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THE CLINICAL VALUE OF MONITORING GOLD LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS UNDERGOING CHRYSOTHERAPY

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A thesis submitted

for the degree of Doctor of Philosophy

Council for National Academic Awards

Department of Life Sciences

Trent Polytechnic

Nottingham

June 1988

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The inscription at the foot of the title page is the Chinese word for Gold. The word is constructed of three characters which represent what early Chinese alchemists believed were the efficacious medicinal properties of the element. Thus, whilst together the characters mean Gold, individually they connote 'Longevity', 'Immortality' and 'Resurrection'.

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ABSTRACT

In this study of patients with rheumatoid arthritis undergoing chrysotherapy, good correlations were demonstrated between total, free and protein bound gold and between these parameters and salivary and urinary gold levels. The possibility of monitoring efficacy of toxicity of treatment was established using a combination of total and free serum gold levels in the form of ratios and correlation plots with regression. Circadian rhythmicity of urinary gold excretion was demonstrated and further relationships were demonstrated between urinary gold, zinc and copper excretion rates. Relationships were also shown between immunological proteins and complement and the various gold fractions in serum, saliva and urine. Although the differential binding of gold to various protein fractions was demonstrated, analysis of samples stored at different temperatures revealed different protein binding properties for gold at the different temperatures. Gold distribution amongst the protein fractions is too sensitive to changes in temperature to be used routinely to monitor treatment. Finally it was demonstrated that the gold and thiol moieties of disodium aurothiomalate have different fates following intramuscular injection for

whilst free plasma thiomalate peaked between 30 and 45 minutes after injection and was no longer detected in urine after 4 hours, the gold reached a peak in plasma 60 to 120 minutes post injection and was still present in urine in significant amounts after 4 hours. The urinary recovery of free thiomalate revealed that 82-98% of the injected thiol moiety remained in the body in some form following the clearance of the free plasma levels.

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CHAPTER 1

CHAPTER 1

1. INTRODUCTION

The management of rheumatoid arthritis (RA) by gold therapy (also referred to as chrysotherapy and aurotherapy) is based on more than fifty years experience (see page 11). During this time the use of gold drugs in arthritis patients has been hindered by the inability to relate any easily measurable parameter to the potential efficacy or toxicity of the treatment.[1] Toxic symptoms can occur and are often severe (page 16), indeed, instances of death have been recorded as a result of the therapeutic side effects of chrysotherapy.

1.1. PATHOGENESIS OF RHEUMATOID ARTHRITIS

Rheumatoid arthritis may be broadly defined as a sub-acute or chronic, non-suppurative, inflammatory polyarthritis initially affecting the peripheral joints, usually in a symmetrical nature.[2] The disease exhibits a prolonged course of exacerbation and remission and may be accompanied by distressing early morning stiffness and signs of systemic disturbance such as anaemia, weight loss and a raised

erythrocyte sedimentation rate. In 70-80% of patients with RA, blood tests show positive results for 'Rheumatoid Factor' and there are characteristic (but not unique) histological appearances in the synovium and sometimes in subcutaneous nodules. However, difficulty of diagnosis arises because so many cases occur with either incomplete or atypical features and because patients may be seronegative to start with and only later convert to seropositive states. Furthermore, discrimination of RA as a single disease from conditions such as the 'seronegative arthropathies' (eg. psoriatic arthritis) and from chronic juvenile polyarthritis is often difficult.[2]

The need for a generally accepted definition of the disease led the American Rheumatism Association (ARA) to devise a classification which would ensure uniformity of diagnosis in different centres.[3,4,5] Although no great difficulty was encountered in defining the essential clinical characteristics of 'classical' RA, it was found necessary to allow for less typical cases in categories described as 'definite', 'probable' and 'possible' and to list twenty reasons for excluding individual cases from all these diagnostic categories. Approximately 1.5 million people are affected with RA in Great Britain, many cases of which are mild but none the less the

disease constitutes a serious social and economic problem.

1.1.i. Intrinsic Factors associated with Rheumatoid Arthritis

Age at Onset

Approximately 70% of cases occur between the ages of 25-54 years, but the disease can start at any age. In a study conducted by the Empire Rheumatism Council [6] the mean age at onset was found to be 42 in males and 41 in females. In 1957, Short [7] concluded that males of all ages over 15 years are equally susceptible, but in females a marked increase in disease frequency occurs between the ages of 50 and 55 years, followed by a decrease at 60 and over. Such studies however are limited by the unconfirmed assumption that the patients studied are representative of RA occuring in the general population.

Sex

Population surveys have confirmed that the signs and symptoms of RA are about three times as common among women as among men but the incidence of radiological evidence of erosive arthritis is equal in the two sexes, although the disease is usually more severe in females.[8-10]

Heredity

In discussing the evidence for a genetic factor in 1960, Blumberg[11] concluded that a familial influence had been demonstrated both for RA and the rheumatoid factor[11-14] but suggested that there was no proof of transmission by a single gene. This was challenged by Bunim et al. in 1964[15] who investigated the incidence of the disease in various Red Indian tribes. These workers suggested that in order to avoid selection bias the only satisfactory method of studying the problem is by surveys of whole populations rather than just comparing the incidence in relatives of affected and non-affected individuals. Such studies have revealed little if any evidence of the effects of heredity on the incidence of either the disease or its associated serological manifestations.

The understanding of the development of RA was improved following the demonstrations that certain HLA antigens were strongly associated with this disease. These antigens include HLA-Dw4,[16] DR1, DR2, DR3,[17] DR4[18] and DRw53 (MT3).[19] No association of RA with HLA-A or B locus antigens was found in early studies[20,21] although later reports suggested other HLA antigen associations.[22]

The frequency of DR4 in patients with RA is

within the range of 50-75% whilst the range for controls is 25-35%. It has been suggested that this evidence argues for the existence of an RA factor which is co-transmitted or associated with HLA. Furthermore it has been proposed that this factor could be DR4 itself or a component of susceptibility in linkage disequilibrium with HLA-DR4.[23] It has also been suggested however, that the presence of DR4 alone does not produce an increased risk of RA [24,17] and DR3 [17,25] and DR5 [26] have also been proposed.

Toxicity of treatment has been found in some studies to be associated with HLA-B8 and DR3. These side effects have included proteinuria and thrombocytopenia but an association of the most frequent side effects, ie skin rash and HLA antigens, has been found in only a few studies.[25]

The above findings should be interpreted in the context of the known familial tendency to develop RA however and in the small family studies so far undertaken the evidence for genetic linkage between HLA and RA remains weak.[23,24] It seems likely that the susceptibility to and development of RA are not due to one factor alone, but to several which may include environmental factors.

1.1.ii. Extrinsic Factors associated with Rheumatoid Arthritis

Population surveys in America and Europe have revealed no definite relationship to climate. Radiological evidence of erosive arthritis was most common in Jamaica although severe disease was less common there than in Europe and America.[27] These studies indicate that the disease is widespread and appears to occur with an approximate similar frequency in the different countries studied. The prevalence in these populations varies from 1-2% in adults using the ARA criteria.[4] The belief that RA is associated with cold wet climates has been disproved by two studies of American Indian tribes living in opposite climatic conditions.[28] No significant difference was found in the prevalence of RA in these tribes. No race appears to be immune from disease although it occurs with apparent less frequency in some populations.[29-32]

1.1.iii. Rhythmicity of Rheumatoid Arthritis Symptoms

The distressing early morning stiffness associated with RA is well documented.[2,33-35] Recent studies have demonstrated that the disease activity as measured by estimation of joint pain, stiffness,

articular index and grip strength[36] and by domiciliary self-measurement of finger joint size[37] has circadian rhythms resulting in the well known problems associated with the early morning time of day.

1.2. IMMUNOLOGIC PATHOGENESIS AND AETIOLOGY

The antigenic stimulus that initiates the immune response and subsequent inflammation in RA is unknown.

There is an increased prevalence of histocompatibility antigens in patients with RA and this has been discussed in Section 1.1.i. The genetic mechanism that promotes the development of RA is unknown but HLA-Dw4 may impart a genetic susceptibility to an unidentified environmental factor, such as a virus, that may initiate the disease process. Although no virus particles have ever been identified, it is likely that an antigenic stimulus leads to the appearance of an abnormal IgG. This IgG results in the production of rheumatoid factor and to the eventual development of rheumatoid disease.[38,39] (See Fig. 1) Synovial lymphocytes produce an IgG that is recognised as foreign and stimulates an immune response within the joint giving rise to the production of 7SIgG, 7SIgM and 19SIgM anti-immmunoglobulins i.e.

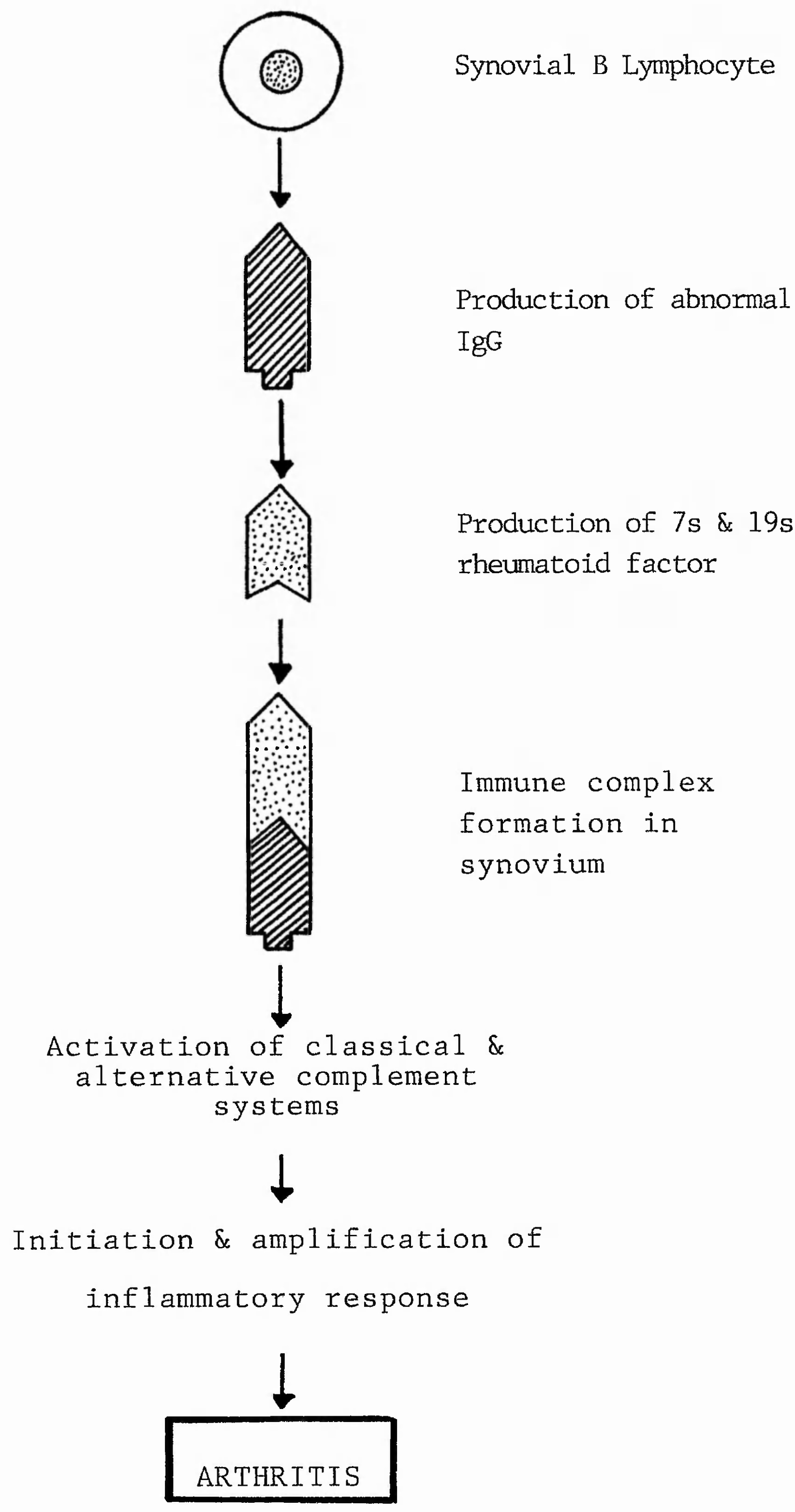


Figure 1. Hypothetical Immunopathogenesis in Rheumatoid Arthritis

rheumatoid factors. The presence of IgG aggregates on IgG rheumatoid factor complexes results in activation of the classic complement system. Activation of the complement system in turn gives rise to a number of inflammatory phenomena including histamine release, the production of factors chemotactic for polymorphonuclear leukocytes and mononuclear cells and membrane damage with cell lysis. Rheumatoid factors do not initiate the inflammatory process that causes the rheumatoid disease but they probably perpetuate and amplify that process by their effects on complement activation.[38]

1.3. THE MANAGEMENT OF RHEUMATOID ARTHRITIS

Once RA has been diagnosed the basic approach to the management of the disease is a complete patient evaluation. The type of treatment depends on the signs of disease activity, the severity of the patient symptoms, the disease duration and on the laboratory and radiological features. Treatment is based on relieving the patient's pain and stiffness and in this the use of drugs forms only a part of the management. Splints and physiotherapy are used to improve the function of joints and surgery may be undertaken for certain joint problems mainly late on in the disease.

For descriptive purposes drugs used in the management of RA may be conveniently divided into the following categories:

1. Analgesics.
2. Non-steroidal analgesic anti-inflammatory (NSAID).
3. Long-acting drugs with apparent 'anti-rheumatic' or disease modifying activity.[40] (Eg Antimalarials, penicillamine, gold(1) salts [38,40] and salazopyrine.[41,42])
4. Non-specific immunomodulatory and cytotoxic agents (eg Azathiaprine, levamisole and cyclophosphamide).
5. Corticosteroids.
6. Miscellaneous and ancillary drugs (eg muscle relaxants, iron and anti-depressants).

The mechanisms of action of many of these compounds are unknown. Many are toxic or have significant side effects and interaction with other drugs is common. Therefore, as a general rule the least number of drugs necessary, the least toxic drug available and the lowest dose required is used to achieve the therapeutic aim of maximum benefit with minimal toxicity.

The large number of NSAID available (eg Aspirin, Indomethacin, Flurbiprofen etc) alleviate the symptoms

of pain often within hours but do not alter the course of the disease. Although these 'first-line' drugs may produce a variety of biological effects their modes of action have no unifying hypothesis to explain their activity. Interruption of the arachidonic acid pathway by inhibition of prostaglandin synthetase or cyclo-oxygenase has been proposed as a major mode of action but effects can also be demonstrated on oxidative phosphorylation, lysosomal and cell membranes, granulocyte and macrophage chemotaxis, mobility and function and other membrane and protein systems (eg Kinins). Although there is seldom need to use more than one agent with a similar action, a common exception is the use of one NSAID for daytime symptoms and a long acting one for night pain and morning stiffness.

A major disadvantage of NSAID is their high incidence of gastro-intestinal side effects, the most important being mucosal ulceration and haemorrhage. Several attempts have been made to overcome the gastric irritability caused by these drugs, including enteric coating of tablets or combination with antacids, rectal administration of suppository (eg Indomethacin), slow release drugs (eg Indocid R) and the formulation of 'prodrugs' which are given in inactive (and theoretically less irritant) forms which are converted

to the active form at a site distant from the stomach (eg Sulindac, Fenbufen and Benorylate).

If it is judged that the disease is causing erosive changes or further progression of already established disease then long acting drugs with apparent anti-rheumatic activity are considered.[40] The term 'second-line' is often used for these drugs which have an accepted slow-developing, long-lasting effect in RA and which include Penicillamine, Salazopyrine, antimalarials (eg Hydroxychloroquine) and gold(1) salts (see Chapter 1.4.). These drugs appear to have a fundamental action on disease (and do not just suppress manifestations) but all possess potentially serious side effects (see page 16) which make them truly 'second-line' drugs. Invariably NSAID are given concurrently with these drugs for alleviation of pain.

Since most drugs have a narrow therapeutic ratio it is important to adjust the 'standard' dose for each individual by adequate consideration of both pharmacokinetic and pharmacodynamic factors. For example penicillamine chelates iron so that concurrent administration will reduce absorption of both drugs and subsequent withdrawal of iron may lead to penicillamine toxicity.

Since rheumatoid arthritis is associated with

evidence of both over-activity of humoral immunity and suppression of some facets of cell mediated immunity both 'immunosuppressive' and 'immunostimulant' drugs (also termed immunomodulatory or 'third-line' drugs) have been considered for management of the disease. However, these do not correct recognised immunological abnormalities. Most of them are cytotoxic agents which affect the immune system by interfering with proliferation or differentiation of lymphocytes. None of these drugs are specific however and their ameliorating effect on RA may well be produced by actions other than those on immune cells. There are other drugs which are capable of altering immune function and which may prove to be of future interest in treatment (eg Frentizole, Cyclosporin A and Interferon).

Although marked therapeutic benefit may be obtained from the anti-inflammatory and immuno-suppressive activity of corticosteroids the effects of these steroids are widespread and potentially devastating. Apart from the many systemic side effects there is the possibility that steroids may also hasten joint destruction and influence the distribution of rheumatic disease so their use is justified only in exceptional circumstances. These include exceptionally severe exacerbations which are

not remitting with rest, intra-articular injections of corticosteroids and NSAID, failure to control disabling symptoms in workers, threat to elderly patients of confinement to bed and life or sight threatening visceral disease such as severe pericarditis, polyarteritis or scleritis. In each of these circumstances a slow-acting anti-rheumatic agent is commenced simultaneously with a view to a gradual withdrawal of corticosteroid therapy when a remission has been obtained. Disorders that respond to glucocorticoids may also respond to ACTH therapy and consequently ACTH is sometimes used during introduction of the slow-acting second-line therapy in active RA.

No method of treatment has proved capable of producing complete and permanent remissions of peripheral RA in all cases and the conviction that chrysotherapy alone has been successful in permanently arresting the disease[43] remains in doubt. Gold(1) salts gained a reputation for being dangerous during the early years of their use and are still avoided by some physicians. However, since gold therapy has been demonstrated to lead to improvement in most patients and clinical experience has lessened the dangers of its toxicity[44], gold(1) salts now have an important role in the therapeutic management of RA for many

patients.

Current Practices

In 1988 the common management practice is probably to begin the control of symptoms of RA with an NSAID. If the disease progresses salazopyrine might be introduced as the first second-line drug. If this fails to control the symptoms or produces side effects either gold, penicillamine or hydroxy-chloroquine may be introduced. NSAID are given concurrently with these second-line drugs but whilst waiting for the second-line drug to take effect some clinicians may also additionally use low dose prednisolone (5mg daily). For severe systemic diseases with patients resistive to these drugs an immunomodulatory or cytotoxic drug (ie third-line) may be introduced.

1.4. <u>TREATMENT OF RHEUMATOID ARTHRITIS USING GOLD SALTS</u>

1.4.i. <u>A Brief History of Gold Salts</u>

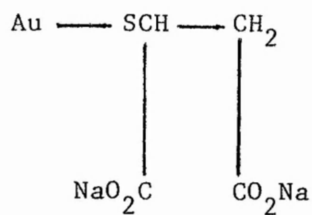
Although gold salts have been used to treat RA for almost sixty years, gold was first used in China as a general elixir of life in the quest for immortality by alchemists and physicians as long ago as 700 BC. (The Chinese word for gold consists of three characters which individually connote longevity, immortality and resurrection.[45] See title page). By 400 AD it appears that gold was being taken as treatment for various pathological conditions including rheumatism.[46,47] The introduction of gold compounds for modern treatment of RA probably owes its origin to Koch[48] (1890) who reported the <u>in vitro</u> tuberculostatic properties of gold cyanide and concluded that it was the gold and not the cyanide that was effective. In 1927 Lande reported on the use of aurothioglucose in bacterial endocarditis and other conditions (many of which may have been rheumatic fever) and noted especially relief of joint pain.[48] Later Forestier[49] called attention to the value of aurothioglucose in RA. Detailed drug trials by Hartfall,[50] Freyberg[51] and Lockie[52] emphasised the benefits of gold salts in the treatment of RA.

The use of gold did not become widely acceptable until the double blind trial by the Empire Rheumatism Council in 1961 [53] which demonstrated that a course of intramuscular injections of sodium aurothiomalate (resulting in a total dose of 1g of gold) was superior to a placebo. It is from this study that the conventional regimen for sodium aurothiomalate is derived.[54] Since then further controlled studies have indicated that gold salts can provide effective and long lasting symptomatic relief of RA.[55-58] Nowdays gold salts are usually continued as maintenance therapy after the total of 1g has been reached so long as the patient does not suffer side effects.

1.4.ii. Gold Drugs in Current Use

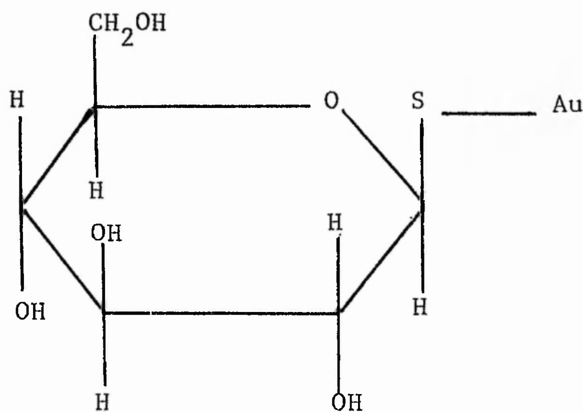
Gold(0), gold(1) and gold (111) are the principal oxidation states of gold. The three salts of gold(1) such as the halides are unstable in the presence of moisture and disproportionate to elemental gold and gold(111). However, gold(1) can be stabilised by complexation to ligands such as thiolates.[59]

The commonly used drugs are water soluble gold(1) thiolates such as gold sodium thiomalate (Myochrysin)



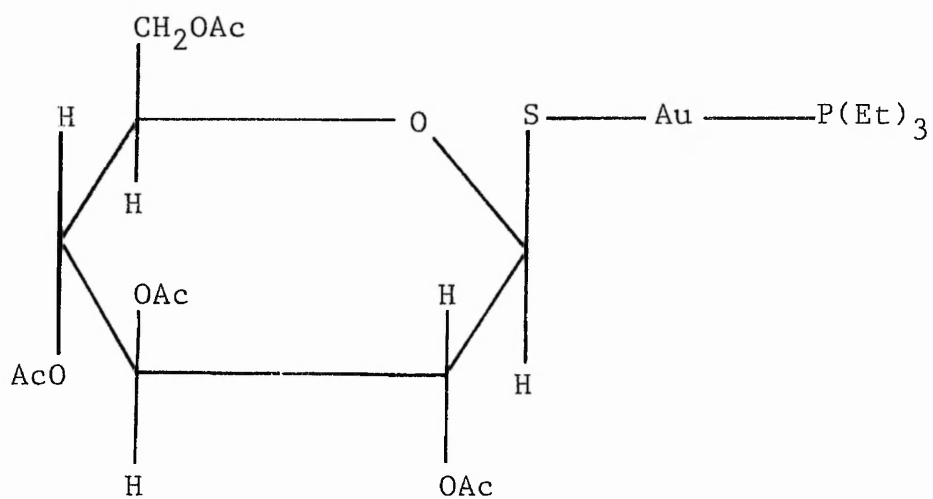
Gold (I) Thiomalate

Na_2 (AuTM) (MYOCHRYSIN)



Gold (I) Thioglucose

AuTG (SOLGANOL)



$\underline{Et_3\ PAupATG}$ (AURANOFIN)

and gold sodium thioglucose (Solganol)[44,59] which have a high ratio of therapeutic effectiveness to toxicity[60] and Auranofin (complexed with triethyl-phosphine). Gold sodium thiomalate (or sodium auro-thiomalate) is prepared in a 50% aqueous solution and is administered intramuscularly. Aurothioglucose is also administered by intramuscular injection but as a water-soluble 50% solution prepared as a suspension in oil. The Auranofin complex contains 29% elemental gold and is administered orally. Intravenous administration of these compounds is contraindicated because of toxicity.[44]

1.4.iii. Drug dosage

Gold drugs are used as an adjunct for carefully selected suitable patients when basic therapy, ie analgesic or non-steroidal anti-inflammatory drugs, rest and physical therapy has failed. In general the higher the dose of the gold drug the more side effects but an increase in dosage is usually tried if treatment at the standard dosage fails.[44] The aims of the therapy are to build a level of gold in the body of the patient sufficient to produce a remission and to maintain that level until a permanent remission is established.[43]

The drug regimes operated by physicians are various but are usually based on the study by the Empire Rheumatism Council (1961).[53,54]

At the Nottingham City Hospital two different gold regimes are followed. In one regimen a test dose of 10 mg sodium aurothiomalate is given by intramuscular injection in order to assess patient suitability, followed by weekly injections of 20 mg until response occurs, at which point the frequency of injections is reduced to fortnightly or monthly. In the second regimen a test dose of 10 mg is used then an initial dose of 20 mg followed by weekly injections of 50 mg until response occurs, when the frequency of the injections is reduced to fortnightly or monthly.

1.4.iv. Side effects of drug treatment

Considerable experience in the management of RA by chrysotherapy has lessened the dangers of gold toxicity but despite this the incidence of adverse reactions remains high when compared with some of the other drugs used for management of rheumatoid diseases. These adverse responses affect 25-50% of patients treated.[61-65] Proteinuria occurs in approximately 10% of patients taking gold salts and is

usually mild but may be severe enough to cause nephrotic syndrome.[44,66,67] In rare cases, doses of gold drugs have led to destruction of the proximal tubules of the kidneys and to tubular necrosis (resembling other heavy metal poisonings) leading to fatality.[59] Other complications include thrombocytopenia (the most common haematologic abnormality associated with gold therapy, affecting 1-3% of patients),[44,67] pulmonary hypersensitivity reactions, nitroid reactions, hepatic toxicity,[67] dermatitis, corneal chrysiasis and gold encephalopathy.[54] Other more common side effects include fever, diarrhoea, mouth sores, stomatitis and rash.[44] A summary of the diagnosis and management of adverse reactions from gold compounds was made by Gottlieb and Gray [69] in 1978 and by Mullin [44] in 1982. It has been established that when gold toxicity occurs the RA is quiescent but patients who fail to receive benefit from chrysotherapy do not experience toxicity.[43]

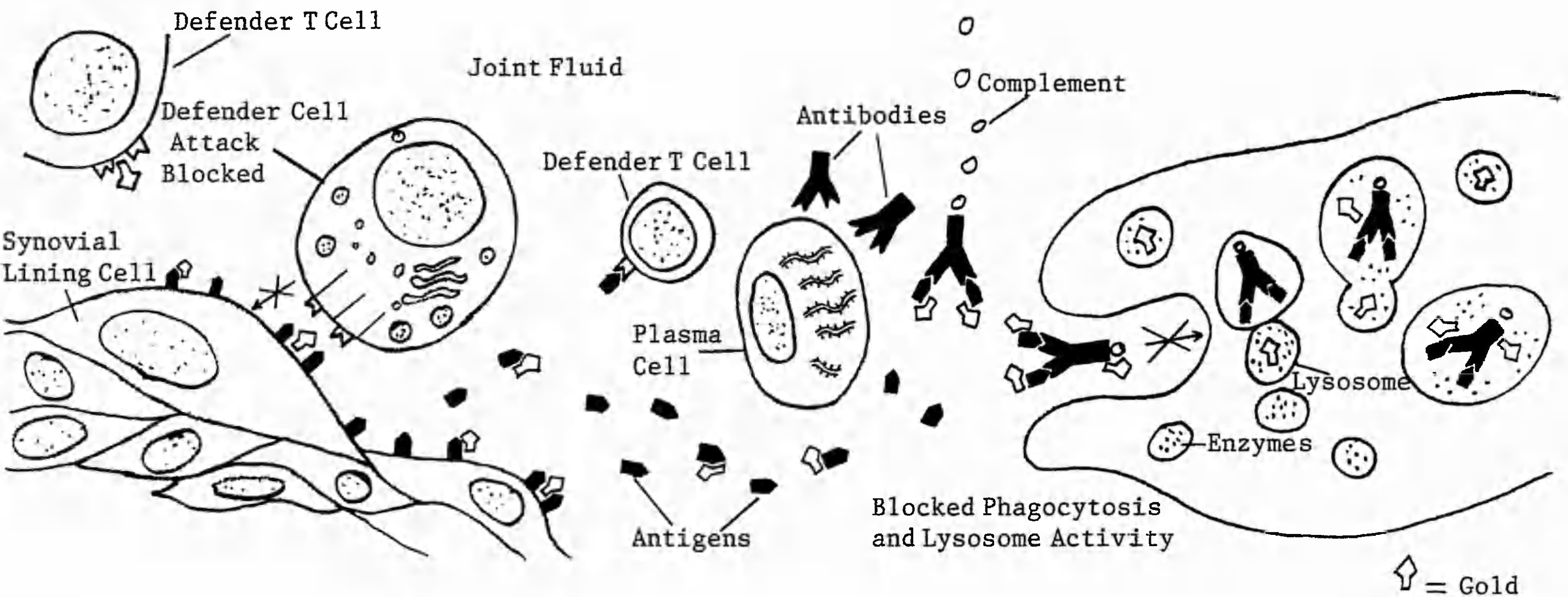
1.4.v. Mode of action of gold salts

Although the use of gold salts is well established their mode of action remains unclear. It has been demonstrated that gold is taken up by synovial tissue macrophages and accumulates in the

lysosomes,[70] which become progressively dense to the electron microscope as more gold accumulates. These gold laden lysosomes have been named aurosomes [59] and their presence has led to speculation that gold might act either by lysosome stabilisation as does hydrocortisone,[71] or by the inhibition of the lysosomal enzymes, acid phosphatase, β-glucuronidase and cathepsin, which occurs in vitro.[72] It is the activity of the lysosomal enzymes which contributes to the degradation of the joints by destruction of articular cartilage.[59]

These mechanisms presuppose a site of action of gold within the synovial membrane and cavity [73] but information on the deposition in the body during chrysotherapy suggests that the majority of the gold is selectively concentrated in the lymph nodes, liver and bone marrow with relatively small concentrations in articular structures.[59,74] However, selective concentration of the majority of gold outside of the synovial membrane and cavity does not necessarily imply therapeutic activity in these areas of higher deposition.

Other possible actions of gold include strong inhibition of sulphydryl systems, alteration of collagen by increasing cross-linkages, suppression of histamine release, prevention of prostaglandin



1.4.v. Figure 1. The Possible Sites of Action of Gold Salts

A schematic representation of gold binding to cell surface structures (antigens and antigen receptors) to inhibit their reactions. Gold also binds to antigens and immune complexes which ultimately blocks phagocytosis and inhibits the lysosomal enzyme release and activity. Other possible actions not depicted include inhibition of thiol-dependent proteases (eg cathepsins), inhibition of neutrophils and superoxide ion or oxyradical generation, alteration of human monocyte structure and function, inhibition of endothelial cell proliferation, decrease in rheumatoid factor production, modulation of T lymphocyte function, prevention of myeloperoxidase induced inactivation of α-1-proteinase inhibitor of ADP or collagen-induced platelet aggregation.

synthesis and moderate suppression of cellular immunity.[75]

Whilst the afforementioned actions are of an inhibitory nature, recent evidence suggests that gold compounds might also act as cell stimulators by activation of cellular transduction pathways in processes involving the promotion of protein kinase C in the association with plasma membranes.[76]

1.4.vi. Metabolism and Distribution of Sodium Aurothiomalate

Jellum[77,78] showed that sodium aurothiomalate dissociates immediately following intramuscular injection both in animals and in humans to form free thiomalate and protein bound gold in the blood followed by the appearance of free thiomalate in the urine. More recent work has demonstrated that a small unbound gold moiety exists rather than all the gold being bound to protein.[79] Further animal work by Jellum[80] indicated that approximately 50% of the thiol is excreted in the urine whilst the remainder is bound to tissue membranes and cells.

The gold is widely dispersed throughout the body but concentrates in the lymph nodes, liver, bone

marrow, adrenal gland, spleen and kidney.[59] Low concentrations have also been found in articular structures,[74] endocrine glands, muscle and skin,[81] the lens of the eye [82] and in hair and nails.[83] Whole blood contains proportionally lower gold levels than serum or plasma because most of the gold present in the circulation is bound to albumin (85-95%) and to a lesser extent the remaining protein fractions [84-93] and an unbound or 'free' moiety.[79]

The serum half-life of radiolabelled aurothiomalate is 6 days following injection.[94] Significant amounts of gold are excreted in the urine and faeces. From 50-85% is eliminated in the urine whilst 15-50% is removed in the faeces.[95] The daily urinary gold levels decrease successively following injection whilst the daily faecal content is erratic.[96]

1.5. AIMS AND OBJECTIVES OF THE THESIS

In assessing the value of monitoring gold levels in certain body fluids of patients with RA undergoing chrysotherapy, there is a need to study the gold levels in serum, urine and saliva.

The existence of rhythmic patterns has been demonstrated for certain other metallic ions in serum and plasma along with circadian rhythmicity of

urinary excretion of metallic ions. (See page 26)
Furthermore, metallic ions have been detected in saliva and circadian rhythms have been demonstrated for certain endocrine hormones in this fluid. (See page 28)

It is therefore clearly necessary to demonstrate any existence of circadian rhythmicity for gold in serum, urine and saliva and to be aware of these rhythms when assessing the possible significance of the gold levels.

Any rhythmic pattern will be studied in relationship to the urinary excretion of copper and zinc and to the serum levels of immunoproteins and acute phase reactants. Furthermore, the toxic side effects of drug treatment on certain of these parameters will be studied. Other aspects of treatment will be studied with respect to the free thiomalate part of the gold drug. The possible relationships between free thiomalate and urinary and serum gold levels will be examined.

The aims and objectives of this work will be carried out by following four major studies.

Study 1

There are conflicting reports regarding the distribution of gold in plasma and serum. Most investigators have demonstrated that gold is bound to albumin,[84-93] but whereas some have measured amounts of gold bound to the protein globulins (α, β and γ)[84,85,97] others have not.[98] Likewise, whilst it is generally accepted at present that a free or unbound fraction of gold exists.[1,84,90,93] Some investigators have been unable to confirm this.[78,80,99] It has been established that with several drugs the unbound rather than the total or the protein bound concentration often shows a better correlation with pharmacological response.[100-102] It has been suggested that similarly unbound serum gold might be a more useful parameter for monitoring patients during chrysotherapy.[90,93] One aim will be to establish the distribution of gold in the serum of patients with RA commencing gold salt therapy and in patients already established on gold therapy. Serum gold levels (total, free and protein bound) will be analysed and the possibility of any relationship between gold distribution and toxic side effects will be investigated.

Methods of assaying free serum gold have either

been unsuccessful or have given inconsistent results probably as a consequence of technical problems encountered at the very low levels of free gold expected. Some methods have not been suited to handling groups of specimens and have not had the necessary speed of analysis to cope with the reactive characteristics of gold in blood or with the changing nature of plasma proteins with time.[85] Furthermore, the redistribution of gold among the protein fractions during separation and the reactive properties of the metallic ions with the reagent chemicals have made the assay difficult.

Membrane ultrafiltration[103] is a method which has been used successfully in studies where rapidity of analysis without addition of potentially competitive buffer components and electrolytes has been required. This work has included studies of protein binding to drugs,[104,105] analysis of free tryptophan in plasma[106] and the protein binding of sex hormones.[107] Ultrafiltration has been demonstrated to be at least equivalent to the equilibrium dialysis method of separation but simpler to carry out.[108] The ultrafiltration method of analysis of the gold will be used extensively in this study.

When the distribution of gold in the different serum fractions has been established the project will

examine the gold binding properties of the protein globulins. The possibility that changes occur in individual protein fractions as a result of gold toxicity will be investigated.

Study 2

Although considerable effort has been applied for many years to the investigation of gold in the treatment of RA there is still no agreement as to the relationship between gold levels in physiological fluids and the potential efficacy or toxicity of the treatment. One area of investigation has been the relationship between serum and urinary gold where in the main, the various reports have been contradictory.

Freyberg *et al.*[51] and Lawrence[99] found no correlation between serum and urine gold levels. Smith *et al.*[109] found that poor therapeutic effect was related to high urinary gold excretion and that toxic reactions were related to a low excretion, but Krusius *et al.*[110] and Billings *et al.*[111] concluded that although serum gold levels correlated with therapeutic efficacy, urine levels did not.

It is evident that the collection times of urine specimens have differed in studies, for whilst some investigators have analysed random urine specimens

collected when need arose[111] others have studied complete 24 hour collections.[110,112] Also the methods of reporting the gold levels have differed. For example Krusius _et al_.[110] and Arden-Jones _et al_.[112] have reported urine gold excretion in milligrams per 24 hours, whilst Billings _et al_.[111] have reported gold excretion in random urine specimens as micrograms of gold per gram of creatinine.

A possible explanation for the contradictory reports is that gold excretion might exhibit circadian rhythmicity. The existence of some intrinsically rhythmic process which alters the flow of urine has been known since the experiments of Roberts[113] in 1860. It is now well established that urine flow has an endogenous circadian rhythm.[114-117] The term diurnal has often been used in the past and is still sometimes used, for the rhythm with a period of one day, but this is an ambiguous term as it is used to distinguish day from night as well as being applied to fluctuations confined to the working day.[117] The term circadian was introduced by Halberg[116] in 1959 to correct this, 'circadian' indicating a rhythm with a period of around 24 hours.[114] Investigators studying human circadian rhythms have standardised the conditions of their experiments to a greater or lesser extent. Food and fluid intake, urine

collection intervals and posture have been standardised in the past in an attempt to remove or regularise the major interfering influences on the excretory patterns but more recently successful studies have been made without standardised conditions.[117]

Circadian rhythmicity has been demonstrated in the excretion of electrolytes[114,118] and heavy metals such as mercury[119] and lead[120] indicating different but regular peaks of excretion throughout the 24 hour daily cycle.

The second major aim of this study is to establish whether periodicity in gold excretion exists. The existence of excretory rhythms would cause variations in daily random urine gold levels and if these rhythms were different from creatinine rhythms of excretion, then the calculation of results as excretion per gram of creatinine would compound the inconsistency in these values obtained by previous workers.[111]

The role of copper in inflammatory processes has recently been of interest particularly because of the abnormally high serum copper levels[121,122] and synovial fluid copper levels[123] found in patients with RA.[124] Serum zinc levels have been demonstrated to be lowered in RA.[125] Urinary copper and zinc

levels will be measured along with gold in the above study and the possibility of rhythmic excretion of the metals will be investigated. Any likely relationship between the excretion rates of these metals will be examined.

Study 3

Circadian variations have been demonstrated in serum levels of copper[126,127] and iron[128] and the first part of the third study will be an investigation of the possibility that total, free and protein bound gold are present in different concentrations at various times of the day and night in the serum of patients with RA receiving gold drugs. Gold will also be assayed in urine specimens obtained during the same time period.

Gold has been attributed to have several effects on the immune responses. These include stimulation of immunoglobulin secreting cells, inhibition of the activation of the classical and alternate complement pathways (see 1.2. and 1.4.v.) and the inhibition of numerous cell-mediated immune responses to various mitogens and antigens.[129] Lorber et al.[130] noted a significant fall in γ-globulins at 6 months of gold therapy and reported increased affinity of gold at

higher gold levels for various immunoglobulins, including IgG, IgM, complement and complexes of these proteins.[97] Gottlieb *et al*.[131] similarly showed significant depression of γ-globulins and suppression of IgA, IgG and IgM at 6 and 12 months of gold therapy, but Mouridsen *et al*.[132] were unable to show alteration in the metabolic rate of these proteins after 2-5 months of therapy. It is evident that so far reports from this area of investigation have been contradictory and inconclusive.[133]

Circadian rhythmicity of serum immunoglobulins in sick[134] and healthy subjects[135] has been studied and periodicity has been demonstrated in each group for serum IgA, IgG and IgM. The serum specimens obtained in this study will also be analysed for the immunoproteins IgA, IgG and IgM and for acute phase reactants associated with inflammation (complement factors and α_1 acid glycoprotein). The results will be analysed for periodicity in the levels of these parameters and for the possibility of correlation between these and levels of serum gold. Any correlation between these levels and urinary gold, copper and zinc will also be studied along with any possible correlation with efficacy of treatment.

Circadian rhythms have been demonstrated in saliva for cortisol[136] and testosterone.[137] The presence

of metallic ions in saliva has also been demonstrated, including sodium and potassium,[138] lithium,[139] calcium and inorganic phosphate[140] and copper, silver, tin, mercury and zinc.[141] Around the times of venous section and micturition in this study, salivary specimens will be collected and assayed for gold. The combined results will be analysed for possible relationships between salivary gold and serum gold levels, salivary gold and urine gold and salivary gold and immunoproteins. Once again the data will be examined for any correlation between salivary gold and efficacy or toxicity of the treatment.

Study 4

The commonly used gold drugs are water soluble thiolates[44,59] (page 12) mainly because it has been observed that only salts of gold are effective in long-term clinical studies.[142] The clinical effects, course of action and toxicity of these salts are similar to those of their thiol compounds also known to be effective anti-rheumatic agents. These include D-Penicillamine,[143] levamiscle,[144] 5-thiopyridoxine[145,146] and pyrithioxine. It may be possible that at least part of the effectiveness and toxicity of gold salts may be related to the sulphydryl group used for their solubilisation.

It has been demonstrated that disodium aurothiomalate dissociates to form free thiomalate and protein bound gold in the blood followed by free thiomalate in the urine,[77,78] although recent work has reported a free gold fraction also.[79] While the absorption and excretion of the gold moiety have been widely investigated, similar information on the thiol ligand is lacking.

The aim of the final study is to examine the distribution of free thiol and total gold both in plasma and urine after the initial gold injection and investigate any relationships between the two moieties. (The study will be conducted in association with Dr. Susan Rudge at the St. Bartholomews Hospital, London.)

CHAPTER 2

CHAPTER 2

2. PATIENTS AND METHODS

2.1. PATIENTS

All subjects studied in this project were suffering from rheumatoid arthritis and were treated with disodium aurothiomalate. Patients were recommended for the studies by medical staff and were selected from those attending the Nottingham Gold Clinic who fulfilled the following criteria :-

i) Have a diagnosis of RA according to ARA criteria

ii) Aged between 16 and 65 years

iii) Have a disease duration between 6 months and 5 years

iv) Have failed to respond to analgesic or anti-inflammatory drugs

v) Have been prescribed gold therapy

vi) Have given consent to the study

Exclusions :-

i) Patients currently on corticosteroids

ii) Patients who have received Penicillamine during the preceding 3 months

Full approval was given to this study by the Nottingham City Hospital Ethical Committee.

2.2. STUDY METHODS

The project consisted of 4 main studies.

2.2.i. Study 1. The Distribution of Gold in Serum

Part A

Two groups of patients were investigated in this study.

Group 1

Fifty four suitable subjects stabilised on gold therapy had 10 ml of blood withdrawn by venous section immediately prior to the next maintenance gold injection. Serum was separated from the clotted blood and stored at 4°C within 30 minutes of blood collection.

Group 2

Fifteen patients commencing gold therapy had blood withdrawn weekly for 9 weeks prior to the next gold injection and the serum was stored at 4°C within 30 minutes of venous section as for Group 1 patients.

Specimen Analysis

All serum specimens were analysed for total gold and free gold. From these two values the protein bound gold (PBG) levels were calculated.

Total Gold

The total gold levels in the serum specimens were assayed by flame atomic absorption spectrometry (Section 2.3.).

Free Gold

Free, or unbound, serum gold was measured by flame atomic absorption in the protein free ultrafiltrates obtained using Amicon Centriflo membrane cones (Section 2.3.).

Sera from 20 patients were filtered and analysed in duplicate in order to test the method precision (Precision Study).

Statistical Analysis of Results

All the statistics were calculated using a Commodore Series 3032 computer with the appropriate statistical program.

Group 1 Patients

Frequency distribution plots were made for total, free and protein bound gold results.

The results were also analysed by the Pearson correlation method with linear regression (Section 2.4).

Correlation coefficients (r) were calculated for total gold against free and total gold against protein bound gold.

Group 2 Patients

Results obtained from this patient group were analysed as for Group 1 patients. Frequency distributions were plotted for the three gold fractions and Pearson correlation with linear regression was applied to all the results. Correlation coefficients were calculated for total against free gold and total against protein bound gold.

A single correlation with regression was plotted for the combined results from the two groups of subjects both for total against protein bound and for total against free gold.

Part B

As a follow up study to Part A, 10 patients exhibiting side effects of medication demonstrated by cutaneous rash had blood withdrawn during the presentation of the toxic symptoms and then later, when these symptoms had subsided.

The specimens were analysed as for Part A of the study and the results were included in the single

correlation with regression plots from Part A.

Part C. Differential Protein Bound Gold Fractions

Fifty five serum protein concentrates obtained by ultrafiltration in Part A were analysed for differential albumin and globulin gold levels using cellulose acetate membrane electrophoresis followed by flameless atomic absorption spectrometry (Section 2.3.)

Ten of the original serum samples were divided into 3 parts and stored for one week at room temperature, 4°C and minus 20°C. At the end of this period all the specimens were analysed as above for differential protein gold binding values together with total, free and total protein bound gold in order to investigate the effects of storage temperature on the protein bound gold values.

2.2.ii. Study 2. The Rhythmicity of Urinary Gold, Copper and Zinc Excretion

Thirty suitable subjects, of which 5 were in-patients at the Nottingham City Hospital and 25 were out-patients, were admitted to the study. Twenty one subjects were female and 9 were male.

Each patient was tested before admission to the

study to assess their capability of measuring urine volumes accurately. Whilst a small number required some coaching and envigilated practice, a good standard of accuracy was achieved in the main by the group in the measurement of volume. By the time of the study each patient was able to measure urine volumes to the nearest one millilitre using the equipment provided.

Urine Collection Equipment

All patients were given the following equipment which was supplied for the study in a hold-all.

1. Full study instructions including my day and night telephone number.
2. Data sheets. One data sheet for each of the study days.
3. Pen.
4. Sterile urine containers.
5. Measuring (or collecting) jug.
6. A 500 ml and a 250 ml measuring cylinder.

The information required on the data sheets was as follows :-

1. Personal details. (Name, age, sex)
2. Other medication and times of administration.
3. Time of micturition.
4. Total urine volume passed for each micturition.

DOMICILIARY GOLD STUDY (3-4 Days)

1. Pass urine into jug or measuring cylinder.
2. Measure urine volume to nearest 1 ml in measuring cylinder.
3. Record urine volume on data sheet.

   N.B. One data sheet for each day.
4. Record time of micturition on data sheet.
5. Pour aliquot of urine into container.
6. Label container with Name, Date, Volume, Time.
7. Record any special comments. Eg. Alcohol, or further medication.
8. Refrigerate specimen.
9. Reminder. Fill in name on data sheet.

