 2.5×10^{-3}M as elemental gold) blocks the aggregation of washed human platelets by thrombin and also the release of ^{14}C-serotonin.

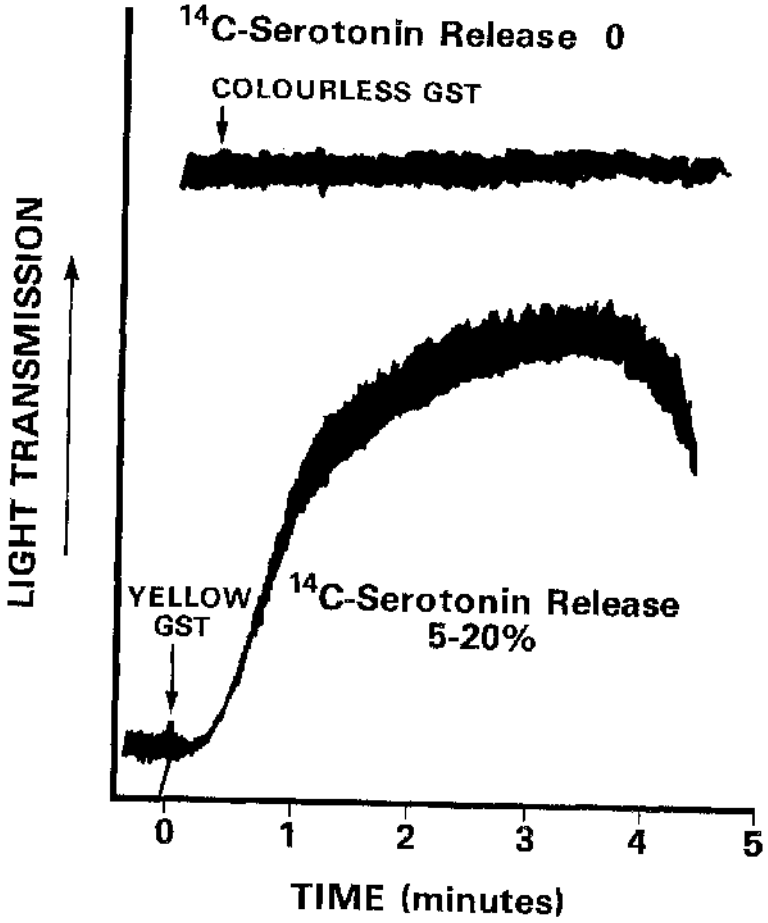


FIGURE 61: The addition of the yellow solution of gold sodium thiomalate (yellow G.S.T.) (1.3×10^{-3} - 6.4×10^{-3}M) to washed human platelets causes platelet shape change within 30 seconds followed by platelet aggregation and release of ^{14}C-serotonin. Deaggregation of platelets occurs within 2-4 minutes.
The addition of colourless gold sodium thiomalate (colourless G.S.T.) (1.8×10^{-5} - 6.4×10^{-3}M) added to washed human platelets does not result in any aggregation nor release reaction.

Similarly, gold thioglucose and gold sodium thiosulphate in equimolar concentrations of elemental gold ($1.8x10^{-5}$ - $6.4x10^{-3}$M) and disodium thiomalate in equimolar concentrations of thiomalate to the concentrations of thiomalate in gold sodium thiomalate ($1.8x10^{-5}$ - $6.4x10^{-3}$M) did not cause platelet aggregation nor release of internal granules.

2,3,4,6-tetra-o-acetyl-1-thio-β-D-glucopyranosato-S-(triethylphosphine) gold (Auranofin) in the concentration range $6.4x10^{-7}$ - $5.1x10^{-5}$M did not cause platelet aggregation nor release of internal granules as measured by ^{14}C-serotonin radioactive counting (figure 62). Platelet shape change did occur within 30 seconds following the addition of $5.1x10^{-5}$M of Auranofin, but no aggregation or release occurred (figure 62). When a gold concentration of Auranofin ($5.1x10^{-4}$M) which is greatly in excess of the pharmacological serum concentrations achieved during gold therapy ($6.4x10^{-7}$ - $4.1x10^{-5}$M) was added to the platelets, shape change took place over 3 minutes, followed by a light transmission pattern consistent with platelet clumping (figure 62). ^{51}Cr release did not indicate any evidence of lysis.

Mechanism of Platelet Aggregation by the
Yellow Solution of Gold Sodium Thiomalate

Creatine phosphate/creatine phosphokinase (CP/CPK) converts adenosine diphosphate (ADP) to adenosine tri-phosphate (ATP) (225). Prior addition of 100 µl of CP/CPK to the washed human platelet suspensions blocks the platelet aggregation induced by the yellow solution of gold sodium thiomalate ($1.3x10^{-3}$ - $6.4x10^{-3}$M). Adenosine

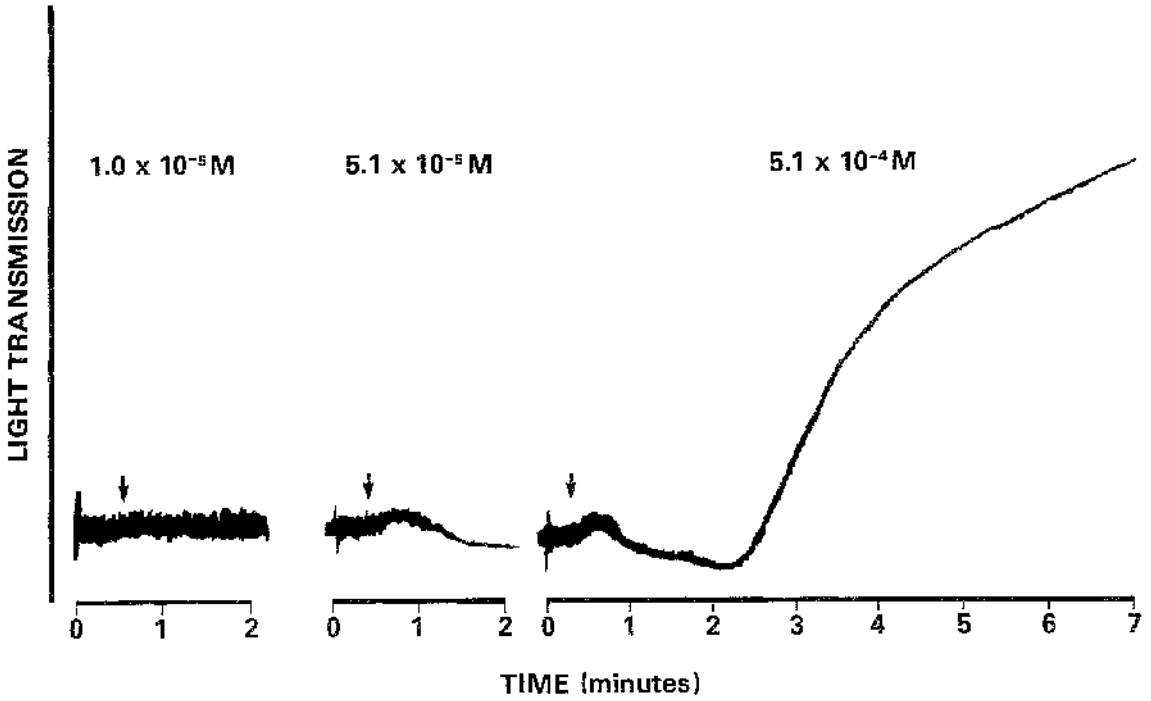


FIGURE 62: The Effect of 2,3,4,6-tetra-o-acetyl-1-thio-β-D-glucopyranosato-S-(triethylphosphine) gold (Auranofin) on Washed Human Platelets.

Auranofin concentrations less than $5.1x10^{-5}M$ (as elemental gold) had no effect on washed human platelets. At a concentration of $5.1x10^{-5}M$, shape change took place but no release. At concentrations of $5.1x10^{-4}M$ shape change occurred over 3 minutes followed by a pattern consistent with platelet clumping.

monophsphate (A.M.P.) is a competitive inhibitor of ADP induced platelet aggregation (226). Prior addition of adenosine monophosphate (A.M.P.) in excess (10^{-3}M) to a washed human platelet suspension blocks the effect of the yellow solution of gold sodium thiomalate (1.3×10^{-3} - 6.4×10^{-3}M) on platelet aggregation. Thus when A.D.P. is eliminated by CP/CPK or competitively blocked from its platelet surface membrane receptor by excess A.M.P., platelet aggregation induced by the yellow solution of gold sodium thiomalate does not occur. A synergistic effect on the aggregation of washed human platelets was observed for subaggregating concentrations of A.D.P. (5×10^{-6}M) added to subaggregating concentrations of the yellow solution of gold sodium thiomalate (2.5×10^{-4} - 1.3×10^{-3}M) (figure 63). No such synergistic effect was noted for the colourless solution of gold sodium thiomalate and ADP.

Thus the yellow solution of gold sodium thiomalate causes platelet aggregation, whereas the colourless solution of gold sodium thiomalate has no effect on platelets. Since the platelet aggregation and ^{14}C-serotonin release induced by the yellow solution can be blocked by agents which prevent the action of ADP on platelets, and since ADP and the yellow solution of gold sodium thiomalate act synergistically on platelet aggregation, it appears that the aggregation of washed human platelets by the yellow solution of gold sodium thiomalate is ADP dependent.

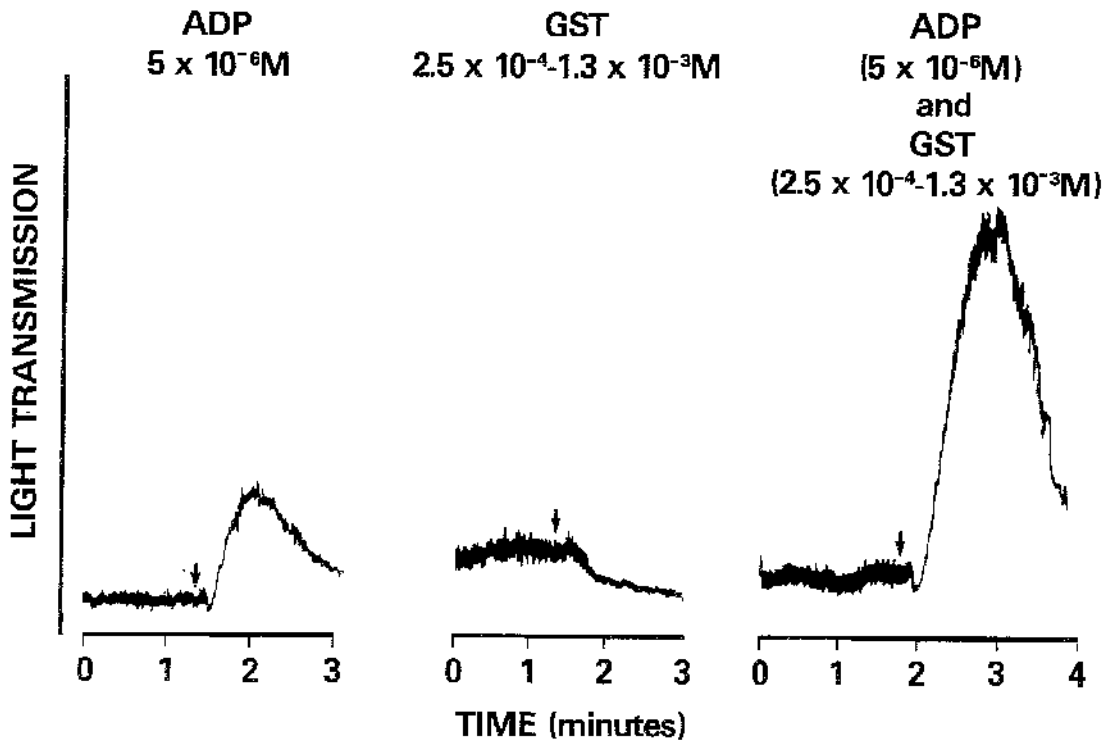


FIGURE 63: The yellow solution of Gold Sodium Thiomalate (GST) in subaggregating concentrations ($2.5x10^{-4}$ - $1.3x10^{-3}$M as elemental gold) produces a synergistic aggregation of washed human platelets with subaggregating concentrations of adenosine diphosphate (ADP - $5x10^{-6}$M).

