THE NATURALLY OCCURRING INFLAMMATORY ARTHROPATHIES OF THE DOG.

A Clinical, Pathological and Immunological Study, Including a Consideration of the Comparative Aspects of these Diseases in Man and the Dog.

(IN TWO VOLUMES)

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by

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Dedicated to all those full-time University members of staff (and their wives) who dare to study for a post-graduate degree.

The Moving Finger writes; and having writ,
Moves on; nor all thy Piety nor Wit
Shall lure it back to cancel half a line,
Nor all thy Tears wash out a Word of it.

Omar Khayyam (11th Century).

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SUMMARY

A group of joint diseases characterised by a marked synovitis has been identified in the dog and distinguished from the degenerative arthropathies. These were designated the inflammatory arthropathies and divided into five groups:- Group I - rheumatoid arthritis, Group II - arthritis associated with systemic lupus erythematosus, Group III - infectious arthritis, Group IV - arthritis associated with bacterial endocarditis, and, Group V - idiopathic (including enteropathic) arthritis. A total of 82 dogs were included in the study, 21 in Group I, 4 in Group II, 12 in Group III, 7 in Group IV and 38 in Group V. Various criteria were used to identify each of these types of inflammatory arthropathy and were based on those used in the human patient. The equivalent of the human diseases, rheumatoid arthritis, systemic lupus erythematosus and enteropathic arthritis have been identified in the dog and provide naturally occurring models for further study.

The study included assessments of the clinical, radiographical, haematological, biochemical, microbiological, immunological and pathological features. Synovial fluid analyses are also reported. Details of treatment are given.

Most of the canine rheumatoid arthritis patients showed a non-septic symmetrical polyarthritis, sometimes with systemic illness (pyrexia, anorexia, lethargy) with destructive bony changes visible on the joint radiographs. Other lesions besides those of the joints were identified in some cases. The cases of systemic lupus erythematosus were characterised by a polyarthritis associated with autoimmune haemolytic anaemia, nervous disease or albuminuria. The dogs with simple infectious arthritis had

involvement of one or two joints only and systemic signs were rare. Bacterial organisms were cultured from the joints in all cases. Some of these dogs were presented with only a mild lameness associated with a "low-grade" joint infection. Those dogs with arthritis and bacterial endocarditis generally had systemic signs and a cardiac murmur. Often, several joints were involved but not as many as in the rheumatoid and idiopathic cases. In addition to a septic arthritis, most of the dogs with bacterial endocarditis had a non-septic (possibly immune complex) synovitis. Sometimes bony destructive lesions were seen on the radiographs of infected joints. Dogs with idiopathic arthritis most often showed a non-destructive, bilaterally symmetrical, non-septic polyarthritis and the majority also exhibited systemic illness. The idiopathic group comprised those dogs which did not satisfy the criteria for the other types of inflammatory joint disease. Several of these dogs showed lesions of other body systems. Two dogs classed as cases of enteropathic arthritis had a polyarthritis associated with an ulcerative colitis.

Routine haematological and biochemical examinations revealed several abnormalities in all five groups of dog, e.g. anaemia, elevated erythrocyte sedimentation rate, elevated blood enzymes, raised blood globulin and proteinuria.

The histopathological examinations of the joints demonstrated a marked synovitis in all groups of dog. Villous hypertrophy of the synovial membrane was most often seen in the rheumatoid dogs. The production of a granulation (pannus) tissue associated with cartilage and bone destruction was most commonly seen in the rheumatoid and

infectious groups of dog. The direct immunofluorescent technique was used to study the immunopathology of the synovitis in the five groups. Evidence for locally produced immune complexes was obtained in all groups. Evidence for the deposition of circulating immune complexes in the blood vessel walls of the synovium was obtained in some of the rheumatoid, bacterial endocarditis and idiopathic dogs.

An important part of the study was the development and assessment of laboratory tests for the identification of certain autoantibodies in the dog, particularly antinuclear antibody and rheumatoid factor. The most useful tests were the indirect immunofluorescent test for antinuclear antibody, using frozen rat liver sections as the substrate and the modified Rose-Waaler test using sheep red blood cells coated with dog antibodies, for rheumatoid factor. Antinuclear antibody was detected in significant titre in dogs with systemic lupus erythematosus and rheumatoid factor in the majority of dogs with rheumatoid arthritis. However, significant levels of antinuclear antibody and rheumatoid factor were found in dogs with a variety of diseases other than inflammatory joint disease and low titres of these autoantibodies were found in the normal dog population. Certain commercial tests available for the detection of antinuclear antibody and rheumatoid factor in man were unreliable in the dog. A deoxyribonucleic acid binding radio-immunoassay for the detection of anti-deoxyribonucleic acid antibodies was not applicable to the dog because of non-specific binding factors. The LE-cell reaction proved to be an insensitive test in the dog.

The direct Coombs' (antiglobulin) test was used to show red blood cell autoantibodies in the dog and the direct antiglobulin consumption

test to show white blood cell autoantibodies. Positive Coombs' tests were noted in the rheumatoid, systemic lupus erythematosus and idiopathic groups of dog. White blood cell autoantibodies were only demonstrated in two dogs, one from the rheumatoid group and one from the systemic lupus erythematosus group.

Treatment varied between the different groups of dog. Prednisolone was the most effective drug for the rheumatoid, systemic lupus erythematosus and idiopathic groups. Gold therapy was helpful in a few of the rheumatoid dogs. The non-steroidal anti-inflammatory drugs were not very effective. Drainage tubes were inserted into the infected joints of the Group III dogs and allowed local irrigation with antibiotic solutions; systemic antibiotics were also used in these dogs. All cases of bacterial endocarditis died or had to be destroyed on humane grounds, despite antibiotic therapy.

The public health implications of these diseases in the dog are also discussed.

INTRODUCTION

Nosology, like every human endeavour, has practical ends - the recognition, investigation, prevention and treatment of disease - but classification for one purpose is not the same as classification for another.

E.G.L. Bywaters 1976

Joint disease in the dog has not been studied by pathologists, microbiologists and immunologists to anything like the extent it has been in the human patient. There is an inadequate appreciation of the underlying pathological processes in canine joint disease and the relationship of these to the clinical syndromes. In recent years, several attempts have been made to clarify joint disease in the dog but often these have been based directly on the human situation and are confused and incomplete.

In the 1930's and early 1940's when the science of bacteriology was at the height of its impact and popularity, the classification of arthritis and rheumatism in the human was exceedingly simple - pyogenic arthritis, serum sickness, rheumatic fever, gout, osteoarthritis and rheumatoid (or chronic infectious) arthritis. However, in the late 1940's and early 1950's a significant increase in research into these diseases was achieved, particularly in the field of immunology, leading to a dramatic change in the concepts of how rheumatic diseases should be classified. There are recognised at the present time over one hundred different causes of arthritis and rheumatism in the human patient (Shulman 1976).

This thesis reports the investigation of a group of joint diseases characterised by an inflammatory reaction within the synovial membrane - the group known as the"inflammatory arthropathies". The study has been made on clinical cases and has included detailed clinical, pathological, microbiological and immunological investigations.

Several types of canine inflammatory arthropathy have been identified.

An important aspect has been the determination of diagnostic criteria for the recognition of these different types of inflammatory joint disease in the canine patient.

TYPES OF JOINT DISEASE RECOGNISED IN THE DOG

In order to appreciate the group of dogs under investigation,it is helpful to realise the types of joint pathology recognised in this species. The classification of canine arthropathies given below is based on that previously used by the author (Bennett 1979).

1. MECHANICAL DISRUPTION

This follows severe traumatic injuries to joints producing luxation, subluxation and instability and/or fractures of the bony components of the joint. A traumatic arthritis will invariably be part of the pathological response.

2. NEOPLASIA

Neoplastic articular disease is rare in the dog but can affect joints in one of three ways:-

(a) tumours can originate from tissues within the joint

(b) tumours can arise from extra-articular structures and erode into the joint cavity

(c) tumours can metastasize to joint structures.

The commonest primary joint neoplasm is the "synovial sarcoma" (malignant synovioma) (Bennett 1979; Cotchin 1954; Lieberman 1956).

3. OSTEOCHONDROSIS

This is a disease which affects both growth plate cartilage and articular cartilage and involves a disturbance of endochondral ossification (Olsson 1976). Osteochondritis dissecans refers to the joint disease produced when the articular cartilage is affected.

4. DYSPLASIA

Abnormal growth and development of joints is well recognised in the dog e.g. hip dysplasia(Riser 1975),congenital elbow luxation (Campbell 1969) and congenital shoulder luxation (Vaughan and Jones 1969).

5. METABOLIC

Several different metabolic disorders can produce joint lesions. Nutritional secondary hyperparathyroidism and rickets for example, result in the resorption of bone from the epiphyses and in articular cartilage abnormalities(Pepper,Bennett,Brown and Taylor 1978). Hypervitaminosis A in cats produces bone deposition around the joints of the spine and limbs(Bennett 1976; English and Seawright 1964; Seawright, English and Gartner 1967; 1970)

Joint changes have been described in mucopolysaccharidosis of the cat (Cowell, Jezyk, Haskins and Patterson 1976; Jezyk, Haskins, Patterson, Mellman and Greenstein 1977) and dog (Dr.V.Perman - personal communication). Extensive deposits of bone are found around the joints of the limbs and spine and in the cat these appear similar to those of hypervitaminosis A.

It is thought that the disease is due to specific enzyme defects in the metabolism of the connective tissue mucopolysaccharides. Excessive amounts of mucopolysaccharide are excreted in the urine.

A primary connective tissue disease of dogs and mink has been reported(Arlein 1947; Hegreberg and Padgett 1967; Hegreberg, Padgett, Gorham and Henson 1969· Hegreberg, Padgett, Henson and Ott 1966; Wall 1947) which resembles the Ehlers-Danlos syndrome of man, a heritable disorder characterised by fragility of the skin and peripheral blood vessels, hyperextensibility and laxity of the skin and hyperextensibility of the joints,sometimes with synovial effusion. This genetically linked disease involves an abnormal metabolism of connective tissue.

6. ISCHAEMIC NECROSIS

This can arise as a complication of articular fractures where separated bony fragments can lose their blood supply and become necrotic within the joint. Ischaemic necrosis of the femoral head is thought to be the pathology involved in Legg-Calve-Perthes' disease (Bouckaert and Mattheeuws 1973; Lee 1974; Lee and Fry 1969; Ljunggren 1967).

7. PULMONARY HYPERTROPHIC OSTEOARTHROPATHY

Although this is primarily a disease of bone, changes have been reported in the joint structures, mainly oedema and fibrosis of the articular capsule (Brodey, Sauer and Medway 1963; Holling,Brodey and Boland 1961).

8. ARTHRITIS

Although this term strictly means inflammation of a joint, it is used in a broader sense to cover a whole range of joint diseases. Particular pathological terms are given to the latter and often several terms are applied to the same disorder e.g. osteoarthritis suggesting inflammation and osteoarthrosis implying degeneration refer to the same clinical disease where both inflammatory and degenerative changes can be identified. These terms should not be taken in their literal pathological sense since any particular joint disorder can show a variety of different pathological changes. It is usual, for example, to see both inflammatory and degenerative changes in most types of arthritis and one reason for this is the many different anatomical components of a joint which not only vary in the way they react to a disease process but are also limited in their possible responses.

A classification of arthritis in the dog is given below:-

(a) Traumatic arthritis

This is usually restricted to the type of joint reaction produced by a single acute injury to the joint(Ghadially and Roy 1969; Roy, Ghadially and Crane 1966). The lesions include stretching,tearing and laceration of the joint capsule and/or ligaments, fracture of intra-articular bone, damage to articular cartilage and tearing/displacement of menisci, and subluxation and instability of the joint.
A synovitis is present and there is usually a variable amount of haemorrhagic or serous effusion within the joint cavity. The changes

in the synovial membrane include hyperplasia and hypertrophy of the lining cells and infiltration of the supporting layer with inflammatory cells,primarily polymorphonuclear leucocytes, lymphocytes and macrophages. Areas of haemorrhage and deposits of haemosiderin are often present within the synovial membrane. Inflammatory changes may also be seen within the fibrous capsule and ligaments. A joint "sprain" is strictly an example of traumatic arthritis.

(b) Haemophilic arthritis

This is a very rare form of athritis in the dog(Swanton 1959). The clotting defect is similar to that of man and is transmitted as a sex-linked recessive characteristic. Affected dogs show recurring, painful and occasionally swollen joints associated with haemorrhage into the joint cavity and within the joint tissues. On gross examination the synovial membrane appears thickened with haemorrhages visible. Histologically,haemorrhages, haemosiderin deposits and hyperplasia/hypertrophy of the lining layer are usually evident. Articular cartilage damage,intra-articular adhesions and capsular fibrosis have also been recorded.

(c) Osteochondritis dissecans

This is a disease which has only comparatively recently been described in the dog(Arbesser 1974; Jones and Vaughan 1970; Olsson 1974; 1975a,b; 1976; Robins 1970; Vaughan and Jones 1968). The abnormality occurs in certain well-defined areas within particular joints and involves an excessive growth of articular cartilage which fails to

become ossified and resorbed. The affected cartilage becomes necrotic in its deepest layers and cracks and fissures pass from the deeper layers to the joint surface along which synovial fluid reaches subchondral bone and necrotic material reaches the joint cavity. Mild inflammatory changes occur in the synovial membrane; there is hyperplasia and hypertrophy of the lining layer with occasional accumulations of mononuclear inflammatory cells in the supporting layer. A separate fragment of cartilage is generally formed producing a cartilage flap or loose body within the joint. The areas affected in the dog are the caudal portion of the humeral head, the craniomedial part of the coronoid process of the elbow, the anconeal process of the elbow, the medial condyle of the humerus, either condyle of the femur but particularly the lateral and the medial ridge of the tibial tarsal bone.

The aetiology of osteochondritis dissecans is uncertain but a rapid growth rate is certainly believed to be a contributing factor (Reiland 1975).

(d) Osteoarthritis

Of the joint diseases recognised in the dog, this is by far the most common and has attracted most research interest. Osteoarthritis can affect any joint of the dog and is either primary where there is no apparent initiating cause or secondary where some other joint abnormality is present and leads to the development of the osteoarthritic changes. The secondary form of the disease is the commonest in the dog and examples include osteoarthritis secondary to hip dysplasia (Henricson, Norberg and Olsson 1966; Riser 1975), osteochondritis dissecans(Olsson

1975b; 1976), anterior cruciate rupture of the stifle joint (Gilbertson 1975a,b; Henricson and Olsson 1959; Hickman 1964; Paatsama 1952; Pond 1971; Pond and Campbell 1972; Singleton 1960; Strande 1967; Tirgari 1972, 1978) and patellarluxation (Bennett,Bauer and Maddock 1932; De Angelis 1971; Kodituwakku 1962; Leonard 1971). Possible examples of primary osteoarthritis are described by Campbell (1968:1971),Tirgari (1974) and Tirgari and Vaughan(1973;1975).

All the tissues which comprise a synovial joint may be affected by the osteoarthritic disease process. Articular cartilage shows loss of surface chondrocytes and flaking and fibrillation with eventual erosion and ulceration. Chondrocytes tend to form "cell-nests", so-called clumping,often associated with deep vertical clefts in fibrillated cartilage. Certain well-defined biochemical changes also occur in osteoarthritic articular cartilage and these include increased hydration, increased galactosamine - glucosamine molar ratio, and a greater extractability of proteoglycan aggregate with high molarity calcium chloride solution. The presence of marginal proliferations of bone (osteophytes,exotoses,lipping,spurs) is a consistent feature of osteo-arthritis. Osteophyte development is associated with the appearance of numerous blood vessels and can be recognised histologically in the experimental canine, anterior cruciate sectioning model as early as three days after severing the ligament(Gilbertson 1975a,b). This compares to a much later appearance of biochemical cartilage changes at three weeks. Sclerosis of subchondral bone is usually associated with loss of articular cartilage and the extreme stage of this is eburnation. Subchondral bone cysts are not uncommonly seen in man particularly in osteoathritic hip joints but are extremely rare in the

dog. Osteophyte production, subchondral sclerosis and cyst formation are all part of the process of bone remodelling. In addition, there is a general remodelling process of the internal architecture of the epiphysis such that it can, with time, take on a completely different shape.

Hypertrophy and hyperplasia of the synovial membrane is also a feature of osteoarthritis with accumulations of mononuclear inflammatory cells in the supporting layer, especially perivascularly. Occasionally a marked inflammatory infiltrate is seen, again principally mononuclear cells and these responses may in some cases represent reaction to "foreign material" such as hydroxyapatite crystals (Dieppe, Crocker, Tyler and Chapman 1976) or degenerative cartilage (Lloyd-Roberts 1953) released into the joint cavity. Fibrous thickening of the joint capsule regularly occurs and various changes in menisci may be present e,g. fibrillation, calcification, splitting, shredding, tearing and osseous metaplasia. Various vascular changes also occur such as hyperaemia of the bone marrow and vascular tufts can be seen penetrating the bone/cartilage interface. Vascular proliferation occurs in association with osteophyte formation, hyperaemia of the articular capsule is generally present and an impaired venous drainage from the bones of osteoarthritic joints is demonstrable by intraosseous phlebography.

(e) Miscellaneous

Other,quite rare joint diseases in the dog have been reported e.g. haemarthrosis producing synovial changes similar to those seen in haemophilia occurring in cases of multiple myeloma with a hyperviscosity

syndrome (Barrett 1977).

(f) Inflammatory arthropathies

This is a group of arthritic conditions in the dog which can be distinguished from the disorders described above and which are characterised by a marked inflammatory reaction within the synovial membrane. In addition, degenerative changes also occur within the articular cartilage associated with proteolytic enzyme release or the production of synovial granulation tissue(pannus). Various criteria need to be considered in distinguishing this group of diseases from other types of arthritis. It is possible to identify several different types of inflammatory arthropathy in the canine species and it is this group of disorders which are investigated in this thesis.

CANINE INFLAMMATORY ARTHROPATHIES

REVIEW OF THE LITERATURE

As writers become more numerous, it is natural for readers to become more indolent.

Oliver Goldsmith 1728-1774

INFECTIOUS ARTHRITIS(SEPTIC,SUPPURATIVE)

Inflammatory joint disease in the domestic animal has traditionally always been associated with infection and in the early texts, the pathogenesis of almost every recognised type of joint disease was explained by an infectious agent (Hutyra,Marek and Manninger 1938). But even in some quite recent texts(Jubb and Kennedy 1970)there is still the belief that inflammatory joint disease is always caused by a straight-forward infection. The following is quoted from Jubb and Kennedy's book "Arthritis in animals is almost always due to infection in the joint; this statement admits the possibility that non-infectious arthritis may occur such as that which,occurring in hyperimmunised horses, is suggested to be of the nature of a hypersensitivity". The terms septic and suppurative arthritis are used when pyogenic (pus-producing) organisms are responsible; the term infectious arthritis covers all inflammatory joint conditions caused by any pathogenic micro-organism.

