"PARAMETRIC IMAGING IN THE EVALUATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS"

BY

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ABSTRACT

The major difficulty in clinical assessment of patients with inflammatory disease, and in the evaluation of consequent anti-inflammatory drug treatment has hitherto been the impossibility of quantifying the inflammatory process. Clinical methods have proved too rough and ready, with unacceptable levels of error even with so-called objective measurements, while laboratory investigation by scintigraphy has been of only limited value since it has previously evolved around static imaging.

The introduction of dynamic parametric functional imaging, which allows separation of the vascular and bony phases of accretion of radioactive material in scintigraphy permits the accurate objective quantification of the degree of inflammatory response and, as here demonstrated, makes it possible to evaluate the modification of that response by anti-inflammatory medication.

It is also shown also possible to compare clinical and objective responses in given patients and (perhaps in some respects more importantly) to make objective

comparisons of the in vivo anti-inflammatory effects of a wide range of drugs marketed and prescribed for the treatment of the arthritides.

By this experimental model the anti-inflammatory effects of the drugs Indomethacin, Benoxaprofen and Piroxicam have been compared in a suitably controlled trial in adjuvant induced inflammation in rats. In this study it has been shown that neither of the latter two drugs is as effective as Indomethacin in the doses quoted, and the role of Indomethacin is confirmed as the standard agent against which other non-steroidal anti-inflammatory medications should be tested in anti-inflammatory drug trials.

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PREFACE

This study was stimulated by a paper presented at a local Medical Society Meeting describing the comparative effects of anti-inflammatory drugs on bone damage or erosions in the adjuvant arthritic rat. (The adjuvant arthritic rat has for some 20 years been one of the standard models for evaluation of these types of drugs.) Surprisingly, the interpretation in that study was entirely subjective and arbitrary.

It seemed to me that it should be possible objectively to quantify this effect. My clinical work with Dr. Murdo McLeod and Dr. Alec Houston in the Nuclear Medicine Department at the Royal Naval Hospital, Haslar, had convinced me of the advantages, particularly the objectivity and the reproducibility, of radio-nucleide studies in arthritis.

This study was therefore set up objectively to quantify the inflammatory response in the adjuvant arthritic rat.

I am most grateful to Ms Joanna Hase for her endurance in producing the final typescript.

1. INTRODUCTION

Adjuvant arthritis in rats, in which traditionally most reliance is placed on the volume changes in the foot as the arthritic lesion progresses, has been used for the last 20 years as a predictive test for anti-inflammatory activity in man.

Killed Mycobacterium tuberculosis in light mineral oil (Freund's adjuvant) is injected intradermally into the plantar surface of one paw. Over the next 8 to 10 days the inflammatory exudate in the foot is readily measurable and is known as the A lesion. From day 10 to day 30 a secondary wave of inflammation occurs both in the injected paw (called the B lesion) and the non-injected hind paw (C lesion). This has been shown to be immune mediated (Pearson and Wood 1959) [21]. In fact the arthritis becomes a systemic polyarthritis most analgous to Reiter's disease in man with some features common to rheumatoid arthritis (Newbould 1963) [19].

Assay against adjuvant induced arthritis has been shown to be predictive for clinical activity, for it is known that the standard non-steroidal anti-inflammatory drugs (Prostaglandin synthetase inhibitors) are active in this model, though these compounds are not capable of modifying the progressive disease in either animals or man. A literature search and discussion with various pharmaceutical firms indicated that no published work was available on

radio-nucleide functional imaging of adjuvant arthritic joints in the early analysis of anti-inflammatory drug effect, although there were many studies on the use of radio-nucleides in classical rheumatoid arthritic patients using static techniques (Collins et al 1971; Berry and Huskisson 1974; Deohar et. al. 1973) [8,5,9].

Evaluation of anti-inflammatory drug effects has progressed little in the last two decades. The methods used have evolved from the early work of Stoerk and colleagues (1954) [27], later progressed by Pearson and his colleages (1956, 1959) [20,21]. These authors show that the intradermal injection of an emulsion containing Mycobacterium tuberculosis produced a form of reactive arthritis in 10 to 15 days. This arthritis had many of the features of rheumatoid arthritis and thus was a reproduceable model for the study of drug effect based on certain measurements such as hind foot volume. Subsequent work by Ward and Jones (1962) [29] refined the technique as a statistically valuable method.

The method in most common use today involves the intradermal injection of 0.05 ml of a fine suspension of dead tubercle bacilli in liquid paraffin into the plantar surface of the right hind foot of the rat. The development of the arthritis follows a persistent pattern. Immediate swelling of the injected foot is followed by slow progression for 8 to 10 days and thereafterwards the development of a remote polyarthritis from 10 to 30 days in the non-injected sites,

particularly the opposite hind paw knee joint. The arthritis has many of the features of both rheumatoid and Reiter's reactive arthritis. In these conditions the non-steroidal anti-inflammatory drugs are known to have an ameliorating effect. Clearly further progression of these techniques is desirable, particularly as the interpretation is subjective.

More recently studies have been conducted using X-ray analysis to determine the extent of bone damage (erosions) in the adjuvant model. As is usual with X-ray analysis, the interpretation is difficult and not readily quantifiable. Benslay and Nickander (1981) [4] showed that anti-inflammatory drugs had an inhibiting effect on bone damage when compared with controls covering a wide range of inflammatory compounds. The bone damage was assessed by observation of joint space narrowing, periosteal elevation and erosions, using an arbitrary scale.

A percentage inhibition score was produced in comparative studies with controls and this was considered to represent an advance in assessing the effectiveness of the drug. However, reproducibility was uncertain because the visual interpretation of the X-ray was subjective and in different hands too much inter-observer error would intrude.

This present investigation was started in 1982 to evaluate the use of scintigraphy in animals for the assessment of efficiency of anti-inflammatory drugs. There have been numerous publications on the use of radio-nucleides in the

arthritidies, for example by Dick et al (1970, 1976) [11,10], and Hoffer and Genant (1976) [14]. Initially the procedure was complex and took many hours which negated its true value. It was however, even at the earlier stage, useful in the detection of synovitis. From the mid-1960s however, when Andros and others (1965) [2] introduced Technetium 99m into nuclear medicine, it became possible to visualise inflammed joints more readily. Technetium offered two main advantages, those of a short half life and of lack of high energy particles, resulting in low radiation exposure. This allows repeated small dosage examinations to be made for serial assessments of changes in synovial tissues, and of bony change within damaged joints.

It seems that arthritis is associated with periarticular elevation of the periosteum and sub-chondral bone erosions followed by increased osteoblastic activity in reaction to inflammation. Clearly if both aspects of inflammation, that is synovial inflammation (which is represented by increased blood flow around the joint) and osteoblastic bony turnover can be visualised, a quantitative assessment may then be made against controls of the effect of treatment or the progression of the disease process. The method used in this study was functional or parametric imaging, which measures the rate of change of tracer within an organ rather than the amount of tracer within the organ at any one time.

2. FUNCTIONAL IMAGING

Kaihara and his co-workers (1969) [16] were the first to offer the new concept of functional imaging in the rapidly advancing world of nuclear medicine. They reasoned that if one could demonstrate the rate-of-change values for an elapsed time period of radionucleide take up in any organ it would be more meaningful than a static image gathered over the same period.

By 1973 Weiner and others [30] had, with the improvement in isotopes available, (with particular regard to organ specificity) advanced the technique, particularly in renal studies. They had also recognised its application in other organ studies.

Since that time functional imaging has become one of the standard methods of assessment in nuclear medicine. The increasing use of the technique by clinicians, confirms the role of nuclear medicine as the link between particular clinical problems and their solution using organ specific radionucleides.

Whaley and others (1968) [32] worked on methods of quantification of joint inflammation showing isotope pickup to be a function of severity of joint inflammation, that is micro-vascular flow and bony turn-over. They additionally suggested that because Technetium was not actively concentrated in diseased synovium, the display of isotope

(which in their study was a static image) reflected the enhanced vascularity of the synovial membrane and other joint tissues. Dynamic and functional studies have advanced to improve the concept.

Macewan (1979) [17] progressed the argument in his study of the knee by predicting in the difficult condition of chondromalacia patella that this was a three phase condition of increased, normal and absent micro-vascular blood flow. This indicated that there was an inflammatory precursor stage in osteoarthrosis of shortlived duration. It was noted in this study that even the absent blood flow image showed as "hot" on dynamic imaging. It was the ability to divide the two functions, i.e. microvasculature and bony turnover that was significant.

In dynamic radionucleide studies (Houston 1984; Macleod 1984) [15,18] a sequence of images (each of which is a frame) is stored in the computer at regular known time intervals. Each image is recorded at a previously determined elapsed time from the introduction of isotope into the body. Dynamic function is expressed as a function of rate of change of these images.

Functional imaging is the method by which this dynamic sequence may be represented by a single visual display. It was designed to compress into this single image a series of elapsed time images and has the obvious advantage of synthesising large amounts of data and of identifying

processes not visualised by inspection of single incidents. Regional information or area specific information is often obscured by total organ measurement. The main advantage of functional imaging is that it will identify focal areas of abnormality. This ability to identify areas of damage or change is the key to the study in that it can show both vascular and bone change in a standard model identifying the two elements of the inflammatory process.

The image or frame is stored as a visual array of picture elements or pixels. Each contains a value representing the number of counts collected in the study region over a specific time scale. There is a sequence of frames in the study (usually 20 in number) which is stored in a 64 X 64 array. Because interpretation through any single dixel (which is defined as the summation into graph form of the pixels of a specific area in each frame of the study) would make quantification difficult due to insufficient statistical data, they are summated into a 16 X 16 matrix.

If one can find some numerical value or parameter for each dixel an image may be formed to display that parameter across the field of view. This is a functional image (or parametric image). A simple example will explain the concept. If a 3 frame study is taken collected as a 3 X 3 array, the counts of each pixel will be collected (Figure 2.1).

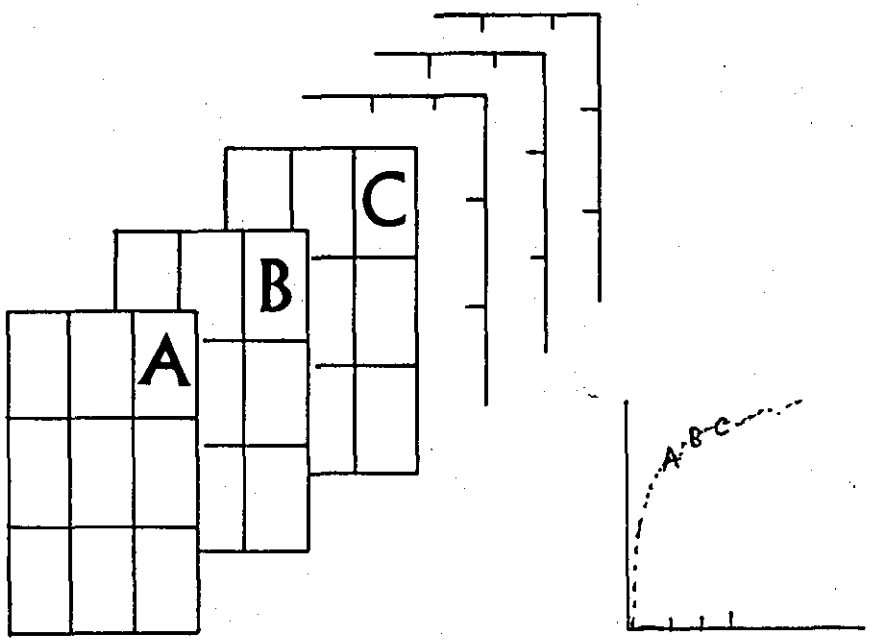


Figure 2.1

Each pixel displays the percentage accretion of the isotope at that moment in a region of interest towards the dixel (100% accretion) and may be represented as a point on the amplitude curve (e.g. Figure 6.1).

Each pixel indicates the percentage accretion of the isotope at that moment in a region of interest towards the final total (100%) accution - the dixel. It may be represented as a point on an amplitude curve (see Figure 6.1 (p.37)).

One dixel may peak earlier than another. This may be viewed as shades of colour, for example white peaks first, then grey, and lastly black (or other colours may be used). The aim of the exercise is thus to separate regions of different rates of uptake in the image which thus identifies regions of different physiological function - which is why clinicians also call it functional.

To apply functional imaging to a particular clinical problem successfully, it is first necessary to identify the patho-physiological functions involved and then ascertain if they can be measured using an appropriate mathematical function. For example, the uptake of bone scanning radionucleide follows the form:

$$d(t) = A(1-E^{\text{Lambda X t}}).$$

This equation contains 2 constants, A and Lambda (used in the study of inflammatory arthritis to describe 2 physiological parameters). These are any given area A equal to the number of osteoblasts and Lambda equivalent to the microvasculature which brings the substance there.

The isotope used is 99M Technetium MDP. This particular isotope is chosen because it has been shown to be bone specific.