(c) Electron Microscopy and Energy Dispersive Analysis

Electron micrographs of platelets treated with yellow gold sodium thiomalate solution ($1.3x10^{-3}$ - $6.4x10^{-3}$M) had numerous pseudopodia and were consistent with platelets which had undergone aggregation (figure 64). Fibrillar gold containing particles 100-700 nm in length were identified: (1) in the process of being phagocytosed; (2) within intracellular vesicles; and (3) in the surrounding medium. Energy dispersive spectroscopic analysis revealed the presence of gold and sulphur within these particles (figure 65). In figure 65 the gold is identified as the Lα peak at 9.627-9.712 KEV and the $M^{\alpha\beta}$ peak at 2.125-2.211 KEV. The M peak spans 1.664-2.806 KEV which overlaps the phosphorous peak (K at 2.013-2.146 KEV) and the sulphur peak (K at 2.307-2.474 KEV). Based on the intensity of x-ray counts obtained over 445 live-seconds it was shown that sulphur was present in significant amounts above the background level, but no phosphorous. In contrast, platelets treated with the colourless solution of gold sodium thiomalate ($1.8x10^{-5}$ - $6.4x10^{-3}$M) were oval in shape and did not appear to have aggregated. These platelets contained numerous scattered particles < 40 nm in diameter which were distinct from glycogen granules (figure 66). Occasional particles were membrane bound. Energy dispersive spectroscopic analysis confirmed that these particles also contained gold and sulphur (figure 67). No gold was detected in platelets treated with gold thioglucose, gold sodium thiosulphate, Auranofin or disodium thiomalate (figure 68-73)

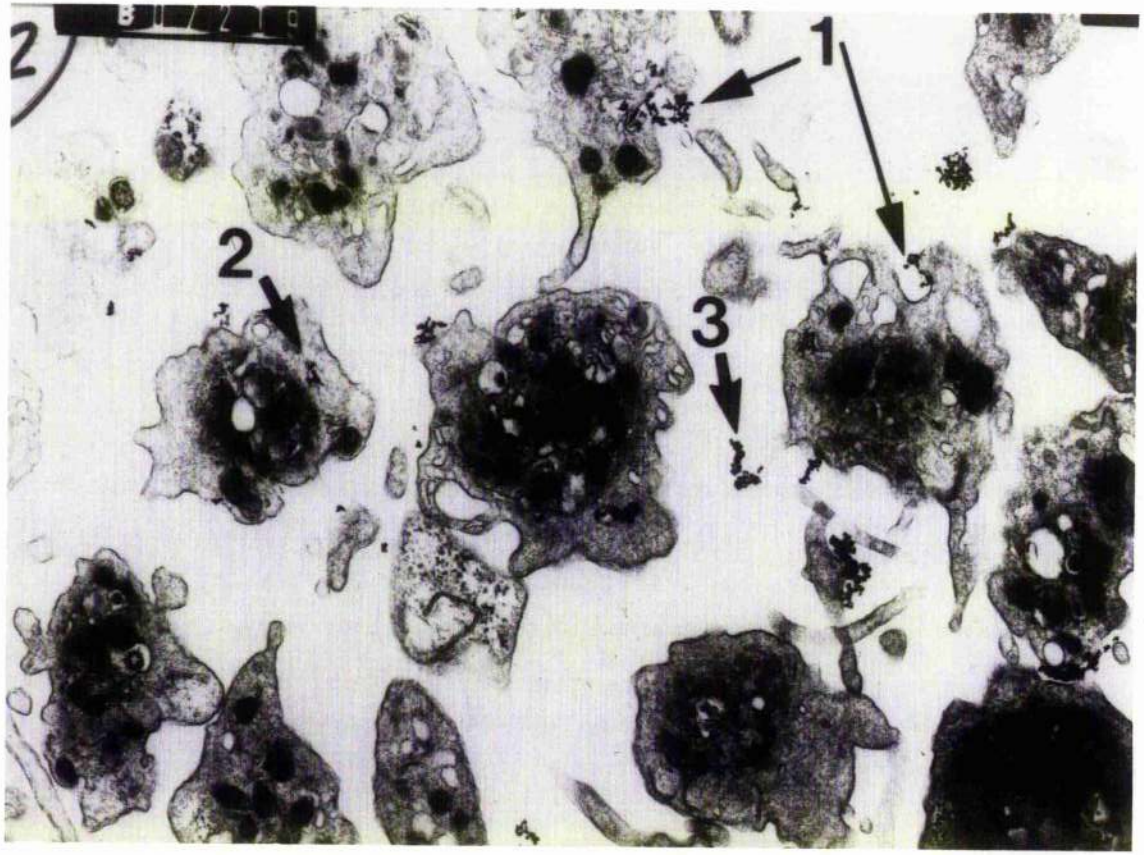
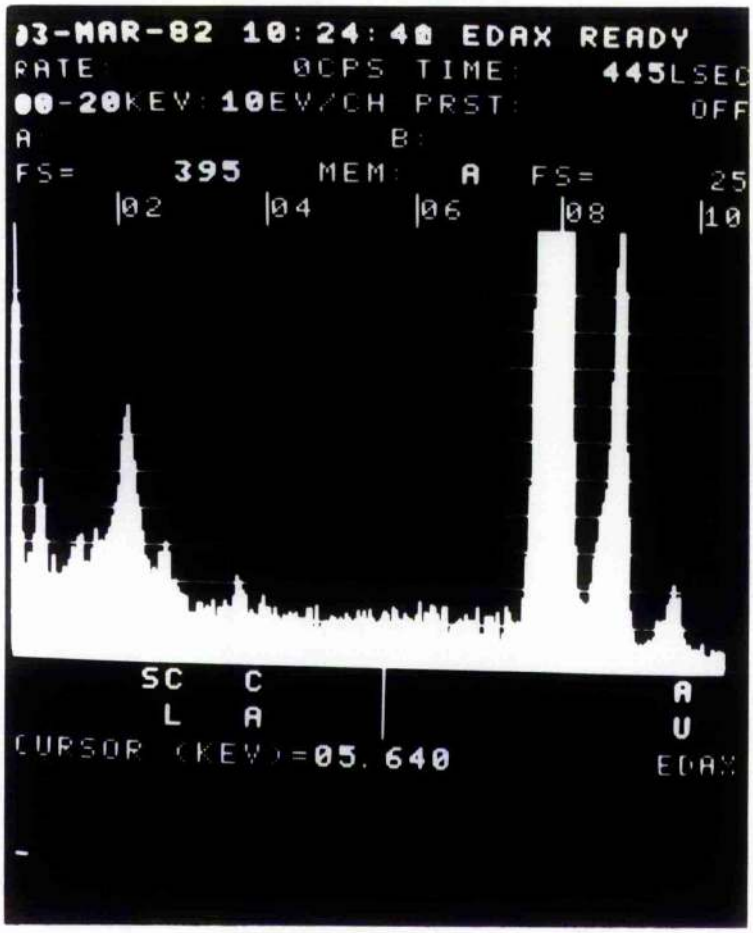


FIGURE 64: Electron micrograph (magnification x 7000) of washed human platelets treated with the yellow solution of gold sodium thiomalate (2.5×10^{-3}M as elemental gold). Particulate material 100-700 nm in length is seen (1) being phagocytosed (2) within intracellular vesicles and (3) in the surrounding media.



AUROTHIOMALATE
(yellow)

FIGURE 65: Energy dispersive spectroscopic analysis of the particulate matter seen in washed human platelets treated with the yellow solution of gold sodium thiomalate (2.5×10^{-3}M as elemental gold), (see figure 64). The graphs show 2 gold peaks. (a) Lα at 9.627-9.712 KEV and (b) Mαβ at 2.125-2.211 KEV (N.B. the M peak spans 1.664-2.806 KEV which overlaps the phosphorous peak (K at 2.013-2.146 KEV) and the sulphur peak (K at 2.307-2.474 KEV).

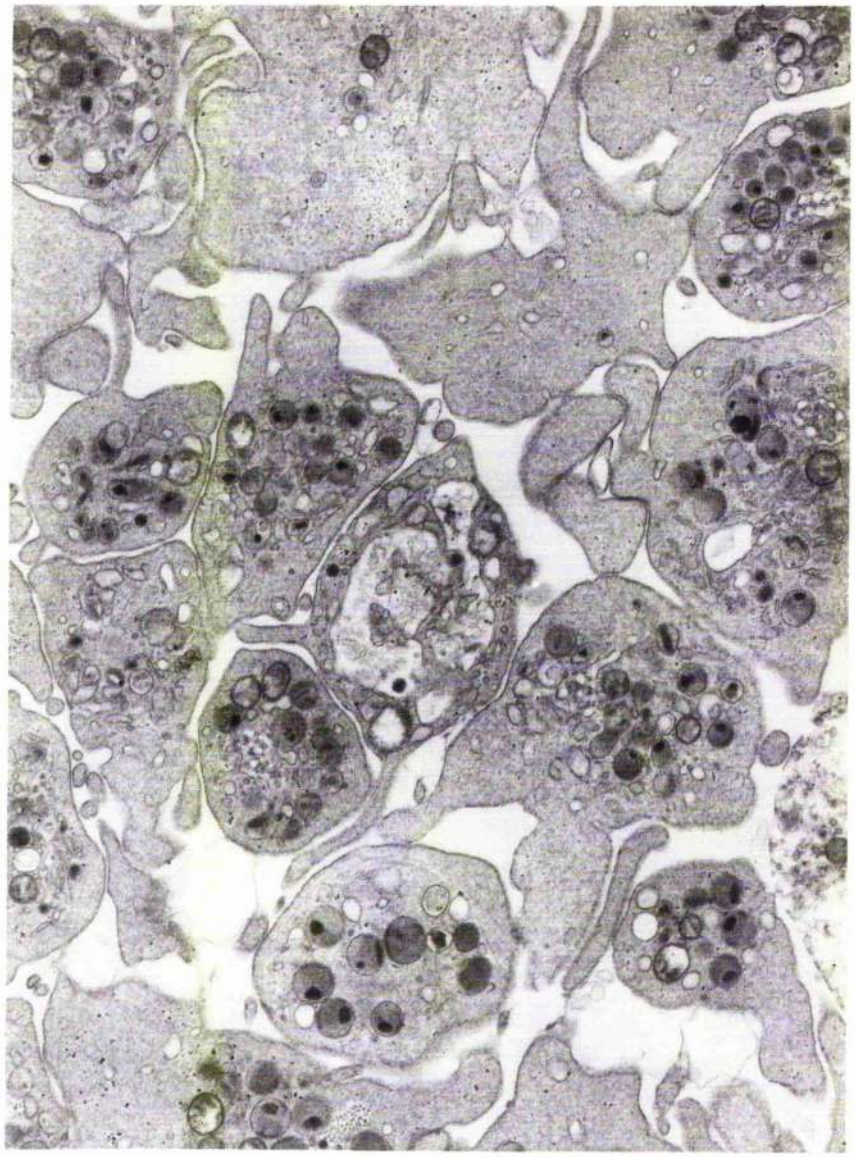
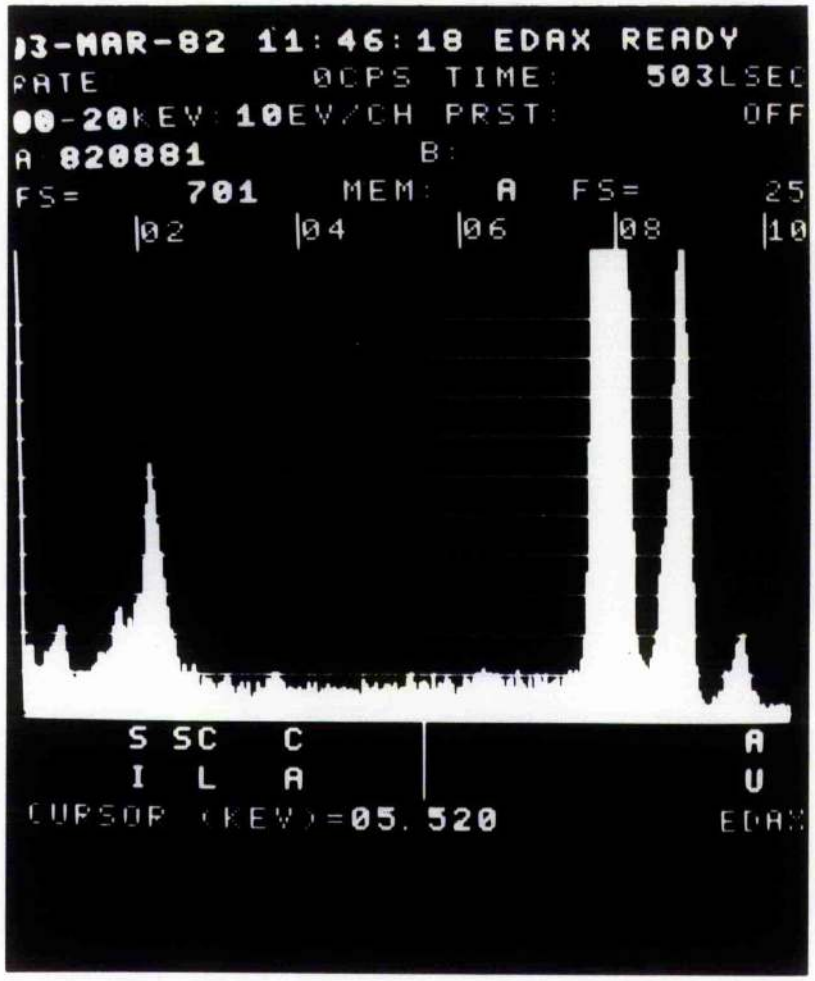


FIGURE 66: Electron micrograph (magnification x 11,000) of washed human platelets treated with colourless gold sodium thiomalate (2.5×10^{-3}M as elemental gold). Numerous scattered particles less than 40 nm in diameter are seen within the platelets. Some of these particles are membrane bound, (see platelet in centre of figure).



AUROTHIOMALATE
(colourless)

FIGURE 67: Energy dispersive spectroscopic analysis of the particulate matter (< 40 nm) seen within washed human platelets treated with colourless gold sodium thiomalate (2.5×10^{-3}M as elemental gold), (see figure 66). The graph shows two gold peaks. (a) Lα at 9.627-9.712 KEV and (b) Mαβ at 2.125-2.211 KEV. (N.B. the M peak spans 1.664-2.806 KEV which overlaps the phosphorous peak (K at 2.013-2.146 KEV) and the sulphur peak (K at 2.307-2.474 KEV).