Infectious arthritis is believed to be rare in the dog(Putnam and Archibald 1968) and is seen mainly in the larger breeds(Pedersen and Pool 1978). The pathology involves a marked synovitis characterised by hyperaemia,oedema and infiltration with polymorphonuclear cells (Putnam and Archibald 1968). Damage can occur to small blood vessels resulting in haemorrhage and areas of focal necrosis. Rupture of the synovial membrane can occur which allows purulent exudate to infiltrate the periarticular tissues. Destruction of articular cartilage may result from the release of proteolytic enzymes and the production of pannus tissue. In advanced cases the infection,associated with pannus

formation, can extend into the subchondral bone and even the metaphysis causing widespread joint destruction; ankylosis is a possible sequel.

Staphylococcus and Streptococcus are said to be the most commonly encountered organisms and bacteria may gain entrance to the joint by penetrating wounds or via the blood-stream (Pedersen and Pool 1978; Putnam and Archibald 1968). Sources of infection in cases of blood-borne disease, have included foci of sepsis in the skin, the umbilicus of neonates, the heart valves or endocardium, the bone and the urogenital tract (Pedersen and Pool 1978). Streptococcal arthritis in the dog has also been reported by Stofseth, Thompson and Neu (1937) and in the fox by Benedict, Wisnicky and McCoy (1941).

A case of polyarthritis in the dog from which Brucella organisms were isolated has been reported (Clegg and Rorrison 1968). Another suspected case of Brucella arthritis has been published (McErlean 1966) and although no organisms were isolated from this dog, Brucella serum agglutinins were present in high titre. In none of these cases was a detailed pathological examination of the joints recorded. Hall (1974) reported the isolation of B.abortus organisms from a urine sample taken from a dog with generalised stiffness. No joint examinations were recorded. Brucella canis was isolated from discospondylitic lesions in three dogs although there was no suggestion that any of the limb joints were involved (Henderson Hoerlein, Kramer and Meyer 1974).

Tubercular arthritis can be a feature of tuberculosis in the dog (Hjärre 1939; Olsson 1957). Again, detailed pathological descriptions

of the articular lesions in this species are lacking, but it is the human type of Mycobacterium tuberculosis which is most commonly isolated from the dog.

Two cases of polyarthritis with ankylosing spondylitis were reported in the dog associated with low titres of circulating antibodies to Erysipelothrix insidiosa, but the organism was not isolated from the joints of these dogs. The authors described the condition as rheumatoid arthritis (vide infra) without attaching very much importance to the erysipelas antibodies (Sikes, Hayes, Prestwood and Smith 1970). However, erysipelas infection associated with arthritis is reported in the dog (Hutyra et al 1938

An important syndrome in the dog is polyarthritis associated with bacterial endocarditis (Bennett, Gilbertson and Grennan 1978; Caywood, Wilson and O'Leary 1977). The joint pathology includes thickening of the synovial membrane with haemorrhages, pannus formation and destruction of cartilage and bone. The synovitis is characterised by hyperplasia and hypertrophy of the synovium with an infiltration of polymorphonuclear leucocytes, lymphocytes, plasma cells and macrophages. Occasionally, microabscesses and areas of necrosis are found and fibrin deposits are common. Several other organs can be involved in the disease process, e.g. kidneys, brain, spleen. The pathogenesis is thought to involve the embolic spread of infection from the heart lesion to various organs including the joints, although an immune complex type of hypersensitivity reaction has also been suggested (Gutman, Striker, Gilliland and Cutler 1972). Two further cases of polyarthritis associated with a possible bacterial endocarditis have been described and are discussed later (Lewis and Borel 1971; Lewis and Hathaway 1967). Three cases were reported

by Pedersen and colleagues (1976b) although very few details are given other than that joints were sterile and the articular pathology was of a non-erosive type. Other cases of lameness in dogs with bacterial endocarditis have been explained by muscle lesions (Knight, Kelly and Wordley 1972; Nielsen and Nielsen 1954; Shouse and Meier 1956). The routine inspection of joints is only rarely done at post-mortem examinations and thus articular involvement in a disease process may be missed in several instances - the three cases of bacterial endocarditis reported by Murdoch and Baker (1977) all had lameness and one even had swelling of and pain in several of its joints but no pathological examination of the joints in any of the dogs is reported.

Bacterial polyarthritis was mentioned by Lewis (1965) but no details were provided. Joint infections due to Mycoplasma species are mentioned in the dog (Pedersen and Pool 1978) but case-reports are lacking. Polyarthritis associated with bacterial L-forms has also been identified in dogs (Pedersen and Pool 1978).

Fungal arthritis has been reported in the canine species. Maddy (1958) reported bone and joint lesions in several dogs infected with Coccidioides immitis. Polyarthritis associated with Cryptococcus neoformans infection was described in a single dog by Kavit (1958); little information is given about the pathology but periarticular inflammation was the main lesion. Blastomycosis (i.e. infection with Blastomyces dermatitidis) can also cause joint lesions in dogs. This fungal disease occurs in the North American continent, particularly around the Great Lakes. The arthritis is associated with the presence of fungal organisms in the joint and these can be seen in a synovial fluid smear (Dr. J. F. Prestcott -

personal communication). Generally, other lesions such as pneumonitis are also present (Maksic 1968). Sporotrichosis (infection with Sporotrichum schenckii) producing inflammatory joint lesions has been described in dogs in France (Hutyra et al 1938).

A case of visceral leishmaniasis was reported in a dog by Thorson, Bailey, Hoerlein and Seibold (1955). The dog was lame with joint pain but no post-mortem examination of the joints was carried out. A similar case has recently been seen at the University of Davis, California,and examination of synovial fluid smears confirmed the presence of the protozoan organism within the joints (Dr. N. C. Pedersen - personal communication).

No reports of viral isolations from diseased joints of dogs have been found. However, some workers have identified intracytoplasmic structures in synovial membrane cells taken from canine rheumatoid arthritic patients, which could have been caused by viral injury (Dr. C. D. Newton - personal communication; Newton, Allen, Halliwell and Schumacher 1974). There is also some evidence that canine systemic lupus erythematosus,a multisystem disease which includes polyarthritis, could have a viral aetiology (Lewis, André-Schwartz, Harris, Hirsch, Black and Schwartz 1973; Quimby, Gebert, Datta, André-Schwartz, Tannenberg, Lewis, Weinstein and Schwartz 1978). Viral infections associated with polyarthritis in the human patient are not uncommon e.g. viral hepatitis, influenza, infectious mononucleosis, rubella, mumps, measles and smallpox (Schmid 1972).

NON-INFECTIOUS ARTHRITIS

Ideally, confirmation of a diagnosis of infectious arthritis is made by identifying an organism in the joint fluid and/or joint tissues. For the absolute proof that there is a relationship between the isolated organism and the disease process, Koch's postulates should be fulfilled. However, there are various reasons why attempts to isolate a micro-organism from an infected tissue might fail even though the organism is present (Van Pelt and Langham 1966). This is particularly true of viruses, mycoplasmas and bacterial L-forms which all require specialised techniques and culture media for their identification.

There are a few early reports of non-infectious inflammatory joint disease in the dog. One of the first was by Muller and Glass (1926) who described an inflammatory arthropathy in this species and referred to it as rheumatic arthritis. However, very few details are given by these authors. Hare (1927) applied the term "rheumatoid" to chronic arthritis that he observed in 14% of old horses. The illustrations of the lesions and the inclusion of such entities as spavin and ringbone indicated that the lesions were not equivalent to rheumatoid arthritis of man but were mainly examples of osteoarthritis. However, Hare (1928) did describe a non-septic "rheumatic disease" of dogs which was characterised by fever and acute painful fibrositis ("muscular rheumatism") or less frequently by painful swelling of the joints ("articular rheumatism"). Such diseases were thought to lead to a chronic arthritis (osteoarthritis). Hoare (1915) described acute articular rheumatism (also called rheumatic fever) in the horse, ox, dog and pig. This was again a non-septic arthritis which usually affected several joints simultaneously, often

in a bilaterally symmetrical and shifting fashion. The pathological descriptions included a serous synovitis often with fibrin deposits. The synovial membrane was often swollen, hyperaemic, opaque and reddish in colour and the synovial villi were vascular and enlarged. The articular cartilage became involved subsequent to the soft tissues and demonstrated softening, erosion and ulceration. No pannus tissue was described. Tendons could also be affected and cardiac lesions (endocarditis, pericarditis and myocarditis), pneumonia and pleuritis were also described. Congestion of the pharyngeal mucous membranes and sometimes also the gastrointestinal mucosae was noted in some animals and meningitis was reported as a rare complication. These animals were invariably pyrexic and "rheumatic nodes and nodules" were also reported. A subacute form of rheumatic fever was also described and a chronic articular rheumatism was mentioned as a sequela to the acute form; the latter was probably osteoarthritis. "Muscular rheumatism" was also reported in several species including the dog (Hoare 1915). Articular rheumatism (also called rheumatoid arthritis) was again described in the dog by O'Connor (1950). This disease was characterised by a "shifting" polyarthritis, associated with fever, periods of remission followed by relapses and loss of articular cartilage in some chronic cases. The condition was said to be "fairly common" in bovine, canine and porcine species.

Unfortunately, in the early literature, there has been confusion over the use of the terms "rheumatoid arthritis", "rheumatic fever", "rheumatism" and "rheumatic disease" which makes evaluation of these publications very difficult. Even in more recent years the term rheumatoid arthritis has been used indiscriminately in the dog without

any justification (Brewster 1969; Fennell 1969a,b; Mann 1969; Pond and Lee 1969). Chronic erysipelas polyarthritis in pigs has also been described as rheumatoid arthritis; the pathology of the chronic form is strikingly similar to rheumatoid arthritis of man and the joints are often sterile (Collins and Goldie 1940; Sikes, Neher and Doyle 1956).

In medical rheumatology, the terms rheumatoid arthritis and rheumatic fever refer to specific disease syndromes and several well-defined criteria are used in their diagnosis in the human patient (Boyle and Buchanan 1971; Ropes, Bennett, Cobb, Jacox and Jessar 1959). The terms should only be reserved for equivalent diseases in the veterinary species and not used as "blanket terms" to describe joint problems of obscure origin. However, the terms rheumatism, rheumatic disease, muscular rheumatism, articular rheumatism etc. are more general terms often applied to connective tissue disorders of unknown origin. Rheumatism may be defined somewhat vaguely as "a disease marked by inflammation of the connective tissue structures of the body, especially the muscles and joints and by pain in these parts" (Dorland 1965). The term "collagen disease" was introduced to cover a certain group of connective tissue disorders (Klemperer, Pollack and Baehr 1942). "Connective tissue disease" is used interchangeably with "collagen disease" and basically these terms refer to conditions characterised by vascular inflammatory lesions of a diffuse nature, by fibrinoid change and by degenerative and inflammatory changes in connective tissue. The connective tissue diseases in man generally include rheumatoid arthritis, systemic lupus erythematosus, progressive systemic sclerosis, dermatomyositis and poly-arteritis nodosa (Boyle and Buchanan 1971). Miller, Osborne and Hurvitz (1975) introduced the term "immunologically mediated canine polyarthritis"

to the veterinary literature and defined this as an "inflammation of joints associated with immunologic mechanisms"and was used to include rheumatoid arthritis and systemic lupus erythematosus

1. CANINE RHEUMATOID ARTHRITIS

The use of the term rheumatoid arthritis by O'Connor(1950) has been referred to above. The term was used again by Misener and Stanton (1951) although very few details of their single case are given. Another single case of rheumatoid arthritis in the dog was reported by Baumgarten and Siegmund (1952). These authors reported "rarefaction of the bones at the joints" on radiography. Radiographic evidence of bone destruction was also reported in several joints of a Pekingese dog by Liu,Suter,Fischer and Dorfman(1969). The radiological features included loss of mineralisation as well as "punched-out" areas of rarefaction. Some joints were so badly affected that luxation and disruption of the joints had occurred. A pathological examination of affected joints was carried out in this dog. Destruction of articular cartilage and its replacement by a rough, irregular pannus of granulation tissue was described. The synovial membrane was thickened with villous proliferation and a fibrinous exudate was evident. Histological examination of the synovial membrane showed varying amounts of collagenous connective tissue with hypertrophy and hyperplasia of the lining cells. The supporting connective tissue contained congested capillaries and numerous inflammatory cells,mainly lymphocytes,plasma cells and macrophages. Proliferative villi and pannus were seen in the joint spaces and in some areas pannus was replacing the articular cartilage and destroying subchondral bone; fibrous ankylosis was evident in some areas of certain joints.

Two further cases of canine rheumatoid arthritis were reported by Sikes and his colleagues (1970). The pathological findings were similar to those described by Liu et al(1969). In addition,oedema of the synovial membrane and thrombosis of some of the synovial blood vessels were described. Interestingly,these two dogs were reported as also having ankylosing spondylitis. The synovial intervertebral joints showed synovitis with connective tissue proliferation,articular cartilage destruction and ankylosis. Ossification of the annulus fibrosus forming bridges of bone across the intervertebral discs was described. Metaplasia of the discs with ectopic bone and trabeculae and marrow space formation was also evident. In some cases marrow spaces within the discs were continuous with those of the vertebral bodies on either side. Some ossification of the spinal ligaments had also occurred. It is true that proliferative bony changes on the vertebral bodies are common in mature dogs and in many instances are not associated with clinical signs(Hansen 1952; Pommer 1933). However, the pathological lesions described by Sikes and co-workers,particularly those of the joints between the articular processes,could suggest a similar pathogenesis to those changes seen in the peripheral joints of their two cases. Although the spine can be involved in rheumatoid arthritis in man(Bland 1967; Bland,Davis,London,van Boskirk and Duarte 1963; Boyle and Buchanan 1971) it is usually only the cervical spine and there is generally very little osteophytosis. Ankylosing spondylitis in man is regarded as a distinct entity(Boland and Present 1945; Boyle and Buchanan 1971; West 1949) which affects the diarthrodial joints of the spine but also the intervertebral discs and sacro-iliac joints (Cruickshank 1960). It is however, also possible for patients with ankylosing spondylitis to show peripheral joint involvement(Sharp 1962)

and the pathology in these cases is very similar to that of rheumatoid arthritis.Patients with ankylosing spondylitis are generally negative for serum rheumatoid factor(Kellgren and Ball 1959). Sikes et al(1970) were the first workers to attempt the identification of rheumatoid factor in dogs; their two reported cases were positive for circulating rheumatoid factor although they used a human latex particle agglutination test,and tested only one normal control dog for comparison. These two dogs also showed low titres of antibodies against Erysipelothrix insidiosa but the significance of this was in doubt.

Another case of canine rheumatoid arthritis was reported by Lewis and Borel(1971). These workers identified rheumatoid factor in high titre but also showed LE-cells and a high titre of antinuclear antibody. There are several features of this case which could question the validity of the diagnosis. At a re-examination of the dog,a B-haemolytic Streptococcus was cultured from the blood and from two joints. At post-mortem examination there were lesions involving a number of organs besides the joints including the heart where an acute necrotising valvulitis was described although no record of a bacteriological culture from the lesion is mentioned. It has been widely reported that bacterial endocarditis with polyarthritis in the human patient can be associated with both circulating rheumatoid factor and anti-nuclear antibody(Bacon,Davidson and Smith 1974; Gutman et al 1972; Messner,Laxdal,Quie and Williams 1968; Williams and Kunkel 1962). A similar phenomenon has been reported in the dog(Bennett et al 1978). The joint pathology reported by Lewis and Borel included villous proliferation and hypertrophy of the synovial membrane,fibrin deposition, congestion of the synovium with perivascular plasmacytosis, necrotising

arteritis and disseminated areas of focal necrosis of collagen within the joint capsules. Occasionally,thrombosis of small blood vessels and mineralisation of necrotic areas were seen. The only evidence of articular cartilage destruction was the radiographic,somewhat subjective, report of a diminished joint space in the carpal and tarsal joints. It is also possible that this case may have been an example of systemic lupus erythematosus with a superimposed secondary infection,since many different body systems were involved and there were high levels of circulating antinuclear antibody. In man it is not unusual to find lupus patients with rheumatoid factor as well as antinuclear antibody in the blood(Kellgren and Ball 1959; Singer 1961; Ziff 1957). However it is also true that some human rheumatoid patients have circulating antinuclear antibodies(Alexander,Bremner and Duthie 1960; Hall,Bordawil, Bayles,Mednis and Golins 1960; Ward,Johnson and Holborow 1964).

Another case of polyarthritis in the dog was described from Boston which at post-mortem examination had an endocarditis and this time organisms were isolated from the heart lesion(Lewis and Hathaway 1967). This case was presented as canine systemic lupus erythematosus although the joint disease was confusingly described as rheumatoid arthritis. The joint lesions included a synovitis characterised by a diffuse mononuclear cell infiltration with areas of haemorrhage,hyperaemia,necrosis and fibrin deposition. There were also areas showing polymorphonuclear cells. Pannus formation was present associated with articular cartilage erosion and subchondral fibrosis. Several other organs showed lesions of various types,most of which could be explained by a subacute/chronic bacterial endocarditis. This dog was positive for LE-cells and circulating antinuclear antibody. Both these case-reports from Boston are difficult

to evaluate. It is certainly possible that more than one pathological process was involved in these dogs although the syndrome in both animals could be explained by a bacterial endocarditis. However, the way in which a bacterial endocarditis is initiated is unknown and it has been suggested that some damage needs to occur to the heart wall before a bacteraemia or septicaemia will colonise the endocardium. Various theories as to how this initial damage may be produced have been suggested and certain "stress situations" have been used to experimentally produce heart lesions in dogs (Trautwein,Brass,Kerstein, Ernst, Schneider,Schulz, Amtsberg, Bisping, Kirchoff and Schole 1973). Other disease processes may thus damage the endocardium by creating a stress situation or even by some direct affect and predispose it to bacterial infection. Bacterial endocarditis could arise secondary to a connective tissue disorder such as rheumatoid arthritis or systemic lupus erythematosus and this is not uncommon in human patients(Cardoe 1978; Hejtmancik, Wright, Quint and Jennings 1964). It is also well documented that the joints of patients with rheumatoid arthritis or systemic lupus erythematosus are particularly susceptible to secondary infection.

Yet another case of endocarditis with septic polyarthritis where the possibility of rheumatoid arthritis is discussed has been described (Schiefer, Hurov and Seer 1974). Certainly, in this patient the classic vegetative thrombus type of lesion normally associated with bacterial endocarditis in the dog was absent. Unfortunately no pathological examination of the joints was carried out and no tests for rheumatoid factor or antinuclear antibody were performed. Interestingly this dog exhibited a diffuse fibrosis of the lungs with pulmonary

emphysema. It was suggested that the primary pathological changes were in the joints which initiated a generalised hypersensitivity reaction causing the pulmonary lesions. It is true that a number of lung diseases can be associated with rheumatoid arthritis in man(Boyle and Buchanan 1971; Walker 1978) including pulmonary fibrosis(Brannan, Good, Divertie and Baggenstoss 1964; Stack and Grant 1965; Thompson and Leathart 1965) but most of these lesions can occur in patients without rheumatoid arthritis. The pathogenesis of pulmonary fibrosis might involve the deposition of immune complexes in the lung capillaries(Tomasi, Fudenberg and Finby 1962) and any antigen/antibody reaction such as that associated with infection, could be involved.