Alarcon-Segovia and co-workers (1967) [1] demonstrated that Technetium had many advantages over other isotopes used. He identified higher uptake in joints affected by the inflammatory process than in normal (controlled) or degenerated joints. In addition to its advantages of short half life, pure gamma emission and minimal radiation exposure, its rapid distribution and absence of allergenic reaction, which is important in the study model, were also emphasised.

This was shown to be better than iodinated serum albumen, Iodine 131 tagged in localisation of inflamed joints (Weiss et al 1965) [31]. The early studies used Technetium perchenetate; subsequently polyphosphates were used, (Subramanian et al 1972) [28]. These showed that these polyphosphates offered significant advantages on previously used isotopes in respect of bone specificity. Pendergass and his colleagues (1973) [22] however found certain difficulty in reproducing Weiss's results and considered that diphosphonate, which is known to chemiabsorb into hydroxyapatite crystals were more stable than polyphosphates chemically.

They considered that methylene diphosphonate would be, if suitably labelled, more specific in imaging. From 1973 it has been the most widely used isotope allowing good reproduceability and producing an excellent marker of osteoblastic activity with high accumulation of Technetium. Osteoclastic regions show low uptake in tracer studies.

It can be deduced therefore that a sound mathematical method allied to a good radionucleide will allow this functional imaging to proceed in a satisfactory and reproducible fashion. Macewen as stated earlier used this technique in the study of the pathological knee and was able to show that the quantitative hot spot seen in the affected area was due to one or other of the parameters of inflammation.

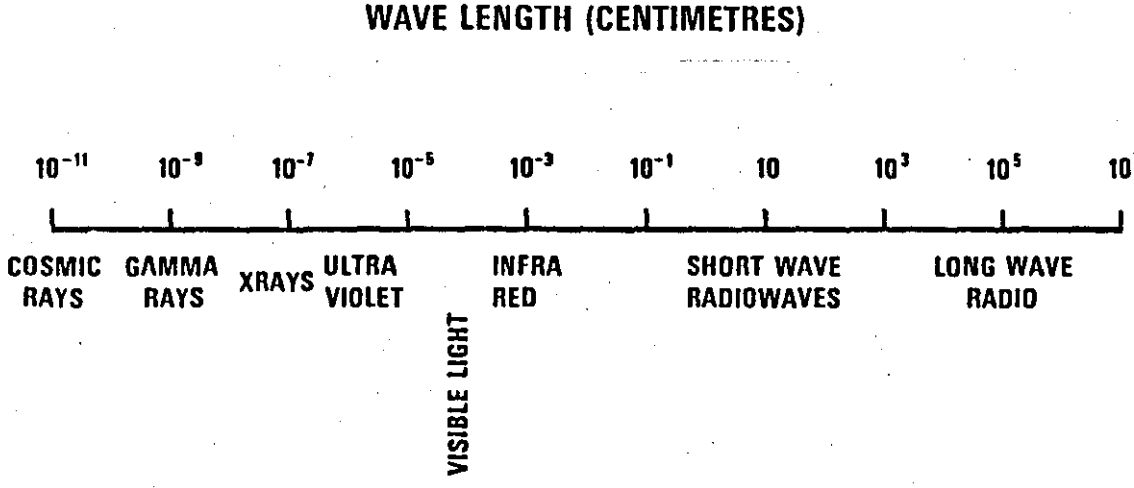
It was decided in this study therefore that the combination of the two techniques allowed the best possible method to obtain a quantifiable mathematical end result which could be used as a marker in producing a list of drugs in order of their effectiveness.

3. THE EQUIPMENT

Nuclear medicine is a 20th century phenomenon. The first Nobel prize for Physics was awarded for the work on the X-ray (X at that time to signify unknown) by William Roentgen.

Subsequently Rutherford conducted a large series of experiments in the identification of radiation emitted by various radioactive materials. He was responsible eventually for identifying Alpha (helium nuclei), Beta (electrons) and Gamma (short wave length electro-magnetic radiation) rays.

Scientific advance with the development of the cyclotron made it possible to produce radioactive isotopes and now over 1500 isotopes are known of which less than 20 have found their way into nuclear medicine. These are characterised by a short half life, rapid excretion, specificity to an organ and no allergenic interreaction. Almost all the diagnostic studies carried out in nuclear medicine utilised Gamma rays (electro-magnetic radiation). Magnetic radiation is classified by its wavelength and Gamma rays come at the far left-hand end of the spectrum, between 10^{-11} and 10^{-9} Angstrom units. The visible spectrum is in the region of 10^{-5} to 10^{-3} Angstrom units and is shown in the spectrum of radiation (Figure 3.1).



The Electromagnetic Spectrum which stretches from the Radiowave lengths through visible light and into the Cosmic Ray Region.

The Spectrum is also measured in Angströms which is defined as a unit of wave length of Electromagnetic Radiation equal to 10^{-7} mm Symbol A.

Figure 3.1 The electro-magnetic spectrum

The isotope used in this experiment is 99M Technetium as its Diphosphonate. The superscript M indicates that the elemental nucleus is metastable. Such nuclei are said to be excited, returning to the ground state by releasing energy as Gamma ray emission. (99M Technetium is not wholly a Gamma emitter but also produces a very small amount of X-ray emission which is not sufficient to influence the experimental validity).

Electro-magnetic radiation, the passage of Gamma rays through matter, produces ionisation with scattering, photo-electric absorption and Compton scattering (defined as the "billiard ball" effect of small ions hitting each other). The most important is ionisation, with the production of free electrons and positive ions. Most detection systems, other than scintillation, use this characteristic to detect radiation, for example, the Geiger counter. Scintillation utilises the phenomenon that certain organic and inorganic materials produce visible light for variable periods following excitation. Television picture tubes (Cathode-ray tubes) are the widest known application of this in everyday life. In nuclear medicine, scintigraphy is limited to the use of sodium iodide (NaI) to assess photo-electric absorption.

Alone, sodium iodide is not a particularly good scintillator but its capacity is enhanced tenfold if 0.5% thallium iodide is added to it. This is the basis of the commercial scintillator.

The equipment used in this study was the Siemens ZLC camera system which incorporates a large sodium iodide crystal giving a wide field of view (Figure 3.2). It has the ability to eliminate distortion in imaging, a common feature in previous cameras such as the Anger camera.

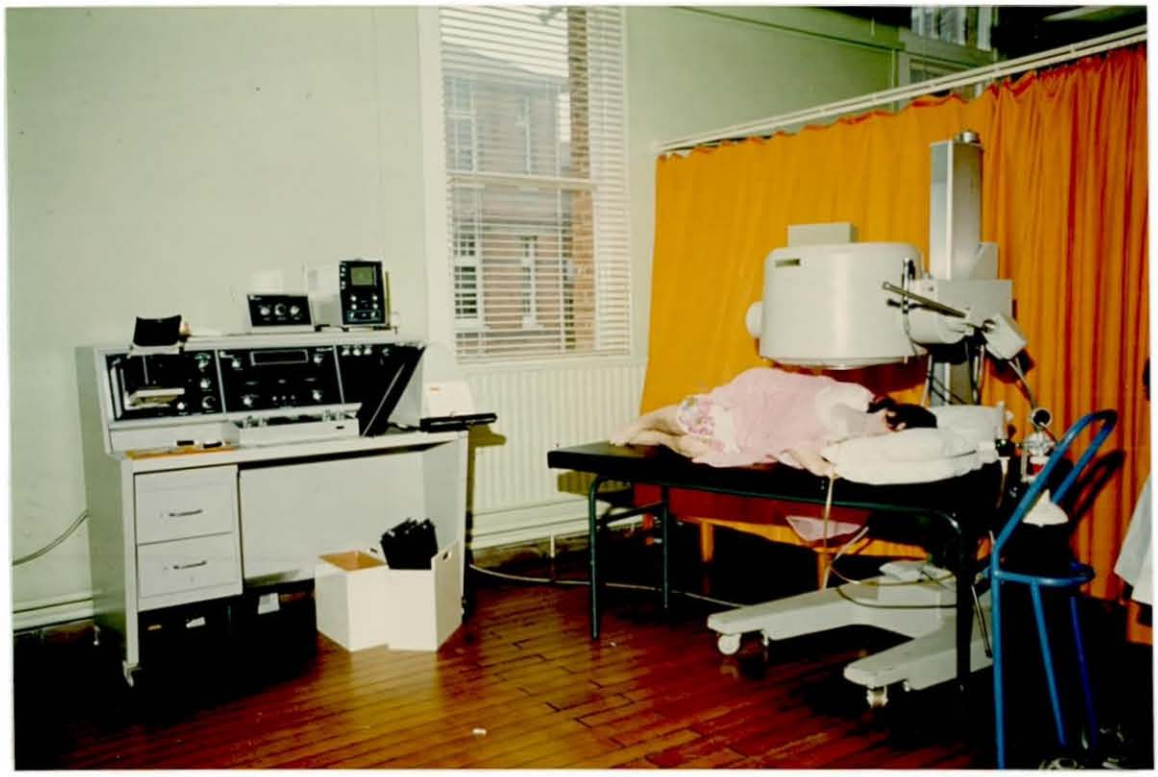


Figure 3.2 Siemens ZLC camera

4. THE DRUGS INVOLVED

The most commonly used preparations in the management of the inflammatory arthritides are termed "the non-steroidal anti-inflammatory drugs" (NSAIDS). Over the last few years, a plethora of novel compounds, and many many copies of these compounds, all based on the carboxylic acid radical, have been marketed. Each drug makes significant claims on its own behalf for increased efficacy over each or any of its rivals.

The majority of trials on these compounds in clinical practice rely on the subjective observation of patients. These observations are published in clinical journals and have been shown to be reasonably accurate predictors of significance over many years.

The majority of the papers work on statistical significance and when the calculation of probability "P" reaches a value of 0.05 that level is significant and the drug is declared effective. What is uncertain, however, is whether the patient who is receiving the drug is convinced himself of the value of 0.05! He would probably prefer that the treatment be considered less statistically significant and more clinically significant.

The whole purpose of this study is to find an objective a priori marker of activity which is reproducible without recourse to subjective criteria.

To illustrate the point, perhaps the most fundamental change that has taken place in the understanding of pain to illustrate the point as a clinical syndrome is the belated recognition that it is not a sensation but more an unpleasant emotional experience. This concept of pain was first invoked by Benedictus Spinoza (1632-1677), the Dutch philosopher, who classified it as a localised form of sorrow - that is, a primary emotion.

Several important clinical consequences arise from this observation, the main one being that when attempting to assess the efficacy of the treatment of pain, one is entirely dependent on the patient's report of his own introspective emotional experience.

It can be seen therefore from this evaluation that it is almost impossible to make a coherent statement that pain intensity must bear a direct relationship to the amount of tissue disturbance that is provoking it (Williams 1984) [33].

The drugs used are called anti-pyretic analgesics and are considered to exert their main clinical effect at a peripheral site (Higgs et al 1974) [13]. Classically they have been shown to modify the nociceptive response induced by Bradykinin (nociceptive signifying responding to tissue abnormality).

Activation of the kinin-forming system is induced by injury. The hyperaemia, oedema and pain of an inflammatory response can all be mediated by Bradykinin and the peptide can be identified in inflammatory exudates in synovial fluids in arthritic joints, that is, peripherally. The non-steroidal anti-inflammatory drugs are recognised for their efficiency in the relief of pain of superficial origin, or where there is evidence of tissue damage, e.g. in the arthritides and in the non-articular rheumatoid conditions.

Interest has centred on the role of these drugs in inhibiting the enzymatic synthesis of prostaglandins from long chain fatty acids. Vane, Smith and Willis with various co-workers (e.g. Salmon et al) (1978) [25] show that the anti-pyretic analgesics inhibited this biosynthesis. Over the next few years it became established that (1) all human cells, with the possible exception of erythrocytes, have enzymes for the production of prostaglandins; (2) that they are always released when cellular damage occurs and are shown to be inflammatory exudates. Present evidence is they are not stored so must be synthesized de novo; (3) the anti-pyretic analgesics inhibit the synthesis of prostaglandins but other drugs do not.

In human studies the subdermal injections of prostaglandins E1 and E2 will produce oedema and lower the pain threshold but will not produce spontaneous pain. It is the addition of bradykinin or histamine which causes the intense pain reaction. The prostaglandins appear, therefore, to be

sensitizers of the peripheral nerves to other pain mediators. The diagram (Figure 4.1) shows the prostaglandin cascade and where the various drugs are presumed to act on this progression down from the phospholipids in the cell membranes to the release of prostaglandins E2 and F2 at the end of the arachidonic acid cascade.

It can be seen from the diagram that the majority of the non-steroidal anti-inflammatory drugs exert their effect, which is by the direct inhibition of microsomal enzymes, cyclo-oxygenase just after the arachidonic acid radical. However, total inhibition at this level will cause a degree of reflux down through the cyclic ednoperoxide chain into the leucotriene pathway.