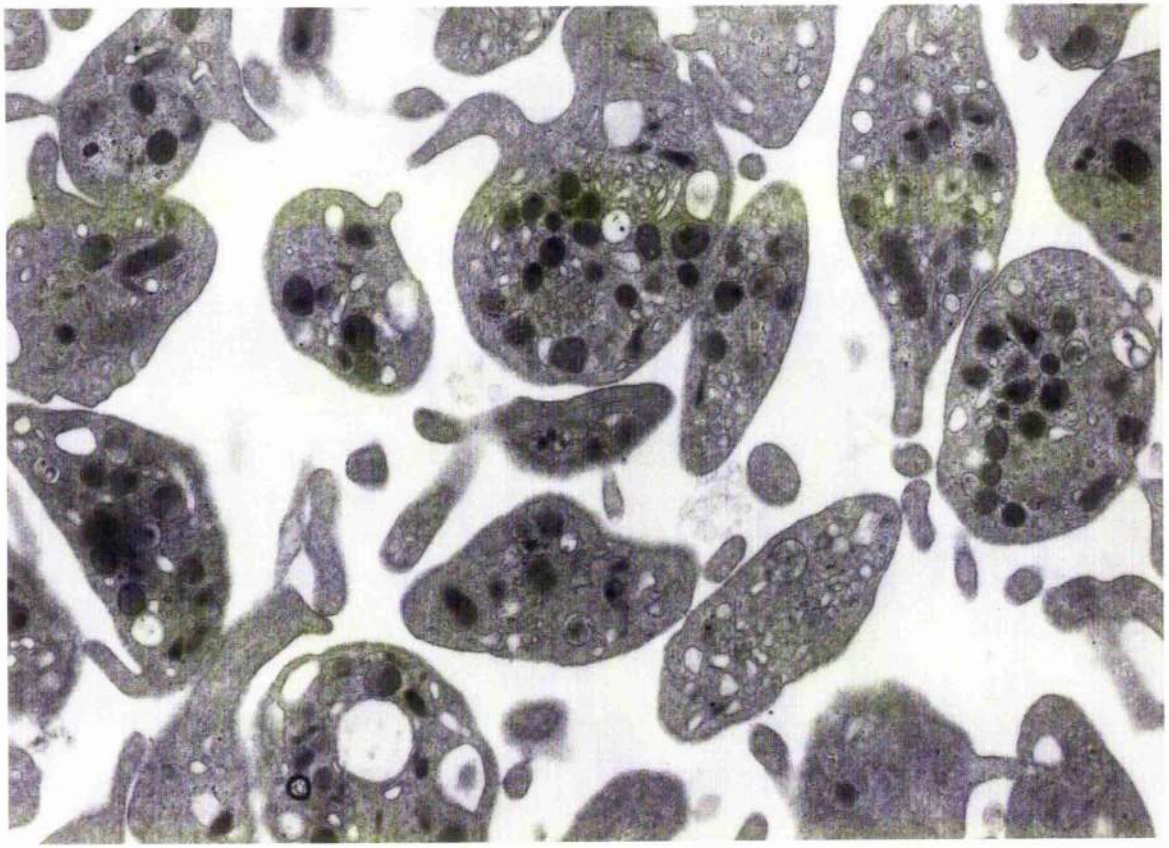
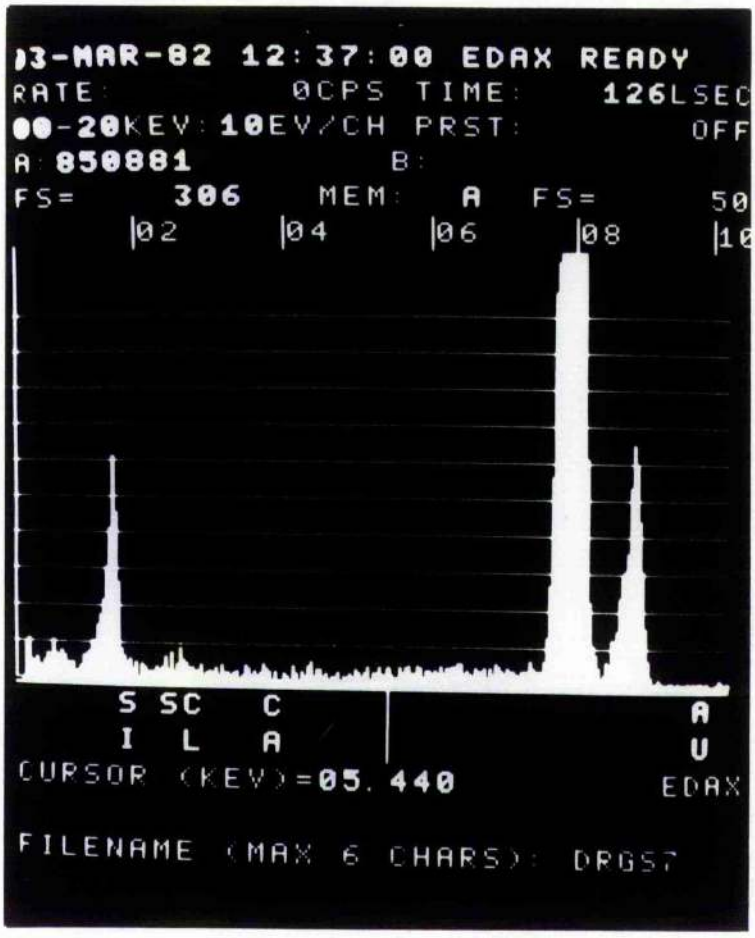


FIGURE 68: Electron micrograph (magnification x 11,000) of washed human platets treated with gold thioglucose (2.5×10^{-3}M as elemental gold). No particulate matter other than normal structures was detected within these platelets.



AUROTHIOGLUCOSE

FIGURE 69: Energy dispersive spectroscopic analysis of the organelles of washed human platelets treated with gold thioglucose (2.5×10^{-3}M as elemental gold, see figure 68). The presence of gold was not detected within any of the platelets.

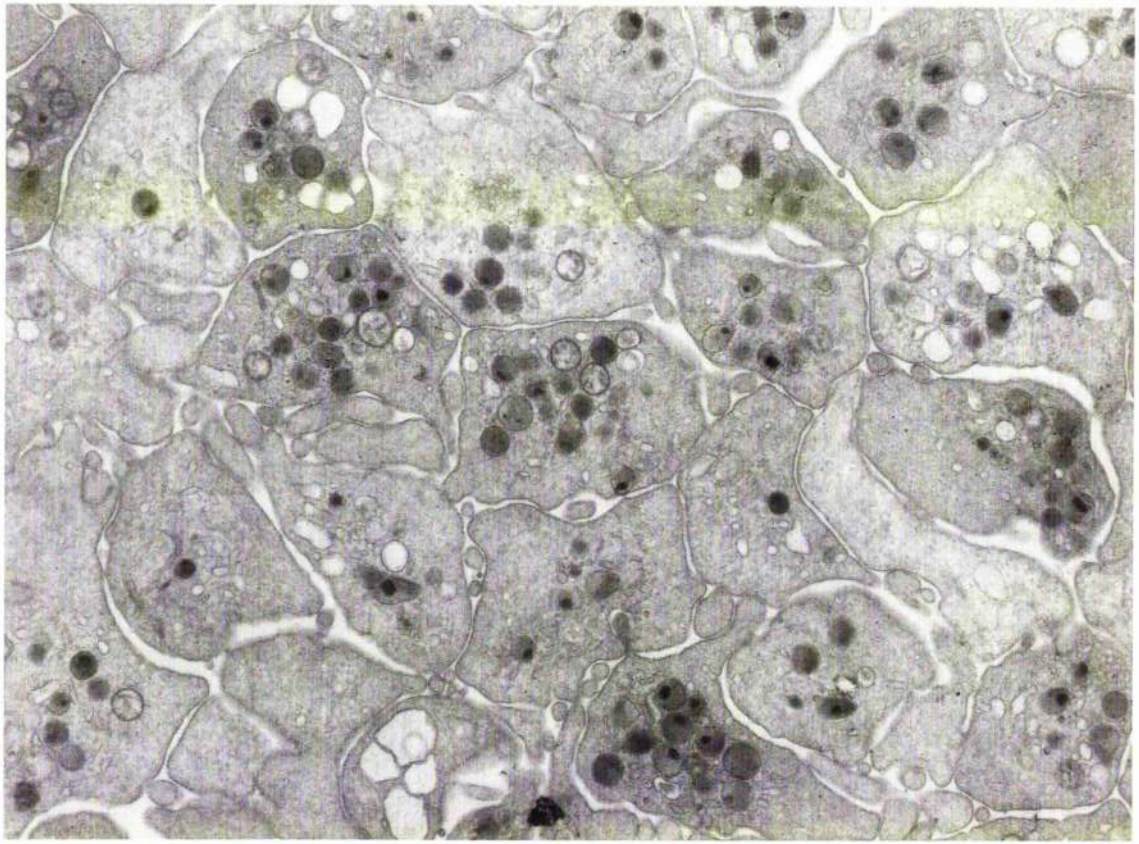
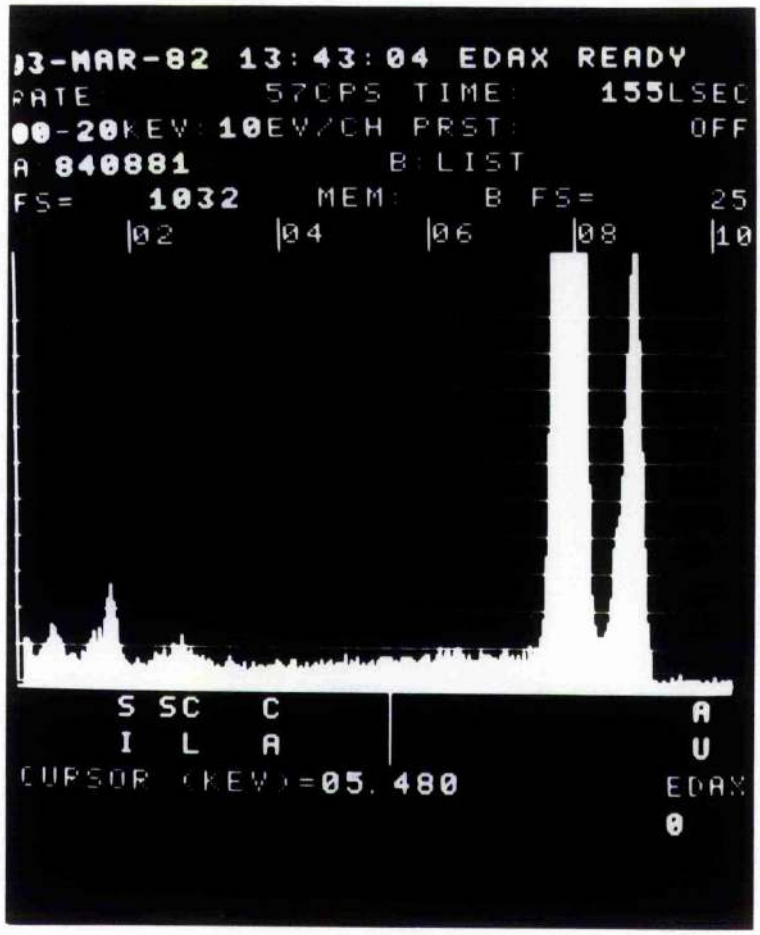


FIGURE 70: Electron micrograph (magnification x 9,100) of washed human platelets treated with gold sodium thiosulphate (2.5×10^{-3}M as elemental gold). No particulate matter other than normal structures was detected within these platelets.



AUROTHIOSULPHATE

FIGURE 71: Energy dispersive spectroscopic analysis of the organelles of washed human platelets treated with gold sodium thiosulphate (2.5×10^{-3}M, as elemental gold, see figure 70). The presence of gold was not detected within any of these platelets.

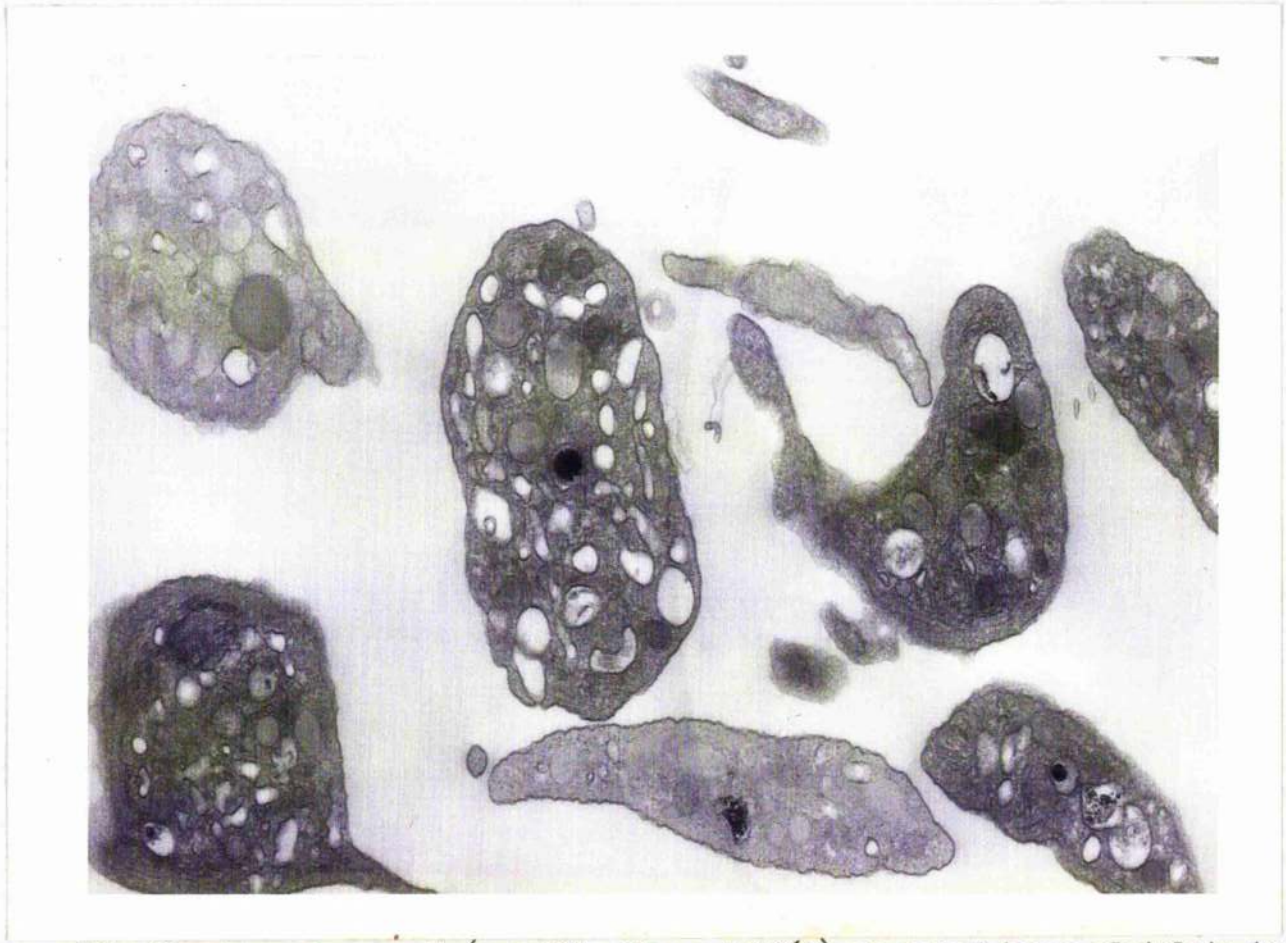
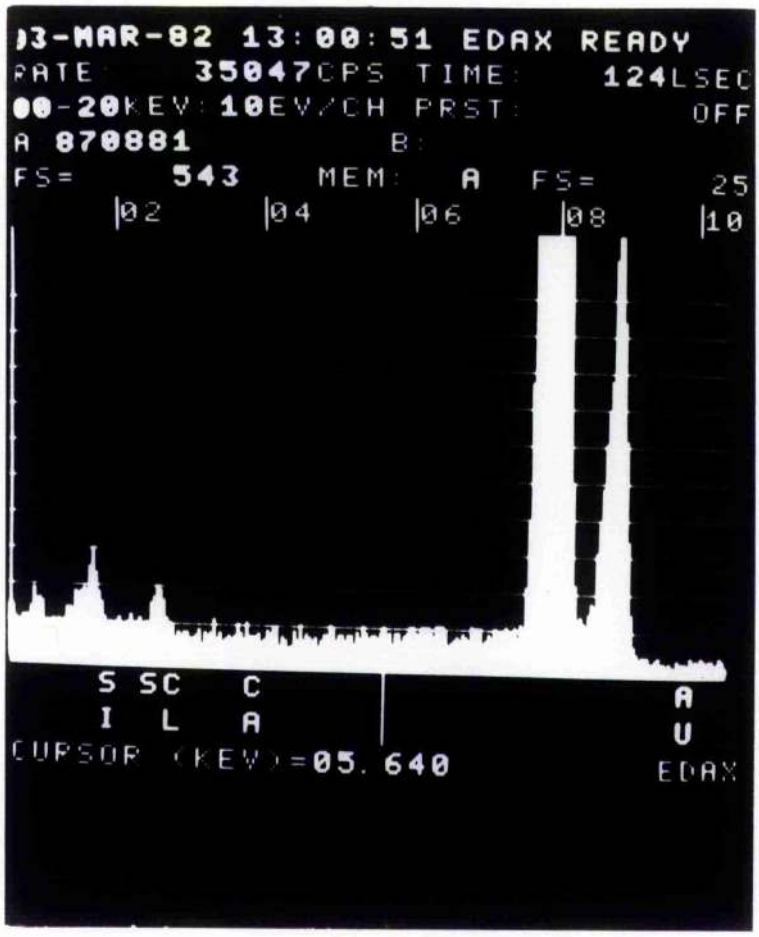


Figure 72: Electron micrograph (magnification x 15,960) of washed human platelets treated with 2,3,4,6-tetra-o-acetyl-1-thio-β-D-glucopyranosato-S-(triethylphosphine) gold (Auranofin, 5.1×10^{-5}M, as elemental gold). No particulate matter, other than normal structures was detected within these platelets.