The term rheumatoid arthritis was again used rather loosely to describe polyarthritis in 6 dogs, 3 of which were positive for LE-cells (Lewis 1965). No clinical or pathological details of these dogs are given.

The only acceptable case of rheumatoid arthritis ever reported in the U.K. appeared in the early 1970's (Halliwell, Lavelle and Butt 1972). This dog showed marked rarefaction of the epiphyses of several joints on radiography indicating bone destruction. The presence of rheumatoid factor in the blood was confirmed.

Another report of canine rheumatoid arthritis in Europe was made by Wentink, Stokhof and Goudswaard from Utrecht in 1974. This dog showed polyarthritis with fever and there was clinical and electro-cardiological evidence of heart disease which was diagnosed as "pancarditis". Subcutaneous nodules were present on the extensor surfaces of the elbows.

Antinuclear antibody at a low titre was detected in only one of five blood samples although rheumatoid factor was positive in three of five samples and at a high titre in two. No abnormalities were reported in the joint radiographs. The clinical features could be consistent with another case of bacterial endocarditis although there was clinical improvement following prednisolone therapy. Unfortunately, no pathological studies were carried out. A year earlier, Wentink published a review of the literature on rheumatoid arthritis in the dog but reported no further original cases(Wentink 1973).

The first clear-cut case-report of canine rheumatoid arthritis in the U.S.A. where strict diagnostic criteria were imposed was made by Newton,Allen,Halliwell and Schumacher(1974). This dog was positive for rheumatoid factor and showed destructive changes of articular cartilage and bone in several joints. The synovial membrane from affected joints was thickened and folded with an extensive infiltration of lymphocytes and plasma cells, with some polymorphonuclear cells in the supporting layers. Haemosiderin-filled macrophages were plentiful. Fibrovascular proliferations (pannus) extended from the synovial membrane and were associated with cartilage and bone resorption. These authors regarded their case as being more typical of juvenile rheumatoid arthritis (or Still's disease) as seen in children (Ansell and Bywaters 1959; 1962; Bywaters 1967; Calabro and Marchesano 1967a; Norcross, Lockie and MacLeod 1958; Schlesinger,Forsyth,White,Smellie and Stroud 1961; Still 1897). The main factors suggesting this were the presence of fever,lymphadenopathy and the rather low titre of rheumatoid factor in the dog's blood. Fever is common in juvenile rheumatoid arthritis (Calabro and Marchesano 1967b; Grossman, Ozoa and Arya 1965) as is lymphadenopathy sometimes also with

splenomegaly (Ansell and Bywaters 1963). Rheumatoid factor is also often absent (Bywaters, Carter and Scott 1959; Sievers, Ahvonen, Aho and Wager 1963; Toumbis, Franklin, McEwen and Kuttner 1963)although the use of a highly sensitive agglutination test can detect many more positives (Edstrom 1958; McEwen, Ziff, Carmel, Di Tala and Tanner 1958). Following their original single case-report, the Pennsylvanian group of workers later produced a report of 10 cases of canine rheumatoid arthritis (Halliwell 1975; Newton, Lipowitz,Halliwell, Allen, Biery and Schumacher 1976). The pathological lesions of the joints were similar to those described in their first case, but in addition included areas of haemorrhage and necrosis in the synovial membrane and fibrin deposits. All dogs were positive for circulating rheumatoid factor although some only showed low titres. An additional 8 cases of rheumatoid arthritis seen in the dog at the University of Pennsylvania were reported in a recent review article by Halliwell (1978). Several other review articles on canine rheumatoid arthritis have appeared from the Pennsylvanian School (Biery and Newton 1975; Lipowitz and Newton 1975; Newton and Lipowitz 1975).

A single case of canine rheumatoid arthritis was described by Rudy, Hohn and Harrison in 1972. This dog had shown lameness for two weeks when presented. Bony destructive changes were present on the radiographs and both radio-carpal and tarso-metatarsal joints showed instability. Histological examination of synovial membrane biopsies revealed a proliferative synovitis with foci of plasma cells and lymphocytes beneath the hypertrophic lining layer. Rheumatoid factor was present in this dog but the details of the test used, other than that it was a latex fixation test, were not given.

A report of eight cases of rheumatoid arthritis in the dog has been made from Cornell University (Alexander, Begg, Dueland and Schultz 1976). No detailed accounts of the joint pathology are given although radiographic evidence of bony destruction is described. Unfortunately, reproduction of some of the radiographs in this publication is poor. Another case report from the Cornell School has been published (Scott 1975). Several joints were affected in this dog and the radiographs showed evidence of subchondral bone rarefaction and narrowing and irregularity of joint spaces in addition to soft tissue swelling and areas of proliferative periostitis. A high serum rheumatoid factor titre was found in this dog. Histological examination of a synovial membrane biopsy revealed lining cell hyperplasia and an infiltration of plasma cells, lymphocytes and polymorphonuclear leucocytes into the supporting layer.

Pedersen, Pool, Castles and Weisner (1976a), from the University of California, Davis, reported eight rheumatoid dogs showing destructive changes in several joints. These authors reported similar pathological features to those already discussed. These included villous hypertrophy of the synovial membrane with hypertrophic lining cells and a mononuclear inflammatory infiltrate in the supporting layer. Fibrin deposits were common. Pannus tissue was present over the articular cartilage in several joints and there was erosion of cartilage and subchondral bone in many instances, especially at the joint margins. Fibrous ankylosis of the carpal and tarsal joints was seen in some dogs. In addition, they described involvement of tendon sheaths and the appearance of granulation tissue arising from the marrow cavity and eroding through the subchondral bone and articular cartilage. Many of the dogs in this series

however were negative for rheumatoid factor(5 of 7 dogs tested). There was no suggestion of an infectious process in any of the dogs. A review article by two of the Californian workers stated that canine rheumatoid arthritis occurs mainly in the small or toy breeds of dogs with an average age of onset of approximately 4 years (Pedersen and Pool 1978).

2. CANINE SYSTEMIC LUPUS ERYTHEMATOSUS

This disease syndrome was first reported in the U.S.A. by Lewis, Schwartz and Henry (1965). Systemic lupus erythematosus is a multisystem disease i.e. there is simultaneous or sequential involvement of different body systems or components in the disease process. Of the 7 cases reported by these authors,all showed haemolytic anaemia associated with red blood cell autoantibodies, 6 had thrombocytopaenia and 6 had some evidence of renal disease. Two dogs also showed intermittent lameness although no pathological investigations of the joints were undertaken. Six of these cases were positive for the presence of LE-cells and 2 also showed circulating rheumatoid factor although the test used for the latter was not specified. Later the same year, further cases of systemic lupus erythematosus in the dog were reported from the Angel Memorial Animal Hospital in Boston(Lewis 1965). This publication was mainly reporting an evaluation of the LE-cell phenomenon in the dog and did not give detailed case-reports. Another publication from this centre appeared describing 7 cases of canine systemic lupus erythematosus (Lewis,Schwartz and Gilmore 1965) but these would appear to be the same cases described by Lewis,Schwartz and Henry(1965). In these earlier reported cases of canine lupus,the main features were haemolytic anaemia, thrombocytopaenia and glomerular lesions; joint lesions although recog-

nised were not a prominent finding. The case of polyarthritis associated with systemic lupus erythematosus reported by Lewis and Hathaway(1967) has already been discussed above with the suggestion that this could be a case of bacterial endocarditis. A few years later, 8 cases showing positive LE-cell tests were reported from the Californian Veterinary School(Schalm and Ling 1970). In these dogs, haemolytic anaemia and thrombocytopaenia were not significant findings and glomerulonephritis was suspected in only one case. All these dogs did show lameness,some with joint swellings and some with muscle wastage. Three of these cases were attributed to systemic lupus erythematosus but the remainder were not and it is not clear how this distinction was made. In some cases it is difficult to know whether there was multi-system involvement in the disease process. It is also interesting that one lame dog had a heart murmer,pyrexia,responded to antibiotic treatment and died suddenly after corticosteroid treatment had been instigated; no post-mortem examination was carried out. This again could have been a case of bacterial endocarditis with joint involvement. In fact, 2 other dogs in this series could have been cases of bacterial endocarditis.

The first publication suggesting the occurence of canine systemic lupus erythematosus in Europe was from France(Cassan 1968). This investigator described 51 dogs with a variety of non-specific clinical signs; 3 dogs showed polyarthritis. However, only 2 of these 51 dogs demonstrated a significant level of antinuclear antibody in their serum and neither of these dogs had joint involvement. Another report of lupus in the dog in France appeared in the early 1970's (Lapras and Oudar 1971a;b). Most of the description and all the illustrations of the disease were in

fact taken from Dr. Lewis's publications. However, the French workers did mention 50 dogs "all showing, to various degrees, symptoms resembling those of DLE (systemic lupus erythematosus) (chronic nephritis with albuminuria, polyarthritis, heart disease, eczematous dermatitis)" of which only 4 were positive for antinuclear antibody. None of these four dogs had polyarthritis. More recently, four Alsatian dogs with pyrexia and polyarthritis were reported from the Veterinary School at Lyon (Monier, Schmitt, Perraud, Fleury, Giand and Lapras 1978). These dogs also showed wasting and albuminuria and two dogs also had skin lesions. High levels of antinuclear antibody in the blood were detected in all four dogs but no positive LE-cell preparations were recorded. Two dogs also had low titres of serum rheumatoid factor. These authors diagnosed a "lupus-like syndrome" in these dogs. Unfortunately no pathological investigations of the joints were carried out.

A single case-report of a dog with anaemia and thrombocytopaenia was described as systemic lupus erythematosus by Alexander, George and Moffa (1975). This dog was positive for LE-cells, antinuclear antibody and rheumatoid factor. The methods used to identify the latter two autoantibodies were not specified and the results were not quantitated. This dog was negative for red cell autoantibodies by the Coombs' anti-globulin test.

The only publication suggesting the existence of canine systemic lupus erythematosus in the U.K. is that of Jones and Darke (1975). They reported 6 dogs with autoimmune haemolytic anaemia, based on the papain test demonstrating red cell autoantibodies. Three of these dogs showed evidence of joint disease and one of these was diagnosed as systemic

lupus erythematosus. The test used by these authors to detect antinuclear antibodies was a human, commercial latex slide agglutination test, the usefulness of which is in doubt for the canine species (see Section VI). The criteria used by Jones and Darke to diagnose systemic lupus are unclear; seven of their dogs, for example, were positive with the latex test although only one of these was thought to be suffering with systemic lupus erythematosus.

Various review articles have appeared in which the features of canine systemic lupus erythematosus are described (Anon 1972; Dear 1970; Lapras 1972; Lewis 1972; 1974). Unfortunately, Dr. Lewis persistently describes rheumatoid arthritis as the joint disease found in canine systemic lupus erythematosus. The pathology described by this author is certainly similar to that expected in rheumatoid arthritis of man i.e. a marked synovitis with pannus formation leading to destruction of articular cartilage and subchondral bone (Allison and Ghormley 1931; Boyle and Buchanan 1971; Collins 1949; Fisher 1929; Hoffa and Wollenberg 1908; Nichols and Richardson 1909; Parker and Keefer 1935). However, this type of destructive joint pathology is only rarely seen in human systemic lupus erythematosus (Cruickshank 1959). It is more usual to find a non-erosive type of joint inflammation (Boyle and Buchanan 1971; Dubois and Tuffanelli 1964).

More recently, 29 dogs with polyarthritis were reported as having systemic lupus erythematosus (Pedersen, Weisner, Castles, Ling and Weiser 1976b). The pathology was described as a non-infectious, non-erosive arthritis. The synovial membrane was thickened and infiltrated with varying proportions of inflammatory cells. The primary infiltrating

cell was the neutrophil, particularly in the superficial layers,although mononuclear cells were also seen especially adjacent to blood vessels. Loss of the synovial cell layer was occasionally seen in severe cases and a fibrinous exudate often covered the synovium. Villous hyperplasia was not a feature and pannus formation and marginal erosions of subchondral bone were absent. Fibrosis of the deep supporting layers were seen in long-standing cases. This pathology is certainly more consistent with that seen in human systemic lupus erythematosus. However, it is again not clear how the authors were diagnosing systemic lupus erythematosus. For instance, only 19/29 dogs had a positive LE-cell test, only 10/15 tested had anti-deoxyribonucleic acid antibodies and only 7/29 were positive for red cell autoantibodies by the Coombs' antiglobulin test. The test used to detect anti-deoxyribonucleic acid antibodies in these dogs may also be of dubious value (see Section VI). The authors stated that "a number of these dogs had other systemic abnormalities" but it is not clear just how many. Another case of non-erosive polyarthritis associated with systemic lupus erythematosus was reported from the Davis School,this time with a concomitant polymyositis (Krum,Cardinet, Anderson and Holliday 1977). This dog was positive for LE-cells and for a non-specified antinuclear antibody test. One of the cases reported by Pedersen et al(1976b) also had a polymyositis. Muscle disease does occur in systemic lupus erythematosus (and rheumatoid arthritis) in man but the pathological features are often minor and non-specific (Adams, Denny-Brown and Pearson 1962; Boyle and Buchanan 1971). Rarely, a more serious, diffuse myopathy occurs in systemic lupus erythematosus(Sibrans and Holley 1967) although its occurrence may be related to certain drugs used in treating lupus patients (Perkoff, Silber, Tyler, Cartwright and Wintrobe 1959; Whisnant, Espinosa, Kierland and Lambert 1963). There

is another syndrome in man where muscle involvement is the primary complaint and the pathological changes are more consistent with those described by Krum and his colleagues (1977)i.e. myofibre necrosis and phagocytosis, myofibre regeneration, perivascular and interstitial infiltration of macrophages, lymphocytes and plasma cells, Type I and II myofibre degeneration and vacuolation. This condition is known as dermatomyositis although various classifications of it have been described (Pearson 1966) and one type is characterised principally by a polymyositis. Arthritis occurs in about one third of patients with this disease and in some cases it can resemble rheumatoid arthritis (Pearson 1966). Approximately 25% of patients with dermatomyositis have positive tests for antinuclear antibody (Beck 1963) but tests for LE-cells are usually negative (Barwick and Walton 1963; Diessner, Howard, Winkelmann, Lambert and Mulder 1966; Venters and Good 1963). Rheumatoid factor is also present in some patients (Pearson 1959; Shy and Silverstein 1965; Singer 1961).

In a recent review article, Halliwell (1978) reported 18 cases of canine systemic lupus erythematosus diagnosed at the University of Pennsylvania. Of these, 10 exhibited lameness often in the form of a polyarthritis. Other clinical signs included anaemia, fever, proteinuria, thrombocytopaenia, leucopaenia, skin disease and signs of nervous disturbance (seizures). All 18 dogs had positive LE preparations and/or significant titres of antinuclear antibody in the blood.

In the 1960's when Dr. Lewis and his colleagues first recognised canine systemic lupus erythematosus, affected dogs were acquired for breeding and establishing a colony of dogs in which genetic and environ-

mental factors related to the disease could be analysed. A report on these dogs appeared in the early 1970's (Lewis and Schwartz 1971) describing serological abnormalities including positive LE-cell tests, positive indirect immunofluorescent tests for antinuclear antibodies and positive bentonite agglutination tests for rheumatoid factor. However, no clinical signs related to systemic lupus erythematosus had been seen in these dogs. Very recently, a variety of clinical signs were described in some of the colony dogs (Quimby, Jensen, Nawrocki and Scollin 1978) although there is no specific mention of arthritis. It should be realised that these dogs had been extensively in-bred before clinical signs appeared and it is relevant to consider just how important the in-breeding itself might have been in creating the pathological changes.

3. CANINE POLYARTERITIS NODOSA

Polyarteritis nodosa is another connective tissue disease of man. It is a true polyarteritis and may affect any part of the blood vessel. Fibrinoid necrosis is a prominent feature of the early lesion and is accompanied by an intense polymorphonuclear inflammatory reaction. Necrosis may involve the entire circumference of the vessel or be limited to a focal area within the wall. Lymphocytes and plasma cells are prominent in the peripheral zone of the lesion and thrombosis of affected vessels may occur with resulting infarction. Fibrosis of the vessel wall occurs during the healing phase. Various classifications of polyarteritis nodosa have been suggested in the human patient(Moskowitz, Baggenstoss and Slocumb 1963; Zeek 1952; 1953). Incidental pathological lesions similar to the above have been described in a number of veterinary

species including the dog (Jubb and Kennedy 1970). A single clinical case of polyarteritis nodosa has been described in the dog, one feature of which was lameness and at post-mortem examination, congestion of the synovial membranes in the hip and stifle joints was reported (Lewis et al 1965). This disease was also reviewed with reference to the veterinary species by Lapras (1972).

4. CANINE SJØGREN'S SYNDROME

This condition was first described in the human patient by the Swedish ophthalmologist Henrik Sjøgren (1933). It is usually characterised by keratoconjunctivitis sicca ("dry eye"), xerostomia ("dry mouth") and some form of connective tissue disease, usually rheumatoid arthritis but systemic lupus erythematosus, progressive systemic sclerosis, polymyositis or polyarteritis nodosa have all been reported (Bloch, Buchanan, Wohl and Bunim 1965). An extensive lymphoid infiltration into the lacrimal and salivary glands is seen. Affected patients may show circulating rheumatoid factor (Bunim, Buchanan, Wertlake, Sokoloff,Bloch, Beck and Alepa 1964), antinuclear antibody (Beck, Anderson, Bloch, Buchanan and Bunim 1965) and LE-cell preparations may be positive(Bloch et al 1965). Organ specific autoantibodies directed against salivary tissue are also present (Bertram and Holberg 1965; Feltkamp and van Rossum 1968; Morgan 1954). Thyroid autoantibodies may also be found and some patients have clinical lymphocytic (Hashimoto's) thyroiditis (Kesslar 1968).

Keratoconjunctivitis in the dog associated with systemic lupus erythematosus and lymphocytic thyroiditis has been reported by Quimby

and his colleagues(1978). It is not clear in this publication whether the authors were reporting just 2 dogs in the lupus colony,or whether they were also reporting 2 "field" cases of Sjøgren's syndrome. Very few details are given but certainly in one case there was a lymphocytic infiltration of the nictitating and lacrimal tear glands as well as of the mandibular salivary gland. Two dogs had circulating antibodies against the nictitating tear gland but not the lacrimal gland. Arthritis was not reported in these dogs.