Prostaglandin E1 and E2 Alpha which are the end results of the cascade shown promote osteoclastic bone resorption and therefore may well be the reason for the osteoporosis and erosive changes from the synovial pannus overgrowth at the affected joints. Robinson and his co-worker (1975) [24] confirmed in vitro experiments that all bone resorption was prostaglandin E2 mediated and additionally that it was entirely secreted from the rheumatoid synovial tissue in the experimental model. It follows that the adequate suppression of the E series prostaglandin synthesis by the anti-inflammatory anti-pyretic drugs may reduce or prevent bone destruction in the rheumatoid arthritides and give a reasonable model for treatment.

Simple diagram of the Prostaglandin Cascade which is the mediator of pain and inflammation

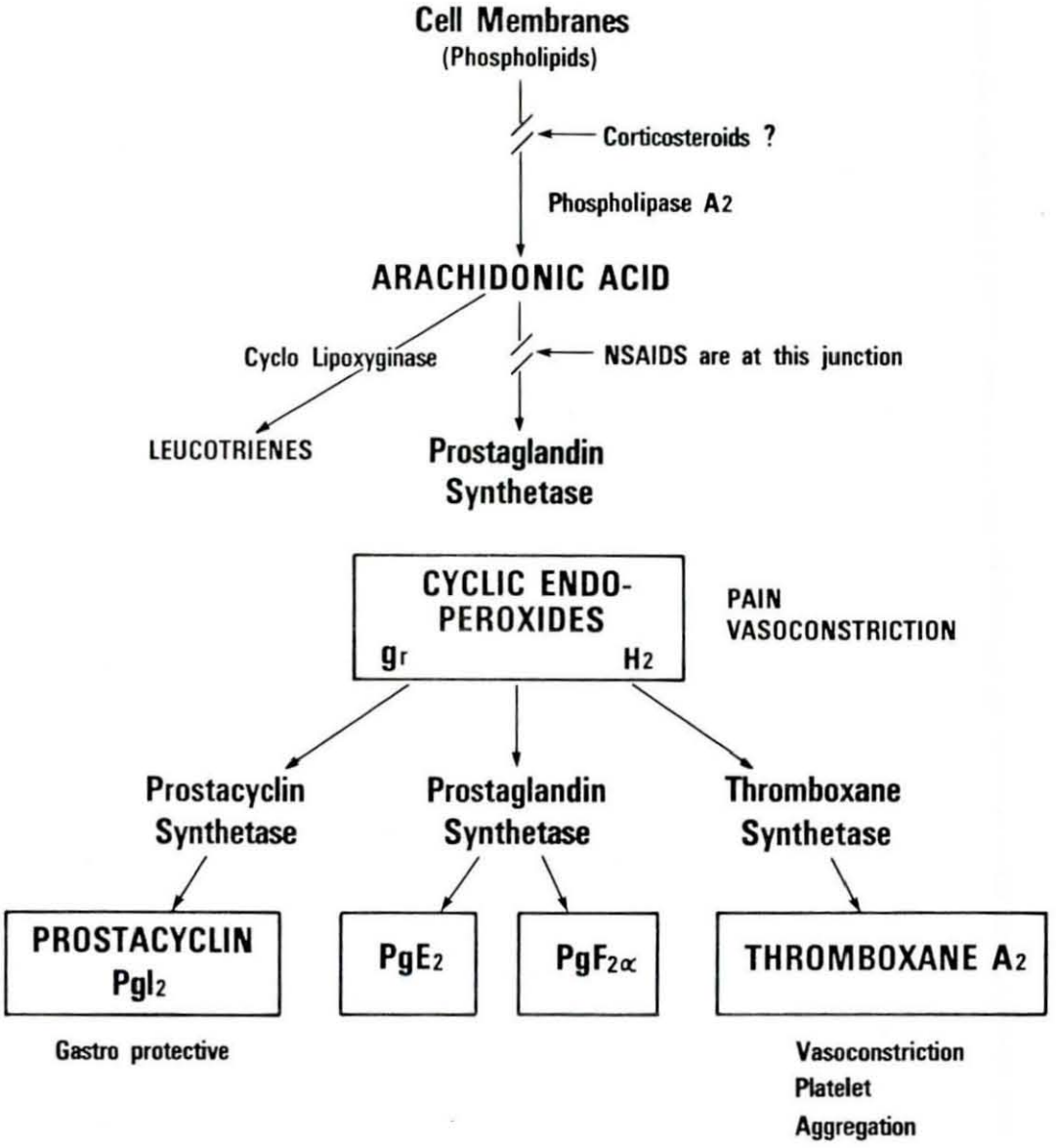


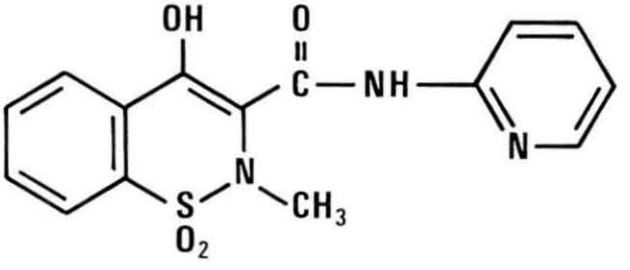
Figure 4.1 Simple diagram of the Prostaglandin Cascade (which is the mediator of pain and inflammation)

Benslay and Nickander (1981) in their study on the comparative effects of Benoxaprofen on the inhibitory action of non-steroidal anti-inflammatory drugs on the prostaglandin system, indicated that in high dosage, all the prostaglandin inhibitors were capable of causing bone regeneration or osteoblastic activity and this could be considered disease modifying.

The inhibitory action of these non-steroidal anti-inflammatory drugs on the prostaglandin system is complex and probably only accounts for the short-term effects of the drugs used. Other mechanisms may also be involved in achieving longer term benefits. In addition, evidence that some of these drugs inhibit prostaglandin biosynthesis, suggests the possibility of developing drugs capable of inhibiting production at the site of inflammation without interfering with the gastro-protective function of those prostaglandins which act on the gastric mucosa and are beneficial, i.e. the prostacyclins.

The three drugs used were chosen to cover the entire spectrum of the non-steroidal anti-inflammatory drugs as discussed. The first one was Piroxicam (Figure 4.2), which is 4 Hydroxy-2 Methyl-N (2 Pyridyl)-2H-1, 2-Benzothiazine, 1, 1-Dioxide. It is not a true Carboxylic acid as are the other two drugs used in the study. It is an acid by virtue of the enolization of the 4 Hydroxy substituent. In addition to considerable potency, Piroxicam was considered to have pharmaco-kinetic properties.

PIROXICAM



Piroxicam is 4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1, 2-benzothiazine 1, 1-dioxide.

Figure 4.2 The structure of Piroxicam

In laboratory animals, there is no spontaneously occurring counterpart of the human arthritic process. Therefore, to evaluate potential anti-inflammatory agents, many animal models have been devised, each mimicking one or more of the cardinal signs of inflammation. Piroxicam is potent in inhibiting adjuvant induced oedema of the rat foot with a potency considered equal to that of Indomethacin and greater than that of the majority of others within the generic group. It is also an anti-pyretic in the rat. It was considered effective at 10 mgs. per Kilogram.

Piroxicam is intrinsically active as an anti-inflammatory agent and does not depend upon adrenal stimulation to produce its anti-inflammatory effects. This intrinsic activity is most clearly demonstrated in anti-oedema studies in rats in which, both in those adrenalectomised and those not, the activity was exactly the same.

In laboratory animals, Piroxicam has a long plasma half-life, it is smoothly absorbed and is eliminated from plasma with a half-life of 40-45 hours. Consequently it is claimed that plasma concentrations are remarkably stable between dosing and that single daily doses can lead to plateau concentrations maintained for a full 24 hours. By virtue of its long half-life, plasma concentrations are stable at about 3 to 5 micrograms/ml. and it is hypothosised that the lack of pharmaco-kinetic interaction between it and other drugs is probably the result of this low plasma concentration. In all species, Piroxicam is

extensively metabolized with less than 5% being excreted unchanged. It is an effective inhibitor of Prostaglandin biosynthetase. It seemed therefore that as it was comparable in its pharmaco-kinetic claims with Indomethacin (which has been considered by many to be the bench-mark drug by which all others are evaluated), it is worthy as one of the newer ones, of being included in the study.

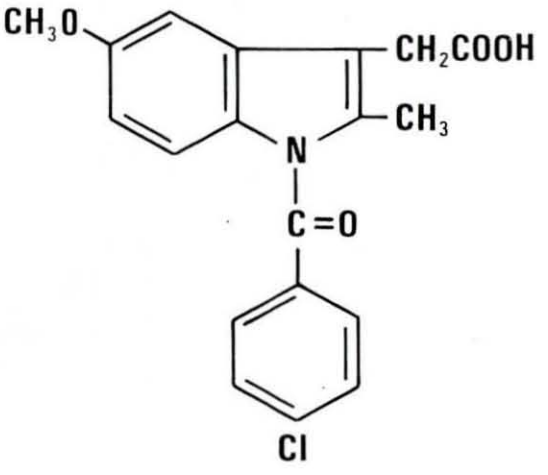
The second drug used was Indomethacin (Rhymer 1979) [23], (Figure 4.3) which has been used in the treatment of rheumatic disorders for over 15 years. It has analgesic, anti-inflammatory and anti-pyretic actions. It has now become one of the reference agents used in the field of Prostaglandin synthetase inhibition.

Indomethacin is 1-(p-Chlorobenzoil)-5-Methoxy-2-Methylindole-3-Acetic Acid. As shown in the diagram, its structural formula is C_{19}, H_{16}, NO_4 Cl.

Indomethacin is well absorbed after oral administration in all animals, and peak plasma levels occur in 0.5 to 2 hours. Following oral administration the absorption of the drug is rapid and complete. In general, peak plasma concentrations of 2 to 3/ugrams/per ml are achieved within 1 to 2 hours but concomitant ingestion of food reduces and delays the concentrations without reducing the amount absorbed.

About 60% of a normal dose is excreted in the urine and 40% is excreted in the faeces after biliary secretion. It is a

INDOMETHACIN



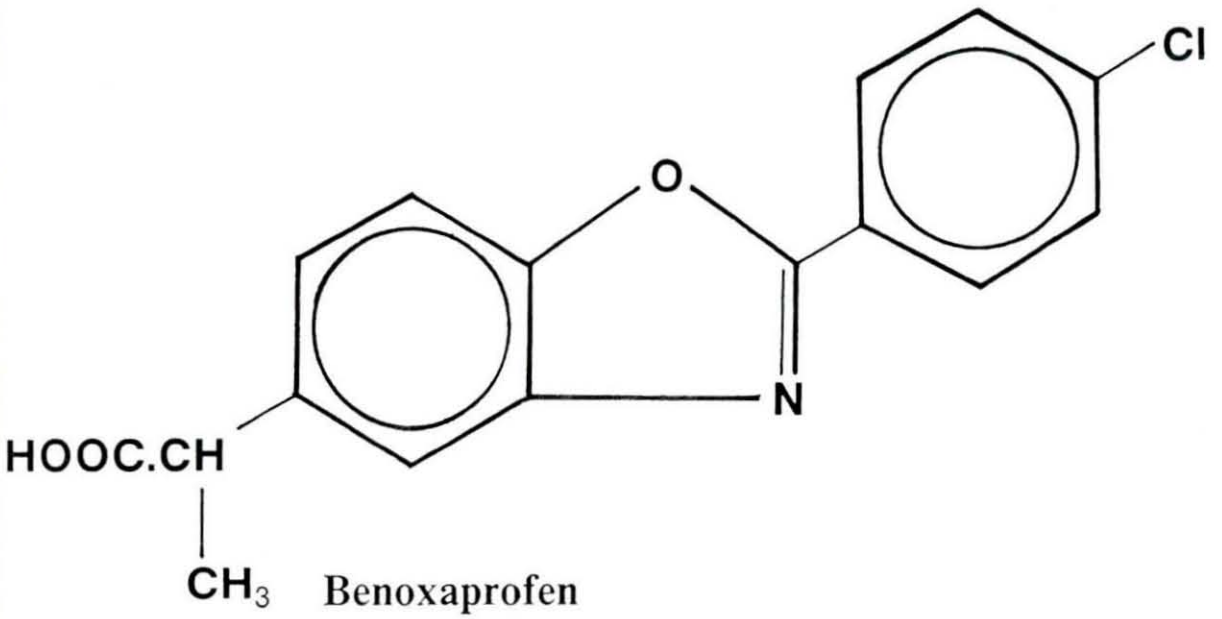
Indomethacin is 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid. Its empirical formula is $C_{19}H_{16}NO_4Cl$; molecular weight 357.78.

Figure 4.3 The structure of Indomethacin

potent inhibitor of the synthesis of Prostaglandins. It is excreted as a conjugate with glucuronic acid. All its metabolites are devoid of anti-inflammatory activity (Shen 1965) [26].