AURANOFIN

FIGURE 73: Energy dispersive spectroscopic analysis of the organelles of washed human platelets treated with 2,3,4,6-tetra-o-acetyl-1-β-D-glucopyranosato-S-(triethylphosphine) gold (Auranofin, $5x10^{-5}$M as elemental gold, see figure 72). The presence of gold was not detected within any of these platelets.

DISCUSSION

For approximately seven years, I have, in common with most rheumatologists, frequently administered gold sodium thiomalate intramuscularly to patients with rheumatoid arthritis. I was thus well acquainted with the familiar yellow colour of the gold sodium thiomalate solution as supplied commercially. It was thus surprising that, in the course of my laboratory research work, I noted that the gold sodium thiomalate solution supplied from the same commercial source (Rhone-Poulenc, Quebec) was colourless. Enquiries revealed that the yellow colour resulted from the heat sterilisation process used to prepare the solution for human clinical use, and that the pharmaceutical industry, although aware of this physical change, did not have any chemical explanation for it. I determined to investigate the chemical basis for this change. I was fortunate, that in the resultant researches I had the expert guidance of Professor Colin J.L. Lock and the technical expertise of his colleagues and staff.

When solid (white powder) gold sodium thiomalate is added to sterile water, to a concentration of 0.13M (as elemental gold) a colourless solution is produced. If this solution is heated at 100^{o}C for 30 minutes as in the standard sterilisation procedure for marketing, a yellow solution results. It is this yellow solution which is available for human use in the treatment of rheumatoid arthritis. The experiments reported in this chapter have established

that not only heating at 100°C for 30 minutes, but ultra-violet irradiation with a 350 nm wavelength results in the change from a colourless to a yellow solution for gold sodium thiomalate in sterile water. The change is accompanied by a decrease in intense ultra-violet absorptions at 230 nm and 270 nm (figure 55) and the formation of a very weak peak in the visible region at 450 nm (figure 56) as a shoulder on the ultra-violet band. The yellow species appears to be a minor component since no significant change can be detected in the ^{1}H spectra. However, long term ultra-violet irradiation of the colourless solution for either 500 minutes at 350 nm or 750 minutes with shorter wavelength light (300 nm) results in a dark brown solution. The ^{1}H N.M.R. spectra of the sample after 750 minutes of ultra-violet irradiation at 300 nm are different (figure 74) and consistent with a significant change in the ligand.

Dr. Peter Sadler of Birkbeck College, University of London, U.K. has reported (210) the separation of a yellow solution phase made by treating gold sodium thiomalate solution with sodium chloride. While visiting McMaster University last year, Dr. Sadler offered several points of good advice and suggested privately that his yellow solution may be identical to the yellow material produced by heat and ultra-violet light. So far I have been unable to reproduce the separation process described in his report (210) and thus have been unable to test the hypothesis.

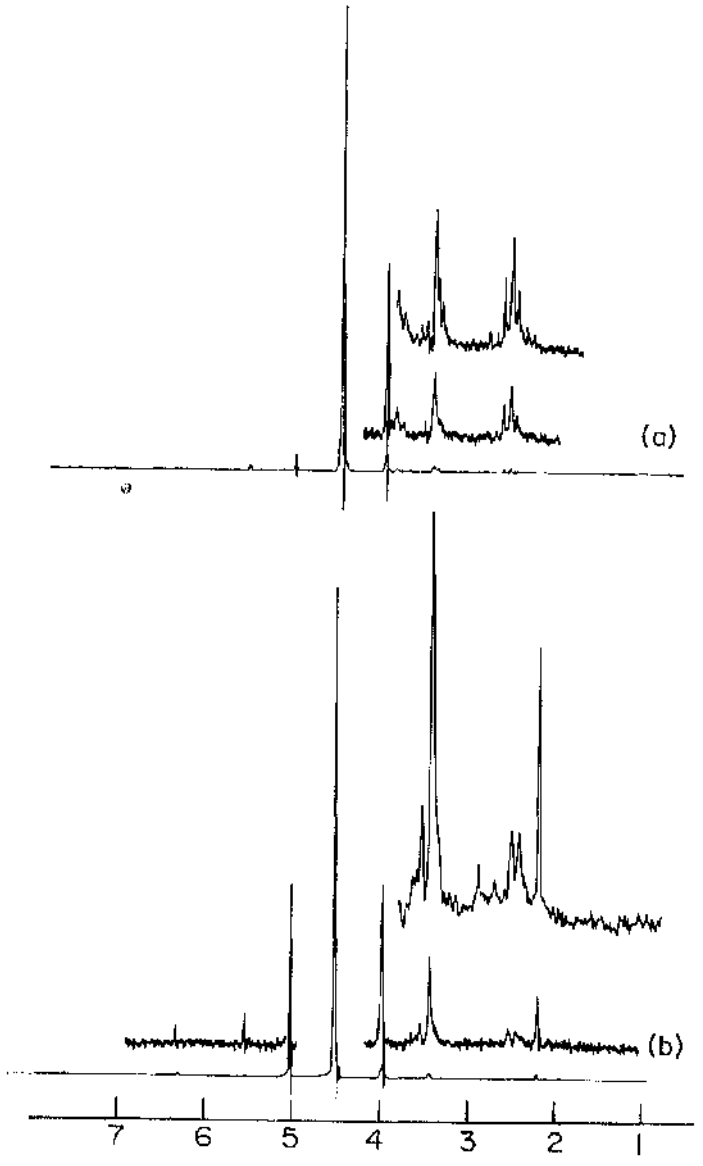


FIGURE 74: Hydrogen-1-nuclear magnetic resonance spectra of (a) colourless gold sodium thiomalate (0.13M) and (b) the same sample after ultra-violet irradiation (300 nm) for 750 minutes.

Intensity is measured on the ordinate. δ, the downfield shift in p.p.m. from tetramethylsilane, is measured on the abscissa. The difference in the patterns of these spectra are consistent with a significant change in the ligand.

Gold sodium thiomalate has been marketed commercially for clinical use for more than 50 years (87). At the time of its introduction, although this colour change in gold sodium thiomalate must have been observed, available chemical expertise was probably insufficient to explain the molecular basis for the change. It can only be presumed that familiarity with this colour change explains the failure of the chemists in the pharmaceutical industry to investigate this further until the present time. The research work in this chapter has rekindled their interest and in addition to the results reported here, many further extensions of this work are planned or are in an early stage of development. These further studies have shown that indeed not only is the yellow solution of gold sodium thiomalate a mixture but that the original solid (white powder) can be separated into two components: one which results in a colourless solution when mixed with sterile water and one which results in a yellow solution when mixed with sterile water. If both of these solutions are heated, the colourless solution remains colourless and the yellow solution becomes a deeper yellow. Full reporting of these details are outside the scope of this thesis particularly as the chemical extraction processes involved are the subject of a patent application by McMaster University on behalf of Professor Colin J.L. Lock, Miss Debra A. Harvey (Ph.D. student) and myself.

In the biological studies the effect of the colourless and yellow solution of gold sodium thiomalate on the mixed lymphocyte culture were consistent with the findings of previous authors (214-217), namely that gold sodium thiomalate over a range of concentrations added on day one of the mixed lymphocyte culture, resulted in inhibition of the mixed lymphocyte response. Thus, the yellow component present in the commercially available gold sodium thiomalate preparation is not the active component responsible for the inhibition of the mixed lymphocyte response and clearly any other changes induced by the heat sterilisation process during marketing (100°C for 30 minutes) are not necessary for the inhibiting effect of gold sodium thiomalate on lymphocytes.

Both the colourless and yellow solutions of gold sodium thiomalate inhibited the effect of the serine esterase thrombin on washed human platelets. (This will be further discussed in Chapter VIII). As with the inhibition of the mixed lymphocyte response, it is evident that the yellow component is not necessary for the inhibition of the serine esterase thrombin.

Electron microscopy and platelet aggregation studies illustrated that at least one difference between the colourless and yellow solutions of gold sodium thiomalate was that the yellow solution contained a particulate component 100-700 nm in diameter, whereas the colourless solution contained particles of less than 40 nm. Energy dispersive analysis revealed that this particulate material found in platelets treated with both colourless and yellow solutions

of gold sodium thiomalate contained gold and sulphur in quantities greater than expected for a normal platelet not treated with gold sodium thiomalate. The gold and sulphur are most likely from the gold sodium thiomalate as has previously been suggested, and the morphology of membrane bound, rod-like particles is also similar to that described in the rabbit synovial tissue by Ghadially (227). It is also apparent that the yellow solution causes an ADP dependent platelet aggregation. This ADP dependent aggregation is analogous to the effect of latex particles on platelets as observed by Movat et al (228). Platelet phagocytosis of latex particles is accompanied by ADP release which leads to platelet aggregation (228) similar to that observed for the yellow solution of gold sodium thiomalate. The localisation of the particulate component of the yellow solution of gold sodium thiomalate in phagolysosomes is not surprising, since it is analogous to the findings of Lewis and coworkers who showed that platelets take up particles into vacuoles which contain acid phosphatase (i.e. phagolysosomes) (229) and that the electron microscopic morphology is similar to that described for other phagocytic cells which have been exposed to gold sodium thiomalate, either in vitro or in vivo (227, 230-232).

It is evident that the thermal sterilisation of gold sodium thiomalate solution causes a chemical change and the resultant formation of a new species with different biological activity from the starting compound, at least in its interaction with the platelet.

The exact chemical structure of gold sodium thiomalte is unknown, but is presumed to be a polymer (201, 202). My colleagues and I do not know the exact nature of either the colourless or yellow form of the compound at present but one could postulate that they are both polymers of different size. It has been suggested that the new species may be an oxidation product since $Au(SCN)^{4-}$ has an absorption at 450 nm (233). This explanation is unlikely as attempts to cause oxidation with dioxygen (O_2) were unsuccessful and Professor Colin J.L. Lock and his colleague Dr. Adam Hitchcock (Institute for Materials Research, McMaster University) have shown that by X-ray Absorption Near Edge Spectroscopy (XANES) (at the Cornell High Energy Synchrotron Source) (figure 75) that the upper limit for Au(III) in the yellow gold sodium thiomalate sample is 2%. A comparison of corresponding XANES and U.V. visible spectra (figure 75 and 56) of a sample of colourless gold sodium thiomalate (3.2×10^{-2}M as elemental gold) irradiated for 500 minutes at 350 nm shows that Au(III) cannot be the cause of the colouration of gold sodium thiomalate. The XANES spectra of $HAu(III)Cl_4$ and gold sodium thiomalate irradiated with 350 nm light for 500 minutes are shown in figure 75. Allowing for the error caused by noise, it is possible to establish that the gold sodium thiomalate samples has an upper limits of 2% Au(III) if the absorption at 11.915 KEV (electron volts) is caused by Au(III). The U.V. visible specta (figure 56) shows that the same sample at 500 minutes has an absorbance (optical density) at 450 nm of about 3.0. The colourless solution of gold sodium thiomalate has