A case of keratoconjunctivitis sicca with xerostomia was reported in an 8 year old Cocker Spaniel bitch by Staman, Goudswaard, Stades and Wouda (1979) and was diagnosed as Sjøgren's syndrome. This dog also had skin and ear disease and these workers reported "hyperlaxidity and crepitation" in the carpal, tarsal and stifle joints. Radiographic examination of these joints showed "a few secondary changes". The dog was positive for circulating antinuclear antibody with a speckled fluorescent pattern. Circulating rheumatoid factor was detected using sheep red blood cells coated with dog IgG. Unfortunately, the titres of antinuclear antibody and rheumatoid factor are not specified. Antibodies against the parotid salivary gland were not demonstrable. Histological examination of the mandibular salivary gland from this dog revealed intralobular infiltration with lymphocytes and plasma cells. Sialoadenitis has been reported in one dog with rheumatoid arthritis but no further details were provided (Pedersen et al 1976a).

5. CANINE ENTEROPATHIC ARTHRITIS

Pedersen and Pool (1978) claimed to have recognised polyarthritis in a single dog with chronic ulcerative colitis and in several dogs with a more fulminating enterocolitis. The pathological changes in the synovial membrane and fluid were said to be indistinguishable from those

described in systemic lupus erythematosus and idiopathic polyarthritis (vide infra). Another case was reported by Quimby and colleagues(1978). Histopathological examination of the colon revealed necrosis and denudation of the mucosa with infiltration of plasma cells, small lymphocytes and occasional eosinophils into the submucosa and tunica propria. The synovial membrane from affected joints showed villous hypertrophy with hyperplasia of the lining cells, a diffuse cellular infiltration which was predominantly plasma cells in some areas and polymorphonuclear cells in others, and fibrosis. No destruction of articular cartilage or bone was reported. This dog, however, was positive for LE-cells, antinuclear antibody and rheumatoid factor. Human patients with enteropathic arthritis are usually negative for these laboratory tests (Haslock 1978).

One of the cases described as systemic lupus erythematosus by Pedersen and his colleagues (1976b) also had an ulcerative colitis. Gastrointestinal disease is recognised in human patients with lupus (Boyle and Buchanan 1971; Harvey, Shulman, Tumulty, Conley and Schoenrich 1954; Kurlander and Kirsner 1964) but it is possible that the case reported by the Californian authors was another example of canine enteropathic arthritis.

6. CANINE IDIOPATHIC INFLAMMATORY JOINT DISEASE

This term undoubtedly reflects the ignorance in our understanding of the aetiology and pathogenesis of this group of inflammatory arthropathies The affected dogs cannot unequivocally be fitted into any of the categories reviewed above but in some cases, insufficient assessment of the patients

is a major reason why the reports have been included in this idiopathic classification.

One case was reported by McDonald and Loomis (1968), although the detailed description is strongly suggestive of an infectious polyarthritis. All limb joints were affected and histological examination showed a severe, acute, purulent inflammatory reaction of the synovial membrane. In the deeper layers, many plasma cells were seen and extensive oedema of the joint capsule was also present. An epidural acute/subacute meningitis was also found. This dog exhibited a severe leucocytosis. Bacterial cultures of cerebrospinal and synovial fluids were negative but antibiotics had been used prior to the sampling for bacteriological investigation. No focus of infection was reported at the post-mortem examination.

Polyarthritis was again reported in a single case by Ajmal and Hayward (1970) and compared with three cases of osteoarthritis. These authors introduced the somewhat vague term, "true inflammatory arthritis" to describe the pathological features of their polyarthritic case. The synovial membrane from the affected joints of this dog showed villous hypertrophy with deposits of fibrin both within and on the surface of the membrane. Necrosis of the lining cells was present in some areas, hyperplasia in others. Polymorphonuclear leucocytes, plasma cells, lymphocytes and macrophages were seen in the supporting layer. Granulation tissue was also present but the articular cartilages did not show obvious lesions. The authors suspected an infection although no organisms were cultured (bacterial and mycoplasmal) and no agglutinins against Erysipelothrix or Brucella antigens could be

demonstrated in the synovial fluid. Again, the animal had been receiving antibiotics prior to the taking of samples for bacterial and mycoplasmal cultures.

Fairly recently a detailed report of 17 cases of polyathritis, all in the Greyhound breed, was published(Huxtable and Davis 1976). The synovial membrane from affected joints showed hyperplasia often with the formation of fibro-vascular villi and a variable inflammatory infiltration of polymorphonuclear cells,plasma cells and lymphocytes. The lymphocytes tended to occur in groups, sometimes extremely dense and confluent. In some sections, there was a severe destructive reaction with masses of organising fibrin overlying the damaged membrane. Foci of recent haemorrhage were sometimes found in these areas. Macrophages and mast cells were also commonly seen and there was often proliferation of vascular fibrous tissue deeper in the membrane. In sections of articular cartilage and adjacent bone, pannus formation at the margins of the joints was often present. The pannus occasionally extended through the cartilage to form subchondral granulation tissue and islands of degenerating cartilage and bone were sometimes seen. The cartilage in andadjacent to such areas showed fibrillation and matrix deficit with chondrocyte clumping. Small numbers of polymorphonuclear cells could occasionally be seen within degenerate cartilage. Severe cartilage degeneration in the absence of pannus formation was very common over most of the articular cartilage, characterised by thinning and fibrillation of the cartilage with clustering of chondrocytes. Tendon sheaths were also affected, showing fibrinous exudate and polymorphonuclear cellular infiltrate. The dogs were negative for LE-cells, antinuclear antibody and rheumatoid

factor, although details of the tests used are not given. No evidence of infection or of crystal deposits within the joints was found and tests for antibodies to Erysipelothrix, Brucella species and Chlamydia species were negative. The authors discarded the possibility of an underlying immunopathological process akin to rheumatoid arthritis or systemic lupus erythematosus because of the absence of extra-articular and vascular lesions. However, this is not a completely acceptable conclusion and certainly some of the cases of canine rheumatoid arthritis reported have had no obvious extra-articular manifestations (Newton et al 1976; Pedersen et al 1976a). It is interesting that all the cases reported by the Australian workers were in the Greyhound breed since an inflammatory joint syndrome has been reported in the U.K. in this breed (Castell 1969). This author described the condition as a peri-arthritis which affected usually one, but occasionally several, joints. However, no aetiological assessment of these cases was carried out and no pathological examination of any diseased joint was performed.

A group of inflammatory arthropathies associated with a chronic infectious disease process elsewhere in the body e.g. dirofilariasis, actinomycosis was reported by Pedersen and his colleagues(1976b). The pathological changes in the synovial membrane are indistinguishable from those seen in cases of systemic lupus erythematosus. A much larger group of dogs suffering a similar arthritis but with no apparent association with other disease processes was also reported by the same investigators(Pedersen et al 1976b).

Jones and Darke (1975) described polyarthritis associated with autoimmune haemolytic anaemia in one dog and with autoimmune haemolytic

anaemia and thrombocytopaenia in another. These were not thought to be cases of systemic lupus erythematosus. No pathological descriptions of the joint lesions are given.

Several dogs with polyarthritis have been reported in France and although the publications were describing the features of systemic lupus erythematosus, there was little evidence to suggest that these polyarthritic dogs were afflicted with lupus (Cassan 1965; Lapras and Oudar 1971a,b). Again, no pathological descriptions are given.

CRYSTAL-INDUCED ARTHRITIS

The classic example of this type of arthritis in man is gout caused by the deposition of sodium urate crystals into the joints (Boyle and Buchanan 1971; Brochner-Mortensen, Cobb and Rose 1963; Sydenham 1850). There is a single case report of naturally occurring gout in the dog (Miller and Kind 1966) but the evidence presented does not totally substantiate the diagnosis. Acute arthritis has been produced experimentally in the dog by the intra-synovial injection of sodium urate crystals (Faires and McCarty 1961).

Pseudogout or chondrocalcinosis, caused by the deposition of crystals of calcium pyrophosphate dihydrate or calcium phosphate dihydrate into the joints (Boyle and Buchanan 1971; McCarty, Kohn and Faires 1962; Moskowitz and Katz 1965; Zitnan and Sitaj 1963) has also been described in the dog. Again, this was a single case report from the U.S.A. (Gibson and Roenigk 1972).

More recently, hydroxy apatite-like crystals have been identified in the synovial fluid of some human patients with "inflammatory exacerbations of osteoarthritis (Dieppe et al 1976). It is suggested that these crystals could initiate a synovitis. A similar phenomenon is thought to occur in the dog (Bennett-unpublished data). Crystal-induced inflammatory arthropathies in the dog are not considered in the present study.

CONCLUSION

From the above discussion it is obvious that different types of inflammatory joint disease do occur in the dog but that considerable confusion exists over their characteristics and recognition. Most of this confusion has been caused by insufficient diagnostic evaluation of cases and by the misuse of terms taken from human rheumatology. In some cases problems have arisen from the failure to appreciate differences in these diseases between man and the veterinary species and also by not considering a complete differential diagnosis. Most of the investigations into the veterinary "rheumatic diseases" have to date been done in the North American continent; no comprehensive studies have so far been carried out in the United Kingdom.

TABLE 1 SUMMARY OF MAJOR PUBLICATIONS RELATED TO THE CANINE INFLAMMATORY ARTHROPATHIES

INFECTIOUS ARTHRITIS	
Clegg and Rorrison (1968)	Brucella abortus infection.
Hall (1974)	Brucella abortus infection.
Henderson et al (1974)	Brucella canis infection of spine. No involvement of synovial joints reported.
Hjärre (1939)	Mycobacterium tuberculosis infection.
Hutyra et al (1938)	Sporotrichum schenckii infection.
	Erysipelothrix infection.
Kavit (1958)	Cryptococcus neoformans infection.
McErlean (1966)	Brucella abortus infection (diagnosis based on positive agglutinin titre).
Maddy (1958)	Coccidioides immitis infection.
Olsson (1957)	Mycobacterium tuberculosis infection.
Pedersen and Pool (1978)	Review article. Staphylococcus and Streptococcus commonest organisms. Bacterial L forms, mycoplasma and fungi also mentioned.
Putnam and Archibald (1968)	Review article. Staphylococcus and Streptococcus commonest organisms.
Stafseth et al (1937)	Streptococcus infection.

TABLE 1 CONTINUED

Thorson *et al* (1955)	*Leishmania* infection.
BACTERIAL ENDOCARDITIS WITH ARTHRITIS	
Bennett *et al* (1978)	2 cases reported. *Streptococcus* and *Escherichia coli* in one case, *Pasteurella multocida* and *Pasteurella pneumotropica* in the other.
Caywood *et al* (1978)	2 cases reported. *Streptococcus* in one case, *Pasteurella multocida* in the other.
RHEUMATOID ARTHRITIS	
Alexander *et al* (1976)	8 cases reported.
Baumgarten and Siegmund (1952)	Early case, not fully evaluated.
Halliwell (1978)	Review article. 16 cases reported, 8 of which reported elsewhere (Newton *et al* 1976).
Halliwell *et al* (1972)	Only case so far reported in U.K.
Lewis and Borel (1971)	*Streptococcus* cultured from blood and 2 joints; endocarditis lesion (?BE). Possibility also of SLE.
Liu *et al* (1969)	Not fully evaluated.
Misener and Stanton (1951)	Early case, not fully evaluated.
Newton *et al* (1974)	Single case reported.

TABLE 1 CONTINUED

Newton *et al* (1976)	10 cases reported. Low titre of RF recorded in most cases.
Pedersen *et al* (1976a)	7 cases reported. 5 negative for RF.
Rudy *et al* (1973)	Single case.
Schiefer *et al* (1974)	Possibility of bacterial endocarditis. Organisms cultured from joints. Pulmonary emphysema and fibrosis present.
Scott (1975)	Single case.
Sikes *et al* (1970)	2 cases reported. Ankylosing spondylitis also. Low titres of antibodies to *Erysipelothrix insidiosa* present.
Wentink *et al* (1974)	Single case reported. No radiographic abnormalities present.
SYSTEMIC LUPUS ERYTHEMATOSUS	
Alexander *et al* (1975)	Single case reported. No joint involvement.
Cassan (1968)	First report in Europe. 2/51 cases possibly SLE. No joint involvement in these 2 cases.
Halliwell (1978)	18 cases of lupus, 7 with polyarthritis, 3 others with shifting lameness.
Jones and Darke (1975)	First report in U.K. but insufficient evaluation of cases. Diagnostic criteria unclear. Usefulness of test for anti-nucleoprotein antibodies in doubt.

TABLE 1 CONTINUED

Krum et al (1977)	Polyarthritis and polymyositis. Possible example of dermatomyositis. Similar case reported by Pedersen et al (1976b).
Lapras and Oudar (1971a, b)	4/50 cases possibly SLE. No joint involvement in these 4 cases.
Lewis et al (1965a)	7 cases reported. Mainly haemolytic anaemia associated with red cell
Lewis et al (1965b)	autoantibodies, thrombocytopaenia and renal disease. 2 dogs had intermittent lameness.
Lewis and Hathaway (1967)	Single case reported. Bacterial endocarditis found at PME.
Miller et al (1974)	3 cases mentioned although very few details given.
Monier et al (1978)	4 cases. Polyarthritis, wasting, albuminuria and skin lesions reported.
Pedersen et al (1976b)	29 cases reported. Diagnostic criteria unclear.
Quimby et al (1978)	Clinical signs reported in colony of inbred SLE dogs. Inbreeding could be significant in explaining lesions.
Schalm and Ling (1970)	3/8 cases reported as SLE. Diagnostic criteria unclear. Lameness was main clinical sign. Some cases suspicious of bacterial endocarditis.
POLYARTERITIS NODOSA	
Lewis et al (1965b)	Only clinical case reported. Joints involved.

TABLE 1 CONTINUED

SJØGREN'S SYNDROME	
Pedersen et al (1976a)	Single case of sialoadenitis with rheumatoid arthritis.
Quimby et al (1978)	2 (?4) cases reported. No joint involvement.
Staman et al (1978)	Single case. Inflammatory disease of joints uncertain.
ENTEROPATHIC ARTHRITIS	
Pedersen and Pool (1978)	Review article. No case reports. Probable case described by Pedersen et al (1976b).
Quimby et al (1978)	Single case reported but positive for LE cells, ANA and RF.
IDIOPATHIC	
Ajmal and Haywood (1970)	Poor evaluation of single case.
Castell (1969)	Peri-arthritis syndrome reported in Greyhounds. No pathological studies.
Huxtable and Davis (1976)	19 cases reported, all in Greyhounds.
Jones and Darke (1975)	2 cases of polyarthritis reported associated with autoimmune haemolytic anaemia and thrombocytopaenia. Evaluation of cases confused.
McDonald and Loomis (1965)	Probable case of infectious polyarthritis.
Pedersen et al (1976b)	15 cases of polyarthritis associated with chronic infectious disease process elsewhere in the body and 19 cases of polyarthritis with no identifiable infectious disease process.

SECTION I

IDENTIFICATION OF DOGS WITH INFLAMMATORY JOINT DISEASE

The selection of criteria requires a proper, empirical balancing of manifestations and laboratory findings in reference to their sensitivity and specificity.

J.A. Boyle and W.W. Buchanan 1971.

A total of 82 dogs with inflammatory joint disease are included in the present study. All the dogs were seen personally by the author at the University of Glasgow Veterinary School between July 1975 and January 1979. The majority of dogs were referred as second opinion cases from surrounding veterinary practices although some came as "first-time" patients presented at the Cargill Clinic for under-privileged people. Over this same period of time a total of 6,750 dogs were referred to the clinical departments of the Hospital for second opinions on a variety of medical and surgical disorders.

The cases of inflammatory joint disease were classified into six groups:-

(a) Group I - cases of rheumatoid arthritis (total 21)

(b) Group II - cases of systemic lupus erythematosus (total 4)

(c) Group III - cases of infectious arthritis (total 12)

(d) Group IV - cases of infectious arthritis associated with bacterial endocarditis (total 7)

(e) Group V - cases of idiopathic inflammatory arthropathy (total 38).

Each dog in a particular group was identified by a number commencing at one and a suffix before each number was used to indicate the group to to which the dog belonged, i.e. Rh for Group I, SLE for Group II, INF for Group III, BE for Group IV and ID for Group V. Thus the 82 dogs in this study were designated as follows:- Rh 1 - Rh 21, SLE 1- SLE 4, INF 1-INF 12, BE 1- BE 7 and ID 1-ID 38.

MAJOR DIAGNOSTIC CRITERIA USED TO IDENTIFY THE DIFFERENT GROUPS OF INFLAMMATORY ARTHROPATHY

Although the identification of criteria for the recognition of these diseases in the dog formed a major part of the thesis and was the result of assessing several different studies as the work progressed, it is relevant at this stage to relate some of the major diagnostic criteria used, in order to introduce the main types of disease which were studied.

RHEUMATOID ARTHRITIS

The diagnosis of rheumatoid arthritis in human patients has caused confusion, so much so that various attempts at standardising the diagnostic criteria used have been made (Boyle and Buchanan 1971; Ropes 1959; Ropes, Bennett, Cobb, Jacox and Jessar 1956; 1957; 1959). It was decided in the present study to use the human criteria (Ropes 1959) for diagnosing rheumatoid arthritis in the dog. These are listed below.

A) Classical Rheumatoid Arthritis

Seven of the following criteria should be satisfied. For criteria 1-5 the joint signs or symptoms must be continuous for at least six weeks.

(i) Morning stiffness.

(ii) Pain on motion or tenderness in at least one joint (observed by a physician).

(iii) Swelling (soft tissue thickening or fluid - not bony overgrowth alone) in at least one joint (observed by a physician).

(iv) Swelling (observed by a physician) of at least one other joint (any interval free of joint symptoms between the two joint involvements may not be more than three months).

(v) Symmetrical joint swelling (observed by a physician) with simultaneous involvement of the same joint on both sides of the body (bilateral involvement of mid-phalangeal, metacarpophalangeal or metatarsophalangeal joints is acceptable without absolute symmetry). Terminal phalangeal joint involvement will not satisfy this criterion.

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

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

(viii)Positive agglutination test demonstration of rheumatoid factor by any method that, in two laboratories, has been positive in not more than 5% of normal controls; or positive streptococcal agglutination test.

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

(x) Characteristic histological changes in synovial membrane with three or more of the following:- marked villous hypertrophy, proliferation of superficial synovial cells

often with palisading, marked infiltration of chronic inflammatory cells (lymphocytes or plasma cells predominating) with tendency to form "lymphoid nodules", deposition of compact fibrin either on surface or interstitially,foci of cell necrosis.

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

B) Definite Rheumatoid Arthritis

This diagnosis requires five of the above criteria. With criteria 1-5 the joint signs or symptoms must be continuous for at least six weeks.

