CYTIDINE DEAMINASE
IN
RHEUMATOID ARTHRITIS

A thesis submitted for the degree
of Doctor of Medicine

Paul Warren THOMPSON MBBS MRCP(UK)

ABSTRACT

Cytidine deaminase (CD) is an cytoplasmic enzyme found in high concentrations in polymorphonuclear leucocytes (polymorphs). In rheumatoid arthritis (RA) huge numbers of polymorphs are attracted to inflamed joints where they lyse releasing their contents into synovial fluid. The thesis describes a series of experiments designed to test the hypothesis that, in RA, CD drains from synovial fluid to blood where serum levels represent an integrated measure of 'acute' inflammation.

An assay was established and evaluated. Studies of CD in joint tissues and body fluids showed that serum levels of CD were raised in RA patients with a gradient running from synovial polymorphs to synovial fluid, across the synovium to blood. The mean synovial fluid CD level was very close to the value predicted using a mathematical model of synovial fluid solute kinetics. Studies of a variety of other enzymes and a selective inhibitor of CD supported the joint polymorph as the source of origin for serum CD in RA. Clinical evaluation revealed a small diurnal variation in serum CD in RA patients probably related to exercise increasing lymphatic drainage from joints. Serum CD was shown to correlate cross-sectionally and longitudinally with other clinical and biochemical measures of joint inflammation in RA, and levels were able to detect the flare produced by the withdrawal of non-steroidal anti-inflammatory drugs from RA patients. The results were discussed according to the principles of measurement theory.

In conclusion, serum CD is a simple, cheap, reproducible and sensitive measure of joint inflammation in RA that reflects polymorph lysis known to be at the centre of the inflammatory process. It supplies the clinician with a serological tool for patient monitoring which is a direct assessment of one aspect of inflammation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

In 1986 I was awarded an Arthritis and Rheumatism Council (ARC) Fellowship to study cytidine deaminase in rheumatoid arthritis. During the Fellowship year I established and evaluated the assay (Chapter III), collected and processed the tissues of origin of serum cytidine deaminase (Chapter IV), assayed the samples for comparison with 'liver' enzymes (Chapter V) and examined the patients for the clinical study (Chapter VIII). Thereafter I was funded by the ARC (Cytidine Deaminase in Arthritis - B92) for a biochemistry technician who took over the sample processing and assays (Chapters VI, IX and X). Further funding from the ARC (Clinical Utility of Cytidine Deaminase in Arthritis - T23) supported a nurse/metrologist who examined the patients for the longitudinal clinical study (Chapter IX).

I would like to thank Professor Harry Currey, Professor David Blake, Professor Donald Moss, Dr John Kirwan, Dr Jon Dixon, Dr Paul Mapp, Dr Jim Archer and Dr David Perrett for their expert criticism and advice. Dr Dilwyn Jones, whose observations started the project, and for undertaking the comparative cytidine deaminase assays (Chapter III) and inhibitor assays (Chapter VI). Professor Donald Moss and Dr Katrine Whitaker for help with the alkaline phosphatase isoenzyme assays, and Dr Brian Houghton and Caroline Clifford for use of the SMAC autoanalyser for the 'liver' enzyme assays (Chapter V). Professor Harry Currey, Professor David Blake, Dr Colin Barnes, Dr J David Perry and Dr Susan Rudge for allowing me to study patients under their care. Dr Steven Evans and Dr Alan Silman for statistical advice, Dr J Rodney Levick for help with the mathematical model (Chapter II), and Dr Roy Pownell and Boots Ltd for undertaking the statistical analysis of circadian rhythm (Chapter VII). Dr Sally Wheatcroft, Sister Fran Pegley, Nurse Sheila Snow and Nurse Mary Huggins for help with sample collection (Chapters IX and X). Professor Paul Dieppe, Dr Ian Griffiths and Dr Howard Bird for encouragement and advice concerning funding. Dr Jon Shipman and Roche for financial support (Chapter X), and the ARC for funding.

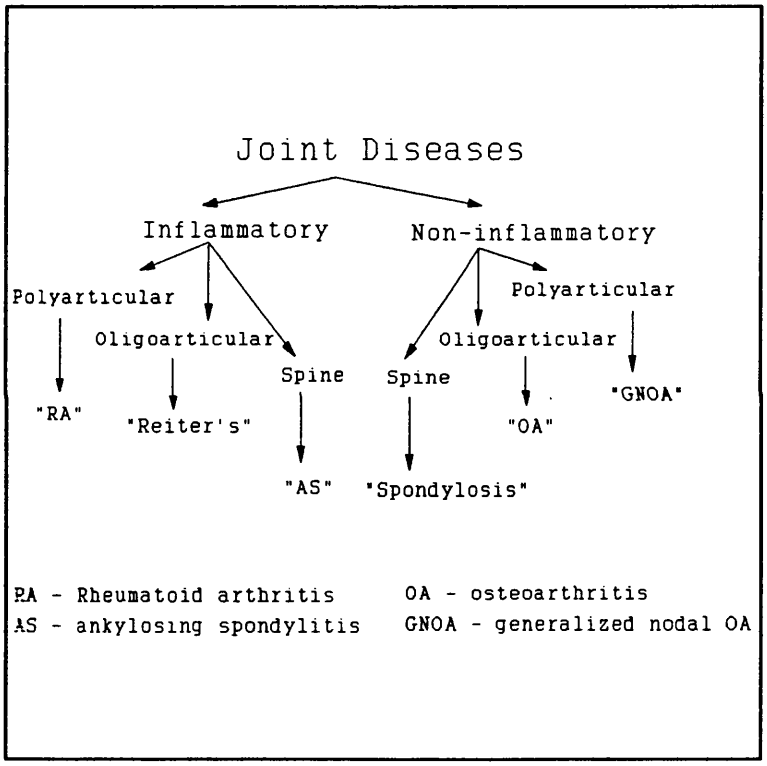
CHAPTER I

INTRODUCTION AND AIMS

1.1 CLASSIFICATION OF JOINT DISEASES

Rheumatology is largely a descriptive speciality and, with a few exceptions where the causal agent is recognised, the classification of joint diseases depends on the presence or absence of joint inflammation and the distribution of joints affected (Figure 1).

Figure 1 Classification of joint diseases.



Thus, arthritis (Greek, arthron = joint, -itis = inflammation) may affect the peripheral joints or the spine resulting in syndromes such as rheumatoid arthritis (RA) and ankylosing spondylitis (AS). In contrast, the arthroses (-osis = a process) are characterised by minimal inflammation and consist of identifiable conditions such as osteoarthrosis (OA) and cervical spondylosis.

It is increasingly recognised that many of these syndromes overlap so that, for example, certain types of OA may be associated with features of inflammation [Dieppe et al, 1980]. Nevertheless, the division of joint disease into inflammatory and non-inflammatory types is at the root of rheumatological dogma. RA is studied here as the archetypal inflammatory polyarthritis.

1.2 RHEUMATOID ARTHRITIS.

1.2.1 Clinical features.

RA is a syndrome characterised by the presence of a number of distinct features (4 or more are needed for diagnosis). The criteria for diagnosis have been recently revised Table I [Arnett et al, 1987].

Table I 1987 criteria for RA.

1. Morning stiffness in and around joints for at least 1 hour.
2. Soft tissue joint swelling observed by a physician in at least 3 of 14 joint groups (R or L: MCP, PIP, wrist, elbow, knee, ankle, MTP).
3. Soft tissue swelling in a hand joint (MCP, PIP or wrist).
4. Symmetrical swelling of 1 joint area in (2) above.
5. Rheumatoid nodule.
6. Rheumatoid factor by a method that is positive in less than 5% of the normal population.
7. Radiological changes on wrists or hands: erosions or juxta-articular osteoporosis.

RA affects about 1.7 million people in the United Kingdom [Lawrence, 1977]. Typically patients are women of childbearing age who develop a widespread, symmetrical, peripheral polyarthritis. The joints become swollen, tender, warm and are stiff after rest. There may be systemic upset with malaise, anaemia and weight loss. Some patients show extra-articular features such as subcutaneous nodules, polyserositis and vasculitis. The onset is variable and the outcome uncertain. Overall 25% remain with normal function, 40% have moderate impairment, 25% considerable impairment and 10% become wheel-chair bound cripples [Masi et al, 1983]. There is little evidence that treatment alters the outcome [Scott et al, 1987].

1.2.2 Pathology.

An affected joint is characterised by an overgrowth of synovial lining forming great seaweed like villi [Gardner, 1972]. Microscopically there is a mononuclear cell infiltration, with aggregates of lymphocytes and plasma cells forming lymphoid follicles and an increase in the intimal cell layer. The resulting 'chronic' inflammatory granulation tissue (pannus) exists in parallel with an 'acute' inflammatory joint effusion containing polymorphonuclear leucocytes (polymorphs) that migrate from the circulation, cross the synovium and accumulate in the synovial fluid.

1.2.3 Pathogenesis.

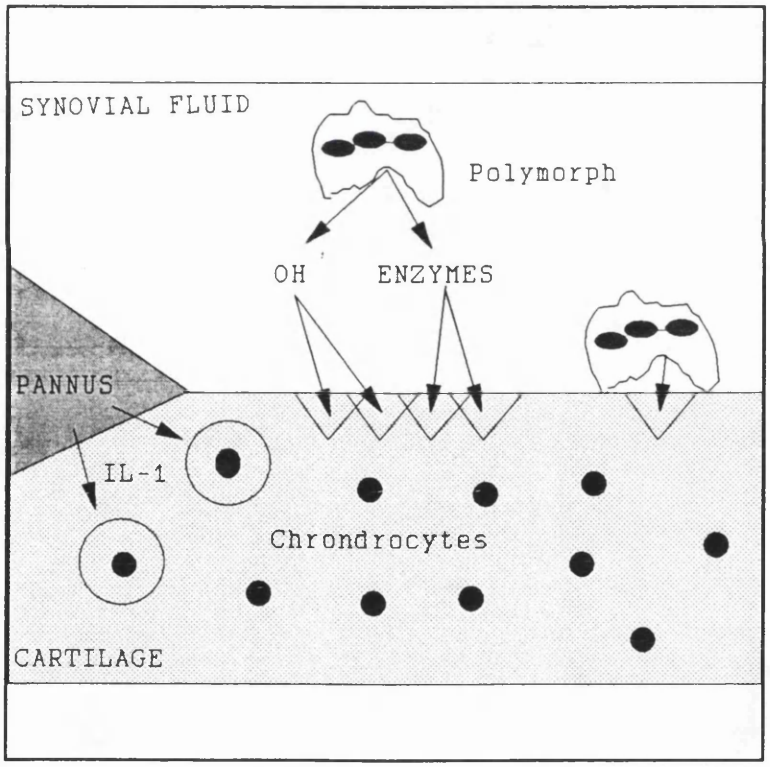
Many of the processes in RA can be explained by the formation of immune complexes. Rheumatoid factors (antibodies directed against the constant fragment, Fc, of immunoglobulins) react with immunoglobulins in the synovial fluid producing immune complexes that fix complement and activate phagocytes [Cochrane, 1967]. Some complexes may form in the circulation and cause extra-articular features in these patients [Melsom, 1984]. The aetiology of rheumatoid factors is unknown but

some abnormality in immune regulation is postulated by most workers in this field. An inflamed rheumatoid joint is typified by the apparent paradox of a 'chronic' inflammatory infiltrate of the synovium occurring in conjunction with an 'acute' inflammatory joint effusion. The ways in which these 2 inflammatory processes lead to cartilage and bone damage will be briefly discussed.

1.2.4 Inflamed synovial membrane (pannus).

Inflammation of the synovial membrane may lead to joint tissue damage in a number of ways that have only recently been recognized (Figure 2).

Figure 2 Pathogenic processes in RA.



Most connective tissues are capable of synthesising and secreting a family of metalloproteinases that are capable of breaking down collagens, elastin and proteoglycans. These tissues also produce a small (Mr 27,500) antiproteinase known

as 'tissue inhibitor of metalloproteinases (TIMP)' [Cawston 1984]. It is thought that these proteinases and inhibitors provide connective tissues with a control mechanism for tissue repair but the details are far from clear. Many forms of synovial pannus share the ability to produce metalloproteinases although often from different cell types, and several metalloproteinases have been localized to the cartilage pannus junction. The production of these enzymes is under the control of a family of cytokine mediators that are potent stimulators of metalloproteinase production. Interleukin-1 (IL-1) is the prototype and is derived from activated macrophages. These mediators are also capable of diffusing through connective tissue and of stimulating chondrocytes to secrete metalloproteinases [Hardingham, 1986]. Therefore pannus mediated cartilage degradation occurs not only by direct invasion where inflamed synovium develops in contact with hyaline cartilage, but also at a distance from the pannus around chondrocytes (Figure 2).

Inflammatory pannus is associated with increased bone turnover although the mechanisms by which this is achieved are less well known than for cartilage. The erosive effects of osteoclasts are usually followed by a repair process due to the coupling effect on osteoblast population. This cellular response accounts for the large number of osteoblasts and the increased bone turnover seen in 'hot' ^{99}Technetium bone scans. The osteoclasts may be seen at the tidemark causing the separation of hyaline cartilage from the damaged subchondral plate and the eroded trabeculae [Bromley and Wooley, 1984]. The balance between bone resorption and repair tips in favour of bone loss despite the increased repair, under the influence of a number of inflammatory mediators such as IL-1, interferon-gamma, osteoblast activating factors, PTH and vitamin D, but the exact sequence of events has yet to be elucidated [Skjodt and Russell, 1985]. Resorption of subchondral bone occurs where pannus is in direct contact with bone causing marginal erosions, and at

a distance, presumably under the influence of diffusing inflammatory mediators, producing peri-articular osteoporosis.

1.2.5 Inflamed synovial fluid and the role of the polymorph

The classic clinical features of joint inflammation (rubor, calor, dolor et turgor) are associated with a joint effusion containing high numbers of polymorphs, so that the polymorph count is considered by many to be the gold standard of joint inflammation [Ropes and Bauer, 1953]. Polymorphs enter the joint from the circulation by migration from synovial capillaries and rapidly transverse the synovium to the joint space under the influence of potent chemotactic molecules such as C5a and leukotriene B4 [Harris, 1985]. The joint is the polymorphs' graveyard and they lyse releasing their contents after taking part in a number of potentially tissue damaging processes. The half-life of a joint polymorph is about 4 hours or less and it has been estimated that a billion cells will enter and die in an averagely inflamed knee joint within 24 hours [Hollingworth et al, 1967].

Under the influence of a number of different stimuli and during lysis, the polymorphs release their granular proteolytic enzymes that are capable of degrading collagens, proteoglycans and elastase [Harris, 1985]. In the synovial fluid these enzymes are inactivated by a family of circulating proteinase inhibitors that diffuse into the joint from the circulation [Cawston, 1984]. In general the levels of inhibitors in joint fluid are in excess of enzyme concentrations so that free, active enzymes cannot be detected. For this reason it has been concluded by many workers that polymorph granular enzymes have little to do with joint damage in inflammatory arthritis [Hadler et al, 1981]. However, many polymorphs release their contents in a microenviroment at the cartilage surface as they frustratedly

try to phagocytose embedded particles (Figure 2). It has suggested that the released enzymes would have uninhibited access to their substrates [Henson, 1971]. Perhaps more importantly it has recently been shown that the cartilage matrix interacts with the binding between enzyme and inhibitor allowing the smaller enzymes to diffuse into the cartilage [Burkhardt et al, 1987]. Furthermore, reactive oxygen species ($OH^{\cdot}$ see Figure 2) are capable of altering proteinase inhibitors; for example, alpha-1-protease inhibitor exposed to stimulated polymorphs fails to bind and inactivate human neutrophil elastase [Johnson and Travis, 1979]. Thus, it is likely that polymorph proteinases play some part in joint destruction as well as causing the pain, swelling and stiffness so characteristic of rheumatoid disease.

1.2.6 Circulating factors

Many of the systemic features of RA are produced in response to circulating messengers released from inflamed joint tissue. The acute phase response is the most well known and is an increased synthesis of a variety of proteins in the liver [Witcher, 1985]. A concomitant fall in other proteins occurs. The role of these proteins and their relationship with one another is poorly understood. Effects on the bone marrow lead to anaemia and changes in platelets as part of the 'anaemia of chronic disorders' [Turnbull,1987] again presumably moderated by circulating factors. The interaction between increased plasma proteins, increased fibrinogen (as part of the acute phase response) and decreased number of red cells causes an increase in the erythrocyte sedimentation rate (ESR).

1.2.7 Treatment.

Our lack of knowledge of the cause of RA has forced us to treat our patients symptomatically. This usually boils down

to treating inflammation and the results of structural damage.

1.2.7.1 Non-steroidal anti-inflammatory drugs (NSAIDs).

NSAIDs are a large group of compounds from several different families that have a short term effect on the clinical features of joint inflammation but have not been shown to have any effect on the systemic response. Their anti-inflammatory effect is mediated by inhibition of prostaglandin synthesis but additional direct effects on the activities on inflammatory cells have been demonstrated. In particular several of these drugs have been shown to inhibit polymorph migration in vivo [Scheja et al 1985; Scheja et al, 1986.]. Despite a considerable clinical effect on inflammation NSAIDs are not thought to have any effect on the production of joint damage [Wright and Amos, 1980].

1.2.7.2 Disease modifying anti-rheumatoid drugs (DMARDs).

DMARDs, such as gold, D-penicillamine, sulphasalazine and chloroquine produce a reduction in the systemic reaction as well as an improvement in clinical joint inflammation. These drugs are slow acting and take up to 6 months to exert their effect [Thompson et al, 1985]. There is a suggestion that treatment with DMARDs slows the progression of joint damage but the case is far from proved and their present use is as medium term anti-inflammatory agents [Goddard and Butler, 1985].

1.2.7.3 Corticosteroids.

These drugs produce a rapid improvement in clinical joint inflammation and the systemic response [McConkey, 1973].

There is evidence that they may reduce the incidence of new joint erosions [Byron and Kirwan, 1986] but they are toxic and difficult to handle.

1.2.7.4 Immunosuppressive drugs.

Azathioprine has been used with good effect in RA. It seems to have a similar effect to classical DMARDs although the onset of action may be a little quicker. More potent drugs such as cylophosphamide and methotrexate are undoubtable effective for extra-articular complications especially vasculitis and their early use is advocated by several workers.

1.2.7.5 Other treatments.

Many novel treatments have been enthusiastically tried often with some success. Most are based on some sort of immune modulation and include total lymphoid irradiation, plasma exchange and cyclosporin A.

1.2.8 Treatment assessment.

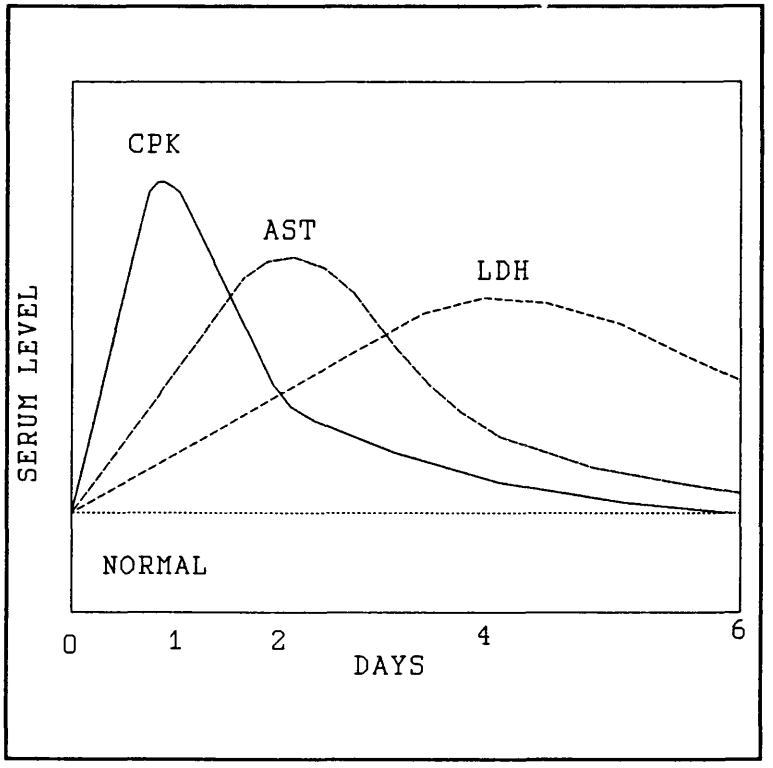
A major problem in the assessment of treatment response in RA is the lack of a 'gold standard' for measuring disease activity. Although of great importance, symptoms and signs are subjective and may be related to structural complications rather than the underlying disease process. Nevertheless many attempts have been made to standardise methods of pain assessment [Huskisson, 1974] and joint examination [Ritchie et al, 1968; Co-operating Clinics, 1965; Lansbury and Haut, 1956; Thompson et al, 1987], and rheumatologists consider such articular indices to be one of the most important assessments in clinical trials [Tugwell and Bombardier, 1982]. Laboratory measures such as the ESR and the acute phase reactants' [Reizenstein, 1979] have been shown to be helpful in the management of patients with inflammatory

exacerbations [McConkey et al, 1972] but they are indirect measures and as pointed out above their relationship to joint inflammation is poorly understood. Recent attempts to determine which of these measures might be most representative [Bull et al, 1986] are to be applauded, and analysis of the pattern of serum biochemical abnormalities [Sitton et al, 1987] may shed light on new aspects of the pathophysiology of arthritis, but there is a need for a marker of joint inflammation that is specific, sensitive, reproducible and makes sound biological sense.

1.3 ENZYMES AS POTENTIAL MARKERS OF TISSUE TURNOVER.

A starting point might be the field of clinical enzymology where intra-cellular enzymes measured in extra-cellular fluids have been used to supply information about tissue events. Any substance that is normally confined within cells or incorporated into tissue structures, but which is released into the extracelluar fluid during cell damage or increased production may act as a marker of tissue turnover. Although many such substances are not enzymes, the principles of clinical enzymology may be applied to illustrate their potential usefulness. The classic sequence of enzyme changes that occur in the serum of patients following myocardial infarction is an example (Figure 3). Specificity relates to tissue distribution of enzymes and has been enormously increased by the advent of immunological assays and the characterisation of isoenzymes. The time course of enzyme appearance in the serum is dependent on molecular size, shape and intracellular position. Creatine phosphokinase (CPK), appearing earlier and showing a more peaked pattern than aspartate transaminase (AST) and lactate dehydrogenase (LDH).

Figure 3 Clinical enzymology of myocardial infarction.



Some progress has been made in quantifying the amount of tissue damage in the acute situation; for example, the area under a curve of serum CPK activity against time has been related to the amount of cardiac muscle damage following myocardial infarction, but this sort of mathematical analysis is still in its infancy [Kaldor, 1983].

In the chronic situation where tissue damage is continuing at a steady rate, at least in the short term, the serum activity of an intracellular enzyme, for example, AST in chronic hepatitis, reflects a balance between input from damaged hepatocytes and removal. Thus, the serum activity will be a direct measure of tissue turnover. While this is undoubtedly an over-simplification the potential for such measures in the assessment of the chronic arthritis is great [Thompson, 1987].

Although the pathogenesis of RA remains obscure much has been learned about the pathological processes. Whether the macrophage precedes the lymphocyte or the polymorph the plasma cell is hotly debated but their co-existence on the battlefield, is not. Thus, measurement of serum levels of enzymes released from these, and other cells involved at the site of inflammation may supply information about different aspects of the disease process. Furthermore, components released into synovial fluid from damaged cartilage, bone and synovium, or resulting from increased production of these tissues, may drain into the blood via the lymphatics allowing serological assessment of the metabolism of these tissues.

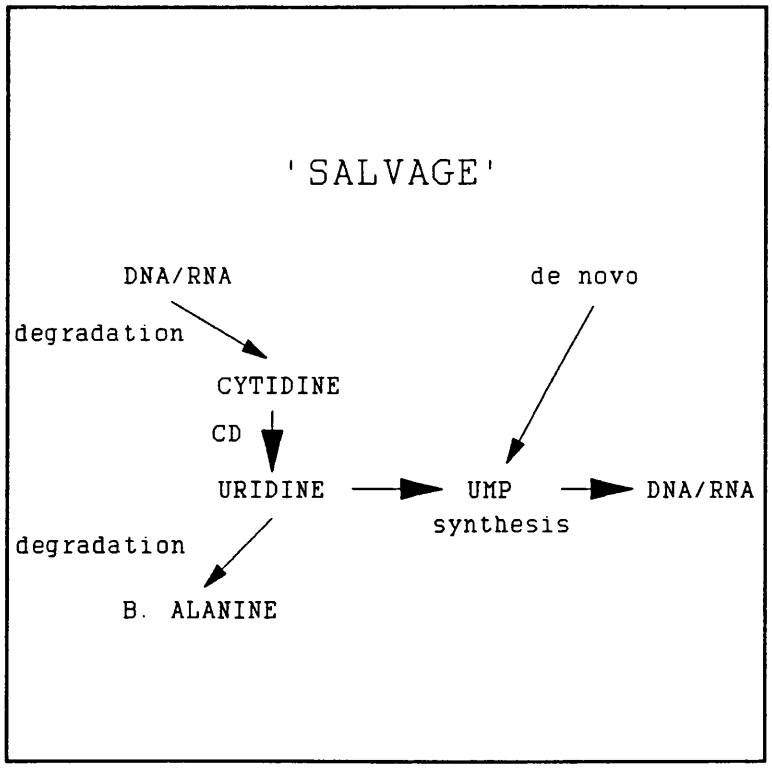
1.4 CYTIDINE DEAMINASE AS A MARKER OF JOINT INFLAMMATION.

Cytidine deaminase (CD) is an intracellular cytoplasmic enzyme that catalyses the hydrolytic deamination of cytidine, deoxycytidine and their analogues to uridine.

$$\text{Cytidine} + H_2O \rightarrow \text{Uridine} + NH_3$$

Although its exact physiological role is unclear, it may provide a 'salvage' pathway for pyrimidine nucleosides during the breakdown and synthesis of nucleic acids (Figure 4) [Jones and Roberts, 1986].

Figure 4 'Salvage' pathways in the metabolism of nucleic acids.



Clinical interest in the enzyme developed when some patients with acute myeloblastic leukaemia proved resistant to treatment with cytosine arabanoside, an anti-leukaemic drug known to be degraded by CD [Steuart and Burke, 1971]. The finding that bone marrow cells in these patients had raised levels of CD led to the suggestion that CD inhibition might be used to augment treatment [Chabot, 1983].

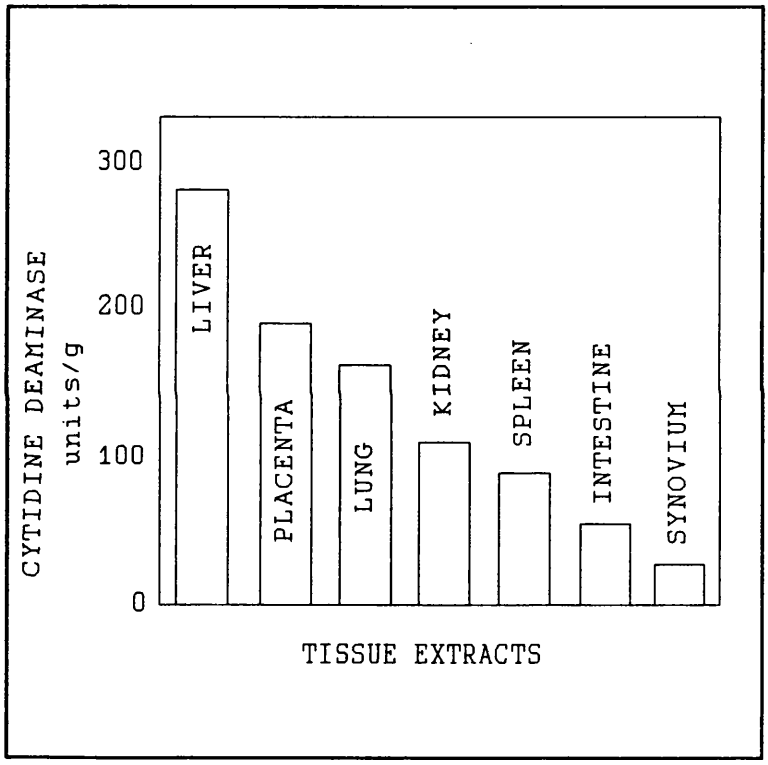
The use of serum CD as a screen for abnormal pregnancy has been the subject of research for Dr D D Jones and colleagues, who have shown that serum CD is a useful serological marker for pre-eclampsia and inter-uterine death although the source of increased serum CD has yet to be elucidated [Bahijri, 1979].

CD is widely distributed in human tissues (Figure 5) with high concentrations in liver and placenta. While direct comparison of concentrations in circulating cells with tissue

extracts cannot be easily made, polymorphs (48 units/10^6 cells) contain approximately 20 times the concentration of mononuclear cells (2 units/10^6 cells), and no CD was detected in erythrocytes [Jones and Roberts, 1986].

In addition to abnormal pregnancy, raised serum CD levels have been found in those conditions where tissues known to contain high levels of CD were damaged (eg. viral hepatitis) or situations where pus (polymorphs) accumulates (eg. cellulitis, septicaemia).

Figure 5 Tissue distribution of CD.



However, normal serum CD levels have been found in conditioned where tissues known to contain little CD were damaged (eg. polymyositis, pancreatitis) or infections without pus (eg. influenza). (Table 2) [Jones and Roberts, 1986].

Table II Serum CD in disease.

RAISED SERUM CD	NORMAL SERUM CD
Hepatitis	Cholestasis
Liver metastases	Cirrhosis
Pyelonephritis	Cystitis
Septacaemia	Pancreatitis
Cellulitis	Myositis
Ulcerative colitis	
Influenza	
Pneumonia	
Osteomyelitis	

1.5 HYPOTHESIS.

In order to explain these findings the hypothesis that CD is released from damaged tissues and diffuses down its concentration gradient into the blood where serum levels might be expected to reflect tissue turnover has been formulated. The high concentration of CD in polymorphs raises the possibility that serum CD might be a marker of polymorph lysis. While several intracellular enzymes are available to monitor liver disease the potential for a biochemical marker of pus seemed high, and may be of use in chronic inflammatory conditions such as RA because huge numbers of polymorphs are attracted to joint cavities where they die releasing their contents. The potential use of serum CD as a measure of polymorph turnover in rheumatoid arthritis is, therefore, the theme of this thesis.

1.6 AIMS.

(i) To review the laws governing the release of macromolecular markers from joint tissues, their distribution in synovial fluid and plasma and the factors affecting deactivation, degradation and elimination.

(ii) To formulate mathematical models of synovial fluid solute kinetics in order to predict the changes anticipated in arthritis.

(iii) To establish and evaluate a biochemical assay of CD with emphasis on measuring repeatability, reproducibility, comparison with a reference laboratory and establishment of a normal range.

(iv) To investigate serum and synovial fluid levels of CD in RA and study the source of the enzyme.

(v) To study the effect of rest, exercise and diurnal variation on serum CD levels.

(vi) To investigate the relationship between serum CD levels and other clinical and serological measures of joint inflammation.

(vii) To investigate the effect of NSAID treatment on serum CD levels in RA.

(viii) To summarise the findings and set the evaluation of CD activity in the overall perspective of the assessment of inflammatory disease, with suggestions for further work.

CHAPTER II

THEORETICAL CONSIDERATIONS

2. THEORETICAL CONSIDERATIONS

2.1 CLINICAL ENZYMOLOGY

2.1.1 Introduction

The hypothesis predicts release of CD from joint polymorphs and diffusion from cells to synovial fluid, across the synovium to blood. Consideration of the factors affecting enzyme activity in body fluids and movement of macromolecules between fluid compartment is, therefore, pertinent.

2.1.2 Enzymes as markers of tissue turnover

Most enzymes are distributed in tissues with steep concentration gradients between intracellular and extracelluar compartments. The coagulation system is a notable exception. Under physiological conditions the relative contribution made to the extracelluar enzyme pool from wear and tear of individual organs depends on the tissue mass and surface area, accessibility to the extracellular compartment and concentration of the enzyme in the tissue. Accordingly, the red cell mass, skeletal and cardiac muscle, liver, central nervous system and bones are important contributors to the normal levels of the various serum enzymes. Altered levels of enzymes detectable in body fluids make useful markers of pathological change in cells and tissues. In the majority of applications of enzymes in diagnosis, the sample analyzed is blood plasma or serum. Interpretation of the results requires an understanding of those factors which underlie the correlation of enzyme levels in plasma with those in their cells of origin.

2.1.3 Factors affecting extracelluar enzyme levels.

Abnormal conditions that results in increased blood enzyme activities include [Kaldor, 1983]:

(i) Tissue injury, eg, myocardial infarction, hepatitis or haemolysis.

(ii) Functional impairment of cells that leads to increased membrane permeability, eg, hypoxia and toxins.

(iii) Increased tissue mass, eg, tumour growth

(iv) Impaired enzyme excretion through a preformed duct, eg, pancreatic duct obstruction..

(v) Enzyme induction, eg, gamma-glutamyl transferase and alcohol abuse.

Decreased enzyme activity in the blood may be caused by the following, although it is a less common event:

(a) Genetic abnormalities resulting in reduced enzyme production.