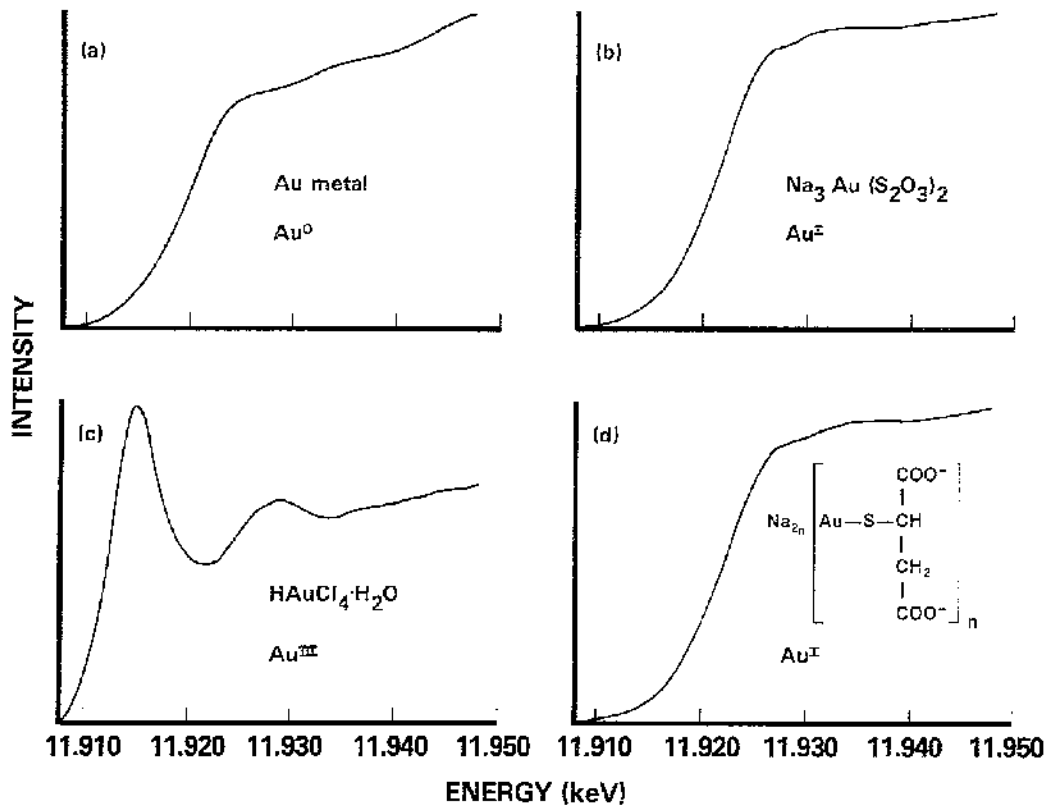


FIGURE 75: X-ray Absorption Near Edge Spectra for various gold compounds. The standard is gold metal (a). A typical Au(I) thiol salt (gold sodium thiosulphate) of known structure is shown in (b), and a standard Au(III) compound showing the characteristic Au(III) absorption at 11.915 KEV is given in (c). The spectrum of U.V. irradiated gold sodium thiomalate (see text) is shown in (d). No trace of a peak characteristic of Au(III) is observed above the background noise error (2%), in the gold sodium thiomalate spectrum (d).

an absorbance of 0.055 at the same wavelength and concentration (figure 55 ii). If we assume that the peak at 450 nm is caused by Au(III) we can estimate the minimum concentration of Au(III) necessary to cause absorption. The absorption at 455 nm in the U.V. visible spectrum of $Au(SCN)^-_4$ (measured at 300K) (233) has a molar extinction coefficient (ε) of 561 and other Au(III) complexes have similar values for the same absorption. Since absorbance = $\varepsilon c \ell$, where c is the concentration in mole L^{-1} and ℓ is the path in centimetres (one cm cells were used) we can write:-

$$3.0 = 561 \times c \times 1$$

Thus the minimum concentration of Au(III) is given by:-

$$c = \frac{3.0}{561} \text{ mole } L^{-1}$$

However, the total gold present is $\frac{12.5}{390}$ mole L^{-1}, therefore the percentage of Au(III) is:-

$$\frac{3.0}{561} \times \frac{390}{12.5} \times 100\% = 16.7\%$$

A maximum Au(III) content of 2% as determined by XANES (figure 75) is therefore inconsistent with a minimum Au(III) content of 16.7%. Therefore the basic assumption, namely that the change in the characteristics present in the yellow solution of gold sodium thiomalate is due to Au(III), is wrong. In conclusion, in these experiments involving U.V. visible irradiation, (figure 56) Au(I) is not oxidised to Au(III).

The experiments reported in this chapter, describe the solutions of gold sodium thiomalate: a yellow solution which is currently marketed and available for human use; and a colourless solution prepared by dissolving 0.13M of solid (white powder) in sterile water. Differences between these two solutions were observed for: spectral absorption visibility; platelet aggregation; and electron microscopy. Similar reactions for the two solutions were identified by: mixed lymphocyte culture inhibition; thrombin enzyme inhibition and; ^{1}H nuclear magnetic resonance (figure 54). These findings indicate that gold sodium thiomalate as currently marketed for human use is a mixture. It is possible that the results of these studies have important clinical consequences. About 60-70% of patients with rheumatoid arthritis respond to treatment with gold sodium thiomalate (108, 109, 110) and 10% show complete remission (124). Unfortunately a serious limiting factor to the continuation of therapy are the adverse effects which occur in as many as 35% of patients (124). If the component of the solution of gold sodium thiomalate which causes the toxic response is different from the component which cause the desirable response, separation of the components of the original solid gold sodium thiomalate may yield a drug with more desirable properties.

SUMMARY

The chemical structure of the most commonly used anti-arthritic gold compound is unknown despite the fact that gold sodium thiomalate has been used in clinical practice for 50 years in the treatment of rheumatoid arthritis. Attempts to determine the structure by X-ray crystallography have resulted in a failure because of the difficulty in obtaining crystals and it is only by inference based on the properties of gold compounds that chemists have suggested that gold sodium thiomalate may be a small polymer, for example a hexamer. Approximately three years ago I noted that samples of gold sodium thiomalate supplied by Rhône-Poulenc, Quebec, for my research purposes were colourless and not the familiar yellow solution known to rheumatologists who treat patients with injectable gold sodium thiomalate. I enquired of the company as to the colour discrepancy and was informed that both the colourless and yellow solution are prepared identically except for one step. If the original solid gold sodium thiomalate (white powder) is dissolved in sterile water to 50 mg of compound per ml, a colourless solution results. However, as a final sterilisation step in the preparation for human use, the colourless solution is heated at 100°C for 30 minutes and a yellow solution results. Since these compounds have identical concentrations, clearly some change had occurred during the heat sterilisation procedure. In order to determine any physical, chemical or biological changes which might have occurred, the compound was then

subjected to a series of experiments: nuclear magnetic resonance, ultra-violet visible irradiation, x-ray absorption near edge spectroscopy to determine any physical and chemical change; mixed lymphocyte culture response to determine effect on one arm of the immune mechanism; and platelets in order to determine any interaction with the thrombin enzyme and the phagocytic capacity of the platelet.

Nuclear magnetic resonance studies (N.M.R.) demonstrated that there was no significant difference in the spectra of the colourless and yellow solution, but ultra-violet visible irradiation spectra indicated that there was a greater absorption for the yellow solution in the 350-450 nm range. In order to follow the colour change from colourless to yellow, the colourless solution (3.2×10^{-2}M as elemental gold) was irradiated with ultra-violet light (350 nm) over a prolonged time period. Optical observation made in the 400-500 nm regions demonstrated that the yellow colour was caused by an absorption at 450 nm.

Previous authors have shown that gold sodium thiomalate inhibits the mixed lymphocyte response. In my experiments, addition of both the colourless and yellow solutions of gold sodium thiomalate in the concentration range 3.8×10^{-5} - 3.0×10^{-4} (as elemental gold) inhibited the mixed lymphocyte response. Thus the changes which occur during the heat sterilisation process are not necessary for this property of gold sodium thiomalate.

In platelet studies, both the colourless and yellow solutions of gold sodium thiomalate were shown to inhibit platelet aggregation by the serine esterase thrombin. Therefore, the heat sterilisation process (100°C for 30 minutes) was not essential to produce this property. However, a difference was observed when the colourless and yellow solutions of gold sodium thiomalate (1.3×10^{-3} - 6.4×10^{-3}M as elemental gold) were added to washed human platelets. The colourless solution had no effect on platelets, but the yellow solution produced an ADP dependent platelet aggregation. When these platelets were examined by electron microscopy, an irregular particulate component 100-700 nm in diameter was identified in platelets treated with yellow gold sodium thiomalate. In platelets treated with colourless gold sodium thiomalate, a particulate component measuring less than 40 nm was observed. Energy dispersive analysis demonstrated that these particles from both sources contained gold and sulphur. Gold thioglucose, gold thiosulphate, Auranofin$_{TM}$ and disodium thiomalate in equimolar concentrations had no effect on producing platelet aggregation nor was any particulate component identified in platelets treated with these compounds.

The differential properties of the colourless and yellow solution of gold sodium thiomalate for some investigations, described above, contrasted by persistence of certain properties following heat sterilisation (e.g. identical effect on mixed lymphocyte culture), clearly indicate that gold sodium thiomalate as currently marketed for human use is a mixture.

This observation has possible important consequences. The beneficial response to gold sodium thiomalate is seen in 60-70% of patients with rheumatoid arthritis but 35% of patients experience a toxicity. If the component of the compound which causes some or all of the adverse effects is different from that which results in the beneficial effects, separation of the components of the solution may yield a drug with more desirable properties.

CHAPTER VIII

THE IN VITRO AND IN VIVO INHIBITION OF THROMBIN BY GOLD SODIUM THIOMALATE

- INTRODUCTION
- MATERIALS
- METHODS: IN VITRO STUDIES
- RESULTS: IN VITRO STUDIES
- METHODS: IN VIVO STUDIES
- RESULTS: IN VIVO STUDIES
- DISCUSSION
- SUMMARY

"Ah, but a man's reach
should exceed his grasp,
Or what's a heaven for."

Robert Browning (1812-1889)

INTRODUCTION

The observation that gold sodium thiomalate, as currently marketed, is a mixture has been a major focus of my research interest and that of my colleagues over the past 2½ years but it is not the only major observation which has been encountered in the course of my research on the anti-arthritic gold complexes. In 1980 while investigating the interaction of various gold compounds with human platelets, I discovered that, of all the anti-arthritic gold complexes currently available, only gold sodium thiomalate inhibited the thrombin enzyme. It was immediately obvious that this effect of the gold sodium thiomalate had possible far reaching implications and certainly required further investigation. In the pursuit of these investigations I am indebted to the following people for their advice and criticism: Professor J. Fraser Mustard, professor of pathology (former Vice-President of Health Sciences); Professor Raelene Kinlough-Rathbone, professor of pathology; Professor Jack Hirsh, Chairman of the Department of Medicine: Professor W. Watson Buchanan, Regional Co-ordinator of Rheumatology, and Professor Colin J.L. Lock, Institute for Materials Research, all of McMaster University. I am also indebted to Mrs. Diana Somers (Ph.D. student, Department of Pathology) who taught me the surgical technique for my animal experiments.

Although gold sodium thiomalate has been used in the treatment of rheumatoid arthritis since the early 1930's (87) its definitive mechanism of action is unknown. Numerous mechanisms so far identified both <u>in vitro</u> and <u>in vivo</u> have been postulated as being contributory to the mode of action of the gold compounds in acute inflammatory rheumatoid arthritis. Among these actions are the inhibitory effects

of the gold thiol complexes on the hydrolytic enzymes (234, 235) such as the serine esterase enzymes elastase and cathepsin G (236, 237). Thus my initial findings that gold sodium thiomalate inhibited the action of thrombin *in vitro* should not have come as a surprise since thrombin is also a serine esterase, but I have as yet found no references in the medial literature other than my own (238 - 240) that gold sodium thiomalate inhibits the action of thrombin *in vitro* and *in vivo*. This chapter outlines some of the results and research techniques used in the investigation of the action of the anti-arthritic gold complexes* on the thrombin enzyme.

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*Details of the effect of the separated components of original gold sodium thiomalate solid (as already discussed in chapter VII) on the thrombin enzyme cannot be given as these details are the subject of a patent application in Canada and the U.S.A. by McMaster University, on behalf of Professor Colin J.L. Lock, Miss Debra A. Harvey, (Ph.D. student) and the author of this thesis.

MATERIALS

Gold sodium thiomalate (Myochrisine$_{TM}$) was supplied gratis by Rhone-Poulenc, Quebec, as the yellow solution in vials as currently marketed for human use. Gold thioglucose and thiomalic acid were obtained from the Sigma Chemical Co., St. Louis, Missouri, U.S.A. Gold sodium thiosulphate was prepared in the Bio-inorganic medicine laboratories at McMaster University after the method of Brown (204). 2,3,4,6-tetra-ortho-acetyl-1-thio-β-D-glucopyranosato-S-(triethyl-phosphine) gold(Auranofin$_{TM}$) was supplied gratis by Smith, Kline and French, Philadelphia, U.S.A. Disodium thiomalate was prepared by the addition of 1 molar sodium hydroxide (NaOH) to thiomalic acid 19 mg/ml until a pH of 7.75 was obtained. Auranofin is insoluble in water. For the platelet studies a stock solution was prepared by dissolving 17 mg of compound in 0.5 ml of absolute alcohol (C_2H_5OH) followed by the addition of 0.5 ml of water to achieve a concentration of 5.1×10^{-4} M expressed as elemental gold. Suitable alcohol control concentrations were used for each dilution of Auranofin added to the platelet suspensions.

All gold compounds listed in this chapter are expressed as molar quantities of elemental gold.

Bovine thrombin was obtained from Miles Laboratories, Kankakee, Illinois, U.S.A. Atropine was obtained from Alcon Laboratories, Mississauga, Ontario. Ketamine hydrochloride was obtained from Parke-Davis, Scarborough, Ontario and pentobarbitol sodium was obtained from Abbott Laboratories, Don Mills, Ontario. Bovine albumin (Pentax Fraction V) was obtained from Miles Laboratories, Kankakee, Illinois,

U.S.A. Radioactive serotonin (^{14}C-serotonin) was obtained from Amersham/Searle, Arlington Heights, Illinois as 5-hydroxytryptamine-3$^{\alpha}$-^{14}C-creatine sulphate, 57 mCi/mmol, and radioactive chromium (^{51}Cr) are $Na_2{}^{51}CrO_4$, 100-400 mCi/mg of Cr was obtained from New England Nuclear, Lachine, Quebec. Sodium heparin was obtained from Harris Laboratories, West Hill, Ontario. Plastic catheters used in animal studies, P.E.90 and P.E.190 polyethylene, were obtained from Clay Adams Co., New York, U.S.A.

METHODS: IN VITRO STUDIES

PREPARATION OF WASHED HUMAN PLATELETS

Washed human platelet suspensions were prepared by the method of Mustard et al (220). Briefly, 129 ml of blood was obtained by anti-cubital vein puncture from volunteers. Volunteers were selected who were on no medications and had taken no medication for two weeks. The blood was immediately transferred in equal volumes to 3 plastic centrifuge tubes (50 ml), each containing 7 ml of acid-citrate-dextrose (ACD) anticoagulant solution, as described by Aster and Jandle (221). The whole blood-ACD mixture was gently but rapidly mixed to prevent clotting. The suspension was then centrifuged at 37°C in an R.C.3 Sorval centrifuge at 1200g for 8 minutes. The platelet-poor plasma was discarded and the platelets were suspended in Tyrodes solution containing 0.35% albumin and 50 units/ml of heparin (220,222). Apyrase prepared according to Molnar and Lorand (223), with a nucleotidase activity of 5.3 units of adenosine diphosphate/mg and 4.2 units of adenosine triphosphate/mg was included in the Tyrodes albumin solution at a concentration of

10 µl/ml (220,224). Platelets were incubated in this first washing solution of Tyrodes albumin with 2 µCi of ^{14}C-serotonin and 200 µCi of disodium chromate/mg for 30 minutes. This first washing solution was then centrifuged at 1200g for 10 minutes, the supernatant discarded and the platelets resuspended in Tyrodes albumin solution (second washing solution) for 10 minutes. The second washing solution of platelets was centrifuged at 1200g for 10 minutes and the supernatant discarded. The platelets were suspended in a final suspension of Tyrodes albumin and the platelet count adjusted to 500,000/mm^3. The platelet suspensions were stored at 37°C in a water bath prior to use.