The third drug was Benoxaprofen which is 2-[2-(4-chlorophenyl) benzoxazol-5-yl] propionic acid (Figure 4.4). It was a new non-steroidal anti-inflammatory agent which was shown to share the actions of analgesia and antipyresis with some evidence of Prostaglandin synthesis inhibition (Cashin et al 1977) [6] in common with other drugs within the carboxylic acid group. Claims were made that because its moderate inhibition of Prostaglandin synthesis was not consistent with its high degree of potency in suppressing adjuvant-induced arthritis in rats, it may have had some additional inhibitory factor. This additional alternative action was considered most important if it was to be used as an anti-rheumatic agent. The claim was that it suppressed the rheumatoid arthritic disease process.

This particular drug has subsequently been taken off the approved drug list because it was considered to have additional damaging effects on liver and kidney and was responsible for some deaths in the elderly. However, at the time the study started, this drug was still in use and it was felt worthwhile evaluating the claimed effects on bony turnover in respect of erosions and on blood flow in respect of synovial disease (which were in fact validated using this new technique). In the results section it is



Benoxaprofen

2-[2-(4-Chlorophenyl)benzoxazol-5-yl]propionic acid

$C_{16}H_{12}ClNO_3$=301.7

Figure 4.4 The structure of Benoxaprofen

clear that this assessed additional efficacy is in fact not borne out, though indeed subsequent to the study and prior to the withdrawal of the drug, major doubts were expressed as to its novel additional activity as claimed by its manufacturers. This confirms the impression that a formal objective test such as functional imaging can in fact refute or confirm many of the claims made by drug manufacturers in respect of additional properties in their drugs.

5. THE EXPERIMENT

The present studies were designed to identify and compare anti-inflammatory effects using scintigraphic evidence of both bone damage and synovial blood flow in the adjuvant arthritic rat.

Beginning on the 15th day of the adjuvant induced disease, non-steroidal anti-inflammatory drugs of the 3 groups (I, II and III) were administered daily and continued until the 30th day. This procedure is called the Established Adjuvant Arthritic Assay.

On day 30, scintigraphic evaluation was carried out using the functional imaging technique already described on groups of 5 rats, each consisting of one normal control, one control rat with untreated adjuvant arthritis and 3 adjuvant arthritic rats treated respectively with Indomethacin, Benoxaprofen and Piroxicam (II, III and I).

Because of claims by Bekemeier (1982) [3] that seasonal variations were noted in hind paw volume in the experimental rat with the lowest values obtained in January and February and the highest values in June to August, the experiment was carried out over a 7 month period from January to July to see whether or not in functional imaging terms any change could be demonstrated. Bekemeier saw no change in the amount of inhibition achieved by Phenylbutazone on hind paw oedema but it was considered,

for the sake of completeness and because this was an entirely objective evaluation, that one from each group should be assessed in January, March, April, May and June. Adjuvant arthritis was induced in adult male rats (Sprague-Dawley 160-200 grams) by a single subplantar injection of Freund's complete adjuvant.

The suspension (0.1 ml) was injected into the subplantar region of the hind paw. Hind paw volumes were determined by mercury displacement. On day 14 both hind paws were measured and those displaying at least 0.5 ml swelling in the uninjected paw were selected for the treatment groups. The rats were then randomly assigned into groups of four, a fifth normal untreated control animal being then added to each group. From the next (i.e. the 15th day of the adjuvant induced arthritis) non-steroidal anti-inflammatory drugs of the groups mentioned (Indomethacin, Benoxaprofen and Piroxicam) were administered daily for a further 15 days (therefore until day 30) so that of the 5 rats in each group one was the non-arthritic control, one arthritic rat was left untreated as the second control and each of the other three arthritic rats received a different study drug.

The method of administration is the standard means by which all non-steroidal anti-inflammatory drugs (NSAIDS) are given to trial rats.

The drugs were administered orally suspended in 1% Carboxymethyl cellulose. Suspensions were prepared using

a tissue mixer model SDT (Tekmar & Co.). Both adjuvant controls and normal non-adjuvant controlled rats were administered the 1% Carboxymethyl cellulose vehicle alone.

Drug doses were as follows:-

Drug I - Piroxicam, dosage 10 mgm per kilogram

Drug II - Indomethacin, dosage 2 mgm per kilogram

Drug III - Benoxaprofen, dosage 5 mgm per kilogram

This procedure is called the Established Adjuvant Arthritic Assay.

On the 30th day scintigraphic evaluation was carried out using functional imaging on the groups of five. Each animal was anaesthetised using 15 mgms of veterinary Nembutal administered by intra-peritoneal injection. A femoral venous catheter was then inserted and secured. 15 millicuries of 99M Technetium MDP was injected via the catheter and washed through with 1 ml of normal saline (to prevent accumulation at the injection site, a problem encountered by Dick and his co-workers (1970)). In this study no significant accumulation was noted except in rat 4 where blood flush was seen to interfere slightly with the amplitude of the curve but not the shape or fit. Counting was started immediately after the injection was washed through and continued for exactly 20 minutes.

At 20 minutes the accumulated data was assessed as follows.

Each colour change through these 3 phases is standardised to a factor of 10.

Each picture display is however standardised to its own maximum uptake, in whichever dixel is hottest, and cannot be compared with another giving a purely qualitative evaluation of the dixel counts (which can be seen in Appendix A) for each joint studied. It was felt that to show individual pictures of each joint would be unnecessarily repetitive so a standard for all groups was chosen.

In group 2, which comprised the adjuvant arthritic untreated group of animals, the amplitudes show more variation related to the time of year, as shown by Bekemeier (1978) [3] worst in January and February and at their least in the Summer months. However, the rate constant as the assessed blood flow (microvasculature) is remarkably constant. In comparative human studies examples of which are shown later, an inflammed knee will have an increased amplitude and the rate constant will approach 2.0.

In group 3, the Indomethacin treated group, all the results equate towards normal with a mean rate constant (Lambda of 54).

In the qualitative evaluation it is shown that both the amplitude and rate constant values are reduced but

comparison in visual terms cannot be made without reference to figures shown in the Appendix. They are in fact virtually indistinguishable from the normal. It is known that Indomethacin can cause aseptic necrosis (or ischaemic damage) to bone following long-term ingestion in the human. No evidence of this was demonstrated in the short-term animal studies at the dosage per kilogram (10 mg) used. In group 3, that is the Benoxaprofen treated group, amplitude values show significant reduction towards the normal level, the asymptote for the hips being 44, the normal controls averaged 28.0, the rate constant value is also reduced to 0.6 which equates to normal levels (0.5) and shows this drug to be an effective anti-inflammatory approximately equal in value to Piroxicam which is studied in group 4 and shown to have an amplitude of 41 and a rate constant value not dissimilar to that of the other two treated groups. They do not equate, however, in absolute values to the Indomethacin group which was the most effective.

Indomethacin has become, in anti-inflammatory Drug Trials, one of the standards against which all of the others are tested. In this study it can be seen that neither of the other study groups is quite as efficient in reducing the blood flow which has been shown to be an accurate marker of synovial activity, which is the most significant change in the arthritic model.

6. RESULTS

The normal untreated group determines the standards for the major parameters to be studied, that is, bony turnover and microvasculature, displayed as amplitude and rate constant (Lambda).

The joint numbers 1-4 in each study are in order, the left hip, the right hip, the left knee and the right knee. It can be seen that the amplitude values in all 7 controls are of the same order of magnitude (Table 6.I) (Appendix). The red line on each graph is a computerised "best fit" to the plotted curves (white) (Figure 6.1) and can be shown to be very accurate, thus reducing the route mean square deviation, which is a form of mobile standard deviation along the whole line of the asymptote. It is known that the dynamic curve for bone, using 99 M Technetium is close to its asympote (maximal amplitude) at 20 minutes. This is of particular value because the study period in each of the models is exactly 20 minutes. In the simple compartmental model proved by Charkes (1980) [7], the value of Lambda in bone should be constant. However, a certain amount of variation may take place as a function of compartmental overlap after a certain period. A diagram of the compartmental model is shown and discussed in Chapter 2.

As a result, the normal amplitude and rate constant (Lambda) pattern must be established for a given skeletal region in each study model. Subsequent deviations from

NORMAL CONTROL

RAT	JOINT	AMPLITUDE	RATE CONSTANT	RMS DEVIATION	ERROR
1	1	48.15	.519	1.683	0
1	2	25.72	.601	1.069	0
1	3	6.81	.615	.623	0
1	4	15.56	.488	.574	0
13	1	39.97	.801	.739	0
13	2	28.96	.501	1.678	0
13	3	5.69	.458	.434	0
13	4	5.17	.526	.378	0
20	1	40.65	.551	1.049	0
20	2	44.74	.527	1.436	0
20	3	7.85	.451	5.283	0
20	4	7.91	.508	.314	0
27	1	25.22	.607	6.469	Blood surge
27	2	24.23	.873	.861	0
27	3	1.38	.322	2.471	0
27	4	1.95	.652	.174	0
28	1	29.07	.691	.575	0
28	2	27.45	.670	.663	0
28	3	4.17	.416	2.693	0
28	4	2.66	.435	.183	0
29	1	38.47	.641	5.202	0
29	2	28.65	.529	.856	0
29	3	4.28	.556	8.388	0
29	4	4.06	.358	.289	0
30	1	55.13	.606	2.333	0
30	2	39.14	.584	1.034	0
30	3	6.79	.377	1.407	0
30	4	6.01	.349	.420	0

Table 6.I Amplitude, accretion rate and standard deviation (RMS) for normal control rats.

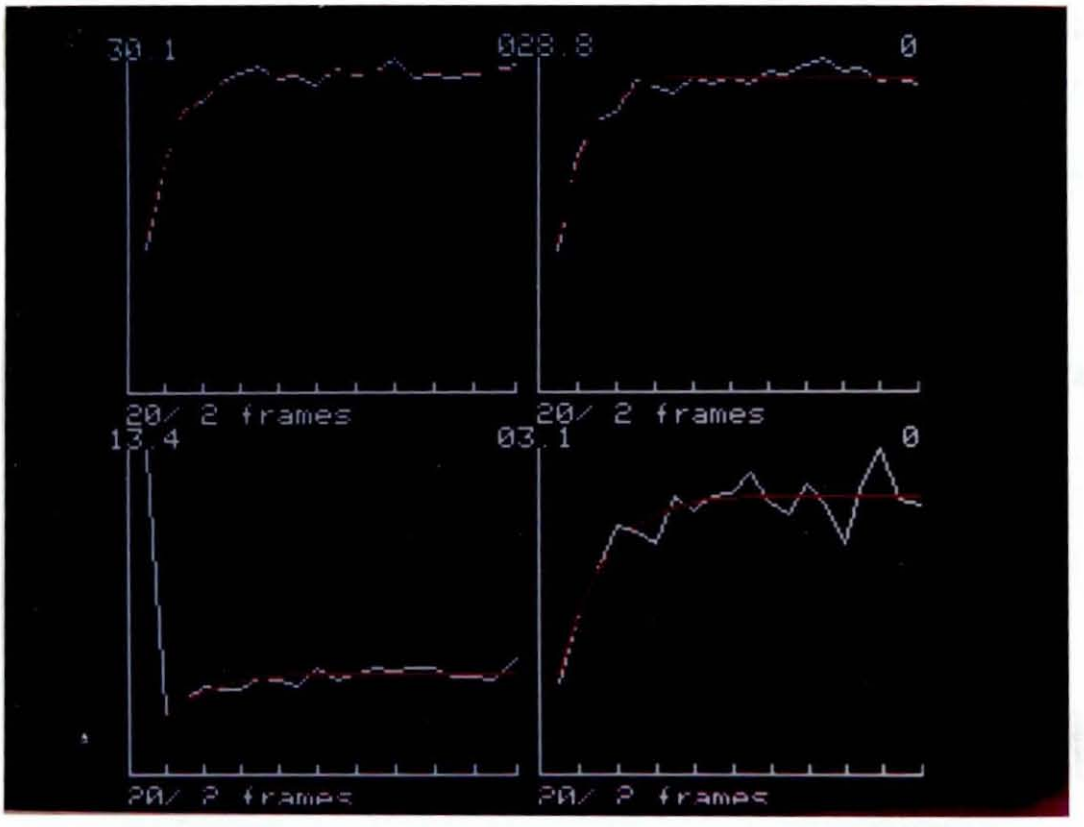


Figure 6.1 shows amplitude curves in normal (untreated) control rat: the hips on the top line and the knees on the lower line graphs.

this established normal level can then be identified and related to specific lesions.

In this study normal control amplitude values for the rat's hips are shown in Figures 6.1 (upper line) and the left and right knees (bottom line). Figure 6.2 shows an adjuvant control with the same joint distribution and demonstrates an increase of greater than a factor of 5. These curves are highly comparable and standardised.