(b) Tissue atrophy.

(c) Malnutrition.

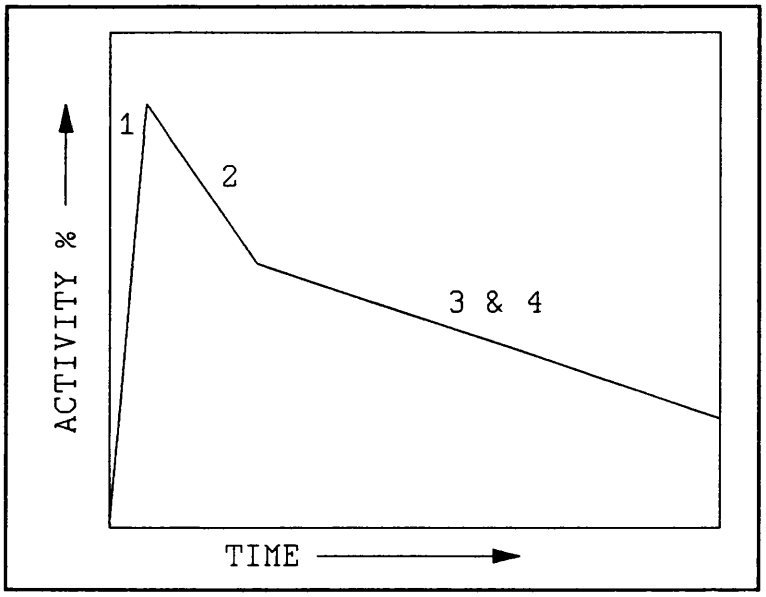
(d) Circulating enzyme inhibitors.

2.1.4 Enzyme elimination.

The activity of an enzyme in the blood is dependent not only on the rate of release of enzymes from tissues, but also the rate of inactivation and removal from the blood.

The fate of a labelled homologous tissue enzyme injected into the circulation has been divided into 4 phases [Schmidt and Schmidt, 1976] (Figure 6).

Figure 6 Enzyme elimination.



Phase 1 is heralded by the injection, phase 2 is marked by a rapid decrease of enzymic activity in the blood due to redistribution of enzyme in the extracelluar spaces. Phases 3 and 4 are characterised by a steady decline in enzyme activity due to degradation and elimination. Using this technique most investigators have found that enzyme disappearance follows first order kinetics, that is, the elimination rate is directly proportional to the plasma enzyme level [Dawson, et al 1969]. This phase of the disappearance curve is thought to be related to the action of circulating proteolytic enzymes, oxidants, fatty acids toxins and the reticulo-endothelial system [Friedel et al, 1976]. Amylase passes freely into the urine [Levitt et al, 1969] but most enzymes are too large to be eliminated by this route. In general, the rates of clearance of enzymes from the circulation have a negligible influence on changes in serum enzyme levels in disease [Moss, 1981].

2.1.5 Enzyme Inhibitors.

Some enzymes are inactivated in extracelluar fluid by specific inhibitors. This usually occurs with enzymes that

are toxic to tissues. Leucocyte elastase is an example. This enzyme is released by activated phagocytes from specific granules. It has potent tissue destroying properties but is rapidly and irreversibly bound by circulation α_1-protease inhibitor. While the enzyme inhibitor complex can be detected by immunological means in situations of phagocyte activation [Adeyemi et al, 1986] these patients' sera show little increased circulating enzyme activity [Cooke et al, 1984], reducing the usefulness of serum leucocyte elastase activity as a measure of polymorph activation.

2.2 SYNOVIAL FLUID SOLUTE KINETICS

2.2.1 Formation of synovial fluid.

William Hunter considered that the 'dewy fluid' of synovial joints was derived from synovial capillaries [Hunter, 1743] and indeed synovial fluid is an ultrafiltrate of plasma across a leaky semipermeable membrane into which hyaluronate is secreted [Bauer et al, 1930]. In the normal knee joint synovial fluid is formed at a rate of ~0.3mL/min/m^2 [Levick, 1984]. The rate of production may be many times normal in inflamed rheumatoid joints. Flows of 5mL/hour have been observed following knee joint aspiration [Palmer and Myers, 1968], although in steady state conditions flows might be less [Levick, 1983].

Studies of the ratio between synovial fluid and plasma concentrations of proteins suggests that the blood/joint barrier is a 2 membrane structure. Permeability to molecules entering the joint increases with inflammation with proportionally greater increases in permeability to larger proteins [Kushner and Somerville, 1971; Levick, 1981].

2.2.2 Joint effusion volumes.

Measurement of effusion volumes using isotope dilution techniques has revealed that the intracapsular distribution volume of inert solutes is approximately twice the synovial fluid volume obtained by aspiration. In one study of 11 RA knee effusions the mean aspiration volume was 33mL, mean and the mean intraarticular distribution volume of ^{131}I-labelled serum albumin was 106 mL [Wallis et al, 1985]. While some of this discrepancy may by due to loculation of synovial fluid, it is likely that aspirated volume underestimates the physiological joint cavity. Since tight junctions are not observed between synovial lining cells, synovial fluid may be considered a fluid phase extension of the extracelluar matrix in synovial tissues [Hadler, 1981].

2.2.3 Removal of synovial fluid from joints

The subintimal lymphatic capillaries are thin walled, blind ended tubes of endothelium with terminal saccular dilatations. They do not communicate directly with the joint cavity but form draining vessels with valves. From the knee these empty into lymphatic trunks which accompany the femoral vascular bundle and empty into the iliac nodes [Elves, 1978]. The net filtration from the microcirculation into the joint cavity is balanced, in the steady state, by the removal of fluid via the lymphatic system. Insoluble particles, proteins and water are absorbed by the synovial lymphatic plexus [Antonas et al, 1973; Bauer et al, 1933]. The flow of lymph is powered mainly by joint motion [Adkins and Davies, 1940; Bauer et al, 1933; Levick, 1980], so it is likely that fluid balance in the immobile joint can only be achieved at the expense of increased volume and pressure, particularly if the limb is dependent. This may account for the large volume of fluid found in the ankle joints of cattle [Levick, 1984]. After prolonged vigorous exercise the synovial fluid volume of normal joints increases suggesting that the net filtration

volume may exceed the capacity of the lymphatic pump [Ekholm and Norback, 1951].

The removal of ^{131}I-labelled albumin injected into human arthritic joints suggests that the clearance rate follows first order kinetics and is greater in RA effusions than OA effusions. A mean clearance rate of 4.3 mL/hour was recorded for 11 RA patients undertaking normal activity [Wallis et al, 1985].

2.3 MARKER CONCENTRATION IN BODY FLUIDS

If enzyme markers of tissue turnover are to be clinically useful in arthritis it is of fundamental importance to determine the relationship between the marker concentration in synovial fluid and blood and the rate of input of the marker from the tissue in question.

2.3.1 Marker concentration in synovial fluid.

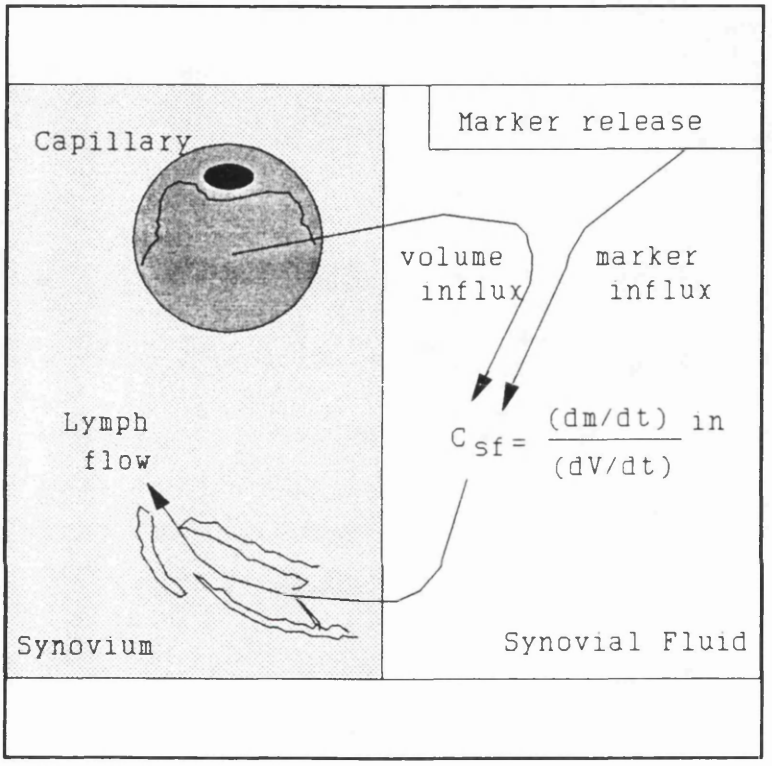
There are reasons to favour measuring marker concentrations in synovial fluid. It is fairly easy to obtain, examination of a sample allows investigation of a single joint and locally produced markers are likely to be more concentrated than blood levels because of dilution. But is marker concentration in synovial fluid a valid quantitative measure of arthritic tissue turnover?

To answer this the physiology of synovial fluid circulation and the movement of macromolecules between fluid compartments must be examined (Figure 7).

Marker macromolecules released from joint tissues into synovial fluid will be distributed throughout the joint cavity. In steady state conditions when the volume of the joint effusion remains the same because the lymphatic drainage balances the formation of synovial fluid, the

concentration of the marker in the fluid will depend not only on the rate of release of tissue marker but also on the rate of turnover of synovial fluid. As the latter may vary many fold in arthritis, the concentration of a marker in synovial fluid is not a direct measure of tissue turnover in the joint.

Figure 7 Synovial fluid solute kinetics.



2.3.1.1 Mathematical model

Let the net synovial fluid volume (V) inflow be dV/dt, where t is time. In the steady state volume balance is preserved by lymph flow L therefore:

$$dV/dt = L \qquad \text{(i)}$$

Let the concentration of marker in lymph be C_L, therefore, marker leaves the joint at rate,

$$C_L \times L \qquad \text{(ii)}$$

Let the net rate of entry of marker mass m into the joint (influx minus local degradation or cellular uptake) be $(dm/dt)_{in}$. The law of conservation of mass requires that influx equals efflux in a steady state, therefore:

$$(dm/dt)_{in} = C_L \times L \qquad \text{(iii)}$$

Let the marker concentration in synovial fluid be C_{sf}. If transport of fluid and solute from joint cavity to subsynovial lymph vessel is convective (bulkflow) and involves negligible molecular sieving (Negligible molecular sieving implies that the marker must be small relative to the size of the interstitial meshwork [Levick, 1984]), then:

$$C_{sf} = C_L \qquad \text{(iv)}$$

But, from (iii):

$$C_L = (dm/dt)_{in}/L$$

Substituting (i) and (iv) into the above expression:

$$C_{sf} = (dm/dt)_{in}/(dV/dt) \qquad \text{(v)}$$

Thus marker concentration in synovial fluid equals the ratio of net marker influx to net volume influx [Levick, 1981]. (For very large particles a sieving coefficient may quantify (iv) and so appear in the denominator of (v)).

Equation (v) shows clearly that marker concentration in synovial fluid depends as much on volume turnover (dV/dt) as on tissue turnover (dm/dt).

2.3.2 Marker concentration in plasma.

Blood is easier to sample than synovial fluid, with little inconvenience to the patient. Marker molecules enter the

circulation via the thoracic duct. In steady state conditions, when the concentration in blood is constant, the input from all joints will be balanced by elimination from the blood. For many markers, including most cytoplasmic enzymes, the rate of elimination is directly proportional to the blood concentration and is not altered by the arthritic processes (first order kinetics) [Kaldor, 1983]. The blood volume is fairly constant and is not affected by arthritic disease to any great extent, so that blood marker concentration is directly proportional to the marker input from all joints and represents an integrated measure of tissue turnover in all joints.

2.3.2.1 Mathematical model

Let the marker concentration in plasma be C_p, and the volume of plasma V_p, marker molecules enter the blood at a rate that is the sum of the inputs from each joint, that is, $\Sigma(dm/dt)_{in}$.

Let the elimination of marker from the plasma by whatever route, be $(dm/dt)_e$.

In steady state conditions when the concentration of marker in the plasma is constant, then:

$$\Sigma(dm/dt)_{in} = (dm/dt)_e \qquad \text{(vi)}$$

The elimination rate by any of these mechanisms will vary with the plasma concentration.

Let f be a function relating elimination rate to plasma concentration,therefore:

$$\Sigma(dm/dt)_{in} = (dm/dt)_e = f.C_p \qquad \text{(vii)}$$

To proceed further the nature of f, the function describing elimination, must be defined. In the case of released enzymes like post-infarct LDH (and presumably synovial fluid cytidine deaminase), marker is eliminated from plasma with first order kinetics in transient (cf steady state) situations, that is:

$$C_p = C_o^{exp-t/r} \qquad \text{(viii)}$$

Where C_o is marker concentration at time (t) zero, and r is the elimination rate constant. Differentiation of (viii) gives:

$$dC_p/dt = -C_p/r$$

Substitution of $C_p = m/V_p$ (where m is total marker mass and V_p is plasma volume) gives:

$$dm/dt.V_p = -C_p/r$$

which becomes:

$$-(dm/dt)_e = -Cp.V_p/r \qquad \text{(ix)}$$

Here -dm/dt is the elimination rate $(dm/dt)_e$, the minus sign signifying an elimination process. Substituting (ix) into the law of conservation of mass (equation (vii)) gives, after re-arrangement:

$$C_p = r.\Sigma(dm/dt)_{in}/V_p \qquad \text{(x)}$$

Thus, the plasma concentration of marker depends on integrated marker influx from all joints; on plasma volume; and on elimination time constant. Because arthritis directly affects $\Sigma(dm/dt)_{in}$ but has little direct effect on V_p or r, therefore:

$$C_p \quad \alpha \quad \Sigma(dm/dt)_{in} \qquad \text{(xi)}$$

Plasma marker concentration is directly proportional to tissue turnover in all joints. It thus provides a

quantitative guide to marker production rate and hence tissue turnover.

2.3.3 Predicted CD activity in synovial fluid.

Applying some figures to these calculations in order to estimate the CD concentration in an inflamed joint and the contribution made by that joint to the blood.

In a moderately inflamed rheumatoid knee joint with a polymorph count of 10×10^9 cells/L and synovial fluid volume of 30mL, and assuming that the halflife of a synovial fluid polymorph is 4 hours [Hollingsworth et al, 1967], the consumption of polymorphs in 24 hours will be approximately 0.5×10^9 cells.

A polymorph contains about 4.7×10^{-5} units CD in its cytoplasm [Jones and Roberts, 1986], therefore approximately 23500 units of CD would be released into the knee joint synovial fluid during the 24 hour period, that is, $(dm/dt)_{in}$ = 23500/24 unit/hour = 979 units/hour)

Assuming a volume flux of 5mL/hour in an inflamed rheumatoid knee, that is, dV/dt = 5mL/hour

Applying equation (v) and assuming no local enzyme inhibition, then the estimated synovial fluid CD concentration is:

$$C_{sf} = 979/5 = 196 \text{units/mL} \qquad (x)$$

Note that the concentration is independent of the joint effusion volume.

2.3.4 Predicted CD activity in plasma

The estimated plasma CD concentration, C_p, is an integrated measure of input from all joints, equation (x). For simplicity CD input from only one joint (a monoarthitis) is assumed, so that:

$$\Sigma(dm/dt)_p = (dm/dt)_{in} \qquad \text{(xi)}$$

From equation (x) the concentration of CD in the plasma, C_p, is dependent on the input from the knee ($(dm/dt)_{in}$ = 1958 units/hour), volume of plasma (V_p = 3000mL) and the elimination constant, r.

Assuming first order kinetics, $r = 0.693/T_{1/2}$ where $T_{1/2}$ is the half-life of the marker [Kaldor,1983]. Therefore,

$$C_p = 979 \times 0.693/T_{1/2} \times 3000 \qquad \text{(xii)}$$

Unfortunately, the half-life of human CD is unknown so the calculation cannot be carried further at present.

CHAPTER III

CYTIDINE DEAMINASE ASSAY

3. CYTIDINE DEAMINASE ASSAY

3.1 INTRODUCTION

CD catalyses the hydroxylation of cytidine with the formation of uridine and ammonia.

$$\text{Cytidine} + H_2O \xrightarrow{\text{CD}} \text{Uridine} + NH_3$$

CD activity can be assessed by measuring the decrease in the substrate, cytidine or the increase in the products, uridine and ammonia. Estimation of the nucleosides requires the use of radioactive isotopes [Malathi and Silber, 1971] or high performance liquid chromatography (HPLC) [Richards et al, 1988]. Measuring liberated ammonia by colorimetry is easier and does not require sophisticated equipment.

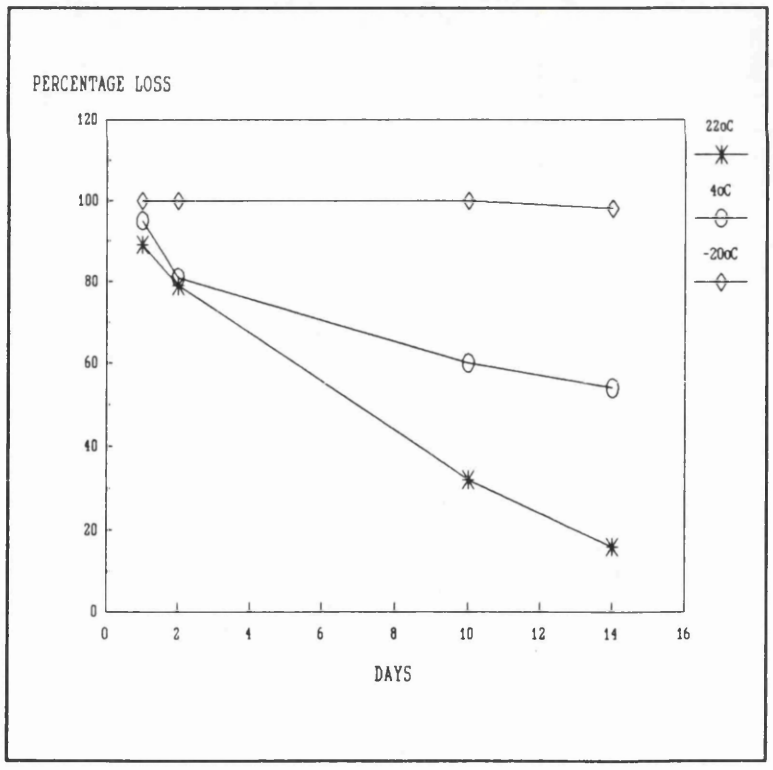
Several fixed time colorimetric techniques have been described [Jones and Roberts, 1986; Jones et al, 1982; Williams, and Jones, 1975]. They have been applied to the estimation of CD activity [Jones et al, 1982] and a method that utilizes the Berthelot reaction without protein precipitation after 18 hour incubation at 22°C has been recommended [Jones and Roberts, 1986] (see appendix I). A shorter method involving a 4 hour incubation at 37°C [Targett-Adams et al, 1975] has been described but uses a kinetic technique involving the oxidation of $NADH_2$ during the enzymic amination of α-ketoglutamate. The Berthelot reaction method with or without protein precipitation, modified Bertholet using sodium salicylate and the kinetic method for ammonia estimation produce similar results [Bahijri, 1979] but the modified Bertholet reaction has the advantage of a stable colour complex without the tedious process of protein precipitation or continuous monitoring.

For the purposes of CD research at the Bone and Joint Research Unit the modified Bethelot reaction without protein

precipitation was chosen following a 4 hour deamination incubation at 37°C. The 4 hour incubation time allowed assays to be set up in the morning and read during the afternoon of the same day. Furthermore, it was easier to maintain the incubation temperature at 37°C than 22°C during hot weather.

Jones and Roberts [1986] have recommended that samples taken for CD assay should be assayed immediately or stored at -20°C because of loss of activity with storage over time. The deterioration is related to temperature and is significant at 22°C (approximately room temperature). Figure 8 shows the percentage loss in enzyme activity with storage at different temperatures over 2 weeks (mean of 10 samples) [Jones and Roberts, 1986].

Figure 8 The percentage loss in CD activity with storage at different temperatures (mean results, n=10).



This chapter reports the results of a study designed to evaluate this short assay method undertaken in the Bone and

Joint Research Unit in comparison with assays using an established method at the reference laboratory (Bronglais Hospital) together with assessment of a reference range. Evaluation included the analysis recommended by Bland and Altman [1986], assessment of intrabatch and interbatch variation, the establishment of a normal reference range and the effects of short and long term storage.

3.2 METHODS

3.2.1 Assay (see Appendix I for details).

The method was a modification of that of Jones [Jones et al, 1982] which involved: (i) Deamination:

$$\text{Cytidine} + H_2O \xrightarrow[\text{CD and pH 9.2}]{} \text{uridine} + NH_3$$

(ii) Ammonia estimation:

$$NH_3 + \text{phenol/nitroprusside/hypochlorite} \xrightarrow[40\text{ minutes, }37^\circ C]{} \text{BLUE}$$

The deamination step was carried out at 37°C for 4 hours at the test laboratory and at 22°C for 18 hours at the reference laboratory.

3.2.2 Samples.

All samples were obtained at the study hospital and consisted of sera from clotted blood spun at 1000g for 10 minutes. Samples were divided into 1 mL aliquots, coded and stored at -20°C within 4 hours of venepuncture. Selected aliquots were packed in solid carbon dioxide and sent to Aberystwyth by the British Rail Red Star service and arrived frozen within 18 hours of departure in all cases. All samples were assayed

in blind fashion within 4 weeks of venepuncture. Blood was taken from normal hospital personnel and patients with RA.

3.2.3 Short term storage at room temperature

10 synovial fluid samples from RA knee effusions were collected and separated into 5 aliquots which were allowed to stand at in a room maintained at 22°C. At 0, 1, 2, 4 and 24 hours the aliquots were spun at 1000g for 10 minutes and the supernatants immediately frozen at -20°C. All aliquots were assayed in duplicate within 1 week of freezing in the same batch.

3.2.4 Long term storage at -20°C.

17 synovial fluid aliquots samples from RA patients were assayed within 4 hours of collection and after storage at -20°C for 6 months.

3.2.5 Analysis

Comparison of the results of the two methods made use of scattergrams and estimation of the correlation coefficient (r) [Snedecor and Cochran, 1980]. Estimation of the correlation coefficient, commonly used to compare 2 methods, gives information concerning the relationship between the results but does not help sort out how much the new measure differs from the old. An alternative analysis [Altman and Bland, 1983] has been described for a medical readership [Bland and Altman, 1986]. The technique allows estimation of:

- 3.2.5.1 Bias (mean difference between the methods).
- 3.2.5.2 Limits of agreement.
- 3.2.5.3 Precision of the limits of agreement.

3.2.5.4 The relationship between the difference and the mean.

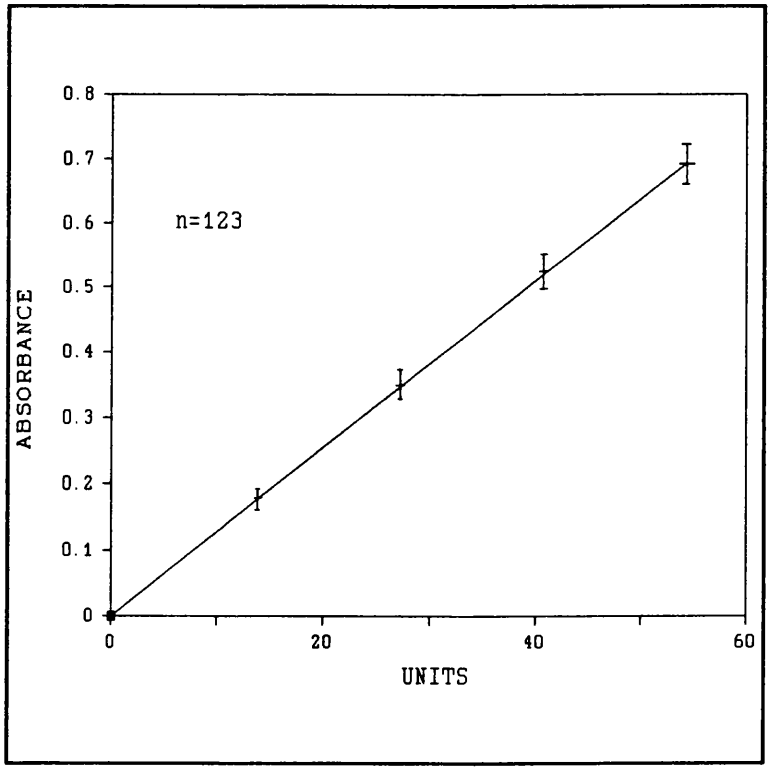
Intrabatch and interbatch variation and reproducibility of the standards was assessed by the coefficient of variation [Snedecor and Cochran, 1980]. Results in men and women were compared using Student's t test.

3.3 RESULTS.

3.3.1 Calibration curve.

Figure 9 shows the mean (SD) results for 123 sets of standards from 41 different assay runs using the same stock ammonia solution. The coefficient of variation for the 13.5, 27.0, 40.5 and 54.0 units standards were: 9.7%; 6.6%; 5.2%, and; 4.2% respectively.

Figure 9 Standard curve (mean(SD) units).



3.3.2 Agreement with reference laboratory.

A scattergram of the results of 120 samples assayed at both laboratories is shown in Figure 10. The coefficient of correlation was $r=0.96$, $p<0.0001$.

Figure 10 A scattergram of the CD assay results of the London Hospital versus Bronglais Hospital.

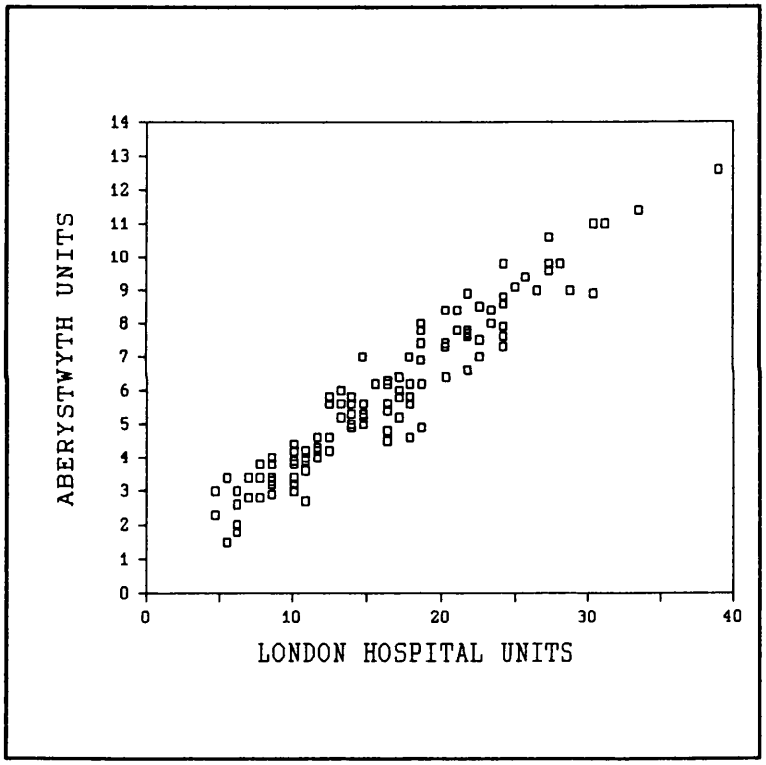


Figure 11 shows a scattergram of the CD results from the London Hospital versus the results from Aberystwyth corrected for temperature using the equation published by Jones: $CD_{37oC} = CD_{22oC} \times 3.25$ [Jones and Roberts, 1986].

Figure 11 A scattergram of the CD assay results from London Hospital versus Bronglais Hospital, corrected for temperature.

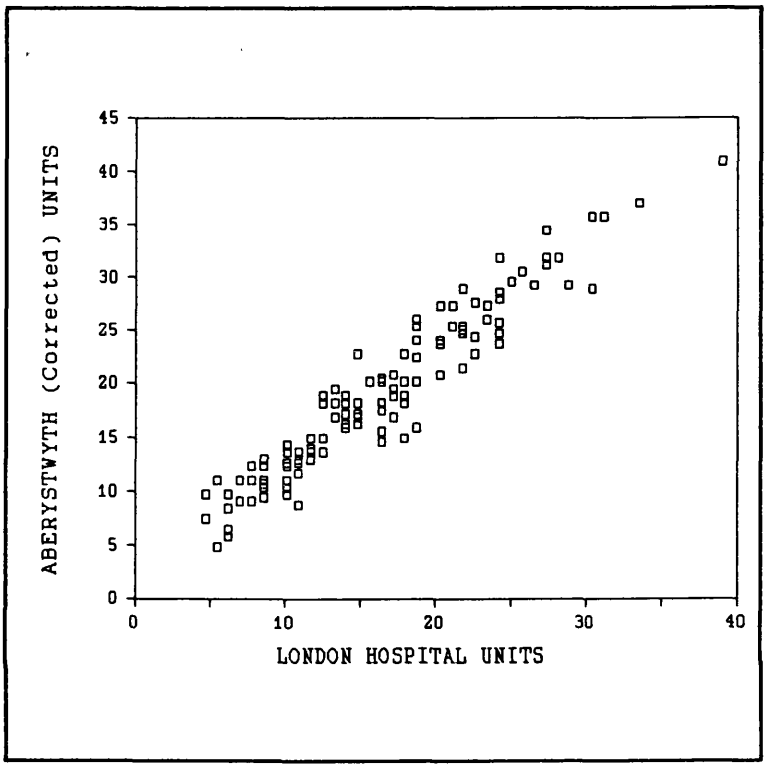


Figure 12 shows the plot of the difference between reference laboratory and the study laboratory results against the mean results. The results were randomly distributed. The mean (SD) difference between them, (bias, d) was -2.7(4.4) units.

The addition of d to the results from the study laboratory removed the bias between the measures (mean difference between the measures = 0) and is shown in Figure 13 with the limits of confidence (d + 2SD to d - 2SD).

Figure 12 Bias.

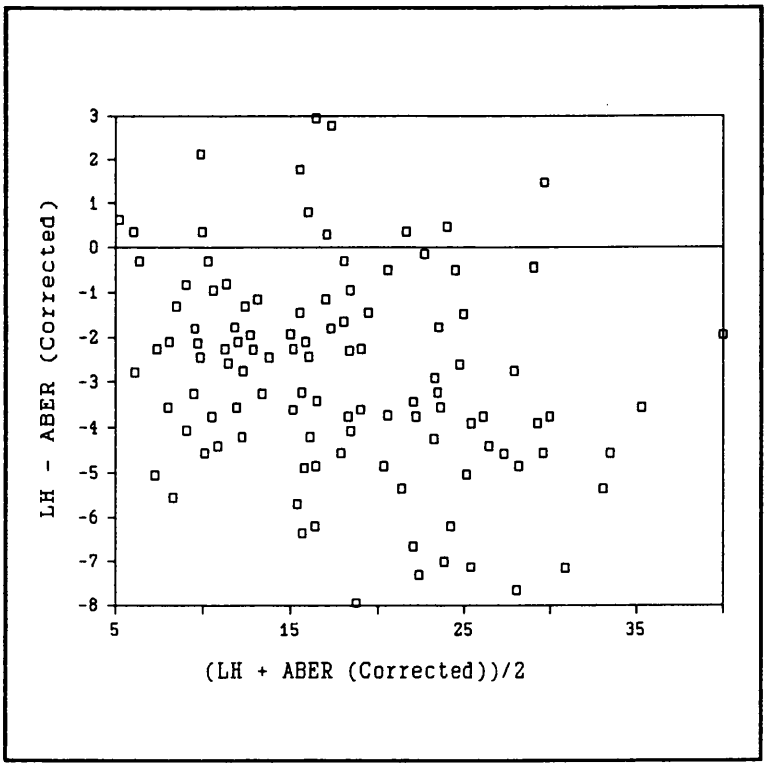
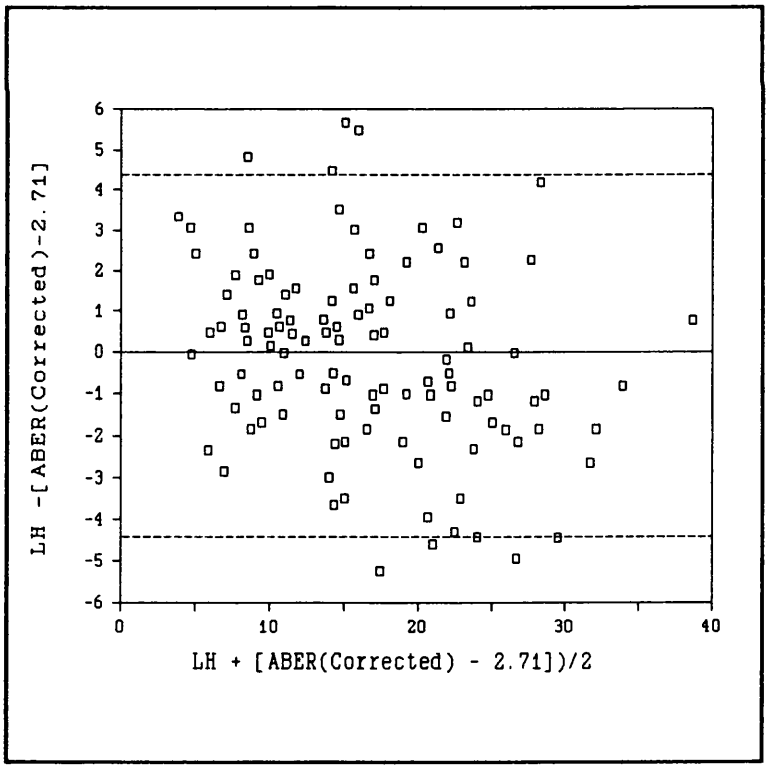


Figure 13 Limits of confidence.