   Important. Record TIME and DATE and VOLUME on specimen and data sheet.

If you have any problems, please telephone Michael Heath :-

Home : 632754

Work : 608111 Exn. 2802/2399

URINE GOLD STUDY

Patient Name

Age

Sex

Study Date

Current Medication

DAY/DATE

Spec. No	Volume	Time	Comments	FOR LABORATORY USE ONLY				
				Gold	Cu	Zn	Crea	Mins

General Comments

Further Medication

If you have any queries please contact :-

Michael Heath
Work: 608111 Exn 2802
Home: 632754

5. General comments. (Eg. Excessive fluid intake since previous micturition and changes in alcohol habits during study.)

Study Method

All subjects (25 domiciliary and 5 in-patient) passed urine when aware of need (ie. voluntary micturition rather than micturition at fixed intervals) and collected aliquots of all urine specimens passed over a period of not less than 4 days, in the sterile labelled bottles supplied. The urine volumes were recorded on the data sheets together with the times of micturition.

As preservatives were not added to the urine containers, where subjects were not able to store specimens at 4°C (to reduce bacterial action), specimens were either delivered to the laboratory or collected from the patients home and then refrigerated.

Specimen Analysis

All urine specimens were analysed for gold, copper and zinc levels by flame atomic absorption spectrometry (Section 2.3.) and for creatinine content using an Instrumentation Laboratory 508 Analyser (page 58).

From the results obtained, excretion rates were

calculated for gold, copper, zinc and creatinine and from the recorded data of urine volumes the rates of water excretion were calculated.

Statistical Analysis of Results

i) For each patient study period histograms were plotted of excretion rates (gold, copper, zinc, creatinine and water) against time.

ii) Results were analysed for periodicity using the sine wave technique of Fort and Mills.[147] (Section 2.4.)

iii) Pearson correlations with regression (see page 68) were calculated and plotted for gold excretion against creatinine excretion and gold excretion against water excretion both for the patient group as a whole and for individual patients.

2.2.iii. Study 3. The Patterns of Gold Levels in Serum, Urine and Saliva and the Relationship of Immunoproteins and Acute Phase Reactants to these Parameters

A further twenty suitable subjects collected aliquots of all measured urine specimens passed over 4 days and recorded the times of micturition as for Study 2. All patients were given instruction sheets

(see page 42), the standard urine collection equipment (page 36) and data sheets (page 43).

On one day of the study during a 24 hour period, each patient was bled by venous section whilst at home as close to 10.00 hours, 16.00 hours and 22.00 hours as possible. Approximately 8-10 ml of blood was allowed to clot, whilst a further 2-5 ml was preserved in disodium ethylenediamine tetra acetic acid (EDTA). On the same day at or around these times each subject collected at least 5 ml of saliva into a sterile universal. The clotted specimens of blood were centrifuged within two hours of collection and the serum withdrawn and stored at 4°C. The specimens collected into EDTA were also centrifuged within two hours of collection, but in this case the resultant plasma samples were stored at minus 70°C until analysis.

The saliva specimens were frozen to minus 20°C within two hours of collection.

In cases where subjects were unable to store urine samples at 4°C, they were collected at the end of each day and refrigerated in the laboratory.

Specimen Analysis

The freezing of saliva followed by thawing and centrifugation enables a clear, debris-free volume of

Blood/Urine/Saliva Study

1. Pass urine into jug or measuring cylinder.
2. Measure urine volume to nearest 1 ml in measuring cylinder.
3. Record urine volume on data sheet.
4. Record time of micturition on data sheet.
5. Pour aliquot of urine into container.
6. Label container with Name, Date, Volume and Time.
7. Record any special comments. Eg. Alcohol, further medication.
8. Refrigerate specimen.
9. Collect saliva specimen into container and label with time and date at 10.00 h, 16.00 h and 22.00 h.
10. If you have gold teeth fillings rinse out mouth with water prior to collection of saliva.

If you have any problems please phone Michael Heath :-

Home: 632754

Work: 608111 Exn. 2802/2399

BLOOD/URINE/SALIVA GOLD STUDY

Name | Age | Sex

Date | Current Medication

Day

Spec. No	Volume	Time	Comments	URINE GOLD	TOTAL S. GOLD	FREE GOLD	PBG	SALIVA GOLD	MINS

sample to be obtained. These clear specimens together with the urine specimens and the serum specimens were analysed for gold levels by flame atomic absorption spectrometry. Free gold in serum was similarly assayed following ultrafiltration and the protein bound fraction calculated from the total and free serum levels. All specimens of serum and saliva were analysed for gold in duplicate.

The serum specimens were further analysed for immunoglobulins (IgA, IgG, IgM) and complement factors (C3 and C4) using the centrifugal turbidimetric method on the Union Carbide Centrifichem (See page 60) and for IgG rheumatoid factor and C-reactive protein by a solid phase enzyme linked immunosorbent assay (ELISA) and a latex enhanced turbidimetric technique on the Centrifichem (page 62) respectively.

The deep-frozen specimens of plasma were thawed and immediately analysed for C3d degradation products using double decker rocket immunoelectrophoresis in agarose (page 60).

Analysis of Results

Histograms were plotted for each study period of urinary gold excretion rates against time. The results for serum gold levels (total, free and protein bound), salivary gold levels and the immuno-

logical parameters measured were imposed onto the urine plots for each patient.

In this way it was possible to investigate the patterns of gold in urine, serum and saliva at different times of day and night and also to examine any relationships between these parameters and immunoproteins and acute phase reactants.

2.2.iv. Study 4. Free Thiomalate in Serum and Urine

This study was arranged in conjunction with Dr. Susan Rudge at the City Hospital, Nottingham. All thiomalate analyses were performed by Mr. David Perrett at St. Bartholomews Hospital, London also in conjunction with Dr. Rudge.

Sixteen suitable patients were admitted to the study. Following an intramuscular test dose, each patient had weekly injections of gold for six months. A full clinical assessment was carried out on each patient on entry to the study by a single observer.[148,149]

Following their first dose of disodium aurothiomalate, all patients had urinary thiomalate and gold levels assayed and plasma thiomalate and gold levels (Total, free and protein bound). The fate of the thiolate moiety was examined with respect to the

fate of the gold part of the drug.

Sampling Procedure

After an overnight fast, basal venous blood and urine samples were taken at 09.00 hours. An intra-muscular injection of 20 mg disodium aurothiomalate was given and blood samples were obtained by vene-puncture at 15, 30, 45, 60, 90 and 240 minutes. Each specimen was divided into two parts, one a clotted specimen and the other into bottles containing EDTA.

All specimens were centrifuged. The serum from the clotted specimens was refrigerated at 4°C for later gold analysis. The plasma specimens from the EDTA samples were treated with sulphosalicylic acid to precipitate proteins and the samples re-centrifuged. The supernatants were rapidly frozen in solid CO_2 and stored at minus 20°C until analysis for thiomalate.

Urine samples for the periods between 0 and 1 hour, 1 and 2 hours, 2 and 4 hours and 4 and 24 hours were collected. The volumes of the urines were recorded and the specimens frozen at minus 20°C until analysis for thiomalate and gold. A specimen report sheet is shown on page 48.

Specimen Analysis

Gold

Urine and total serum gold analyses were performed using flame atomic absorption spectrophotometry. Free gold levels in serum were assayed similarly following ultracentrifugation and the protein bound gold levels calculated from the total and free gold levels.

Free Thiomalate

Concentrations of free thiomalate were measured by high performance liquid chromatography and electrochemical detection. (See page 62).

Analysis of Thiomalate and Gold Results

Simultaneous plots were made for free plasma thiomalate and total serum gold against time after injection. Similarly plots were made for individual patient urinary free thiomalate levels and urinary gold levels against time after injection.

The urinary recovery (within the first 24 hours) for the injected dose of thiomalate was calculated as a percentage.

Time after injection of aurothiomalate (mins)	Plasma Thiomalate (nmol/1)	Total Serum Gold (µmol/1)	Free Serum Gold (µmol/1)	PBG (µmol/1)	Urinary Thiomalate (µmol/1)	Urinary Gold (µmol/1)
0						
15						
30						
45						
60						
90						
120						
240						

2.2.iv. Figure i. Specimen Report Sheet for Thiomalate Study

2.3. ANALYTICAL METHODS

All the analytical methods used in this study were subject to critical quality control regimes and all exhibited precision and accuracy within acceptable limits.

2.3.i. Total Serum Gold

Gold was determined in serum by a simple dilution of the sample with double distilled water followed by analysis on an IL (Instrumentation Laboratory) flame atomic absorption spectrometer, of the model type 251.[150]

Standardisation of the method was by dilutions of a stock standard of 1000 µg/ml of gold dissolved in aqua regia. In order to ensure linearity of the method three levels of standard were used, namely 5.0, 2.5 and 1.0 µmol/l. In order to overcome the depression of the results due to the presence of sodium, potassium and chloride, (the matrix interference) the standards were diluted in a solution containing sodium, potassium and chloride to give values to each of the standards of 140, 5.0 and 100 mmol/l respectively. The instrumental parameters were as follows :-

Light Source	Gold hollow cathode lamp
Lamp Current	5 mAmps
Wavelength	242.8 nm
Slit Width	320 μm
Burner Head	Boling
Flame Description	Air/Acetylene (Oxidising)

Precision

The coefficients of variation (CV) for the within batch standards were 1.0 μmol/l CV 5%, 2.5 μmol/l CV 4% and 5.0 μmol/l CV 4%.

General Precautions

For this method and all other atomic absorption methods used in this work, it was essential to ensure that all glassware and tubes were scrupulously clean. To secure this all glassware was washed in 10% nitric acid followed by washing in double distilled water prior to specimen analysis.

2.3.ii. Free (unbound) Serum Gold

Free gold was measured by flame atomic absorption spectrometry following ultrafiltration of the serum specimens.

Ultrafiltrates of serum were obtained using Amicon

Centriflo Membrane Cones (type CF25) which are an inert, non-cellulosic polymer laminated on a tough inert substrate. All specimens were centrifuged in individual cones, with a maximum volume of 7 ml, at 4°C ensuring that 100G relative centrifugal force (RCF) was not exceeded. Excessive RCF causes protein leakage and can even burst the filter.

The resulting ultrafiltrates were tested for pervading proteins using Boehringer BM test strips, which are sensitive towards albumin (sensitivity limit 6 mg/100 ml) prior to analysis for gold. A total of 5% of all specimens centrifuged were found to contain traces of protein. These were discarded.

All ultrafiltrates were analysed for gold in duplicate and all duplicates gave answers which were within 10% of each other.

Post Script

At the time this study was conducted the BM test strips were the only method available to test the ultrafiltrates for traces of albumin. Some time later the Pharmacia radio immunoassay method for detecting trace amounts of albumin became available.

Twenty four residual specimens of ultrafiltrate were still available from the previous studies having been stored at 4°C. These specimens were analysed for

albumin by the Pharmacia method. In no specimen was a level of albumin greater than 0.8 mg/l detected.

In order to examine the albumin content of freshly prepared ultrafiltrates rather then retro-spectively as above, 20 specimens of serum were centrifuged in membrane cones and similarly analysed. Albumin was detected in 5 of the 20 specimens, but at levels less than 0.8 mg/l. Gold associated with albumin at these concentrations would not significantly alter the gold levels detected in the various serum fractions in the earlier studies.

Pharmacia Albumin RIA-100 Method

This method is a double antibody radioimmunoassay. Albumin in the sample competes with a fixed amount of I^{125} labelled albumin for the binding of the specific antibodies. Bound and free albumin are separated by addition of a second antibody immunoadsorbent followed by centrifugation and decanting. The radio-activity in the pellet is then measured. The radio-activity is inversely proportional to the quantity of albumin in the sample.

2.3.iii. Differential Protein Bound Gold

Serum was firstly analysed for total gold content (T) as in 2.3.i. Serum proteins were next collected in a concentrated form following ultracentrifugation at 4°C using the Amicon Centriflo Membranes. The ultrafiltrates were analysed for free gold (F) as in 2.3.ii.

A dense application of the protein concentrate was made to a cellulose acetate strip in an electrophoresis tank containing a barbitone buffer, pH 8.9 and a current of 2.5 mAmps was applied to the strip. After 30 minutes the strip was removed from the tank and stained in a 0.2% (w/v) Ponceau S solution made up in 3% trichloracetic acid.[157] The now visible bands of albumin and α, β and γ globulins were cut from the strip and individually dissolved in 500 µl of 50% acetic acid. A portion of the strip not containing protein was treated in a similar manner. Five solutions were now present :-

1. Strip alone.
2. Strip plus albumin plus any bound gold.
3. Strip plus α globulins plus any bound gold.
4. Strip plus β globulins plus any bound gold.
5. Strip plus γ globulins plus any bound gold.

Gold analyses were performed on these solutions using an IL 455 Flameless Atomiser in conjunction with the IL 251 Atomic Absorption Spectrometer.[152] The instrumental parameters are shown below :-

IL 251

Light Source	Hollow cathode gold lamp
Lamp Current	5 mAmp
Wavelength	242.8 nm
Slitwidth	320 μm
Analysis Mode	Single beam - Absorption

IL 455

Integration Time	1/16 sec				
Purge Gas	Nitrogen				
Flow Rate	18 SCFN				
Operation Mode	Auto				
Sample Size	25 μl				
Temperature Settings	75	225	450	600	3600°C
Time Settings (×5)	4	4	4	4	2 sec

From the readings obtained on the IL 455 and together with the value for total protein bound gold (P), the gold values for the individual protein fractions were calculated.

Example :

$$P = T - F \text{ µmol/l}$$

	Albumin	α	β	γ	Blank
IL 455 Reading	20	5	60	100	0
Gold Level (µmol/l)	$\frac{P \times 20}{40}$	$\frac{P \times 5}{40}$	$\frac{P \times 60}{40}$	$\frac{P \times 100}{40}$	

where Total Gold (T) = 40 µmol/l

Precision

Replicate analyses were performed for all samples. All duplicate results were within 5% of each other, provided that specimens were stored at the same temperatures.

Replicate analyses of a pooled protein concentrate also gave a CV of 5%.

2.3.iv. Urine Gold

Gold in urine was assayed using an IL Atomic Absorption Spectrometer by direct aspiration of the urine sample. Because of the varying amounts of sodium and chloride in the large numbers of urine specimens analysed, it was not possible to overcome the interferences caused by these atoms by diluting

standards with saline (ie. impossible to match matrix of standards with that of sample). Instead the corrections were made using the automatic background correction mode of the instrument.

The deuterium background correction technique makes use of the fact that a UV continuum source will behave toward non-specific absorption in the same way as a line source and it will thus enable non-specific absorption corrections to be made. By arranging for the continuum source to be out of phase with the sample beam, non-specific absorption can be measured and automatically subtracted from the total absorption measurement.[152]

The method was standardised using 3 standards (5.0, 2.5 and 1.0 μmol/l) diluted with double distilled water from a stock standard. The instrumental parameters were as for 2.3.i.

Precision

The within batch precision for the 3 standards was :-

5.0 μmol/l Standard CV = 3%

2.5 μmol/l Standard CV = 4%

1.0 μmol/l Standard CV = 4.6%

2.3.v. Urine Copper and Zinc

Urine copper and zinc were assayed in a similar manner to urine gold by direct aspiration of the urine samples on an IL 251 Atomic Absorption Spectrometer. The methods were standardised using 3 levels of standard (5.0, 2.5 and 1.0 μmol/l) copper and zinc stock solutions diluted with double distilled water.

As for urine gold the automatic deuterium background correction was employed in order to correct for differences in the matrices between the samples and the standards.

The instrumental parameters are shown below :-

	Copper	Zinc
Hollow cathode lamp	Cu	Zn
Lamp current	5 mA	5 mA
Wavelength	324.7 nm	213.8 nm
Slit Width	320 μm	320 μm
Burner	Boling	Boling
Fuel	Acetylene	Acetylene
Oxidant	Air	Air
Flame	Oxidising	Oxidising
Aspiration Rate	5 ml/min	5 ml/min

Precision

The reproducibility of the 3 standards was as follows :-

Copper : 5.0 µmol/l Standard CV = 4.0%

2.5 µmol/l Standard CV = 4.0%

1.0 µmol/l Standard CV = 4.5%

Zinc : 5.0 µmol/l Standard CV = 4.4%

2.5 µmol/l Standard CV = 5.0%

1.0 µmol/l Standard CV = 5.4%

Accuracy

A urine quality control manufactured by General Diagnostics with a mean copper value of 4.2 µmol/l and a mean zinc value of 2.5 µmol/l was assayed after every nine samples.

Copper : Mean = 4.12 CV = 4.1%

Zinc : Mean = 2.60 CV = 5.4%

2.3.vi. Urine Creatinine

Urine specimens were analysed for creatinine using an IL 508 analyser with a sampling rate of 100 tests per hour. The method employed is a kinetic procedure based on the Jaffé reaction. The principle of this familiar method is that creatinine reacts

with picrate in alkaline solution to form a creatinine picrate complex which is orange-red in colour. This method is susceptible to interference from other components in the sample called chromogens, which react in a similar way to form coloured complexes with alkaline picrate.

The approach made in this method to overcome these interferences uses the observation that most interfering compounds in the Jaffé reaction react more slowly than creatinine. Consequently the absorbance change of the reaction is measured over a period of 16 seconds beginning 25 seconds after the mixing of the sample and reagent at 525 nm. In this way creatinine is measured before the chromogen reaction takes place.

The method was calibrated using IL proprietary creatinine standard and quality controlled using Ortho urine controls from General Diagnostics.

Accuracy

Ortho urine control :

Mean Creatinine level = 8.59 μmol/l

CV = 3.8%

2.3.vii. Serum Immunoglobulins

IgA, IgG, IgM, C_3 and C_4

The method used was a turbidimetric reaction using ICL sheep anti-human antisera (nephelometric grade) on a Centrifichem centrifugal analyser.[153] The antigen excess reactions were detected by kinetic analysis over 3-5 minutes.

The methods were standardised using Atlantic Antibodies Calibrator 1 and 3 and quality controlled using Ortho normal control serum. The CV's obtained for the methods were less than 5%.

C3d Degradation Products

The method of choice was "double decker" rocket immunoelectrophoresis in agarose.[154] The method is a type of immunoselection using anti C3d antisera supplied by Dako Ltd. Because the antisera also react with native C3 this native moiety is removed by precipitation in anti C3c containing gel. The remaining C3d fragments form 'rockets' in a second gel. The heights of the 'rockets' are measured and these are proportional to the levels of C3d. As yet there is no international standard available and so the units are expressed as units/ml.

In house standards are prepared from zymosan

(yeast extract activated complement) treated serum from which the majority of C3c has been removed by precipitation with 11% polyethylene glycol (PEG).

The precision for in house standard assays is better than 10% CV.

IgG Rheumatoid Factor

The method used was an enzyme linked immuno-absorbent assay (ELISA).[155] This is a more specific test for RA than the classical haemaglutination or latex assays.

The basics of the methods are that human IgGFc fragments are coated passively onto microtitre plate wells. The residual protein binding sites are blocked by coating with bovine serum albumin (BSA) and the plates washed. Diluted patient serum is added for 1 hour at 37°C.

The plates are washed and the IgG binding detected using alkaline phosphatase conjugated $F(ab^1)2$ fragments of affinity purified anti-human IgGFd fragment following incubation for 1 hour at 37°C.

The plates are re-washed and the colour developed using p-nitrophenyl phosphate as substrate and an incubation for 20 minutes at room temperature.

The results are expressed as a ratio of test to normal controls. Normal controls are assayed on each

plate and an elevated index is represented by a ratio of greater than 2.5. All fragments are prepared in the laboratory using sheep antisera, which are obtained from the Immunodiagnostic Research Labs. (IDRL) in Birmingham. The quality control values give a better than 10% CV.

C-Reactive Protein (CRP)

This method employs latex enhanced turbidimetry as fluid phase turbidimetry and lacks the sensitivity to measure normal levels of CRP (Less than 20 mg/l).

Anti-human CRP is passively coated to latex beads and the resulting agglutination when serum is added is measured kinetically on the Centrifichem. The precision of the method gives a CV of less than 5%.

This method has been developed in house in the department of immunology and has so far not been published.

2.3.viii. Serum and Plasma Thiol

The thiol study was arranged in conjuction with Dr. Susan Rudge at the Nottingham City Hospital. Dr. Rudge liaised with and transported all the specimens to Mr. David Perrett at St. Bartholomews Hospital, London, who completed all the thiol

2.3. Table 1. Summary of the Analytical Methods

TEST	METHOD	CV
Serum Gold (Total)	Flame atomic absorption (FAA)	5%
Serum Free Gold	FAA following ultra-filtration	10%
Urine Gold	FAA with deuterium background correction	(1)3%(2)4%(3)4.6%
Urine Copper	FAA with deuterium background correction	(1)4%(2)4%(3)4.5%
Urine Zinc	FAA with deuterium background correction	(1)4.4%(2)5%(3)5.4%
Urine Creatinine	Automated kinetic Jaffé	3.8%
IgA, IgG, IgM, C3 & C4	Turbidimetry on centrifugal analyser (CA)	5%
C3d	Double decker rocket immunoelectrophoresis	10%
IgG Rheumatoid Factor	ELISA	10%
C-Reactive Protein	Latex enhanced turbidimetry. Kinetic on CA	5%
Plasma Thiol	High performance liquid chromatography	11.1%
Urine Thiol	High performance liquid chromatography	3.7%

analyses using a method of reversed-phase liquid chromatography followed by electrochemical detection.[156]

The method was standardised using dilutions of a stock thiomalate standard (1.0 mmol/l) prepared in 100 mmol/l phosphoric acid. The working standards were prepared freshly for each run and were in the range 100 µmol/l to 100 nmol/l.

The overall reproducibility in plasma was 11.1% and for urine specimens the CV was 3.7%.

2.4. STATISTICAL METHODS AND DEFINITIONS[157]

2.4.i. Standard Deviation (SD)

$$SD = \sqrt{\frac{\Sigma(\bar{x} - x)^2}{N - 1}}$$

where Σ = Sum of

$\bar{x}$ = Arithmetic Mean = $\Sigma x/N$

x = Observed values

$(\bar{x} - x)$ = Deviation of a value from the mean $\bar{x}$

N = Number of observations

2.4.ii. Standard Error

The standard error helps to determine the potential degree of discrepancy between the sample

mean and the population mean. It is estimated by dividing the standard deviation by the square root of the number of cases.

In this study the standard error (SE) for the individual points was calculated using the following formula :-

$$SE = \theta \times \sqrt{\left(1+\frac{1}{n}\right) + \frac{(x-\bar{x})^2}{\Sigma(x-\bar{x})^2}}$$

where
θ = Standard deviation
n = Number of observations
x = Observed values
$\bar{x}$ = Arithmetic Mean
Σ = Sum of

2.4.iii. Coefficient of Variation (CV)

This figure gives an estimate of the error of a sample expressed as a percentage.

$$CV = \frac{SD}{Mean} \times 100\ \%$$

2.4.iv. Skewness

Skewness is the degree to which a distribution of cases approximate a normal curve. It measures deviations from symmetry. The measure of skewness is sometimes called the third moment and will take on the value of zero when the distribution is a completely symmetric bell-shaped (Gaussian) curve. A positive value indicates that the cases are clustered to the left of the mean with most of the extreme values to the right. A negative value indicates clustering to the right.

2.4.v. Kurtosis

Kurtosis is a measure of the relative peakedness or flatness of the curve defined by the distribution of cases. A normal distribution usually has a kurtosis of zero. If the kurtosis is positive, then the distribution is more peaked (narrow) than would be true for a normal distribution, while a negative value means that it is flatter. Kurtosis is sometimes called the fourth moment.

In this study the statistical program calculated a figure for a normal distribution along with the value of kurtosis for the data supplied.

2.4.vi. Correlation Analysis

Bivariate correlation provides a single number which summarizes the relationship between two variables. These correlation coefficients indicate the degree to which variation (or change) in one variable is related to variation (change) in another.

A correlation plot for two sets of variables is called a scattergram and is a graph of data based on two variables, where one variable defines the horizontal axis and the other defines the vertical axis.

Regression Lines

One way to reduce detail on a scattergram is to draw a straight or curved line through the points in such a manner that it approximates the pattern of points. The closer the data points fall to such a line that best summarizes the relationship, the stronger the correlation between the two variables. The most common statistical procedure for fitting a line to a scattergram based on interval-level variables is called least-squares regression. When the objective is to locate the best-fitting straight line the regression is called linear regression.

Pearson Correlation Coefficient

A measure of the goodness of fit of the linear regression line is the Pearson Correlation Coefficient symbolised by r. When there is a perfect fit (no error) :

$$r = +1.0 \text{ or } -1.0$$

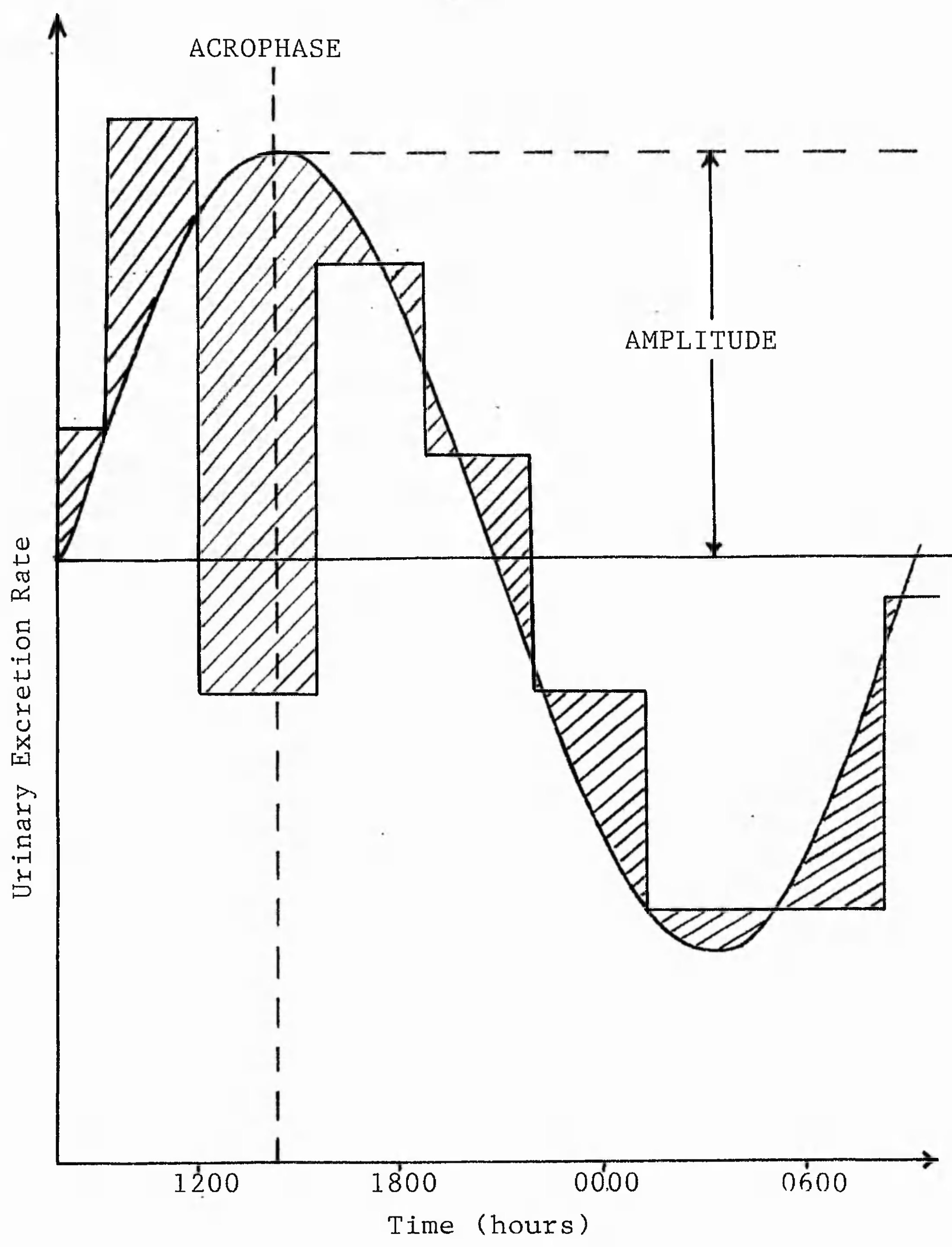
When the linear regression line is a poor fit to the data, r will be close to zero.

Pearson correlation coefficients (r) were assayed on the departmental computer at the Biochemistry Department, City Hospital, Nottingham. Mathematically, r is defined as the ratio of covariation to the square root of the product of the variation X and the variation in Y, where X and Y symbolize the two variables.

2.4.vii. Analysis of Circadian Variation

A histogram offers the most accurate graphical representation of the average rates of excretion when sampling intervals are irregular as in these studies. This is because the alternative mid-point representation produces distortion of actual behaviour when there are long collections of urine.[158] (See Figure 1.)

A sine wave curve was fitted to the data



2.4.vii. Figure 1. Analysis of Circadian Variation

obtained from the urine studies.
Departure from perfect fit was assessed by taking the differences between predicted and observed areas (Figure 1.), squaring them and summing the squares. Sine wave curves were fitted until this sum was reduced to a minimum to obtain the 'best fitting curve'.[147] The sum of the squared deviations of the determined areas from those predicted by the curve was divided by the number of urine specimens minus three, since three parameters are required to define a sine curve. Dividing the estimate of the goodness of fit by an estimate of the total variance, (that is, the sum of the squared differences of the determined areas from the 24 hour mean) gave a variance ratio. From this the probability that the fit of the sine curve could be a chance phenomenon was determined.

Data was processed at the Queens Medical Centre, Nottingham, using a computer program originally developed by Dr. David Minors at the Manchester University Medical School. The program based on the methods of Fort and Mills modified from that of Halberg[158] and called the Zero Amplitude Test, calculated the joint significance value (p) for the amplitude and acrophase of the sine curve.

Definitions associated with Circadian Rhythmicity

Rhythm	- A regularly oscillating process.
Mesor	- The 24 hour mean level of a fitted sine wave.
Acrophase	- The time of the peak on the best fitting sine curve.(See Figure 1.)
Amplitude	- Half the distance from trough to peak in a sinusoid curve. (See Figure 1.)
'p' Value	- Significance level. (See page 70) 'p' values greater than 0.05 (5%) are classed as insignificant.

CHAPTER 3

CHAPTER 3

3. RESULTS

3.1. STUDY 1. THE DISTRIBUTION OF GOLD IN SERUM

Part A

Group 1 Patients

The values obtained for total gold, free and protein bound gold (PBG) in the serum of the 54 patients stabilised on gold therapy are shown in the Appendix (7.1. Table 1.).

The results for the 20 duplicate analyses of free gold are shown in Table 1. In each pair of duplicate assays the CV was better than 10%.

Analysis of Results

a) Frequency Distribution

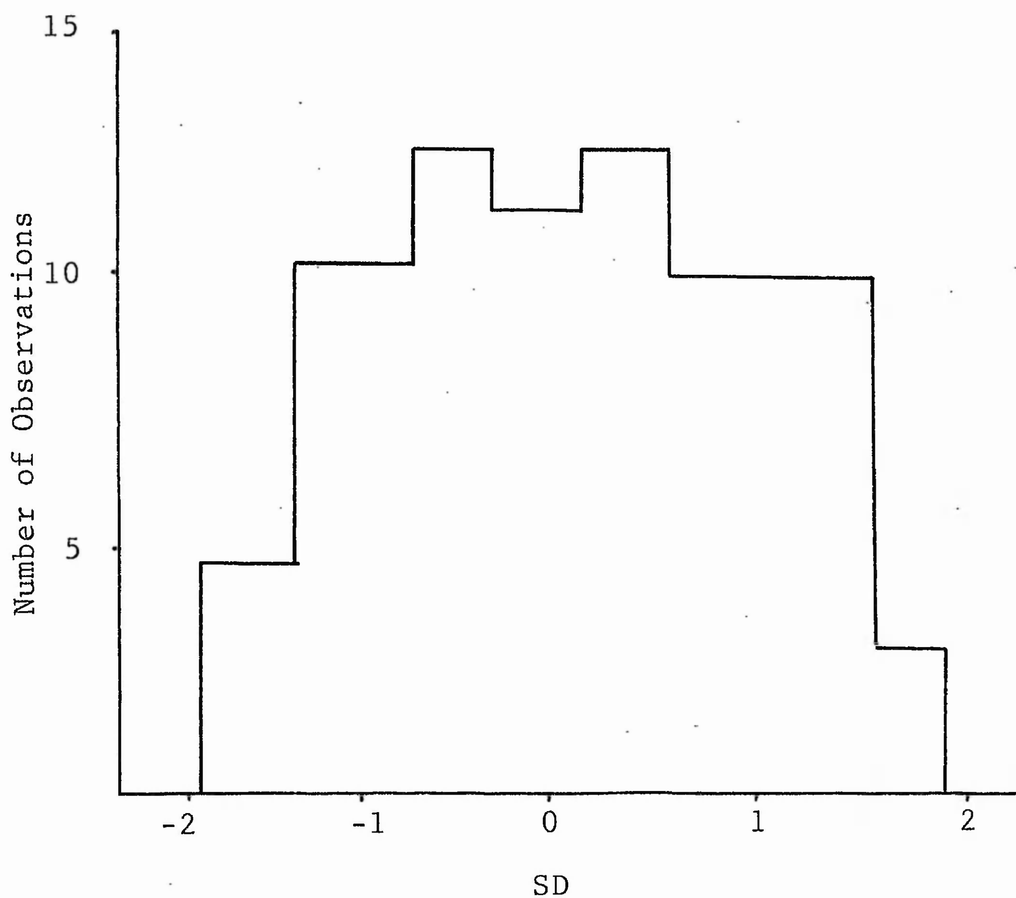
The frequency distribution for total, free and protein bound gold were calculated as standard deviations from the mean and plotted as histograms (Figures 1,2 and 3). The distribution for each gold fraction demonstrated a slight skew to the left of the mean whilst the estimated values of kurtosis

3.1. Table 1. Duplicate Analyses of Serum Free Gold

Patient	Sample	Free Gold Level µmol/l
J.S.	A	1.80
	B	1.75
P.T.S.	A	1.20
	B	1.12
H.O.	A	1.50
	B	1.39
H.A.E.	A	0.66
	B	0.70
E.F.	A	0.90
	B	0.84
E.J.B.	A	1.46
	B	1.42
D.B.	A	0.59
	B	0.57
T.D.	A	0.80
	B	0.83
M.D.	A	1.04
	B	1.00
C.S.	A	1.00
	B	1.00
P.T.S.	A	1.20
	B	1.16
J.H.	A	0.46
	B	0.50
J.C.	A	0.70
	B	0.72
P.J.S.	A	1.12
	B	1.15
B.F.	A	0.25
	B	0.27

3.1. Table 1. continued

Patient	Sample	Free Gold Level μmol/l
D.A.	A	0.52
	B	0.54
M.A.J.	A	1.00
	B	1.08
R.A.W.	A	1.26
	B	1.30
M.H.	A	1.22
	B	1.18
E.C.	A	0.55
	B	0.59

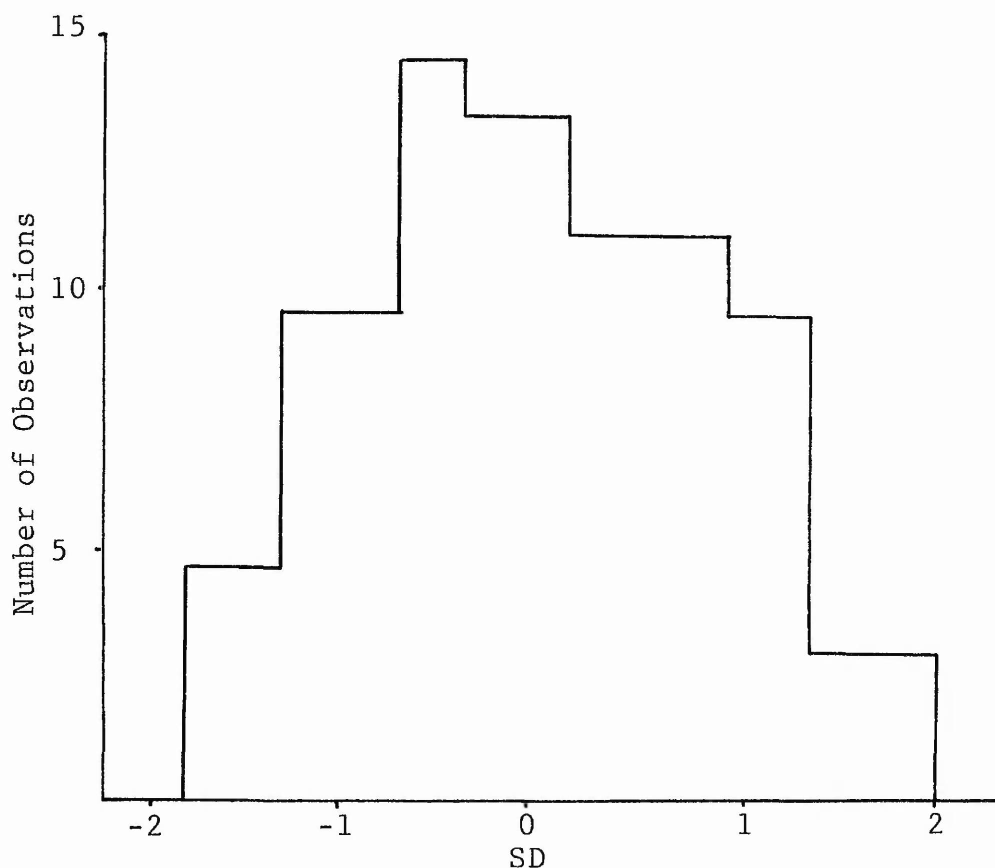


3.1. Figure 1. Frequency Distribution of Total Gold Values

n = 54

Coefficient of Skewness = 0.26 (0) (should be)

Estimate of Kurtosis = 2.52 (3)

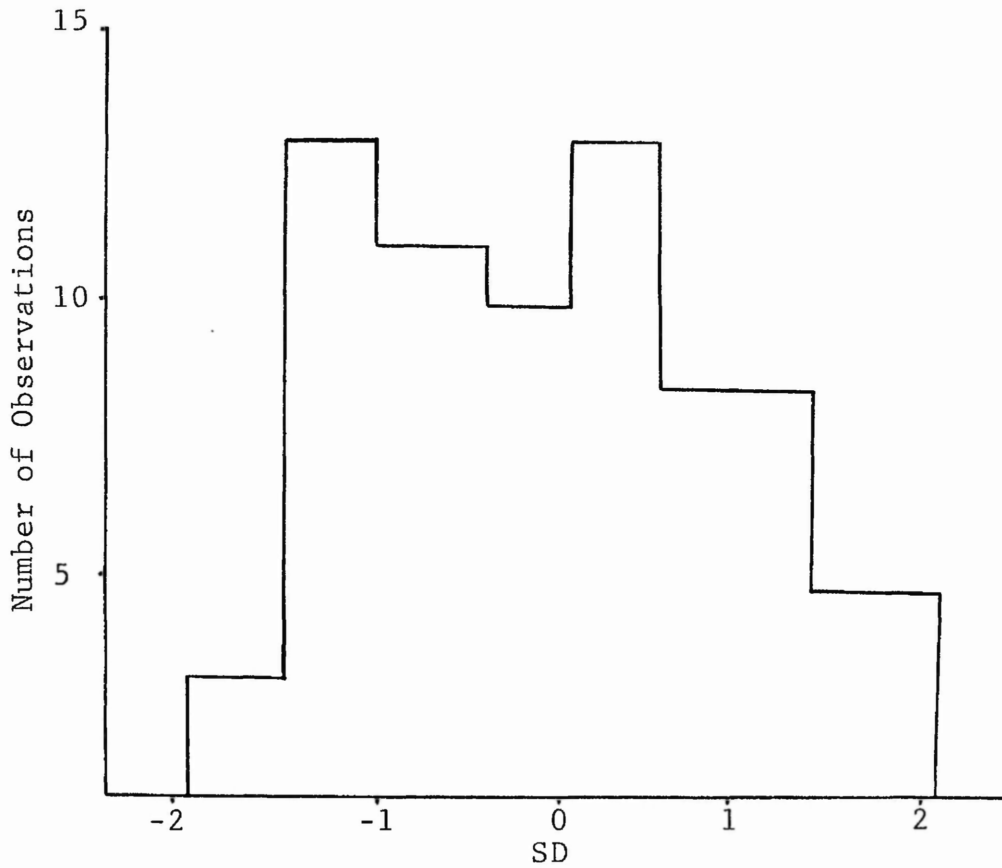


3.1. Figure 2. <u>Frequency Distribution of Protein Bound Gold Values</u>

n = 54

Coefficient of Skewness = 0.24 (0) (should be)

Estimate of Kurtosis = 2.54 (3)



3.1. Figure 3. <u>Frequency of Distribution of Free Gold Values</u>

n = 54

Coefficient of Skewness = 0.28 (0) (should be)

Estimate of Kurtosis = 2.51 (3)

demonstrated that for each gold fraction the curve representing the distribution was more peaked than for a normal distribution.

b) <u>Pearson Correlation</u>

Good correlation was demonstrated between total and protein bound gold ($r = 0.998, p = <0.0001$, Figure 4.) and between total and free gold ($r = 0.909$, $p = <0.0001$, Figure 5).

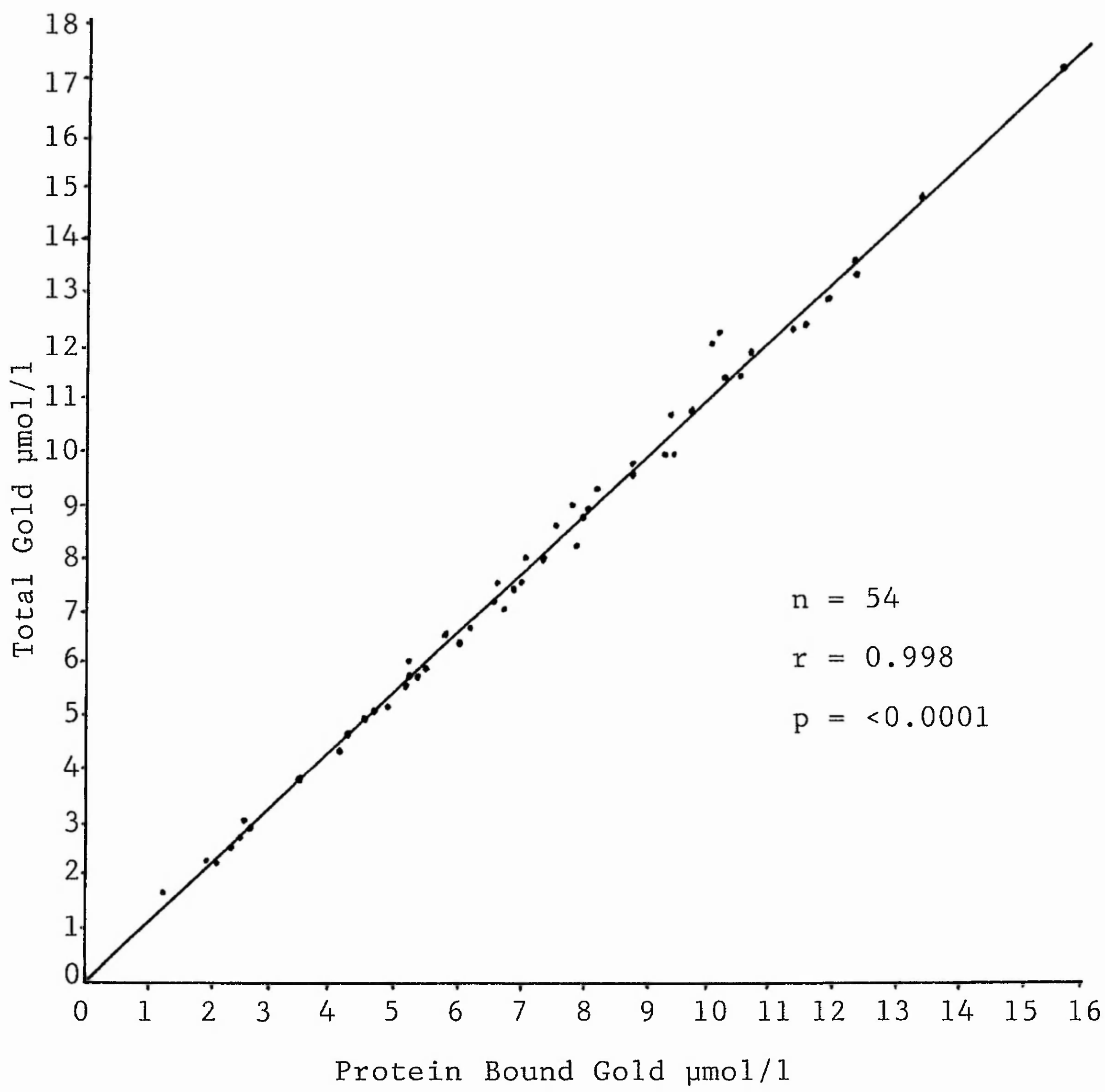
Group 2 Patients

The values obtained for total, free and PBG for the 15 patients who were commencing therapy are shown in the Appendix (7.1. Table 3.).