The rate constant values showed remarkable consistency indicating that microvasculature in this model produces a constant rate of flow in all joints, the volume changes purely relating to the size of the joint. The average mean rate constant is approximately 0.5 which is accepted as the normal for this rat study.

In human studies the accepted normal rate constant (bearing in mind Charkes' statement that it has to be evaluated for each study group) is 1.0. The root mean square deviation (RMS) which is best described as a mobile standard deviation shows remarkably little variance in any of the normal control animals.

Their qualitative display in this series of results of the normal, shown in Figure 6.3 has a colour display of amplitude in the left-hand picture and a colour display of rate constant in the right of each group. The colour scaling is from white as the maximum pick up in each study down to blue through red, yellow and green.

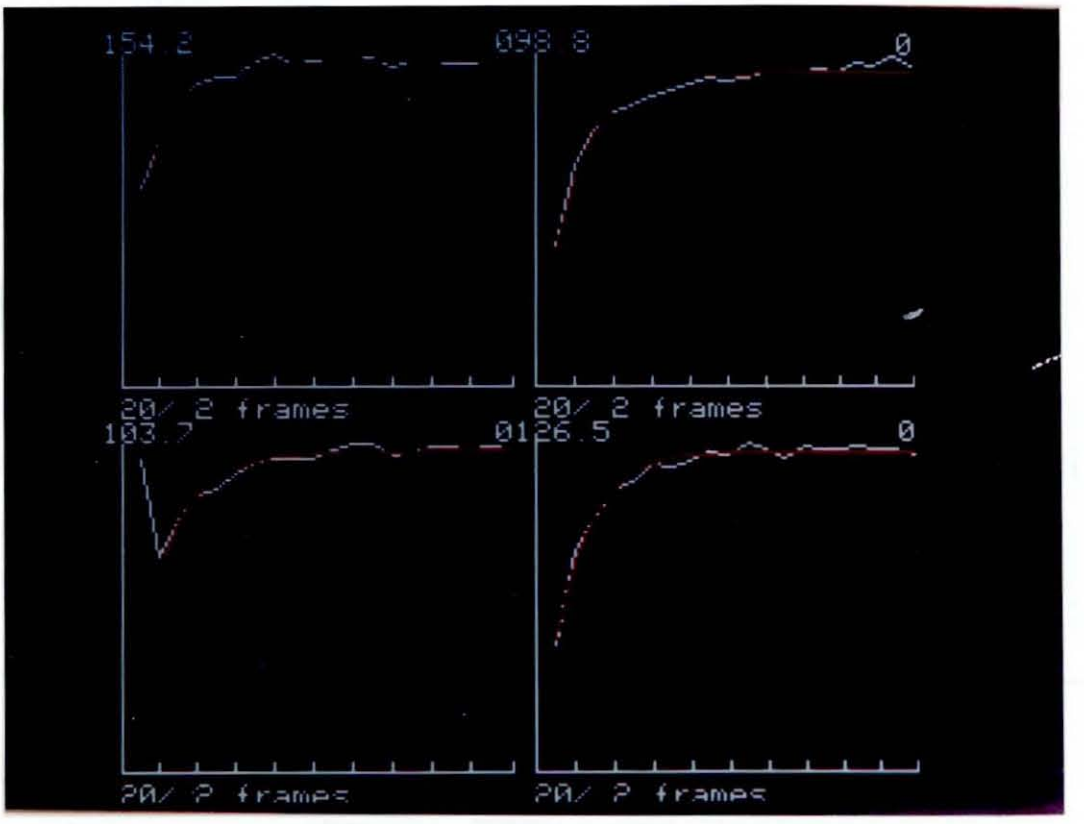


Figure 6.2 shows an untreated adjuvant control with the hips on the upper line and the knees on the lower line graph showing an increase of greater than a factor of 5.

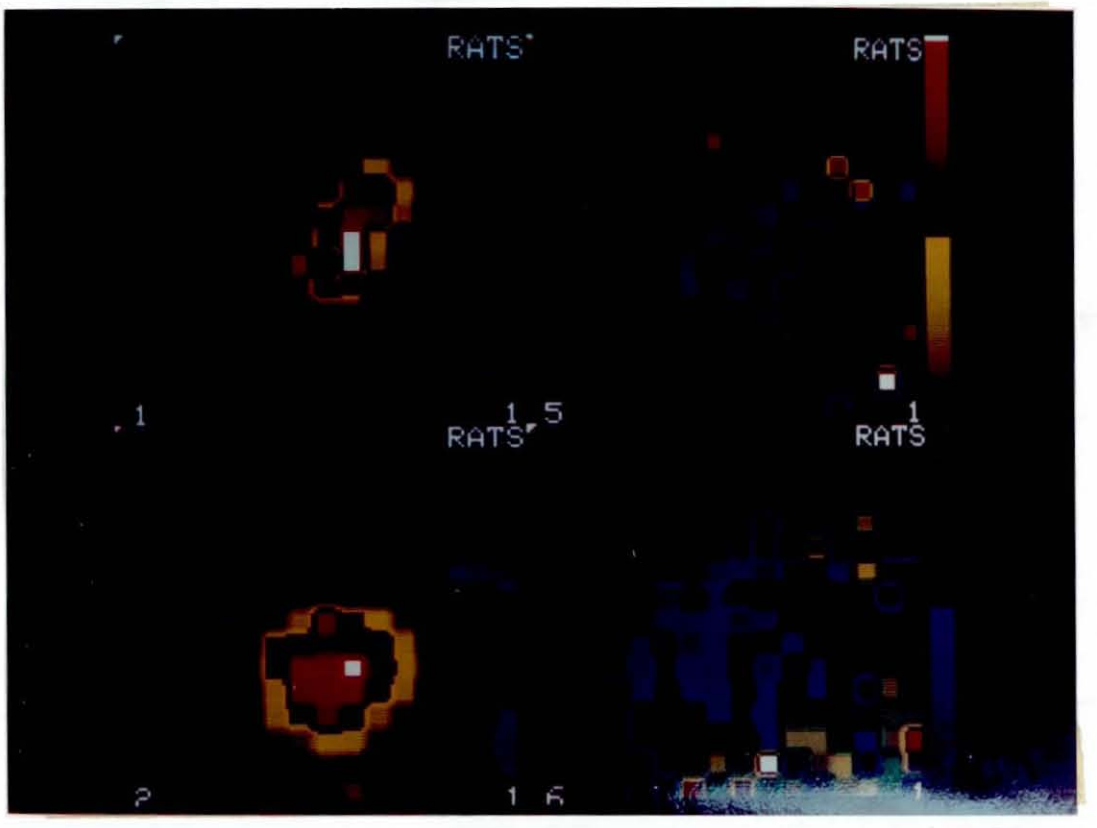


Figure 6.3 A a functional image of a normal (upper) and untreated adjuvant (lower) rat.

A whole body image of the animal (Figure 6.4) allowed the 4 joints being studied (i.e. the left knee, right knee, left hip and right hip) to be isolated as regions of interest (ROI) in the computer. Table 6.II shows the square area of these regions of interest in pixel size. The advantages of using circumscribed regions of interest is that only the joints involved are analysed. Background radiation and specific organ involvement (such as the excreting kidney and rapidly filling bladder) are eliminated. Failure to eliminate this extraneous radiation would have the effect of reducing the signal recorded. Four animals did not have their regions of interest specifically isolated because of technical faults on the ZLC 70 gamma camera. They were counted instead on the PHO gamma IV camera which has no zoom facility. However, even in this slightly modified study, the uptake curves were identical in shape and they were considered valid within the experiment.

So consistent are the findings in each of the 5 models (normal control, adjuvant arthritic control and drug treated groups I, II and III (Figures 6.1, 6.5, 6.12)) that one example of each suffices to demonstrate the typical values of all (Tables 6.III - 6.VI). They include the amplitude of uptake (asymptote), the rate constant (Lambda), the root mean square deviation (which is a mobile standard deviation along the whole curve of amplitude) and finally a functional image as a photographic image.

RATS: SQUARES FOR FUNCTIONAL IMAGING

TOP LEFT HAND CORNER OF SQUARE (16^2)

RAT	COMPUTER NO	LEFT HIP X	LEFT HIP Y	RIGHT HIP X	RIGHT HIP Y	LEFT KNEE X	LEFT KNEE Y	RIGHT KNEE X	RIGHT KNEE Y	COMMENT
1	555	28	37	72	38	14	20	89	21	
2	666	48	46	89	44	29	29	101	27	
3	777	59	65	95	66	50	47	113	50	
4	888-82	64	58	104	60	51	40	113	40	
5	999-82	65	61	105	62	50	46	113	49	R knee mainly out of view Square contains part of hip
6	1000-82	38	64	82	67	26	48	95	47	
7	1111-82	38	66	77	68	16	54	95	56	
8	1222-82	23	60	67	62	8	43	92	45	
9	1333-82	27	53	75	54	8	37	85	38	
10	1444-82	31	58	72	66	19	39	87	46	
11	1555-82	31	46	77	54	28	22	86	34	
12	1666-82	39	63	85	63	28	46	97	42	
13	1777-82	35	63	79	64	12	49	98	47	
14	1888-82	16	59	61	70	8	36	79	53	
15	1999-82	20	56	70	59	1	41	86	41	
16	2000-82	25	55	67	56	15	32	77	36	
17	2111-82	30	69	71	66	22	45	76	42	
18	2222-82	34	75	81	73	18	53	88	48	
19	2333-82	20	53	63	51	9	34	70	31	
20	2444-82	25	62	72	60	2	47	88	39	
21	2555-82	20	61	68	64	2	42	85	38	
22	2666-82	46	50	94	52	30	34	103	29	
23	2777-82	53	43	104	46	38	26	113	27	R knee partly out of view
24	2888-82	38	48	84	50	15	34	95	28	
25	2999-82	32	44	79	49	10	28	97	30	
26	3000-82	29	39	78	47	9	19	95	30	
27	3111-82	53	42	77	61	49	25	96	60	
28	3222-82	51	59	78	74	49	43	97	70	
29	3333-82	55	60	82	75	51	43	99	70	
30	3444-82	51	55	75	74	50	36	92	73	
31	111	21	51	66	58	14	28	82	41	
32	222	45	39	88	40	31	21	106	21	
33	333	1	104	41	93	1	78	45	69	L hip mainly out of view L knee totally out of view Arbitrary background region chosen

N.B. 444 is a static view
1444-82 1st frame at different orientation from rest
(1st frame is not used in calculations anyway)
RATS 27-30 done on PHO GAMMA IV (unzoomed)

Table 6.II Square areas of regions of interest by pixel size

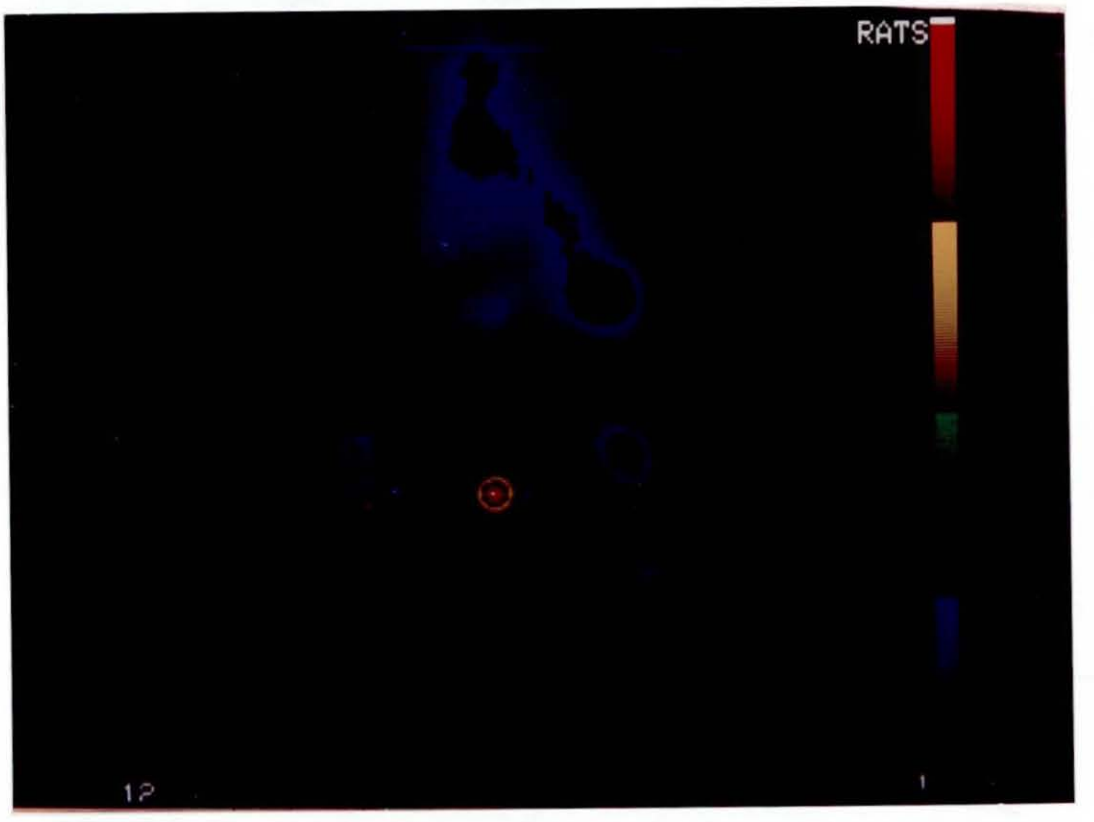


Figure 6.4 shows a whole body image in an adjuvant (untreated) model, from which regions of interest (ROI) can be selected. (The bright areas indicate the hips and knees in the animal - the central and brightest zone is the rapidly filling bladder.)