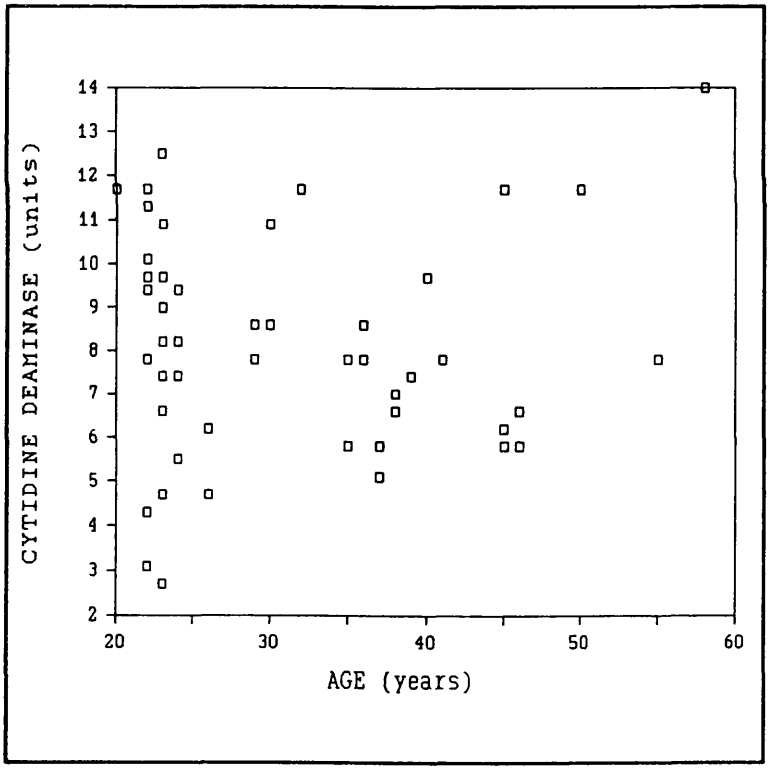
3.3.3 Intrabatch and interbatch variation.

The intra-batch coefficient of variation for samples of mean (SD) CD activity, 11.3 (1.0), and 26.6 (1.1) were, 8.5 percent, 4.1 percent respectively (n=19). The inter-batch variation for samples of mean (SD) CD activity, 10.2 (1.0) units, 17.5 (1.2) units and 31.7 (1.7)units were, 9.8 percent, 6.9 percent and 5.4 percent respectively (n=26).

3.3.4 Reference range.

The mean (SD) CD activity for 53 normal controls was 8.2 (2.5) units and the distribution at different ages shown in Figure 14.

Figure 14 Reference range.

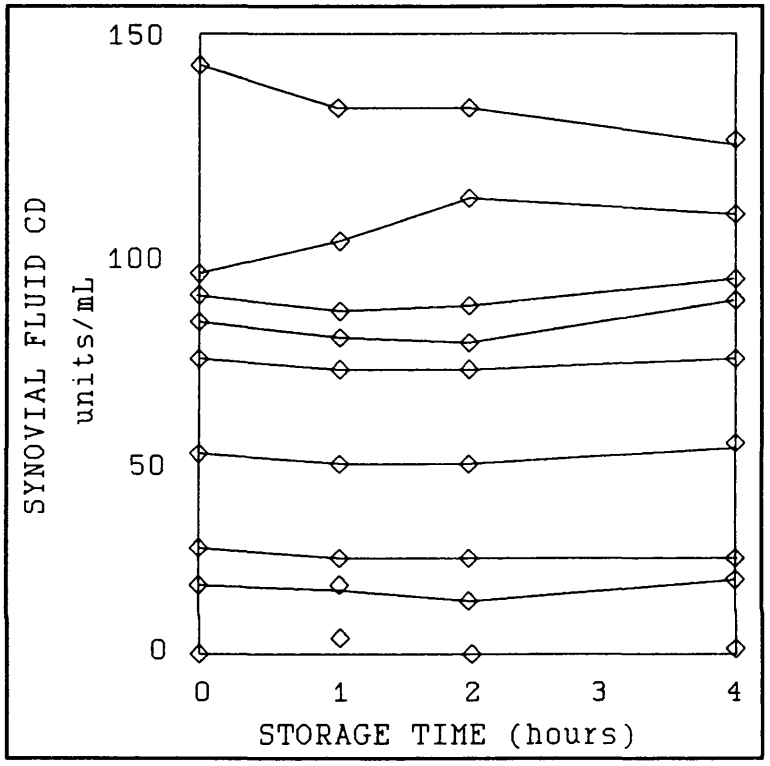


The mean (SD) CD activity in 31 men and 22 women was 8.7 (2.6) and 7.5 (2.1) respectively ($p>0.05$, t test).

3.3.5 Storage

Short term storage at room temperature and long term storage at -20oC are shown in Figures 15 and 16.

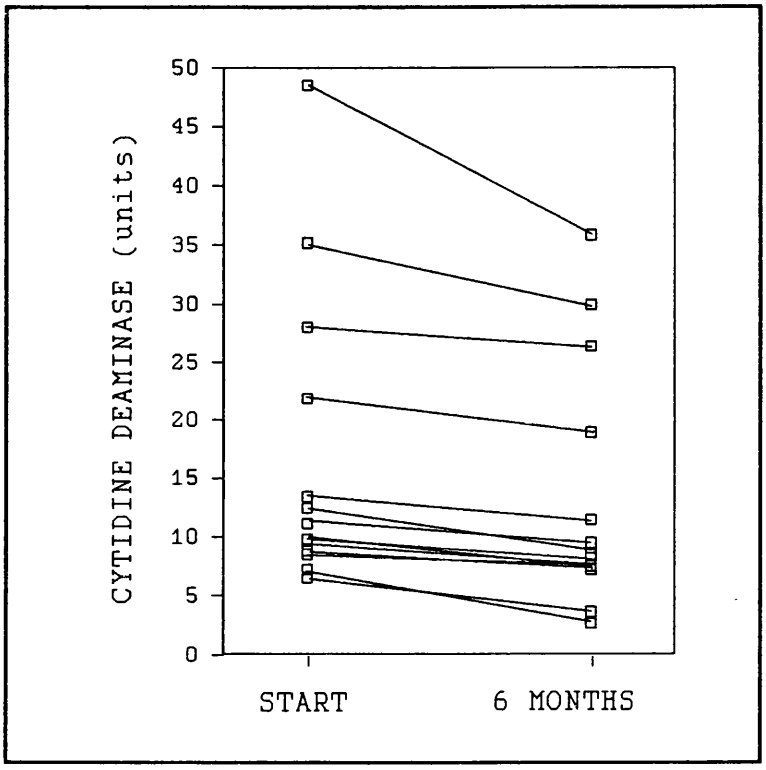
Figure 15 The effect of short term storage at 22°C on CD activity.



3.4 DISCUSSION

The results suggest that the modified CD assay can be established in a modest laboratory using simple equipment and produce measurements that are clinically useful. They support the suggestion that the analysis of Bland and Altman supplies information about the relationship between different methods that is not apparent from simple correlation.

Figure 16 The effect of long term storage at -20°C on CD activity.



3.4.1 Bias.

The reason for the lower results given by the study method (mean difference (bias) = 2.71 units) was discovered to be variation in the ammonia concentration used to make up the standards. The conversion factor reported by Jones [Jones and Roberts, 1986] has been confirmed during an 18 month period in an external quality control scheme (Lewis J, Jones DD. Personal communication).

3.4.2 Limits of agreement.

The limits of agreement suggest that the result obtained by the study method may be up to 7.1 units above or 1.7 units below the result obtained by the reference method. This discrepancy is not obvious from the scattergram shown in Figure 10 or the high correlation (r=0.96) between the methods. Patients with rheumatoid arthritis have serum CD

levels ranging from 5 to 50 units. In this context the limits of agreement are sufficiently small to allow the methods to be used interchangeably without significant loss of clinically relevant information.

3.4.3 Variation.

The intrabatch and interbatch variation reported here is in keeping with other reports [Jones and Roberts, 1986; Jones et al, 1982]. The variation was greater at low levels of CD activity and related to a standard deviation of about 1.0 units, the lower mean values producing greater variation. This suggests that the error is fixed and is most likely to be related to inaccuracy in aliquoting the serum samples.

3.4.4 Normal range.

No difference between sexes or at different ages in the reference population was found but the very elderly have not been studied and may show different values.

3.4.5 Storage

The results suggest that short term storage of synovial samples at room temperature and long term storage at -20°C produces little effect on the CD activity of the samples. Taken with the data of Jones, therefore, is has not been necessary to calculate a correction factor for use in the studies that follow.

3.4.6 Improvements.

The problem of sampling inaccuracy may be overcome using a manual diluter (Finnpipette Diluter, Labsystems) that flushes each serum sample with buffer or substrate. However, as the aim of the study was to test a system that used basic equipment the data have not been reported.

The variation in the standard curves resulting from differences in ammonia concentration of the stock solutions was corrected following the production of a composite 'standard curve' which was stored on a computer programme. Subsequently, each batch of samples was run with an internal control instead of a set of standards. In this way variation between laboratories has been reduced and bias eliminated.

CHAPTER IV

ORIGIN OF SERUM CYTIDINE DEAMINASE

4. ORIGIN OF SERUM CYTIDINE DEAMINASE

4.1 INTRODUCTION

This chapter describes a series of studies designed to test the hypothesis that CD is released from dead and damaged synovial fluid polymorphs and drains from the joint into blood.

4.2 METHODS.

4.2.1 Samples.

Samples of blood, synovial fluid and synovium were collected from patients with OA and definite or classical RA [Ropes et al, 1958] during routine venepuncture, joint aspiration or joint replacement at The London Hospital.

Blood was taken into ethylenediaminetetra-acetic acid (EDTA)or plain glass tubes. Synovial fluid samples collected without anticoagulant were centrifuged at 1000g for 10 minutes and the supernatant separated. In some cases a recorded volume of synovial fluid was centrifuged and the pellet which adhered to the bottom of the tube separated from the supernatant.

All samples were stored at -22°C within 4 hours of processing.

4.2.2 Cell counts.

Synovial fluid leucocyte counts were performed with a haemocytometer (Hawksley BS 748) on samples collected into EDTA, and differential cell counts performed on stained smears of EDTA synovial fluid by the author without knowledge of the fluid CD activity.

The CD activity in the cellular component of a unit volume of synovial fluid was calculated from the following equation.

$$\text{CD activity in the cells from 1 mL synovial fluid} = \frac{\text{Total CD of synovial fluid sample}}{\text{Volume of synovial fluid in mL}}$$

4.2.3 Tissue processing.

Synovium and cellular pellets were homogenised with distilled water in a tissue grinder. The extract was ultrasonicated for 10 minutes at 8 kHz and then centrifuged to remove debris.

4.2.4 Cytidine deaminase assay.

Samples were thawed within 4 weeks of freezing and analysed without knowledge of the patient's diagnosis or condition. Estimations of CD activity were carried out as previously described [Chapter III]. For fluids the activity is expressed as units/mL, for tissues as units/gm wet weight and for cells as units/10^6cells.

4.2.5 Analysis.

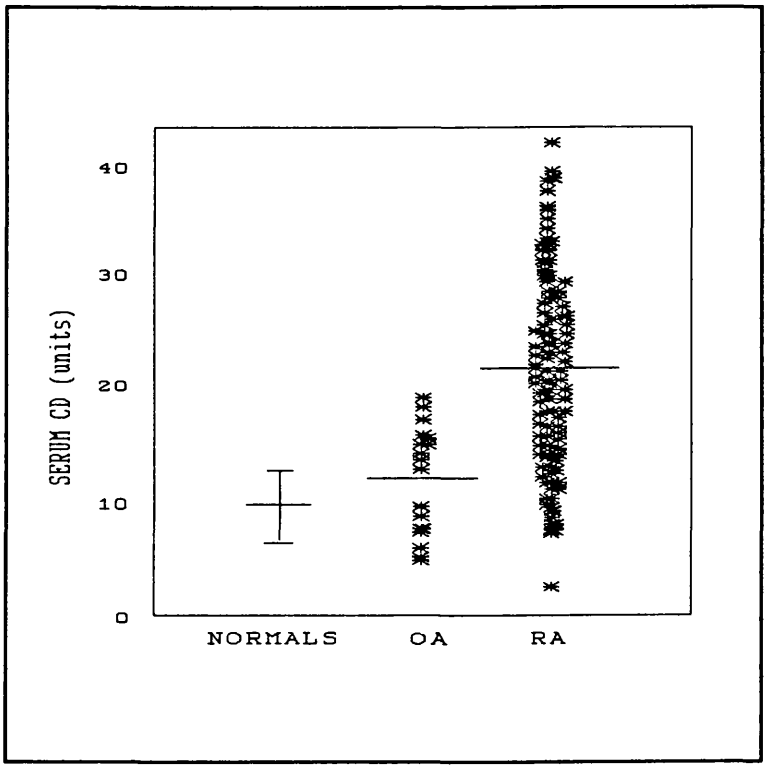
Analysis employed the techniques of linear regression and Student's t test.

4.3 RESULTS.

4.3.1 Serum.

There was a large variation in the serum CD activity among the 88 RA patients tested (Figure 17). The mean CD activity (17.9 units/mL) was significantly higher than in both OA patients (9.8 units/mL, n=19), a reference population of Welsh students (7.8 units/mL, n=208), and our reference range of 8.2 units/mL ($p<0.01$).

Figure 17 Serum CD in arthritis.



4.3.2 Synovial fluid.

Synovial fluid CD activity was markedly raised in 39 RA patients compared with 11 OA patients (Figure 18).

There was significant positive correlation between synovial fluid CD and the synovial fluid polymorph count (r=0.93) (Figure 19).

Figure 18 Synovial fluid CD.

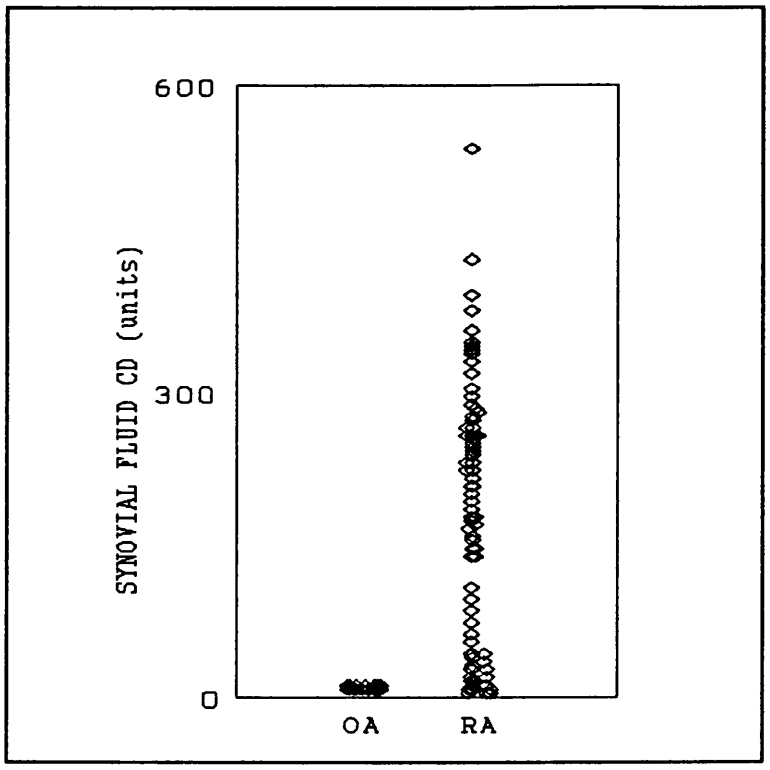
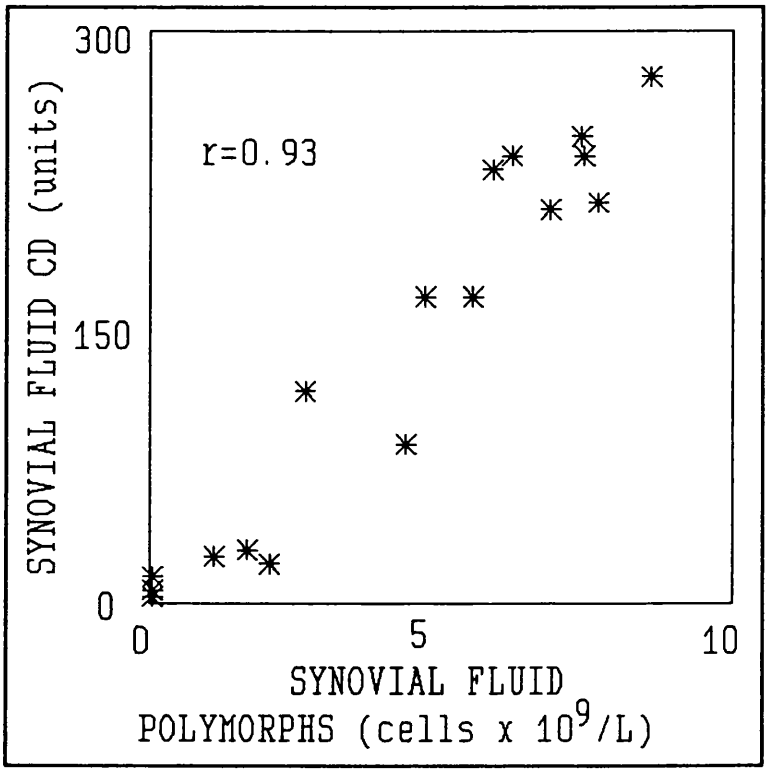
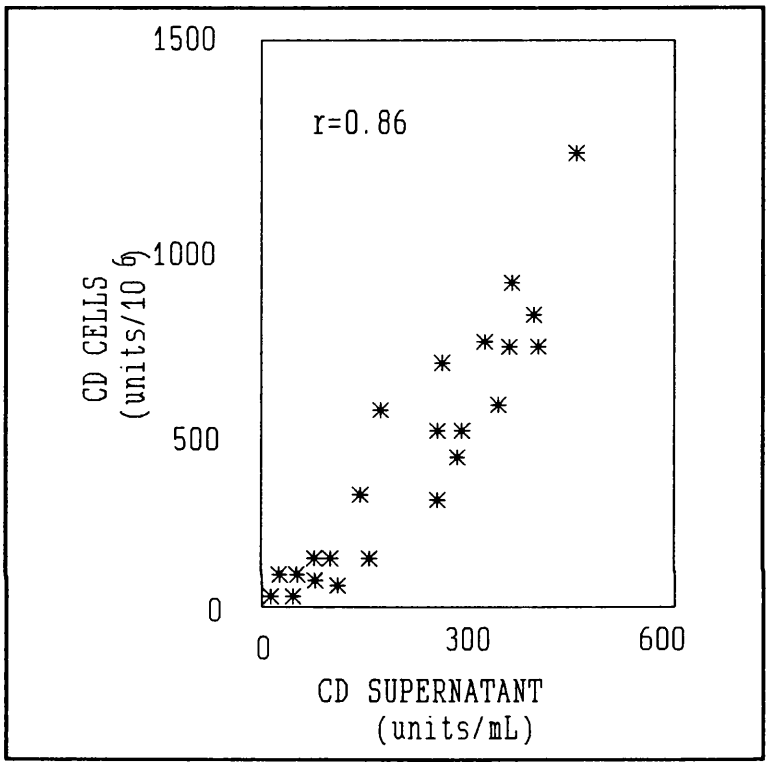


Figure 19 Scattergram of synovial fluid CD versus polymorph count.



The CD activity of the cellular components of a unit volume of synovial fluid (mean = 940 units/mL) was greater than the corresponding supernatant CD activity (mean = 561 units/mL) ($p<0.05$), and the cellular and supernatant CD activities correlated strongly ($r=0.86$) (Figure 20).

Figure 20 Scattergram of cell pellet versus supernatant CD.



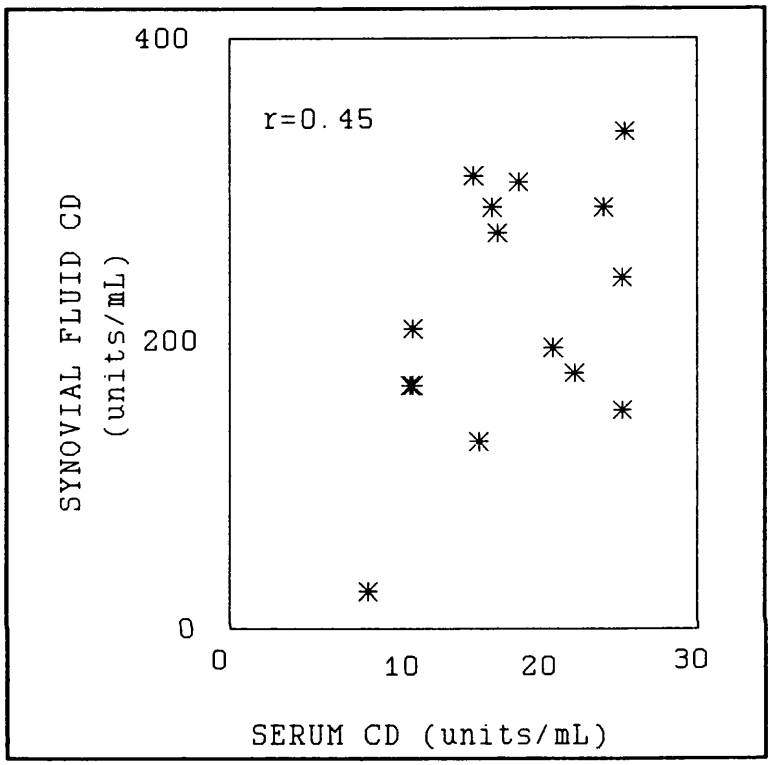
4.3.3 Synovial fluid/serum ratios.

Synovial fluid CD activity was lower than corresponding serum activity for the OA patients (mean synovial fluid/serum ratio = 0.6, n = 11), but up to 22 times higher for the RA patients (mean synovial fluid/serum ratio = 13.1, n = 16). Figure 21 shows weak positive correlation between serum and synovial fluid levels in RA patients ($r=0.45$).

4.3.4 Synovium.

For the 9 samples of rheumatoid synovium that were processed the mean(SD) CD activity was 172(156) units/g.

Figure 21 Scattergram of serum versus synovial fluid CD.



4.4 DISCUSSION.

The data support the hypothesis that CD is released from synovial fluid polymorphs and drains via the lymphatics into the blood.

4.4.1 Synovial fluid.

The high CD levels in RA synovial fluid and the close relationship with polymorph count (r=0.93), long considered as the marker of acute inflammation [Ropes and Bauer, 1953]

suggests that synovial fluid CD is a good measure of acute inflammation of a single joint.

The mean synovial fluid CD concentration for the 39 RA patients was 170 units/mL. This is of the same order as the theoretical concentration calculated of 196 units/mL.

4.4.2 Serum.

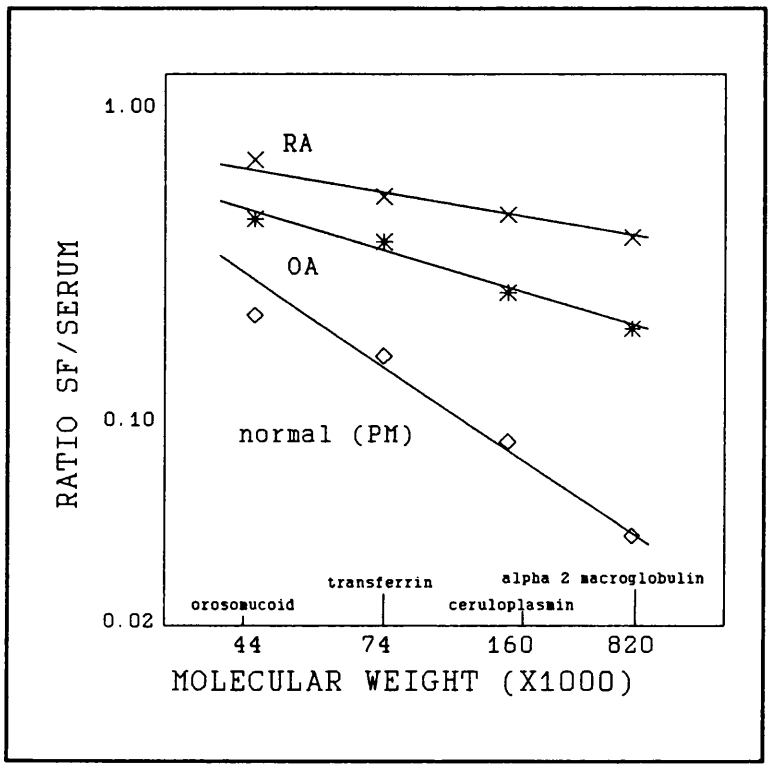
The wide range and higher CD concentrations in the RA patients sera than OA or controls is in keeping with the hypothesis.

The slightly higher mean serum CD activity for the OA patients compared with the normal volunteers may be accounted for by demographic difference between the groups. Although we did not record the ages of patients or normal volunteers the former were generally elderly females while the latter were male students. However, we have not found differences between the sexes or with age in a reference population and it may be that the difference reflects mild inflammation in the OA group [Dieppe et al, 1980].

4.4.3 Synovial fluid/serum ratios - origin from the joint in RA.

Analysis of the ratio between synovial fluid and serum levels of the enzymes was made in order to determine whether an enzyme was originating from a joint and draining to the blood or defusing into the joint from the blood. This technique [Kushner and Somerville, 1971] was originally used to study the permeability of the synovial membrane in health and disease. The ratio synovial fluid/serum concentration for a number of molecules known not to be produced in a joint can be plotted against their molecular weights to produce a straight line (Figure 22).

Figure 22 Synovial fluid/serum ratio plotted against molecular weight for 4 proteins.



This suggests that permeability across the synovial membrane is related to molecular size. The slope of the line is increased in RA suggesting that inflammation increases permeability. Substances that give synovial fluid/serum ratios of greater than unity do not fit on the line and suggest that they are produced in the joint. While the technique can be further refined (see [Levick, 1981]), comparison of the synovial fluid/serum ratios for different enzymes is used here to supply information on their likely site of origin.

Kushner and Sommerville found the ratio of synovial fluid/serum for transferrin, a protein manufactured solely by the liver and of similar size to CD, varied from 0.56 in OA patients to 0.84 in RA patients [Kushner and Sommerville, 1971]. A ratio of 0.6 for the CD synovial fluid/serum ratio

of OA patients agrees well with this, suggesting diffusion of background CD from blood to synovial fluid. In contrast, the synovial fluid/serum CD ratio for RA patients (mean = 13.1) reflect high levels of CD in the synovial fluid compared with serum and suggests that CD originates from inflamed joints and drains (presumably via the lymphatics) into the blood.

4.4.4 CD gradient - origin from synovial fluid polymorphs.

The cellular components of the RA synovial fluid contained higher CD activity than the corresponding supernatant, but RA synovial CD activity was no greater than that of 15 normal postmortem specimens previously studied (mean(SD) = 146(78) units/g [Jones and Roberts, 1986]). This suggests a CD concentration gradient running from cells to fluid to synovium to blood.

It is concluded from these data that, in RA, CD is released from synovial fluid polymorphs into synovial fluid where the CD concentration is a good measure of the joint polymorph count. CD drains down its concentration gradient (either directly or, more likely, via lymphatics) into the blood.

In normal controls and OA patients there is a low background of CD that does not originate from joint polymorphs and presumably reflects CD release from polymorphs at other sites and/or other tissues as part of the normal cellular turnover.

CHAPTER V

CYTIDINE DEAMINASE AND 'LIVER' ENZYMES

5. CYTIDINE DEAMINASE AND 'LIVER' ENZYMES

5.1 INTRODUCTION.

Serum CD is raised in those liver diseases that are characterised by hepatic necrosis suggesting that CD is released from damaged liver cells and diffuses into the bloodstream in similar fashion to alanine transaminase (ALT) and aspartate transaminase (AST) [Jones and Roberts, 1986]. A case has been made for involvement of the liver in RA as part of the systemic manifestations of the disease [Kendall et al, 1970]. Therefore, a hepatic origin for circulating CD in RA might be anticipated.

The concept of 'rheumatoid liver' is mainly based on the abnormal serum activity of alkaline phosphatase (ALP) [Lehman et al, 1964; Kendall et al, 1970a; Webb et al, 1975; Fernandes et al, 1979; Spooner et al, 1982; Thompson et al, 1986], 5-nucleotidase (5NT) [Farr et al, 1973], gamma-glutamyl transferase (GGT) [Lowe et al, 1978; Spooner, 1982] and lactate dehydrogenase (LDH) [Vesells et al, 1962; Dawes et al, 1986] in about one third of RA patients. However, in uncomplicated RA there are no clinical manifestations of chronic liver disease, only mild non-specific changes on liver biopsy [Rau, 1976; Movitt et al, 1953; Rau et al, 1975; Otto et al, 1976; Dietrichson et al, 1976; Mills et al, 1980] and persistently normal levels of serum bilirubin and transaminase enzymes [Lefkovits and Farrow, 1955]. The exceptions are Sjogren's syndrome which is associated with abnormal bromsulphthalein excretion in about 18 percent of cases and Felty's syndrome in which nodular regenerative hyperplasia has been reported [Steiner, 1959] and may present with bleeding from oesophageal varices [Blendis et al, 1978].

In view of these inconsistencies a series of studies have been performed designed to determine whether or not CD and 'liver enzymes' originate from the liver in RA.

5.2 PATIENTS AND METHODS.

5.2.1 Patients.

Outpatients with either definite or classical RA [Ropes et al, 1958] were studied at the London Hospital, Whitechapel over a 2 year period. Altogether 175 patients (mean(SD) age=58.2(13.4) years, F:M ratio=3.9:1) took part in a number of separate studies. The age and sex distribution in the study groups were not significantly different from the parent population and are shown for each study in the text. The data from a study of serum and synovial fluid 5NT on 66 patients with RA (mean(range) age=54.4(24-77), F:M=3.5:1) reported by Kendall et al [Kendall et al, 1973], were reanalysed.

5.2.2 Samples

Samples were processed as described previously [Chapter III].

5.2.3 Biochemical assays.

Samples were thawed within 4 weeks of freezing and analysed without knowledge of the patients' condition. Serum ALP isoenzymes were studied qualitatively using polyacrylamide gel electrophoresis [Moss, 1982] and quantitatively using multipoint heat inactivation [Moss and Whitby, 1975] techniques. GGT, AST, ALT and LDH were analysed by standardized automated methods.

5.2.4 Analysis

Serum enzyme activity was expressed as multiples of the upper limit of the normal range taken as +/- 2 standard deviations

from the mean. Thus, in a normally distributed population, 2.5% of patients would be expected to be above the upper limit of normal by chance alone. The ALP results have been corrected for age using the figures published by Whitaker et al [1977].

Analysis of the ratio between synovial fluid and serum levels of the enzymes was made in order to determine whether an enzyme was originating from a joint and draining to the blood or defusing into the joint from the blood as previously described. Group means were compared using Student's t tests and the relationship between different measures expressed as Spearman's correlation coefficient.

5.3 RESULTS.

The serum levels of the enzymes in the study groups are shown in Figure 23.

The number of RA patients studied, their age, sex ratio, number of patients with serum levels greater than the upper limit of normal and mean (SD) levels expressed as multiples of the upper limit of normal are shown in Table III.

Electrophoresis and heat inactivation excluded the presence of carcinoplacental, intestinal and immunoglobulin complexed isoenzymes.

Figure 23 Serum enzymes in study groups.