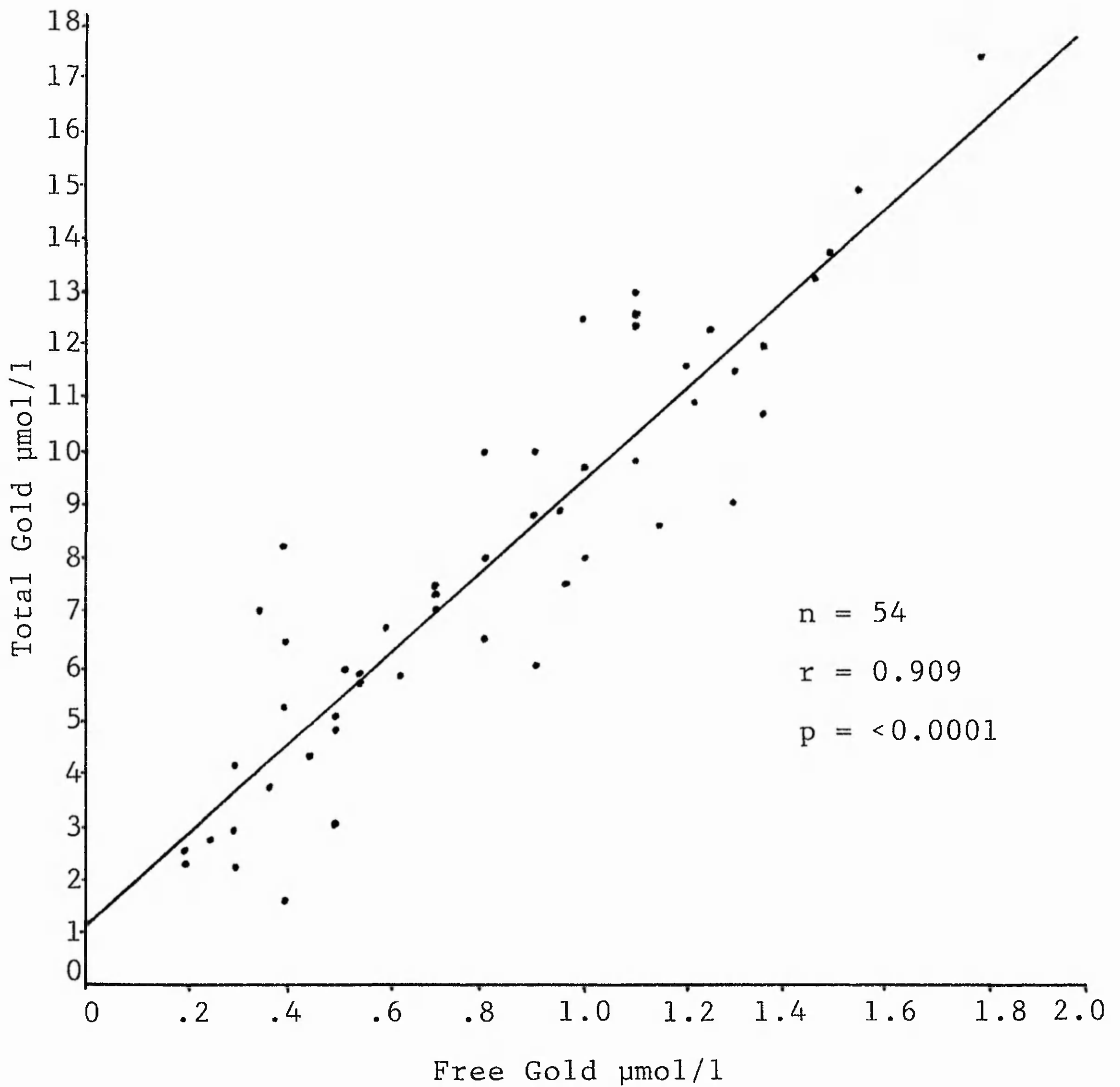
Analysis of Results

a) For each patient continuous plots were drawn on the same graph for total, free and PBG for each week of the 9 week study (See Figure 6.).

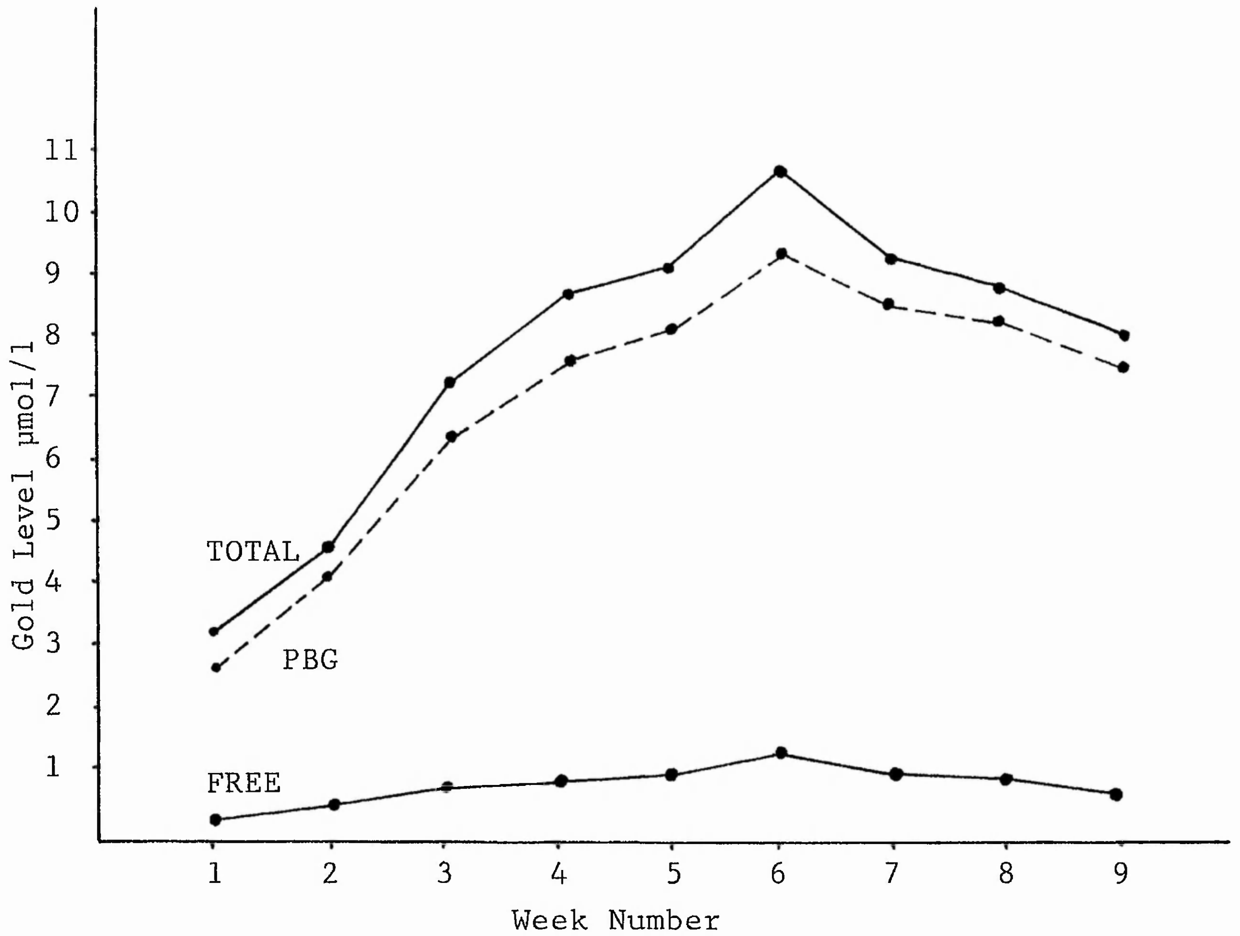
Observed by eye each patient demonstrated consistently similar distributions of total, free and PBG throughout the study. These results wère analysed mathematically by the Pearson Correlation technique.



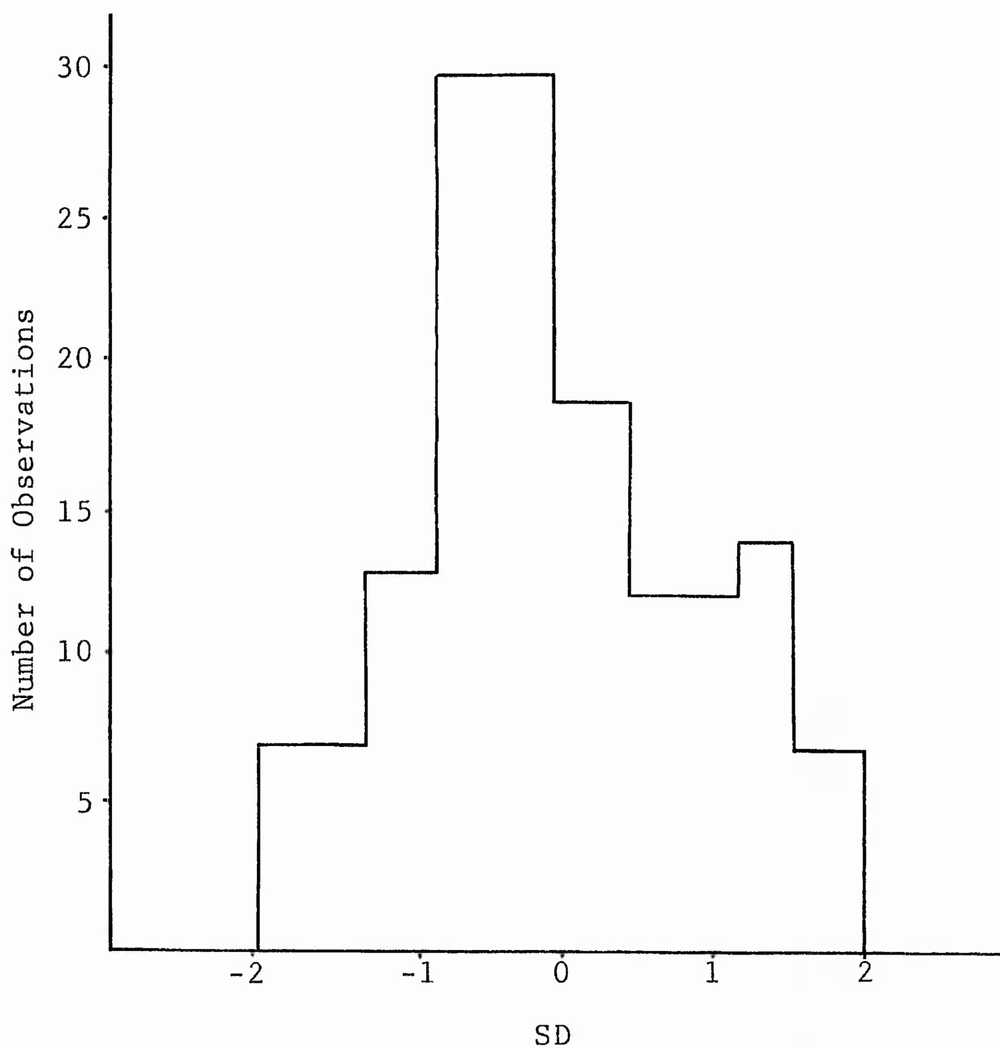
3.1. Figure 4. Total against Protein Bound Gold Correlation



3.1. Figure 5. Total against Free Gold Correlation



3.1. Figure 6. Continuous Plot of Total, Free and PBG over the 9 Week Study Period for Patient Z.F.

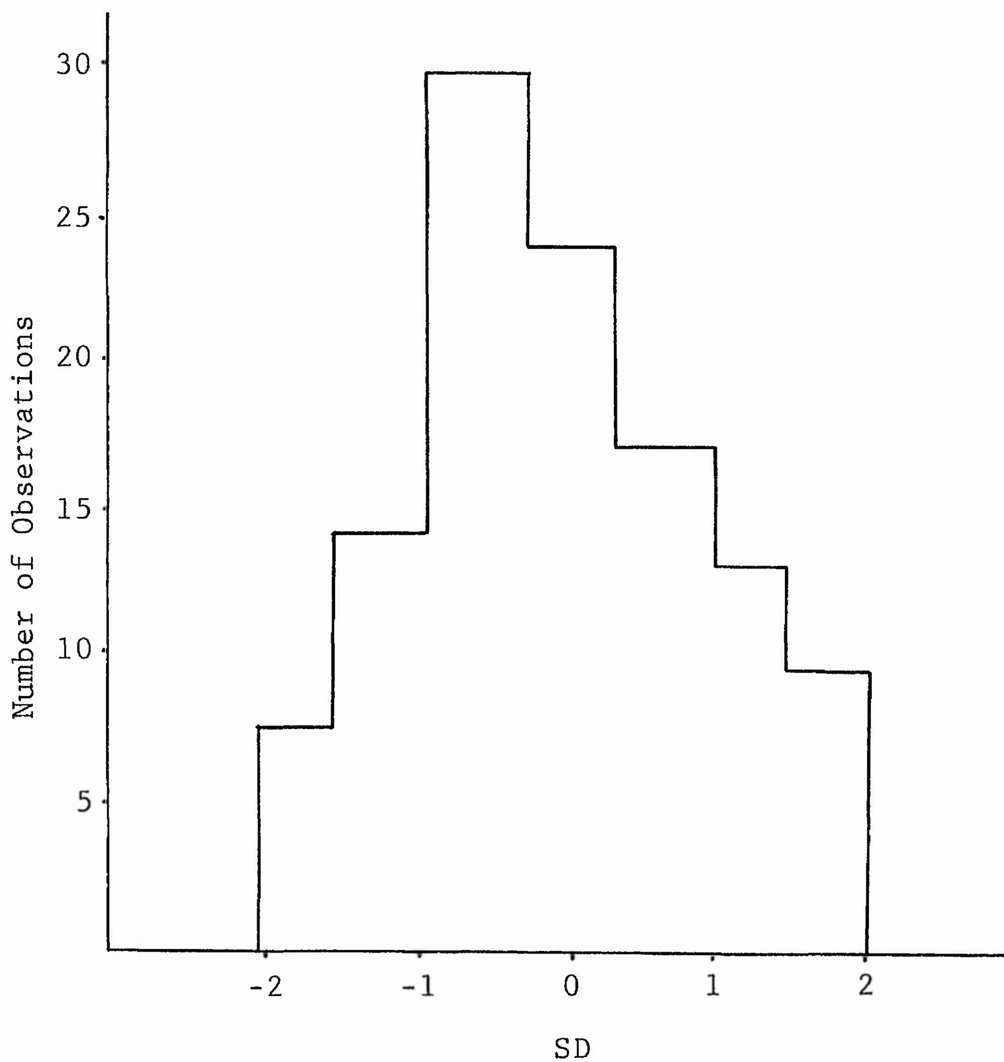


3.1. Figure 7. Frequency Distribution of Total Gold Values for Study 2 Patients

n = 135

Coefficient of Skewness = 0.30 (should be 0)

Estimate of Kurtosis = 2.56 (should be 3)

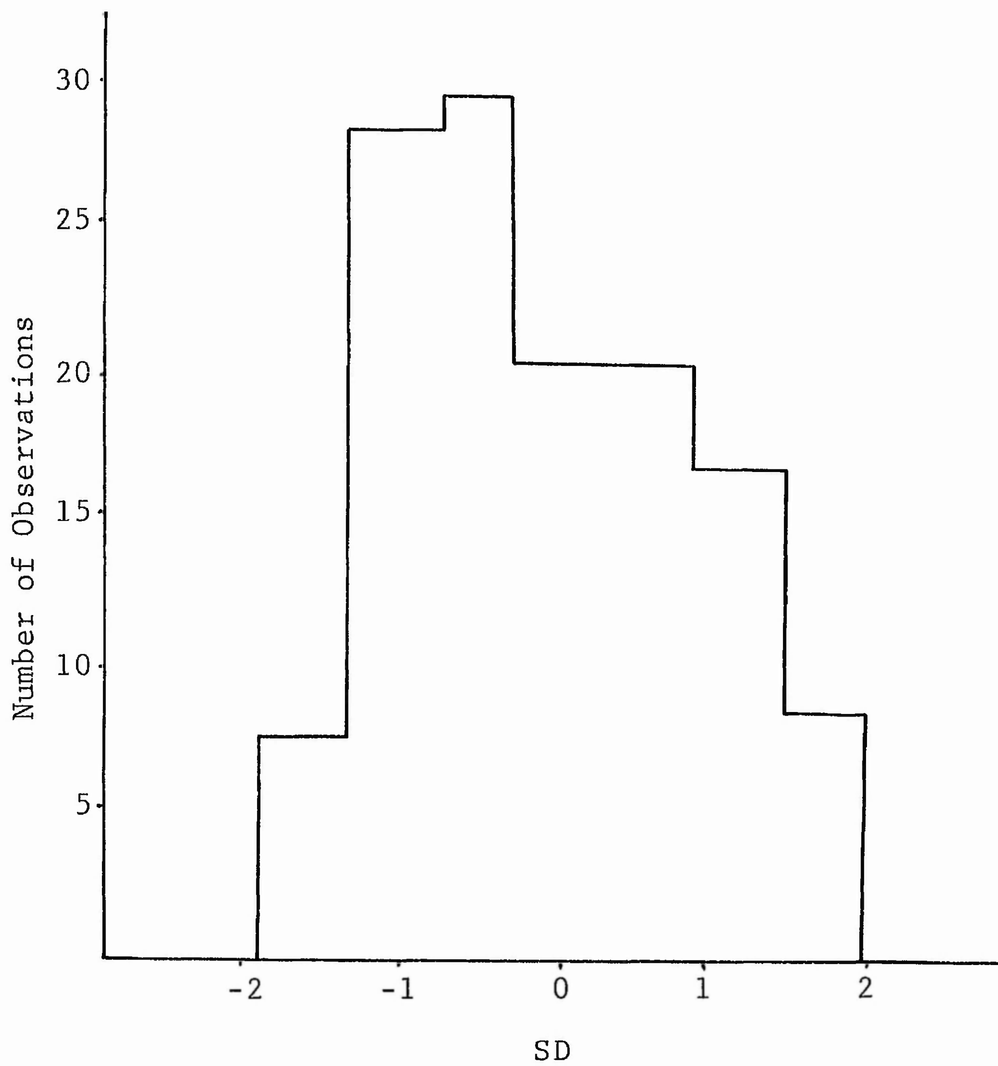


3.1. Figure 8. Frequency Distribution of PBG Values for Study 2 Patients

n = 135

Coefficient of Skewness = 0.30 (0)

Estimate of Kurtosis = 2.60 (3)



3.1. Figure 9. <u>Frequency Distribution of Free Gold Values for Study 2 Patients</u>

n = 135

Coefficient of Skewness = 0.35 (0)

Estimate of Kurtosis = 2.52 (3)

b) <u>Frequency Distribution</u>

The frequency distribution for total, free and PBG were calculated as standard deviations from the mean and plotted as histograms (Figures 7, 8 and 9.).

Each gold fraction demonstrated a skew to the left of the mean whilst the estimated kurtosis values indicated that each distribution curve was more peaked than a normal distribution curve.

c) <u>Pearson Correlation</u>

<u>Total Gold against PBG</u>

Good correlation was demonstrated between total gold and PBG for the combined results of the Group 1 and Group 2 patients ($n = 189$, $r = 0.996$, $p = <0.0001$).

However, the addition of the line of linear regression to the plots reveals that of the 189 points plotted, only 7 are above the regression line (Figure 10.), which intercepts the Y axis at 0.59 µmol/l. The recalculation and plot of the correlation with regression for the results minus these 7 values reveals good correlation once again ($n = 182$, $r = 0.998$, $p = <0.0001$) but this time the line of linear regression has an intercept of 0.09 on the X axis (See Figure 11.).

The addition of lines representing plus and minus the standard error (SE) of the individual points

to the regression line shows the 182 points inside these limits whilst the addition of the 7 omitted points to the graph places then outside the ±SE lines (Figure 12.).

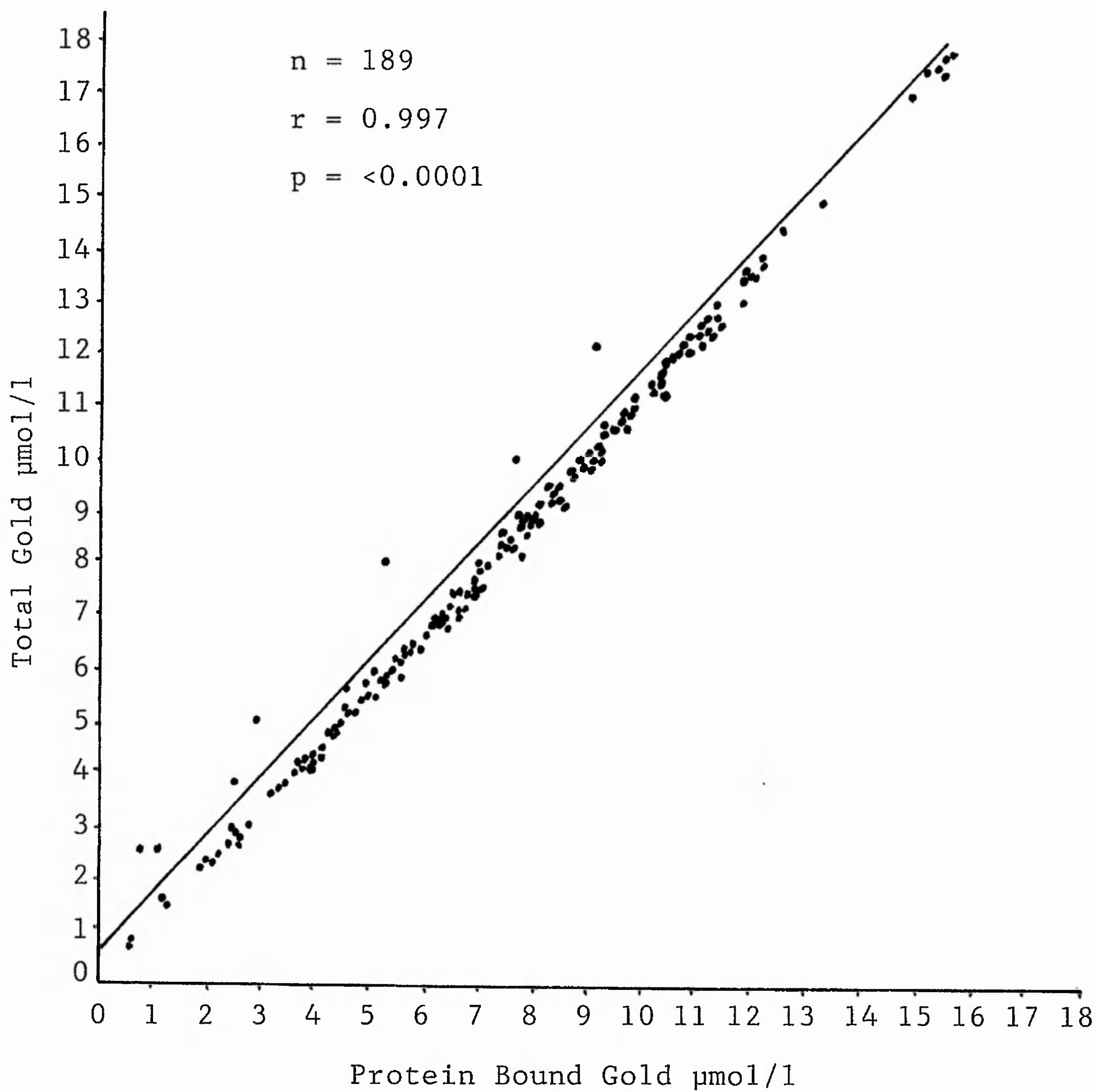
Total Gold against Free Gold

Good correlation was demonstrated between total gold and free gold for the combined results of the Group 1 and Group 2 patients ($n = 189$, $r = 0.845$, $p = <0.0001$). It is evident from the correlation plot with linear regression (Figure 13.) that there are 7 outlying points.

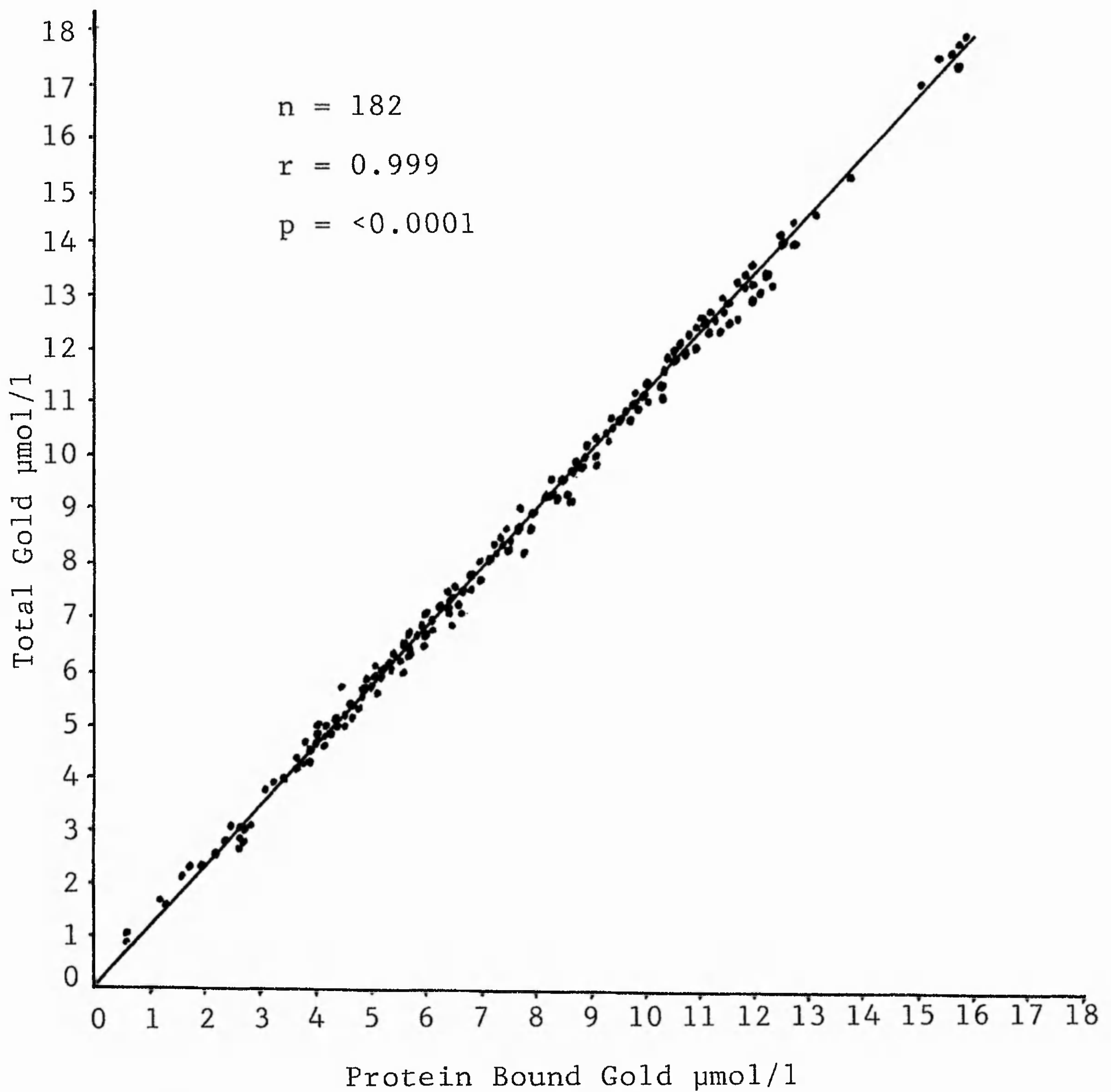
Recalculation of the graph minus the 7 outliers improves the correlation ($n = 182$, $r = 0.947$, $p = <0.0001$) and lowers the intercept of the regression line on the Y axis (Figure 14.).

The addition of lines plus and minus the SE of the individual points to the regression line reveals 182 points inside these limits whilst the addition of the 7 omitted points places them significantly outside of these limits (Figure 15.).

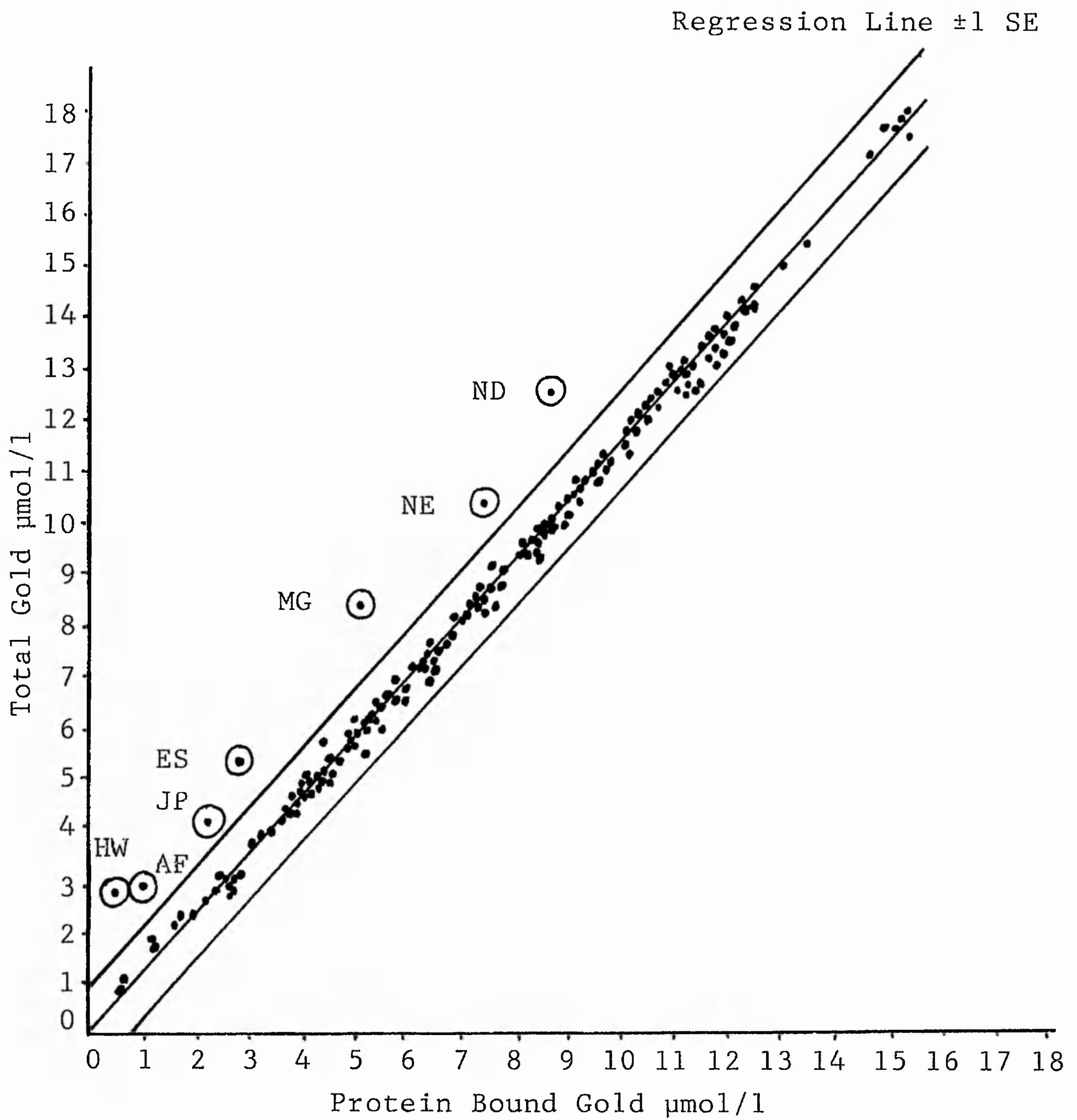
Investigation of the medical histories of the 7 patients with results outside ±SE of the regression lines revealed that at these times 6 of the patients presented with side effects of gold salt treatment,



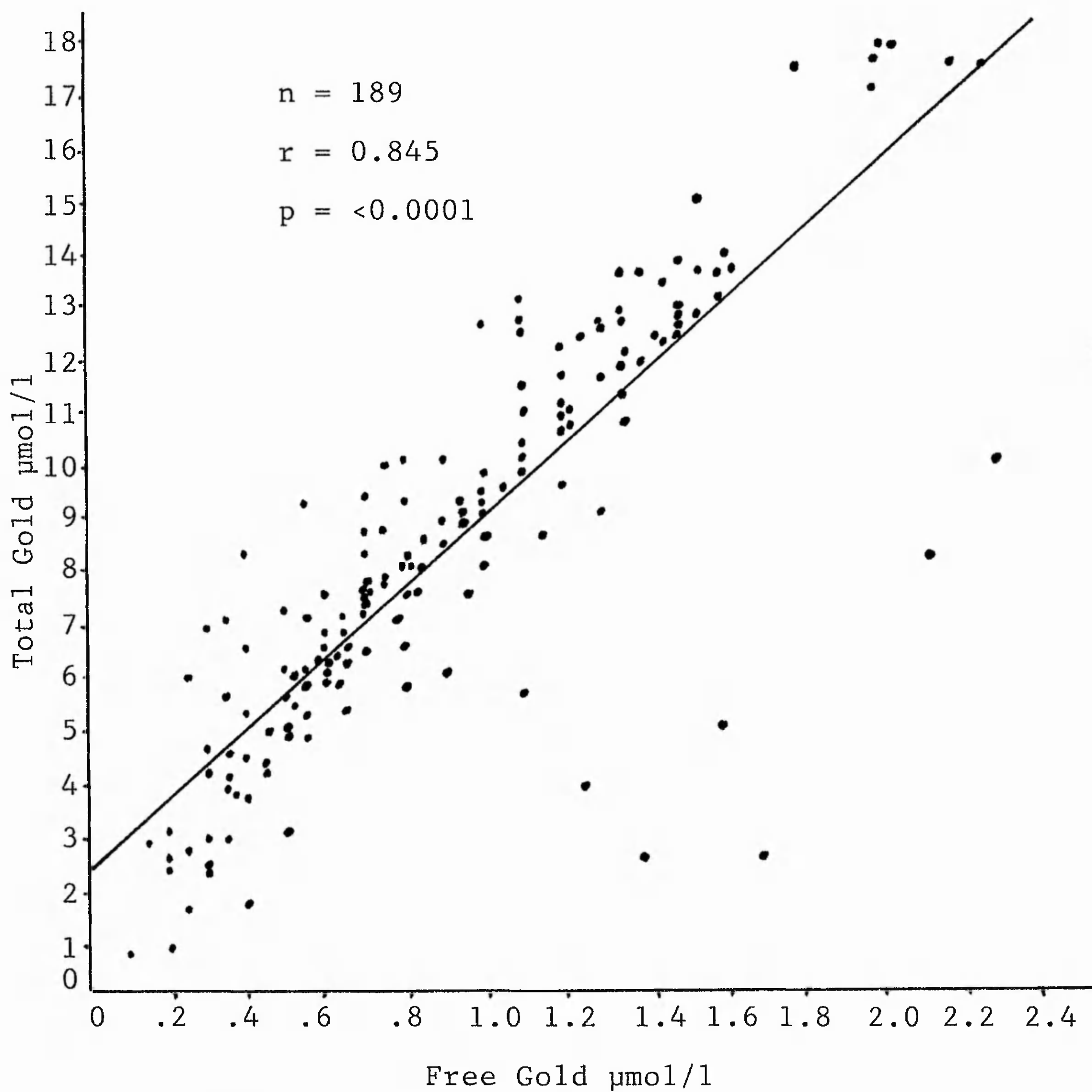
3.1. Figure 10. Total against Protein Bound Gold Correlation



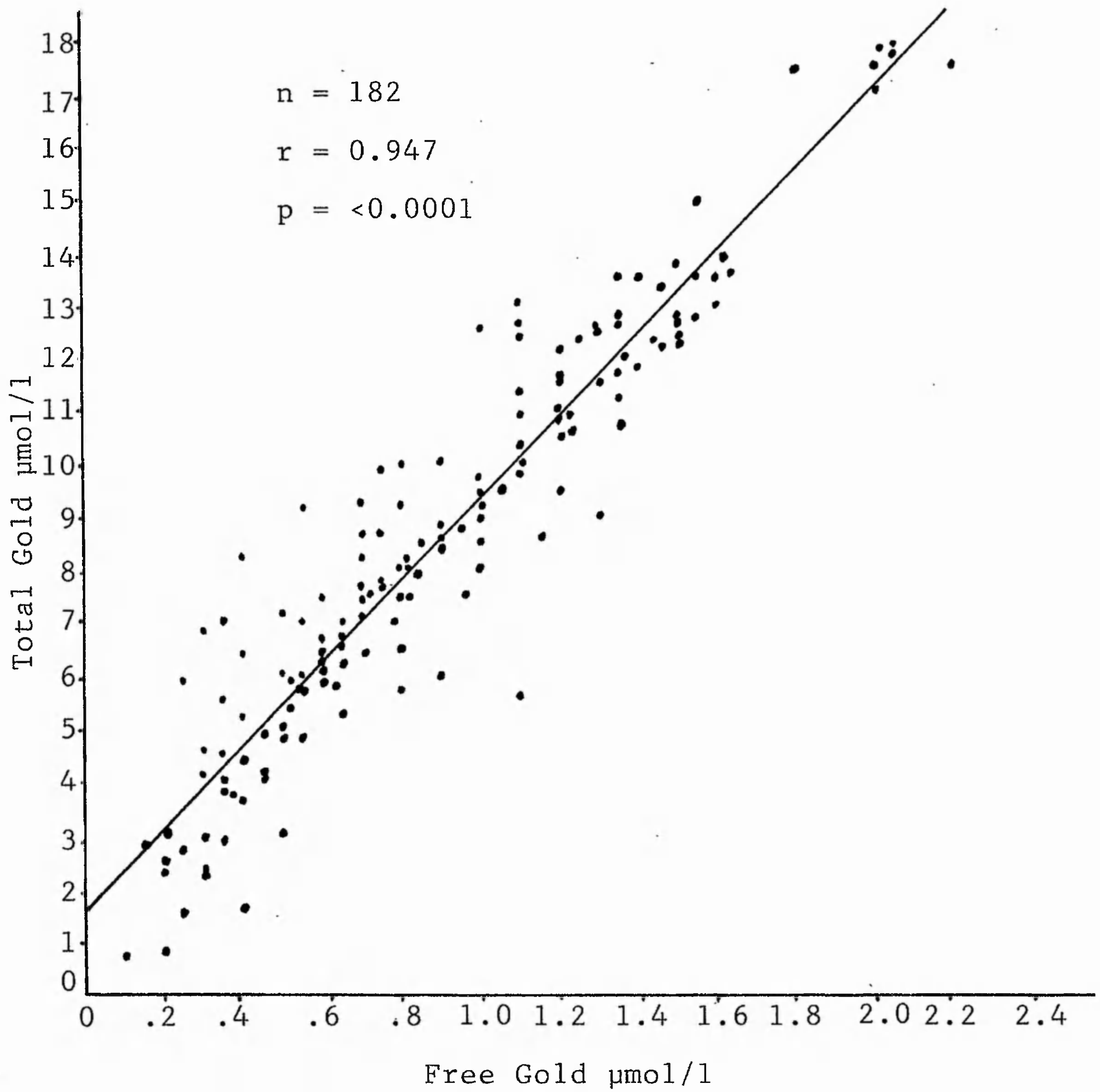
3.1. Figure 11. <u>Total against Protein Bound Gold Correlation</u>



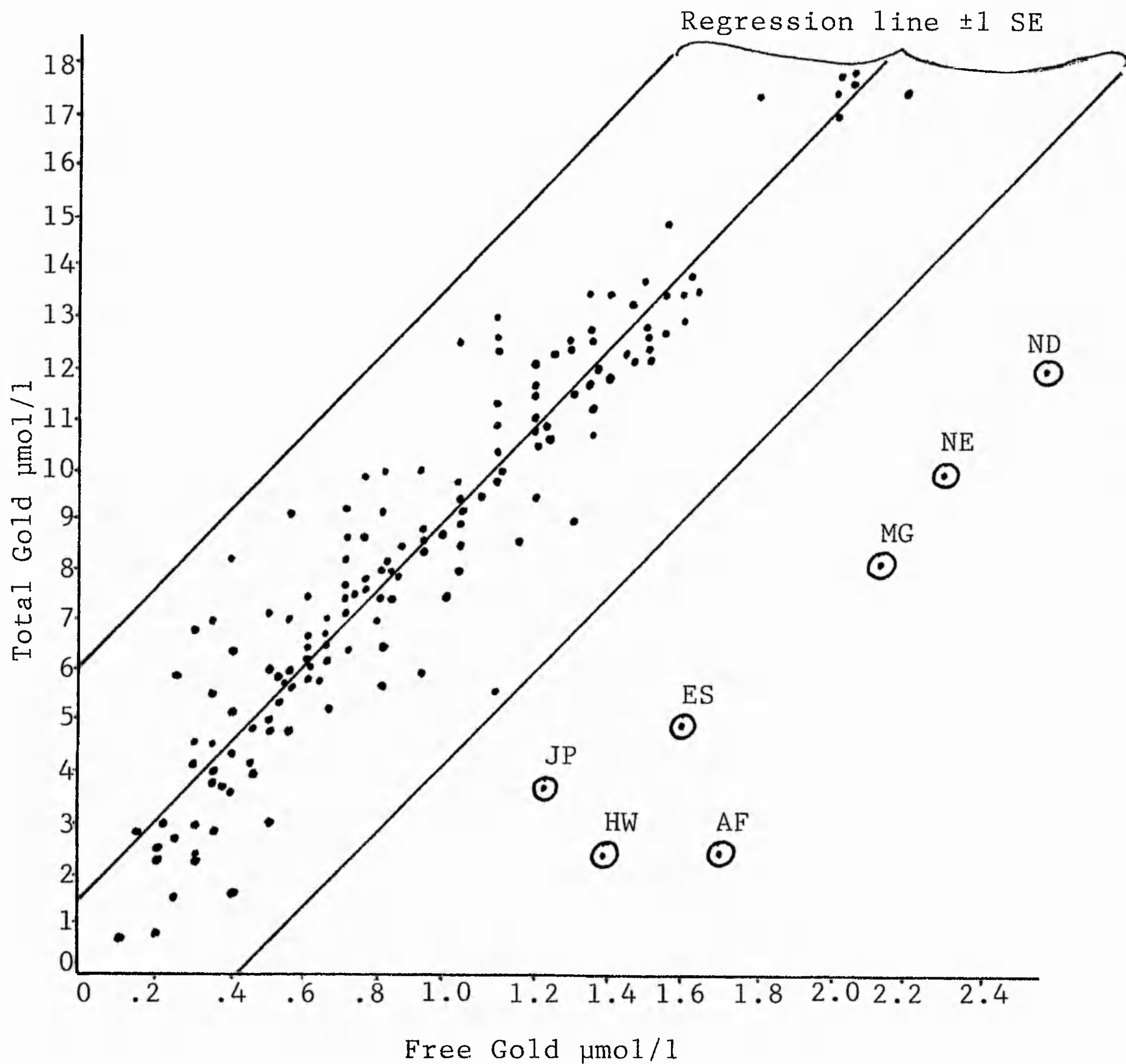
3.1. Figure 12. <u>Total against Protein Bound Gold Correlation</u>



3.1. Figure 13. Total against Free Gold Correlation



3.1. Figure 14. Total against Free Gold Correlation



3.1. Figure 15. Total against Free Gold Correlation

whilst the information on the seventh patient was not available (See Table 2.). Retrospective analysis of all other patient notes failed to reveal any other instance of side effects of the treatment during the study.

d) Further Relationships between Total, Free and Protein Bound Gold

Apart from the 7 instances noted above, the free gold fraction in 182 results represented less than 12% of the total serum gold. In these 7 cases where the plots fell outside the limits set on the regression line, there was a higher free to total gold ratio and a lower PBG to total gold ratio.

Free Gold to Total Gold Ratios

The range for 182 paired results for the free gold to total gold ratio was 0.04 to 0.17. The mean value was 0.10 (Mean ±2 SD = 0.06 to 0.14). The ratios for the 7 outliers were in the range 0.21 to 0.65 (See Table 3.).

Protein Bound Gold Ratios

The range for the 182 paired results for the PBG to total gold ratio was 0.82 to 0.95 with a mean value of 0.90 (Mean ±2 SD = 0.86 to 0.94). The ratios

PATIENT	CLINICAL OBSERVATION
J.P.	RASH
H.W.	RASH
A.F.	THROMBOCYTOPENIA
E.S.	RASH
M.G.	RASH
N.E.	RASH
N.D.	INFORMATION NOT AVAILABLE

3.1. Table 2. Clinical Observations of Patients Showing Abnormal Correlation Plots

Patient Outliers	Free to Total Gold Ratio
E.S.	0.32
H.W.	0.55
N.D.	0.21
M.G.	0.26
J.P.	0.32
N.E.	0.27
A.F.	0.65
Range for 182 paired results within ±2 SD of regression line (0.04 - 0.17)	

3.1. Table 3. Free to Total Gold Ratios for the 7 Outliers together with the Range for the Non-outlying Results.

Patient Outliers	PBG to Total Gold Ratio
E.S.	0.68
H.W.	0.45
N.D.	0.77
M.G.	0.63
J.P.	0.65
N.E.	0.76
A.F.	0.35
Range for 182 paired results within ±2 SD of regression line (0.82 - 0.95)	

3.1. Table 4. Protein Bound Gold Ratios for the 7 Outliers together with the Range for the Non-outlying Results.

for the 7 outliers were in the range 0.35 to 0.77 (See Table 4.).

It is evident from the previous observations that it is the ratio of free and PBG to the total serum gold levels which reflects the presentation of side effects and not just the amount of total or free gold.

e) Total Serum Gold Values during Toxic Side Effects

Whilst there was a very noticeable change in the free fraction at times of toxicity, examination of the serial total gold values for each patient (Table 5.) reveals no noticeable or unexpected changes in these levels.

Part B (Part A follow-up study)

The results for total, free and protein bound gold in the serum of blood withdrawn from 10 patients exhibiting side effects of medication, both during and after presentation of toxic symptoms are shown in Table 6. The specimens were taken whilst a rash was present and thence variously 2 to 4 weeks after the initial observation of a cutaneous rash had disappeared.

