SOME APPLICATIONS OF GAMMA EMITTING RADIOISOTOPES TO THE STUDY OF THE NORMAL AND DISEASED DIARTHRODIAL JOINT

W. Carson Dick

M.B., Ch.B., M.R.C.P.(Lond.), D.Obst.R.C.O.G.

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Roma, Alison and John Dick.

A STUDY ON THE RELATIONSHIP BETWEEN ISOTOPE STUDIES AND CLINICAL METHODS AS INDICES OF INFLAMMATORY ACTIVITY.
Ann. Rheum. Dis. (in press)
Dick, W.C., Grayson, M.F., Woodburn, A., Nuki, G. and Buchanan, W.W.

DERIVATION OF KNEE JOINT SYNOVIAL PERFUSION USING THE XENON (133 Xe) CLEARANCE TECHNIQUE (1970)
Ann. Rheum. Dis. 29:131-134
Dick, W.C., St. Onge, R.A., Gillespie, F.C., Downie, W.W., Nuki, G., Gordon, I., Whaley, K., Boyle, J.A. and Buchanan, W.W.

ADRENERGIC CONTROL OF CANINE SYNOVIAL PERFUSION IN EXPERIMENTALLY INDUCED OSTEOARTHRITIS AND SYNOVITIS.
Research in Veterinary Science (in press)
Dick, W.C., Provan, C. and Pond, M.

EFFECT OF ANTI-INFLAMMATORY DRUG THERAPY ON CLEARANCE OF 133 Xe FROM KNEE JOINTS OF PATIENTS WITH RHEUMATOID ARTHRITIS (1969)
Brit. Med. J. 3:278-280.
Dick, W.C., Dick, P.H., Nuki, G., Whaley, K., Boyle, J.A., Shenkin, A., Downie, W.W. and Buchanan, W.W.

EFFECT OF SYNOVECTOMY ON THE CLEARANCE OF RADIOACTIVE XENON (133 Xe) FROM THE KNEE JOINT OF PATIENTS WITH RHEUMATOID ARTHRITIS (1970)
Journal of Bone and Joint Surgery 52B:70-76
Dick, W.C., Shenkin, A., Freeman, P., Nuki, G. Whaley, K. and Buchanan, W.W.

SIMPLE RADIOISOTOPIC METHOD FOR THE STUDY OF SMALL JOINT INFLAMMATION IN RHEUMATOID ARTHRITIS: RADIOACTIVE TECHNETIUM (99 mTc) UPTAKE IN THE PROXIMAL INTERPHALANGEAL JOINTS AND THE EFFECTS OF ORAL CORTICOSTEROIDS.
Ann. Rheum. Dis. (in press)
Collins, K., Nuki, G., Deodhar, S.D. and Dick, W.C.

THE ARTICULAR SCAN IN PATIENTS WITH RHEUMATOID ARTHRITIS: A POSSIBLE METHOD OF QUANTITATING JOINT INFLAMMATION USING RADIO-TECHNETIUM (1968)
Clin. Sci. 35:547-552
Whaley, K., Pack, A.I., Boyle, J.A., Dick, W.C., Downie, W.W Buchanan, W.W. and Gillespie, F.C.

I have personally presented some of the data contained in this thesis to the following meetings:-

COMMUNICATIONS

SOME ASPECTS OF THE SYNOVIAL BLOOD FLOW IN THE NORMAL AND DISEASED JOINT
to Scottish Society of Experimental Medicine, Glasgow. October, 1967.

MEASUREMENT OF SYNOVIAL BLOOD FLOW IN NORMAL AND DISEASED JOINTS
to Heberden Society, Glasgow. May, 1968.

THE ARTICULAR SCAN IN PATIENTS WITH RHEUMATOID ARTHRITIS
to Heberden Society, London. November, 1968.

ISOTOPE STUDIES AS AN OBJECTIVE AND QUANTITATIVE INDEX OF THE DEGREE OF INFLAMMATORY INVOLVEMENT IN VARIOUS ARTHRITIDES
to British Orthopeadic Association, Glasgow. April, 1969.

QUANTITATIVE EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY DRUGS
to Glasgow Post-Graduate Medical Board and Trust for Education and Research in Therapeutics, Glasgow. April, 1969 (by invitation).

A RADIOISOTOPE TRACER METHOD OF MEASURING SYNOVIAL BLOOD FLOW AND ITS CLINICAL APPLICATIONS
to 2e Congres Francais d'electronique medicale et de genie biologique, Nancy. June, 1969 (by invitation).

THE DEVELOPMENT AND EVALUATION OF A METHOD OF MEASURING SYNOVIAL BLOOD FLOW: THE EFFECT OF ANTI-INFLAMMATORY DRUGS, VASOREACTIVE AGENTS AND SYNOVECTOMY,
to International Society of Rheumatology, Prague. Oct. 1969.

EFFECT OF ISOPRENALINE AND NORADRENALINE ON AN INDIRECT MEASURE OF SYNOVIAL BLOOD FLOW IN DOGS
to Medical Research Society, London. November, 1969.

ASSESSMENT OF THE RELATIVE VALUE OF CLINICAL METHODS OF EVALUATION AND ISOTOPE TECHNIQUES IN ANTI-INFLAMMATORY TRIALS IN RHEUMATOID ARTHRITIS.
to Heberden Society, Stoke Mandeville. February, 1970.

The work reported in this thesis forms the basis of an invited chapter in "Modern Trends in Rheumatology", Butterworths, (in press) and a proportion of the data reported in this thesis has been published or accepted for publication in the following journals:-

PUBLICATIONS

CLINICAL STUDIES ON INFLAMMATION IN HUMAN KNEE JOINTS: XENON (133 Xe) CLEARANCE CORRELATED WITH CLINICAL ASSESSMENT IN VARIOUS ARTHRITIDES AND STUDIES ON THE EFFECT OF INTRA-ARTICULARLY ADMINISTERED HYDROCORTISONE IN RHEUMATOID ARTHRITIS (1970).
Clin. Sci. 38:123-133.
Dick, W.C., Whaley, K., St. Onge, R.A., Downie, W.W., Boyle, J.A., Nuki, G., Gillespie, F.C. and Buchanan, W.W.

STUDIES ON THE RELATIONSHIP BETWEEN SYNOVIAL PERFUSION AND INERT GAS CLEARANCE RATES IN THE CANINE STIFLE JOINT.
Ann. Rheum. Dis. (in press)
Dick, W.C., St. Onge, R.A., Krasner, N., Unsworth, J., Whaley, K. and Buchanan, W.W.

SOME ASPECTS IN THE QUANTITATION OF INFLAMMATION IN JOINTS OF PATIENTS SUFFERING FROM RHEUMATOID ARTHRITIS.
Arch. Phys. Med. (in press)
Buchanan, W.W., Nuki, G., Whaley, K., Deodhar, S.D. and Dick, W.C.

RADIOACTIVE XENON (133 Xe) DISAPPEARANCE RATES FROM THE SYNOVIAL CAVITY OF THE HUMAN KNEE JOINT IN NORMAL AND ARTHRITIC SUBJECTS (1968)
Ann. Rheum. Dis. 27:163-166.
St. Onge, R.A., Dick, W.C., Bell, G. and Boyle J.A.

MEASUREMENT OF JOINT INFLAMMATION: A RADIOISOTOPIC METHOD (1970)
Ann. Rheum. Dis. 29:135-137
Dick, W.C., Neufeld, R.R., Prentice, A.G., Woodburn, A., Whaley, K., Nuki, G. and Buchanan, W.W.

THE SYNOVIAL PERFUSION OF CLINICALLY NORMAL KNEE JOINTS IN PATIENTS WITH RHEUMATOID ARTHRITIS
Ann. Rheum. Dis. (in press)
Porter, B.B., Nuki, G., Buchanan, W.W. and Dick, W.C.

PREFACE

The clinical work upon which this thesis is based was carried out over the past four years at the Centre for Rheumatic Diseases and the University Department of Medicine, Royal Infirmary, Glasgow. The experimental animal work was performed at the Wellcome Surgical Research Institute, Glasgow. I am particularly grateful to Professor E.M. McGirr and Dr. W.W. Buchanan for their help, encouragement and advice. I would also like to express my gratitude to the following:- Dr. F.C. Gillespie of the Regional Department of Physics and Bio-engineering for instructing me initially in the general principles of the physical and radioisotopic methods and for helpful advice throughout; Dr. A.M. Harper for criticism and advice in the animal experiments and for extending the facilities of the Wellcome Surgical Research Institute; to my colleagues Dr. J.A. Boyle, Dr. K.Whaley and Dr. G. Nuki for helpful criticism; to Mr. J.G. Littlejohn of the Wellcome Surgical Research Institute for invaluable technical assistance in the animal experiments; to Dr. M. Grayson, Merck, Sharp and Dohme Ltd., for obtaining computer facilities for data

analysis; to Mr. Paul Kent for assistance in the preparation of the figures and tables and to Mrs. Cathleen Sweeney for typing the thesis. I acknowledge my indebtedness to the librarians of the Royal College of Physicians, Glasgow and I would also like to thank the patients attending the Centre for Rheumatic Diseases and the medical students who so generously co-operated in these studies.

To facilitate the reading of the thesis, introductory paragraphs to the sections of each chapter are printed in yellow and separate summaries are presented in blue. The references are arranged in alphabetical order.

SYMPATHETIC CONTROL OF SYNOVIAL BLOOD VESSELS
to Heberden Society and Ligue Francais Contre Rheumatisme, Paris. May, 1970.

RECEPTOR CONTROL OF THE SYNOVIAL MICROCIRCULATION: MONITORING BY AN INERT GAS CLEARANCE TECHNIQUE
to International Symposium on Venous Disorders, Sardinia. October, 1970. (by invitation - proceedings to be published).

C O N T E N T S

CHAPTER 7 (contd.) Page

INTRODUCTION

One of the outstanding problems facing the rheumatologist is that of quantitatively measuring change in disease activity. The methods which are currently employed generally require to be assessed qualitatively by either the patient or the physician and therefore possess both inter- and intra-observer errors. In general, the criteria described by Galen namely, tumor, dolor, rubor and calor remain the basis for modern clinical assessment.

My first approach to the problem of quantitating joint inflammation was to study the disappearance of a radioisotope from the joint cavity. Radioactive xenon (133 Xe) was selected as the most suitable radioisotope for this purpose.. This is an inert lipid soluble small molecular weight gas which possesses a low emission energy and which, unlike ionised radioisotopes such as radioactive sodium, is not actively transported across cell membranes but simply diffuses through tissues in accordance with simple physical laws. Over 95% of circulating radioactive 133 Xe is exhaled in the expired air

reducing recirculation into the monitored area.

In Chapter 2, I describe observations on the clearance rates of 133 Xe from normal and diseased human knee joints. In this chapter the techniques of injection and of counting are described. Since the joint is a complex arrangement of tissues with different microvascular arrangements it was important to determine the tissue subserving isotope clearance. I conducted human studies and animal experiments which are described in this chapter which demonstrate that the route of clearance from the articular cavity is through the venule of the synovial membrane and not through cartilage and bone blood vessels. A method for constant monitoring of femoral flow rate is introduced which has not previous been employed to my knowledge. During these experiments it became apparent that the clearance rate was related to blood flow. The relating constant was the partition coefficient of the isotope for synovial tissue with respect to blood and in vitro experiments were conducted to measure this. In Chapter 2 the validity of expressi of clearance rates in terms of blood flow is critically discussed and normal canine, normal human and inflamed human synovial perfusion rates are presented.

Having established the relationship between clearanc

rates and synovial perfusion, I proceeded to investigate the reproducibility of the technique in normal and diseased joints. The day to day variability, the site and technique of injection, the effect of large injection volumes and of the presence of a knee joint effusion and the influence of the age and sex of the subject were studied and the results are recorded in Chapter 3.

In the course of these studies it became clear that this technique would detect changes in synovial blood flow and accordingly I decided to apply the method to determine the nature of the innervation of the vascular supply to the synovial membrane. My initial studies were conducted in dogs and the results are described in Chapter 4. I have been able to demonstrate functionally by the response of the xenon clearance rate to the local installation of exogenous catecholamines the presence of both α and β adrenergic receptors in synovium. Similarly, it was possible to demonstrate muscarinic receptors employing methacholine and atropine. In this chapter I also describe experiments with histamine, serotonin, atropine and a new alpha-adrenergic blocking agent,

thymoxamine.

I was able, during the course of the animal experiments, to ascertain the dose of the various pharmacological substances which was required to produce effect locally, but not systemically, and this knowledge enabled me to carry out parallel studies in normal and diseased human joints the results of which are reported in Chapter 5. In addition, in this chapter, I describe studies on the effects of heat and of exercise on human synovial vasculature.

Chapter 6 describes studies on the clinical significance of the xenon clearance method with particular reference to early diagnosis of arthritis, to clinical evaluation of anti-inflammatory drugs and to synovectomy.

In an attempt to devise a more sensitive index of change in disease activity than was afforded by the 133 Xe clearance technique, I decided to study the uptake by the joint of intravenously administered radioactive technetium (99 mTc) and in Chapter 7 I describe a new quantitative method for joint scans with this isotope. I also describe a simplified method for determining change in joint inflammation using external directional counting. Employing analysis of variance

and co-variance techniques in a double-blind crossover clinical trial of various anti-inflammatory agents, I have been able to demonstrate that this new simple isotope method compares favourably with established clinical methods. It is possible that this isotopic technique, which has a high degree of reproducibility, may prove to be of value in clinical trials of anti-inflammatory compounds.

Thus far in this thesis the studies described have been dictated by a logical sequence of thought. With the aid of serendipity, however, a further study was conducted which evolved from the observation that the clearance rate of intra-articularly injected charged particles differed from the clearance rate of ^{133}Xe. The results of this study are reported in the concluding section of Chapter 7 in which the presence of an active anion transport mechanism from the joint cavity across the synovial cell to the synovial blood vessels is postulated.

CHAPTER I

HISTORICAL

The subject of this thesis is the application of the clearance rate of radioactive xenon (133 Xe) and the uptake of intravenously administered radioactive technetium (99 mTc) to the study of the physiology, pharmacology and pathology of the diarthrodial joint. The work reported in the thesis began in 1967 and there were no previous reports in the literature relevant to the first and very few relevant to the second of these techniques. In general, therefore, the historical background to individual points is discussed in the relevant sections. Frequent reference is however made to three main topics, namely, the anatomical arrangement of the synovial vasculature, the "pathophysiology" of the joint and the "clearance model". Accordingly, I have presented a historical account of the relevant aspects of these topics in this chapter.

A REVIEW OF THE RELEVANT ASPECTS OF THE ANATOMY, PATHOLOGY AND PATHOPHYSIOLOGY OF THE VASCULAR SUPPLY TO THE SYNOVIAL MEMBRANE OF THE HUMAN KNEE JOINT AND A REVIEW OF THE BASIS OF RADIOISOTOPIC DETERMINATION OF BLOOD FLOW

ANATOMY:

(i) Macroscopic

The arrangement of the vasoular supply to the epiphysis and knee joint structures in the adult is well documented and complex (Scapinelli, 1968). Blood is supplied from the femoral artery, the profunda femoris, the popliteal, the anterior and the posterior tibial arteries by their geniculate branches. These branches are commonly the descending branch of the lateral circumflex femoral, the medial and lateral, superior and inferior, and the middle geniculates, the anterior and posterior recurrent tibial vessels and the circumflex fibular vessels. These vessels combine to form a rich anastomotic network consisting of a superficial and a deep component. From the deep network myriads of arterial twigs conduct blood to the synovial membrane and epiphysis. The venous drainage corresponds to the arterial supply contributing to the popliteal and femoral trunk. It is noteworthy that the anastamoses round the knee has several connections with the cruciate anastamoses which links the popliteal vessels with the iliac vascular system. Thus the synovial membrane should possess an adequate blood supply in all positions

of the joint. Movement therefore should not jeopordise the circulation.

(ii) Microscopic

The articular surface of the bone is covered with hyaline cartilage 2 to 4 mm. in thickness. Light microscopy discloses four layers: calcified cartilage with Haversian systems and scanty blood vessels, the radial zone, the transitional zone and the tangential zone, which borders on the joint cavity. The latter three zones are completely avascular. At its free edges this articular cartilage merges with the synovial membrane This membrane is composed of two distinct layers. Externally there is a layer of dense fibrous tissue into which the ligaments and muscles of the joint are inserted. The blood supply of this is said (Branemark, 1969) to be distinct from that of the internal layer. The internal layer varies in composition in different areas of the joint. Bordering on the joint cavity specialised cells (synoviocytes) of two main types have been described (Barland et al., 1962): Ghadially and Roy 1966): the type 'A' cell possesses numerous lysosomal vacuoles and is probably phagocytic whereas the type 'B' cell possesses a relatively larger amount of endoplasmic reticulum and is thought to be the site of synthesis of the hyaluronate

protein, which, together with a dialysate of plasma, constitutes the synovial fluid of the joint. Many cells are intermediate in type and may perform both functions.

Beneath these lining cells Key (1928) describes three types of connective tissue, areolar, fibrous and adipose. In this area are displayed the synovial blood vessels, approximately 1 to 3 microns beneath the surfac (Norton and Ziff, 1969).

"Circulosus Vasculosus Articuli et Epiphyseosus" (Harris 1933)

Synovial tissue has a complicated system of peripheral vessels which are closely connected with the vasculature of the epiphysis (Davies and Edwards, 1948 ; Kelly, 1968). William Hunter (1743) first described the blood supply to the synovial membrane and called it "circulus articuli vasculosus". A period of confusion followed the suggestion by Heuter (1866) that the synovial capillaries lay naked on the surface until Testut (1880), Hagentorn (1882) and Hammar (1894) demonstrated that this was not so. Davies (1946), however, emphasised the point that the proximity to the surface which confused Heuter was of considerable importance in joint disease. Capillaries and venules can be seen lying immediately beneath the one to three

layers of synovial cells (Norton and Ziff, 1969 : Schumacher, 1969). Policard (1936) remarked upon the variation in abundance of blood supply, noting a paucity of vessels in aponeurotic areas, and he also drew attention to the numerous precapillary arterioles in the more profusely endowed areas. Davies and Edwards (1948) also described regional variations in vasculature. They note two or three vascular systems in areolar areas which run parallel to the synovial surface and which freely intercommunicate. The most superficial plexus is composed of arterioles and venules which give off capillary loops immediately under the synovial lining. A proportionately reduced supply is noted in areas possessing fibrous connective tissue. Several investigators have noted the presence of arterio-venous shunts (Scapinelli, 1958 : Bucciante, 1960).

The villus vascular system comprises a central arteriole and venule which fails to reach the tip of the villus. In fat pads and at the articular margins the vessels break up in a tree-like fashion with no capillary loop system. The ligaments and intra-articular discs are poorly supplied with blood vessels.

The articular margins of the joints, where synovial

tissue blends into avascular cartilage, is a site of vascular anastamoses, where the synovial vessels freely intercommunicate with those of the epiphysis in a series of arcades. The blood supply to the epiphysis was first clearly described by Lexer (1904) who recognised periosteal, diaphyseal, metaphyseal and epiphyseal systems. Harris (1929, 1933) stressed the independence of the diaphyseal and epiphyseal blood supply and coined the term "circulus vasculosus articuli et epiphyseosus". Anseroff (1934) could only demonstrate periosteal, diaphyseal and epiphyseal systems and he also noted that in many cases there was only one femoral diaphyseal nutrient vessel. This vessel nearly always entered in the middle third of the femoral shaft. Laing (1953) also found only one nutrient vessel in many cases. Rogers and Gladstone (1950) investigated the nutrient supply to the distal femoral epiphysis and demonstrated osteal branches of genicular vessels entering supracondylar, condylar and intercondylar groups of foramina. Much of the venous drainage of bone would appear to leave at the bone ends (Steinbach et al., 1957 ; Morgan, 1959 and Cuthbertson et al., 1965). In the adult, as opposed to the child there is no metaphyseal plate and

the diaphyseal and epiphyseal systems communicate.

Thus it would appear that the blood supply to the synovial membrane, epiphyseal bone and intra-articular structure forms a unified system distinct from the fibrous capsule and extra-articular structures. The numerous sources of supply and their voluminous anastamoses should ensure an adequate synovial blood flow in all positions of the joint. The venous system follows the arterial supply.

SUMMARY

The macroscopic and microscopic anatomy of the vascular supply of the synovial tissue and related structures is complex and many alternative sources of supply are available through the knee joint and the cruciate anastamoses. The peripheral vasculature of the synovium itself varies in amount and arrangement according to the type of supporting connective tissue and numerous anastamotic connections are present between the epiphyseal and synovial blood vessels. No single vessel which supplies or drains synovial tissue alone is accessible.

THE PATHOPHYSIOLOGY OF THE SYNOVIAL MEMBRANE

The pathophysiology of the synovial membrane has been studied in the past by many workers with conflicting results. One recurrent difficulty has been the lack of availability of reproducible quantitative techniques.

That the mechanism of removal of colloidal and particulate carbon was not mediated by blood flow was demonstrated by Key (1926). Working with acidic dyes Engel (1940, 1941) and Engel and Forrai (1943) showed that synovial permeability was selective. They also demonstrated reduced blood to synovial fluid filtration after sympathectomy and they recorded the unexpected finding that synovial permeability was reduced in inflammation. This was not confirmed by later workers.

Synovial permeability or "leakage" has been studied by several investigators. Intravenously injected bacteria (Lewis and Cluff, 1956 ; Bauer, Ropes and Waine, 1940 ; Shaffer and Bennett, 1939), colloidal iron and trypan blue (Kling, 1938) and egg or horse serum protein (Bennett and Schaffer, 1939) have all been detected promptly in the joint cavity. The concentration of protein was shown to be higher in the

joint cavity than in either aquaeous humour or cerebrospinal fluid. Electrolytes and glucose enter the synovial fluid freely (Bauer et al., 1940) and small proteins enter more readily than do the larger globulins (Hamerman and Barland, 1966 ; Schur and Sandson, 1963). On the other hand, employing immunofluorescent techniques, the quantity of globulin in synovial membrane does not appear to be elevated (Rodman et al., 1967). In a preliminary study, Schumacher (1969) has presented evidence which suggests that joint movement may increase synovial leakage. The basis of the phenomenon of synovial leakage is not clearly understood however.

The clearance of intra-articularly administered phenolsulphonphthalein (P.S.P.) and phenol red from the animal knee joint has been studied extensively and in particular the effects of anti-rheumatic drugs have been assessed on this model. Seifter and Baeder (1954) and Seifter, Baeder and Begany (1949) showed reduction of permeability by corticosteroids and increase in permeability with hyaluronidase and investigated the effects of endocrine ablation on this system. Other workers (Paul, Hodges, Knouse and Wright, 1952 ; Hidalgo McClure, Henderson, Whitehead and Smyth, 1952) were

unable to reproduce this effect of corticosteroids on synovial permeability. Bertolani, Lorenzini and Bonati (1951), Bianchi (1953) and Sharp (1963) demonstrated the effect of drugs on the phenylsulphonylphthalein clearance and Bianchi (1953) defined the limitations of this method of study. He noted that it depended upon renal function and the permeability of peri-articular connective tissue. Nakamura, Asai, Sonozaki and Nagano (1967) investigated the rate of phenylsulphonylphthalein clearance from human knee joints. They demonstrated that the clearance rate was influenced by physical activity and by local inflammation and that it could be decreased by external heating applied to the skin over the knee and by intra-articular corticosteroid therapy. Branemark et al., (1963a,1966) have studied intra-articular and skin temperature. They observed that the normal joint's response to heat was a rise in intra-articular temperature and the reverse was true when a cold pack was applied over the patellar region. This they termed a "positive reaction". The converse of this situation obtained in rheumatoid joints, a "negative reaction". This observation has not been

confirmed by other workers (Cosh, 1970). Hollander et al., (1949a and b; 1951 : Horvath and Hollander 1949) have studied the use of skin and intra-articular temperature in the assessment of joint inflammation. They showed a correlation with disease activity in rheumatoid joints and alteration of temperature with vasereactive substances and heat applied to the skin over the joint. They also demonstrated a parallel drop in joint temperature, the synovial fluid white cell count and the clinical status following corticosteroid therapy. They interpret their results to indicate alteration in tissue metabolism in addition to circulatory changes. These results were confirmed by Gualtieri and Lanzi (1964).

More recently infra-red energy emmission from the joints has been utilised to construct two dimensional thermograms (Boas, 1964 : Haberman, Ehrlich and Levenson, 1968 : Ring and Cosh, 1968) which reflect blood flow, inflammation and local tissue metabolism. Anecdotal evidence of correlations with disease severity and with therapeutic effects have been reported in preliminary studies. Calorimetric methods (Stewart, 1911 : Hollander, et al. 1949a and b : 1951) have

obvious limitations as measures of blood flow. As Greenfield, Shepherd and Wheelan (1951) have pointed out, it is impossible to transform heat changes into units of blood flow without knowing the temperature of the blood entering and leaving the relevant segment.

Venous occlusion plethysmography was employed to study the normal human knee joint by Barcroft, Bonnar and Edholm (1947) and by Bonney, Hughes and Janus (1952 The principle of the method was introduced by Brodie an Russell (1905). Despite supplementary techniques including adrenaline iontophoresis and "rapid cooling", Bonney et al. were unable to separate synovial blood flow from blood flow through other superficial and deep knee segment structures. It appears unlikely that any modification of plethysmography will be able to do this, considering the complexity of the anatomy of the vascular supply to the synovium previously described (p. 7).

In the past few years, external counting of gamma emmitting radioisotopes has been employed to investigate the pathophysiology of the joint. Several radioisotopes, including radioactive sodium (22_{Na} or 24_{Na})

(Jacox, Johnson and Koontz, 1952 : Harris and Millard, 1956 : Harris, Millard and Banerjee, 1958 : Davison and Wisham, 1958 : Scholer, Lee and Polley, 1959 : Laing and Kim, 1960 : Harris, 1961 : Wisham and Dworecl 1966), radioactive iodine (^{131}I) (Verhaege and Lebeurr 1954 : Laing and Kim, 1960 :Doering and Miehlke, 1961 Tesarek and Kolar, 1964 : Scoccianti, 1966 : Hernborg, 1968 and 1969), radioactive gold (Verhaege and Lebeurr 1954) and radioiodinated serum proteins (Ahlstrom, Gedda and Hedberg, 1956 : Kuipers, Francke and Robert, 1956 : Rodnan and McLachlan, 1960) have been injected intra-articularly and their subsequent clearai studied by external directional counting over the joint

Following intra-articular injection, the count rate over the joint can be expressed as the sum of exponential components. For the first 40 minutes after injection clearance of all these substances is commonly monoexponential ($Q = Qoe^{-kt}$) and when the count rate is plotted on semilogarithmic graph paper against time a straight line is obtained (Scholer, Lee and Polley, 1959 : Hernborg, 1968 and 1969). From this line the biological half life ($T\frac{1}{2}$ value, mins of the isotope within the joint can be calculated,

this being simply the time taken for the original count rate to halve. Some workers (Harris and Millard, 1956: Harris, Millard and Banerjee, 1958 : Davison and Wisham, 1958 : Harris, 1961) employ the slope of the line (k) which can be expressed by the relationship:

$$k = \frac{\log_e C1 - \log_e C2}{0.4343\ (t_1 - t_2)}$$ where C1 = concentration at t_1, C2 = concentration at t_2

$$= \frac{0.693}{T\frac{1}{2}}$$

In some studies (Harris, Millard and Banerjee, 1958) an extremely fast clearance rate has been noted for a few minutes after injection. In these studies two lines could be fitted to the semilogarithmic plot of the results and these are therefore biexponential ($Q = Qo^{e^{-k_1 t_1}} + Qo^{e^{-k_2 t_2}}$). The second slower component is of the same order of magnitude as is the single exponential usually found. The early transient fast component has also been found in clearance studies in other tissues (Larsen, Lassen and Quaade, 1966 : Sjersen 1967) and there is some evidence to suggest that it is an injection artefact (Sjersen, 1967). This question is examined in greater detail in a later part of this thesis (p. 96).

The clinical results of those clearance studies are remarkably uniform considering the widely different

chemical and physical properties of the tracer substances employed. Clearance from inflamed joints is consistently faster than from normal joints (Ahlstrom, Gedda and Hedberg, 1956 ; Harris, Millard and Banerjee, 1958 ; Davison and Wisham, 1958 ; Scoccianti, 1966 and Hernborg, 1968). In the reports in which two joints were studied the one which was more severely involved clinically exhibited the faster clearance rate (Harris, Millard and Banerjee, 1958 ; Hernborg, 1968). The clearance rate can be increased by the external application of heat (Harris, 1961) and by an intra-arterial injection of "Priscoline", an adrenolytic and sympatholytic agent (Davison and Wisham, 1958). When an inflamed joint, possessing a fast clearance rate is treated with intra-articular corticosteroid therapy, the clearance rate is reduced (Harris, Millard and Banerjee, 1958 ; Scholer, Lee and Polley, 1959 ; Scoccianti, 1966). In contradiction to the fast clearance rates noted in inflammation is the work by Hernborg (1969). He showed that the clearance rate of $Na^{131}I$ from the osteoarthritic femoral head was reduced when compared with normal joints although the reverse was true in the knee (Hernborg, 1968). Venous engorgement of the femoral head has been demonstrated in

osteoarthritis (Phillips, 1966) and this may explain these low clearance rates.

Fast clearance rates have been recorded in inflammatory arthritides (Harris, Millard and Banerjee, 1958 ; Scholer, Lee and Polley, 1959 ; Scoccianti, 1966) and also in osteoarthritis (Davison and Wisham, 1958 ; Scoccianti, 1966 ; Hernborg, 1968 and 1969). The finding of increased clearance rates in osteoarthritis is of particular interest since this condition is commonly considered to be primarily a degenerative process. This question is examined in greater detail in a later part of this thesis (page 223).

In another approach to the study of joint pathophysiology by gamma emitting radioisotopes, the radioisotope is injected intravenously and a photoscan display system is employed to determine the local accumulation of radioactivity. The detector head moves across the joint in a scanning fashion and counts are recorded as dots. A map of varying concentrations of radioactivity is assembled, the number of dots in any region being proportional to its concentration of radioactivity. Recording may be accomplished in colour form for clarity. Several gamma emitting radioisotopes have been employed in scanning studies of human joints. These include radioactive calcium (47_{Ca})

(Danielsson, Dymling and Heripret, 1964 : Bauer, 1968), radioactive strontium (85_{Sr}) (Bauer and Scoccianti, 1961 : Danielseon, Dymling and Heripret, 1964 : Fellander and Lindberg, 1966 : Holopainen and Rekonen, 1966 : Kettunen and Rekonen, 1968 : Rekonen and Holopainen, 1968 : Lovgren, 1969 : Bauer and Smith, 1969), radioiodinated human serum albumin (Weiss, Maxfield, Murison and Hidalgo, 1966 : Stasser and Thrift, 1968), radioiodinated human plasma fibrinogen (Hashimoto, Okado, Satah and Shiokawa, 1969) radioiodinated rabbit antibody to human fibrinogen (Jacox, Spar, Farrer and Rubin, 1969) and radioactive technetium (99_{mTc}) (Weiss, Maxfield, Murison and Hidalgo, 1966 : Alarcon-Segovia et al., 1967a and b : Green and Hays, 1969 : McCarty, 1970a and b).

In all of these studies uptake has been higher over inflamed than over normal joints (Holopainen and Rekonen, 1966 : Weiss, Maxfield, Murison and Hidalgo, 1966 : Bauer, 1968 : Green and Hays, 1969 : Hashimoto, Okado, Sata and Shiokawa, 1969 : Jacox, Spar, Farrer and Rubin, 1969 : McCarty, 1970). This finding has been consistently demonstrated regardless of the individual pathological diagnosis of

the inflamed joint thus demonstrating the non-specifity of the technique. The studies with radioactive calcium and with radioactive strontium have been of particular value in the study of bone disease. Radioactive strontium scans have been shown to be positive prior to the appearance of radiological sclerosis in the sacoiliac joints (Lovgren, 1969).

Thus the study of joint pathophysiology has become increasingly sophisticated, particularly within the last few years and the methods of study are becoming more precise. Most of the work with radioisotopes is in the early stages and much work remains to be carried out in this field. No method presently available is applicable to the measurement of synovial blood flow and no attempt has yet been made to express the results obtained by photoscan systems quantitatively.

SUMMARY

The various methods of study which have been applied to the pathophysiology of the joint are described. These include temperature methods, plethysmography and the removal or accumulation of various radioactive or non-radioactive tracer substances. No method has proved entirely satisfactory for all purposes and, in particular, no method is available for the measurement of synovial blood flow.

REVIEW OF THE DEVELOPMENT OF "CLEARANCE" STUDIES AS A MEASURE OF BLOOD FLOW

For many years a variety of indicator substances have been employed in the measurement of blood flow. The first substances to be used thus were dyes which could be measured spectrophotometrically, but hydrogen, ascorbic acid and even heat or "coolth" have been employed. The development of radioactive tracer substances is merely an extension of the same technique in principle, but in practice the availability of these substances has greatly extended the applicability to blood flow measurement. This is principally due to their range and versatility. This subject has been reviewed by McGirr (1952) and more recently by Veall (1968).

The basis of the indicator method of measuring blood flow lies in the principle of conservation of material. Kety (1960) delineated the basic equation:

$$Qa = Qi + Qe + Qv + Qm \quad \text{---------- (1)}$$

where

Qa = amount of indicator entering the tissue in a given period

Qi = amount retained in the tissue

Qv = amount leaving the tissue in venous blood

Qe = amount excreted by other routes

and Qm = amount metabolised by the tissue,

However, over a short time interval where

F = flow in ml./min.

$$Qa(t) = C_{(a)}t \cdot F \cdot \Delta t \quad \text{---------- (2)}$$

(i.e. arterial inflow = arterial concentration x blood flow rate) and

$$Qv(t) = Cv(t) \cdot F \cdot \Delta t \quad \text{---------- (3)}$$

(i.e. venous outflow = venous concentration x blood flow rate), provided that the tracer is uniformly distributed. The relationship to blood flow measurement thus becomes obvious. In the Fick (1870) principle, the classical technique for the measurement of cardiac output, Qi is assumed to be negligible and the inert tracer is administered by constant infusion so that Ca remains uniform. After equilibration Cv becomes constant and Qe is measured over time Δt.

Substituting (2) and (3) in (1)

$$F = \frac{Qe}{(Ca-Cv)\Delta t} \quad \text{---------- (4)}$$

where F = flow rate through the organ.

This technique measures total blood flow including shunts.

The Fick principle was modified by Kety and Schmidt (1948) employing nitrous oxide as an inert

tracer to measure cerebral blood flow. Qe and Qm are zero, since the tracer is neither metabolised nor excreted and Qi (tissue uptake) is inserted in (4). Qi cannot be measured directly so it is assumed that Ci (tissue concentration) is proportional to venous concentration after blood/tissue equilibrium is reached. The relating constant is the partition coefficient (λ) which is the ratio, at equilibrium, between the solubility of the tracer in tissue, to its solubility in blood. For this to be valid the tracer must be freely diffusible in the relevant tissue. Since the period of measurement should cover the time it takes for the tissue to reach saturation, the venous concentration will not be constant. Thus it is necessary to integrate the a-v difference over the entire period of measurement. Thus the formula for blood flow becomes:

$$F(\text{ml/ml/min.}) = \frac{\lambda Cv}{\int_0^\infty (Ca-Cv)\,dt} \quad \text{-------} \quad (5)$$

The introduction of radioisotopes which can be injected into a tissue and monitored by external counting has allowed modifications of these methods (Kety, 1949). Provided that a freely diffusible tracer is introduced into a single homogeneous tissue then:

$$C(t) = C(o)e^{-kt} \quad \text{----------} \quad (6)$$

where Co = initial count rate

Ct = count rate at time t

and k = clearance constant.

This equation is merely that which describes any monoexponential function.

Kety tested this theoretical model injecting 5 μCi of ^{24}Na into muscle. The count rate in each case, when plotted semilogarithmically against time, yielded a straight line, thus validating the theoretical model. The slope of the graph obtained allowed k, the clearance constant, to be calculated since:

$$k = \frac{\log C1 - \log C2}{0.4343(T2-T1)}$$

where C_1 and C_2 are counts per minute at T_1 and T_2 respectively and the time interval (T_2 - T_1) was of sufficient duration to allow a reliable average slope to be drawn on the graph. Minor modifications (Lassen et al., 1964) have been made to be mathematics but the method remains substantially unchanged. One modification, which facilitates the calculation of the results, is the use of the "half-time" ($T_{\frac{1}{2}}$ value - minutes) which can be readily obtained from the graph. The clearance constant (k) is related to the half time

thus

$$k = \frac{0.693}{T_{\frac{1}{2}}} \quad \text{---------- (7)}$$

(Wisham, Yalow and Freund, 1951). A further modification of more fundamental importance was the conversion of the clearance constant to units of blood flow (Thorburn et al., 1963 : Lassen, 1964).

Provided that the relevant tissue is homogeneous, that the tracer isotope is freely diffusible and that re-circulation is either minimal or accountable, the clearance constant is related to the 'perfusion' (capillary blood flow) of the tissue by the expression

$P = k\lambda$ (Veall, 1968) -------- (8)

where P = perfusion in ml. per ml. per minute.

k = the clearance constant

and λ = the tissue blood partition coefficient.

This expression can be re-written as:

$$P = \frac{69.3}{T_{\frac{1}{2}}} \cdot \lambda \text{ ml/100 ml/min.} \quad \text{------- (9)}$$

Thus capillary blood flow can be determined in a tissue by measuring only the rate of change of tissue activity, in arbitrary units, and the partition coefficient. The terms "perfusion", "nutritive perfusion" and "capillary blood flow" are employed synonymously to indicate that flow through non-nutritive

shunts will not be detected by this method (Friedman, 1968).

The most recent modification of the measurement of blood flow by this interstitial method is based on work by Ingvar and Lassen (1962) and by Hoedt-Rasmussen et al. (1966). Two exponential functions with very different k values are obtained in interstitial isotopic studies in brain. These workers have analysed this situation theoretically and concluded that the different k values may be related to white and grey matter respectively. Thus, provided there are two adjoining tissues with markedly different perfusion rates, blood flow values can be derived for each, employing this technique. This has now been applied to skin (Sjersen, 1967) to prostatic (Andersson et al., 1967) and to renal (Thorburn et al., 1963) blood flow.

SUMMARY

The gradual development of blood flow measurement by indicator techniques is delineated. With each modification of the standard equation, which is based on the principle of conservation of material, the applicability and range of the techniques have been extended. Of particular relevance to this thesis, tissue perfusion can be derived from the rate change of activity of an injected radioisotope and from a knowledge of the partition coefficient, under suitable circumstances.

CHAPTER II

In this chapter the physical and biological attributes of radioactive xenon (133 Xe) as a capillary flow indicator are outlined and the technique employed in the 133 Xe studies is introduced.

In preliminary animal experiments the route of clearance of intra-articularly injected 133 Xe is shown to be blood flow dependent. The route of clearance is delineated more precisely in a further series of animal experiments, approximately 90% of the isotope leaving the joint being recoverable in the femoral vein. Neither the biological system employed nor the technique for constant monitoring of femoral venous blood flow have previously been described to the best of my knowledge.

Synovial perfusion in the canine joint is derived from the clearance rate of intra-articularly injected 133 Xe and the in vitro measurement of the diffusion rate and the partition coefficient of 133 Xe for synovium with respect to blood. The problems of the clearance model are studied with

particular reference to the use of this technique in the present context and the validity of the expression of the results in terms of perfusion is discussed.

Preliminary studies are described, the results of which demonstrate that the clearance rate of 133 Xe from the diseased joint greatly exceeds the clearance rate from the normal joint.

It is more difficult to demonstrate the route of clearance of the isotope from the human than from the animal joint. However, the dependence of clearance on blood flow is demonstrated by simple tourniquet studies so designed as to exclude the possibility of appreciable clearance through osseous nutrient vessels and the qualitative identification of 133 Xe in femoral venous blood is described. The results of a study in which the isotope was introduced under direct vision into synovial tissue are also described and it is proposed that there is sufficient theoretical and factual evidence to support the conclusion that the route of clearance from the human joint is similar to that which obtains in greyhounds.

The clearance model is then applied to the results of the human studies and synovial perfusion is again derived from the clearance rate of the isotope and the diffusion rate and the partition coefficient of 133 Xe for human synovial tissue with respect to blood. Synovial perfusion in the rheumatoid joint was found to be of the order of five times the value obtained in the normal.

RADIOACTIVE XENON

Physical Characteristics

The isotope employed in the studies which are the subject of the first part of this thesis was radioactive xenon (^{133}Xe).

The radioactive gas Xenon (^{133}Xe) has been employed previously in the measurement of blood flow through several organs (Bain and Harper, 1968). Xenon is a chemically inert gas, three times as soluble as oxygen and one seventh as soluble as carbon dioxide at body temperature. The isotope $^{133}_{54}Xe$ decays to stable cesium with a half life of 5.27 days emitting a negative beta particle of maximum energy 0.347 MeV. which is absorbed by less than 1 mm. of tissue. The nucleus formed by beta decay may reach a stable state either by emitting a gamma ray (0.081 MeV) or by the process of internal conversion with the emission of a K, X-ray (0.03 MeV) (Ball et al., 1962). These energies are sufficiently low to allow shielding with only 3/16th inch of lead. The low energy and short half life result in minimal radiation hazard to both patient and investigator.

Dosimetry Estimates

Under the circumstances of the isotope studies performed in the following human studies with 133 Xe, the mean energy deposited locally is less than 0.15 MeV. Assuming 133 Xe to

be uniformly distributed throughout the synovial membrane and assuming that the membrane is of the order of 1cm. thick in rheumatoid arthritis, then the dose rate to the membrane will be 10 millirad/μCi of 133 Xe. The doses employed were of the order of 10μCi (i.e. 100 millirads) and the effective biological half life of the isotope proved to be only of the order of 45 minutes, leaving a wide safety margin (Harper et al., 1964), well beneath the radiation dose delivered during routine diagnostic radiology. The radiation dose delivered during a standard radiographic examination of chest is of the order of 200 millirads and comparative figures for an intravenous pyelogram and for a screening examination of stomach without image intensification are 2.25 rads and 90 rads respectively. At the surface of bone the dose rates would be, at the very most, 50% of the dose to the synovial membrane. The partial pressure of 133 Xe will be maximal at the surface of the membrane and will descend towards the bone because of the intervening synovial blood vessels. One millimetre below the surface of bone or cartilage the dose rates will be less than 10% of the above value. Whole body and gonadal doses are negligible.

Biological Characteristics

Xenon is thus a safe isotope to employ in human studies.

Furthermore, its high solubility in air results in over 95% of the circulating isotope being discharged into the alveoli in one circulation through the lungs (Ball et al., 1962). Consequently only negligible amounts are available to re-circulate into the joint and counts recorded over the joint do not require to be corrected for this. This facilitates the calculation of perfusion (vide infra page 55). At body temperatures, 133 Xe is both chemically and biologically inert, which also facilitates the calculation of blood flow since no metabolic "shifts" or exchanges need be considered as are necessary with, for example, radioactive sodium (Gosselin, 1966). Xenon (133 Xe), being lipid soluble and of small molecular weight, is freely diffusible in biological media and is not limited by membrane permeability. Its distribution in the body is therefore subject only to physical laws of gas transfer.

ANIMAL EXPERIMENTS

Feasability Studies

The following preliminary animal experiments were undertaken to study the clearance of intra-articularly administered 133 Xe.

Approximately 100 µCi of 133 Xe dissolved in 1 ml. of 0.9% sterile sodium chloride were injected into the medial aspect of the stifle joint of an anaesthetised adult greyhound dog. A lightly collimated directional counter was then placed six inches from the lateral aspect of the joint. The directional counter (Ekco N599D) incorporated a 1" x 1½" thallium activated sodium iodide crystal and photomultiplier, and was connected through an Ekco ratemeter (Ekco 1750) to a double channel chart recorder (B24) which recorded the count rate per minute graphically at a paper speed of 20 mm. per minute. Monitoring began two minutes after injection and was continued for 45 minutes. The graph was sampled at one minute intervals and the absolute count rate was plotted on to semilogarithmic graph paper as a function of time. Straight lines were obtained in this way from which the halving time ($T\frac{1}{2}$ value, mins.) could be easily determined. The count rate was noted to fall exponentially following injection of the isotope,

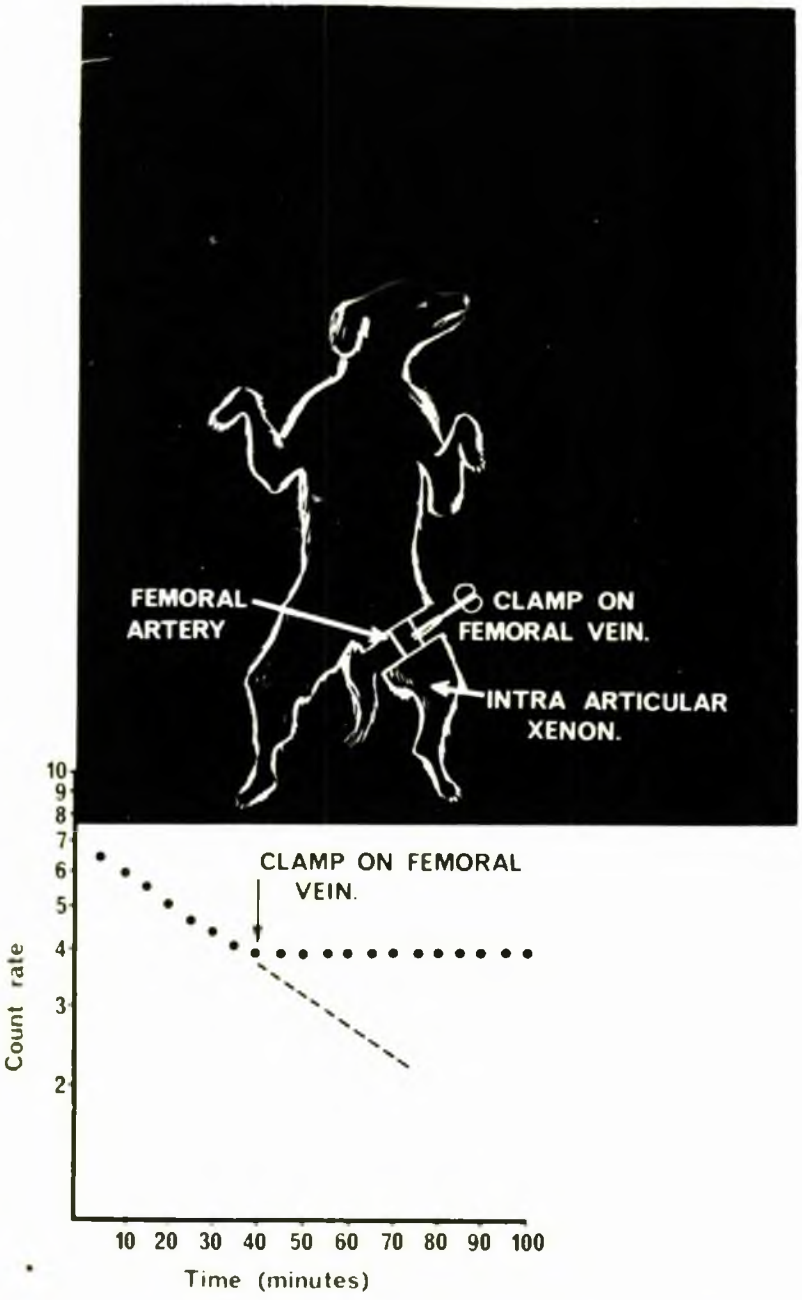


Fig. I

Legend: The leg was severed at the level of the femoral trochanter leaving only the femoral artery and vein connecting the hind limb to the trunk. The count rate following intra-articular injection of 133 Xe was monitored by external directional counting and plotted semilogarithmically against time. When the femoral vein was clamped no further appreciable clearance occurred.

demonstrating that the isotope was leaving the joint. This experiment was repeated on a further two occasions employing different dogs and the $T\frac{1}{2}$ value (mins.) was calculated in each case. The same experimental method was employed in a fourth dog. However, on this occasion, prior to the injection of the isotope, the relevant hind limb was disarticulated at the hip and all the tissues were severed, with haemostatic control, leaving only the femoral artery and vein connecting the hind limb to the body (Fig. 1). The isotope was injected and counting performed in the usual manner. After 15 minutes, when the rate of clearance was established, the femoral vein was clamped. After a further 15 minutes inguinal and para-aortic lymph nodes were removed and placed in a well type scintillation counter.

Results

The $T\frac{1}{2}$ values calculated in each study were 65, 73, 58 and 90 minutes from the four graphs. Figure 1 shows the result obtained in the fourth experiment when the femoral vein was clamped. It can be seen that the clearance, which had been proceeding at a constant rate, stopped abruptly at that point and no further appreciable loss of radioactivity occurred. When the lymph glands

were placed in a well type scintillation counter, no activity was detectable.

Discussion

Total cessation of clearance of the radioisotope when the circulation ceased implies that, under the conditions of this experiment, the route of clearance of intra-articularly administered 133 Xe was blood flow dependent. Failure to detect activity in the regional lymph nodes suggests that no significant radioactivity was lost to the lymphatic system in this experiment. The latter point is less securely based than the former since it may be argued that, whereas alternative vascular routes were ligated and thus closed, this was not true of the alternative lymphatic channels. A point against this objection is that at least some activity might have been expected to clear through the main lymphatic channels, although from the design of this experiment it is not possible to answer this objection fully. Further animal studies were therefore undertaken.

MEASUREMENT OF SYNOVIAL PERFUSION IN DOGS

Introduction:

Most methods for the determination of blood flow measure total flow to or from an organ or tissue. It is more difficult to derive a measure of flow through the capillary bed which reflects effective tissue perfusion (Friedman, 1968). The only acceptable methods of assessing flow through any particular capillary bed are to observe it directly or to employ some flow dependent process which takes place only in the microcirculation (Gosselin, 1966).

In the present study, an attempt was made to validate the use of the isotopic clearance method as a measure of synovial tissue perfusion in animals. Clearance of a small molecular weight substance by a tissue is mediated by several variables including the chemical and physical properties of the substances (Kety, 1951 : Jacox et al., 1952 : Laing and Kim, 1960 : Hollander et al., 1961 : Perl et al., 1965) and the structure and perfusion of the tissue (Kety, 1949 : Pappenheimer et al., 1951 : Pappenheimer, 1953 : Renkin, 1955 : Barlow et al., 1961 : Perl, 1962 : Landis and Pappenheimer, 1963 : Thorburn et al., 1963 : Perl and Chinard, 1968). When an inert lipid soluble gas

is employed particularly relevant measurable variables include the diffusion rate of the gas in the tissue (Krogh, 1919 : Kety, 1951 : Renkin, 1955 : Perl et al., 1965 : Unsworth and Gillespie, 1969), the partial pressures developed (Gillespie, 1968) and the solubility of the gas in the tissue with respect to its solubility in blood (Conn, 1961). Many workers (Thorburn et al., 1963 : Lassen et al., 1964 : Larsen et al., 1966 and Sjersen, 1967) have employed the clearance method to derive tissue perfusion. In each case those variables which affect clearance other than perfusion are either minimised or are incorporated into the method. In the present study three factors in particular are quantitated namely, the route of clearance of the tracer from its site of injection, the diffusion rate of the tracer and the partition coefficient of the tracer in the relevant tissue.

MATERIALS AND METHODS

The in vivo studies were conducted in a surgical theatre in the Wellcome Surgical Research Centre, Glasgow. Studies were performed on the stifle joints of adult greyhound dogs weighing from 25 to 31 kg. Anaesthesia was induced with thiopentone sodium (20 mg./kg.) and a cuffed endotracheal tube was passed into the trachea. Outflow was connected to a Starling respiratory pump and inflow to a Boyle's anaesthetic machine. Anaesthesia was maintained with a 5:3 mixture of nitrous oxide and oxygen and was monitored by periodic determinations of blood gases. A catheter placed in the brachial artery was connected to a three way mercury manometer which provided continual recordings of blood pressure and pulse rate and a slow intravenous infusion of 0.9% NaCl was continued throughout each experiment.

Monitoring Technique

Exercising care to avoid the presence of air bubbles, one ml. of 133 Xe (approximately 100 μ Ci) was withdrawn from the isotope reservoir into a syringe. Another one ml. syringe was fitted with a $\frac{15}{16}$" guage needle and the needle was carefully inserted into the lateral aspect of the stiffle joint. Confirmation of the siting of the

needle was achieved by the aspiration of synovial fluid. To minimise the effects of injection trauma (page 95), the needle point was left in situ for five minutes. The syringe was then removed and replaced with the syringe containing the isotope solution which was slowly injected into the joint cavity. In no case was the injection volume in excess of 0.5 ml. The empty syringe was then removed and that containing synovial fluid was again fitted to the needle and remained in place throughout the study.

Counting Technique

The count rate was monitored and graphed semilogarithmically in the same manner, employing the same equipment as in the previous experiment (page 39).

ROUTE OF CLEARANCE

Tourniquet Studies

In one study a tourniquet, placed around the thigh, was inflated 15 minutes after injection, on one occasion to mid-pulse pressure and on another to a level greater than the systolic blood pressure. On each occasion the tourniquet remained inflated for 8 minutes and the count rate was monitored for ten minutes following deflation.

A similar protocol was followed in three further studies when a narrow (2") tourniquet was placed immediately

proximal to the stiffle joint and therefore distal to the site of emergence of the femoral nutrient vessels from bone.

Femoral Vein Sampling and Free Flow Determinations of Femoral Flow

Two isovolumetric isotope solutions were withdrawn from the isotope reservoir and the count rate of each was determined. One dose was added to a known volume of 0.9% NaCl in a glass flask. The count rate of the empty syringe was immediately determined and the activity added to the flask was calculated by subtraction. The flask was sealed with all air excluded and the contents thoroughly mixed. Two mls. of the standard were pipetted into a 2 ml. counting vial which was sealed. The count rate was obtained by placing the vial in a well type scintillation counter (Ekco M5400A/1) and background count rate was subtracted. From the count rate, and from a knowledge of the activity delivered into a known volume of 0.9% NaCl, a figure for 100% dose could be easily obtained, in units of counts per ml.

When the dog was in position following induction of anaesthesia, the femoral vein was exposed in the groin. Careful blunt dissection was employed and no major vascular tributaries were severed. A 1/2" diameter plastic T-tube fitted with a three way tap was introduced into the vein

and secured. At this point the dog received 10,000 units of heparin intravenously. Femoral flow was determined by collecting all the blood flowing through the T-tube in 30 seconds. A mean of three readings was taken and the blood was immediately replaced. At the conclusion of the study femoral flow rate was again determined in the same manner.

The isotope solution from the second syringe was injected into the stiffle joint. Immediately following injection the count rate of the empty syringe was determined employing precisely the same counting geometry as had been utilised in counting the other syringes. The activity delivered into the joint cavity could be obtained by subtraction. The detector was then positioned over the joint and counting commenced in the usual manner (page 39). The clearance curve was plotted on to semilogarithmic graph paper. From 2 to 46 minutes after injection femoral vein samples were obtained at two minute intervals. Two syringes were connected to the three way tap. 4 mls. of blood were withdrawn into one and 2 mls. into the other. The 2 mls. volume was removed and pipetted into the standard 2 mls. counting vials which were immediately sealed with all air excluded. The blood withdrawn was isovolumetrically replaced with 0.9% NaCl

and finally the 4 mls. of blood in the opposite syringe was flushed through the T-tube. The counting vials were placed in the well type scintillation counter and the activity, corrected for background, was determined in counts per ml. Multiplying by femoral flow rate, these counts could then be expressed as % of 100% dose per minute and cumulative % per minute could be determined.

To relate these figures to the activity lost from the joint the straight line obtained on semilogarithmic graph paper was produced back to time zero. This figure was designated 100% and the absolute count rate obtained at any time thereafter could be expressed as a % of 100% dose. This could easily be converted to % per minute and could then be compared with the femoral vein activity.

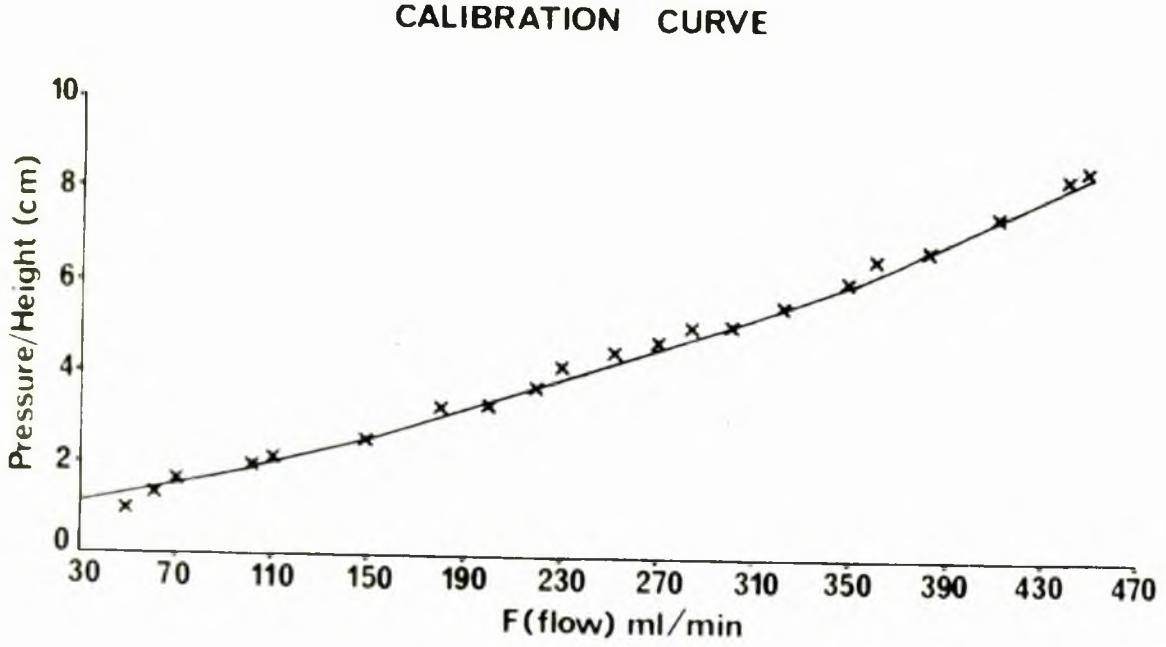


Fig. 2

Legend: Calibration curve for femoral flow rate. The difference in height of the blood columns in the inflow and outflow tracts (difference B-A Fig.3) is plotted on the vertical axis. The femoral flow rate measured by free flow (mean of duplicate readings at flow increments of 20 ml./min. over the range 50 to 400 ml./min.) is plotted on the horizontal axis.

CONSTANT MONITORING OF FEMORAL VEIN ACTIVITY AND FLOW RATE

Twelve inches of $^{1}/_{4}$" brass tubing was shaped into a coil with a single loop of 2" internal diameter. Two inches from each free end a vertical sidearm was inserted and each of these was connected to 18" of graduated plastic tubing. The coil was siliconised before each experiment and was calibrated in vitro using heparinised dog's blood (P.C.V.40) at 37°C. The blood was conducted through the coil from a reservoir placed two feet above the coil and collected in a graduated measuring cylinder from the coil outflow tract. By partially clamping the tubing proximal to the coil the flow rate could be adjusted. The flow rate was measured by collecting all the blood flowing into the graduated cylinder in a known time, the mean of duplicate readings being taken at each flow rate. While free flow was established, the difference in height of the columns of blood in each vertical sidearm of the coil was recorded and these were plotted against the flow rates to derive a flow curve (Fig. 2). Each set of readings shown were determined at flow increments of 20 ml./min. over a range of 50 to 400 ml./min.

The coil, with its polythene tubing extensions, was fitted around a 2" thallium activated sodium iodide crystal and photomultiplier (Ekco P1062) with the inflow and outflow

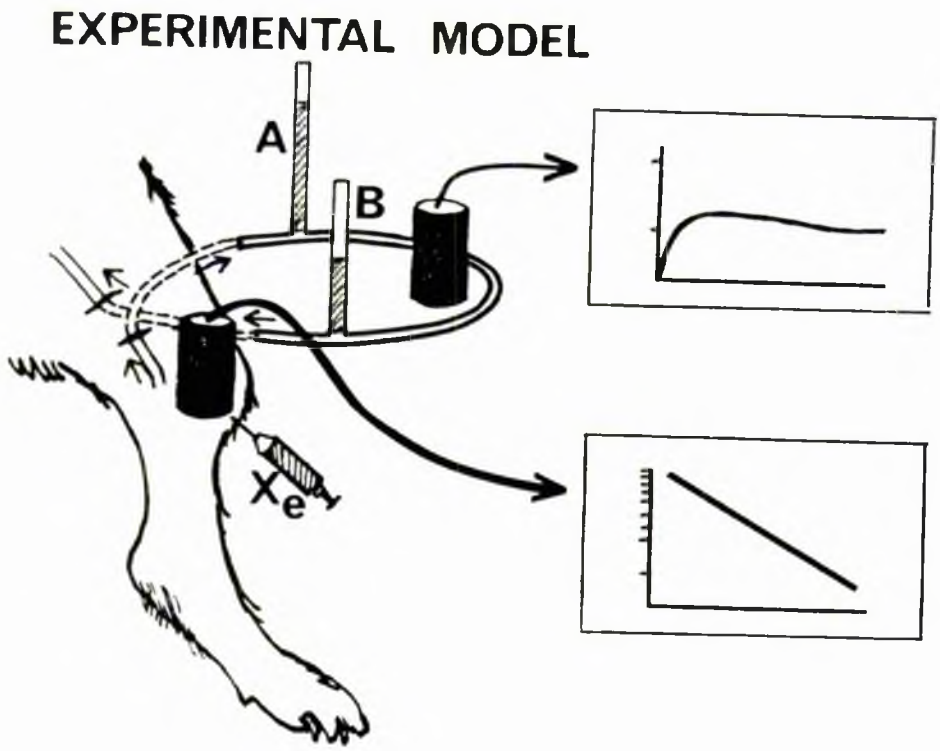


Fig. 3

Legend: The experimental model employed for continuous
recording of femoral vein activity and flow rate throu
the coil and isotope clearance from the joint. Femoral
venous blood was conducted through the siliconised bra
coil which was moulded round a scintillation detector.
A linear plot of femoral venous activity against time
shown in the top graph. A second detector was employ
to monitor activity over the joint and a semilogarithm
plot of activity against time is shown in the lower gr

tracts with their vertical sidearms emerging through apertures in the lead. Pulses were fed from the photomultiplier to a pulse height analyser and rate-meter (Ekco N600B) and recorded on the Rikadenki chart recorder. The coil was filled with standard isotope solution made up as previously described (page 46) and the count rate, less background was determined. This was expressed as 100% dose in the coil. The coil was emptied and washed and the inflow and outflow tracts were carefully inserted and advanced at least 3" proximally and distally into the exposed femoral vein. The arrangement was checked to ensure that blood would flow horizontally and that no acute angles were presented to impede flow.

The femoral flow rate could be derived in vivo by reading the difference in height between outflow and inflow blood columns and from the flow curve and could be monitored continuously (Fig. 3). The absolute coil count rate was also monitored continuously following injection of 133 Xe into the stiffle joint and could be expressed as % of 100% dose by multiplying by contemporaneously obtained femoral flow rate. Comparison with decrement in knee activity was obtained as described previously (page 48). 8 studies were

conducted with the coil in place.

At the conclusion of six experiments lymph glands were removed from the paraaortic and inguinal regions and placed in the well type scintillation counter. At the conclusion of three coil experiments the plastic tubing was removed from the proximal end of the femoral vein. Femoral flow after its passage through the coil was measured by free flow, while the height difference between inflow and outflow blood columns was noted. Thus the in vitro calibration curve could be checked in vivo.

MEASUREMENT OF DIFFUSION AND PARTITION COEFFICIENTS

Specimens for the determination of the diffusion and partition coefficients of 133 Xe in synovial tissue were obtained from the opposite stifle joints of the dogs at the conclusion of the experiments with the coil. The joint was exposed through an elliptical incision and the patello-tibial ligament was divided. The patella was reflected and blocks of tissue 1 to 2 cm. in area and 5 mm. in depth were removed from different areas within each joint. Synovial fluid specimens were removed from the aspirate obtained at the beginning of the experiments.

Diffusion Coefficient

The diffusion coefficient was determined by the method of Unsworth and Gillespie (1969). If one face of a medium of thickness L cm. containing a dissolved gas at uniform partial pressure Po is suddenly exposed to and maintained at zero partial pressure, it can be shown by simple diffusion theory that the partial pressure P(xt) at a point x cms. from the other face after t seconds is given by:-

$$P_{(x,t)} = \sum_{n=o}^{\infty} \frac{4Po}{n\pi} \sin \frac{n\pi}{2} e^{n^2 \beta t} \cos n\alpha x \quad \text{---} \quad (1)$$

where $\beta = \frac{-k\pi^2}{4L^2}$

and $\alpha = \frac{\pi}{2L}$

and $k(cm^2\ sec^{-1})$ is the diffusion coefficient of the gas

in that medium.

For large values of t only the first term is important and the partial pressure is given by:-

$$P_{(xt)} = \frac{4Po}{\pi} e^{\beta t} \cos \alpha x \qquad (2)$$

Thus for large values of t the partial pressure has a cosine profile decreasing exponentially. The halving time is given by:-

$$T\tfrac{1}{2} = \frac{-en\ 0.5}{\beta} = \frac{2.77\ L^2}{k\ \pi^2} \qquad (3)$$

If the gas is a radioactive gamma emitter, the mean partial pressure can be monitored with a collimated scintillation detector. Samples were introduced into a temperature controlled vessel. For the synovial fluid samples a level pool 2 to 3 mm. deep was employed. For synovial tissue, slices of the order of 1 mm. were placed on an impermeable glass base.

133 Xe (approximately 100 μCi) was introduced into the vessel and, after sufficient equilibration time, the space above the samples was flushed continuously with humidified air. A collimated sodium iodide detector monitored the remaining activity in the sample.

From the $T\tfrac{1}{2}$ value of the semilogarithmic plot of the count rate against time and the sample thickness, the diffusion coefficient was determined (Equation 3).

Partition Coefficient

The partition coefficient (λ) of 133 Xe for animal synovial tissue with respect to blood, that is the ratio at equilibrium in vitro of the concentration of 133 Xe (μCi/ml.) in tissue to its concentration in blood (μCi/ml.), was determined in the following manner. Each sample was placed in a solution of 133 Xe in saline in an air free glass test tube. The test tubes were sealed and left at 37°C. for 24 hours to equilibrate. The sample was then removed, freed of excess liquid and at the same time 2 mls. of saline were also removed from the test tube. Each was then placed in a sealed counting tube and their relative activities determined. The concentration of activity was calculated and from these results and from published data (Conn, 1961) the partition coefficient of 133 Xe for synovial tissue with respect to blood was calculated.

CALCULATION OF PERFUSION

In each study clearance from two to forty-five minutes after injection was monoexponential and is given by:-

$$Q = Qoe^{-kt} \qquad (1)$$

This result was predicted theoretically and confirmed experimentally by Kety (1949) studying radioactive sodium clearance from muscle.

From the general form of the Fick equation

$$\frac{dQ}{dt} = P(Ca-Cv) \qquad (2)$$

where $\frac{DQ}{dt}$ = moles removed from the tissue of unit volume v in unit time t.

P = Perfusion (ml./min.)

and Ca , Cv = concentration in pre- and post-capillary blood in moles/ml.