Only these two types of rheumatoid arthritis,i.e. classical and definite,were diagnosed in the dog although two additional types are recognised in man - probable rheumatoid arthritis and possible rheumatoid arthritis. A list of several exclusions has also to be considered in the human patient, in particular systemic lupus erythematosus, rheumatic fever and infectious arthritis. These exclusions were also considered with the canine patient. When diagnosing classical and definite rheumatoid arthritis in the dog, the same recommendations regarding the listed criteria as applied to man were considered but, in addition, of the three criteria (vii), (viii), and (x) at least two had to be satisfied. These three criteria are probably most typical of rheumatoid arthritis. The diagnostic

criteria for canine rheumatoid arthritis were thus more strict than for the human disease but this was deliberate in order to ensure that the disease was not "over-diagnosed" in the dog.

SYSTEMIC LUPUS ERYTHEMATOSUS

The diagnosis of systemic lupus erythematosus in the human patient provides a major problem since this disorder can mimic almost any other disease process and because of this has been called the "great impersonator". The following is quoted from Davies, Atkins, Josse and Hughes (1973) - "Epidemiological and therapeutic studies in systemic lupus erythematosus are limited by a lack of a precise definition of the disease". The American Rheumatism Association (ARA) has recently published criteria for the classification of the human disease based on a multi-centre analysis (Cohen, Reynolds, Franklin, Kulka, Ropes,Schulman and Wallace 1971). Out of 57 features analysed from a total of 696 cases, 14 criteria were selected as being of significance and the presence of 4 or more of these was considered to be compatible with systemic lupus erythematosus. These criteria were:-

(i) Facial erythema

(ii) Discoid lupus

(iii) Raynaud's phenomenon

(iv) Alopecia

(v) Photosensitivity

(vi) Oral/nasal ulceration

(vii) Arthritis without deformity

(viii)LE-cells (2 or more)

(ix) False positive serological tests for syphilis

(x) Proteinuria (3.5g/day)

(xi) Cellular casts

(xii) Pleuritis/pericarditis

(xiii)Psychosis/convulsions

(xiv) Haemolytic anaemia/leucopaenia (<4000/cmm)/thrombocytopaenia (<100,000/cmm).

The detection of antinuclear antibody (ANA) by the fluorescent antibody technique or anti-deoxyribonucleic acid antibodies by radioimmunoassay binding techniques in lupus patients was not assessed in the study of Cohen and fellow workers (1971). Davies and his colleagues (1973) evaluated their cases of systemic lupus erythematosus diagnosed by the deoxyribonucleic acid binding activity with the ARA criteria and found a 91.7% correlation. The criteria were also supported by Lie and Rothfield (1972) who defined systemic lupus erythematosus as a disease involving at least two systems in addition to causing haematological abnormalities and being associated with positive LE-cell preparations and/or positive indirect immunofluorescent tests for ANA. Only 4% of their lupus patients did not meet the proposed criteria. The criteria however, were criticised on several accounts by Fries and Siegel (1973):- (a) testing of the criteria had not been performed on a population other than the one used to derive the criteria, (b) the population used showed certain unusual features, (c) no information was given regarding ANA, (d) the wording of some criteria was unsatisfactory, (e) several criteria were closely related and dependent on each other, (f) the criteria were not applied to patients in remission or in the early stages of the

disease (g) the criteria could not separate out cases of drug-induced systemic lupus erythematosus (h) the sensitivity of individual criteria was low. These authors claimed many false positive and negative lupus patients in their assessment of the criteria; they suggested the use of the computer to assess stored patient information and to create "simulated" patient populations with repetitive revision and re-testing of proposed criteria in order to finally select the best.

Some authors in the U.S.A. have listed criteria for the diagnosis of canine systemic lupus erythematosus and have grouped these into major and minor categories (Alexander et al 1975; Halliwell 1978). Unfortunately, there is lack of uniformity and some criteria listed as major by one authority are listed as minor by another. The author recently visited the U.S.A. and it was apparent that systemic lupus erythematosus was being "over-diagnosed" in the dog because of a failure to use rigid criteria. There was also a great variation in the diagnostic criteria used between different centres.

The criteria used to diagnose canine systemic lupus erythematosus in the present thesis are listed below and have been based on reports of the diagnosis of lupus in both human and canine patients :-

(i) The simultaneous or sequential involvement of several different body systems in the disease process. Systemic lupus erythematosus is a multi-system disease in the dog (Halliwell 1978; Lewis 1972; 1974; Lewis Schwartz and Gilmore 1965; Lewis Schwartz and Henry 1965)just as it is in the human (Boyle and Buchanan 1971; Haserick 1955; Hill 1957). The main manifestations of lupus in the dog

are as follows:-

(a) A symmetrical polyarthritis causing variable lameness with joint pain and swelling;

(b) Autoimmune haemolytic anaemia which can produce various signs such as pale mucous membranes, weakness and lethargy;

(c) Immune mediated thrombocytopaenia resulting in petechial or ecchymotic haemorrhages or an even more massive haemorrhage;

(d) Leucopaenia which could be immune based;

(e) Glomerulonephritis often of the membranous type and typified by proteinuria leading to hypoalbuminaemia, loss of body weight and condition and oedema. Renal failure may ensue with raised blood urea,vomiting, collapse etc.;

(f) Skin disease. Skin lesions are many and varied and often bizarre. It is difficult to define the typical changes associated with the disease (Halliwell 1978) but some generalisations can be made. The lesions are often symmetrical with a predilection for the head and ears. Sometimes they may be more extensive and involve the feet and mucocutaneous junctions. They are not usually ulcerative but are erythematous and/or atrophic and crusting is common. Because of the non-specific nature of the skin lesions it can be difficult to distinguish significant lesions which are part of the lupus disease complex from other types of skin disease.

(g) Fever which may be intermittent or continuous;

(h) Central nervous signs. These can be varied but convulsions and meningeal signs,particularly pain, are not uncommon;

(i) Pleuritis, associated with pleural effusion and respiratory signs.

Continuous assessment of suspected lupus cases is necessary since it may take several months for additional body systems to become involved in the disease syndrome. It is also true that pathological involvement of a body system could be present without any clinical signs referable to it. It is, of course, also possible that simultaneous disease of several body systems in an individual is merely coincidental and not representative of a single disease process.

(ii) The presence of ANA in the blood of affected patients, detected by the indirect immunofluorescent test.

(iii) The presence of positive LE-cell preparations.

(iv) In cases where glomerulonephritis is present the demonstration of typical glomerular lesions in biopsy or autopsy specimens. In addition, deposits of complement and immunoglobulin should be demonstrable in the glomeruli by the direct immunofluorescent test (Freedman and Markowitz 1962a,b;Lachmann,Müller-Eberhard, Kunkel and Paronetto 1962; Svec, Blair and Kaplan 1967; Vasquez and Dixon 1957).

(v) In cases where skin manifestations are present, the demonstration of bound complement and immunoglobulin at the epidermal-dermal junction in skin biopsies/autopsies

(Cormane, Ballieux, Kalsbeck and Hijmans 1966; Ten Have-Opbrock 1966; Tan and Kunkel 1966). These deposits probably represent immune complexes of antinuclear antibody and nuclear antigens and occur in both normal skin and skin showing lesions. Circulating skin autoantibodies are not found in systemic lupus erythematosus.

(vi) In cases with haemolytic anaemia, red cell autoantibodies should be demonstrable by the direct (Coombs') antiglobulin test on a suspension of erythrocytes collected from the patient (Meacham and Weisberger 1955; Mongan, Leddy, Atwater and Barnett 1967). In some patients it is possible to show red cell autoantibodies in the absence of haemolysis or anaemia.

(vii) In cases with thrombocytopaenia, autoantibodies against platelets should be demonstrable, in the patient's serum, by the platelet-factor III or platelet migration inhibition tests (Dameshek 1958).

(viii) In cases with leucopaenia, autoantibodies against white blood cells may be shown by for example, the antiglobulin consumption test (Michlmayr, Asamer, Huber and Huber 1972; Scastny and Ziff 1971).

(ix) In dogs with joint involvement, deposits of immunoglobulin and complement can be demonstrated in synovial membrane biopsies or autopsies.

For the diagnosis of <u>definite</u> canine systemic lupus erythematosus, criteria(i)and(ii)had to be present together with the immunological features described in criteria (iv), (v), (vi), (vii), (viii) and (ix)

if any of these clinical manifestations were diagnosed. A diagnosis of probable systemic lupus erythematosus was made if only criteria (i) and (ii) were satisfied. A diagnosis of lupus was always excluded if criteria (i) and (ii) were not satisfied. The presence or absence of LE-cells was not regarded as a significant criterion. Obviously, a diagnosis of systemic lupus erythematosus was excluded if the disease signs could be explained by an alternative pathological process such as bacterial endocarditis.

INFECTIOUS ARTHRITIS

Inflammatory arthropathies were only classified as cases of infectious arthritis if organisms were actually cultured from the synovial fluid and/or synovial membrane. Cases in which bacterial organisms were seen in stained smears of synovial fluid but where no growth was obtained on culture were not classified into this group. Only bacterial infections of joints were classed as cases of infectious arthritis.

Cases of infectious arthritis secondary to acquired open wounds of the joint or secondary to surgical procedures performed upon the joint were not included in the present study. Such cases are not common in the canine patient.

INFECTIOUS ARTHRITIS ASSOCIATED WITH BACTERIAL ENDOCARDITIS

Positive bacterial cultures were obtained from affected joints in the majority of these dogs. The presence of an endocarditis lesion was confirmed at post-mortem examination in all cases. A positive bacterial culture was obtained from the heart lesion at autopsy in all cases.

As well as an infectious arthritis in these dogs, most cases demonstrated a non-septic inflammatory arthropathy.

IDIOPATHIC

This group of dogs was formed by all those animals which could not be classified into any of the other groups discussed above. Several sub-groups were identified, one of the most significant being dogs with an enteropathic arthritis where an ulcerative colitis was confirmed by a biopsy procedure.

SECTION II

CLINICAL AND ANAMNESTIC ASSESSMENT OF DOGS WITH INFLAMMATORY JOINT DISEASE

Physical ills are the taxes laid upon this wretched life; some are taxed higher, and some lower, but all pay something.

Lord Chesterfield to the Bishop of Waterford 1757.

METHODS

Most cases were referred directly to the author for assessment although some initially went to other clinicians within the Hospital, particularly the physicians who then transferred the case to the author. The history of each case was obtained from the owner and sometimes also from the referring veterinary surgeon. Every dog was personally examined by the author,both initially and during any subsequent follow-ups. Complete, detailed clinical examinations were always performed. Sometimes other clinicians, with a particular speciality,were asked to examine the patient e.g. for auscultation of the heart or chest,to perform electrocardiographical and electrophonographical examinations, to check ocular abnormalities, to assess skin lesions and to advise on renal complications.

All dogs were hospitalised at the time of their first presentation for detailed investigations. The period of hospitalisation varied from 1-4 weeks.

RESULTS

The clinical features of all the dogs are summarised in Tables 2-9 and Fig. I.

GROUP I (RHEUMATOID) (TABLES 2,3,4 & 5; Fig. 1).

Several different breeds of dog were represented in this group, the majority being the smaller and medium sized breeds. The average age was approximately six years with a range from 9 months to 13 years. Most dogs came within the age range 4-7 years when first presented (Fig.I). No sex predominance was apparent.

Over one third of the dogs (8/21) were presented with systemic illness as well as lameness. Systemic signs included pyrexia, lethargy and inappetence or anorexia. Five animals had some clinical evidence of respiratory disease and one had a severe tonsillitis/pharyngitis. Two dogs had subcutaneous swellings over bony prominences.

In all but two cases, there was evidence of simultaneous involvement of several different joints, often in a bilaterally symmetrical fashion. Dogs Rh 1 and 8 had clinical disease limited to a single joint only. Signs of joint disease included pain on manipulation, particularly at the extremes of joint motion, synovial effusion within the joint and soft tissue thickening around the joint. The ability to detect synovial effusion and periarticular thickening was obviously much easier with some joints than others. Joint heat was apparent in 5 dogs.

Over half the dogs had a history of lameness of a gradual onset (12/21). The remainder had shown an acute, sudden onset lameness and these were the animals which most often had systemic signs. Seventeen of the dogs had stiffness when first rising after rest and in most cases this was the owners' main reason for seeking veterinary assistance. Seven animals demonstrated an obvious limp in one particular leg. Eleven had difficulty in walking but only two were so severely affected that ambulation was impossible. The joint problem had generally been present for several weeks prior to presentation at the Hospital, the duration ranging from 10 days to 6 years. Most of the animals had a history of a single "attack" of the joint problem with persistence and sometimes progression of the clinical disease. Seven animals had an initial lameness which apparently resolved, or improved, but which was followed by a relapse.

The most commonly affected joints were in order of frequency, the stifle, carpus, hip, elbow and hock. Seven dogs also showed involvement of the digital joints (principally the metacarpo/metatarso-phalangeal and proximal interphalangeal joints). A bilaterally symmetrical involvement of joints was a feature of most cases. Three dogs exhibited spinal pain suggesting a possible involvement of certain intervertebral joints.

Other signs included restriction of joint movement (six cases), articular crepitus (seven cases) and enlargement of peripheral lymph nodes (four cases). Obvious muscle atrophy was apparent in eight dogs and three of these showed disproportionate atrophy of the temporal muscles. Three dogs had bilateral medial patellar luxation, probably congenital in origin. Two dogs developed a rupture of the anterior

cruciate ligament of one stifle joint during the course of the disease. A single dog showed instability of the digital joints and another had bilateral intertarsal subluxation. The latter was a Shetland Sheepdog which is a breed thought to be predisposed to intertarsal instability (Campbell, Bennett and Lee 1976). Carpal instability was diagnosed in two dogs. Recurrent diarrhoea was reported in three dogs.

GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS) (TABLES 2,3,6 & 7)

Only four dogs in the present study satisfied the criteria for systemic lupus erythematosus. Three were classified as definite canine systemic lupus erythematosus; dog SLE 2 was diagnosed as a probable case. The owners of all four cases complained principally of a generalised stiffness in their dogs following a period of inactivity. It was difficult to detect obvious abnormalities in most of the limb joints of these dogs, although three had some synovial effusion within both stifle joints. Joint pain was only detected in dog SLE 4. Cases SLE 1 and 3 had evidence of anaemia and SLE 2 showed anorexia, lethargy and loss of weight. Dog SLE 4 had a history of central nervous disease characterised by head pressing and occasional fits during which the dog exhibited collapse, loss of consciousness and "paddling" of the limbs; no nervous signs were seen during the period of hospitalisation. Three of the dogs had exhibited a gradual onset of stiffness, the fourth a sudden onset and the problem in all cases had been present some weeks prior to presentation at the Hospital. Two cases were female, one a speyed female, and the other a male. All dogs were adult.

GROUP III (INFECTIOUS) (TABLES 2,3,6 & 7; Fig. 1)

All twelve dogs in this group were of the larger breeds and the majority were male. Only one animal was less than one year of age, the average age of this group being approximately 4½ years. Only three of the animals showed systemic illness in the form of fever, loss of appetite and dullness, although a fourth dog showed lethargy and another inappetence. Some of the dogs had clinical evidence of possible foci of infection elsewhere in the body, e.g. cutaneous and oral mucosal ulceration, periodontal disease, tonsillitis, pharyngitis, vaginal or preputial discharges, skin abscessation, infected bone adjacent to the involved joint. One dog had been accidentally shot and a piece of lead-shot had become lodged within the joint. Two dogs had been involved in dog fights and acquired penetrating wounds into the affected joints. There was a history of trauma to the diseased joint in 7 dogs.

All but two dogs had infection of a single joint - shoulder (1 case), elbow (2 cases), carpus (3 cases), hip (2 cases) and stifle (2 cases). Case INF 3 had involvement of two joints (right shoulder and left stifle) although only one joint was examined bacteriologically (see Section VIII) and case INF 1 suffered infection of two joints but at different times (right stifle and right hock). Nine of the dogs had a history of a sudden onset lameness and all twelve animals were presented with an obvious limp in the affected limb. The duration of the problem prior to presentation ranged from one day to six months, although most had shown lameness for several weeks.

Joint pain during manipulation was a feature of all cases except one. Excess synovial fluid and soft tissue swelling were also

common features and joint heat was detected in over half the dogs. Restriction of joint motion was present in 3 cases and muscle atrophy was prominent in five. Two dogs showed oedematous swelling of the affected limb.

GROUP IV (BACTERIAL ENDOCARDITIS) (TABLES 2,3,6 & 7; Fig. 1)

Like the previous group, all affected dogs were of the larger breeds and the male sex again predominated. All seven animals showed systemic upset as well as lameness and a pansystolic cardiac murmur was detected in all but one dog during thoracic auscultation. Phonocardiographic recordings confirmed the presence of the cardiac murmur in dogs BE 1, 2, 3, 4, 5 and 7. Cutaneous ulceration was present in 2 dogs and a subcutaneous swelling was palpable in dog BE 1. Retinal abnormalities were present in three dogs; case BE 2 showed multiple small rounded and discrete grey areas probably representing retinal exudates, case BE 3 showed areas of retinal haemorrhage and case BE 5 showed both these lesions on ophthalmoscopic examination. Five dogs showed weight loss and all 7 had enlargement of the peripheral lymph nodes.

Three animals were presented with an obvious limp, one could only walk with great difficulty and 2 were unable to stand and walk at all. Unlike Group III, several joints in each animal were affected, the commonest being stifle, hock and hip joints. Involvement of elbow, carpal and shoulder joints was also recorded. However, many joints in the majority of these animals were not clinically diseased. All cases had a history of being unwell prior to developing a lameness of sudden onset. The dogs had been ill and lame for some time prior to presentation.

Synovial fluid effusion, soft tissue swelling, joint pain and joint heat were common articular abnormalities. Muscle atrophy was obvious in 3 cases and limb oedema developed in cases BE 1, 2 and 4. Case BE 2 developed rupture of the anterior cruciate ligament in the left stifle joint to which infection had previously localised and cases BE 1 and 7 had evidence of spinal pain.

GROUP V (IDIOPATHIC) (TABLES 2,3,8 & 9; Fig. 1).

This group comprised dogs of a variety of different breeds; five dogs were Shetland Sheepdogs but this is one of the most commonly represented breeds in the general hospital population at Glasgow (Campbell, Bennett and Lee 1976). The male sex predominated (26M, 5F, 7FS). The age range extended from 15 months to 11 years with an average of about 5 years. Most dogs when first presented were within the age range 1-3.5 years (Fig. 1).

Over two thirds of the dogs showed systemic illness (pyrexia,ancrexia, inappetence, lethargy) in addition to the lameness. Several of these dogs also showed tachypnoea which in some cases may have been explained by the fever and/or the joint pain. Pale mucous membranes were recorded in 5 dogs and 12 dogs had some evidence of respiratory disease such as nasal discharge, rhonci/rales on thoracic auscultation and a persistent cough. A cardiac murmur was detected in 8 animals and 5 dogs had extensive ulceration of the lingual and buccal mucous membranes accompanied by the production of excess, foetid and discoloured saliva. Four dogs showed severe inflammation of the tonsils and pharynx and 8 had dental disease, principally excessive tartar deposits and periodontal disease.