Platelet Aggregation Studies

Platelet aggregation was studied by a modification of a turbidimetric method as previously described (220). Briefly, light transmission of 1 ml suspensions of washed platelets was measured on a Payton Aggregation Module (Payton Associates, Scarborough, Ontario, Canada) and recorded on a Rikadenki Pen Recorder (Kogyo, Japan). Platelet shape change and aggregation were recorded following the interaction with test compounds (e.g. gold complexes) and aggregating agents (e.g. ADP).

Platelet Rich Plasma and Platelet Poor Plasma

Whole blood (9 parts) anticoagulated with 3.8% sodium citrate (1 part) was centrifuged at 1200g for 3 minutes in an R.C.3 Sorval Centrifuge in order to obtain platelet rich plasma. Platelet poor plasma was obtained by centrifuging platelet rich plasma at 1200g for 10 minutes.

Thrombin Clotting Time

Thrombin clotting time of platelet rich plasma and platelet poor plasma were measured in a Hyland Clotek System (Hyland Laboratories, Malton, Ontario). This is an automatically timed instrument which operates by causing a steel ball bearing (6 mm diameter) to oscillate in a glass test tube (6x75mm) containing 300 µl of plasma. Following the addition of thrombin (5 units/ml final concentration) a fibrin clot forms which coats the bearing and stops the free oscillation of the ball, thus stopping the timer. In the standard thrombin clotting time using the Hyland Clotek System, calcium chloride is used. Since the gold sodium thiomalate and the calcium chloride form a precipitate, the calcium chloride has been omitted from these experiments. Under these conditions the control thrombin clotting time was in the order of 17 secs. (see table 18).

RESULTS: IN VITRO STUDIES

Bovine thrombin (0.025-0.25 units/ml) added to washed human platelets caused platelet aggregation and release of ^{14}C-serotonin from platelet granules (figure 60). Prior addition (0.5-240 minutes) or simultaneous addition of gold sodium thiomalate (1.8×10^{-5} - 2.5×10^{-3}M as elemental gold) to the 1 ml platelet suspension inhibited the effect of the thrombin enzyme on the washed human platelets (figure 60). As seen from the figure 60, the differences observed were dramatic and since the observation was consistent with 30 separate experiments, statistical analysis was not required. If the gold sodium thiomalate was added after the addition of the thrombin to the platelet suspension, inhibition did not occur. The inhibitory effect of gold sodium thiomalate on the thrombin induced aggregation, was independent of the duration of incubation of the gold sodium thiomalate with the platelets.

Gold thioglucose and gold sodium thiosulphate in equimolar concentrations of elemental gold and equimolar disodium thiomalate (i.e. equimolar concentration of thiomalate to the thiomalate in gold sodium thiomalate) did not inhibit the effect of thrombin induced aggregation on washed human platelets. Auranofin$_{TM}$ in the concentration range 1.3×10^{-7} - 5.1×10^{-4}M, as elemental gold, did not inhibit the action of thrombin on washed human platelets.

The effect of gold sodium thiomalate on the thrombin (5 units/ml) clotting time of platelet rich plasma and platelet poor plasma are

shown in Table 18 (A representative experiment has been shown as an example). All concentrations of gold sodium thiomalate 2.1×10^{-4}M (as elemental gold) and greater, induced prolongation of the thrombin time of both platelet rich plasma and platelet poor plasma. Concentrations of gold sodium thiomalate of 2×10^{-2}M (as elemental gold) and greater, extended the thrombin clotting time to infinity (Table 18). Gold thioglucose and gold sodium thiosulphate in equimolar concentrations of elemental gold and equimolar disodium thiomalate (i.e. as equimolar concentrations of thiomalate to the thiomalate in gold sodium thiomalate) did not prolong the thrombin clotting time of platelet rich plasma or platelet poor plasma. Similarly Auranofin$_{TM}$ in the concentration range (1.3×10^{-7} - 5.1×10^{-4}M as elemental gold) did not prolong the thrombin clotting time of platelet rich plasma or platelet poor plasma. The prolongation of the thrombin clotting time of platelet rich plasma and platelet poor plasma by gold sodium thiomalate was independent of the duration of incubation of the compound with the plasma suspension but was related to the concentration of the gold sodium thiomalate used, as shown in Table 18.

TABLE 18. THE EFFECT OF GOLD SODIUM THIOMALATE ON THE THROMBIN CLOTTING TIME IN VITRO

GOLD SODIUM THIOMALATE (Moles of Elemental Gold)	THROMBIN TIME	
	PLATELET POOR PLASMA (Seconds ± SEM)	PLATELET RICH PLASMA (Seconds ± SEM)
C	17.7 ± 0.4	16.5 ± 0.2
2.1×10^{-4}	18.7 ± 0.1	17.4 ± 0.3
4.2×10^{-4}	19.6 ± 0.2	18.3 ± 0.6
1.0×10^{-3}	24.6 ± 0.4	22.2 ± 0.7
2.1×10^{-3}	33.2 ± 0.9	30.2 ± 0.9
4.2×10^{-3}	60.7 ± 1.7	51.7 ± 1.4
2.1×10^{-2}	∞	∞

Representative experiment. Each thrombin time sampled 5 times

S.E.M. - Standard error of the mean

∞ = Infinity

METHODS: IN VIVO STUDIES

Male New Zealand rabbits (2.5 - 3.5 kilograms) used in the study were housed singly and maintained at McMaster University animal facilities in a controlled temperature and humidity environment and fed rabbit chow and water adlibitum.

In order to study the effect of gold compounds on the action of thrombin in vivo, experiments to measure platelet survival and the weight of thrombus formation, were studied in an experimental model of intra aortic thrombosis by an indwelling aortic catheter method (figure 76). The experiment was carried out over 12 days. Animals were given daily injections of drug on days 1 to 5 and the indwelling aortic catheter was surgically inserted on day 8. Animals were sacrificed on day 12. In order to obtain a relative uniformity of serum gold by day 8 (i.e. day of catheter insertion) for each gold complex administered, pilot dosage studies with 8 rabbits in each group were undertaken. Using equimolar concentration of the gold compounds at 0.13M as elemental gold in 0.5 ml of sterile water given intramuscularly daily from day 1 to 5, the following mean serum gold levels (± SEM) could be achieved: gold sodium thiomalate treated, 1.0×10^{-4}M ± 6.7×10^{-6}M; gold thioglucose treated 1.1×10^{-4}M ± 4.7×10^{-6}M' gold sodium thiosulphate treated 1.6×10^{-4}M ± 5.6×10^{-6}M. Control animals were given 0.5 ml of sterile water intramuscularly daily for 5 days. Disodium thiomalate was administered as 0.13M thiomalate in 0.5 ml of sterile water. Five identical experiments with appropriate control groups were carried out as follows:

(a) 4 gold sodium thiomalate treated and 4 water treated, (b) 4 gold sodium thiomalate treated and 4 water treated, (c) 5 gold sodium thiomalate treated and 5 water treated (d) 5 water treated, 5 gold thioglucose treated and 5 gold thiosulphate treated, (e) 5 water treated and 5 disodium thiomalate treated. Some animals succumbed to anaesthesia induction leaving 19 water treated, 12 gold sodium thiomalate treated, 5 gold thioglucose treated, 5 gold thiosulphate treated and 4 disodium thiomalate treated available for analysis to date. Serum gold was measured on day 12. Platelet counts were measured on days 1, 8, 9, 10, 11 and 12. Haemoglobin and white blood cell count were measured on day 1 and day 8 in experiments (a), (b) and (c) and on day 12 in experiments (d) and (e).

In all experiments platelet survival studies were started on day 8 immediately after catheter insertion. Briefly, washed rabbit platelets were prepared from stock New Zealand rabbits by the method of Ardlie et al (220) radiolabelled in the first wash with 150 μCi of $Na_3{}^{51}CrO_4$ per 50 ml of rabbit whole blood and incubated for 1 hour at 20°C. The platelets were resuspended in rabbit platelet poor plasma ($2x10^6$/cu.mm). Four ml (75 μCi) of the labelled platelet suspension was injected into the marginal ear vein of experimental rabbits for platelet survival and thrombus weight studies. Platelets were allowed to circulate for 2 hours, at which time a blood sample was taken to determine recovery of radioactivity in the circulation. The radioactivity of this sample was assigned a value of 100% for the purpose of platelet survival determination.

Additional rabbit whole blood samples were taken at 2, 19, 22, 43, 67 and 91 hours after the 2 hour recovery sample. Radioactivity in these samples was expressed as a percentage of the radioactivity in the initial 2 hour recovery sample. Mean platelet survival was calculated using the gamma function developed by Murphy et al (241).

At time zero (immediately after recovery samples were taken) the rabbits were anaesthetised with pentobarbitol sodium (35-45 mg/Kg) intravenously. Twenty centimetres (cm) polyethylene catheters (P.E. 190 Clay-Adams, New York), sealed at both ends, were surgically inserted into the aorta in all animals via the right femoral artery. The distal end extended to 1 cm above the diaphragm where it was free to oscillate with the arterial blood pulsation (Figure 76). A continuous vessel wall injury results from this procedure and experimental thrombi form along the length of the vessel (Figure 77). The catheters were left in situ until day 12, when the animals were then killed by perfusion fixation. For perfusion the animals were anaesthetised with Ketamine hydrochloride 100 mg intramuscularly, pentobarbitol sodium 20 mg/Kg and atropine 0.1 mg subcutaneously. The left femoral and left carotid arteries were isolated and a saline filled polyethylene catheter (P.E.90) was inserted in each (Figure 78,79). The animals were heparinized with 1000 units of sodium heparin and then perfused with oxygenated Locke's Ringer containing heparin 1 unit/ml via the left carotid artery and exsanguinated via the

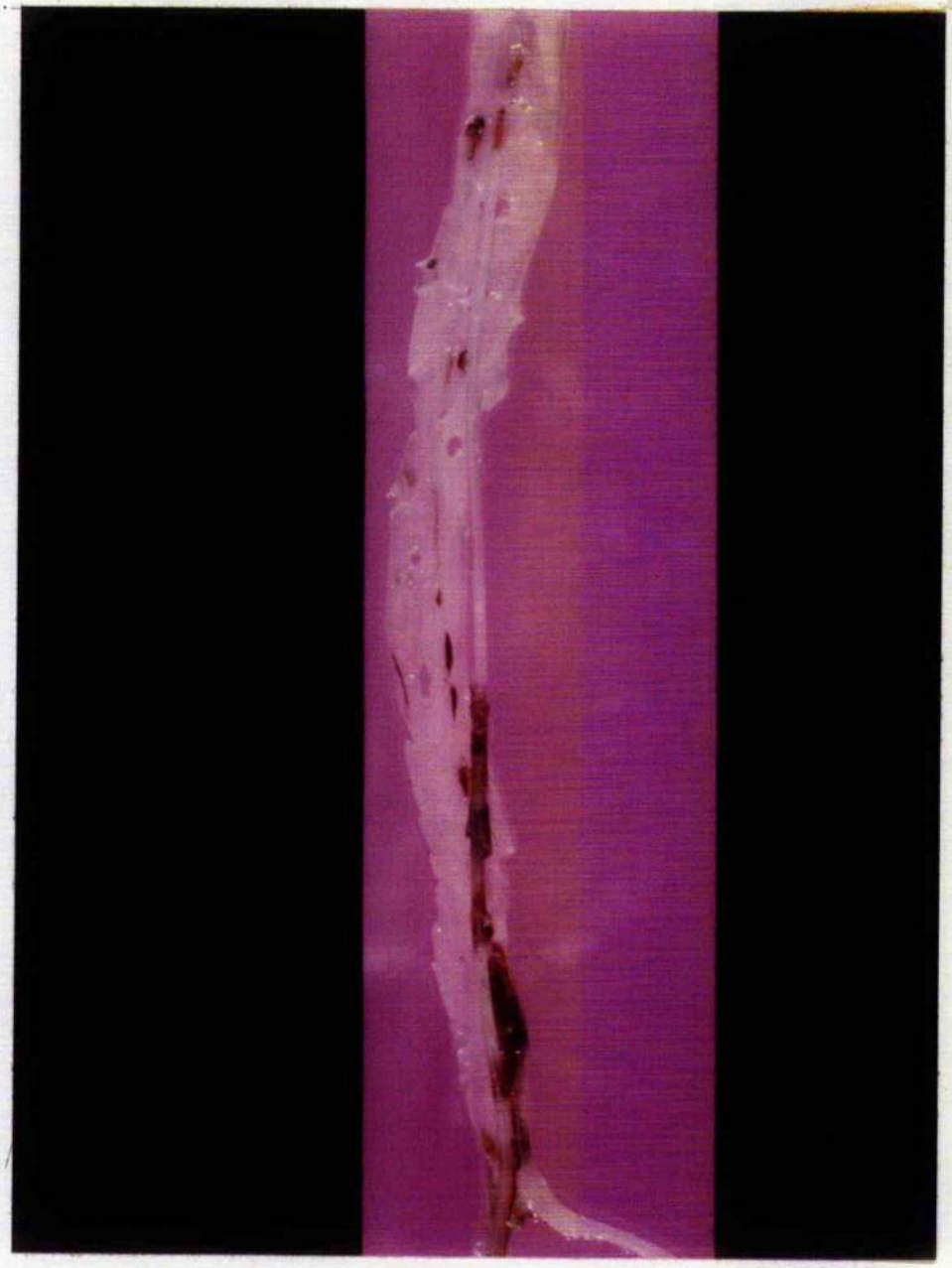

FIGURE 76: Twenty centimetre polyethylene catheter (P.E.190) inserted into a rabbit aorta via the right femoral artery. The distal end extends to 1 centimetre above the diaphragm where it is free to oscillate with the arterial pulsation.