Since recirculation of 133 Xe is negligible (Ca=0) and:

$$\frac{dQ}{dt} = - PCv \qquad (3)$$

There is evidence that equilibration is rapid and that the venous exit condition ($Cv = \frac{Ci}{\lambda}$) holds (Perl, 1962) and $Ci = \frac{Q}{v}$ where Q = number of moles in tissue, therefore by substitution:-

$$\frac{dQ}{dt} = - \frac{PQ}{v\lambda} \qquad (4)$$

Rearranging and Integrating

$$Q = Q_o e^{-(P/\lambda v)t} \qquad (5)$$

and $P = k.\lambda.v.$ (6)

The slope of the semilogarithmic plot (k) =

$$\frac{\log_e c_1 - \log_e c_2}{0.4343\ (t_1 - t_2)}$$

where c_1 and c_2 are counts at t_1 and t_2 respectively

$$= \frac{0.693}{T_{\frac{1}{2}}}$$

therefore:-

$$P = \frac{0.693.\lambda.100}{T_{\frac{1}{2}}} \text{ ml./100ml./min.}$$

$$= \frac{69.3\ .\lambda}{T_{\frac{1}{2}}} \text{ ml./100ml./min.} \qquad (7)$$

TABLE I

$T\frac{1}{2}$ values obtained from the semilogarithmic plots of activity recorded over the joint against time before and after the intervention of tourniquet inflation. The studies in which a narrow tourniquet was employed are depicted by an asterisk.

$T\frac{1}{2}$ Values (mins.)	
Before	After
72	70
74	72
91	90
82	81
64	60
56	58
69*	73*
55*	50*
74*	75*
78*	76*
71*	68*
88*	85*

* Denotes the experiments in which a narrow tourniquet was applied immediately proximal to the joint.

RESULTS

Route of Clearance

In four preliminary studies, clearance commenced within seconds of injection and could be described by a single exponential over the time period 30 secs. to 45 mins. after injection. The half lives ($T\frac{1}{2}$ values, mins.) of the semilogarithmic plots of count rate against time were 65, 60, 68 and 72 minutes.

When a tourniquet was inflated either to mid-pulse or to over-systolic blood pressure clearance ceased, and recommenced at the original rate on release of the tourniquet. The $T\frac{1}{2}$ values before and after inflation of the tourniquet were closely similar. This experiment was repeated on six occasions and the results were found to be consistent (Table 1). Precisely similar results were obtained when the narrow tourniquet was placed immediately above the joint, namely cessation of clearance on inflation of the tourniquet and recommencement at the original rate on release (Table 1).

The results obtained when the percentage of 100% dose leaving the stiffle joint was compared with the percentage of 100% dose recovered either in samples or in the coil experiments are shown in Table 2. Only the figures for 10, 20, 30 and 40 minutes are shown, results

TABLE II

'CUMULATIVE % OF 100% DOSE CLEARED FROM STIFLE JOINT

and

'CUMULATIVE % OF 100% DOSE ACCOUNTED FOR IN FEMORAL VEIN

at

'10, 20, 30 and 40 MINS. AFTER INJECTION

	10 mins.		20 mins.		30 mins.		40 mins.	
	Joint	Fem. vein	Joint	Fem. vein	Joint	Fem. vein	Joint	fem. vein
Dog No.2	11.9	11.3	19.4	16.7	25.0	19.2	30.6	20.4
6	12.5	12.6	20.3	20.7	28.6	29.5	32.2	35.3
13	1.9	1.1	17.3	12.0	23.0	18.3	30.8	24.9
16			33.7	30.1	55.9	52.8	70.6	72.9
18	5.4	7.6	10.7	14.3	18.2	22.3	24.6	30.6
19	4.7	2.4	5.9	5.1	35.1	28.6	61.4	51.7
21	9.0	7.2	15.5	13.9	23.0	20.2	25.0	25.8
22_L	5.1	7.1	16.0	16.5	33.1	25.9		
22_R	12.5	8.7	27.0	22.2	35.4	28.4	48.8	38.8
Mean	7.9	7.2	18.4	16.8	30.8	27.2	40.5	37.5
	92		91		88		93	

% of counts cleared from joint which can be detected in femoral vein.

at other times being entirely in accord with these. At ten minutes after injection the mean cumulative percentage of 100% dose cleared from the joint (7.9) was closely similar to the mean value obtained for femoral venous activity (7.2). Similarly, at 20 mins. the respective values were 18.4 and 16.8; at 30 mins. 30.8 and 27.2 and at 40 mins. 40.5 and 37.5. The percentages of activity cleared from the joint which could be accounted for in the femoral vein at 10, 20, 30 and 40 minutes were 92%, 91%, 88% and 93% respectively.

The results for femoral flow rate when determined by free flow at the beginning of the experiment were 120, 142, and 135 ml. per minute and at the end of the experiment the values were 145, 168 and 146 ml. per minute. A mean of all six values was employed in calculating the results in that experiment. However, it was clear that the femoral flow rate was not constant and accordingly the method of continuous recording of flow rate was developed. In calculating the results in the coil experiments the individual flow rates obtained contemporaneously with the respective coil count rates were employed. The flow curve obtained when the difference in height of the inflow and outflow vertical sidearm blood

columns was plotted against the experimentally determined flow rate is shown in Fig. 2. Each point shown represents the mean of duplicate readings at each flow rate. When the femoral flow rate derived from the height difference in vivo was compared with free flow determinations of femoral flow rate at the conclusion of the experiments, the derived values were 110, 160 and 140 ml./min. and the corresponding free flow readings were 100, 155, and 145 ml. per minute respectively.

Diffusion and Partition Coefficients

The values obtained for the diffusion coefficient of 133 Xe in animal synovial tissue were of the order of 1.0×10^{-5} cm^2 sec^{-1} at 37°C, the range of values obtained being 0.8 to 1.3×10^{-5} cm^2 sec^{-1}, whereas values for the diffusion coefficient of the isotope in synovial fluid were of the order of 1.5×10^{-5} cm^2 sec^{-1} (range 1.3 to 1.6 cm^2 sec^{-1} at 37°C.). These values are similar to those obtained in other biological tissue and fluid media (Unsworth and Gillespie, 1969).

The values obtained for the partition coefficient of 133 Xe for synovial tissue with respect to blood ranged from 0.87 to 1.1 (mean 1.0) and this value also accords with partition coefficients obtained in other biological

tissues (Conn, 1961 : Larsen et al., 1966).

Calculation of Synovial Perfusion in Animal Synovial Tissue

From Equation 7 (page 56), utilising the value 1.0 for the partition coefficient and the $T_{\frac{1}{2}}$ values obtained in these studies, synovial perfusion rates in these experiments ranged from 0.72 to 1.26 ml./100 ml./min. (mean 0.91 ml./100 ml./min.).

DISCUSSION

These results support the conclusion that intra-articularly administered Xenon (^{133}Xe) diffuses into the synovial membrane and is cleared by the subsynovial blood vessels to the femoral vein.

Thus the results of the preliminary tourniquet studies demonstrated that no clearance occurred when the tourniquets remained inflated. Furthermore, no clearance occurred when the narrow tourniquet placed distal to the site of emergence of the femoral diaphyseal vessels was inflated. Had any significant clearance been occurring through cartilage and thence through the blood vessels in bone, it is unlikely that a tourniquet in this position would completely arrest clearance. Further evidence that clearance does not occur through cartilage is provided by the slow diffusion rate of the isotope. Cartilage is avascular and it has been shown that in the absence of flow the diffusion of 133 Xe in tissue over 2 mm. distance takes longer than the time involved in these studies (Sjersen, 1967). Cartilage is over 2 mm. in thickness and should therefore present a formidable barrier to the isotope. On the other hand, since the capillaries of the synovial membrane are only microns removed from the surface (Schumacher, 1969 : Norton and Ziff, 1969), it can

easily be shown that over 50% of the injected isotope would diffuse across this distance in the time of these studies. That this is indeed so, is demonstrated by the fact that clearance commences within seconds of injection.

In the sample and coil experiments approximately 90% of the activity clearing from the joint could be accounted for at any time up to 40 minutes of injection in the femoral vein. Thus, at most, only 10% is available to clear by all other channels. The discrepancy may be partly attributable to clearance through other venous channels and to loss of isotope by diffusion from vessels between the joint and the monitoring system. Passive diffusion out of the area under the counter is invalidated by the slow diffusion rate of the isotope and since the needle was allowed to remain in situ closed by synovial fluid passage up the needle track is unlikely to have been a major factor. That clearance in lymphatic vessels is not a major factor is suggested by the work of Hollander et al. (1961) and of Stone and Miller (1949) and in this study no activity was detected in excised lymph nodes. Those instances in which femoral vein accountancy exceeded joint clearance are almost certainly related to lack of

quantitative precision in this relatively crude biological system. In two experiments (Dog 6 and Dog 18), the discrepancy persists throughout, suggesting a systematic error.

It would appear, therefore, that the clearance of intraarticularly administered 133 Xe is mediated by, and subject to, similar conditions which apply to any clearance model. One advantageous difference encountered in the present system however is that tissue distension due to deposition of the tracer solution is unlikely to be a major factor when the solution is introduced into the synovial cavity.

The clearance method for the determination of capillary flow has been employed by many workers (Kety, 1949 : Thorburn et al., 1963 : Lassen et al., 1964 : Larsen et al., 1966 : Sjersen, 1967). Since it is an indirect method, certain assumptions are necessary. Criticism has been levelled at those assumptions, (Gosselin, 1966). Clearance can easily be shown to be flow related but to what extent is it mediated by partial pressure gradients, diffusion or membrane permeability?

Gillespie (1968) has studied the influence of partial pressure gradients on tissue clearance of an

inert radioactive gas. He concluded that in an infinite homogeneous tissue the partial pressure at any stage during clearance could be assumed to be approximately uniform, except at relatively tiny regions surrounding the arterial ends of the capillaries. He also noted that significant errors could arise if tissue adjacent to the region under study possessed markedly different flow rates, for example grey and white matter in brain. This situation does not obtain in the case of the synovial tissue. As to the question of tissue homogeneity, this is a more complex problem. No tissue is histologically homogeneous. Accordingly, homogeneity in the context of clearance studies must be defined functionally. A small (m.m.) section of tissue which possesses a characteristic diffusion and partition coefficient and from which inert gas clearance is monoexponential is assumed to be functionally homogeneous (Perl et al., 1965).

The extent to which inert gas clearance is diffusion dependent over micron distances has been closely studied. Pappenheimer (1953) demonstrated very rapid equilibration rates for small molecular

weight substances. Kety (1951) also concluded that diffusion was not a limiting factor and he derived equilibration rates for inert gases in tissue to be of the order of seconds. Perl and Chinard (1968) have studied this problem and noted that under certain circumstances "large scale diffusion" (over mm.) could exert an appreciable effect. "Small scale diffusion" (over microns) is allowed for in the clearance model by the inclusion of λ (the partition coefficient). He proposed a method for calculating the importance of large scale diffusion and employing this method and the relevant values for diffusion coefficient, partition coefficient, flow rate and other system parameters employed in the present model, large scale diffusion is not a major factor in the clearance of 133 Xe from the joint cavity.

Membrane permeability is also an important aspect of clearance. In particular lipid insoluble substances such as radioactive sodium are likely to be affected by this (Gosselin, 1966). However, for inert freely diffusible lipid soluble small molecular weight tracers such as radioactive 133 Xe, on current concepts of membrane permeability, the predominant process is likely to be one of passive diffusion.

Thus it would seem reasonable to accept the assumptions involved in the clearance method in the context of this study. Figures for synovial perfusion of the order of 1.0 ml./100 ml./min. seem reasonable in the light of flow in other supporting connective tissues (Sjersen, 1967 ; Lassen et al., 1964 Larsen et al., 1966). However, since this is the first time that it has been possible to measure this, no direct comparisons are possible.

HUMAN STUDIES

Preliminary Studies in Normal and Diseased Human Subjects

Studies were then performed in normal volunteers and in patients afflicted with rheumatoid arthritis to determine the clearance rates from the human knee joint.

Patient Studies

133 Xe studies were performed on 14 clinically active knee joints of six patients with "definite" or "classical" rheumatoid arthritis (Ropes et al.1959). In all but one knee joint articular erosions were present on radiological examination and all had marked synovial hypertrophy clinically. At aspiration none had more than 5 ml. of synovial fluid in the joint. The mean age of these patients was 56.8 years (range 46 to 62 years). Four were male. Seven studies were performed on four healthy male volunteers aged between 22 and 26 years. None had a history of disease or trauma of the knee joint and in all the knee X-rays and sheep's cell agglutination tests (Ziff, 1957) were normal.

Injection Technique

All injections were performed in a surgical theatre, with the patient lying supine. The knee was extended and immobilised with sand bags. The room temperature ranged from 65 to 70°C. and an extraction fan was constantly in operation to reduce the concentration in

air of the radioactive gas exhaled by the patient. A fine guage needle was introduced into the joint cavity by a lateral infra-patellar approach and when an effusion was present this was aspirated as fully as possible. A prepared syringe containing the isotope dissolved in less than 1.5 ml. of 0.9% sterile sodium chloride was then attached to the needle and the solution was introduced into the joint cavity. In one normal volunteer the volume of diluent was 20 ml. to examine the effect of volume on the 133 Xe clearance. The needle was withdrawn and the wound was sealed with adhesive.

Preparation and Dose of 133 Xe

The reservoir of 133 Xe (Amersham) was kept outside the surgical theatre. In the first experiment on the human knee (the author's) a dose of 100 μCi was chosen. In this experiment it was found that sufficient counts should be present with the directional counter touching the skin over the patella, employing only 10 μCi and this dose was used successfully in all studies thereafter. The dose was prepared directly from the reservoir for each study and air bubbles were carefully excluded from the syringe owing

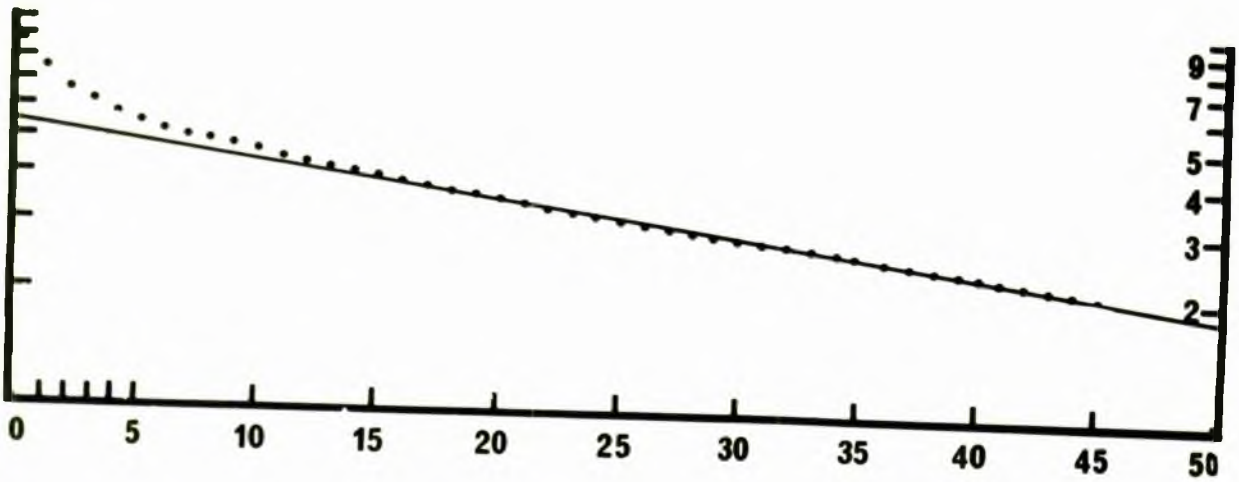


Fig. 4

Legend: Semilogarithmic plot of the disappearance curve of 133 Xe from the knee joint cavity of a patient with rheumatoid arthritis. Activity is plotted semilogarithmically on the vertical x-axis, against time in minutes on the horizontal axis.

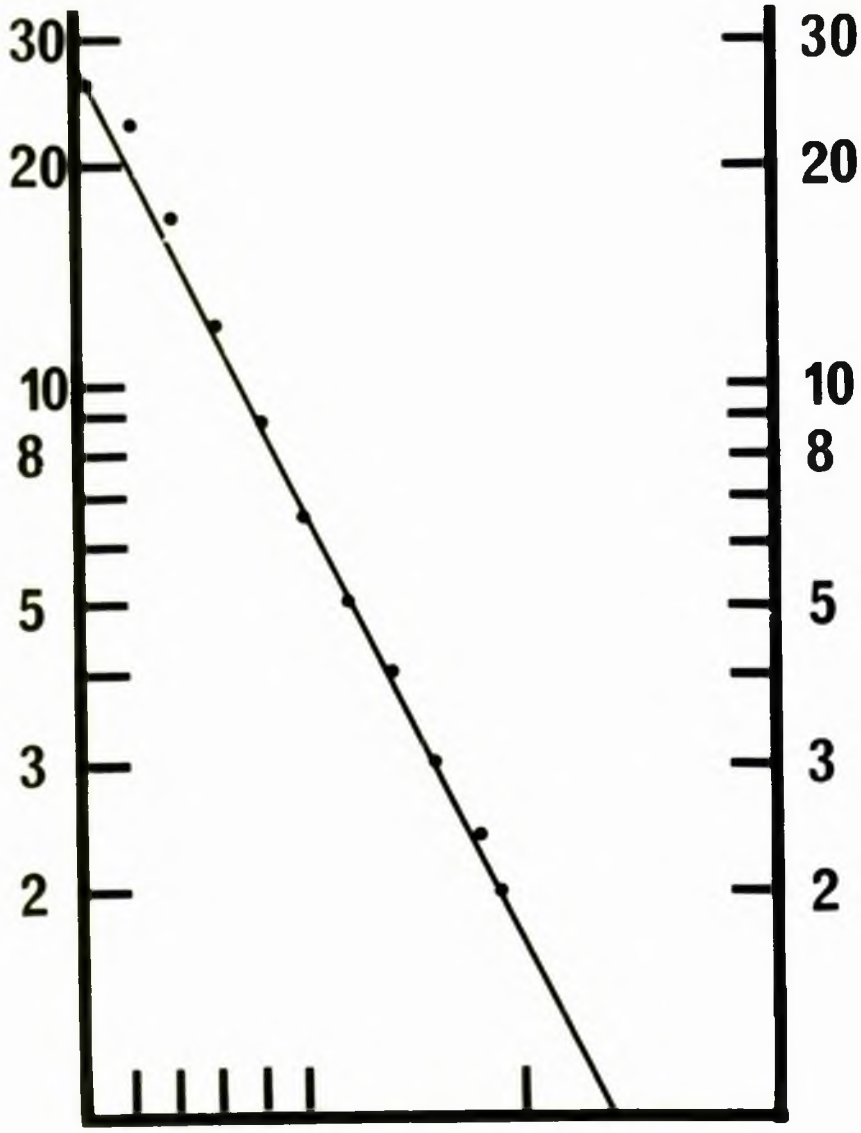


Fig. 5

Legend: Semilogarithmic plot of the difference between the points plotted in the first fifteen minutes after injection of 133 Xe and the straight line produced back to the vertical axis in Fig.1.

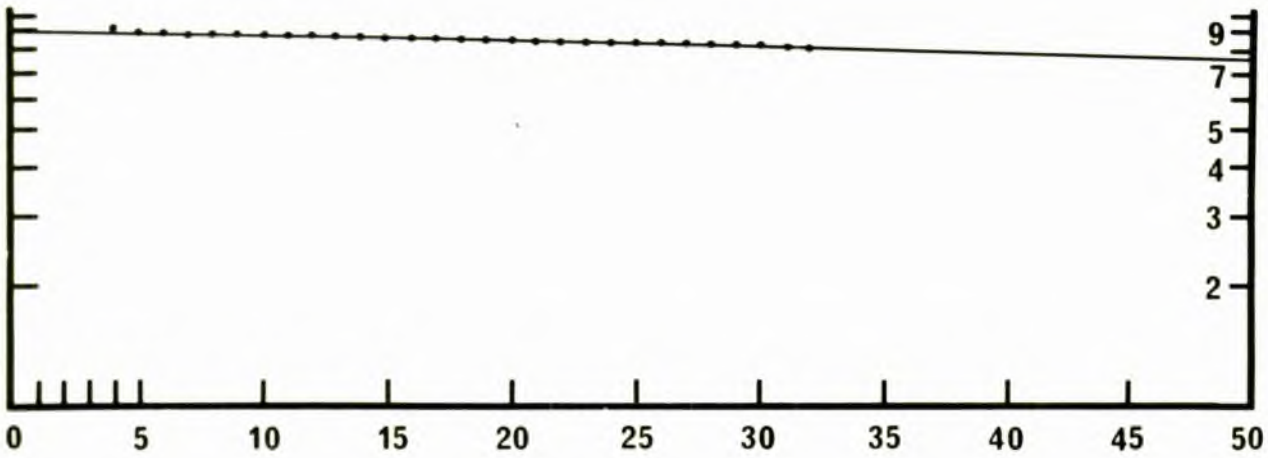


Fig. 6

Legend: Semilogarithmic plot of a disappearance curve of 133 Xe from the normal knee joints. The graph can be described by a single exponential.

to the high solubility of the radioactive gas in air.

Counting Technique

Counting was performed using a lightly collimated 1" x 1½" thallium activated sodium iodide crystal connected to an Ecko ratemeter and direct writing pen recorder. The crystal was directed at the medial aspect of the joint using the upper border of the patella as a reference point. Counts per minute were within the range 1,000 to 10,000 the back ground being of the order of 10 to 30 counts per minute. Counting commenced two minutes after injection and was continued for 30 to 45 minutes at a paper speed of 600mm/hour. The graph was sampled at one minute intervals for count rate and the results, minus the background, were transposed onto semilogarithmic graph paper as a function of time. The biological half life ($T\frac{1}{2}$ value in minutes) was then calculated from the semi-logarithmic plots of the results.

Results

The results are shown in Table 3 and Figs. 4 to 6. A slow monoexponential clearance pattern (Fig. 6) was observed in all but one normal subject. In that subject the volume of diluent was 20 ml., to determine the effect

of the volume injected and the $T\frac{1}{2}$ value obtained in that case was 66 minutes. This was by far the lowest value obtained in any normal subject. In that normal volunteer the shape of the curve obtained also differed from the pattern of clearance of the other normal subjects. When plotted onto semi-logarithmic paper, two exponential functions could be clearly discerned. . . . The $T\frac{1}{2}$ value of the initial fast component was 3.6 minutes (henceforth designated $T^{1}\frac{1}{2}$) and that of the slower second component was 66 minutes. The slower $T\frac{1}{2}$ value was included in the calculation of the mean (217.85 mins.) and standard error (72.6 minutes) of the results obtained in normal subjects.

The results in the rheumatoid subjects differed markedly from the results obtained in normal subjects. Whereas only one normal subject displayed a bi-exponential clearance, the results in all the rheumatoid subjects could be resolved into two exponentials very similar in pattern and in order of magnitude to the bi-exponential graph obtained in the normal subject. The short initial component could be described by a $T^{1}\frac{1}{2}$ value. The straight line of the second component was extrapolated back to the

TABLE III

Biological half lives ($T\frac{1}{2}$ values in minutes) of disappearance curves of 133 Xe from Human Knee Joint Cavities

	Patients with Rheumatoid Arthritis		Normal subjects
	$T^{1}\frac{1}{2}$ +	$T\frac{1}{2}$ *	$T\frac{1}{2}$
Biological $T\frac{1}{2}$ values (mins.)	3.0	42.0	630
	3.4	61.2	120
	3.6	19.2	150
	2.0	24.5	66($T^{1}\frac{1}{2}$+ = 3.6
	3.2	40.0	264
	3.0	57.0	120
	3.2 (3.0)	29.5 (28.0)	175
	2.6 (2.4)	35.0 (30.0)	
	1.8	50.0	
	6.4	50.0	
	2.7	26.6	
	2.2	27.0	
Range	1.8 - 6.4	19.2 - 61.2	66-630
Mean	3.09	38.5	217.85
Standard Error	0.34	3.97	72.6

$0.01 > p > 0.001$

+ $T^{1}\frac{1}{2}$ is the biological half life of the fast exponential curve in patients with rheumatoid arthritis.

* $T\frac{1}{2}$ is the biological half life of the slow exponential curve in patients with rheumatoid arthritis and the slow mono-exponential curve in normal subjects.

vertical axis. The count rate in the initial minutes, minus the corresponding figure on this extrapolated line, was then plotted semilogarithmically against time and yielded a straight line from which the $T^{1}\frac{1}{2}$ value could be readily calculated (Fig. 5). This is the same technique as was employed for the bi-exponential normal graph. The mean and standard error of the $T^{1}\frac{1}{2}$ values obtained in the rheumatoid patients was 3.09 ± 0.34 minutes. The $T\frac{1}{2}$ values of the slower component in the rheumatoid subjects, obtained directly from the first semilogarithmic plots, gave a mean value of 38.5 minutes, ($\pm$ standard error 3.97 minutes) and the results obtained in the rheumatoid patients for this slow component were significantly faster ($0.01 > p > 0.001$) by a student t-test) than the values obtained in normal subjects (Table 3).

Discussion

Thus the rate of clearance of intra-articularly administered 133 Xe from the knee joint is considerably faster in rheumatoid subjects than in normal volunteers. Indeed, inspection of the results shows that there was no overlap whatever between the groups. It was

possible that the difference observed was explicable on the basis of the difference in the mean ages of the groups studied. However, in view of the magnitude of the difference, this seemed unlikely.

In normal subjects the semilogarithmic plots obtained yielded a straight line and could therefore be described by the equation:

$$y = Ke^{-kt}$$

$$\text{where } k = \frac{0.693}{T\frac{1}{2}}$$

However, in rheumatoid subjects the graphs obtained were bi-exponential, i.e.:-

$$y = Ke^{-kt} + K^1e^{-k^1t}$$

$$\text{where } k^1 = \frac{0.693}{T^1\frac{1}{2}}$$

The reason for this discrepancy was not immediately apparent and furthermore the constancy of the difference could not be assumed since the results obtained in the normal subject injected with 20 ml. of diluent was bi-exponential. It was also noteworthy that the $T\frac{1}{2}$ value obtained in that normal subject was very much less than those obtained in all other normal subjects. This value was included in the calculation of the mean of the normal results because the numbers studied were so small and since it could

only militate against over-optimistic interpretation of these early results. However, this one result did suggest that the volume of diluent had an effect on the result and prompted further studies to elucidate this problem (page 90).

Scholer et al., (1959) demonstrated a rise in the concentration of deuterium and of radioactive sodium (^{24}Na) in the blood during the clearance of these substances from the joint. Accordingly, in one rheumatoid patient, femoral vein blood was removed during the 133 Xe study and placed in a well type scintillation counter. 133 Xe was qualitatively identified in this sample. From this and from previous experimental work in animals (page 57), it seemed reasonable to propose that the monoexponential clearance of 133 Xe from the joint was accounted for by passage of the isotope into the blood stream. It also seemed likely that the faster clearance rates observed in rheumatoid patients could be accounted for by a similar mechanism and that the shorter biological half life represented greatly enhanced perfusion of the inflammed synovium.

DERIVATION OF SYNOVIAL PERFUSION IN THE HUMAN JOINT

It was not possible to employ the same experimental protocol to demonstrate the route of clearance of 133 Xe in the human. Furthermore, it is impossible to extrapolate directly from the results obtained in normal dogs to the diseased human diarthrodial joint. Accordingly, studies were designed in an attempt to obtain some evidence, albeit indirect, that the route of clearance in the human was also blood flow dependent and that the clearance model could be applied to results obtained in human studies.

ROUTE OF CLEARANCE OF 133 Xe FROM THE DIARTHRODIAL JOINT CAVITY IN MAN

TOURNIQUET STUDIES

Method

133 Xe studies were conducted in the usual manner in ten patients with "classical" rheumatoid arthritis (Ropes et al., 1959), five of whom had been subjected to the operation of anterior knee synovectomy more than one year prior to these 133 Xe studies. The mean age of these patients was 63.2 years (range 42-71 years). Two were male and all had articular erosions on radiological examination. When the clearance rate

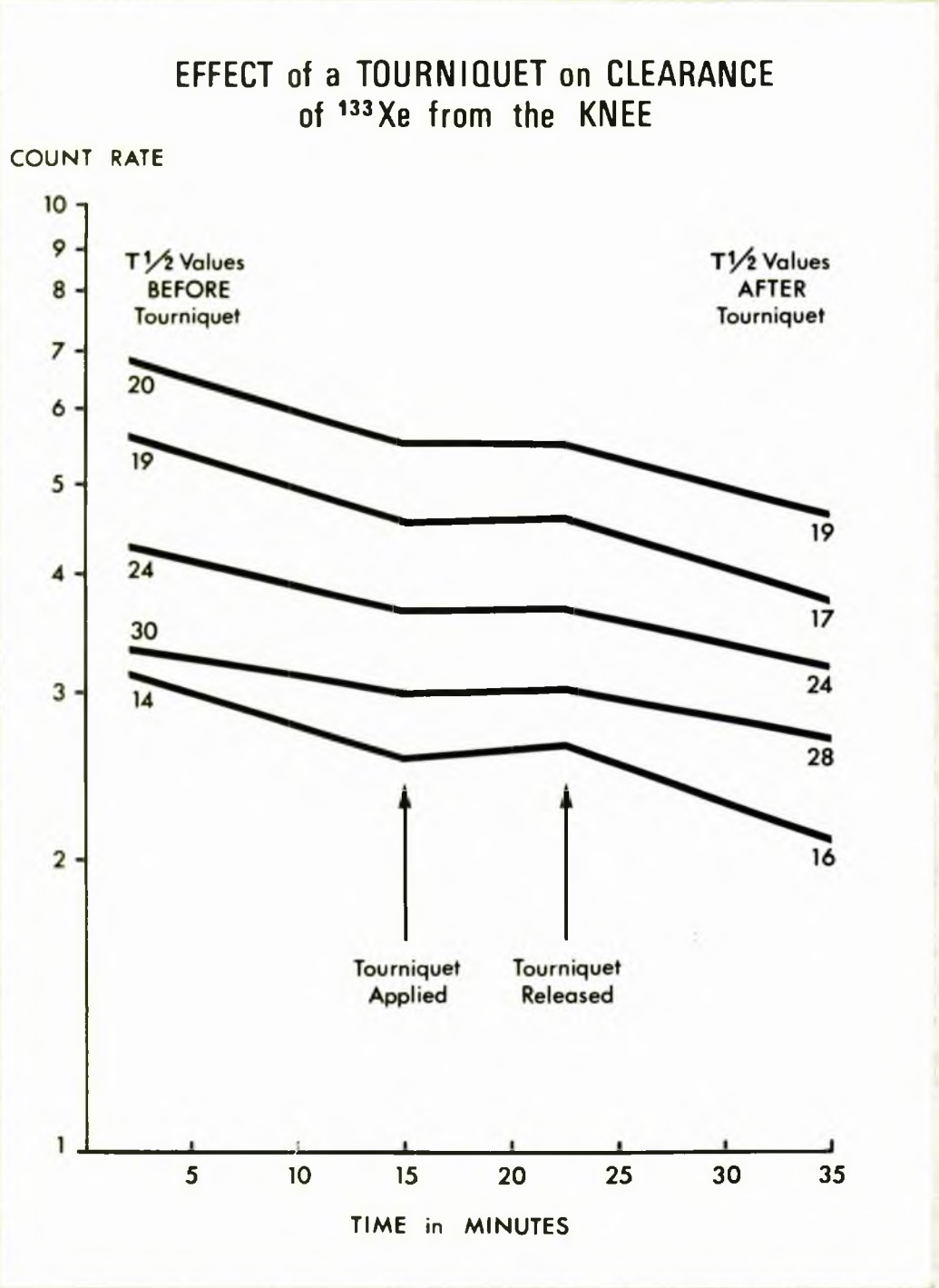


Fig. 7

Legend: The effect of tourniquet inflation on the clearance of intra-articularly administered 133 Xe from post-synovectomised knee joint of five patients with rheumatoid arthritis. The $T\frac{1}{2}$ values prior to inflation were 20, 19, 24, 30 and 14 minutes and the corresponding $T\frac{1}{2}$ values following deflation were 19, 17, 24, 28 and 16 minutes.

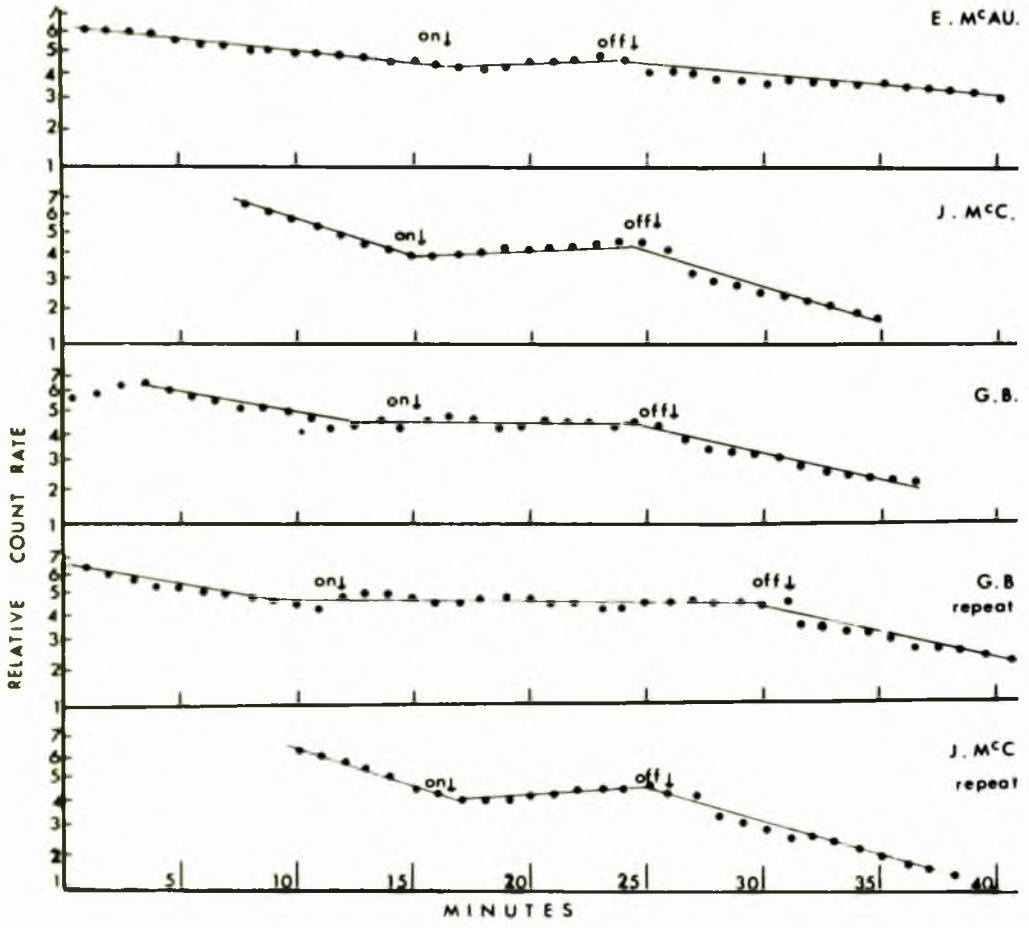


Fig. 8

Legend: The effect of tourniquet inflation on the clearance of intra-articularly administered 133 Xe from the unoperated knee joints of five patients with rheumatoid arthritis. The $T_{\frac{1}{2}}$ values before inflation were 70, 16, 46, 40 and 26 minutes and following tourniquet deflation the corresponding values were 72, 14, 49, 36 and 25 minutes respectively.

had been established a narrow (2" in width) tourniquet, positioned immediately above the upper border of the joint, was inflated to a pressure mid-way between the individual's systolic and diastolic blood pressure. After an interval of 5 to 10 minutes, the tourniquet was deflated and the clearance rate monitored for a further 20 minutes.

Results and Interpretation

The $T\frac{1}{2}$ values obtained before and after the intervention of the tourniquet are shown in Figs. 7 and 8. The values obtained beforehand in both operated (20, 19, 24, 30 and 14 minutes) and unoperated (70, 16, 46, 40 and 26 minutes) knees were similar to the corresponding values obtained following tourniquet deflation (19, 17, 24, 28 and 16 minutes and 72, 14, 49, 36 and 25 minutes respectively) and in each individual study the two values obtained were closely similar. In no case was appreciable clearance of the isotope observed during the time of tourniquet inflation.

These results show that isotope clearance can be interrupted by venous occlusion in both synovectomised and unoperated knees of patients with rheumatoid arthritis. The position of the tourniquet, below the

exit from bone of the nutrient vessels allowed separation of clearance in diaphyseal vessels from clearance superficial to bone. No appreciable clearance was occurring through the diaphyseal vessels, since flow in these veins would not have been affected by occlusion below the nutrient foramen (page 11).

INJECTION OF 133 Xe UNDER DIRECT VISION INTO HUMAN SYNOVIAL TISSUE

Method

A 52 years old female patient with "classical" rheumatoid arthritis (Ropes et al., 1959) whose knee joint exhibited marked involvement with clinically obvious synovial hypertrophy agreed to participate in this study. She had previously accepted the recommendation that she would benefit from synovectomy. Preoperatively, her knee score (page 112) was +12 and the $T\frac{1}{2}$ value obtained four days preoperatively was 23 minutes. At the operation of anterior knee synovectomy, the joint cavity was opened. A solution of 133 Xe (20 μCi in 0.1 ml. of sterile 0.9% NaCl) was injected under direct vision approximately 2 to 3 cm. into synovial tissue at the periphery of the joint, as

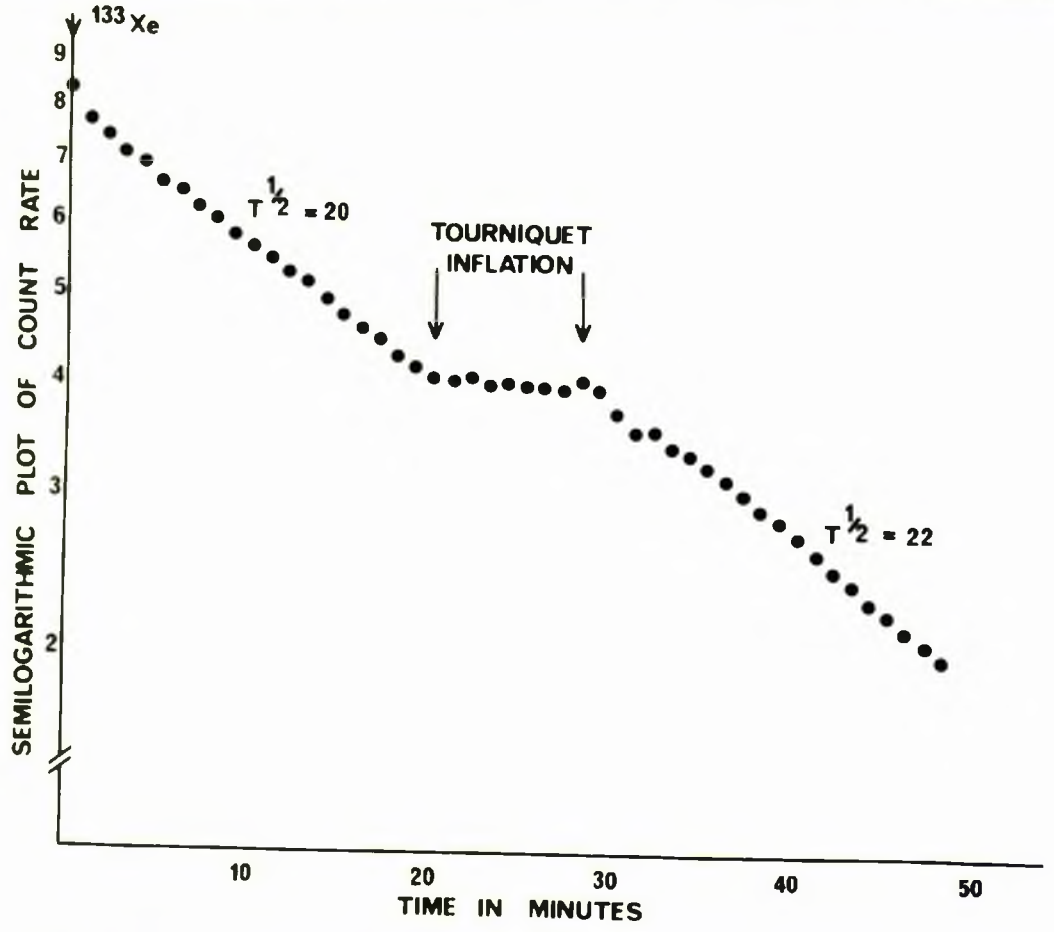


Fig. 9

Legend: 133 Xe was injected at operation under direct vision into synovial tissue. During monitoring of clearance rate a narrow tourniquet placed around the lower third of thigh was inflated for 8 minutes. The $T_{\frac{1}{2}}$ value prior to inflation was 20 minutes and following deflation 22 minutes.

far removed from cartilage as possible. The cavity was quickly packed with saline soaked swabs and the capsule and skin wounds were approximated and clamped. The swabs employed were placed under the directional counter at the conclusion of the experiment and the count rate obtained was less than 4% of the count rate obtained over the joint. The isotope clearance rate was monitored in the usual manner and when this was established a narrow tourniquet placed round the lower third of thigh was inflated to 90 mm. Hg. Inflation was maintained for 8 minutes and the clearance rate was monitored throughout this time and for 20 minutes following deflation.

Results and Interpretation

The semilogarithmic plot of count rate is shown in Fig. 9. The $T\frac{1}{2}$ value of the initial period is 20 minutes. No appreciable clearance occurred during tourniquet inflation and the $T\frac{1}{2}$ value following deflation is 22 minutes.

Both of these $T\frac{1}{2}$ values accord well with results obtained in other patients exhibiting a similar degree of clinical involvement and with the value obtained preoperatively. Cessation of clearance on venous

occlusion implies that clearance is flow dependent.

In this experiment the isotope was introduced directly into synovial tissue whereas in all other studies injection is intra-articular. In the latter situation diffusion must occur across an intervening avascular layer prior to clearance in small blood vessels. The similarity between the clearance rates obtained by these different methods is accounted for by proximity to the surface of these small vessels with consequent reduction in the intervening distance to be traversed by the isotope (Norton and Ziff, 1969).

Clearance through cartilage and diaphyseal vessels was totally excluded by the design of this experiment. Indeed, clearance by all routes other than the synovial vessels was excluded since the wound was closed throughout the study.

DIFFUSION RATE AND PARTITION COEFFICIENT IN NORMAL AND DISEASED HUMAN SYNOVIUM

Methods

Specimens of synovial tissue, synovial fluid and cartilage were obtained at the operation of synovectomy from eight patients with rheumatoid arthritis and from seven necropsies in young patients who had never been noted to have articular disease. The superficial

1 to 2 mm. of synovium were carefully dissected from each specimen and the synovium, synovial fluid and cartilage were separately homogenised. Thereafter the methods employed for the determination of diffusion and partition coefficients were those previously described (pages 51 and 54).

Results and Interpretation

The values for the partition coefficients of 133 Xe in all the specimens were closely similar, the ranges of values obtained being almost identical. The mean value for both normal and rheumatoid synovium was 1.0 (range 0.87 to 1.1) and the mean value for all of the cartilage and synovial fluid specimens was 1.02 (range 0.91 to 1.22). These accord with values obtained in similar biological tissue (Conn, 1961). The mean value of 1.0 was employed in the derivation of synovial perfusion throughout this thesis. It should be emphasised that since it is not possible to determine individual partition coefficients in each 133 Xe study, the assumption that this narrow range of values obtains throughout is necessary. Although this assumption is commonly accepted (Thorburn et al., 1963 : Lassen et al., 1964 : Larsen et al., 1966 : Sjersen, 1967), absolute values quoted for perfusion must be interpreted with this in mind.

The values obtained for the diffusion rate of 133 Xe in synovial tissue were within the range of values obtained in the animal tissues. The mean value was 1.0×10^{-5} cm^2 sec^{-1} for synovial tissue, 1.5×10^{-5} cm^2 sec^{-1} for synovial fluid and 0.8×10^{-5} cm^2 sec^{-1} for cartilage. These values are similar to those obtained in other biological tissue. The same arguments which apply in considering diffusion in the animal model obtain therefore in man (page 64). The proximity of capillaries to the cavity allows rapid equilibration and the early establishment of clearance. On the other hand the slow diffusion rate renders clearance by any other route unlikely on theoretical grounds alone.

DERIVATION OF SYNOVIAL PERFUSION IN MAN

Method

133 Xe studies (page 67) were conducted in 18 healthy male volunteers. Particular care was exercised to exclude a history of disease or trauma to the knee joint. Careful clinical examination of the knee joint revealed no abnormality. Radiological examination of knees, hands and feet and serological tests for rheumatoid factor were negative. Their ages ranged from 20 to 68 years. Twelve were male University students between the ages of 20 and 28 years and the remaining six had non-articular complaints, their ages ranging from 42 to 68 years (mean 52.2 years).

Twelve further studies were conducted in patients with "classical" rheumatoid arthritis (Ropes et al., 1959), all of whom had severe clinical involvement of the knee and in all of whom articular erosions were present on radiological examination. None were receiving corticosteroid therapy. All were post-menopausal females, their ages ranging from 58 to 64 years (mean 60.3 years). As is the case with all studies reported in this thesis, both patients and control subjects volunteered to participate with full knowledge of the content and implications of the proposed

TABLE IV

Synovial Perfusion (ml./100 ml./min.) in 12 Patients with Rheumatoid Arthritis and in 18 Normal Subjects

Rheumatoid Arthritis	Normal Subjects	
Age Range 58-64 years n = 12	Age Range 20-28 years n = 12	Age Ra 40-68 n = 7
1.22	0.49	0.21
0.81	0.36	0.27
1.69	0.15	0.57
2.10	0.11	0.6
2.31	0.58	0.4
2.77	0.11	0.3
4.08	0.26	0.3
1.92	0.46	
1.51	1.05	
2.04	0.58	
1.39	0.40	
1.82	1.00	
Mean 1.97	0.46	0.3
± S.E.M. 0.24	0.09	0.0
$p < 0.001$	$0.6 > p > 0.5$	

All normal subjects v. rheumatoid arthritis $p<0.001$.

study.

The relationship employed for the derivation of synovial perfusion (S.P.) was: (Equation 7, page 56).

$$S.P. = \frac{69.3}{T\frac{1}{2}} k \lambda \text{ ml./100 ml./min.}$$

The value employed for λ was unity.

Results

The results are shown in Table 4. The synovial perfusion values obtained in the two groups of normal subjects (mean 0.46 ± S.E.M. 0.09 ml./100 ml./min. and mean 0.39 ml./100 ml./min. ± S.E.M. 0.06 ml./100 ml./min.) were not significantly different ($0.6 > p > 0.5$). The mean value of all normal subjects (0.43 ± S.E.M. 0.06 ml./100 ml./min.) was, however, significantly lower ($p < 0.001$) than the mean value obtained in the patients with rheumatoid arthritis (1.97 ± 0.24 ml./100 ml./min.).

Discussion

The evidence presented in this section supports the conclusion that intra-articularly injected 133 Xe is cleared from the joint cavity in man, as in the greyhound, by the synovial vessels.

Apart from theoretical considerations based upon the site of injection (page 61), the diffusion rate of the isotope in tissue (pages 64 and 79) and the proximity to

the joint cavity of the capillaries, certain facts support this assertion. The isotope has been qualitatively identified in femoral venous blood. Tourniquet studies (page 74) confirm that clearance is flow dependent and that no appreciable clearance occurs through diaphyseal vessels. Furthermore, when other routes are excluded (page 76) clearance occurs at a rate consistent with that obtained in similarly afflicted joints. The relevant values for diffusion rate and partition coefficient have been determined and accord with those obtained in similar tissues by other workers. Whereas the order of magnitude of the diffusion rate is such as to render extremely unlikely any effect of biological variation of diffusion upon clearance, the same is not true of the partition coefficient. Although the range of partition coefficient values obtained in these studies is small, the acceptance of uniformity of partition coefficient must be more cautious. This point must be constantly remembered in assessing the validity of absolute values of perfusion in all clearance models.

The clearance method for the derivation of perfusion is indirect, and therefore certain assumptions are necessary. In the present context no other method

is available with which to compare the results. The values obtained for normal synovial perfusion are of a realistic order of magnitude when compared with perfusion rates of other supporting connective tissues (Lassen et al., 1964 ; Larsen et al., 1966 ; Sjersen, 1967). The difference obtained between normal perfusion and perfusion of rheumatoid synovium accords with the pathology of the disease. It would seem reasonable to accept comparative figures for perfusion in normal and diseased states as being biologically meaningful and to cautiously accept the absolute values obtained until another method becomes available for confirmation.

SUMMARY

1. 133 Xe studies are simple to perform and expose neither patient nor investigator to undue radiation hazard.

2. 133 Xe is cleared from the joint cavity by synovial vessels to the femoral vein in animals and probably also in the human subject.

3. The diffusion and partition coefficients of 133 Xe in synovial tissue have been determined.

4. Clearance can be described by a single exponential for the first 40 minutes following injection and the rate of clearance is proportional to the synovial perfusion rate.

5. Synovial perfusion rates in the stifle joint of dogs, in normal human knee joints and in the knee joints of patients with rheumatoid arthritis have been derived.

6. The synovial perfusion rate in patients with rheumatoid arthritis greatly exceeds normal.

CHAPTER 3

It is mandatory to establish the confidence limits which can be ascribed to any new technique. Particula problems associated with the 133 Xe clearance method, highlighted in the preliminary studies reported in Chapter 2, were the variability of the clearance rate in the first few minutes after injection and the effec of large diluting volumes on the clearance rate. In Chapter 3 the results of studies designed to investigate these problems are reported. In particular, the influence of the method and of the site of injection and the effect of the diluting volume injected and of the presence of a knee joint effusion are reported.

The reproducibility of the derivation of $T\frac{1}{2}$ value is quantitated, and the results obtained by prolonged monitoring of the count rate are recorded. The variability of clearance rate with time and with the age and sex of the subject is examined.

TABLE I

$T\frac{1}{2}$ Values Derived from 5 to 40 mins. and from 90 to 120 mins. after Injection

Rheumatoid Arthritis		Normal Dogs	
5-40 mins.	90-120 mins.	5-40 mins.	90-120 mins
18	70	89	90
32	96	70	72
41	74	82	80
45	88	42	41
51	90	46	46
		54	59
		59	63
		63	97
		61	85
		68	111
		81	98
		43	81
		42	102
		50	79
		35	65
		73	91
		78	88
		66	106
		68	92
		62	95

* The paired values below the line represent those studies in which a separate late exponential was detected.

PROLONGED MONITORING OF CLEARANCE RATE

In one normal subject (the author's) the clearance rate was monitored for over four hours. The $T\frac{1}{2}$ value obtained from both the early and late segments of the graph were identical (600 mins.).

In five male patients with "classical" or "definite" rheumatoid arthritis (Ropes et al., 1959) the clearance rate was monitored for over 120 minute In each one the early clearance rate ($T\frac{1}{2}$ values 18, 41, 45 and 51 minutes) persisted for approximately 4(minutes. Thereafter the clearance rate gradually altered the change being discernable between 50 and 8 minutes after injection. The $T\frac{1}{2}$ values of the late exponentials were 70, 96, 74, 88 and 90 minutes respectively (Table 1).

In twenty experiments in normal dogs monitoring was continued for over four hours. In five of those studies no late exponential could be separately discerned. In thirteen studies a late exponential could be clearly defined which was characterised by a different $T\frac{1}{2}$ value (Table 1). In the remaining two studies the $T\frac{1}{2}$ value calculated from the later phases was greater than the original $T\frac{1}{2}$ but could not be clearly defined as significantly different (i.e. with

the range of the reproducibility studies - Table 10).

It is possible that these slower late exponentials represent clearance from more peripheral areas of the synovium, but there is insufficient information to assert this confidently. Clearly, however, the term "synovial perfusion" in the context of this thesis can only be ascribed to clearance within 40 minutes of injection.

STUDIES TO INVESTIGATE THE PATTERN OF CLEARANCE GRAPHS OBTAINED

It has previously been noted (page 7o) that on some occasions only a single exponential could be fitted to the results whereas on other occasions an early separate exponential could be clearly discerned. Two clues were offered by the pilot studies performed (page 7o). In the normal subjects when the volume of isotope diluent was 20 ml. the resulting semilogarithmic plot could be described by two exponentials whereas when the volume was less than 2 ml. only a single exponential was observed. Secondly, two exponentials were found more frequently in studies on diseased joints than in normal joints. In further studies it was also noted, in a qualitative uncontrolled manner, that whenever difficulty was experienced with the initial injection and whenever the initial synovial fluid aspirate contained fresh blood, then the result was more likely to be biexponential. The first exponential in these studies could be either slower or faster than the second but was always of less than 15 minutes' duration. To investigate this, further studies were conducted to examine the effect of volume and the effect of injection technique on the clearance

rate of intra-articularly administered 133 Xe.

THE EFFECT OF VOLUME OF INJECTION ON THE CLEARANCE OF INTRA-ARTICULARLY ADMINISTERED 133 Xe

Introduction

Rupture of the joint cavity following injection of large volumes intra-articularly is not uncommon (Jayson, 1970) and although this is not usually accompanied by clinical disability, nevertheless it was felt necessary to conduct further studies in animals rather than in human subjects.

The effect of the volume of injection was studied in different ways. One experiment was performed during which 133 Xe was injected consecutively on four occasions into the same joint cavity. On each occasion the identical dose of isotope was employed but the diluting volume was progressively increased. As a control for this experiment identical doses with constant diluting volumes were employed in the opposite joint on four successive occasions. In a further experiment femoral vein samples and the femoral venous flow rate were obtained in addition to clearance rate from the joint following the injection of a large diluting volume and the results were compared with those obtained previously (page 58) when a smaller volume of diluent was employed.

Materials and Methods

Four experiments were conducted on the stifle joints of adult greyhound dogs weighing 29, 27, 33 and 28 kg. The anaesthetic employed and the injection (page 39) and counting (page 44) techniques have been described. The clearance rate from one joint was monitored on four successive occasions. The dose of 133 Xe administered on each occasion was closely similar but on the first occasion the diluting volume was 0.5 ml., on the second 2.5 ml., on the third 4 ml. and on the fourth 6 ml. The half lives ($T\frac{1}{2}$ values, mins.) were calculated from each of the four 15 minute monitoring periods following injection. In the opposite joint the same protocol was followed but on each occasion the volume injected was only 0.5 ml. Four experiments were conducted employing this protocol. In a further six studies a standard volume (6 ml.) of 0.9% sterile NaCl was injected on four successive occasions without the addition of 133 Xe. This sequence was adhered to in all six experiments.

In one additional experiment in which the diluting volume was 4 ml., a T-tube placed in the femoral vein allowed the determination of femoral venous flow rate prior to injection. By removing venous samples at

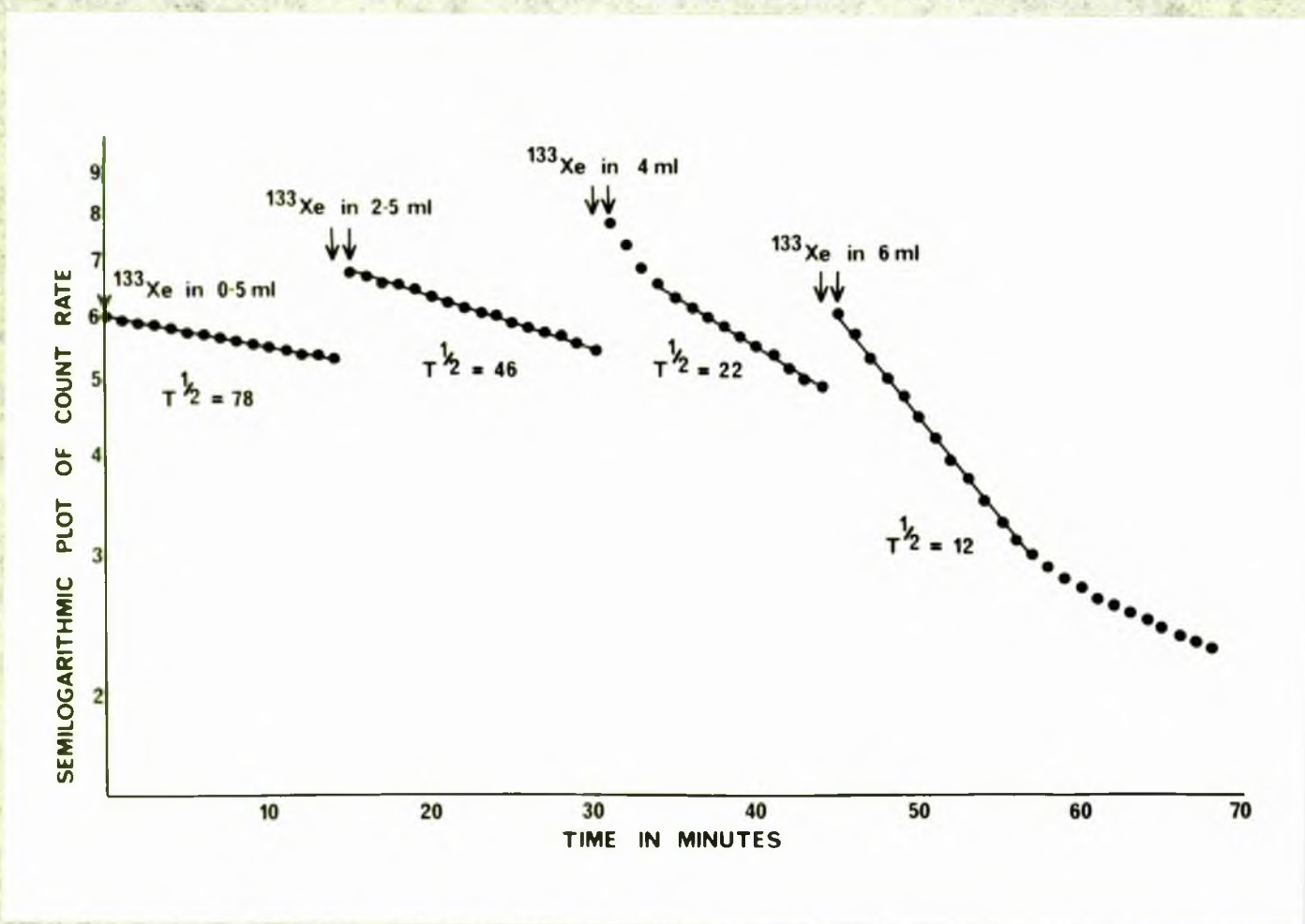


Figure 1

Legend: Effect of increasing diluent volume on the 133 Xe clearance rate.

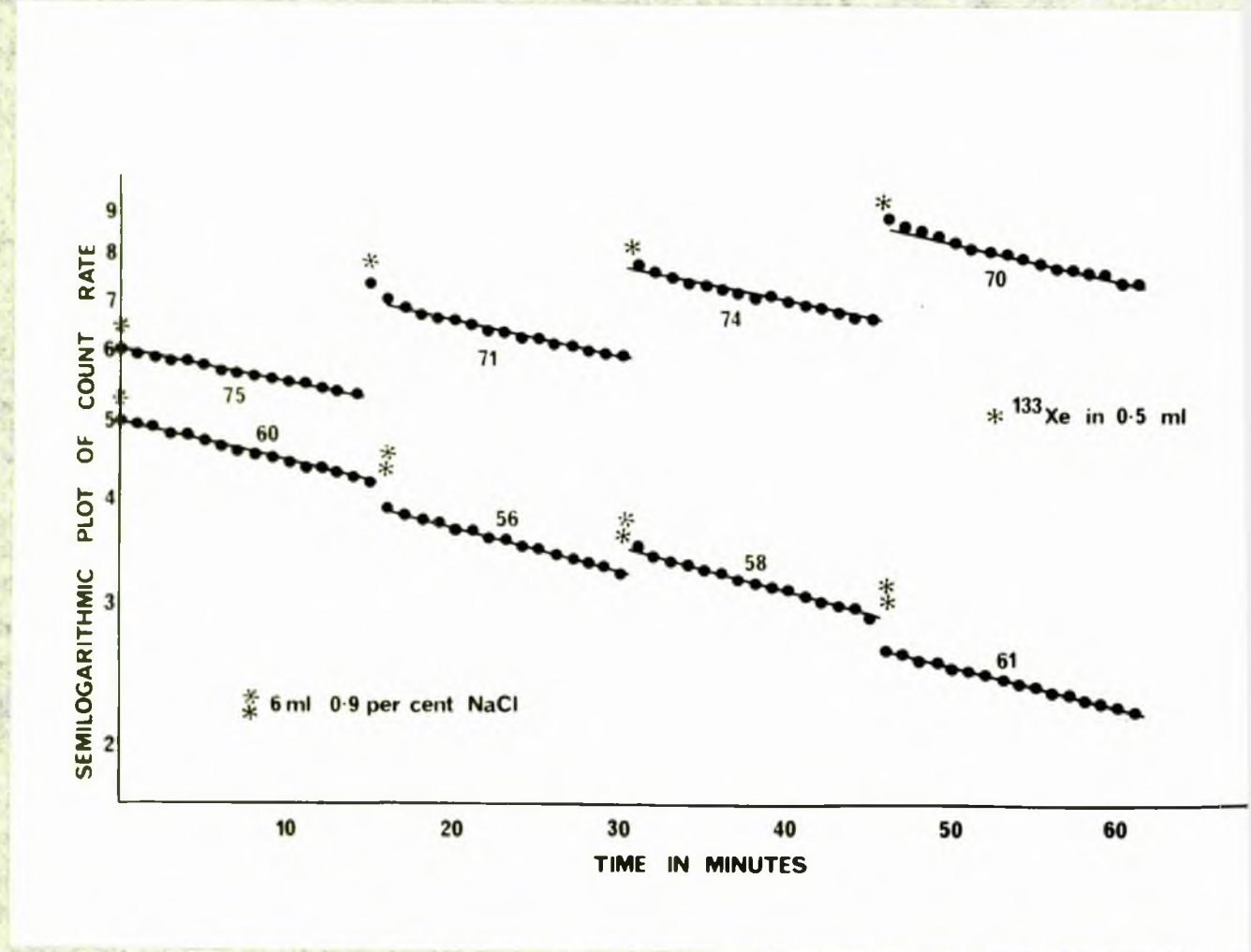


Figure 2

Legend: Effect of repeated injections of a constant dose and volume of 133 Xe and of injections of a larg volume of 0.9% NaCl with additional isotope.

TABLE II

Effect of Increasing Volumes of Diluent on the Clearance of 133 Xe.

		133 Xe in:-			
	Exp.	0.5 ml.	2.5 mls.	4 mls.	6 mls
$T\frac{1}{2}$ values (mins.)	1.	78	46	22	12
	2.	55	43	34	20
	3.	64	56	38	25
	4.	52	43	26	17

TABLE III

Effect of Repeated Administration of 133 Xe in a Constant Volume on the Clearance Rate

		133 Xe in:-			
	Exp.	0.5 mls.	0.5 mls.	0.5 mls.	0.5 m
	1.	75	71	74	70
$T\frac{1}{2}$ values (mins.)	2.	62	62	60	58
	3.	60	63	58	55
	4.	47	45	45	48

5, 10, 15, 25, 30, 25 and 40 minutes after injection femoral venous activity could be compared with the activity lost from the joint (page 58).

Results

The results are shown in Figs. 1 to 3 and Tables 2 to 5.

In all four experiments, when the diluting volume was increased so also was the clearance rate (Fig. 1). The $T\frac{1}{2}$ values at each monitoring period are shown in Table 2. The baseline $T\frac{1}{2}$ values were 78, 55, 64 and 52 minutes. The respective values following injection of 2.5 ml. were 46, 43, 56 and 43 mins.; following injection of 4 ml. were 22, 34, 38 and 26 minutes and following the injection of 6 ml. were 12, 20, 25 and 17 minutes respectively (Table 2). Two exponentials could be fitted to the semilogarithmic plots of the experiments in which 4 ml. and 6 ml. were injected (Fig. 1).

In the control studies, where the diluting volume was constant, no alteration in clearance rates was observed. The $T\frac{1}{2}$ values obtained throughout each experiment were closely similar (Fig. 2 : Table 3). On each occasion when 6 ml. of 0.9% NaCl alone were injec during monitoring of the clearance rate, no marked or consistent alteration in the $T\frac{1}{2}$ values before and after

TABLE IV

Effect of the Intra-articular Administration of a Constant Volume of 0.9% NaCl without Additional 133 Xe on the Clearance Rate

		Volume Injected:-			
	Exp.	6 mls.	6 mls.	6 mls.	6 mls.
	1.	60	56	58	61
	2.	45	43	45	40
$T\frac{1}{2}$ value (mins.)	3.	76	79	74	80
	4.	54	54	47	46
	5.	50	46	47	43
	6.	63	60	66	68

TABLE V

Cumulative % of 100% Dose Cleared from the Stifle Joint
and
Cumulative % of 100% Dose Accounted for in Femoral Vein.

Time after Injection	Joint	Femoral Vein	% of Counts cleared which can be detected in Femoral Vein
5	20.7	3.6	17
10	32.8	5.9	18
15	43.1	8.5	20.
25	60.3	12.3	20
30	64.7	20.9	32
35	67.0	35.3	53.
40	72.4	36.8	51.

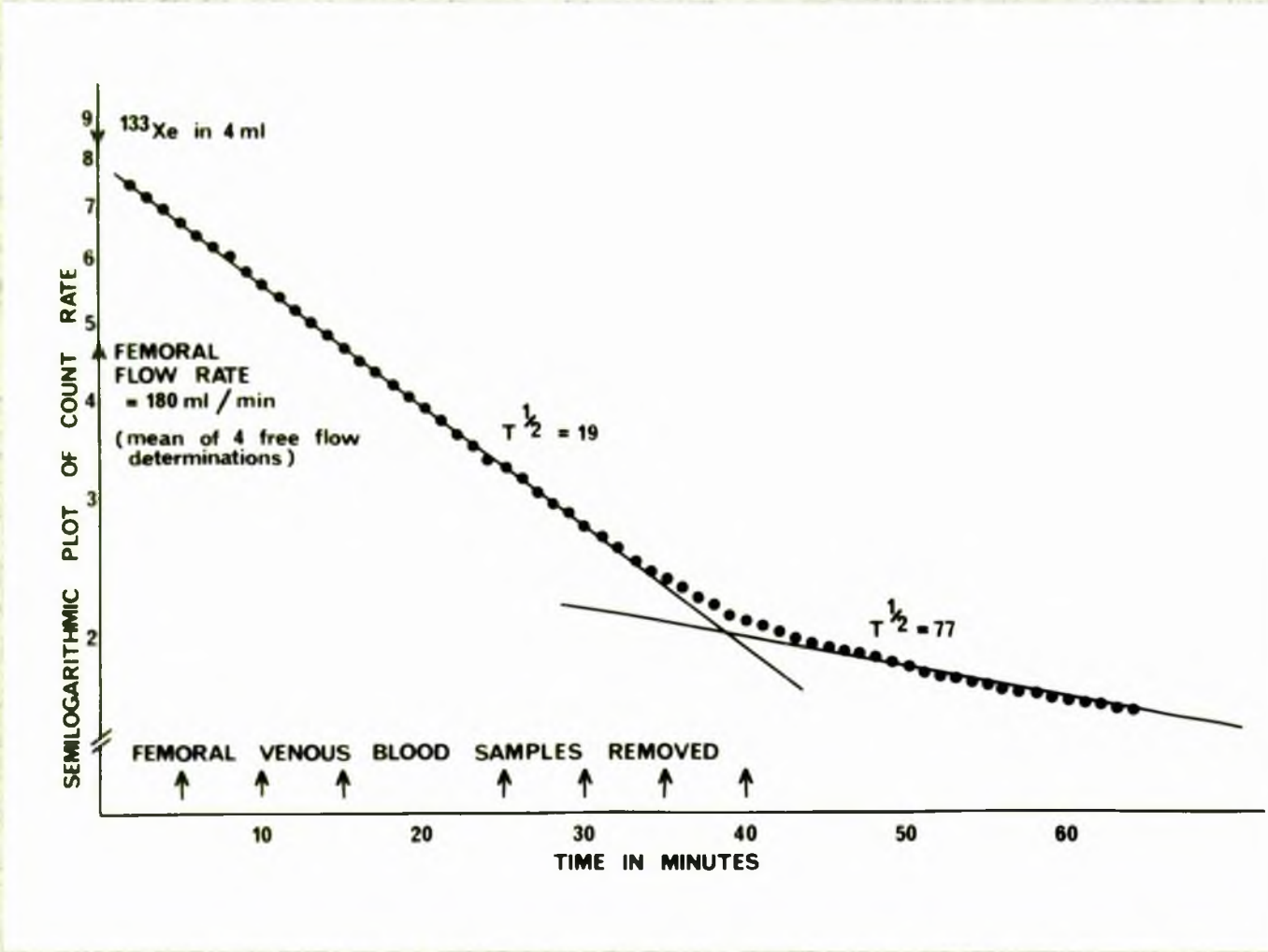


Figure 3

Legend: The graph obtained following injection of 4 ml. of 133 Xe solution intra-articularly.

injection was observed (Fig. 2 : Table 4).