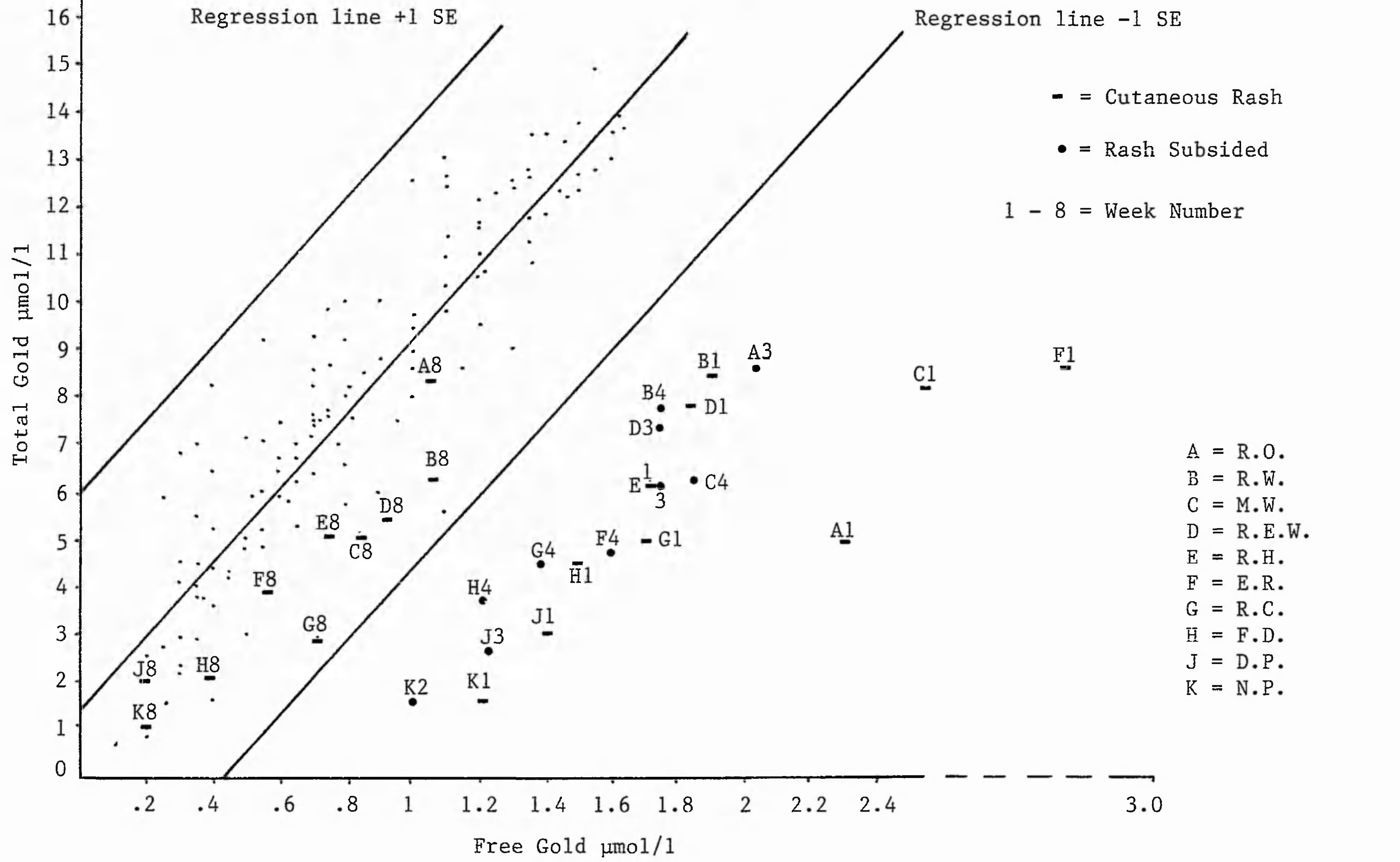
Name	Gold Fraction	Gold Values µmol/l								
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
J.P.	Total	0.70	2.80	*3.90	7.70	12.50	13.75	12.60	7.00	7.50
	PBG	0.60	2.65	2.65	6.95	11.20	12.25	10.25	6.45	6.70
	Free	0.10	0.15	1.25	0.75	1.30	1.50	1.35	0.55	0.80
H.W.	Total	0.80	*2.55	4.55	6.20	8.75	13.50	8.55	7.90	7.50
	PBG	0.60	1.15	4.25	5.55	7.80	11.90	7.55	7.06	6.80
	Free	0.20	1.40	0.30	0.65	0.95	1.60	1.00	0.84	0.70
A.F.	Total	0.70	*2.60	6.80	7.65	12.75	13.75	12.50	7.05	7.40
	PBG	0.60	0.90	6.50	6.90	11.45	12.25	11.15	6.50	6.60
	Free	0.10	1.70	0.30	0.75	1.30	1.50	1.35	0.55	0.80
E.S.	Total	4.00	*5.00	7.60	10.80	9.20	6.40	8.20	9.00	9.40
	PBG	3.65	3.40	6.95	9.60	8.40	5.80	7.50	8.00	8.40
	Free	0.35	1.60	0.75	1.20	0.80	0.60	0.70	1.00	1.00
M.G.	Total	4.90	5.40	6.20	6.70	6.30	*8.40	7.50	6.40	6.00
	PBG	4.45	4.90	5.60	6.05	5.70	5.30	6.70	5.80	5.45
	Free	0.45	0.50	0.60	0.65	0.60	2.20	0.80	0.60	0.55
N.E.	Total	6.00	9.25	*10.05	12.10	11.35	13.50	13.55	12.75	11.20
	PBG	5.40	8.55	7.75	10.90	10.25	12.15	12.00	11.20	9.85
	Free	0.60	0.70	2.30	1.20	1.10	1.35	1.55	1.55	1.35

* Indicates toxicity diagnosed by overt clinical signs.

3.1. Table 5. Serum Gold levels for 6 patients exhibiting toxic side effects of treatment

These results were added to the regression line plus and minus the SE which was produced in Part A (Figure 15.) and are shown in Figure 16. In all cases the points fell outside the SE limits imposed on the regression line. Furthermore the results obtained following the disappearance of the rash also remained outside these limits, although in all but one case the points moved closer to the regression line (ie. the free gold to total gold ratio became lower). In this one case the points remained in the same position.

Serum analysed 8 weeks after the initial diagnosis of toxicity revealed that for all 10 patients the correlation plots for total against free gold and total against PBG had returned within the limits on the regression line.



3.1. Figure 16. Total against Free Gold Correlation including 10 Patients exhibiting Side Effects

3.1. Table 6. Part B: Serum Gold Results for Patients both During and After Presentation of Toxic Side Effects

Name	Total Gold µmol/l	Free Gold µmol/l	PBG µmol/l	Clinical Conditions	Week
N.P.	1.5	1.2	0.3	Cutaneous Rash (CR)	1
N.P.	1.5	1.0	0.5	Rash Subsided (RS)	2
N.P.	1.0	0.2	0.8	RS	8
E.R.	8.4	3.0	5.4	CR	1
E.R.	4.5	1.6	2.9	RS	4
E.R.	4.0	0.6	3.5	RS	8
R.H.	6.0	1.7	4.3	CR	1
R.H.	6.0	1.7	4.3	RS	3
R.H.	4.5	0.7	3.8	RS	8
R.W.	8.2	1.9	6.3	CR	1
R.W.	7.6	1.7	5.9	RS	4
R.W.	6.0	1.0	5.0	RS	8
R.E.W.	7.7	1.8	5.9	CR	1
R.E.W.	7.2	1.7	5.5	RS	3
R.E.W.	5.4	0.9	4.6	RS	8
F.D.	4.2	1.5	2.7	CR	1
F.D.	3.5	1.2	2.3	RS	4
F.D.	2.0	0.4	1.6	RS	8
R.C.	5.0	1.7	3.3	CR	1
R.C.	4.2	1.4	2.8	RS	4
R.C.	3.2	0.7	2.5	RS	8
D.P.	2.8	1.4	1.4	CR	1
D.P.	2.5	1.2	1.3	RS	3
D.P.	2.0	0.2	1.8	RS	8
M.W.	8.0	2.5	5.5	CR	1
M.W.	6.2	1.8	4.4	RS	4
M.W.	5.0	0.9	4.2	RS	8
R.O.	10.0	2.3	7.7	CR	1
R.O.	8.5	2.0	6.5	RS	3
R.O.	7.9	1.0	6.9	RS	8

Part C

Differential Protein Bound Gold Fractions

The results for 5 patients who had specimens analysed for differential albumin and globulin gold levels using the method described in Section 2.3.iv. are shown in Table 7. The results of further patients are shown in the Appendix.

It was observed that protein bound gold represented on average 88% of the total serum gold. The remaining 12% was free gold. In all but 4 patients the major part of the protein bound fraction was bound to albumin. This was equivalent to 60% of the protein bound gold. In the other 4 cases the major part of the gold was bound to the alpha globulin fraction. Although gold was generally detected bound to all protein fractions, on occasions it was observed that in certain patients some protein fractions did not bind gold (See Table 7.).

The results for the 10 samples which were divided into 3 parts and stored at room temperature, 4°C and minus 20°C prior to analysis are presented in

Table 8.

Whilst the total, free and PBG levels remain the same within experimental error (5%), it is evident from the results that the albumin and globulin gold levels vary as a result of different storage temperatures by amounts outside of the expected method CV. The variation in values appears to be random and follows no regular trend. In some cases patients have gold bound to globulins at one temperature, whilst there is none at all in specimens stored at a different temperature.

3.1. Table 7. Differential Protein Bound Gold (Au) Fractions

Name	Total Au µmol/1	Free Au µmol/1	PBG µmol/1	Albumin Au µmol/1	α Globulin Au µmol/1	β Globulin Au µmol/1	γ Globulin Au µmol/1
J.S.	6.0	0.9	5.1	2.8	1.2	0	1.1
M.W.	1.6	0.4	1.2	0.8	0.2	0	0.2
J.W.	8.7	1.2	7.5	4.8	0	2.7	0
E.E.	12.3	1.3	11.0	5.0	1.7	3.3	1.0
F.O.N.	6.7	0.4	6.3	4.9	0	0	1.4

3.1. Table 8. Differential Protein Bound Gold (Au) Fractions for Specimens stored at Different Temperatures

Name	Storage Temp	Total Au	Free Au	PBG	Albumin Au	α Globulin Au	β Globulin Au	γ Globulin Au
		μmol/l	μmol/l	μmol/l	μmol/l	μmol/l	μmol/l	μmol/l
J.S.	Room (22°C)	6.0	0.9	5.1	2.8	1.2	0	1.1
	4°C	5.8	0.5	5.3	2.2	1.6	0.8	0.7
	Minus 20°C	6.0	0.5	5.5	1.9	1.8	1.4	0.4
M.W.	Room (22°C)	1.6	0.4	1.2	0.8	0.2	0	0.2
	4°C	2.0	0.3	1.7	1.0	0.2	0.2	0.3
	Minus 20°C	2.0	0.3	1.7	1.0	0.4	0	0.3
J.W.	Room (22°C)	8.7	1.2	7.5	4.8	0	2.7	0
	4°C	8.6	0.9	7.7	4.6	0.4	2.0	0.7
	Minus 20°C	8.5	0.8	7.7	0.6	1.8	1.8	1.0
E.E.	Room (22°C)	12.3	1.3	11.0	5.0	1.7	3.3	1.0
	4°C	11.9	0.9	11.0	4.5	2.0	3.0	1.5
	Minus 20°C	11.9	0.9	11.0	4.4	2.1	3.2	1.3
E.O.N	Room (22°C)	6.7	0.4	6.3	4.9	0	0	1.4
	4°C	6.4	0.5	5.9	4.0	0.4	0.3	1.2
	Minus 20°C	6.5	0.4	6.1	4.2	0.3	0.6	1.0
H.W.	Room (22°C)	5.3	0.4	4.9	3.0	0.6	0.4	0.9
	4°C	5.1	0.2	4.9	3.5	1.4	0	0
	Minus 20°C	5.1	0.2	4.9	3.3	0.9	0.5	0.2
M.P.D	Room (22°C)	10.0	0.9	9.1	4.0	1.1	2.0	2.0
	4°C	10.2	0.6	9.6	3.5	1.3	2.7	2.1
	Minus 20°C	10.0	0.4	9.6	5.8	1.9	1.3	0.5

3.1. Table 8. continued

Name	Storage Temp	Total Au µmol/1	Free Au µmol/1	PBG µmol/1	Albumin Au µmol/1	α Globulin Au µmol/1	β Globulin Au µmol/1	γ Globulin Au µmol/1
Z.F.	Room (22°C)	10.8	0.8	10.0	5.0	2.8	2.0	0.2
	4°C	10.8	0.9	9.9	4.0	2.5	3.4	0
	Minus 20°C	10.8	0.4	10.4	6.2	3.6	0.3	0.2
S.A.	Room (22°C)	9.0	1.0	8.0	6.0	0.8	0.4	0.8
	4°C	9.5	1.5	8.0	6.1	1.9	0	0
	Minus 20°C	9.5	1.5	8.0	3.3	1.3	3.2	0.2
D.B.	Room (22°C)	5.7	0.5	5.2	3.9	0.4	0.6	0.3
	4°C	5.7	0.5	5.1	4.1	0.3	0.8	0
	Minus 20°C	5.4	0.4	5.0	3.2	0.6	0.2	1.0

3.2. STUDY 2. THE RHYTHMICITY OF URINARY GOLD, COPPER AND ZINC, CREATININE AND WATER

The results obtained from the analysis of urine specimens from 30 suitable subjects, for gold, copper and zinc, creatinine and water are described below.

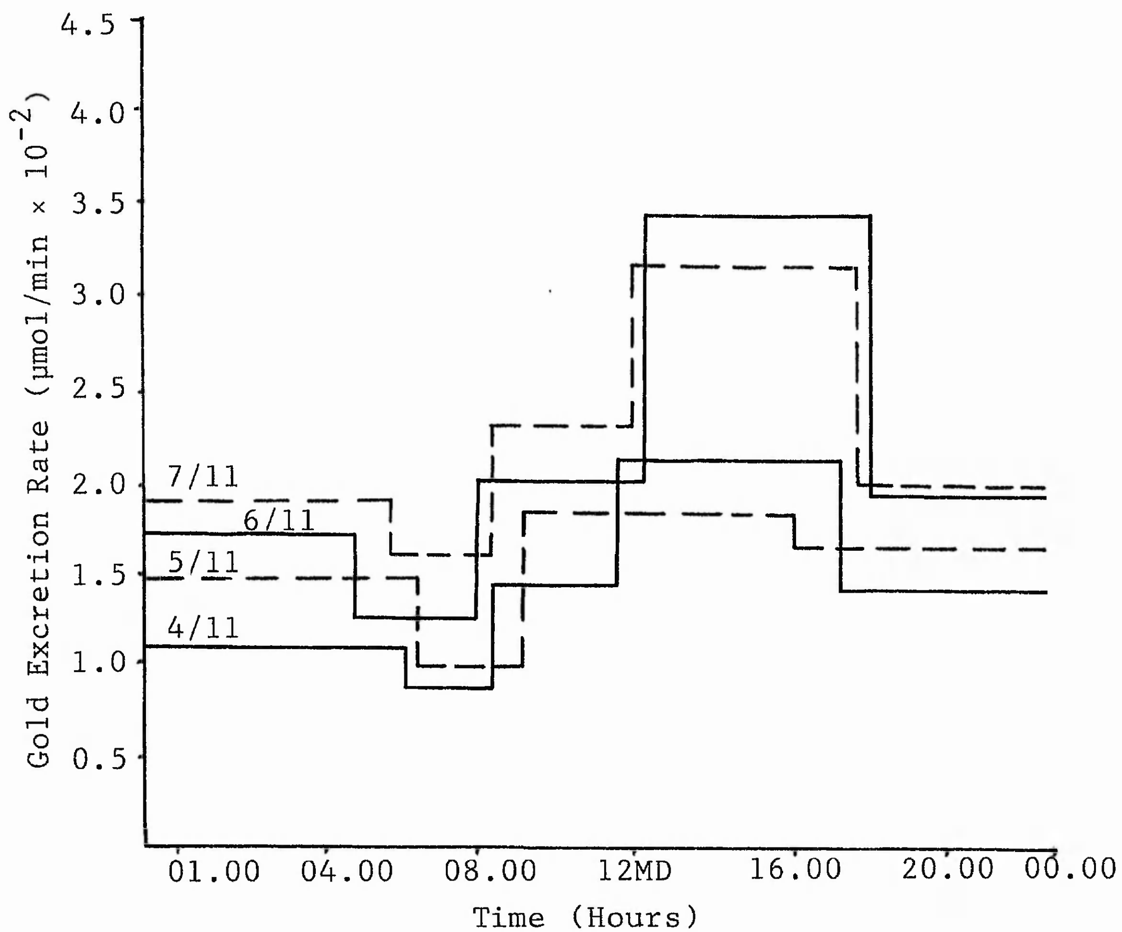
3.2.i. Histogram Analysis

Urine Gold

The individual histograms of gold excretion for 28 of the 30 subjects studied, described regular patterns of excretion throughout the 4 day study. Twenty four patients demonstrated biphasic patterns, four monophasic and two demonstrated tri-phasic patterns of excretion. An example of a biphasic pattern is shown in Figure 1. where the peaks of excretion occur at 03.02 and 16.12 hours. The results of further subjects are presented in the Appendix. (Section 7.2.)

Urine Copper

Twenty of the 30 patients studied demonstrated regular patterns of excretion as shown by histogram representation. Of these 20 subjects, 15 demonstrated



3.2. Figure 1. Histogram of Gold Excretion Rate for Patient E.W.

The histogram for patient E.W. demonstrates regular biphasic patterns for gold excretion.

peaks of excretion which coincided with the excretion rate of gold. The 8 subjects who did not have regular excretion rates of copper, failed to excrete any copper at all in many of the urine specimens collected. The histogram representation of copper excretion for patient E.W. is presented in Figure 2. whilst further results are shown in the Appendix.

Urine Zinc

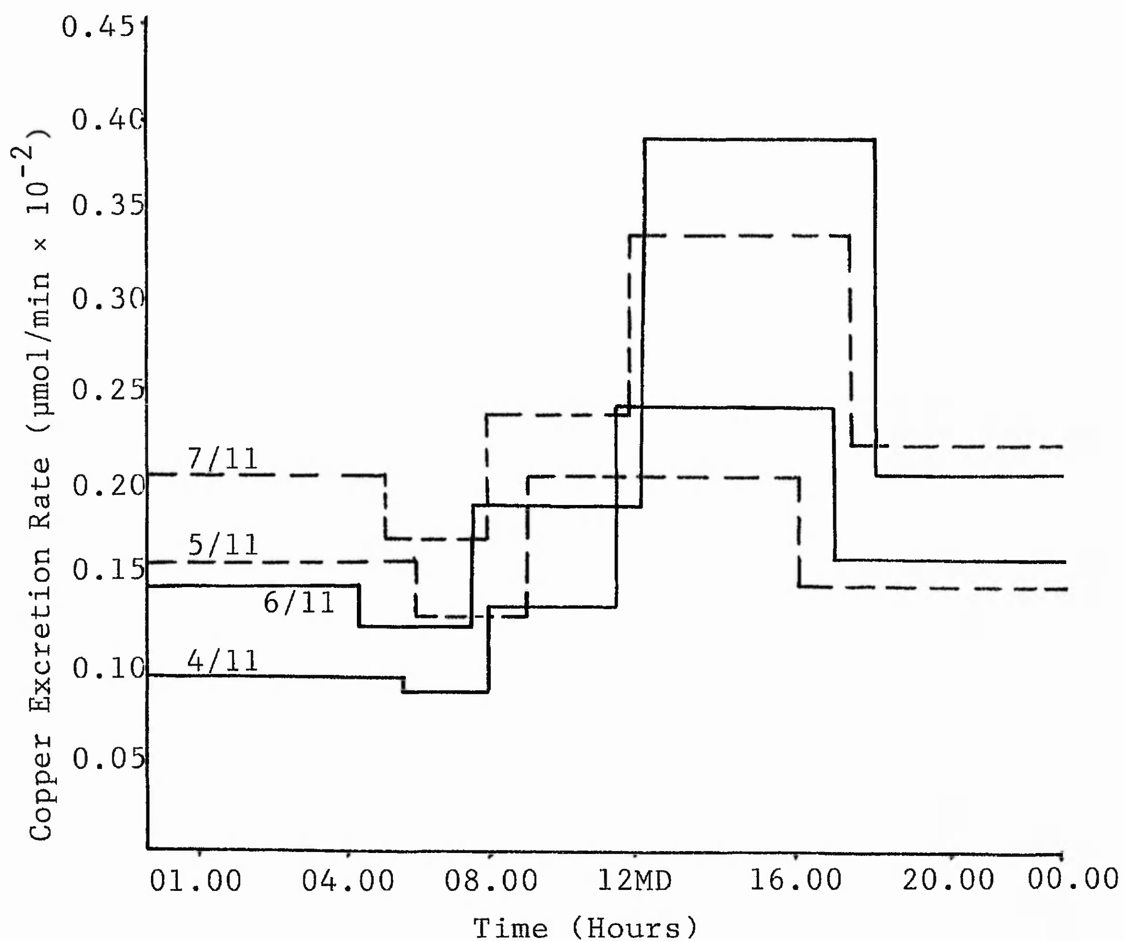
Twenty eight of the 30 patients studied demonstrated regular excretion rates for zinc with similar times of peak excretion to those of gold (Figure 3.). Further results are presented in the Appendix.

Urine Water (Volume)

Twenty eight patients demonstrated histograms of water excretion with peaks and troughs similar to those for gold excretion for the same days (Figure 4.). Further results are presented in the Appendix.

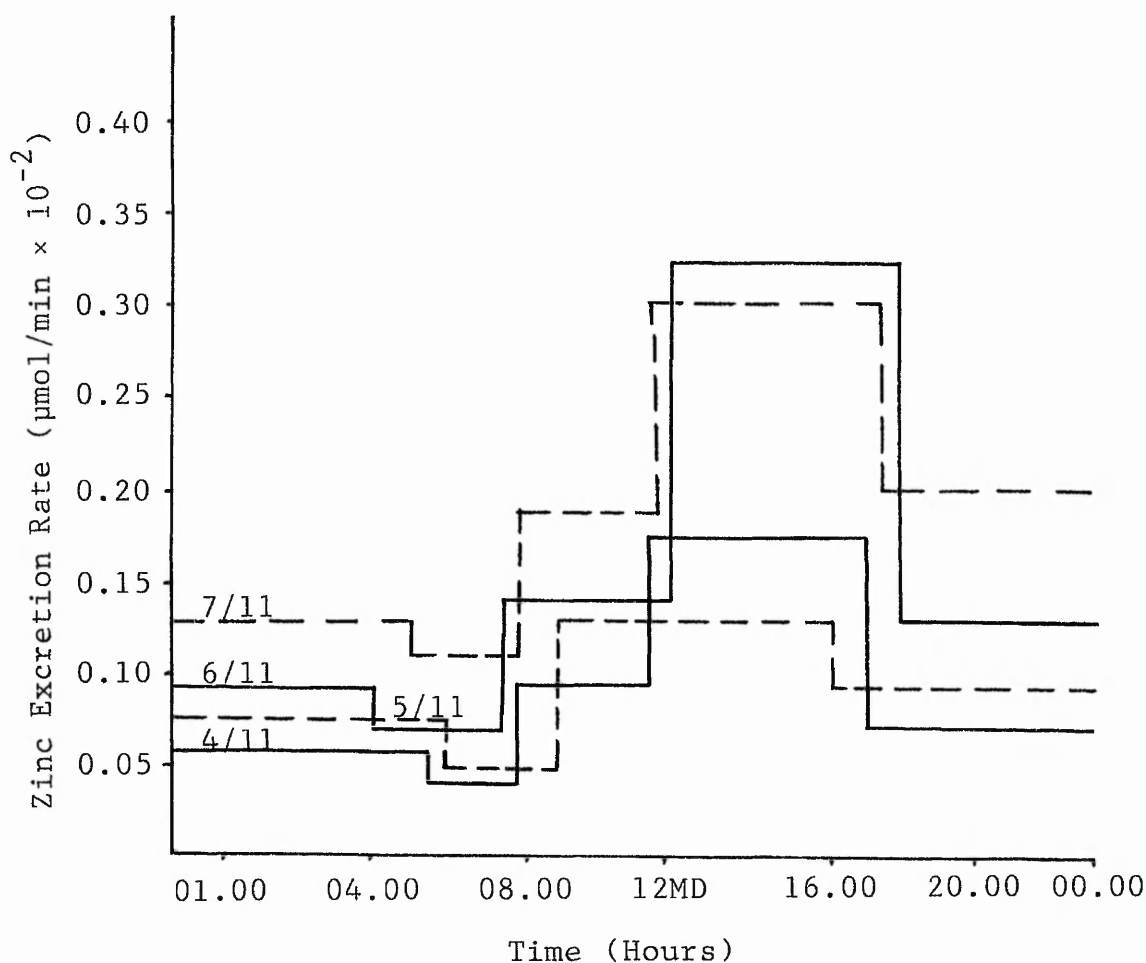
Urine Creatinine

Although individual patients showed regular patterns of excretion throughout the study group, only 6 subjects demonstrated excretion rates and times similar to those of gold and water. Patient E.W., presented in Figure 5., demonstrates biphasic



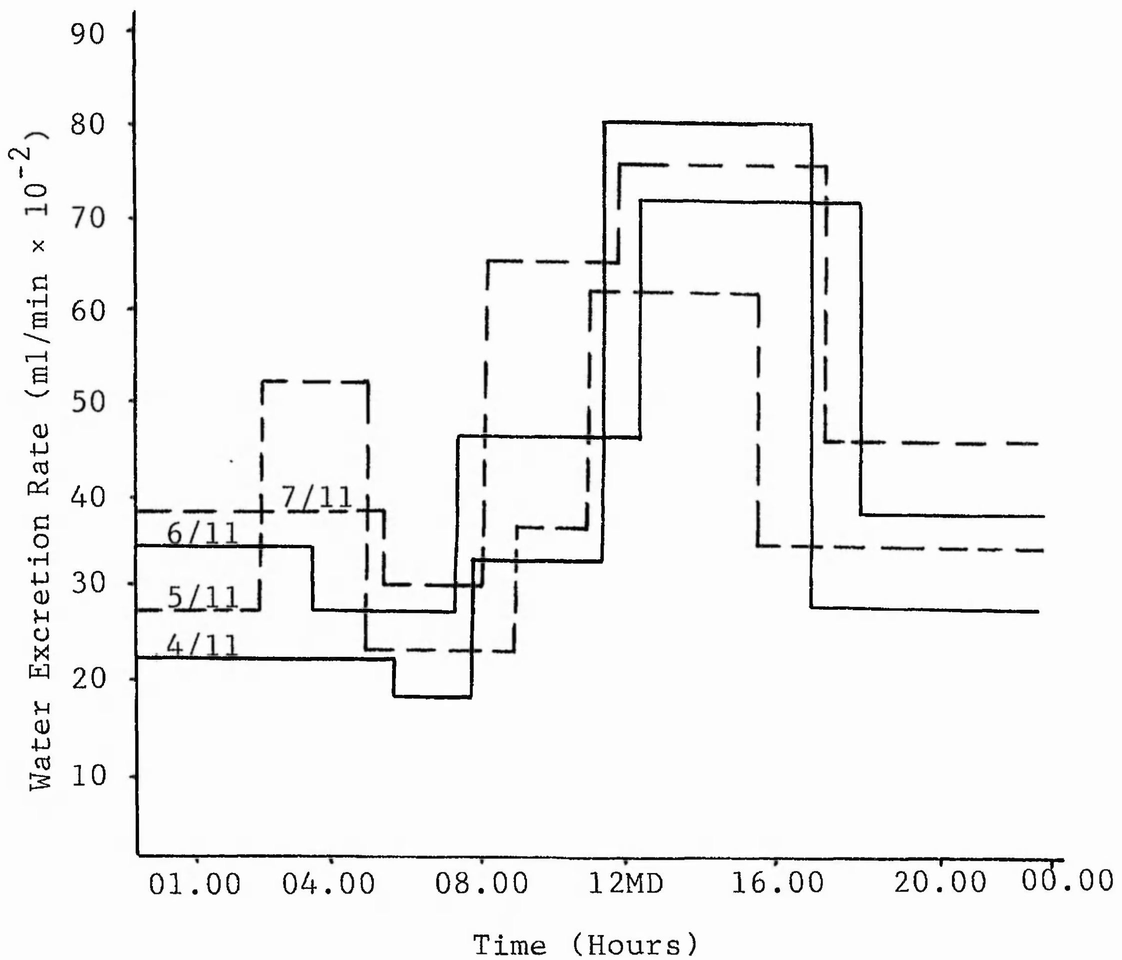
3.2. Figure 2. Histogram of Copper Excretion Rate for Patient E.W.

The histograms for this subject demonstrate biphasic patterns of copper excretion with similar times of peak excretion to the excretion of gold (Figure 1.)



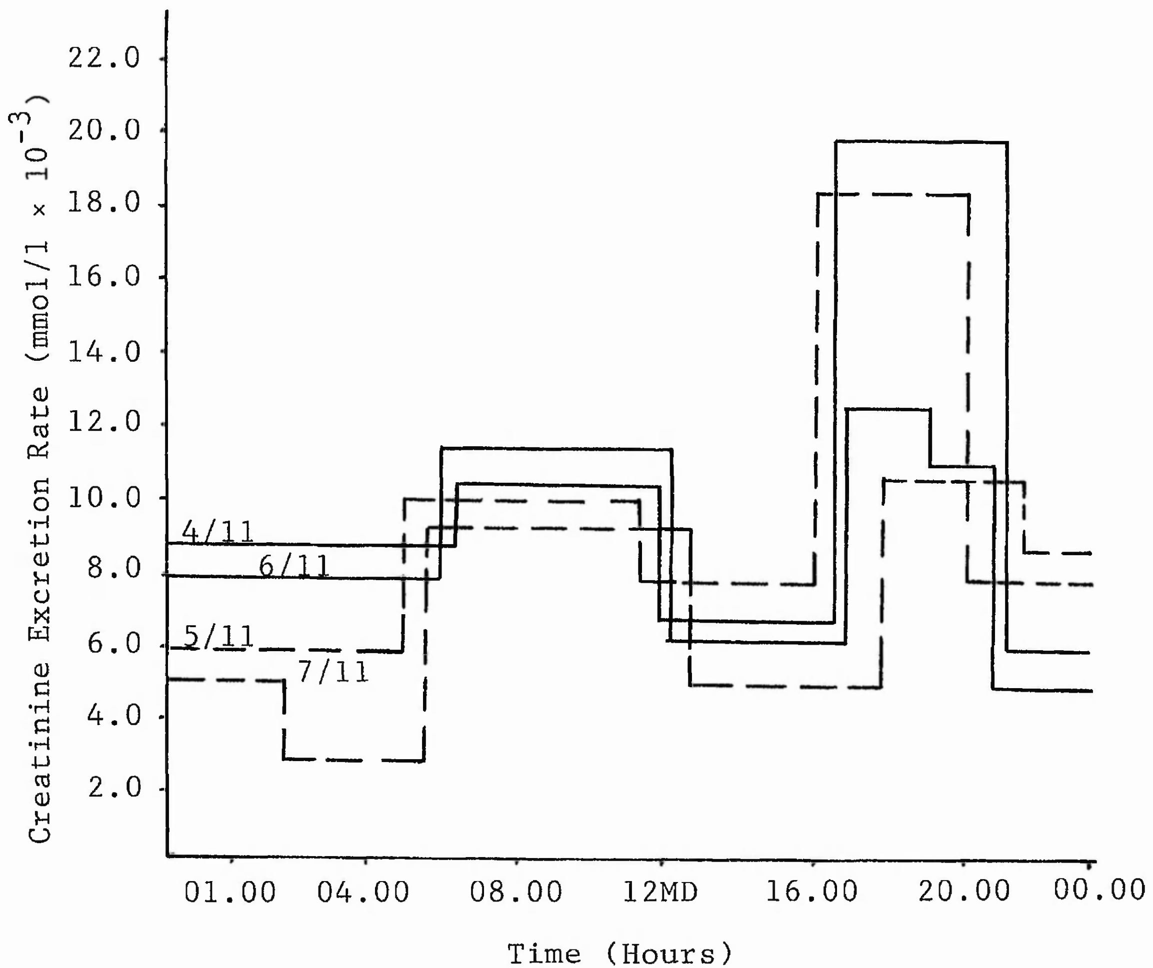
3.2.Figure 3. Histograms of Zinc Excretion Rates for Patient E.W.

Biphasic patterns are demonstrated which are similar to those of gold excretion (Figure 1.).



3.2. Figure 4. Histogram of Water Excretion Rate for Patient E.W.

The histograms for patient E.W. demonstrate regular biphasic patterns of water excretion.



3.2.Figure 5. Histograms of Creatinine Excretion Rate for Patient E.W.

The histograms for patient E.W. demonstrate biphasic patterns of creatinine excretion. These patterns are dissimilar to those of gold and water excretion. (Figures 1. and 4.)

patterns of excretion which are dissimilar to the water and gold patterns for this patient presented in Figure 4. and Figure 1. Further results are displayed in the Appendix.

3.2.ii. Sinewave Analysis

Gold Excretion

Each of the 28 subjects who demonstrated regular excretion rates for gold by histogram representation also demonstrated individual statistically significant rhythmicity of gold excretion when the results were analysed by the sine wave technique. Seven examples are presented in Table 1., whilst further results are presented in the Appendix.

Copper Excretion

The 20 patients with regular histogram representation of copper excretion all demonstrated individual statisitically significant rhythmicity for copper excretion rates. The remaining 10 patients did not demonstrate significant rhythms of excretion. Table 1. shows 6 patients with significant rhythms and one patient with no rhythmic excretion rate. Further results are presented in the Appendix.

Zinc Excretion

The 28 patients demonstrating regular excretion rates of zinc by histogram analysis also demonstrated significant individual rhythmicity by sine wave analysis. Seven examples are shown in Table 1., whilst further results are presented in the Appendix.

Water, Creatinine and Gold Excretion

The 28 patients who demonstrated individually significant rhythmicity of gold excretion also demonstrated rhythmicity for water and creatinine excretion. It is apparent from the results that whilst for all individual subjects the acrophases for gold and water excretion rates are similar, only 6 patients have similar acrophases for gold and creatinine excretion rates. In Table 2. is presented 6 examples of patients with similar individual acrophases for gold and water excretion and one example of a patient with similar acrophases for gold, water and creatinine.

3.2. Table 1. Sine Wave Analysis Results for Gold, Copper and Zinc Excretion

Name	Gold Excretion				Copper Excretion				Zinc Excretion			
	M	A	Acr	p	M	A	Acr	p	M	A	Acr	p
E.W.	0.85	0.46	16.12	0.03	0.71	0.16	16.10	0.02	0.68	0.52	16.20	0.04
W.W.	0.82	0.71	20.58	0.05	0.07	0.06	20.42	0.04	0.09	0.04	20.50	0.05
B.R.	0.95	0.35	16.53	0.04	2.90	8.10	16.44	0.04	0.94	0.63	16.42	0.05
I.R.	1.31	0.42	17.13	0.03	1.00	0.70	17.20	0.04	0.82	0.71	17.12	0.04
A.G.	1.45	0.66	16.18	0.05	0.09	0.07	16.24	0.03	0.05	0.03	16.14	0.05
P.A.	1.56	0.86	01.29	0.03	0.08	0.03	01.20	0.03	0.08	0.04	01.19	0.03
F.W.*	1.47	0.95	23.24	0.05	0.72	0.24	13.14	0.95	0.84	0.80	23.16	0.05

M = Mesor (μmol/min × 10^{-2})

A = Amplitude (μmol/min × 10^{-2})

Acr = Acrophase (hours)

*Patient F.W. demonstrates no significant rhythm for the excretion of copper.

3.2. Table 2. Sine Wave Analysis Results for Gold, Water and Creatinine Excretion

	Gold Excretion				Water Excretion				Creatinine Excretion			
Name	M	A	Acr	p	M	A	Acr	p	M	A	Acr	p
E.W.	0.85	0.46	16.12	0.03	1.42	0.74	16.25	0.04	2.08	0.97	21.04	0.04
W.W.	0.82	0.71	20.58	0.05	1.54	0.89	20.32	0.05	3.02	1.24	16.84	0.05
B.R.	0.95	0.35	16.53	0.04	1.69	0.76	16.04	0.03	2.16	2.32	20.21	0.05
I.R.	1.31	0.42	17.13	0.03	2.14	1.04	17.01	0.02	3.19	0.80	15.85	0.04
A.G.	1.45	0.66	16.18	0.05	1.92	0.94	16.52	0.05	2.87	0.94	12.34	0.04
P.A.	1.56	0.86	01.29	0.03	1.98	0.92	02.00	0.03	3.24	0.89	04.10	0.03
E.F.*	1.47	0.95	23.24	0.05	2.01	1.42	23.58	0.05	3.01	1.01	23.30	0.06

M = Mesor A = Amplitude Acr = Acrophase

Units:	Gold	Water	Creatinine
Mesor	$\mu mol/min \times 10^{-2}$	ml/min	mmol/min
Amplitude	$\mu mol/min \times 10^{-2}$	ml/min	mmol/min
Acrophase	Hours	Hours	Hours

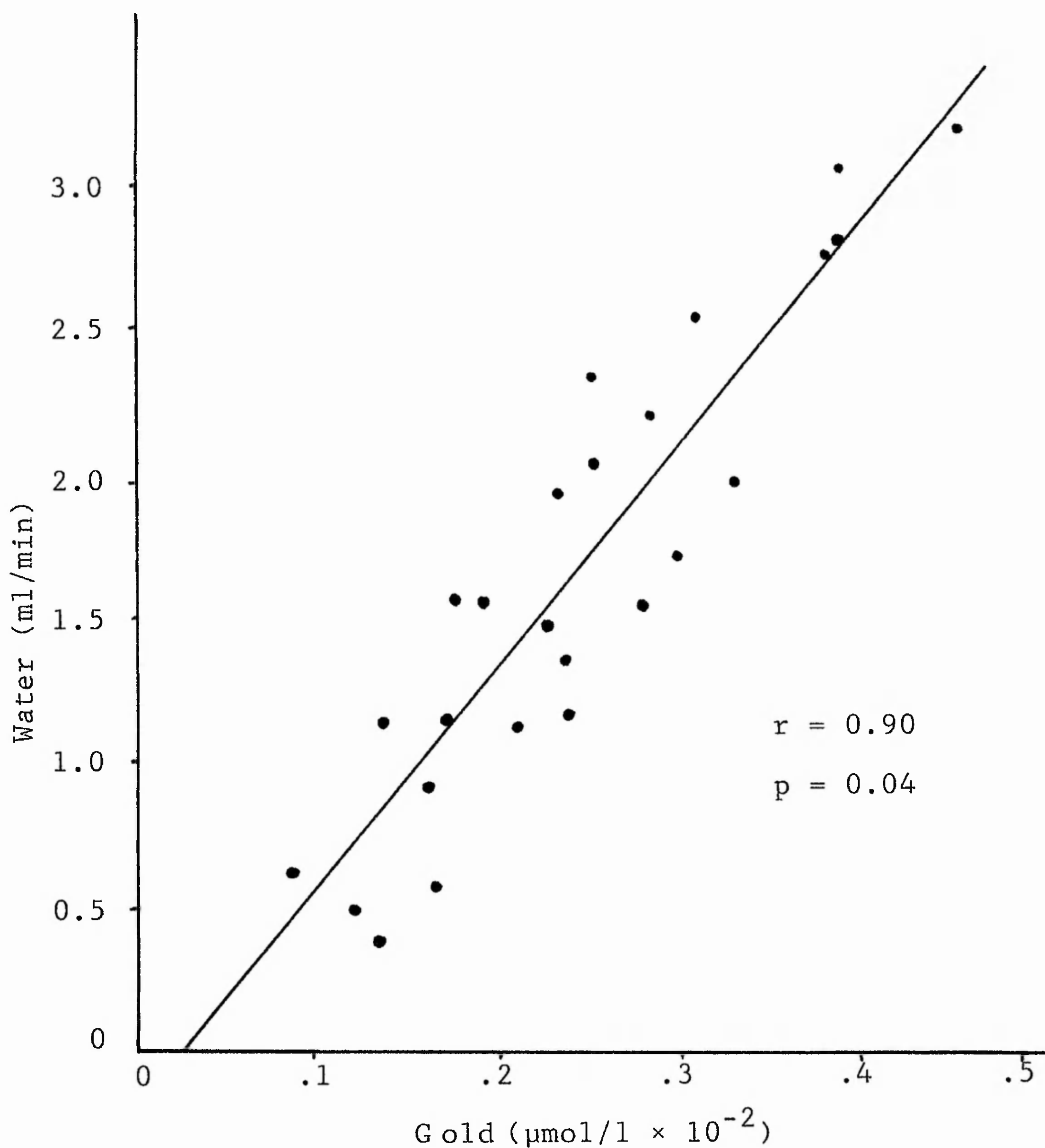
Six examples are shown of patients with similar individual acrophases for gold and water excretion and one example of a patient (E.F.*) with similar acrophases for gold, water and creatinine excretion.

3.2.iii. Correlation Studies

Pearson correlation analysis was applied to the group results for gold excretion against water excretion excluding the 2 patients who did not exhibit rhythmicity. From the results obtained it is evident that correlation was poor ($r = 0.32$, $p = 0.10$, $n = 561$). Individual patient correlation plots however, showed good correlation with all correlation coefficient values greater than 0.85 and p values of 0.05 or less (See Table 3. and Figure 6.).

There was no apparent correlation for the patient group of creatinine excretion rates against water excretion rates (r = less than 0.25, p = greater than 0.1) and there was bad correlation for 22 patients when plotted individually against water excretion (r = less than 0.20 for all subjects and p = greater than 0.10).

Six patients showed individually good correlation for creatinine against gold excretion (r = greater than 0.85 in all cases and p values 0.05 or less as presented in Table 4.).



3.2. Figure 6. Correlation of Gold against Water Excretion Rates for Patient E.W.

3.2. Table 3. Correlation Values for Individual Patient Plots of Gold against Water Excretion

Name	Correlation Coefficient (r)	p Value	No. of Observations (n)
E.W.	0.90	0.04	25
W.W.	0.86	0.05	15
B.R.	0.86	0.04	15
I.R.	0.91	0.04	20
A.G.	0.89	0.05	20
P.A.	0.88	0.04	15
F.W.	0.96	0.03	29
P.A.G.	0.98	0.03	30
M.D.	0.89	0.05	15
M.G.	0.90	0.04	12
J.C.	0.92	0.04	12
N.E.	0.91	0.04	15
T.D.	0.86	0.05	16
B.W.	0.90	0.05	20
T.B.	0.95	0.04	28
H.A.E.	0.87	0.04	21
D.A.	0.91	0.04	23
D.A.B.	0.98	0.02	33
D.G.	0.91	0.05	16
H.W.	0.92	0.05	19
F.A.W.	0.89	0.05	26
M.G.	0.88	0.04	26
L.E.	0.90	0.04	12
P.J.S.	0.94	0.03	27
J.H.	0.90	0.05	13
M.O.D.	0.88	0.04	18
S.S.	0.92	0.04	19
M.P.	0.91	0.04	33

3.2. Table 4. Correlation Values for Individual Patient Plots of Creatinine against Gold Excretion showing Good Correlation

Name	Correlation Value (r)	p Value	n
P.A.G.	0.87	0.04	30
B.W.	0.90	0.05	20
T.B.	0.86	0.05	28
H.W.	0.92	0.04	19
F.A.W.	0.88	0.04	26
J.H.	0.91	0.04	13

3.3. STUDY 3. THE PATTERNS OF GOLD LEVELS IN SERUM, URINE AND SALIVA AND THE RELATIONSHIP OF IMMUNOPROTEINS AND ACUTE PHASE REACTANTS TO THESE PARAMETERS

3.3.i. Urine

Of the 20 subjects studied, 18 demonstrated rhythmicity in the excretion of gold, both by histogram and sine wave analysis. (See Appendix)

3.3.ii. Serum

Eighteen of the 20 subjects demonstrated similar variations in both total and free serum gold at different times of the day. Fourteen patients had both total and free gold levels higher at 10.00 and 22.00 hours than at 16.00 hours and the other 4 patients had lower levels at these times.

The differences in the levels were all outside the possible differences due to the CV of the method. In Table 1. are 5 examples of patient results together with the values for the CV of each method. Further results are presented in the Appendix.

3.3. Table 1. Comparison of Patients Results at Different Times of Day and Night for Total Serum, Free, Protein Bound and Salivary Gold Levels together with the Relative Urinary Gold Levels

Patient	Time	Total Serum Gold µmol/l	Free Gold µmol/l	PBG µmol/l	Salivary Gold µmol/l	Relative Urine Gold Levels
J.C.	10.00	13.0	1.3	11.7	0.5	Low
	16.00	12.0	1.1	10.8	0.1	High
	22.00	13.0	1.4	11.6	0.6	Low
W.D.	10.00	13.0	1.9	11.1	0.6	High
	16.00	15.5	2.2	13.5	0.9	Low
	22.00	14.0	2.0	11.8	0.6	High
N.E.	10.00	13.0	2.2	10.8	0.5	High
	16.00	15.0	2.8	12.3	0.9	Low
	22.00	14.5	2.4	12.2	0.6	High
I.G.	10.00	14.5	2.4	12.1	0.6	High
	16.00	16.0	3.0	13.1	1.1	Low
	22.00	13.0	2.7	10.3	0.7	High
M.D.	10.00	15.0	3.5	11.5	0.6	Low
	16.00	13.0	3.1	9.9	0.4	High
	22.00	15.0	3.4	11.6	0.6	Low

CV for Total Gold 1.9%
Free Gold 3%
Salivary Gold 9%

3.3.iii. Saliva

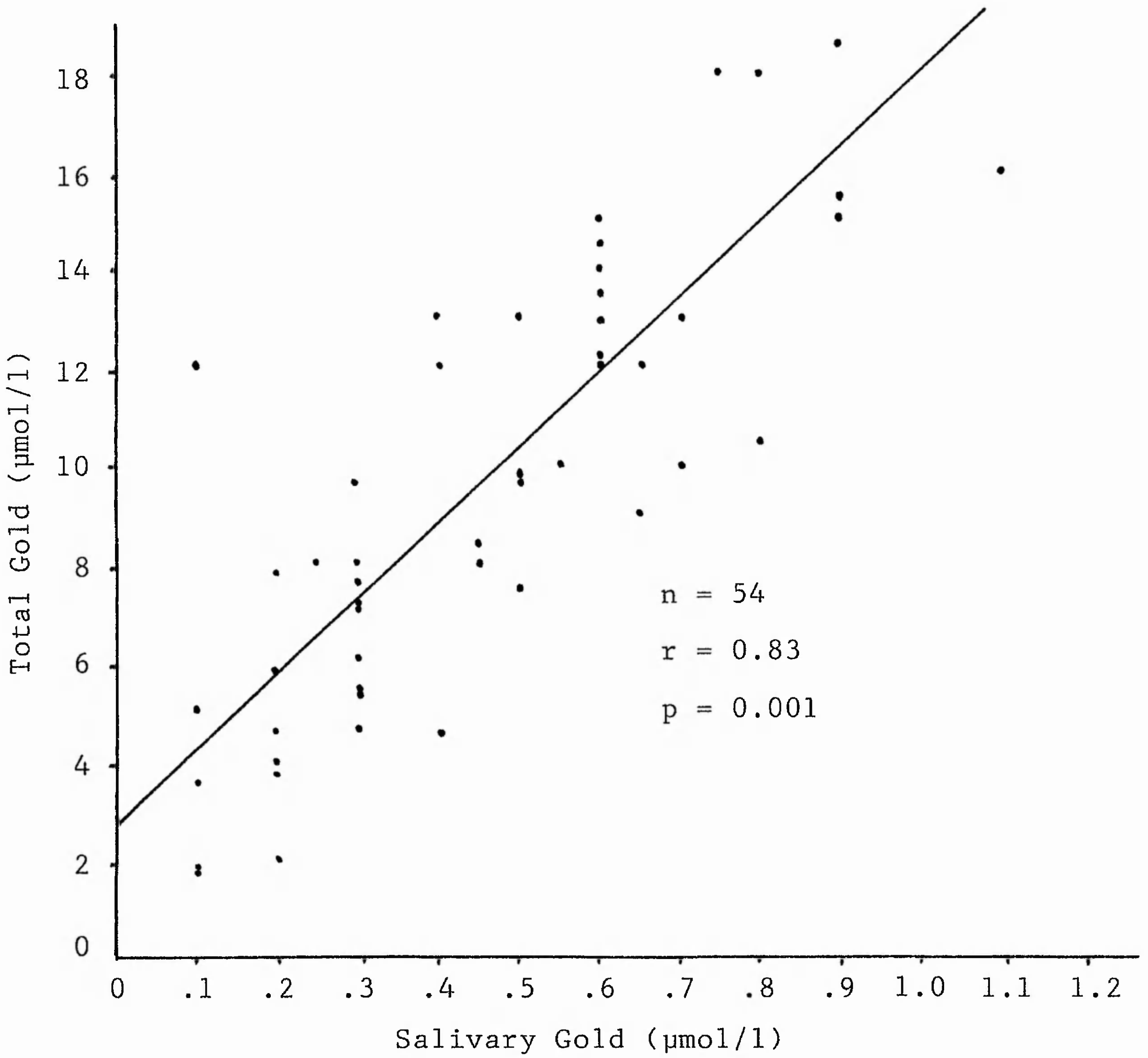
Gold was detected in all the salivary specimens. Variations were observed in all the gold levels at different times of the day, which were outside the CV of the method. These variations reflected the similar differences observed in the total and free gold levels in the serum from blood taken at the same time as the saliva was collected (See Table 1. and Appendix).