Skin disease, mainly seen as a pruritus and/or ulceration was apparent in 6 dogs; a further 2 animals showed subcutaneous swellings and 6 dogs had excessive dandruff. Ocular discharges in one or both eyes were recorded in 7 dogs, often associated with some degree of conjunctival inflammation. Retinal abnormalities were found during ophthalmoscopic examination of 5 dogs and these were mainly haemorrhages or grey focal areas where the normal retinal pigmentation had been lost; areas of increased reflectivity were seen in 2 of these dogs. Polydipsia was noted in 4 dogs, vomiting and/or diarrhoea in 6 and weight loss in 2. Seven animals had enlargement of the peripheral lymph nodes. Other features included one dog with a preputial discharge, 2 dogs with haemorrhages within the oral mucosae, three dogs with testicular neoplasia, 2 dogs with splenomegaly, one dog with ascites, one dog with ventricular extrasystoles in the electrocardiograph, one dog with an anal sac abscess and one dog with hypopyon and uveitis showing lowered intra-ocular pressures in both eyes.

Over half the dogs were presented with an obvious limp in an individual limb, 13 dogs had great difficulty in walking and 3 were completely unable to stand. A history of stiffness after rest and/or exercise was reported in 16 of the dogs. Joint pain upon manipulation was present in all but 7 of the 38 dogs. Heat was felt in some joints of several animals. Soft tissue thickening and synovial effusion were appreciated in many cases and a few animals had reduced joint movement and/or crepitus. The duration of the lameness varied from a few days to several months and many dogs had shown periods of remission followed by a relapse (16 dogs had suffered more than one "attack" of the joint problem prior to presentation at the Hospital). All but 5 dogs had the

history of a sudden onset locomotory problem. Most of the dogs in this group showed clinical disease of several joints, generally in a bilaterally symmetrical fashion. The most commonly affected joints were the stifle, elbow and carpus. The hock and hip joints were less frequently affected and only 5 dogs showed obvious clinical disease of the digital joints.

Ten dogs had obvious muscle atrophy, 6 of which demonstrated disproportionate atrophy of the temporal muscles. Spinal pain was detected in 10 dogs, suggesting a possible involvement of the vertebral joints. Four dogs developed oedema of a single limb and 2 dogs showed involvement of the extensor tendon sheaths of the forelegs and hindlegs. One dog developed a rupture of the anterior cruciate ligament of the left stifle joint and 3 dogs had bilateral medial patellar luxation when presented.

TABLE 2

SHOWING DETAILS OF BREED, SEX AND AGE OF

ALL THE DOGS WITH INFLAMMATORY JOINT DISEASE.

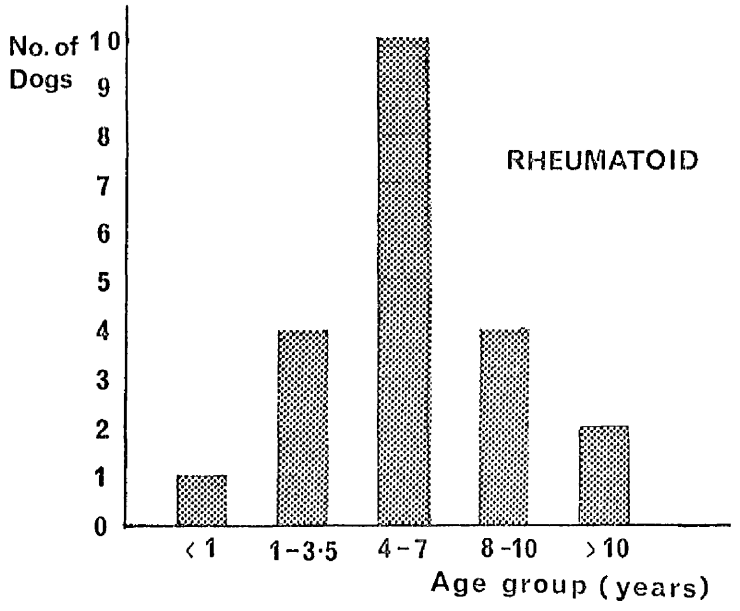
	Rh 1	Rh 2	Rh 3	Rh 4	Rh 5	Rh 6	Rh 7	Rh 8	Rh 9	Rh 10	Rh 11	Rh 12	Rh 13	Rh 14	Rh 15	Rh 16	Rh 17	Rh 18	Rh 19	Rh 20	Rh 21
BREED	Min. Pood.	Coll. X	Gold. Retr.	Dach.	Cock. Span.	Bor. Coll.	Cairn	Rou. Coll.	Lurch.	Cock. Span.	Bear. Coll.	Rou. Coll.	Peke.	Terr. X	Bor. Coll.	Lab.	R.H. Terr.	Terr. X	Shetl. Shpd.	Irish Sett.	Dob. X
SEX	M	M	FS	M	M	M	MN	F	F	M	M	M	F	FS	F	M	F	F	M	F	FS
AGE (YEARS)	8	5	7	4.5	4.5	6	13	2	0.75	1.5	8	4	5	8	5.5	9	2	11	6.75	3	6.5
	SLE 1	SLE 2	SLE 3	SLE 4			INF 1	INF 2	INF 3	INF 4	INF 5	INF 6	INF 7	INF 8	INF 9	INF 10	INF 11	INF 12			
BREED	Pood.	Als.	Cairn	O.E.S.			Dalm.	Box.	Dob.	Trail Hnd.	Rou. Coll.	Afgh. Hnd.	Bor. Coll.	S.H G.P	Lab. X	Grey Hnd.	Als.	Dob.			
SEX	F/S	M	F	F			M	F	M	M	M	M	M	F	FS	F	F	M			
AGE (YEARS)	8	6	3	4			8.5	0.38	6	3	4	0.29	9	1	4	10	6.5	2.75			
	BE 1	BE 2	BE 3	BE 4	BE 5	BE 6	BE 7														
BREED	Als.	Als.	Box.	Als.	Lab.	Als.	Box.														
SEX	M	F	M	M	M	M	M														
AGE (YEARS)	1.17	10	10	8	6	1	1.33														
	ID 1	ID 2	ID 3	ID 4	ID 5	ID 6	ID 7	ID 8	ID 9	ID 10	ID 11	ID 12	ID 13	ID 14	ID 15	ID 16	ID 17	ID 18	ID 19	ID 20	
BREED	Terr. X	Grey Hnd.	Gold. Retr.	Terr. X	Dalm.	Terr. X	Terr. X	Als.	Box.	Bull Mast.	Whip.	Als.	Shetl. Shpd.	Coll. X	Pood.	Irish Sett.	Rou. Coll.	Shetl. Shpd.	Terr. X	Als.	
SEX	F	M	M	FS	M	M	M	M	M	M	M	M	F	FS	FS	M	M	M	FS	M	
AGE (YEARS)	6	2	2.5	11	8	9	1.5	1.5	1.25	3.5	1.5	8	5	8	10	1.17	10.5	11	8	10	
	ID 21	ID 22	ID 23	ID 24	ID 25	ID 26	ID 27	ID 28	ID 29	ID 30	ID 31	ID 32	ID 33	ID 34	ID 35	ID 36	ID 37	ID 38			
BREED	Coll. X	Cock. Span.	Sp. Sp.	Als.	Gold. Retr.	Lab.	Dob.	Shetl. Shpd.	C.K. C.Sp.	Scott. Terr.	Lab.	W.H. F.T.	O.E.S.	W.H. F.T.	Shetl. Shpd.	Shetl. Shpd.	Cock. Span.	Irish Sett.			
SEX	F	F	M	FS	M	M	M	M	M	M	M	M	M	FS	M	FS	M	F			
AGE (YEARS)	1.5	6	1.33	3	1	4	6	1.5	7	11	8	6	2	4	3	6	3	3.5			

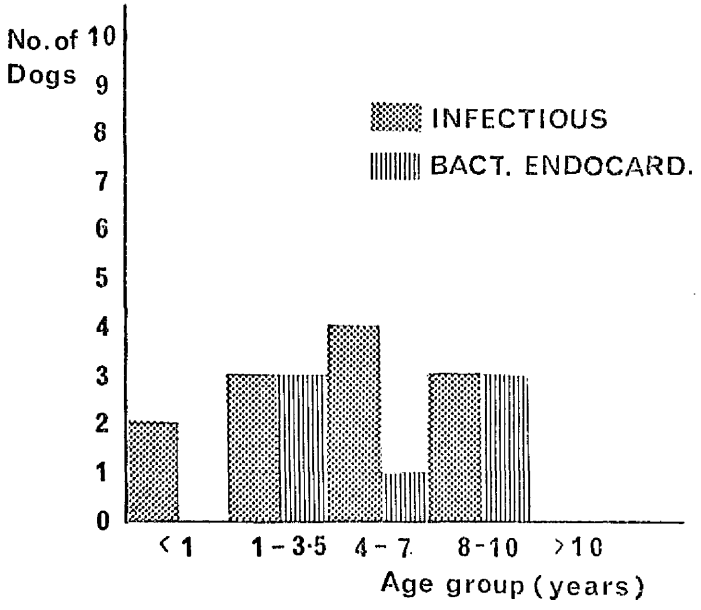
		SEX				AVERAGE AGE	AGE RANGE
GROUP I	RHEUMATOID	10M	1MN	7F	3FS	5.76 years	0.75 - 13 years
GROUP II	SYSTEMIC LUPUS ERYTHEMATOSUS	1M		2F	1FS	5.25 years	3 - 8 years
GROUP III	INFECTIOUS	7M		4F	1FS	4.62 years	0.29 - 10 years
GROUP IV	BACTERIAL ENDOCARDITIS	6M		1F		5.36 years	1.17 - 10 years
GROUP V	IDIOPATHIC	26M		5F	7FS	5.19 years	1.17 - 11 years

TABLE 3 SUMMARISING DETAILS OF SEX AND AGE OF ARTHRITIC DOGS IN EACH OF THE 5 GROUPS

FIGURE 1

HISTOGRAMS SHOWING AGE DISTRIBUTION IN THE GROUP I, GROUP III, GROUP IV AND GROUP V DOGS.


No. of Dogs
10
9
8
7
6
5
4
3
2
1
0
RHEUMATOID
< 1
1-3·5
4-7
8-10
> 10
Age group (years)


No. of Dogs
10
9
8
7
6
5
4
3
2
1
0
INFECTIOUS
BACT. ENDOCARD.
< 1
1-3·5
4-7
8-10
> 10
Age group (years)

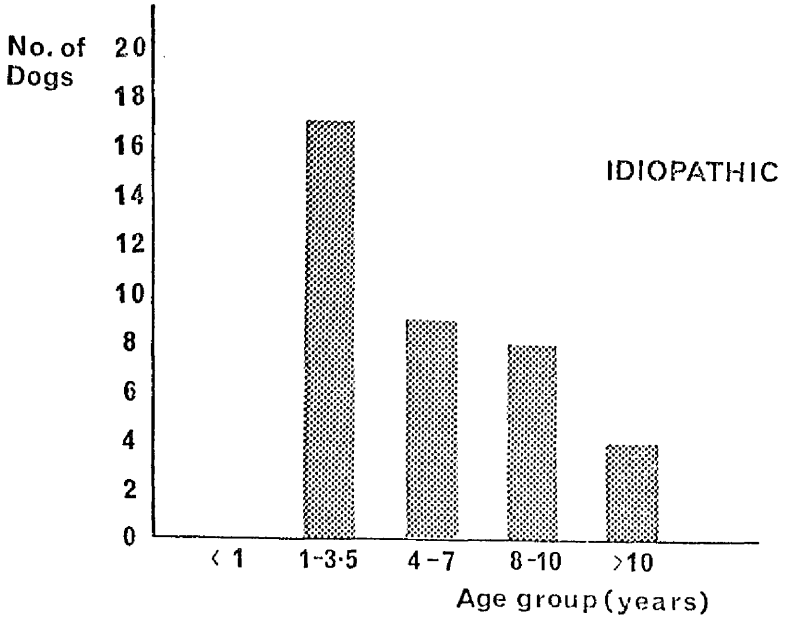

No. of Dogs
20
18
16
14
12
10
8
6
4
2
0
IDIOPATHIC
< 1
1-3·5
4-7
8-10
>10
Age group (years)

TABLE 4

SUMMARISING THE MAIN CLINICAL FEATURES (ASSOCIATED WITH THE MUSCULOSKELETAL SYSTEM) OF THE GROUP I (RHEUMATOID ARTHRITIS) DOGS.

A + score indicates the presence of that particular clinical feature. No score indicates that the clinical feature was not present.

ACL - anterior cruciate ligament rupture

(T) - temporal muscle atrophy prominent

M - multiple (more than 8 joints)

L - left

R - right

F - foreleg

H - hindleg

CLINICAL FEATURES REFERABLE TO THE MUSCULOSKELETAL SYSTEM

DOG IDENTIFICATION	Obvious Limp	Unable to Walk	Difficulty in Walking	Stiff after Rest	Stiff after Exercise	Joint Effusion	Artic. Soft Tissue Swell.	Joint Pain	Joint Heat	Joint Instabil.	Restrict. Joint Motion	Joint Crepitus	Muscle Atrophy	No. Jts. with Pain/Swelling	JOINTS CLINICALLY AFFECTED: Shou.	Elb.	Carp.	Hip	Stifle	Hock	MC/MT Phal.	Int. Phal.	Involv. Tend. Sheath	Spinal Pain	Limb Oedema	Duration	No. of attac.	Onset
Rh 1	+						+	+	+	Carp.	+			1			L									5m	1	Su.
Rh 2			+	+		+	+	+		ACL RH	+	+	+	4			L R		L R							9m	2	Gr.
Rh 3				+	+		+	+			+	+		5		L	L R	R	R							6m	2	Gr.
Rh 4			+	+		+	+	+	+			+	+ (T)	8			L R	L R	L R	L R						6wk	2	Su.
Rh 5				+										4			L	L R	R					+		6wk	1	Gr.
Rh 6	+			+			+			Dig.	+	+		M			R		R		L R F H	L R F H				2m	1	Su.
Rh 7	+			+		+	+	+						3			L R		R							3yr	1	Gr.
Rh 8				+			+						+ (T)	1			L									10dy	1	Su.
Rh 9		+				+	+	+	+				+ (T)	M	L R	L R	L R	L R	L R	L R	L R F H	L R F H		+		3wk	2	Su.
Rh 10			+			+	+	+						7		L R	L R		L R	R						1m	1	Su.
Rh 11			+	+						ACL LH			+	M		L			L R		L R F H	L R F H				6yr	2	Gr.
Rh 12	+		.	+			+	+						3			L	L R								6m	1	Gr.
Rh 13		+				+	+	+		Bil. Pat.		+		4		L R			L R							1m	2	Gr.
Rh 14	+			+	+			+		Bil. Pat.				3			L		L R							6m	1	Gr.
Rh 15			+	+				+					+	M		L R	L R	L R	L R	L R	L R F H	L R F H				10m	1	Gr.
Rh 16			+	+				+						4			L R	L R						+		2wk	1	Gr.
Rh 17			+	+		+	+	+						M	L R	L R	L R	L R	L R	L R	L R F H	L R F H				4wk	1	Su.
Rh 18			+	+	+	+	+	+		Bil. Pat.				8		L R	L R		L R	L R						3wk	2	Su.
Rh 19			+	+		+	+	+		Bil. Int-tar.	+	+	+	6			L R		L R	L R						10m	1	Gr.
Rh 20	+		+	+		+	+	+	+		.			M			L				L R F H					3m	1	Su.
Rh 21	+		+	+		+	+	+	+		+	+	+	M		L R	L R		L R	L R	L R F H	L R F H				7m	1	Gr.

TABLE 5

SUMMARISING THE MAIN CLINICAL FEATURES (OTHER THAN THOSE ASSOCIATED WITH THE MUSCULOSKELETAL SYSTEM) OF THE GROUP I (RHEUMATOID ARTHRITIS) DOGS.

A + score indicates the presence of that particular clinical feature. No score indicates that the clinical feature was not present.

CLINICAL FEATURES (EXCEPT MUSCULOSKELETAL)

DOG IDENTIFICATION	Pyrexia	Anorexia Inappet.	Dull	Tachypn.	Tachyca.	Bradyca.	Pale M.M.	Cardiac Murm.	Rhonci Rales	Nasal Disch.	Haem. Oral M.M.	Ulc. Oral M.M.	Excess Salivat.	Lymph-adenop.	Cough	Tonsil. Pharyn.	Period. Dis.	Dental Car/Abs.	Pruritus	Alopecia	Ulc. Skin	Subcut. Swelling	Excess Dandru.	Hyper-kerat.	Ocular Disch.	Nuclear Scler.	Catar.	Retinal Abnorm.	Prep/Vag. Disch.	Polydip.	Loss of Weight	Vomit / Diarrh.	Previous Illness	Additional Inform.
Rh 1															+										+									
Rh 2									+	+					+		+																Lame RH	
Rh 3																																		
Rh 4	+	+	+	+				+						+		+	+					+	+								+			
Rh 5		+	+																															
Rh 6																							+											Work. dog
Rh 7																	+		+							+							Skin Allerg. Seminoma	
Rh 8	+	+	+	+						+														+	+									
Rh 9	+	+	+				+							+	+														+		+			
Rh 10	+	+	+	+									+																					
Rh 11																						+												
Rh 12			+														+		+	+			+									+		
Rh 13						+																											Loss of eye - sinus	
Rh 14				+					+						+										+									
Rh 15																																		
Rh 16	+	+	+	+																														
Rh 17	+	+	+																										+					Work. dog Pregnant
Rh 18	+	+	+											+													+							
Rh 19			+								+																	+				+		
Rh 20																																	Dystocia (caesarean)	
Rh 21														+																		+		Splenic enlargement

TABLE 6

SUMMARISING THE MAIN CLINICAL FEATURES (ASSOCIATED WITH THE MUSCULOSKELETAL SYSTEM) OF THE GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS) GROUP III (INFECTIOUS) AND GROUP IV (BACTERIAL ENDOCARDITIS) DOGS.

A + score indicates the presence of that particular clinical feature. No score indicates that the clinical feature was not present.

Key as for Table 4.