Table 5 shows the results of femoral venous activity and activity lost from the joint each expressed as % of 100% dose as has been described (page 48). The activity lost from the joint which could be accounted for in the femoral vein at 5, 10, 15, 25, 30, 35 and 40 minutes was 17%, 18%, 20%, 20%, 32%, 53% and 51% . The percentage obtained in previous experiments (page 58) at 10, 20, 30 and 40 minutes were 92%, 91%, 88% and 93% respectively. The semilogarithmic plot of count rate against time (Fig. 3) differs from the plots obtained in other studies with lower diluting volumes. Two exponentials can be detected as is shown in Fig. 3, the second commencing between 30 and 45 minutes after injection. The first exponential is markedly faster than is commonly encountered and the second is of a similar order of magnitude to the first exponential usually observed (pages 51, 71, and 82).

Discussion

It is clear from these results that whereas volume alone does not affect the clearance rate within this experimental design, the combination of isotope and high diluting volume does exert a profound influence on

the clearance rate. It is possible that one of the non-perfusion variables which affect clearance is affected. For example, higher hydrostatic pressures and partial pressures may individually or collectively contribute to the increased clearance rate in the early stages after injection resulting in an additional exponential. It seems more likely, however, that perfusion itself is indeed increased. It is extremely interesting that the activity lost from the joint which could be accounted for in the femoral vein was markedly lower than in all other studies in which the injection volume was 0.5 ml. In addition, the clearance rate from the joint is notably faster over the first 30 minutes following injection and only thereafter does the slope accord with those obtained in the other studies (Fig. CHART). The shape of the graph is similar to that obtained in the human subject in whom the injection volume was 20 ml. (page 69). These findings suggest that clearance is occurring through unusual routes under these circumstances. One possible explanation would be that additional vascular channels become available in response to either higher pressure or higher volumes within the joint cavity. Alternatively, it is possible that rupture of the

joint cavity occurs in response to the increased volume and that the isotope is then "leaking" from the area under the counter. If this were true, however, a similar result should have been obtained when a large volume of saline (Fig. 2) was injected alo

Regardless of the precise mechanism involved, it is clearly necessary to maintain the diluting volume employed in clearance studies to a minimum.

EFFECT OF THE METHOD OF INJECTION ON THE CLEARANCE RATE OF INTRA-ARTICULARLY ADMINISTERED 133 Xe

The qualitative observation that an early exponential, variable in time and direction, might be related to the facility of injection prompted the following study.

Method

The method of injection was altered. The needle was introduced into the joint cavity and any free fluid present was aspirated. If no free fluid was present, a very small (less than 1.0 ml.) amount of sterile 0.9% Na Cl was injected and immediately withdrawn to check that the needle point was free in the joint cavity. A period of five minutes (more than the mean $T^{1}/_{2}$ value PAGE 71) wa then allowed to elapse, with the patient lying comfortat to allow recovery from the immediate effects of knee injection. The knee was then immobilised with sandbags and the isotope was introduced very gently and slowly

TABLE VI

The $T^{1}_{\frac{1}{2}}$ values obtained by the original and by the amended method of injection

Original Method n = 12	Amended Method n = 8
3.0	1.3
3.4	1.1
3.6	0.8
2.0	1.4
3.2	1.4
3.0	0.8
3.2	1.3
2.6	1.5
1.8	
6.4	
2.7	
2.7	
Mean 3.09	1.2
± S.E.M. 0.34	0.096

$p = < 0.001$

(mean injection time 60 seconds) into the joint cavity. The needle was carefully withdrawn and counting commenced in the usual manner. This procedure was followed in thirty consecutive, normal (3) and rheumatoid (27), 133 Xe studies.

Results

No $T^{1}\frac{1}{2}$ value was calculable in 2 normal and in 19 rheumatoid subjects.

The $T^{1}\frac{1}{2}$ value in the remaining normal subject was 0.8 minutes.

The mean and standard error of the $T^{1}\frac{1}{2}$ values in the other 8 rheumatoid subjects was 1.2 ± 0.096 minutes. This differs significantly ($p < 0.001$) from the $T^{1}\frac{1}{2}$ values obtained previously (page 71) by the original method (Table 6).

Discussion

Some workers (Harris and Millard, 1956 : Harris et a 1958 : Scholer et al., 1959 : Scoccianti, 1966 : Hernborg, 1969) have observed a monoexponential clearance pattern of different substances from the rheumatoid knee joint, whereas in other studies the pattern of clearance curve obtained was similar to the

biexponential curves reported in this thesis (Harris et al., 1958). As a further complication, the clearance rate in the first few minutes following injection may be not increased but almost horizontal, thereafter clearance becoming established in the usual manner.

In other tissues including skin (Sjersen, 1967), brain (Harper et al., 1964 : Hoedt-Rasmussen et al., 1966) and kidney (Thorburn et al., 1963) bi- or multi-exponential clearance patterns have been obtained consistently and found to be biologically meaningful. Andersson et al. (1967), studying prostatic blood flow, have employed the early fast component in the calculation of their results. Sjersen (1967) considered that the initial fast component obtained following intradermal injection of 133 Xe is an injection phenomenon. Larsen (1966), in the study of adipose tissue, supports this assertion and he stated that the slope of this early fast component could be altered by alteration in injection technique. No evidence to explain this initial fast component has been advanced in any tissue as yet and therefore great caution is required in its interpretation.

It seems unlikely that the $T^{1}\frac{1}{2}$ value obtained in these articular studies has any biologically important meaning. It is inconstant in time and in direction and can be altered by alteration of the method of injection.

The precise explanation of this phenomenon is not immediately apparent. One possibility, suggested by the relationship to freshly blood-stained aspirate, is that some 133 Xe was being introduced directly into small blood vessels. However, the effect of such a "bolus" injection would probably have been of extremely brief duration. A second possibility is that the isotope was introduced into a relatively high pressure area, for example fibrous capsule, and that elevated local hydrostatic pressure was resulting in early acceleration of the rate of clearance of the isotope. That this is unlikely in the human studies is suggested by the ease of injection which was mandatory in every case for verification of the position of the needle. Also in many cases synovial fluid was aspirated from the joint. However, the results of the succeeding study shows that this is indeed a possible mechanism. A further possibility

discussed in a later part of this thesis (page 152 et seq.) is that local histamine or catecholamine release consequent upon tissue damage accelerates or retards the early phase of clearance. Early diffusion and mixing of the isotope may also be important. Although $T^{1}/_{2}$ values were much less frequently obtained and were of markedly reduced duration in later work reported in this thesis, the only points on the clearance graphs which were utilised in deriving $T^{1}/_{2}$ values were those following injection by over three minutes.

THE EFFECT OF THE SITE OF INJECTION ON THE CLEARANCE OF 133 Xe

At the conclusion of one 133 Xe clearance study in a greyhound, while the animal remained under anaesthetic, the patello-tibial ligament was severed and the patella was reflected, exposing the joint cavity. 133 Xe (0.1 ml.) was then injected successively into four separate areas adjoining the joint cavity. Between each injection, saline soaked swabs were packed into the joint cavity, the patella was replaced and the wound approximated with artery forceps. The 133 Xe clearance rate was then

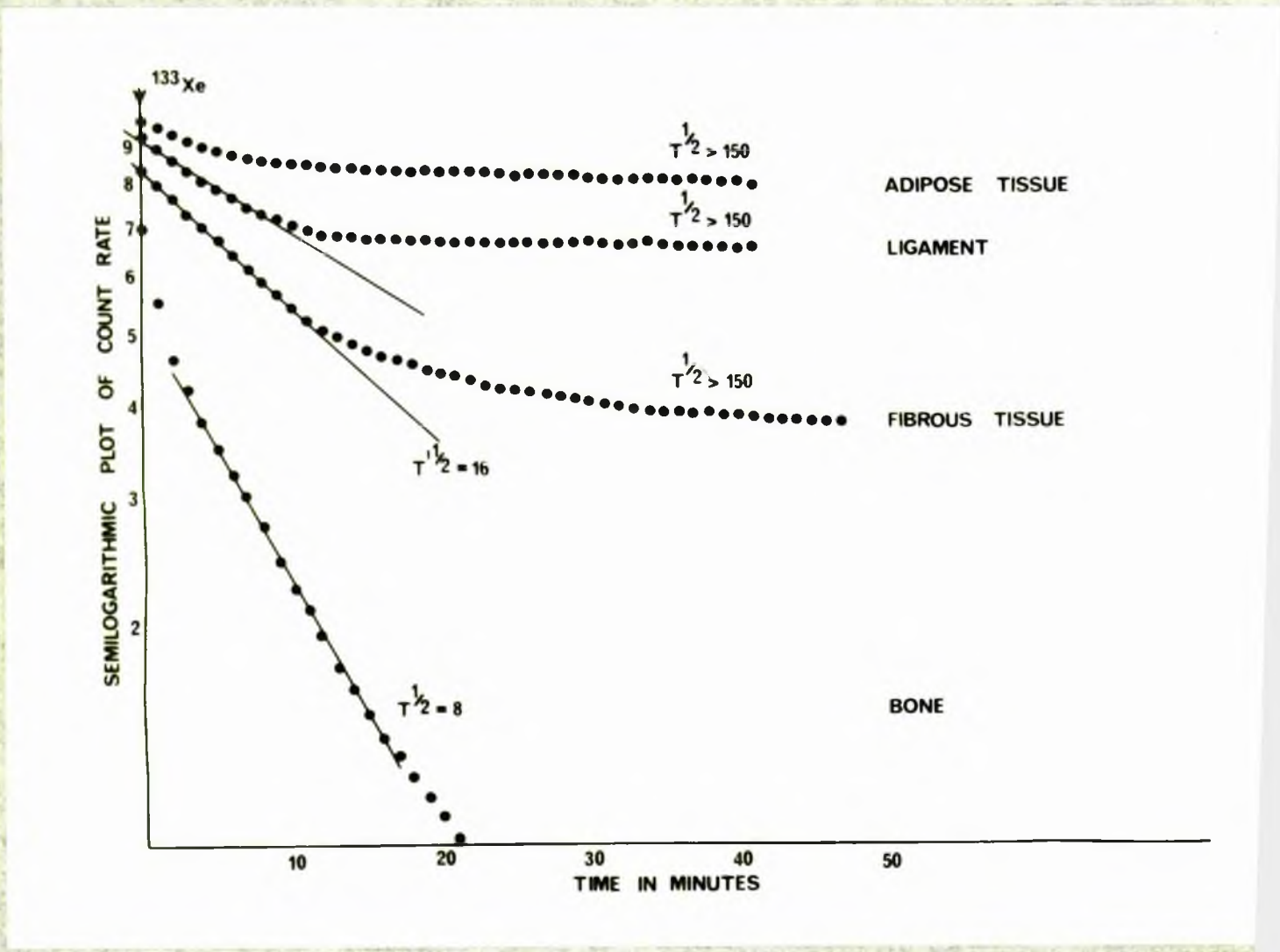


Figure 4

Legend: Clearance rates of 133 Xe injected into adipose tissue, ligament fibrous tissue and bone.

monitored for 20 minutes before the wound was again opened and the experiment repeated employing a different site of injection. The sites selected for study were the medial femoral condyle, the patellotibial ligament, an area of fibrous tissue and an area of adipose tissue. The injected area was removed at the conclusion of the study and the tissue identity confirmed histologically in each case. The pressure required to inject the isotope solution into the first three areas was noticeably greater than the pressure required to inject the isotope into the joint cavity.

Results

The half lives of the initial clearance rate is designated $T^{1}\frac{1}{2}$ and that of any succeeding exponential is recorded as $T\frac{1}{2}$ value (page 70).

The results of the $T^{1}\frac{1}{2}$ and of the $T\frac{1}{2}$ values are shown in Fig. 4. In the case of bone, ligament and fibrous tissue, the early clearance rate was markedly faster than the results commonly obtained when the isotope is introduced into the joint cavity. In the case of clearance from adipose tissue, fibrous tissue and ligament, the $T\frac{1}{2}$ values are greater than those

obtained in clearance from the joint cavity (pages 57), denoting a slower clearance rate. The $T\frac{1}{2}$ value of clearance from adipose tissue accords with 133 Xe clearance rates obtained in studies on human adipose tissue (Larsen et al., 1966). The initial fast clearance rate in adipose tissue was considered to be insufficient duration to justify calculation of a $T^{1}\frac{1}{2}$ value. The $T\frac{1}{2}$ value of clearance from bone is markedly less than all other clearance rates. Bone blood flow is of the order of 10 ml./100 ml./min. (Copp and Shim, 1965) which is in accord with the $T\frac{1}{2}$ value obtained in this study (8 mins. = 8.5 ml./ 100 ml./min.).

There being only one study in each group limits the confidence which can be employed in interpreting these results. The $T^{1}\frac{1}{2}$ values obtained in fibrous and ligamentary tissue does suggest an association with tissue composition. The early faster clearance rate may well be associated with a local increase in hydrostatic pressure. These results do, however, underline the importance which must be attached to ensuring that the needle point lies free in the joint cavity when performing the 133 Xe clearance studies. Whereas the facility of injection will assist in

excluding injection into bone, ligament or fibrous capsule, it is not possible to exclude injection into adipose tissue in this way. Aspiration of synovial fluid will certainly confirm the intra-articular position of the needle point but whereas this is not difficult in the dog and commonly in rheumatoid joints, the same can not be said for normal human subjects. When this is not possible the injection and easy withdrawal of a volume of sterile 0.9% NaCl will assist in localisation and was mandatory in the studies reported in this thesis.

Thus three possible mechanisms which may affect the pattern of clearance have been to some extent characterised. It is possible that many other factors may contribute to clearance in the early period immediately following injection. However, the remaining studies in this study were conducted with particular attention to the technique of injection which was standardised throughout, and any free fluid in the joint cavity was removed prior to the injection of 133 Xe.

Studies were then designed to examine the possible effects of certain other factors which might be expected to exert an influence on the 133 Xe clearance rate.

TABLE VII

The $T\frac{1}{2}$ values obtained prior to and following aspiration of a knee effusion in 14 rheumatoid patients. The volume aspirated in each case i noted. The correlation coefficient between the volume aspirated and the difference in $T\frac{1}{2}$ value was 0.354

	$T\frac{1}{2}$ Value (mins.)			
	Before Aspiration	After Aspiration	Difference	Volume Aspirat
	72	60	12	44
	58	50	8	52
	52	48	4	63
	32	28	4	52
	152	125	27	61
	34	14	20	70
	49	29	20	72
	43	36	7	55
	27	23	4	12
	63	35	28	35
	39	37	2	21
	56	38	18	60
	69	66	3	38
	68	50	18	30
Mean	58.14	45.64	12.30	47.
S.E.M.	8.19	7.19	2.449	4.8

T (paired) = 5.104 r = 0.354

p = < 0.001 p = > 0.1

EFFECT OF A KNEE JOINT EFFUSION ON THE CLEARANCE OF INTRA-ARTICULARLY ADMINISTERED 133 Xe

The following study was conducted to determine the effect of the presence of a knee joint effusion upon the 133 Xe clearance rate.

Subjects and Methods

Fourteen post-menopausal female patients were selected who were suffering from "classical" or "definite" rheumatoid arthritis (Ropes et al. 1959) and who had clinically obvious knee effusions. Their mean age was 50.8 years and the age range was 46 to 60 years. All patients were informed of the object of the study prior to seeking their agreement to participate. The 133 Xe studies were performed in the manner previously described (page 95) and one hour later the knee joint was aspirated to completion with full aseptic precautions. The volume of aspirate was measured. On no occasion was fresh blood observed macroscopically in the aspirate. The 133 Xe studies were then repeated in an identical manner.

Results

The $T\frac{1}{2}$ values obtained on the two occasions are shown in Table 7 together with the volume of synovial fluid aspirated from each knee joint. It can be seen that the mean $T\frac{1}{2}$ value obtained prior to aspiration

(mean 58.14± S.E.M. 8.19 minutes) was significantly higher ($p < 0.001$) than the mean value obtained following aspiration (mean 45.64± S.E.M. 7.19 minutes). The reduction in $T\frac{1}{2}$ value in each patient was not related to the amount of fluid aspirated. The correlation coefficient was 0.354 ($p \Rightarrow 0.1$).

Discussion

These results show that the presence of a knee joint effusion reduces the rate of clearance of 133 Xe from the knee joint. This accords well with theoretical considerations. Thus, the further the isotope is from the effluent blood flow the more the diffusion coefficient will operate. Since the diffusion rate of 133 Xe is slow (page 59), the rate of clearance will be reduced.

Of practical importance however is the fact that relationship between the volume of the aspirate and the degree of reduction in clearance rate is not significant ($r = 0.354$). Thus, the presence of a knee joint effusion introduces an avoidable variable into the 133 Xe studies. Accordingly, wherever possible, an effusion which was present was aspirated as completely as possible prior to the 133 Xe studies reported hereafter.

REPRODUCIBILITY OF 133 Xe CLEARANCE RATE

Variability of results is encountered in any biological system whether the method of monitoring is subjective or objective. Whereas it is certainly possible to reduce biological variation by attention to technique, it is not possible to remove it. The only alternative, therefore, is to examine the variation encountered, to quantitate it and to interpret all future results accordingly. The following studies were designed to examine the inherent variability of the 133 Xe clearance rate in normal and diseased human subjects and in the greyhound.

REPRODUCIBILITY OF LINE-FITTING

The only aspect of the calculation of the $T\frac{1}{2}$ values which is open to inter- and intra-observer error is the process of fitting a straight line to the semi-logarithmic plots of the results. It became obvious in the course of these 133 Xe studies that, provided the knee remained absolutely immobilised throughout the monitoring period and the counting geometry was maintained, semilogarithmic plots of the count rate clearly described a straight line. However, in several cases, doubt existed and accordingly the inter-

TABLE VIII

The $T\frac{1}{2}$ values (mins.) obtained when the same results were calculated by the same observer on different occasions

INTRA-OBSERVER ERROR

	Occasion 1	Occasion 2
	65	60
	30	32
	10	12
Results from	46	42
rheumatoid	22	20
knees	27	25
n = 9	21	24
	36	32
	46	41

Standard Error of the difference 1.07 mins.

	260	245
	170	185
Results from	220	265
normal knees	125	150
n = 9	360	290
	125	145
	330	195
	250	235
	265	270

Standard Error of the difference 18.7 mins.

TABLE IX

The $T\frac{1}{2}$ values (mins.) calculated from the same results by two different observers

INTER-OBSERVER ERROR

	Observer 1	Observer 2
	17	18
	69	62
	17	15
	17	18
Results from	17	15
rheumatoid	50	50
knees	9	13
n = 14	17	17
	23	17
	47	52
	40	46
	18	22
	42	31
	20	17

Standard error of the difference 1.31 mins.

	130	145
	170	130
	260	190
Results from	150	180
normal	190	160
knees	280	330
n = 10	310	250
	220	210
	265	180
	110	125

Standard error of the difference 14.44 mins.

and the intra-observer errors were quantitated.

Method

To determine the inter-observer error, the counts recorded in 14 rheumatoid subjects in whom the semilogarithmic plots of count rate against time did not describe a straight line were re-plotted and the $T\frac{1}{2}$ values calculated separately by two different observers. Similarly, $T\frac{1}{2}$ values were determined by two observers from the counts recorded from 10 normal subjects which likewise did not describe a straight line when plotted semilogarithmically against time. To quantitate the intra-observer error, the results obtained in 9 studies of patients with rheumatoid arthritis and in 9 studies on normal subjects were plotted "blind" on two occasions, one week apart, by the same observer.

Results

The results of the $T\frac{1}{2}$ values obtained are shown in Tables 8 and 9. The standard error of the difference of the inter-observer was 1.31 minutes and that of the intra-observer error was 1.07 minutes for the rheumatoid graphs. However, the standard error of the difference of the inter-observer error was 14.44 minutes and that of the intra-observer error was 18.7 minutes in the case of the normal graphs.

Discussion

It can be seen from these results that the inter- and intra-observer for the normal graphs is greatly in excess of that recorded in the case of the rheumatoid graphs. This is closely related to the position of the graphs on the semilogarithmic scale (Fig. 6 : page 69). To determine the $T\frac{1}{2}$ of the normal subjects the graph, running almost parallel to the horizontal axis, must be produced for some considerable distance and this is not the case in the faster clearance graphs of the rheumatoid subjects (Fig 4, p 69. Also it must be appreciated that the graphs employed to determine these errors were the most irregular graphs obtained in the course of these studies. However, the wide variation found in the normal subjects must be constantly remembered in considering the results of the succeeding studies. The error is very small in the case of the results obtained in studies on patients with rheumatoid arthritis.

EFFECT OF REPEATED ADMINISTRATION OF ISOTOPE INTO THE SAME JOINT CAVITY

In experiments previously reported (page 90), 133 Xe was injected consecutively four times into the stifle joint and the clearance rate monitored between

TABLE X

Reproducibility of $T\frac{1}{2}$ values of 133 Xe clearance: 12 studies showing the effect on $T\frac{1}{2}$ values of a second injection of 133 Xe given about 15 minutes after the initial dose.

$T\frac{1}{2}$ (minutes) after 1st injection of 133 Xe	$T\frac{1}{2}$ (minutes) after 2nd injection of 133 Xe
150	150
150	150
100	100
63	56
55	51
55	52
41	39
32	27
30	27
28	27
28	25
13	16

Standard error of the difference = 0.82 minutes (Calculated from lower 10 figures in the Table).

each injection for 15 minutes. This experiment was repeated four times and on each occasion the $T\frac{1}{2}$ values obtained (Table 3) were similar.

The same protocol was followed in twelve rheumatoid patients. In each patient approximately 10 μCi of 133 Xe dissolved in 0.2 ml. of 0.9% NaCl were injected into the joint cavity on two consecutive occasions with an intervening control monitoring period of 15 minutes. Again the $T\frac{1}{2}$ values obtained following each injection were similar (Table 10). The standard error of the difference was only 0.82 mins. However, clearly no result can be considered to be significant unless it is at least outwith the range of the difference of the values quoted (7 minutes). This is particularly relevant in the studies on the effect of pharmacological agents reported later in this thesis (pages 152 et seq).

VARIATION WITH TIME OF INJECTION ON THE CLEARANCE OF 133 Xe

Morning and Evening Studies

In eight rheumatoid subjects studies were performed at 9 a.m. and again at 6 p.m. The $T\frac{1}{2}$ values obtained are shown in Table 11.

TABLE XI

$T\frac{1}{2}$ values obtained when 133 Xe studies were performed at 9 a.m. and at 6 p.m. in six patients with rheumatoid arthritis

	$T\frac{1}{2}$ values (minutes)	
Patient	9 a.m.	6 p.m.
1	46	42
2	62	60
3	39	38
4	56	59
5	54	58
6	60	65
mean	52.8	53.6
$\pm$ Standard Error	3.58	4.46

Standard error of difference = 1.49 minutes.

TABLE XII

$T\frac{1}{2}$ values obtained when 133 Xe studies were performed on separate occasions, 24 hours apart, in 7 rheumatoid patients and in one normal subject.

	$T\frac{1}{2}$ values obtained (minutes)	
Subject	Day 1	Day 2
1 (R.A.)	30	25
2 (R.A.)	50	40
3 (R.A.)	30	28
4 (R.A.)	43	47
5 (R.A.)	34	30
6 (R.A.)	30	25
7 (R.A.)	30	34
8 (normal)	120	135

Standard error of the difference of the values obtained in the seven rheumatoid subjects = 1.93 mins.

The standard error of the difference was only 1.49 minutes.

Thus no circadian rhythm was detectable in these abnormal subjects.

Day to Day Variation

In seven patients with rheumatoid arthritis and in one normal subject 133 Xe studies were performed on two occasions twenty-four hours apart. The $T\frac{1}{2}$ values obtained are shown in Table 12. The standard error of the difference was 1.93 minutes.

VARIATION WITH SITE OF INJECTION

Since the diffusion rate of 133 Xe in tissue is slow (1.0×10^{-5} cm^2/sec. in tissue - page 59) the rate of clearance of the isotope is probably relevant only to the areas anatomically closely related to the site of deposition of intra-articularly injected 133 Xe The histology of synovium is by no means uniform (Chap. 1 : page 8) and accordingly it was considered necessary to investigate the variation in clearance rate from different sites within the joint.

Method

Six patients with "classical" or "definite" rheumatoid arthritis were selected. All of these patients had clinical involvement of the knee joint

TABLE XIII

The $T\frac{1}{2}$ values obtained when the isotope was introduced into the joint, on one occasion by a medial approach and on the second occasion by a lateral approach

Patient	Knee Score	Medial Approach	Lateral Approac
1	12	28	27
2	12	46	49
3	12	33	30
4	4	68	65
5	4	57	59
6	4	78	78

Standard error of the difference 1.02 mins.

but the degree of involvement was not uniform. Thus three patients had clinical knee scores (page 112) of +12 and the other three had knee scores of +4. All were female and their mean age was 60.2 years (range 55 to 64 years). In all patients their arthritis was in a "stable" state at the time of study and no alteration in their clinical state or regimen of therapy was noted during this study.

133 Xe studies were performed on two occasions within 24 hours. On the first occasion the isotope was introduced by a medial infra-patellar approach and on the second occasion by a lateral infra-patellar approach. Otherwise the method of injection and the counting conditions were identical.

Results

The $T\frac{1}{2}$ values (in minutes) obtained on the two occasions in each patient are shown in Table 13. It can be seen that the values obtained on each occasion were closely similar regardless of the degree of involvement in the inflammatory process. The standard error of the difference of the values obtained on the two occasions was 1.02 minutes.

Discussion

Thus these results show that the clearance of

intra-articularly administered 133 Xe is similar, regardless of the site of injection. This suggests that, although the pathological process is not uniform, the changes affect the parts of the synovium subserving isotope clearance sufficiently severely to render the rate of clearance of 133 Xe abnormal and sufficiently uniformly to give similar clearance rates.

VARIATION WITH SEX

Method

The results obtained in over 800 studies performed on patients suffering from "classical" rheumatoid arthritis from the inception of the technique until May 1969 were surveyed. From these results, fourteen male and fourteen female subjects were selected who were age-weight- and as far as possible disease-matched. The parameters of disease-matching included evidence of systemic disease by disease duration, erythrocyte sedimentation rate, articular index (Ritchie et al., 1968 - page 242), sheep's cell agglutination titre (Ziff, 1957), haemaglobin and white cell count and evidence of local disease in the joint studied. For the assessment of local disease, a knee score was employed which is utilised throughout the succeeding studies in this thesi

TABLE XIV

The $T\frac{1}{2}$ values obtained when 133 Xe studies were performed in 14 male and 14 female patients who were suffering from rheumatoid arthritis

	Male	Female
	56	57
	48	52
	62	64
	38	36
	26	25
$T\frac{1}{2}$ values	81	86
obtained	65	60
(minutes)	59	61
	32	31
	38	35
	46	43
	42	45
	58	61
	72	73
Mean	51.6	52.1
Standard Error	4.21	4.59

$0.9 > p > 0.8$.

TABLE XV

The mean and standard error of the parameters employed in comparing the $T\frac{1}{2}$ values obtained in male and in female subjects

Matching Parameter	Male	Female
Disease duration (yrs.)	5.6 ± 1.04	5.5 ± 1.12
E.S.R.	52 ± 3.6	50 ± 4.1
Hb	10.8 ± 0.8	10.9 ± 0.7
w.b.c.	6.5 ± 1.1 thousand	6.7 ± 2.06t
SCAT titre	128	128
Articular Index	56 ± 3.6	52 ± 4.1
Knee Score	7.0 ± 1.14	7.5 ± 1.06
Age (years)	55 ± 4.2	57 ± 3.8
Weight (Kg)	78 ± 5.6	72 ± 6.1

A history of pain and of tenderness was elicited and graded. The knee was then subjected to firm pressure over the joint margin and the response graded. Finally the knee was palpated and the degree of swelling was graded. Grading was on a 0 to 3 basis where 0 = "absent" 1 = "slight" 2 = "moderate" and 3 = "severe". The maximum score for any individual joint was therefore +12.

Results

The results are shown in Tables 14 and 15. The $T\frac{1}{2}$ values obtained are similar in the female and male subjects when the other variables are stabilised.

The values obtained in male subjects (mean 51.6 minutes $\pm$ S.E.M. 4.21 minutes) were not significantly different ($0.9 > p > 0.8$) from the values obtained in female subjects (mean 52.1 minutes $\pm$ S.E.M. 4.59 minutes) by a student t-test. The mean and S.E.M. of the parameters employed in comparing the groups of patients are shown in Table 15. It can be seen that the groups of patients were closely similar in all respects. Thus no sex difference is encountered in these 133 Xe studies such as has been reported with radioactive sodium (Jacox et al., 1952).

TABLE XVI.

The $T\frac{1}{2}$ values obtained in 16 rheumatoid patients within the age range 30 to 40 years and in a further 16 patients within the age range 55 to 65 years.

	Age Group	
	30/40 years	55/65 years
	49	47
	76	77
	71	75
	82	80
	26	28
	36	39
	32	31
$T\frac{1}{2}$ values	44	42
obtained	41	43
(minutes)	29	32
	57	53
	53	53
	67	68
	39	37
	51	55
	43	42
Mean	49.75	50.13
Standard Error	4.24	4.21

$0.97 > p > 0.95$

TABLE XVII

The mean and standard error of the mean of the parameters employed in comparing the $T\frac{1}{2}$ values obtained from the different age groups studied.

	Age Group	
Matching Parameter	30 to 40 years n = 16	55 to 65 years n = 16
Disease duration (yrs.)	3.1 ± 1.01	3.3 ± 0.81
E.S.R.	36 ± 4.2	39 ± 2.9
Hb	11.1 ± 0.9	11.4 ± 0.8
w.b.c.	6.9 ± 1.6 thousand	6.2 ± 1.1
SCAT titre	128	128
Articular Index	48 ± 4.2	47 ± 3.9
Knee Score	5 ± 1.2	5.3 ± 1.01
Weight (Kg)	82 ± 4.2	79 ± 3.6

VARIATION WITH AGE

133 Xe clearance studies have been previously reported (page 82) which show that no variation could be demonstrated in normal subjects within the age groups studied. The following investigation was undertaken to exclude the possibility of variation with age in diseased subjects.

Method

In a similar manner to that described for investigation of sex difference, all the results were again scrutinised. Thirty-two patients were selected who were weight-matched and disease-matched. Sex matching was omitted and disease matching was performed on the same basis as has been described above. Sixteen of these patients were between the ages of thirty and forty years and sixteen were between the ages of fifty-five and sixty-five years.

Results

The results are shown in Tables 16 and 17. The $T\frac{1}{2}$ values obtained in the patients aged thirty to forty years (mean 49.75 $\pm$ S.E.M. 4.24 minutes) did not differ significantly ($0.97 > p > 0.95$) from the values obtained in the patients aged between fifty-five and sixty-five years (mean 50.13 $\pm$ S.E.M. 4.21 minutes)

by a paired student t-test. The mean and standard error of the parameters employed in comparing these results are shown in Table 17 where it can be seen that the two groups were similar in other respects. Age, therefore, within the limits of the study, does not seem to influence the clearance rate of 133 Xe.

The rate of clearance of intra-articularly administered 133 Xe from the joint cavity possesses an acceptable degree of inherent biological variation provided that the isotope is delivered in a small diluting volume, that the injection is carefully and accurately accomplished and that any free fluid is aspirated before the commencement of the study. Clearance is then monoexponential for approximately 40 minutes after injection and there is little variation with age, sex, time or site of injection. The error of calculating $T\frac{1}{2}$ values is small in the case of relatively fast clearance rates but becomes considerable when the clearance rate is slow.

SUMMARY

1. Clearance is monoexponential for approximately 40 minutes after injection. Thereafter a slower exponential may occur.

2. Early fast clearance rates may be related to volume of diluent employed or to the method or site of injection. Clearance under these circumstances may occur through routes other than the femoral vein. This inconstant transient phenomenon is unlikely to be biologically important in these studies.

3. The clearance rate is increased following aspiration of an effusion but not in proportion to the volume of aspirate.

4. Reproducibility of line-fitting to derive $T\frac{1}{2}$ values is considerably higher when the clearance rate is fast than when it is slow.

5. Inherent biological variation within short time limits and with age and sex of the subject is small.

CHAPTER 4

The change in the rate of clearance of intra-articularly injected 133 Xe from the normal canine joint following external heating and with local administration of vaso-reactive drugs is reported in this chapter.

The response to the intra-articular injection of exogenous catecholamines and their respective blocking drugs was studied in an attempt to demonstrate the presence of α and β adrenergic receptors in normal synovium. A similar but limited study was conducted in joints which were the seat of experimentally induced osteoarthritis and synovitis and the results of both are reported in this chapter.

The change in clearance rate resulting from the local administration of methacholine and of atropine was studied to investigate the effects of parasympathetic stimulation and studies were conducted to characterise the effects of histamine and of serotonin on the clearance rate. The results of these studies are also reported in this chapter.

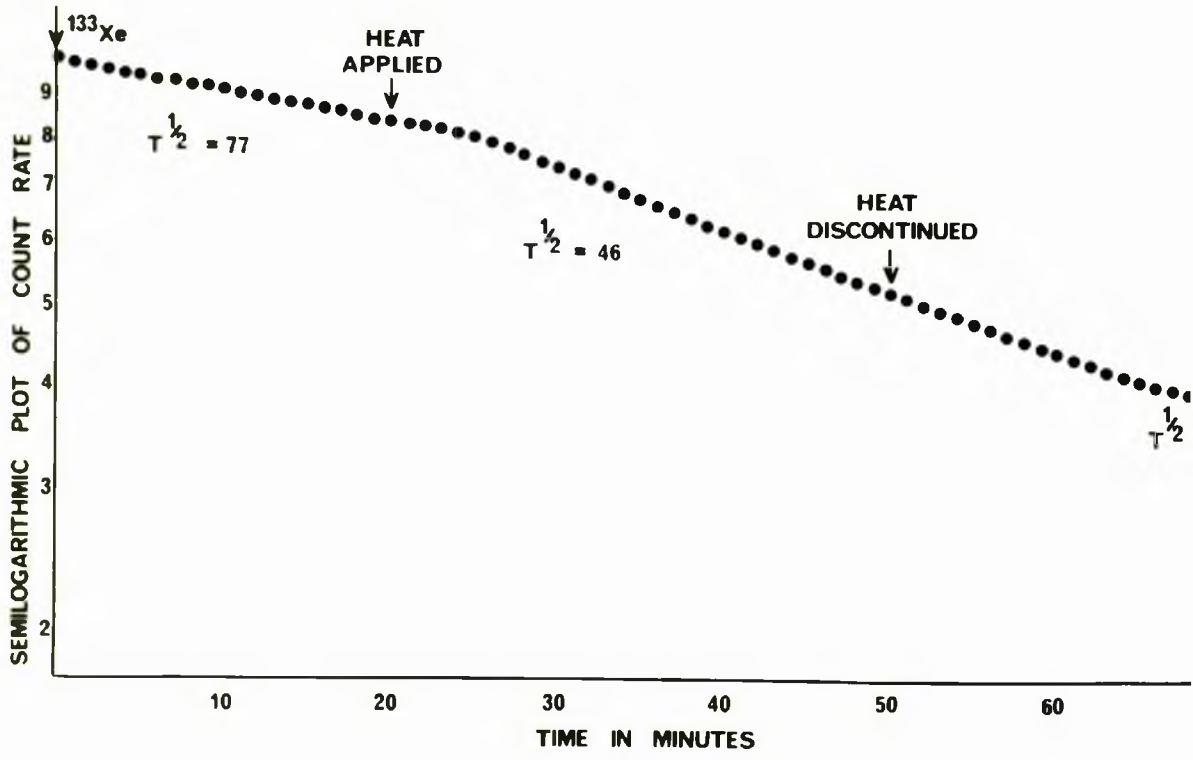


Figure 1

Legend: The effect of externally applied heat on the clearance rate of 133 Xe.

EFFECT OF EXTERNAL HEATING ON THE 133 Xe CLEARANCE RATE

Introduction

The effect of the application of external heat on the rate of clearance of intra-articularly injected 133 Xe from the stifle joint was determined in anaesthatised adult greyhound dogs.

Materials and Methods

Twelve experiments were conducted employing both stifle joints of six greyhounds weighing from 24 to 30 kg. The anaesthetic (Chap. 2 : page 44) the injection (Chap. 3 : page 96) and the counting (Chap. 2 : page 39) technique have been described. The baseline clearance rate was monitored for 20 minutes in each study and thereafter the joint was exposed to external heating with a Philip's short-wave diathermy lamp. The detector was protected from the heat source and the count rate was monitored throughout the heating period (30 minutes) and for a further 20 minutes following removal of the heat source.

Results and Interpretation

An example of the semilogarithmic plot of the result is shown in Fig. 1. Five minutes following the application of heat the clearance rate accelerated and

TABLE I

$T\frac{1}{2}$ values (minutes) before during and after the exhibition of external heat to the canine stifle joint. The time intervals between the application or removal of the heat source and the subsequent change in clearance rate are shown.

	$T\frac{1}{2}$ (mins.) Before Heating	Time (mins.) to Change	$T\frac{1}{2}$ (mins.) During Heating	Time (mins.) to Change	$T\frac{1}{2}$ (Aft Heat
	68	4	42	9	69
	49	3	31	11	48
	52	6	40	20	51
	54	5	32	15	50
	77	5	46	17	74
	70	8	53	16	72
	42	8	25	13	42
	55	4	20	8	55
	54	6	36	20	57
	65	9	51	12	67
	67	5	50	12	64
	60	7	36	14	56
Mean	59.4	5.8	38.5	13.9	58
Range	42-77	3-9	20-53	8-20	42

similarly there was a delay of 8 minutes following the removal of the heat source before the clearance rate began to decelerate and return to its pre-heating value. The $T_{\frac{1}{2}}$ values (mins.) derived from the pre-heating, heating and post-heating periods are shown in Table 1.

These results indicate that the exhibition of external heating results in an increase in clearance rate and therefore in synovial perfusion rate in canine synovium. The increase, while persisting during heating, is transient lasting only from 8 to 20 minutes following the removal of the heat source.

ADRENERGIC CONTROL OF SYNOVIAL PERFUSION

THE PRESENCE OF α AND β RECEPTORS

Introduction

Both functional (Kellgren and Samuel, 1950) and histological (Gardner, 1950 : Boyd, 1954 : Skoglund, 1956 : Polacek, 1961 : Goldie and Wellisch, 1969) techniques have been employed to demonstrate the presence of nerve fibres in human synovial tissue. In addition, Cobbold and Lewis (1956a,b,c) have studied the response of "joint blood flow" to both physical and pharmacological agents in an isolated perfused animal preparation and demonstrated the presence of both sympathetic and parasympathetic responses.

The experimental model employed in the study of the route of clearance of 133 Xe (page 50 : Fig. 3) is particularly suited to the study of the physiological responses of the synovial vasculature to pharmacologically active agents. The only interference with normal mechanisms in the joint is anaesthesia and the introduction of minute volumes of isotope and compounds. In the present study, the monitoring system employed for the detection of the vascular effects of the compounds administered was the 133 Xe clearance rate. The femoral venous flow

rate and femoral venous radioactivity were also monitored.

It is currently believed that a "receptor site" is a specialised area on the protein surface of a muscle cell. When the fibre of the nerve cell is stimulated, a chemical mediator is released across the myoneural junction and combines with this specialised area producing an effect. Adrenergic receptor sites may be demonstrated histochemically (Falck and Owman, 1965) or their presence may be inferred from the effects of selected adrenergic agents and their antagonists on a suitable monitoring system. The latter method is employed in the present study. The compounds employed were selected from the point of view of both specificity and duration of action. Whereas few compounds have exclusively either α or β effects, the peripheral effect of noradrenaline is mainly on α and of isoprenalin mainly on β receptors. Similarly, phentolamine and propranolol act principally as α and β receptor blockers respectively although a direct action of phentolamine on small blood vessels has been demonstrated (Goodman and Gilman, 1965).

The present study was designed to demonstrate the presence of α and β receptors in synovial tissue by

monitoring the effect of both α and β receptor stimulation and inhibition on the 133 Xe clearance rate.

TABLE II

DRUG STUDIES - EXPERIMENTAL PROTOCOL

Group	No. of Experiments	Control Monitoring Period	Drug Administered I	Response Monitoring Period - I	Drug Administered II	Response Monitoring Period - II	Parameters studied All*	Parameters studied $T_{\frac{1}{2}}$ B.P. Pulse only
1	9	15 mins.	Isoprenaline (5μg)	15 mins.	-	-	1	8
2	6	15 mins.	Noradrenaline (5μg)	15 mins.	-	-	1	5
3	7	15 mins.	Propranolol (20μg)	15 mins.	Isoprenaline (5μg)	15 mins.	1	6
4	8	15 mins.	Phentolamine (20μg)	15 mins.	Noradrenaline(5μg)	15 mins.	0	8
5	6	15 mins.	Phentolamine (40μg)	15 mins.	Noradrenaline(5μg)	15 mins.	1	5
6	6	15 mins.	Phentolamine (20μg)	15 mins.	Isoprenaline (5μg)	15 mins.	0	6
7	4	15 mins.	Propranolol (20μg)	15 mins.	Noradrenaline(5μg)	15 mins.	0	4
8	7	15 mins.	Phentolamine (20μg)	15 mins.	-	-	0	7
9	6	15 mins.	Phentolamine (40μg)	15 mins.	-	-	1	5
10	6	15 mins.	Propranolol (20μg)	15 mins.	-	-	1	5
11	9	15 mins.	Isoprenaline (5μg)	15 mins.	Noradrenaline(5μg)	15 mins.	0	9
12	9	15 mins.	Noradrenaline (5μg)	15 mins.	Isoprenaline (5μg)	15 mins.	0	9
13	1	0	Isoprenaline (5μg)	15 mins.	-	-	0	1
14	1	0	Noradrenaline (5μg)	15 mins.	-	-	0	1

The experimental protocol employed in studying the effect of α and β receptor stimulation on the clearance rate of intra-articularly administered 133 Xe.

* "All parameters" denotes continuous monitoring of clearance rates from both test and control stiffle joints, femoral venous flow rate and count rate, blood pressure and pulse rate.

MATERIALS AND METHOD

A series of experiments was undertaken on the stifle joints of adult greyhound dogs at the Wellcome Surgical Research Centre, Glasgow.

The anaesthetic procedure, the injection technique and the counting and monitoring of clearance rate, femoral flow rate and femoral venous activity have been described (Chap. 2 : page 46). The needle remained in place throughout each experiment, closed by a syringe containing sterile 0.9% NaCl, thus providing a convenient route for the administration of the active compounds.

The experimental protocol is shown in Table 2. Fourteen groups of studies were conducted. In all but two groups the experiment commenced with a control period of 15 minutes duration during which baseline values were obtained. The active compounds were administered in the order shown in Table 2 and a monitoring period of 15 minutes was allowed following administration of each compound. In at least one study in each major group, in addition to blood pressure pulse rate and clearance rate from the joint, the femoral venous flow rate and activity and clearance from the opposite stifle joint were also monitored continuously. Clearance rates are expressed as

$T\frac{1}{2}$ values (minutes). In two studies the effect of administering the isotope and active compound simultaneously without a preliminary control monitoring period was studied. The compounds employed on these occasions were isoprenaline (5 µg) and noradrenaline (5 µg).

The pharmacological agents employed in this study were:

Isoprenaline sulphate in normal saline (Boots Pure Drug Co.),

Phentolamine mesylate BP ("Regitine", Ciba Laboratories Ltd.),

Propranolol hydrochloride ("Inderal", Imperial Chemical Indutries), and

l-noradrenaline acid tartrate ("Levophed", Bayer Production Co.).

These preparations were diluted in normal saline to achieve a concentration of 5 µg/0.25 ml. for isoprenaline and noradrenaline and 20 µg/0.25 ml. for propranolol and phentolamine.

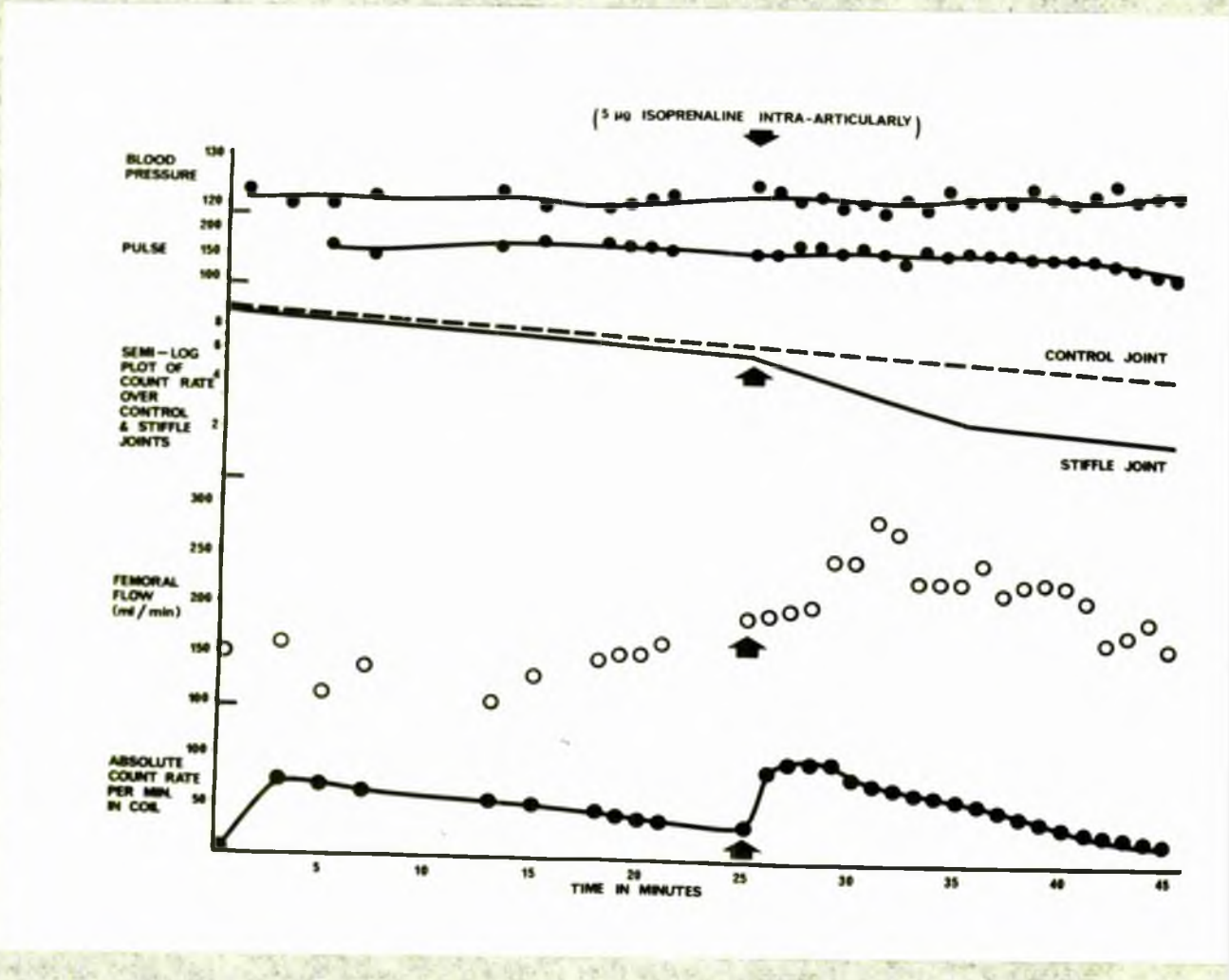


Figure 2

Legend: The effect of intra-articular isoprenaline (5 µg.). Blood pressure, pulse rate and the clearance rate from the opposite joint remain unchanged.

The rate of clearance from the injected join the femoral flow rate and femoral venous activity are all increased following injection.

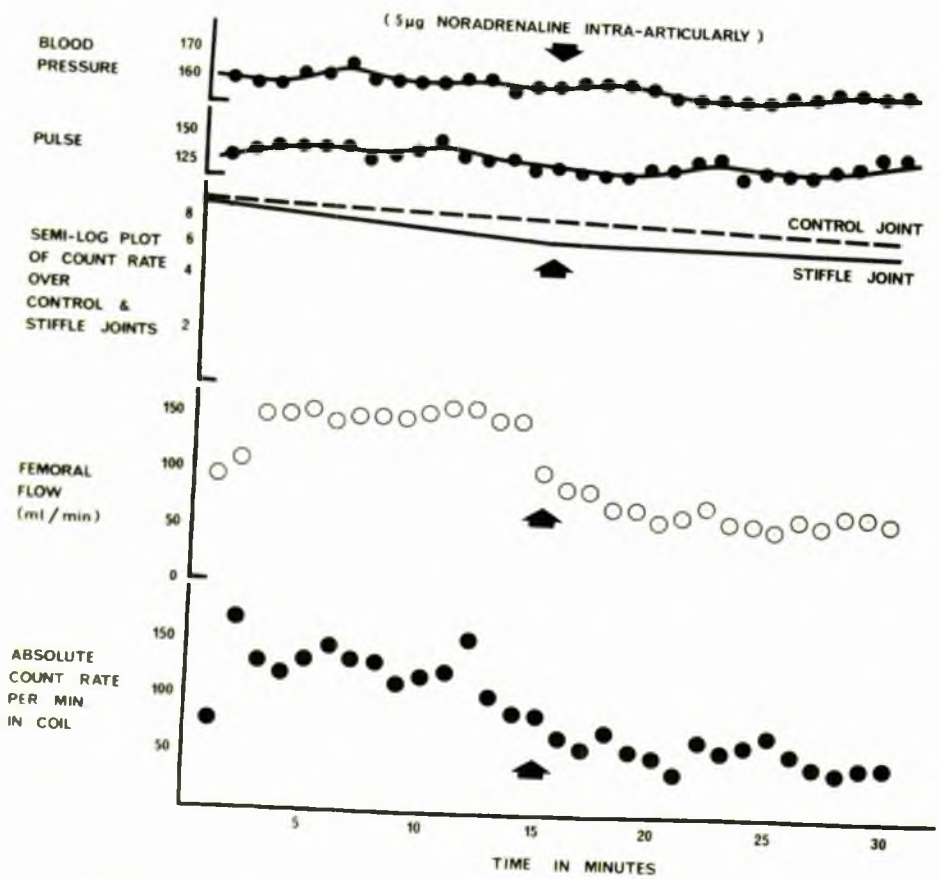


Figure 3

Legend: The effect of intra-articular noradrenaline (5 µ g.). Blood pressure, pulse rate and clearance from the opposite joint remain unchanged.

The rate of clearance from the injected joi the femoral flow rate and femoral venous activity ar all reduced following injection.

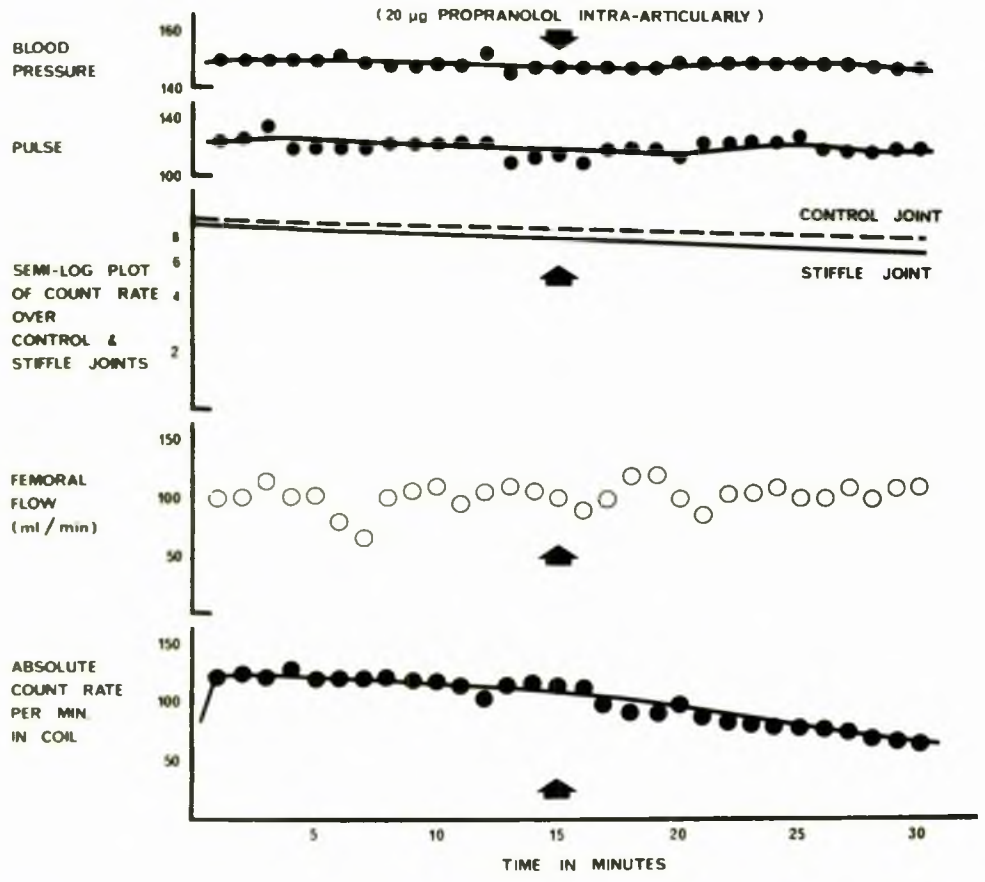


Figure 4

Legend: The effect of intra-articular propranolol (20 µg.). Blood pressure, pulse rate, clearance rate from both opposite and injected joints, femoral flow rate and femoral venous activity all remain unchanged following injection.

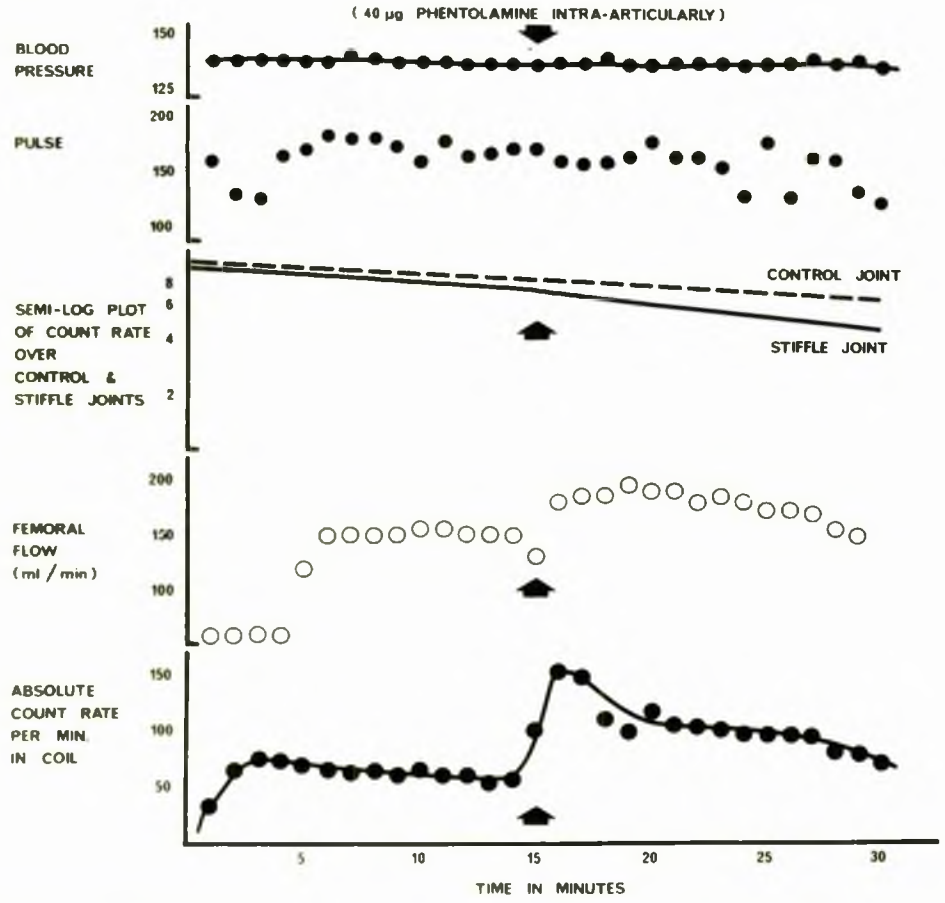


Figure 5

Legend: The effect of intra-articular phentolamin
(40 γ g.). Blood pressure, pulse rate and clearan
from the opposite joint remain unchanged.

The rate of clearance from the injected
joint, femoral flow rate and femoral venous activ
are all increased following injection.

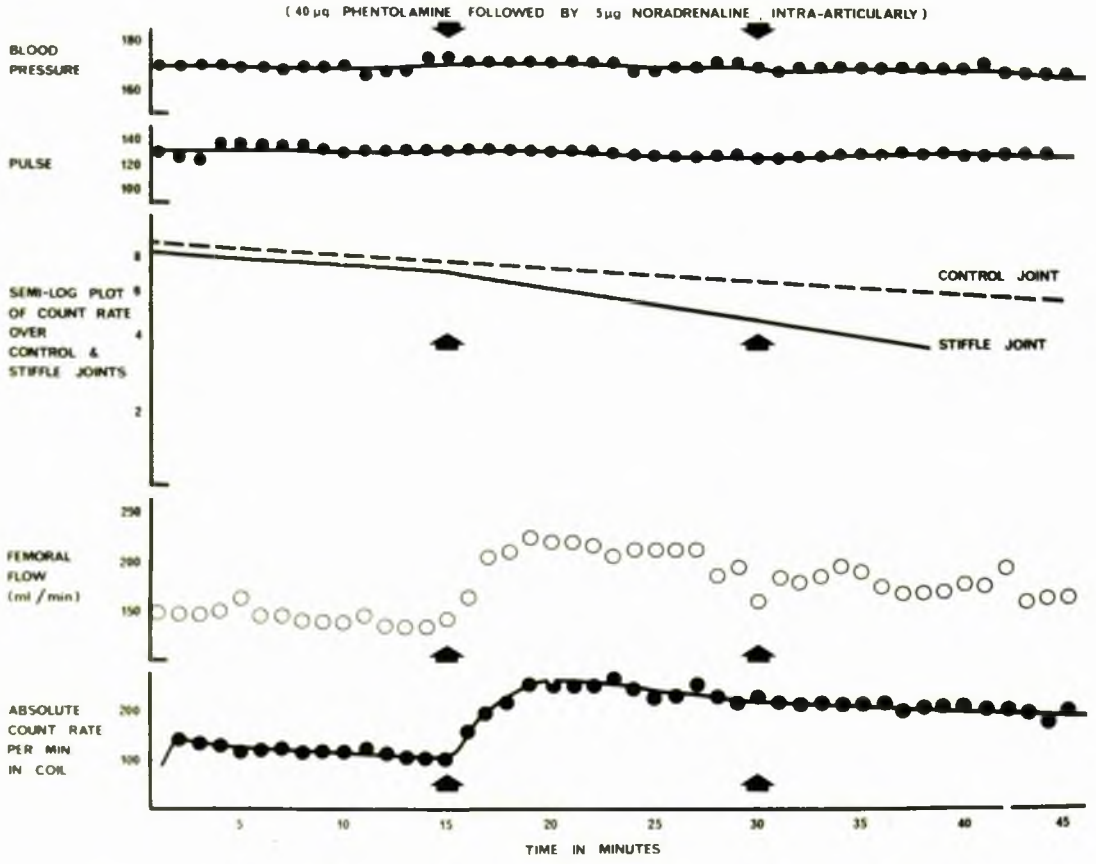


Figure 6

Legend: The effect of intra-articular noradrenalin (5 ug.) following the prior administration of phentolamine (40 µg.).

Blood pressure, pulse rate and clearance from the opposite joint remain unchanged throughout the experiment.

Although the clearance rate from the injected joint, the femoral flow rate and femoral venous activity are all increased following the injection of phentolamine (40 µ g.), there is no subsequent change following noradrenaline (5 µ g.).

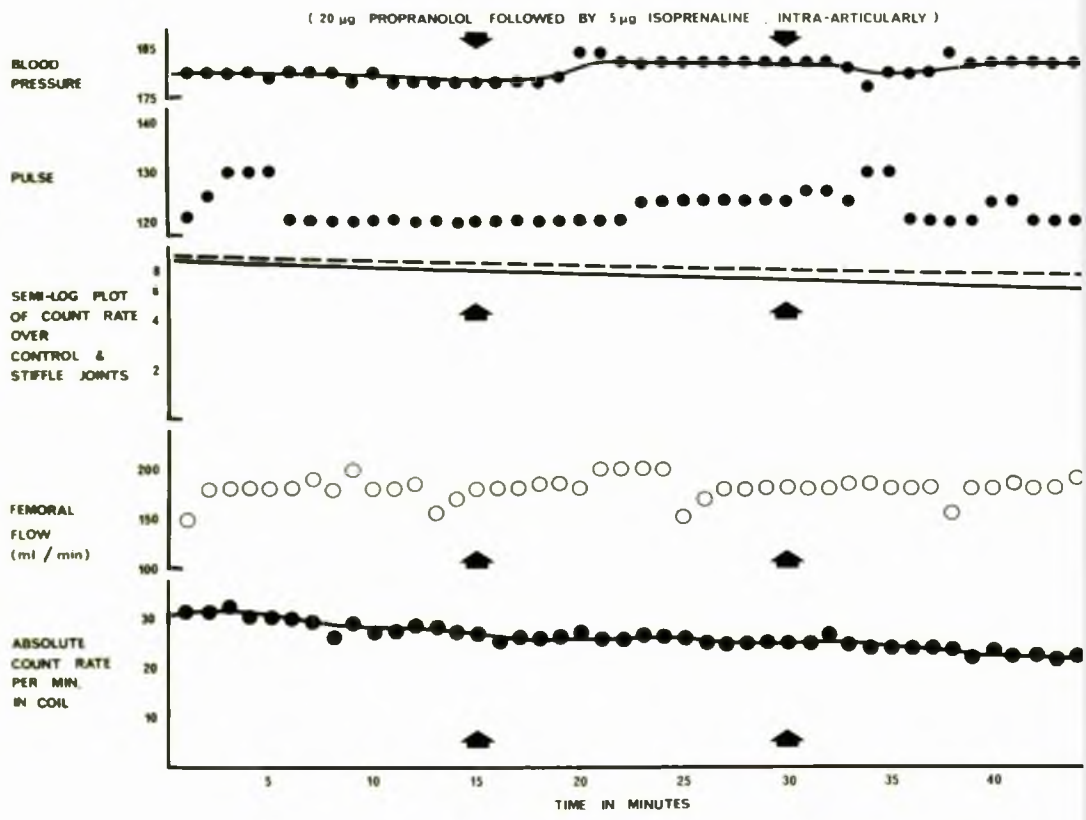


Figure 7

Legend: The effect of intra-articular isoprenaline (5 γ g.) following the prior administration of propranolol (20 γ g.).

Blood pressure, pulse rate, the clearance rate from opposite and injected joints, the femoral flow rate and femoral venous activity remain unchang throughout the experiment.

RESULTS

The effects of intra-articularly administered drugs on the clearance of 133 Xe are shown in Figs. 2 to 7 and Tables 3 to 7.

In Figs. 2 to 7 the values obtained for pulse rate, blood pressure, clearance rates ($T\frac{1}{2}$ values) from both control and test stifle joints, femoral venous flow rate and femoral venous count rate before and after the administration of each drug are shown. It can be seen that there was no alteration in blood pressure, pulse rate or in the clearance rate from the opposite stifle joint in any of these studies. Whenever a change in clearance rate from the test joint was observed, the femoral venous flow rate and femoral venous count rate altered in the same direction.

Following the intra-articular administration of isoprenaline (5 μg), the clearance rate, femoral flow rate and femoral count rate were all increased (Fig. 2). However, no change occurred when isoprenaline (5 μg) was administered after the prior administration of propranolol (20 μg) (Fig. 7).

Following the intra-articular administration of noradrenaline (5 μg), the clearance rate, femoral flow

TABLE III

EFFECT OF β RECEPTOR STIMULATION (ISOPRENALINE) ON ^{133}Xe CLEARANCE RATE - I

	Isoprenaline (5 μg) Alone		Isoprenaline (5 μg) aft Propranolol (20 μg)	
	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after
	90	21	74	74
	72	7	60	60
	35	12	110	110
	200	80	80	80
	200	11	51	51
	100	28	76	76
	95	23		
	66	9		
Mean	107	24	75	75
Range	35-200	7-80	51-110	51-110
No. of experiments showing change	8/8		0/6	

TABLE IV

EFFECT OF β RECEPTOR STIMULATION (ISOPRENALINE) ON ^{133}Xe CLEARANCE RATE - II

	Isoprenaline (5μ g) after Phentolamine (20μ g)		Isoprenaline (5μ g) aft Noradrenaline (5μ g)	
	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after
			100	100
			110	110
	80	21	110	110
	66	42	200	200
	58	11	52	52
	56	22	120	100
	64	31	20	20
	48	27	56	56
			120	51
Mean	62	25.5	99	89
Range	48-80	11-42	20-200	20-200

No. of experiments showing change

6/6 2/9

TABLE V

EFFECT OF α RECEPTOR STIMULATION (NORADRENALINE) ON ^{133}Xe CLEARANCE RATE - I

	Noradrenaline (5 μ g) Alone		Noradrenaline (5 μ g) After Isoprenaline (5 μ g)	
	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after
	60	200	21	100
	72	250	17	110
	85	280	53	110
	55	300	12	52
	76	210	34	100
			9	45
			28	150
			23	44
			11	20
Mean	69.5	248	23	81
Range	55-85	200-300	9-53	20-150
No. of experiments showing change	5/5		9/9	

TABLE VI

EFFECT OF α RECEPTOR STIMULATION (NORADRENALINE) ON 133 Xe CLEARANCE RATE - II

Noradrenaline (5 μg) after Phentolamine (20 μg)		Noradrenaline (5μg) after Phentolamine (40 μg)		Noradrenaline (5 μg) after Propranolol (20 μg)	
$T\frac{1}{2}$ before	$T\frac{1}{2}$ after	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after
34	100	100	100	37	120
24	160	28	28	66	210
44	56	52	52	55	260
11	31	48	48	72	250
28	28	58	58		
37	120				
100	100				
50	120				
Mean 41	89	57	57	57.5	210
Range 11-100	28-160	28-100	28-100	37-72	120-260
No. of experiments showing change					
6/8		0/5		4/4	

TABLE VII

EFFECT OF α and β RECEPTOR ANTAGONISTS ON 133 Xe CLEARANCE RATE

	Propranolol (20 µg.)		Phentolamine (20 µg.)		Phentolamine (40 µg.)	
	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after	$T\frac{1}{2}$ before	$T\frac{1}{2}$ after
	60	60	80	34	160	28
	80	80	110	80	120	100
	51	51	95	24	76	54
	60	60	63	50	65	45
	110	110	60	37	82	56
			60	44		
			11	11		
Mean	72	72	68	40	140	64
Range	51-110	51-110	11-110	11-80	65-160	28-100
No. of experiments showing change	0/5		6/7		5/5	

rate and femoral count rate were all decreased (Fig. 3). However, when noradrenaline (5 μg) was administered following the prior administration of phentolamine (40 μg) no change in these parameters was noted (Fig. 6).

When phentolamine (40 μg) was injected into the joint the clearance rate, femoral flow rate and femoral count rate were all increased (Fig. 5). However, when propranolol (20 μg) was injected no change in these parameters was noted (Fig. 4).