Pearson Correlation

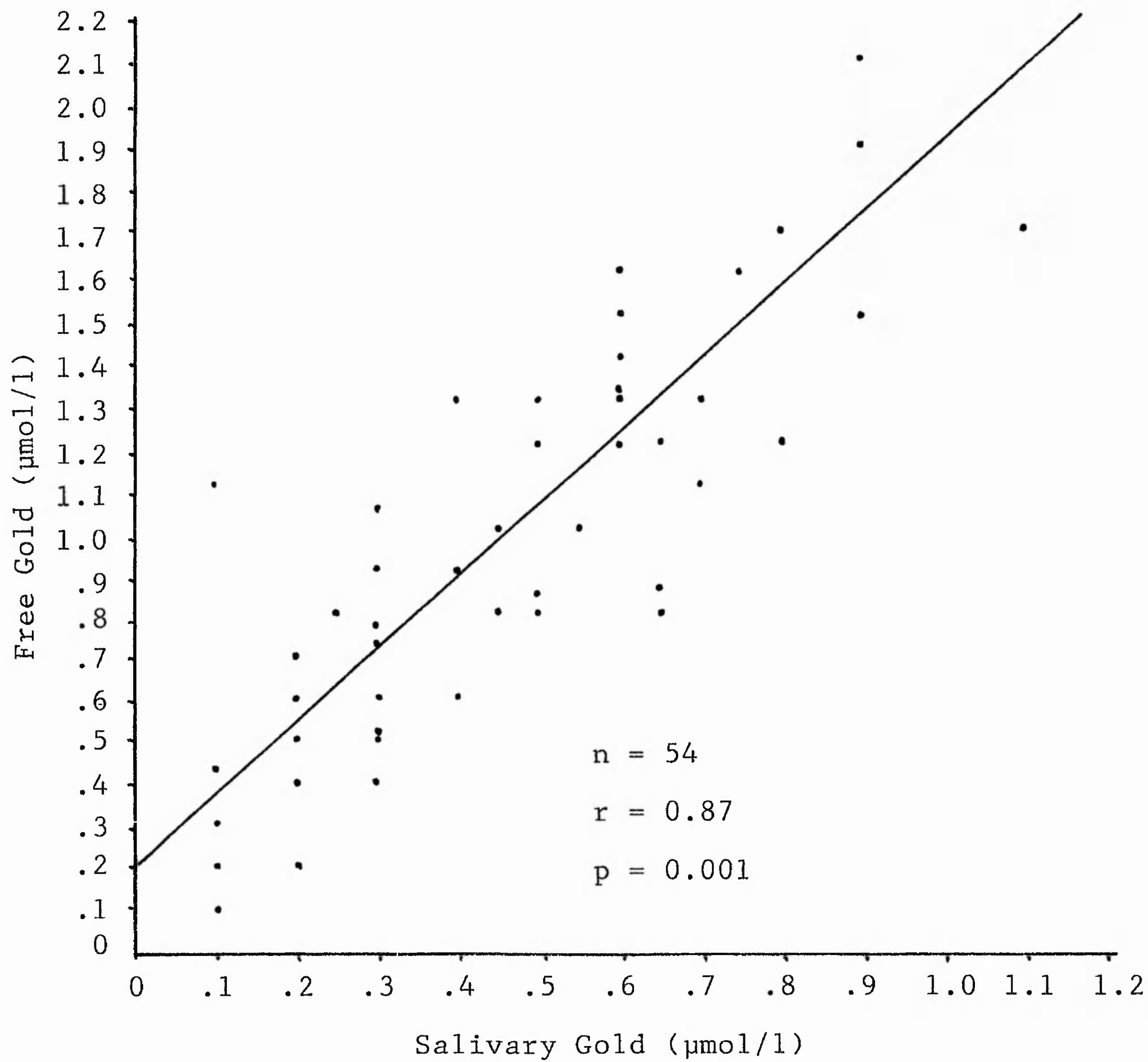
Good correlation was observed for the group results for total serum gold against salivary gold ($r = 0.83$, $p = 0.001$, see Figure 1.), for free serum gold against salivary gold ($r = 0.57$, $p = 0.001$, see Figure 2.) and for protein bound gold against salivary gold ($r = 0.82$, $p = 0.001$, see Figure 3.).

Relationships between Urine, Serum and Salivary Gold

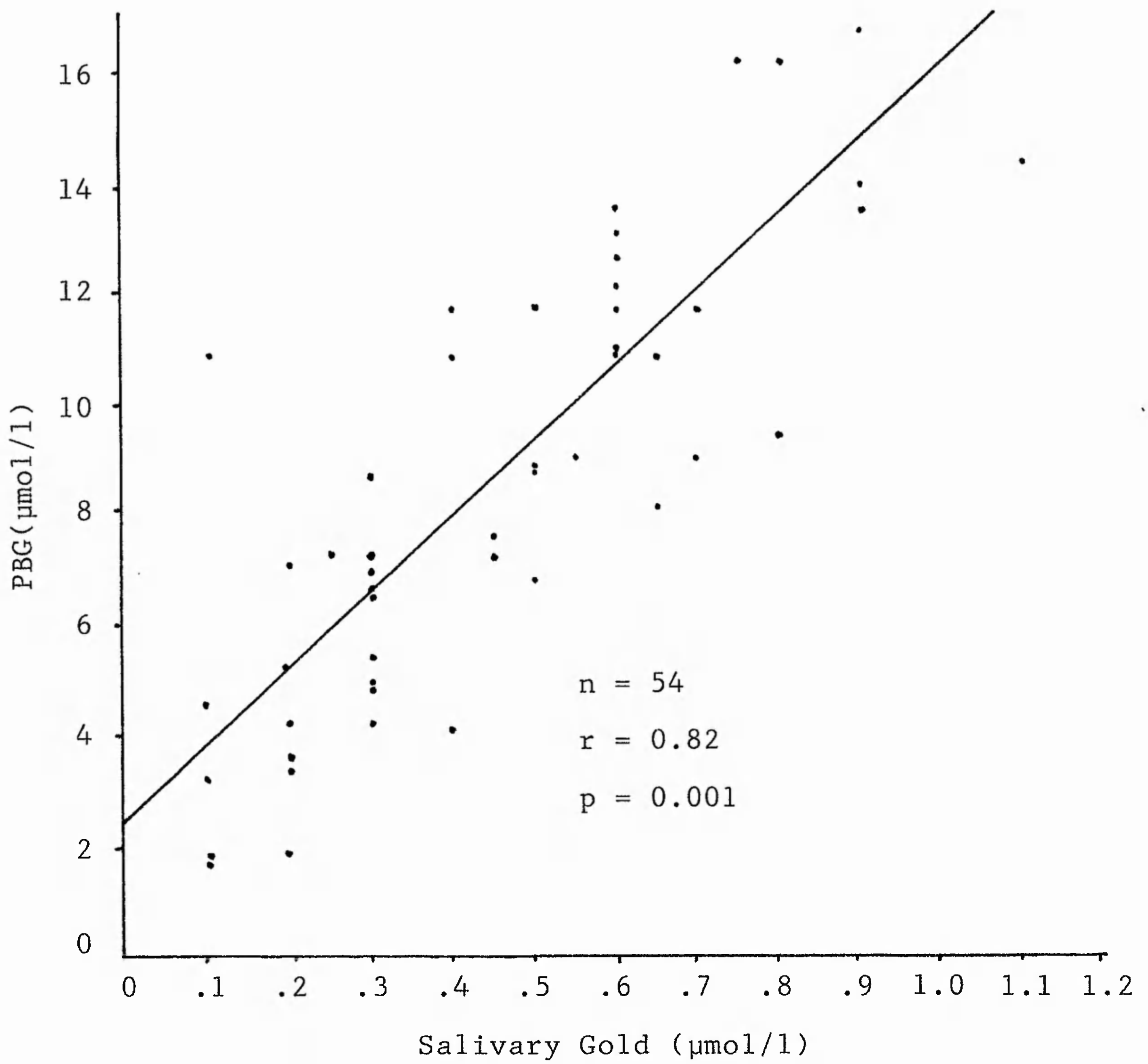
For each patient at times of higher urinary gold excretion rates, it was observed that total serum gold and its free fractions were lower as were salivary gold levels. Conversely, when urine gold levels were low both serum and salivary gold levels were raised. This is illustrated in Figure 4. where



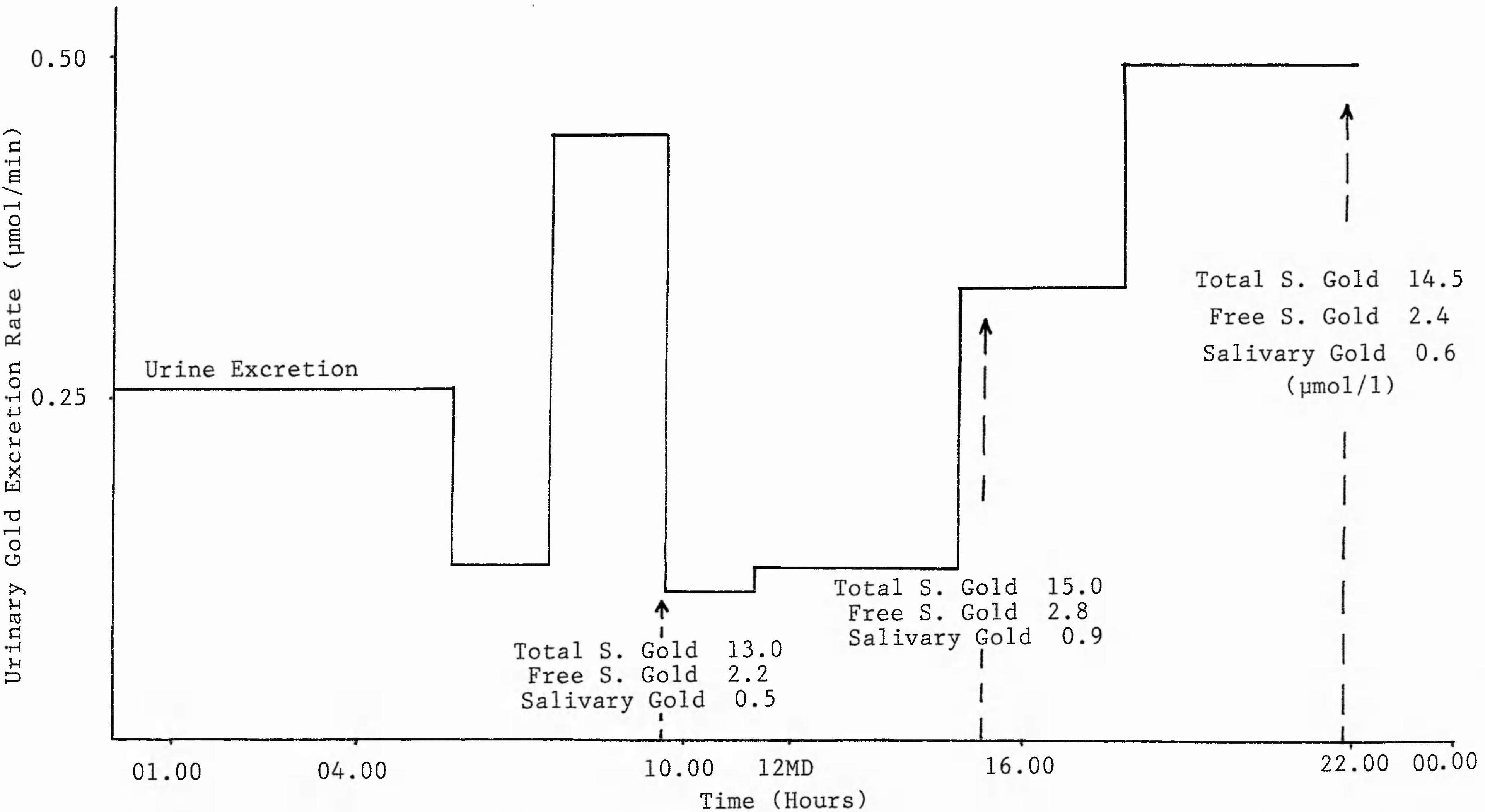
3.3. Figure 1. Correlation of Salivary Gold against Total Serum Gold



3.3. Figure 2. Correlation of Salivary Gold against Free Gold



3.3. Figure 3. Correlation of Salivary Gold against Protein Bound Gold



3.3. Figure 4. Comparison of Urinary Gold Excretion with Blood and Saliva Levels (Patient N.E.)

the urinary excretion rates of gold are plotted for a single day together with serum and salivary gold levels. Further results are displayed in the Appendix.

3.3.iv. The Relationship of Immunoproteins and Acute Phase Reactants to Urine, Serum and Salivary Gold

Complement Factor C3

In 16 of the 18 subjects the serum C3 levels were lowest at the time of peak gold excretion and low serum free gold and salivary gold levels. In the other 2 subjects, C3 was at its lowest when urinary gold excretion was low with accompanying higher serum and salivary gold levels.

Complement Factor C4

The results for C4 were the same as for C3.

Serum Immunoglobulin IgA

These levels were all lowest when the urinary gold levels were highest. At these times the serum and salivary levels were low. The levels of IgA were not at their highest when urinary gold levels were at their lowest however.

Serum Immunoglobulin IgG

Levels of IgG were lowest in 14 out of 18 subjects studied during times of peak urinary gold excretion and low serum and salivary gold levels.

Serum Immunoglobulin IgM

In all 18 subjects IgM was lowest at times of peak gold excretion and low serum and salivary gold levels. The IgM levels were not at their highest when urinary gold was at its lowest.

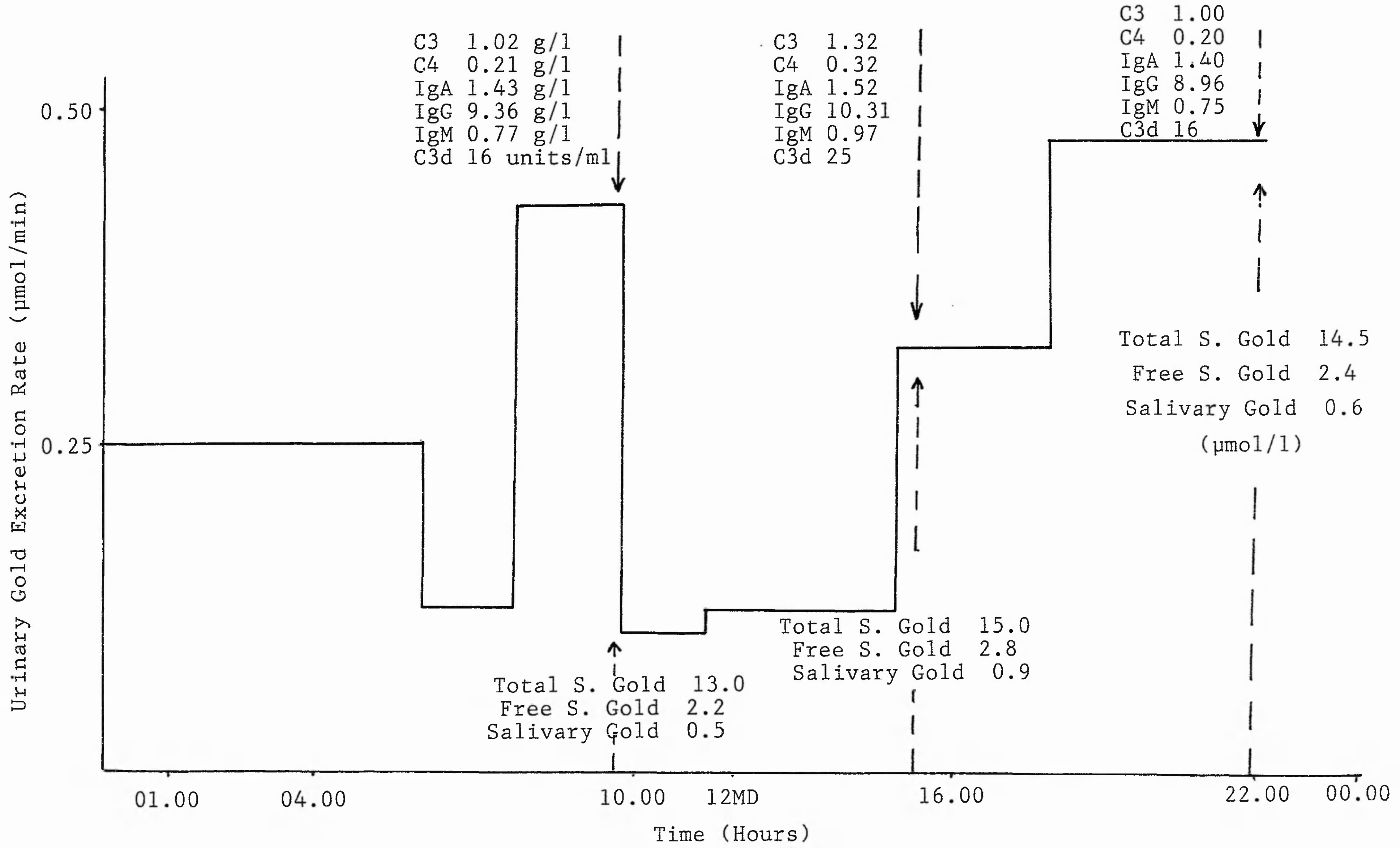
Serum C-Reactive Protein

Inconsistent results were obtained throughout the study group.

Plasma C3d Degradation Products

These levels were at their highest in 13 out of 18 patients during the lowest levels of gold excretion, but whilst serum and salivary gold were high.

A compilation graph is presented in Figure 5. showing the urinary gold excretion rates for one 24 hour period (for patient N.E.) and the serum and salivary gold levels together with the immunoprotein and acute phase reactant levels.



3.3. Figure 5. Compilation of Urine, Serum and Salivary Gold Levels and Blood Immunoproteins and Acute Phase Reactants (Patient N.E.)

A summary of the results for this part of the study (3.3.) is presented in Table 2. Further results are presented in the Appendix.

3.3. Table 2. A Summary of the Relationships between Immunoproteins and Acute Phase Reactants in Blood and Gold Levels in Urine, Serum and Saliva

		Urine Gold	Total Serum Gold	Free Gold	Salivary Gold
C3	Lowest for 16/18	Highest	Lowest	Lowest	Lowest
C4	Lowest for 16/18	Highest	Lowest	Lowest	Lowest
IgA	Lowest	Highest	Lowest	Lowest	Lowest
IgG	Lowest for 14/18	Highest	Lowest	Lowest	Lowest
IgM	Lowest	Highest	Lowest	Lowest	Lowest
CRP	Inconsistent	-	-	-	-
C3d	Highest for 13/18	Lowest	Highest	Highest	Highest

3.4. STUDY 4. FREE THIOMALATE AND GOLD IN PLASMA, SERUM AND URINE

The results for subject B.C. are presented in Table 1. Further results are shown in the Appendix.

Plasma Thiomalate

Free thiomalate was detected in the plasma of 15 of the 16 patients studied. Peak plasma levels were detected between 30 and 45 minutes after injection of aurothiomalate and became undetectable by 4 hours.

Urine Thiomalate

Free thiomalate was found in the urine of all 16 patients. The maximum excretion usually occurred during the first hour. In 10 subjects thiomalate was undetectable after 4 hours. The percentage of the total dose of aurothiomalate administered, excreted in the urine in 24 hours as free thiomalate, ranged from 2.2 to 17.6% (See Table 2.).

Serum Gold

Serum gold levels (total, free and PBG) reached a maximum between 60 and 90 minutes after aurothiomalate injection in 14 patients and after 120 minutes in 2 patients. Unlike plasma thiomalate levels,

3.4. Table 1. Free Thiomalate and Gold in Serum and Urine (Patient B.C.)

Time after Injection of Aurothiomalate (mins)	Plasma Thiomalate (nmol/l)	Total Serum Gold (µmol/l)	Free Serum Gold (µmol/l)	PBG (µmol/l)	Urinary Thiomalate (µmol/l)	Urinary Gold (µmol/l)
0	0	1.7	0.1	1.6		
15	252	11.1	1.1	10.0		
30	282	12.6	1.6	11.0		
45	318	13.4	1.6	11.8		
60	216	13.6	1.6	12.0	0.84	0.85
90	48	11.7	1.2	10.5		
120	UD	11.5	1.1	10.4	0.09	0.45
240	UD	11.0	1.0	10.0	0.07	0.35

UD = Undetectable

3.4. Table 2. Molar Recovery of Injected Dose as Free Thiomalate within 24 Hours

Patient	Urinary Recovery %
B.C.	2.2.
B.L.	2.2
N.H.	5.4
A.H.	3.6
J.B.	8.2
B.T.	3.0
E.W.	12.1
D.S.	16.8
M.H.	12.0
E.J.B.	11.2
J.S.	17.6
I.C.H.	6.4
E.T.	15.4
M.R.	8.6
E.W. (2)	6.3

total gold levels still remained at levels which were greater than 80% of the maximum after 4 hours.

Urine Gold

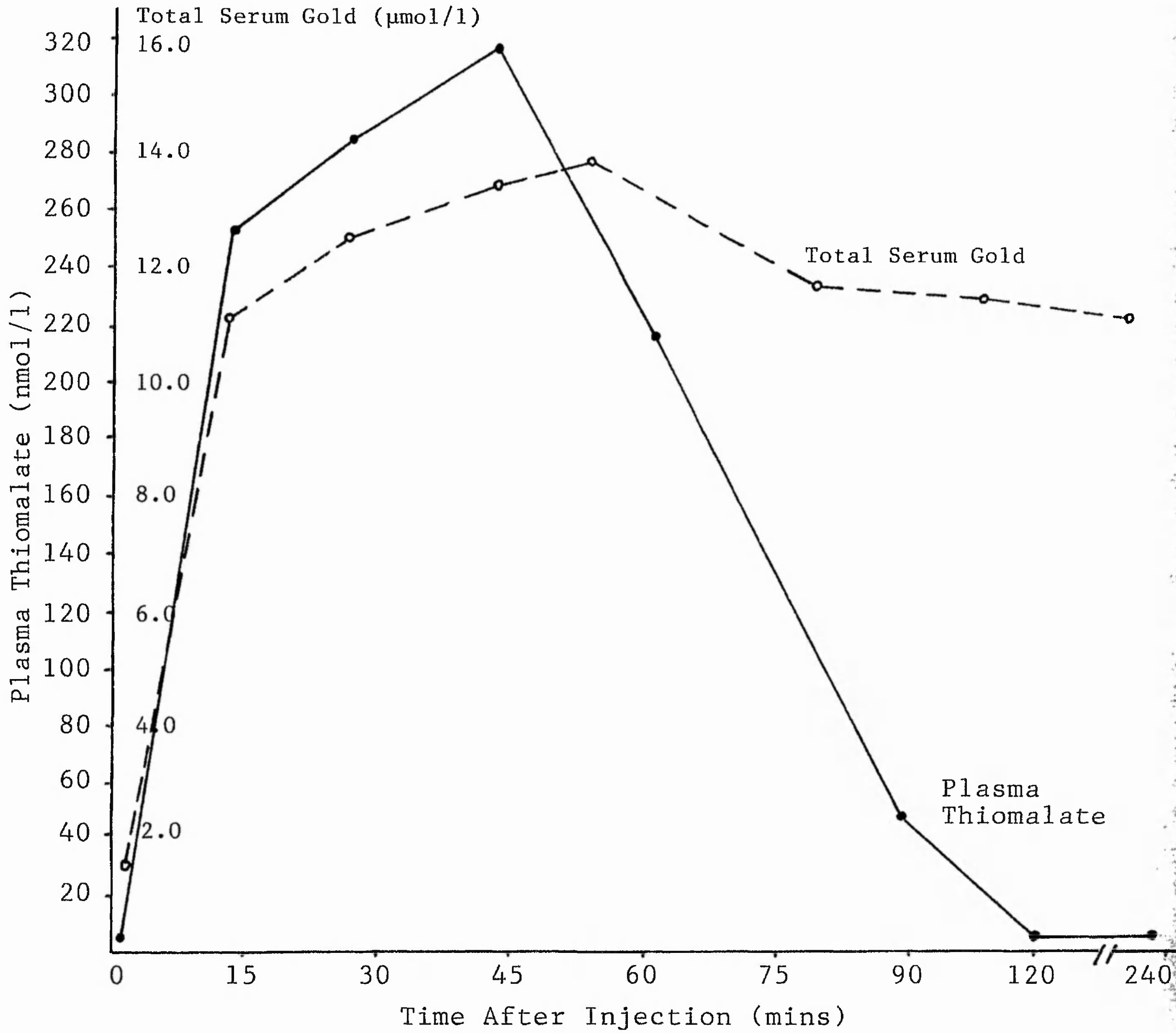
In all subjects the peak excretion of gold occurred when the serum levels were at their highest.

Whilst thiomalate peaked early (30 to 45 minutes after injection) in plasma and was cleared in the majority of subjects from the urine after 4 hours, the gold moiety peaked somewhat later after injection (60 to 90 minutes and 120 minutes) and was present in the urine in significant amounts after 4 hours.

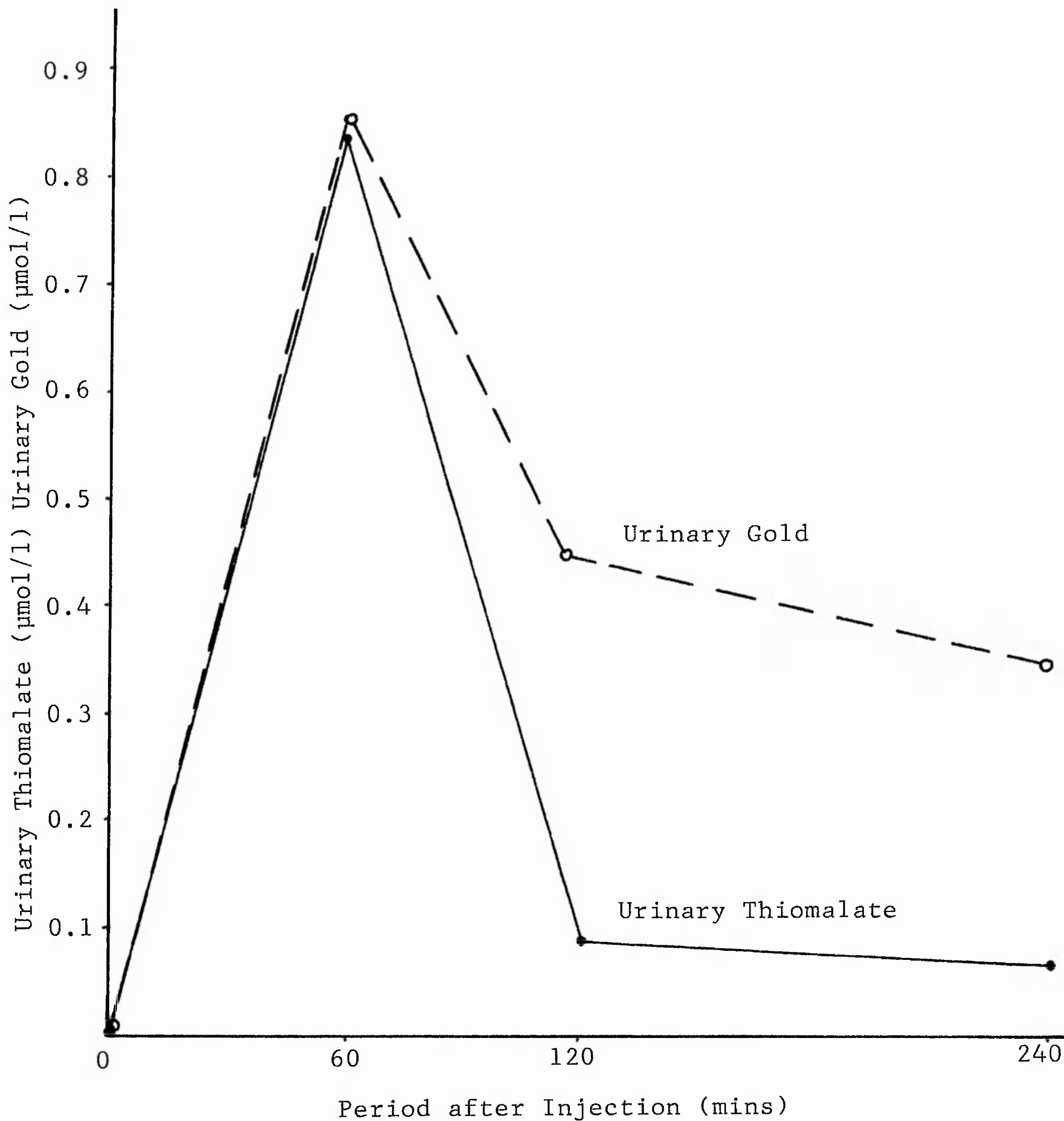
Figure 1. shows plasma thiomalate levels compared with gold levels and Figure 2. demonstrates urinary excretion of thiomalate and gold in a typical patient (B.C.).

PUBLISHED WORK

Three papers from this work have been published in the Annals of Rheumatic Diseases and are presented in the pocket of the back cover.



3.4. Figure 1. Plasma Concentration of Thiomalate and Serum Levels of Total Gold (Patient B.C.)



3.4. Figure 2. Urinary Excretion of Thiomalate and Gold

CHAPTER 4

CHAPTER 4

4. CONCLUSIONS

A summary of the conclusions is presented in Table 1.

4.1. STUDY 1 THE DISTRIBUTION OF GOLD IN SERUM

Part A

The results obtained from 54 patients stabilised on gold therapy (Group 1) and 15 patients who were commencing therapy (Group 2) and who were subsequently studied for the following 8 weeks, demonstrate that there is good correlation between total and free gold and total and protein bound gold in serum.

In patients who did not present with side effects of medication, the free gold fraction represented less than 12% of the total serum gold. On 7 occasions when patients demonstrated toxicity of treatment, there was a higher free to total gold ratio and a lower protein bound to total gold ratio.

This was demonstrated graphically by correlation plots with linear regression for the 189 series of results collected. In cases showing no side effects, all plots of total against free gold and total against

PBG fell within plus or minus the standard error of the individual plots on the regression line. In the 6 instances where patients presented with side effects, the plots fell significantly outside the plus or minus standard error limits applied to the line of regression.

At no time during the study did any plots from patients with toxicity fall inside the regression limits or did any plots from patients not exhibiting side effects fall outside the regression limits.

Despite the obvious changes in free gold values at times of toxicity there were no obvious variations from the expected total gold levels for each patient.

<u>Part B</u>   <u>The Follow-up Study to Part A</u>

It can be concluded from this study of 10 further patients exhibiting toxicity that whilst there is a higher free to total gold ratio and a lower PBG to total gold ratio during the presentation of side effects of gold treatment, the return to a normal distribution of gold in the serum fractions proceeds more slowly than the disappearance of the cutaneous rash. Furthermore, the distribution of gold in serum does not return to the non-toxic pattern until some weeks after the disappearance of the physical symptoms of toxicity.

Part C Differential Protein Bound Gold Fractions

It was observed that 88% of the total serum gold was bound to protein whilst the remainder was free gold.

In 51 patients it was demonstrated that 60% of the protein bound gold was bound to the albumin fraction. In 4 other cases it was observed bound mainly to the alpha globulin fraction. Although gold was generally detected bound to all the protein fractions, in certain patients particular proteins did not bind gold.

It is evident from the results obtained from examination of the differential protein binding properties of serum stored at room temperature, 4°C and minus 20°C prior to analysis, that whilst the total protein bound gold levels remained the same, there was random variation in the gold binding properties of the different protein fractions associated with the different storage temperatures.

4.2. STUDY 2. THE RHYTHMICITY OF URINARY GOLD, ZINC, COPPER, WATER AND CREATININE

Examination of the results from 30 patients by histogram and sine wave analysis revealed that there was a significant individual circadian rhythm of gold excretion for 28 patients.

The same patients demonstrated individual rhythmicity of zinc excretion with similar individual acrophases to those of gold excretion.

However, 66% of the patients studied (20) demonstrated individual rhythmicity of copper excretion of which 75% (15 patients) demonstrated acrophases which coincided with those of gold. In other words, one half of the patients studied had individual rhythmic patterns of copper excretion which had similar acrophases for gold excretion.

The 28 patients also demonstrated rhythmicity for water and creatinine excretion. However, it is evident from the results that whilst for individual subjects the acrophases for gold and water excretion are similar, only 6 patients have similar acrophases for gold and creatinine excretion.

4.2.i. Correlation Studies

Whilst the group correlation for urinary gold against water excretion was poor, there was good correlation for the individual patient plots of gold against water.

Water did not correlate with creatinine excretion either for the patient group or for individual patients, apart from 6 cases who showed individually good correlation both for water against creatinine and gold against creatinine excretion.

It can be concluded generally that the rhythmic properties of gold excretion are related to those of water excretion but not to creatinine.

4.3. STUDY 3. THE PATTERNS OF GOLD LEVELS IN SERUM, URINE AND SALIVA AND THE RELATIONSHIP OF IMMUNOPROTEINS AND ACUTE PHASE REACTANTS TO THESE PARAMETERS

4.3.i. Serum, Saliva and Urinary Gold Levels

Eighteen of 20 patients studied demonstrated variations in total and free gold in serum at different times of the day. Variations were also detected in the salivary specimens which were collected at the same time as the blood specimens and these reflected the similar differences observed in the serum gold fractions.

The relationship between saliva and serum gold levels was further enhanced by the correlation studies which showed good correlation between the group saliva results and the group results for total, free and PBG respectively.

Observing the gold results for serum,saliva and urine it is clear that a relationship exists between the gold levels in all three fluids. For each patient studied, at times of higher urinary gold excretion rates, it was observed that total serum gold and its free fractions were lower along with the

salivary gold levels. Conversely when urine gold levels were low both serum and salivary gold levels were raised.

4.3.ii. Immunoproteins and Acute Phase Reactants in Relation to Gold Levels in Serum, Urine and Saliva

Whilst the relationships between C-reactive protein and gold in serum, urine and saliva were inconsistent in the majority of patients, at times when the complement factors (C3 and C4) and immunoglobulins (IgA, IgG and IgM) were lowest, the urinary gold excretion rates were highest accompanied by the lowest levels of total and free serum gold and salivary gold. The levels of the C3 degradation products were highest in a majority of the patients during lowest urinary gold excretion rates and highest total and free serum gold and salivary gold levels.

4.4. STUDY 4. FREE THIOMALATE AND GOLD IN PLASMA,SERUM AND URINE

Free thiomalate was detected in the plasma of 15 of the 16 patients studied and in the urine of all 16 subjects.

The fates of the thiol and gold moieties differed in that free plasma thiomalate peaked early after injection of the drug (30 to 45 minutes) and disappeared from the urine after 4 hours, whilst the gold reached a peak in the serum later after the injection (60 to 120 minutes) and was still present in the urine in significant amounts after 4 hours.

The urinary recovery of the free thiomalate as a percentage of the injected dose within 24 hours reveals that 82.4% to 97.8% of the injected thiol moiety remains within the body in some form following the clearance of the free plasma levels.

Table 1. Summary of Conclusions

	Conclusion	Study
1.	There is good correlation between Total, Free and PBG in serum.	1A
2.	The normal Free fraction represents less than 12% of the total gold.	
3.	Patients exhibiting toxic side effects have HIGHER free to total gold ratios and LOWER PBG to total gold ratios.	
4.	There is no recognisable alteration in total gold values at times of toxicity.	
5.	Following toxicity the return to a normal distribution of gold in the serum fractions proceeds more slowly than the disappearance of the cutaneous rash.	1B
6.	Protein Bound Gold represents 88% of the Total serum gold.	1C
7.	The majority of PBG is bound to albumin in serum (62%).	
8.	There is random variation in the binding properties of proteins when stored at different temperature.	
9.	There is a significant individual circadian rhythm of urinary gold excretion between patients.	2
10.	Urinary gold levels closely resemble those of zinc but only 50% of those of copper.	
11.	Rhythmic patterns of gold excretion are related to water excretory rhythms but not to creatinine.	
12.	There is a relationship between the gold levels of serum, saliva and urine. Higher gold excretion is associated with lower total serum gold, free gold and salivary gold. Conversely lower gold excretion is associated with higher serum and salivary levels.	3
13.	There is an apparent relationship between C3, C4, IgA, IgG and IgM and gold levels in serum, saliva and urine.	

Table 1. continued

14.	The thiol and gold moieties of aurothiomalate have different fates following injection. Plasma was cleared of free thiol after 4 hours.	4
15.	A mean value of 90% of the thiol moiety remains within the body in some form 24 hours following injection.	

CHAPTER 5

CHAPTER 5

5. DISCUSSION

The current management of RA by gold therapy is based on almost sixty years experience. Over this period of time ideas and practices have changed frequently but although the present drug regimes operated by physicians are various, they are usually based on the study by the Empire Rheumatism Council (1961)[53,54]. However, despite the considerable experience gained in the use of gold drugs, the incidence of adverse reactions to treatment remains high when compared to some of the other drugs which are used in the management of rheumatoid disease. Consequently throughout this period of usage of gold drugs, considerable work has been undertaken in attempts to relate any consistent, easily measurable parameter to the potential efficacy or toxicity of the treatment. Much of this work has been devoted to the study of urinary and serum gold levels, but the results of these studies in many cases have been contradictory or inconclusive, such that in 1979 Danpure *et al.*[84] reported that 'the clinical value of monitoring total plasma gold levels is still conjectural'.

The aims of this thesis have been to re-assess the value of monitoring gold levels in various body fluids of patients with RA treated with sodium aurothiomalate and to investigate the possible causes of the uncertain findings of previous workers. In order to assess the value of gold analyses the possible relationships between the gold levels of the various body fluids and the levels of certain other clinical parameters have been studied. Furthermore, variations in blood levels have been established which can probably be related to the toxic side effects of treatment.

5.1.i. The Distribution of Gold in Serum

This study demonstrates that for patients commencing gold therapy and for those stabilised on gold drugs, there is under normal conditions (ie during the absence of side effects) a definite, consistent relationship between the total, free and protein bound gold levels in serum. This is demonstrated by the good correlation shown between total and free gold and total and PBG.

Although some investigators accept that a free or unbound fraction of gold exists in serum [84,90,93] there are those who have been unable to confirm

this.[78,80,99] Less than 12% of the total serum gold was demonstrated to be unbound in this study, in agreement with Campion (1974) [159] and also Herrlinger (1982) [160] who detected slightly lower levels at around 7%. Lorber *et al*. (1983) [90] recorded even lower values for free gold at less than 2% of the total gold concentration. It is likely that this study has succeeded in producing free gold samples and assaying free gold in serum, where others in the past have failed, due to the availability of ultra-filtration methods. Earlier studies perhaps have not been suited to handling large specimen numbers and have been too slow analytically to cope with the changing nature of serum proteins with time and the reactive characteristics of gold in blood.[85] Although ultrafiltration membranes are fragile and susceptible to protein leakage when incorrectly treated, all filtrates produced in this study were tested for the presence of proteins and only analysed for gold when shown to be protein-free. For this reason and because duplicate analyses using different membranes gave good precision, it is unlikely that the free gold values were the result of protein bound gold contamination. Danpure (1977) [84] has suggested that free gold is not aurothiomalate, but a gold metabolite such as a complex with cysteine and/or glutathionine

in which form the gold may be released from the tissues, but the evidence of the good correlation at all levels of free gold with total gold and PBG would suggest that the gold is from the aurothiomalate source rather than from tissues.

The biological effect of a drug is invariably influenced by the form in which it circulates.[90] For many drugs the active part has been demonstrated to be the unbound fraction rather than the total or protein bound fractions.[161] It has further been established that with several drugs the unbound or free part shows a better correlation with pharmacological response[100-102] and subsequently it has been suggested that unbound serum gold might be a more useful parameter for monitoring patients during chrysotherapy.[90,93]

As previously discussed, this study demonstrated a close relationship between the total, free and PBG present in serum. The correlation with regression for total against free gold showed that all patients proceeding through treatment and not presenting with any side effects had plots which were within plus or minus the standard error for the plots on the regression line. The important observation from this study however, is that during the presentation of toxic side effects by 6 patients, as a result of

higher levels of free gold in serum, there was a higher free to total gold ratio resulting in a lower PBG to total gold ratio. At these times the regression plots for total against free gold were positioned significantly outside the standard error limits set for the normal patient results. This change in the distribution of gold was distinctive and at no time did points fall outside the limits when there were no side effects, or remain inside during recorded episodes of toxicity (ie no false positives or negatives).

The types of toxicity recorded were cutaneous rash and one case of thrombocytopenia. On no occasion was proteinuria recorded in the patient notes at the times of observed alterations in the total and free gold distributions. Furthermore, proteinuria was not recorded during any study when the plots for total against free gold were positioned within the plus or minus confines of the standard error.

Further investigations of patients exhibiting toxicity revealed that the return of the gold fractions to a normal distribution did not occur until 4 to 6 weeks after the abatement of the side effects. At the present time medication is suspended until the disappearance of side effects. The observation that free gold levels do not return to 'normality' until

some time after the abatement of cutaneous rash, raises two important questions :-

1. Is toxicity really over when treatment is restarted?
2. If not, is the patient likely to suffer more adverse side effects as a result of premature commencement of therapy?

The incidences of toxicity were diagnosed retrospectively. A future prospective study should follow patients from the outset of chrysotherapy and investigate whether the change in the distribution of gold in the serum occurs before or after the overt appearance of side effects.

It is evident from these studies that in combination with the total serum levels, free gold levels could be used as a diagnostic aid in monitoring toxic side effects. This could take the form of continuous plots of total against free gold around the regression line plus or minus the standard error (See Section 3.1. Figure 15.) or a simple free to total gold ratio calculation. A calculated result outside the range of 0.04 to 0.17 (See Section 3.1. Table 3.) is likely to indicate the presence of toxicity of treatment.

A further observation made during this study was that at times of the presentation of toxic side

effects, there were no significant abnormalities in the total gold levels for any patient, although the free gold levels were high. In an area of research where the findings of previous workers have been contradictory, these results argue against those of Krusius *et al*. (1970)[110] who related high plasma gold levels to toxic side effects and of Jessop and Johns (1973)[162] who related incidences of skin rashes to high total serum gold levels, but support the findings of Mascarenhas *et al*. (1972)[92] and Lorber *et al*. (1973)[163] in terms of total gold levels. Indeed, in the cases where toxicity was diagnosed in this study, the incidents occurred early on in the treatment schedule when the total gold levels were comparatively low (See Section 3.1. Table 5.).

Patients exhibiting side effects in the study represent 11% of all patients studied. The usual figures for toxicity are 25-33% of all patients. There are several possible explanations for this low incidence of toxic reactions in the population studied :-

1. The initial group of 54 subjects were selected on the basis that they were stabilised on therapy and might therefore be expected to have a lower incidence of toxic reactions.

2. The average weekly gold dose for each patient in the study was 20 mg as opposed to the more usual 50 mg per week. This regime is used at this hospital because of the apparent lower rates of toxicity.

3. The 15 patients studied in Group 2 were followed for 9 weeks after commencing therapy. The usual figure of 25 to 30% for patients exhibiting side effects is normally applied to subjects studied during 12 months of therapy, thus it is likely that with a longer study period, the incidence of toxic reactions in the group of 15 would be greater.

The study supports the suggestions of earlier investigators regarding the possible therapeutic usefulness of measuring free gold [90,93] and demonstrates the existence of the free gold moiety and its close relationship to total and PBG in response to those who have doubted its presence [78,80,99] and possibly indicates why workers have found that whole blood gold [164], total serum or PBG alone have no relationship with the clinical response to therapy.[92,94,111,162]

5.1.ii. Differential Protein Bound Gold Fractions

The major part of the gold in serum was detected bound to the albumin protein fraction. This was in agreement with the studies of previous workers [1,85-89,160,165,166] and is an uncontroversial aspect of the study. The areas of investigation which are open to conjecture concern the actual levels of gold bound to albumin and the globulin fractions. Levels of albumin bound gold have been reported as representing 85 to 95% of the total PBG by some workers [86,88,89,92] and from 70 to 80% by others.[1,85,87] In this study the mean albumin gold level for 51 patients was 60%, a figure somewhat less than those described.

The analytical techniques employed in the protein bound studies are diverse and for this reason comparisons are difficult. For example, in some studies [1,160] eluates from chromatographic columns have been analysed for albumin and total globulin gold levels. These methods are imprecise and there is a possibility that the albumin eluates might be contaminated with globulin which would give the higher levels of albumin gold associated with these methods. Furthermore, as the levels of gold are low, these studies have relied on neutron activation or radioactive labelling techniques, neither of which is

particularly suited to meeting the requirements of rapidity and ease for handling large numbers of samples.

The method used for this study was developed in house but based on that of Kamel *et al*. (1977)[85] which by virtue of its speed, sensitivity and reliability, meets all the necessary requirements for accurate and precise assays. Using this method (See Section 2.3.iv.) discrete differential gold levels were obtained for albumin and alpha, beta and gamma globulin as opposed to the almost semi-quantitative methods described above. Apart from the contradictory results caused by differences in the analytical methods obtained by previous workers, further difficulties in comparisons arise because many studies have not used the direct measurement of gold in human serum. Mascarenhas *et al*. (1972)[92] and Danpure (1974/76)[167,165] used human serum mixed *in vitro* with gold compounds. Danpure (1974)[167] also used foetal calf and rat serum *in vitro* whilst McQueen and Dykes (1969)[168] used rabbit plasma *in vivo*. Rat serum was also used *in vivo* by Lawson *et al*. (1977).[169]

An important observation of this study was that the reactive nature of the proteins necessitated the rapid separation of serum from samples and the

maintenance of a constant temperature up to the electrophoresis stage of analysis. The analyses following different temperatures of storage reveal that whilst reproducible results can be obtained from duplicate specimens stored at the same temperature, the results are altered considerably by storage of specimens at different temperatures. This suggests that gold is only loosely bound to the various protein fractions and is easily displaced by changes in temperature, rather than being tightly bound as suggested by Danpure et al. (1979) [1], but who based their assumptions in part, on results obtained following the in vitro study of aurothiomalate mixed with fresh serum.