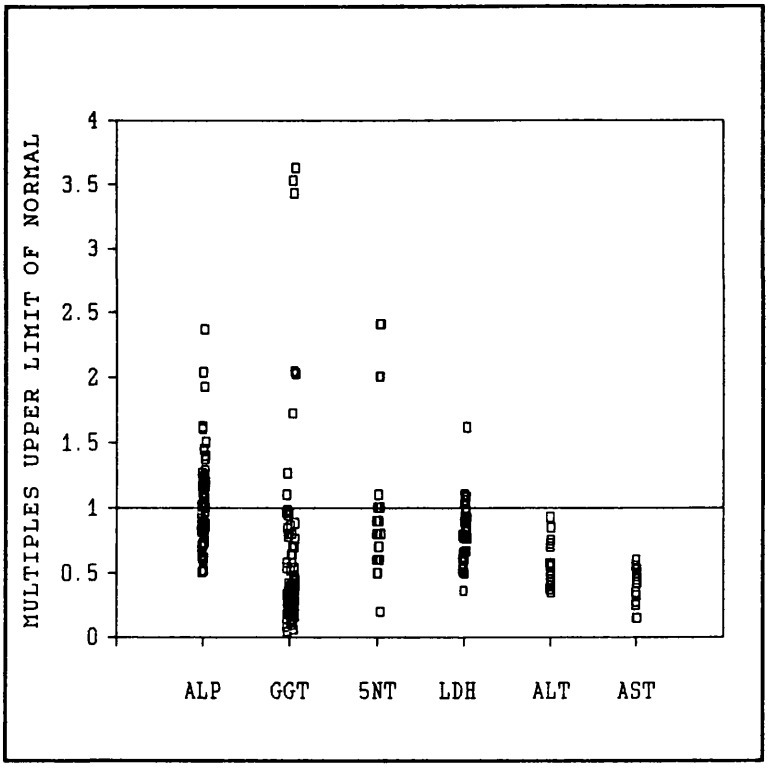
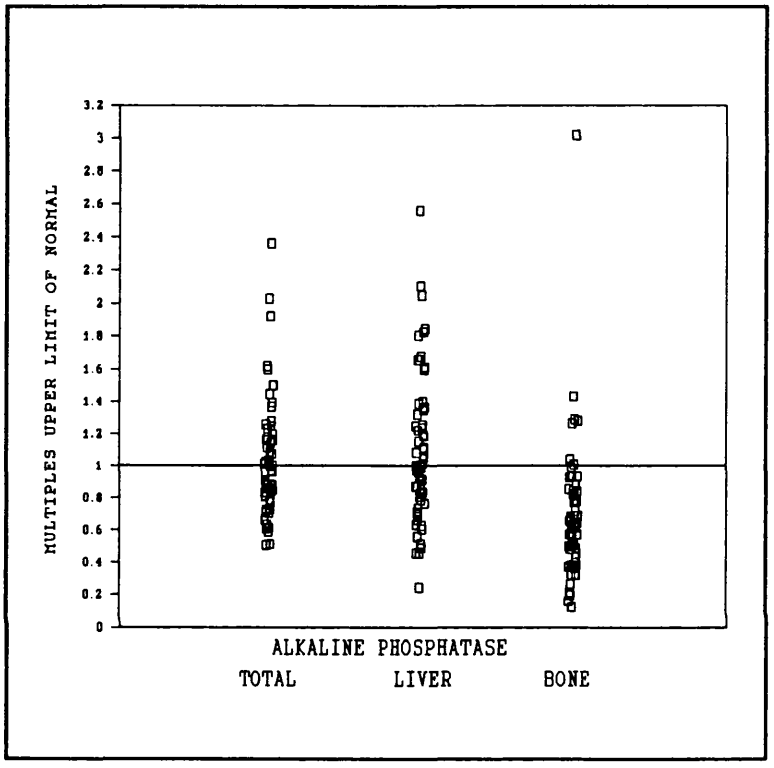


Table III Demographic data, percentages of patients and multiples of the upper limit of normal for 6 enzymes.

	ALP	**5NT**	**LDH**	**GGT**	**AST**	**ALT**
Number	91	66	45	113	25	25
Mean Age (years)	58	54	58	58	53	53
F:M	4.3	3.5	3.5	4.0	5.0	5.0
>Upper Limit	48%	35%	11%	7%	0%	0%

The serum levels of ALP bone and liver enzymes of 60 RA patients (mean(SD) age=58.1(13.8), F:M=3.6:1) corrected for age and expressed as multiples of the upper limit of normal are shown in Figure 24.

Figure 24 ALP isoenzymes in RA.



30 patients (50%) had raised levels of liver isoenzyme (mean(SD) 1.09(0.45) multiples of the upper limit of normal) compared with only 7 patients (12%) who had elevated levels of bone isoenzyme (mean(SD) 0.67(0.42) multiples of the upper limit of normal).

Serum ALP was raised in 11 out of 20 patients who had simultaneous estimations of ALP and GGT, but GGT was raised in only 2, both of whom also had raised ALP.

The synovial fluid/serum ratios for the enzymes studied are shown in Table IV.

Table IV Synovial fluid/serum enzyme ratios.

	CD*	ALP	5NT	LDH
Number	16	51	26	14
AGE (years)	-	59	-	58
F:M	-	3.4	3.5	3.5
SF/serum Ratio	13.1	0.54	7.0	4.2

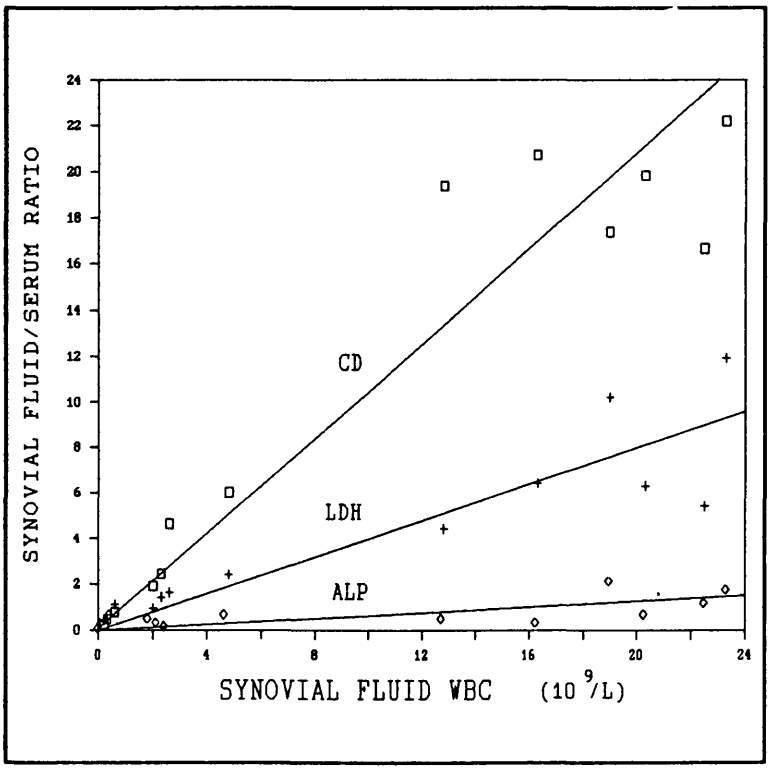
* from Chapter IV

The ratios for CD, 5NT and LDH were all greater than unity while ALP was 0.54. There was close correlation between synovial fluid leucocyte count LDH level ($r=0.94$, $n=13$, $p<0.001$).

The relationship between synovial fluid/serum ratio and synovial fluid leucocyte count is shown in Figure 25.

There was moderate correlation between serum C-reactive protein and total serum ALP ($r=0.52$, $p<0.01$, $n=22$), CRP and liver ALP isoenzyme ($r=0.53$, $p<0.01$, $n=22$), but not between CRP and bone ALP isoenzyme ($r=-0.17$, $p>0.05$, $n=22$). Serum GGT correlated only weakly with CRP ($r=0.26$, $p<0.05$, $n=93$).

Figure 25 Synovial fluid/serum ratio and leucocyte count.



5.4 DISCUSSION.

The data confirm that serum ALP, LDH and GGT are raised in RA patients and the serum transaminase levels are normal. The results suggests that hepatic necrosis does not normally occur, ALP and GGT reflect the acute phase response, CD and LDH are a measure of polymorph lysis, and measurement of serum 5NT may give information about the in vivo activity of synovial lining cells. The hypothesis that serum CD originates from joint polymorphs and not the liver is supported.

The findings are in keeping with other studies. Raised serum LDH in RA has been previously reported [Vesell et al, 1962; Dawes et al, 1986] while serum bilirubin and transaminases are almost invariably normal [Mills and Sturrock, 1982]. In this study 48% of the patients had raised ALP levels and only 7% elevated levels of GGT. Other authors have reported 18%,

25%, 35%, 46% and 51% patients with raised serum ALP [Webb et al, 1975; Lowe et al, 1978; Fernandes et al, 1979; Spooner et al, 1982; and Rau et al, 1976], and 19%, 24% and 77% with raised serum GGT [Fernandes et al, 1979, Spooner et al, 1982; Lowe et al, 1978]. Furthermore, the number of patients with raised ALP without a concomitant rise in GGT varied between 82% in this study to 64%, 33% and 0% [Spooner et al, 1982; Fernandes et al, 1979; and Lowe et al, 1978]. The methods and limits of normal used in these studies are shown in Table V.

Table V Studies of 'liver' enzymes in RA.

AUTHOR	ENZYME	LIMIT	% ABOVE LIMIT
Rau [1976]	ALP	Not given	51
Thompson	ALP	Variable*	48
Spooner [1982]	ALP	Variable*	46
Fernandes [1979]	ALP	100iu/L	35
Lowe [1978]	ALP	13KA units	25
Webb [1975]	ALP	15KA units	18
Lowe [1978]	GGT	28iu/L+	77
Spooner [1982]	GGT	35iu/L	24
Fernandes [1979]	GGT	45iu/L	19
Thompson	GGT	50iu/L	7

* Adjusted for age and sex
\+ Adjusted for sex

KA King Armstrong units

The differences are most marked for GGT where there is an inverse relationship between the upper limit of normal and the percentage of patients with raised GGT. Similarly the early studies of ALP using King Armstrong units report a smaller percentage of patients with elevated levels than the later studies that used the Scandinavian method [Committee on Enzymes of the Scandinavian Society of Clinical Chemistry,

1974] and adjusted for age and sex. Therefore, the differences may arise from variations in the assay methods or limits for the upper range of normal.

Present evidence suggests that different ALPs predominate in the tissues: placenta, intestine, liver, bone and kidney [Moss, 1982]. These ALPs exhibit tissue specific characteristics which derive from the existence of distinct structural genes for placental and intestinal ALP and from post translational modification in the other cases. The ALPs of bone, liver and kidney should therefore not be thought of as isoenzymes by strict definition. Further identifiable forms of ALP are produced by the formation of complexes with immunoglobulins [Negmine et al, 1975; Crofton et al, 1975; De Broe et al, 1979; Hattori et al, 1979], or may be associated with cancers [Stolbach et al, 1969; Nathanson et al, 1971]. These different forms of ALP can be interfere with the multipoint heat inactivation method used to quantify the bone and liver ALP fractions of serum [Moss and Whitby, 1975]. The heat-stability characteristics of intestinal ALP are similar to those of liver but differentiation can be made by a electrophoresis or inhibition by phenylalanine. The presence of the very heat stable placental ALP or the cancer related (Regan) ALPs alters the shape of the heat inactivation curve and can therefore be detected during the analysis of bone and liver ALPs [Moss and Whitby, 1975]. Thus it is necessary to undertake a qualitative preliminary electrophoresis before quantitative analysis of bone and liver ALPs can be made. In this study electrophoresis and heat inactivation excluded the presence of carcinoplacental, intestinal and immunoglobulin complexed isoenzymes suggesting that the serum ALP was predominantly of bone and liver type.

The results of ALP isoenzyme studies suggest that liver type ALP accounts for most of the increases in total ALP. Seven patients showed raised bone type isoenzyme possibly reflecting osteoblastic activity relating to coincidental

osteomalacia or Paget's disease although the incidence of osteomalacia in RA patients who have not sustained fractures is very small [Wordsworth et al, 1984].

A ratio of 0.54 for the ALP synovial fluid/serum ratio suggests diffusion of ALP from blood to synovial fluid. In contrast, the synovial fluid/serum ratios of 5NT and LDH reflect high levels of these enzymes in the synovial fluid compared with serum and suggests that they originate from inflamed joints and drain (presumably via the lymphatics) into the blood. Vesells [Vesells et al, 1962] reported that synovial fluid from patients with RA showed the polymorph LDH isoenzyme pattern on gel electrophoresis. Taken with the close correlation between synovial fluid polymorph count this suggests that LDH also originates from joint polymorphs. In contrast the work of Kendall and colleagues [Kendall et al, 1973] suggests that 5NT originates from the synovial lining layer and not synovial fluid cells. They found lower levels of 5NT in synovial sediment compared with supernatant and high levels in desquamated synovial lining cells.

The traditional view of liver involvement in RA is of a 'non-specific hepatitis' in response to the systemic 'rheumatoid process' [Mills and Sturrock, 1982]. Although RA patients take a number of potential hepatotoxic drugs no relationship between alterations in hepatic function and use of non-steroidal anti-inflammatory drugs, disease modifying drugs, steroids or immunosuppressive agents have been demonstrated except occasional hepatotoxic reactions resulting in raised serum transaminases (see below). William's group have reported positive correlation between the sicca syndrome and serum ALP in RA patients and have suggested that hepatobiliary dysfunction occurs as a result of antibodies to salivary tissue cross-reacting with bile duct antigens [Fernandes et al, 1979]. However, biliary damage would be expected to result in intrahepatic cholestasis and in increases in both ALP and GGT as these

enzymes occupy similar sites on the bile duct membrane. Proportionally greater rises in GGT would be anticipated because of its greater sensitivity in reflecting biliary damage [Penn and Worthington, 1983]. As most studies in RA have found that a significant proportion of RA patients with raised ALP have normal or only mildly raised GGT with no cases of raised serum bilirubin, and liver biopsy studies have not recorded signs of bile duct damage or cholestasis [Lefkovits and Farrow, 1955; Webb et al, 1975; Dietrichson et al, 1976], it seems unlikely that this hypothesis can account for the enzyme abnormalities.

If ALP is not related to hepatobiliary disease or drug toxicity then why are serum levels raised in patients with active RA? One possibility is that secretion of ALP and GGT is stimulated by inflammatory mediators such as interleukin-1, and act as part of the acute phase response [Whitcher, 1984]. Both enzymes can be induced by a variety of external and internal stimuli [Penn and Worthington, 1983; Moss, 1982]. This view is supported by the correlation of ALP liver isoenzyme and GGT with CRP reported here. Similar correlations with other acute phase reactants have been reported. Kendall [Kendall et al, 1970b] observed more active disease in 15 RA patients with raised ALP compared with a group with normal ALP levels. Akesson et al [1980] reported correlation between ESR or serum orosomucoid in 182 RA patients, and a recent study showed correlation of serum ALP with plasma viscosity in 56 RA patients taking non-steroidal anti-inflammatory drugs and 17 RA patients not taking these drugs [Doube et al, 1989]. Furthermore, sequential measurements have shown that serum ALP levels fall following treatment with steroids [Kendall et al, 1970b]. Similar findings in polymyalgia rheumatica [Sheehan et al, 1985] and ankylosing spondylitis [Kendall et al, 1973; Sheehan et al, 1983; Thompson et al, 1986] support the concept of these enzymes acting as non-specific acute phase reactants in patients with inflammatory diseases.

The normal levels of AST and ALT reported here are in keeping with all the studies referenced so far suggesting that hepatic necrosis does not occur to any significant degree. While liver biopsy studies have found portal tract mononuclear infiltration [Lefkovits and Farrow 1955, Webb et al, 1975: Dietrichson et al, 1976] these findings are the same as those found in an aging population [Mooney et al, 1985] and do not include hepatic necrosis. It is likely that hepatic necrosis is not normally found in RA patients. However, raised liver transaminases have been reported in RA patients taking salicylates [Russell et al, 1971] and sulphasalazine [Farr et al, 1985] suggesting that their measurement may be useful in monitoring for drug hepatotoxicity.

In conclusion, the results suggest that in RA hepatic necrosis does not normally occur and the transaminases may be useful in monitoring drug toxicity. Serum ALP and GGT reflect the acute phase response and may useful in monitoring the clinical course of the disease. Serum CD and LDH are a measure of polymorph lysis in all joints and may offer a specific measure of one aspect of joint inflammation, while measurement of serum 5NT may give information about the in vivo activity of synovial lining cells.

CHAPTER VI

SELECTIVE INHIBITION OF LIVER CYTIDINE DEAMINASE

6. SELECTIVE INHIBITION OF LIVER CYTIDINE DEAMINASE

6.1 INTRODUCTION

In an attempt to differentiate between CD of different human tissue origin, Jones and Roberts studied heat inactivation of human CD [Jones and Roberts, 1986]. CD was found to be heat resistant to 60°C. At 70°C there was more inhibition of CD from placenta and polymorphs than other tissues but the differences were too small to be of clinical utility. Recently 2 molecular forms of CD have been isolated from human polymorphs (type A) and liver (type B) using dye ligand [Stoller et al, 1978] and HPLC techniques. An inhibitor has been developed that can differentiate between CD extracted from human blood polymorphs and liver [D D Jones, personal communication]. Studies undertaken by Jones have shown less than 5% inhibition of CD of polymorph origin (n=35) compared with greater than 92% inhibition of CD from human liver cells. At the time of writing the biochemical details of the inhibitor are confidential. This study applied the inhibitor of polymorph CD to compare enzyme type in patients with RA and a group of patients with raised serum liver enzymes from a variety of causes.

6.2 PATIENTS AND METHODS

Serum was obtained from 22 RA patients (mean(SD) age=55(14) years, F:M=19:3) and 47 patients with raised levels of 'liver' enzymes (mean(SD) age=44(20) years, F:M=23:24). CD levels were assayed in the presence and absence of the inhibitor. ALP, AST and ALT were also measured.

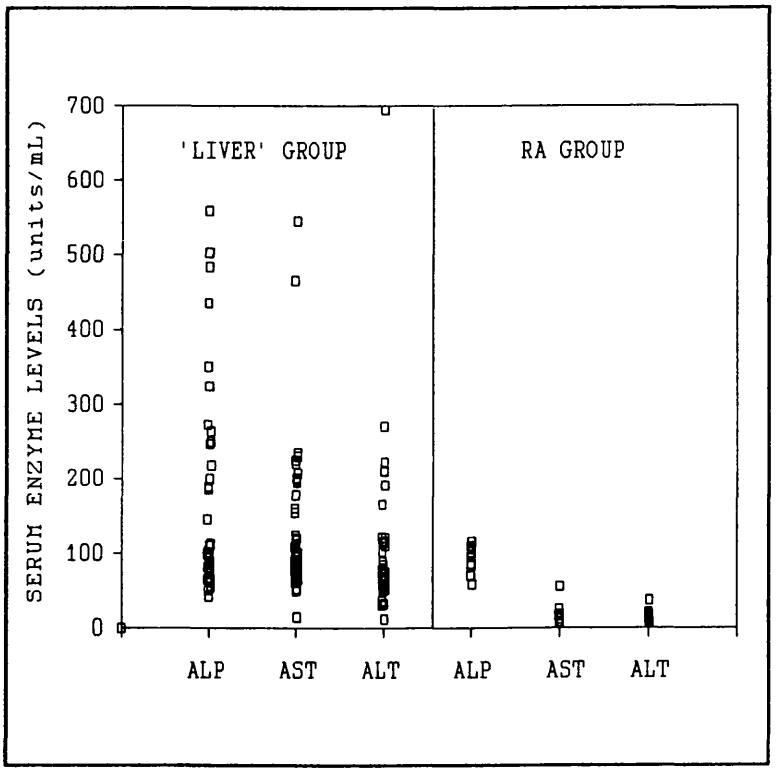
6.3 RESULTS

Table VI shows the diagnoses for the patients with raised 'liver' enzymes and Figure 26 shows the serum levels of ALP, AST and ALT for both groups.

Table VI Diagnoses of 'liver' group patients.

DIAGNOSIS	N
Crohn's disease	1
sprue	1
cholecystitis	1
chest pain	1
SLE	1
cardiac failure	1
nephrotic syndrome	2
renal transplant	2
post op	2
myelodysplasia	2
carcinoma	3
hepatitis	3
diabetes	3
cardiac surgery	3
leukaemia	5
renal failure	7
unknown	13

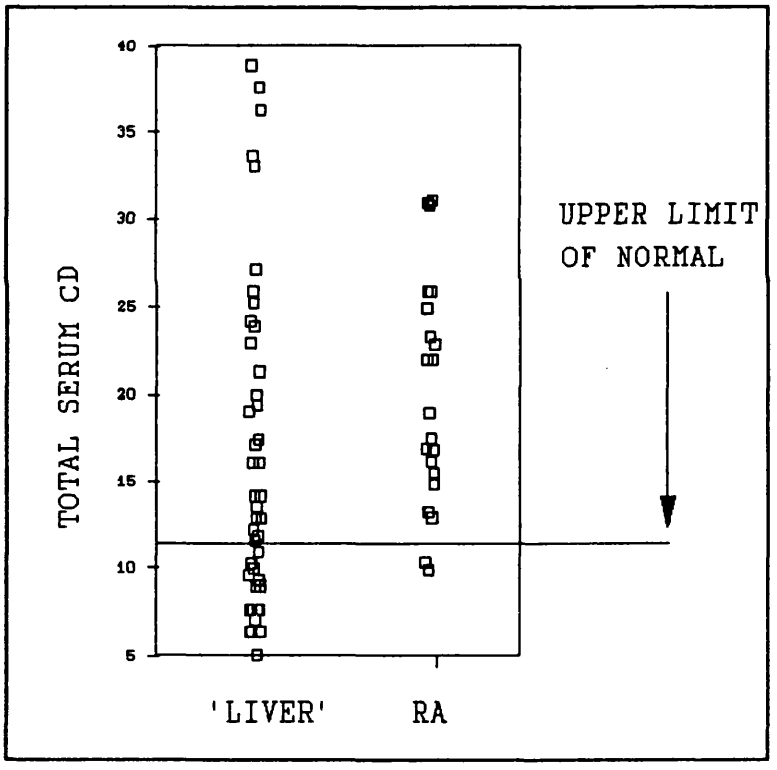
Figure 26 Serum levels of ALP, AST and ALT in RA and 'liver' group patients..



The serum levels of these enzymes were significantly higher in the 'liver' enzyme group (mean(SD) ALP=298(578) iu/L, ALT=187(273) iu/L and AST=134(238) iu/L) compared with the RA group (mean(SD) ALP=94(17) iu/l, $p<0.05$; ALT=16(12) iu/L, $P<0.01$; AST=16(7) iu/L, $P<0.01$).

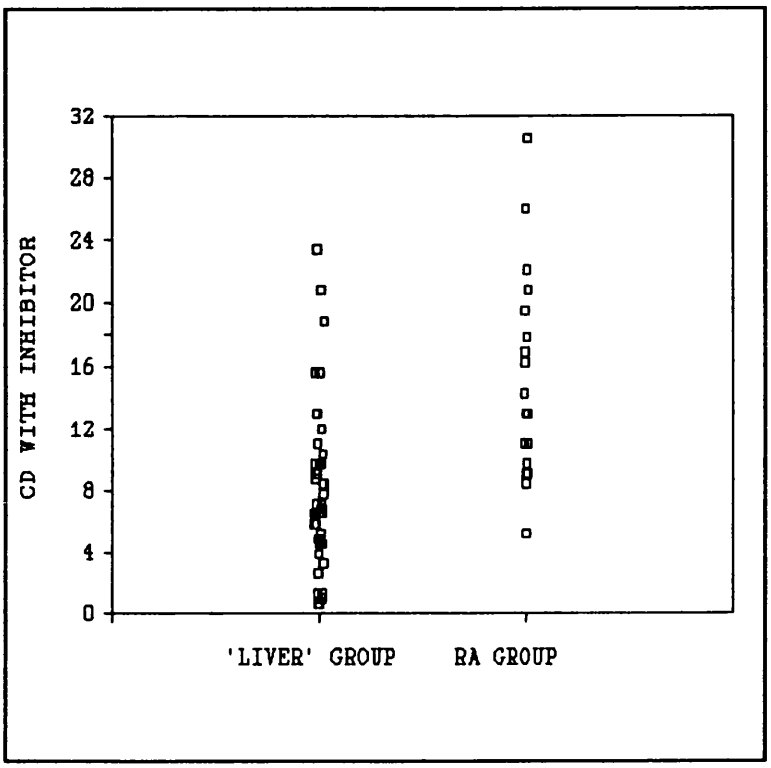
Figure 27 shows both groups of patients had raised serum levels of CD and there was no significant difference between them although the 'liver' enzyme group showed a larger range (RA group CD mean(SD)=20(7) u/mL; 'liver' enzyme group CD mean(SD)=16(9) u/mL, $P=0.16$).

Figure 27 Total serum CD in RA and 'liver' group enzymes.



Serum level of CD in the presence of the inhibitor was significantly higher in the RA group (mean(SD)=16(7) u/mL compared with the 'liver' group (mean(SD)=8(5) u/mL, $p<0.05$) (Figure 28).

Figure 28 Serum CD with inhibitor in RA and 'liver' group patients.



There was significant correlation of:

(i) total CD with CD in the presence of inhibitor in the RA group ($r=0.68$, $p<0.01$) but not in the 'liver' group;

(ii) total CD minus CD in the presence of the inhibitor and AST ($r=0.34$, $p<0.01$) in the 'liver' enzyme group, and;

(iii) total CD minus CD in the presence of the inhibitor and total CD in the 'liver' enzyme group.

6.4 DISCUSSION

The search for inhibitors of CD started in the early 1960s after the discovery that ß-D-arabinofuranosyl derivatives of nucleosides showed cytotoxic activity against some animal

tumours [Pizer and Cohen, 1960]. In particular, 1-ß-D-arabinofuranosyl cytosine (cytosine arabinoside) was developed as a useful anti-leukaemic drug [Evans et al, 1961]. The intra-cellular metabolism of this agent in tumour cells is known to proceed by successive phosphorylation steps and degradation at the level of the nucleotide and nucleoside by cytidine and deoxycytidylate deaminase respectively [Chabner et al, 1975]. Steuart and Burke [1971] found a correlation between responsiveness to cytosine arabinoside and intra-cellular levels of CD precipitating a search for non-toxic CD inhibitors that would provide a rational basis for combination therapy with cytosine arabinoside in some human malignancies. Tetrahydrouridine is a potent inhibitors of CD and has been shown to potentiate the anti-leukaemic activity of cytosine arabinoside in mice [Neil et al, 1970]. It has been suggested that potent CD inhibitors such as 3,4,5,6 tetrahydrouridine exert their influence by tight binding to the enzyme due to structural resemblance to hypothetical transition state intermediates formed in the deamination of cytidine [Stoller, 1977] but a detailed account is beyond the scope of this text. During these experiments CD was extracted from a number of sources, ranging from E. coli to cultured HeLa cells [Conway and Cooke, 1939; Greenstein et al, 1946; Creasey, 1963; Camiener and Smith, 1965; Wentworth and Wolfenden, 1975; Mekras et al 1986; Jones and Roberts, 1986]. The properties of these enzymes with CD activity vary between species and tissues suggesting that there may be several distinct forms.

The 'liver' enzyme patients represented an heterogenous group with a variety of diagnoses and often multiple organ disease. While they all showed liver damage reflected by raised ALP, ALT and AST, it seems probable that CD from other tissues made up some of the serum level. Despite this, application of the inhibitor to serum produced significantly more inhibition of CD from the 'liver' enzyme group than the RA group. This suggests that a greater contribution to the serum CD pool was

made from hepatocytes in the 'liver' group than in RA patients, supporting the concept than CD originates from polymorphs in RA patients.

The results reported here support the concept that CD is of predominantly polymorph origin in RA, and suggest that the inhibitor can differentiate between liver damage and polymorph mediated inflammation.

CHAPTER VII

CIRCADIAN RHYTHM OF SERUM CYTIDINE DEAMINASE.

7. CIRCADIAN RHYTHM OF SERUM CYTIDINE DEAMINASE.

7.1 INTRODUCTION

It is not known why clinical measures of disease activity in RA such as grip strength, pain, stiffness and articular index show greatest activity in the early hours of the morning [Lee et al, 1974; Harkness et al, 1982], but similar changes in blood total protein count, α-1-antitripsin, orosomucoid, ceruloplasmin and transferrin [Harkness et al, 1982; Bruguerolle et al, 1986] suggests that there is diurnal variation of inflammatory markers possibly relating to circulating cortisol levels, although similar changes in CRP, plasma viscosity, serum sulphydryl and serum histidine have not been demonstrated [Harkness et al, 1982; Bruguerolle et al, 1986; Sitton et al, 1984]. Other factors may be important, for example diurnal variation in the ESR can be explained by food ingestion [Mallya et al, 1982].

This chapter reports a study that was designed to assess circadian variation in serum CD in a group of RA patients studied at rest and during activity. CRP was also measured as a control marker of inflammation known not to show circadian rhythm [Harkness et al, 1982].

7.2 METHODS

With Ethics Committee approval and written informed consent, 8 female and 3 male inpatients of mean age 62 years (range 40 - 71 years) with definite or classical RA [Ropes et al, 1958] volunteered for the study. Patients with infections or recent changes in treatment were excluded. Patients were randomly allocated to 24 hours bedrest or normal ward activities on the first day, crossing to the other regimen for the second day. Blood was taken at 06.00, 12.00, 18.00 and 24.00 hours on each day. During the rest day the patients stayed in bed but were allowed up to the toilet. During the activity day

the patients were encouraged to get up after the 06.00 venepuncture and attended physiotherapy during the morning.

Samples were collected and processed as previously described [Chapter III].

Data, or data transformations (as logs and percentages of the 24 hour mean), were assessed by analysis of variance [Snedecor and Cochrane, 1980] and by fitting sine curves with an assumed period of 24 hours by least squares regression techniques [Nelson et al, 1979]. The amplitude of the sine waves so derived were tested against zero, that is with the null hypothesis that the data did not vary significantly with time of day. The interday differences were compared using Student's t test.

7.3 RESULTS

There was no significant circadian variation of CRP levels. CD values following activity, but not at rest, showed circadian rhythm ($p=0.048$) (Figure 29) with a mesor (24 hour mean level) of 17.1 units and an amplitude of 1.1 units.

The circadian variation defined as the fitted curve's acrophase (peak) to bathyphase (trough) difference as a percentage of the 24 hour mean, was 12.3%. The acrophase occurred at 12.08 hours and the bathyphase at 00.08 hours.

There were no statistically significant interday differences for CRP (Table VII). The serum CD was higher at 12.00 hours on the exercise day compared with the rest day ($p=0.048$) but there were no other significant differences (Table VII).

Figure 29 Circadian rhythmicity of CD.

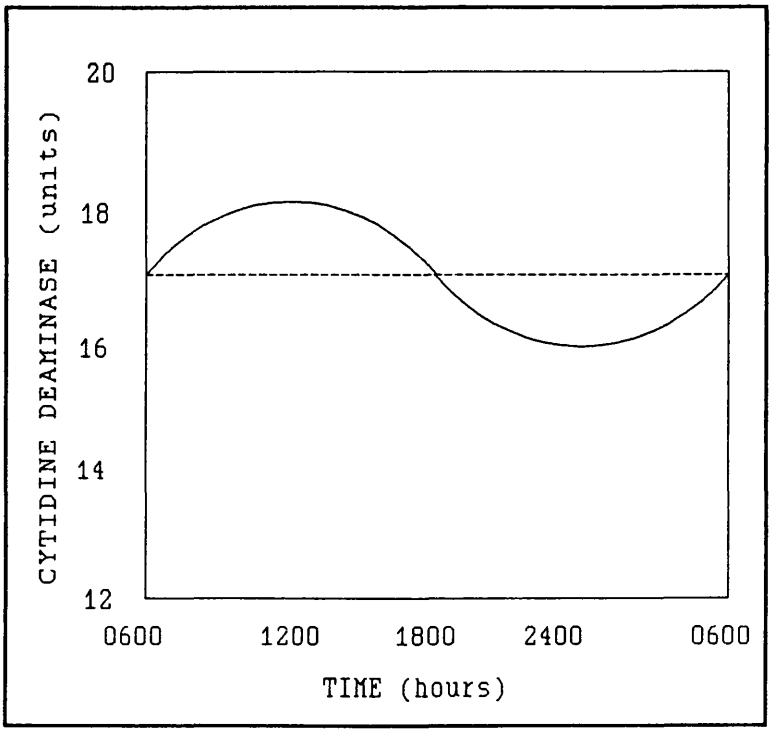


Table VII CD and CRP results at 6 hourly intervals in 10 patients.

C-REACTIVE PROTEIN (mg/L)

TIME	REST		EXERCISE	
	MEAN	SD	MEAN	SD
06.00	4.4	3.6	5.0	4.1
12.00	4.4	3.7	5.5	4.9
18.00	4.5	4.0	5.5	4.7
24.00	4.6	4.0	5.4	4.5

CRP - Normal range 0.8 - 8.0 mg/L

CYTIDINE DEAMINASE (units)

TIME	REST		EXERCISE	
	MEAN	SD	MEAN	SD
06.00	15.1	8.3	17.0	13.1
12.00	14.5	6.9	18.2	10.5
18.00	15.7	6.6	17.1	8.9
24.00	16.8	8.5	16.1	7.4

CD - Normal range 3 - 13 units

7.4 DISCUSSION.

The results suggest that serum CD levels follow a circadian rhythm in ambulatory hospital RA inpatients but not if the patients rested in bed, while CRP show no such variation in keeping with published findings [Harkness et al, 1982; Bruguerolle et al, 1986]

A common pattern of change in human biological systems is a cycle repeated every 24 hours. One way of studying such circadian rhythms is the fitting of a sine wave. The technique is useful in supplying the time of the maximum and minimum values allowing comparison between rhythms, but may artificially reduce the amplitude of the variation and excludes other potential patterns. These possible shortcoming should be born in mind when considering the interpretation of any chronobiological study [Pownall, 1984].