The $T\frac{1}{2}$ values obtained before and after the administration of each drug in the other studies of each group in which no coil was inserted are shown in Tables 3 to 7. In all experiments the direction of change was identical to that noted above with the exception of the results obtained when noradrenaline (5 μg) was administered following the prior administration of phentolamine (20 μg). In six of eight instances there was a distinct alteration with noradrenaline which did not occur when the dose of phentolamine employed was 40 μg (Table 6). When isoprenaline (5 μg) was injected following the prior administration of phentolamine (40 μg) the $T\frac{1}{2}$ values were markedly reduced and when noradrenaline (5 μg) was administered following the prior administration of propranolol the $T\frac{1}{2}$ values were greatly increased

(Tables 4 and 6 respectively). When isoprenaline (5 γ g) was injected after noradrenaline (5γ g) the isoprenaline effect was obtained in only two of nine experiments, whereas when noradrenaline (5 γ g) was injected following isoprenaline (5γ g), the noradrenaline effect was noted on all occasions (Tables 4 and 5).

The $T\frac{1}{2}$ values obtained when isoprenaline (5γ g) was administered mixed with 133 Xe and when noradrenaline (5 γ g) was administered with 133 Xe were 16 minutes and 170 minutes respectively.

DISCUSSION

The responses obtained in this study are qualitatively similar to those obtained in other tissues (Goodman and Gilman, 1965 : Glover and Hutchison, 1965 : Gosselin, 1966 : Brick et al., 1968). Thus, clearance rate, femoral venous count rate and femoral venous flow rate are all increased following the intra-articular injection of isoprenaline, suggesting an increase in synovial perfusion. These effects are not apparent following the prior administration of propranolol denoting the β receptor blocking action of this substance (Brick et al., 1966). However, the isoprenaline effects are demonstrable following the prior administration of the α blocking drug phentolamine (Eisenfeld et al., 1967 : Foster, 1969). Conversely the clearance rate, femoral venous flow rate and femoral venous count rate were all reduced by noradrenaline, demonstrating predominant α effect of this drug at the dose level of 5γ g. Again these effects were noted following the prior administration of propranolol. The results when noradrenaline was administered following the prior administration of phentolamine were of interest.

When the dose of phentolamine was 20 μg six of eight studies showed some response to noradrenaline. However, when the dose of phentolamine was 40 μg no response to noradrenaline was observed. These findings suggest that the blocking action of phentolamine on the α effect is dose related. Phentolamine given intravenously produces vasodilatation and cardiac stimulation (Goodman and Gilman, 1965). The blood pressure response varies with the relative contributions of the two effects. This drug also increases the peripheral blood flow, predominently in the skin, but with the doses usually employed in man this is often much less than that due to local anaesthetic nerve block. The dilatation is due primarily to a direct action on vascular smooth muscle; however, with higher doses of phentolamine α adrenergic blockade may also be involved, thus demonstrating a dose related effect. It would appear therefore that in low dosage phentolamine probably acts mainly on vascular smooth muscle while in high dosage it acts as an α adrenergic blocker.

When isoprenaline (5 μg) was injected following the prior administration of noradrenaline (5 μg) the effect of isoprenaline was only rarely apparent.

However, when noradrenaline (5 µg) was injected following the prior administration of isoprenaline (5 µg) the effect of noradrenaline could be clearly demonstrated. No conclusion can be drawn as to the relative potency of these substances from this data, however, since the molar concentrations at the receptor sites are not known. Isoprenaline, for example, by increasing flow will tend to reduce its local concentration, the converse effect obtaining in the case of noradrenaline. It has been suggested (Stephenson, 1956) that the population of receptors is not homogeneous in its interaction and that in spite of its higher affinity, isoprenaline has a lower efficacy than noradrenaline. Alternatively, it may be that low concentrations of noradrenaline may be taken up more avidly than any concentration of isoprenaline, or indeed, than a higher concentration of noradrenaline itself (Foster, 1967).

There was no response to the intra-articular administration of propranolol however when phentolamine (40 µg) was injected, the clearance rate, femoral flow rate and femoral count rate were all increased. This suggests that the synovial vessels possess a vaso-constrictor tone, supporting the conclusions of Cobbold

and Lewis (1965abc). It remains possible however that phentalomine exerts a direct effect on peripheral vessels in these circumstances.

Criticism has been levelled at the use of a control period prior to the administration of a drug. Gosselin (1966) states that if the control period is employed one must then propose that all of the injected drug reaches the isotope-containing tissue quickly and uniformly. To investigate this criticism, in two studies the isotope was injected simultaneously with the drug. In both studies the effect of the drug could be clearly seen to be established within ten minutes after injection. Furthermore, the resulting $T\frac{1}{2}$ values were in the same range as those obtained in the other studies in which the drug was injected only after a control period. It would seem therefore that diffusion of the pharmacological agents employed in this study was not a limiting factor.

The results obtained in this study of the response of synovial blood vessels to the sympathomimetic agents support the conclusion that the synovial blood vessels are under sympathic control and also suggest the presence of both α- and β-receptor sites in the synovium

Although no final conclusions regarding the relative importance of α and β receptors in synovium, it is interesting that noradrenaline appeared to be more potent than isoprenaline and that phentolamine, but not propranolol, exerted an effect alone. This may indicate that α receptors predominate in the control of synovial blood vessels.

SUMMARY

1. Local injection of isoprenaline results in an increase in clearance rate which is abolished by pre-treatment with propranolol but not with phentolamine demonstrating the presence of β receptors in synovium.

2. Local injection of noradrenaline results in a decrease in clearance rate which is abolished by pre-treatment with phentolamine but not with propranolol demonstrating the presence of α receptors in synovium.

3. Femoral venous accountancy of activity lost from the joint persists through pharmacologically induced changes in the clearance rate.

4. At the doses employed in these studies no systemic effects were observed.

5. Phentolamine injection causes an increase in clearance rate which may be due either to its direct effect on small blood vessels or to its α receptor blocking action.

ADRENERGIC CONTROL OF CANINE SYNOVIAL PERFUSION IN EXPERIMENTALLY INDUCED OSTEOARTHRITIS AND SYNOVITIS

Introduction

In the course of clinical, radiological and pathological studies conducted in collaboration with Mr. M. Pond at the Veterinary Hospital, Glasgow on experimental osteoarthritis in dogs, observations were made which throw some light on the adrenergic control of inflammed synovial tissue.

The joint disease is produced in a normal stifle joint by surgically dividing the anterior (cranial) cruciate ligament through a two millimetre (2 mm.) incision alongside the straight patellar ligament. The opposite stifle joint serves as a control. No splint is applied to the limb and the dogs are allowed to exercise for three hours each day.

The immediate result of the surgical procedure is gross joint instability which is followed very rapidly by a synovial effusion. This effusion is readily appreciated as a warm fluctuant swelling on either side of the straight patellar ligament but after about one month it becomes obscured by thickening of the joint capsule and periarticular soft tissues. As the disease process continues into the third month an irregular proliferation of osteophytes can be

palpated along the attachments of the joint capsule.

The degree of lameness and joint instability steadily decreases after a two to three week period following the surgery.

Histologically, in addition to marked osteophyte formation, a progressive vascular synovitis is observed from the second to the sixth post-operative month.

MATERIALS AND METHOD

The animals were anaesthetised with sodium thiopentone and a 5:3 mixture of O_2:NO_2 with supplementary trilene. A fine guage needle was inserted into the joint cavity and the position confirmed by aspiration of synovial fluid. The needle remained in place, closed by a saline filled syringe, throughout each study. Approximately 50 Ci of 133 Xe (Amersham) in an air free solution of 0.9% NaCl were injected into the joint cavity. Volume accountancy was strictly adhered to throughout each experiment and at no time did the volume exceed the amount of synovial fluid initially aspirated by more than 50%. The counting techniques and derivation of $T\frac{1}{2}$ values has been described (Chap. 2 : page 44.

Both the diseased and the contralateral control joints of each dog were studied. Following the determination of baseline count rate, various adrenergic compounds were administered intra-articularly through the indwelling needle. Each time an active compound was administered, a small volume of isotope solution was administered with it. The pharmacological agents employed in this study were:-isoprenaline sulphate B.P. (Boots Pure Drug Co. Ltd.); l-noradrenaline acid tartrate

("Levophed" Bayer Products Co.); propanolol hydrochlorid
("Inderal" Imperial Chemical Industries Ltd.) and phentolamine mesylate B.P. ("Rogitine" Ciba Laboratories Ltd.). These compounds were diluted in 0.9% NaCl to achieve the desired concentration.

TABLE VIII

Baseline $T\frac{1}{2}$ values and duration of disease

Dog	$T\frac{1}{2}$ values (mins.)		Duration of disease (mnths.)
	Normal Jt.	Diseased Jt.	
1	140	49	7
2	68	14	6
3	120	23	5
4	89	9	4

RESULTS AND INTERPRETATION

The baseline $T\frac{1}{2}$ values obtained in each experiment were markedly reduced on the affected side, regardless of the duration of the disease. This implies an increased synovial perfusion rate. The fastest clearance rate was obtained in the stifle joint showing the most acute disease when this was judged clinically by assessing the degree of lameness, joint instability, warmth, soft tissue hypertrophy and bony proliferation. This was the joint studied after the shortest post-operative period. The slowest clearance rate in a diseased joint was found in the one judged clinically to be the least acutely affected This was the joint studied after the longest post-operative period (Table 8).

Although higher doses of active compound were required the response of the clearance rate to the intra-articular administration of the pharmacological agents was qualitatively similar in both the normal and the affected joints. Thus the clearance rate was increased by isoprenaline in a dose of 2.5 μg in the normal and 10 μg in the affected joints. Conversely, the clearance rate was reduced by noradrenaline in a

TABLE IX

$T\frac{1}{2}$ values (mins.) obtained before and after drug administration

Isoprenaline				Isoprenaline:Propranolol			
Normal		Diseased		Normal		Diseased	
Dose 2.5 ug		10 ug		Dose 2.5 ug:80 ug		10 ug:40 u	
Before	After	Before	After	Before	After	Before	Aft
120	15	23	13	19	22	105	11
26	13	19	12	59	57	20	2
140	14	28	10	30	30	140	14
65	20	49	17	21	20	60	6
88	24	20	7	46	46	45	4
100	30	50	14	32	30	48	4

Noradrenaline				Noradrenaline:Phentolamine			
Normal		Diseased		Normal		Diseased	
Dose 25 ug		10 ug		Dose 2.5 ug:40 ug		10 ug: 80 u	
Before	After	Before	After	Before	After	Before	Aft
25	70	29	150	50	52	20	2
80	150	42	150	40	41	42	4
65	90	22	60	38	39	60	6
38	60	10	70	52	54	38	3
33	75	15	51	26	28	38	3
48	73	35	68	64	62	30	3

TABLE X

$T\frac{1}{2}$ values (mins.) obtained before and after the administration of Phentolamine

Normal Dose 40 ug		Diseased 80 ug	
Before	After	Before	After
68	26	57	40
60	32	60	24
140	72	26	30
82	51	14	19
76	43	9	7
91	64	30	28
88	52	21	22
110	80	19	9
73	36		
75	42		
66	38		
71	41		

* Studies in which phentolamine did not cause an increase in clearance rate.

dose of 2.5 μg in the normal and 10 μg in the affected joint. A dose related effect was observed in one study in which first 2.5 and then 10 μg of noradrenalin were administered consecutively. The $T_{\frac{1}{2}}$ value was increased from 29 to 35 minutes on the first occasion and from 35 to 68 minutes on the second. These responses were not obtained when the catecholamines were administered following an adequate dose of their respective blocking agents, propanolol and phentolamine (Table 9). Again, however, the dose of the blocking agent required was higher on the inflamed side. When phentolamine (40 μg) was administered to the normal joints the clearance rate was increased. However, in 5 of 8 studies in inflamed joints, employing 80 μg of phentolamine, no increase in clearance rate was observed (Table 10).

Thus, although quantitative differences between the responses to isoprenaline, noradrenaline and the combination isoprenaline:propanolol and noradrenaline: phentolamine were obtained, qualitatively the responses were similar. The positive responses to these agonists and the abolition of these responses by specific blockin agents, suggests the presence of both α and β

adrenergic receptors in the control of normal and inflamed synovial perfusion. Increase in clearance rate following phentolamine may be related to a direct effect of the compound upon small blood vessels, or to abolition of a resting adrenergically mediated vasoconstrictor tone. Failure of this response in the inflamed joints may indicate loss of basal vasoconstrictor tone.

SUMMARY

1. Quantitative differences in the dose of agonist or blocking agent required to produce the same effect in normal and inflamed synovium were discovered, higher doses being required in the case of the latter.

2. Qualitatively similar responses to isoprenaline and propranolol and to noradrenaline and phentolamine as agonist and blocking agents were obtained in normal and inflamed synovium, demonstrating the presence of both α and β receptors.

3. Qualitatively different responses to the injection of phentolamine alone were obtained. In the normal joints an increase in clearance rate was obtained but no change occurred even with the exhibition of larger doses in five of the diseased joints suggesting loss of α adrenergicall mediated basal vasoconstrictor tone in these inflamed joints.

PHARMACOLOGICAL STUDIES ON THE EFFECT OF THYMOXAMINE ON PERIPHERAL BLOOD VESSELS: MONITORING BY THE 133 Xe CLEARANCE TECHNIQUE

Introduction

Since Alquist coined the terms α and β recepto in 1948, a great deal of work has been performed on the adrenergic control of peripheral blood vessels in many tissues. It has been established that skin and other superficial tissues contain predominantly α receptors (Brownlee, 1966) whereas in the heart, and in muscle vessels, β receptors are most conspicuous (Brownlee, 1966). The recent availability of a specific α adrenergic blocking agent, thymoxamine ("Opilon", Wm. Warner and Co. Ltd.) which acts reversibly by competitive inhibition (Birmingham and Szolcsanyi, 1965 : Birmingham, Ernest and Newcombe, 1969) affords the opportunity of selectively studying α receptor function. Thymoxamine, unlike phentolamine, has not been shown to possess a direct effect on small blood vessels. Whereas, in the previous section (page 132), increased clearance rates produced by phentolamine could have resulted either from the blocking or the direct effect of the compound, similar results obtained with thymoxamine could more confidently be related to the blocking action thereby strengthening the case for the existen

of basal vasoconstrictor tone. In the present study this compound has been employed in the study of adrenergic control of the peripheral blood vessels in canine synovium.

The acquisition of intimate knowledge of the peripheral circulation has been limited by the availability of suitable monitoring techniques. The introduction of radioisotopes has greatly extended the monitoring techniques available. The system for the detection of drug induced changes in the peripheral blood vessels used in this study was the rate of clearance of intra-articularly injected Xenon (^{133}Xe). The effect of thymoxamine alone was studied and thereafter to further characterise the result the effects of isoprenaline and noradrenaline alone and in combination with thymoxamine were examined.

MATERIALS AND METHODS

The experiments were conducted in a surgical theatre in the Wellcome Surgical Research Centre, Glasgow. Both stifle joints of adult greyhound dogs weighing from 20 to 28 Kg. were studied. Anaesthesia was induced with sodium thiopentone (20 mg./Kg.) and maintained with a 5.3 mixture of nitrous oxide and oxygen and additional (1%) trilene. A catheter placed in the brachial artery permitted continuous recording of blood pressure and pulse rate.

Injection Technique

A 15/16" guage needle was inserted into the joint cavity by a medial infra-patellar approach and confirmation was achieved by aspiration of clear synovial fluid in every case. If the aspirate was blood stained, no further studies were performed in that joint. An air-free volume of less than 0.5 ml. of isotope solution was carefully injected into the joint cavity and the needle remained in situ throughout each experiment closed by a syringe containing 0.9% sodium chloride, thus providing a convenient route for the administration of the pharmacological agents. Each time an active compou

TABLE XI

Group	Number of studies	^{133}Xe Baseline Clearance	First Compound Administered	First Monitoring Period	Second Compound Administered	Second Monitoring Period
1	12	15 mins.	Thymoxamine 5-80 µg.	15 mins.		
2	6	15 mins.	Isoprenaline 2.5 µg.	15 mins.		
3	6	15 mins.	Noradrenaline 2.5 µg.	15 mins.		
4	6	15 mins.	Thymoxamine 40-200 µg.	15 mins.	Isoprenaline 2.5 µg.	15 mins.
5	8	15 mins.	Thymoxamine 60 µg.	15 mins.	Noradrenaline 0.5-10 µg.	15 mins.
6	8	15 mins.	Thymoxamine 20-100 µg.	15 mins.	Noradrenaline 2.5 µg.	15 mins.
7	6	15 mins.	Noradrenaline 1.0 µg.	15 mins.	Thymoxamine 40-200 µg.	15 mins.

Legend:

The experimental protocol showing the order and dose ranges of the compounds administered.

was administered, a small (< 0.25 ml.) volume of 133 Xe was injected with it and throughout each experiment volume accountancy was scrupulously adhered to. At no time did the total volume injected exceed the amount of synovial fluid aspirated initially by more than 50%.

The counting techniques and derivation of $T\frac{1}{2}$ values have been described (Chap. 2 : Page 44). As the clearance rate becomes slower, the semilogarithmic plots approach the horizontal and the errors of calculating the $T\frac{1}{2}$ values become magnified. Accordingly all values in excess of 150 mins. are recorded simply as $T\frac{1}{2}$ => 150 minutes in this paper.

The experimental protocol is shown in Table11. The effects of thymoxamine, isoprenaline and noradrenaline alone were studied (Groups 1 to 3). Isoprenaline was administered following the prior administration of thymoxamine (Group 4). Increasing doses of noradrenaline were administered following a fixed dose of thymoxamine (Group 5), and increasing doses of thymoxamine were administered prior to a fixed dose of noradrenaline (Group 6). Increasing doses of thymoxamine were also administered following a fixed dose of noradrenaline (Group 7).

Pharmacological Agents

The pharmacological agents employed in this study were: thymoxamine (OPILON, Wm. Warner and Co. Ltd.), l-noradrenaline acid tartrate (LEVOPHED, Bayer Production Co.) and isoprenaline sulphate in normal saline (Boots Pure Drug Co.). These compounds were diluted in sterile 0.9% sodium chloride solution to achieve the desired concentrations.

TABLE XII

THE EFFECT OF THYMOXAMINE, ISOPRENALINE AND NORADRENALINE ON THE ^{133}Xe CLEARANCE RATE

	Thymoxamine (Group 1)			Isoprenaline 2.5 µg. (Group 2)		Noradrenaline 2.5 µg. (Group 3)	
	$T\frac{1}{2}$ (mins.) Before	Dose (µg.)	$T\frac{1}{2}$ (mins.) After	$T\frac{1}{2}$ (mins.) Before	After	$T\frac{1}{2}$ (mins.) Before	After
	42	40	26	58	16	66	89
	54	40	28	39	6	54	101
	23	40	17	44	21	38	120
	80	30	38	46	19	47	98
	48	30	28	64	28	42	86
	53	30	31	73	31	49	125
	89	20	42				
	68	20	30				
	44	10	26				
	56	10	38				
	*34	5	30*				
	48	5	16				
Mean	53		30	54	20	49	103
Range	23-89		16-38	39-73	6-31	38-66	86-125
No. showing change		11/12 (1 equivocal)		6/6		6/6	

TABLE XIII

THE EFFECT OF ISOPRENALINE FOLLOWING THYMOXAMINE ON THE ^{133}Xe CLEARANCE RATE (GROUP 4)

$T\frac{1}{2}$ (mins.)

	Baseline	Following Thymoxamine		Following Isoprenalin (2.5ug.)
	56	37		20
	72	46	40 g.	26
	39	28		9
	46	31		16
	68	38	200 g.	21
	55	34		22
Mean	54	36		19
Range	39-72	28-46		9-26
No. showing change	6/6		6/6	

RESULTS

The results are shown in Figures 8 to 10 and Tables 12 and 13.

In eleven of the twelve studies in Group I (thymoxamine alone) the clearance rate following drug administration greatly exceeded the baseline value (Table 12). No clear relationship to the dose employed was noted. An equivocal result was obtained in the remaining study, the $T\frac{1}{2}$ values prior to and following injection being 34 and 30 minutes respectively. In Groups 2 and 3 the results were consistent throughout: in all six studies with isoprenaline (2.5 µg.) the clearance rate following drug administration exceeded the baseline values and in all six studies with noradrenaline (2.5 µg.) the clearance rate was reduced following injection (Table 12). Change in clearance rate was abrupt and marked in every instance. When isoprenaline 2.5 µg. was administered following thymoxamine (Group 4) a marked increase in clearance rate ensued in all studies (Table 13). In three of these studies the dose of thymoxamine was 40 µg. and in the remaining three studies the dose employed was 200 µg.

The results obtained when increasing doses of

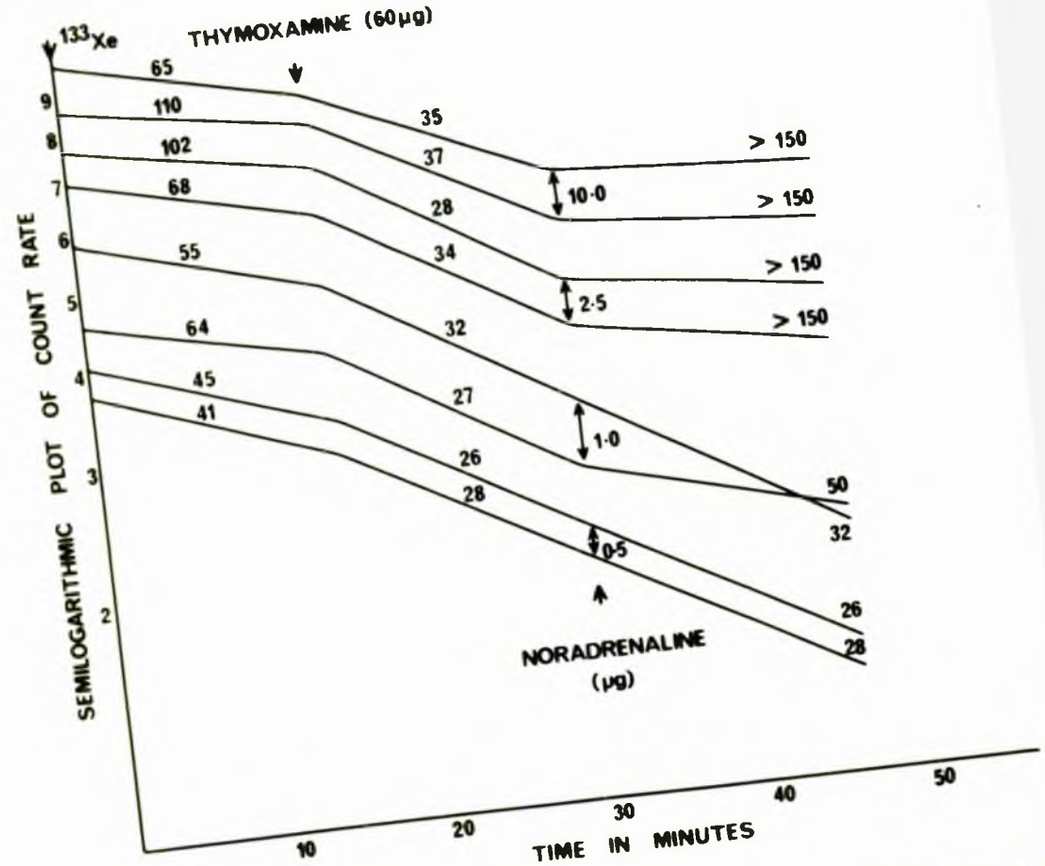


Figure 8

Legend: The effect of increasing doses of noradren
following a fixed dose of thymoxamine (Gro

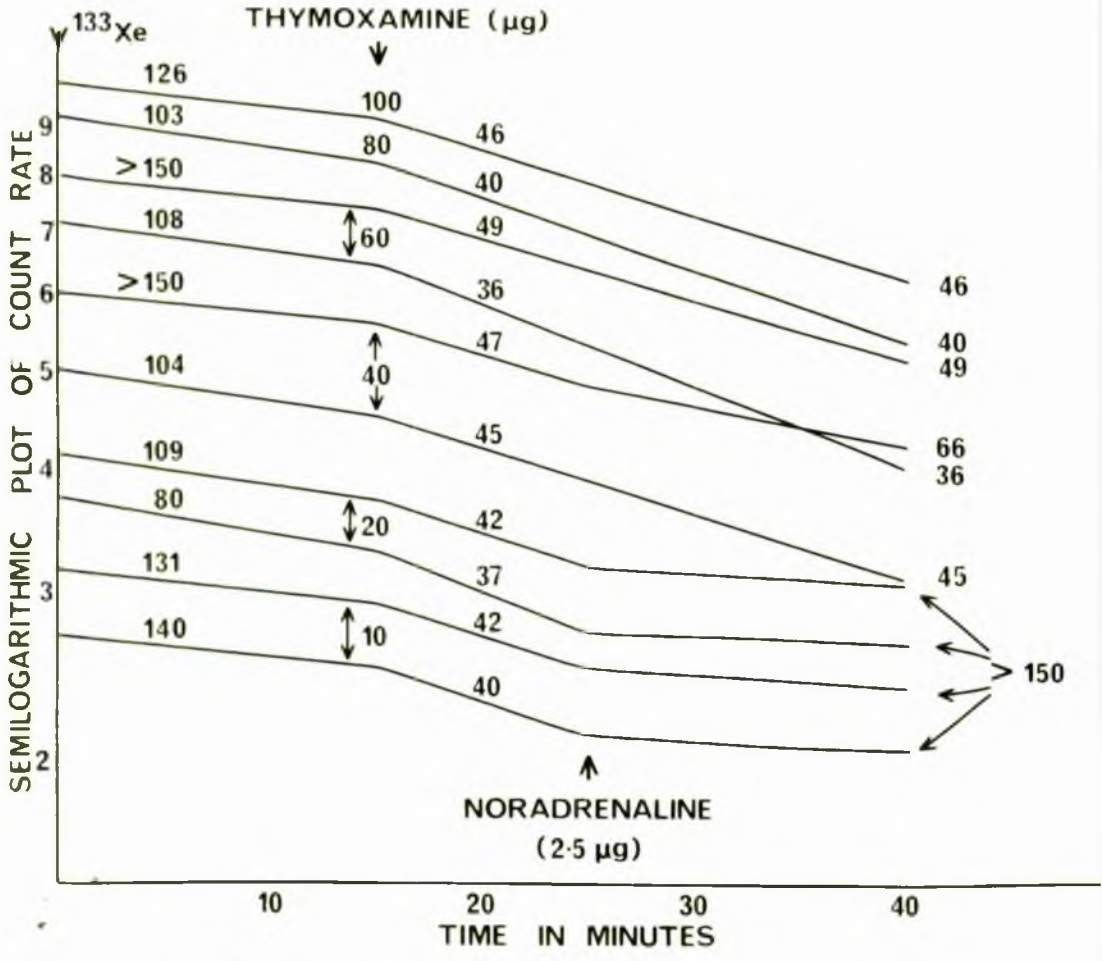


Figure 9

Legend: The effect of increasing doses of t
prior to a fixed dose of noradrenal
(Group 6).

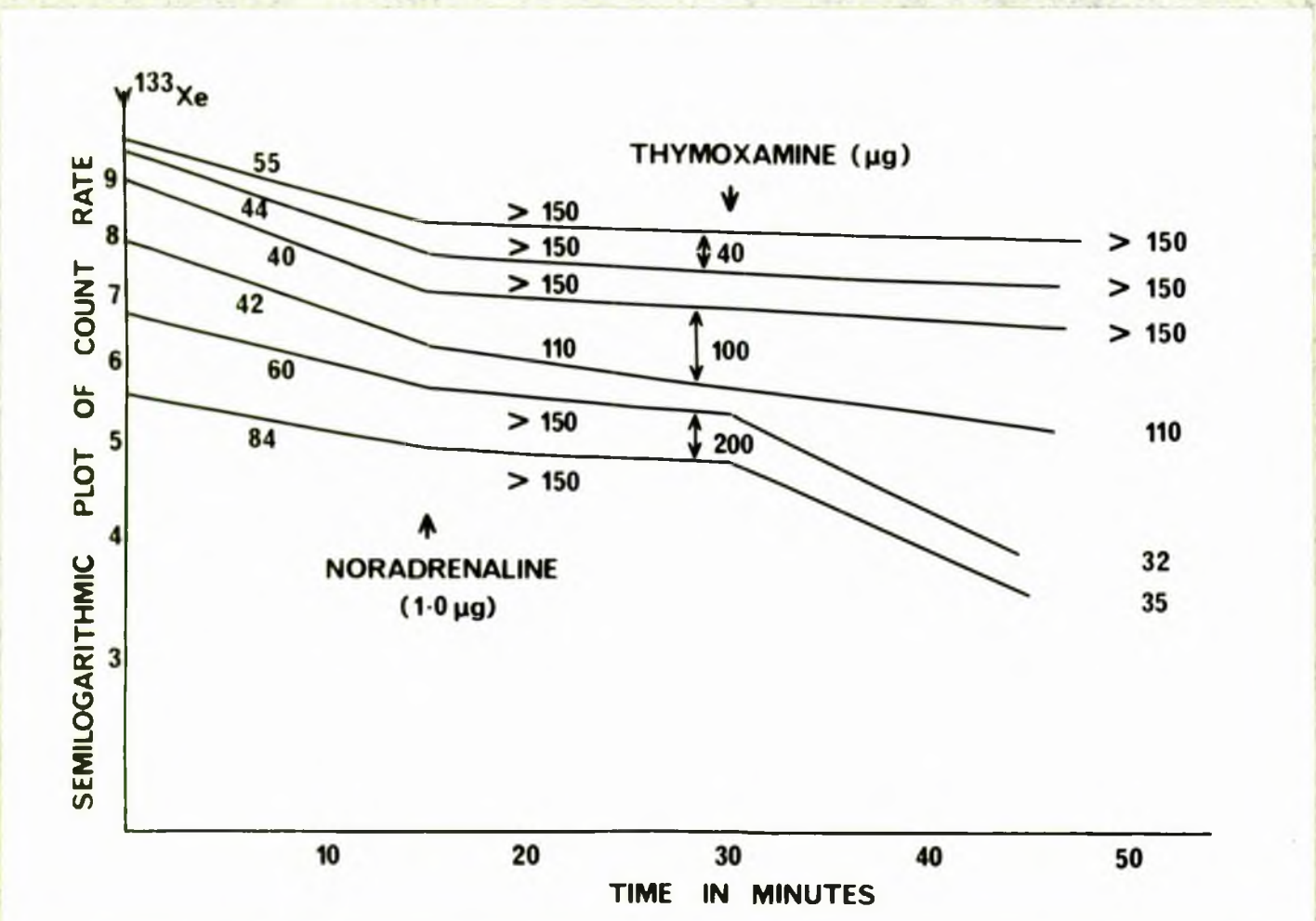


Figure 10

Legend: The effect of increasing doses of thymoxami
following a fixed dose of noradrenaline
(Group 7).

noradrenaline were administered following a fixed dose (60 µg.) of thymoxamine (Group 5) are shown in Figure 8. A dose related response was obtained. Thus when the dose of noradrenaline was 0.5, no change in clearance rate was obtained. When the dose of noradrenaline was 2.5, or 10 µg. the clearance rate was reduced following the injection of noradrenaline. (Variable responses were obtained at a dose of 1 µg.). When increasing doses of thymoxamine were administered prior to a fixed dose (2.5 µg.) (Group 6) of noradrenaline again a dose related response was obtained (Fig. 9). Thus when the dose of thymoxamine was 10 or 20 µg. the clearance rate was subsequently abruptly and markedly reduced by the administration of noradrenaline. However, when the dose of thymoxamine employed was 80 or 100 µg. no such subsequent noradrenaline response was obtained. Variable responses were noted when the dose of thymoxamine employed was either 40 or 60 µg.

When increasing doses of thymoxamine were administered following the prior administration of noradrenaline (1.0 µg.) (Group 7) no response was noted until the dose of thymoxamine employed was 200 µg In two studies in which this dose was employed the clearance rate was abruptly and markedly increased following thymoxamine injection (Fig. 10).

DISCUSSION

The monitoring system employed in this study to demonstrate pharmacologically induced changes in the peripheral vasculature was the rate of clearance of intra-articularly administered 133 Xe. Although the confidence which can be ascribed to the expression of results in absolute units of perfusion cannot be complete since the method is indirect and since no other method is available to confirm the results, major changes in clearance rate such as are encountered in the present study, occurring coincidentally with drug administration, may be justifiably attributed to pharmacologically induced changes in perfusion (Gosselin, 1966). The results of the present study may be interpreted to indicate that whereas noradrenaline reduces synovial perfusion, the intra-articular administration of both isoprenaline and thymoxamine are attended by an increase in clearance rate and therefore in synovial perfusion rate. The results with noradrenaline and with isoprenaline, are easily understood in terms of the known effects of these compounds on other vascular beds (Goodman and Gilman, 1965). However, interpretation of the effect of thymoxamine is less obvious. Increase in clearance rate and in perfusion rate may be the

result of a direct effect of the compound on small blood vessels. Although this effect has been demonstrated with phentolamine however, no conclusive evidence is available to support this assertion in the case of thymoxamine. The more likely possibility supported by the conclusions of other workers (Birmingham, Akubne and Szolcsanyi, 1967 : Turner, Harrison and Schoenfeldt, 1969) is that the compound releases these peripheral vessels from a basal α adrenergically mediated vasoconstrictor tone.

The results of this study also demonstrate that whereas thymoxamine has no influence on β adrenergically mediated isoprenaline response, the compound will abolish the α mediated effect of noradrenaline. The effect is dose related and can be modified by either increasing the dose of the agonist or reducing the dose of the blocking agent. Furthermore, the noradrenaline effect can be reversed by the administration of a large dose of thymoxamine. These results may be adduced in support of the contention that the mode of action of thymoxamine is by competitive antagonism (Birmingham and Szolcsanyi, 1965). The dose ratio at which the modifying effect

of thymoxamine was demonstrable varied in the different groups of studies. When thymoxamine was administered first (Groups 5 and 6) the dose ratio obtained was of the order of 60 (thymoxamine) to 1 (noradrenaline). However, when the compound first administered was noradrenaline the corresponding ratio was 200:1. Variation in affinity of the compounds for the receptor binding sites may explain this discrepancy.

It would seem from the results of this study that the 133 Xe clearance technique provides a suitable model for the in vivo study of pharmacologically induced changes in perfusion. The method is particularly attractive since it involves only minimal interference with the physiological state of the animal.

SUMMARY

1. The rate of clearance of intra-articularly injected 133 Xe provides a simple model for the in vivo study of drug induced changes in synovial perfusion.

2. Intra-articularly administered thymoxamine results in an increased clearance rate. This may be the result of a previously undetected direct effect of the compound on peripheral blood vessels but is more likely to be due to abolition of resting α adrenergically mediated basal vasoconstrictor tone.

3. The increase in clearance rate mediated by an β receptor stimulating compound (isoprenaline) persists following the prior administration of thymoxamine.

4. Thymoxamine prevents, and reverses, the reduction in clearance rate mediated by an α receptor stimulating compound (noradrenaline). This effect is dose related.

PHARMACOLOGICAL STUDIES WITH PARASYMPATHETIC COMPOUNDS, HISTAMINE AND SEROTONIN ON CANINE SYNOVIAL PERFUSION MONITORED BY THE 133 XENON CLEARANCE TECHNIQUE

The effect of parasympathetic compounds, histamine and serotonin upon peripheral blood vessels has been intensively studied particularly in pharmacological test systems which are more or less divorced from the in vivo situation. The opportunity of following peripheral circulatory events in vivo by external monitoring of minute volumes of radioactive tracer substances is now available (Chap. 2). Following the intra-articular injection of radioactive 133 Xe the clearance rate from the joint provides an index of synovial tissue perfusion with minimal further interference to the anaesthetised animal.

This monitoring system was utilised to determine the effects of methacholine, isoprenaline, mepyramine malleate, histamine, serotonin and noradrenaline on canine synovial perfusion measured by the 133 Xe clearance technique. The effect of atropine, a substance which antagonises many of these compounds, was also studied.

MATERIALS AND METHODS

Experiments were conducted on both stifle joints of anaesthetised adult dogs weighing from 20 to 35 kg. at the Wellcome Surgical Research Institute, Glasgow. Twenty-two of the dogs were greyhounds and 10 were mongrels. Anaesthesia was induced with sodium thiopentone (20 mg./kg.) and maintained with a 5:3 mixture of NO_2:O_2 and additional 1% trichloroethylene.

Injection and counting technique

A 15/16th" gauge needle was inserted into the stifle joint by a medial infrapatellar approach and confirmation of the position of the needle point was achieved by the aspiration of synovial fluid in all studies. Any experiment in which the initial aspirate was macroscopically blood stained was abandoned. The needle remained in place throughout each study, closed by a syringe containing 0.9% NaCl, thus providing a convenient route for the administration of isotope and active compound solutions. On each occasion that an active compound was administered a small volume (<0.2 ml.) of 133 Xe solution was injected with it. The counting techniques and derivation of $T\frac{1}{2}$ values have been described (Chap. 2 : page 44).

TABLE XIV

Group	No. of Studies	133 Xe Injected	Control Monitoring Period (15 mins.)	First Compound Administered (µg.)	Monitoring Period (15 to 20 mins.)	Second Compound Administered (µg.)	Monitoring Period (15 mins.)
1	10		——→	Methacholine (0.1 to 5.0)	——→		
2	9		——→	Noradrenaline (0.25 to 2.5)	——→		
3	6		——→	Isoprenaline (2.5)	——→		
4	19		——→	Thymoxamine (10 to 200)	——→		
5	7		——→	Histamine (0.025 to 25)	——→		
6	10		——→	Serotonin (0.1 to 5.0)	——→		
7	9		——→	Atropine (0.1 to 10.0)	——→		
8	10		——→	Methacholine (0.1 to 50)	——→	Methacholine (0.5 to 100)	——→
9	6		——→	"Vasodilator"	——→	Isoprenaline (2.5 to 30)	——→
10	6		——→	Atropine (0.5 to 5.0)	——→	Atropine (0.5 to 5.0)	——→
11	6		——→	Serotonin (0.1 to 10.0)	——→	Atropine (0.1 to 120)	——→
12	10		——→	Methacholine (1.0 to 100)	——→	Atropine (0.5 to 10)	——→

TABLE XIV (contd.)

Group	No. of Studies	133 Xe Injected	Control Monitoring Period (15 mins.)	First Compound Administered (µg.)	Monitoring Period (15 to 20 mins.)	Second Compound Administered (µg.)	Monitoring Period (15 mins.)
13	35		——→	Atropine (2.5 to 10.0)	——→	Methacholine (1.0 to 2500)	——→
14	7		——→	Methacholine (5.0) + Atropine (1.0 to 100)	——→		
15	5		——→	Atropine (5.0 to 120)	——→	Noradrenaline (0.25 to 2.5)	——→
16	6		——→	Atropine (2.5 to 5.0)	——→	Isoprenaline (2.5 to 5.0)	——→
17	14		——→	Atropine (1.0 to 5.0)	——→	Serotonin (0.1 to 10.0)	——→
18	12		——→	Atropine (1.0 to 100)	——→	Histamine (0.1 to 100)	——→
19	17		——→	Thymoxamine (5.0 to 250)	——→	Histamine (0.025 to 25)	——→
20	10		——→	Mepyramine (5.0 to 100)	——→	Histamine (1.0)	——→

Legend:

The dose ranges employed and the order of administration of the compounds studied.

$T\frac{1}{2}$ values derived from almost horizontal slopes are unreliable and accordingly all values in excess of 150 mins. are simply described as $T\frac{1}{2} \Rightarrow 150$ mins.

Experimental protocol

The experimental protocol with the concentration of active compounds and their order of administration is shown in Table 12. Twenty groups of studies were conducted. In groups 1 to 7, the effects of atropine, methacholine, serotonin, histamine, isoprenaline, noradrenaline and thymoxamine on the clearance rate of 133 Xe were monitored following a control monitoring period. In groups 8 to 20 the effects of combinations of those compounds were studied in the dose ratios indicated.

Pharmacological agents

The compounds employed in this study were: atropine sulphate (Antigen Ltd.), methacholine chloride ("Amechol" Macarthy's Ltd.), serotonin creatinine sulphate (Glasgow Royal Infirmary Pharmacy Department), histamine acid phosphate (Evans Medical Ltd.), isoprenaline sulphate ("Suscardia", Boots Pure Drug Co. Ltd.), l-noradrenaline acid tartrate ("Levophed", Bayer Production Co. Ltd.), mepyramine malleate (Anthisan May and Baker) and thymoxamine ("Opilon", Wm. Warner & Co. Ltd.). These were diluted in sterile 0.9% NaCl solution to achieve the desired concentration.

THE EFFECT OF METHACOLINE WITH AND WITHOUT ATROPINE ON THE CLEARANCE RATE.

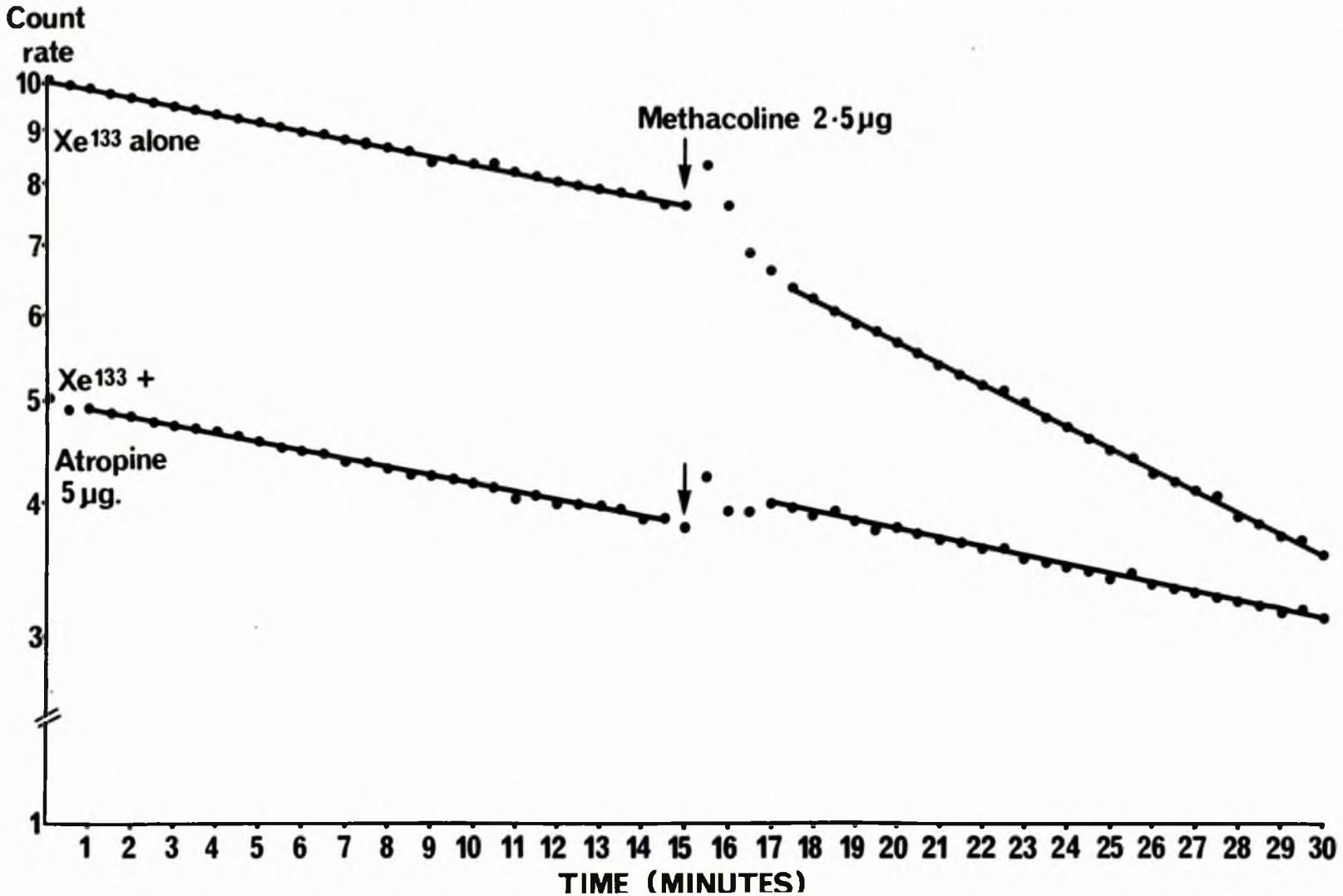


TABLE XV

Methacholine			Noradrenaline			Isoprenaline		
Dose (µg.)	$T\frac{1}{2}$ Before (min.)	$T\frac{1}{2}$ After (min.)	Dose (µg.)	$T\frac{1}{2}$ Before (min.)	$T\frac{1}{2}$ After (min.)	Dose (µg.)	$T\frac{1}{2}$ Before (min.)	$T\frac{1}{2}$ After (min.)
5.0	45	10	2.5	94	150	2.5	150	20
2.5	35	17	2.5	80	150	2.5	130	15
2.5	32	13	2.5	75	150	2.5	102	13
1.25	30	22	2.5	42	88	2.5	48	10
1.0	62	24	2.5	29	51	2.5	33	21
1.0	44	33	2.5	22	44	2.5	30	6
1.0	42	28	2.5	8	48			
1.0	38	16	2.5	7	48			
0.1	89	83*	0.25	18	95			
0.1	46	47*						
No. showing change >25%	8 of 10			9 of 9			6 of 6	

* No. showing change <25%

Legend:
The effect of various doses of methacholine, noradrenaline and isoprenaline on the 133 Xe clearance rate (groups 1 to 3).

RESULTS

The results are shown in Fig. 11 and Tables 15 to 26. Fig. 11 shows a representative example of the alteration in clearance rate following intra-articular injection of methacholine, and the absence of change when methacholine was administered following a prior injection of an appropriate dose of atropine.

The $T\frac{1}{2}$ values obtained, before and after methacholine in doses ranging from 0.1 µg. to 5.0 µg. are shown in Table 15. Although no significant change in the clearance rate attended the administration of 0.1 µg., a marked increase was observed at higher dose levels. In doses in excess of 0.1 µg. no obvious dose related response was obtained. In all of nine studies in which noradrenaline (0.25 to 2.5 µg.) was administered, the clearance rate was markedly reduced following the injection, the degree of reduction again being unrelated to the dose administered (Table 15). Indeed, a marked reduction in clearance rate was obtained with the lowest dose of the compound. In all six studies the clearance rate was increased

TABLE XVI

Thymoxamine			Histamine			Serotonin		
Dose (µg.)	$T\frac{1}{2}$ Before (min.)	$T\frac{1}{2}$ After (min.)	Dose (µg.)	$T\frac{1}{2}$ Before (min.)	$T\frac{1}{2}$ After (min.)	Dose (µg.)	$T\frac{1}{2}$ Before (min.)	$T\frac{1}{2}$ After (min.
200	120	47	25.0	43	20	5.0	30	20
100	150	38	2.5	62	35	1.5	40	23
100	126	46	2.5	64	33	1.0	45	17
80	103	40	2.5	81	31	1.0	44	28
60	150	49	0.1	51	16	1.0	38	30*
60	108	36	0.05	29	18	1.0	18	9
40	150	47	0.025	53	31	0.1	95	45
40	124	45				0.1	52	38
40	104	45				0.1	45	26
40	90	49				0.1	44	25
40	85	53						
40	82	38						
40	75	33						
40	70	30						
40	68	41						
20	109	42						
20	80	37						
10	140	40						
10	131	42						
No. showing change > 25%	19 of 19			7 of 7			9 of 10	

*No. showing change < 25%

Legend: The effects of various doses of thymoxamine, histamine and serotonin on the 133 Xe clearance rate (groups 4 to 6).

following the injection of isoprenaline (2.5 µg.) (Table 15).

The effect of thymoxamine (10 to 200 µg.) is shown by an increase in clearance rate in all of the 19 studies, the degree of change again being unrelated to the dose administered over the dose range studied, and similarly an increase in clearance rate attended the administration of histamine (0.025 to 25.0 µg., seven studies) and of serotonin (0.1 to 5.0 µg., nine of ten studies) (Table 16). In the remaining serotonin study the increase in clearance rate obtained was only 21%.

The two points on the graph immediately following the injection of methacholine are displaced from the straight lines obtained before and after injection. This was noted to occur frequently with all of the compounds studied and $T_{\frac{1}{2}}$ values reported in all drug studies in this thesis refer to the steady state periods characterised by straight line graphs of more than 10 minutes duration.

The duration of effect of methacholine, isoprenaline, histamine and serotonin ranged from 10 to 18 minutes whereas the duration of effect of noradrenaline ranged from 12 to 32 minutes. In all

TABLE XVII

$T_{\frac{1}{2}}$ Values (min.)

Dose (µg.)	Before	After	% Change
10.0	34	34	0
5.0	27	19	21
5.0	27	51	49
2.5	56	54	3
2.5	56	50	10
1.0	82	26	68
1.0	46	24	48
1.0	72	40	55
0.1	36	20	44
0.1	88	31	64
0.1	110	36	67

Legend:

The effect of atropine on the clearance rate of 133 Xe (group 7).
At doses of less than 1.0 µg. the clearance rate was increased (6 of 6). Only one of five cases with higher doses showed a change in $T_{\frac{1}{2}}$ value in excess of 25% and in that case the clearance rate was reduced.

TABLE XVIII

Methacholine following Methacholine

1st Dose (µg.)	2nd Dose (µg.)	$T\frac{1}{2}$ (min.) Before 2nd Dose	$T\frac{1}{2}$ (min.) After 2nd Dose
50.0	100.0	35	24
10.0	50.0	45	35*
5.0	10.0	34	32*
2.5	5.0	46	34
1.25	2.5	70	28
1.0	2.5	66	35
1.0	2.5	33	24
0.5	1.0	50	35
1.0	1.0	21	11
0.1	0.5	83	50
No. showing change >25%		8 of 10	

* No. showing change <25%

Isoprenaline following Vasodilator Compounds

Dose (µg.) and Compound First Administered	Dose (µg) of Isoprenaline	$T\frac{1}{2}$ (min.) Before Isoprenaline	$T\frac{1}{2}$ (min.) After Isoprenaline
Histamine 25 µg.	10	20	26*
Histamine 20 µg.	2.5	26	20*
Isoprenaline 10 µg.	30	31	29*
Isoprenaline 2.5 µg.	5	20	20*
Methacholine 250 µg.	10	30	31*
Methacholine 2.5 µg.	10	42	16
		1 of 6	

Legend: Effect of methacholine following methacholine and of isoprenaline following vasodilator compounds on the 133 Xe clearance rate (groups 8 and 9).

studies the duration of effect was related to the administered dose of the compound, the effect being more prolonged with higher doses of each compound.

When atropine was administered in low doses (0.1 to 1.0 γ g.) the clearance rate was increased in all of six studies whereas variable responses were obtained at higher doses. Increase, reduction or lack of change in clearance rate at doses in excess of 1.0 γ g. did not seem to be related to the doses employed (Table 17). In only one of these studies employing doses in excess of 1.0 γ g. was there a change in $T\frac{1}{2}$ value of more than 25% and in that study the $T\frac{1}{2}$ value was increased, denoting a reduction in clearance.

When methacholine was administered following a prior injection of methacholine in the doses indicated (Table 18) the clearance rate was increased in eight of ten studies by more than 25%, however, in only one of six studies in which isoprenaline was administered following the previous injection of a vasodilator was there any reduction in $T\frac{1}{2}$ value. In that study a small dose of methacholine had been administered as the vasodilator drug.. The compounds

TABLE XIX

Atropine following Atropine				Atropine following Serotonin			
1st Dose (µg.)	2nd Dose (µg.)	$T\frac{1}{2}$ (min.) Before 2nd Dose	$T\frac{1}{2}$ (min.) After 2nd Dose	Dose (µg.) Serotonin	Dose (µg.) Atropine	$T\frac{1}{2}$ (min.) Before Atropine	$T\frac{1}{2}$ (min.) After Atropine
1.0	5.0	81	80*	5.0	120	26	29*
2.5	5.0	33	42*	10.0	100	21	15
5.0	5.0	28	31*	2.5	30	28	18
0.5	1.0	49	43*	1.0	5.0	53	39
1.0	0.5	43	43*	0.1	5.0	35	27*
0.5	0.5	43	41*	1.0	0.1	33	21
No. showing change >25%		0 of 6				4 of 6	

*No. showing change <25%

Legend: Effect of atropine following atropine and atropine following serotonin on the 133 Xe clearance rate (groups 10 and 11).

TABLE XII

Atropine Dose (ug.)	Methacholine Dose (ug.)	$T_{\frac{1}{2}}$ (min.) After Atropine	$T_{\frac{1}{2}}$ (min.) After Methacholine	Results / Dose Ratio Atropine/ Methacholine
5.0	1.0	150	150	B 5:1*
5.0	1.0	80	80	B 5:1*
5.0	1.0	31	33	B 5:1*
2.5	1.0	50	48	B 2.5:1*
10.0	5.0	44	48	B 2:1*
5.0	5.0	150	150	B 1:1*
5.0	5.0	80	78	B 1:1*
5.0	5.0	31	28	B 1:1*
2.5	2.5	68	70	B 1:1*
2.5	2.5	36	37	B 1:1*
2.0	2.5	70	24	NB 1:1.25
2.0	2.5	30	26	B 1:1.25*
2.5	5.0	70	51	NB 1:2
10.0	25.0	48	48	B 1:2.5*
10.0	25.0	36	36	B 1:2.5*
3.0	10.0	47	45	B 1:3.3*
2.5	10.0	51	53	B 1:4*
2.5	10.0	53	42	B 1:4*
5.0	25.0	78	82	B 1:5*
5.0	25.0	37	19	NB 1:5
5.0	25.0	23	13	NB 1:5
10.0	100.0	48	34	NB 1:10
5.0	50.0	150	150	B 1:10*
5.0	50.0	82	80	B 1:10*
3.0	50.0	45	35	B 1:17*
5.0	100.0	80	35	NB 1:20
10.0	150.0	60	39	NB 1:30
3.0	100.0	35	24	NB 1:33
2.5	100.0	73	42	NB 1:40
2.5	100.0	53	34	NB 1:40
2.5	100.0	39	26	NB 1:40
2.5	100.0	84	43	NB 1:40
10.0	500.0	69	37	NB 1:50
10.0	500.0	34	18	NB 1:50
5.0	2500.0	150	24	NB 1:500

No. showing change > 25%

* No. showing change < 25%

/ Change in $T_{\frac{1}{2}}$ value of > 25% has been arbitrarily designated "no block" (NB) and change of less than 25% has been termed "block" (B), see text for explanation.

Legend: The effect of methacholine following a prior dose of atropine (group 13) on the 133 Xe clearance rate.

and doses employed are shown in Table 18.

When atropine was administered following a prior dose of atropine in the doses indicated in Table 19 there was no change in clearance rate in four of six studies. In the remaining two studies a slight increase and a slight decrease in $T\frac{1}{2}$ value was observed, neither change exceeding 25%. When atropine was administered following the prior administration of serotonin in the doses indicated in Table 19 the clearance rate was markedly increased in four of six studies. In the remaining studies the changes in $T\frac{1}{2}$ value were less than 25%.

The results of the studies in which methacholine was administered following atropine in varying dose ratios are shown in Table 20. In this, and in all other studies in which the blocking action of a compound was studied , change in $T\frac{1}{2}$ value of less than 25% was deemed to indicate no change and was designated "block" (B). Conversely when the $T\frac{1}{2}$ value altered by more than 25% following drug administration this was taken to indicate change and was designated "no block" (N.B.). It can be seen from Table 20 that dose ratios of atropine to methacholine in excess of 1:1 resulted in no change in clearance rate, whereas

TABLE XXI

Atropine following Methacholine

Dose (mg.) Me.	Dose (µg.) At.	Dose Ratio At./Me.	$T_{\frac{1}{2}}$ (min.) After At.	$T_{\frac{1}{2}}$ (min.) After Me.
100.0	2.5	1:40	44	73
100.0	2.5	1:40	24	43
25.0	5.0	1:5	31	23
2.5	10.0	4:1	28	70
2.5	5.0	2:1	78	150
2.5	2.0	1:1.25	24	35
2.5	1.0	1:2.5	24	30*
2.5	0.5	1:5	34	22
1.0	2.5	2.5:1	28	33*
1.0	2.5	2.5:1	16	10

No. showing change > 25% 8 of 10

* No. showing change < 25%

Atropine and Methacholine Administered Together

Dose (µg.) At.	Dose (µg.) Me.	Dose Ratio At./Me.	$T_{\frac{1}{2}}$ (min.) Before Injection	$T_{\frac{1}{2}}$ (min.) After Injection
100.0	5.0	20:1	36	37*
25.0	5.0	5:1	38	36*
10.0	5.0	2:1	42	52*
10.0	5.0	2:1	50	48*
3.0	5.0	1:1.7	33	42*
1.0	5.0	1:5	44	23

1 of 6

Legend: Effect of atropine following methacholine, and of simultaneous administration of atropine and methacholine (groups 12 and 14).

KEY - At. = Atropine
Me. = Methacholine

dose ratios of less than 1:17 resulted in all of ten studies in a distinct methacholine effect. Variable responses were obtained at intermediate dose ratios. In six studies (Table 21) methacholine and atropine were administered together in various dose ratios. In the studies in which the dose ratio of atropine to methacholine exceeded 1:1.7 no change in clearance rate of more than 25% was noted and in the study in which the dose ratio was 1:5 a distinct increased clearance rate was obtained.

When atropine was administered following the prior administration of methacholine the $T\frac{1}{2}$ values following atropine were increased in five of ten studies and reduced in the remaining three studies in which change of more than 25% was recorded (Table 21). No obvious relationship to the respective doses of the compounds was noted. When noradrenaline was injected following the prior administration of atropine in all dose ratios studied, the noradrenaline effect of reduction in clearance rate was observed. Even in the study, in which the dose ratio employed was 400:1 a reduction in clearance rate was also noted following noradrenaline. In one study when isoprenaline was injected following atropine in the dose ratio of

TABLE XXII

Noradrenaline following Atropine					Isoprenaline following Atropine				
Dose (µg.) At.	Dose (µg.) Nora.	$T\frac{1}{2}$ (min.) After At.	$T\frac{1}{2}$ (min.) After Nora.	Result	Dose (µg.) At.	Dose (µg.) Iso.	$T\frac{1}{2}$ (min.) After At.	$T\frac{1}{2}$ (min.) After Iso.	Result
100	0.25	15	27	NB	5.0	2.5	27	31	B*
120	2.5	29	51	NB	2.5	2.5	31	22	NB
100	2.5	27	70	NB	2.5	5.0	22	11	NB
25	2.5	7	48	NB	5.0	2.5	20	8	NB
5	1.0	22	44	NB	5.0	2.5	45	16	NB
					5.0	2.5	48	9	NB
No. showing change >25%			5 of 5					1 of 6	

* No. showing change <25%

Legend:

Effect of noradrenaline following atropine and of isoprenaline following atropine on the 133 Xe clearance rate (groups 15 and 16).

TABLE XXIII

Dose (ug.) Atropine	Dose (ug.) Serotonin	$T\frac{1}{2}$ (min.) After Atropine	$T\frac{1}{2}$ (min.) After Serotonin	Results ≠
5.0	0.1	56	73°	B 50:1*
5.0	0.1	51	48	B 50:1*
5.0	0.1	39	43	B 50:1*
3.0	0.1	21	52°	B 30:1
2.5	0.1	54	35	NB 25:1
5.0	0.5	43	39	B 10:1*
1.0	0.1	29	29	B 10:1*
1.0	0.1	28	33	B 10:1*
5.0	1.0	45	61°	B 5:1*
5.0	1.0	39	17	NB 5:1
5.0	2.5	39	17	NB 2:1
5.0	5.0	73	26	NB 1:1
5.0	5.0	41	23	NB 1:1
1.0	10.0	28	21	NB 1:10

No showing change > 25%

*No.showing change < 25%

≠ Dose ratios atropine:serotonin

0 reduction in clearance rate 25%.

Legend: The effect of serotonin following a prior dose of atropine on the 133 Xe clearance rate (group 17).

TABLE XXIV

Dose (ug.) Atropine	Dose (ug.) Histamine	$T_{\frac{1}{2}}$ (min.) After Atropine	$T_{\frac{1}{2}}$ (min.) After Histamine	Results /
5.0	0.1	42	37	B 50:1*
2.5	0.1	43	41	B 25:1*
10.0	1.0	17	20	B 10:1*
5.0	1.0	37	26	NB 5:1
10.0	2.5	34	17	NB 4:1
2.0	1.0	26	24	B 2:1*
1.0	1.0	31	33	B 1:1*
100.0	100.0	74	34	NB 1:1
5.0	5.0	29	20	NB 1:1
3.0	5.0	41	23	NB 1:1.7
2.5	10.0	28	19	NB 1:4
2.5	25.0	43	26	NB 1:10

No. showing change > 25% 7 of 12

* No. showing change < 25%

/ Dose ratio atropine:histamine

Legend: Effect of histamine following atropine on the 133 Xe clearance rate (group 18).

TABLE XXV

Dose (µg.) Thymoxamine	Dose (µg.) Histamine	$T\frac{1}{2}$ (min.) After Thymoxamine	$T\frac{1}{2}$ (min.) After Histamine	Results †
10.0	0.025	19	22	B 400:1*
100.0	0.5	17	24	B 200:1*
10.0	0.05	22	20	B 200:1*
5.0	0.025	30	26	B 200:1*
250.0	2.5	49	49	B 100:1*
250.0	2.5	44	44	B 100:1*
5.0	0.05	32	23	NB 100:1
200.0	2.5	52	47	B 80:1*
200.0	2.5	50	32	NB 80:1
5.0	0.1	26	18	NB 50:1
100.0	2.5	45	17	NB 40:1
80.0	2.5	38	21	NB 32:1
25.0	1.0	37	26	NB 25:1
40.0	2.5	45	22	NB 16:1
20.0	2.5	37	21	NB 8:1
100.0	25.0	24	26	B 4:1*
10.0	5.0	20	13	NB 2:1

No. showing change >25%

* No. showing change <25%

† Dose ratios thymoxamine:histamine

Legend: Effect of histamine after thymoxamine of the 133 Xe clearance rate (group 19).

atropine to isoprenaline of 2:1 no response to isoprenaline was obtained (Table 22). However, when that experiment was repeated on three occasions isoprenaline injection was followed by a marked vasodilator response and when the dose ratio employed was 1:1 or 1:2 the clearance rate was increased following isoprenaline.

When atropine was administered prior to serotonin in a dose ratio of atropine to serotonin of less than 5:1 the serotonin induced increase in clearance rate was obtained. In dose ratios in excess of 5:1 there was an increase in clearance rate in only one of eight studies (Table 23). In three studies a distinct reduction in clearance rate was noted. When histamine was administered following the prior administration of atropine in dose ratios of atropine to histamine in excess of 10:1 no increase in clearance rate was obtained whereas in all studies in which the dose ratio was less than 1:1 the clearance rate was increased (Table 24). Variable responses were obtained at intermediate dose ratios. When histamine was administered following thymoxamine in the dose ratios of thymoxamine to histamine in excess of 100:1 no change in clearance rate was noted (Table 25). However, when

TABLE XXVI

Control $T_{\frac{1}{2}}$ (mins.)	Dose Mepyramine (ug.)	Dose Histamine (ug.)	$T_{\frac{1}{2}}$ (mins.) After Mepyramine	$T_{\frac{1}{2}}$ (mins.) After Histamine
68	5.0	1.0	65*	25
46	10.0	1.0	48*	28
52	10.0	1.0	49*	34
58	20.0	1.0	54*	20
64	40.0	1.0	64*	48
63	60.0	1.0	60*	42
40	80	1.0	45*	30
69	80	1.0	43	29
48	100	1.0	50*	26
65	100	1.0	48	30
Change > 25%	2 of 10		10 of 10	

* Change < 25%

Legend: Effect of mepyramine malleate and of histamine on the 133 Xe clearance rate.

the dose ratio was less than 100:1 the clearance rate was increased in nine of eleven studies. When mepyramine malleate was administered prior to histamine, even in dose ratios of 100:1 the histamine effect was clearly apparent. Although no change attended the administration of low doses of mepyramine an increase in clearance rate was noted with doses of 80 and 100γ g. (Table 26).

DISCUSSION

Although many variables may contribute to the clearance rate, including the physical characteristics of the tracer substance and the structure and perfusion of the tissue (Chap. 2), it would seem reasonable to ascribe abrupt changes in clearance rate occurring reproducibly following drug administration to pharmacologically induced changes in perfusion (Gosselin, 1966). The point is strengthened when predictable dose related responses of large dimensions can be demonstrated. Increase in $T\frac{1}{2}$ value and therefore reduction in clearance rate and perfusion rate are interpreted as vasoconstrictor responses and conversely reduction in $T\frac{1}{2}$ value is interpreted as vasodilator response in this study. As shown in Fig. 11 the points on the clearance graph immediately following drug administration may be erratically distributed and are neglected in calculating $T\frac{1}{2}$ values. All $T\frac{1}{2}$ values quoted in this thesis are calculated only from straight lines of at least 10 minutes' duration. The factors contributing to the points immediately following injection are complex and include isotope and drug distribution, electronic adjustment to the

new count rate and rate of onset of drug effect. The reproducibility of the clearance rate of intra-articularly injected 133 Xe has been determined (Chap. 3). In the present study change in excess of 25% was taken to be significant. This value is in excess of the range of the differences of 14 duplicate $T\frac{1}{2}$ values previously obtained (Chap. 3 : Table 10).

The responses obtained with appropriate doses of methacholine, noradrenaline and isoprenaline administered alone are easily interpretable in the light of their effects on other vascular beds (Goodman and Gilman, 1965). The methacholine effect was found to last from 10 to 15 minutes. In nine cases out of ten when methacholine was injected 15 minutes after a prior dose of methacholine, vasodilation occurred. However, in five of six cases when isoprenaline followed a previously administered vasodilator, no further increase in clearance rate attended the administration of isoprenaline. It is possible that maximal vaso-dilation had occurred with the first drug administration. This is in accordance with the observation of Brownlee (1966) that the vessels maximally dilated with a drug could no longer respond to isoprenaline. It seems

likely that failure of response to isoprenaline is related both to the identity and to the dose of the compound previously injected. In the single study in which a response was obtained, the compound previously administered was methacholine. The dose in that instance was only 2.5µg. whereas no further effect of isoprenaline had been demonstrable following a much larger dose of methacholine. It may be that the duration of action of the higher dose was more prolonge That thymoxamine administration causes an increase in perfusion has been interpreted by other workers as indicating the abolition of basal α adrenergically mediated vasoconstrictor tone (Birmingham et al., 1967; Myers et al., 1968).

Similar conclusions are suggested by the decrease in $T\frac{1}{2}$ values occurring after thymoxamine in the present study. The vasodilator effect of histamine on the peripheral vasculature in other tissues (Dale and Laidlaw, 1919 : Diana et al., 1968) is reflected in the increased clearance rates observed in the present study. Results in other systems on the vascular effects of serotonin have yielded conflicting results in the past. In doses of the order

of 100 to 1,000 γg. subcutaneously injected this compound caused intense vasoconstriction of skin blood vessels (Greaves and Shuster, 1967). When infused intra-arterially in doses of 2 to 16 γg./min. transient vasodilation for two minutes was followed by vasoconstriction and Walshe (1967) concluded that in muscle vessels both constrictor and dilator responses were present, the latter predominating. In the present study only vasodilator responses were obtained, clearance returning to the original rate after 15 to 30 minutes.

The results with atropine are of particular interest In high doses variable responses were obtained whereas with lower doses vasodilation was noted in all studies. Though this is the opposite response which one would expect with a muscarinic blocking agent, therapeutic doses of atropine may result in dilated skin blood vessels (Goodman and Gilman, 1965). Ashford (1962) found that doses of atropine one-tenth of the dose required to block acetylcholine caused excitation and he suggested that atropine may be stimulating the receptors when it first combines with them. When both high and low doses of atropine were administered

following atropine, no change in clearance rate was observed. It is possible that following the initial stimulatory dose of atropine, additional atropine in the receptor area might not be excitatory. When atropine was administered after a previous dose of methacholine, vasoconstrictor responses were obtained in five of the 10 studies; but when atropine followed serotonin in four studies out of six vasodilation occurred. In the first set of experiments atropine could be reversing residual methacholine effects, while in the second set atropine could still be exciting the yet unstimulated parasympathetic receptors. Serotonin may have a sensitizing effect on atropine as it has been found to have with norepinephrine and histamine (De la Lande, 1966).