A consequence of the loose binding of gold to serum proteins is that unless the temperature of the specimen can be controlled closely from the time of venous section until the time of analysis, then an accurate and reproducible assay of the gold distribution is impossible.

One purpose of this particular study was to investigate the possibility that changes might occur in the distribution of gold amongst the protein fractions as a result of toxic side effects of treatment. The demonstration of the temperature sensitivity of the protein binding of gold indicates

that accurate monitoring of these levels would be very difficult. Under these circumstances it would be difficult to relate changes in gold binding characteristics to toxicity rather than to sporadic temperature changes.

5.2. THE RHYTHMICITY OF URINARY GOLD, ZINC, COPPER, WATER AND CREATININE

5.2.i. Gold, Water and Creatinine Excretion

The histogram and sine wave analysis of the results for this study indicate that significant individual patient rhythms exist for the excretion of gold in 28 of 30 patients. These are similar to those for water excretion but only similar to creatinine excretion rates in 6 of the 28 subjects. The correlation studies reveal that the excretion of gold is probably related to the excretion of water (or to urine flow) but not to the excretion of creatinine.

These findings are important when considering the possible reasons for the contradictory findings of other workers and can be used to explain some of these disparities. Workers have examined the excretion of gold in 24 hour urine volumes [51,99,110] but have been unable to demonstrate any correlation between

serum and urinary gold levels and any correlation between urine gold levels and therapeutic efficacy. Other investigators have measured gold levels in random urine specimens and have reported the excretion as gold per gram of creatinine.[111] The existence of urinary gold rhythmicity suggests the likelihood of the existence of patterns of serum gold levels and this is positively indicated in Study 3 (Section 4.3.i.). It should be more appropriate therefore to compare the gold levels of urine passed around the times of blood collection rather than to use 24 hour specimens. Furthermore, if efficacy or toxicity of treatment is reflected by changes in gold excretion, then it is likely that these alterations could be more easily detected by observation of changes in individual excretory patterns, rather than in the total 24 hour excretion. Measurement of 24 hour urinary gold levels would mask any subtle alterations in excretory patterns.

Similarly, because of the acrophases and troughs associated with circadian rhythmicity, the previous attempts to compare random urine specimens collected at different times on different days were bound to fail. In addition, as gold and creatinine excretory patterns have been shown to be in the main different, the calculation of gold excretion in terms of grams

of urinary creatinine will compound inconsistencies in results.[111]

<u>Patient Compliance</u>

Essential to this study was the accuracy with which the subjects complied with the instructions and protocol. Using the experience gained in other studies[117] it was possible to bring co-operative patients to a suitable standard of accurate urine volume measurement and time recording very quickly. Also, because renal rhythmicity is affected by posture[114,117] subjects attempted to maintain similar activities from day to day during the study and to indicate on the data sheet any occasion when their activity was abnormal (eg lying down or very active).

Despite the attempts to standardise the study days, 2 of the 30 patients studied did not demonstrate any regular patterns of excretion, but because of their good compliance in reporting on the data sheets, the reasons for this were evident. One patient was a poor sleeper and drank large quantities of tea both during the day period and often in the early hours of the morning. Tea has diuretic properties and therefore the general patterns of micturition were continually altered. Additionally, the postural changes associated with night-time rising to make the

tea also contributed to loss of rhythmicity. The second patient commenced steroid therapy during the study and was thus disqualified from the patient analyses.

Circadian Rhythmicity - General Points

A major difficulty in the study of human circadian rhythms lies in our ignorance of their precise origin.[170] A current working hypothesis is that circadian and other rhythms are due to a relatively small number of mechanisms with examples at all levels of evolution and that these mechanisms are a property of individual cells and tissues. There is evidence that in some instances, circadian rhythmicity may be the result of multiple coupled high-frequency oscillators.[171,172] In brief general terms, the search for these oscillators has taken three main directions :-

1. Simple biochemical systems.

   One possibility is that the oscillation might be derived from a pure biochemical system which given appropriate reactants tends to oscillate. The biochemical oscillators are well documented natural phenomena but it still remains to be proved whether they are implicated as an important factor in chronobiology.

2. DNA-Transcription theory.

This suggests that some circadian oscillations are due to a serial transcription of DNA resulting in a time controlled synthesis of enzymes, the 'chronon' or polycistronic complex of DNA taking 24 hours for complete transcription of a circadian cycle.[173] It has been postulated that the cell membranes rather than the nucleus has a key role in the 'biological clock'.[174] (See 3.)

3. The cell membrane as oscillator.

One suggestion is that the oscillator lies in or close to the cell membrane and is effective in modifying cellular transport processes.

Rosenwasser and Adler (1986)[172] concluded that the normal co-ordinated functioning of the circadian timing system must be dependent on complex interactions between neural and neuroendocrine systems and that the effects of stimuli which affect the coherence of circadian rhythms, such as light and pharmacological agents, seem to be mediated by the same central transmitter systems. It has been suggested further, that the pineal gland translates such basic environmental information as light and heat into signals which alter most neuroendocrine mechanisms [175,176] and that this is accomplished by means of the circadian

synthesis and release of melatonin (N-acetyl-5-methoxy-tryptamine) which is the most studied and best known pineal neurohormone. Melatonin is synthesised and released upon activation of pineal beta-adrenergic receptors in man and most vertebrates during the nocturnal dark hours and maintains a consistently regular circadian rhythm.[177,178]

Renal Rhythms and Urine Flow

The control of renal rhythms is largely unknown, (and it may be premature with the knowledge that is presently at our disposal to postulate their possible origins) however, it is accepted that the amount of substance excreted in the urine will be determined by the amount filtered at the glomeruli, which depends upon the plasma levels and the glomerular filtration rate (GFR) and also the amount reabsorbed or secreted by the rest of the nephron.[170] The GFR has a small rhythmic amplitude and an assessment of its importance has not been systematically carried out.

An important factor in determining urinary excretion is the reabsorbtive and secretory activities of the tubule. Several hormones and other factors are known to modify these tubule processes.[170] In conditions of controlled and restricted water intake, the solute load comprising mainly sodium, potassium

and chloride may be an important factor in determining the rate of renal water excretion (ie flow rate). This close association of urinary volume with electrolyte excretion would seem to justify the contention that when other circumstances are reasonably constant, the circadian rhythmicity of urine flow is osmotically determined.[179] In addition, the circadian rhythms of these electrolytes have been shown to demonstrate a close phase relationship both with each other and with the diurnal rhythm of water excretion.[117,180,181] In other words, under certain circumstances, the factors governing electrolyte excretion are likely to be indirectly responsible for water excretion.

An explanation of the system controlling sodium and potassium excretion is the renin-angiotensin-aldosterone system.[179] Renin, a proteolytic enzyme, is released from the juxtaglomerular apparatus in the kidney. This enzyme hydrolyses the substrate angiotensinogen, (an $alpha_2$ globulin plasma protein) to give a vasoconstrictor substance called angiotensin I (a decapeptide). The next step occurs mainly in the lungs, where angiotensin I is hydrolysed by the enzyme angiotensinase to produce angiotensin II (an octapeptide), another vasoconstrictor. It is thought that[179] angiotensin in the plasma controls the output

of aldosterone from the adrenal cortex, which in man firstly increases the elimination of potassium and hydrogen ions and decreases the excretion of sodium without any change in GFR. More recent studies have demonstrated that sodium excretion by the kidney is modulated by the relationship between dopamine,[182] a vasodilating and natriuretic agent[183] and nor-adrenaline which inhibits sodium excretion not only by its haemodynamic effect, but also by increasing sodium reabsorption in the proximal convoluted tubule and perhaps also in the loop of Henle.[184] It has also been suggested that angiotensin II can contribute through its direct intrarenal effect, to the reduced sodium excretion during daily activities.[185]

As it seems likely that urine flow is closely associated with, and possibly governed by, electrolyte excretion[179-181] the factors influencing this excretion and its circadian rhythmicity will probably influence the rhythmicity of water excretion. Although rhythmicity has been demonstrated for plasma renin, angiotensin and aldosterone,[181,186-188] it is believed that aldosterone cannot play an important part in the origin of the rhythms for sodium and potassium (and subsequently water) due to the phase relationship between the excretory rhythms of these electrolytes and the reversed phase relationship

between the rhythms of potassium and hydrogen ions.[181,185]

When water intake is varied, variations in the production of anti-diuretic hormone (ADH) by the posterior pituitary (which increases water reabsorption in the distal tubules and collecting ducts) are thought to be a more probable influence on water excretion.[170,179] There are reports that ADH secretion displays circadian rhythmicity but the nature of the rhythm is unclear.[170]

Finally the role of the nervous system is probably of minor importance in the origin of these rhythms, as normal rhythms for sodium and water excretion have been demonstrated for patients, during bedrest, who have undergone renal transplantation with denervation of the kidneys.[117,181,189]

Creatinine Excretion

Creatinine and water are dealt with differently by the kidney for whilst water is passively absorbed in the tubules creatinine is not reabsorbed, although it is excreted by the tubules. Because creatinine is completely filtered at the glomerulus with subsequent non-reabsorption, it is used widely in clinical practice to give an estimation of GFR. Also, because of its endogenous nature and because its

excretion is constant over 24 hours, it has been used as a check to ensure complete 24 hour urine collections. For this reason creatinine values have been used to correct certain random urine values in order to give an impression of the 24 hour value (eg. calcium/creatinine, gold/creatinine ratios), but this study would suggest that this method is probably ill-founded.

<u>Summary</u>

Since rhythmicity has been demonstrated in the excretion of electrolytes [114,118] and certain heavy metals [119,120] and since the distressing early morning stiffness associated with RA [2,33,34] has also been shown to be rhythmic,[36,37] it is not surprising that gold excretion exhibits some form of rhythmicity.

The individual gold rhythms demonstrated were shown to be closely related to urine flow, which in turn is probably influenced in its rhythmicity by the excretion of sodium and potassium and by secretion of ADH.

The results reveal little relationship between gold rhythmicity and creatinine rhythmicity possibly because the mechanisms affecting creatinine are not the same as those affecting water, electrolytes and gold.

5.2.ii. Zinc and Copper Excretion

All patients who exhibited regular patterns of gold excretion also demonstrated patterns of zinc excretion, with similar individual acrophases to those of gold.

Approximately 98% of the zinc in plasma is bound to proteins.[190] This zinc is associated principally with albumin and α_2 macroglobulin[191,192] whilst the unbound fraction is associated with free amino acids.[190] Gold is also bound principally to albumin and to a lesser extent α_2 globulins (and other globulins) and it is possible that the unbound zinc fraction having been filtered at the glomerulus, is excreted in a similar manner to the gold with respect to urine flow, resulting in the similar acrophases for the two metallic ions.

It is not surprising that many of the patients studied (66%) exhibited rhythmicity of copper excretion, since it has been established for some time that plasma copper levels show rhythmic patterns[126,127,193] and more recent work has confirmed the rhythmicity of urinary copper excretion demonstrated in these studies.[193] However, only 50% of the subjects studied had individual acrophases

which were similar to those of gold excretion.

Plasma copper has different protein binding properties to gold and zinc. Total plasma or serum copper is present in three principal forms. Caeruloplasmin is an α_2 globulin with oxidase activity which is synthesised in the liver and increases in serum as an acute-phase reactant. It accounts for 80 to 90% of all serum copper.[194] The remaining copper is also an acute-phase reactant and is released from liver stores as albumin and amino acid complexes, which serve in its transportation and utilisation.[195] The amino acid complexes represent the non protein bound fraction which is filtered at the glomeruli in the kidneys.

In these studies 34% of the patients did not demonstrate rhythmicity of copper excretion. It is evident from the results that on these occasions there were periods when there was no excretion of copper at all. At these times, it is likely that the circulating copper was virtually all protein bound.

It is possible that the unbound fraction of copper is excreted by the same mechanism as gold and zinc. However, differences in excretory patterns may be the result of the different protein binding characteristics for copper. The majority of zinc is 'loosely' bound to albumin[196] whilst the majority of

serum copper is 'avidly' bound to α_2 macroglobulin[194, 196,197] and it might be that this avid binding restricts the availability of non-bound copper for renal filtration.

Relationships between Serum Zinc, Copper and Rheumatoid Arthritis

There is an interaction between zinc and copper in serum such that total serum copper/zinc ratios have been used to assess the body zinc status.[198] Because the majority of zinc is bound to albumin and copper to α_2 globulin, any changes in the concentrations of albumin and ceruloplasmin in plasma or in the affinity of these proteins for zinc and copper can lead to alterations in the concentrations of zinc and copper in plasma.[196]

It has been established that both serum zinc and copper levels are abnormal in patients with RA. Whilst they are both acute phase reactants, zinc levels are lowered[125] and copper levels are raised[121, 122] following onset of the disease. In animals zinc levels are lowered in response to the release of the low-relative molecular mass polypeptide 'leucocyte endogenous mediator' (LEM) from granulocytes, since this substance mediates the sequestration of a portion of the circulating zinc by the liver.[199,200]

In man, LEM has been implicated with both inflammatory diseases such as RA and infective disorders.[201,202,203] It has been shown further in animal studies that the rise in total serum copper is accompanied by a fall in liver concentration and it has been suggested that this is a physiological response to RA.[197] It has been suggested that RA results from a disturbance in copper homeostasis and that the reactivity of aurothiomalate toward albumin bound copper causes elevated serum copper levels which perturbs synovial cell metabolism and further triggers the disease.[204,205]

5.3. THE PATTERNS OF GOLD LEVELS IN SERUM, URINE AND SALIVA AND THE RELATIONSHIP OF IMMUNOPROTEINS AND ACUTE PHASE REACTANTS TO THESE PARAMETERS

5.3.i. Serum, Saliva and Urinary Gold Levels

Eighteen of the 20 patients studied showed similar variations in total and free serum gold levels at different times of day and night (10.00, 16.00 and 22.00 hours). Similar variations in the total salivary gold levels were also shown for specimens collected at the same time as the blood samples. Fourteen patients had higher total and free serum and

salivary gold levels at 10.00 and 22.00 hours than at 16.00 hours and 4 had lower levels at these times. All the variations recorded were outside the possible differences obtainable in results due to the CV of the assay method. Statistical analysis of the results showed a correlation between total salivary gold and total serum gold, total salivary gold and free gold and total salivary gold and PBG. Furthermore, for each patient at times of higher urinary gold excretion rates, total serum gold and its fractions were low, as were salivary gold levels. Conversely, when urine gold levels were low both serum and salivary gold levels were raised. The urinary excretion of gold has already been shown to be rhythmic and it is possible that serum and salivary gold level variations are also rhythmic, but it is not possible to establish this conclusively in this study, using just three time points in a 24 hour day.

It has been suggested on page 169 that the mechanisms for urinary gold rhythmicity might be governed by urine flow (and consequently by the factors affecting this process). Furthermore, it is accepted that the amount of solute excreted in the urine will be determined by the amount filtered at the glomeruli, which is dependent upon the plasma levels and the GFR and also the amount reabsorbed or

secreted by the rest of the nephron.[170] The variations in serum gold levels at different times of the day and night demonstrated in this study, confirm the verity of this statement and adds to the evidence already submitted in this work with regard to the control of urinary gold rhythms by urine flow. As free serum gold represents the filterable fraction of serum gold by healthy kidneys, it is likely that the increase in urine gold may be derived from this serum fraction. There is, however, a decrease in the protein bound level of gold in serum at the same time, which is not filterable, but which might release gold to the depleted free fraction allowing further excretion of gold.

Further Comments on Salivary Gold Levels

The possibility of utilising saliva for clinical tests attracts attention as a result of its ease of collection, for samples can be collected repeatedly without stress or trauma. This original study demonstrating the presence of gold patterns in saliva adds gold to the list of other ions detected in previous studies by other workers (sodium and potassium,[138] lithium,[139] calcium and inorganic phosphate,[140] copper, silver, tin, mercury and zinc[141] and circadian rhythmicity of zinc.[203]).

Apart from the possible source of contamination from gold fillings in teeth (of which there were none in these studies), the risk of contamination of saliva is minimal.

This study did not investigate the free or protein bound nature of the gold measured in specimens of saliva. Albumin is known to be present in saliva and it is thought that its presence is largely the result of contamination by traces of blood or gingival fluid.[206] The recent introduction of more sensitive assays has resulted in the reporting of immunoreactive sex hormone binding globulin (SHBG)[207] and corticosteroid binding globulin[208] in saliva.

This work has demonstrated a good relationship between gold levels in urine, serum and saliva and consequently it should be possible to substitute salivary gold levels for serum gold levels. However, from the results of Study 1 (Section 3.1.) it is evident that no single serum gold parameter can reflect efficacy of treatment or toxicity. In order to monitor the treatment, it is necessary to plot total gold against free gold in serum and therefore although collection of saliva does not incur patient stress, salivary gold levels alone are not likely to be a useful early indicator of side effects. A future study should investigate the nature of salivary gold

with respect to its possible association with protein and examine the correlation plots for total serum against salivary gold in an effort to establish any changes in correlation relative to side effects.

5.3.ii. Immunoproteins and Acute Phase Reactants in Relation to Gold Levels in Serum, Urine and Saliva

The signs and symptoms of RA exhibit periodicity[34,37] as does the immune response to challenge.[209] Circadian rhythmicity has also been demonstrated for serum immunoglobulins in patients affected with allergic rhinitis, atopic asthma and chronic urticaria.[118] Certain relationships between serum gold and immunoglobulins and complement factors are well documented,[97,129-133,210-214] but whilst there is a general concensus amongst workers that gold suppresses immunoglobulin synthesis and inhibits complement components, certain studies remain inconclusive and contradictory.[133]

This study demonstrates that in blood specimens collected at 3 different times of the day, patterns exist for serum complement factors C3 and C4 and immunoglobulins IgA, IgG and IgM. These patterns

might be circadian in periodicity but more sample periods in the 24 hour day are necessary to confirm this.

Relationships were also demonstrated between these immunological parameters (IP's) and the levels of gold in serum, saliva and urine, for at times when serum levels of complement factors and immunoglobulins were low, they were accompanied by low levels of total and free serum gold and low levels of salivary gold. At these times however, the urinary gold excretion rates were high. The converse of these findings was also true (ie high serum levels of IP's were accompanied by high gold levels in serum and saliva, but low urinary gold levels). The levels of C3 degradation products were also, in the main, highest at times of the lower gold excretion rates and higher serum and salivary gold levels.

These findings indicate that despite the recognised effects of gold induced immunological factor supression and although the mean levels for the IP's are lowered, within a 24 hour period higher levels of IP's are associated with higher serum gold levels (with the related raised salivary gold and lower urinary gold levels).

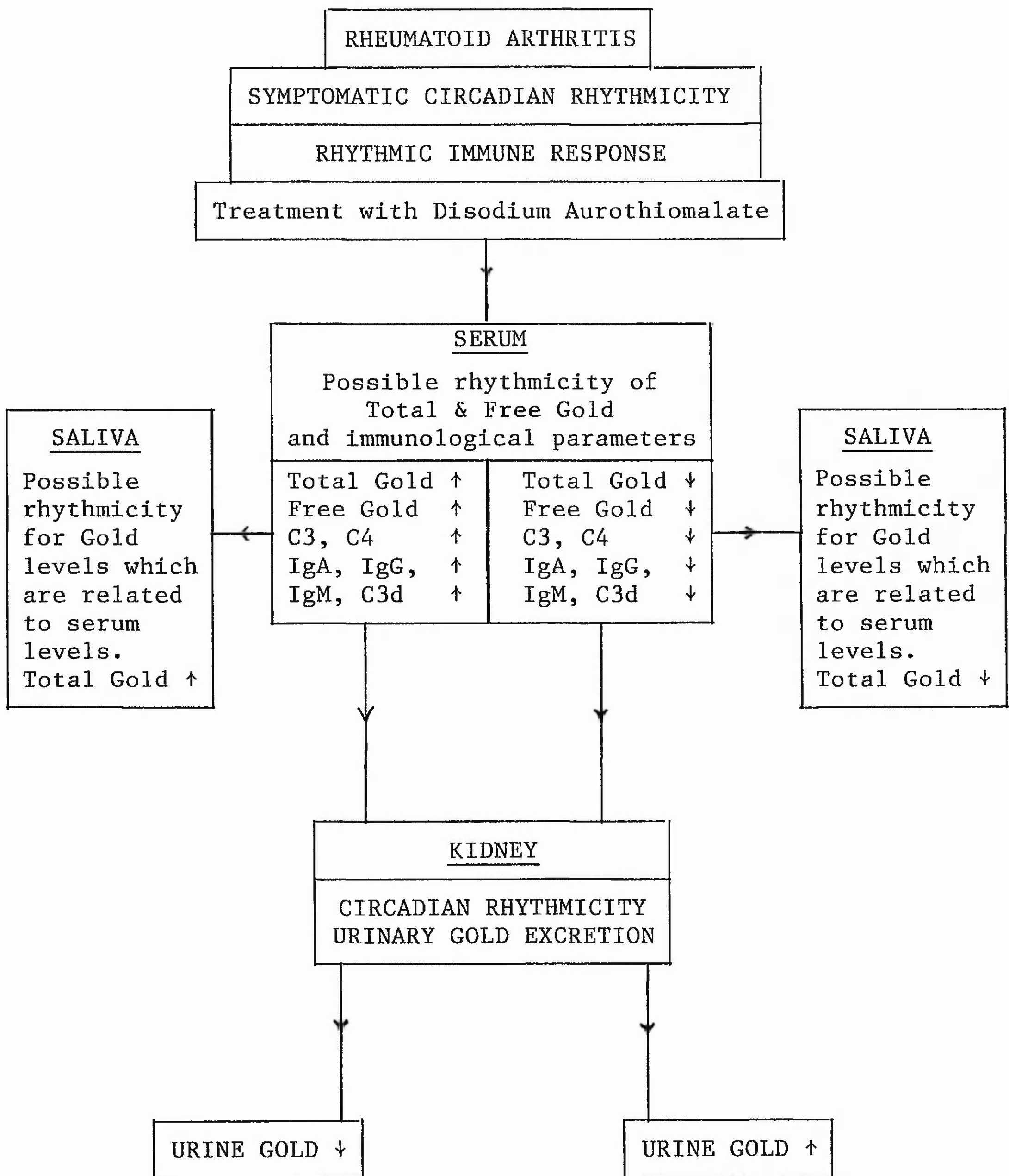
Lorber _et al_. (1978)[97] suggested that there is an increased affinity of gold at higher serum gold levels for various immunoglobulins including IgG and

IgM and complement and since the active uptake of such immune complexes by phagocytic cells has been demonstrated, it has been suggested further that the increased binding of gold at higher gold levels may enhance the access of gold to those cells involved in phagocytosis of such complexes.[97]

It is unlikely that the factors controlling the rhythmic excretion of gold and those responsible for the possible rhythmicity of serum IP's are related, but there may well be an interaction between the gold and the immunoproteins at the higher gold levels.[213] This would result in an alteration in the distribution of gold in the proteins.

A scheme for some of the possible rhythmic events and relationships is presented in Figure 1.

As the excretion of zinc was similar in periodicity to gold then the relationship demonstrated for urine gold will apply to zinc excretion rate and also to those patients who presented similar acrophases for copper excretion.



5.3. Figure 1. Some Possible Rhythmic Events and Relationships associated with Rheumatoid Arthritis

5.4. FREE THIOMALATE AND GOLD IN PLASMA, SERUM AND URINE

Disodium aurothiomalate dissociates to form free thiomalate and protein bound [77,78] and free gold [79] in the blood following intramuscular injection. This is followed by the appearance of free thiomalate and free gold in the urine.

It was demonstrated in this study that the fates of the thiol and gold moieties differed in that whilst free thiomalate peaked in the plasma within 30 to 45 minutes of the injection and disappeared from the urine after 4 hours post administration, the gold moiety reached a peak in the serum later at 60 to 120 minutes post injection (this latter recently supported by Blocka et al., 1986)[215] and was still present in the urine in significant amounts after 4 hours. It was evident from recovery studies of the free thiol moiety assayed in the urine, that after 24 hours 82 to 98% of the injected thiol dose remains within the body in some form following the clearance of free thiol from the plasma. It has been suggested that this remaining fraction is bound to serum protein,[216] tissue membranes and cells.[78]

It is recognised that only thiol-containing gold compounds are active as anti-rheumatic agents [142] and

it has been suggested that thiomalate might be the active metabolite in gold therapy.[80,217,218] Since free thiomalate disappeared from the urine after 4 hours post injection in this study, it is possible that at this time there was no circulating free thiol in the plasma. It is unlikely therefore, that the free thiol moiety can have any lasting therapeutic effect on the disease condition. However, it may be that the protein bound fraction which represents the majority of circulating thiol releases the moiety at metabolically active sites.[216]

It has been suggested that the free thiomalate found in plasma is an impurity of the drug, but work by Rudge _et al_. (1984) [216] has demonstrated conclusively that the circulating thiomalate is a metabolite.

Although attention is now being given to the suggestion that thiomalate and thiosulphate ligands are active anti-rheumatic agents, it should be remembered that gold compounds have been in use for centuries and it was not until 1913 that thiols were first introduced as gold(1) stabilising ligands.[93]

This study shows that there is little relationship between the gold and thiol moieties after intramuscular injection of disodium aurothiomalate and

demonstrates that whilst free thiol disappears from the urine and plasma very quickly, free serum gold (and also total and PBG) continues to be detected in serum for at least one week following injection along with urinary levels (See also Section 3.1. Study 1 & 3.2. Study 2.). This study only measured free thiomalate and despite claims that the majority of the thiomalate is bound to protein [216] this view is not shared by all investigators.[78]

5.5. THE VALUE OF MONITORING GOLD LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS UNDERGOING CHRYSOTHERAPY

Over the last 60 years gold drugs have been used with varying degrees of success in treating patients with RA. Toxic symptoms do occur as a result of treatment, but the multitude of studies employed to test the efficacy or toxicity of this treatment have produced contradictory and inconclusive results. Consequently one must consider if there is indeed any value in monitoring gold levels in these patients.

Results from this series of studies have described relationships between gold in serum, urine and saliva and between these levels and other serum and urinary parameters. Importantly, it has been demonstrated

that montoring certain gold levels in patients with RA undergoing chrysotherapy can be of probable value in monitoring the progress of the treatment.

The simple specimen measurement of total serum or urinary gold is clearly not useful in diagnoses and it is unfortunate that salivary gold with its advantages of easy, stress-free collection, is of no value on its own despite its close correlation with serum levels.

The demonstration of circadian rhythmicity of urinary gold excretion has presented reasons for the contradictory findings in early work. However, changes in free to total gold ratios in serum which have been shown to be related to onset of toxicity (see below) are likely to be reflected in alterations in urinary patterns (as the free gold represents the kidney filterable fraction), but continuous urinary assessment would be an unsuitable form of monitoring patients who would be unlikely to sustain the required subject compliance throughout the necessary weeks of the study.

There might be a change in the differential protein binding properties of gold during the presentation of side effects but the method of analysis is too sensitive to temperature change to give consistently precise results.

The most effective form of diagnosing toxicity developed in these studies is the calculation of free to total gold ratios in serum and the continuous plots of total and free gold values as a correlation with regression (with limits of plus or minus the standard error). These studies were made retrospectively, but a prospective study using these parameters and perhaps total gold to salivary gold ratios might prove to be the diagnostic tool which has eluded workers for the past sixty years.

5.6. FURTHER RESEARCH

There are aspects of the work presented in this thesis which indicate areas in which further studies might be of value:

5.6.i. The Distribution of Gold in Serum

In this study the incidences of toxicity were diagnosed retrospectively. A future prospective study should follow patients from the outset of chrysotherapy and investigate whether the changes in the distribution of gold in the serum occur before or after the overt appearance of side effects. Changes in serum gold distribution following the presentation of rash etc

might indicate that these changes occur as an effect of toxicity rather than as a cause.

5.6.ii. Salivary Gold Levels

This study did not investigate the free or protein-bound nature of the gold measured in the specimens of saliva. A future study should investigate the nature of salivary gold with respect to its possible association with proteins and prospectively examine the correlation plots of total serum gold against salivary gold in an effort to establish any possible changes in correlation in relation to side effects.

Awareness should be maintained for Sjogren's syndrome (which can be associated with RA), in which there is salivary gland enlargement and difficulty for the patient to produce salivary specimens.

CHAPTER 6

CHAPTER 6

6. REFERENCES

1. Danpure, C.J., Fyfe, D.A. & Gumpel, J.M. (1979) Ann. Rheum. Dis. 38, 364-370

2. Currey, H.L.F. (1978) In 'Copemans Textbook of the Rheumatic Diseases' 5th Ed. Churchill Livingstone 261-273

3. Ropes, M.W., Bennett, G.A., Cobb, S., Jacox, R. & Jessar, R.A. (1957) Ann. Rheum. Dis. 16, 118-125

4. Ropes, M.W., Bennett, G.A., Cobb, C. , Jacox, R. & Jessar, R.A. (1959) Ann. Rheum. Dis. 18, 49-53

5. Kellgren, J.H., Jeffrey, M.R. & Ball, J. (1963) In 'The Epidemiology of Chronic Rheumatism' Blackwell Scientific Pubs. Oxford 1, 326-327

6. Empire Rheumatism Council (1950) Ann. Rheum. Dis. 9 (Suppl.) 1-94

7. Short, C.L., Bauer, W. & Reynolds, W.E. (1957) In 'Rheumatoid Arthritis' Cambridge Mass. Harvard Univ. Press

8. Miall, W.E., Ball, J. & Kellgren, J.H. (1958) Ann. Rheum. Dis. 17, 263-272

9. Laine, V., De Graaf, R. & Lawrence, J.S. (1961) Atti del X Congresso della Legra Internazionale contro il Reumatismo 1, 31-32

10. Lawrence, J.S. (1961) Ann. Rheum. Dis. 20, 11-17

11. Blumberg, B.S. (1960) Arth. & Rheum. 3, 178-185

12. Lawrence, J.S. & Ball, J. (1958) Ann. Rheum. Dis. 17, 160-168

13. Bremner, J.M., Alexander, W.R.M. & Duthie, J.J.R. (1959) Ann. Rheum. Dis. 18, 279-284

14. De Blécourt, J.J., Polman, A. & De Blécourt-Meindersma, T. (1961) Ann. Rheum. Dis. 20, 215-223

15. Bunim, J.J., Burch, T.A. & O'Brien, W.M. (1964) Bull. Rheum. Dis. 15, 349-350

16. Stastny, P. (1974) Tissue Antigens 4, 571-579

17. Legrand, L., Lathrop, G.M., Bardin, T., Marcelli-Barge, A., Dryll, A., Debeyre, N. & Ryckewaert, A. (1987) Ann. Rheum. Dis. 46, 395-397

18. Stastny, P. (1978) N. Eng. J. Med. 298, 869-871

19. Otha, N., Nishimura, Y.K. & Tanimoto, K. (1982) Hum. Immunol. 5, 123-132

20. Lies, R.B., Messner, R.P. & Troup, G.M. (1972) Arth. Rheum. 15, 524-529

21. Seignalet, J., Clot, J., Sany, J. & Serre, H. (1972) Vox Sang. 23, 468-473

22. Ryder, L.P., Anderson, E. & Svejgaard, A. (1979) 'HLA and disease registry' 3rd Report Copenhagen : Munksgaard

23. Jaraquemada, D., Ollier, W., Awad, J., Young, A., Silman, A., Roitt, I.M., Corbett, M., Hay, F., Cosh, J.A., Maini, R.N., Venables, P.J., Ansell, B., Holborow, J., Reeback, J., Currey, H.L.F. & Festenstein, H. (1986) Ann. Rheum. Dis. 45, 627-636

24. Read, A., Grennan, D.M. & Dyer, P. (1982) Dis. Markers 1, 271-282

25. Van de Putte, L.B.A., Speerstra, F., Van Riel, P.L.C.M., Boerbooms, A.M.Th., Van't Pad Bosch, P.J.I. & Reekers, P. (1986) Ann. Rheum. Dis. 45, 1004-1006

26. Carette, S., Lang, J.Y., Mathieu, J.P., Roy, R. & Morissette, J. (1987) Arth. & Rheum. 30,2, 233 (letter)

27. Lawrence, J.S., Behrend, T., Bennett, P.H., Bremner, J.M., Burch, T.H., Gofton, J., O'Brien, W. & Robinson, H. (1966) Ann. Rheum. Dis. 25, 425-432

28. O'Brien, W.M., Bennett, P.H., Burch, T.A. & Bunim, J.J. (1968) Arth. & Rheum. 10, 163-179

29. Shichikawa, K., Mageda, A., Komatsubara, Y., Yamamota, T. *et al*. (1966) Ann. Rheum. Dis. 25, 25-31

30. Blumberg, B.S., Block, K.J., Black, R.L. & Dotter, C. (1961) Arth. & Rheum. 4, 325-341

31. Hall, L. (1966) East Africa Med. J. 43, 161-170

32. Black, J.S., Lewis, H.E., Thacker, C.K.M. & Thould, A.K. (1963) Brit. Med. J. 2, 1018-1024

33. Scott, J.T. (1960) Ann. Rheum. Dis. 19, 361-368

34. Kowanko, I.C., Pownall, R., Knapp, M.S., Swannell, A.J. & Mahoney, P.G.C. (1981) Br. J. Clin. Pharmacol. 11, 477-484

35. Rhind, M., Unsworth, A. & Haslock, I. (1987) Brit. J. Rheum. 26, 126-130

36. Harkness, J.A.L., Richter, M.B., Panayi, G.S., Van de Pette, K., Unger, A., Pownall, R. & Geddawi, M. (1982) Brit. Med. J. 284ii, 551-554

37. Kowanko, I.C., Knapp, M.S., Pownall, R. & Swannell, A.J. (1982) Ann. Rheum. Dis. 41, 453-455

38. Fye, K.H. & Sack, K.E. (1982) In 'Basic and Clinical Immunology' 4th Ed. Lange Med. Pub. 435-436

39. Axford, J.S., Lydgard, P.H., Isenberg, D.A., Mackenzie, L., Hay, F.C. & Roitt, I.M. (1987) Lancet Dec. 26, 1486-1488

40. Bresnihan, B. (1982) Irish Med. J. 75, 1, 7-9

41. Watkinson, G. (1986) Drugs 32, 1, 1-11

42. McConkey, B. (1986) Drugs 32, 1, 12-17

43. Smith, R.T., Peak, W.P., Kron, K.M., Herman, I.F. & Deltorro, R.A. (1958) J.A.M.A. 7, 1197-1204

44. Mullin, T.M. (1982) Rheum. Arth. 72, 205-217

45. Mahdihassan, S. (1981) Am. J. Chin. Med. IX, 2, 108-111

46. Needham, J. (1976) In 'Science and Civilisation in China' Cam. Univ. Press 5, Part 3, Section 33, Chapter 5, 117-167

47. Gutzlaff, C. (1837) J. R. Asiatic Soc. 4, IX, 154-173

48. Rodnan, G.P. & Benedek, T.G. (1970) Arth. Rheum. 13, 145-165

49. Forestier, J. (1935) J. Lab. Clin. Med. 20, 827-840

50. Hartfall, S.J., Garland, H.G. & Goldie, W. (1937) Lancet 2, 784-788

51. Freyberg, R.H., Block, W.D. & Levey, S. (1941) J. Clin. Invest. 20, 401-412

52. Lockie, L.M., Norcross, B.M. & Riordan, D.J. (1958) Jama. 167, 1204-1207

53. Research Sub-Committee of the Empire Rheumatism Council (1961) Ann. Rheum. Dis. 20, 315-333

54. Kaye, R.L. (1982) J. Rheumatol (suppl. 8) 9, 124-131

55. Kaye, R.L. & Pemberton, R.E. (1976) Arch. Int. Med. 136, 1023-1028

56. Co-operating Clinics Committee of the American Rheumatism Association (1973) Arth. Rheum. 16, 353-358

57. Sigler, J.W., Bluhm, G.B. & Duncan, H. (1974) Ann. Int. Med. 80, 21-26

58. McKenzie, J.M.M. (1981) Rheum. & Rehab. 20, 198-202

59. Shaw, C.F. III (1979) Inorg. Perspectives in Biol. and Med. 2, 287-355

60. Freyberg, R.H. (1966) In 'Arhtritis and Allied Conditions' 7th Ed. Hollander, J.L. (Ed.) Lea & Febiger, Philadelphia 302-332

61. Almeyda, J. & Baker, H. (1970) Brit. J. Derm. 83, 707-711

62. Davis, P. (1979) J. Rheum. 5, (suppl.) 18-24

63. Gibbons, R.B. (1979) Arch. Int. Med. 139, 343-346

64. Kean, W.F. & Anastassiades, T.P. (1970) Arth. & Rheum. 22, (5) 495-501

65. Penneys, N.S. (1979) J. Am. Academy Derm. 1, 315-320

66. Vaamonde, C.A. & Hunt, F.R. (1970) Arth. & Rheum. 13, 826-834

67. Silverberg, D.S., Kidd, E.G. & Shnitka, T.K. (1970) Arth. & Rheum. 13, 812-825

68. Howrie, D.L. & Gartner, J.C. (1982) J. Rheum. 9, 5, 727-729

69. Gottlieb, N.L. & Gray, R.G. (1978) J. Anal. Toxicol. 2, 173-184

70. Persellin, R.H. & Ziff, M. (1966) Arth. & Rheum. 9, 57-65

71. Weissmann, G. & Thomas, L. (1963) J. Clin. Invest. 42, 661-669

72. Ennis, R.S., Granda, J.L. & Posner, A.S. (1968) Arth. & Rheum. 11, 756-764

73. Grahame, R., Billings, R., Lawrence, M., Markes, V. & Wood, P.J. (1974) Ann. Rheum. Dis. 35, 556-559

74. Gottlieb, N.L., Smith, P.M. & Smith, E.M. (1972) Arth. & Rheum. 15, 16-22

75. Gilman, A.G., Goodman, L.S. & Gilman, A. (1980) In 'The pharmacological basis of therapeutics' 6th Ed. 699-705, 710-711, 713-715, 1627

76. Parente, J.E., Wong, K. & Davis, P. (1986) J. Rheum. 13, 5, 846-848

77. Jellum, E., Munthe, E., Goldal, G. & Aaseth, J. (1979) Scand. J. Rheum. (suppl.) 28, 28-36

78. Jellum, E. & Munthe, E. (1980) Ann. Rheum. Dis. 39, 155-158

79. Lorber, A., Kunishima, D.H., Harralson, A.F. & Simon, T.M. (1983) J. Rheumatol. 10, 568-573

80. Jellum, E. & Munthe, E. (1982) Ann. Rheum. Dis. 41, 431-432

81. Gottlieb, N.L. (1979) J. Rheum. (suppl. 5) 2-6

82. Gottlieb, N.L. & Major, J.C. (1978) Arth. & Rheum. 21, 704-708

83. Gottlieb, N.L., Smith, P.M. & Penneys, N.S. (1974) Arth. & Rheum. 17, 56-62

84. Danpure, C.J. (1977) J. Physiol. 267, 25-26

85. Kamel, H., Brown, D.H., Ottaway, J.M. & Smith, W.E. (1977) Analyst. Sept., 102, 645-657

86. Ward, R.J., Danpure, C.J. & Fyfe, D.A. (1977) Clin. Chim. Acta. 81, 87-97

87. Francois, P.E., Goldberg, I.J.L., Lawton, K., Al-Aui, D.T. & Redding, J.H. (1978) Ann. Clin. Biochem. 15, 324-325

88. Van de Stadt, R.J. & Abbo-Tilstra, B. (1980) Ann. Rheum. Dis. 39, 31-36

89. Pederson, S.M. (1981) Biochem. Pharmacol. 30, 3249-3252

90. Lorber, A., Vibert, G.J., Harralson, A.F. & Simon, T.M. (1983) J. Rheumatol. 10, 4, 563-567

91. Smith, P.M., Smith, E.M. & Gottlieb, N.L. (1973) J. Lab. & Clin. Med. 82, 930-937

92. Mascarenhas, B.R., Granda, J.L. & Freyberg, R.H. (1972) Arth. & Rheum. 15, 391-402

93. Pederson, S.M. (1986) Ann. Rheum. Dis. 45, 712-717

94. Gerber, R.C., Paulus, H.E. & Bluestone, R. (1972) Arth. & Rheum. 15, 625-629

95. Gottlieb, N.L., Smith, P.M. & Smith, E.M. (1972) Arth. & Rheum. 15, 582-592

96. Gottlieb, N.L. & Bjelle, A. (1977) Scand. J. Rheumatol. 6, 225-230

97. Lorber, A., Bovy, R.A. & Chang, C.C. (1972) Nature New Biol. 236, 250-252

98. Schattenkirchner, M. & Grosbenski, Z. (1977) Atomic Abs. Newsletter 16, 84-88

99. Lawrence, J.S. (1961) Ann. Rheum. Dis. 20, 341-351

100. Hinderling, P.H. & Ganet, E.R. (1976) Pharma cokinet Biopharm. 4, 199-230

101. Weser, J.K. & Sellers, E.M. (1976) N. Eng. J. Med. 294, 311-316

102. Bett, R.J., Himmelstein, K.J. & Patton T.F. (1979) Cancer Treat. Rep. 63, 1515-1521

103. Whitlam, J.B. & Brown, K.F. (1981) J. Pharm. Sci. 70,2,146-150

104. Wijkstrom, A. & Westerland, D. (1983) J. Pharm. & Biomed. Analysis 1,3, 293-299

105. Wittfoht, W., Duwe, K., Kuhnz, W. & Nau, H. (1984) Clin. Chem. 30,6,878-879

106. Knott, P.J. & Curzon, G. (1972) Nature 239, 452-453

107. Lee, I.R., Greed, L.C. & Hahnel, R. (1984) Clin. Chim. Acta. 137, 131-139

108. Sophianopoulos, J.A., Durham, S.J., Sophianopoulos, A.J., Ragsdale, H.L. & Cropper, W.P. Arch. Biochem. Biophys. 187, 1, 132-137

109. Smith, R.T., Pea, W.P., Kron, K.M., Del Toro, R.A. & Goldman, M. (1958) J. Amer. Med. Ass. 167, 1197-1204

110. Krusius, F.E., Markkanen, A. & Peltola, P. (1970) Ann. Rheum. Dis. 29, 232-235

111. Billings, R., Grahame, R., Marks, V., Wood, P.J. & Taylor, A. (1975) Rheum. & Rehab. 14, 13-18

112. Arden-Jones, R., Ahmed, M.U. & Chan, B.W.B. (1971) Brit. Med. J. 1, 610 (letter)

113. Roberts, W. (1860) Edinb. Med. J. 5, 817-825

114. Conroy, R.T.W.L. & Mills, J.N. (1970) In 'Human Circadian Rhythms' Churchill, London Chap. 4, 47-67

115. Sirota, J.H., Baldwin, D.S. & Villereal, H. (1950) J. Clin. Invest. 29, 187-192

116. Halberg, F. (1959) Z. Vitam-Horm.-u Fermentforsch 10, 225-296

117. Heath, M.J. (1981) M.Phil. Thesis, Univ. of Nottingham

118. Dossetor, J.B., Gorman, H. & Beck, J.C. (1964) Metabolism 13, 1439-1455

119. Molyneux, M.K.B. (1966) Ann. Occup. Hyg. 9, 95-102

120. Molyneux, M.K.B. (1964) Brit. J. Indust. Med. 21, 203-209

121. Scudder, P.R., Al-Timini, D., McMurray, W., White, A.G., Zoob, B.C. & Dormandy, T.L. (1978) Ann. Rheum. Dis. 37, 67-70

122. Brown, D.H., Buchanan, W.W., El-Ghobarey, A., Smith, W.E. & Teape, J. (1979) Ann. Rheum. Dis. 38, 174-176

123. Scudder, P.R., McMurray, W., White, A.G. & Dormandy, T.L. (1978) Ann. Rheum. Dis. 37, 71-72

124. Banford, J.C., Brown, D.M., Hazelton, R.A., McNeil, C.J., Sturrock, R.D. & Smith, W.E. (1982) Ann. Rheum. Dis. 41, 458-462

125. Bird, H.A. (1983) Ann. Rheum. Dis. 42, 474-475

126. Nielsen, A.L. (1944) Acta. Med. Scandinav. 118, 87-91

127. Much-Petersen, S. (1950) Scand. J. Clin. Lab. Invest. 2, 48-52

128. Hoyer, K. (1946) Arhus. Diss.

129. Harth, M. (1979) J. Rheumatol. (suppl.5) 7-11

130. Lorber, A., Simon, T.M., Leeb, J. & Carroll, P.E. (1975) J. Rheumatol. 2, 401-410

131. Gottlieb, N.L., Kiem, I.M., Penneys, N.S. & Schultz, D.R. (1975) J. Lab. Clin. Med. 86, 962-972

132. Mouridsen, H.T., Baerentsen, O., Rossing, N. & Jensen, K.B. (1974) Arth. Rheum. 17, 391-396

133. Leibfarth, J.H. & Persellin, R.H. (1981) Agents & Actions 11,5,458-472

134. Cricchio, I., Arcara, G., Abbate, G., Ferrar, T., Tarantino, R. & Romano, S. (1980) In 'Recent advances in chronobiology of allergy and immunology' Eds. Smolenski, M.H., Rheinberg, A. & McGovern, J.P. Series: Advances in the Bio-sciences Vol. 28, Pergamon Press, Oxford

135. Halberg, F., Duffert, D. & Mayersbach, H. von. (1977) Chronobiologia 4, 114-115

136. Price, D.A., Close, G.C. & Fielding, B.A. (1983) Arch. Dis. Child. 58(6), 454-456

137. Cambell, I.T., Walker, R.F., Riad-Fahmy, D. & Wilson, D.W. (1983) Chronobiologia 9(4), 389-396

138. Bauer, F., Balant, L., Zender, R. & Humair, L. (1983) Eur. Heart J. 4(1), 64-70

139. Kolomazuik, M., Hronek, J., Janonsek, I., Musil, F., Suva, J., Svejnohova, D. & Vyletal, O. (1983) Cesk. Psychiatr. 79, 111-117 (Eng. Abstr.)