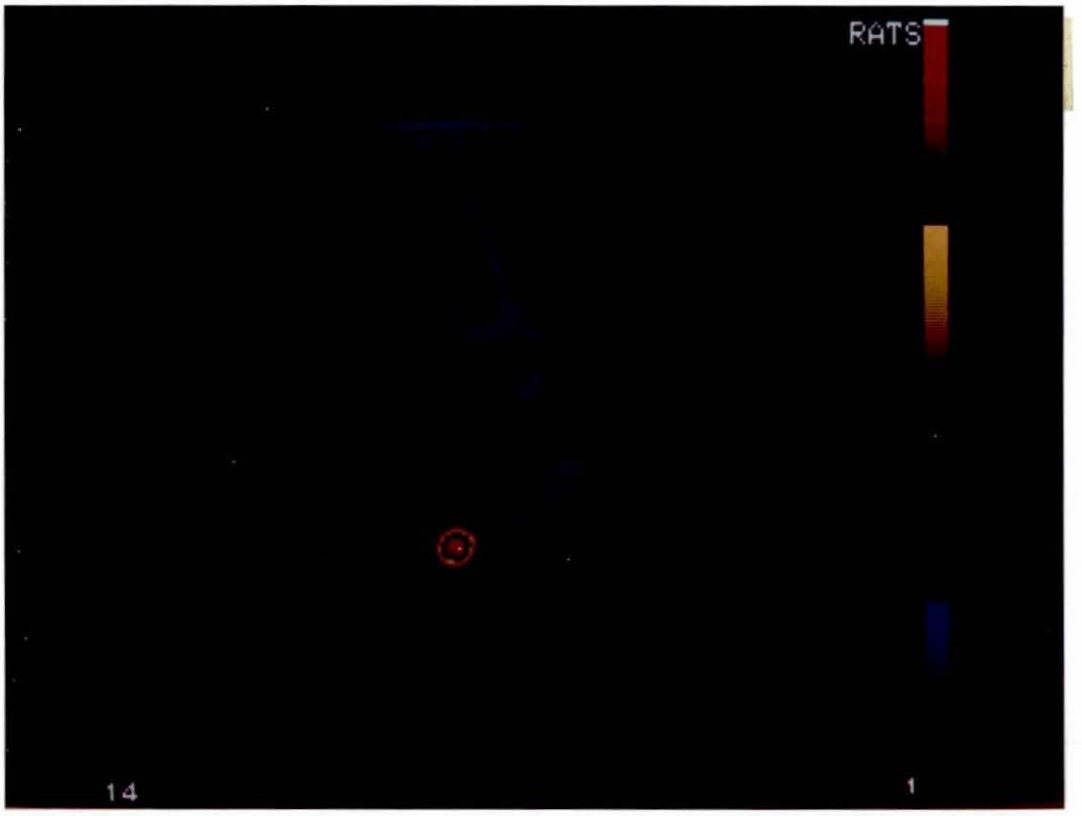


Figure 6.5 An Indomethacin whole body image from which regions of interest were chosen for the study.

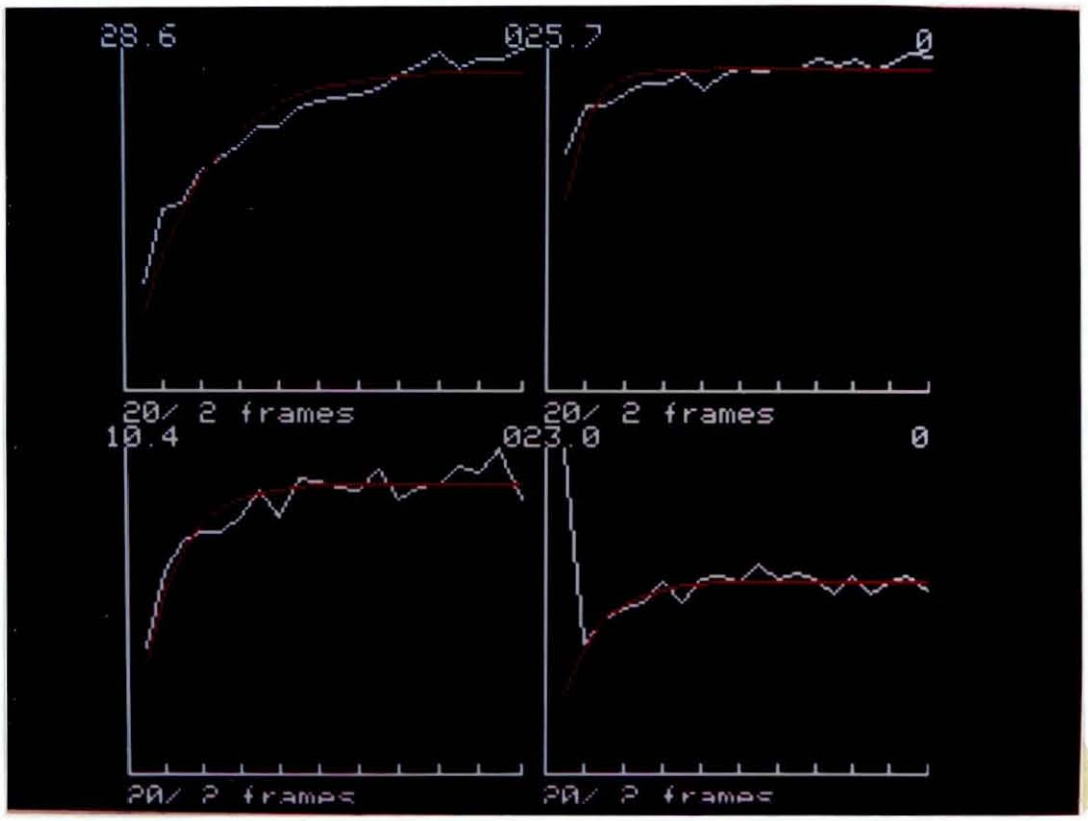


Figure 6.6 Indomethacin treated amplitude curves showing a tendency towards normal (see Figure 6.1).

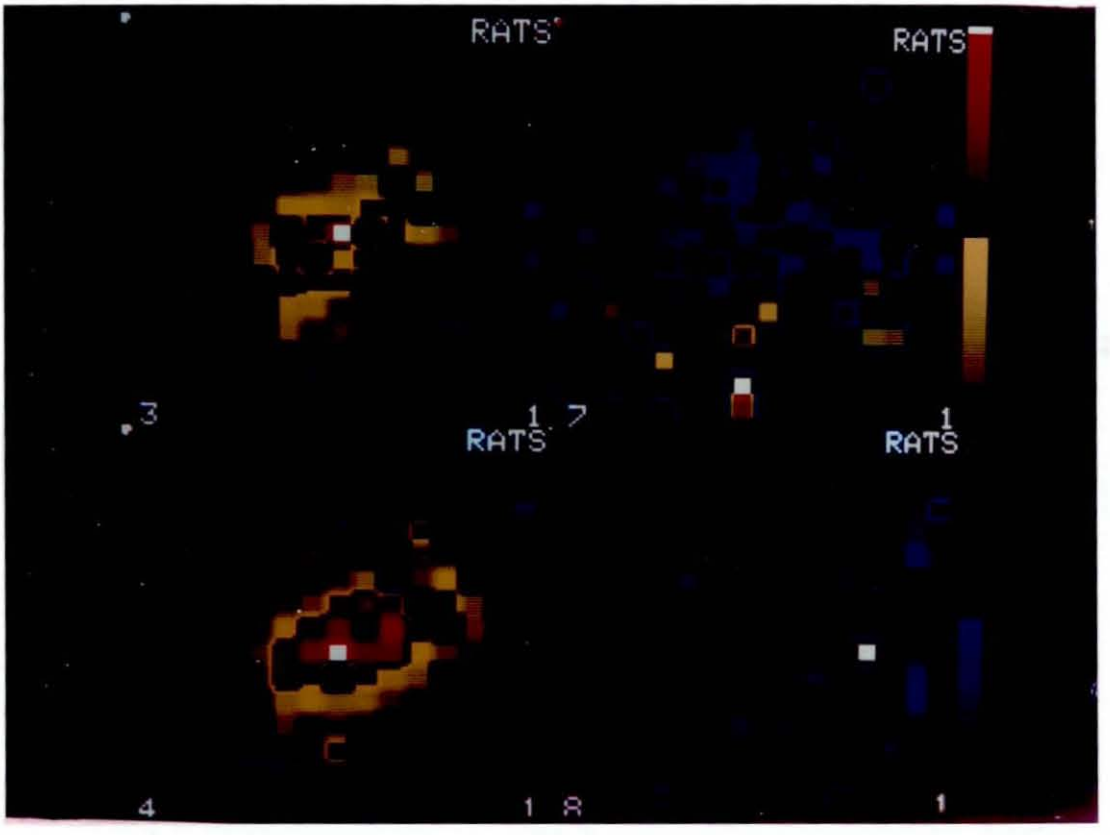


Figure 6.7 Comparative functional images of Benoxaprofen against Indomethacin showing amplitude on the left-hand images and rate constant on the right-hand images.

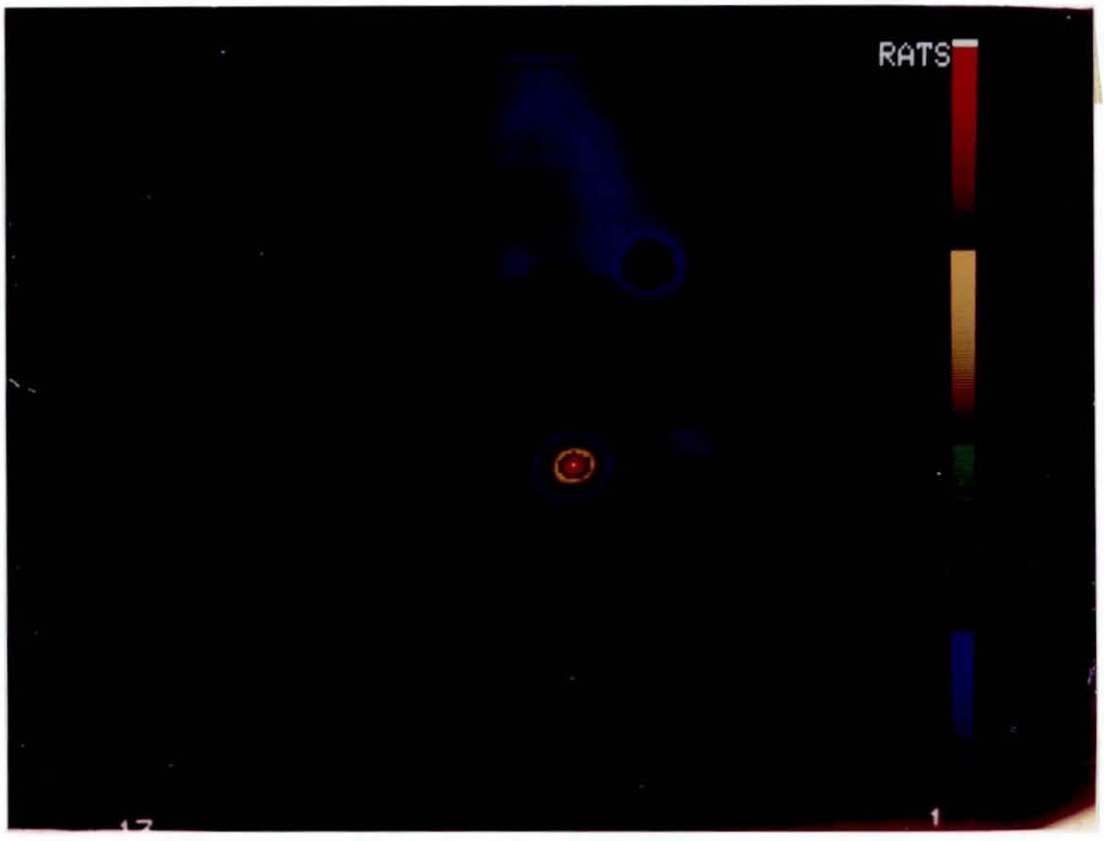


Figure 6.8 Benoxaprofen treated whole body image from which regions of interest were chosen for the study. (The bright central region is the bladder.)