When methacholine was administered after atropine, the methacholine vasodilatory effect could be prevented consistently at dose ratios of one to one and greater while ratios of one to 17 or less did not block. When atropine and methacholine were administered together a block occurred at dose ratios of two to one and greater. Atropine, a

muscarinic receptor antagonist, would be expected to block methacholine, and it acted as an effective blocker for at least 35 to 45 minutes. Any modifying effect of atropine on the vasoconstriction induced by noradrenaline must be extremely weak. Noradrenaline induced vasoconstrictor responses were obtained even at a dose ratio of 400 to one. Interestingly, atropine was found to inhibit in a dose ratio of two to one the vasodilatory effect of the β adrenergic receptor stimulator isoprenaline. This result however was unrepeatable and did not occur at other dose ratios. Although the explanation of this unusual response is not immediately apparent, the other results suggest that atropine does not exert a modifying influence on isoprenaline. Atropine may, however, abolish the vasodilator effect of histamine on peripheral blood vessels (Goodman and Gilman, 1965). When administered prior to histamine in a dose ratio of ten to one or greater, no increase in clearance rate subsequently attended histamine administration. This result may be interpreted to suggest that at least some component of the histamine response is mediated by receptors susceptible to atropine blockade. It is extremely interesting that the histamine effect persisted despite the prior administration of large doses of antihistamine.

again suggesting that histamine is not acting simply through "histamine receptors". Increase in clearance rate with large doses of mepyramine may be the result of histamine release by this compound. A modifying effect of thymoxamine on the histamine vasodilator response in a dose ratio of greater than 100 to 1 was noted. This weak histaminolytic effect has been noted by other workers (Brownlee, 1966 : Birmingham et al., 1967). Atropine is the stronger blocking agent of the two because the dose ratio of atropine necessary to block histamine was at least ten times lower than the ratio of thymoxamine needed to block histamine. Atropine was also found to block serotonin at dose ratios higher than 5 to 1, which is the same order of magnitude as the dose ratio required to block histamine. It is possible that atropine blocks through the same pathways for both drugs. The methacholine blocking action of atropine is clearly more efficient than its weak effect on histamine and serotonin. It is interesting that vasoconstrictor responses were obtained when serotonin was injected following atropine. This may indicate that serotonin

constrictor responses are also present in synovial tissue, but are only seen when the over-riding vasodilatory responses are blocked.

The results of this study suggest the presence of muscarinic receptors in canine synovial tissue. Both histamine and serotonin elicit vasodilator responses, which are modified by the prior administration of atropine. A weaker histaminolytic effect of thymoxamine has been demonstrated but mepyramine malleate failed to modify the effect of histamine. This suggests that histamine may be acting through muscarinic receptors rather than specific "histaminic" receptors in canine synovium. The methacholine vasodilator response may be inhibited either by prior administration or concurrent administration of atropine. The administration of atropine alone, in low doses excites a vasodilator response but variable results are obtained with higher doses. Noradrenaline induced vasoconstriction occurs despite prior treatment with atropine and atropine failed to exert a modifying affect on isoprenaline induced vaso-dilation.

SUMMARY

1. The effect of various compounds on the rate of clearance of intra-articularly administered 133 Xe as an indirect measure of synovial perfusion was studied.

2. Vasodilator responses were obtained with methacholine, histamine, serotonin, thymoxamine, isoprenaline with high doses of mepyramine and with low doses of atropine. Higher doses of atropine yielded conflicting results. Vasoconstrictor responses were obtained with noradrenaline.

3. Atropine injected following serotonin commonly resulted in vasodilator responses whereas following methacholine variable results were obtained.

4. Atropine abolished the response to methacholine and to a lesser extent, to serotonin and histamine. Thymoxamine was weakly histaminolytic. Mepyramine failed to prevent histamine induced vasodilatation. It is proposed that histamine may be acting through cholinergic rather than specific "histaminic" receptors in canine synovium.

5. Atropine did not modify the vasodilator response to isoprenaline and did not affect the vasoconstrictor response to noradrenaline.
6. The results suggest the presence of muscarinic receptors in canine synovial tissue.

CHAPTER 5

PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES IN NORMAL AND INFLAMED HUMAN KNEE JOINTS

In this chapter the physiological responses of human synovial vasculature to physical agents and to pharmacological stimuli are documented. Most of this work is founded upon results obtained previously in animal experiments reported in Chapter 4.

The changes in synovial perfusion induced in both normal and inflamed joints by external heating, exercise and the intra-articular injection of histamine are discussed.

The presence of adrenergic receptors in both normal and inflamed human synovial tissue is demonstrated and the differences between the responses of the normal and diseased joints are discussed.

STUDIES ON THE SYMPATHETIC CONTROL OF NORMAL AND DISEASED SYNOVIAL BLOOD VESSELS: THE EFFECT OF AND RECEPTOR STIMULATION AND INHIBITION, MONITORED BY THE 133 XENON CLEARANCE TECHNIQUE

ADRENERGIC CONTROL OF HUMAN SYNOVIAL PERFUSION

Introduction

This study represents an attempt to define functionally, by the response of the 133 Xe clearance rate to intra-articularly administered exogenous catecholamines, the presence of adrenergic receptors in both normal and abnormal human synovial tissue. The work is a logical extension of previous work performed in animals (Chap. 4 : page 122) and the methods employed are similar. In particular, the similarities between the responses obtained in normal human and canine and in inflamed human and canine joints were studied.

TABLE I

Numbers of Studies using Different Drugs Injected into the Knee Joints of Normal Subjects and of Patients with Osteoarthritis (O.A.) and Rheumatoid Arthritis (R.A.)

	Normal	O.A.	R.
Isoprenaline 2.5 g.	8	25	2
Propranolol 40 g.	8	17	2
Propranolol 40 g. with isoprenaline 2.5 g.	8	15	2
Noradrenaline 2.5 g. without prior heating	1	6	
Noradrenaline 2.5 g. after application of external heat to joint	7	4	
Phentolamine 40 g.	8	20	2
Phentolamine 40 g. with noradrenaline 2.5 g.	5	20	2
Phentolamine 40 g. with noradrenaline 5 g.	3	0	

MATERIALS AND METHODS

Patients and Control Subjects

In all, 275 studies were performed (Table 1). Sixteen normal subjects volunteered to participate. All were male University students between the ages of 22 and 30 years and none gave a history of disease or of trauma to the knee joint. Careful clinical examination of the knee joints revealed no abnormality. Thirty-nine patients who had clinical and radiological evidence of osteoarthritis of the knee joints were studied. Their ages ranged from 39 to 72 years (mean 58.2 years) and 12 were male. Serological tests for rheumatoid factor were negative and in all the erythrocyte sedimentation rate was less than 15 mm. per hour (Westergren). Sixty-four patients with "classical" rheumatoid arthritis (Ropes et al., 1959) were studied. In all, serological tests for rheumatoid factor were positive and the erythrocyte sedimentation rate exceeded 20 mm. per hour (Westergren). All of these patients had clinical and radiological evidence of knee joint involvement by the disease process at the time of study. Their mean age was 42.3 years (range 28 to 69 years); nineteen were male.

All patients and control subjects volunteered to participate in the study with full knowledge of its conten

Injection and Counting Technique

The method of injection, with aseptic precautions, has been described previously (Chap. 3 : page 96). To obtain reproducible results, it is of particular importance to ensure that the needle point is free in the joint cavity and to wait for approximately five minutes following the insertion of the needle before injecting the isotope solution. In the present study the joint was not exercised following injection and the dose of 133 Xe administered was approximately 20μ Ci. The needle, which between injections was attached to a sterile syringe, containing either sterile 0.9% Na Cl or synovial fluid, remained in situ throughout the study providing a convenient route for the repeated intra-articular administration of drugs with minimal discomfort to the patient.

In feasibility studies on normal subjects the drugs were administered without additional isotope. The expected responses to exogenous catecholamine injection were not obtained consistently but when these studies were repeated employing the same dose of the drug but with the concurrent administration of a small amount (less than 0.05 ml.) of additional isotope solution

a change in clearance rate was observed in every case. The problem is discussed by Gosselin (1966) and is due to the placement of isotope and drug in different sites. In the present study, whenever a drug was administered a small amount of isotope solution was injected with it. In twelve studies on knees with different clearance rates, including two normal subjects, following the control monitoring of clearance rate a second dose of 133 Xe was injected and the count rate again monitored for fifteen minutes. The $T\frac{1}{2}$ values obtained on each occasion are shown in Table 2. The standard error of the difference was 0.87 minutes.

The count rate was monitored using a collimated detector (EKCO M5400A/1) incorporating a 1" x 1½" T.I. sodium iodide scintillation crystal which was aligned 2" from, and directly above, the patella. Pulses were fed into a pulse height analyser (EKCO M5010) and ratemeter (EKCO M5183A) and recorded on a direct writing pen recorder (Goerz Servoscribe RE511). At the completion of each study the count rate minus background (of the order of 0.1% count rate) was plotted onto semilogarithmic graph paper as a function of time. The halving times ($T\frac{1}{2}$ values, minutes) were obtained

from these straight line graphs.

Pharmacological Agents

The pharmacological agents employed in this study were Isoprenaline sulphate B.P. in normal saline (Boots Pure Drug Co.), Phentolamine mesylate B.P. ("Rogitine" CIBA Laboratories Ltd.), Propranolol hydrochloride ("Inderal" Imperial Chemical Industries Ltd.) and l-nor-adrenaline acid tartrate ("Levophed" Bayer Products Co.). These preparations were diluted in sterile 0.9% NaCl to give a final concentration of 5 μg/ml. for isoprenaline and noradrenaline and 80 μg/ml. for propranolol and phentolamine. In three studies on normal subjects the dose of noradrenaline employed with 40 μg. of phentolamine was 5 μg. whereas in the other five in that group the dose of noradrenaline was reduced to 2.5 μg. (Table 1). Otherwise, and with the exception of the repeat studies in which the doses employed are separately recorded, the doses of drugs used were as follows:- isoprenaline 2.5 μg., noradrenaline 2.5 μg., propranolol 40 μg. and phentolamine 40 μg.

Experimental Protocol

In all cases, a control monitoring period of approximately 15 minutes followed the intra-articular

injection of the isotope. Thereafter a catecholamine was administered, together with a further small amount of isotope and the count rate monitored for another 15 minutes.

In the normal subjects both knees were studied. In the first knee either isoprenaline or noradrenaline was injected as described and then the opposite joint was studied using the appropriate blocking drug. Following the control period, either propranolol or phentolamine was administered and the count rate monitored for 10 to 15 minutes; finally the stimulating and corresponding blocking agent were administered together and the clearance rate followed for a further 15 minutes. Thus, if isoprenaline was administered into the left knee joint, then first propranolol and then a mixture of propranolol and isoprenaline were injected into the right knee.

In the case of arthritic patients it was frequently not possible to study both joints, either because the patients found it uncomfortable to remain immobile for the necessary time or because of asymmetry of joint involvement. Accordingly, the administration of isoprenaline, noradrenaline, propranolol or phentolamine

alone was considered to represent a separate study. Likewise, the administration of a blocking agent, followed later by a mixture of blocking agent and catecholamine was considered to represent a separate study and the results are expressed in this way. In all, isoprenaline alone was administered in eight studies of normal subjects, in 25 studies of patients with osteoarthritis and in 24 studies of patients with rheumatoid arthritis (Table 1). The number of studies in each group in which propranolol, propranolol with isoprenaline, noradrenaline, phentolamine and noradrenaline with phentolamine were administered is similarly shown in Table 1.

One limitation inherent in the 133 Xe clearance technique is the difficulty of demonstrating a reduction in an already slow perfusion rate. The semilogarithmic plots tend towards the horizontal and the error of plotting and of determining the $T\frac{1}{2}$ value increases. Accordingly, all $T\frac{1}{2}$ values in excess of 150 minutes in this study are simply recorded as > 150. For the same reason it was not possible to demonstrate directly the effect of noradrenaline in normal subjects and in some diseased patients whose baseline $T\frac{1}{2}$ values

were high. In such instances, therefore, prior to injection of noradrenaline, the knee joint was subjected to external heating. Following the control monitoring period the subject was placed in a sitting position with the knee joint equidistant between the electrodes of a short wave diathermy machine (Siemens Ultratherm 6089B). The control was set to maximum heat for between five and ten minutes. When the subject began to feel uncomfortably warm, heating was discontinued and the patient returned to the original counting position. In no case did the time to return to the original position exceed 1 minute. The count rate was monitored for over 10 minutes and, thereafter noradrenaline was injected and the study continued. In one subject the waning of the increased clearance rate due to heating was followed without interference for twenty minutes. The semilogarithmic plot obtained is shown in Fig. 2.

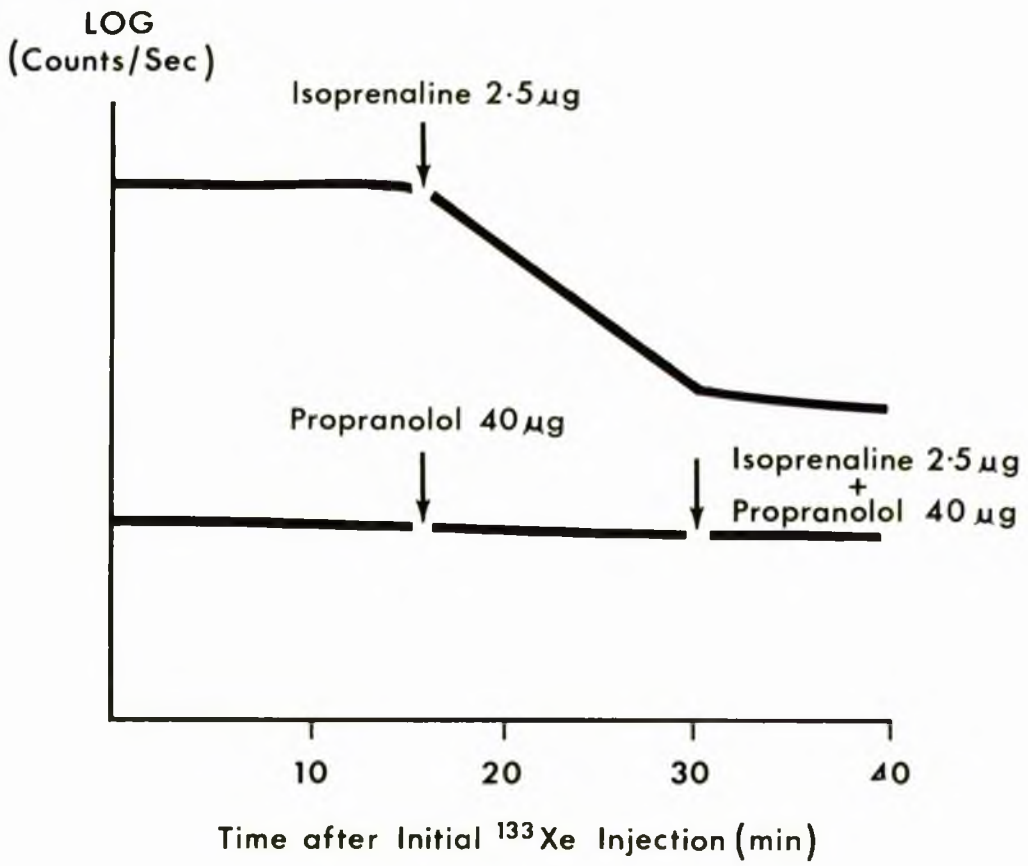


Fig. 1

Legend: Semi-logarithmic plots of count rate per second against time showing, in normal subjects, the effects of intra-articular isoprenaline, propranolol and isoprenaline with propranolol on the clearance of 133 Xe from the knee.

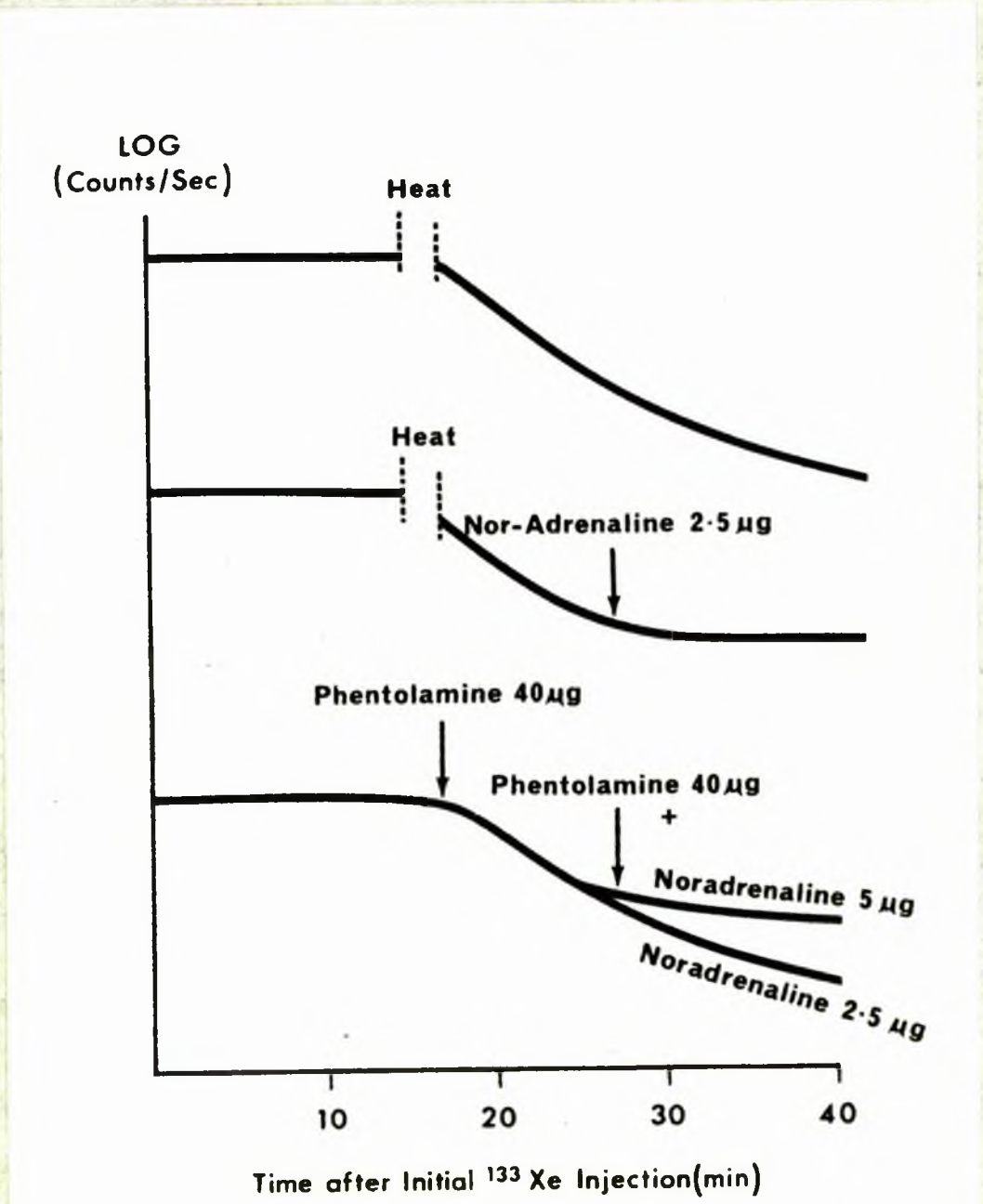


Fig. 2

Legend: Semi-logarithmic plots of count rate per second against time showing, in normal subjects, t effects on 133 Xe clearance from the knee of exter heat, intra-articular noradrenaline, phentolamine phentolamine with noradrenaline in different dose ratios.

TABLE II

Reproducibility of $T\frac{1}{2}$ values of 133 Xe clearance: 12 Studies showing the effect on $T\frac{1}{2}$ values of a second injection of 133 Xe given about 15 mins. after the initial dose

$T\frac{1}{2}$ (minutes) after 1st injection of 133 Xe	$T\frac{1}{2}$ (minutes) after 2nd injection of 133
150	150
150	150
100	100
63	56
55	51
55	52
41	39
32	27
30	27
28	27
28	25
13	16

Standard error of the difference = 0.82 minutes.
(Calculated from lower 10 figures in the table).

RESULTS

The results are shown in Figs. 1 and 2 and Tables 2-6.

The $T\frac{1}{2}$ values obtained from each monitoring period when 133 Xe alone was introduced into the joint cavity on two occasions consecutively are shown in Table 2. The standard error of the difference was only 0.82 minutes.

Examples of the responses of the clearance rate in normal subjects to each of the drugs administered are shown in Figs. 1 and 2. It can be seen that isoprenaline (2.5 μg.) markedly increased the clearance rate whereas this effect was not apparent following the prior administration of propranolol (40 μg.). The injection of propranolol (40 μg.) alone was attended by no alteration in clearance rate (Fig. 1). The effect of external heating alone is depicted in Fig. 2, where it can be seen that the effect is transient and that return to baseline values is attended by a smooth curve on the semilogarithmic plot of the count rate against time. On the other hand, when noradrenaline (2.5 μg.) is injected in the post-heating period the clearance rate is abruptly and markedly reduced at that point. When phentolamine

TABLE III Normal Subjects. Effect of the intra-articular injection of various drugs, and of external heating, on the $T\frac{1}{2}$ values of 133 Xe clearance from the knee joint.

Isoprenaline (2.5μg)		Propranolol (40μg)		Isoprenaline (2.5μg) with and after Propranolol (40μg)		Noradrenaline (2.5μg) and heating			Phentolamine (40μg)		Noradrenaline (2.5μg) with and after phentolamine (40μg)		Noradrenaline (5μg) with and after phentolamine (40μg)	
						$T\frac{1}{2}$ (min.)								
$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)	$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)	$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)	Before heating	After heating	After norad.	$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)	$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)	$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)
>150	7	>150	>150	>150	>150	>150	40	>150	>150	60	70	70	74	110
>150	9	>150	>150	>150	>150	>150	55	>150	>150	74	65	65	60	112
>150	12	>150	>150	>150	>150	>150	55	>150	>150	35	40	40	35	100
>150	19	>150	>150	>150	>150	>150	65	>150	>150	40	40	44		
>150	22	>150	>150	>150	>150	140	60	>150	>150	40	30	35		
>150	30	>150	>150	>150	>150	130	58	>150	>150	65				
>150	40	140	140	140	140	130	80	>150	145	70				
>150	42	130	130	130	130	105	-	>150	135	30				
Proportion of studies showing change														
8/8		0/8		0/8 (all block)		8/8			8/8		0/5 (all block)		3/3	

TABLE IV **Patients with Osteoarthritis.** Effect of the intra-articular injection of various drugs, and of external heating, on the $T\frac{1}{2}$ values of 133 Xe clearance from the knee joint.

Isoprenaline (2.5μg)		Propranolol (40μg)		Isoprenaline (2.5μg) with and after Propranolol (40μg)		Noradrenaline (2.5μg) and heating			Phentolamine (40μg)		Noradrenaline (2.5μg) with and after Phentolamine (40μg)	
						$T\frac{1}{2}$ (min.)						
$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)	$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)	$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)	Before heating	After Heating	After norad.	$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)	$T\frac{1}{2}$ before (min.)	$T\frac{1}{2}$ after (min.)
>150	30	>150	>150	>150	>150	150	80	>150	105	65	70	70
>150	17	115	110	110	110	105	50	>150	100	45	65	65
>150	12	100	100	100	105	90	65	>150	98	30	60	60
>150	22	95	95	95	95	75	60	>150	82	70	45	45
>150	47	92	90	80	84	56	-	>150	75	60	42	40
>150	32	86	87	70	70	50	-	>150	73	35	40	40
150	19	82	80	52	50	46	-	>150	58	42	40	40
110	22	72	70	50	50	42	-	>150	58	33	38	90*
110	26	50	50	42	21	38	-	>150	56	40	38	60
95	25	50	52	35	8*	38	-	>150	52	40	37	80
88	20	42	42	30	20				41	38*	36	96
85	16	35	35	31	31				40	36	35	35
78	31	32	30	28	30				36	36	34	54
56	22	30	31	26	12				36	37	33	33
55	23	28	28	16	20				32	34	30	29
50	50*	24	26						31	30	30	30
48	6	16	16						25	26	26	28
45	16								24	24*	24	50
42	17								18	20	20	50
40	12								17	17*	17	20
39	10											
34	17											
28	16											
26	31*											
16	20											
Proportion of studies showing normal responses												
22/25		0/17		11/15 (block)		10/10			10/20		13/20 (block)	

* studies subsequently repeated; see Table 6.

40 μg. was injected the clearance rate was increased (Fig. 2): subsequently, whereas the injection of 5 μg. of noradrenaline with 40 μg. of phentolamine was attended by a reduction in clearance, no change in the clearance rate was noted with 2.5 μg. of noradrenaline plus 40 μg. of phentolamine.

The $T_{\frac{1}{2}}$ values obtained in the studies on normal subjects are shown in Table 3. In all of eight normal subjects a response was obtained to isoprenaline (2.5 μg.) but not to propranolol (40 μg.) or to propranolol (40 μg.)/isoprenaline (2.5 μg.) combination. In all studies, noradrenaline (2.5 μg.) resulted in a marked reduction in clearance either administered alone (1 study) or following external heating (7 studies). In all of eight studies the injection of phentolamine (40 μg.) resulted in an increase in clearance. In all of five studies where the dose ratio of noradrenaline to phentolamine was 2.5 μg.:40 μg. no response was obtained, whereas when the dose ratio was 5 μg.: 40 μg. all of three studies showed a reduction in clearance.

The results obtained in the studies on patients with osteoarthritis are shown in Table 4. In 22 of 25 studies response to isoprenaline (2.5 μg.) was obtained

TABLE V Patients with Rheumatoid Arthritis. Effect of the intra-articular injection of various drugs, and of external heating, on the $T_{\frac{1}{2}}$ values of 133 Xe clearance from the knee joint.

Isoprenaline (2.5μg)		Propranolol (40μg)		Isoprenaline (2.5μg) with and after Propranolol (40μg)		Noradrenaline (2.5μg) and heating			Phentolamine (40μg)		Noradrenaline (2.5μg) with and after Phentolamine (40μg)	
						$T_{\frac{1}{2}}$ (min.)						
$T_{\frac{1}{2}}$ before (min.)	$T_{\frac{1}{2}}$ after (min.)	$T_{\frac{1}{2}}$ before (min.)	$T_{\frac{1}{2}}$ after (min.)	$T_{\frac{1}{2}}$ before (min.)	$T_{\frac{1}{2}}$ after (min.)	Before heating	After heating	After norad.	$T_{\frac{1}{2}}$ before (min.)	$T_{\frac{1}{2}}$ after (min.)	$T_{\frac{1}{2}}$ before (min.)	$T_{\frac{1}{2}}$ after (min.)
150	20	>150	>150	120	120	150	75	>150	>150	100	100	100
140	16	120	120	100	105	110	80	>150	>150	49	56	55
140	52	100	100	95	95	110	60	>150	150	40	50	50
140	17	95	95	91	91	110	50	>150	110	30	49	50
130	30	90	91	84	85	82	-	>150	110	36	48	48
120	36	87	84	80	81	62	-	110	100	40	42	86*
110	35	78	80	55	35	54	-	>150	80	50	40	40
100	50	55	55	55	55	48	-	>150	62	20	40	40
85	31	55	55	46	46	40	-	130	58	48	38	40
82	82	48	46	45	35	36	-	>150	58	12	36	35
52	20	45	45	42	40	27	-	140	56	56	36	80
50	17	42	42	39	19	25	-	140	46	38	30	30
44	18	39	39	30	30	22	-	>150	46	30	30	32
38	38*	30	30	30	17*				42	42*	29	42
35	8	29	29	29	8				36	36	29	60
34	34	26	30	25	25				27	29	27	50
33	10	25	25	20	20				27	27	23	72
30	31	22	20	22	22				27	29	20	20
28	18	21	22	18	18				23	23*	19	18
26	26*	20	18	20	22				21	19	12	65 *
24	8	15	20						14	12*	12	14
24	24											
23	16											
22	22											
						Proportion of studies showing normal responses						
16/24 (and 1 equivocal response).		0/21		15/20 (block)		13/13			11/21 (and 1 equivocal response).		12/21 (block)	

* studies subsequently repeated; see Table 6.

and no response to propranolol (40 µg.) was obtained in any of 17 studies. In 4 of 15 studies response to isoprenaline (2.5 µg.) was obtained when administered with propranolol (40 µg.). In all studies in osteoarthritis response to noradrenaline (2.5 µg.) was obtained, in six studies directly and in four studies following heating. However, only 10 of 20 patients demonstrated an increase in clearance rate following phentolamine (40 µg.) and in all of these the baseline $T\frac{1}{2}$ value was over 50 minutes. In 13 of 20 studies no response to noradrenaline (2.5 µg.) given with phentolamine (40 µg.) was obtained but in the remainder (7 studies) the clearance rate was reduced.

In the studies on patients with rheumatoid arthritis (Table 5), 16 of 24 responded to isoprenaline (2.5 µg.), there was one equivocal response, and the remainder showed no increase in clearance rate. No response to propranolol (40 µg.) was obtained in any of 21 studies. In five studies the clearance rate was increased following the injection of propranolol (40 µg.) and isoprenaline (2.5 µg.) together, but in another 15 studies there was no such change. Reduction

TABLE VI **Repeat studies on patients with osteoarthritis and rheumatoid arthritis, using varying doses of drugs.**

a) **Isoprenaline**

Patient	Diagnosis	Dose of Drug (μg)	T½ before (min.)	T½ after (min.)
1	O.A.	2.5	50	50
		2.5	30	29
		10	29	12
2	O.A.	2.5	26	31
		2.5	39	39
		10	39	18
3	R.A.	2.5	38	38
		2.5	29	29
		10	29	12
4	R.A.	2.5	26	26
		2.5	20	21
		10	21	11

b) **Isoprenaline with propranolol**

Patient	Diagnosis	Dose of Drug (μg) Isopren.	Propran	T½ before (min.)	T½ after (min.)
5	O.A.	2.5	40	35	8
		2.5	40	42	14
		1	80	39	39
6	R.A.	2.5	40	30	17
		2.5	40	36	20
		1	80	38	38

c) **Phentolamine**

Patient	Diagnosis	Dose of Drug (μg)	T½ before (min.)	T½ after (min.)
7	O.A.	40	41	38
		40	36	36
		200	36	35
8	O.A.	40	24	24
		40	38	38
		200	38	38
9	O.A.	40	17	17
		40	26	26
		200	26	26
10	R.A.	40	23	23
		40	20	20
		200	20	21
11	R.A.	40	42	42
		40	48	46
		200	46	47
12	R.A.	40	14	12
		40	24	24
		200	24	23

d) **Noradrenaline with phentolamine**

Patient	Diagnosis	Dose of Drug (μg) Noradren.	Phentol	T½ before (min.)	T½ after (min.)
13	O.A.	2.5	40	38	90
		2.5	40	30	72
		1	80	34	34
14	R.A.	2.5	40	42	86
		2.5	40	31	70
		1	80	34	34
15	R.A.	2.5	40	12	65
		2.5	40	24	53
		1	80	20	20

in clearance rate was obtained in all of 13 studies following the injection of noradrenaline (2.5 µg.), in 9 studies administered alone and in 4 administered following heating. In 9 of 21 studies no increase in clearance rate was observed following the injection of phentolamine (40 µg.), these studies being characterised by baseline $T\frac{1}{2}$ values of less than 60 minutes. When phentolamine (40 µg.) and noradrenaline (2.5 µg.) were administered together no response was obtained in 14 studies. However, reduction in clearance rate was observed in the other seven studies.

Repeat Studies

Certain studies in osteoarthritic and rheumatoid knee joints were repeated and the results are shown in Table 6.

Repeat studies were carried out on two patients with osteoarthritis in whom no response to isoprenaline (2.5 µg.) had been obtained on the first occasion. In one patient the $T\frac{1}{2}$ value before injection was 50 minutes and was unchanged after the injection. On the second occasion, using the same dose of isoprenaline, the values before and after injection were 30 and 29 minutes respectively. 10 µg. of isoprenaline were then injected and the $T\frac{1}{2}$ value was reduced from 29

minutes to 12 minutes. In the second patient the respective $T\frac{1}{2}$ values before and after injecting 2.5 μg. of isoprenaline were 26 and 31 minutes and 39 and 39 minutes, and with 10 μg. of isoprenaline, 39 and 18 minutes.

In one osteoarthritic patient response to isoprenaline (2.5 μg.) was obtained when the drug was administered with propranolol (40 μg.). The $T\frac{1}{2}$ values were 35 and 8 minutes respectively. On the second occasion, employing the same dose ratio, the respective values were 42 and 14 minutes, but when the dose of isoprenaline was reduced to 1 μg. and that of propranolol increased to 80 μg. the respective values were 39 and 39 minutes, denoting the blocking action of propranolol.

In three patients, whose initial study had shown lack of response to phentolamine (40 μg.) ($T\frac{1}{2}$ values 41 and 38, 24 and 24, 17 and 17 minutes respectively), the study was repeated with the same dose of phentolamine. Again no response was obtained ($T\frac{1}{2}$ values 36 and 36, 38 and 38, 26 and 26 minutes respectively) and when the study was repeated employing 200 μg. of phentolamine still no response was obtained ($T\frac{1}{2}$ values 36 and 35, 38 and 38, 26 and 26 minutes respectively).

In one study where noradrenaline (2.5 µg.) effect had been apparent despite the coincidental administration of phentolamine (40 µg.), the $T\frac{1}{2}$ values were 38 and 90 minutes respectively. When this study was repeated employing the same doses the $T\frac{1}{2}$ values were 30 and 72 minutes, but when the study was repeated on a third occasion with only 1 µg. of noradrenaline to 80 µg. of phentolamine the $T\frac{1}{2}$ values were 34 and 34 minutes denoting the α blocking action of phentolamine.

Similarly the $T\frac{1}{2}$ values obtained in repeat studies in rheumatoid arthritis are shown in Table 6. When isoprenaline 2.5 µg. was administered, on the first occasion the $T\frac{1}{2}$ values were 38 minutes before and 38 minutes after injection in one patient and 26 minutes before and 26 minutes after in another. On the second occasion the results were 29 and 29 minutes, and 20 and 21 minutes respectively. In both patients, however, when the dose of isoprenaline was increased to 10 µg., change in $T\frac{1}{2}$ values were observed, in the first patient from 29 to 12 minutes and in the second patient from 21 to 11 minutes.

In another study the isoprenaline effect was noted when 2.5 µg. were administered in conjunction with 40 µg.

of propranolol. The $T\frac{1}{2}$ values were 30 and 17 minutes. The same result was obtained when the study was repeated employing the same dose ratio, the $T\frac{1}{2}$ values being 36 and 20 minutes. When the study was repeated in the same patient employing only 1 µg. of isoprenaline to 80 µg. of propranolol, the $T\frac{1}{2}$ value before injection (38 minutes) was identical to that obtained after the injection.

When phentolamine (40 µg.) was injected intra-articularly in three studies, on the first occasion the basal $T\frac{1}{2}$ values (23, 42 and 14 minutes) were similar to the values obtained after injection (23, 42 and 12 minutes respectively). These studies were repeated and again the $T\frac{1}{2}$ values prior to injection (20, 48 and 24 minutes) were close to the post-injection values (20, 46 and 24 minutes respectively). On a third occasion, the dose of phentolamine employed was 200 µg. and again there was no difference between the values before (20, 46 and 24 minutes) and after (21, 47 and 23 minutes) respectively, the injection.

In two studies, noradrenaline (2.5 µg.) and phentolamine (40 µ g.) were administered and in both cases a response to noradrenaline had been obtained,

the changes in $T\frac{1}{2}$ values before and after injection being 42 to 86 minutes and 12 to 65 minutes respectively. This response was confirmed when the studies were repeated; the corresponding changes in $T\frac{1}{2}$ values were 31 to 70 minutes and 24 to 53 minutes respectively. On a third occasion, noradrenaline (1 μ g.) and phentolamine (80 μ g.) were administered together and in both studies the $T\frac{1}{2}$ values prior to injection (34 and 20 minutes) were identical to those obtained after injection, denoting the α receptor blocking action of phentolamine.

DISCUSSION

The 133 Xe clearance technique offers certain advantages which permit measurements otherwise impossible *in vivo*. The technique is simple, requiring only accurate placement of the isotope and careful injection technique. The clearance rate is reproducible, the standard error of the difference of repeated observations being only 0.8% minutes and the $T_{\frac{1}{2}}$ value provides an indirect measure of nutritive or capillary perfusion (Friedman, 1968 : Chapter 2). This may be more relevant to cellular mechanisms than the determination of total flow to or from a tissue. Finally, the low energy of gamma emission (80 KeV) and the high air:blood partition coefficient renders the radiation exposure very much less than that incurred by standard radiographic techniques (Bentivoglio et al., 1963 : Harper et al., 1964), particularly at the dose levels employed in this study. One advantage which is peculiarly relevant to the joint is that the isotope is introduced into a cavity and local pressure differences (Gosselin, 1966) are not, therefore, likely to present a major problem. The validity of the clearance model in the present context has been discussed (Chapter 2).

The results of the present study indicate that both α and β adrenergic receptors are functionally active in the control of synovial perfusion in normal subjects. Thus the increase in perfusion mediated by isoprenaline is prevented by the prior administration of propranolol, a β blocking agent. Similarly, the reduction in perfusion which attends the administration of noradrenaline is not apparent following the prior administration of the selective α blocking agent phentolamine. That the mechanism of this effect is related to the respective doses employed is demonstrated by the response of the normal subjects to noradrenaline and phentolamine. When the dose ratio was 2.5 μg. to 40 μg. respectively, then the effect of noradrenaline was not apparent. However, when the dose of noradrenaline was doubled to achieve the ratio 5 μg. to 40 μg., then the noradrenaline effect was obtained in every case.

The response of normal subjects to phentolamine alone was of particular interest; in all of them, synovial perfusion was markedly increased by the administration of this blocking agent. Phentolamine has been shown to possess a direct effect on blood

vessels at low concentration and a specific blocking effect at high doses. The molar concentrations of phentolamine in the synovium were not determined in this study and accordingly it is impossible to be certain whether this was "high" or "low". However, from the fact that the α blocking action was apparent at the dose employed, it seems reasonable to suggest that the increase in perfusion was consequent upon the α blocking action. If this is so, then the fact that an increase in perfusion was obtained suggests that the normal synovial perfusion rate is under α-adrenergic control. This has been shown to obtain in isolated animal preparations (Cobbold and Lewis, 1956a and c: Chap. 4).

The results obtained in both patient groups were less homogeneous. Thus, whereas in normal subjects all patients responded to the intra-articular injection of 2.5 γg. of isoprenaline, no response was obtained in three patients with osteoarthritis and in seven patients with rheumatoid arthritis. Similarly, in four patients with osteoarthritis and in five with rheumatoid arthritis, propranolol failed to block the isoprenaline response, and in seven patients with

osteoarthritis and in seven with rheumatoid arthritis phentolamine failed to block the noradrenaline response. When repeat studies employing the same dose levels were performed in a selection of these patients at a later date, although the initial $T\frac{1}{2}$ values were not identical the same results were obtained. Subsequently, however, each study was repeated employing a higher dose of the catecholamine, or a different catecholamine to blocking substance dose ratio and these unusual results were found to be dose related. Thus in those patients who failed to respond to isoprenaline in a dose of 2.5 μg., response was obtained when the dose of isoprenaline was 10 μg. Similarly if propranolol (40 μg.) failed to block the response to isoprenaline (2.5 μg.) the response was prevented when the dose of propranolol was 80 μg. and that of isoprenaline only 1 μg.; if phenolamine 40 μg. failed to block the response to noradrenaline 2.5 μg., then the blocking action was demonstrable when the dose of phentolamine was 80 μg. and that of noradrenaline only 1 μg.

The precise mechanism of this dose-related difference between normal and diseased subjects is not immediately apparent. Since the abnormalities were present in both

groups of patients, the mechanism cannot be specific to either disease entity. It is noticeable, however, that these abnormalities tended to occur in patients whose initial perfusion rates were high (Tables 4 and 5). It is possible, therefore, that the local concentration of these drugs is quickly reduced by an elevated perfusion rate. However, the relationship is not absolute and there were several studies where high baseline perfusion rates were associated with normal responses. On the other hand, particularly in the rheumatoid subjects, it is possible that elevated synovial fluid protein levels result in a degree of protein binding and inactivation of the catecholamines. This explanation seems less satisfactory in the osteoarthritis group since here synovial fluid protein levels are less likely to be abnormal. In both osteoarthritis and rheumatoid arthritis, clearance rates will be shown to be related to a clinical index of the degree of inflammatory involvement of the joint at the time of study (Chapter 6). It is possible that the differences between normal and abnormal responses are related to the perfusion rate only indirectly, through the degree of inflammatory involvement of the joint; in this case, tissue volume and

vascularity, synovial fluid protein content and the diffusion rates of the respective drugs, may all be involved. It is interesting to compare these findings with those obtained previously in normal and inflamed canine joints (Chapter 4). The results in normal human subjects and in normal canine studies are entirely consistent throughout. Similarly the results in rheumatoid arthritis and in osteoarthritis are similar to those obtained in experimental synovitis in dogs. The similarities are particularly striking in the respective responses to phentolamine. It would seem therefore that the unusual responses obtained in diseased joints are a feature of the inflammatory response regardless of the underlying pathological mechanism.

Failure of response to phentolamine in the diseased groups in these human studies was not related to the dose employed, since at repeat studies employing a higher dose no increase in clearance rate was obtained. Furthermore, in three studies in osteoarthritis and in two in rheumatoid arthritis, failure of response of clearance rate to phentolamine was followed by undoubted α blocking effect on noradrenaline. The failure of response to phentolamine

was more closely associated with high perfusion rates than were the other abnormalities (Tables 4 and 5). It would thus seem reasonable to propose that basal α vasoconstrictor tone, which is a feature of normal synovium, is lost in both osteo-arthritis and rheumatoid arthritis in those patients with high baseline perfusion rates.

In most tissues the α and β receptor populations are not equally balanced. Thus in skin, α receptors predominate, whereas in heart β receptors are more prominent. While no final conclusions are justified on the basis of the purely qualitative data obtained in the present study, the consistency and degree of the responses to noradrenaline and the results with phentolamine might be interpreted to suggest that α receptor influence is greater than that of the β receptors in synovium.

SUMMARY

1. The half-life ($T\frac{1}{2}$) of the disappearance curve of 133 Xe from the knee joint has been used as an indirect measure of synovial perfusion.

2. The effects of intra-articularly administered isoprenaline and noradrenaline and their respective blocking agents, propranolol and phentolamine, on the $T\frac{1}{2}$ values have been investigated in normal subjects and in patients with osteoarthritis and rheumatoid arthritis.

3. In normal subjects, isoprenaline increased the clearance rate and this effect was blocked by propranolol. Noradrenaline decreased the clearance rate and this effect was blocked by an adequate dose of phentolamine.

4. The injection of phentolamine, in normal subject caused an increase in synovial perfusion, suggesting the presence of a basal

   α -constrictor tone.

5. In the patients with osteoarthritis and rheumato arthritis quantitative differences were found in the responses to isoprenaline and noradrenaline and their respective blocking agents.

6. There appeared to be a qualitative difference in the case of phentolamine; vasoconstrictor tone may be lost in inflamed joints.

THE EFFECTS OF HEAT, EXERCISE AND HISTAMINE ON SYNOVIAL PERFUSION IN NORMAL SUBJECTS AND IN PATIENTS WITH RHEUMATOID ARTHRITIS

Introduction

Physical methods, including the exhibition of heat and judicious use of exercise programmes are widely employed in the management of all forms of arthritis. The rationale underlying their use is not clear, principally because no quantitative methods of assessment are available. In the present study the effects of those physical treatments and of an intra-articular injection of histamine on synovial perfusion measured by the 133 Xe clearance rate are examined.

MATERIALS AND METHODS

Subjects Studied

Fifteen healthy male subjects volunteered to participate and both knee joints were studied on different occasions. Their ages ranged from 25 to 31 years (mean 27.6 years). None gave a history of disease or trauma of the knee joints.

Twenty patients with "classical" rheumatoid arthritis, all of whom had active involvement of the knee joints, were selected for study. Their mean age was 42.6 years (range 32 to 61 years). Six were male.

All patients and control subjects volunteered to participate in this study with full knowledge of its content.

Injection and Counting Technique

All injections into the knee joint were performed in a surgical theatre. The room temperature ranged from 65 to 70°C. and an extraction fan was constantly in operation to reduce the concentration in air of the radioactive gas exhaled by the subject.

The subject lay comfortably in a supine position and the knee was extended and carefully placed into a position whence it could be removed and replaced accurately. The knee was then prepared with full

aseptic precautions and when an effusion was present this was aspirated prior to commencing the study.

A syringe containing an air free solution of approximately 50 μCi of 133 Xe in 1 ml. of sterile 0.9% NaCl was then prepared. A fine gauge needle was introduced into the joint cavity by a medial infrapatellar approach and five minutes later the isotope solution was slowly injected.

The count rate per minute was monitored using a collimated detector (EKCO M.5400 A/1) with a 1" x 1½" thallium activated sodium iodide crystal. The detector was alligned 2" from, and directly above the patella. Pulses were fed into an EKCO rate-meter (M.5180) and pulse height analyser (M.5010) and recorded on a KEINZLE automatic printer. At the completion of each study the count rate, minus the background, was plotted onto semilogarithmic graph paper as a function of time. Since each semi-logarithmic plot was mono-exponential, biological half lives ($T\frac{1}{2}$ values) could readily be obtained from the graph. As the semilogarithmic plots approach th horizontal, the errors inherent in calculating $T\frac{1}{2}$ values become magnified. Accordingly, all slopes with $T\frac{1}{2}$ values over 150 minutes are expressed simply

TABLE VII

EXPERIMENTAL DESIGN - NORMAL SUBJECTS

NUMBER OF SUBJECTS	NUMBER OF STUDIES	NUMBER IN EACH GROUP	EXPERIMENT						EFFECT OF
			133 Xe injected						
			Baseline Count Rate	Heat	Monitoring Count Rate				
		6	10 mins.	5 to 10 mins.	20 mins.				HEAT
			Baseline Count Rate	Exercise	Monitoring Count Rate				
15	30	8	10 mins.	5 mins.	20 mins.				EXERCISE
			Baseline Count Rate	Histamine 1µg.	Monitoring Count Rate				
		4	10 mins.		20 mins.				
			Baseline Count Rate	Histamine 10µg.	Monitoring Count Rate	6	Count Rate		
		12	10 mins.		20 mins.		25 mins.		HISTAMINE
							Exercise	Count Rate	
						6	5 mins.	20 mins.	

TABLE VIII

EXPERIMENTAL DESIGN - RHEUMATOID ARTHRITIS

NUMBER OF PATIENTS	NUMBER OF STUDIES	NUMBER IN EACH EXPERIMENT	133 Xe injected		
			Baseline Count Rate	Heating	Monitoring Count Rate
		12	15 mins.	5 to 10 mins.	20 mins.
			Baseline Count Rate	Exercise	
20	40	16	15 mins.	5 mins.	20 mins.
			Baseline Count Rate	Histamine 10 ug.	Monitoring Count Rate
		6	15 mins.		20 mins.
			Baseline Count Rate	Histamine 250 ug.	Monitoring Count Rate
		6	15 mins.		20 mins.

as $T\frac{1}{2} = > 150$ in this study.

Control subjects and Patients Studied

The experimental design employed in the human studies is shown in Tables 7 and 8.

Thirty knee joints of 15 normal subjects were studied (Table 7). In the first group (six studies) the effect of external heat was examined and in the second (eight studies) the effect of exercise alone was studied. In the third group (four studies) the dose of histamine employed was 1 γ g. whereas in the fourth group (12 studies) the dose was 10 γ g. In six of the last group, following the monitoring period of the effect of histamine, the joint was exercised and the alteration in clearance rate was monitored. The other six in the fourth group were continuously monitored following the injection of histamine, for a total period of 45 minutes and thus served as controls for the effect of exercise following histamine.

Forty knee joints of 20 rheumatoid patients were studied (Table 2). In 12 studies the effect of external heating was monitored and in 16 studies the effect of exercise was studied. In the remaining 12

studies the effect of 10 μg. and of 250 μg. of histamine (histamine acid phosphate: Evans Medical Ltd.) was monitored.

Each time the drug was administered intra-articularly to any control subject a small (<0.25 ml.) amount of 133 Xe was added to the solution and injected with the drug.

Method of Heating the Knee Joint

The patient rose from the theatre table carefully to avoid altering the position of the detector. He was then placed in a sitting position with the knee joint equidistant between the electrodes of a short wave diathermy machine (SIEMENS ULTRATHERM 608 GB). The control was set to position 6 (maximum heat) for not less than 5 minutes. Between 5 and 10 minutes after heating commenced, the patient began to feel uncomfortably warm and at that point heating was discontinued and the patient returned to the theatre table with a minimum of delay (vide infra). The knee joint was then carefully replaced in its original position relative to the detector. In order to confirm that the intra-articular tissues were being heated, intra-articular temperatures were taken on three occasions with an elektrolaboratoriet thermometer

(SIEREX TE 3). In these cases the intra-articular temperature rose by 1.1, 1.3 and 0.9°C respectively.

Method of Exercising the Knee Joint

In normal subjects a standard pattern of exercise was followed. The subject rose from the theatre table and stood in front of a platform 18" in height. "Step ups" commenced at as fast a rate as the subject would tolerate and continued for 5 minutes. The patient then returned to the theatre table and the knee joint was carefully repositioned under the detector (vide infra).

In the patients with rheumatoid arthritis it was not possible to adhere to a standard exercise regimen since the severity of knee joint involvement varied and since the patient was frequently limited by dysfunction of other joints. Accordingly each patient was subjected to knee joint exercise to tolerance. In the most severely affected patients active exercise of the knee joint against a system of dead weights (½ to 5 lbs.) was conducted. In the least affected patients "step ups" were performed in a similar manner to that described above. In all cases the exercise involved moving the knee joint through as wide a range as the disease would permit.

Medical Qualifications:

1. M.B., Ch.B., Glasgow University, 1965.

2. D.Obst.R.C.O.G., Royal College of Obstetricions and Gynaecologists (London), April, 1967.

3. M.R.C.P. (London), Royal College of Physicians (London), July, 1968.

Medical Appointments:

1. House Physician to Dr. A. H. Imrie at the Royal Infirmary, Glasgow, August 1965 - February 1966.

2. House Surgeon to Mr. Galloway at the Victoria Infirmary, Glasgow, February 1966 - August 1966.

3. House Surgeon to Dr. D. McKay-Hart at Stobhill General Hospital, Glasgow, August - February 1967.

4. Locum appointments in General Practice, February 1967 - May 1967.

5. Senior House Officer in Medicine at the Centre for Rheumatic Diseases, Glasgow, May 1967 - August 1968.

Present Appointment:

6. Registrar in Medicine with an interest in Rheumatology - the University Department of Medicine, Royal Infirmary, Glasgow.

SUMMARY

This thesis describes clinical and laboratory investigations into the physiology and pathology of the diarthrodial joint employing both Xenon (133 Xe) and Technetium (99 mTc) as radioactive tracer substances.

In Chapter 1 a short historical outline of the appropriate topics is presented. The subjects covered in this chapter are the anatomy of the blood supply to the synovium, the methods of study previously employed to investigate the pathophysiology of the joint and the development of blood flow measurement with the aid of radioisotopes.

The physical and biological attributes of 133 Xe, the basic technique of intra-articular injection of the isotope and the external monitoring system employed are described in Chapter 2. The radioactive xenon which is cleared from the joint may be accounted for in the femoral venous blood and the animal experiments designed to provide evidence of this are reported. Further direct evidence in animals and indirect evidence in man, to show that the principle route of clearance of intra-articularly

injected 133 Xe is by the synovial venous blood, is also presented in Chapter 2. The diffusion and partition coefficients of 133 Xe for canine and human synovium with respect to blood were determined in vitro and the validity of the derivation of synovial perfusion from the clearance rate of 133 Xe and the partition coefficient is discussed critically. Synovial perfusion rates in normal canine and human joints and in joints of patients suffering from rheumatoid arthritis are documented.

Chapter 3 is devoted to the reproducibility and inherent biological variation associated with the measurement of the $T\frac{1}{2}$ value of intra-articularly injected 133 Xe. The influence of the method and the site of injection and the effect of increasing injection volumes and of the presence of a joint effusion are described. The errors involved in line fitting to determine the $T\frac{1}{2}$ value are quantitated. The day to day variation in the clearance rate and the effect of the age and of the sex of the subject are reported.

In Chapter 4 the effect of physical and pharmacological agents upon the clearance rate of

^{133}Xe from the canine joint are described. The response of the clearance rate to the local administration of exogenous catecholamines was studied and by employing suitably selected and adrenergic receptor stimulating and inhibiting compounds it was possible to demonstrate functionally the presence of both and receptors in synovium. The results of these studies and of studies with a new adrenergic blocking agent, thymoxamine, are reported in Chapter 4. The presence of muscarinic receptors was likewise inferred from the response of the clearance rate to local administration of methacholine and atropine and the results of these experiments and of experiments upon the effect of serotonin are presented. The results of studies conducted with histamine, mepyramine malleate and atropine which suggest that histamine acts in the joint to some extent through cholinergic receptors are presented and discussed in Chapter 4.

A limited study was conducted in diseased canine joints to study the effect of exogenous catecholamines administered locally upon the clearance rate. The results of these studies

suggest that both qualitative and quantitative differences exist between normal and inflamed joints. These studies are also described in Chapter 4.

In Chapter 5 the results of similar studies conducted in normal human subjects, in patients with osteoarthritis and in patients with rheumatoid arthritis are presented. The responses obtained to local administration of stimulating and inhibitory adrenergic compounds in normal subjects are similar to those obtained in the normal canine joint. The presence of both and receptors could be deduced from these results. The responses in diseased joints, whether afflicted with osteoarthritis or with rheumatoid arthritis were both qualitatively and quantitatively different from normal joints and were similar to the results obtained in the inflamed canine joint. The response of the clearance rate to the exhibition of external heating, to exercise and to local injection of histamine is documented. Again differences between normal and inflamed joints were apparent in the response to heating and to histamine injection. Exercise, however, caused a reduction in clearance rate in both normal and diseased subjects.

In Chapter 6 certain clinical applications of the determination of the clearance rate of intra-articularly injected 133 Xe are explored. Synovial perfusion rates in rheumatoid arthritis, osteoarthritis, Reiter's syndrome, psoriatic arthritis, gout and infectious arthritis are recorded and the relationship to a clinical index of local disease severity is examined. It is demonstrated that a joint judged clinically to be uninvolved may yet display evidence of involvement expressed by an abnormally fast clearance rate. Although change in disease activity during therapy with corticosteroids and with indomethacin was reflected in a reduction in clearance rate the same did not apply to salicylate therapy and the results of a crossover drug trial demonstrating this are reported in Chapter 6 in addition to the results of studies on the effect of synovectomy on the clearance rate. The clinical implications of these findings are discussed.

In an attempt to devise a more sensitive index of change in disease activity than was afforded by the 133 Xe clearance technique, studies were conducted employing intravenously administered radioactive

technetium (99 mTc) and the results of these studies are reported in Chapter 7. Preliminary studies conducted with joint scans demonstrated the possibility of quantitating these scans by reference to a joint "phantom" and the results of these are reported in terms of % of 100% uptake. Although the degree of uptake was related to the severity of involvement of the joint, the error of the method proved to be unacceptably high. A simpler method of quantitation employing lower tracer doses is described in Chapter 7. This method proved to possess a more acceptable degree of reproducibility and proved to be capable of reflecting change in disease activity with both steroidal and non-steroidal compounds in both large and small joints although the advantage of localisation of activity within the joint was lost. Pilot studies with this method are reported in Chapter 7 and two controlled clinical trials with widely employed anti-inflammatory agents and with a newly introduced compound, Ibuprofen, are also reported in which the isotope studies were performed in conjunction with standard methods of clinical assessment. The results

of 99 mTc studies in normal subjects, in patients with osteoarthritis and in patients with rheumatoid arthritis of whom a group had previously been subjected to the operation of synovectomy are reported in terms of "% uptake" in Chapter 7.

The thesis concludes with a small study upon the rates of clearance of different anions compared with the rate of clearance of simultaneously administered 133 Xe both in the presence of, and in the absence of systemic administration of potassium perchlorate. The results suggest the presence of a transport mechanism for anions from the joint cavity across the synovial membrane to the synovial veins.

On all occasions the time between the various manoeuvres was reduced to a minimum by practice and on each occasion the time taken to change positions was noted. In the case of the normal subjects the mean time to change position was 31 seconds (range 20 to 55 seconds). In the case of the rheumatoid subjects the time to change positions was much longer (mean 96 seconds range 28 to 180 seconds).

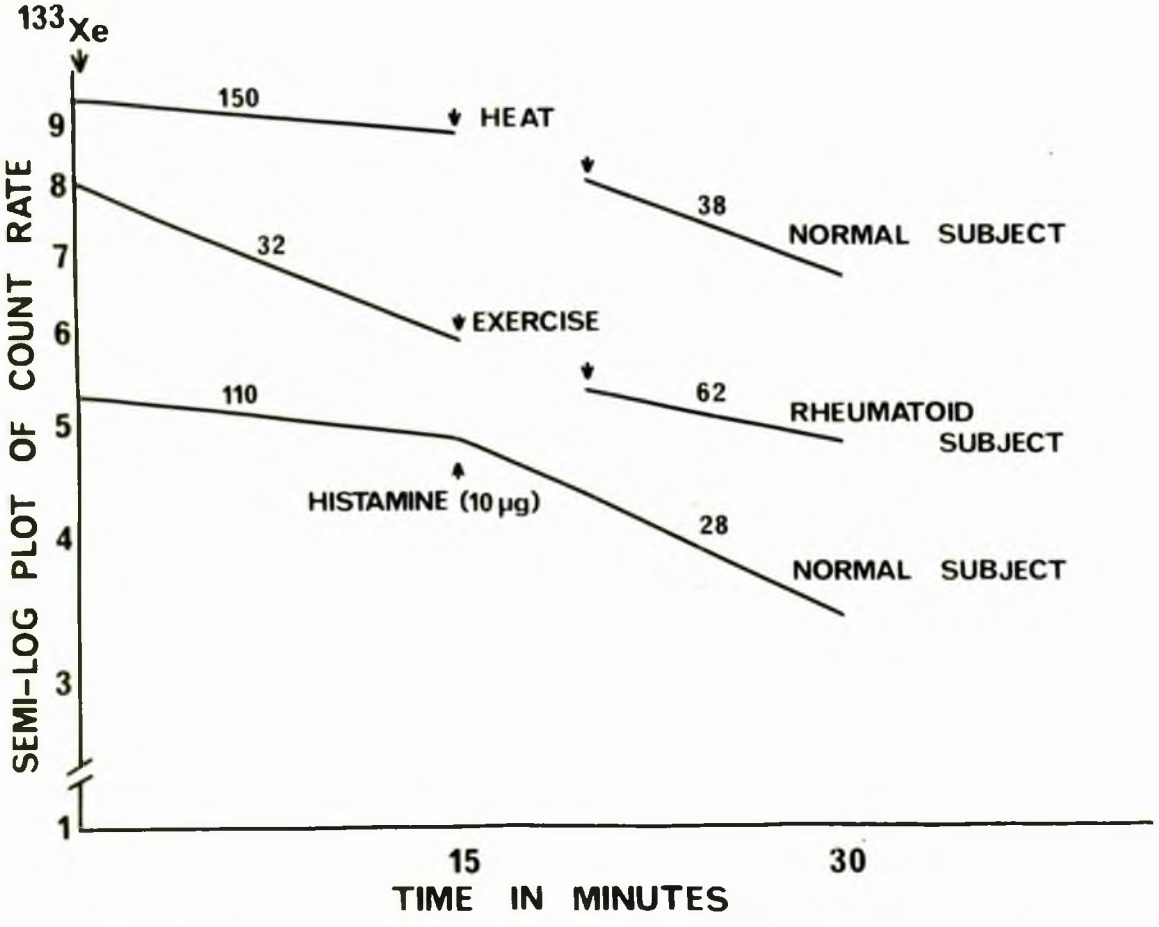


Fig. 3

Legend: Examples of the responses of the clearance rate to heating, exercise and the intra-articular administration of histamine.

TABLE IX

EFFECT OF HEAT ON 133 Xe CLEARANCE RATE

KEY	NORMAL SUBJECTS		RHEUMATOID ARTHRITIS	
	Before	After	Before	After
/ Studies in which intra-articular temperatures were measured	>150	90	95	50
	>150	50	87	63
	>150	38	84	60
	>150	45	79	53 /
* Inconsistent results	120	66	78	46
	110	80	62	42
			51	53
			42	43 /
			36	37
			30	*18
			23	23 /
			18	20
Number studied	6		12	
Mean	-	61.5	57.0	42.5
Range	110->150	38-90	18-95	18-63
Number showing change	6/6		7/12	

EFFECT OF EXERCISE ON 133 Xe CLEARANCE RATE

NORMAL SUBJECTS		RHEUMATOID ARTHRITIS	
Before	After	Before	After
>150	>150	86	99
>150	>150	85	128
>150	>150	77	105
>150	>150	72	94
>150	>150	71	98
150	>150	65	106
140	150	63	85
140	145	61	88
		*55	*50
		50	86
		*46	*48
		43	60
		32	62
		29	72
		26	49
		18	56
8		16	
-	-	55.0	80.5
140->150	145->150	18-86	48-128
0/8		14/16	

RESULTS

HUMAN STUDIES

The effect of Heat

The $T_{\frac{1}{2}}$ values prior to heating the knee joint in normal subjects (range 110 to > 150 minutes) were markedly higher than were the corresponding values following the exhibition of external heating (range 38 to 90 minutes) (Table 9). A change in the clearance rate was noted in all studies (Fig. 3). These results are in accord with those obtained previously in the normal canine joint (Chap. 4).

In the patients with rheumatoid arthritis, however, the response to heating was not uniform. Thus a marked change in clearance rate was observed in seven studies whereas no change whatsoever occurred in a further five. It is noteworthy (Table 9) that of the studies in which the initial $T_{\frac{1}{2}}$ value exceeded 60 minutes change was noted on each occasion whereas when the initial $T_{\frac{1}{2}}$ value was less than 60 minutes change was noted in only one of six studies. In that study the clearance rate following heating ($T_{\frac{1}{2}}$ 18 mins.) was faster than the pre-heating value ($T_{\frac{1}{2}}$ 30 mins.). Intra-articular temperatures were determined in two of the studies in which no change in clearance rate

TABLE X

EFFECT OF EXERCISE ON 133 Xe CLEARANCE RATE FOLLOWING THE PRIOR ADMINISTRATION OF INTRA-ARTICULAR HISTAMINE.

	EXERCISED SUBJECTS $T_{\frac{1}{2}}$ VALUES (MINS.)			CONTROL SUBJECTS $T_{\frac{1}{2}}$ VALUES (MINS.)		
	Following Histamine 10 mins.	Following Exercise 15 mins.	% Change	Following Histamine 10 mins.	Following Histamine 15-45 mins.	% Change
	79	108	127%	35	55	63%
	20	98	490%	70	84	120%
	23	120	521%	28	48	171%
	60	145	242%	55	62	112%
	53	110	207%	80	93	116%
	42	91	216%	46	58	126%
Number studied	6			6		
Mean	46	112	300%	52.5	66.6	118%
Range	20-79	91-145		28-80	48-93	
Number showing change	6/6			6/6		

following heating was observed. and the rises in temperature following heating were 0.9 and 1.1°C. respectively.

Effect of Exercise

The $T\frac{1}{2}$ values in normal subjects prior to and following exercise (range 140 to > 150 and 145 to > 150 minutes respectively) were similar and no obvious change was noted in the clearance rate on any occasion.

In six normal subjects histamine was administered intra-articularly prior to exercise. The $T\frac{1}{2}$ values following the injection of histamine (range 20 to 79 minutes) were markedly lower than the corresponding values obtained following exercise (range 91 to 145 minutes). The mean percentage increment was 300%. In a further six normal subjects, who also received histamine intra-articularly, monitoring was continued for the same time period but these subjects were not exercised. The $T\frac{1}{2}$ values immediately following histamine injection (range 28 to 80, mean 52.5 minutes) were lower than the $T\frac{1}{2}$ values recorded from 15 to 45 minutes after injection (range 48 to 93, mean 66.5 minutes). The mean percentage increment was 118% (Table 10).

TABLE XI

EFFECT OF INTRA-ARTICULARLY ADMINISTERED HISTAMINE ON 133 Xe CLEARANCE RATE

	HISTAMINE (10 μg.) NORMAL SUBJECTS $T\frac{1}{2}$ (mins.)		HISTAMINE (10 μg.) RHEUMATOID ARTHRITIS $T\frac{1}{2}$ (mins.)		HISTAMINE (250 μg.) RHEUMATOID ARTHRITIS $T\frac{1}{2}$ (mins.)	
	Before	After	Before	After	Before	After
	>150	80	83	80	89	41
	>150	20	81	83	82	22
	>150	23	75	74	80	38
	>150	60	72	73	74	39
	>150	85	*66	*42	73	46
	>150	70	65	64	64	52
	>150	55	48	51	51	38
	>150	53	40	42	44	46
	140	35	37	38	38	36
	130	42	32	33	33	34
	125	46	30	28	29	29
	110	28	26	29	18	19
Number studied	12		12		12	
Mean	-	50.0	54.5	53.0	56.5	36.5
Range	110->150	20–85	26–83	28–83	18–89	19–52
Number showing change	12/12		1/12		7/12	

In patients with rheumatoid arthritis, the $T\frac{1}{2}$ values before exercise (range 18 to 86 minutes) were lower than were the corresponding values following exercise (range 48 to 128 minutes)(Table 9). Change in clearance rate was noted in 14 of the 16 studies (Fig. 3).

Effect of Histamine

In four normal subjects who received 1 γ g. of histamine intra-articularly no change in clearance rate was observed. The $T\frac{1}{2}$ values beforehand were 95, 135, 150 and >150 minutes and the corresponding values following injection were 95, 135, 150 and >150 minutes respectively. When the dose of histamine was raised to 10 γ g. the $T\frac{1}{2}$ values following injection (range 20 to 85 minutes) were lower than the pre-injection values (range 110 to >150 minutes). Change in clearance rate was observed in all studies (Fig. 3) (Table 11).

In patients with rheumatoid arthritis who received 10 γ g. of histamine intra-articularly $T\frac{1}{2}$ values before injection (range 26 to 83 minutes) were similar to those obtained after injection (range 28 to 83 minutes). In only one of these studies was there a change in clearance rate. In that study the $T\frac{1}{2}$

value obtained following injection was 42 which was increased when compared with the pre-injection value (66 minutes). When the dose of histamine was increased to 250 γ g. change in clearance rate was noted in seven of 12 studies. In all of these studies the baseline $T\frac{1}{2}$ value exceeded 50 minutes. In the remaining five studies no change in clearance rate attended the administration of 250 γ g. of histamine and in all of these studies the baseline $T\frac{1}{2}$ values were less than 45 minutes (Table 11).

DISCUSSION

In the present study the effect of external heating in normal subjects and in several rheumatoid patients was to reduce the $T\frac{1}{2}$ value, denoting an increase in synovial perfusion. However, in those rheumatoid subjects whose control $T\frac{1}{2}$ values were less than 60 minutes, implying a fast resting synovial perfusion rate, no change was noted with external heat. That the method of heating did in fact result in an increased intra-articular temperature was confirmed by direct measurement in two of these subjects. Several workers (Hollander and Horvath, 1949b : Horvath and Hollander, 1949 : Hollander et al. 1951 : Branemark et al., 1963 and 1966 ; Ring and Cosh 1968) have studied the response of normal and rheumatoid joints to external heating. Branemark et al. (1963, 1966) describe a pattern of response to heating which differs in normal and in rheumatoid subjects. Unfortunately, however, continuous monitoring of intra-articular temperature was not feasible in the present study and consequently no conclusions can be drawn on this point.