140. Tokueva, L.I. (1983) Stomatologiia (Mosk) 62(1), 62-64 (Eng. Abstr.)

141. Nilner, K. & Glantz, P.O. (1982) Swed. Dent. J. 6(2), 71-77

142. Jaffe, I.A. (1980) Clinics in Rheum. Dis. 6, 633-645

143. Andrews, F.M., Camp, A.V., Day, A.T., Freeman, A.M., Golding, D.N., Golding, J.R., Hill, A.G.S., Lewis-Faning, E. & Lyle, W.H. (1973) Lancet 1, 275-280

144. Huskisson, E.C., Dieppe, P.A., Scott, J., Trapwell, G., Balme, H.W. & Willoughby, D.A. (1976) Lancet 1, 393-395

145. Camus, J.P., Crouzet, J., Prier, A. & Bergevin, H. (1978) Revue de Rheumatisme 45, 95-100

146. Huskisson, E.C., Jaffe, I.A., Scott, J. & Dieppe, P.A. (1980) Arth. & Rheum. 23, 106-110

147. Fort, A. & Mills, J.N. (1970) Nature 226, 657-658

148. Mallya, R.K. & Mace, B.E. (1981) Rheum. & Rehab. 20, 14-17

149. Ritchie, D.M., Boyle, J.A. & McInnes, J.M. (1968) Am. J. Med. 147, 393-406

150. The I.L. Atomic Absorption Application Laboratory (1972) Clinical applications of atomic absorption/emission spectroscopy. 51-52

151. Varley, H., Gowenlock, A.H. & Bell, M. (1980) In 'Practical Clinical Biochemistry' William Heinemann Med. Books Ltd. London & Interscience Books Inc. New York, Vol. 1, 559-561

152. Instrumentation Laboratory Inc. Analytical Instrument Division (1975) Atomic absorption methods manual. Vol. 1

153. Centrifugal Analysers in Clinical Chemistry (1980) Ed. Price, C.P. & Spencer, K. Eastbourne, East Sussex : New York, N.Y. : Praeger

154. Brandslund, I., Siersted, H.C., Svehag, F-E & Teisner, B. (1981) J. Immunol. Methods 44, 63-71

155. Powell, R.J., Leyland, A.M., Pound, J.D. & Bossingham, D.H. (1985) J. Rheumatol. 12,3, 427-430

156. Rudge, S.R., Perrett, D., Drury. P.L. & Swannell, A.J. (1983) J. Pharm. & Biomed. Analysis 1,2, 205-210

157. Nie, N.H., Hull, C.H., Jenkins, J.G., Steinbrenner, K. & Bent, D.H. (1975) In 'Statistical package for the social sciences' 2nd Ed. McGraw-Hill Book Co. Eds. Bowman, K.J. & Cahill, M. Chapter 18, 266-281

158. Halberg, F. (1970) Circulation 42, 786-798

159. Campion, D.A., Olsen, R., Bohan, A. & Bluestone, R. (1974) J. Rheum. 1(suppl.), 1-112

160. Herrlinger, J.D. & Weikert, W. (1982) Z. Rheumatol. 41, 230-234

161. Goldstein, A. (1949) Pharmacol. Rev. 1, 102-165

162. Jessop, J.D. & Johns, R.G.S. (1973) Ann. Rheum. Dis. 32, 228-232

163. Lorber, A., Atkins, C.J., Chang, C.C., Lee, V.B., Starrs, J. & Bovy, R.A. (1973) Ann. Rheum. Dis. 32, 133-139

164. Dahl, S.L., Coleman, M.L., Williams, H.J., Altz-Smith, M., Kay, D.R., Paulus, H.E., Weinstein, A. & Kaplan, S. (1985) Arth. & Rheum. 28,11, 1211-1218

165. Danpure, C.J. (1976) Biochem. Soc. Trans. 4, 161-163

166. Rodgers, A.I.A., Brown, D.H., Smith, W.E., Lewis, D. & Capell, H.A. (1982) Anal. Proc. (London) 19, Part 2, 87-88

167. Danpure, C.J. (1974) Biochem. Soc. Trans. 2, 899-901

168. McQueen, E.G. & Dykes, D.W. (1969) Ann. Rheum. Dis. 28, 437-442

169. Lawson, K.J., Danpure, C.J. & Fyfe, D.A. (1977) Biochem. Pharmacol. 26, 2417-2426

170. Mills, J.H., Minors, D.S. & Waterhouse, J.M. (1981) In 'Principles and practices of Human Physiology' Ed. Edholm, D.G. & Weiner, J.S. Academic Press, London. Chapter 9, 451-501

171. Simpson, H.W. (1976) Essays Med. Biochem. 2, 115-187

172. Rosenwasser, A.M. & Adler, N.T. (1986) Neuroscience & Biobehavioural Reviews 10, 431-448

173. Ehret, C.F. (1974) Adv. Biol. Med. Phys. 15, 47-77

174. Ashkenazi, I.E. & Hortman, H.H. (1975) Chronobiologia Suppl. 1, Abstr. Int. Conf. Int. Soc. Chronobiol. 12th., Abstr. No. 6

175. Axelrod, J., Fraschini, F. & Velo, G.P. Eds. (1982) Proc. NATO Adv. Study, Erice, Italy, Plenum Press, New York, N.Y.

176. Reiter, R.J. Ed. (1984) In 'The Pineal Gland' Raven Press, New York, N.Y.

177. Birau, N. & Schloot, W. Eds. (1981) In 'Melatonin : Current Status and Perspective' Pergamon Press, Oxford

178. Maestroni, G.J.M., Conti, A. & Pierpaoli, W. (1987) Ann. NY, AcAD. Sci. 496, 67-77

179. Bell, G.H., Davidson, J.N. & Emslie-Smith, D. (1972) In 'Textbook of Physiology and Biochemistry'. 8th Ed. Churchill Livingstone - Edinburgh & London.

180. Minors, D.S., Mills, J.H. & Waterhouse, J.M. (1976) Int. J. Chronobiol. 4, 1-28

181. Koopman, M.G., Krediet, R.T. & Arisz, L. (1985) Neth. J. Med. 28, 416-423

182. Weinberger, M.H., Luft, F.C. & Henry, D.P. (1982) Clin. Exp. Hypertens. 174, 719-735

183. Lee, M.R. (1982) Clin. Sci. 62, 439-448

184. DiBona, G.F. & Sawin, L.L. (1982) Amer. J. Physiol. 243, 576-580

185. Bultasova, H., Veselkova, A., Brodan, V. & Pinsker, P. (1986) Endocrin. Exp. 20, 359-369

186. Veglio, F., Dietrandrea, R., Ossola, M., Vignani, A. & Angeli, A. (1987) Chronobiologia 14, 21-25

187. Brandenberger, G., Simon, C. & Follenius, M. (1987) Life Sciences 40, 2325-2330

188. Stern, H., Sowers, J.R., McGiuty, D., Beahm, E., Littner, M., Catania, R. & Eggena, P. (1986) J. Hypertension 4, 543-550

189. Koene, R., Van Liebergen, F. & Wijdeveld, P. (1973) Clin. Neph. 1(4), 226-227

190. Giroux, E.L. & Henkin, R.I. (1972) Biochim. Biophys. Acta. 273, 64-72

191. Parisi, A.F. & Vallee, B.L. (1970) Biochemistry 9, 2421-2426

192. Foote, J.W. & Delves, H.T. (1984) Analyst 109, 709-711

193. Bhattacharya, R.D. (1986) Arch. Env. Health 41,5, 302-305

194. Gubler, C.J., Lahey, M.E., Cartwright, G.E. & Wintrobe, M.M. (1952) Am. J. Physiol. 171, 652-658

195. Osterberg, R. (1974) Nature 249, 382-383

196. Hinks, L.K., Colmsee, M. & Delves, H.T. (1982) Analyst 107, 815-823

197. Koskelo, P., Kekki, M. & Virkkunen, M. (1966) Acta. Rheum. Scand. 12, 261-266

198. Abdulla, M. (1979) Lancet 1, 616 (letter)

199. Pekarek, R.S., Wannemacher, R. & Beisel , W.R. (1972) Proc. Soc. Exp. Biol. & Med. 140, 685-688

200. Kampschmidt, R.F., Upchurch, H.F., Eddington, C.L. & Pulliam, L.A. (1973) Amer. J. Physiol. 224, 530-533

201. Wannemacher, R.W., Dupont, H.L., Pekarek, M.C., Schwartz, A., Hornick, R.B. & Beisel, W.R. (1972) J. Infect. Dis. 126, 77-86

202. Wannemacher, R.W., Pekarek, R.S., Klainer, A.S., Bartelloni, P.J., Dupont, H.L.,Hornick, R.B. & Beisel, W.R. (1975) Infect. & Immun. 11, 873-875

203. Snowden, J. & Freeland, J.H. (1978) Fed. Proc. 37, 890 (Abstract)

204. Rafter, G.W. (1987) Med. Hypothesis 22, 245-249

205. Cashin, C.H., Lewis, E.J. & Burden, T. (1985) Brit. J. Rheum. 24, 137-145

206. Hattingh, J. (1979) S. African J. Sci. 75, 184-186

207. Hammond, G.L., Langley, M.S. & Robinson, P.A. (1985) J. Steroid Biochem. 23, 451-460

208. Hammond, G.L. & Langley, M.S. (1986) Acta. Endocrinologica 112, 603-608

209. Cove-Smith, J.R., Kabler, P., Pownall, R. & Knapp, M.S. (1978) Brit. Med. J. 7, 253-254

210. Littman, B.H. & Schwartz, P. (1982) Arth. & Rheum. 25,3, 288-296

211. Stuckey, B.G.A., Hanrahan, P.S., Zilko, P.J. & Owen, E.T. (1986) J. Rheum. 13,2, 468-469 (letter)

212. Wildhagen, K., Seidel, J. & Deicher, H. (1987) Scand. J. Rheum. 16, 217-220

213. Lorber, A., Simon, T. & Leeb, J. (1978) Arth. & Rheum. 21, 785-791

214. Strong, J.S., Bartholomew, B.A. & Smyth, C.J. (1973) Ann. Rheum. Dis. 32, 233-237

215. Blocka, K.L.N., Paulus, H.E. & Furot, D.E. (1986) Clin. Pharm. 11, 133-143

216. Rudge, S.R., Perrett, D. & Swannell, A.J. (1984) Ann. Rheum. Dis. 43, 698-702

217. Rudge, S.R., Perrett, D., Swannell, A.J. & Drury, P.L. (1984) J. Rheum. 11,2, 150-152

218. Rudge, S.R., Perrett, D. & Swannell, A.J. (1984) Ann. Rheum. Dis. 43, 66-69

CHAPTER 7

APPENDIX

7.1. Table 1. Study Group 1 Patients and Results

Name	Age	Sex	Total Serum Gold µmol/l	Free Gold µmol/l	Protein Bound Gold µmol/l
B.W.	52	F	2.5	0.2	2.3
T.B.	48	M	2.3	0.2	2.1
B.F.	36	M	2.6	0.2	2.4
H.E.	54	M	4.1	0.3	3.8
P.B.	50	F	2.9	0.3	2.6
H.W.	42	F	2.2	0.3	1.9
J.P.	54	M	6.9	0.3	6.6
F.D.N.	46	F	3.6	0.3	3.3
E.S.	43	F	8.2	0.4	7.8
J.S.	39	F	6.4	0.4	6.0
M.W.	46	F	5.2	0.4	4.8
J.W.	51	F	1.6	0.4	1.2
E.E.	54	F	4.2	0.4	3.8
M.P.	38	F	4.1	0.4	3.7
J.H.	44	F	3.0	0.5	2.5
D.S.	50	F	4.8	0.5	4.3
S.A.	55	F	5.0	0.5	4.5
D.A.	44	F	5.8	0.5	5.3
E.C.	51	M	5.7	0.5	5.2
D.B.	56	M	5.6	0.5	5.1
D.A.B.	41	F	6.7	0.6	6.1
N.E.	50	F	5.7	0.6	5.1
H.A.E.	56	M	7.5	0.7	6.8
D.G.	60	F	7.1	0.7	6.4
H.O.	49	F	13.7	1.5	12.2
J.C.	44	F	7.4	0.7	6.7
T.D.	60	M	10.0	0.8	9.2
H.O.D.	60	F	8.0	0.8	7.2
M.G.	59	F	6.5	0.8	5.7
E.W.	35	F	10.0	0.9	9.1
F.A.W.	48	M	8.8	0.9	7.9
E.F.	37	F	10.0	0.9	9.1
P.A.	52	F	6.0	0.9	5.1
M.J.T.	50	F	6.0	0.9	5.1
E.P.S.	37	F	7.4	0.9	6.5
B.P.	58	M	8.8	0.9	7.9
C.S.	42	M	8.0	1.0	7.0
M.A.J.	46	F	9.7	1.0	8.7
M.P.	59	F	12.5	1.0	11.5
C.L.P.	61	F	13.0	1.1	11.9
J.H.L.	52	F	12.6	1.1	11.5
S.S.	48	F	12.4	1.1	11.3

7.1. Table 1. continued

Name	Age	Sex	Total Serum Gold	Free Gold	Protein Bound Gold
L.E.	45	M	9.8	1.1	8.7
P.J.S.	57	F	8.5	1.1	7.4
P.T.S.	52	M	11.6	1.2	10.4
M.H.	62	M	10.8	1.2	9.6
E.R.	42	M	11.2	1.2	10.0
R.A.W.	48	F	11.5	1.3	10.2
P.A.B.	60	M	9.0	1.3	7.7
M.S.	51	F	11.9	1.3	10.6
M.C.L.	55	F	10.6	1.3	9.3
E.J.B.	47	F	13.2	1.4	11.8
I.C.H.	50	F	14.8	1.5	13.3
J.S.	49	F	17.4	1.8	15.6

7.1. Table 1A. Information Available for the NSAID Taken Concurrently with Gold Treatment by Study Group 1 Patients

Name	Drug	Dose
L.S.	Lederfen	600mg (Daily)
I.G.	Indocid	25mg (x2 Daily)
J.C.	Indocid (Suppository)	50mg (x2 Daily)
M.D.	Indocid	25mg (as required)
F.R.	Alrheumat	2 tabs (x4 Daily)
	Indocid R	75mg (at Night)
J.D.	Indocid R	75mg (Daily)
N.E.	Indocid (Suppository)	100mg (at Night)
P.A.	Sulindac	200mg (x3 Daily)
E.W.	Brufen	400mg (x3 Daily)
B.W.	Naprosyn	500mg (x2 Daily)
	Soluble Aspirin	2 tabs (x3 Daily)
T.B.	Indocid	25mg (x2 Daily)
B.F.	Benoral	10ml (x2 Daily)
	Naprosyn (Suppository)	500mg (at Night)
H.E.	Indocid (Suppository)	100mg (at Night)
P.B.	Benoral	10ml (x2 Daily)
H.W.	Indocid R	75mg (at Night)
J.P.	Diclofenac	50mg (x3 Daily)
F.D.N.	Brufen	400mg (x3 Daily)
E.S.	Sulindac	200mg (x3 Daily)
J.S.	Indocid R	75mg (at Night)
M.W.	Benoral	10ml (x2 Daily)
J.W.	Indocid	25mg (x2 Daily)
E.E.	Indocid (Suppository)	100mg (at Night)
M.P.	Brufen	400mg (x3 Daily)
J.H.	Diclofenac	50mg (x3 Daily)
D.S.	Lederfen	600mg (Daily)
S.A.	Indocid	25mg (x2 Daily)
D.A.	Indocid R	75mg (at Night)
E.C.	Benoral	10ml (x2 Daily)
D.B.	Indocid R	75mg (at Night)
D.A.B.	Indocid (Suppository	100mg (at Night)
N.E.	Indocid	75mg (at Night)
H.O.D.	Indocid	25mg (as required)

7.1. Table 2. Study Group 2 Patients and Results

Name	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	
A.W.	0.7	2.8	6.8	7.65	12.75	13.75	12.5	7.05	7.4	TG μmol/1
	0.6	2.65	6.5	6.9	11.45	12.25	11.15	6.5	6.6	PBG μmol/1
	0.1	0.15	0.3	0.75	1.3	1.5	1.35	0.55	0.8	FG μmol/1
E.S.	4.0	5.0	7.6	10.8	9.2	6.3	8.2	9.0	9.4	TG
	3.65	3.4	6.95	9.6	8.4	5.7	7.5	8.0	8.4	PBG
	0.35	1.6	0.75	1.2	0.8	0.6	0.7	1.0	1.0	FG
M.P.D.	6.4	10.3	11.0	12.4	13.0	13.5	12.75	11.5	11.5	TG
	5.7	9.2	9.8	11.1	11.4	12.1	11.4	10.3	10.3	PBG
	0.7	1.1	1.2	1.3	1.6	1.4	1.35	1.2	1.2	FG
Z.F.	3.0	4.5	6.9	8.1	8.5	10.0	9.5	9.2	8.0	TG
	2.8	4.15	6.2	7.3	7.65	8.9	8.3	8.2	7.18	PBG
	0.2	0.35	0.7	0.8	0.85	1.1	1.2	1.0	0.82	FG
H.W.	0.8	2.55	4.55	6.2	8.75	13.5	8.55	7.8	7.4	TG
	0.6	1.15	4.25	5.55	7.8	11.9	7.55	7.0	6.7	PBG
	0.2	1.4	0.3	0.65	0.95	1.6	1.0	0.8	0.7	FG
J.P.	0.7	2.8	3.9	7.7	12.5	13.75	12.6	7.0	7.5	TG
	0.6	2.65	2.65	6.95	11.2	12.25	10.25	6.45	6.7	PBG
	0.1	0.15	1.25	0.75	1.3	1.5	1.35	0.55	0.8	FG
M.G.	4.9	5.3	6.2	6.7	6.2	8.4	7.4	6.4	6.0	TG
	4.45	4.8	5.6	6.05	5.6	5.3	6.6	5.8	5.45	PBG
	0.45	0.5	0.6	0.65	0.6	2.1	0.8	0.6	0.55	FG

7.1. Table 2. continued

Name	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	
E.H.	6.2	10.0	10.5	11.8	12.4	11.7	13.5	13.8	13.5	TG
	5.6	8.9	9.3	10.4	10.9	10.35	11.9	12.2	11.9	PBG
	0.6	1.1	1.2	1.4	1.5	1.35	1.6	1.6	1.6	FG
A.M.	5.85	7.2	8.2	10.5	12.6	12.7	17.0	17.5		TG
	5.25	6.5	6.0	9.3	11.1	11.2	15.0	15.3		PBG
	0.6	0.7	2.2	1.2	1.5	1.5	2.0	2.2		FG
N.D.	6.0	9.0	9.2	9.5	12.1	12.4	12.6	12.0	12.8	TG
	5.45	8.05	8.2	8.45	10.7	10.9	11.1	9.45	10.75	PBG
	0.55	0.95	1.0	1.05	1.4	1.5	1.5	2.55	2.05	FG
S.H.B.	5.5	7.0	7.2	9.8	9.8	12.2	12.3	17.2	17.5	TG
	5.0	6.35	6.5	8.7	8.7	10.8	10.8	15.7	15.5	PBG
	0.5	0.65	0.7	1.1	1.1	1.4	1.5	2.0	2.0	FG

TG = Total Gold

PBG = Protein Bound Gold

FG = Free Gold

7.1. Table 3. Differential Protein Bound Gold Fractions (μmol/1)

Name	Total Gold	Free Gold	PBG	Albumin Gold	αGlobulin Gold	βGlobulin Gold	γGlobulin Gold
B.W.	2.5	0.2	2.3	1.8	0.2	0	0.3
F.O.N.	3.6	0.3	3.3	1.6	0.4	0.4	0.9
D.A.B.	6.7	0.6	6.1	4.0	0.2	0.9	1.0
D.G.	7.1	0.7	6.4	3.7	1.3	1.4	0
J.P.	7.0	0.35	6.65	4.05	0.6	0.6	1.45
J.H.	3.0	0.5	2.5	1.4	0	0.6	0.5
J.S.	17.4	1.8	15.6	13.2	1.1	0.9	0.4
L.E.	9.8	1.1	8.7	5.2	0	0.5	3.0
J.H.L.	12.6	1.1	11.5	6.3	4.2	0	1.0
M.A.J.	9.7	1.0	8.7	4.9	0.4	1.3	2.1
C.S.	8.0	1.0	7.0	6.0	0.3	0.3	0.4
E.J.B.	13.2	1.4	11.8	5.8	1.2	2.8	2.0
T.B.	2.3	0.2	2.1	0.9	1.2	0	0
B.F.	2.7	0.25	2.45	1.0	1.2	0.25	0
M.W.	5.2	0.4	4.8	3.4	0.5	0.2	0.7
H.A.E.	7.5	0.7	6.8	4.9	0	1.5	0.5
J.C.	7.4	0.7	6.7	5.2	0.7	0.5	0.3
T.D.	10.0	0.8	9.2	7.0	0	1.0	1.2
M.O.D.	8.0	0.8	7.2	6.3	2.3	2.0	2.0
S.S.	12.4	1.1	11.3	8.0	0	2.0	1.3
P.J.S.	8.6	1.15	7.45	5.05	2.0	0	0.4
J.W.	1.6	0.4	1.2	0.8	0	0	0.4
M.P.	4.2	0.45	3.75	1.0	2.1	0.4	0.25
D.S.	4.8	0.5	4.3	2.2	0.8	0.2	1.1
D.A.	5.8	0.5	5.3	4.6	0.4	0.3	0
E.C.	5.8	0.55	5.25	5.0	0	0	0.25
D.B.	5.7	0.55	5.15	2.0	2.6	0.25	0.3

7.1. Table 3. continued

Name	Total Gold	Free Gold	PBG	Albumin Gold	αGlobulin Gold	βGlobulin Gold	γGlobulin Gold
J.S.	6.4	0.4	6.0	3.6	2.0	0.4	0
I.C.H.	14.8	1.5	13.3	10.1	0.2	0.4	2.65
E.J.B.	13.2	1.4	11.8	6.2	3.0	0.6	2.0
M.C.L.	10.6	1.3	9.3	5.3	0.5	3.0	0.5
M.S.	11.9	1.3	10.6	8.0	0.7	0.3	1.6
T.D.	10.0	0.8	9.2	7.4	0	0.5	1.2
M.G.	6.5	0.8	5.7	3.6	1.2	0.2	0.8
E.W.	10.0	0.9	9.1	6.2	1.0	1.0	0.9
F.A.W.	8.8	0.9	7.9	5.5	0.3	2.1	0
P.A.B.	9.0	1.3	7.7	4.1	0.6	2.0	1.0
R.A.W.	11.5	1.3	10.2	6.4	0	3.0	0.8
E.R.	12.3	1.25	10.05	5.05	2.1	2.5	0.4
M.H.	10.8	1.2	9.6	7.6	0.2	0.3	1.5
P.T.S.	11.6	1.2	10.4	7.3	0	0.3	2.0
E.F.	3.6	0.4	3.2	1.8	0	0	1.4
P.A.	6.0	0.9	5.1	4.0	0.2	0.6	0.3
M.J.T.	6.0	0.9	5.1	3.2	0.8	0.9	0.2
E.P.S.	7.4	0.9	6.5	3.0	0	1.5	2.0
B.P.	8.9	0.95	7.95	6.2	1.25	0.5	0
H.O.	13.75	1.5	12.25	8.25	1.5	2.5	0
D.G.	7.1	0.7	6.8	4.6	2.2	0	0
N.E.	5.7	0.6	5.1	2.1	0	1.8	1.3
R.P.	3.1	0.2	2.9	1.7	0.4	0.2	0.6

7.2. Table 1. Raw Data from 4 Patients showing Excretion Rates for Gold, Copper, Zinc, Water and Creatinine

Sample	Volume	Time	Gold	Copper	Zinc	Water	Creatinine
		Hours	$\mu mol/l\ 10^{-2}$	$\mu mol/l\ 10^{-2}$	$\mu mol/l\ 10^{-2}$	$ml/min\ 10^{-2}$	$mmol/min\ 10^{-2}$
M.J.T. Day 1							
1	270	07.30	2.40	0.30	1.00	75	1.4
2	270	09.25	3.05	0.32	1.54	235	1.4
3	160	10.30	3.45	0.35	1.81	246	1.3
4	160	11.50	2.60	0.24	1.14	200	1.2
5	90	17.15	0.55	0.05	0.34	27	1.3
6	220	20.45	1.89	0.19	1.01	104	1.6
7	320	23.35	1.13	0.10	0.61	188	1.5
Day 2							
1	520	07.00	2.00	0.31	0.85	116	1.0
2	200	09.35	1.80	0.28	0.80	129	1.0
3	140	12.25	1.20	0.20	0.60	82	1.6
4	190	17.15	1.50	0.25	0.82	65	1.3
5	170	23.15	1.60	0.25	0.84	47	1.2
Day 3							
1	470	07.50	1.83	0.54	0.84	91	2.0
2	140	09.55	2.02	0.56	0.87	112	1.6
3	150	13.50	0.83	0.32	0.34	63	2.3
4	120	15.45	1.36	0.42	0.72	104	2.0
5	100	20.25	1.21	0.39	0.70	35	1.4
6	80	22.20	1.32	0.41	0.76	69	1.2

7.2 Table 1. continued

Sample	Volume	Time	Gold	Copper	Zinc	Water	Creatinine
Day 4							
1	320	07.15	1.56	0.44	0.74	59.8	1.20
2	80	09.55	0.65	0.20	0.26	50.0	1.10
3	140	15.00	1.24	0.30	0.60	45.9	2.00
4	70	16.30	1.32	0.34	0.63	77.7	1.60
5	260	20.00	1.23	0.29	0.56	123.8	1.70
6	210	20.50	1.26	0.32	0.59	42.0	1.10
7	210	23.20	1.40	0.40	0.71	14.0	1.30
W.W.							
Day 1							
1	250	04.30	2.19	0.16	0.30	68.5	0.98
2	360	13.55	0.98	0.10	0.06	63.7	1.03
3	350	18.45	1.56	0.13	0.12	120.7	0.70
4	250	22.05	5.59	0.31	0.56	147.1	0.86
Day 2							
1	455	04.00	4.10	0.14	0.40	128.2	0.99
2	355	13.05	1.04	0.09	0.00	65.1	1.21
3	250	17.35	1.76	0.10	0.18	92.6	0.94
4	370	20.35	2.61	0.12	0.24	217.6	0.86
5	400	22.55	6.93	0.16	0.78	266.7	0.74
Day 3							
1	650	04.45	2.41	0.15	0.26	185.7	0.90
2	375	12.51	1.16	0.09	0.10	77.3	1.26
3	450	17.35	1.74	0.10	0.15	157.9	1.11
4	210	22.18	2.58	0.16	0.26	73.7	0.96

7.2. Table 1. continued

Sample	Volume	Time	Gold	Copper	Zinc	Water	Creatinine
Day 4							
1	550	06.27	1.57	0.12	0.142	112.5	0.84
2	260	11.55	0.77	0.04	0.064	76.9	1.19
3	465	20.45	1.25	0.08	0.124	89.4	1.00
4	180	22.32	2.21	0.16	0.243	147.1	0.87
E.F.							
Day 1							
1	50	11.00					
2	65	14.45	1.20	0.10	0.234	28.9	1.44
3	90	20.10	0.83	0.07	0.108	27.7	1.30
4	85	21.40	2.36	0.23	0.217	94.4	1.20
Day 2							
1	85	02.15	0.79	0.09	0.136	30.9	1.20
2	85	06.52	0.83	0.12	0.163	30.7	1.42
3	50	09.05	1.64	0.28	0.477	44.7	1.03
4	60	13.15	0.85	0.14	0.330	24.0	1.21
5	120	16.10	1.68	0.20	0.384	68.5	1.09
6	45	18.30	1.35	0.29	0.408	32.1	1.14
7	110	20.35	2.15	0.26	0.334	88.0	0.98
8	85	23.45	1.65	0.27	0.434	44.7	0.99
Day 3							
1	80	06.15	0.66	0.00	0.226	20.5	1.31
2	70	10.45	1.62	0.18	0.596	25.9	1.10
3	60	14.30	1.20	0.13	0.376	26.7	1.28
4	110	17.25	1.38	0.15	0.235	37.3	1.09
5	350	23.45	3.3	0.13	0.256	135.0	1.14

7.2. Table 2. continued

Sample	Volume	Time	Gold	Copper	Zinc	Water	Creatinine
Day 4							
1	260	07.15	1.85	0.12	0.237	57.8	1.29
2	60	10.15	1.38	0.20	0.217	33.3	1.16
3	40	13.15	1.35	0.08	0.400	22.2	1.31
4	170	18.50	1.48	0.05	0.304	50.7	1.09
5	230	23.45	2.41	0.07	0.179	77.9	1.02
Day 5							
1	175	07.05	1.53	0.07	0.159	39.8	1.25
2	80	10.10	1.51	0.26	0.035	18.2	1.10
3	150	16.30	1.92	0.07	0.118	39.5	1.20
4	130	20.45	1.85	0.15	0.343	51.2	1.09
5	260	23.45	4.69	0.14	0.804	144.0	1.11
F.A.W.							
Day 1							
1	160	18.45	0.51	0.11	0.300	38.1	1.30
2	100	20.00	0.42	0.40	1.160	133.3	1.30
Day 2							
1	140	07.15	0.153	0.04	0.023	20.7	1.40
2	100	13.05	0.286	0.11	0.143	28.6	1.20
3	110	16.50	0.547	0.20	0.227	66.7	1.30
4	110	20.25	0.429	0.17	0.248	56.4	1.50
5	120	23.20	0.473	0.19	0.363	64.9	1.30

7.2. Table 2. continued

Sample	Volume	Time	Gold	Copper	Zinc	Water	Creatinine
Day 3							
1	250	07.15	0.312	0.07	0.124	53.8	1.4
2	130	12.05	0.327	0.08	0.157	44.9	1.5
3	140	16.30	0.686	0.29	0.299	96.6	1.2
4	160	22.00	0.179	0.04	0.179	48.5	1.1
Day 4							
1	70	07.15	0.055	0.02	0.024	12.6	1.2
2	60	11.45	0.216	0.08	0.167	22.6	1.6
3	190	17.05	0.527	0.18	0.221	61.3	1.3
4	190	22.05	0.164	0.06	0.291	63.3	1.1
Day 5							
1	230	06.30	0.254	0.08	0.103	44.7	1.3
2	40	11.50	0.108	0.06	0.830	12.5	1.8
3	190	16.30	0.265	0.07	0.122	67.9	1.2
4	190	19.30	0.412	0.11	0.190	105.6	1.2

7.2. Table 2. Sinewave Analysis Results for Gold, Copper and Zinc Excretion

Name	Gold Excretion				Copper Excretion				Zinc Excretion			
	M	A	Acr	p	M	A	Acr	p	M	A	Acr	p
E.R.	1.12	0.52	16.14	0.02	0.12	.051	18.00	0.03	0.64	0.36	16.32	0.03
R.H.	1.23	0.64	17.24	0.04	0.16	.073	17.20	0.15	0.96	0.05	17.25	0.03
M.W.	1.84	0.90	19.18	0.04	IE	IE	IE	0.16	0.98	0.04	19.18	0.04
E.F.	1.55	0.71	23.40	0.03	0.15	.072	23.36	0.04	0.30	0.17	23.36	0.04
D.P.	0.92	0.44	16.28	0.03	0.1	.042	16.27	0.04	0.06	0.02	16.20	0.04
J.W.	0.85	0.42	21.09	0.04	IE	IE	IE	0.09	0.10	0.04	21.15	0.05
J.S.	0.94	0.44	04.26	0.15	IE	IE	IE	0.10	0.08	0.05	04.26	0.04
E.E	1.62	0.79	17.28	0.03	0.09	.063	17.13	0.02	0.07	0.03	17.29	0.04
F.O.N.	1.36	0.62	20.19	0.04	IE	IE	IE	0.10	0.14	0.06	20.30	0.02
I.G.	2.12	0.10	19.36	0.02	IE	IE	IE	0.15	0.94	0.43	19.28	0.04
S.A.	0.81	0.39	16.28	0.05	0.08	.042	16.24	0.03	0.08	0.04	16.15	0.04
D.B.	0.79	0.35	16.24	0.20	0.14	.067	18.30	0.03	0.09	0.06	16.09	0.03
M.Y.	0.99	0.44	17.19	0.04	0.21	.091	17.22	0.04	0.76	0.39	17.21	0.03
W.W.	2.37	1.12	22.55	0.04	0.13	.058	18.09	0.03	0.24	0.11	12.50	0.04
R.C.	1.12	0.62	21.06	0.03	0.16	.09	21.13	0.05	0.32	0.16	21.12	0.02
R.O.	1.36	0.61	20.50	0.05	IE	IE	IE	0.10	0.09	0.05	20.42	0.04
H.W.	1.42	0.73	09.14	0.02	0.09	.062	09.18	0.04	0.86	0.37	09.12	0.05
M.D.D.	1.29	0.61	07.16	0.04	0.1	.049	10.25	0.04	0.09	0.04	08.55	0.04
M.J.T.	1.62	0.85	08.50	0.02	0.31	.17	10.00	0.04	0.79	0.37	09.00	0.04
Z.F.	1.11	0.54	22.11	0.04	IE	IE	IE	0.14	0.21	0.10	22.14	0.04
F.A.W.	0.32	0.05	16.30	0.04	.124	.060	16.20	0.04	0.23	0.16	16.40	0.03

M = Mesor (μmol/min 10^{-2})
A = Amplitude (μmol/min 10^{-2})
Acr = Acrophase (hours)
IE = Irregular Excretion

7.3. Table 1. Comparison of patient results at different times of day and night for Total Serum, Free and Protein Bound Gold and Salivary Gold Levels together with the relative Urinary Gold Levels

Patient	Time Hours	Total Serum Gold	Free	PBG	Salivary Gold	Relative Urine Gold
1	10.00	1.8	0.1	1.7	0.1	Higher (H)
	16.00	2.0	0.4	1.6	0.2	Lower (L)
	22.00	1.9	0.2	1.7	0.1	H
2	10.00	3.7	0.3	3.4	0.1	H
	16.00	3.9	0.6	3.3	0.2	L
	22.00	3.6	0.5	3.1	0.1	H
3	10.00	4.5	0.7	3.8	0.2	H
	16.00	4.7	0.9	3.8	0.3	L
	22.00	4.5	0.7	3.8	0.2	H
4	10.00	9.4	1.0	8.4	0.5	H
	16.00	9.8	1.3	8.5	0.7	L
	22.00	9.6	1.0	8.6	0.5	H
5	10.00	7.8	0.6	7.2	0.3	L
	16.00	7.7	0.5	7.2	0.2	H
	22.00	7.8	0.8	7.0	0.45	L
6	10.00	11.5	1.2	10.3	0.6	H
	16.00	11.8	1.4	10.4	0.65	L
	22.0	11.6	1.3	10.3	0.6	H
7	10.00	8.0	0.7	7.3	0.25	H
	16.00	8.2	1.0	7.2	0.45	L
	22.00	8.0	0.9	7.1	0.3	H
8	10.00	13.0	1.3	11.7	0.5	L
	16.00	12.0	1.1	10.8	0.1	H
	22.00	13.0	1.4	11.6	0.6	L
9	10.00	13.0	1.9	11.1	0.6	H
	16.00	15.5	2.2	13.5	0.9	L
	22.00	14.0	2.0	11.8	0.6	H
10	10.00	13.0	2.2	10.8	0.5	H
	16.00	15.0	2.8	12.3	0.9	L
	22.00	14.5	2.4	12.2	0.6	H
11	10.00	14.5	2.4	12.1	0.6	H
	16.00	16.0	3.0	13.1	1.1	L
	22.00	13.0	2.7	10.3	0.7	H

7.3. Table 1. continued

Patient	Time	Total Serum Gold	Free	PBG	Salivary Gold	Relative Urine Gold
12	10.00	15.0	3.5	11.5	0.6	L
	16.00	13.0	3.1	9.9	0.4	H
	22.00	15.0	3.4	11.6	0.6	L
13	10.00	13.2	1.0	12.2	0.5	H
	16.00	13.6	1.6	12.0	0.6	L
	22.00	13.0	1.2	11.8	0.5	H
14	10.00	14.5	1.9	12.6	0.9	H
	16.00	15.0	2.1	12.9	0.9	L
	22.00	14.8	1.5	13.3	0.6	H
15	10.00	18.0	1.2	16.8	0.8	H
	16.00	18.2	1.9	16.3	0.9	L
	22.00	18.0	1.7	16.3	0.8	H
16	10.00	5.2	0.4	4.8	0.3	L
	16.00	5.0	0.3	4.7	0.1	H
	22.00	5.3	0.5	4.8	0.3	L
17	10.00	8.0	0.4	7.6	0.25	H
	16.00	8.2	0.8	7.4	0.45	L
	22.00	8.0	0.5	7.5	0.3	H
18	10.00	12.0	1.1	10.9	0.1	H
	16.00	12.8	1.6	11.2	0.7	L
	22.00	12.0	1.3	10.7	0.4	H
19	10.00	7.0	0.3	6.7	0.1	No noticable
	16.00	7.0	0.3	6.7	0.1	difference at
	22.00	7.0	0.4	6.6	0.2	these times
20	10.00	5.8	0.4	5.4	0.2	No noticable
	16.00	5.8	0.4	5.4	0.3	difference at
	22.00	5.8	0.5	5.3	0.3	these times

Units for Serum and Salivary Gold Levels µmol/l

7.3. Table 2. Relationships between Immunoproteins at different times of the day

Patient	Time hours	C3	C4	IgA	IgG	IgM	C3d	CRP	Relative Urine Gold
1	10.00	1.02	0.21	1.43	0.39	0.77	16	22	H
	16.00	1.41	0.36	1.48	10.42	0.97	25	23	L
	22.00	1.00	0.18	1.39	9.62	0.84	17	22	H
2	10.00	1.72	0.28	3.47	19.86	0.81	11	38	H
	16.00	1.94	0.34	4.00	20.14	0.98	14	36	L
	22.00	1.78	0.30	3.91	19.84	0.86	12	36	H
3	10.00	1.47	0.40	4.01	20.54	1.06	10	20	H
	16.00	1.54	0.34	4.62	21.04	1.24	14	<20	L
	22.00	1.46	0.30	4.32	19.87	1.09	12	20	H
4	10.00	1.09	0.22	3.53	10.32	0.70	16	20	H
	16.00	1.18	0.24	4.21	10.00	1.00	15	20	L
	22.00	1.04	0.20	3.41	10.00	0.82	15	21	H
5	10.00	1.31	0.24	2.06	11.61	1.29	20	<20	L
	16.00	1.28	0.21	1.96	10.95	1.10	14	22	H
	22.00	1.29	0.23	2.00	11.40	1.31	19	21	L
6	10.00	1.10	0.26	1.99	10.76	1.23	11	<20	H
	16.00	1.12	0.28	2.50	10.98	1.41	13	<20	L
	22.00	1.10	0.24	2.12	10.49	1.30	10	<20	H
7	10.00	1.06	0.22	3.21	9.48	0.65	12	19	H
	16.00	1.12	0.26	3.34	9.04	0.71	10	20	L
	22.00	1.09	0.21	3.24	9.01	0.69	9	20	H
8	10.00	1.62	0.36	5.00	22.46	1.43	14	23	L
	16.00	1.53	0.30	4.64	20.21	1.04	10	22	H
	22.00	1.63	0.35	4.98	23.58	1.41	13	22	L
9	10.00	1.10	0.31	1.94	10.27	0.33	11	20	H
	16.00	1.32	0.36	2.58	10.48	0.36	11	21	L
	22.00	1.20	0.32	2.12	10.24	0.31	11	21	H
10	10.00	1.02	0.21	1.43	9.36	0.77	16	37	H
	16.00	1.32	0.32	1.52	10.31	0.97	25	36	L
	22.00	1.00	0.20	1.40	8.96	0.75	16	36	H
11	10.00	1.12	0.24	1.75	9.44	0.26	11	<20	H
	16.00	1.30	0.28	1.78	9.68	0.37	16	<20	L
	22.00	1.14	0.25	1.72	9.46	0.25	13	20	H

7.3. Table 2. continued

Patient	Time	C3	C4	IgA	IgG	IgM	C3d	CRP	Relative Urine Gold
12	10.00	1.34	0.28	5.32	18.94	0.30	17	<20	L
	16.00	1.16	0.21	5.16	18.45	0.24	14	<20	H
	22.00	1.29	0.27	5.40	18.84	0.31	16	<19	L
13	10.00	0.95	0.23	2.27	11.66	0.30	13	20	H
	16.00	0.97	0.26	2.48	12.00	0.36	16	20	L
	22.00	0.88	0.20	2.27	11.65	0.28	10	20	H
14	10.00	1.32	0.29	4.72	19.40	0.32	11	21	H
	16.00	1.46	0.36	5.14	18.39	0.38	10	23	L
	22.00	1.36	0.31	4.91	19.32	0.30	11	23	H
15	10.00	0.88	0.20	1.96	9.69	0.28	12	22	H
	16.00	0.94	0.26	2.04	10.00	0.32	14	<20	L
	22.00	0.90	0.21	1.91	9.64	0.27	10	9	H
16	10.00	1.88	0.34	3.84	17.68	0.64	12	23	L
	16.00	1.80	0.31	3.08	17.01	0.52	10	22	H
	22.00	1.89	0.38	3.74	17.66	0.66	13	23	L
17	10.00	1.00	0.22	2.29	12.02	0.34	15	21	H
	16.00	1.19	0.28	2.47	13.21	0.42	17	24	L
	22.00	0.98	0.24	2.25	12.46	0.38	16	22	H
18	10.00	1.10	0.22	2.13	12.25		17	<20	H
	16.00	1.32	0.26	2.36	11.14		17	<20	L
	22.00	1.20	0.23	2.20	11.62		16	21	H
19	10.00		No appreciable difference						
	16.00		No appreciable difference						
	22.00		No appreciable difference						

7.4. Table 1. Free Thiomalate and Gold in Plasma, Serum and Urine

Name	Time after injection of Aurothiomalate (mins)	Plasma Thiomalate (nmol/l)	Total Serum Gold (µmol/l)	Free Serum Gold (µmol/l)	PBG (µmol/l)	Urinary Thiomalate (µmol/l)	Urinary Gold (µmol/l)
B.L.	0	-	-	-	-	-	-
	15	0	6.7	0.65	6.05	-	-
	30	250	8.6	0.85	7.75	-	-
	45	395	9.1	1.00	8.10	-	-
	60	350	9.6	1.10	8.50	0.88	0.40
	90	200	9.5	1.00	8.50	-	-
	120	56	9.4	0.90	8.50	0.08	0.30
	180	UD	8.6	0.80	7.80	0.07	0.35
N.H.	0	-	-	-	-	-	1.00
	30	300	12.1	1.30	10.80	-	-
	60	610	15.6	1.80	13.80	1.32	1.40
	90	500	14.0	1.60	12.40	-	-
	180	350	11.9	1.20	10.70	0.90	0.60
	300	280	-	-	-	0.40	0.55
	420	UD	10.6	1.10	9.50	0.10	0.45
A.H.	0	-	-	-	0.25	0	1.25
	15	UD	2.8	0.30	2.50	-	-
	30	360	7.9	0.80	7.15	-	-
	60	350	5.4	0.60	4.80	0.90	2.00
	90	320	10.0	1.30	8.50	0.80	2.65
	180	300	10.4	1.10	9.30	-	-
	300	UD	8.0	0.80	7.20	0.09	2.50
	420	UD	7.7	0.70	7.00	-	-

7.4. Table 1. continued

Name	Time after injection of Aurothiomalate	Plasma Thiomalate	Total Serum Gold	Free Serum Gold	PBG	Urinary Thiomalate	Urinary Gold
J.B.	0	0	8.55	0.9	7.65	3.2	0.65
	15	375	10.6	1.2	9.4	-	-
	30	340	13.1	1.5	11.6	-	-
	45	180	16.45	1.9	14.55	-	-
	60	140	16.6	2.1	14.5	0.9	1.35
	90	50	16.1	1.7	14.4	-	-
	120	-	-	-	-	UD	0.85
	140	UD	15.8	1.6	14.2	-	-
	240	-	-	-	-	UD	0.75
B.C.	0	0	1.7	0.1	1.6	-	-
	15	250	11.1	1.3	9.8	-	-
	30	360	12.6	1.5	11.1	-	-
	45	200	13.4	1.6	11.8	-	-
	60	75	13.6	1.65	11.95	3.8	0.85
	90	UD	11.7	1.2	10.5	1.2	0.45
	120	-	-	-	-	0.2	0.35
	240	UD	11.0	1.2	9.8	-	-
B.T.	0	0	10.1	1.1	9.0	0.99	1.05
	15	280	23.3	2.4	20.9	-	-
	30	345	25.3	2.6	22.7	-	-
	45	482	26.4	2.7	23.7	-	-
	60	300	28.3	3.1	25.2	0.42	2.05
	90	209	26.1	2.7	23.4	-	-
	120	52	25.8	2.5	23.3	-	-
	240	UD	25.3	2.5	22.8	0.06	2.05

7.4. Table 1. continued

Name	Time after injection of Aurothiomalate	Plasma Thiomalate	Total Serum Gold	Free Serum Gold	PBG	Urinary Thiomalate	Urinary Gold
E.W.	0	0	4.1	0.3	3.8	0	0.5
	15	400	4.4	0.4	4.0	-	-
	30	498	5.0	0.5	4.5	-	-
	45	300	5.2	0.5	4.7	-	-
	60	186	5.8	0.6	5.2	4.63	0.68
	90	25	5.8	0.6	5.2	-	-
	120	UD	5.6	0.4	5.2	-	-
	140	UD	5.4	0.4	5.0	-	-
	180	-	-	-	-	1.14	0.45
	300	-	-	-	-	0.28	0.2
D.S.	0	0	0	0	0	-	-
	15	78	6.2	0.7	5.5	-	-
	30	100	8.6	0.9	7.7	-	-
	45	290	9.0	1.0	8.0	-	-
	60	250	9.4	1.2	8.2	4.0	0.45
	90	200	9.3	1.1	8.2	-	-
	120	UD	9.0	1.0	8.0	2.4	0.3
	140	UD	8.2	0.8	7.4	-	-
	180	-	-	-	-	1.74	0.32
M.H.	0	80	4.0	0.4	3.6	0	0.4
	15	200	10.0	1.1	8.9	-	-
	30	395	10.6	1.2	9.4	-	-
	45	210	11.0	1.3	9.7	-	-
	60	100	12.8	1.4	11.4	4.7	0.65
	90	25	12.4	1.3	11.1	1.0	0.6
	120	UD	12.2	1.2	11.0	-	-
	140	UD	12.0	1.1	10.9	-	-
	180	-	-	-	-	UD	0.55

7.4. Table 1. continued

Name	Time after injection of Aurothiomalate	Plasma Thiomalate	Total Serum Gold	Free Serum Gold	PBG	Urinary Thiomalate	Urinary Gold
E.J.B.	0	UD	2.0	0.2	1.8	-	0.45
	15	UD	4.2	0.5	3.7	-	-
	30	UD	5.3	0.5	4.8	-	-
	45	UD	5.6	0.6	5.0	-	-
	60	UD	6.2	0.6	5.6	2.3	0.62
	90	UD	6.6	0.8	5.8	2.5	0.7
	120	UD	7.4	0.9	6.5	0.8	1.2
	140	UD	7.0	0.7	6.3	-	-
	240	-	-	-	-	UD	0.7
J.S.	0	0	3.1	0.2	2.9	0	0.5
	15	75	3.4	0.25	3.15	-	-
	30	235	3.8	0.4	3.4	-	-
	45	175	4.1	0.4	3.7	-	-
	60	210	5.2	0.5	4.7	4.21	0.72
	90	UD	5.6	0.5	5.1	2.5	0.74
	120	UD	6.2	0.7	5.5	1.74	0.94
	140	UD	5.5	0.5	5.0	UD	0.8
I.C.H.	0	0	10.0	1.1	8.9	0	1.05
	15	330	14.2	1.6	12.6	-	-
	30	330	18.1	1.9	16.2	-	-
	45	192	18.6	2.0	16.6	-	-
	60	150	19.2	2.2	17.0	2.9	2.4
	90	18	19.0	1.9	17.1	0.83	2.34
	120	UD	18.7	2.0	16.7	UD	2.00
	140	UD	18.5	1.9	16.6	-	-

7.4. Table 1. continued

Name	Time after injection of Aurothiomalate	Plasma Thiomalate	Total Serum Gold	Free Serum Gold	PBG	Urinary Thiomalate	Urinary Gold
E.T.	0	0	1.05	0.1	0.95	-	-
	15	60	6.6	0.6	6.0	-	-
	30	185	8.0	0.82	5.18	-	-
	45	160	8.3	0.8	7.5	-	-
	60	160	9.1	1.0	8.1	4.0	0.75
	120	UD	8.9	0.85	8.05	2.6	0.4
	240	UD	8.75	0.85	7.9	1.1	0.6
M.R.	0	0	-	-	-	-	-
	15	260	5.7	0.6	4.1	-	-
	30	430	9.2	0.9	8.3	-	-
	60	600	10.6	1.1	9.5	-	-
	90	480	13.2	1.3	11.0	3.0	1.3
	180	30	12.2	1.2	11.0	0.4	0.95
	300	120	11.4	1.0	10.4	0.2	0.5
	420	UD	11.3	1.1	10.2	0.02	0.45
E.W.	0	0	-	-	-	-	-
	15	450	5.05	0.5	4.55	-	-
	30	610	9.6	0.9	8.7	-	-
	60	430	9.9	0.9	9.1	1.62	0.75
	90	300	10.4	1.05	9.35	1.14	0.8
	180	90	10.6	1.1	9.5	0.3	1.85
	300	UD	9.9	0.9	9.0	0.09	1.4
	420	UD	8.0	0.85	7.15	-	-

7.4. Table 1. continued

Name	Time after injection of Aurothiomalate	Plasma Thiomalate	Total Serum Gold	Free Serum Gold	PBG	Urinary Thiomalate	Urinary Gold
P.J.S.	0	0	6.0	0.5	5.5	0	0.8
	15	75	6.4	0.6	5.8	-	-
	30	305	6.8	0.7	6.1	-	-
	45	279	7.4	0.8	6.6	-	-
	60	170	7.6	0.8	6.8	2.9	0.85
	90	82	8.2	0.9	7.3	0.83	1.05
	120	UD	8.0	0.7	7.3	UD	0.8
	140	UD	7.5	0.7	6.8	-	-

UD = Undetectable
PBG = Protein Bound Gold

7.5.i. Diagnostic Criteria for Rheumatoid Arthritis 1958 Revision (ARA)

Classical Rheumatoid Arthritis

This diagnosis requires seven of the following criteria. In criteria 1 to 5 the joint signs or symptoms must be continuous for at least 6 weeks. (Any one of the features listed under 'Exclusions' will exclude a patient from this category.).