Most workers attribute circadian rhythms in clinical and serological measures of inflammation to a cyclical variation in circulating cortisol levels that peak early in the morning. It has been suggested that the morning exacerbation of symptoms experienced by many RA patients is related to these changes [Unger, 1984] although the definitive criteria for proof of a causal relationship have never been met [Pownall et al, 1984]. However, the results presented here suggest that exercise is an important factor in the diurnal variation of serum CD. In patients with RA there is a steep gradient between inflamed synovial fluid CD and serum CD (up to 20:1) [Thompson et al, 1986] suggesting that CD originates from joints and drains into the blood. Small proteins such as CD, are cleared from joints via synovial lymphatics and the lymphatic flow is powered mainly by joint motion [Levick, 1987]. It follows that RA patients would be expected to have higher levels of serum CD after exercise than while resting. The inpatients studied underwent a period of moderately vigorous physiotherapy between 08.30 and 10.00 hours but

spent the rest of the day according to the ward routine which involves little ambulatory activity. The peak in serum CD levels around noon, and the lack of circadian variation during either day for CRP, which originates from the liver and is, therefore, unaffected by joint motion, is in keeping with this hypothesis.

The number of circulating polymorphs shows a circadian rhythm with a peak in the early evening in resting RA patients [Harkness et al, 1982] as well as normal controls [Pownall, 1984]. The pattern of CD variation shown here does not reflect this cycle suggesting that there is not an exercise related alteration in the numbers of blood polymorphs undergoing lysis in joints.

While statistically significant, the cyclical differences in serum CD were small (mean difference between acrophase and bathyphase=2.1 units). In the context of RA, with serum CD levels up to 50 units (normal 3-13 units) these differences are unlikely to be clinically important although workers studying small difference in serum CD levels, such as those produced by the withdrawal of non-steroidal anti-inflammatory drugs [Thompson et al, 1988], should endeavour to collect samples under controlled conditions.

CHAPTER VIII

CROSSECTIONAL CORRELATIONS OF SERUM CYTIDINE DEAMINASE WITH MEASURES OF INFLAMMATION

8. CROSSECTIONAL CORRELATIONS OF SERUM CYTIDINE DEAMINASE WITH MEASURES OF INFLAMMATION

8.1 INTRODUCTION.

8.1.1 Articular indices of joint inflammation.

Many rheumatologists consider that joint examination is the most important assessment of patients with RA [Bombardier et al, 1982]. Several articular indices have been developed to standardise the clinical measurement of joint signs but there is little agreement about which joints should be selected or how they should be examined. For example: the Ritchie index [Ritchie et al, 1968], recommended by the EULAR Standing Committee on International Clinical [Lequesne, 1980], grades the tenderness of groups of joints; the index in common use in North America described by the Cooperating Clinics of the American Rheumatism Association (the ARA index) [Co-operating clinics, 1965] counts joints that are tender and/or swollen; and the Lansbury index [Lansbury, 1956] scores the severity of inflammation, weighted for joints surface area (Table VIII).

All have been shown to be sensitive to changes in a patient's condition, but which is the best measure of joint inflammation? The clinical measurement of inflammation relies on the assessment of tenderness, swelling, warmth, and redness by observation and palpation [Buchanan and Tugwell, 1983]. As any of the 187 synovial joints may be affected by the rheumatoid process [Gardner, 1972] a full articular index might examine every joint for each of these 4 classic signs of inflammation.

Table VIII Articular Indices.

RITCHIE ARTICULAR INDEX

Sum of the grades of tenderness (0, 1, 2 and 3 - see Table IX) elicited by applying firm pressure over the joint margin (or by passive movements in the case of the cervical spine, talocalcaneal and midtarsal joints) of the elbows, wrists, hips, knees, ankles, talocalcaneal and midtarsal joints examined as single joints, and the temporo-mandibular, cervical spine, sterno-clavicular, acromio-clavicular, MCPs, PIPs, and MTPs examined as single units.

ARA ARTICULAR INDEX

Count of 'clinically active' joints defined as the presence of tenderness on pressure and/or pain on passive movement, and/or swelling other than bony proliferation. Joints examined bilaterally - temporo-mandibular, sterno-clavicular, acromio-clavicular, shoulder, elbow, wrist (radio-carpal, intercarpal and carpo-metacarpal as one unit), MCPs (5 units), IP thumb, PIPs and DIPs (8 units), hip, knee, ankle (mortice), tarsus (including subtalar, transverse tarsus and tarso-metatarsal as one unit), MTPs (5 units), IP great toe, and toes (PIPs and DIPs together) (5 units).

LANSBURY ARTICULAR INDEX

Calculation of the percentage of the total possible inflammation by adding the scores for severity of inflammation of each joint (defined as minimal, slight, moderate or maximal) weighted for joint surface area. Joints examined bilaterally are the same as for the ARA index but in addition each carpo-metacarpal, transverse carpal, PIP and DIP of the toes, transverse intertarsal, tarso-metatarsal, talonavicular-calcaneocuboid and talocalcaneal are examined separately

In theory articular indices might be constructed by counting the number of joints with one or more signs of inflammation or any combination of signs. Further indices might be produced by weighting for the severity of the signs, or by weighting for joint size. Thus, an almost infinite number of articular indices might be constructed. In practice, by recording separately the data for each of the signs of inflammation for every joint that could be examined by an observer and using computer analysis to combine the signs in different ways, choose different joints, weight for the severity of the signs and weight for joint surface area it has been possible to produce a large number of different articular index scores for RA patients.

8.1.2 The acute phase response.

One well recognised feature of rheumatoid disease activity is the acute phase response [Witcher, 1985]. It is thought to be stimulated by interleukin-1 released from activated synovial cells and, thus, reflects inflammation. The issue is complex, however, and the relationship between the acute-phase reactants and different aspects of inflammation remains unclear [McConkey et al, 1972]. Nevertheless, treatment with corticosteroids [McConkey et al, 1973] and DMARDs [Kirwan and Currey, 1983] produces a decrease in the levels of acute-phase reactants in those patients who subsequently show clinical improvement.

Of the reactants that can be measured, CRP is at present the choice. CRP shows a fast response, short half life, large incremental change and constant catabolic rate in different sorts of inflammation. It is a more specific and sensitive measure of the acute-phase response than is the erythrocyte sedimentation rate, the plasma viscosity, the haptoglobin value, or the orosomucoid level [Witcher, 1985].

8.1.3 Erythrocyte Sedimentation Rate.

The ESR is a measure of inflammation that has stood the test of time. It is still the most commonly used laboratory parameter despite problems of reproducibility, specificity and practicality [Wright et al, 1980]. The ESR is a composite index reflecting many changes in the blood but is mainly affected by the plasma proteins, fibrinogen and number of red cells [Turnbull, 1987]. This feature renders it non-specific but probably accounts for its ability to best represent a spectrum of different inflammatory measures [Bull, 1986].

A new test purporting to measure total joint polymorph turnover might be expected to correlate with clinical measures of joint inflammation and measures of the acute phase response. This chapter discusses the relationship between CD and a number of articular indices of joint inflammation, serum CRP and the ESR.

8.2 PATIENTS AND METHODS.

8.2.1 Patients.

Thirty patients with definite or classic RA according to the ARA criteria [Ropes et al, 1958], were examined by the author. The patients' ages ranged from 38 to 75 years (mean 57.0) with mean duration of disease 15.5 years (range 4-34 years). Twenty-seven patients were receiving NSAIDs, 3 were taking simple analgesic drugs, 7 were taking DMARDs, 3 were receiving corticosteroids, and 5 were taking immunosuppressant drugs. The manifestation of RA in these patients represented a wide range of joint inflammation and joint deformity.

8.2.2 Clinical parameters.

Three signs of inflammation (tenderness, swelling, and warmth) were sought in the peripheral joints. The following joints were examined bilaterally: the temporomandibular, sternoclavicular, acromioclavicular, shoulder, elbow, distal radio-ulnar, carpus, each carpometacarpal (5), each metacarpophalangeal (5), each proximal interphalangeal (4), each interphalangeal of the thumb, the hip, knee, ankle (mortice), subtalar, midtarsal, each metatarsophalangeal (5), and the interphalangeal joint of the great toe.

Joints were graded on a scale of 0-3 for tenderness (measured by firm pressure applied to the joint line, according to the method of Ritchie et al [Ritchie et al, 1958]), soft tissue swelling (measured by observation and palpation), and temperature (measured with the dorsum of the observer's fingers), according to the criteria delineated in Table IX.

In addition, the metacarpophalangeal, metatarsophalangeal, and proximal interphalangeal joints of the hands were examined as single units yielding additional scores for tenderness (according to the Ritchie method).

The cervical spine was examined for pain on active movement. The hip and subtalar joints were examined for pain on passive movement because of the anatomic difficulty of assessing tenderness at these sites.

Data for the intraarticular surface area of peripheral joints was taken from that published by Lansbury and Haut [1956], so that indices could be weighted for joint size.

SCORE*	TENDER	SWELLING	WARMTH
0	NOT TENDER	NONE	COOL
1	TENDER	PROBABLE SWELLING	PROBABLE WARMTH
3	TENDER WITH WINCE	DEFINITE SWELLING	DEFINITE WARMTH
4	TENDER WINCE AND WITHDRAW	TENSE SWELLING	HOT

*Each joint was examined for tenderness (or pain on movement), soft tissue swelling and warmth. For each sign a score of 0-3 was awarded according to the criteria shown above. Each joint had a possible minimum score of 0 and maximum score of 9 (ie. score of 3 for tenderness, plus score of 3 for swelling, plus score of 3 for warmth). A grade of 1, 2 or 3 indicated that a sign was present; a grade of 0 indicated a sign was absent.

Blood samples were taken at the time of examination. Samples were analysed in blinded fashion for CD by the method previously described and CRP by the turbidometric method, using a Hyland Laser Nephelometer [Ritchie, 1967].

8.2.3 Articular Indices.

The results were entered into a microcomputer spreadsheet programme (Lotus 123) and combined in various ways to produce articular indices for each patient.

8.2.3.1 Varying the signs of inflammation

Tenderness (T), soft tissue swelling (S), and warmth (W) were considered absent (Grade 0) or present (grades 1,2,or 3) in each joint. Simple numerical counts were made (prefixed) "N") of joints with tenderness, swelling, and warmth (Table X, column 3, rows 1-3).

Counts of joints possessing either of any 2 or any 1 of the 3 signs of inflammation were calculated (Table X, column 3, rows 4-7), as were simple joint counts based on the simultaneous presence of any 2, or all 3 signs of inflammation (Table X, column 3, rows 8-11).

8.2.3.2 Grading the signs of inflammation

All the combinations of signs described above were analyzed using the score for each joint on graded observation. These "graded indices" (prefixed "G"), thus, weighted the combination of different joints according to the severity of their signs (Table X, column 4).

8.2.3.3 Weighting for joint size

Weighting for joint surface area was obtained using the surface area in square centimetres, of each inflamed joint, identified from figures published by Lansbury and Haut [1956]. The "area weighted indices" were labelled with the prefix "A" (Table X, column 5).

Table X Articular indices*.

SIGNS	ABBREVIATION	SIMPLE COUNTS	GRADED INDICES	AREA WEIGHTED INDICES
Tenderness	(T)	N(T)	G(T)	A(T)
Swelling	(S)	N(S)	G(S)	A(S)
Warmth	(W)	N(W)	G(W)	A(W)
Tenderness and/or Swelling	(T/S)	N(T/S)	G(T/S)	A(T/S)
Tenderness and/or warmth	(T/W)	N(T/W)	G(T/W)	A(T/W)
Swelling and/or warmth	(S/W)	N(S/W)	G(S/W)	A(S/W)
Tenderness and/or swelling and/or warmth	(T/S/W)	N(T/S/W)	G(T/S/W)	A(T/S/W)
Tenderness and swelling	(T+S)	N(T+S)	G(T+S)	A(T+S)
Tenderness and warmth	(T+W)	N(T+W)	G(T+W)	A(T+W)
Swelling and warmth	(S+W)	N(S+W)	G(S+W)	A(S+W)
Tenderness and swelling and warmth	(T+S+W)	N(T+S+W)	G(T+S+W)	A(T+S+W)

*Simple joint counts, labelled with the prefix 'N', were produced by counting the number of tender (T), swollen (S), warm (W), or combinations of (T), (S), and (W), joints (column 3). Graded indices, labelled with the prefix 'G', added the scores for severity of each sign (column 4) and the area weighted indices, labelled with the prefix 'A', were obtained by adding the surface area for each joint (from figures published by Lansbury and Haut [1956]) for each joint with the signs indicated (column 5). Each index was produced for all joints examined ('AJ' indices) and a restricted number of joints described by Egger et al [1985] ('RJ' indices), thus, a total of 66 articular indices were produced.

8.2.3.4 Selecting different combinations of joints

All the indices described in 1-3 above were calculated after selecting all joints examined (AJ indices) or, as advocated by Egger et al [1985], a restricted number of joints (RJ indices). These were the interphalangeal joint of the thumbs, individual proximal interphalangeal and metacarpophalangeal joints, individual metatarsophalangeal joints, the wrists (radio-ulnar and carpus), elbows, ankles (mortice), and the knees.

8.2.3.5 Described articular indices

The Ritchie, ARA, and Lansbury indices were calculated from appropriate observations. A modified Ritchie index, advocated by Hart et al [1985], in which tenderness is not graded, was also calculated.

8.2.3.6 Statistical analysis.

Comparisons were made between the articular index scores, CD, CRP and ESR levels using the Pearson correlation coefficient (r). The matrix of r values was examined for trends of changing values of r with the inclusion or exclusion of the index parameters studied.

8.3 RESULTS.

8.3.1 Described Indices.

The Ritchie index ($r=0.42$) and the Hart modified Ritchie index ($r=0.45$) correlated weakly with CD but did not reach statistical significance when adjusted for 70 tests. Both the ARA index ($r=0.65$) and Lansbury index ($r=0.67$), however, showed highly significant correlation ($p<0.0001$).

A correlation matrix for CD with the 33 'AJ' articular indices is shown in Table XI, and for the 33 'RJ' articular indices in Table XII.

Table XI CD and 'AJ' indices r values.

SIGNS*	SIMPLE COUNTS	GRADED INDICES	AREA WEIGHTED INDICES
(T)	0.61	0.53	0.54
(S)	0.46	0.47	0.38
(W)	0.46	0.57	0.58
(T/S)	0.62	0.54	0.48
(T/W)	0.62	0.59	0.55
(S/W)	0.49	0.55	0.47
(T/S/W)	0.61	0.57	0.48
(T+S)	0.50	0.45	0.53
(T+W)	0.46	0.54	0.65
(S+W)	0.38	0.50	0.53
(T+S+W)	0.35	0.50	0.57

For 30 patients the significance levels for the correlation coefficients after correction for 66 teats are: $r>0.56$, $p<0.05$. $r>0.58$, $p<0.02$. $r<0.60$, $p<0.01$.

*For key see Table X.

Simple joint counts more closely correlated with CD values when tender joints (T) were included in the index, compared with the results when swollen joints (S) and warm joints (W), either alone or in combination were included.

Table XII CD and the 'RJ' indices r values.

SIGNS*	SIMPLE COUNTS	GRADED INDICES	AREA WEIGHTED INDICES
(T)	0.46	0.28	0.49
(S)	0.39	0.35	0.50
(W)	0.25	0.37	0.36
(T/S)	0.46	0.35	0.41
(T/W)	0.41	0.35	0.42
(S/W)	0.35	0.40	0.46
(T/S/W)	0.45	0.38	0.38
(T+S)	0.42	0.26	0.65
(T+W)	0.38	0.42	0.49
(S+W)	0.33	0.34	0.44
(T+S+W)	0.37	0.42	0.55

For 30 patients the significance levels for the correlation coefficents after correction for 66 teats are: $r>0.56$, $p<0.05$. $r>0.58$, $p<0.02$. $r<0.60$, $p<0.01$.

*For key see Table X.

Grading the severity of the signs or weighting for joint surface area resulted in lower correlations with CD values for indices including tender joints (T) or swollen joints (S) but increased the correlation for indices including warm (W) joints. This difference was maintained with the combination indices.

For most indices, restricting the number of joints included in the indices (RJ indices, Table XII) did not importantly

reduce the correlation with CRP values, compared with the selection of all joints (AJ indices). The highest correlation was with the Lansbury index. A correlation matrix for the laboratory measures is shown in Table XIII.

Table XIII Correlation between ESR, CRP and CD.

	ESR	CRP	CD
ESR	1.00	0.63	0.48
CRP			0.68

8.4 DISCUSSION.

The results suggest that serum CD correlates moderately with clinical assessments of joint inflammation. However, the considerable difference in correlation between CD and the 4 described articular indices suggests that they are measuring different aspects of joint pathology. Consideration of the results obtained with the 66 new indices sheds light on the underlying reasons for these differences.

8.4.1 Simple joint counts (N).

Both Savage [1958] and Copeman [1964] considered joint tenderness to be the most reliable measure of joint inflammation. Their view is supported by the higher correlation of CD values with the indices that included tenderness compared with those counting combinations of only swelling and warmth. Nevertheless, soft tissue joint swelling can be identified by palpation and observation or semi-quantified using jeweller's rings [Boardman and Hart, 1967] and measurement of the reduction in finger joint swelling has been shown to differentiate between the anti-inflammatory and analgesic effects of drugs [Webb et

al, 1973]. The addition of swelling as an alternative sign of inflammation might have been expected to improve correlation with CD. This was the case for the indices that selected either of 2 or any of 3 signs. However counting the simultaneous presence of tenderness, swelling or warmth may exclude truly inflamed joints with only one sign, possibly explaining the lowered correlation with CD of these indices.

8.4.2 Grading the severity of the signs (G).

The lower correlation of CD levels with indices in which joints were graded for tenderness, compared with simple counts of tender joints, is in keeping with the higher correlation of CD values with the Hart modified Ritchie index (which omits grading) compared with the Ritchie index. This may represent differences in the strength of the pain stimulus (usually firm digital pressure over the joint margin) and variation in a patient's response when tested at different sites. Furthermore, there is no reason to suppose the arbitrary differences between grades represent linear differences in joint inflammation. The results support Hart's contention that grading is an important source of error in the Ritchie articular index.

8.4.3 Area weighted indices.

Area weighting decreased correlation compared with simple joint counts with the indices that selected either of 2 or any of 3 signs of inflammation but increased the correlation of those indices that selected the simultaneous presence of 2 or 3 signs. This might be because the addition of a second sign to identify inflamed joints tends to exclude joints that were tender because of mechanical damage rather than current inflammation, or swollen because of bony changes rather than synovitis. This reduces sensitivity in that marginally inflamed joints are likely to be omitted but increases specificity which would be amplified by weighting.

8.4.4 Selection of joints.

The optimum balance between information lost and reliability obtained by excluding some joints from an articular index was a restricted range of easily examined joints, as proposed by Egger et al [1985]. The reduced joint indices were found to show at least as high a correlation as the all joint indices, which suggests that they may be equally effective as measures of inflammation.

The low correlation of both the Hart modified Ritchie and the original Ritchie indices with levels of CD, compared with the simple counts of tender joints and graded tenderness indices respectively, suggests that selecting groups of joints as single units decreases the ability of an index to measure inflammation and, in conjunction with the grading of tenderness, seems to be the reason for the poor correlation between the Ritchie index and the CD value.

Joint selection may also play a part in the different correlations of the Lansbury and ARA indices.

8.4.5 The Lansbury index.

The Lansbury index which combines the grade of severity of tenderness and/or swelling and/or warmth weighted for joint surface area showed closest correlation with CD.

8.4.6 CRP and ESR

The correlation between CRP and ESR is in keeping with other studies [Crockson and Crockson, 1974]. CD correlated with these laboratory measures at levels that indicate measurement of related but not identical phenomena. The relationship suggests that about one half of the variance of CD, CRP or ESR results can be explained by the other measures.

CHAPTER IX

LONGITUDINAL CHANGES IN SERUM CYTIDINE DEAMINASE

9. LONGITUDINAL CHANGES IN SERUM CYTIDINE DEAMINASE

9.1 INTRODUCTION

RA is characterised by exacerbations and remissions that only broadly fit into any one pattern [Masi et al, 1983]. It is not possible to predict a naturally occurring change in patients' condition and the effects of multiple drugs are difficult to interpret. Any test that measures inflammation might be expected to alter over time in keeping with other measures. This chapter discusses the relationship between CD and a number of clinical and laboratory measures of joint inflammation measured in individual patients over a 12 month period in a group of RA outpatients.

9.2 PATIENTS AND METHODS

Ten patients with definite or classical RA [Ropes et al, 1958] attending one outpatients department were identified (mean(SD) age=60(12) years, F:M=7:3). Patients were monitored at monthly intervals by a single observer who was not responsible for their management. The following assessments were made using the forms shown in Appendix II: Current medications; pain (10 centimetre visual analogue scale); duration of early morning stiffness in minutes (EMS), and; the RJ.A(T+S) articular index [Thompson et al, 1986]. At each visit blood and urine samples were taken and the following tests performed: full blood count, ESR, CRP and CD, and urine dipsticks for protein and blood. The data were tabulated graphically.

9.3 RESULTS

The raw data for each patient are shown graphically in Figures 30 to 39. The upper graph illustrates changes in the clinical assessments, and the lower graph changes in the laboratory variables.

Figure 30 Patient 1.

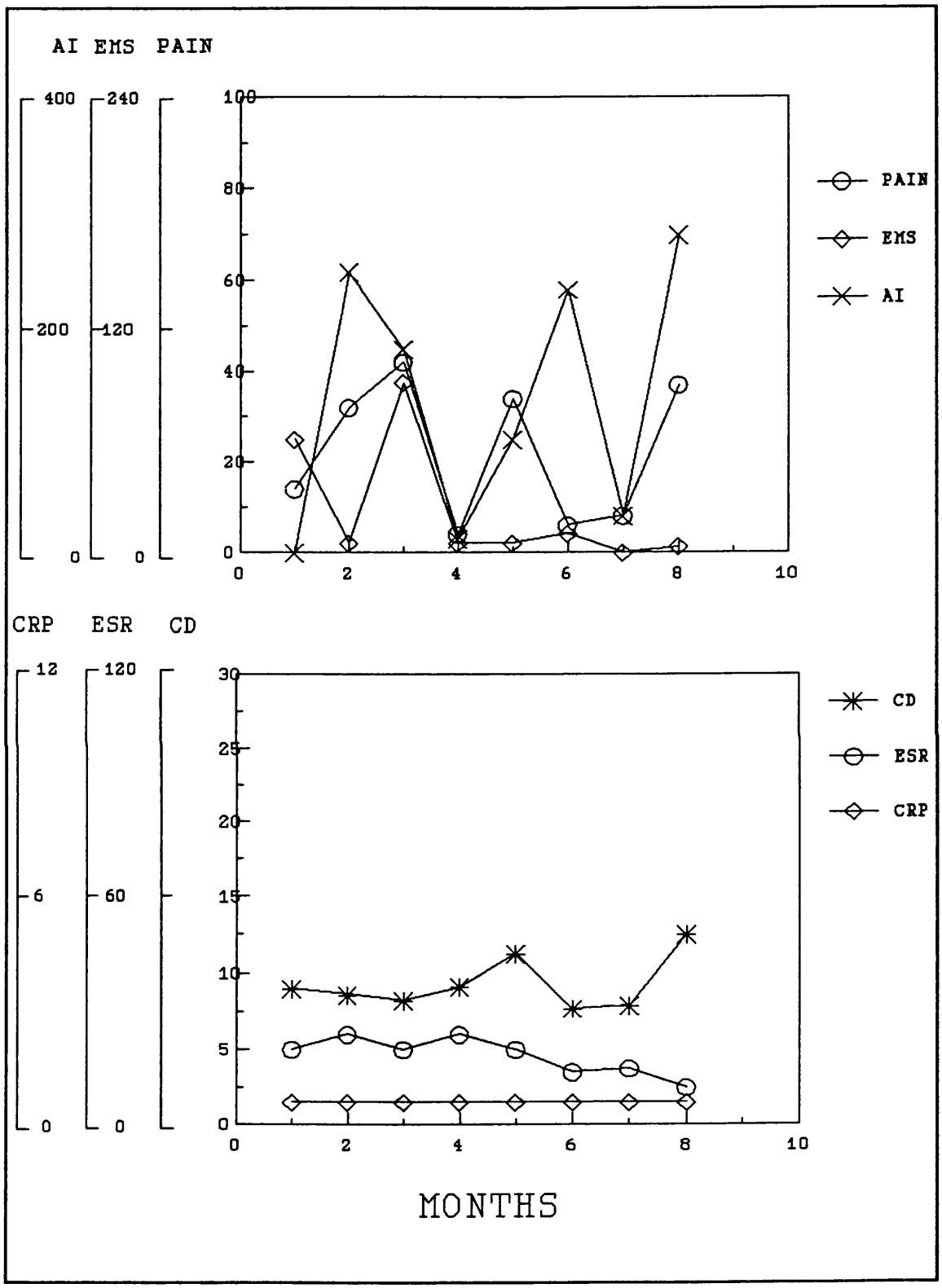


Figure 31 Patient 2.

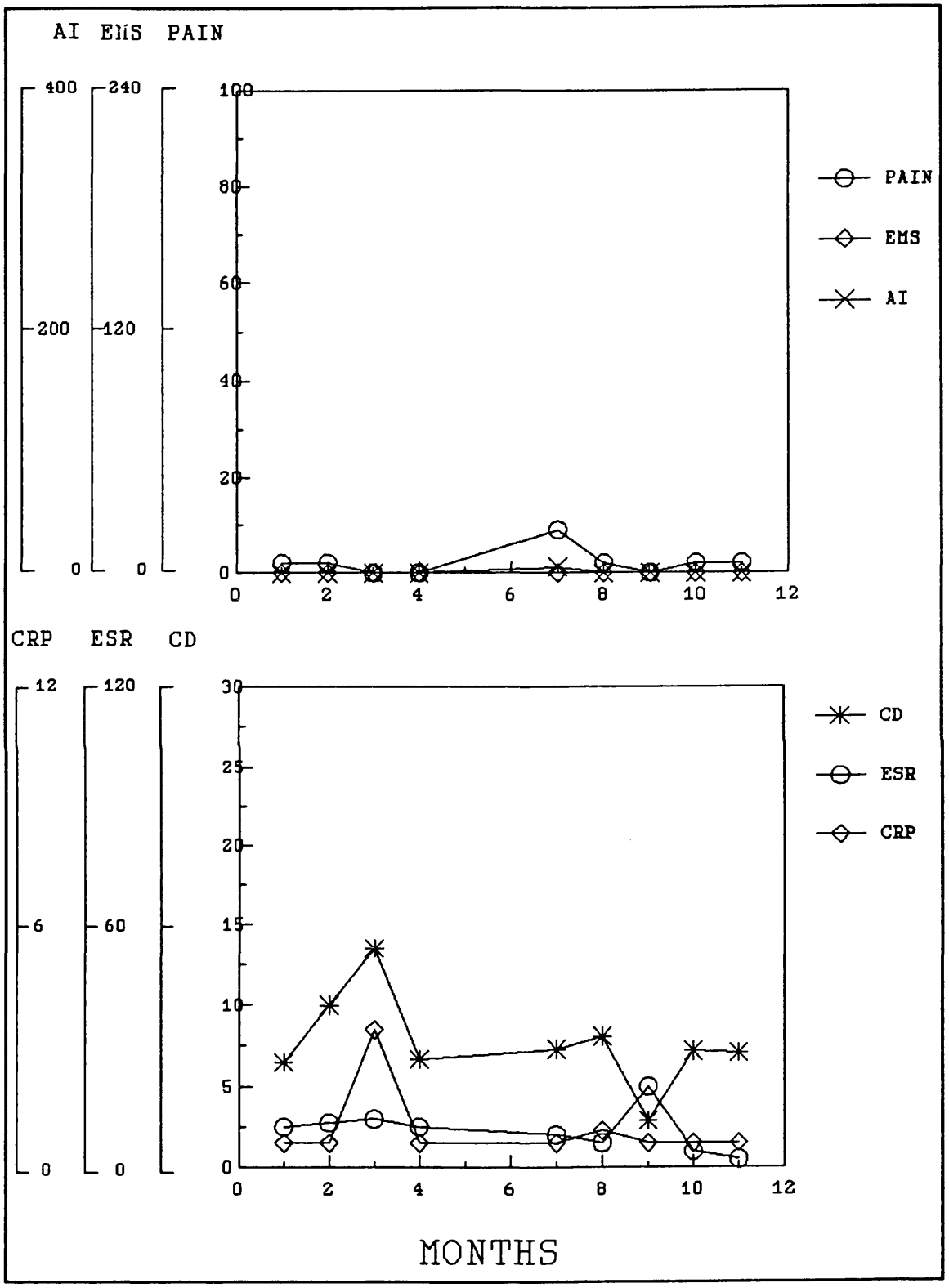


Figure 32 Patient 3.

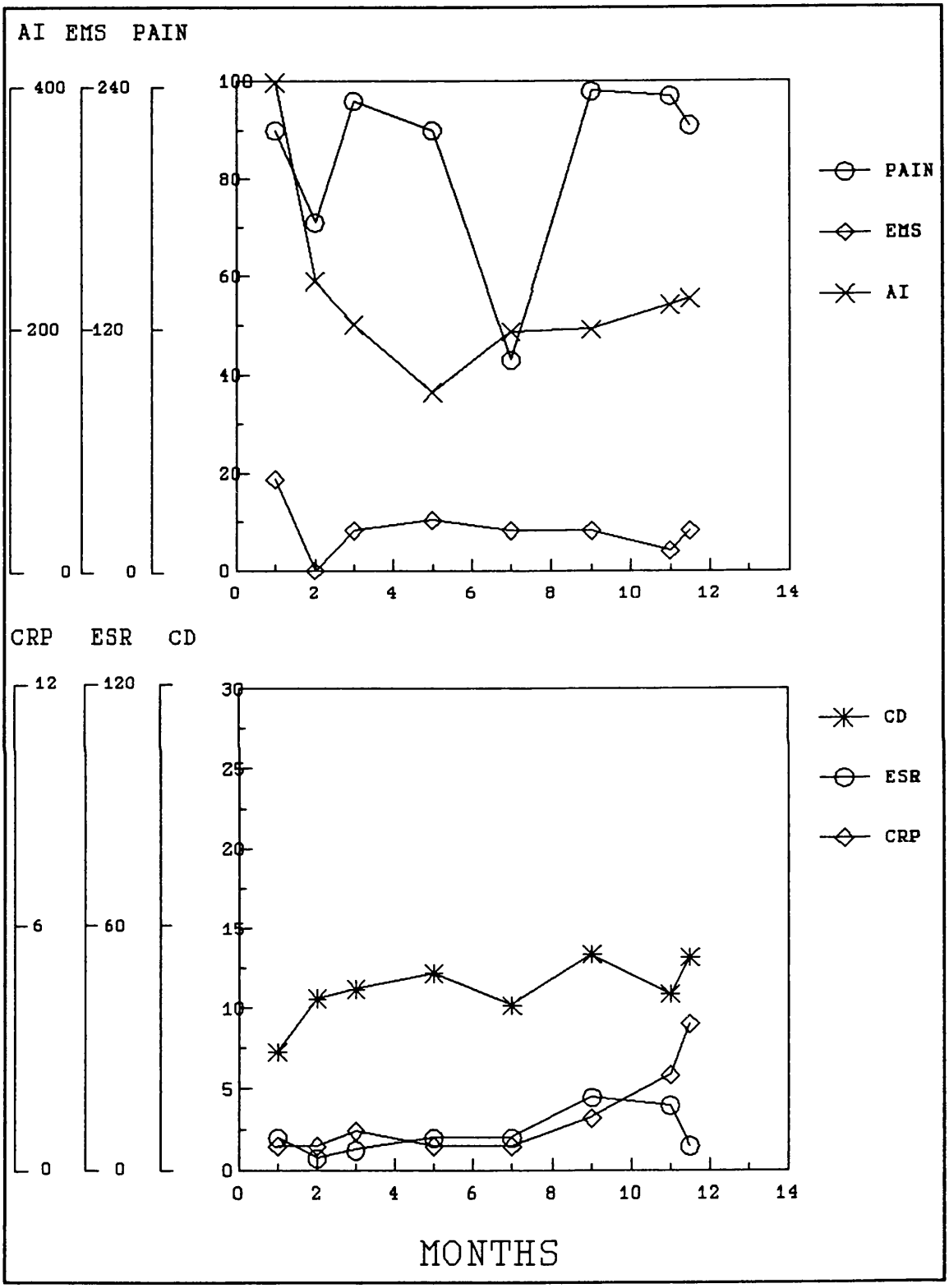


Figure 33 Patient 4.