In all but two rheumatoid subjects who were

subjected to exercise in this study, the $T\frac{1}{2}$ values obtained following exercise were higher than those obtained beforehand. Thus, exercise has the effect of reducing synovial perfusion in most patients with rheumatoid arthritis. It is unfortunate that the design of the present study permitted only the measurement of a complicated response system. Thus either the exercise per se, or the unavoidable positional changes involved could have accounted for this effect. Intra-articular pressure changes are complex (Palmer and Myers, 1968) and it is possible that the reduction in perfusion is related to a rise in intra-articular pressure. In several subjects no change in $T\frac{1}{2}$ value was noted following exercise. However, the method of calculation of $T\frac{1}{2}$ values of almost horizontal semilogarithmic plots imposes severe restrictions on the interpretation of these results. Indeed it is not possible to demonstrate directly a further reduction in slow perfusion rates by this method. Therefore, to investigate further the effect of exercise on normal subjects, the perfusion rate was first increased by the prior administration of histamine (10 γg.). When the subject was exercised thereafter a marked increase in $T\frac{1}{2}$ value was noted

implying a reduction in synovial perfusion. The increase in $T\frac{1}{2}$ values with exercise greatly exceeded increase attributable to the waning of the effect of histamine. It is probably therefore that exercise has the same effect in normal subjects as in rheumatoid patients, namely to reduce synovial perfusion.

A dose related response to histamine was noted in both normal subjects and in patients with rheumatoid arthritis in this study. However, the dose levels required to elicit a response differed in the two groups. Thus in normal subjects, although no response was obtained with a dose of 1 γ g., all of 12 subjects responded to 10 γ g. with a reduction in $T\frac{1}{2}$ value and therefore an increase in perfusion. However, change in clearance rate in response to 10 γ g. of histamine was obtained in only one patient with rheumatoid arthritis and it is noteworthy that the baseline $T\frac{1}{2}$ value of this patient was 66 minutes suggesting low resting synovial perfusion. When, however, 250 γ g. of histamine were administered change in clearance rate was noted in seven of 12 studies. All of the studies in which the clearance rate was increased by histamine were characterised by

baseline $T\frac{1}{2}$ values in excess of 50 minutes implying low resting synovial perfusion. Furthermore in each of the other five studies which exhibited a response to 250 µg. of histamine, the baseline $T\frac{1}{2}$ value was below 50 minutes implying fast resting perfusion. The explanation of these findings is not clear, but it is interesting that rheumatoid subjects whose control $T\frac{1}{2}$ value was less than 50 to 60 minutes, indicating high resting synovial perfusion failed to respond to both external heating (in all but one study) and to intra-articular histamine. That the 133 Xe clearance rate does not impose a methodological limitation is demonstrated by the fact that $T\frac{1}{2}$ values of less than 25 are recorded in normal subjects and in initially slowly perfused rheumatoid subjects. Furthermore, one rheumatoid patient with a high resting perfusion rate did respond to external heating.

A clue to the explanation of the findings with histamine may be afforded by consideration of the differences in the drug responses between normal and inflamed joints noted previously both in animals (Chap. 4) and in man (Chap. 5 : page 173). Thus

the dose of α and β adrenergic compounds required to elicit a similar response in inflamed joints was consistently higher as is the case with histamine in the present study. The ratio of tissue volume and vascularity to receptor concentration and drug diffusion rates and local binding effects may all contribute.

These factors however cannot be invoked to explain the lack of response to external heating. It is possible that when the disease is associated with a high resting synovial perfusion rate, the peripheral vasculature undergoes changes which render it to some degree autonomous. However, since the numbers involved in the present study are small final conclusions are not justifiable. No rationale either for or against the exhibition of heat in rheumatoid arthritis is afforded by the results of this study. Conceivably, however, the reduction in pathologically elevated perfusion rates occasioned by exercise may exert a beneficial effect.

CONCLUSIONS

1. Both α and β adrenergic receptors are functionally active in the control of synovial perfusion in normal subjects and in rheumatoid and osteoarthritis. α receptor control probably predominates.
2. The evidence suggests the presence of basal α adrenergic vasoconstrictor tone.
3. A quantitative difference in the response to adrenergic agents exist in inflamed synovium. Higher doses are required to elicit the response.
4. Normal basal α adrenergic vasoconstrictor tone may be lost in inflamed joints.
5. Exercise reduces synovial perfusion in rheumatoid arthritis and probably also does so in normal subjects.
6. Both intra-articular histamine and the exhibitio of external heat increase synovial perfusion in normal subjects.
7. Initially slowly perfused rheumatoid patients respond to heating and to higher doses of histamine in the same way, that is by an increas in synovial perfusion.
8. When the resting perfusion rate is markedly elevated in rheumatoid arthritis, no response to heat or to histamine is obtained.

CHAPTER 6

In this chapter I have explored some clinical applications of the 133 Xe clearance technique. Synovial perfusion rates in various inflammatory arthritides such as rheumatoid arthritis, Reiter's syndrome, psoriatic arthritis, gouty arthritis and Behcet's syndrome and in "degenerative" arthritis are compared to the values obtained in normal subjects. The relationship between the $T\frac{1}{2}$ value of the 133 Xe clearance rate and a clinical index of the severity of joint involvement is explored and the application of the method to the early diagnosis of arthritis is evaluated.

The results of studies with steroidal and non-steroidal anti-inflammatory agents are reported and the sensitivity of the 133 Xe clearance method is compared with that of better established clinical methods of assessment.

Finally the effect of the operation of anterior synovectomy of the knee upon synovial perfusion was studied in an attempt to devise a satisfactory method of assessment of that operation and the results are discussed.

SYNOVIAL PERFUSION MEASURED BY THE 133 XENON CLEARANCE TECHNIQUE IN VARIOUS ARTHRITIDES

Introduction

In Chapter 2 it was noted that synovial perfusion rates are elevated in patients with rheumatoid arthritis. In this section the results obtained in larger numbers of patients with rheumatoid arthritis are reported and observations were extended to include patients suffering from various other arthritides.

SYNOVIAL PERFUSION RATES IN VARIOUS DISEASE STATES

Patients Studies

133 Xe studies were performed on 130 knee joints of patients with "classical" or "definite" rheumatoid arthritis. The mean age of these patients was 48.2 years (range 35 to 71 years) and 34 were male. In all cases the relevant knee joint was involved clinically or showed radiological evidence of previous involvement in the form of articular erosions.

Forty-four knee joints of 30 patients with osteoarthritis were studied, all of whom had clinical or radiological evidence of involvement of the knee joint. In none were tests for rheumatoid factor positive and in all the erythrocyte sedimentation rate was normal. Their mean age was 62.4 years (range 40 to 78 years) and four were male. None of these patients was suffering from any other disease and in particular diabetes mellitus and hypertension were excluded.

Twenty-nine studies were performed on the knee joints of 16 patients with Reiter's disease in all of whom the knee joint was clinically involved. All

had urethritis conjunctivitis and a sero-negative polyarthritis and four had keratodermia blenorrhagica. Their mean age was 30.1 years (range 18 to 47 years).

Fifteen studies were conducted in fourteen patients with psoriatic arthritis. In all cases there was prominent involvement of the distal interphalangeal joints and in eleven patients there was either clinical or radiological evidence of sacroiliitis. Both skin and nail changes were present in all of these subjects and the treatment of the dermatological manifestations was being supervised by the Dermatology Departments of the Royal Infirmary, the Western Infirmary or the Victoria Infirmary in Glasgow. In nine, the knee joint studied was involved clinically but in the remaining six the knee joint was neither involved clinically at the time of study nor was there either clinical or radiological evidence of past involvement.

Four studies were performed in two male patients aged 45 and 55 years who had clinical and radiological evidence of gouty arthritis of the relevant knee joint. One of these studies was performed during an acute episode of gout in the joint.

RESULTS

The results are shown in Table 1. The mean ± 2 S.Ds. of the entire normal groups was 0.38 ± 0.44 ml/100 ml/min. The values (ml/100 ml/min.) obtained for patients with rheumatoid arthritis (mean 2.57 ± S.E.M. 0.13), psoriatic arthritis (mean 1.86 ± S.E.M. 0.37) and with Reiter's disease (mean 1.98 ± S.E.M. 0.27) were significantly higher than the values obtained in normal subjects (mean 0.38 ± S.E.M. 0.03). There were however no significant differences between the values obtained in psoriatic arthritis, in Reiter's disease and in the rheumatoid arthritis patients. The mean value for the osteoarthritic knees (1.01 ± S.E.M. 0.1 ml/100 ml/min.) was significantly higher ($p < 0.001$) than the values obtained in the normal group but was significantly lower than the values obtained in either rheumatoid arthritis or Reiter's disease.

The values obtained in the patients with psoriatic arthritis (mean 1.86 ± S.E.M. 0.38 ml/100 ml/min.) are individually recorded (Table 2). It can be seen that in two of the six patients whose knee joints were termed "not clinically involved" based on the absence of pain, stiffness, heat, swelling or tenderness both at

the time of study and in the past history, the perfusion rates were elevated over the normal mean + 2 S.Ds. whereas in the other four studies in that group normal values were obtained.

The perfusion values obtained in patients with chronic gouty arthritis (0.44, 0.4 and 0.13 ml/100 ml/min.) were low but the value obtained in the patient whose knee joint was the seat of acute gouty arthritis at the time of study was 1.54 ml/100 ml/min.

The values obtained in patients with septic arthritis (1.92 and 2.26 ml/100 ml./min.) in tuberculous arthritis (1.65 and 2.04 ml/100 ml/min.) and in Behcet's syndrome (2.9 ml/100 ml/min.) were outwith two standard deviations from the mean of the normal group.

DISCUSSION

Thus it can be seen that high perfusion values were by no means confined to patients suffering from rheumatoid arthritis.

The values obtained in Reiter's syndrome, infectious arthritis and Behcet's syndrome were of the same order of magnitude as the values obtained in rheumatoid arthritis. Since all of these diseases are characterised by chronic inflammation, of which vascular changes are an integral part, these findings are in accord with current pathological concepts.

The marked differences between the results obtained in quiescent and acute gouty arthritis are of interest but the numbers studied are insufficient to support specific conclusions.

Whereas in psoriatic arthritis all of the values obtained in symptomatic knees were elevated, the results in the clinically uninvolved joints were not concordant. In two of six studies the perfusion rates obtained were outwith the normal range (mean + 2 S.Ds. of the normal group). It is interesting in this context that capillary abnormalities in uninvolved skin of psoriatic patients have been reported (Davis et al., 1968).

It is possible that a similar mechanism obtains in the synovium.

The results obtained in osteoarthritis are also interesting. Osteoarthritis is considered by many non-rheumatologists to be a hypertrophic degenerative pathological process. The results of this study are at variance with this concept. Thus, the mean value obtained in osteoarthritis (mean 1.01 ± S.E.M. 0.1 minutes) was significantly higher ($p < 0.001$) than the mean value of the normal group. Inspection of the individual results revealed blood flow values of 2.0, 2.0, 2.2, 2.3 and 2.8 ml./100 ml./minutes in individual osteoarthritic knees. This would seem to indicate that, in some situations in osteoarthritis, the synovial perfusion rate may be as high as those recorded in the inflammatory arthritides. This finding is in accord with those of Davison and Wisham (1958) who showed high clearance rates of radioactive sodium from osteoarthritic knees and with the finding of elevated intra-articular temperatures in this disease (Hollander et al., 1956). All of these observations tend to suggest the presence of an vascular lesion of the synovium in osteoarthritis

in addition to the well established cartilage disorder.

The results of this study show that elevated synovial perfusion rates are a non-specific finding and occur regardless of the underlying pathological process. These results support the concept that a vascular derangement is a feature of the inflammatory response in the synovium.

THE RELATIONSHIP BETWEEN THE $T_{\frac{1}{2}}$ VALUE AND CLINICAL ASSESSMENT OF THE JOINT AT THE TIME OF STUDY.

Method

The diagnostic categories of the patients studied in the previous section were reviewed and more stringent standards were imposed for the purposes of the present study. Thus only patients with "classical" as opposed to "classical or "definite" rheumatoid arthritis were retained and only the results obtained in those patients who demonstrated both clinical and radiological evidence of osteo-arthritis of the knee were analysed. All four patients with Reiter's syndrome who had keratodermia blenorrhagica were selected and four further patients who had a seronegative polyarthritis, involving the knees, ankles and sacroiliac joints, conjunctivitis, urethritis or balanitis and evidence of tendinous calcification were accepted as unequivocally satisfying the diagnostic category of Reiter's syndrome.

Patients Studied

Forty-three studies performed on the knee joints of thirty-nine patients with "classical" rheumatoid

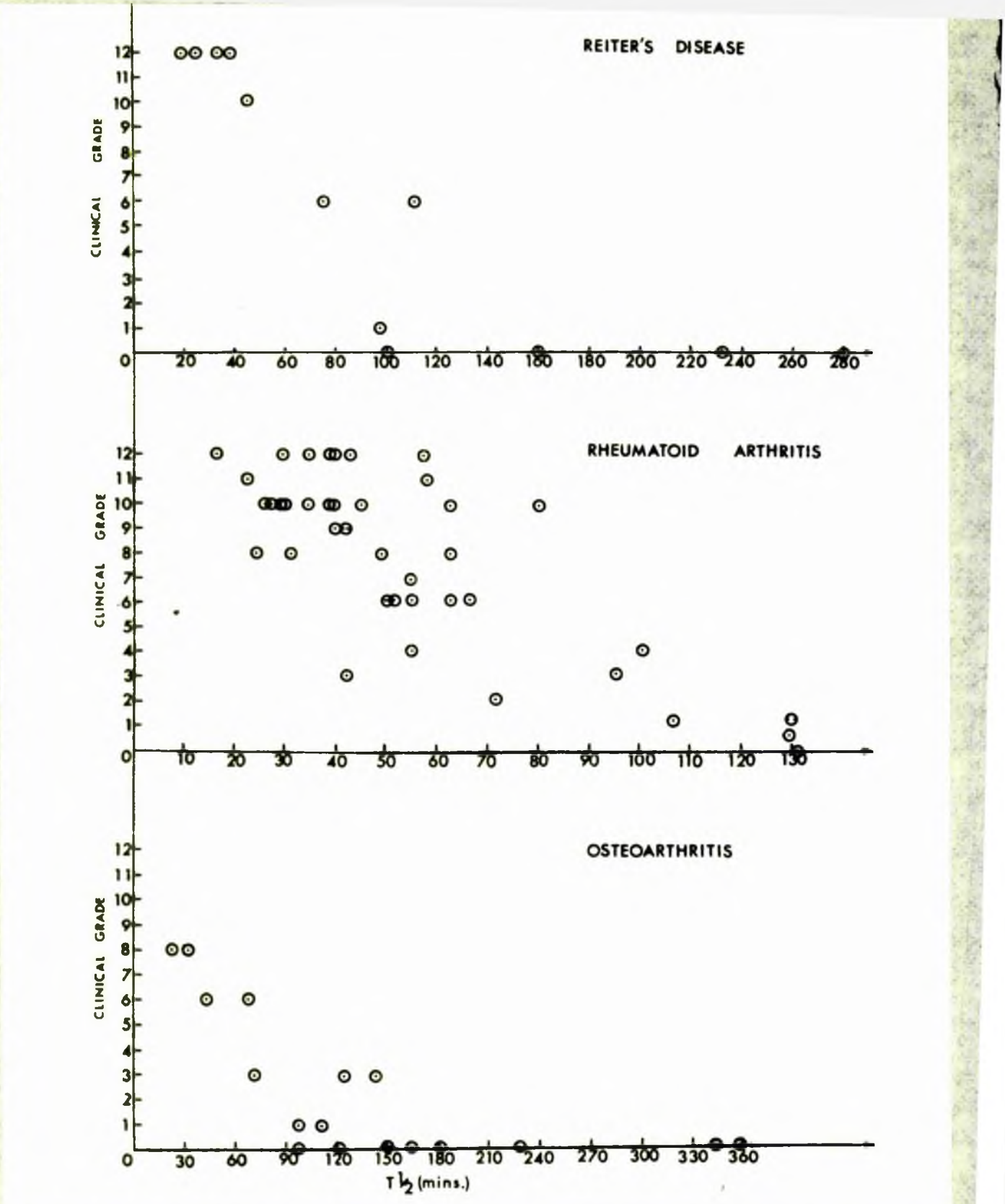


Figure 1

The relationship between the $T\frac{1}{2}$ value and the clinical knee score in the individual disease groups studied.

arthritis were suitable for further analysis. The mean age of those patients, of whom twelve were male, was 55.7 years (range 40 to 69 years). Similarly, the results from eighteen studies performed on nine patients with osteoarthritis were analysed further. Four of these patients were male and the age range was 45 to 78 years (mean 60.9 years). Twelve studies performed in eight male patients with Reiter's syndrome were analysed. The mean age of these patients was 18 to 43 years (mean 33.4 years).

The injection technique (page 96) and counting technique (page 175) have been described and the method of clinical assessment of the joint has been detailed (page 112).

Results and Interpretation

Figure 1 illustrates the relationship between the clinical index of inflammation and the $T\frac{1}{2}$ value obtained in these studies. When the clinical index is high, the $T\frac{1}{2}$ value is low and conversely when the index is low the $T\frac{1}{2}$ value is high. The correlation coefficient when all diseases were considered together was 0.76. The relationship was

individually analysed in each of the disease groups.

The correlation coefficients obtained were 0.82 for Reiter's syndrome, 0.75 for rheumatoid arthritis and 0.21 for osteoarthritis.

A correlation coefficient of 0.82 does not of course imply a causal relationship between the variables and indeed indicates that only approximately 65% of the correlation is accounted for by the variables studied. Thus 35% remains to be accounted for by other factors. However, these results do support the previous observations of high clearance rates (low $T\frac{1}{2}$ values) in inflamed joints (page 71). It would seem reasonable to suggest that the rate of clearance does reflect to some extent the degree of inflammatory involvement at the time of study. Indeed, considering that the clinical index involves the estimation of four parameters, all of which have to be assessed qualitatively by patient and/or clinician, it would be surprising if this index correlated more closely with a single quantitative measurement.

SYNOVIAL PERFUSION OF CLINICALLY NORMAL KNEE JOINTS IN PATIENTS WITH RHEUMATOID ARTHRITIS

In recent studies on the pathogenesis of rheumatoid joint inflammation, Hollander and his co-workers have injected immune gamma globulin molecules or IgG fragments isolated from serum into clinically uninvolved joints of patients with rheumatoid arthritis (Restifio et al., 1965 : Hollander et al., 1966 : Quismorio et al., 1968 : Hollander and Rawson, 1968). Inflammatory reactions were induced by these injections and the suggestion was made that fragments related to the IgG molecule might be the antigen which induces the production of rheumatoid factor.

The question may be posed as to whether a "clinically uninvolved joint" is also a joint unaffected by rheumatoid pathology. Sokoloff and Gleason (1954) demonstrated in a small series of necropsy examinations of rheumatoid patients that the sternoclavicular joints showed histological changes characteristic of rheumatoid arthritis although in none of the cases was there a record of

the joint having been involved clinically. However, it was not indicated that evidence of involvement had been sought in the sternoclavicular joint specifically during life and, as suggested by the authors themselves, routine clinical examination is unlikely to elicit involvement of these joints when arthritis of other larger joints is attracting attention.

In this study patients with "classical" rheumatoid arthritis (Ropes et al., 1959) were selected who did not have evidence of clinical involvement of the knee joint in the disease process. These patients were subjected to 133 Xe studies to determine whether the rate of synovial perfusion in these "clinically uninvolved" joints was normal.

Patients studied

Twenty-two patients were studied. Each had "classical" rheumatoid arthritis, all having articular erosions, a peripheral symmetrical polyarthritis of more than six months' duration and positive serological tests for rheumatoid factor. Sixteen were female and their ages ranged from 35 to 66 years.

No patient gave a history of pain, swelling,

stiffness or dysfunction of the relevant knee joint. On careful clinical examination by several clinicians independently there was no evidence of swelling either due to effusion or to synovial hypertrophy or abnormal warmth of the overlying skin and there was no joint tenderness or restriction of movement. All the patients had normal radiographs of that knee joint.

The method of injection (page 96), counting techniques (page 175) and derivation of synovial perfusion (page 55) have been described.

All patients volunteered to participate in the study with full knowledge of its content.

TABLE III

The $T\frac{1}{2}$ values and synovial perfusion rates recorded using ^{133}Xe clearance technique in 22 clinically uninvolved kn in patients with rheumatoid arthritis. Also shown are th mean values and ranges obtained in 40 knee joints of norm subjects and in 82 clinically involved knee joints of patients with rheumatoid arthritis

Patient		$T\frac{1}{2}$ value (mins.)		Synovial Peri ml/100 ml/mi
1		35		1.98
2		38		1.82
3		46		1.51
4		50		1.39
5		52		1.33
6		56		1.24
7		68		1.02
8		82		0.85
9		92		0.75
10		95		0.73
11		105		0.66
12		106		0.65
13		150		0.46
14		160		0.43
15		180		0.39
16		190		0.36
17		240		0.29
18		260		0.27
19		320		0.22
20		325		0.21
21		360		0.19
22		465		0.15
	Mean	158	Mean	0.77
	S.E.M.	25.9	S.E.M.	0.12
40 normal subjects	Mean	252	Mean	0.28
	Range	95-630	Range	0.11-0.7
82 rheumatoid knee joints (knees clinically involved		43		1.61
		10-134		0.52-6.93

RESULTS

The results are shown in Table 3. In seven studies the synovial perfusion exceeded 1 ml./100 ml./minute ($T\frac{1}{2}$ value < 70 mins.). In a further five patients the values obtained were between 0.5 and 1.0 ml./100 ml./minute ($T\frac{1}{2}$ values between 82 and 106 minutes).

Figure 3 shows the distribution of the $T\frac{1}{2}$ values obtained in the clinically uninvolved joints and also the $T\frac{1}{2}$ values for two other groups, namely, unselected patients with "classical" or "definite" rheumatoid arthritis attending the Centre for Rheumatic Diseases and a series of 40 normal volunteers who had no evidence of joint disease.

The range of $T\frac{1}{2}$ values in the rheumatoid patients was 10 to 134 minutes (synovial perfusion 0.52 to 6.93 ml./100 ml./minute). The range in the normal joints was 95 to 630 minutes (synovial perfusion 0.11 to 0.73 ml./100 ml./minute). In the rheumatoid group only two patients out of 82 had a $T\frac{1}{2}$ value in excess of 100 mins. and in the normal group only one knee of 40 had a $T\frac{1}{2}$ value of less than 100 minutes.

DISCUSSION

Rheumatoid arthritis is a polyarthritis and the distribution of clinically involved joints in any particular patient appears to be quite random although certain joints are affected more frequently than others. Joints which do not appear to be involved on one examination may be inflamed at a later date and, though perhaps less commonly, joints obviously inflamed on one occasion may be quiescent at a subsequent examination. The question therefore arises as to whether any diarthrodial joint may be considered to be uninvolved in rheumatoid arthritis. No histological information is presently available on this point.

Several workers (Sokoloff and Bunim, 1957 ; Branemark et al., 1963b; Kulka, 1964) have presented evidence which suggests that an early feature of the pathology in rheumatoid arthritis is an abnormality of small blood vessels. It seemed appropriate therefore to employ a method of measuring synovial perfusion in this study.

The results obtained in the clinically normal

knee joints of patients with "classical" rheumatoid arthritis show that in seven of the twenty-two knees examined the synovial perfusion rate was markedly abnormal the levels being of the same order of magnitude as those found in a series of clinically affected joints in rheumatoid patients. In a further five knees the synovial perfusion rate was greater than the values commonly encountered in normal subjects.

All patients with rheumatoid arthritis attending the Centre for Rheumatic Diseases with clinically uninvolved knee joints were not studied: the 22 who were examined were not chosen for any reason other than convenience, freely given consent and availability. The study therefore is "highly selected". In selecting only patients with "classical" rheumatoid arthritis, however, there is likely to have been a bias in favour of more advanced and generalised joint involvement.

The results of the previous study (page 224) showing that there was a relationship between clinical involvement and synovial perfusion are not at variance

with these results. Since the correlation was not linear ($r = 0.75$) then approximately only 50% of the relationship was accounted for by the variables studied. The results of the present study represent a proportion of the remaining 50%. The results of the study clearly demonstrate that a joint which is normal clinically may yet show evidence of involvement in the disease process expressed as an abnormally high synovial perfusion rate.

THE EFFECT OF INTRA-ARTICULAR ADMINISTRATION OF HYDROCORTISONE ON THE 133 XENON CLEARANCE RATE.

Either systemic or local corticosteroid therapy exerts a powerful non-specific suppressive effect on the inflammatory process. Since the clearance rate of 133 Xe from the joint cavity bears a relationship to the degree of joint inflammation at the time of study it could be expected that changes in the state of the joint induced by corticosteroid therapy would be paralleled by changes in clearance rate. A preliminary study was therefore conducted to determine whether local treatment with high doses of hydrocortisone would induce a change in the rate of clearance of intra-articularly injected 133 Xe.

Patients studied

Twenty-five patients suffering from "classical" or "definite" rheumatoid arthritis were selected for study. Their ages ranged from 42 years to 61 years (mean 49.6 years) and all had clinical and radiological involvement of the relevant knee joint. Nine were male.

Methods

133 Xe studies were performed in the usual manner and the $T\frac{1}{2}$ value was calculated as has been previously

described (page 69). At the conclusion of the 133 Xe studies 100 mg. of hydrocortisone hemisuccinate were injected into the joint cavity and the 133 Xe studies were repeated, firstly at 24 hours after injection and then again at one week. One patient failed to attend for the third 133 Xe study one week after injection. Prior to each 133 Xe study clinical knee assessments were performed in the manner previously described (page 112) by the same observer. This study was not "double blind", both assessor and patient being aware of the treatment administered.

The $T\frac{1}{2}$ values and the results of the clinical knee assessments, obtained prior to the injection were compared with those obtained at 24 hours and the 24 hour values were compared with the results of the same studies repeated one week later. Statistical analysis was performed using the paired Student t-test.

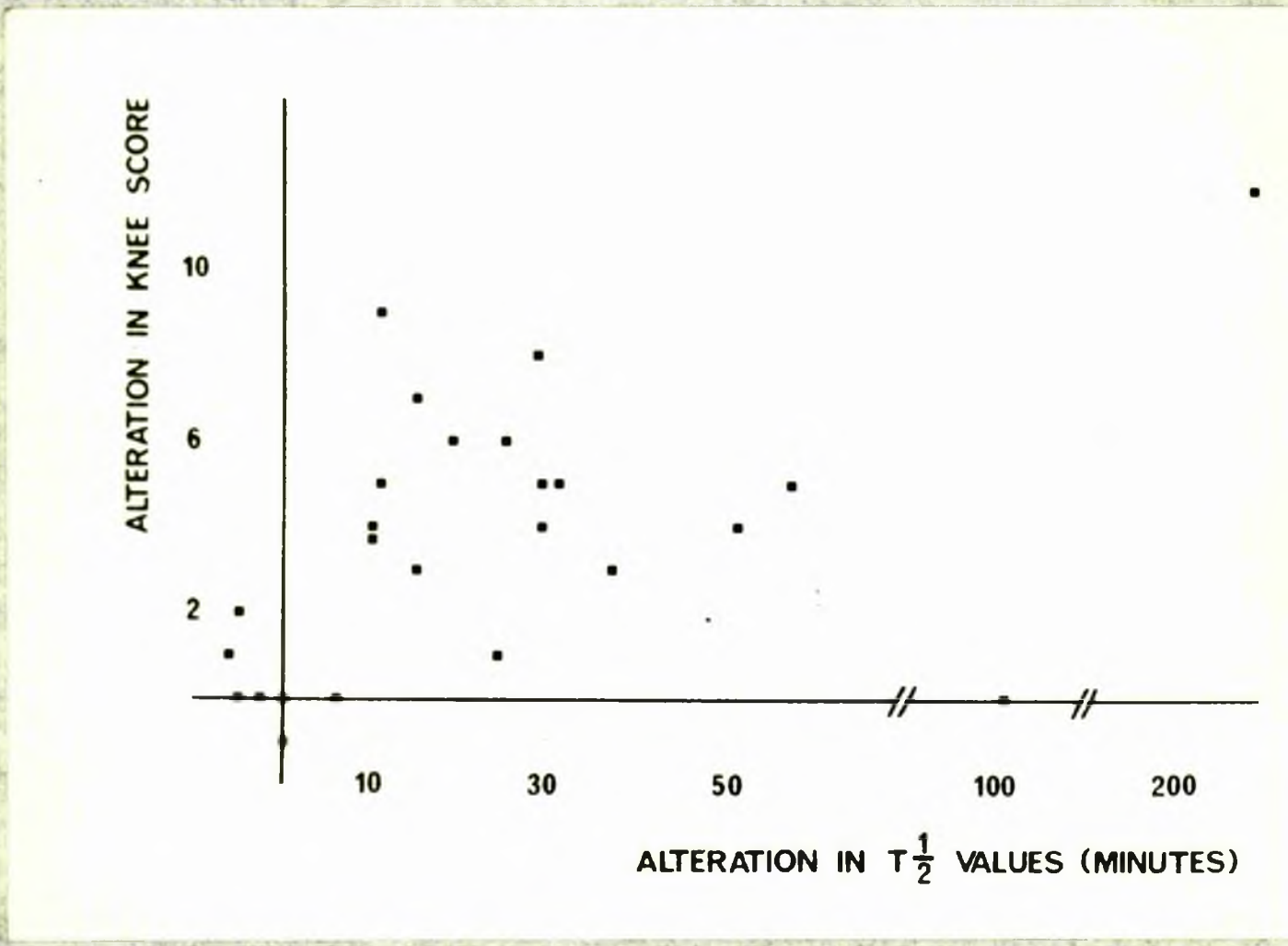


Figure 3

Legend: The relationship between the change in $T\frac{1}{2}$ valu
and the change in clinical knee score before and after
intra-articular corticosteroid therapy.

RESULTS

The mean and standard error of the $T\frac{1}{2}$ values 24 hours following the administration of hydrocortisone (mean 62.4 ± S.E.M. 10.27 minutes) were significantly higher ($0.01 > p > 0.001$) than the corresponding values prior to the injection (mean 34.56 ± S.E.M. 3.89 minutes). The values obtained for clinical knee assessment 24 hours after the injection (mean 5.7 ± S.E.M. 0.72) were significantly lower ($p < 0.001$) than the pre-injection values (mean 9.4 ± S.E.M. 0.57 minutes). In 19 patients the $T\frac{1}{2}$ values fell, in two cases they were the same and in four cases higher values were obtained 24 hours after the treatment. The correlation coefficient between the alteration in $T\frac{1}{2}$ value and the change in the clinical index was 0.52 (Fig. 3).

There was no significant difference between the $T\frac{1}{2}$ values (mean 62.44 ± S.E.M. 10.27 minutes) or the values obtained for the clinical score (mean 5.7 ± S.E.M. 0.72 minutes) at 24 hours and the corresponding values obtained one week after the injection (mean 52.42 ± S.E.M. 4.79 minutes and mean 5.8 ± S.E.M. 0.75 minutes respectively) in the 24 patients who completed the trial ($0.8 > p > 0.7$ and $0.95 > p > 0.9$ respectively).

DISCUSSION

Although this preliminary study was not double blind and did not incorporate a placebo, the numbers of patients studied, the level of significance achieved and the high degree of objectivity in the method of measurement all support the conclusion that intra-articularly administered hydrocortisone in high doses reduces the rate of clearance of 133 Xe from the knee joint implying a reduction in synovial perfusion. It is particularly interesting that those patients whose $T\frac{1}{2}$ value failed to be reduced were those who did not obtain clinical benefit from the injection. Failure of response to local installation of corticosteroid has been noted by others (McCarty & Hogan, 1964)
and it has been established that locally administered corticosteroid may induce a "crystal synovitis"

This may explain the results recorded here.

The alteration in $T\frac{1}{2}$ value is related to the change in clinical knee assessment. The correlation coefficient between these parameters was 0.52.

It is impossible to determine from the foregoing results which of these indices is the more sensitive and it is likely that they are complementary parameters each reflecting, primarily, different aspects of the same process. This relationship between improvement as judged by clinical index and by $T_{\frac{1}{2}}$ value suggested that the 133 Xe clearance rate might be of value in the objective assessment of anti-inflammatory drug effect, thus prompting the succeeding study.

THE EFFECT OF NON-STEROIDAL ANTI-INFLAMMATORY DRUG THERAPY ON THE $T_{\frac{1}{2}}$ VALUE

Introduction

The exhibition of large doses of an anti-inflammatory agent results in a reduction in the rate of 133 Xe clearance.

The "test" of any new method of assessment is to evaluate it in a relevant clinical situation. Thus in the context of an index of anti-inflammatory effect it is essential to show that meaningful results can be obtained under the conditions of a clinical trial. Accordingly, 133 Xe clearance rates were measured in conjunction with several established indices of anti-inflammatory effect, in such a situation, employing widely used anti-inflammatory agents.

TABLE IV

Patient No.	Week 1	Week 2	Week 3
1	Indomethacin	Placebo	Salicylate
2	Placebo	Indomethacin	Salicylate
3	Salicylate	Indomethacin	Placebo
4	Placebo	Indomethacin	Salicylate
5	Indomethacin	Salicylate	Placebo
6	Placebo	Salicylate	Indomethaci
7	Salicylate	Placebo	-
8	Indomethacin	Salicylate	Placebo
9	Indomethacin	Salicylate	Placebo
10	Indomethacin	Salicylate	Placebo
11	Salicylate	Placebo	Indomethaci
12	Placebo	Indomethacin	-
13	Salicylate	Placebo	Indomethaci

Sequence of administration of salicylate indomethacin and placebo in the thirteen patients studied.

MATERIALS AND METHODS

Thirteen patients with classical rheumatoid arthritis (Ropes et al., 1959) entered a three week double blind trial during which they received three course of treatment each lasting for one week: Enteric-coated sodium salicylate ("Entrosalyl", Cox-Continental Ltd.) 1.2 g. four times per day, Indomethacin ("Indocid", Merck, Sharp and Dohme Ltd.) 25 mg. four times per day and lactose as placebo. The order of treatment was allocated randomly and the sequence is illustrated in Table 4 where it can be seen that 4 patients commenced on placebo, 5 patients commenced on indomethacin and 4 patients commenced on aspirin.

Two patients (7 and 12) failed to complete the trial. One patient (case 7) completed one week's salicylate therapy and one week on placebo but on the third day of the period on indomethacin therapy she developed severe headaches and took no further medication. The second patient (case 12) completed the first two weeks of the trial successfully, during which he received indomethacin and placebo but failed

to attend for assessment at the conclusion of the third week.

This study was performed in a double blind fashion, the physician assessing the patient's response being unaware of which treatment was being given. The patients were assessed at the end of each week's treatment. All female patients were post-menopausal and all the patients volunteered to participate in this study with full knowledge of its content.

133 Xe studies (page 94 were performed at each attendance on one knee joint, selected for study at the beginning of the trial on the basis of severity of clinical involvement.

Prior to each 133 Xe study, clinical knee assessments were performed by the same observer on each occasion. At the same time the patients were questioned regarding side effects and the articular index (Ritchie et al., 1968) was performed as a measure of total articular status. This index is based on the response of the patient to firm pressure over the joint margin, the response being graded as follows:

0 = no pain; +1 = patient complains of pain;

+2 = patient complains of pain and winces;

+3 = patient complains of pain, winces and withdraws. Certain joints are examined by passive movement, e.g. cervical spine, hips and mid-tarsal joints, and some joints are considered as single units, e.g. tempero-mandibular joints, cervical spine, the sterno- and acromio-clavicular joints, the metacarpo- phalangeal and proximal interphalangeal joints in each hand and the mid-tarsal and metatarso phalangeal joints in each foot. The maximum possible score for an individual patient is +78. This articular index has been shown to have an acceptable degree of intra-observer reproducibility; the mean intra-observer error difference in 18 patients examined by the same observer within 30 minutes is of the order of 1.2 score units and the standard error of this difference is 1.1 score units (Ritchie et al., 1968). This articular index has also been shown to compare favourably with the articular score employed by the Co-operating Clinics Committee of the American Rheumatism Association (1965), the correlation co-efficient being 0.89.

TABLE V

Mean and S.E.M. of the values obtained on placebo, salicylate and indomethacin therapy of the articular index, total knee score and $T\frac{1}{2}$ of the 133 Xe clearance from the knee.

	$T\frac{1}{2}$ (minutes)	Total Knee Score	Articular Index
Placebo (n = 13)	24.7±3.1	7.6±0.8	18.6±2.0
Salicylates (n = 12)	25.3±1.3	4.6±1.1	8.6±2.1
Indomethacin (n = 12)	32.3±3.9	3.8±0.9	10.3±2.9

TABLE VI

The individual $T\frac{1}{2}$ values (in minutes) recorded while the patients received placebo, salicylate and indomethacin therapy

	Placebo	Salicylate	Indomethacin
1.	18	19	19
2.	20	26	24
3.	16	31	50
4.	48	22	55
5.	23	24	32
6.	20	27	23
7.	43	27	-
8.	14	28	52
9.	30	17	20
10.	16	38	22
11.	13	21	23
12.	35	-	42
13.	25	23	25
Mean	24.7	25.3	32.3
S.E.M.	3.1	1.6	3.9

RESULTS

The results are shown in Tables 5, 6 and 7. Table 5 shows the mean and S.E.M. of the values for the half-life ($T\frac{1}{2}$ minutes) of the 133 Xe clearance, the knee scoreand the articular index while the patients received placebo, salicylate and indomethacin respectively. It can be seen that the $T\frac{1}{2}$ values while the patients received indomethacin (mean 32.3 $\pm$ S.E.M. 3.9) were significantly higher by a paired student's t-test ($p < 0.05$) than the values obtained while the patients received placebo (mean 24.7 $\pm$ S.E.M. 3.1). The $T\frac{1}{2}$ values while the patients received salicylates (mean 25.3 $\pm$ 1.3 SE.M.) were slightly, but not significantly, higher than the placebo values ($p > 0.8$). There was no significant difference between the $T\frac{1}{2}$ values on salicylate or indomethacin ($p > 0.2$) therapy. The $T\frac{1}{2}$ values are individually recorded in Table 6.

The values obtained for total knee score while the patients received placebo (mean 7.6 $\pm$ S.E.M. 0.8) were significantly higher as tested by a paired student's t-test than the values obtained while the patients received either salicylates ($p < 0.001$) or

–	PLACEBO					SALICYLATES					INDOMETHACIN				
	Pain	Tenderness	Stiffness	Swelling	TOTAL	Pain	Tenderness	Stiffness	Swelling	TOTAL	Pain	Tenderness	Stiffness	Swelling	TOTAL
1	3	0	2	3	8	0	0	0	1	1	0	0	1	1	2
2	3	2	2	2	9	2	2	2	1	7	1	2	1	2	6
3	2	0	0	1	3	0	0	0	1	1	0	0	0	1	1
4	1	2	1	1	5	1	2	0	1	4	1	0	0	0	1
5	1	2	2	1	6	0	0	0	2	2	0	0	0	1	1
6	2	2	0	2	6	1	0	0	0	1	0	0	0	0	0
7	3	3	3	3	12	2	1	2	2	7	-	-	-	-	-
8	1	0	2	3	6	0	0	2	2	4	0	1	2	2	5
9	3	3	2	2	10	3	3	3	3	12	3	1	3	2	9
10	1	3	1	2	7	0	0	0	1	1	2	3	0	1	6
11	3	3	3	2	11	2	0	2	2	6	2	2	2	2	8
12	1	1	1	1	4	-	-	-	-	-	1	0	0	0	1
13	3	3	3	3	12	2	3	2	2	9	1	1	2	2	6
Mean	2.1	1.8	1.7	2.0	7.6	1.1	0.9	1.1	1.5	4.6	0.9	0.8	0.9	1.2	3.8

TABLE VII The values obtained for the individual components of the knee score while the patients were receiving placebo, salicylate and indomethacin therapy.

indomethacin ($p < 0.001$) but there was no difference ($p > 0.8$) when the values obtained on salicylates and on indomethacin were compared. Table 7 shows the results obtained for each component of the knee score. Both salicylate and indomethacin significantly reduced pain, tenderness and stiffness. However, whereas indomethacin significantly reduced the degree of swelling as compared with placebo ($p < 0.01$), salicylate therapy did not ($p > 0.1$). There was, however, no significant difference between the degree of reduction of swelling achieved by salicylate compared with indomethacin therapy ($p > 0.4$). No significant correlation was found between the total knee score, or any of its individual components, and the $T\frac{1}{2}$ value.

The values for the articular index (Table 5) while the patients received placebo (mean 18.6 $\pm$ S.E.M. 2.0) were significantly higher by a paired student's t-test than the corresponding values when they were treated with either salicylates (mean 8.6 $\pm$ S.E.M. 2.1)($p < 0.001$) or indomethacin therapy (mean 10.3 $\pm$ S.E.M. 2.9)($p < 0.02$). There was no significant difference between the values obtained

while on salicylate or on indomethacin therapy ($p > 0.4$).

Only one patient failed to complete this trial on account of side effects (case 7). She developed severe headaches while receiving indomethacin. Five of the eleven patients on sodium salicylate complained of mild dyspepsia which required no therapy and two patients developed tinnitus and diminution in hearing during the last two days of salicylate therapy. Whilst on indomethacin therapy four patients complained of headache, two of those patients also experiencing mild dyspepsia.

DISCUSSION

"The properly controlled quantitative approach holds the only real hope for dealing with the oncoming flood of new drugs" (Beecher, 1959). This statement made 10 years ago remains true today. In rheumatoid arthritis difficulties lie in the inherent variability of the disease process and in the lack of availability of methods for measuring the response to drug therapy. These difficulties are more fully discussed elsewhere in this thesis (page 264).

In the present study the relevance of 133 Xe studies to assessment of the anti-inflammatory effect of drug therapy was investigated under the conditions of a clinical trial. Sodium salicylate, indomethacin and placebo were each given for one week in a double blind trial. Although the $T\frac{1}{2}$ values showed a significant change with indomethacin, there was no significant change with sodium salicylate. The dose of sodium salicylate was adequate (5 g. per day) and indeed two patients developed salicylism. Furthermore, both local (knee score) and general (articular index) clinical indices demonstrated that sodium salicylate exerted significant clinical benefit. Since the

133 Xe clearance did not show changes with the "sheet anchor" of drug treatment, this technique will not be of value on its own in the clinical assessment of the short term response of rheumatoid arthritis to anti-inflammatory therapy when salicylates are employed as "standard treatment". Further evidence to support this assertion is presented in Chapter 7.

The results are of some interest however since indomethacin significantly altered the $T\frac{1}{2}$ values whereas sodium salicylate did not. There are several possible explanations of this phenomenon. Sodium salicylate may exert a weaker anti-inflammatory effect than dose indomethacin in these doses. In experimental animals Winter (1964) showed that indomethacin exerted a more powerful inhibitory effect on carrageenin oedema than sodium salicylate. However, clinical experience (Sunshine, 1964 ; Pinals and Frank, 1967) suggests that these drugs in adequate anti-inflammatory doses are approximately equipotent, although Pitkeathly et al.,(1966) demonstrated a greater improvement in grip strength with indomethacin than with sodium salicylate. It is interesting to note that, in this study, indomethacin but not sodium salicylate reduced swelling significantly.

However, all other clinical indices showed significant change with both drugs.

A further possibility is that indomethacin exerts a more selective effect on the vascular component of inflammation than does sodium salicylate. This would explain the alteration in a parameter which measures blood flow. There is evidence that indomethacin exerts an effect on cerebral blood vessels (Vecchio and Fontana, 1965). Sicuteri et al. (1965) noted that indomethacin decreased cerebro-spinal fluid pressure and elevated the retinal artery pressure and concluded that indomethacin has a vaso-reactive effect on cerebral blood vessels. It may be that the 133 Xe technique is recording similar changes on the peripheral vascular system in the knee.

These results demonstrate that the rate of clearance of 133 Xe from the knee joint and the clinical indices of inflammation may be altered by indomethacin therapy. However, the beneficial effect of salicylates, as measured by the clinical indices, is not attended by an alteration in the 133 Xe clearance rate.

EFFECT OF SYNOVECTOMY ON THE SYNOVIAL PERFUSION RATE MEASURED BY THE 133 Xe CLEARANCE RATE

The operation of synovectomy, introduced into the therapeutic armamentarium early this century, is employed as empirically today as it was then. "As long as this proof is lacking we shall, in my opinion, have to regard this treatment (Synovectomy) like all the others in rheumatoid arthritis, as an empirical one and we shall have to discuss the best way to obtain evidence of the influence of this procedure on the natural history of the disease" (Gosling 1969).

One major obstacle to the investigation of this problem has been the lack of suitable quantitative methods which are biologically meaningful. Since the 133 Xe clearance rate provides a measure of synovial perfusion, it seemed reasonable to study the effect of synovectomy on this parameter, in an attempt to provide such a method.

The present study was designed to investigate the effect of synovectomy on synovial perfusion measured by the 133 Xe clearance technique in patients with rheumatoid arthritis.

PATIENTS AND METHODS

Two groups of patients were studied. The first group, comprising eight patients with "classical" rheumatoid arthritis (Ropes et al., 1959) had 133 Xe studies performed before synovectomy at one to three months afterwards and at one year following operation. The mean age of these patients was 52.4 years (range 35 to 61) and five were male. The second group, comprising 21 patients with "definite" or "classical" rheumatoid arthritis had 133 Xe studies performed between two and three years (mean 2.9 years) following the operation of anterior synovectomy of the knee. In all of these patients the sheep's cell agglutination test was positive and X-rays of the knee joints showed articular erosions. Sixteen were female and the mean age was 54.6 years (range 26 to 71 years). Clinical assessment of the joint was performed by the same observer prior to each study and the patient was asked whether the result of the operation was "excellent", "satisfactory", "poor" or "bad". 133 Xe studies were performed in the usual manner and the synovial perfusion rate was calculated from the $T\frac{1}{2}$ value (Equ. 7 : page 55)

Technique of Operation

The operation of synovectomy of the knee was

performed by the same surgeon under pneumatic tourniquet control through an elongated medical para-patellar incision. The synovium and menisci were removed and the cruciate and collateral ligaments and popliteus tendon were cleared of granulation tissue. Synovium from the posterior aspect of the joint was removed with the knee in full flexion using small bone nibblers. The patella was left intact in all cases. The joint was closed in two layers with a suction drain. Guarding plaster was applied and the knee was manipulated at two weeks under general anaesthesia.

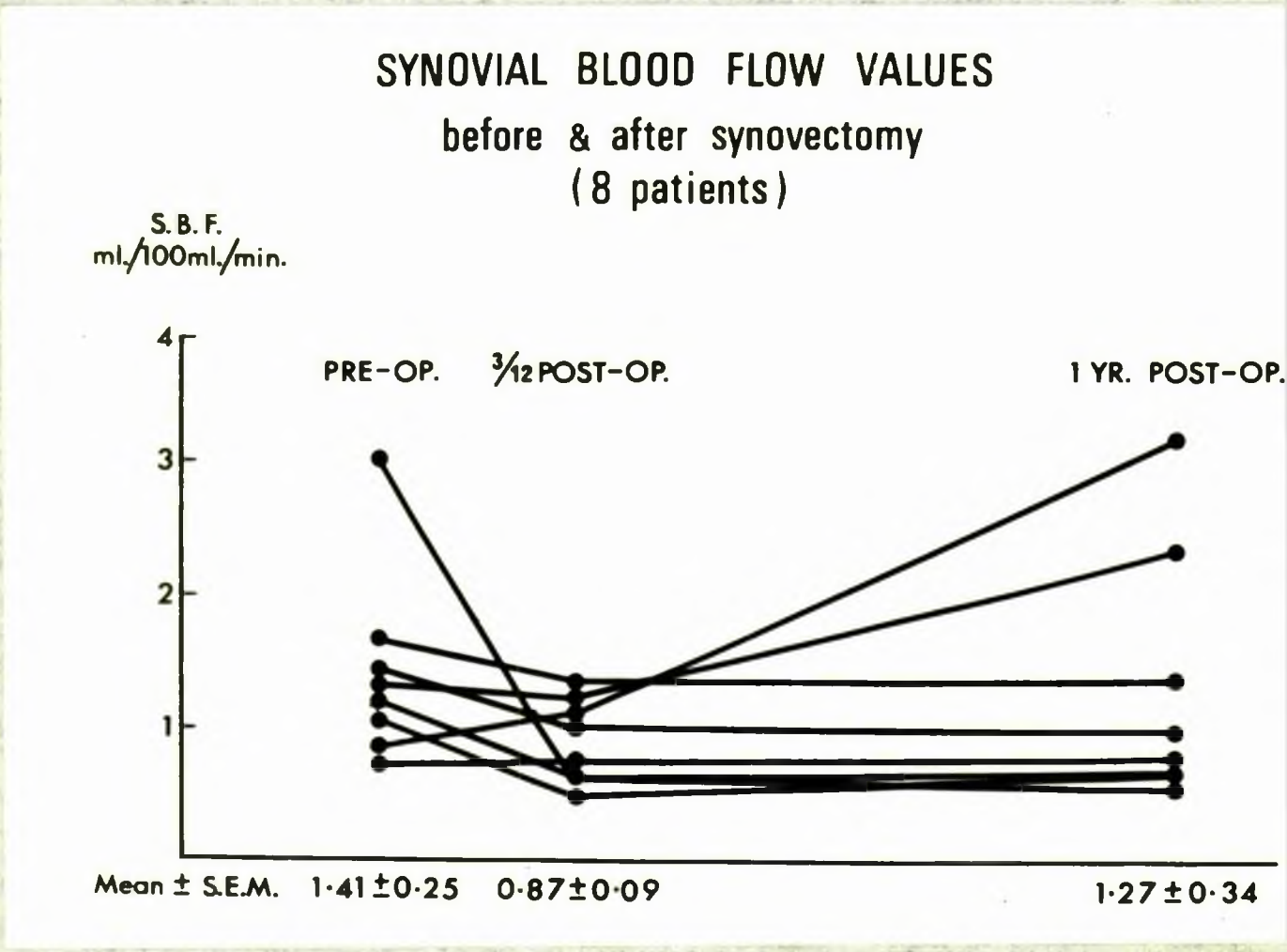


Figure 4

Legend: Synovial blood flow values in 8 rheumatoid patients before, 3 months following and one year following the operation of synovectomy.

RESULTS

Group I

Figure 4 shows the synovial perfusion values obtained before one to three months after and one year after operation. The values one to three months following operation (mean 0.87 ± S.E.M. 0.09 ml/100 ml/min.) are significantly lower ($p < 0.05$) than the values obtained before operation (mean 1.41 ± S.E.M. 0.25 ml/100 ml./min.). However, the values obtained one year after operation (mean 1.27 ± S.E.M. 0.34 ml/100 ml/min.) did not differ significantly from the pre-operative values ($p > 0.4$) nor from the one to three months post-operative values ($p > 0.2$) by a paired student t-test. All eight patients recorded the results of their operation as "satisfactory". The values for knee score both one to three months (mean 3.2 ± S.E.M. 0.2 units) and one year post-operatively (mean 3.6 ± S.E.M. 0.3 units) were significantly lower ($0.05 > p > 0.01$ and $0.05 > p > 0.01$) respectively, than the pre-operative values (mean 6.7 ± S.E.M. 0.6 units).

Group II

Figure 5 shows the synovial perfusion values obtaine two to three years post-operatively. Included for

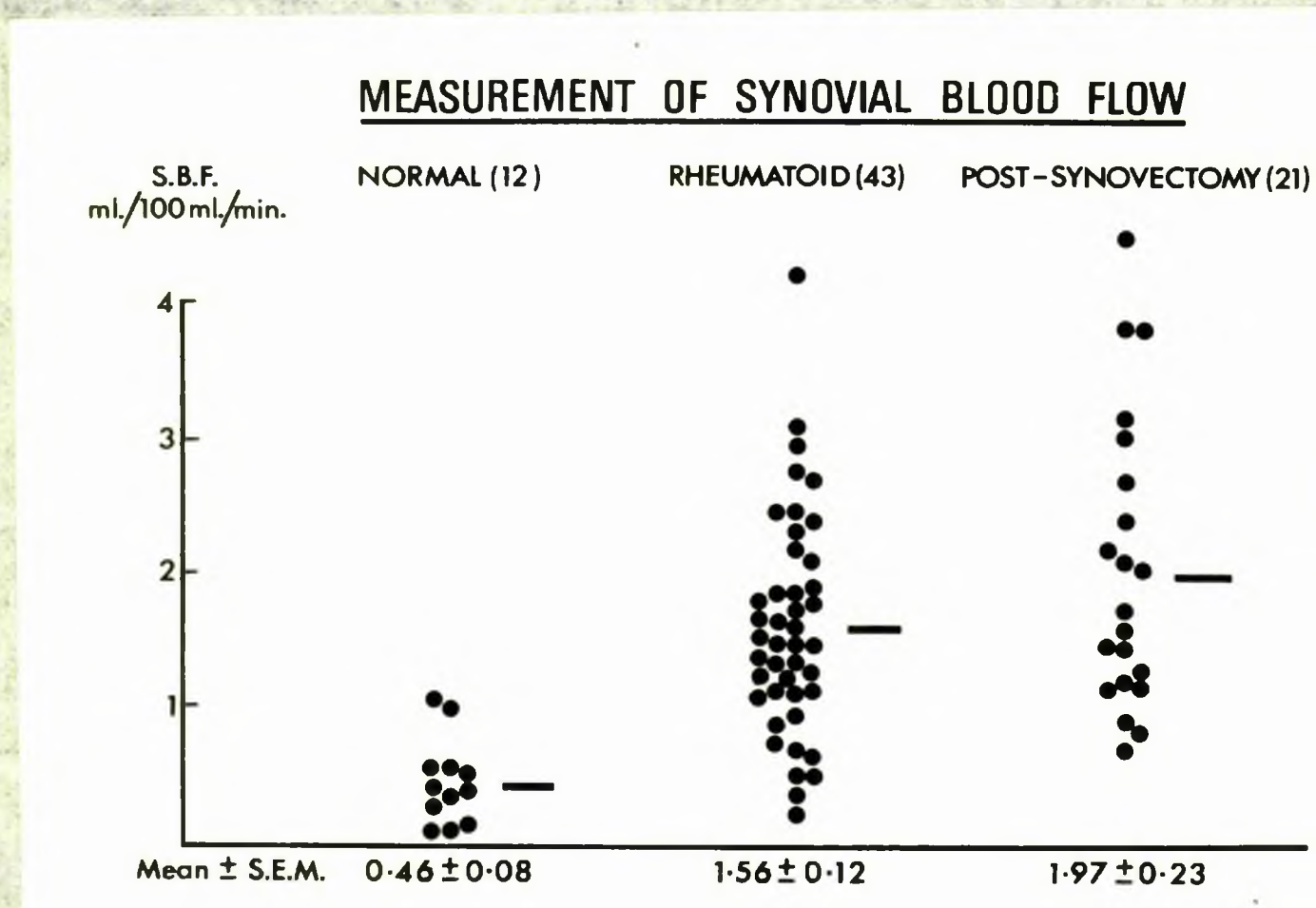


Figure 5

Legend: Synovial blood flow values 2 to 3 years followin
synovectomy compared with those obtained in normal subjec
and in unoperated patients with rheumatoid arthritis.

comparison are synovial perfusion values, derived from normal subjects and 43 patients with rheumatoid arthritis who were not subjected to operation, reported previously (page 285). The perfusion values obtained post-operatively (mean 1.97 ± S.E.M. 0.23 ml/100 ml/min.) are of the same order of magnitude as the values in non-operated rheumatoid arthritis. More refined statistical analysis is not justified since the groups of patients are not matched for clinical involvement.

The relationship between the clinical index of inflammatory involvement and the relevant $T\frac{1}{2}$ values were analysed. The 'r' value obtained was 0.32 which is not significant.

Four of these patients considered that the result of their operation was "bad", the remaining 17 recording the result as "satisfactory".

DISCUSSION

The clinical results of synovectomy of the knee are well documented (Speed, 1925 : Wolcott, 1927 : Heyman, 1928 : Inge, 1938 : Ghormley et al.1941: London, 1955 : Carruthers, 1960 : Aidem and Baker, 1964 : Gariepy et al., 1966 : Marmor, 1966 : Stevens and Whitefield, 1966 : Vainio, 1966 : Barnes and Mason, 1967 : Branemark et al., 1967 : Lembo, 1967 : Mikklesen, 1967 : Ramadier et al., 1967 and Paradies, 1969) and have been recently reviewed by Paradies, 1969. Most series record a "satisfactory" figure of over 80% in terms of pain relief, although a variable degree of reduction in range of motion is recorded in a significant percentage of cases. Paradies (1969) records a progressive diminution in the percentage of "satisfactory" results as the length of follow-up increases. Thus, at 2 years, 65% in his series were "satisfactory" however at 3 years, only 40% remained "satisfactory". The results of a series which included long-term follow-ups (London, 1955) also seems to demonstrate a similar tendency to recurrence of disease activity with increasing time after operation. Thus, at the clinical level, there is some evidence to suggest recurrence of disease activity following

synovectomy of the knee.

Experimental synovectomy (Key, 1925) has been less intensively studied. Suleimanov (1964) demonstrated that granulation tissue, with disturbed vascularity, grows in following experimental animal synovectomy and Lindstrom (1966) showed that, following "atraumatic" synovectomy in rabbits complete regeneration of normal tissue could occur but in "traumatic" cases granulation tissue, with wide tortuous blood vessels, grew in. The caloric response in traumatic cases was abnormal. Branemark et al. (1967) demonstrated abnormal tissue following synovectomy and Mitchell and Cruess (1967), employing tritiated thymidine, showed that metaplasia of existing mesenchyme, rather than hyperplasia of remaining synovial tissue, contributed to the re-growth of villi, in the space of 30 days following synovectomy in rabbits.

However, some workers (e.g. Marmor, 1966) have presented evidence that regenerated synovium and its vasculature may be normal in human subjects. Thus it seemed reasonable to study the effect of synovectomy on the clearance of 133 Xe which provides a measure of blood flow.

The results of this study are of some interest.

The values obtained one to three months following operation were significantly lower than were the pre-operative values presumably reflecting reduction in vascularity of the regenerating tissue. At first sight it may seem surprising that the values were not lower post-operatively considering that the great mass of synovium is removed at operation. However, this method does not measure total volume of vascular tissue. It is an index of the degree of vascularity of the tissue close to the site of deposition of the isotope. Furthermore, there is evidence that tissue re-growth following synovectomy occurs within a few weeks (Mitchell and Cruess, 1967), and it seems probable that these results include some degree of regeneration. The results one year following operation however were similar to those obtained pre-operatively. This suggests that the tissue through which the isotope is clearing is vascularly abnormal. Experiments previously reported (page 75) suggest that the route of clearance of 133 Xe from the synovectomised joint is similar to that which obtains in unoperated rheumatoid arthritis. Thus it would appear that the re-growth

"synovial tissue" is vascularly abnormal. It is interesting to note that all patients considered th results of their operation to be "satisfactory" and this impression is borne out by the results of the clinical knee scores. It is possible therefore th the results of the 133 Xe studies are anticipating clinical change. The number studied is small, however, and firm conclusions are not yet justifie

The values obtained in the second group of patients are clearly different from those obtained normal joints. It is interesting to note how well they accord with values obtained for patients with rheumatoid arthritis who had not been subjected to synovectomy. The 43 values for rheumatoid patient were obtained simply by studying every patient suffering from "classical" or "definite" rheumatoi arthritis with clinical involvement of the knee jo who presented as an outpatient at this hospital be September, 1967 and February, 1968. The clinical score was performed in all these cases in the same manner and by the same physician who examined clin the knee joints of the 21 patients who had a knee synovectomy. In the case of the 43 patients the

correlation coefficient between the clinical knee score and the relevant $T\frac{1}{2}$ value was 0.76 (Chapter 6, page 226). However, in the case of the 21 synovectomised knees reported in this study (Fig. 5) the correlation coefficient was 0.32. Although once again refined statistical analysis of these two figures is not valid for the reasons mentioned above, the difference between a good correlation in the one case and none at all in the other is sufficient to suggest that a meaningful difference does exist. However, the reason for this discrepancy is obscure. It may be that abnormalities or delay in the re-growth of the nerve supply following synovectomy (Goldie et al., 1969) may have an effect on the post-operative clinical score. However, there is insufficient evidence on this point at present.

These isotope studies suggest that the operation of synovectomy has a limited "life span" and this is in accord with clinical findings in long term follow up studies (London, 1955 : Paradies, 1969). It would seem reasonable to suggest that the re-growth of vascularly abnormal synovium occurs in the early months post-operatively. This vascular abnormality may be the precursor of florid inflammatory change to follow

or alternatively it may well have no clinical relevance in terms of patient disability and relapse. It will be interesting to see on long term follow up studies whether the 133 Xe clearance technique will prove to possess predictive power.

SUMMARY

1. Synovial perfusion is elevated in the inflammatory arthritides and in some patients with osteo-arthritis. The elevation is therefore non-specific

2. The increase in 133 Xe clearance rate from the joint is related to the severity of involvement at the time of study in the inflammatory arthritides.

3. The synovial perfusion rate may be elevated in joints of patients with rheumatoid arthritis despite absence of clinical signs of involvement in the disease process. The method may therefore prove to be of value in the early diagnosis of arthritis.

4. The clearance rate may be reduced by hydrocortisone administered intra-articularly and by oral indomethacin but not by oral malicylate therapy. The sensitivity of the 133 Xe clearance method is probably less than that of the standard clinical methods of assessment.

5. Synovial perfusion, although reduced in the early months following synovectomy, returns to its pre-operatively elevated level within one year. Synovial perfusion rates are elevated between 2 and 3 years following operation. It is possible that the 133 Xe clearance method may be of value in the assessment of the results of this operation.

CHAPTER 7

In an attempt to discover a more sensitive objective method of assessment of change in the severity of the inflammatory process than was afforded by the 133 Xe clearance method, I studied the localisation of radioactive technetium (99 mTc) in the joint following intravenous injection.

A method of quantitation of articular scans is described which discriminated between severely involved, moderately involved and normal joints. The method, however, was time consuming, required high tracer doses and did not possess an entirely satisfactory degree of reproducibility.

A second simpler method for quantitating isotope uptake was studied, which required lower tracer doses and possessed a higher degree of reproducibility. Studies are reported which demonstrate that differences between normal and inflamed, large and small joints may be detected by this method. Change in disease activity consequent upon the exhibition of corticosteroid therapy could also be detected.

Thereafter two studies designed to assess the sensitivity of 99 mTc uptake when compared with clinical methods of assessment are reported, the results of which suggest that this radio-isotopic method compares favourably with the better established techniques.

99 mTc uptakes in synovectomised and unoperated patients with rheumatoid arthritis and in patients with osteoarthritis are compared with those obtained in normal subjects and the relationship between 99 mTc uptake, 133 Xe clearance rate and clinical evaluation is examined.

Finally, the fortuitous obsefvation that the rate of clearance of ionised radioisotopes differed from the rate of clearance of 133 Xe prompted the final study reported in this thesis. Currently synovial fluid is considered to be composed of an ultrafiltrate of plasma to which is added hyaluronic acid and protein by the synoviocytes. The results of this study suggest that the position may be more complex and that active transport mechanisms exist across the synovial membrane.

THE ARTICULAR SCAN IN PATIENTS WITH RHEUMATOID ARTHRITIS

Introduction

"Measure what is measurable and what is not measurable make measureable". Galileo.

This adage is particularly relevant to the study of the connective tissue diseases. Lack of reliable quantitative indices finds expression in conflicting results of drug trials (O'Brien, 1967) and in ignorance of the effect of widely applied surgical procedures (page 250).

Rheumatoid arthritis remains an incurable disease and its management must include the use of non-specific suppressive agents. The great majority of pharmacological agents in this class are drugs of relatively small effect. The difficulty of assessing any such drug is compounded by the spontaneous alterations in activity which are a feature of this disease and whereas there is no difficulty in detecting major changes in disease activity clinically, the small changes produced by these drugs present a major problem to the assessor.

Anti-inflammatory effect or spontaneous change in disease activity may be detected either in terms of subjective or objective methods of assessment. In an exhaustive series of meticulously controlled studies Beecher (1959) has validated the use of subjective methods of assessment provided only that they be susceptible of quantitation. Indeed, it is clearly better to employ a relevant subjective than an irrelevant objective method. The methods which are currently employed in this field include clinical indices based on pain and tenderness (Co-operating Clinics of the American Rheumatism Association, 1965 McCarty et al., 1965 : Lansbury, 1966 : Mainland, 1967 : Ritchie et al., 1968), alteration in grip strength (Wright, 1959 : Ingpen, 1968) or in joint circumference (Boardman and Hart, 1967), duration of morning stiffness and aspirin count, articular and skin temperature changes (Hollander et al., 1951 : Hollander and Moore, 1956 : Branemark et al., 1966 : Haberman et al., 1968 : Goldie, 1969), synovial fluid white cell count and enzyme levels, rate of absorption of phenylsulphonylphthalein from the joint cavity (Nakamura et al., 1967) and alteration in haemoglobin, erythrocyte sedimentation rate and other blood

constituents. No single method has proved to be entirely satisfactory and it is still common practice to employ several methods concurrently (Steinbrocker et al., 1949).

The availability of radioisotopes has widened the scope of applicable techniques. The various radioisotopes which have been injected intra-articularly and the clearance rates of which have been related to the severity of local disease are reviewed in Chapter 1. Apart from the disadvantage of local injection and the restricted applicability to only the particular joint injected, the clearance rate of 133 Xe did not change following the exhibition of large doses of oral salicylates for one week, suggesting that the 133 Xe clearance rate would be of only limited value in the assessment of non-steroidal anti-inflammatory agents. Radioisotopes, however, may be utilised in a different manner. The local accumulation of radioactivity following intravenous injection of the isotope may be monitored. The isotope which has been employed most frequently in this way is radioactive technetium (99 mTc

The value of radioactive technetium (99 mTc) as a scanning agent has been studied in many situations

(McCready, 1967 ; Anon., 1968). Amongst the many organs qualitatively displayed by the isotope are the stomach and salivary glands (Harden et al., 1967a and the heart cavity (Witcofski and Bolliger, 1965), the brain (Davis et al., 1966), extra-cranial tumours (Whitley et al., 1966), the placenta (Larson and Nelp, 1965) and the inflamed joint (Weiss et al., 1966 ; Alarcon-Segovia et al., 1967a,b:McCarty et al., 1970a and b , Sholkoff and Glickman, 1969.).

Clearly purely qualitative display will present difficulties in the detection of small changes and accordingly in the present study an attempt was made to derive a means of quantitating radioactive technetium articular scans.

MATERIALS AND METHODS

Radioactive Technetium

99 mTc was obtained as the pertechnetate by elution with 10 ml. of sterile physiological saline solution from a column generator loaded with molybdenum-99. The eluate was sterilised by autoclaving and delivered to the Centre for Rheumatic Diseases at noon. All studies were performed between the hours of noon and 4 p.m. owing to the short physical half life (6 hours) of the isotope. This short half life, in addition to the lack of beta emission (99 mTc decays by emitting a gamma ray of emission energy 140 keV) renders this an ideal scanning agent with a wide margin of safety (Smith, 1965). A 1 millicurie dose administered intravenously results in a total body exposure of 17 millirads.

Patients Studied

Twenty-four patients with "classical" or "definite rheumatoid arthritis were studied, all of whom had marked involvement of the joint studied. Four of these patients were submitted to repeat examination one week after the initial scan was performed to

determine the variability of the method. All female patients were post-menopausal. The mean age of these patients was 50.1 years (range 39 to 66 years) and eight were male.

Two members of the medical staff served as controls and scanning studies were performed on their elbows, writs, hands and knees. Four hours before scanning, each subject received 600 mg. of potassium perchlorate, orally, to block the uptake of 99 mTc by the stomach and thyroid gland.

A dose of approximately 1 mCi of 99 mTc-pertechnetate was given to each patient and control subject and the relevant joint was scanned 25 minutes after the administration of the dose. In the first six patients serial scans were conducted over a period of 3 hours to determine the optimum scanning time and counting conditions.

Visualisation

The pattern of radioactivity in the joint was visualised with a Picker-Magnascanner V, scanning in the antero-posterior position. The line spacing and scan speed varied with each joint; in the knee, a line spacing of 1 cm. and scan speed of 100 cm./min.

were found convenient, whereas for the wrist joint a line spacing of 0.5 cm. was required. The time taken for each scan was approximately 10 to 15 minutes depending upon the joint being scanned.