1. Morning stiffness.
2. Pain on motion or tenderness in at least one joint (observed by a physician).
3. Swelling (soft tissue thickening or fluid - not bony overgrowth alone) in at least one joint (observed by a physician).
4. Swelling (observed by a physician) of at least one other joint (any interval free of joint symptoms between the two joint involvements may not be more than 3 months).
5. Symmetrical joint swelling (observed by a physician) with simultaneous involvement of the same joint on both sides of the body (bilateral involvement of mid-phalangeal, metacarpophalangeal, or metatarsophalangeal joints is acceptable without

absolute symmetry). Terminal phalangeal joint involvement will not satisfy this criterion.

6. Subcutaneous nodules (observed by a physician) over bony prominences, on extensor surfaces, or in juxta-articular regions.

7. X-ray changes typical of rheumatoid arthritis (which must include at least bony decalcification localised to or greatest around the involved joints and not just degenerative changes). Degenerative changes do not exclude patients from any group classified as RA.

8. Positive agglutination test - demonstration of the 'rheumatoid factor' by any method that, in two laboratories, has been positive in not more than 5% of normal controls, or positive streptococcal agglutination test.

9. Poor mucin precipitate from synovial fluid (with shreds and cloudy solution).

10. Characteristic histological changes in synovial membrane with three or more of the following: Marked villous hypertrophy; proliferation of superficial synovial cells often with palisading; marked infiltration of chronic inflammatory cells (lymphocytes or plasma cells predominating) with tendency to form 'lymphoid nodules'; deposition of compact fibrin, either on surface

or interstitially; foci of cell necrosis.

11. Characteristic histological changes in nodules showing granulomatous foci with central zones of cell necrosis, surrounded by proliferated fixed cells and peripheral fibrosis and chronic cell infiltration, predominantly perivascular.

Definite Rheumatoid Arthritis

This diagnosis requires five of the above criteria. In criteria 1 to 5 the joint signs and symptoms must be continuous for at least 6 weeks. (Any one of the features listed under 'Exclusions' will exclude a patient from this category).

Probable Rheumatoid Arthritis

This diagnosis requires three of the above criteria. In at least one of the criteria 1 to 5 the joint signs or symptoms must be continuous for at least 6 weeks. (Any one of the features listed under 'Exclusions' will exclude a patient from this category).

Possible Rheumatoid Arthritis

This diagnosis requires two of the following criteria and the total duration of joint symptoms must be at least 3 weeks. (Any one of the features listed under 'Exclusions' will exclude a patient from this category).

1. Morning stiffness.
2. Tenderness or pain on motion (observed by a physician) with history of recurrence or persistence for 3 weeks.
3. History or observation of joint swelling.
4. Subcutaneous nodules (observed by a physician).
5. Raised erythrocyte sedimentation rate or C-reactive protein.
6. Iritis.

Exclusions

1. The typical rash of disseminated lupus erythematosus, with butterfly distribution, follicle plugging and areas of atrophy.
2. High concentration of lupus erythematosus cells (four or more in two smears prepared from heparinised blood incubated for not more than 2 h).

3. Histological evidence of periarteritis nodosa, with segmental necrosis of arteries associated with nodular leucocytic infiltration extending perivascularly and tending to include many eosinophils.
4. Weakness of neck, trunk and pharyngeal muscles, or persistent muscle swelling of dermatomyositis.
5. Definite scleroderma (not limited to the fingers).
6. A clinical picture characteristic of rheumatic fever, with migratory joint involvement and evidence of endocarditis, especially if accompanied by subcutaneous nodules or erythema marginatum or chorea. (A raised antistreptolysin titre will not rule out the diagnosis of RA).
7. A clinical picture characteristic of gouty arthritis, with acute attacks of swelling, redness and pain in one or more joints, especially if relieved by colchicine.
8. Tophi.
9. A clinical picture characteristic of acute infectious arthritis of bacterial or viral origin with an acute focus of infection or in close association with a disease of known infectious origin, chills, fever, an acute joint involvement usually migratory initially (especially if these are organisms in the joint fluid or response to antibiotic therapy).

10. Tubercle bacilli in joints or histological evidence of joint tuberculosis.

11. A clinical picture characteristic of Reiter's Syndrome, with urethritis and conjunctivitis associated with acute joint involvement, usually migratory initially.

12. A clinical picture characteristic of the shoulder-hand syndrome, with unilateral involvement of shoulder and hand, with diffuse swelling of the hand followed by atrophy and contractures.

13. A clinical picture characteristic of hypertrophic pulmonary osteo-arthropathy, with clubbing of fingers and/or hypertrophic periostitis along the shafts of the long bones especially if an intrapulmonary lesion is present.

14. A clinical picture characteristic of neuro-arthropathy, with condensation and destruction of bones of involved joints and with associated neurological findings.

15. Homogentisic acid in the urine detectable grossly with alkalinization.

16. Histological evidence of sarcoid or a positive Kveim test.

17. Multiple myeloma as evidenced by marked increase in plasma cells in the bone marrow or by Bence-Jones protein in the urine.

18. Characteristic skin lesions of erythema nodosum.
19. Leukemia or lymphoma, with characteristic cells in peripheral blood, bone marrow or tissues.
20. Agammaglobulinaemia.

The ARA 1987 revised criteria for classification of RA is presented on the following page.

7.5.ii. The American Rheumatism Association 1987 Revised Criteria for the Classification of Rheumatoid Arthritis

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around joints, lasting at least 1 hour before maximal improvement.
2. Arthritis of 3 or more joint areas.	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle and MTP joints.
3. Arthritis of hand joints.	At least one area swollen (as defined above) in a wrist, MCP, or PIP joint.
4. Symmetric arthritis.	Simultaneous involvements of the same joint areas (as defined in 2) on both sides of the body. (Bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry.)
5. Rheumatoid nodules.	Subcutaneous nodules, over bony prominences, or extensor surfaces,

	or in juxta-articular regions, observed by a physician.
6. Serum rheumatoid factor.	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in less than 5% of normal control subjects.
7. Radiographic changes.	Radiographic changes typical of rheumatoid arthritis on postero-anterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localised in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify).

PIPs   Proximal interphalangeal joints

MCPs   Metacarpophalangeal joints

MTPs   Metatarsophalangeal joints

For classification purposes, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least four of these seven criteria. Criteria 1 to 4 must have been present for at least 6 weeks. Patients with two clinical diagnoses are not excluded. Designation as classic, definitive or probable RA is not to be made.

Annals of the Rheumatic Diseases, 1987; **46**, 823–826

Patterns of urinary excretion of gold in pati
rheumatoid arthritis undergoing chrysother

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From the Departments of [1]Clinical Chemistry and [2]Rheumatology and Rehabilit
Nottingham; and the [3]Department of Life Sciences, Trent Polytechnic, Nottinghar

SUMMARY Thirty patients receiving gold therapy for rheumatoid arthritis (R
the urinary excretion of gold. Statistical analysis of all the urine specimens pas
four days by each patient showed that a definite rhythm of gold excretion ex
which is possibly related to water excretion but not to creatinine excretion.
possible reasons for the inability of earlier workers to relate gold excretion
gold status of patients and suggests that as the study of 24 hour excretions
insensitive marker of gold excretion, closer examination of individual patie
excretion could possibly provide a more useful method of analysis.

Key words: circadian rhythms, aurothiomalate.

The management of rheumatoid arthritis (RA) by gold therapy is based on more than 50 years' experience. During this time successful management has been plagued by the inability to relate consistently any easily measurable parameter to the potential efficacy or toxicity of the treatment.

One area of investigation has been the relation of serum gold to urinary gold. The findings of the various investigators, however, have been in the main, contradictory. Freyberg *et al* found no correlation between serum and urine gold levels,[1] and nor did Lawrence.[2] Smith *et al* found that a poor therapeutic effect was related to a high urinary gold excretion and toxic reactions to a low excretion,[3] but Krusius *et al*[4] and Billings *et al*[5] concluded that although serum gold levels correlated with therapeutic efficacy, urine levels did not.

It is evident, however, that the types of urine specimens studied and the methods of reporting gold levels have differed in various studies. For example, Krusius *et al*[4] and Arden-Jones *et al*[6] reported excretion of gold in milligrams per 24 hours, whereas Billings *et al* reported the excretion of gold in random urine specimens as micrograms per gram of creatinine.[5] The aim of this study is to investigate the possibility of the existence of circadian periodicity in the excretion of gold and to compare this with the endoge
and creatinine excretion. Cle
rhythm of gold excretion wo
daily random urine gold level
the rhythms be different
rhythms of excretion then th
as excretion in micrograms
would compound inconsiste

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Patients and methods

Thirty patients receiving g
diagnosed according to the
Association[7] criteria were ad
patients were receiving a
monthly stabilised dose of
and had a disease duration
and five years. Twenty one su
nine were male, and all we
16–65 years. Patients receivi
excluded from the study. Fiv
Nottingham City Hospital
outpatients.

The patients measured th
specimens passed over a
collecting jugs and measurin
provided and placed aliquots
labelled bottles. The volum
forms which were supplied,
of micturition. The procedu
minimum period of four d

ANALYSIS

All urine specimens were analysed for gold content by atomic absorption spectroscopy. Creatinine assays were performed on each specimen using an Instrumentation Laboratory 508 analyser, which adopts the traditional Jaffé alkaline picrate method. From the results, both gold and creatinine excretion rates were calculated. The excretion rates for water were also calculated.

DATA ANALYSIS

The data obtained from the study were analysed as follows: (*a*) histograms were plotted for each patient study period of excretion rates (gold, creatinine, and water) against time; (*b*) results from each patient were analysed for periodicity by the sine wave technique developed by Fort and Mills[9]; (*c*) Pearson correlations were calculated for gold excretion against water excretion and for gold excretion against creatinine excretion both for the patient group and for the individual patients.

Results

HISTOGRAM ANALYSIS

Gold

The individual histograms of gold excretion for 28 of the 30 patients studied described regular patterns of excretion throughout the four day study. Twenty four patients showed biphasic patterns, four monophasic, and two triphasic patterns of excretion.

Patient No 1 shown in Fig. 1 demonstrates a biphasic pattern with its peaks at 03·02 and 16·12 hours.

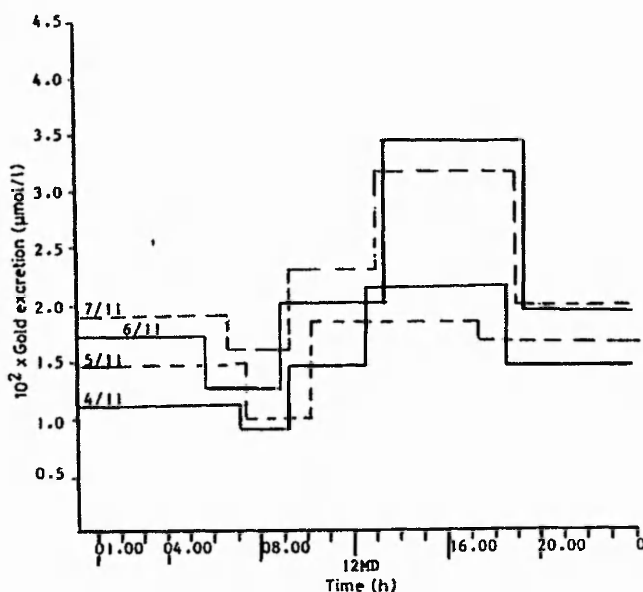

Fig. 1 *The histograms for patient No 1 show regular biphasic patterns of gold excretion.*

Water

The histograms for water excretion sh and troughs similar to those for gold e the same days when analysed by eye. (shown in Fig. 2) demonstrates a biph with peaks at 05·00 and 16·25 hours.

Creatinine

Although individual patterns of creatin

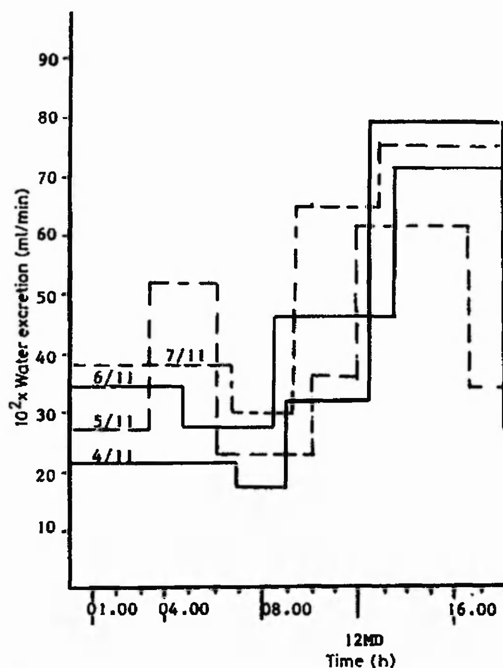

Fig. 2 *The histograms for patient No 1 show biphasic patterns of water excretion.*

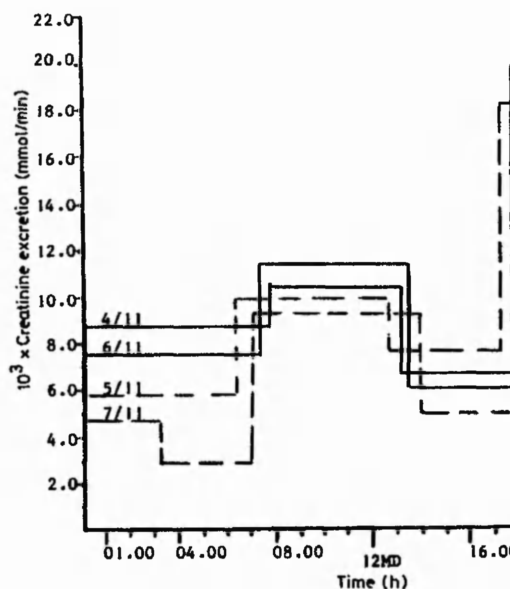

Fig. 3 *The histograms for patient No 1 sho biphasic patterns of creatinine excretion. The are dissimilar to those of gold and water excr*

were evident, thoughout the group only six patients showed excretion rates and times similar to those of gold and water. Patient No 1 shown in Fig. 3 demonstrates biphasic patterns of excretion, but these are dissimilar to the gold and water excretion patterns for this patient. The peaks are at 10·10 and 19·58 hours.

SINE WAVE ANALYSIS

Each of 28 patients showed individual statistically significant circadian rhythmicity of urinary gold excretion. The same subjects demonstrated rhythmicity for water and urinary creatinine excretion. It can be seen from the results that although in individual subjects the acrophases for gold and water excretion were similar, this occurred in only six instances for gold and creatinine excretion. Table 1 shows six examples of patients with similar individual acrophases for gold and water excretion, and one example of a patient with similar acrophases for gold, water, and creatinine excretion.

In the two instances where no regular patterns of excretion were detected one patient commenced steroid medication during the study, and the other had a very high, random (including nocturnal) intake of tea, which has known diuretic properties.

CORRELATION RESULTS

From the results of a group correlation between gold excretion and water excretion it is evident that correlation was poor ($r=0·32$, $p=0·10$, $n=140$). Individual patient correlation plots, however, showed good correlation, with all correlation coefficients greater than 0·85 and with p values less than 0·05 (see Fig. 4). There was no apparent correlation between the patient group creatinine excretion rates and water excretion rates ($r<0·25$, $p>0·1$), and also bad correlation for 22 patients when individually plotted ($r<0·2$, $p>0·1$). Six patients showed good

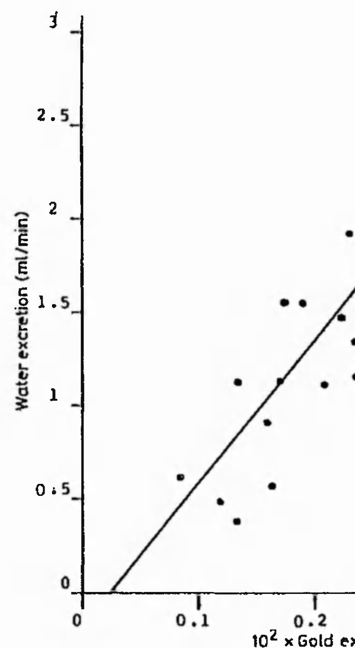

Fig. 4 *Correlation of gold an (Patient No 1.)*

correlation between creati rates.

Discussion

The histogram and sine indicates that statisticall patient rhythms exist for gold. These are similar to t all cases, but similar to minority (six out of 28) Correlation studies show t possibly related to the e

Table 1 *Sine wave analysis results for gold, water, and creatinine excretion*

Patient No	*Gold excretion*				*Water excretion*				*Creatinine*
	*10^2×M** (μmol/min)	*10^2×A** (μmol/min)	*Acr** (h)	*p Value*	*M* (ml/min)	*A* (ml/min)	*Acr* (h)	*p Value*	*M* (mmol/min)
1	0·85	0·46	16·12	0·03	1·42	0·74	16·25	0·04	2·08
2	0·82	0·71	20·58	0·05	1·54	0·89	20·32	0·05	3·02
3	0·95	0·35	16·53	0·04	1·69	0·76	16·04	0·03	2·16
4	1·31	0·42	17·13	0·03	2·14	1·04	17·01	0·02	3·19
5	1·45	0·66	16·18	0·05	1·92	0·94	16·52	0·05	2·87
6	1·56	0·86	01·29	0·03	1·98	0·92	2·00	0·03	3·24
7	1·47	0·95	23·24	0·05	2·01	1·42	23·58	0·05	3·01

*M=mesor; A=amplitude; Acr=acrophase.

kidney, but not related to the excretion of creatinine.

This study suggests possible reasons for the disparities achieved by earlier workers in attempting to relate urinary excretion of gold to the body status using random urine specimens and random specimens corrected for creatinine content. Clearly, random urine specimens from each subject will have different gold levels depending upon the times of collection. It is likely that correcting random urine gold levels for creatinine content will compound inconsistencies in results found between random specimens owing to the existence of creatinine excretory rhythms which are often different from those of gold. If efficacy or toxicity of treatment is reflected in changes in gold excretion it is possible that these alterations are likely to be more readily detected by observation of changes in the excretory rhythms or in the excretory rates (μmol/min) rather than in changes in total 24 hour excretion. Measurement of total 24 hour gold excretion could mask any subtle alterations in excretion rates occurring at different times of the day.

References

1 Freyberg R H, Block W D, Levey S. Metaboli manner of action of gold compounds used in arthritis. Human plasma and synovial fluid cc urinary excretion of gold during and followin gold sodium thiomalate and colloidal gold *Invest* 1941; **20:** 401–12.
2 Lawrence J S. Studies with radioactive gold. 1961; **20:** 341–51.
3 Smith R T, Pea W P, Kron K M, Hermann I F Goldman M. Increasing the effectiveness of rheumatoid arthritis. *J Am Med Assoc* 1958;
4 Krusius F E, Markkanen A, Peltola P. Pl urinary excretion of gold during routine treat toid arthritis. *Ann Rheum Dis* 1970; **29:** 232
5 Billings R, Grahame R, Marks V, Wood P J, and urine gold levels during chrysotherapy arthritis. *Rheumatol Rehabil* 1975; **14:** 13–18
6 Arden-Jones R, Ahmed M U, Chan B W B. C rheumatoid arthritis. *Br Med J* 1971; **i:** 610.
7 Ropes M W, Bennett G A, Cobbs S, Jacox Revision of diagnostic criteria for rheumatoi *Rheum Dis* 1958; **9:** 175–6.
8 Heath M J. The composition of the urine at d day and night in patients who have undergone tation. University of Nottingham, 1981: 16–
9 Fort A, Mills J N. Fitting sine waves to 24 *Nature* 1970; **226:** 657–8.

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Patterns of gold levels in urine, serum, and sa patients with rheumatoid arthritis undergoin chrysotherapy

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SUMMARY Twenty patients undergoing treatment with aurothiomalate for rhe (RA) were studied for the presence of gold in all urine specimens passed over gold in the serum of blood drawn by venous section at 10·00, 16·00, and 22·00 day of the study. Specimens of saliva collected at the same times as the blood spe analysed for (total) gold content. Eighteen patients showed rhythmic urinar Variations were observed in the serum levels for total, free, and protein bound times of the day and night together with similar variations in the salivary total g established that a possible relation exists between urinary gold, serum gold, such that at times of higher urinary gold excretion the serum gold levels (total, bound) and the total salivary gold levels were decreased. Conversely, at times gold excretion serum and salivary gold levels were increased.

Key words: gold therapy, aurothiomalate, circadian rhythms.

The existence of circadian rhythmicity in the urinary excretion of gold in patients with rheumatoid arthritis receiving chrysotherapy has been shown by this group in earlier studies.[1] Circadian variations have been shown for copper in serum by Nielsen[2] and Munch-Petersen[3] and for iron by Hoyer,[4] and therefore a follow up study to our urinary gold work was conducted to examine the possibility of variations in serum levels of gold at different times of day and night and to compare them with urine levels in specimens passed over the same time period. Saliva specimens produced around the times of micturition and venous section were also analysed for gold content. The presence of circadian rhythms in saliva for cortisol has been reported by Price *et al*[5] and for testosterone by Campbell *et al*.[6] The prevalence of metallic ions in saliva has been demonstrated by Bauer *et al* (sodium and potassium),[7] Kolomaznik *et al* (lithium),[8] Tokueva (calcium and inorganic phosphate),[9] and Nilner and Glantz (copper, silver, tin, mercury, and zinc)[10]; hence rhythmic presence of gold was

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Patients and methods

Twenty new patients with RA r apy collected aliquots of all urin over four days and noted the ti and the volume of each specimen study each patient collected sp around the time of micturition. each subject had blood withdraw at 10·00, 16·00, and 22·00 hou saliva specimens were analysed atomic absorption spectrophoto the blood specimens were analy and protein bound gold by AA ultrafiltration of the serum. A analysed in duplicate.

Results

URINE

Eighteen of the 20 patients sho

urinary gold excretion as demonstrated by histogram and sine wave analysis, in agreement with our earlier studies.

BLOOD
The 18 subjects showed variations in total gold levels at different times of day. All variations were outside the possible differences due to the coefficient of variation (CV) of the method (see Table 1). Marked variations in free and protein bound gold were also demonstrated.

SALIVA
Gold was detected in all the salivary specimens collected, and variations were observed in these levels at different times of day which were outside the CV of the method. These variations reflected the similar differences observed in the total, free, and protein bound gold levels in serum for blood taken at the same time as the collection of saliva (see Table 1). There was correlation for the group results for total serum gold against salivary gold ($r=0\cdot82$, $p=0\cdot001$), free serum gold against salivary gold ($r=0\cdot70$, $p=0\cdot001$), and protein bound gold against salivary gold ($r=0\cdot80$, $p=0\cdot001$).

Table 1 *Comparison of patients' results at diffe of day and night for total serum, free, protein bo salivary gold levels together with the relative urin levels*

Patient No	*Time*	*Total serum gold (μmol/l)*	*Free gold (μmol/l)*	*Protein bound gold (μmol/l)*	*Salivar gold (μmol*
1	10·00	13·0	1·3	11·7	0·5
	16·00	12·0	1·1	10·8	0·1
	22·00	13·0	1·4	11·6	0·6
2	10·00	13·0	1·9	11·1	0·6
	16·00	15·5	2·2	13·5	0·9
	22·00	14·0	2·0	11·8	0·6
3	10·00	13·0	2·2	10·8	0·5
	16·00	15·0	2·8	12·3	0·9
	22·00	14·5	2·4	12·2	0·6
4	10·00	14·5	2·4	12·1	0·6
	16·00	16·0	3·0	13·1	1·1
	22·00	13·0	2·7	10·3	0·7
5	10·00	15·0	3·5	11·5	0·6
	16·00	13·0	3·1	9·9	0·4
	22·00	15·0	3·4	11·6	0·6

Coefficient of variation (CV) for total gold 1·9%, for f and for salivary gold 9%.

Discussion

It is evident from the results that each patient studied showed variations in total, free, and protein bound serum gold levels at different times of day and night. Variations in the total salivary gold levels were also shown for specimens collected at the same time as the blood samples. All the recorded were outside the possible obtainable in results due to the coe variation (CV) of the assay method. analysis of the results showed a correlatic

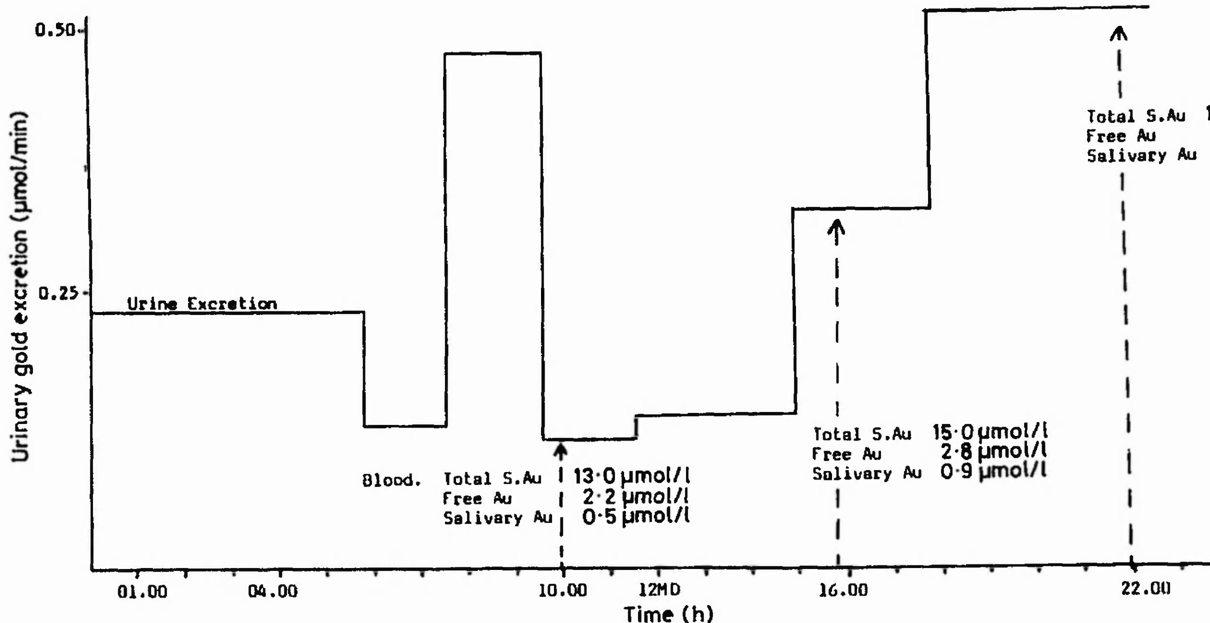

Fig. 1 *Urinary gold excretion rates for one day for patient No 3 together with the serum and salivary gold leve that were collected on the same day.*

total salivary gold and total serum gold, total salivary gold and free gold, and total salivary gold and protein bound gold. Furthermore, for each patient, at times of higher urinary gold excretion rates total serum gold and its fractions were low, as were salivary gold levels. Conversely, when urine gold levels were low both serum and salivary gold levels were raised (Fig. 1). The urinary excretion of gold has already been shown to be rhythmic, and it is possible that serum and salivary gold level variations are also rhythmic, but it is not possible to establish this conclusively in this study using just three time points in a 24 hour day.

As free serum gold represents the filterable fraction of serum gold by healthy kidneys it is possible that the increase in urine gold may be derived from this serum fraction. There is, however, a decrease in the protein bound level of gold in serum at the same time, which is not filterable, but which might release gold to the depleted free fraction allowing further excretion of gold.

The role of salivary gold in these events is being investigated further by this group by a study of the differential gold fractions in saliva.

References

1 Heath M J, Swannell A J, Williams C urinary excretion of gold in patients undergoing chrysotherapy. *Ann Rh*
2 Nielsen A L. Serum copper. III. N *Scand* 1944; **118:** 87–91.
3 Munch-Petersen S. The variations in of 24 hours. *Scand J Clin Lab Inv*
4 Hoyer K. Serumjernets fysiologisk (Dissertation.)
5 Price D A, Close G C, Fielding B circadian rhythms in salivary cortisol *Child* 1983; **58:** 454–6.
6 Campbell I T, Walker R F, Riad- Circadian rhythms of testosterone an of activity-phase shifts and continu *logica* 1982; **9:** 389–96.
7 Bauer F, Balant L, Zender R, Hum and digitalis. *Eur Heart J* 1983; **4:**
8 Kolomaznik M, Hronek J, Janonsc possibilities of assessing lithium in se *Cesk Psychiatr* 1983; **79:** 111–7. (E
9 Tokueva L I. Mixed salivary calcium the rate of saliva excretion and car during the mineralisation period i *(Mosk)* 1983; **62:** 62–4. (English a
10 Nilner K, Glantz P O. The prevale mercury and zinc ions in human sal 71–7.

Annals of the Rheumatic Diseases, 1988; **47,** 18–21

Measurement of 'free' gold in patients receiving disodium aurothiomalate and the association of high free to total gold levels with toxicity

M J HEATH

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SUMMARY Serum from patients with rheumatoid arthritis (RA) receiving disodium aurothion late was analysed for total gold by atomic absorption spectrometry and for unbound (free) g by the same method after ultrafiltration by an inert membrane. It was shown that it is possible obtain reliable free gold concentrations by this method. Good correlations were shown betwe total and 'free' gold and between total and protein bound gold (PBG) for 54 patients with I who were stabilised on gold therapy. Significant correlation was also shown between the sa parameters for a second group of 15 patients starting gold therapy who were bled at wee intervals for nine weeks immediately before medication. A single correlation with regression all patients studied again showed good correlation between total and free gold and between to and PBG. Of the 189 paired values plotted, 182 fell within 2SD of the regression lines for the t plots. Of the seven patients with results outside 2SD of the regression line, six presented with s effects during the study.

Key words: rheumatoid arthritis, unbound gold, gold therapy, chrysotherapy, gold measu ment.

It has been shown that most of the gold given to patients suffering from rheumatoid arthritis (RA) as aurothiomalate is present in the serum bound to albumin and to a lesser extent bound to the remaining protein fractions.[1–8] It has also been established that with several drugs the unbound rather than the total or protein bound concentration shows a better correlation with pharmacological response.[9–11] In consequence it has been suggested that unbound (or free) serum gold might be a useful parameter for monitoring patients during chrysotherapy.[7 12]

Until recently methods of assaying free serum gold have either been unsuccessful or have given inconsistent results, probably as a result of technical problems encountered at the very low levels of free gold. Many methods have not been suited to handling large numbers of specimens and have not been sufficiently fast to cope with the reactive characteristics of gold in blood or with the changing nature of plasma proteins with time.[2] Furthermc the redistribution of gold among the protein fi tions during separation and the reaction of meta ions with chemicals have made the assay diffic

Membrane ultrafiltration has been used succe fully in studies where speed of analysis, with addition of potentially competitive buffer com nents and electrolytes, has been required.[13] T method has been used in studies of protein binc to drugs,[14 15] analysis of free tryptophan plasma,[16] and the protein binding of hormones.[17] Ultrafiltration has been shown to b least equivalent to equilibrium dialysis but simp to carry out.[18] The aims of this study were to expl the possibility of producing an ultrafiltrate of ser containing unbound gold using filtration membra and to investigate the possible relations betw total, free, and protein bound gold (PBG) in serum of patients with RA receiving gold thera

Patients and methods

PATIENTS

Two groups of patients were studied.

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Group 1
Fifty four patients who fulfilled the American Rheumatism Association (ARA) criteria for RA[19] and who were stabilised on gold therapy (disodium aurothiomalate) had 10 ml of blood withrawn by venous section before the next maintenance gold injection. Serum was separated from the clotted blood and stored at 4°C within 30 minutes of blood collection.

Group 2
A further 15 patients with RA who were starting gold therapy had blood withdrawn weekly for nine weeks before the next gold injection. The serum was stored at 4°C within 30 minutes of venous section as for group 1.

SPECIMEN ANALYSIS
All serum specimens were analysed for total gold and free gold. From these two values the PBG levels were calculated.

Total gold
Total gold levels were assayed by flame atomic absorption spectrometry.[20]

Free gold
Ultrafiltrates of serum were obtained using Amicon Centriflo membrane cones (type CF25), which are an inert, non-cellulosic polymer laminated on a tough inert substrate.

All specimens were centrifuged in an individual membrane cone at 4°C at a relative centrifugal force not exceeding 100 *g*. (Excessive relative centrifugal force causes protein leakage.) The resulting ultrafiltrates were tested for pervading proteins using test strips (Boehringer BH). From a total of 189 serum specimens centrifuged, 10 filtrates were discarded after the detection of protein. In these cases there was sufficient serum left to repeat the filtration in new cones.

The protein free ultrafiltrates were analysed for gold by flame atomic absorption spectrometry.

Serum samples from 20 patients were filtered and analysed in duplicate as a test of the method precision (precision study). Each set of duplicate specimens assayed had gold values which were within 10% of each other.

STATISTICAL ANALYSIS OF RESULTS
Results from group 1 patients were analysed by Pearson correlation with linear regression. Correlation coefficients (r) were calculated for total gold against free gold and total gold against protein bound gold.

Results obtained for group 2 patients were analysed by the same technique and for the same values as group 1 results.

A single correlation with regression was plotted for the combined results from groups 1 and 2 both for total against PBG and for total against free gold.

Results

CORRELATION STUDY
Good correlation was shown in group 1 patients

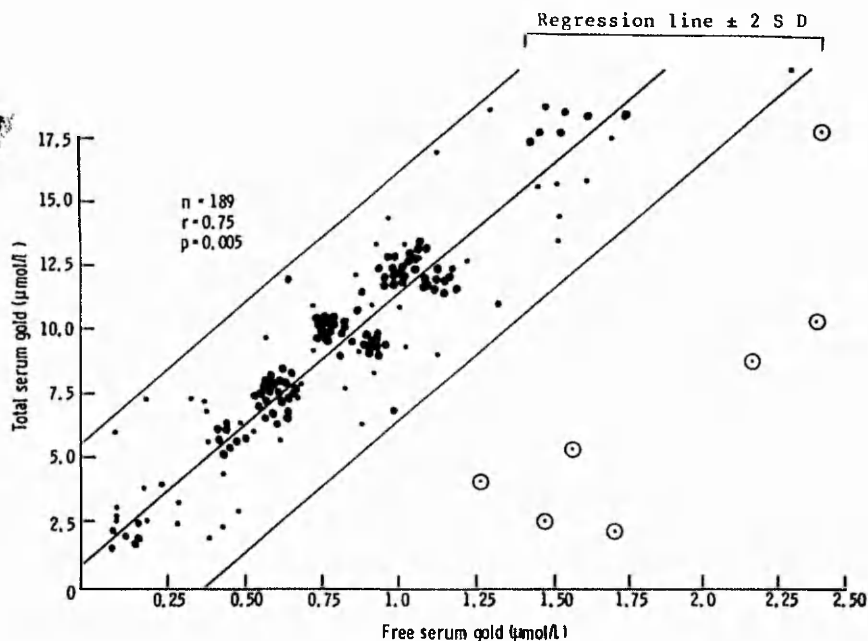

Fig. 1 *Correlation between total and free serum gold including lines within 2SD of the regression line.*

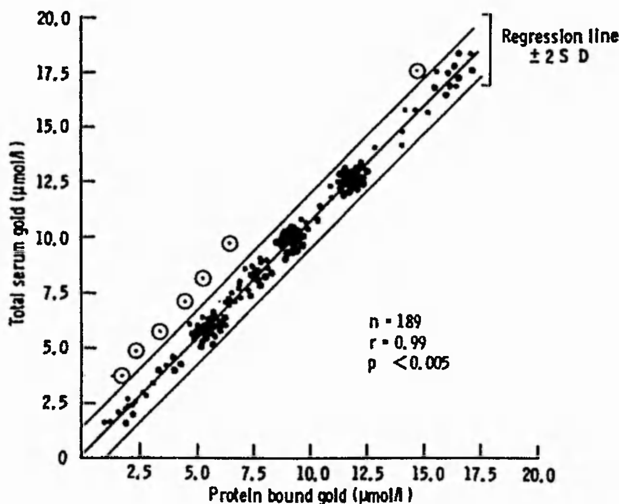

Fig. 2 *Correlation between total and protein bound gold including lines within 2SD of the regression line.*

between total gold and free gold (r=0·80, p=0·005, n=54, where p is the measure for the significance of the results and n is the number of paired results observed) and between total gold and PBG (r=0·99, p=0·005, n=54).

Good correlation was also obtained in group 2 patients between total gold and free gold (r=0·79, p=0·005, n=135) and between total gold and PBG (r=0·99, p=0·005, n=135).

The single correlation with regression for results from all patients in the two study groups (i.e., the initial established group of 54 plus the nine serial analyses for the 15 patients starting therapy) again showed significant correlation both between total and free gold (r=0·75, p=0·005, n=189) and between total and PBG (r=0·99, p=0·005, n=189). When limits of 2SD were added to the plots of the regression lines it was observed that in each case 182 of the 189 plots were within the limits set and seven fell outside the limits (see Figs 1 and 2).

Discussion

When ultrafiltration cones were used analyses were carried out rapidly and with good precision. The results obtained from the precision study show that good reproducibility is possible for free gold in serum using this technique.

Levels of free gold were detected which were up to 12% of the total serum gold levels measured. This is in agreement with the work done by Campion *et al.*[21] The analyses for free gold were performed up to one week after the blood samples were obtained by venous section. The results obtained differ from the studies made by Danpure,[22] who was unable to detect the free gold moiety from 100 mir onwards after gold injection.

It is evident from the results that there is g correlation between serum total gold and free and between total gold and PBG for pat starting gold therapy and for those stabilised therapy. Furthermore, the single correlation regression for the combined results from the groups of patients for total against free gold total against PBG showed that in all but seven c the correlation points were within 2SD of regression lines (see Figs 1 and 2).

Retrospective analysis of patients' notes sho that of these seven patients, five had devel rashes at the time of study and one had tran thrombocytopenia. Data on the seventh pa were not available. No other patient in either g developed side effects over the study period. possible that toxicity may be related to a higher to total serum gold ratio and also to a lower PB total gold ratio.

A study is now in progress in which blood is t from patients receiving gold therapy for RA present with toxic reactions and then again afte disappearance of the reaction when treatme temporarily withheld. Blood is analysed for t free, and protein bound gold and the results ent on the regression plots. In this way it ma possible to show any relation existing betwee toxicity of gold treatment and the distributio gold in the serum.

Patients showing side effects in this study repre 11% of all patients studied as opposed to the usual figure of 25 to 33%. There are several pos explanations for this low incidence of toxic reac in the patient population studied: (*a*) The i group of 54 subjects was selected on the basis they were stabilised on therapy and might ther be expected to have a lower incidence of reactions. (*b*) The average weekly gold dose each patient in the study was 20 mg as oppos the more usual 50 mg/week. This regimen is us this hospital because of the apparently lower rat toxicity. (*c*) The 15 patients studied in group 2 followed up for nine weeks after starting the As the usual figure of 25 to 33% for pat showing side effects is normally applied to sub studied during 12 months of therapy, it is likely with a longer study period the incidence of reactions in the group of 15 would be greater.

The above observations show that it is possib obtain reliable free gold concentrations for pat receiving gold therapy and that measuremen both free and total serum gold levels are likely more useful parameters for monitoring pat during chrysotherapy than total gold alone

addition, this might explain why previous studies have failed to relate total or PBG concentrations alone to toxic reactions.[23]

References

1 Danpure C J, Fyfe D A, Gumpel M J. Distribution of gold among plasma fractions in rheumatoid patients undergoing chrysotherapy compared with its distribution in plasma incubated with aurothiomalate in vitro. *Ann Rheum Dis* 1979; **38:** 364–70.
2 Kamel H, Brown D H, Ottaway J M, Smith W E. Determination of gold in separate protein fractions of blood serum by carbon furnace atomic absorption spectrometry. *Analyst* 1977; **102:** 645–57.
3 Ward R J, Danpure C J, Fyte D A. Determination of gold in plasma and plasma fractions by atomic absorption spectrometry and by neutron activation analysis. *Clin Chim Acta* 1977; **81:** 87–97.
4 Francois P E, Goldberg I J L, Lawton K, Al-Ani D T, Redding H J. Distribution of gold in blood during chrysotherapy. *Ann Clin Biochem* 1978; **15:** 324–5.
5 Van de Stadt R J, Abbo-Tilstra B. Gold binding to blood cells and serum proteins during chrysotherapy. *Ann Rheum Dis* 1980; **39:** 31–6.
6 Pedersen S M. The binding of gold to human albumin in vitro. Intrinsic association constants at physiological conditions. *Biochem Pharmacol* 1981; **30:** 3249–52.
7 Lorber A, Vibert G J, Harralson A F, Simon T M. Unbound serum gold: procedure for quantitation. *J Rheumatol* 1983; **10:** 563–7.
8 Smith P M, Smith E M, Gottlieb N L. Gold distribution in whole blood during chrysotherapy. *J Lab Clin Med* 1973; **82:** 930–7.
9 Hinderling P H, Garret E R. Pharmacokinetics of the antiarrhythmic disopyramide in healthy humans. *J Pharmacokinet Biopharm* 1976; **4:** 199–230.
10 Weser J K, Sellers E M. Drug therapy-binding of drugs to serum albumin. *N Engl J Med* 1976; **294:** 311–6.
11 Bett R J, Himmelstein K J, Patton T F *et al.* Pharmacokinetics of non-protein bound platinum species following administration of cis-dichlorodiammineplatinum (II). *Cancer Treat Rep* 1979; **63:** 1515–21.
12 Pedersen M P. Binding of sodium aurothiomalate to human serum albumin in vitro at physiological conditions. *Ann Rheum Dis* 1986; **45:** 712–7.
13 Whitlam J B, Brown K F. Ultrafiltration in serum protein binding determinations. *J Pharm Sci* 1981; **70:** 146–50.
14 Wijkstrom A, Westerlund D. Plasma protein binding of sulphadiazine, sulphamethoxazole and trimethoprim determined by ultrafiltration. *J Pharm Biomed Anal* 1983; **1:** 293–9.
15 Wittfoht W, Duwe K, Kuhnz W, Nau H. Microscale ultrafiltration technique for determining free drug in 50 μl serum samples. *Clin Chem* 1984; **30:** 878–9.
16 Knott P J, Curzon G. Free trytophan in plasma and brain typtophan metabolism. *Nature* 1972; **239:** 452–3.
17 Lee I R, Greed L C, Hahnel R. Comparative measurements of plasma binding capacity and concentration of human sex hormone binding globulin. *Clin Chim Acta* 1984; **137:** 131–9.
18 Sophianopoulos J A, Durham S J, Sophianopoulos A J, Ragsdale H L, Cropper W P. Ultrafiltration is theoretically equivalent to equilibrium dialysis but much simpler to carry out. *Arch Biochem Biophys* 1978; **187:** 132–7.
19 Ropes M W. Diagnosis criteria for rheumatoid arthritis. *Ann Rheum Dis* 1959; **18:** 49–53.
20 Instrumentation Laboratory Inc., Lexington, Mass, USA. *Clinical applications of atomic absorption/emission spectroscopy.* 1970; **72:** 51–2.
21 Campion D A, Olsen R, Bohan A, Bluestone R. Interaction of gold sodium thiomalate (Myocrysin) with serum albumin. *J Rheumatol* 1974; **1** (suppl): 1–112.
22 Danpure C J. 'Free' plasma gold in rheumatoid patients undergoing chrysotherapy. *J Physiol (Lond)* 1977; **267:** 25–6.
23 Gottlieb H L, Smith P M, Smith E M. Pharmacodynamics of ^{197}Au and ^{195}Au labelled aurothiomalate in blood. *Arthritis Rheum* 1974; **17:** 171–83.