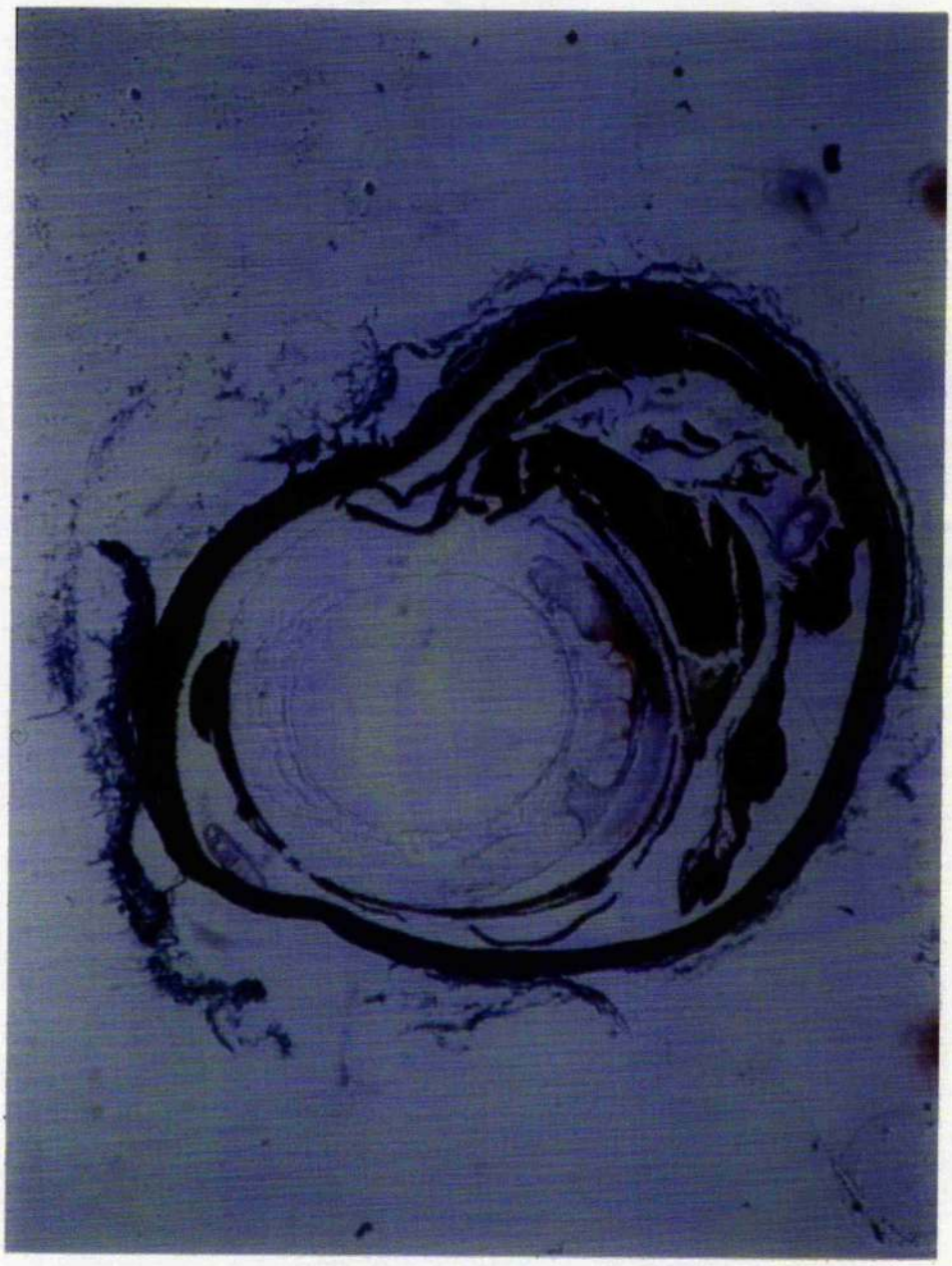

FIGURE 77: Transverse section of rabbit aorta with 20 cm indwelling polyethylene catheter (P.E.190) in situ. Organised thrombus is seen attached to the vessel wall and around the catheter (section stained with Lendrum's martius scarlet blue).

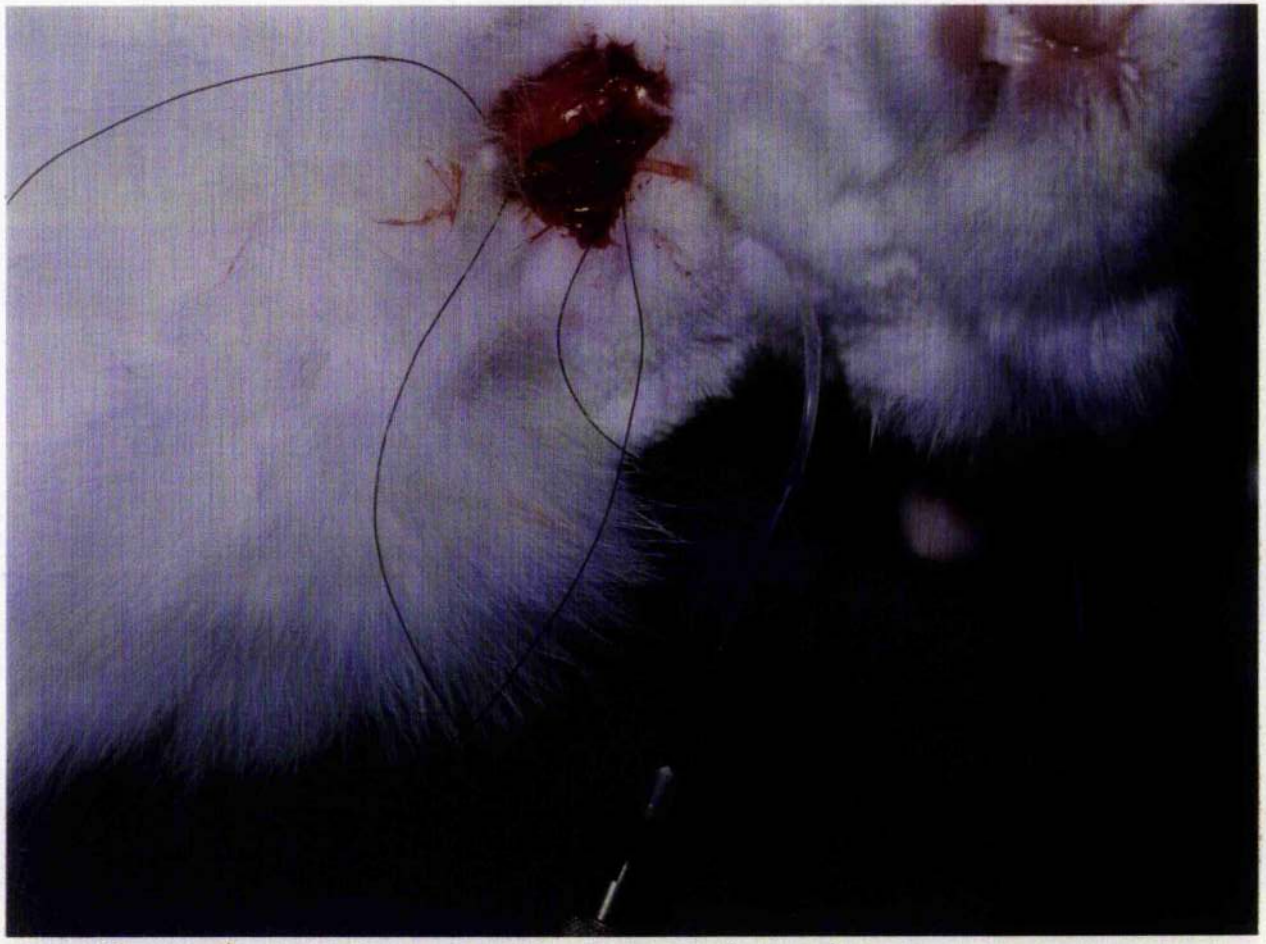

FIGURE 78: Saline filled polyethylene catheter (P.E.90) inserted into the left carotid artery of a rabbit. At the time of sacrifice, the animal is perfused via this catheter with oxygenated Locke's Ringer solution containing heparin 1 unit/ml and exsanguinated via the left femoral catheter (see text and figure 79).

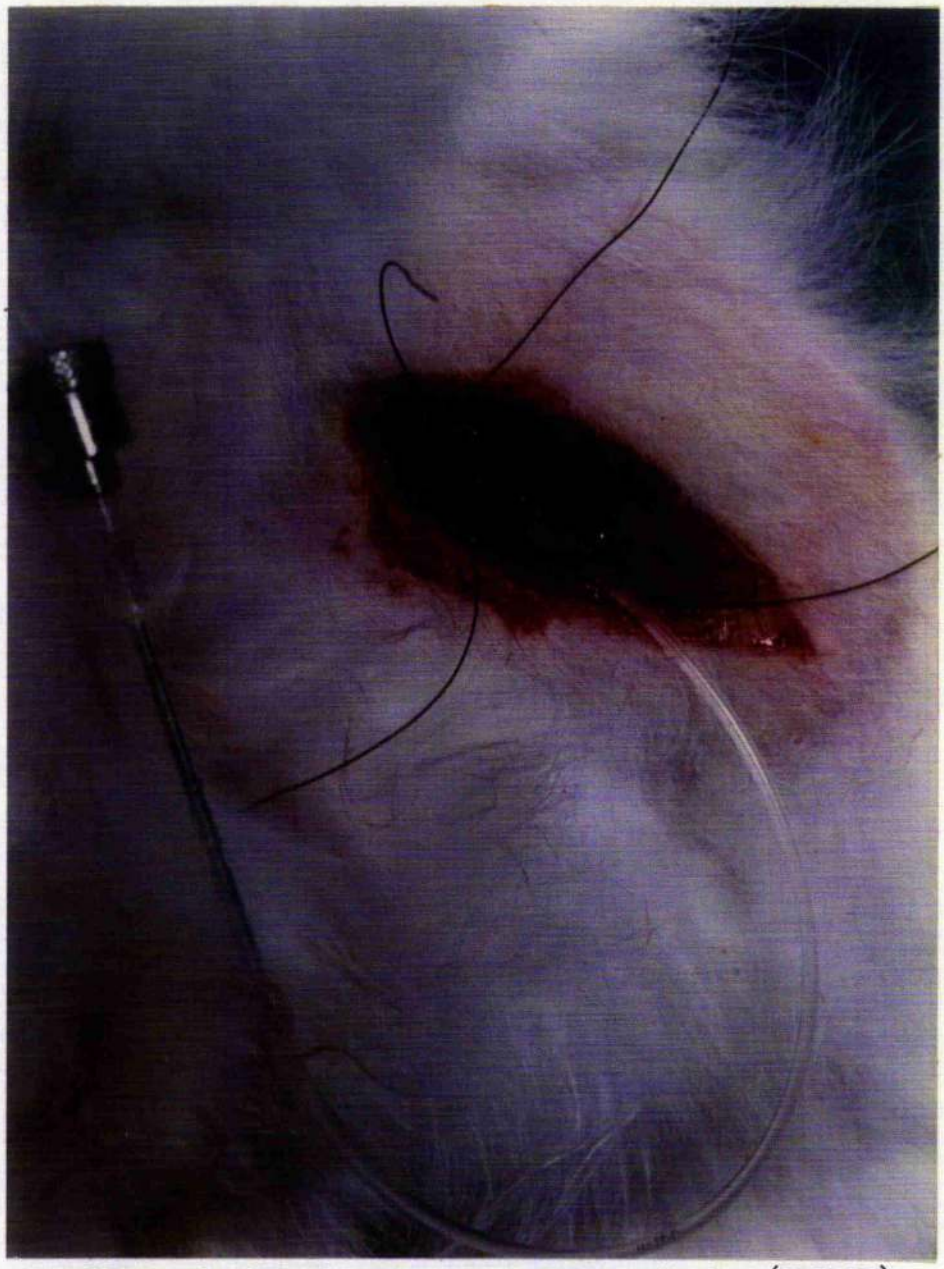

FIGURE 79: Saline filled polyethylene catheter (P.E.90) inserted into the left femoral artery of a rabbit. The animal is exsanguinated via this catheter. (See text and figure 78).

left femoral artery (Figure 80). When the solution draining from the femoral artery was virtually clear of red blood cells the Locke's Ringer solution was replaced by 4% paraformaldehyde in phosphate buffer. The aortae with indwelling catheter and thrombi were removed intact by careful dissection. Six sections of the aorta (2.5 cm per section) with catheter and thrombus in situ were taken from the aortic bifurcation upwards. The thrombi were then dissected free of the vessel wall and catheter, and air dried at room temperature for 1-2 hours before weighing.

Whole Blood Fibrinolysis Assay

In order to determine the effect of gold sodium thiomalate on whole blood fibrinolysis, the following experiment was undertaken.

Ten New Zealand White rabbits (2.5 - 3.5 kilograms) were given gold sodium thiomalate 0.13M in 0.5 ml of sterile water intramuscularly for 5 days. Ten control animals were given 0.5 ml sterile water intramuscularly for 5 days. On day 8 serum gold samples were taken and 2 ml of whole blood was withdrawn for fibrinolysis assay. ^{125}Iodine labelled human fibrinogen (grade L, Kabi Laboratories, Stockholm, Sweden) was used to prepare ^{125}I-fibrin coated test tubes as described by Moroz and Gilmore (242). While blood fibrinolysis was determined using a ^{125}I-fibrin radiometric assay. Briefly, 0.9 ml of rabbit whole blood was collected in 0.1 ml of heparin (final concentration 10 units/ml) and incubated (37^{o}C) in a ^{125}I-fibrin coated test tube (tube A) for one hour. Plain Tyrodes solution (2 ml) containing 10 units/ml of heparin was then added and the whole sample transferred to a clean

FIGURE 80: Anaesthetised rabbit being perfused with oxygenated Locke's Ringer solution via the left carotid artery (figure 78) and exsanguinated via the left femoral artery (figure 79).

uncoated 12 x 75 mm polystyrene tube (tube B). The ^{125}I-fibrin coated tube (tube A) was then rinsed with a further 1 ml of Tyrodes solution containing 10 units/ml of heparin and this was also transferred to tube B. Both the ^{125}I-fibrin coated tube (tube A) and the 12 x 75 mm uncoated polystyrene tube (tube B) were then counted in a gamma counter. Fibrinolysis was expressed as a percentage by determining the radioactivity in the uncoated tube (tube B) divided by the sum of the radioactivity in the ^{125}I-fibrin coated tube (tube A) and the uncoated tube (tube B):-

$$\% \text{ Fibrinolysis} = 100 \times \frac{\text{Tube B}}{\text{Tube A + Tube B}}$$

RESULTS: IN VIVO STUDIES

Haemoglobin, white blood cell count and platelet counts for all study animals were normal on day 1. No change was detected in haemoglobin nor white blood cell values measured on day 8 {experiments (a), (b) and (c)} or on day 12 {experiments (d) and (e)} compared to baseline values observed on day 1.