Quantitation

Quantitation of the uptake of the isotope, as opposed to photographic display of the distribution of radioactivity, was achieved in the following way (Hilditch et al., 1967): the area corresponding to the joint being scanned was drawn on the scanning paper. The uptake by the joint, together with background radioactivity in the tissues and in the atmosphere, was calculated by counting the number of dots enclosed in the scanning area. An estimate of the background count, obtained by counting an area adjacent to the joint area was subtracted from the original count. The final count was then corrected for decay using the midpoint of the scanning time as the theoretical time of scanning. A phantom of the joint, which contained a known percentage of the dose given to the patient, was scanned under conditions identical to those employed when scanning the joint except that the dot factor, which varies the number of dots recorded in proportion to the intensity of radiation received, was increased.

Fig. 1

Legend: The display of 99 mTc in the wrist joint and metacarpophalangeal joints in a patient with rheumato: arthritis.

Fig. 2

Legend: The display of 99 mTc over a knee joint a patient with rheumatoid arthritis.

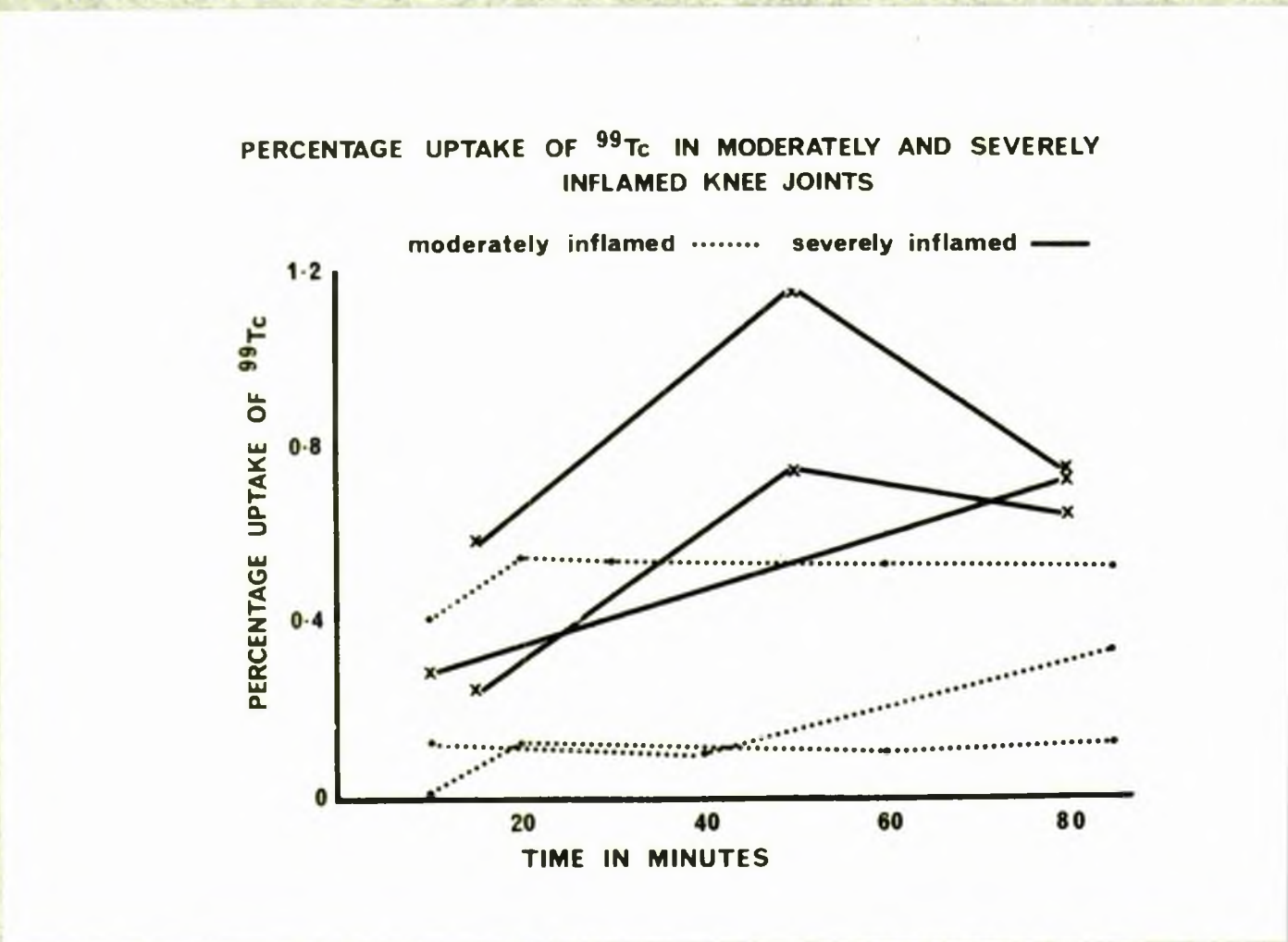


Fig. 3

Legend: Serial scans of knee joints affected by rheumatoid arthritis: ·····, moderately infl
————, severely inflamed. Percentage uptake o
99 mTc is plotted against time.

RESULTS

The results are shown in Figs. I-V and in Table 1. In normal subjects there was virtually no uptake of isotope by any of the joints examined and it was impossible to visualise the joint by scanning.

In contrast to these results, patients with rheumatoid arthritis exhibited a satisfactory uptake of the isotope in all the joints, allowing the joint to be visualised by the scanner. Representative examples of the pattern of display of the isotope in the wrist and hand and in the knee joint are shown in Figs. 1 and 2.

Figure 3 shows the results obtained when serial scans were performed in six patients. Broadly speaking, two patterns emerge. In some patients, an initially rapid uptake is followed by decay of 99 mTc, while in others the uptake appears to be initially les rapid and to increase more slowly. Because of this temporal variation it was necessary to scan all subjects at a standard time and it was decided to scan all joints 25 minutes following injection.

The results of repeated estimations of isotope

TABLE I

Reproducibility of uptake of 99 mTc in wrist joints, at an interval of 1 week

Patient	Scan 1	Scan 2
1	0.12%	0.13%
2	0.087%	0.11%
3	0.089%	0.095%
4	0.078%	0.06%
5	0.085%	0.11%
6	0.12%	0.18%
7	0.081%	0.11%
8	0.10%	0.10%

Coefficient of variation = 25%

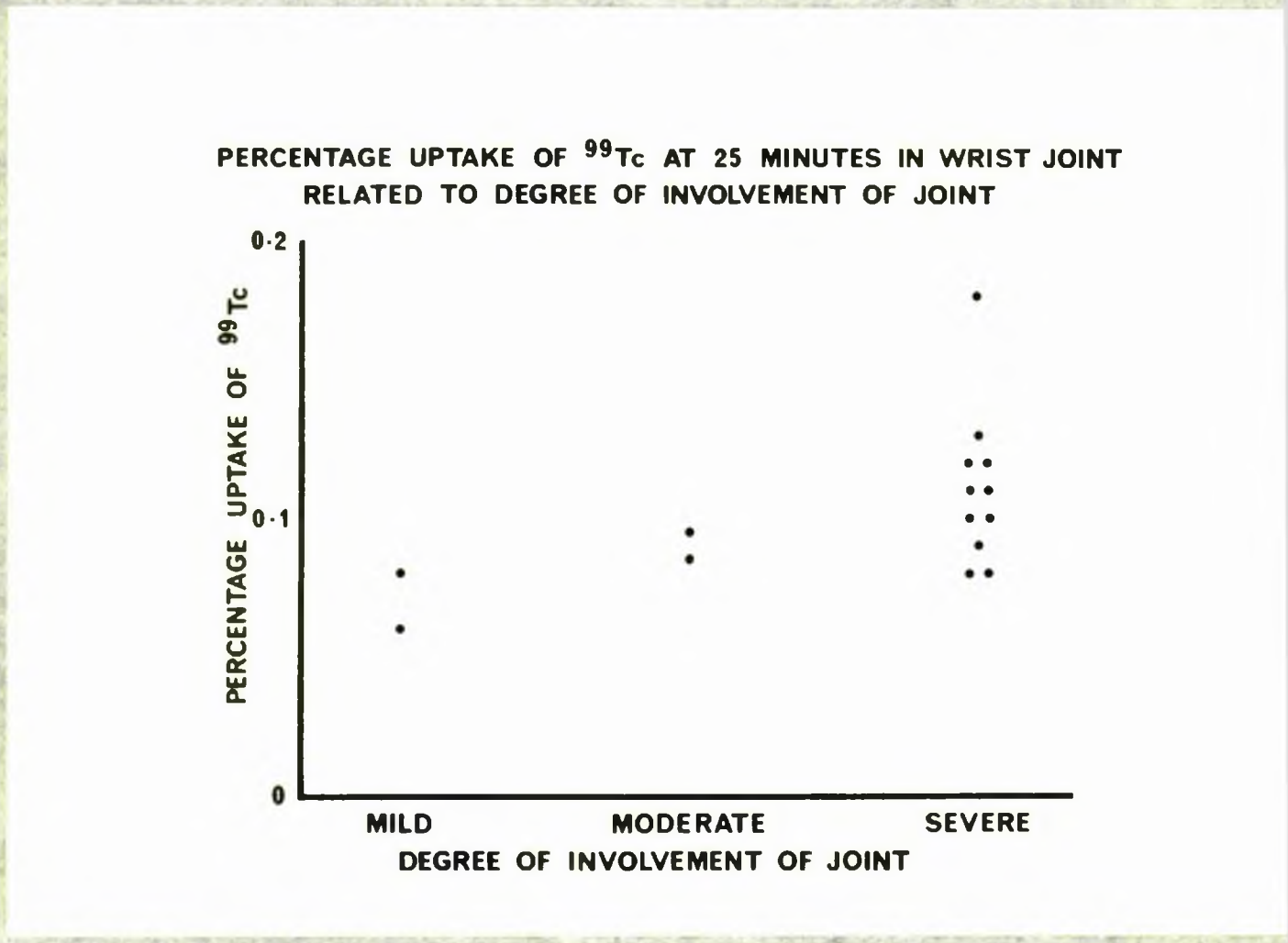


Fig. 4

Legend: Uptake of 99 mTc at 25 min. in wrist joints correlated with degree of inflammation: mild, moderate and severe.

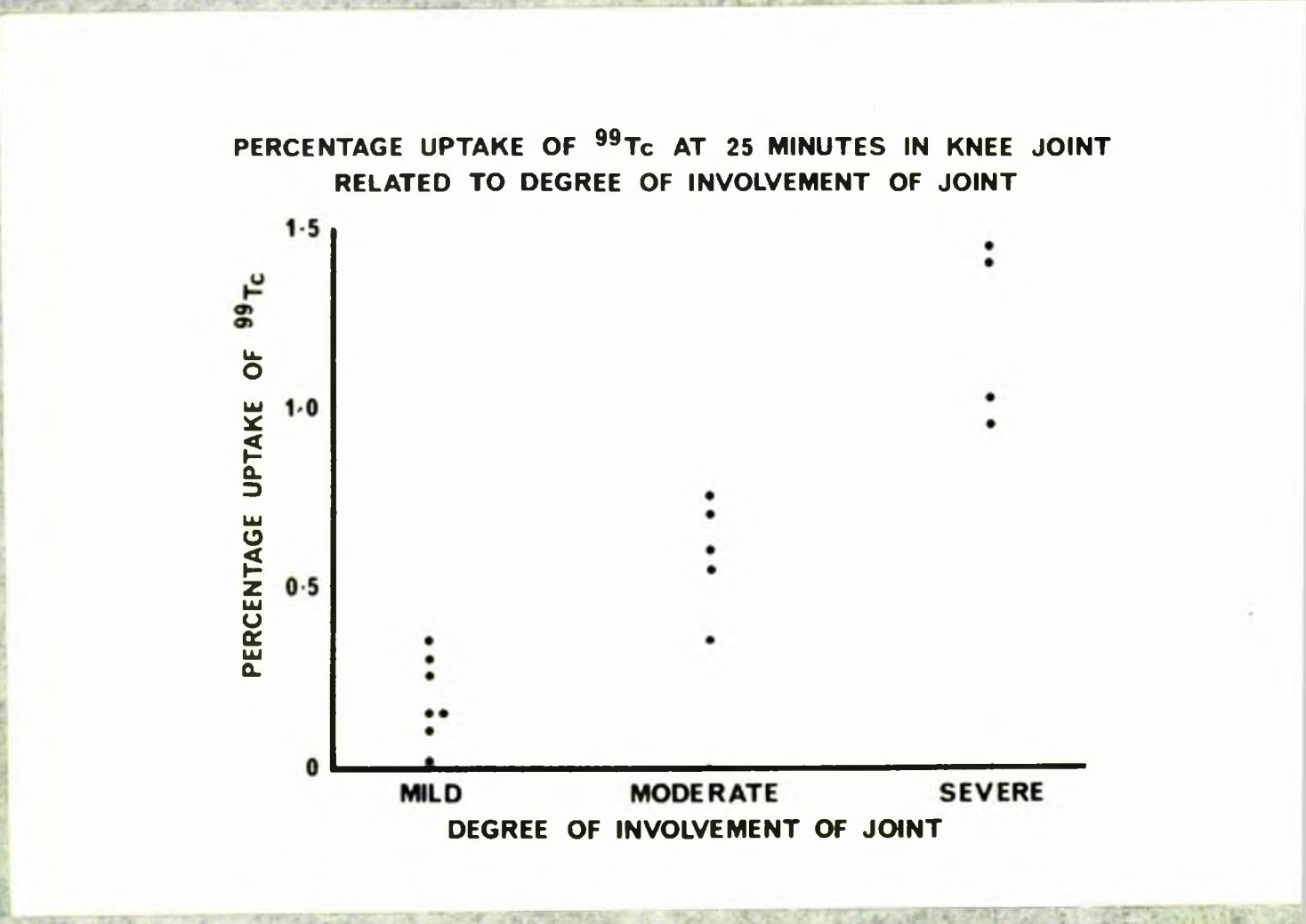


Fig. 5

Legend: Uptake of 99 mTc at 25 min. in knee joint correlated with degree of inflammation: mild, moderate and severe.

uptake by an individual joint in four patients (each wrist joint being scanned on both occasions) are shown in Table 1. The coefficient of variation was 25%.

The percentage of the initial dose taken up by the wrists (Fig. 4) and by the knees (Fig. 5) correlated with the degree of inflammatory involvement of the joint as judged by tenderness of the joint to direct pressure. Clinically three degrees of tenderness were recognised: "mild", "moderate" and "severe". In the wrists the uptake varied from 0.06% when mildly inflamed to 0.18% when the joint was very tender. The knee however showed much higher uptake of 99 mTc and the separation between the three degrees of tenderness was even more marked. The range of uptake for a mildly involved knee was 0.1% to 0.45% of the dose of 99 mTc, for moderately inflamed knees from 0.45% to 0.75% and for severely inflamed knees from 0.85% to 1.45%. The pattern of uptakes shown in Fig. 3 also corresponded to the degree of inflammatory involvement, rapid uptakes being observed in the presence of "severe" inflammation and slow uptakes in the case of the "moderately" inflamed joints.

In order to investigate if the 99 mTc uptake by the joint was purely a reflection of the increased blood flow through the inflamed joint, or whether 99 mTc is actively taken up by the synovial membrane, the following experiment was performed. A patient going for synovectomy of the knee was given 1 mCi of 99 mTc intravenously 25 min. before the tourniquet was put over the patient's leg. At the time when the tourniquet was put on, 20 ml. of heparinized blood were collected from the opposite arm to that in which the 99 mTc had been injected. All the synovium was collected after removal, and the radioactivity of equal quantities of blood and synovial membrane was counted. The ratio of the radioactivity of blood to that of synovial membrane was 1.68. This indicates that 99 mTc is probably not actively trapped by the synovial membrane and that rather, the 99 mTc circulates in the blood through the joint tissues.

DISCUSSION

The present study has shown that radioactivity may be displayed in joints involved in rheumatoid arthritis, after the administration of 99 mTc. The results of the studies designed to compare the activities of the isotope in blood and in synovial membrane suggest that the presence of the isotope in the joint is accounted for not by active synovial concentration of 99 mTc, but rather by the passage of the isotope through the vascular channels in the synovium. If active concentration of isotope by the synovial cells were taking place one would expect that the ratio of isotope activities in equal volumes of synovial membrane and blood at a given time would be greater than unity. That this is not the case favours the alternative explanation of passage of isotope through the vascular channels in the joint. If one accepts this explanation, it is not surprising to find increased values for isotope concentration over joints which are involved in the rheumatoid inflammatory process, for in this disease an enormous increase in the vascular supply of the inflamed synovium occurs.

Tentative support for this hypothesis comes from the observation that the concentration of isotope in the joint at any one time shows a correlation with a rough clinical assessment of the degree of inflammation which is present. It may be that the isotope readings give a truer indication of the extent and the amount of inflammation in the synovium than does the clinical assessment for it is notoriously difficult to make an accurate clinical assessment of the degree of inflammation. This speculation must be tempered with the knowledge that with present techniques the coefficient of variation of the isotope methods is high.

The procedure which this study describes may have value in the assessment of the anti-inflammatory activity of drugs in rheumatoid arthritis for if diminution in inflammation is usually accompanied by diminution in the blood flow to the synovium, then a reduction in the display of 99 mTc in the joint, proportionate to the diminution in inflammation, might be expected.

The technique may also be of value to assess the completeness, or otherwise, of synovectomy,

for presumably if it proved possible to remove entirely the vascular synovium, a dramatic reduction of the concentration of isotope in the joint should be achieved.

SUMMARY

1. In an effort to obtain an objective index of articular inflammation, radioscans have been performed on a variety of joints in twenty-four patients with rheumatoid arthritis of varying severity, 25 minutes after the intravenous administration of 1 mCi of radio-technetium (99 mTc). Localisation of the isotope in the joint was easily demonstrated using a Picker-Magnascanner V.

2. It has proved possible to quantitate the display of the isotope in a joint. The method is sufficiently reproducible for clinical use and the uptake has been found to be a function of the clinical severity of joint inflammation.

3. Further studies have shown that the isotope is not actively concentrated by the diseased synovial membrane and this finding suggests that the display of isotope in an inflamed joint may reflect enhanced vascularity of the synovial membrane and other joint tissues.

QUANTITATION OF RADIOACTIVE TECHNETIUM LOCALISATION IN THE KNEE AND PROXIMAL INTERPHALANGEAL JOINTS BY EXTERNAL DIRECTIONAL COUNTING

In the succeeding two sections of this chapter a simpler method of quantitating 99 mTc uptake by the joints requiring a lower tracer dose is proposed and the reproducibility of this method is quantitated.

QUANTITATION OF RADIOACTIVE TECHNETIUM LOCALISATION IN THE KNEE JOINT BY EXTERNAL DIRECTIONAL COUNTING

Introduction

The photoscan display system, employed in the previous study, yielded a picture of the isotope content of the area over a relatively short period of time and the method of quantitation was tedious. Furthermore, a dose of the order of one millicurie of the isotope was required to achieve optimum scans. This dose precluded frequent repitition of the studies which would be necessary in a clinical trial of a therapeutic agent.

It seemed possible that continuous external directional counting over the knee joint following the intravenous administration of the isotope might overcome these problems. Thus, more information might be derived from the pattern of uptake than was afforded by a single photoscan and with a more sensitiv detecting system a much lower dose of the isotope would be required. Finally, this might be expected to provide a method which would be simpler to quantitate.

MATERIALS AND METHODS

Patients Studied

Studies were performed on 20 clinically active knee joints of patients with classical rheumatoid arthritis (Ropes et al., 1959). Their mean age was 52.8 years (range 42 to 65 years). Five were male. In five patients repeat studies were performed at 24 hours to determine the day to day variability of the method and a further six patients received hydrocortisone (Ultracortenol 10 mg.) intra-articularly, the studies being repeated one week later.

Isotope studies were performed on eight healthy male volunteers aged between 20 and 28 years (mean 24.5 years). None gave a history of disease or trauma to the knee joint. The isotope studies were repeated at 24 hours in two of these subjects to determine the day to day variation in the method.

Clinical knee assessments (page 112) were performed by the same observer prior to each isotope study.

Isotope Studies

The patient reclined comfortably in bed and the

knee was immobilised during the study.

One millicurie in 5 ml. sterile NaCl of 99 mTc obtained daily at noon and studies were performed between then and 4 p.m. because of the short half life of the isotope (6 hours).

Approximately 200 μCi of 99 mTc in 1 ml. of NaCl were administered intravenously into a vein in the antecubital fossa. In early feasability studies it had been noted that excess counts were frequently present over the injection site when compared with the identical area on the opposite arm at the conclusion of the study. Accordingly, great care was taken to ensure that the injection was truly intravenous.

A collimated 1" x 1.5" thallium activated sodium iodide crystal connected through an Ecko ratemeter (M.5180) and pulse height analyser (M.5010) to a Kienzle automatic print-out was positioned directly over the patella touching the skin.

Counts per 40 seconds were taken with the counting conditions adjusted for 99 mTc and counting commenced 120 seconds prior to the injection and

continued until the count rate was declining. The results obtained were plotted onto graph paper and the peak count rate and time to reach the peak were read from the graph.

At the conclusion of the study the count rate over the injection site and over the corresponding area on the opposite arm was measured. If the injection site displayed excess counts the study was disregarded.

TABLE II

NORMAL		RHEUMATOID ARTHRITIS	
Peak Counts	Time to peak (mins.)	Peak Counts	Time to peak (mi
8,900	18	28,000	16
13,100	16	23,000	13
5,700	23	18,000	5
11,800	47	16,800	20
7,500	22	10,100	10
9,300	30	6,400	33
6,100	18	14,000	18
12,000	12	13,400	22
		19,900	9
		7,900	9
		9,300	31
		10,300	15
		5,000	23
		26,400	6
		30,000	13
		12,000	15
		34,900	18
		19,500	20
		24,000	13
		14,000	8
Mean 9,300	23.3	17,145	15.9
S.E.M. 987	3.89	1,882	1.69

Time to peak rheumatoid v. normal subjects $0.1 > p > 0.05$

Peak counts rheumatoid v. normal subjects $0.05 > p > 0.01$

Legend: Time to peak and peak count rates over the knee in normal subjects and in rheumatoid arthritis.

TABLE III

The results obtained when the peak counts were measured in 7 subjects on two occasions with a time interval of 24 hours.

Subjects	1st Occasion	2nd Occasio
Normal	6,300	6,400
Normal	6,400	5,800
Rheumatoid arthritis	13,000	12,700
Rheumatoid arthritis	12,700	13,100
Rheumatoid arthritis	25,000	23,900
Rheumatoid arthritis	18,000	17,700
Rheumatoid arthritis	32,000	32,600

Standard Error of the difference 222 minutes.

TABLE IV

	PRE			POST		
Patient	Knee Score	Peak Counts	Time to peak (mins.)	Knee Score	Peak Counts	Time to peak (mins.)
1	9	22,000	14	3	6,000	13
2	9	10,800	18	1	9,600	12
3	7	14,000	31	4	12,000	14
4	6	19,900	6	5	4,600	13
5	9	30,800	12	1	9,000	18
6	2	13,300	15	6	14,500	18
Mean	7.0	18,466	16.0	3.3	9,283	14.6
± S.E.M.	1.13	3,014	3.41	0.84	1,499	1.09

Knee score pre v. post hydrocortisone	$0.05 > p > 0.01$
Peak counts pre v. post hydrocortisone	$0.05 > p > 0.01$
Time to peak pre v. post hydrocortisone	$0.8 > p > 0.7$

Legend: The values obtained for knee score, peak counts and time to reach peak counts in the six patients prior to, and one week following intra-articular injection of hydrocortisone.

RESULTS

The results are shown in Tables 2, 3 and 4. The peak values obtained in the normal subjects (mean 9,300 ± S.E.M. 987 counts per min.) were significantly lower ($0.05 > p > 0.01$) than the peak values obtained in the rheumatoid patients (mean 17,145 ± S.E.M. 1,882 counts per min.). The time to reach the peak in the normal subjects (mean 23.3 ± S.E.M. 3.89 mins.) did not differ significantly ($0.1 > p > 0.05$) than the time to reach a peak in the rheumatoid subjects (15.9 ± S.E.M. 1.69 mins.) (Table 2).

Table 3 shows the results obtained in the two normal subjects and the five rheumatoid subjects in whom isotope studies were repeated 24 hours later. The standard error of the difference was 222 counts per minute for the peak count rate.

The results of the isotope studies and clinical knee assessments before and after the intra-articular injection of hydrocortisone are shown in Table 4. The means of the peak values and clinical assessments prior to the injection (mean 18,466 ± S.E.M. 3,014 counts per min. and mean 7 ± S.E.M. 1.13 respectively)

differ significantly ($0.05 > p > 0.01$ and $0.05 > p > 0.01$ respectively) from the means of the same parameters one week after the injection (mean 9,283 $\pm$ S.E.M. 1,499 counts per min. and mean 3.3 $\pm$ S.E.M. 2.07 respectively).

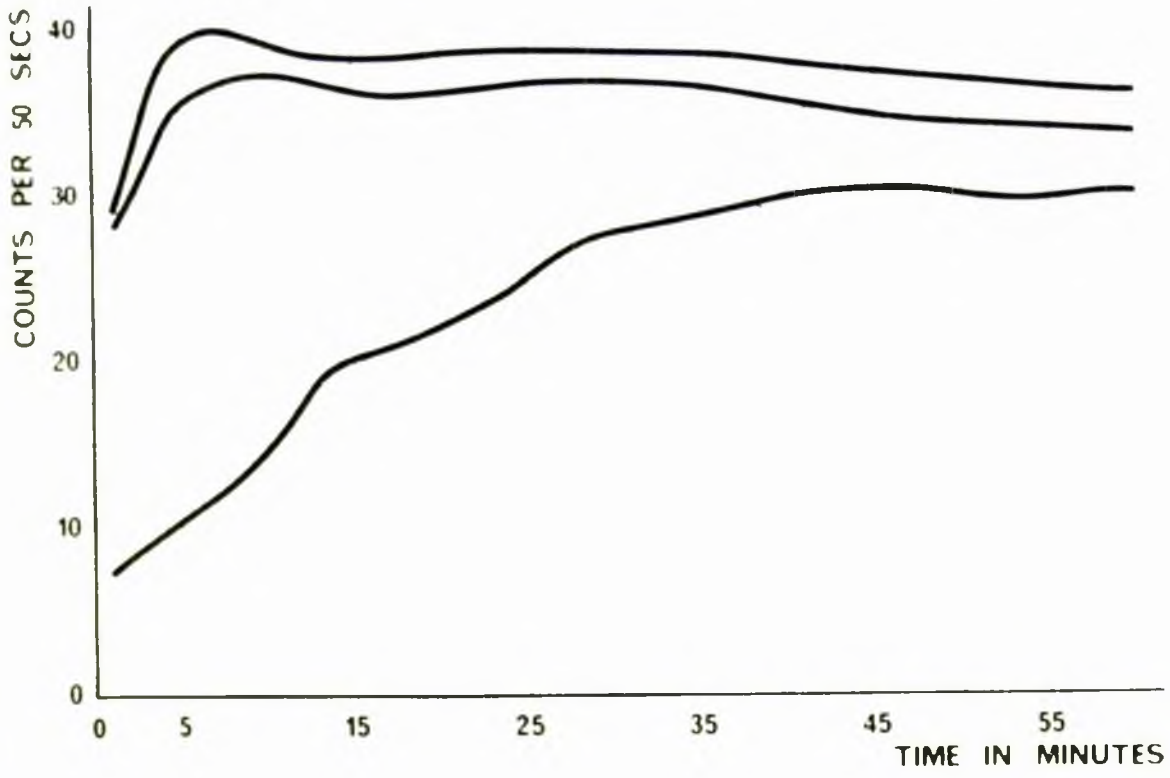
The mean values of the time to reach the peak prior to the injection of hydrocortisone (16 $\pm$ S.E.M. 3.41 mins.) did not differ significantly ($0.8 > p > 0.7$) from the values obtained one week after the injection (14.6 $\pm$ S.E.M. 1.09).

DISCUSSION

In this study a simpler method of measuring the same phenomenon as is reflected by the photo-scan display system is introduced. A major advantage of the revised technique is the reduction in the dose of isotope required.

In early feasibility studies it was noted that in many cases, despite no obvious fault in the injection technique, a significant proportion of the administered dose remained at the injection site. In the most obvious cases this was manifest in the results by a very slow rise to peak count rate. To obviate this problem great care is required when administering the isotope and the injection site should be monitored following each study.

Provided care is taken these isotope studies would seem to possess an acceptable degree of reproducibility. The standard error of the differe in the repeat isotope studies was 222 counts per minute for peak uptake of the isotope.



Legend to Fig.6.

Linear plots of 99mTc.count rate over the knee joint in the same patient on three occassions at weekly intervals.The top graph was recorded while the patient was receiving placebo,the middle one during aspirin therapy and the lower one during corticosteroid therapy.

These results also suggest the possibility that 99 mTc uptake might be a useful adjunct to the objective assessment of the effect of anti-inflammatory drugs. Thus, in this study, 99 mTc peak uptake paralleled the clinical knee assessment when the knee joint was treated with local anti-inflammatory therapy. The dose of the isotope employed in this study (200 μCi) was sufficiently low to allow repeat studies and, indeed, considering the magnitude of the count rate obtained with this dose, it appeared feasible to reduce the dose even further in future studies.

In Fig. 6 are shown results obtained in a single patient who received placebo, aspirin (4 g. per day) and prednisolone (10 mg. per day) orally for one week each. Whereas the graph is lowered only a little by aspirin a marked change is seen after prednisolone. This result supports the validity of studying 99 mTc uptake as a measure of change in joint inflammation.

SUMMARY

A method for the quantitation of joint inflammation based upon the rate of uptake of 99 mTc by the joint is described. The method is reproducible and shows differences between normal and diseased joints. It has been shown to be capable of reflecting the effect of anti-inflammatory therapy.

QUANTITATION OF RADIOACTIVE TECHNETIUM LOCALISATION IN THE PROXIMAL INTERPHALANGEAL JOINTS BY EXTERNAL DIRECTIONAL COUNTING

Introduction

Following intravenous injection elevated count rates of radioactive technetium are recorded over inflamed when compared with normal joints. The time selected for determination of count rate was 15 minutes after injection. Elegant studies on the distribution of intravenously administered radioactive technetium within the joint by McCarty et al., (1970b) have demonstrated that at this time the radioisotope is located intravascularly. At a later time following injection leakage of the tracer occurs into tissue and synovial fluid and count rates at one hour following injection will include the binding of 99 mTc by inflamed tissue protein. Since this introduces a further variable factor which cannot be adequately quantitated it is reasonable to employ early count rates in comparative studies of inflamed and normal joints.

In the present study the count rate at 15 mins. after injection over the proximal interphalangeal joints of the hand was determined in normal subjects

and in patients with rheumatoid arthritis. The effect of oral low dose corticosteroid therapy upon the count rate was also determined in conjunction with more established indices of joint inflammation.

MATERIALS AND METHODS

Patients

Twenty-eight patients with "definite" or "classical" rheumatoid arthritis as defined by the diagnostic criteria of the American Rheumatism Association (Ropes et al., 1959) took part in the study. There were 27 females and one male. The mean age was 56.7 years with a range of 22 to 76 years.

Thirteen healthy volunteers (8 female, 5 male) with a mean age of 23.1 years (range 22 to 35 years) served as controls.

All patients and control subjects volunteered to participate in this study with full knowledge of its content.

Isotope Studies

Approximately 200 μCi (standardised by count rate) of 99 mTc in 1 ml. of sterile NaCl was administered intravenously into a vein in the antecubital fossa of the left arm. The count rate was monitored continuously for 15 minutes over the proximal interphalangeal joint of the index finger

of the right hand. Peak values were then obtained for the other proximal interphalangeal joints, counting for one minute over each joint. The sequence of counting was consistent being from the first to fourth proximal interphalangeal joints, the right hand being counted first.

In previous feasibility studies it had been noted that excess 99 mTc counts were occasionally present over the site of the intravenous injection when compared with the identical area on the opposite arm at the conclusion of the study. Because of this great care was exercised to ensure that the injection was truly intravenous and verification that the total dose of 99 mTc had been administered intravenously was carried out by washing the syringe through with intravenous blood and by comparing counts at the injection site with the corresponding site on the right arm at the end of the study. In no instance were excess counts recorded at the site of injection.

During the isotopic studies the patient's hand was positioned on blocks of adjustable height below a lead shield 0.5 cm. thick with an aperture 2 cm.

in diameter. The joint to be counted was placed directly below the aperture, the counter resting on the shield which was supported by two lead supports 6 cm. high and 12 cm. apart. The counting equipment consisted of a collimated 1 x 1.5 inch thallium activated sodium iodide crystal connected through an Ekco ratemeter (M.5180) and pulse height analyser (M.5010) to a Kienzle automatic print-out. The initial count rate per 10 seconds was 15,000 ± 3% at 16 inches and the background of the order of 50 to 100 counts per 10 seconds. The results were related to the dose administered and expressed as a percentage of the administered dose corrected for counting geometry.

Repeat uptakes of 99 mTc in the proximal interphalangeal joints were measured in 9 patients at 24 hours to determine the reproducibility of the measurement.

The effects of oral prednisolone therapy 5 mg. twice daily on the proximal interphalangeal joint uptakes of 99 mTc were studied in seven patients. The effects of 99 mTc uptakes were compared with

the joint circumference as measured by a plastic spring gauge (Geigy Ltd.) described by Boardman and Hart (1967) and with the grip strength as measured by the mean of three readings over a baseline of 30 mmHg. for each hand. Prior to each isotope study each joint was assessed by the same observer. A history of pain and of stiffness was elicited and graded. Firm pressure was exerted over the joint margin and the response graded. An assessment of the degree of joint warmth and swelling was graded. Grading was on a 0 to 3 basis where 0 = absent; 1 = slight; 2 = moderate and 3 = severe. The maximum score for an individual joint was +15.

TABLE V

REPEAT ^{99m}Tc UPTAKES (%) IN PROXIMAL INTERPHALANGEAL JOINTS IN 9 PATIENTS WITH RHEUMATOID ARTHRITIS AT 24 HOURS (Mean ± S.E.M.)

	Right					Left					Both Hands
	1	2	3	4	Total	1	2	3	4	Total	
^{99m}Tc Uptakes %											
1st Uptake	8.24±0.83	6.75±0.70	6.48±0.82	5.87±0.73	6.85±0.69	6.54±0.64	6.51±0.51	6.82±0.64	5.63±0.63	6.15±0.54	6.23±0.43
2nd Uptake	8.17±0.83	7.04±0.66	6.30±0.68	6.02±0.87	6.89±0.66	6.86±0.73	7.64±0.79	7.26±1.03	7.29±0.82	7.26±0.76	6.92±0.64
Mean ± S.E. Mean of Difference	0.05±0.26	0.28±0.49	0.23±0.43	0.17±0.42	0.12±0.40	0.36±0.40	1.13±0.44	0.44±0.62	1.66±0.56	1.09±0.37	0.66±0.36

TABLE VI

^{99m}Tc UPTAKES (%) IN PROXIMAL INTERPHALANGEAL JOINTS

(mean ± S.E.M.)

Clinical Groups	Right				Left			
	1	2	3	4	1	2	3	4
Normal (13)	3.11 ± 2.9	3.0 ± 0.25	2.93 ± 0.25	2.60 ± 0.23	3.32 ± 0.34	3.07 ± 0.32	2.67 ± 0.21	2.36 ± 0.26
Rheumatoid Arthritis (22)	5.79 ± 0.31	6.61 ± 0.44	5.82 ± 0.36	4.77 ± 0.34	6.23 ± 0.40	6.32 ± 0.41	6.23 ± 0.35	5.24 ± 0.34
Significance t =	5.823	5.999	5.687	4.527	4.849	5.354	7.089	5.739
p =	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

RESULTS

The results are summarised in Tables 5, 6 and 7.

Table 5 summarises the results of repeat 99 mTc uptakes in the eight proximal interphalangea joints in 9 patients at 24 hours. It can be seen from the table that in all eight joints the mean of the differences and the standard errors of the difference are acceptably small. The mean difference for eight studies was 0.66 with a standard error of the difference of 0.36.

Table 6 summarises the results of the mean and S.E.M. of the 99 mTc uptakes in the proximal interphalangeal joints of each hand in 13 non-arthritic healthy controls and in 22 patients with rheumatoid arthritis. From the table it can be seen that the uptake in all the joints was significantly higher ($p < 0.001$) in the rheumatoid patients. No significant difference was noted in any of the mean uptakes in the proximal interphalangeal joints in the normal patients. However, in the patients with rheumatoid arthritis the mean uptakes in the first three right proximal

TABLE VII

99 mTc UPTAKES (%) AND JOINT CIRCUMFERENCE (mm) OF PROXIMAL INTERPHALANGEAL JOINT BEFORE AND AFTER ONE WEEK'S ORAL PREDNISOLONE THERAPY (Mean and S.E.M.)

		Right					Left					Both Hands
		1	2	3	4	Total	1	2	3	4	Total	
99 mTc Uptakes %	Before	6.23±0.12	7.50±0.46	6.34±0.43	4.28±0.38	6.09±0.26	6.07±0.31	6.17±0.16	6.40±0.22	5.06±0.32	5.89±0.18	5.99±0.16
	After	3.94±0.35	5.28±0.55	4.50±0.34	3.74±0.20	4.37±0.28	4.96±0.32	5.40±0.36	4.58±0.36	3.88±0.29	4.70±0.25	4.53±0.24
	t	8.14	2.96	5.20	1.17	4.84	3.15	2.07	7.11	3.26	6.59	5.93
	p	0.001	0.05	0.01	-	0.01	0.02	-	0.001	0.02	0.001	0.01
Joint Circumference (mm)	Before	59.86±1.60	62.00±0.62	59.57±1.32	49.71±0.68	57.51±0.80	59.29±1.21	60.43±1.43	57.14±1.10	50.29±1.02	57.27±0.90	57.43±0.65
	After	59.43±1.56	60.14±0.55	57.86±1.14	48.00±1.02	56.44±0.88	58.71±1.34	59.29±1.67	56.29±1.15	49.43±1.32	55.93±1.13	55.80±0.99
	t	1.44	4.04	2.83	2.05	4.34	1.9	1.6	2.1	1.87	2.51	3.36
	p	-	0.01	-	-	0.01	-	-	-	-	0.05	0.02

interphalangeal joints were significantly higher than the mean uptake in the fourth right proximal interphalangeal joint ($p < 0.025$) and in the left hand the mean uptake in the fourth left proximal interphalangeal joint was significantly lower ($p < 0.05$) than the mean uptakes in the first three proximal interphalangeal joints.

The effects of prednisolone 10 mg. per day for one week on the 99 mTc uptakes and joint circumferences of the proximal interphalangeal joints are summarised in Table 7. With the exception of the fourth right and second left proximal interphalangeal joints, the 99 mTc uptakes were significantly reduced with prednisolone therapy, as tested by a student's t-test for paired values.

In each proximal interphalangeal joint the mean of the differences before and after prednisolone therapy exceeds the mean of the differences observed in the reproducibility study, with the exception of the left fourth proximal interphalangeal joint where the mean of the differences in the prednisolone trial was 1.18 $\pm$ S.E.M. 0.36, whereas in the reproducibility study it was 1.66 $\pm$ S.E.M. 0.56. This casts doubt

on the validity of the significance value for the fourth left proximal interphalangeal joint.

When each joint was considered individually significant reduction in joint circumference was observed in only the second right proximal interphalangeal joint. However, when means of the four joints in each hand and the mean of all eight joints were considered the differences were statistically significant (Table 7).

The mean and S.E.M. of three readings for grip strength in the right and left hands was 76.29 ± 10.42 mmHg. and 88.71 ± 11.96 mmHg. respectively and after prednisolone therapy the respective values were 94.14 ± 9.77 mmHg. and 107.7 ± 13.08 mmHg. Although increased in both hands the differences were not statistically significant in either hand as tested by the student's t-test for paired values.

There was no significant correlation between any of the clinical features studied, joint circumference or grip strength and 99 mTc uptake in any of the fingers or individually in the means of these parameters in either hand.

DISCUSSION

Rheumatoid arthritis is an inflammatory polyarthritis affecting both large and small joints. Radioactive isotopes have mostly been employed to assess inflammation in large joints. McCarty and his colleagues (1970a and b) have scintiphotographs of small joints in the hands demonstrating increased localisation of radioactive technetium in inflamed tissue. If, as seems likely, the major application of radioactive technetium in arthritis is to provide a method of assessment of anti-inflammatory drug effect, then it is clearly essential that it be susceptible of quantitation, that it be reproducible, that clinically acceptable doses of the tracer be used and that it shows changes with conventional doses of anti-inflammatory compounds.

In the present study an extremely low tracer dose of radioactive technetium (less than 200 μCi) was employed and clear differences in uptakes between normal and inflamed proximal interphalangeal joints, when considered individually or as a mean

for each or both hands, were detected. In order to obtain adequate definition in technetium scintiphotography, large doses of radioactive technetium of the order of millicuries are necessary (McCarty et al., 1970a and b). This method affords the opportunity of defining localisation of radioactive technetium within the joint, but the high doses required severely limit the number of repeat observations possible. Although the advantage of definition within the joint is sacrificed with the present technique, nevertheless the very small doses of the isotope employed allow frequent repeat observations such as are required to follow anti-inflammatory drug effect without exposing either patient or physician to undue radiation hazard.

Repeat observations within twenty-four hours were associated with an acceptably high degree of reproducibility. Changes following one week's oral treatment with low dose corticosteroid therapy were clearly demonstrated when the means of the four proximal interphalangeal joints of each hand and the means of all eight proximal

interphalangeal joints were considered. That it is probably preferable to consider the mean values for either or both hands rather than for individual joints is suggested by the fact that changes were not demonstrable in two of the joints studied.

Changes were observed in joint circumference before and after corticosteroid therapy. These changes were significant when the means of the values for either hand or for the mean of both hands were considered. In only one individual joint, however, was the change significant. This finding accords with the suggestion that it is preferable to consider the mean values rather than the values for individual joints. Although no significant correlation was found between any of the clinical parameters of inflammation, joint circumference, or grip strength in individual joints or in mean values for either or both hands and the technetium uptake, there was a relationship ($r = 0.69$ $p < 0.05$) between the degree of change of both technetium uptake and joint circumference before and after corticosteroid

therapy. McCarty et al. (1970b) have shown a high correlation between scintiphotography and clinical assessment. The reason for the discrepancy between their results and those in the present study is not immediately apparent. These workers have also shown that isotope studies may be positive prior to clinical expression of disease. It is possible that the isotope studies are therefore more sensitive than is clinical assessment.

The difference in grip strength before and after corticosteroid therapy was not statistically significant. In this study, therefore, the changes exerted by corticosteroid therapy were most apparent in the isotope studies and less obviou either joint circumference or grip strength. It is possible that the technetium count rate provides a more sensitive index of change with anti-inflammator drug therapy than is afforded by joint circumference or grip strength.

SUMMARY

A simple radioisotopic method for the study of inflammation in the proximal interphalangeal joints in patients with rheumatoid arthritis is described. The tracer dose of radioactive technetium is extremely small permitting frequent repeat observations. The method is reproducible and shows differences between normal and inflamed joints. Elevated uptakes can be reduced by low dose corticosteroid therapy. The method may prove to be more sensitive than joint circumference and grip strength.

STUDIES ON THE RELATIONSHIP BETWEEN RADIOACTIVE TECHNETIUM COUNT RATE AND CLINICAL METHODS AS INDICES OF INFLAMMATORY ACTIVITY

Although the 99 mTc count rate is elevated in inflamed joints and may be reduced by anti-inflammatory therapy, the sensitivity of the method in relationship to currently employed methods of assessment remained to be established. Two studies were conducted to this purpose. The basic protocols were similar, each study comprisin a crossover clinical trial in which selected patients each received sequential courses of anti-inflammatory therapy of one week's duration. At the conclusion of each course of therapy a standard set of assessment methods were completed and the results by each method were compared. In the first pilot study the numbers studied were small and the assessor and patient were aware of the identity of the treatments. Well establishe drugs were employed. In the second study the number of patients studied was larger and the tri was double-blind. This study incorporated a newly introduced anti-inflammatory compound ibuprofen (Brufen : Boots Pure Drug Co. Ltd.).

THE EFFECTS OF INDOMETHACIN AND OF SALICYLATE THERAPY IN RHEUMATOID ARTHRITIS

Introduction

In this preliminary investigation the effects of indomethacin and of sodium salicylate upon patients with rheumatoid arthritis were monitored by standard clinical assessment methods in conjunction with both radioactive 133 Xe clearance rates and determination of 99 mTc count rate.

TABLE VIII

	Indomethacin	Sodium Salicylate	Placebo
Week 1	2	4	4
Week 2	4	3	3
Week 3	4	3	3

Legend: The number of patients receiving each treatmen
in each of the three periods.

MATERIALS AND METHODS

Ten patients with "classical" rheumatoid arthritis (Ropes, Bennett, Cobb, Jacox and Jessar, 1959) took part in a triple crossover clinical tri over a period of 3 weeks. Four patients were mal and the six female patients were post-menopausal. The age range was 39 to 58 years. Each patient volunteered to participate in the trial after explanation of its content and implications.

All patients received three successive treatments of a week each on sodium salicylate (Entrosalyl) 5 g. per day, indomethacin (Indocid) 100 mg. per day and lactose as placebo, all given as 4 divided doses in non-identical capsules. The order of treatments administered is summarised in Table 8. At the conclusion of each week's treatment the patients were all assessed by the same observer. Each patient was asked general and then specific questions designed to elicit both the efficacy and side effects of the previou week's treatment. An articular index (Ritchie et al., 1968) was performed, and measurements were made of grip strength (Wright, 1959) and of circumference of the finger joints using a millimetre gauge (Boardman and Hart, 1967).

One knee joint, selected for a study at the first assessment on the basis of severity of involvement, was then examined. A history of pain and of stiffness was elicited. The degree of tenderness, measured as the response to firm pressure over the joint margin was tested and the degree of joint swelling was also subjectively assessed. Grading for each of these 4 criteria was on a four point scale where 0 = absent, 1 = mild, 2 = moderate and 3 = severe.

A narrow bore needle was inserted into the joint cavity and as much synovial fluid as possible was withdrawn. Approximately 50 μCi of 133 Xe in 0.5 ml. of sterile sodium chloride were injected into the joint cavity and the needle withdrawn. The wound was sealed with adhesive. A detector incorporating a 1" x 1.1/2" thallium activated sodium iodide scintillation crystal and photo-multiplier (Ekco M5400A/1) was positioned over the joint. Immediately afterwards approximately 200 μCi of radioactive technetium as per-technetate in solution were injected into a vein in the antecubital fossa. The dose was standardised by the count rate per minute. A double channel counting system

allowed the separate monitoring of 133 Xe and 99 mTc through the same detector. Pulses from the detector were fed through 2 pulse height analysers (Ekco 5010), the settings of one being adjusted to the 99 mTc peak and the other to the 133 Xe peak. Counting was achieved through two ratemeters (Ekco M5183A) and, in the case of 99 mTc, a Kienzle printer. The 133 Xe counts were displayed linearly on graph paper by a direct writing pen recorder (Potentiometerschreiber, RE 511).

Counting commenced three minutes before the intravenous injection of 99 mTc and approximately two minutes after the intra-articular injection of 133 Xe and was continued for 30 to 45 minutes. The 133 Xe graph was sampled at one minute intervals and plotted on semilogarithmic graph paper as a function of time. A straight line was obtained in each case from which the half-time ($T\frac{1}{2}$) in minutes for the clearance of 133 Xe from the knee joint could be obtained following corrections for background and for overlap of the higher energy 99 mTc activity onto the 133 Xe channel. The 99 mTc print-out was also sampled at one minute

intervals and plotted linearly as a function of time. Monitoring was continued until a peak count rate for 99 mTc had been obtained. At the end of each study the count rate over the site of injection of 99 mTc was compared with that obtained over the equivalent area on the opposite arm. In any study in which a marked discrepancy was found, indicating faulty injection technique, the results from that patient were rejected. Four studies were unsuitable for analysis for this reason.

Patients were asked at the end of the three weeks of the trial to give a single preference for one of the three treatments.

TABLE IX

Patient	Articular Index			Knee score			Joint sizes (mm.)						Grip strength (mm.Hg)						^{133}Xe Clearance $T_{\frac{1}{2}}$ (mins)			99 mTc peak counts/min.		
	I	S	P	I	S	P	I		S		P		I		S		P		I	S	P	I	S	P
							L	R	L	R	L	R	L	R	L	R	L	R						
1	28	30	46	3	4	10	48	42	44	44	65	60	80	60	90	65	50	30	39	23	18	12,700	18,000	16,500
2	5	3	28	6	7	8	46	48	40	41	49	52	60	46	30	40	60	40	23	38	33	8,700	6,500	19,300
3	13	66	62	2	11	10	48	42	62	60	60	58	50	80	20	40	10	30	60	26	22	12,600	47,000	46,000
4	30	14	38	9	5	11	60	50	45	42	55	50	110	80	70	90	60	100	50	68	36	9,400	6,200	11,500
5	17	3	29	3	1	6	40	46	42	44	42	44	70	80	60	80	40	50	49	50	53	13,600	12,200	32,000
6	20	33	40	4	9	11	48	53	48	53	46	55	75	60	45	80	65	70	60	28	26	28,500	33,000	45,500
7	18	28	38	8	8	8	46	52	40	50	49	58	65	20	80	30	90	30	18	20	22	22,000	26,000	30,000
8	13	2	31	6	1	9	36	38	36	44	38	42	90	120	110	100	40	70	25	20	19	17,300	15,600	24,500
9	56	50	60	7	6	9	55	58	63	65	61	63	15	15	20	35	10	15	34	30	18	17,400	16,300	18,000
10	15	28	42	5	8	10	58	46	62	42	46	44	90	110	60	55	45	55	28	26	20	12,400	31,300	43,800

Key: I = Indomethacin; S = Salicylate; P = Placebo; L = Left; R = Right.

TABLE X

Patient	Articular index	Knee score	Joint size	Grip strength	^{133}Xe T½	99 mTc peak count	Consistency of assessment methods	Patient's preference
1	I	I	I = S	S	I	I	4/6 best on I	I
2	S	I	S	I	S	S	4/6 best on S	S
3	I	I	I	I	I	I	6/6 best on I	I
4	S	S	S	I	S	S	5/6 best on S	S
5	S	S	I=S=P	I	I=S=P	S	Inconsistent	No decision
6	I	I	I=S=P	Opposite results each hand	I	I	4/6 best on I	I
7	I	I=S=P	S	P	P	I	Inconsistent	P
8	S	S	I	S	I	S	4/6 best on S	S
9	S	S	I	S	I	S	4/6 best on S	S
10	I	I	P	I	I	I	5/6 best on I	I

Key: I = Indomethacin; S = Salicylate; P = Placebo

THE CONSISTENCY OF THE RESULTS OBTAINED BY EACH METHOD OF ASSESSMENT IN EACH PATIENT AND THE PATIENTS' OVERALL PREFERENCES.

RESULTS

The individual values obtained on each treatment are shown in Table 9. This trial was in part an attempt to compare different methods of assessing the effect of treatment and Table 10 shows how consistent were the 6 techniques employed in each patient. In one patient (No.3) all 6 methods indicated that the same treatment was best. In two patients 5 of the 6 methods gave the same result, whilst the results of 4 of the 6 methods coincided in 5 patients. Thus in 8 out of 10 patients at least 4 of the 6 assessment techniqu gave the same answer. Of the individual methods, articular index and the radiotechnetium test corresponded to this answer in all 8 patients, whilst the xenon clearance test differed from this in 2 and joint sizes only corresponded in 3 of the 8 patients.

Also shown in Table 10 are the decisions which each patient made as to the single most effective treatment. In 8 of the 10 patients this decision reflected the majority preference indicated by the assessment methods. One patient (No.7) selected placebo as the single most effective treatment and in that patient the assessment methods were totally

TABLE XI

Dummy Variable	VALUES ASSIGNED WITH EACH DRUG GIVEN			
	Salicylate	Indomethacin	Placebo	Comparison te
d_1	+ 1	- 1	0	Salicylate a; indomethaci
d_2	- 1	- 1	+ 2	

Legend: Values assigned to the Dummy Variables used in the analysis of covariance.

discordant. In the final patient (No.5) who was unable to select a single most effective treatment, 3 out of the 6 methods of assessment suggested that salicylate was best. These were the articular index, knee score and peak 99 mTc count rate.

The results were further analysed statistically. Each of the 6 methods of assessment was considered separately, using analysis of variance techniques to decide whether there were significant differences in value of any of the methods when patients were given the 3 treatments. As shown in Table 8, the triple crossover design in 10 patients did not produce a balanced design due in part to rejection of results of 99 mTc studies and 2 patients started on indomethacin whilst 4 each started on salicylate and placebo. The statistical method used took account of this imbalance in the design. Dummy variables (Quenouille, 1953) were used to test the treatment effects of salicylate against indomethacin (d_1) and placebo against active drugs (d_2). These two comparisons were chosen to be orthogonal and form together the total variance between treatments. Table 11 describes the values assigned to d_1 and d_2.

TABLE XII

SOURCE	D.F.	SUM OF SQUARES				
		$(A\ I)^2$	$A\ I \times d_1$	d_1^2	$A\ I \times d_2$	d_2^2
Patients	9	5,073	0	0	0	0
Periods	2	179	4.1	0.6	4.1	0.6
Residual	18	4,031	37.9	19.4	351.9	59.4
Total	29	9,283	42.0	20.0	356.0	60.0

Source	D.F.	Sum of Squares	Mean Square	F-Ratio	Significance
Due to d_1	1	74.0	74	0.6	N.S.
Due to d_2	1	2,083.2	2,083	17.8	$p < 0.001$
New Residual	16	1,874.0	117		
Old Residual	18	4,031			

AN EXAMPLE OF THE ANALYSIS EMPLOYED. FIGURES RELATE TO VALUES OBTAINED WITH THE ARTICULAR INDEX (A I)

TABLE XIII

	Corrected Mean Scores			Significance Tests			
	Indomethacin	Salicylate	Placebo	Placebo v Active Drugs		Indomethacin v Salicylate	
				t_{16}	P	t_{16}	P
Articular index	21.6	25.5	41.3	4.2	< 0.001	0.8	$\simeq 0.4$
Knee score	5.3	6.0	9.2	3.7	< 0.005	0.5	$\simeq 0.6$
Joint size	48.6	48.0	51.6	1.7	$\simeq 0.1$	0.2	$\simeq 0.8$
Grip strength	68.2	60.1	48.2	3.0	< 0.01	1.3	$\simeq 0.2$
Xenon T½	38.2	33.0	26.9	2.1	$\simeq 0.05$	1.0	$\simeq 0.3$
99mTc peak counts	15,400	21,200	28,700	3.5	< 0.005	1.7	$\simeq 0.1$

CORRECTED MEAN VALUES OBTAINED WITH EACH METHOD OF ASSESSMENT, WHILE THE PATIENT RECEIVED INDOMETHACIN, SALICYLATE AND PLACEBO. SIGNIFICANCE TESTED USING STUDENT'S t WITH 16 DEGREES OF FREEDOM (t_{16}).

Analysis of covariance were performed separately for each of the 6 assessment criteria using the dummy variables and separating out the effects due to patient variation and to period effect leaving a residual variation measuring treatment effect alone. Regression coefficients measuring d_1 and d_2 were determined and their significance tested in the analysis of variance. A two-tailed significance level was assigned to each drug comparison by using the square root of the F-ratios obtained in the regression analysis and entering tables of Student's t-test with 16 degrees of freedom. Table 12 shows an example of one analysis using the figures for articular index.

Table 13 shows the significance levels obtained by these analysis. Also shown in Table 13 are the overall mean values for each assessment method, corrected for period effect by the use of the relevant regression coefficients. It can be seen that for the comparison of placebo versus active drugs, all the parameters except joint size showed a significant improvement in favour of the active drugs. This was more marked for the measurements of articular index ($p < 0.001$), knee score and

TABLE XIV

	Mean differences		σ^2	$\sqrt{\frac{2\sigma^2}{10}}$	Significance Tests			
	P - I	P - S			t_{16} P - I	p	t_{16} P - S	p
Articular index	19.7	15.8	117	4.8	4.1	< 0.001	3.3	< 0.005
Knee score	3.9	3.2	6.2	1.11	3.5	< 0.005	2.9	$\simeq 0.01$
Joint size	3.0	3.6	24.7	2.23	1.3	$\simeq 0.2$	1.6	< 0.2
Grip strength	20.0	11.9	187	6.1	3.3	< 0.005	2.0	< 0.1
Xenon $T'_{\frac{1}{2}}$	11.3	6.1	120	4.9	2.3	< 0.05	1.2	< 0.3
99mTc peak counts	13,300	7,500	58,350,000	3,420	3.9	< 0.005	2.2	< 0.05

Key: P = Placebo; S = Salicylate; I = Indomethacin.

MEAN DIFFERENCES & SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE VALUES OBTAINED FOR EACH ASSESSMENT METHOD WHEN EACH ACTIVE DRUG WAS COMPARED INDIVIDUALLY WITH PLACEBO.

technetium peak count ($p < 0.005$), whilst grip strength reached a significance of $p < 0.01$ and xenon clearance times reached $p < 0.05$. The tests of indomethacin versus salicylate did not reach conventional statistical significance for any of the parameters, although the technetium peak count came near to this at $p = 0.1$. With the exception of joint size, however, the mean scores for all the methods on indomethacin treatment were better than those on salicylate.

Further analysis were made to compare placebo against each of the two active drugs individually. This was done by forming a set of t-tests derived from the residual squares (σ^2) in each analysis of covariance, using the value $\frac{2\sigma^2}{n}$ as the standard error of the difference between the means, where n = the number of patients on which each mean score was based. Table 14 shows these results. Indomethacin was shown to be significantly better than placebo by all the methods except joint size with xenon clearance times the least significant of the five. Salicylate was better than placebo according to articular index, knee score and technetium peak count, but no significant difference was

shown using joint size, grip strength or xenon clearance times.

DISCUSSION

In the present clinical trial the results obtained by two new methods of assessment were studied in conjunction with previously established methods. The results clearly show that the 133 Xe clearance method offers at best only poor discrimination when compared with standard clinical methods, as has been noted previously (Chapter 6). Thus, although a difference at the 5% level of significance was demonstrable between placebo and active drug therapy by this technique, the standard methods articular index, knee score and grip strength distinguished the effects of treatment at the 1% level or better. Since the xenon clearance time reflects synovial tissue perfusion alone (Chapter 2)which is only one feature of the total inflammatory response, this result is not altogether surprising.

The change in joint circumference provided even poorer discrimination than did the results of the xenon studies. Although joint size has been shown to change with adequate anti-inflammatory therapy by some workers (Boardman and Hart, 1967), alteration attributable to drug effect has been less obvious in

other clinical trials (Pitkeathley, Baherjee, Harris and Sharp, 1966). There are many possible explanations for this discrepancy. Most of the joint swelling in the patients in this study represented irreversible change and probably could not be modified by short term anti-inflammatory therapy.

On the other hand, the simple determination of peak count rate following an intravenous injection of a measured dose of radioactive technetium seems to offer an index which more closely mirrors clinical change. Thus, the same order of levels of significance achieved when active therapy was compared with placebo by means of the articular index ($p < 0.001$), knee score ($p < 0.005$) and grip strength ($p < 0.01$) was also seen with the technetium peak count rate ($p < 0.005$). Furthermore, the technetium peak count rate gave the same treatment preference as did the articular index for all 10 patients and both these measures corresponded to the majority trend in the 8 patients showing consistent results (Table 10), whilst the xenon clearance test fell short of this.

In 8 of the 10 patients the result by the radio-technetium peak count rate agreed with the patients'

own treatment preference. Criticism may be levelled at the use of patient preference in a trial which is not blind, but the high level of agreement of the patients' choice with both subjective and objective measurements in this study provides some support for its inclusion.

The mean scores for technetium peak count, articular index, knee score and grip strength all showed better values on indomethacin than on salicylate, and although none of the parameters showed a statistically significant difference the nearest approach to this was the technetium peak count ($p = 0.1$). Although most authorities consider indomethacin and aspirin in these doses to be roughly equipotent (Sunshine, Laska, Meisner and Morgan, 1964 : Pitkeathly and others, 1966 : Pinals and Frank, 1967) there is some evidence to suggest that indomethacin exerts a more powerful anti-inflammatory effect than aspirin (Gaspardy, Gaspardy Balint and Kemeny, 1966). The trend of the findings in the present study agree with this.

The results of this small but objective study therefore show that the measurement of peak count

rate of radioactive technetium is capable of demonstrating anti-inflammatory effect in a short term clinical trial of non-steroidal anti-inflammatory drugs. The direction and the degree of change of this measurement compare favourably with results obtained by other clinical methods.

SUMMARY

Ten patients with "classical" rheumatoid arthritis entered a triple crossover clinical trial of anti-inflammatory treatment using sodium salicylate 5 g. per day, indomethacin 100 mg. per day and placebo, each treatment being administered for one week. Six methods of assessment were employed including an articular index, joint size, grip strength, a knee score and two new methods of assessment derived from isotope studies. Peak uptake by the knee following intravehous administration of radioactive technetium and the half life of intra-articularly injected xenon were determined each week. The articular index, grip strength, knee score and radiotechnetium peak count rate discriminated change in the inflammatory process as a result of treatment better than did joint size or xenon clearance measurements.

A STUDY ON THE EFFECTS OF ANTI-INFLAMMATORY DRUG THERAPY IN RHEUMATOID ARTHRITIS: THE RELATIVE EFFICACY OF SEVEN METHODS OF ASSESSMENT

Introduction

In this study both subjective and objective methods of assessment were employed in a double-blind four week crossover trial of placebo, aspirin, prednisolone and a newly introduced anti-inflammatory compound, Ibuprofen (Brufen, Boots Pure Drug Co. Ltd.) in order to derive information on both the clinical efficacy of the drugs and the relative efficiency of the methods of assessment.

MATERIALS AND METHODS

Patients Studied

Twenty-four patients with "classical" or "definite" rheumatoid arthritis (Ropes et al., 1959) entered the trial. The mean age was 47.7 years (range 18 to 67 years) and eleven were female.

Each patient received aspirin ("Entrosalyl", Cox-Continental Ltd., 4 g. per day), ibuprofen ("Brufen", Boots Pure Drug Co. Ltd., 1.2 g. per day) prednisolone (10 mg. per day) and lactose as placebo The order of treatment was randomised and the compounds were administered, orally, in identical capsules, for one week each.

At the conclusion of each week's treatment the patients were reviewed. An articular index (Ritchie et al., 1968) which provides a measure of joint tenderness and which correlates with the articular index of the Co-operating Clinics of the American Rheumatism Association was performed. This index takes only two to three minutes to perform and both the inter- and the intra-observer errors have been determined. Although the inter-observer error is large, precluding the comparison

of results obtained by different observers, the intra-observer error is only 1.2 score units.

One knee joint, selected for study at the first assessment on the basis of severity of involvement, was then examined. The joint margin was subjected to firm pressure and the response graded on a 0 to 3 basis where 0 = absent, 1 = slight, 2 = moderate and 3 = severe.

The circumference of the proximal inter-phalangeal joints and the inter-phalangeal joint of the thumb in the right hand were measured using the Geigy apparatus, the totals for all five joints being recorded. Grip strength (sum of three readings) in the right hand was determined using a modified sphygmomanometer cuff and baseline level of +30 mmHg.

The patient then lay comfortably and the knee joint which had previously been examined was exposed. Employing full aseptic precautions 0.5 ml. of a solution of radioactive xenon (133 Xe) in sterile 0.9% NaCl (Amersham, England) was carefully injected into the joint cavity. Immediately thereafter approximately 200 μCi (standardised by count rate) of radioactive technetium (99 mTc) were injected

intravenously. A lightly collimated thallium activated sodium iodide crystal and photomultiplier (Ecko M5400A/1) positioned vertically above and 2" from the patella conducted the pulses through two pulse height analysers (Ecko M5010) which were set at the counting conditions for 133 Xe and 99 mTc respectively. Recording was accomplished through two ratemeters (Ecko M5183A) and the 133 Xe counts were displayed upon a Servoscriber RE511 chart recorder, the 99 mTc counts being displayed on a Kunzle digital print-out. The counts were corrected for background (of the order of 1% of count rate) and overlap (of the order of 18%) and the 15 minute count rate for 99 mTc, and the half-life ($T\frac{1}{2}$ value, minutes) of the semilogarithmic plot of the 133 Xe were determined.

At the conclusion of these studies the patients were asked to assess numerically their clinical status the previous week. The grades allowed were 0, 1, 2, 3 and 4 ranging from 0 = excellent to 4 = very bad.

TABLE XV

Clinical and laboratory data after one week's therapy with placebo, ibuprofen, aspirin and prednisolone

(Mean ± SEM)

	Placebo	Ibuprofen	Aspirin	Prednisolone
General assessment	3.79 ± 0.09	1.96 ± 0.25	1.75 ± 0.12	0.79 ± 0.23
Articular index	40.67 ± 2.65	30.71 ± 2.66	26.63 ± 1.95	17.63 ± 1.64
Knee tenderness	2.83 ± 0.08	1.96 ± 0.14	1.42 ± 0.12	0.46 ± 0.12
Joint circumference (mm)	294.76 ± 5.09	288.24 ± 4.52	286.56 ± 5.03	279.52 ± 4.85
Grip strength (mm/Hg)	148.96 ± 23.45	156.76 ± 21.55	156.76 ± 22.18	212.88 ± 27.81
^{99m}Tc Uptake (c per min)	32,333.00 ± 5,294.65	33,250.00 ± 5,020.79	24,500.00 ± 6,523.04	15,983.00 ± 4,183.14
Xe^{133} Disappearance rate (min)	29.1 ± 2.84	31.0 ± 5.5	32.9 ± 4.8	48.0 ± 5.13

TABLE XVI

Results of Statistical Analyses (Student's t test on paired samples)

	Placebo v ibuprofen	Placebo v aspirin	Placebo v prednisolone
General assessment	t = 7.6949 P <0.001	t = 13.8804 P <0.001	t = 20.1759 P <0.001
Articular index	t = 2.9347 P <0.05	t = 6.2717 P <0.001	t = 8.6191 P <0.001
Knee tenderness	t = 6.3069 P <0.001	t = 9.6757 P <0.001	t = 16.3658 P <0.001
Joint circumference	Not significant	Not significant	t = 4.6791 P <0.001
Grip strength	Not significant	Not significant	t = 4.4830 P <0.001
Xe^{133} disappearance rate (min)	Not significant	Not significant	t = 3.4890 P <0.05
^{99m}Tc uptake	Not significant	Not significant	t = 8.0396 P <0.001

RESULTS

The results are shown in Tables 15 and 16.

The mean value of the patients' score for general assessment while on placebo (3.79 ± 0.09) was significantly higher ($p < 0.001$) than while on ibuprofen (1.96 ± 0.25), aspirin (1.75 ± 0.12) and prednisolone (0.79 ± 0.23). There was no significant difference in the mean scores while on ibuprofen and aspirin, but the mean score while on prednisolone was significantly lower ($p < 0.001$) than the mean scores while on ibuprofen and aspirin.

The mean value obtained for the articular index while the patients received placebo (40.7 ± 2.65) was significantly higher ($p < 0.05$) than the mean values obtained with ibuprofen (30.7 ± 2.66) and also ($p < 0.001$) with aspirin (26.6 ± 1.95) and prednisolone (17.6 ± 1.64). The values obtained on prednisolone differed significantly from the values obtained on aspirin ($p < 0.01$) and on ibuprofen ($p < 0.001$) but there was no significant difference between the values obtained on ibuprofen and on aspirin.

Similar results were obtained with the knee tenderness score. The mean value on placebo (2.8 ± 0.08) was significantly higher ($p < 0.001$) than th values obtained on ibuprofen (1.9 ± 0.14), aspirin (1.4 ± 0.12) and prednisolone (0.46 ± 0.12). The values obtained on prednisolone were significantly lower than those obtained on aspirin ($p < 0.01$) or ibuprofen ($p < 0.05$) and the values obtained on aspirin were significantly lower ($p < 0.05$) than on ibuprofen.