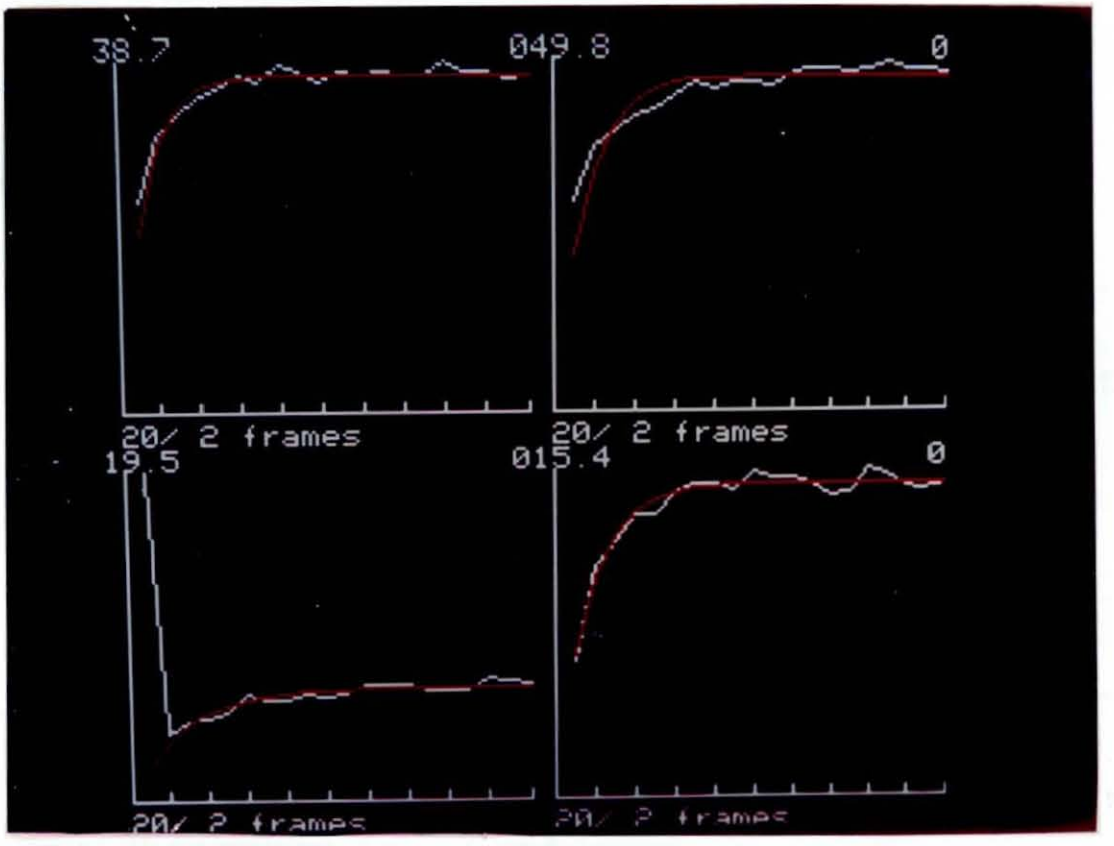


Figure 6.9 Benoxaprofen treated amplitude curves for knees and hips (upper and lower graph images) which tend towards normal (see Figure 6.1)
(Note: the amplitude has been reduced from a mean of 156 (adjuvant control) to 38.7/49.8 in the hips (L&R) and leans towards normal.)

Figure 6.10 Piroxicam whole body image from which regions of interest (ROI) were chosen.

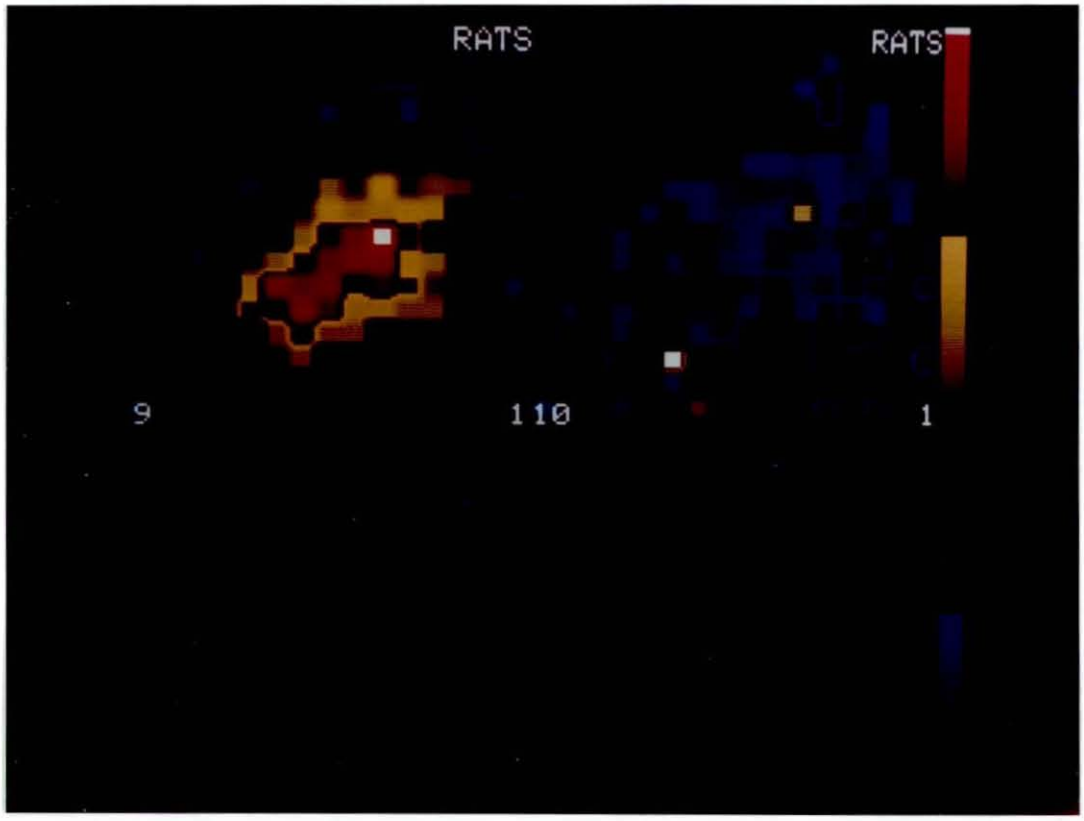


Figure 6.11 Functional image of Piroxicam treated rat showing amplitude in the left-hand image and rate constant in the right.

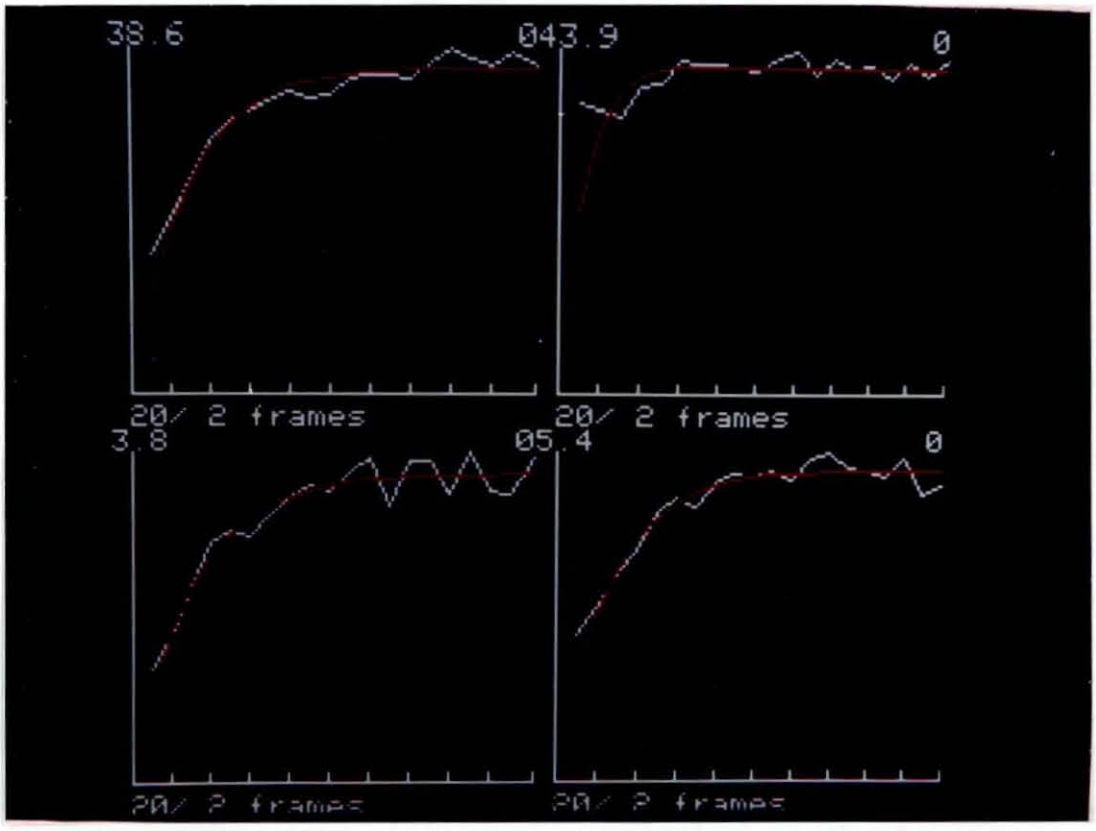


Figure 6.12 is Piroxicam amplitude (asymptotic) curves which can be compared directly with the normal curves in Figure 6.1 and are seen to tend towards normal.

ADJUVANT CONTROL

RAT	JOINT	AMPLITUDE	RATE CONSTANT	RMS	ERROR
2	1	53.89	.886	1.352	0
2	2	15.87	.676	1.639	0
2	3	4.84	.310	.244	0
2	4	6.37	.346	.326	0
6	1	16.44	1.992	.456	0
6	2	32.03	.405	.796	0
6	3	6.57	.730	3.482	0
6	4	31.33	.353	.392	0
10	1	55.86	.321	13.797	0
10	2	57.07	.444	2.000	0
10	3	9.78	.434	1.673	0
10	4	14.40	.611	.462	0
16	1	65.16	.449	3.387	0
16	2	45.69	.430	1.890	0
16	3	36.55	.381	3.440	0
16	4	46.49	.529	1.410	0
17	1	151.43	.731	3.922	
17	2	94.43	.555	2.263	
17	3	101.86	.504	13.372	
17	4	128.96	.530	1.922	
22	1	47.49	.429	1.083	
22	2	57.56	.580	1.646	
22	3	37.93	.281	2.593	
22	4	80.88	.364	2.619	

Table 6.III Amplitude, accretion rate and standard deviation (RMS) for untreated adjuvant control rats.

INDOMETHACIN

RAT	JOINT	AMPLITUDE	RATE CONSTANT	RMS	ERROR
4	1	29.02	.425	1.440	0
4	2	25.47	6.688	21.154	0
4	3	6.38	.549	.291	0
4	4	10.97	.416	1.277	0
11	1	28.84	.599	2.827	0
11	2	16.58	.324	.880	0
11	3	7.03	.416	30.929	0
11	4	5.12	.533	.502	0
15	1	17.74	.456	1.172	0
15	2	37.03	.474	2.557	0
15	3	2.70	.394	.276	0
15	4	6.85	.931	.445	0
19	1	27.11	.280	1.442	0
19	2	24.48	.911	1.161	0
19	3	9.37	.490	.502	0
19	4	13.68	.521	3.949	0
24	1	53.69	.412	1.910	
24	2	53.44	.818	3.869	
24	3	8.70	.472	.542	
24	4	18.25	.738	7.838	

Table 6.IV Amplitude, accretion rate and standard deviation (RMS) for Indomethacin treated rats.

BENOXAPROFEN

RAT	JOINT	AMPLITUDE	RATE CONSTANT	RMS	ERROR
3	1	38.45	.859	1.238	0
3	2	27.99	.800	.933	0
3	3	6.01	.419	.378	0
3	4	9.13	.831	.307	0
7	1	21.03	.641	1.839	0
7	2	7.46	.989	.295	0
7	3	3.87	.449	2.481	0
7	4	7.58	.993	.374	0
9	1	28.68	.465	1.460	0
9	2	17.76	.400	1.420	0
9	3	2.78	.472	.191	0
9	4	6.94	.605	.422	0
14	1	42.59	.519	1.855	0
14	2	40.01	.511	1.967	0
14	3	6.45	.460	4.609	0
14	4	10.91	.830	.514	0
18	1	37.55	.794	.990	0
18	2	40.07	.653	2.243	0
18	3	6.71	.378	3.903	0
18	4	14.75	.605	.377	0
23	1	53.83	.464	1.560	0
23	2	41.53	.520	1.569	0
23	3	5.89	.425	3.113	0
23	4	6.45	.517	.275	0

Table 6.V Amplitude, accretion rate, and standard deviation (RMS) in Benoxaprofen treated rats.