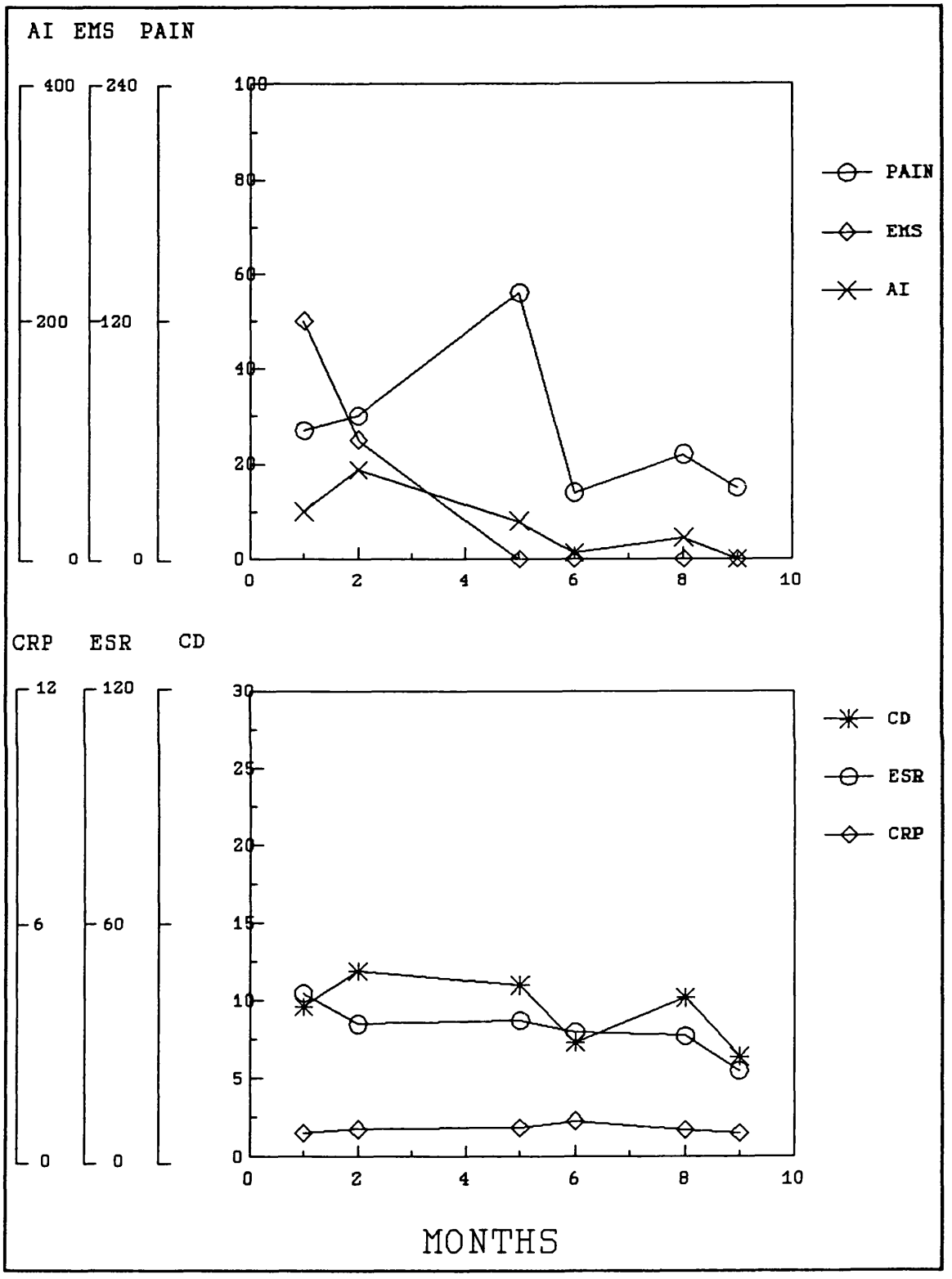


Figure 34 Patient 5.

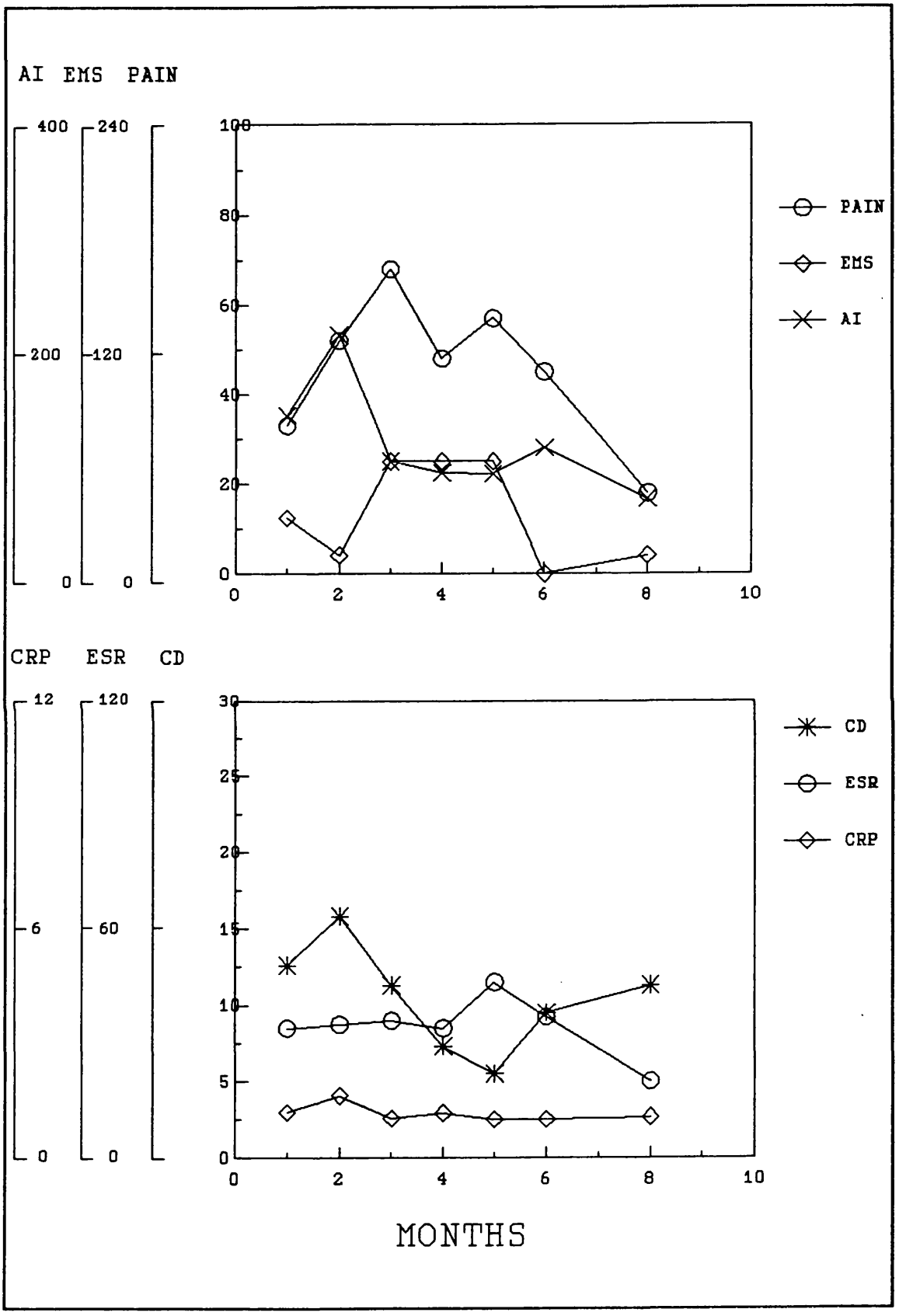


Figure 35 Patient 6.

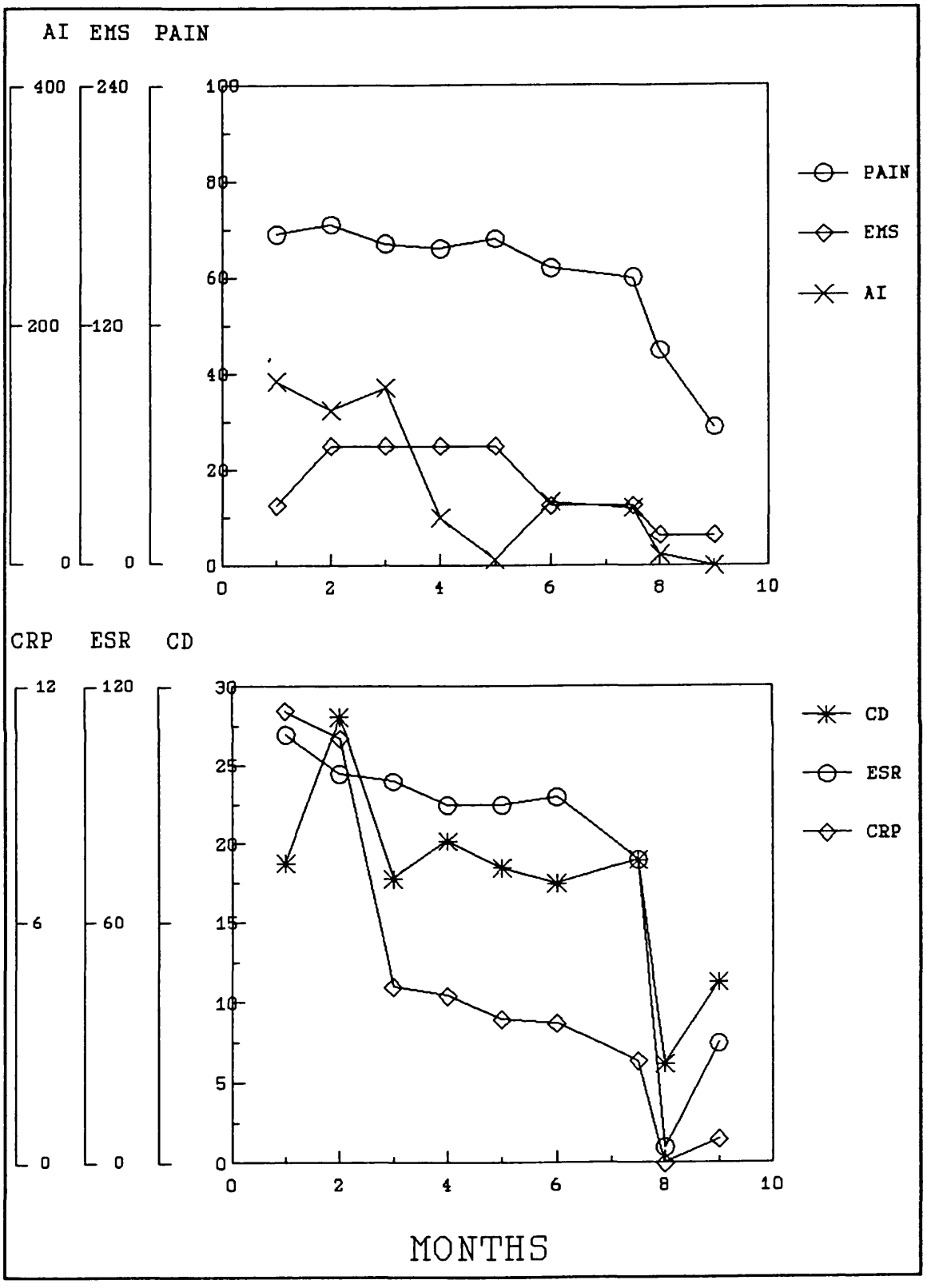


Figure 36 Patient 7.

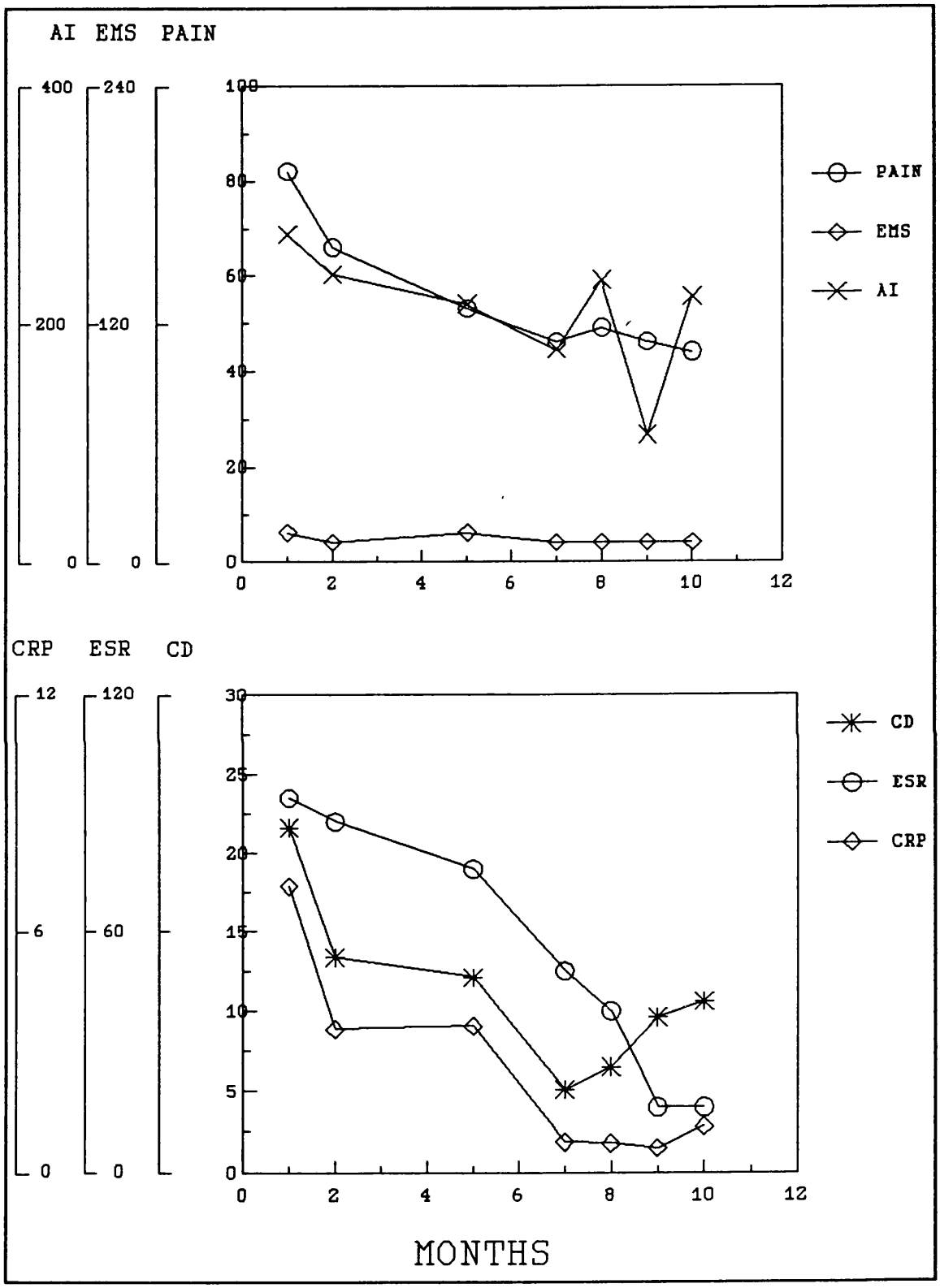


Figure 37 Patient 8.

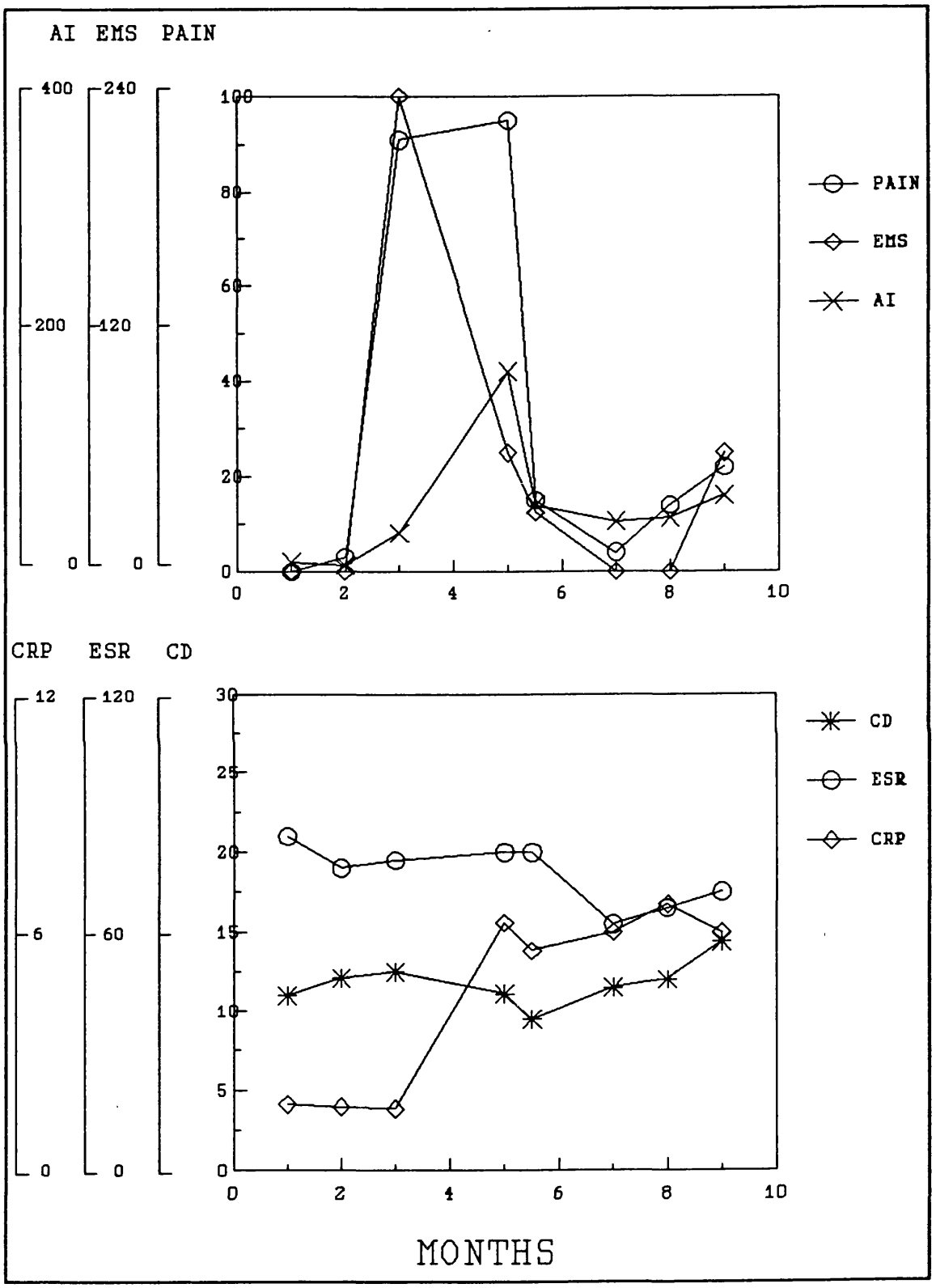


Figure 38 Patient 9.

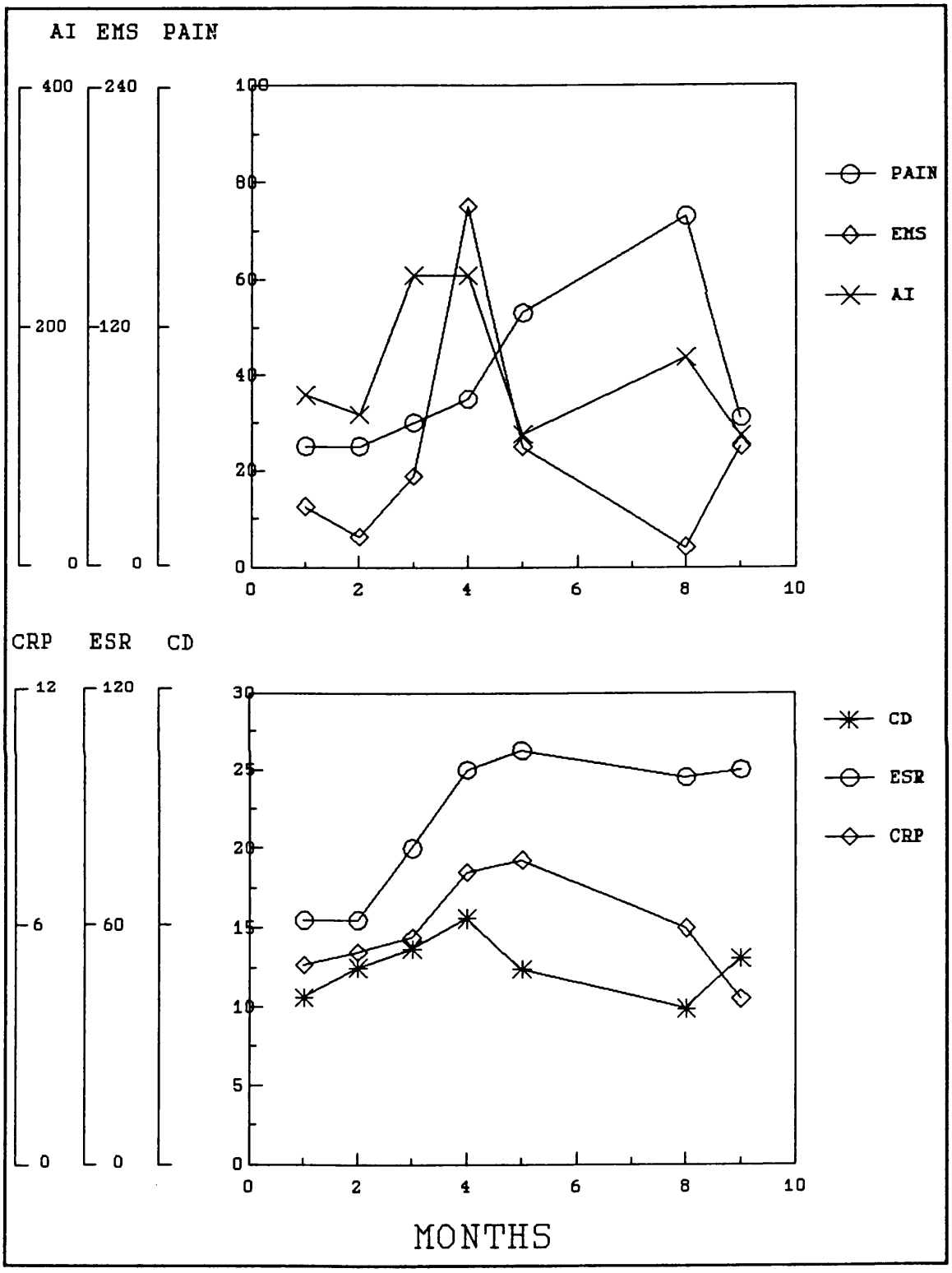
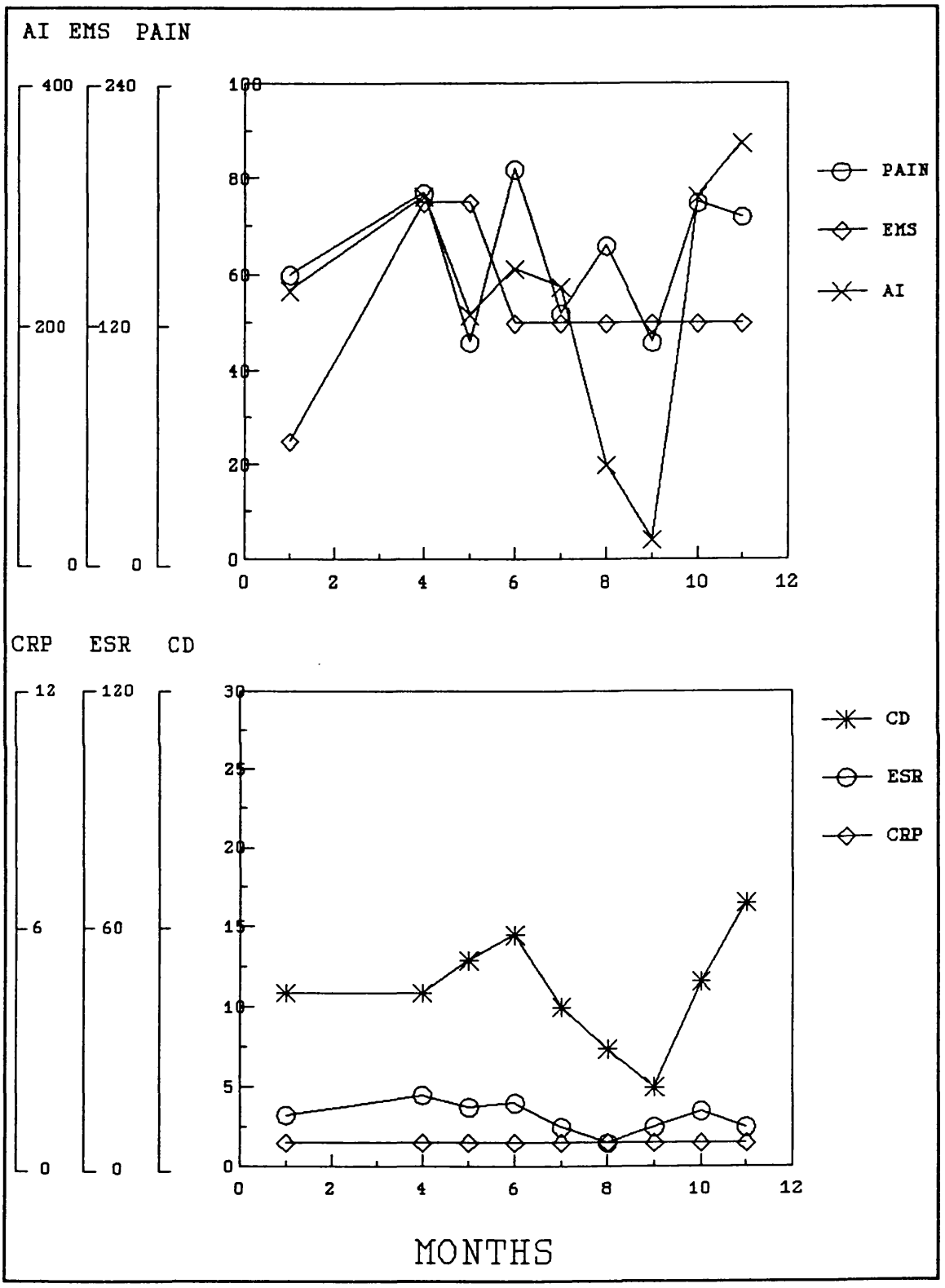


Figure 39 Patient 10.



Three broad patterns of change were seen in the clinical assessments:

(i) steady state (patients 1,2 and 3);

(ii) an improvement (patients 4,5,6 and 7), and;

(iii) a flare (patients 8,9 and 10).

In general the clinical changes were accompanied by parallel changes in the laboratory variables with some notable exceptions (compare the rise in CRP with the fall in ESR for patient 8, and the second rise in CD with the minimal change in ESR and CRP for patient 10).

Changes in the laboratory tests showed less variability than the clinical measures on a visit to visit basis (eg. patient 1) but produced large and consistent changes during improvement (eg. patient 6) or a flare (eg. patient 9).

The magnitude of these changes was greatest for CRP (range 0.06-12.00 nM/L), less for ESR (4-108 mm/hr) and least for CD (6.2-28.1 units/mL).

9.4 DISCUSSION

The results suggest that serum CD levels show low random variation but change in parallel with clinical and biochemical measures of inflammation.

While the clinical measures showed similar overall trends, there was considerable variation between them on a visit to visit basis. This may reflect poor observer reproducibility rather than true changes in patients' condition. Several workers have shown that subjective assessments of pain, EMS and articular index suffer from observer variation that would be unacceptable in a laboratory test, eg many articular

indices have coefficients of variation of over 30%. [Thompson and Kirwan, 1986; Hart et al, 1985]. These effects can be minimized by using a single observer and validated methods such as the pain visual analogue scale [Huskisson, 1974] and an articular index which omits grading of tenderness shown to be a major cause of variability in the Ritchie index [Hart et al, 1985]. Nevertheless, the unstable baseline produced by the large fluctuations from visit to visit is likely to reduce the sensitivity of the clinical measures to detect true changes in the degree of joint inflammation and response to treatment.

In contrast the biochemical measures show less fluctuations between visits and moderate correlation between changes over time. The reproducibility of these measures is much better than the clinical measures with coefficients of variation of less than 10%. The longitudinal correlation between the measures suggests that they are measuring aspects of the same event, but it is interesting to note some discrepancies. Patient 8, for example, shows a significant rise in CRP level at 3 months that is sustained for the rest of the study. This change is not accompanied by similar changes in the other measures. Indeed, the ESR shows a tendency to fall. So does this patient exhibit improvement or deterioration? This is a fundamental problem in the management of RA patients. What can be said about this case is that there was a flare in the acute phase response that was not accompanied by alterations of polymorph lysis in joints. Furthermore, because the ESR fell there was not a significant alteration in serum fibrinogen levels unless the effects of increased fibrinogen was offset by contrary changes in plasma proteins or red cell adhesiveness. This may be of no more help to the patient but it is one step closer to mechanisms of joint damage.

CHAPTER X

CHANGES IN SERUM CD FOLLOWING AN INDUCED FLARE OF INFLAMMATION

10. CHANGES IN SERUM CD FOLLOWING AN INDUCED FLARE OF INFLAMMATION

10.1 INTRODUCTION.

To be a useful measure of inflammation serum CD should be able to detect clinically important changes in a patient's condition. NSAIDs produce improvement in clinical measures of inflammation in RA patients but have little effect on the acute phase response or the ESR. Recent evidence suggests that, in addition to an effect on prostoglandin synthesis [Ferreira and Vane, 1974], NSAIDs have a direct effect on polymorph migration [Scheja et al, 1985; Scheja et al, 1986] suggesting that they might reduce polymorph turnover and decrease serum CD as well as reducing clinical measures of joint inflammation.

This chapter describes a study that was designed to evaluate the ability of serum CD to detect changes in joint inflammation when NSAID therapy was temporarily withdrawn from patients with RA, and to compare the pattern of response with existing clinical and laboratory measures.

10.2 PATIENTS AND METHODS.

10.2.1 Patients.

With Ethical Committee approval and written informed consent, ten volunteers with classical or definite RA [Ropes et al, 1958] (mean (SD) age = 63 (14) years, F:M = 6:4) were invited to participate. All patients had been receiving one NSAID in the same daily dosage for at least one month and a variety of other drugs that were not altered during the study. Patients a and e were receiving D-penicillamine, patient j gold and no patients were taking corticosteroids. Naproxen was taken by patients b and f, benorylate by patients c and d, and ibuprofen, fenbufen, ketoprofen, diclofenac and indomethacin

by patients i, g, a, h and e respectively. Patient j withdrew after 2 days because of intercurrent infection requiring hospitalisation.

Patients were visited every morning at about the same time at home or their place of work, for 13 days (day 0 - day 12) except day 6 (a sunday), by the same observer trained in clinical assessment and venepuncture.

The patients were studied in groups of 3, 3, 3 and 4 during separate 13 day periods. At each visit pain (10 centimetre visual analogue scale (VAS)), duration of morning stiffness in minutes (EMS), articular index, and drug therapy were recorded and blood was taken.

10.2.2 Treatment.

Patients were randomized to treatment with either: 6 days of their normal NSAID followed by 6 days of no NSAID (Group A, patients a, b, c, d and e) or; 6 days of no NSAID followed by 6 days of their normal NSAID (Group B, patients f, g, h, i and j), in open fashion. They were encouraged to take paracetamol tablets (500mg) up to 4 grams per day in divided doses as analgesia during the no NSAID period and to record all drugs taken each day on forms provided.

10.2.3 Samples.

The serum was separated within 4 hours of venepuncture, coded and stored at -20°C for subsequent measurement of CD activity by the method previously described and for CRP by the turbidometric method employing a Hyland Laser Nephelometer [Ritchie, 1967]. The assays were performed without knowledge of patients' treatment.

10.2.4 In vitro samples.

Normal serum was incubated with naproxen, ibuprofen, ketoprofen, diclofenac and indomethacin at 1, 2, 20 and 200 times therapeutic serum levels to determine whether the assay was affected in vitro by the NSAIDs.

10.2.5 Statistics.

The difference between the groups and the differences between the peak results during the flare and baseline levels on day 5 for group A and day 12 for group B, and were compared using Student's t test.

10.3 RESULTS.

10.3.1 Demography.

Groups A and B were similar with respect to mean(SD) age (A=65(14), B=61(16) years), sex ratio (F:M, 3:2) and mean(SD) values for pain (A=42(25), B=60(18) mm), EMS (A=49(64), B=65(52) minutes), Ritchie index (A=19(14), B=20(10), and CD levels (A=5.8(2.2), B=6.3(2.1) units), but Group B (18mg/L) had lower mean CRP levels than Group A (33mg/L), $p<0.01$).

10.3.2 In vitro samples.

The presence of 5 NSAIDs in vitro, even in high concentration, had no effect on CD assay results.

The pattern of individual daily CD results were similar for all patients except patient b (Figures 40 and 41).

Figure 40 Group A.