The values for grip strength on prednisolone differed significantly ($p < 0.001$) from the values obtained on aspirin, ibuprofen and placebo. Howeve there was no significant difference between the values on aspirin and on ibuprofen, or between the values on either drug and on placebo.

The values obtained for joint circumference while the patients received placebo were significan higher ($p < 0.001$) than the corresponding values obtained during the period of prednisolone treatmen However, there were no significant differences between the values obtained while the patients

received either aspirin or ibuprofen and either the placebo values or the prednisolone values.

There was a significant difference ($p < 0.05$) between the $T\frac{1}{2}$ values of the 133 Xe clearance rates obtained during placebo treatment and during prednisolone treatment. However, there were no significant differences between the $T\frac{1}{2}$ values on either aspirin or ibuprofen and the placebo values.

The mean value for 99 mTc count rate obtained on placebo therapy was significantly higher than the mean value obtained on prednisolone ($p < 0.001$) and aspirin ($p < 0.05$) but was not significantly different from the value obtained on ibuprofen. The difference between the ibuprofen and aspirin values was not significant.

DISCUSSION

This study was designed to provide comparative information on two inter-related topics, namely, the efficacy of the drugs administered and the reliability of the methods of assessment employed. The form of the study was a double-blind crossover short term clinical trial. The incorporation of a period of placebo administration has been criticised on ethical grounds. The minimal requirement in this situation is freely given consent by the inform patient. It is then the responsibility of the trialist to provide adequate and reliable informatio upon which the clinician can base a valid conclusion on the efficacy of the compounds studied. The inclusion of a period of placebo administration permits some degree of standardisation and in addition safeguards against the result: drug x = drug y = nothing. A great deal of knowledge is now available on the placebo reaction as a consequence of the meticulously controlled work of such workers as Beecher (1959), Lasagne (1955), Joyce (1961) and others. The placebo reaction is an unwelcome but inevitable feature of

drug trials and, in my opinion, renders the incorporation of a period of placebo administration mandatory. A further problem encountered in short term clinical trials is that of "carry over" effect of one compound into the succeeding assessment period. Assessment at the conclusion of a full seven days' therapy affords some protection against this effect which is further reduced by random allocation to treatments. This problem however is only minimised in this way and can neither be eliminated nor even quantitated without the aid of mathematical methods requiring the assistance of computer analysis.

The results of this study clearly show the effec of low dose corticosteroid therapy in rheumatoid arthritis. The difference between the placebo and the prednisolone values is significant by any of the methods of assessment, thus underlining the relative unimportance of the method of assessment employed in the evaluation of a drug of major effect. The biological difference obtained is large enough to counteract a large degree of insensitivity or lack of specificity in the monitoring system employed.

The situation, however, is rather different when the results obtained with placebo, salicylate and ibuprofen are considered. The difference between placebo and the two active compounds is indicated by the significantly lower values obtained for general assessment, articular index and knee tenderness. It is noteworthy that the values obtained by all three methods while the patients were receiving salicylate were lower than were the corresponding values obtained for ibuprofen. With only knee tenderness however were the differences significant. The count rate of 99 mTc is significantly higher on both ibuprofen and placebo, than on salicylate therapy. However, three factors may have contributed to this result: the method may not be sensitive enough to detect small changes; the number studied may have been too small; or the compound may indeed be less effective than aspirin as has been suggested previously (Boardman et al., 1967).

The measurement of joint circumference, grip strength or 133 Xe clearance rate were not significantly different while the patients were receiving placebo, salicylate or ibuprofen,

although in all cases the results showed a tendency to improvement with the active drugs. That the 133 Xe clearance rate, changes less with salicylate than with either indomethacin or corticosteroid therapy has already been noted (Chapter 6). Failure of joint circumference and grip strength to change significantly during adequate anti-inflammatory therapy may be related to the presence of irreversible changes in the rheumatoid hand. However, since these methods cannot be relied upon to show changes with standard treatments in rheumatoid arthritis, it is difficult to justify their retention in the assessment armamentarium in short term studies of non-steroidal anti-inflammatory compounds.

Certain conclusions are suggested by the results of this study. Prednisolone therapy can be shown to be effective in the treatment of rheumatoid arthritis by any method of assessment. Objective, semi-objective and subjective assessment methods are available to demonstrate the efficacy of salicylate therapy. That ibuprofen is effective in the treatment of

rheumatoid arthritis is suggested by subjective and semi-objective methods of assessment. However, consideration of the biological significance of all of the results suggests that it is less effective than high dose salicylate therapy. It is possible that the determination of 99 mTc count rate and of joint tenderness best demonstrate this point. The methods of assessment, joint circumference, grip strength and 133 Xe clearance rate cannot be relied upon to demonstrate anti-inflammatory effect on non-steroidal compounds and are less sensitive than the 99 mTc count rate, articular index and joint tenderness and the patient's own general assessment of joint pain. These conclusions are in accord with those obtained in the smaller pilot study previously reported. Clearly, the precise value of 99 mTc studies as routinely employed indices of anti-inflammatory effect must await confirmation of these results by other laboratories. However, the conclusions suggested by the studies reported in this thesis are promising.

SUMMARY

In a quadruple crossover double-blind clinical trial of prednisolone, salicylate, ibuprofen amd placebo involving twenty-four patients, six methods of assessment in addition to patient preference were employed concurrently.

The results suggest that the order of anti-inflammatory potency of these treatment periods was prednisolone aspirin ibuprofen placebo and further suggest that the discriminatory power of the methods articular index, knee tenderness and 99 mTc count rate exceeded that of grip strength, joint circumference or 133 Xe clearance rate.

RADIOACTIVE TECHNETIUM COUNT RATE IN ARTHRITIS RELATED TO THE CLINICAL DEGREE OF INVOLVEMENT AT THE TIME OF STUDY AND TO THE 133 Xe CLEARANCE RATE

Introduction

In this chapter studies have previously been reported on the relationship between clinical findings and the 133 Xe clearance rate and between clinical findings and the 99 mTc count rate over the joint at 15 minutes following intravenous injection of technetium.

In the present study the relationship between the 99 mTc count rate and clinical assessment of the joint at the time of study was investigated in larger numbers of patients (group 1) with osteoarthritis and with rheumatoid arthritis, some of whom had been subjected to the operation of anterior knee synovectomy. The 99 mTc count rate at 15 minutes in a large number of normal subjects was determined for comparison with the disease groups.

In a further group of patients (group 2) with rheumatoid arthritis the relationships between clinical assessment of the joint and concurrent estimations of 99 mTc count rate and 133 Xe clearance rate were also investigated.

MATERIALS AND METHODS

GROUP 1

Patients Studied

131 knee joints of 66 patients with "classical" or "definite" rheumatoid arthritis (Ropes et al., 1959) were studied. 54 were female and the mean age of these patients was 51.4 years (range 17 to 71 years). 26 of these patients had previously been subjected to the operation of anterior knee synovectomy at varying time intervals (mean 32.8 months - range 8 to 61 months) prior to these studies

38 studies were conducted on the knee joints of 19 patients with osteoarthritis. All exhibited both clinical and radiological evidence of involvement of the relevant joint and serological tests for rheumatoid factor were negative. 16 of these patients were female and their mean age was 65.8 years (range 54 to 92 years).

All of these patients were selected from the Outpatient Clinic at the Centre for Rheumatic Diseases, Glasgow, on the basis of certainty of diagnostic category, availability for study and freely given consent by the patient. No selection

on the basis of severity of involvement was imposed and these studies were conducted between December, 1969 and April, 1970.

The results of these studies were compared with the results of 20 identical studies performed in volunteer hospital personnel and in medical students. None of these subjects had either past or present clinical evidence of involvement of the joint in any traumatic or inflammatory disease process. Fourteen of these subjects were female and their mean age was 29 years (range 18 to 48 years). Both knee joints of each subject were studied.

Repeat studies were conducted at 24 hours in 9 normal subjects and 11 patients with rheumatoid arthritis and within 30 minutes in 3 normal subjects and 5 patients with rheumatoid arthritis to determine the reproducibility of the method.

Clinical score

Clinical knee assessments ("clinical score") were conducted by the same physician prior to each isotope study. A history of pain and of stiffness was elicited and graded. The knee joint margin was subjected to firm pressure and the response

graded. An assessment of soft tissue swelling (whether due to fluid or to synovial hypertrophy) was graded. Grading was on a 0 to 3 basis where 0 = absent, 1 = slight, 2 = moderate and 3 = severe. The maximum total score for an individual joint was therefore +12.

Technetium Studies

The isotope was administered intravenously, the dose being standardised by count rate (10,000 counts per minute ± 2% at 16 inches from the detector = 50 μCi) and the count rate over the joint was monitored by a thallium activated sodium iodide scintillation crystal and photo-multiplier (Ekco M.5400A/1) placed vertically above and 1/4" from the skin over the patella. Pulses were fed through a pulse height analyser and ratemeter (Ekco M.5183A) and displayed on a Kunzle digital print-out. Monitoring commenced two minutes after injection and was continued for 15 minutes. The count rate was recorded at that time in all studies and expressed as a percentage of the administered dose following corrections for counting geometry and background (2% count rate). At the conclusion of each study the count rate over

the site of injection of 99 mTc was monitored and compared with the count rate on the same area of the opposite arm to exclude extravascular deposition of some of the dose (page 281).

Repeat Studies

In 9 normal subjects and in 11 patients with rheumatoid arthritis identical studies were performed on a second occasion 24 hours later. In a further three normal subjects and five patients with rheumatoid arthritis, immediately following the first isotope study a further dose of approximately 250 μCi was injected intravenously and the count rate again monitored for 15 minutes. The second dose was also standardised by count rate (50,000 counts per minute ± 2% at 16 inches from the detector) and therefore both the first and the second 15 minute count rates over the joint could be related to the count rate administered. For comparison, the second readings were converted to an equivalent of 10,000 counts per minute at 16 inches.

GROUP 2

Patients Studied

A further group of 16 females and 6 male patients with "classical" rheumatoid arthritis (Ropes et al., 1959) were studied. Their ages ranged from 36 to 62 years with a mean of 45.7 years. Each was carefully selected for study on the basis of severity of involvement in addition to the criteria applied in selecting the first group and consequently the period of time required for their collection was longer (January, 1969 to August, 1969). Clinical assessment was performed by the same physician prior to each study in the manner previously described.

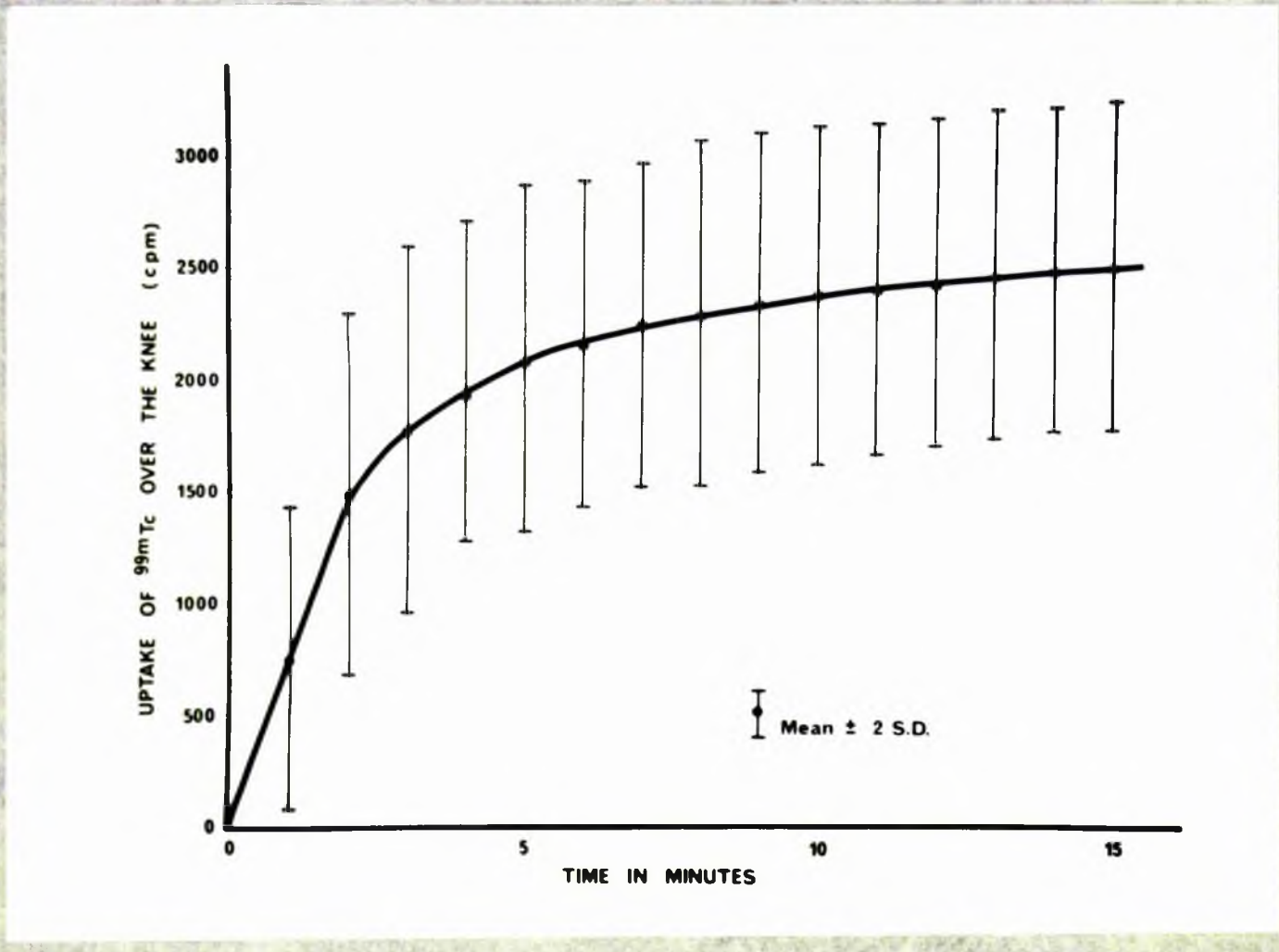
Concurrent 133 Xe and 99 mTc Studies

Immediately following the performance of the clinical knee score the patient was positioned lying supine with the knee joint exposed and extended. Employing full aseptic precautions a narrow gauge needle was inserted into the joint cavity by a medial infra-patellar approach and any free fluid present was aspirated. A sterile airfree solution of 133 Xe in 0.5 ml. of sterile

0.9% NaCl was slowly injected into the joint cavity. The needle was withdrawn and the wound sealed with adhesive tape.

The detector was positioned vertically above and 3 inches from the skin over the patella. The 99 mTc solution (50,000 counts per minute $\pm$ 1% at 12 inches from the detector $\simeq$ 400 μCi) was injected intravenously and the 99 mTc count rate was monitored for 15 minutes through the system previously described. The 133 Xe pulses were conducted through a second pulse height analyser and ratemeter (Ekco M.5010) suitably adjusted to the counting conditions for 133 Xe and were displayed on a direct writing pen recorder (Smith's Servoscribe 2).

After corrections for background (1% count rate) isotope decay and overlap of the higher energy 99 mTc onto the 133 Xe channel, the 99 mTc counts were plotted linearly against time and the 15 minute count rate was recorded while the 133 Xe counts were plotted semilogarithmically against time and the $T\frac{1}{2}$ values were obtained from these graphs.



Legend to Fig.7

The mean and 2 standard deviations fron the mean of the results obtained in all the normal subjects.

TABLE XVII REPRODUCIBILITY OF ^{99m}Tc UPTAKES IN NORMAL SUBJECTS AND RHEUMATOID ARTHRITIS PATIENTS

		^{99m}Tc Uptake (CPM) over the joint on:-			
		Two Successive Days		Same Day	
		I	II	I	II
Normal Subjects:	1	2397	2538	2645	2807
	2	3002	2690	3118	2997
	3	2662	2787	2451	2398
	4	2205	2531		
	5	2811	2658		
	6	1981	2071		
	7	2755	2419		
	8	2862	2607		
	9	2917	2859		
S.E. of the difference		36		32	
Rheumatoid Arthritis:	1	3592	3702	3929	4112
	2	3820	3679	3381	3458
	3	4689	4938	4129	4019
	4	2870	3158	2877	2963
	5	3522	3851	3788	3515
	6	2932	3222		
	7	4135	4419		
	8	3868	3528		
	9	4218	4538		
	10	3961	3611		
	11	4875	4588		
S.E. of the difference		24		37	

TABLE XVIII

CLINICAL SCORE MEAN ± S.E.M.

Subjects	Number	Pain	Tenderness	Swelling	Stiffness	Total	^{99m}Tc Uptake ($X10^{-2}$ per cent dose) at 15 mins. Mean ± S.E.M.
Normal subjects	40	-	-	-	-	-	57.8 ± 1.3
Osteoarthritis	38	1.75 ± 0.18	1.68 ± 0.20	1.15 ± 0.15	0.63 ± 0.12	5.2 ± 0.5	63.5 ± 2.3
Rheumatoid arthritis (unoperated)	80	1.78 ± 0.12	1.66 ± 0.13	1.08 ± 0.10	0.65 ± 0.08	5.17 ± 0.34	90.6 ± 3.2
Rheumatoid arthritis (synovectomised)	51	1.12 ± 0.16	1.04 ± 0.16	1.00 ± 0.12	0.72 ± 0.10	3.9 ± 0.42	78.0 ± 3.7

STATISTICAL ANALYSIS

Total Clinical Score

Osteoarthritis v Rheumatoid Arthritis (unoperated) - $t = 0.04$ $p > 0.1$

Osteoarthritis v Rheumatoid Arthritis (synovectomised) - $t = 1.91$ $0.1 > p > 0.05$

Rheumatoid Arthritis (unoperated) v Rheumatoid Arthritis (synovectomised) - $t = 2.28$ $0.05 > p > 0.01$

^{99m}Tc Uptake

Normal subjects v Osteoarthritis - $t = 1.98$ $0.1 > p > 0.05$

Normal subjects v Rheumatoid Arthritis (unoperated) - $t = 5.28$ $0.001 > p$

Normal subjects v Rheumatoid Arthritis (synovectomised) - $t = 3.59$ $0.001 > p$

Osteoarthritis v Rheumatoid Arthritis (unoperated) - $t = 5.52$ $0.001 > p$

Osteoarthritis v Rheumatoid Arthritis (synovectomised) - $t = 3.08$ $0.01 > p > 0.001$

Rheumatoid Arthritis (unoperated) v Rheumatoid Arthritis (synovectomised) - $t = 2.52$ $0.05 > p > 0.01$

RESULTS

The results are shown in Fig. 7 and Tables 17 to 20.

The mean ± 2 standard deviations from the mean 99 mTc count rate at increasing times after injection are shown in Fig. 7. Table 17 shows the paired values obtained on the same and on successive days in normal subjects and in patients with rheumatoid arthritis.

The mean and S.E.M. of the 99 mTc uptake in the different disease groups studied, together with the values obtained in normal subjects are shown in Table 18. The values in synovectomised (mean 78.0 ± S.E.M. 3.7) and in unoperated patients with rheumatoid arthritis (mean 90.6 ± S.E.M. 3.2) were significantly higher than the values obtained either in patients with osteoarthritis (mean 63.5 ± S.E.M. 2.3) or in normal subjects (mean 57.8 ± S.E.M. 1.3). There was no significant difference between the values in normal subjects and in osteoarthritis, but the values obtained in synovectomised patients were significantly lower than were the values obtained in unoperated patients with rheumatoid arthritis.

TABLE XIX - CORRELATION BETWEEN CLINICAL FINDINGS AND ^{99m}Tc COUNT RATE (Group 1)

	^{99m}Tc Count Rate at 15 mins v				
	Pain	Tenderness	Swelling	Stiffness	Total
Osteoarthritis	r = 0.43 0.01>p>0.001	r = 0.28 0.1>p>0.05	r = 0.36 0.05>p>0.01	r = 0.24 p > 0.1	r = 0.43 0.01>p>0.001
Rheumatoid Arthritis (unoperated)	r = 0.35 0.01>p>0.001	r = 0.48 0.001>p	r = 0.53 0.001>p	r = 0.24 0.05>p>0.01	r = 0.52 * 0.001>p
Rheumatoid Arthritis (synovectomised)	r = 0.39 0.01>p>0.001	r = 0.16 p>0.1	r = 0.39 0.01>p>0.001	r = 0.06 p>0.1	r = 0.34 0.05>p>0.01

* r (^{99m}Tc count rate v total score in excess of 6)

= 0.34 (0.05<p<0.01 N = 36).

The values obtained for total clinical score in both osteoarthritis (mean 5.2 ± S.E.M. 0.51) and unoperated patients with rheumatoid arthritis (mean 5.17 ± S.E.M. 0.34) were significantly higher than the values obtained in synovectomised patients with rheumatoid arthritis (mean 3.9 ± S.E.M. 0.42). There was no significant difference between the values obtained in osteoarthritis and in unoperated patients with rheumatoid arthritis. The mean and S.E.M. of the individual components of the clinical score are also shown in the table.

The relationship between the total clinical score and its individual components and the 99 mTc uptake at 15 minutes after injection are shown in Table 19. In osteoarthritis the relationships between pain and uptake ($r = 0.43$), swelling and uptake ($r = 0.36$) and total score and uptake ($r = 0.43$) were significant. The relationships between tenderness and uptake ($r = 0.28$) and between stiffness and uptake ($r = 0.24$) were not significant. The corresponding 'r' values in synovectomised patients with rheumatoid arthritis for pain ($r = 0.39$), swelling ($r = 0.39$) and total

TABLE XX - VALUES FOR CLINICAL SCORE, ^{99m}Tc COUNT RATE, $T\frac{1}{2}$ VALUE FOR ^{133}Xe CLEARANCE RATE

(GROUP 2 RHEUMATOID ARTHRITIS N = 22)

Clinical Score (mean ± S.E.M.)					15 minute ^{99m}Tc Count Rate x $10^{-2}/40$ secs. (mean ± S.E.M.)	^{133}Xe $T\frac{1}{2}$ values (mins) (mean ± S.E.M.)
Pain	Tenderness	Swelling	Stiffness	Total		
2.4 ± 0.15	1.9 ± 0.19	1.7 ± 0.23	1.8 ± 0.26	7.8 ± 0.63	29.09 ± 1.9	33.9 ± 4.1

Total Clinical Score v ^{99m}Tc Count Rate $r = 0.89$ $0.001 > p$

Total Clinical Score v ^{133}Xe $T\frac{1}{2}$ Value $r = 0.63$ $0.01 > p > 0.001$

^{99m}Tc Count Rate v ^{133}Xe $T\frac{1}{2}$ Value $r = 0.58$ $0.01 > p > 0.001$

score ($r = 0.34$) and uptake were also significant and again the relationships between tenderness ($r = 0.16$) and stiffness ($r = 0.06$) and uptake were not significant. In unoperated patients with rheumatoid arthritis the relationships between pain ($r = 0.35$), tenderness ($r = 0.48$), swelling ($r = 0.53$) stiffness ($r = 0.24$) and total score ($r = 0.52$) and uptake were all significant. The relationship between the total scores in unoperated patients with rheumatoid arthritis which gave a total of more than 6 and the 99 mTc uptake was significant ($r = 0.34$).

The results obtained in the second group of patients with rheumatoid arthritis are shown in Table 20. The mean and S.E.M. of the values for pain, tenderness, swelling, stiffness and total knee score were 2.4 ± 0.15; 1.9 ± 0.19; 1.7 ± 0.23; 1.8 ± 0.26 and 7.8 ± 0.63. The relationships between clinical knee score and 99 mTc count rate ($r = 0.89$), between clinical score and $T\frac{1}{2}$ value ($r = 0.63$) and between $T\frac{1}{2}$ value and 99 mTc count rate ($r = 0.58$) are significant.

DISCUSSION

Although other radioactive isotopes have been employed in articular scanning studies (Danielsson et al., 1963 : Fellander and Lindberg, 1966 : Holopainen and Rekonen, 1966 : Bauer, 1968 : Kettunen and Rekonen, 1968 : Bauer and Smith, 1969), radioactive technetium offers the particular advantage of low radiation hazard (Smith, 1965 : Anon., 1968 : McCarty, 1970a). This hazard may be even further reduced by the very low doses (50 Ci) employed in the present study which is made possible by the use of external directional counting as opposed to articular scanning. The technique sacrifices the advantage of localisation of uptake within the joint. Provided however that only quantitative information on the joint as a whole is required, for example in following anti-inflammatory drug effect, the advantage of repeated measurement may outweigh this disadvantage. The count rate over the joint is reproducible both from day to day and when repeat measurements are made on the same day (Table 1). At the time chosen for determinatio of count rate (15 mins.) it would appear from the elegant studies of McCarty et al., (1970a) that these isotope studies are providing a measure of

vascularity.

McCarty et al., (1970a) have studied the distribution and protein binding of 99 mTc and have attributed the basis of "early" and "late" maximal visualisation (page 271) to vascularity on the one hand and to the binding ability of inflamed tissue protein on the other. The term "vascularity' includes the vascularity of juxta-articular bone, peri-articular soft tissue, skin and patella in addition to synovium. The relative contributions of these various components remains to be determined. However, from the marked changes induced by anti-inflammatory therapy (page 322), it would seem reasonable to propose that a large proportion of the total is contributed by synovium and peri-articular tissue.

Since counting equipment and conditions vary, each laboratory must establish its own normal range and to this purpose we have studied a large number of normal subjects. The mean and two standard deviations at increasing time intervals following injection are shown. In an analogous manner to that employed in radioisotope thyroid tests the count rate has been expressed as a percentage of the

administered dose. If results are expressed as percentage uptake then comparable values should be obtained by different laboratories. This may prove to be an advantage of the present technique in routine clinical practice.

The patients studied in the first group, although selected in terms of attendance at a Rheumatology Centre, were not thereafter further selected in terms of disease severity. Inclusion in this group was determined by freely given consent, availability and certainty of diagnostic category. The values obtained for 99 mTc uptakes in both synovectomised and unoperated patients with rheumatoid arthritis were significantly higher than the values obtained either in osteoarthritis or in normal subjects. Although the mean value obtained in osteoarthritis was higher than that obtained in normal subjects this difference was not significant. Assuming that the 99 mTc count rate reflects vascularity, these studies are in accord with the pathologies of the respective diseases. Thus it could be anticipated that such a parameter would be elevated in the disease with the most marked inflammatory component.

It is interesting to compare these findings with the results obtained with the 133 Xe clearance technique (Chapter 6). In both the difference between normal subjects and patients with rheumatoid arthritis is marked. In both there is a difference between patients with rheumatoid and with osteo-arthritis. However, whereas with the 133 Xe technique there is a significant difference between normal subjects and patients with osteoarthritis, the difference is not significant by the 99 mTc method. It should first be emphasised that direct comparison between the two studies is not possible since there were differences between them both in terms of time of study and in terms of patient selection. However, both studies incorporated large numbers of patients which would to some extent reduce the differences and the assessing physician was the same in both studies. A further difference between the results obtained by these different isotopic techniques lies in the studies on synovectomised patients with rheumatoid arthritis. Whereas the results obtained by the 133 Xe clearance technique demonstrated no difference between operated and unoperated patients, the results obtained by the 99 mTc method show reduced values in operated

patients. Again, however, the studies cannot be directly compared for similar reasons. These differences however may be related to the differences in the two techniques. Whereas on the one hand the 133 Xe clearance technique provides a measure only of synovial tissue perfusion, the results obtained by the 99 mTc method provide an index of vascularity of all tissues beneath the detector. It is therefore possible for example that in osteoarthritis, synovial perfusion may be elevated producing an increased 133 Xe clearance rate, but the peri-articular tissue may be less severely involved resulting in less elevated 99 mTc uptakes. Similarly, in synovectomised patients with rheumatoid arthritis the synovium may be vascularly abnormal producing an increased 133 Xe clearance rate, but the peri-articular tissues may be less severely involved resulting in 99 mTc uptake which is pathologically elevated but lower than that obtained in unoperated patients. There is however no means of establishing this hypothesis at present and although attractive, the idea must be regarded as conjectural.

The relationship between the individual components of the clinical knee score and the 99 mTc uptake was also studied. In both osteo-arthritis and in synovectomised patients with rheumatoid arthritis the relationships between pai and uptake, swelling and uptake and total score an uptake were significant whereas the relationships between tenderness and uptake and between stiffnes and uptake were not. In unoperated patients with rheumatoid arthritis, in which the numbers studied were larger, all relationships were significant. These results suggest that both pain and swelling are more discriminating variables with regard to 99 mTc uptake than are either tenderness or swelling. The better relationship to swelling is easily understood in terms of 99 mTc uptake as an index of vascularity. The reason for the relationship to pain is less obvious. It is possible that the same chemical mediators of inflammation which produce pain also produce an increase in vascularity, however, there is insufficient information on this topic at present to sustain valid conclusions. It should be noted, however, that although statistically significant the 'r' values obtained in this group of patients are of the order of 0.4 to 0.5. This suggests

that only approximately 16 to 25% of the relationship is accounted for by the variables studied (r^2 = coefficient or "index" of determination). The biological significance of these results is therefore limited and other variables neither studied nor identified are of importance.

The second group of patients reported in this study were selected rather differently from the first. Thus in addition to the criteria previously applied the knee joint was obviously severely involved in the disease process. The difference in severity of disease is shown by the difference in their mean total scores (5.17 group 1 and 7.8 group 2). Furthermore, although the assessing physician was the same, the studies were not conducted contemporaneously. There were differences in the methodology due to the necessity of employing higher doses in the double isotope studies. However, it would have been possible to apply a correction factor for this. In balance it was felt to be unjustifiable to include the results obtained with the first group reported.

Although direct comparison is invalid, the 99 mTc

count rates obtained were of a higher order of magnitude than those obtained in normal subjects when suitably corrected for counting geometry and the $T\frac{1}{2}$ values were also less than those obtained in normal subjects (Chap. 6), denoting increased clearance rates and synovial perfusion rates in this group of patients. The relationship between the $T\frac{1}{2}$ value and the clinical score ($r = 0.63$) is significant and accords well with that obtained in a previous study (Chap. 6 : page 227). The relationship between the 99 mTc count rate and $T\frac{1}{2}$ value is also significant. Surprisingly, the 'r' value between total clinical score and 99 mTc count rate ($r = 0.89$) is very much higher than that obtained in the patients in the first group studied. McCarty et al., (1970b) have also noted a highly significant relationship between clinical assessment and 99 mTc "photoscans". Why then should the relationship between these variables be so different in different studies? In the present study the striking difference between the two groups of patients is the severity of local disease shown by the difference in mean total knee scores. However, when those knee scores from the first group in

excess of 6 were separately related to 99 mTc uptake the 'r' value (0.34) was no higher than for the group as a whole. The difference therefore cannot be directly related to severity alone. Since pain and swelling appeared to be the more discriminating variables in the first group studied, a further possibility remained that a difference existed in the relative contributions of the discriminating components of the total score in the two groups. However, consideration of each component of both groups as a percentage of the total score reveals that this is not the case. It is not possible to explain the discrepancies from the facts available. It would seem however that the 133 Xe clearance rate, 99 mTc studies and the clinical score each provide information on different but related aspects of the inflammatory response. Each must be considered complementary and not to replace the other. The 99 mTc uptake will probably be of most value in assessment of disease severity and of response to treatment.

SUMMARY

1) 99 mTc uptakes in synovectomised and unoperated patients with rheumatoid arthritis were higher than those obtained either in osteoarthritis or in normal subjects.

2) 99 mTc uptakes were lower in synovectomised than in unoperated patients with rheumatoid arthritis.

3) The values for pain, swelling and total clinical score were more closely related to 99 mTc uptake than were the values for tenderness or stiffness.

4) There is a relationship between 133 Xe $T\frac{1}{2}$ value, 99 mTc uptake and clinical score. The degree of significance of the relationship varies with different groups of patients studied.

ANION TRANSPORT FROM THE SYNOVIAL CAVITY

In the concluding section of this thesis I have employed 133 Xe and 99 mTc together in an attempt to delineate the mechanism of removal of anions from the articular cavity. The results show that an anion transport mechanism across the synovial membrane exists and suggest that the mechanism is an active one.

DOUBLE ISOTOPE STUDIES WITH 133 Xe, 99 mTc AND 131 I ON THE MANNER OF REMOVAL OF SMALL MOLECULAR WEIGHT ANIONS FROM THE JOINT CAVITY

Introduction

Although the clearance rates of several small molecular weight substances from the synovial cavity have previously been studied (Harris and Millard, 1956 : Laing and Kim, 1960 : Hernborg, 1969 and Chapter 1), the mechanisms involved have received less attention. Whereas there is some evidence to support the relationship between the 133 Xenon clearance rate and local tissue perfusion (Chapter 2), the assumptions involved in relating the clearance rates of the ionised radioisotopes and blood flow is less securely based. Laing and Kim (1960) have noted that the clearance rate of the anion ^{131}Iodine was faster than the clearance rates of the cations ^{24}Na or ^{42}K but were unable to explain these differences.

In the present study the clearance rates of ionised radioisotopes have been compared to the clearance rate of simultaneously injected inert 133 Xe both in the presence and in the absence of potassium perchlorate in an attempt to define further the mechanisms involved.

MATERIALS AND METHODS

Experiments were conducted on both stifle joints of anaesthetised adult greyhound dogs weighing from 20 to 36 kg. at the Wellcome Surgical Research Institute. Anaesthesia was induced with sodium thiopentone (20 mg/kg.) and maintained through a Starling respiratory pump and Boyle's anaesthetic machine with a 5:3 mixture of nitrous oxide and oxygen and 1% trichloroethylene. Monitoring was accomplished by periodic determinations of blood gases, pulse rate and blood pressure. When the animal was anaesthetised and in position, a fine guage needle was introduced into the stifle joint cavity and the position checked by the aspiration of synovial fluid. The volume of synovial fluid aspirated was noted and the volume of subsequent isotope solutions was adjusted to approximately equal the volume aspirated.

A detector (Ekco 5590) was positioned directly above and 1 cm. from the skin over the patella and pulses were fed into a pulse height analyser and ratemeter (Ekco 1750) which was suitably adjusted to the counting conditions for the different isotopes

studied and continuously recorded on a Rikadenki chart recorder (B.24). Room background (of the order of 1 to 2% of the total count rate) was recorded at the beginning and end of each experiment and overlap of the higher energy radioisotopes onto the lower energy channel was calculated at the beginning of each experiment. The radioisotopes employed (133 Xe, 24 Na, 131 I and 99 mTc) were obtained from the Western Infirmary, Glasgow.

In three preliminary experiments, 133 Xe was injected in the first with 24 Na, in the second with 99 mTc and in the third with 131 I and the count rates of each isotope pair were monitored alternately for 10 minutes each from 0 to 60 mins. after injectio

The design of succeeding experiments with the isotope pairs 133 Xe: 131 I and 133 Xe: 99 mTc were identical. Both isotopes were injected into the first stifle joint studied and the count rates of each monitored alternately for 8-10 minutes, for 50 to 60 minutes. Potassium perchlorate (1g. in 50 ml.) was then injected intravenously and 30 minutes later identical doses of both isotopes were injected into the opposite stifle joint. The

count rates of each were again monitored alternately for 8-10 minutes, for 50 to 60 minutes. $T_{\frac{1}{2}}$ values were obtained from the semilogarithmic plots of the count rates. In four experiments with the isotope pair 133 Xe : 99 mTc, the animal died between the periods of study of each joint. A total of 13 experiments with 133 Xe : 99 mTc alone, 9 experiments with 133 Ce : 99 mTc following perchlorate, 6 experiments with 133 Xe : 131 I alone and 4 experiments with 133 Xe : 131 I following perchlorate were conducted in this manner.

TABLE XXI

TIME AFTER INJECTION (Mins.)

EXPERIMENT		WITHOUT PERCHLORATE						WITH PERCHLORATE					
		0–10	10–20	20–30	30–40	40–50	50–60	0–10	10–20	20–30	30–40	40–50	50–60
1	133 Xe	38	–	45	–	50	–	30	–	33	–	–	–
	99 mTc	–	27	–	32	–	36	–	35	–	–	–	—
2	133 Xe	–	37	–	43	–	51	40	–	40	–	–	–
	99 mTc	21	–	25	–	39	–	–	42	–	–	–	–
3	133 Xe	–	43	–	73	–	–	42	–	56	–	58	–
	99 mTc	32	–	31	–	–	–	–	150	–	120	–	–
4	133 Xe	–	57	–	65	–	–	–	44	–	59	–	–
	99 mTc	29	–	25	–	–	–	43	–	57	–	–	–
5	133 Xe	50	–	50	–	–	–	–	40	–	44	–	–
	99 mTc	–	32	–	–	–	–	48	–	48	–	–	–
6	133 Xe	–	30	–	29	–	32	–	26	–	33	–	29
	99 mTc	21	–	20	–	16	–	38	–	41	–	41	–
7	133 Xe	–	55	–	53	–	–	–	42	–	69	–	78
	99 mTc	19	–	21	–	–	–	56	–	72	–	80	–
8	133 Xe	–	52	–	40	–	58	–	25	–	36	–	35
	99 mTc	14	–	15	–	13	–	38	–	35	–	37	–
9	133 Xe	–	46	–	46	–	42	–	29	–	24	–	27
	99 mTc	8	–	16	–	20	–	32	–	32	–	41	–
10	133 Xe	150	–	150	–	–	–						
	99 mTc	–	38	–	–	–	–						
11	133 Xe	27	–	150	–	30	–						
	99 mTc	–	17	–	19	–	–						
12	133 Xe	110	–	110	–	–	–						
	99 mTc	–	76	–	–	–	–						
13	133 Xe	80	–	78	–	84	–						
	99 mTc	–	51	–	54	–	54						

Legend: The $T\frac{1}{2}$ values derived alternately at the time periods after injection indicated for 133 Xe and 99 mTc both with and without a prior injection of perchlorate.

RESULTS

The results are shown in Tables 21 and 22.

The $T\frac{1}{2}$ value obtained in the three preliminary experiments were 57 and 81 minutes for 133 Xe and 24 Na, 42 and 30 minutes for 133 Xe and 131 I, and 69 and 23 minutes for 133 Xe and 99 mTc respectively. Thus whereas the clearance rate of 24 Na was slower than that of 133 Xe, the converse result was obtained for both 131 I and 99 mTc, both clearance rates being faster than that of simultaneously administered 133 Xe.

Table 21 shows the $T\frac{1}{2}$ values obtained alternately for 133 Xe and for 99 mTc at the time periods 0 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50 and 50 to 60 minutes after injection in the 13 experiments in which these isotopes were injected alone and in the 9 experiments in which the isotopes were injected into the opposite stifle joint following an intravenous injection of perchlorate. In the absence of perchlorate the $T\frac{1}{2}$ values obtained at all times for 99 mTc were less than those obtained for 133 Xe denoting faster clearance of the anion.

TABLE XXII

TIME AFTER INJECTION (Mins.)

EXPERIMENT		WITHOUT PERCHLORATE						WITH PERCHLORATE					
		0–10	10–20	20–30	30–40	40–50	50–60	0–10	10–20	20–30	30–40	40–50	50–60
1	133 Xe	60	–	62	–	61	–	51	–	52	–	50	–
	131 I	–	41	–	40	–	41	–	60	–	68	–	68
2	133 Xe	–	33	–	38	–	46	–	40	–	42	–	42
	131 I	22	–	24	–	24	–	46	–	50	–	50	–
3	133 Xe	32	–	34	–	34	–	44	–	46	–	46	–
	131 I	–	16	–	18	–	–	–	48	–	50	–	55
4	133 Xe	60	–	64	–	66	–	52	–	53	–	55	–
	131 I	–	16	–	29	–	31	–	60	–	68	–	74
5	133 Xe	80	–	77	–	–	–						
	131 I	–	50	–	50	–	–						
6	133 Xe	–	65	–	62	–	62						
	131 I	46	–	46	–	49	–						

Legend: The $T_{\frac{1}{2}}$ values derived alternately at the time periods after injection indicated for 133 Xe and 131 I, both with and without a prior injection of perchlorate.

Conversely, however, following a preliminary injection of perchlorate the $T\frac{1}{2}$ values for 133 Xe were less than those obtained for 99 mTc denoting slower clearance of the anion.

Table 22 shows the $T\frac{1}{2}$ values obtained alternately for 133 Xe and for 131 I at the same time periods following injection in 6 studies in which the isotopes were injected without and in 4 studies in which they were administered following the prior administration of perchlorate.

Whereas the $T\frac{1}{2}$ values for 131 I are all less than the corresponding values for 133 Xe in the absence of perchlorate, when perchlorate had previously been injected, the converse was found in all cases, the $T\frac{1}{2}$ values for 133 Xe being less than those obtained for 131 I. Similarly, therefore, the clearance rate of 131 I exceeds that of 133 Xe in the absence of, and is less than that of 133 Xe in the presence of perchlorate.

DISCUSSION

The results of this study show that whereas the rate of 24 Na clearance from the articular cavity is reduced when compared with simultaneously monitored 133 Xe, the clearance rates of both 99 mTc and 131 I are faster than the 133 Xe clearance rate. These results are in accord with those of Laing and Kim (1960) who demonstrated slower clearance of cations than anions.

However, when the clearance rates of 131 I and 99 mTc are compared to that of simultaneously administered 133 Xe following perchlorate the rate of clearance of both anions was reduced when compared with that of the inert 133 Xe. These results suggest the presence of an anion transport mechanism from the articular cavity through the synovial membrane to the synovial blood vessels. Although anion movement in these studies is clearly taking place in the direction of a concentration gradient perchlorate inhibition supports the contention that this is an active process. It remains possible, however, that the anion transport

system is dependent upon active cation transport. This possibility remains to be studied at a more fundamental laboratory level.

The discovery of one transport mechanism in the joint may well presage the delineation of others and lead to a fuller understanding of ion fluxes across the synovial membrane.

SUMMARY

The rate of clearance of the cation 24 Na was slower than the clearance rate of the inert reference radioisotope 133 Xe.

The clearance rates of both anions, 131 I and 99 mTc, exceeded the 133 Xe clearance rate in the absence of, and were slower than the 133 Xe clearance rate in the presence of pre-treatment with potassium perchlorate.

These results suggest the presence of an anion transport mechanism from the joint cavity.

REFERENCES

Ahlquist, R.P. (1948)
Amer. J. Physiol. 153:586.

Ahlstrom, S., Gedda, P.O. and Hedberg, H. (1956)
Acta Rheum. Scand. 2:129.

Aidem, H.P. and Baker, L.D. (1964)
JAMA 187:4.

Alarcon-Segovia, D., Trujeque, M., Tovar, E. and Adame, M.A. (1967a)
Arthr. Rheum. 10:262.

Alarcon-Segovia, D., Trujeque, M., Tovar, E. and Adame, M.A. (1967b)
Proc. of the Fourth Panamerican Congress of Rheumatology, Mexico City. Oct. 1967.
Excerpta Medica Congress Series No. 165.

Andersson, L., Dahn, I., Nelson, C. and Norgren, A. (1967)
Invest. Urol. 5:140.

Anon. (1968)
Lancet 1:131.

Anseroff, N.J. (1934)
Zietschrift fur Anatomie und Entwicklungsgeschichte 103:793.

Ashford, A. Penn, G.B. and Ross, J.W. (1962)
Nature (Lond.) 193:1082.

Bain, W.H. and Harper, A.M. (1968)
"Blood flow through organs and tissues"
Livingstone Ltd., Edinburgh.

Ball, W.C. (Jr.), Stewart, P.B., Newsham, L.G.S.
and Bates, D.V. (1962)
J. Clin. Invest. 41:519.

Barcroft, H., Bonnar, W.M. and Edholm, O.G. (1947)
J. Physiol. 106:271.

Barland, P., Novikoff, A.B. and Hamerman, D. (1962)
J. Cell Biol. 14:207.

Barlow, T.E., Haigh, A.L. and Walder, D.N. (1961)
Clin. Sci. 20:367.

Barnes, C.G. and Mason, R.M. (1967)
Ann. Phys. Med. 9:83.

Bauer, W., Ropes, M.W. and Waine, H. (1940)
Physiol. Rev. 20:272.

Bauer, G.C.H. and Scoccianti, P. (1961)
Acta. Orthop. Scand. 31:90.

Bauer, G.C.H. (1968)
J. Bone Jt. Surg. 50A:1681.

Bauer, G.C.H. and Smith, E.M. (1969)
J. Nucl. Med. 10:109.

Beecher, H.K. (1959)
Measurement of subjective responses: Quantitative
effects of drugs.
Oxford University Press. New York.

Bennett, G.A. and Shaffer, M.F. (1939)
J. Exper. Med. 70:277.

Bentivoglio, L.G., Beerel, F., Bryan, A.C., Stewart, P.B., Rose, B. and Bates, D.V. (1963)
J. Clin. Invest. 42:1193.

Bertolani, F., Lorenzini, R. and Bonati, B. (1951)
Acta Vitamina. (Milano) 5:153.

Bianchi, C. (1953)
Brit. J. Pharmacol. 8:130.

Birmingham, A.T. and Szolcsanyi, J. (1965)
J. Pharm. Pharmacol. 17:449.

Birmingham, A.T. Akubue, P.I. and Szolcsanyi, J. (1967)
J. Pharm. Pharmacol. 19:137.

Birmingham, A.T., Ernest, K. and Newcombe, J.F. (1969)
Brit. J. Pharmacol. 35:127.

Boardman, P.L. and Hart, F.D. (1967)
Brit. Med. J. 4:264.

Boardman, P.L., Hart, F.D. and Nuki, G. (1967)
Ann. Rheum. Dis. 26:560.

Boas, N.F. (1964)
Ann. N.Y. Acad. Sci. 121:223.

Bonney, G.L.W., Hughes, R.A. and Janus, O. (1952)
Clin. Sc. 11:167.

Boyd, I.A. (1954)
J. Physiol. 124:476.

Branemark, P-I, Laine, V., Lindstrom, J. and Vainio, K. (1963a)
Acta Rheum. Scand. 9:94.

Branemark, P-I, Lindstrom, J., Jonsson, I., Laine, V. and Vainio, K. (1963b)
Acta Rheum. Scand. 9:284.

Branemark, P-I, Laine, V. and Vainio, K. (1966)
Acta Rheum. Scand. 12:35.

Branemark, P-I, Ekholm, R., Goldie, I. and Lindstrom, J. (1967)
Acta Rheum. Scand. 13:161.

Branemark, P-I. (1969)
in "Early Synovectomy in Rheumatoid Arthritis (p11)"
Proceedings of the symposium on early synovectomy in rheumatoid arthritis, Amsterdam, The Netherlands, April (1967).
Ed. by Higmans, W., Paul, W.D. and Herschel, H.
Exerpta Medica Foundation.

Brick, I., Glover, W.E., Hutchison, K.J. and Roddie, I.C. (1966)
Am. J. Cardiol. 18:329.

Brick, I., Hutchison, K.J., McDevitt, D.G., Roddie, I.C. and Shanks, R.G. (1968)
Brit. J. Pharmac. 34:127.

Brodie, T.G. and Russell, A.E. (1905)
J. Physiol. 32:47.

Brownlee, G. (1966)
Angiology. 17:186.

Bucciante, L. (1960)
Arch. De Vecchi Anat. Patol. 32:117.

Carruthers, F.W. (1960)
Western J. Surg. Obstet. and Gynec. 68:382

Cobbold, A.F. and Lewis, O.J. (1956a)
J. Physiol. 132:379.

Cobbold, A.F. and Lewis, O.J. (1956b)
J. Physiol. 133:467.

Cobbold, A.F. and Lewis, O.J. (1956c)
J. Physiol. 133:472.

Conn, H.L. (Jr.)(1961)
J. Appl. Physiol. 16:1065.

Co-operating Clinics Committee of the American Rheumatism Association (1965)
Arthr. Rheum. 8:302.

Copp, D.H. and Shim, S.S. (1965)
Circulat. Res. 16:461.

Cosh, J.A. (1970)
Personal communication.

Cuthbertson, E.M., Siric, E. and Gilfillan, R.S. (1965)
J. Bone Jt. Surg. 47A:965.

Dale, H. and Laidlaw, P. (1919)
J. Physiol. (London) 52:355.

Danielsson, L.G., Dymling, J.F. and Heripret, G. (1964)
Clin. Orthop. 31:184.

Davies, D.V. (1946)
Lancet 2:815.

Davies, D.V. and Edwards, D.A.W. (1948)
Ann. R. Coll. Surg. Eng. 2:142.

Davis, C.H. (Jr.), Alexander, E. (Jr.), Witcofski, R.L. and Maynard, C.D. (1966)
J. Neurosurg. 24:987.

Davis, M.J. and Lawler, J.C. (1958)
Arch. Dermat. 77:690.

Davison, S. and Wisham, L.H. (1958)
J. Clin. Invest. 37:389.

De. le Lande, I.S., Cannell, V.A. and Waterson, J.A. (1966)
Brit. J. Pharmac. Chemother. 28:255.

Diana, J.N., Schwinghamer, J. and Young, S. (1968)
Amer. J. Physiol. 214:494.

Doering, P. and Miehlke, K. (1961)
Ztschr. f. Rheumafor. 20:137.

Drinker, C.K. (1946)
Ann. N.Y. Acad. Sci. 46-807.

Fisenfeld, A.J., Axelrod, J. and Krakoff, L. (1967)
J. Pharmac. Exp. Ther. 156:107.

Engel, D. (1940)
Quart. J. Exp. Physiol. 30:231.

Engel, D. (1941)
J. Physiol. 99:161.

Engel, D. and Forrai, E. (1943)
J. Physiol. 102:127.

Falck, B. and Owman, C. (1965)
Acta Universitatis Lundensis 7:(suppl.)1.

Fellander, M. and Lindberg, L. (1966)
J. Bone Jt. Surg. 48A:1585.

Fick, A. (1870)
Uber die Messung des Blutquantums in den Herzventrikeln
Verhandl. d. phys. med. Gesellsch Z:XVI (Quoted in its entirety).

Foster, R.W. (1967)
Brit. J. Pharmac. Chemother. 31:466.

Foster, R.W. (1969)
Brit. J. Pharmac. 35:418.

Friedman, J.J. (1968)
Amer. J. Physiol. 214:488.

Gardner, E. (1950)
Physiol. Rev. 30:127.

Gariepy, R., Demers, R. and Laurin, C.A. (1966)
Canad. Med. Ass. J. 94(part 2):1349.

Gaspardy von G., Gaspardy (Jr.), Balint, G. and Kemeny, V. (1966)
Z. Rheumaforsch. 25:199.

Ghadially, F.N. and Roy, S. (1966)
Ann. Rheum. Dis. 25:318.

Ghormley, R.K. and Cameron, D.M. (1941)
Amer. J. Surg. 53:455.

Gillespie, F.C. (1968)
in "Blood Flow Through Organs and Tissues" (page 90)
Ed. Bain, W.H. and Harper, A.M.
E. & S. Livingstone Ltd. Edinburgh and London.

Glover, W.E. and Hutchison, K.J. (1965)
J. Physiol. 177:59p.

Goldie, I. (1969)
Acta Orthop. Scand. 40:382.

Goldie, I. and Wellisch, M. (1969)
Acta Orthop. Scand. 40:143.

Goodman, L.S. and Gilman, A. (1965)
The Pharmacological basis of Therapeutics.
pp. 399-441, 477-521, 546-578
Collier-Macmillan Ltd., London.

Goslings, J. (1969)
in "Early Synovectomy in Rheumatoid Arthritis"
p XIII.
Proceedings of the Symposium on early synovectomy
in rheumatoid arthritis, Amsterdam, The Netherlands,
April, 1967.
Edited by Hijmans, W., Paul, W.D. and Herschel, H.
(Excerpta Medica Foundation).

Gosselin, R.E. (1966)
Am. J. Physiol. 210:885.

Greaves, M. and Shuster, S. (1967)
J. Physiol. 193:255.

Green, P.A. and Hays, M.T. (1969)
12th Int. Congress of Rheumatology, Prague, 1969
Geigy (abstract).

Greenfield, A.D.M., Shephard, J.T. and Whelan, R.F. (1951)
J. Physiol. 112:459.

Gualtieri, G. and Lanzi, F. (1964)
Clinica Ortopedica Dell' Universita Di Milano 77:535.

Haberman, J.D., Ehrlich, G.E. and Levenson, S. (1968)
Arch. Phys. Med. 49:187.

Hagen-Torn, O. (1882)
Arch. f. mikr. anat. 21:591.

Hamerman, D. and Barland, P. (1966)
Bull. Rheum. Dis. 16:396.

Hammar, J.A. (1894)
Arch. f. mikr. anat. 43:266.

Harden, R. McG., Alexander, W.D. and Kennedy, I. (1967a)
Lancet 1:1305.

Harden, R. McG., Hilditch, T.E., Kennedy, I., Mason, D.K., Papadopoulos, S. and Alexander, W.D. (1967b)
Clin. Sci. 32:49.

Harper, A.M., Glass, H.I., Steven, J.L. and Granat, A.H. (1964)
J. Neurol. Neurosurg. Psychiat. 27:255.

Harris, H.A. (1929)
J. Anat. Lond. 64:3

Harris, H.A. (1933)
Bone Growth in Health and Disease
Oxford University Press.

Harris, R. and Millard, J.B. (1956)
Clin. Sci. 15:9.

Harris, R., Millard, J.B. and Banerjee, S.K. (1958)
Ann. Rheum. Dis. 17:189.

Harris, R. (1961)
Arch. Phys. Med. 42:241.

Hashimoto, H., Okada, T., Sato, H. and Shiokawa, Y. (1969)
12th Int. Congress of Rheumatology, Prague, 1969
Geigy (abstract).

Hernborg, J. (1968)
Arthr. Rheum. 11:618.

Hernborg, J. (1969)
Arthr. and Rheum. 12:30.

Heuter, C. (1866)
Virchow's Arch. 36:25.

Heyman, C.H. (1928)
Surg. Gynec. and Obstet. 46:127.

Hidalgo, J., McClure, C.D., Henderson, J.B., Whitehead, R. and Smyth, C.J. (1952)
Proc. Soc. Exp. Biol. N.Y. 80:97.

Hilditch, T.E., Gillespie, F.C., Shimmins, J., Harden, R. McG. and Alexander, W.D. (1967)
J. Nucl. Med. 8:810.

Hoedt-Raemussen, K., Sveinsdottir, E., Lassen, N.A. (1966)
Circulat. Res. 18:237.

Hollander, J.L. and Horvath, S.M. (1949a)
Arch. Phys. Med. 30:437.

Hollander, J.L. and Horvath, S.M. (1949b)
Am. J.M. Sc. 218:543.

Hollander, J.L., Stoner, E.K., Brown, E.M.(Jr.) and DeMoor, P. (1951)
J. Clin. Invest. 30:701.

Hollander, J.L. and Moore, R. (1956)
Ann. Rheum. Dis. 15:320.

Hollander, J.L., Fudenberg, H.H., Rawson, A.J., Abelson, N.M. and Torralba , T.P. (1966)
Arthr. Rheum. 9:675.

Hollander, J.L. and Rawson, A.J. (1968)
Bull. Rheum. Dis. 18:502.

Hollander, W., Reilly, P. and Burrows, B.A. (1961)
J. Clin. Invest. 40:222.

Holopainen, T. and Rekonen, A. (1966)
Acta Rheum. Scand. 12:102.

Horvath, S.M. and Hollander, J.L. (1949)
J. Clin. Invest. 28:469.

Hunter, W. (1743)
Phil. Trans. Roy. Soc. 42:514.

Inge, G.A.L. (1938)
Jama 111:2451.

Ingpen, M.L. (1968)
Ann. Phys. Med. 9:322.

Ingvar, D.H. and Lassen, N.A. (1962)
Acta Physiol. Scand. 54:325.

Jacox, R.F., Johnson and Koontz, R. (1952)
Proc. Soc. Exp. Biol. & Med. N.Y. 80:655.

Jacox, R.F., Spar, I., Farrer, P. and Rubin, P. (1969)
12th Int. Congress of Rheumatology, Prague, 1969
Geigy (abstract).

Jayson, M. (1970)
Personal communication.

Joyce, C.R.B. (1961)
Ann. rheum. Dis. 20:78.

Kellgren, J.H. and Samuel, E.P. (1950)
J. Bone Jt. Surg. 32B:84.

Kelly, P.J. (1968)
J. Bone Jt. Surg. 50A:766.

Kettunen, K. and Rekonen, A. (1968)
Ann. Chir. Gynaec. Fenniae. 57:250.

Kety, S.S. and Schmidt, C.F. (1948)
J. Clin. Invest. 27:476.

Kety, S.S. (1949)
Amer. Heart J. 38:321.

Kety, S.S. (1951)
Pharmacol. Rev. 3:1.

Kety, S.S. (1960)
Meth. Med. Res. 8:228.

Key, J.A. (1925)
J.B.J.S. 7:793.

Key, J.A. (1926)
J. Bone Jt. Surg. 8:666.

Key, J.A. (1928)
"Special cytology"
Ed. E.V. Cowdrey.

Kling, D.H. (1938)
The Synovial Membrane and the Synovial Fluid
Los Angeles : Medical Press.

Krogh, A.S. (1919)
J. Physiol. 52:391.

Kuipers, R.K.W., Francke, C. and Robert, W.N. (1956)
Acta Rheum. Scand. 2:81.

Kulka, J.P. (1964)
Ann. N.Y. Acad. Sci. 116:1018.

Laing, P.G. and Kim, I.D. (1960)
Nature 186:898.

Laing, P.G. (1953)
J. Bone Jt. Surg. 35B:462.

Landis, E.M. and Pappenheimer, J.P. (1963)
"Exchange of substances through capillary walls"
in Handbook of Physiology Sec. 2, Vol. 2
circulation
Ed. W.F. Hamilton and P. Dow. Washington D.C.

Lansbury, J. (1966)
"Methods for evaluating rheumatoid arthritis"
in Arthritis and Allied Conditions 7th Ed.
Ed. J.L. Hollander
Philadelphia Lee and Febiger.

Larsen, O.A., Lassen, N.A. and Quaade, F. (1966)
Acta Physiol. Scand. 66:337.

Larson, S.M. and Nelp, W.B. (1965)
Amer. J. Obstet. Gynec. 93:950.

Lasagna, L. (1955)
Sci. Amer. 193:68.

Lassen, N.A., Lindbjerg, J. and Munck, O. (1964)
Lancet 1:686.

Lembo, E.J.A. (1967)
Bol. Soc. Argent. Ortop. Traum. 32:194.

Lewis, G.W. and Cluff, L.E. (1956)
Bull. Hopkins Hosp. 116:175.

Lexer, E. (1904)
Arch. Klin. Chir. 43:481.

Lindstrom, J. (1966)
Acta Rheum. Scand. 12:175.

London, P.S. (1955)
J. Bone Jt. Surg. 37B:392

Lovgren, O. (1969)
12th Int. Congress of Rheumatology, Prague, 1969
Craigy (abstract).

McCarty, D.J. (Jr.) and Hogan, J.M. (1964)
Arthr. and Rheum. 7:359.

McCarty, D.J., Polcyn, R.E., Collins, P.A. and
Gottschalk, A. (1970a)
Arthr. Rheum. 13:11.

McCarty, D.J., Polcyn, R.E. and Collins, P.A.
(1970b)
Arthr. Rheum. 13:21.

McCready, V.R. (1967)
Brit. J. Radiol. 40:401.

McGirr, E.M. (1952)
Brit. Med. Bull. 8:192.

Mainland, D. (1967)
Arthr. Rheum. 10:71.

Marmor, L. (1966)
Amer. J. Surg. 111:211.

Mikkelsen, O.A. (1967)
Nord. Med. 78:1483.

Mitchell, W.S. and Cruess, R.L. (1967)
Canad. J. Surg. 10:234.

Morgan, J.D. (1959)
J. Bone Jt. Surg. 41B:185.

Myers, K.A., Hobbs, J.T. and Irvine, W.T. (1968)
Cardiovasc. Res. 2:360.

Nakamura, R., Asai, H., Sonozaki, H. and
Nagano, M. (1967)
Ann. rheum. Dis. 26:246.

Norton, W.L. and Ziff, M. (1969)
Arth. and Rheum. 12:589.

O'Brien, W.M. (1968)
Clin. Pharmacol. Therap. 9:94.

Palmer, D.G. and Myers, D.B. (1968)
Arthr. Rheum. 11:745.

Pappenheimer, J.R., Renkin, E.M. and Borrero, L.M.
(1951)
Amer. J. Physiol. 167:13.

Pappenheimer, J.R. (1953)
Pharmacol. Rev. 33:387.

Paradies, L.H. (1969)
in "Early synovectomy in Rheumatoid Arthritis"(p.12
Proceedings of the symposium on early synovectomy i
rheumatoid arthritis, Amsterdam, The Netherlands,
April, 1967
Ed. Hijmans, W., Paul, W.D. and Herschel, H.
(Excerpta Medica Foundation).

Paul, W.D., Hodges, R.G., Knouse, R.W. and Wright,
C.S.(Jr.) (1952)
Proc. Soc. Exp. Biol. N.Y. 79:68.

Perl, W. (1962)
J. Theoret. Biol. 2:201.

Perl, W., Rackow, H., Salanitre, E., Wolf, G.L. and Epstein, R.M. (1965)
J. Appl. Physiol. 20:621.

Perl, W. and Chinard, F.P. (1968)
Circulat. Res. 22:273.

Phillips, R.S. (1966)
J. Bone Jt. Surg. 48B:280.

Pinals, R.S. and Frank, S. (1967)
New Engl. J. Med. 276:512.

Pitkeathly, D.A., Banerjee, N.R., Harris, R. and Sharp, J. (1966)
Ann. rheum. Dis. 25:334.

Polacek, P. (1961)
Acta Anatomica. 47:112.

Policard, A. (1935)
Physiologie Generale des articulations a l'etat normale et pathologique.
Masson Paris.

Quenouille, M.H. (1953)
The Design and Analysis of Experiments p.269
Griffin, London.

Quasimorio, F., Owen, E., Rawson, A.J., Abelson, N.M. and Hollander, J.L. (1968)
Arthr. Rheum. 11:113.

Ramadier, J.O., Achach, P. and Courbon, S. (1967)
Rev. Rheum. 34:413.

Rekonen, A. and Holopainen, T. (1968)
Calcif. Tissue Res. Suppl. 35-35a.

Renkin, E.M. (1955)
Amer. J. Physiol. 183:125.

Restifo, R.A., Lussier, A.J., Rawson, A.J.
Rockey, J.H. and Hollander, J.L. (1965)
Ann. Int. Med. 62:285.

Ring, E.F.J. and Cosh, J.A. (1968)
Brit. Med. J. 4:448.

Ritchie, D.M., Boyle, J.A., McInnes, J., Jasani,
M.K., Dalakos, T.G., Grieveson, P. and
Buchanan, W.W. (1968)
Quart. J. Med. 37:393.

Rodman, W.S., Williams, R.C.(Jr.), Bilka, P.J.
and Muller-Eberhard, H.J. (1967)
J. Lab. Clin. Med. 69:141.

Rodnan, S.P. and Maclachlan, M.J. (1960)
Arthr. Rheum. 3:152.

Rogers, W.M. and Gladstone, H. (1950)
J. Bone Jt. Surg. 32A:867.

Ropes, M.W. (Chairman of a Committee of the
American Rheumatism Association) (1959)
Ann. rheum. Dis. 18:49.
Diagnostic criteria for rheumatoid arthritis
1958 revision.

Scapinelli, R. (1968)
Acta Anat. 70:305.

Scholer, J.F., Lee, P.R. and Polley, H.F. (1959)
Arthr. and Rheum. 2:426.

Schumacher, H.R. (1969)
Arthr. and Rheum. 12:387.

Schur, P.H. and Sandson, J. (1963)
Arthr. and Rheum. 6:115.

Scoccianti, P. (1966)
J. Nucl. Biol. Med. 10:95.

Seifter, J., Baeder, D.H. and Begany, A.J. (1949)
Proc. Soc. Exp. Biol. N.Y. 72:277.

Seifter, J. and Baeder, D.H. (1954)
Proc. Soc. Exp. Biol. N.Y. 87:276.

Sejrsen, P. (1967)
Scand. J. Clin. Lab. Invest. (Suppl.) 99 p.52.

Shaffer, M.F. and Bennett, G.A. (1939)
J. Exp. Med. 70:293.

Sharp, G.W.G. (1963)
Ann. Rheum. Dis. 22:50.

Sholkoff, D.S. and Glickman, M.G. (1969)
Invest. Radiol. 4:207.

Sicuteri, F. (1965)
Proceedings of symposium on non-steroidal anti-inflammatory drugs
Milan. Minerva Medica Turin, Italy. page 159.

Skoglund, S. (1956)
Acta Physiol. Scand. 36 (Suppl.124):1.

Smith, E.M. (1965)
J. Nucl. Med. 6:231.

Sokoloff, L. and Gleason, I.O. (1954)
Amer. J. Clin. Path. 24:406.

Sokoloff, L. and Bunim, J.J. (1957)
J. Chron. Dis. 5:668.

Speed, J.S. (1924)
JAMA 83:1814.

Stasser, N.F. and Thrift, C.B. (1968)
J. Amer. Ger. Soc. 16:539.

Steinbach, H.L., Jergesen, F., Gilfillan, R.S. and Petrakis, N.L. (1957)
Surg. Gynec. and Obstet. 104:215.

Steinbrocker, O., Traeger, C.H. and Batterman, R.C. (1949)
JAMA 140:659.

Stephenson, R.P. (1956)
Brit. J. Pharmac. Chemother 11:379.

Stevens, J. and Whitefield, G.A. (1966)
Ann. rheum. Dis. 25:214.

Stewart, G.N. (1911)
Heart 3:33.

Stone, P.W. and Miller, W.B.(Jr.) (1949)
Proc. Soc. Exp. Biol. et Med. 71:529.

Suleimanov, I. (1964)
Ortop. Traun. Protez. 25:43.

Sunshine, A., Laska, E., Meisher, M. and Morgan, S. (1964)
Clin. Pharm. et Ther. 5:699.

Tesárek, B. and Kolar, M. (1964)
Cas. Lek. Cesk. 103:545.

Testut, L. (1880)
Vaisseaux et nerfs des tissues concontifs, fibreux seruex et osseux.
Thèse Paris G. Mason.

Thorburn, G.D., Kopald, H.H., Herd, J.A., Hollenberg, M., O'Morchoe, C.C.C. and Barger, A.C. (1963)
Circulat. Res. 13:290.

Turner, P. Harrison, J. and Schoenfeldt, R. (1969)
Lancet 1:1238.

Unsworth, J. and Gillespie, F.C. (1969)
Communication at Thomas Graham Memorial Symposium Univ. of Strathclyde, Glasgow. (to be published)

Vainio, K. (1966)
Med. et Hyg. (Geneve) 24:616.

Veall, N. (1968)
Brit. J. Hosp. Med. 2:460.

Vecchio, C. and Fontana, S. (1965)
Proc. of symp. on non-steroidal anti-inflammatory drugs Milan.
Minerva Medica Turin, Italy pages 166-179.

Verhaege, J. and Lebeurre, R. (1954)
Rev. Rheumat. 21:120.

Walsh, J.A. (1967)
Brit. J. Pharmac. Chemother. 30:518.

Weiss, T.E., Maxfield, W.S., Murison, P.J. and Hidalgo, J.U. (1966)
Southern Med. J. 59:484.

Whitley, J.E., Witcofski, R.L., Bolliger, T.T. and Maynard, C.D. (1966)
Amer. J. Roentgenol. 96:706.

Winter, C.A. (1964)
Proceedings of International Symposium on non-steroidal anti-inflammatory drugs. Milan.
Excerpta Medica Int. Congress Series No.82 page 190.

Wisham, L.H., Yalow, R.S. and Freund, A.J. (1951)
Amer. Heart. J. 41:810.

Wisham, L.H. and Dworecka, F.F. (1966)
J. Cardiovasc. Surg. 7:66.

Witcofski, R.L. and Bolliger, T.T. (1965)
J. Nucl. Med. 6:555.

Wolcott, W.E. (1927)
J.B.J.S. 9:67

Wright, V. (1959)
Clin. Sci. 18:17.

Ziff, M. (1957)
J. Chron. Dis. 5:644.