DOG ID		CLINICAL FEATURES REFERABLE TO THE MUSCULOSKELETAL SYSTEM																																
		Obvious Limp	Unable to Walk	Difficulty in Walking	Stiff after Rest	Stiff after Exercise	Joint Effusion	Artic. Soft Tissue Swell.	Joint Pain	Joint Heat	Joint Instabil.	Restrict. Joint Motion	Joint Crepitus	Muscle Atrophy	No. Jts. with Pain/ Swelling	JOINTS CLINICALLY AFFECTED										Involv. Tend. Sheath	Spinal Pain	Limb Oedema	Duration	No. of Attac.	Onset
																Shou.	Elb.	Carp.	Hip	Stifle	Hock	MC/MT Phal.	Int. Phal.											
D	SLE 1			+	+		+				+ 911.Pat.		+	+	2					L R								3wk	1	Su.
	SLE 2				+		+	+							2					L R								4wk	1	Gr.
O	SLE 3				+		+								2					L R								5m	1	Gr.
	SLE 4				+				+						2				L R							+		4m	1	Gr.
G	INF 1	+			+		+	+	+	+					2					R	R							2wk	2	Su.
	INF 2	+							+					+	1				L									2wk	1	Su.
I	INF 3	+		+			+	+	+			+		+	2	R				L							+	3wk	1	Su.
D	INF 4	+				+		+	+	+					1			L										6m	1	Su.
E	INF 5	+													1			R										1m	1	Gr.
N	INF 6	+					+	+	+	+					1					L								5dy	1	Su.
T	INF 7	+					+	+	+	+		+		+	1				R									10dy	1	Su.
I	INF 8	+					+	+	+						1		L											2dy	1	Su.
F	INF 9	+							+						1	L												3m	1	Gr.
I	INF 10	+						+	+	+					1		L										+	1dy	1	Su.
C	INF 11	+						+	+	+		+	+	+	1			L										4m	1	Su.
A	INF 12	+						+	+	+				+	1	R												3m	1	Gr.
T	BE 1	+			+		+	+	+	+				+	M	L R	L R	L R	L R	L R	L R					+	+	3m	1	Su.
I	BE 2	+			+		+	+	+	+	+ ACL LH				6	L	L R		L R	L						+		2wk	2	Su.
O	BE 3		+				+	+	+	+				+	4					L R	L R							1wk	1	Su.
N	BE 4		+				+	+	+	+				+	4				L R	L R							+	1m	1	Su.
	BE 5			+			+	+	+	+					5				R	L R	L R							3wk	1	Su.
	BE 6	+			+	+		+	+	+					4	R			L		L R							4wk	2	Su.
	BE 7				+	+			+						3	R					L R					+		3m	3	Su.

TABLE 7

SUMMARISING THE MAIN CLINICAL FEATURES (OTHER THAN THOSE ASSOCIATED WITH THE MUSCULOSKELETAL SYSTEM) OF THE GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS), GROUP III (INFECTIOUS) AND GROUP IV (BACTERIAL ENDOCARDITIS) DOGS.

A + score indicates the presence of that particular clinical feature. No score indicates that the clinical feature was not present.

DOG IDENTIFICATION	CLINICAL FEATURES (EXCEPT MUSCULOSKELETAL)																																Previous Illness	Additional Inform.
	Pyrexia	Anorexia Inappet.	Dull	Tachypn.	Tachyca.	Bradyca.	Pale M.M.	Cardiac Murm.	Rhonci Rales	Nasal Disch.	Haem. Oral M.M.	Ulc. Oral M.M.	Excess Salivat.	Lymphadenop.	Cough	Tonsil. Pharyn.	Period. Dis.	Dental Car/Abs.	Pruritus	Alopecia	Ulc. Skin	Subcut. Swelling	Excess Cerumen	Hyperkerat.	Ocular Disch.	Nuclear Scler.	Catar.	Retinal Haem.	Prep/Vag. Disch.	Polydip.	Loss of Weight	Vomit./Diarrh.		
SLE 1							+										+									+								
SLE 2		+	+				+																								+			
SLE 3							+							+																				
SLE 4				+																														CNS Signs
INF 1														+																			Gastr. Dermat.	
INF 2												+		+		+									+				+					
INF 3	+	+	+														+				+													
INF 4																				+														Work.dog Kn. trauma
INF 5		+												+			+																	Inf. Skin Wound
INF 6	+	+	+																															Kn. trauma
INF 7																																		Work.dog.Kn. trauma Inf.Fr.Pel.
INF 8			+	+																									+					Work. dog G.S.W.
INF 9																																		
INF 10	+	+	+											+							+										+		Thyroid Neo.	Inf. Decubitus.
INF 11																					+													Dog fight Wound
INF 12																																		Dog fight Wound
BE 1	+		+	+	+			+				+	+	+							+	+									+			
BE 2	+	+	+	+				+						+														+			+			
BE 3	+	+	+					+						+														+			+			
BE 4	+	+	+	+				+						+							+													
BE 5	+	+	+	+	+			+	+	+			+	+		+									+			+						
BE 6	+	+	+	+										+											+					+	+	+	OCD R sh.	Died within 24 hr.
BE 7	+	+	+	+				+						+											+						+			Died within 24 hr.

TABLE 8

SUMMARISING THE MAIN CLINICAL FEATURES (ASSOCIATED WITH THE MUSCULOSKELETAL SYSTEM) OF THE GROUP V (IDIOPATHIC) DOGS.

A + score indicates the presence of that particular clinical feature. No score indicates that the clinical feature was not present.

Key as for Table 4.

CLINICAL FEATURES REFERABLE TO THE MUSCULOSKELETAL SYSTEM

		Obvious Limp	Unable to Walk	Difficulty in Walking	Stiff after Rest	Stiff after Exercise	Joint Effusion	Artic. Soft Tissue Swell.	Joint Pain	Joint Heat	Joint Instabil.	Restrict. Joint Motion	Joint Crepitus	Muscle Atrophy	No. Jts. with Pain/ Swelling	JOINTS CLINICALLY AFFECTED								Involv. Tend. Sheath	Spinal Pain	Limb Oedema	Duration	No. of Attac.	Onset.
																Shou.	Elb.	Carp.	Hip	Stifle	Hock	MC/MT Phal.	Int. Phal.						
DOG IDENTIFICATION	ID 1			+	+				+						M			L	L R			L R F H	L R F H	+	+		3dy	1	Su.
	ID 2			+											8	L R	L R		L R	L R							5dy	1	Su.
	ID 3	+			+	+			+						5		L R		L R	R					+		1yr	1	Gr.
	ID 4	+		+	+		+		+					+ (T)	M	L R	L R	L R	L R	L R	L R	L R F H	L R F H				2wk	1	Su.
	ID 5	+						+	+						2			L		R							2wk	1	Su.
	ID 6	+			+										2	R	R										3wk	1	Su.
	ID 7	+					+	+	+						5			L R	R	L R							2dy	1	Su.
	ID 8	+					+	+	+						1		L										5dy	1	Su.
	ID 9			+				+	+						M	L R	L R	L R		L R	L R				+		10dy	3	Su.
	ID 10	+			+	+	+	+	+	+					2			R		R							3wk	7	Su.
	ID 11			+				+	+						6	L R			L R	L R					+		1m	1	Su.
	ID 12				+				+						5		L R		R	L R							2wk	1	Su.
	ID 13	+			+	+			+						6	L R	L R		L R								2yr	2	Su.
	ID 14				+		+	+	+		ACL LH	+			3			L		L R							18m	4	Su.
	ID 15	+			+	+					Dil.Pat.				4		L R			L R							2wk	1	Su.
	ID 16			+			+	+	+					+	8	L R	L R	L R		L R							3wk	1	Su.
	ID 17	+			+		+	+	+						3					L R	L					+	2wk	3	Su.
	ID 18			+			+	+	+						2		L			L					+		5m	1	Su.
	ID 19			+					+					+ (T)	6	L R	L R			L R					+		7dy	3	Su.
	ID 20	+						+	+						1			L									3wk	1	Gr.

TABLE 8 (CONTINUED)

CLINICAL FEATURES REFERABLE TO THE MUSCULOSKELETAL SYSTEM

DOG IDENTIFICATION	Obvious Limp	Unable to Walk	Difficulty In Walking	Stiff after Rest	Stiff after Exercise	Joint Effusion	Artic. Soft Tissue Swell.	Joint Pain	Joint Heat	Joint Instabil.	Restrict. Joint Motion	Joint Crepitus	Muscle Atrophy	No. Jts. with Pain/ Swelling	JOINTS CLINICALLY AFFECTED: Shou.	Elb.	Carp.	Hip	Stifle	Hock	MC/MT Phal.	Int. Phal.	Involv. Tend. Sheath	Spinal Pain	Limb Oedema	Duration	No. of attac.	Onset
ID 21	+			+		+	+	+					+ (T)	8			L R	L R	L R	L R						5dy	1	Su.
ID 22			+					+					+	4	L R	L R								+		4m	1	Gr.
ID 23		+				+	+	+	+				+ (T)	M	L R	L R	L R	L R	L R	L R	L R F H	L R F H				2wk	1	Su.
ID 24	+			+		+	+	+						4	L		L	R	L							3m	2	Gr.
ID 25		+						+					+	2						L R						3wk	2	Su.
ID 26	+			+			+	+						6		L R	L R			L R					+	5wk	1	Su.
ID 27	+					+	+	+			+			2	R				L						+	3wk	1	Su.
ID 28	+			+									+	0										+		6m	7	Su.
ID 29			+					+						M							L R F H	L R F H	+			8wk	1	Su.
ID 30	+							+						1		L										5dy	1	Su.
ID 31	+													0												1wk	4	Su.
ID 32	+			+			+	+	+	Bil.Pat	+	+		2					L	L				+		6wk	3	Gr.
ID 33			+				+	+	+				+ (T)	M		L R	L R		L R	L R	L R F H	L R F H			+	12dy	1	Su.
ID 34			+			+	+	+	+					7		L R	L	L R	L R							7dy	2	Su.
ID 35		+				+	+	+	+	Bil.Pat			+ (T)	M	L R	L R	R	L R	L R	L R						4wk	6	Su.
ID 36	+								+					1			R									4dy	1	Su.
ID 37				+	+									8		L R	L R		L R	L R				+		1yr	5	Su.
ID 38			+			+	+	+						2			R			R						2wk	2	Su.

TABLE 9

SUMMARISING THE MAIN CLINICAL FEATURES (OTHER THAN THOSE ASSOCIATED WITH THE MUSCULOSKELETAL SYSTEM) OF THE GROUP V (IDIOPATHIC) DOGS.

A + score indicates the presence of that particular clinical feature. No score indicates that the clinical feature was not present.

CLINICAL FEATURES (EXCEPT MUSCULOSKELETAL)

DOG IDENTIFICATION	Pyrexia	Anorexia Inappet.	Dull	Tachypn.	Tachyca.	Bradyca.	Pale M.M.	Cardiac Murm.	Rhonci Rales	Nasal Disch.	[illegible] Oral M.M.	Ulc. Oral M.M.	Excess Salivat.	Lymph-adenop.	Cough	Tonsil. Pharyn.	Period. Dis.	Dental Car/Abs.	Pruritus	Alopecia	Ulc. Skin	Subcut. Swelling	Excess Dandru.	Hyper-Kerat.	Ocular Disch.	Nuclear Scler.	Catar.	Retinal Haemorr.	Resp./Vag. Dischr.	Polydip.	Loss of Weight	Vomit/Diarrh.	Previous Illness	Additional Inform.
ID 1	+	+	+	+						+	+				+																			
ID 2	+	+	+	+																														
ID 3																																		
ID 4	+	+	+											+													+							
ID 5	+		+		+			+											+	+														
ID 6																														+				Splenic Enlargement
ID 7	+		+	+																					+									
ID 8																																		
ID 9																																		
ID 10	+	+	+																															
ID 11																																		
ID 12	+		+																+															
ID 13	+	+	+					+		+							+	+	+	+			+							+				
ID 14	+		+	+	+		+		+	+																								
ID 15		+		+										+												+				+				
ID 16	+	+	+									+	+																					
ID 17	+		+	+								+	+			+	+					+				+					+			
ID 18	+	+	+	+					+													+	+											
ID 19	+	+	+	+	+			+									+						+		+			+				+		
ID 20																	+						+										Test. Neo.	

TABLE 9(CONTINUED)

DOG IDENTIFICATION	CLINICAL FEATURES (EXCEPT MUSCULOSKELETAL)																																	
	Pyrexia	Anorexia Inappet.	Dull	Tachypn.	Tachyca.	Bradyca.	Pale M.M.	Cardiac Murm.	Rhonci Rales	Nasal Disch.	Haem. Oral M.M.	Ulc. Oral M.M.	Excess Salivat.	Lymph-adenop.	Cough	Tonsil. Pharyn.	Period. Dis.	Dental Car/Abs.	Pruritus	Alopecia	Ulc. Skin	Subcut. Swelling	Excess Dandruff	Hyper-kerat.	Ocular Disch.	Nuclear Scler.	Cater.	Retinal Abnorm.	Prep/vag. Disch.	Polydip.	Loss of Weight	Vomit/ Diarrh.	Previous Illness	Additional Inform.
ID 21	+	+	+	+			+	+							+								+											
ID 22	+	+	+	+	+					+	+		+	+					+		+		+		+			+			+			Vent.extra-systol.(ECG)
ID 23	+	+	+	+			+		+	+		+	+						+						+				+					
ID 24	+			+																								+						
ID 25		+	+				+	+						+																+				Splenic Enlargement
ID 26	+	+	+									+	+	+		+																		
ID 27	+	+	+														+	+			+													
ID 28	+	+	+							+						+	+								+							+		
ID 29								+																				+				+	Faec.Blood Test. Neg.	
ID 30				+				+	+								+								+								Ascites Test. Neg.	
ID 31		+																														+	Faec. Blood Anal.Sac Abs.	
ID 32		+	+															+																
ID 33	+	+	+	+	+		+			+			+	+																				
ID 34	+	+	+	+					+			+		+																		+		
ID 35	+	+		+				+	+	+						+									+	+		+						
ID 36	+	+	+																+	+	+													Hypopyon Uveitis
ID 37										+					+																			
ID 38																																+		

DISCUSSION

GROUP I (RHEUMATOID)

A variety of breeds were affected but the small and medium sized dogs predominated. This is similar to the findings of other authorities (Newton et al 1976; Pedersen and Pool 1978; Pedersen et al 1976a). However, the disease has been described in the larger breeds (Alexander et al 1976; Newton et al 1976) and certainly some of the larger breeds were represented in the present study. The disease seems to occur principally in middle aged dogs although can affect immature animals (Pedersen et al 1976a). One dog under a year of age was seen in the present study. In man, the disease can start at any time from 6 months to over 90 years of age but most commonly occurs in middle age (Boyle and Buchanan 1971). No sex predisposition has been noted in the dog although in man the female sex is more commonly affected (sex ratio of 2 or 3:1)(Boyle and Buchanan 1971).

Some of the dogs in the present study showed fever, dullness and loss of appetite and this has been recorded also by other workers (Alexander et al 1976; Newton et al 1976: Pedersen and Pool 1978; Pedersen et al 1976a)although Barrett(1977) regards pyrexia as very uncommon in the rheumatoid canine patient. Fever and malaise, often associated with an acute joint problem are uncommon in the adult human patient although are not infrequently seen in juvenile rheumatoid arthritis (Still's disease) (Boyle and Buchanan 1971).

Affected animals are usually reluctant to move and the lameness is usually generalised or"shifting", of a chronic progressive nature

(Alexander et al 1976; Barrett 1977; Newton et al 1976; Pedersen and Pool 1978; Pedersen et al 1976a; Wentink 1973). Periods of remission with relapses can occur (Barrett 1977). Bilaterally symmetrical involvement of several joints is typical in this disease. Joint pain was detected in most of the dogs although was not often severe. Heat in affected joints was rarely detected. Joint pain has been described as a feature of rheumatoid arthritis in the dog (Alexander et al 1976) although is often absent according to some authorities (Newton et al 1976; Pedersen et al 1976a). Soft tissue swelling around affected joints and synovial effusion within joints are also commonly appreciated in canine rheumatoid arthritis. Most dogs in the present study had a history of stiffness after rest - the equivalent of "morning stiffness" experienced by human rheumatoid patients (Boyle and Buchanan 1971). This stiffness is thought to be related to local oedema of the joint capsule and the ligamentous structures. Normally during the hours of sleep there appears to be a physiological transudation of fluid into these areas because of a lack of effective pumping action of the muscles which clears blood into the larger veins. This transudation of fluid into the soft tissues of an already inflamed joint probably causes further swelling of tissues which are already "stretched" because of the inflammation (Scott 1960). The degree of clinical problem exhibited by the dogs varied greatly and this is again true of the human patient and is probably related to the severity of the pathological processes within the joints.

According to most of the American authorities, secondary damage to collateral ligaments, cruciate ligaments and menisci frequently occurs and is associated with angular deformities and joint laxity.

Dogs Rh 2 and Rh 11 developed cruciate rupture and case Rh 1 showed subluxation of the left carpus, Rh 19 showed bilateral carpal luxation and intertarsal subluxation and Rh 6 had subluxation of several metacarpophalangeal and metatarsophalangeal joints. It is assumed that the inflammatory process affects ligamentous structures causing them to become weakened and thus predisposed to stretching and tearing. A similar phenomenon has also been reported in human rheumatoid patients (Boyle and Buchanan 1971). Three cases in the present study also showed medial patellar luxation but in all these dogs this was a long-standing problem apparently not related to the rheumatoid disease process.

Any peripheral joint can be affected in the dog although the stifle and carpus were the most common. This is again similar to the American reports. In man, the carpus and stifle are also commonly affected although the most consistently involved joint is the metacarpophalangeal and the metatarsophalangeal joint is also regularly involved (Boyle and Buchanan 1971). Seven of the dogs in this study did show involvement of these joints. One dog in the series of ten reported by Newton and his colleagues(1976)also had involvement of the metacarpophalangeal joints.

Involvement of tendon sheaths is a common feature of human rheumatoid arthritis but has not been reported in the dog. No obvious clinical evidence of tendon sheath inflammation was detected in any of the dogs in this study. No published account describing clinical involvement of the spine in canine rheumatoid arthritis has appeared although dogs with rheumatoid disease of the cervical spinal joints have been seen at the Animal Medical Center in New York (Dr.N.C.Pedersen - personal communication). Three dogs

in the present study showed spinal (neck) pain on palpation. In man, rheumatoid arthritis frequently involves the cervical spine (Boyle and Buchanan 1971).

Ankylosis of diseased joints occurs regularly in human patients but was not seen in any of the 21 dogs in the present study. None of the case-reports by the American workers mention ankylosis as the extreme pathological change in diseased joints of the dog although it is described in the review articles by Barrett (1977) and Pedersen and Pool (1978). Ankylosis of a single joint is reported in the only published account of canine rheumatoid arthritis in the U.K. (Halliwell et al 1972). In man, it has been proposed that there are biological variations among individuals which in some favour joint subluxation and instability,and in others promote bony or fibrous ankylosis (Anderson 1975). Some dogs had restricted joint movements and crepitus and muscle atrophy, presumably a disuse atrophy, was often apparent (see Section IX). Muscle atrophy occur in human rheumatoid patients and is mainly due to disuse,but more serious pathological alterations have been described.