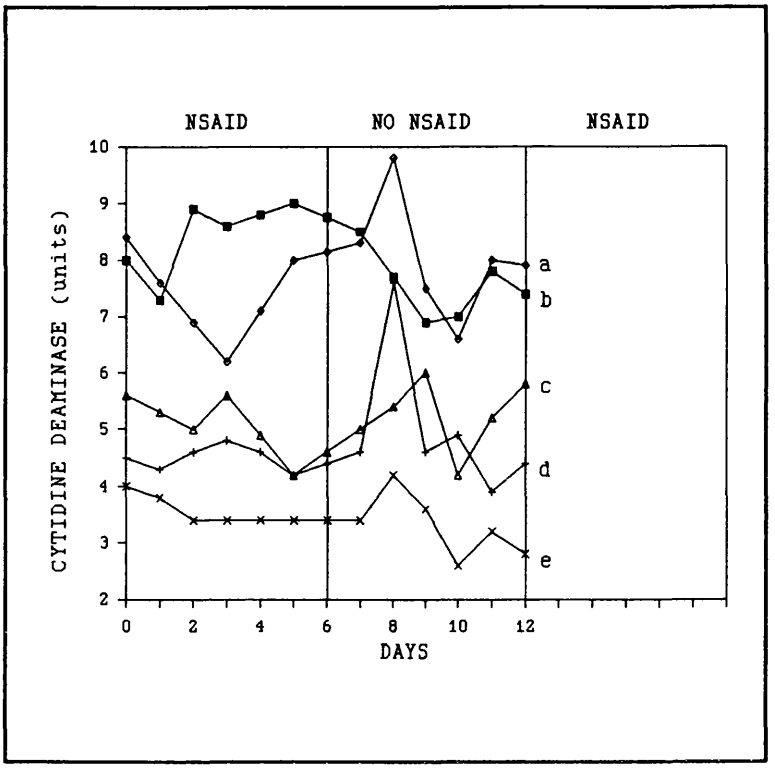
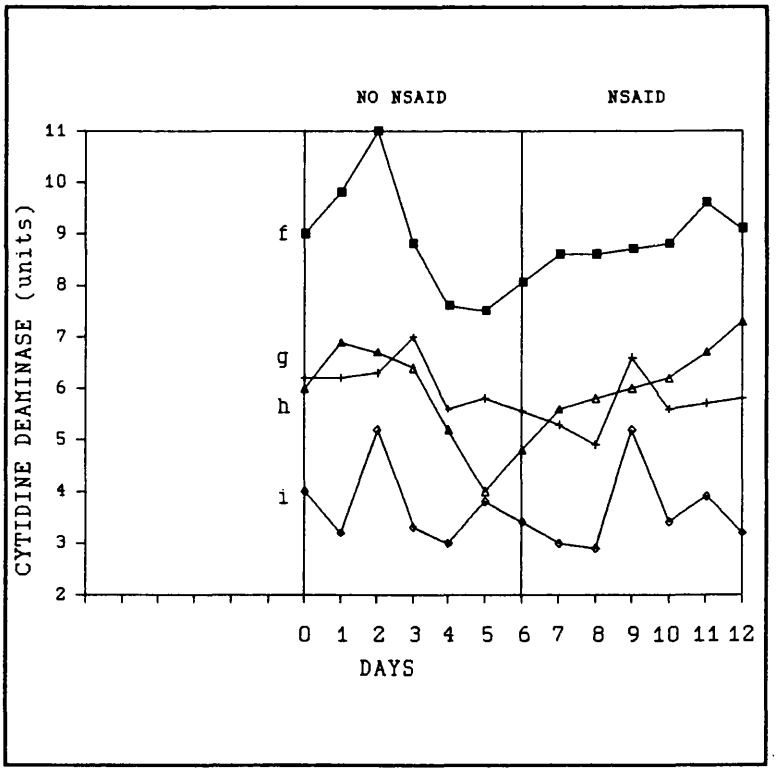
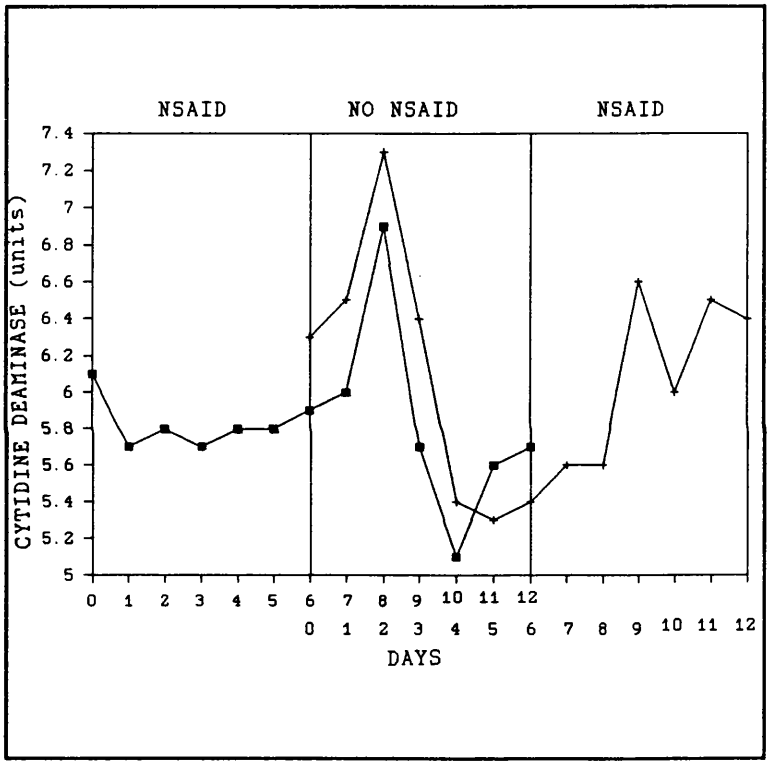


Figure 41 Group B.



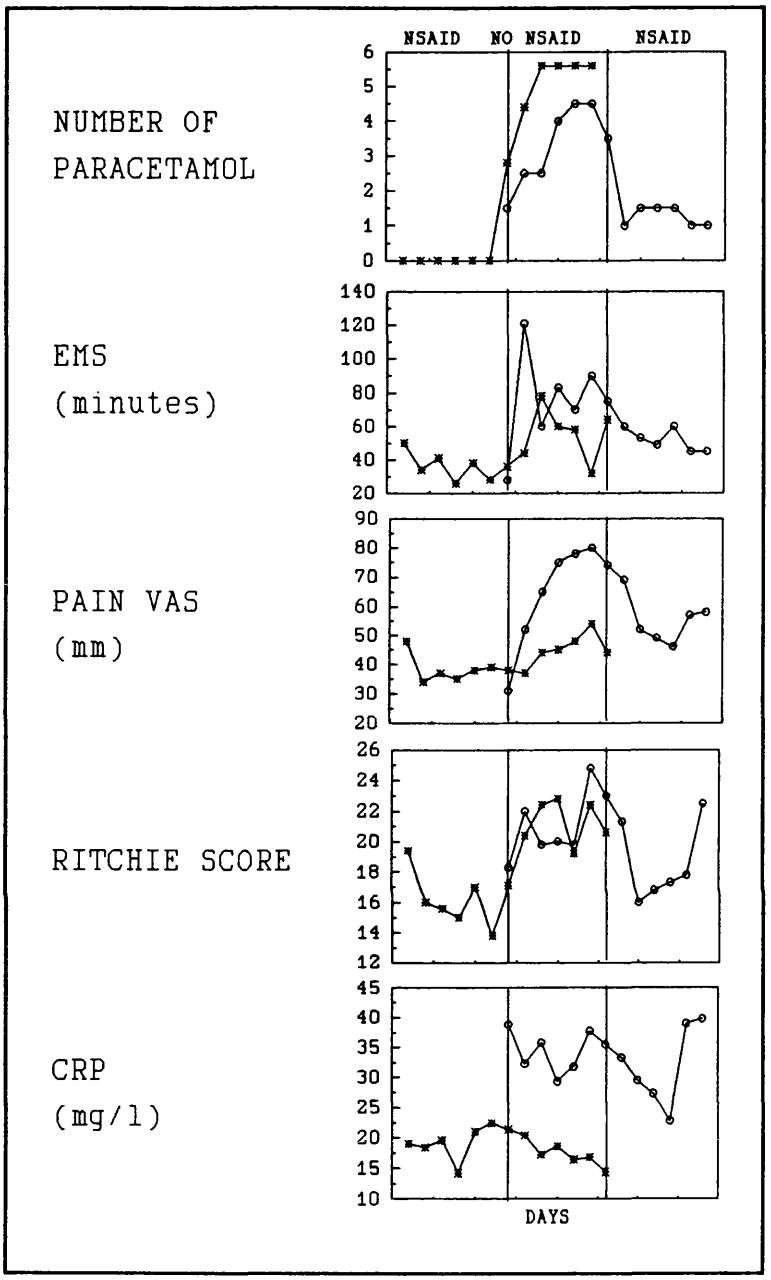
The mean daily CD results for groups A and B are shown in Figure 42. A significant peak ($p<0.05$) occurred 2 days after stopping NSAIDs, and a trough 2 days later ($p<0.05$), followed by a return to the baseline.

Figure 42 Mean CD results.



The flare was detected by all other measures except CRP (Figure 43) but the pattern of response differed from that of CD as there were no troughs and the peaks occurred later.

Figure 43 Mean results for the other measures.



11. DISCUSSION.

The results suggest that serial blood levels of CD can detect the flare produced by the withdrawal of NSAIDs from RA patients, and support the observation that NSAIDs inhibit polymorph turnover in vivo [Scheja et al, 1985].

The study was designed so that daily measurements could be undertaken in the patients' home environment during an induced flare of joint inflammation. The 'flare' technique is a recognised method used to select patients for trials of new NSAIDs [Paulus, 1983]. Ideally all patients would have been monitored for 18 days and received the same NSAID during the first and last 6 day periods and no NSAID during the middle 6 days period. However, in order to minimise the numbers of daily venepunctures patients were divided into the 2 groups. The higher mean CRP level for Group B suggests a difference in the acute phase response in the 2 groups, however, similar differences were not seen with any other clinical or serological measure of inflammation. While this study design complicated statistical interpretation of the results we were mainly interested in the timing and pattern of change in the clinical and serological measurements.

Differences in synovial fluid clearance rates of the NSAIDs might have been expected to affect the timing of the changes. In practice the peaks during the flare for different patients occurred within a few hours of each other, suggesting that such considerations are not of great practical importance when NSAIDs were withdrawn. However, the unstable baseline for all measures when NSAIDs were restarted (Group B) might have reflected fluctuations in blood and synovial fluid levels before steady state kinetics were reached.

Serial CD levels showed similar changes for most of the patients suggesting that the mean pattern was representative for patients as a group. Patient b showed a fall in serum CD during the flare despite changes in the clinical scores. The patient took up to 5 grams per day of paracetamol during the no NSAID period suggesting a possible effect of this drug in high doses on polymorph turnover. This point is further discussed below.

The gradual increase in EMS, pain and Ritchie scores after treatment withdrawal is in keeping with the known inhibitory effect of NSAIDs on prostaglandin synthesis [Ferreira and Vane, 1974]. The pattern of CD response with a sharp early peak and subsequent trough suggests a different mechanism. In vivo studies using skin window techniques have shown inhibition of polymorph migration by all NSAIDs tested which included: diclofenac; indomethacin; naproxen; ibuprofen; and piroxicam [Scheja et al, 1985; Scheja et al, 1986].

It has been estimated that the half-life of a synovial fluid polymorph in RA is 4 hours. In a 30ml effusion containing 25 x 10^9 polymorphs per litre, the daily breakdown in the synovial cavity might exceed a billion cells [Hollingsworth et al, 1967]. However, the kinetics of polymorph release from the marrow and duration in the blood are similar in normal controls and RA patients [Bertino et al, 1963], therefore, at least in the short term, the enormous numbers of polymorphs entering inflamed joints will be balanced by the same number leaving the marrow. Sudden removal of the inhibitory tone of chronic NSAID therapy might allow a large influx of cells into joints. These cells would be rapidly destroyed releasing CD into synovial fluid with the subsequent rise in serum CD activity. The peak at 2 days fits well with the time taken for cell migration to return to normal after NSAID removal in the skin window experiments [Scheja et al, 1985].

The subsequent trough was not anticipated. It might result from a transient depletion of polymorphs available to migrate into the joints following the sudden influx. If so, leucopenia might be anticipated but circulating polymorphs were not measured during this study. An alternative explanation is that paracetamol, taken as escape analgesia, has a stabilizing effect on polymorph activity that has not been previously documented. This is supported in that both patients who were taking benorylate, which yields paracetamol

after ingestion, showed a peak in CD levels during the flare. There is evidence that paracetamol lacks anti-inflammatory action [Boardman and Hart, 1967] but it would be worth while checking its in vivo effect on polymorph migration.

Serial blood levels of CRP showed large daily variation but did not significantly change overall during the study period supporting the belief that NSAIDs do not affect the acute phase response at least in the short term [Ferreira and Vane, 1974].

The open nature of the study probably introduced bias in favour of the clinical measures because the patients and the nurse/metrologist anticipated the flare. This was particularly true for the number of paracetamol taken because patients were actively encouraged to use paracetamol as analgesia during the no NSAID period. In contrast, the CRP and CD assays were carried out without knowledge of the patients' condition or treatment. Any comparison between the clinical and serological measures should recognise this bias and take it into account.

CHAPTER XI

IS CYTIDINE DEAMINASE A USEFUL MEASURE OF INFLAMMATION IN RHEUMATOID ARTHRITIS?

11. IS CYTIDINE DEAMINASE A USEFUL MEASURE OF INFLAMMATION IN RHEUMATOID ARTHRITIS?

The thesis has been structured to investigate the properties of CD according to the principles of measurement theory. Published guidelines [Tugwell and Bombardier, 1982] suggest that a useful measurement tool should: (i) be simple to perform (practical); (ii) show low observer variation (reproducible); (iii) make sound biological sense (biologically valid), and; (iv) detect the smallest clinically important change of interest to the patient and the physician (sensitive).

11.1 PRACTICABILITY.

Serum sampling is a routine procedure frequently carried out in RA patients to monitor potential drug side effects. A 5 mL blood sample needed for duplicate CD assays is unlikely to be difficult to obtain at clinic visits. The alteration of serum CD with exercise suggests that samples should not be taken after vigorous activity but the changes are small and are not of the same order as the changes seen during a response to treatment. Thus strict adherence to a particular time of day is not necessary when taking a blood sample.

Preparation of serum is simple and can be undertaken during routine preparation for other laboratory tests. Samples can be safely processed within 4 hours of collection and longer storage at room temperature will result in only a small loss in activity. Storage at -20°C will produce only a small drop in CD activity after 6 months.

CD is a robust protein that does not require delicate conditions to assay. The spectrophotometric method described is simple to perform, with basic equipment and reagents costs only a few pence for each sample. It can be easily carried out in a modest laboratory by a semi-skilled technician.

However, the manual method described is labour intensive. The assay can be semi-automated using mechanical sampling devices but still requires a 4 hour incubation period for the deamination reaction. Incubation at higher temperatures has been suggested to reduce the time but has not gained acceptance because of the difficulties maintaining a high temperature differential and the danger of protein denaturation [Jones and Roberts, 1986]. The HPLC method requires labour intensive sample processing and the cost of the equipment is high. With the purification of CD the way will be open to produce antibodies allowing the use of highly sensitive techniques for sample analysis, but this is for the future.

11.2 REPRODUCIBILITY.

The intrabatch and interbatch variation of the assay was less than 10 percent. While by no means perfect this represents acceptable reproducibility and is considerably better than for clinical assessments where the variation may be more than 30 percent between observers [Thompson and Kirwan, 1986] and many immunological test with variation around 20 percent. [Haskard D, Personal communication].

There was reasonable agreement between 2 laboratories using slightly different methods, so that, in the context of RA patients, the laboratories could be used interchangeably. This would allow broad comparisons to be made between different units which has important implications for multicentre studies. This is not true for most clinical measures and many standard biochemical measures, for example alkaline phosphatase, where laboratory techniques are not standardised. The between laboratory reproducibility for CD is monitored by a quality control system currently run by Dr D D Jones.

Under stable condition the day to day serum activity of CD remains within close limits. Similarly, the variation between serum CD levels between clinic visits shows low variation when a patient's condition is not changing. This suggests that total joint polymorph lysis, and consequently serum CD levels, do not shown large, random fluctuations often seen, for example, with the ESR.

11.3 BIOLOGICAL VALIDITY.

Published data suggested that CD was found in high concentrations in several tissues but particularly in liver, placenta and polymorphs and was released from these cells during tissue damage. This was in keeping with current concepts of clinical enzymology, and certain predictions were made as to the likely synovial fluid CD concentrations in disease.

The investigations described support the hypothesis that CD leaked out of dead and damaged polymorphs that were accumulating in RA joints and that synovial fluid CD was a good measure of synovial fluid polymorph count. Observed synovial fluid CD levels closely approximated those expected. Evidence was put forward that CD diffused down a concentration gradient from cells to fluid to synovium to the blood, and that raised serum CD found in RA patients originated predominantly from joints.

Serum measurements of other markers were made in an attempt to exclude liver or kidney damage as a source for CD as injury to these organs has been associated with raised serum CD levels. Furthermore, raised ALP and LDH have been reported in RA patients. Data was presented that suggested that raised serum LDH originated from synovial fluid polymorphs in the same way as CD. ALP reflected the acute phase response and hepatocyte damage did not occur because of the normal liver transaminases. Therefore, the evidence suggests that serum CD

does not originate to any significant amount from the liver or kidney in RA patients unless there is concomitant liver disease.

This possibility was further investigated using a selective inhibitor of liver CD. It was shown that serum CD levels of RA patients contained significantly higher concentrations of inhibitor resistant CD than patients with known liver disease from a number of different causes.

Serum CD correlated with other clinical and laboratory measures of joint inflammation at levels that suggest measurement of different aspects of the inflammatory process.

There is, thus, direct laboratory results and strong circumstantial evidence to support the hypothesis that serum CD is an integrated measure of polymorph lysis in all joints and that measurement of serum levels supply meaningful information in RA patients.

11.4 SENSITIVITY.

To the best of the author's knowledge serum CD is the only simple biochemical measure that can detect the flare following the withdrawal of NSAIDs in RA patients. While small, the stable baseline allowed the changes to be documented. This ability to detect a small, but clinically very important change in a patient's condition suggests that serum CD is a sensitive measure of joint inflammation.

The longitudinal changes seen in patients followed for 12 months suggests that serum CD levels are able to detect real changes in RA patients' inflammatory status. The response of serum CD to the introduction of other conventional treatments in RA and the possibility that serum CD might be used as a measure of the effect of potential new drugs on polymorph

lysis is exciting but further evaluation is beyond the scope of this text.

11.5 CONCLUSION.

Serum CD is a simple, cheap, reproducible and sensitive measure of joint inflammation in RA that reflects the turnover of a specific cell type known to be at the centre of the inflammatory process. It supplies the clinician with a serological tool for patient monitoring which is a more direct reflection of inflammation than measurements such as the ESR. Its role as an in vivo marker of polymorph turnover may also prove helpful in elucidating the relationship between joint inflammation and destruction.

APPENDIX I

CYTIDINE DEAMINASE ASSAY

I. CYTIDINE DEAMINASE ASSAY

A. APPARATUS

Plastic disposables were used throughout (Sarstedt clear polystyrene tubes 11.5mm x 75mm, Cat. no. 55.478; and caps, Cat. no. 65.809). Air displacement pipettes of adjustable volume were used to aliquot the substrate and standards which were prepared in plastic disposable containers (Sterelin 25mls and 7mls). Absorbances were determined on a Kontron Uvikon 860 UV/VIS double beam spectrophotometer reading at 630nm.

B. REAGENTS

1. Stock buffers

Buffer A 0.1M Na_2CO_3 (10.6g/L)
Buffer B 0.1M $NaHCO_3$ (8.4g/L)

2. Working buffer

10% buffer A to 90% buffer B.
pH 9.2
Store at 4°C. Stable indefinitely.

3. Substrate

80mg cytidine in 10mls working buffer
Dilute 1:7 with working buffer for use.
Store at 4°C. Stable indefinitely.

4. Phenol reagent

10g phenol, 150mg sodium nitroprusside
Make upto 1L with distilled water.
Store in amber bottles at 4°C. Stable up to 1 month

5. Hypochlorite reagent

5g NaOH, 21.3g Na_2HPO_4
10mls of 10-14% sodium hypochlorite solution
Make upto 1L with distilled water.
Store in amber bottles at 4°C. Stable up to 1 month.

6. Ammonia standards

a. Stock

1.14g/L of distilled water. Store at 4°C. Stable indefinitely.

b. Working

For top ammonia standard dilute stock ammonia solution 1:19 with working buffer. Prepare daily.

For the preparation of other ammonia standards use TABLE XIV.

Table XIV Preparation of ammonia standards.

STANDARD NUMBER	VOLUME (mL) DILUTE STOCK	VOLUME (mL) BUFFER	ENZYME ACTIVITY (units)
1	0	4	0.0
2	1	3	13.5
3	2	2	27.0
4	3	1	40.5
5	4	0	54.0

C. SAMPLES

Serum or plasma samples are suitable but should be separated within 4 hours of venepuncture. Samples were stored at -20°C (stable for several months) or assayed immediately. Haemolysed samples were not used.

D. ASSAY

The method used was that of Jones et al.[3], which involved:

1. Deamination:

$$\text{CYTIDINE} + H_2O \xrightarrow[\text{pH 9.2, 4 hrs, 37°C}]{\text{Cytidine Deaminase}} \text{URIDINE} + NH_3$$

2. Ammonia estimation:

$$NH_3 + \text{PHENOL/HYPOCHLORITE} \xrightarrow[\text{40 mins, 37°C}]{} \text{BLUE COMPLEX}$$

E. PROTOCOL

Cap tubes.

Mix (rotomixer).

Incubate at 37°C for 4 hours.

Table XV Protocol.

REAGENT	VOLUME (μL) TEST	VOLUME (μL) CONTROL	STANDARD
BUFFER	0	150(40)*	0
SUBSTRATE	150(40)	0	0
EXPERIMENT STANDARDS (1-5)	0	0	150(40)
POOLED SERUM	0	0	100(27)
TEST SERUM	100(27)	100(27)	0

*Volumes in brackets are used for the miniassay.

1. Berthelot reaction

a. Large sample volume assay

Add 1.5 mls phenol reagent to each tube (stops reaction), followed by 1.5 mls hypochlorite reagent.

b. Mini-assay

Add 0.4 mls of phenol reagent followed by 0.4 mls hypochlorite reagent.

Cap tubes and mix thoroughly.

Incubate at 37°C for 40 minutes.

Read absorbance at 630 nm, 10mm cell.

Draw standard curve and read results.

One unit of enzyme activity = 10^{-4} uMol NH_3/ml/min. Divide by 3.25 to convert to Aberystwyth units(18 hr incubation at 22°C, [1]).

Normal range 3.2-13.2 units.

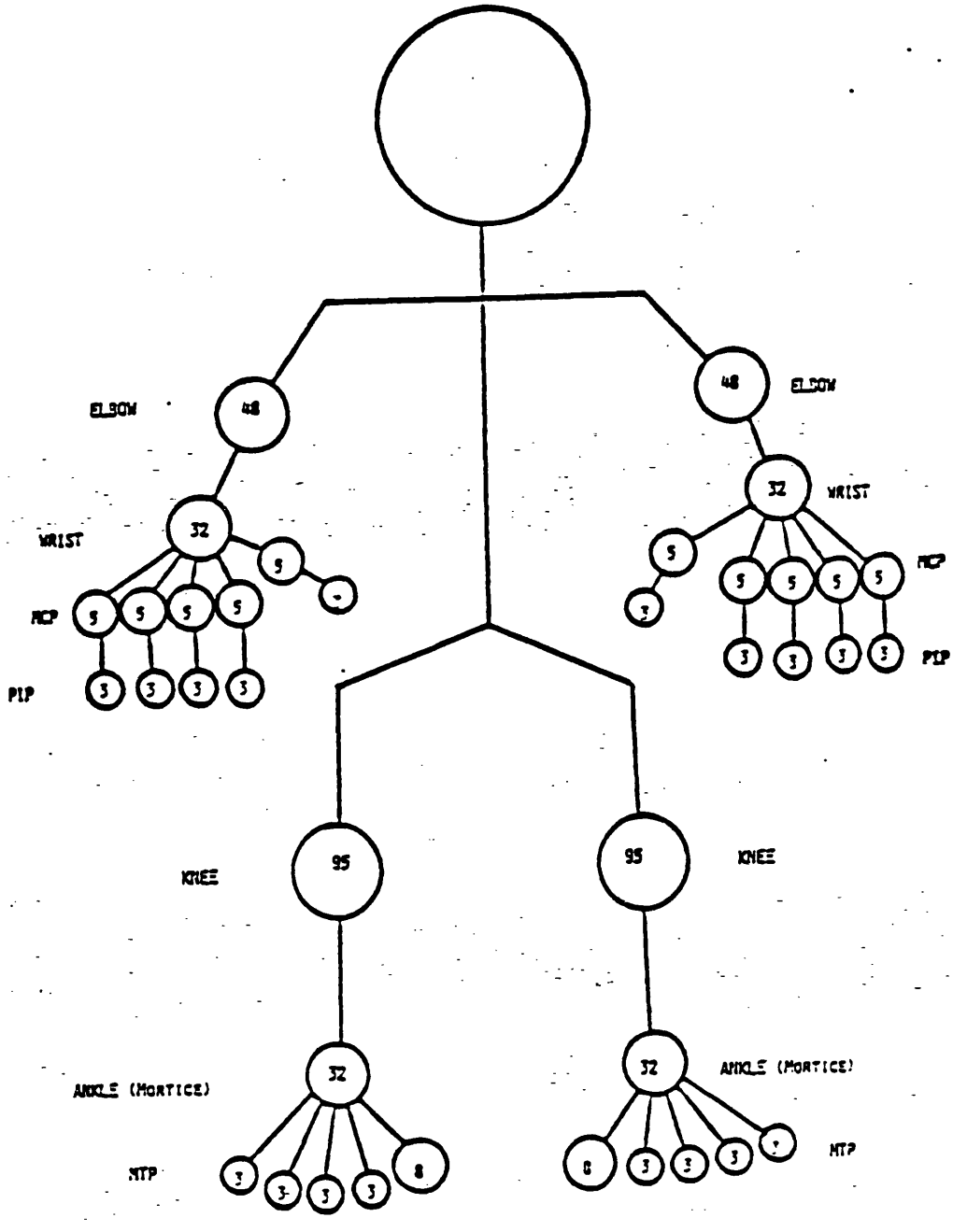
APPENDIX II

METROLOGY FORMS

AND HAVE SOFT TISSUE SWELLING OR EFFUSIONS.
ADD THE ENCIRCLED NUMBERS FOR THE MARKED JOINTS AND WRITE THE TOTAL IN THE BOX.

PATIENT'S NAME.....
OBSERVER.....
DATE.....

TOTAL SCORE



NO PAIN ——— WORST PAIN POSSIBLE

DATE

PATIENT HOSPITAL NO.

DRUGS

SKIN RASH

EMS(minutes)

PAIN (mm)

ARTICULAR INDEX SCORE

URINE PROTEIN (dipstick)

BLOOD Hb ESR

PLATES

WBC CRP

%Pmn CD

NEXT APPOINTMENT

COMMENTS

APPENDIX III

PUBLICATIONS RESULTING FROM THE THESIS

Thompson PW, Kirwan JR, Rudge SR, Houghton BJ, Currey HLF, Eds. Laboratory Markers of Joint Inflammation and Damage. Arthritis and Rheumatism Council Conference Proceedings III. London. 1988.

Thompson PW, Jones DD, Currey HLF. Cytidine deaminase activity as a measure of acute inflammation in rheumatoid arthritis. Ann Rheum Dis 1986;45;9-14

Thompson PW, Kirwan JR. Observer variation and the Ritchie articular index. J Rheumatol, 1986;13;836-7

Thompson PW, Silman AJ, Kirwan JR, Currey HLF. Articular indices of joint inflammation in rheumatoid arthritis. Correlation with the acute phase response. Arthritis Rheum 1987;30;618-23

Thompson PW. Laboratory markers of joint inflammation and damage. Br J Rheumatol, 1987;26;83-5

Thompson PW Kirwan JR, Jones DD, Currey HLF. Serum cytidine deaminase responds to changes in non-steroidal anti-inflammatory therapy in rheumatoid arthritis. Ann Rheum Dis, 1988;47;308-12

Thompson PW, Kirwan JR, Currey HLF. The ability of 28 articular indices to detect an induced flare of joint inflammation in rheumatoid arthritis. Br J Rheumatol 1988;27;375-80

Levick JR, Thompson PW. Intra-articular volume as an important factor governing macromolecular half life in synovial fluid. Ann Rheum Dis 1988;47;701-2

Thompson PW, James IT, Wheatcroft S Pownall R, Barnes CG. Circadian rhythm of serum cytidine deaminase in rheumatoid arthritis patients during rest and exercise. Ann Rheum Dis, 1989;48:502-4.

James IT, Herbert KE, Perrett D, Thompson PW. A rapid and sensitive HPLC assay for cytidine deaminase in serum. J Chromatol, 1989 (in press)

Thompson PW, Houghton BJ, Clifford C, Jones DD, Whitaker KB, Moss DW. The clinical enzymology of rheumatoid arthritis: an alternative interpretation of raised 'liver' enzymes. Lancet, 1989 (submitted).

Thompson PW, James IT, Jones DD. A selective inhibitor of polymorphonuclear leucocyte cytidine deaminase in rheumatoid arthritis. (in preparation)

Thompson PW, Pegley F, James IT. Longitudinal changes in serum cytidine deaminase in rheumatoid arthritis. (in preparation)

II. SCIENTIFIC LETTERS

Thompson PW, Jones DD. Serum lactic dehydrogenase as a marker of joint damage in rheumatoid arthritis. Ann Rheum Dis 1987;46;263

Thompson PW, Jones DD, Currey HLF. Serum cytidine deaminase as a laboratory test for acute inflammation in rheumatoid arthritis. Ann Rheum Dis 1988;47;173-4

III. ABSTRACTS

Thompson PW, Jones DD, Currey HLF. Cytidine deaminase; a measure of synovial inflammation. Br J Rheumatol, 1985;24;203

Thompson PW, Jones DD Currey HLF. Serum cytidine deaminase as an integrated measure of acute joint inflammation. Br J Rheumatol, 1986;25;97-8

Thompson PW, Kirwan JR. A comparison of articular indices in rheumatoid arthritis. Br J Rheumatol, 1986;25;98-9

Thompson PW, Kirwan JR. Raised serum enzymes in arthritis; their source and potential for disease assessment. Br J Rheumatol, 1986;15;31-2

Thompson PW, Whitaker KB, Kirwan JR. Alkaline phosphatase isoenzymes in rheumatoid arthritis. B J Rheumatol, 1986;25;28

Thompson PW, Kirwan JR, Jones DD, Currey HLF. Serial blood levels of cytidine deaminase can detect the flare produced by the withdrawal of non-steroidal anti-inflammatory drugs in rheumatoid arthritis. Br J Rheumatol, 1986;25;79

Thompson PW, Kirwan JR, Currey HLF. The ability of different articular indices to detect an induced flare of joint inflammation in rheumatoid arthritis. Br J Rheumatol, 1987;26(suppl);93

Thompson PW, Wheatcroft S, Barnes CG. Exercise increases serum cytidine deaminase in rheumatoid arthritis. Br J Rheumatol, 1987;26;(suppl 2);16

Thompson PW, Austin C Kirwan JR. Laboratory markers of ankylosis and spondylitis. Br J Rheumatol, 1987;27(supl 1);61

Thompson PW, Pownell R. Circadian variation of serum cytidine deaminase and C-reactive protein in rheumatoid arthritis. Br J Rheumatol, 1988;27(suppl 1);19

Thompson PW, James IT, Joned DD, Houghton BJ. Inhibition of polymorph cytidine deaminase in serum of patients with rheumatoid arthritis or patients with raised liver enzymes. Br J Rheumatol 1988;27 (suppl 2);31

Thompson PW, Kirwan JR, Moss DW. Evidence supporting alkaline phosphatase as an acute phase protein in rheumatoid arthritis. Arthritis Rheum, 1989 (suppl)

IV. PRESENTATIONS

Thompson PW, Jones DD, Currey HLF. Cytidine deaminase: a measure of synovial inflammation?
Presented at the spring meeting of the BSR 1985

Thompson PW, Jones DD, Currey HLF. Serum cytidine deaminase as an integrated measure of acute joint inflammation.
Presented at the annual meeting of the BSR, November 1985

Thompson PW, Kirwan JR. A comparison of articular indices in rheumatoid arthritis.
Presented at the 2nd Annual Meeting of the BSR, November 1985

Thompson PW, Kirwan JR. Raised serum enzymes in arthritis - their source and potential for disease assessment.
Presented at the apring meeting of the BSR 1986

Thompson PW, Jones DD, Currey HLF. Serum cytidine deaminase: a biochemical joint count in rheumatoid arthritis.
Presented to the Royal Society of Medicine, February 1986

Thompson PW, Whitaker KB, Kirwan JR. Alkaline phosphatase isoenzymes in rheumatoid arthritis.
Presented at the 3rd Annual Meeting of the BSR, November 1986

Thompson PW, Kirwan JR, Jones DD, Currey HLF. Serial blood levels of cytidine deaminase can detect the flare produced by the withdrawal of non-steroidal anti-inflammatory drugs in rheumatoid arthritis.
Presented at the 3rd Annual Meeting of the BSR, November 1986

Thompson PW, Kirwan JR, Currey HLF. The ability of different articular indices to detect an induced flare of joint inflammation in rheumatoid arthritis.
Presented at the spring meeting of the BSR, 1987

Thompson PW, Wheatcroft S, Barnes CG. Exercise increases serum cytidine deaminase in rheumatoid arthritis.
Presented at the 4th Annual Meeting of the BSR, November 1987

Thompson PW, Austin C, Kirwan JR. Laboratory markers of ankylosis and spondylitis.
Presented at the 4th Annual Meeting of the BSR, November 1987

Thompson PW, Pownell R. Circadian variation of serum cytidine deaminase and C-reactive protein in rheumatoid arthritis.
Presented at the spring meeting of the BSR, July 1988

Thompson PW, James IT, Jones DD, Houghton BJ. Inhibition of polymorph cytidine deaminase in serum of patients with rheumatoid arthritis or patients with raised liver enzymes.
Presented at the Annual General Meeting of BSR, November 1988

Thompson PW, Kirwan JR, Moss DW. Evidence supporting alkaline phosphatase as an acute phase protein in rheumatoid arthritis. Arthritis Rheum, 1989 (suppl)
Presented at the 53rd meeting of the Americian College of Rheumatology, Cinncinati, 1989.