The mean serum gold levels measured on day 12 (i.e. day of sacrifice) are illustrated in table 19; gold sodium thiomalate - 4.9×10^{-5}M $\pm$ 1.4×10^{-6}M; gold thioglucose - 4.2×10^{-5}M $\pm$ 3.8×10^{-6}M; and gold sodium thiosulphate 4.4×10^{-5}M $\pm$ 2.8×10^{-6}M. There was no significant difference between these values as calculated by the student's t test assuming an alpha level of 0.05 ($t = 1.8-0.4$, $p > 0.05$ not significant).

The average platelet count in normal New Zealand White rabbits is approximately 500,000 to 700,000/mm^3. The lower platelet values illustrated in table 19 (mean values for days 8 to 12) are consistent with the presence of an indwelling aortic catheter resulting in vessel wall damage (Figures 76, 77) and subsequent reduced platelet survival (see table 20). Platelet survival (table 20) was not significantly different in any gold treated group compared to controls as calculated by the student's t test. Thus the effect of gold sodium thiomalate on thrombus weight was not related to an alteration in platelet survival.

TABLE 19

SERUM GOLD AND PLATELET VALUES OF RABBITS WITH INDWELLING AORTIC CATHETERS, TREATED WITH GOLD COMPLEXES OR CONTROL COMPOUNDS

	TREATMENT				
	H_2O	GOLD SODIUM THIOMALATE	GOLD THIOGLUCOSE	GOLD THIOSULPHATE	SODIUM THIOMALATE
N	19	12	5	5	4
SERUM GOLD (moles) ± SEM	–	4.9×10^{-5} ± 1.4×10^{-6}	4.2×10^{-5} ± 3.7×10^{-6}	4.4×10^{-5} ± 2.8×10^{-6}	–
PLATELET COUNT/ mm^3 ±SEM (MEAN VALUES) (DAY 8 to 12)	425,000 ± 32,000	4000,000 ± 26,000	519,000 ± 12,000	477,000 ± 68,000	414,000 ± 44,000

N = Number of rabbits in each category

mm^3 = Cubic millimetres

S.E.M. = Standard error of the mean

TABLE 20

PLATELET SURVIVAL RESULTS OF RABBITS WITH INDWELLING AORTIC CATHETERS TREATED WITH GOLD COMPLEXES OR CONTROL COMPOUNDS

	TREATMENT				
	H_2O	GOLD SODIUM THIOMALATE	GOLD THIOGLUCOSE	GOLD THIOSULPHATE	SODIUM THIOMALATE
N	19	12	5	5	4
PLATELET SURVIVAL (Hours) ± SEM	32.0 ± 5.0	27.6 ± 4.2	28.4 ± 2.0	27.8 ± 2.0	31.0 ± 2.0

N = Number of rabbits in each category

S.E.M. Standard error of the mean

Statistical data was calculated by student's t test

There was no significant difference among the groups for gold treated animals compared to controls (t = 0.7799-0.1857, p > 0.05)

Thrombi do not increase in size in a normally distributed fashion, therefore statistical analysis of thrombus weight results was done on the log transformed data. Actual mean thrombus weights are shown in table 21 as well as the antilog of the mean value of the sum of log thrombus weights from which the statistical analyses were performed. One way analysis of variance confirmed that a difference did exist among the 5 groups. Application of Dunnett's test (243) confirmed that the mean thrombus weight in those animals treated with gold sodium thiomalate was significantly less than the mean thrombus weight in all other groups ($p = 0.035$).

Whole Blood Fibrinolysis Study

The mean serum gold level ($\pm$ SEM) in the 10 rabbits treated with gold sodium thiomalate 0.13M as elemental gold in sterile water intramuscularly was 8.4×10^{-5}M $\pm$ 5.9×10^{-6}M. The percentage fibrinolysis for individual rabbits and the group mean are shown in table 22. There was no significant difference between the percentage fibrinolysis in the 10 animals treated with gold sodium thiomalate, compared to the percentage fibrinolysis observed in the 10 control animals given sterile water (table 22).

TABLE 21

THE WEIGHT (mg) OF EXPERIMENTALLY INDUCED THROMBUS FROM RABBITS WITH INDWELLING AORTIC CATHETERS

	TREATMENT				
	H_2O	GOLD SODIUM THIOMALATE	GOLD THIOGLUCOSE	GOLD THIOSULPHATE	SODIUM THIOMALATE
N	19	12	5	5	4
MEAN THROMBUS WEIGHT (mg)	31.01	14.79	23.60	20.60	23.40
S.D.	16.03	11.09	10.10	7.19	4.22
S.E.M.	4.24	3.32	2.00	2.00	1.73

In view of skewed data due to biological variation, statistical analysis was calculated using log thrombus weight values.

	TREATMENT				
	H_2O	GOLD SODIUM THIOMALATE	GOLD THIOGLUCOSE	GOLD THIOSULPHATE	SODIUM THIOMALATE
ANTILOG OF MEAN VALUE OF SUM OF LOG THROMBUS WEIGHTS	26.92	11.14	21.95	19.74	23.13
95% CONFIDENCE LIMITS	15.03-48.03	4.88-25.46	14.37-33.55	14.40-27.09	19.43-33.55

Statistical comparison of antilogs of mean value of sum of log thrombus weights were calculated by one way analysis of variance and Dunnett's test. The thrombus weight of gold sodium thiomalate treated animals was significantly less than all other treatment groups. ($p < 0.035$)

N = Number of rabbits in each category
mg – Milligrams
SD – Standard deviation
S.E.M. – Standard error of the mean

TABLE 22: Percent Fibrinolysis of Whole Blood from Rabbits Treated with Gold Sodium Thiomalate, Compared to Control Animals Treated with Sterile Water.

Group Treated with Gold Sodium Thiomalate 25mg in 0.5ml sterile water (I.M.) for 5 days		Control Group Treated with 0.5ml sterile water (I.M.) for 5 days	
Rabbit No.	% Fibrinolysis *	Rabbit No.	% Fibrinolysis *
1	3.20	11	3.33
2	3.04	12	3.31
3	4.87	13	2.61
4	3.60	14	5.18
5	3.59	15	4.10
6	3.87	16	3.25
7	3.43	17	3.19
8	5.07	18	2.37
9	3.40	19	4.44
10	4.03	20	2.77
MEAN ± SEM	3.81 ± 0.16	MEAN	3.45 ± 0.20

$t = 1.405$

$p > 0.05$ Not significant

* Each sample measured in duplicate

S.E.M. = Standard error of mean

Statistical analysis was by student's t test

I.M. - Intramuscularly

DISCUSSION

Gold sodium thiomalate has previously been shown to inhibit serine esterase enzymes isolated from the lyosomes of white cells (234-237). This study demonstrates for the first time to my knowledge that gold sodium thiomalate is inhibitory to the serine esterase thrombin in its interaction with, washed human platelets, human platelet rich plasma, human platelet poor plasma, and on the formation of experimental thrombosis in New Zealand White rabbits. Since thrombin is a serine esterase, phylogenetically related to the serine esterases, elastase and cathepsin G, the most likely mechanism of action is an interaction of the gold thiol complex with one or all of the four cysteine-cysteine disulphide bridges of the thrombin molecule (244).

The serum gold levels achieved in patients receiving gold sodium thiomalate for rheumatoid arthritis are usually in the range 1.5×10^{-5}M - 4.1×10^{-5}M, and values as high as 7.6×10^{-5}M may be achieved immediately after an intramuscular injection. The concentration of gold sodium thiomalate (as elemental gold) which inhibit the action of thrombin (0.025-0.25 units/ml) on washed human platelets was clearly within this range. The gold sodium thiomalate concentrations which resulted in prolongation of the thrombin clotting time of platelet rich plasma and platelet poor plasma were much higher (2.1×10^{-4}M and greater) than gold values usually achieved in patients but the thrombin concentration used was 20 times higher than the concentration used in the washed human platelet studies. In the dosage schedule

used in the animals experiments, the serum gold values on day 8 were in the range 1.0×10^{-4}M - 1.6×10^{-4}M and by day 12 had fallen to 4.2×10^{-5}M - 4.9×10^{-5}M (i.e. comparable to values achieved in patients with rheumatoid arthritis). Continuous vessel wall injury has been shown by previous workers from the laboratories of Professor Fraser Mustard and Professor Jack Hirsh, McMaster University, to result in the formation of thrombi in the rabbit aorta and also to shorten platelet survival (245 - 247). As reported in this chapter, the platelet survival was similar in gold treated animals and in control groups; therefore indicating that the reduction in thrombus weight in the gold sodium thiomalate treated animals was not due to changes in platelet survival. The continuous vessel wall injury in the rabbit, as used in the study, results in endothelial damage and platelets adhere to the damaged surface. The localised generation of thrombin results in activation of other platelets to change shape and stick together (248). Thrombin causes fibrinogen in and around the platelet aggregates to polymerise, thus creating a platelet mass stabilised with fibrin (249). Studies to measure the fibrinolytic activity of gold sodium thiomalate were therefore necessary to determine whether this gold complex reduced thrombus weight by enhancing the fibrolytic process. The results shown in table 22 demonstrate that in the whole blood of New Zealand White rabbits, gold sodium thiomalate had no effect on fibrinolysis when compared to control animals. This finding is similar to the results reported by Moroz (250), namely that gold

sodium thiomalate 10^{-4} to 10^{-7}M had no effect on the fibrinolytic activity of human whole blood. The significant reduction in thrombus weight found in the animals treated with gold sodium thiomalate is likely due to the inhibition of thrombin action at the site of vessel wall injury. The reduction in thrombus weight observed in my experiments is thus most likely due to reduced fibrin formation.

Thrombus formation due to vessel wall injury is analogous to the process which occurs in acute vasculitis (251) or glomerulonephritis (252-254), specifically the diffuse and focal proliferative variety of lupus nephritis (253, 254). The endocapillary damage seen in acute-glomerulo-nephritis has been postulated to be due to the presence of soluble immune complexes (252). Whether or not this is a primary event, subendothelial damage does occur which results in activation of platelets to adhere to the damaged endothelial tissue, release their internal granules and perpetuate local platelet aggregation (248, 255). Clearly an agent which reduces the deposition of fibrin and hence micro-thrombi (252-254) could be a major breakthrough in the management of glomerulonephritis (252) and acute vasculitis (251).

That gold sodium thiomalate inhibits the action of thrombin <u>in vitro</u> and <u>in vivo</u> poses a number of problems for future study. Vascular thrombo-embolic disorders constitute one of the major causes of death in Western society today. It has been suggested previously that patients with rheumatoid arthritis are in some way

protected from such disorders as myocardial infarction (256) and also deep venous thrombosis after surgical trauma (257, 258). It has been assumed that somehow this is related to the pharmacotherapy of the inflammatory joint disease and especially to non-steroidal anti-inflammatory drugs. However, no conclusive evidence that these agents are responsible is available and this may be due to the failure to consider gold sodium thiomalate treatment either as the major agent involved or as a major adjunct to this effect. It would certainly be worthwhile carrying out studies in the future to assess such a possibility. As noted above, equally important in the area of inflammatory joint disease, would be the consideration of the effect of gold sodium thiomalate on acute glomerulonephritis. Glomerulonephritis is widely considered to be due in many cases to vascular microthrombi caused by immune complex deposition (251-253). If such thrombi could be avoided there may be considerable protection against renal functional deterioration.

Research in thrombo-embolism has been slowly progressing for many years but no one has yet reached "El Dorado", perhaps the sign post to their goal is also "gold"!

SUMMARY

Gold sodium thiomalate (1.8×10^{-5}M - 2.5×10^{-3}M as elemental gold) inhibited the effect of bovine thrombin (0.025-0.25 units/ml) on washed human platelets suspended in Tyrodes albumin 0.3%. The thrombin clotting time of human platelet rich plasma and platelet poor plasma was prolonged in a dose related manner by gold sodium thiomalate in concentrations of 2.1×10^{-4}M (as elemental gold) and greater. Gold thioglucose, gold sodium thiosulphate, Auranofin, and disodium thiomalate had no effect on thrombin in the above test procedures.

In order to study the effect of gold compounds on the action of thrombin in vivo, experiments to measure platelet survival and the weight of thrombus formation, were studied in an experimental model of intra aortic thrombosis by an indwelling aortic catheter method. Platelet survival was reduced in all gold compound treated and control animals with indwelling aortic catheters. Thrombus weight was reduced in animals treated with gold sodium thiomalate compared to animals treated with sterile water, gold thioglucose, gold sodium thiosulphate, and disodium thiomalate. In vivo studies demonstrated that gold sodium thiomalate had no effect on whole blood fibrinolysis.

These studies indicate that gold sodium thiomalate is the only gold complex which inhibited thrombin in vitro. Gold sodium thiomalate treated rabbits had a reduced weight of experimentally induced intra-aortic thrombi compared to animals

treated with sterile water or equimolar concentrations of gold thioglucose, gold sodium thiosulphate and disodium thiomalate. Finally, the reduction in thrombus weight in the animals treated with gold sodium thiomalate was not reflected by changes in platelet survival nor fibrinolysis.

The serum gold levels achieved in the in vivo experiments was in the range $5x10^{-5}$M to $1.0x10^{-4}$M. These values are comparable to levels which can be achieved in human subjects immediately after a gold injection. Clearly an agent such as gold sodium thiomalate which is capable of thrombin inhibition requires further study as to its value in the management of thrombo-embolic diseases and conditions such as vasculitis and acute lupus nephritis where microthrombi play a role.

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