PIROXICAM

RAT	JOINT	AMPLITUDE	RATE CONSTANT	RMS	ERROR
5	1	40.99	.720	1.782	0
5	2	38.26	.752	1.659	0
5	3	9.53	.461	.391	0
5	4	1.47	1.992	.456	0
8	1	23.47	.549	.559	0
8	2	26.28	.632	1.132	0
8	3	3.53	.462	.769	0
8	4	6.89	.878	.449	0
12	1	26.72	.577	.779	0
12	2	40.86	1.094	.706	0
12	3	5.24	.471	1.265	0
12	4	5.79	.932	.342	0
21	1	36.74	.363	1.482	0
21	2	42.30	.832	3.320	0
21	3	3.53	.329	.177	0
21	4	5.17	.376	.241	0
25	1	47.34	.583	3.871	
25	2	37.91	.736	.790	
25	3	5.15	.405	.730	
25	4	9.79	.744	.282	
26	1	32.77	.986	.896	0
26	2	50.85	.528	2.027	0
26	3	3.75	.175	.107	0
26	4	11.68	.263	.393	0

Table 6.VI Amplitude, accretion rate and standard deviation (RMS) in Piroxicam treated rats.

7. DISCUSSION

Joint scintigraphy was introduced in the 1960s by Weiss [31] and by Alarcon-Sergovia [1], who showed that the uptake of Technetium 99m pertechnetate was increased in inflamed joints as compared to normal ones.

The localisation of these isotopes in inflamed joints results from increased synovial vascularity associated with synovitis. Synovitis is the hallmark of inflammatory arthritis and the producer of prostaglandins at local and peripheral joint sites which are the mediators of pain and inflammation. The main isotope used nowadays is 99m Technetium M.D.P., a bone seeking nucleide.

In synovitis, two phases of increased uptake are recognised, an early vascular phase, within minutes, and a delayed phase at about 20 minutes when it is known that in dynamic studies the uptake curve for bone is close to its asymptote, equating to the maximal amplitude.

Abnormal static scintigraphy with 99m Technetium M.D.P. correlates well with clinically apparent synovitis and may even exceed the accuracy of the Physician in diagnosing clinically detectable synovitis. At times therefore, joint scans can predict sites where clinically apparent synovitis later develops. Any inflamed joint may produce a positive scintiscan.

Studies of rheumatoid arthritis, and, in the animal model, adjuvant arthritis, have been carried out showing abnormal uptake to be lessened or normalised by effective treatment with non-steroidal anti-inflammatory drugs (NSAIDS). Scans have also been useful for following the effects of treatment in rheumatoid arthritis.

Whereas the majority of studies carried out in hospitals and in research establishments relate to static imaging, in this study the imaging is early and active (dynamic). Diphosphonate was used because the increased uptake was considered to result from its chemadsorption on to hydroxyapatite crystals in juxta-articular cancellous bone which in most joint disease becomes abnormally vascular. Additionally, synovial vascularity also contributed to abnormal uptake. These two modalities are seen in this study as the amplitude (or asymptote) and the rate constant (or acretion rate). In human knees, using Technetium M.D.P., the rate constant levels of normality can be assessed and in the normal study the rate constant for normal knees is set at 1.0. In an inflammed knee the rate constant will approach 2.0 and in an ischaemic knee (which may be an osteoarthritic knee) will be below 1.0, tending towards 0.5.

Photographs of each of these three models in the human are shown to exclude any doubt and to help differentiate between the three forms of arthritis (and thus rule out any confusion). Figure 7.1 shows normal knees, with effectively

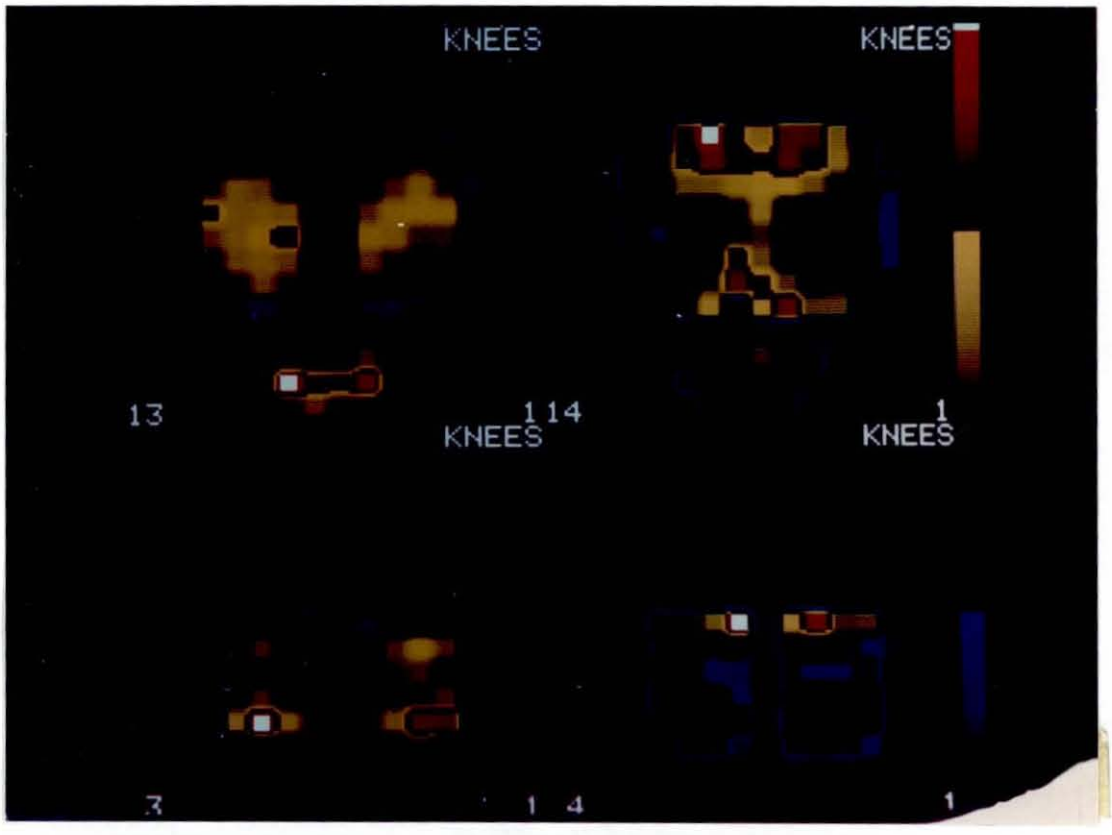


Figure 7.1 shows on the upper line a normal knee with normal amplitude (left) but slightly increased rate constant (Lambda) (right), and on the lower line a normal knee with normal amplitude and rate constant.

normal amplitude and normal rate constant levels (Lambda). Each knee is maximised in counts to its hottest spot. Figure 7.2 shows increased amplitude and increased rate constant which is typical of the inflammatory model in the animal studies in this series of experiments. Figure 7.3 shows increased amplitude and decreased rate constant equivalent to an ischaemic condition, and clearly cold, such as would be seen in the osteoarthritic (degenerative) model.

In this experimental study evaluation of novel compounds used as anti-inflammatory drugs in the arthritic complaints, have been evaluated and questions asked in respect of their true comparison with each other.

The apparent determination of member firms of the Pharmaceutical Industry to compare their drugs in the most favourable light against other products from other firms is accepted as a natural event. Because of this, and in particular because of their enthusiastic claims made on behalf of one of the drugs in this study, questions which had to be asked were as much as to the objectivity of the design as well as the interpretation in animal and clinical trials. Certainly dosage rates of drugs appear to be chosen to show the better points of the in-house drug against competitors!

Drugs have hitherto been evaluated by a series of classical tests amongst which the adjuvant (Freunds') complete arthritic rat hind paw volume study is just one.

Figure 7.2 Inflammatory model showing increased amplitude (left) and increased rate constant (right) in both upper and lower knees.

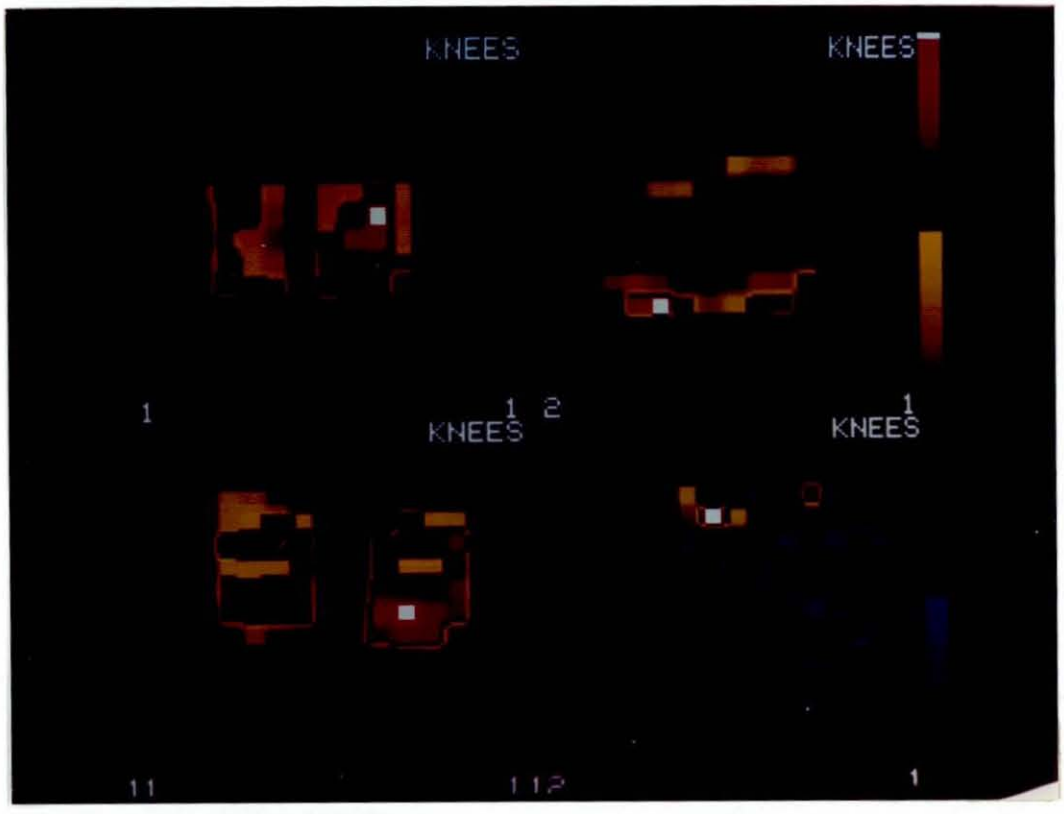


Figure 7.3 Comparison of upper inflammatory model with increased amplitude (left) and increased rate constant (right) with lower degenerative (osteoarthritic) model with increased amplitude (left) but decreased rate constant (right).

The true relevance of some of these tests should have been established far earlier as seen by the deaths of 70 elderly patients due to side effects, the subject of recent unwelcome publicity.

This study uses an absolutely objective test, that is, functional imaging, which is reproducible from each group of animal studies. The significance of this technique, which is truly pharmaco-dynamic, extends far beyond these specific effects, i.e. amplitude and rate constant results into the whole field or organ specific studies using a variety of isotopes.

Weiner (1973) [29] and Kaihara (1969) [10] before him saw the value of these techniques in renal and hepatic studies using such isotopes. In pharmaco-dynamic studies, the technique is used, for example, in the renal transplant field. Because of its great flexibility it can identify parenchymal change long before biochemical parameters show any abnormality. By facilitating the objective comparison of drug effectiveness a far more specific watch can be kept on side effects, titrated against established anti-inflammatory activity.

The balance of effect against side effects, which today is based on subjective or clinical evaluation by the medical practitioner, can thus be made more scientific and indeed, considerably easier.

These particular advantages for the patient and for the physician are enhanced since the objectivity of the test permits quality control over some of the more extravagant claims made for any drug formulation in this field.

Functional imaging thus permits the objective assessment of drug effects both desirable and detrimental.

8. CONCLUSIONS

1. This test model is valid for the mathematical evaluation of drug effect, with particular correlation to blood flow (Lambda) or rate constant values. The pattern is indistinguishable from the normal human curves.

2. Amplitude or acretion rate is similarly mathematically evaluable.

3. Because of the timing of the study, the problems of bone to ECF leakage of isotope is not relevant. This compartment model can therefore be considered an excellent research model.

4. The claim of bone effect on osteoblastic/osteoclastic activity with particular reference to Benoxaprofen (III) have not been borne out.

5. The test has a potential to become a significant screen for pharmaco-dynamic activity of novel compounds in the anti-inflammatory analgesic field.

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