REFERENCES

REFERENCES

Adeyemi EO, Hull RG, Chadwick VS, Hodgson HJF. Circulating human leucocyte elastase in rheumatoid arthritis. Rheumatol Int, 1986;6:57-60.

Adkins ESO, Davies DV. Absorption from the joint cavity. Q J Exp Physiol, 1940;30:147-54.

Akesson A, Berglund K, Karlsson M. Liver function in some common rheumatic diseases. Scand J Rheumatol, 1980;9:81-8.

Altman RD, Gray R. Inflammation in osteoarthritis. Clin Rheum Dis, 1985;11:353-65.

Altman DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. Statistician, 1983;32:307-17.

Antonas KN, Fraser JRE, Muirden KD. Distribution of biologically labelled radioactive hyaluronic acid injected into joints. Ann Rheum Dis, 1973;32:103-11.

Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum, 1988;31:315-24.

Bahijri S. Human serum enzymes during pregnancy. PhD Thesis. Aberstwyth. 1979.

Bauer W, Bennett GA, Marble A, Claflin D. Observations on normal synovial fluid of cattle. I. The cellular constituents and nitrogen content. J Exp Med, 1930;52:835-48.

Bauer W, Short CL, Bennett GA. The manner of removal of proteins from normal joints. J Exp Med, 1933;57:419-33.

Bertino JR, Hollingsworth JW, Cashmore AR. Granulocyte kinetics in rheumatoid effusions stained by a biochemical label. Trans Ass Amer Phycns, 1963;76:63-71.

Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet, 1986;(i):307-10.

Blendis LM, Lovell D, Barnes CG, Ritland S, Caltan D, Veslin P. Oesophageal varaceal bleeding in Felty's syndrome associated with nodular regenerative hyperplasia. Ann Rheum Dis, 1978;37:183-6.

Boardman PL, Dudley Hart F. Clinical measurement of the anti-inflammatory effects of salicylates in rheumatoid arthritis. Brit Med J, 1967;4:264-8.

Bombardier C, Tugwell P, Sinclair A, Dok C, Anderson G, Watson Buchanan W. Preference for endpoint measures in clinical trials: results of structured workshops. J Rheumatol, 9:798-801, 1982.

Bromley M, Woolley DE. Chondroclasts and osteoclasts at subchondral sites of erosion in the rheumatoid joint. Arthritis Rheum, 1984;27:968-75.

Bruguerolle B, Bouvenot G, Arnaud C, Levy F, Mechkouri M, Bartilin R, Peronne R. Rythmes circadiens des proteines dites de l'inflammation chez sujet sain. Revue du Rhumatisme, 1986;53:313-6.

Buchanan WW, Tugwell P. Traditional assessments of articular diseases. Clin Rheum Dis, 1983;9:515-29.

Bull BS, Westengard JC, Smith PF et al. Ranking of laboratory tests by consensus analysis. Lancet, 1986;2:377-80.

Burkhardt H, Kasten M, Rauls S, Renkopf F. Interference of cartilage surface with interaction of granulocyte elastase with α_1-proteinase inhibitor. Rheumatol Int, 1987;7:133-8.

Byron MA, Kirwan JR. Corticosteroids in rheumatoid arthritis: Is a trial of their disease modifying potential feasible? Ann Rheum Dis, 1986;46:171-3.

Camiener GW, Smith CG. Studies of the enzymatic deamination of cytosine arabinoside. I. Enzyme distribution and species specificity. Biochem Pharm. 1965;14:1405-16., 1965

Cawston TE. The control of connective tissue breakdown - a role for metalloproteinase inhibitors. Current Med Lit, 1984;3:127-9.

Chabner BA, Myers CE, Coleman CN, Johns DG. The clinical pharmacology of anti-neoplastic agents. New Engl J Med, 1975;292:1107.

Chabot GG, Bouchard J, Monparler RL. Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside. Biochem Pharmacl, 1983;32:1327-8.

Co-operating Clinics Committee of the American Rheumatism Association: A seven-day variability study of 499 patients with peripheral rheumatoid arthritis. Arthritis Rheum, 8:302-335, 1965.

Cochrane CG. The arthus phenomenon - a mechanism of tissue damage. Arthritis Rheum, 1967;10:392-6.

Cockel R, Kendall MJ, Becker JF, Hawkins CF. Serum biochemical values in rheumatoid disease. Ann Rheum Dis, 1971;30:166-170.

Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended methods for the determination of four enzymes in blood (Second Report). Scand J Clin Lab Invest, 1974;33:291-306.

Conway EJ, Cooke R. Blood ammonia. Biochem J, 1939;33:457-78.

Cooke LA, Hay FC, Isenberg DA. Elastase activity in serum and synovial fluid of patients with connective tissue disorders. J Rheumatol, 1984;11:666-71.

Copeman WSC. Textbook of the Rheumatic Diseases. Third Edition. London, Livingstone, 1964.

Creasey WA. Studies on the metabolism of 5-iodo-2-deoxycytidine in vitro. Purification of nucleoside deaminase from mouse kidney. J Biol Chem, 1963;238:1772-6.

Crockson RA, Crockson AP. Relationship ot the erythrocyte sedimentation rate to viscosity and plasma proteins in rheumatoid arthritis. Ann Rheum Dis, 1974;33:53-6.

Crofton PM, Smith AF. The properties and clinical significance of some electrophoretically slow forms of alkaline phosphatase. Clin Chem Acta, 1978; 83:235-47.

Dawes PT, Fowler PD, Jackson RJ, Collins M, Shadforth MF, Stone R, Scott DL. Prediction of progressive joint damage in patients with rheumatoid arthritis receiving gold or D-peinicillamine therapy. Ann Rheum Dis, 1986;45:945-9.

Dawson DM, Alper CA, Seidman J, Mendelsohn J. Measurement of serum enzyme turnover rates. Ann Int Med, 1969;70:799-805.

De Broe ME, Mets TE, Loroux-Roels GG, Wieme RJ. Occurence of immunoglobulin G-alkaline phosphatase complexes in human serum. Ann Intern Med, 1979;90:30-5.

Dieppe PA, Huskisson EC, Willoughby D. The inflammatory component of osteoarthritis. In: The Aetiopathogenesis of Osteoarthrosis. Ed. Nuki G. Pitman Press. London. 1980. pp117-22.

Dietrichson O, From A, Christofferson P, Juhl E. Morphological changes in liver biopsies in patients with rheumatoid arthritis. Scand J Rheumatol, 1976;5:65-9.

Doube A, Davies J, Davis M, Maddison PJ. Influence of non-steroidal anti-inflammatory drugs and disease activity on serum alkaline phosphatase levels in rheumatoid arthritis, osteoarthritis and polymyalgia rheumatica. Ann Rheum Dis (in press).

Eberl DR, Fasching V, Rahlfs V, Schleyer I, Wolf R: Repeatability and objectivity of various measurements in rheumatoid arthritis: A comparative study. Arthritis Rheum, 19:1278-1286, 1976.

Egger MJ, Huth DA, Ward JR, Reading JC, Williams HJ. Reduced joint count indices in the evaluation of rheumatoid arthritis. Arthritis Rheum 28:613-9. 1985.

Ekholm R, Norback B. On the relationship between articular changes and function. Acta Orthop Scand, 1951;21:81-98.

Elves MW. The immunobiology of joints. In: The Joints and Synovial Fluid. Ed: Sokoloff L. Academic Press. New York. 1978. pp332-406.

Epstein O, Thomas HC, Sherlock S. Primary biliary cirrhosis is a dry gland syndrome with features of chronic graft-versus-host disease. Lancet, 1980;i:1166-7.

Evans JS, Musser EA, Mengel GD, Forsblad KR, Hunter JH. Antitumor activity of 1-β-arabinofuranosylcytosine. Proc Soc Expt Med, 1961;106:350.

Farr M, Symmons DPM, Bacon PA. Raised serum alkaline phosphatase and aspartate transaminase levels in two rheumatoid patients treated with sulphasalazine. Ann Rheum Dis, 1985;44:798-800.

Farr M, Kendall MJ, Shuttleworth R, Meynell MJ, Hawkins CF. Source and significance of 5-nucleotidase in synovial fluid. Ann Rheum Dis, 1973;32:326-30.

Fernandes L, Sullivan S, Mcfarlane IG, Wojcicka BM, Warnes TW, Eddleston ALWF, Hamilton EBD, Williams R. Studies on the frequency and pethogenesis of liver involvement in rheumatoid arthritis. Ann Rheum Dis, 1979,38:501-6.

Ferreira SH, Vane JR: Inhibition of protaglandin biosynthesis and the mechanism of action of non-steroidal anti-inflammatory agents. In: Future Trends in Inflammation I. Eds. Velo GP, Willoughby DA, Giroud JP, 1974. Padua-London. Piccin Medical Books. p.171.

Frank O, Klenmayer K. Die alkalische serumphosphatase bei erkankungen des rheumatischen formenkreises und ihre beinflussung durch kortikosteroide. 2 Rheumaforsh, 1968;27:142.

Friedel R, Bode R, Frautschold I. Verteilung Heterologen. Homologen und autologen enzyme nach intravenoser injektion. J Clin Chem Clin Biochem, 1976;14:129-36.

Gardner DL. The Pathology of Rheumatoid Arthritis. William Clowes and Sons. London. 1972. pp79.

Goddard D, Butler R. Rheumatoid arthritis - the treatment controversy. Macmillan Press. London. 1984.

Greenstein JP, Carter CE, Chalkley HW, Leuthardt FM. Enzymatic deamination and dephosphorylation of ribosnucleic and desoxyribosnucleic acids. J Nat Ca Inst, 1946;7:9-27.

Hadler NM. The biology of the extracellular space. Clin Rheum Dis, 1981;7:71-97.

Hadler NM, Johnson AM, Spitznagel JK, Quinet RJ. Protease inhibitors in inflammatory synovial effusions. Ann Rheum Dis, 1981;40:55-9.

Hardingham TE. Mechanisms of cartilage destruction in osteoarthritis. Curr Med Lit, 1986;5:1-4.

Harkness JAL, Richter MB, Panayi GS et al. Circadian variation in disease activity in rheumatoid arthritis. Br Med J, 1982;284:551-4.

Harris ED. The pathogenesis of rheumatoid arthritis. In: Textbook of Rheumatology. Eds: Kelly WN, Harris ED, Ruddy S, Sledge CB. 2nd Edn. WB Saunders. Philadelphia. 1985. pp890-94.

Hart FD, Clark CJM: Measurement of digital joint swelling in rheumatoid arthritis. Lancet, i:775, 1951.

Hart LE, Tugwell P, Watson Buchanan W, Norman GR, Grace EM, Southwell D. Grading of tenderness as a source of interrater error in the Ritchie articular index. J Rheumatol, 12:716-717, 1985.

Hasselbacher P (Ed). The biology of the joint. Clin Rheum Dis, 1981;7:1-287.

Hattori Y, Yamamoto K, Taniguchi B. Formation of an alkaline phosphatase-immunoglobulin G complex in human sera. Clin Chem Acta, 1979;97:243-52.

Henson PM. The immunologic release of constituents from neutrophil leukocytes. II - mechanisms of release during phagocytosis and adherence to non-phagocytosable surfaces. J Immunol, 1971;107:1547-57.

Hollingsworth JW, Siegel ER, Creasey WA. Granulocyte survival in synovial exudate of patients with rheumatoid arthritis and other inflammatory joint diseases. Yale J Biol Med, 1967;39:289-96.

Hunter W. Of the structure and diseases of articulating cartilages. Philos Trans R Soc London, 1743;42:514.

Huskisson EC. Measurement of pain. Lancet, 1974;ii:1127-31.

Iannauzzi L, Dawson N, Zein N, Kushner I. Does any therapy slow radiographic deterioration in rheumatoid arthritis? New Engl J Med, 1983;309:1023-7.

Johnson D, Travis J. The oxidative inactivation of human α_1-proteinase inhibitor: further evidence for methionine at the reactive centre. J Biol Chem, 1979;254:4022-6.

Jones DD, Bahrjri S, Roberts EL, Williams GF. Activity of cytidine deaminase during pregnancy. Brit J Obstet Gynae, 1982;89:314-7.

Jones DD, Roberts EL. Enzymes of DNA Metabolism in Clinical Diagnosis. 2nd Edn. 1986. Aberystwyth, Chancery Publications.

Kaldor G. Clinical Enzymology. Praeger Press. New York. 1983.

Kendall MJ, Bold AM, Farr M, Hawkins CF. 5-nucleotidase in the serum and synovial fluid of patients with rheumatoid disease. Lancet, 1971;2:1012-13.

Kendall MJ, Cockel R, Becker J, Hawkins CF. Rheumatoid liver? Br Med J, 1970;i:221.

Kendall MJ, Lawrence DS, Shuttleworth GR, Whitfield AGW. Haematology and biochemistry of ankylosing spondylitis. Brit Med J, 1973;2:235-7.

Kendall MJ, Cockel R, Becker J, Hawkins CF. Raised alkaline phosphatase in rheumatoid arthritis. An index of liver dysfunction? Ann Rheum Dis, 1970;29:537-540.

Kirwan JR. Currey HLF. Rheumatoid arthritis: Disease modifying antirheumatic drugs. Clin Rheum Dis 9:581-99, 1983.

Kushner I, Somerville JA. Permeability of human synovial membrane to plasma proteins. Arthritis Rheum, 1971;14:560-70.

Lansbury J, Baier HN, McCracken S: Statistical study of variation in systemic and articular indices. Arthritis Rheum, 5:445-456,1962.

Lansbury J. Clinical appraisal of the activity index as a measure of rheumatoid activity (letter). Arthritis Rheum 11:599-604, 1968.

Lansbury J, Haut DD: Quantitation of the manifestations of rheumatoid arthritis. 4. Area of joint surfaces as an index to total joint inflammation and deformity. Am J Med Sci, 232:150-155, 1956.

Larsen A, Dale K, Eek M. Radiological evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiologia Diagnosis, 1977;18:481-491.

Lawrence JS. Rheumatism in Populations. Heineman. London. 1977.

Lee P, Baxter A, Dick WC, Webb J. An assessment of grip strength in rheumatoid arthritis. Scand J Rheumatol, 1974;3:17-23.

Lefkovits AM, Farrow IJ. Liver in rheumatoid arthritis. Ann Rheum Dis, 1955;14:162-8.

Lehman MA, Kream J, Brogna D. Acid and alkaline phosphatase activity in the serum and synovial fluid of patients with arthritis. J Bone and Joint Surg, 1964;46:1732-8.

Lequesne M: European guidelines for clinical trials of new antirheumatic drugs. EULAR Bull (suppl), 9:171-175, 1980.

Levick JR. Contributions of the lymphatic and microvascular systems to fluid absorption from the synovial cavity of the rabbit knee. J Physiol London, 1980;306:445-61.

Levick JR. Permeability of rheumatoid and normal human synovium to specific plasma proteins. Arthritis Rheum, 1981;24:1550-60.

Levick JR. Synovial fluid dynamics: the regulation of volume and pressure. In: Studies in Joint Disease. Eds: Holborow EJ, Maroudas A. London. Pitman. 1983. Vol2. pp153-240.

Levick JR. Blood flow and mass transport in synovial joints. The cardiovascular system IV: The Microcirculation. In: Handbook of Physiology. Eds: Renkin EM, Michel CC. Bethesda. American Physiological Society. 1984. pp917-47.

Levick JR. Synovial fluid and trans-synovial flow in stationary and moving joints. In: Joint Loading - Biology and Health of Articular Structures. Ed: Helminen. 1987 J Wright and Son. Potters Bar.

Levick JR, Thompson PW. Intra-articular volume as an important factor governing macromolecular half life in synovial fluid. Ann Rheum Dis 1988;47;701-2

Levitt MO, Rapoport M, Cooperbrand SR. The renal clearance of amylase in renal insufficency, acute panceatitis and macroamylasaemia. Am Int Med, 1969;71:919-25.

Lowe JR, Pickup ME, Dixon JS, Leatham PA, Rhind VM, Wright V, Downie WW. Gamma glutamyl transpeptidase in arthritis: a correlation with clinical and laboratory indices of disease activity. Ann Rheum Dis, 1978;37:428-31.

Malathi VG, Silber R. Effects of murine viral leukemia on spleen nucleoside deaminase: purification and properties of the enzyme from leukemic spleen. Biochim Biophys Acta, 1971,238;377-87.

Mallya RK, Berry H, Mace BEW, De Beer FC, Pepys MB. Diurnal variation of erythrocyte sedimentation rate related to feeding. Lancet, 1982;i:389-90.

Masi AT, Feigenbaum SL and Kaplan SB. Arthicular patterns in the early course of rheumatoid arthritis. Amm J Med (Suppl), 1983;74:16-26.

McConkey B, Crockson RA, Crockson AP, Wilkinson AR. The effect of some anti-inflammatory drugs on the acute-phase proteins in rheumatoid arthritis. Q J Med 42:785-91. 1973.

McConkey B, Crockson RA, Crockson AP. The assessment of rheumatoid arthritis. a study based on measurements of the serum acute-phase reactants. Q J Med, 1972;41:115-25.

Mekras JA, Boothman DA, Greer SB. Use of 5-trifluromethldeoxycytidine and tetrahydrouridine to circumvent catabolism and exploit high levels of cytidine deaminase in tumours to achieve DNA- and target directed therapies. Can Res, 1985;45:5270-80.

Melsom RD. Circulating immune-complexes in rheumatoid arthritis. MD Thesis, Cambridge, 1984.

Mills PR, Macsween RNM, Dick WC, More IA, Watkinson G. Liver disease in rheumatoid arthritis. Scott Med J, 1980;25:18-22.

Mills PR, Sturrock RD. Clinical associations between arthritis and liver disease. Ann Rheum Dis, 1982;41:295-307.

Mooney H, Roberts R, Cooksley WGE, Halliday JW, Powell LW. Alterations in the liver with ageing. Clinics Gastroenterol, 1985;14:757-71.

Moss DW. Alkaline phosphatase isoenzymes. Clin Chem, 1982;28:2007-16.

Moss DW, Whitby LG. A simplified heat-inactivation method for investigating alkaline phosphatase isoenzymes in serum. Clin Chem Acta, 1975;61:63-71.

Moss DW. Diagnostic enzymology: some principles and applications. Hospital Update, 1981:999-1010.

Movitt ER, Davis AE. Liver biopsy in rheumatoid arthritis. Am J Med Sci, 1953;226:516-20.

Negamine M, Ohkuma S. Serum alkaline phosphatase isoenzymes linked to immunoglobulin G. Clin Chem Acta, 1975;65:39-46.

Nathanson L, Fishman WH. New observations on the Regan isoenzyme of alkaline phosphatase in cancer patients. Cancer, 1971;27:1388-97.

Neil GL, Moxley TE, Manak RC. Enhancement by tetrahydrouridine of 1-β-arabinofuranosyl cytosine (cytarabine) oral activity in L1210 leukaemic mice. Cancer Res, 1970;30:2166-72.

Nelson W, Tong YL, Halberg F. Methods for Cosinor-rhythmometry. Chronobiologia, 1979;6:305-23.
O'Driscoll S, O'Driscoll M. Osteomalacia in rheumatoid arthritis Ann Rheum Dis, 1980;39:1-6.

Otto W, Klugmann HJ, Geiler G. Supplementary comment: liver affectation in rheumatoid arthritis. In: Eberl R, Rosenthal M, Eds. Organic Manifestations and Complications in Rheumatoiod Arthritis. Stuttgart. Schatteuer, 1976:171-3.

Palmer DG, Myers DB. Some observations of joint effusions. Arthritis Rheum, 1968;11:745-55.

Paulus HE. Rheumatoid arthritis - anti-inflammatory drugs. Clin Rheum Dis, 1983;9:571-80.

Penn R, Worthington DJ. Is serum gamma-glutamyltransferase a misleading test? Br Med J, 1983;286:531-5.

Pizer LI, Cohen SS. Metabolism of pyrinidinbe arabinonucleasides and cytonucleasides in Escherichia coli. J Biol Chem, 1960;235:2387.

Pownall R, Knapp M, Maloney JD, Kowanko IC. Circadian variations in disease activity and endogenous corticosteroids. In: Glukokortokoide, Forschung und Therapie. Eds: Fehm HL, Graupe K, Kobberling J. Perimed Fachbuch, Erlangen. 1984. pp 168-177.

Pownall R. Biological rhythms in cell-mediated immunity: their relevance in rheumatology. In: Chronobiology and Chronotherapeutics. Eds: Buckler JW, Coffey L. Br J Clin Pract, 1984;(Suppl33):20-3.

Pruzanski W, Saito S, Ogryzlo MA. The significance of lysozyme (muramidase) in rheumatoid arthritis. 1. Levels in serum and synovial fluid. Arthritis Rheum, 1970;13:389-99.

Qureshi AR, Wilkinson JH. The fate of circulating lactate dehydrogenase in the rabbit. Clin Sci Mol Med, 1976;50:1-14.

Rau R. The liver in rheumatoid arthritis. In: Eberl R, Rosenthal M, Eds. Organic Manifestations and Complications in Rheumatoiod Arthritis. Stuttgart. Schatteuer, 1976:155-69.

Rau R, Pfenninger K, Boni A. Proceedings: Liver function tests and liver biopsies in patients with rheumatoid arthritis. Ann Rheum Dis, 1975;34:198-9.

Reizenstein P. The haematological stress syndrome. Br J Haematol, 1979;43:329-34.

Richards DA, Sherwood RA, Ndebele D, Rochs BF. Determination of plasma cytidine deaminase activity by high performance liquid chromatography. Chromatography, 1988;2:148-51.

Ritchie TF. A simple, direct and sensitive technique for the measurement of specific proteins in a dilute solution. J Lab Clin Med, 1967;70:512-7.

Ritchie DM, Boyle JA, McInnes JM, Jasani MK, Dalakos TG, Grieveson P, Buchanan WW. Clinical studies with an articular index for the the assessment of joint tenderness in patients with rheumatoid arthritis. Q J Med, 1968;37:393-406.

Rodnan GP, Benedek TG, Panetta WC. The early history of synovia (joint fluid). Ann Int Med, 1966;65:821-42.

Ropes MW, Bauer W. Synovial Fluid Changes in Joint Diseases. Cambridge, MA: Harvard Univ. Press, 1953.

Ropes MW, Bennett GA, Cobb S, Jacox R, Jessar RA. 1958 revision of diagnostic criteria for rheumatoid arthritis. Bull Rheum Dis, 1958;9:175-6.

Russell AS, Sturge RA, Smith MA. Serum transaminases during salicylate therapy. Br Med J, 1971;2:428-9.

Savage O. Criteria for measurement in chronic diseases. A report on a symposium on clinical trials. Kent. Pfizer, 1958. pp 34-42.

Scheja A, Forsgren A, Ohlsson K, Wollheim FA. Reduced in vivo leucocyte migration and elastase and lysozyme concentrations in skin chamber experiments with piroxicam in healthy volunteers. Clin Exp Rheumatol, 1986;4:43-7.

Scheja A, Forsgren A, Marsal L, Wollheim F. Inhibition of in vivo leucocyte migration by NSAIDs. Clin Exp Rheumatol, 1985;3:53-8.

Schmidt F, Schmidt FW. Clinical enzymology. FEBS Lett, 1976;62:62-79.

Scott DL, Symmons DPM, Coulton BL, Popert AJ. The long term outcome of treating rheumatoid arthritis: results after 20 years. Lancet, 1987;i:1108-11.

Sheehan NJ, Slavin BM, Kind PRN, Mathews JA. Increased serum alkaline phosphatase activity in ankylosing spondylitis. Ann Rheum Dis, 1983;42:563-5.

Sitton NG, Taggart AJ, Dixon JS, Surrall KE, Bird HA. Circadian variation in biochemical assessments used to monitor rheumatoid arthritis. Ann Rheum Dis, 1984;43:444-50.

Sitton NG, Dixon JS, Bird HA, Wright V. Serum biochemistry in rheumatoid arthritis, seronegative arthropathies, osteoarthritis, SLE and normal subjects. Brit J Rheumatol, 1987;26:131-5.

Skjodt H, Russell G. Mediators of bone destruction and repair in chronic arthritis. Current Med Lit, 1985;4:97-101.

Snedecor GW, Cochran WG. Statistical Methods. 7th Edn. 1980. USA. The Iowa State University Press.

Spooner RJ, Smith DH, Bedford D, Beck PR. Serum gamma-glutamyltransferase and alkaline phosphatase in rheumatoid arthritis. J Clin Pathol, 1982;35:638-641.

Steiner PE. Nodular regenerative hyperplasia. Am J Pathol, 1959;35:943-51.

Steuart CD, Burke PJ. Cytidine deaminase and the development of resistance to arabinosyl cytosine. Nature New Biol, 1971;233:190.

Stolbach LL, Krant MJ, Fishman WH. Ectopic production of an alkaline phosphatase isoenzyme in patients with cancer. N Engl J Med, 1969;281:757-62.

Stoller RG, Myers CE, Chabner BA. Analysis of cytidine deaminase and tetrahydrouridine interaction by use of ligand techniques. Biochem Pharm, 1977;27:53-9.

Sukenik S, Henkin J, Zimlichman S, et al. Serum and synovial fluid levels of serum amyloid A protein and C reactive protein in inflammatory and non-inflammatory arthritis. J Rheumatol, 1988;15:942-5.

Targett-Adams L, Jones DD, Williams GF. A rapid method for the determination of deoxycytidylate deaminase activity in pregnancy serum. Clin Chem Acta, 1975;63:377-82.

Thompson PW, Kirwan JR, Rudge SR, Houghton BJ, Currey HLF, Eds. Laboratory Markers of Joint Inflammation and Damage. Arthritis and Rheumatism Council Conference Proceedings III. London. 1988.

Thompson PW, Jones DD, Currey HLF. Cytidine deaminase activity as a measure of acute inflammation in rheumatoid arthritis. Ann Rheum Dis 1986;45;9-14

Thompson PW, Kirwan JR. Observer variation and the Ritchie articular index. J Rheumatol, 1986;13;836-7.

Thompson PW, Silman AJ, Kirwan JR, Currey HLF. Articular indices of joint inflammation in rheumatoid arthritis: Correlation with the acute phase response.
Arthritis Rheum 1987;30;618-23

Thompson PW. Laboratory markers of joint inflammation and damage. Br J Rheumatol, 1987;26;83-5

Thompson PW Kirwan JR, Jones DD, Currey HLF. Serum cytidine deaminase responds to changes in non-steroidal anti-inflammatory therapy in rheumatoid arthritis. Ann Rheum Dis, 1988;47;308-12

Thompson PW, Kirwan JR, Currey HLF. The ability of 28 articular indices to detect an induced flare of joint inflammation in rheumatoid arthritis. Br J Rheumatol 1988;27;375-80

Thompson PW, James IT, Wheatcroft S Pownall R, Barnes CG. Circadian rhythm of serum cytidine deaminase in rheumatoid arthritis patients during rest and exercise. Ann Rheum Dis, 1989;48:502-4.

Thompson PW, Jones DD. Serum Lactic Dehydrogenase as a marker of joint damage in rheumatoid arthritis. Ann Rheum Dis 1987;46;263

Thompson PW, Jones DD, Currey HLF. Serum cytidine deaminase as a laboratory test for acute inflammation in rheumatoid arthritis. Ann Rheum Dis 1988;47;173-4

Thompson PW, Kirwan JR. A comparison of articular indices in rheumatoid arthritis. Br J Rheumatol, 1986;25;98-9

Thompson PW, Kirwan JR. Raised serum enzymes in arthritis; their source and potential for disease assessment. Br J Rheumatol, 1986;15;31-2

Thompson PW, Whitaker KB, Kirwan JR. Alkaline phosphatase isoenzymes in rheumatoid arthritis. Br J Rheumatol, 1986;25;28

Thompson PW, Austin C Kirwan JR. Laboratory markers of ankylosis and spondylitis. Br J Rheumatol, 1987;27(supl 1);61

Thonar EJ-MA, Lenz ME, Klintworth GK et al. Quantification of keratan sulfate in blood as a marker of cartilage catabolism. Arthritis Rheum, 1985;28:1367-75.

Tugwell P, Bombardier C. A methodologic framework for developing and selecting endpoints in clinical trials. J Rheumatol, 1982;9:758-62.

Turnbull A. Anaemia in rheumatoid arthritis: does it matter? Reports on the Rheumatic Diseases (Series 2). Arthritis and Rheumatism Council's Publications. London. 1987.

Unger A. Biological rhythms in the humoral immune system: their possible significance in rheumatology. In: Chronobiology and Chronotherapeutics. Eds: Buckler JW, Coffey L. Br J Clin Pract, 1984;(Suppl33):24-7.

Vesells ES, Osterland KC, Bearn AG, Kunkel HG. Isoenzymes of lactic dehydrogenase; their alterations in arthritic synovial fluid and sera. J Clin Invest, 1962;41:2012-9.

Wallis WJ, Simkin PA, Nelp WB, Foster DM. Intraarticular volume and clearance in human synovial effusions. Arthritis Rheum, 1985;4:441-9.

Ward JR, Niethammer TA, Egger MJ. Can we just measure signal joints? Controversies in the Clinical Evaluation of Analgesic Anti-inflammatory Antirheumatic Drugs. Edited by HE Paulus, GE Ehrlich, E Lindenlaub. New York, FK Schattauer-Verlag, 1980. pp103-10.

Watson Buchanan W: Assessment of joint tenderness, grip strength, digital joint circumference and morning stiffness in rheumatoid arthritis. J Rheumatol, 9:763-766, 1982.

Webb J, Whaley K, Macsween RNM, Nuki G, Carson Dick W, Watson Buchanan W. Liver disease in rheumatoid arthritis and Sjogren's syndrome. Ann Rheum Dis, 1975;34:70-81.

Webb J, Downie WW, Dick WC, Lee P. Evaluation of digital joint circumference measurements in rheumatoid arthritis. Scand J Rheumatol, 2:127, 1973.

Wentworth DF, Wolfenden R. On the interaction of 3,4,5,6-tetrahydrouridine with human liver cytidine deaminase. Biochem, 1975;14:5099-105.

Whitaker KB, Whitby LG, Moss DW. Activities of bone and liver alkaline phosphatases in serum in health and disease. Clin Chem Acta, 1977;80:209-20.

Williams JD, Scott DL, DeBrito FB, Willoughby DA, Huskisson EC. Rheumatoid inflammation and joint destruction: cause and effect of parallel phenomena? Agents and Actions, 1986;18:538-43.

Williams GF, Jones DD. Deoxycytidylate deaminase in pregnancy. Brit Med J, 1975;2:10-12.

Witcher JT. Interleukin 1 and acute phase proteins. Br J Rheumatol, 1985;24(suppl):21-4.

Woo P. Acute phase proteins and rheumatic disease. Cur Med Lit, 1986;5:97-102.

Woodland J, Chaput de Saintonge DM, Evans SJW, Sharman VL, Currey HLF. Azathioprine in rheumatoid arthritis: double blind study of full versus half doses versus placebo. Ann Rheum Dis, 40:355-359, 1981.

Wordsworth BP, Vipond S, Woods CG, Mowat AG. Metabolic bone disease among inpatients with rheumatoid arthritis. Br J Rheumatol, 1984;23:251-7.

Wright V, Amos R. Do drugs change the course of rheumatoid arthritis? Brit Med J, 1980;280:964-6.

Wright V, Bird H, Dixon J, Pickup ME. Erythrocyte Sedimentation Rate (ESR). In. Controversies in the Clinical Evaluation of Analgesic Anti-inflammatory Antirheumatoid Drugs. Eds: Paulus HE, Ehrlich GE, Lindenlaub E. 1980. New York. FK Schattauer Verlag.

Yasmineh WG, Pyle RB, Nicologg DM. Rate of decay and distribution volume of MB isoenzyme of creatine kinase, intravenously injected into the baboon. Clin Chem, 1976;22:1095-7.

Wright V, Bird H, Dixon J, Pickup ME. Erythrocyte Sedimentation Rate (ESR). In. Controversies in the Clinical Evaluation of Analgesic Anti-inflammatory Antirheumatoid Drugs. Eds: Paulus HE, Ehrlich GE, Lindenlaub E. 1980. New York. FK Schattauer Verlag.

Yasmineh WG, Pyle RB, Nicologg DM. Rate of decay and distribution volume of MB isoenzyme of creatine kinase, intravenously injected into the baboon. Clin Chem, 1976;22:1095-7.