Some human patients give a history of an upper respiratory tract infection prior to the joint problem (Lewis-Faning 1950) and this appears to be especially so when the onset of articular disease is acute and fulminating (Short, Bauer and Reynolds 1957). Five of the dogs in this study had some clinical evidence of a respiratory disease and one had a severe pharyngitis/tonsilitis and of these six animals, four had a sudden onset joint problem. Subcutaneous swellings were found in dogs Rh 4 and Rh 11; subcutaneous swellings other than pressure sores have not been reported elsewhere in the dog. Subcutaneous nodules are found

in approximately one third of human patients (Kellgren and Ball 1959) and are most classically seen over the olecranon. In dog Rh 11 the swelling was over the olecranon, in the other dog the swelling was associated with the acromium process of the scapula. A generalised lymphadenopathy was seen in four dogs and this has also been noted by other authorities (Barrett 1977; Newton et al 1976; Pedersen et al 1976a). Enlargement of the peripheral lymph nodes does occur in human rheumatoid patients but is most commonly seen in Still's disease (Boyle and Buchanan 1971).

Three dogs (Rh 12, 19 and 21) had a history of gastrointestinal upset in the form of recurrent diarrhoea. Gastrointestinal involvement in human rheumatoid patients is rare but has been associated with amyloidosis (Sinclair and Cruickshank 1956). There has also been recent suggestions that an abnormal gut flora may be an important aetiological factor in rheumatoid arthritis (Anon 1979c) (see Section VIII). The single case of canine rheumatoid arthritis reported by Halliwell and his colleagues (1972) also exhibited diarrhoea. Three of the dogs in the present study also had an ocular discharge with some conjunctival congestion but the classic ocular diseases seen in human rheumatoid patients were not diagnosed - conditions such as keratoconjunctivitis sicca, episcleritis, scleritis and scleromalacia perforans (Boyle and Buchanan 1971). Several dogs in this study also had some form of skin disorder and other cases reported in the literature have also had skin lesions (Halliwell et al 1972; Newton et al 1974; 1976). The main skin abnormality seen in the human patient is the development of subcutaneous nodules although necrosis of the skin secondary to vasculitis or vascular intimal hyperplasia has been described (Boyle and Buchanan 1971).

GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS)

Only four dogs were placed in this group and thus generalisations are difficult. All four dogs were presented with a history of stiffness after rest and case SLE 1 was only able to walk with extreme difficulty.

Dogs SLE 1 and SLE 3 showed pale mucous membranes and laboratory investigations revealed lowered haemoglobin levels and red blood cell counts associated with the presence of red cell autoantibodies in both cases (see Section VII). Although dog SLE 2 was also anaemic, no red cell autoantibodies were demonstrated. Dog SLE 2 also had a persistent proteinuria associated with weight loss, and although a glomerulonephritis was suspected, permission for a renal biopsy was refused. Case SLE 4 had, in addition to the joint problem, involvement of the central nervous system.

The many review articles on canine systemic lupus erythematosus (e.g. Lewis 1972; 1974) report clinical signs related to autoimmune haemolytic anaemia, thrombocytopaenia purpura, symmetrical polyarthritis, glomerulonephritis, exfoliative dermatitis and lymphocytic thyroiditis. These various manifestations can occur simultaneously or sequentially in various combinations. In the original series reported by Lewis and co-workers (1965), most of the seven dogs had signs referable to haemolytic anaemia, thrombocytopaenia and renal disease. These included pale mucous membranes, icterus, ecchymoses and petechiae in the skin and mucosae, epistaxis, subconjunctival haemorrhages, splenomegaly, weakness and emaciation. However, three dogs also had a history of lameness and one showed swelling and pain of both elbow joints. Lameness attributable to systemic lupus erythematosus was reported in a series

of dogs by Schalm and Ling (1970). A much larger series of lame dogs associated with systemic lupus erythematosus was reported by Pedersen and colleagues (1976b) although the diagnostic criteria used were far less rigid than those of the author's. The age of the dogs in this series ranged from 8 months to 8 years (mean 3.5 years) and the larger breeds, particularly Alsatians and dogs of the sporting breeds were most frequently affected. Several joints were normally involved and the animals walked with difficulty; in some cases only a few joints (or even a single joint) were affected and often these dogs had a migratory lameness. Remission of signs, followed by a relapse was common. Swellings of the joints were noted in about half the dogs and in the acute cases there was heat in the periarticular tissues. Synovial effusion was reported in both the acutely and chronically affected animals. The dogs were often pyrexic and transient diarrhoea was often noted. Peripheral lymph node enlargement, weight loss and muscle atrophy were other features. A number of dogs also had signs related to other systems such as skin disease, anaemia, pleuritis, renal disease and myelopathy. Halliwell (1978) reported 18 cases of probable canine lupus. Of these, 7 showed polyarthritis and another 3 had a history of shifting lameness; lameness was the most common presenting sign in this series and was either of an acute or chronic nature. Various other clinical features were reported e.g. fever, proteinuria, various types of skin lesion, anaemia, thrombocytopaenia, leucopaenia, nervous signs and lymphadenopathy. The ages ranged from 9 months to 10 years and various breeds as well as mongrels were represented.

A suspected case of canine systemic lupus erythematosus with polymyositis as well as polyarthritis was reported by Krum and his colleagues (1977). The four dogs reported by Monier et al (1978) exhibited polyarthritis, fever, wasting and proteinuria. Two of these dogs also had diffuse alopecia and hyperkeratosis and immunofluorescent examination of the skin demonstrated IgG deposits at the dermal-epidermal junction, although the illustrations are not convincing. Immunofluorescent studies in human systemic lupus erythematosus reveal the presence of gamma globulin and complement deposits, usually at the dermal-epidermal junction in 90% of cases with skin lesions (Hughes 1978a). Similar fluorescent patterns are seen in clinically unaffected skin in up to 50% of patients. These deposits probably represent complexes of antinuclear antibody and nuclear antigen (deoxyribonucleic acid) (Tan and Kunkel 1966).

In man, systemic lupus erythematosus can produce widespread clinical manifestations in the skin, heart, kidneys, joints, blood, central nervous system, lungs, gastrointestinal tract and many other organs (Boyle and Buchanan 1971; Haserick 1955; Hill 1957). Human lupus does have a strong predilection for females - some 80-90% of all cases are female (Siegel, Reilly, Lee, Fuerst and Seelenfreund 1964). This does not appear to be true of the dog from the reported cases although it is mentioned as a feature of the disease in several review articles (e.g. Lewis 1972;1974). However, of the 7 cases reported by Lewis et al (1965)only 4 were female, of the 29 reported by Pedersen et al (1976b) only 10 were female and of the 18 reported by Halliwell (1978),9 were female. It has been suggested that ovariectomy gives protection against systemic lupus erythematosus (Dr.F.Quimby- personal communication)and therefore the routine speying of the canine population could upset the sex incidence figures. In the

human patient, the disease is most common between the ages of 30 and 50 although can occur at any age (Maddock 1965). Fever is often present and lymphadenopathy is regularly encountered (Dubois and Tuffanelli 1964). Joint involvement in the human patient may be no more than a mild, transient arthralgia. In others, a symmetrical polyarthritis with an affinity for the small joints of the hands, wrists, elbows, shoulders and knees may be present (Dubois and Tuffanelli 1964). Tendon sheaths can also be affected (Cruickshank 1959).

GROUP III (INFECTIOUS)

Reports of bacterial arthritis in the dog are rare. The twelve cases in the present study were all in the larger breeds of dog and three were young animals. Pedersen and Pool (1978) also regard the larger breeds of dog as being more susceptible to septic arthritis. The male sex predominated. All but two dogs showed involvement of a single limb joint only.

Only two of the animals were presented with fever although Barrett (1977) regards pyrexia as a consistent feature of acute septic arthritis and reports that recurrent fever may be seen in the chronic cases. Similarly, Putnam and Archibald (1968) report fever as a fairly regular accurrence in cases of infectious arthritis.

It is stated that infection may gain entrance into a joint by penetrating wounds or via the blood-stream (Pedersen and Pool 1978). Frequent sources of infection for the latter method have included foci

of sepsis in the skin, the umbilicus of neonates, bone, teeth, pharynx, prostate, anal sacs, uterus etc. (Barrett 1977; Pedersen and Pool 1978). In many cases however, it can only be assumed that the joint infection is blood-borne since the association between septic arthritis and a focus of infection elsewhere in the body cannot be definitely confirmed.

In the present study, in addition to examples of joint infection arising from penetrating wounds and possibly from the localisation of infection from the blood-stream, there were also cases of joint sepsis probably arising from the extension of infection affecting structures adjacent to the joint.

Cases INF 1, 4, 6 and 9 were thought to be blood-borne infections but there were no obvious primary foci of sepsis although INF 1 did have a history of gastritis and a non-ulcerative dermatitis. Cases INF 2,3 and 5 were again thought to be examples of blood-borne infections and in these dogs possible foci of infection were identified. Case INF 2 had a tonsillitis and pharyngitis with ulceration of the oral mucosa as well as a purulent vaginal discharge; case INF 3 showed excessive dental tartar and periodontal disease as well as cutaneous ulceration and dog INF 5 had an infected skin wound as well as severe periodontal disease.

Case INF 7 had a fractured, infected pelvis, together with infection of the surrounding musculature and it is likely that the hip joint became secondarily involved. Dog INF 10 had an infected decubitus over the involved joint (left elbow) and infection may have again spread directly into the joint from the surrounding infected tissues.

Both INF 11 and 12 had been involved in dog fights and sustained penetrating wounds over the joints which subsequently became infected. It is likely that infection was introduced directly into the joint by penetrating teeth wounds, although dog INF 12 also had an extensive osteomyelitis of the proximal right humerus presumably arising from the penetrating wounds sustained during the dog fight and it is possible that the joint became infected by an extension from the bone rather than by a direct wound. Case INF 8 had several gun-shot wounds and a piece of lead shot had lodged in the infected elbow joint and it is obviously likely that the lead shot introduced the infection directly into the joint.

Most cases of septic arthritis in human patients are thought to arise from blood-borne infections (Oldham 1937; Willkens, Healey and Decker 1960) although direct penetration into the joint from the outside or extension of infection into the joint from neighbouring structures can also occur (Cutler 1938; Smith and Ward 1966).

Six of the dogs in the present study had also encountered some form of trauma to the affected joint. INF 4, a working dog, had "sprained" the left carpus 2 or 3 weeks previously and was initially lame at this time for a couple of days. Dog INF 6 had shown a transient lameness two weeks before presentation, after boisterous play with its litter-mates and dog INF 7, another working dog, had been butted by a sheep resulting in an undisplaced fracture of the right ilium which became infected some days later when the lameness altered from a right hindleg limp to complete carriage of the leg. Case INF 8, a working gun-dog, had obvious damage to the joint caused by the gun-shot and dogs INF 11 and 12 obviously

suffered trauma to the joint during their dog fights.

In addition to the dogs reported here, several animals have been presented at the Hospital with open wounds of a joint, particularly the hock joint, resulting from road traffic accidents. Any age or breed of dog can be affected and many of the hock cases are complicated by fractures and/or ligament/tendon injuries. In these cases, the wounds are cleaned and both local and systemic antibiotics are used. These cases rarely develop into septic arthritis problems despite the obvious potential for the direct introduction of infection into the joint.

In man, septic arthritis is characterised by a sudden onset lameness with involvement of one, or sometimes two, joints (Ansell 1978; Clark 1963). Involvement of three or more joints occurs in less than 5% of patients (Argen, Wilson and Wood 1966; Kelly, Martin and Coventry 1970; Russell and Ansell 1972). There is usually joint pain, heat and swelling and there may be a history of trauma. Clinically, the knees,wrists and elbows are most often involved and tenosynovitis is frequent (Boyle and Buchanan 1971) although the hip joint is commonly infected in the infant (Samilson, Bersani and Watkins 1958). Predisposing factors include intra-articular injections, debilitating illnesses such as diabetes, rheumatoid arthritis and systemic lupus erythematosus (Quismorio and Dubois 1975), regional enteritis (London and Fitton 1970), neuropathic joints (Martin, Root, Kim and Johnson 1965), leukaemia (Douglas, Levin and Sokoloff 1964), abdominal surgery (Smith and Ward 1966) and injury (Willkens et al 1960) as well as treatment with corticosteroids. Immunodeficiency disorders are also sometimes important in infantile bone and joint infections (Kuo, Lloyd-Roberts, Orme and Soothill 1975). Primary

septic arthritis in the human affects either sex and all ages but is more common in the young patient (Borella, Goobar, Summitt and Clark 1963; Paterson 1970). Low grade fever and malaise may be evident (Russell and Ansell 1972; Ward, Cohen and Bauer 1960).

GROUP IV (BACTERIAL ENDOCARDITIS)

Like the previous group, these dogs were all of the larger breeds and six of the seven were male. All were mature animals. All the dogs had systemic signs (fever,dullness, poor appetite) and an obvious, pansystolic cardiac murmer was heard in all but one of the dogs.

The exact pathogenesis of canine bacterial endocarditis is unknown but Detweiler and his colleagues (1968) believe that it is secondary to a localised bacterial infection elsewhere in the body and they report the condition in dogs with pneumonia and infections of the mouth,pharynx, tonsils, anal sacs, prostate and skin. It is assumed that there is also some endocardial damage which allows organisms in the blood to localise and colonise the endocardium. From the heart,infected emboli can spread to other tissues including the joints.

Two cases of bacterial endocarditis associated with polyarthritis in the dog were reported by Caywood and his colleagues (1977) and these dogs showed pyrexia and had a history of a shifting lameness and a stiff gait; one of these dogs was a male, the other a bitch. All three dogs reported by Murdoch and Baker (1972) were female,and had exhibited lameness and in one case swelling and pain in a number of joints. Pyrexia was absent in all three at the time of examination although two had a

history of fever. Lameness in cases of canine bacterial endocarditis has been explained by a muscle necrosis associated with septic emboli rather than by a septic arthritis (Knight, Kelly and Wardley 1972; Nielsen, and Nielsen 1954; Shouse and Meier 1956). Petechial haemorrhages in the eyes are regarded as a common feature of canine bacterial endocarditis (Detweiler, Patterson, Luginbühl, Rhodes, Buchanan, Knight and Hill 1968; Lillehei Bobb and Visscher 1950). Two dogs in the present study had retinal haemorrhages. The clinical features of bacterial endocarditis in man are similar to those in the dog and include fever, malaise, petechiae of skin and mucous membranes, subcutaneous nodules, retinal lesions, anaemia, splenomegaly, clubbing, lameness and signs related to renal disease (Bacon et al 1974; Gutman et al 1972; Messner et al 1963; Ruiter and Mandema 1964; Williams and Kunkel 1962). Interestingly, the clinical signs of bacterial endocarditis in the human patient have been confused with systemic lupus erythematosus (Gutman et al 1972; Ruiter and Mandema 1964). One of the dogs in this study was misdiagnosed as lupus and similar confuion has probably occurred elsewhere (see pages 24-26).

GROUP V (IDIOPATHIC)

This was the largest group of dogs in the study. Most dogs were presented with fever, anorexia/inappetence, lethargy and tachypnoea in addition to the lameness. A variety of other clinical signs were recorded but it was difficult to know whether pathology of other body systems was part of the same disease process as that affecting the joints or whether it was just coincidental or even secondary to the debility produced by the joint problem. A similar group of dogs has been described by several authors in the U.S.A.(Barrett 1977; Pedersen and Pool 1978; Pedersen et al 1976b).

Fever is common, often cyclic in nature and the animals may be obviously lame in a particular limb or generally stiff (Pedersen and Pool 1978). The American authorities also state that the smaller distal joints are most often affected, particularly the carpus and hock which is not in total agreement with the cases reported here.

A series of 17 cases (11 female, 6 male) all in young Greyhounds (average age 18 months) was reported by Huxtable and Davis (1976). Fever was not a feature of these cases, and the onset of lameness was insidious. The clinical features varied from mild lameness and restriction in gait to severe joint pain and reluctance to move. Swelling of joints was recorded and the superficial lymph nodes were readily palpable. Only one Greyhound was included in the author's series and none of the American authorities mention a predisposition of this breed to polyarthritis. A condition referred to as periarthritis has been recorded in Greyhounds in the U.K. (Castell 1969). These animals are usually presented acutely ill, depressed, off food, pyrexic and lame. Pain and swelling is usually present in one but sometimes in two or more joints and oedema of the limb may be present.

Pedersen et al (1976b) reported non-septic arthritis in 34 dogs and 15 of these had a chronic infectious disease process somewhere in the body. Included in this group were 3 dogs with bacterial endocarditis, 3 dogs with dirofilariasis, 2 dogs with chronic otitis, one dog with follicular vaginitis, one dog with pyometra, 3 dogs with actinomycosis, one dog with coccidioidomycosis and one dog with canine granulocytopathy syndrome. These authors suggested that the chronic infectious process leads to the formation of immune complexes which then produce the inflam-

matory joint reaction. Some of the dogs in the present study had foci of infection elsewhere in the body e.g. respiratory herpes virus infection, tuberculosis, bacterial pyelonephritis, cystitis and prostatitis, pneumonia (see Section IX). Non-septic joint inflammation has also been reported in human patients with chronic bacterial infections elsewhere and the severity of the joint inflammation is often proportional to the severity of the infectious process (Coggeshall, Bennett, Warren and Bauer 1941).

Pedersen and Pool (1978) described enteropathic arthritis in the dog. These dogs had a polyarthritis associated with a chronic ulcerative colitis or a fulminating enterocolitis. The arthritis tends to "flare-up" simultaneously with the bowel disease and it has been postulated that the bowel and joint diseases share a common aetiology or that products (? antigens) released into the blood from the inflamed bowel have some direct effect on the synovium or lead to an immune complex disease. A single case of canine polyarthritis associated with colonic disease was also reported by Quimby et al (1978) although this dog was positive for LE-cells, antinuclear antibody and rheumatoid factor. Six dogs in Group V showed gastrointestinal disease but in only two cases (ID 29 and 31) was a biopsy of the intestine carried out to confirm an ulcerative colitis. Both these dogs had shown blood in their faeces. A similar relationship is seen in man where approximately 11% of patients with ulcerative colitis show an inflammatory arthropathy(Wright and Watkinson 1965). There is no sex predisposition and it commonly occurs at 30-40 years of age and at the onset it may be monoarticular often the knee or ankle. If the disease spreads to other joints, the pattern of involvement is usually symmetrical. Often the arthritis is only mild but articular symptoms are correlated with the colitis. Synchronous remission and exacerbation of arthritis and

colitis is not uncommon. Colectomy often results in the joint problem resolving itself (Wright and Watkinson 1966) and corticosteroids given in the treatment of the colitis are also effective in controlling the arthritic signs (McEwen,Ling,Kirsner and Spencer 1962). Arthritis also occurs in approximately 5% of human patients with regional ileitis or Crohn's disease (Daffner and Brown 1958; van Patter, Borgen, Dockerty, Feldman, Mayo and Waugh 1954) and as with ulcerative colitis, there is a strong correlation between the activity of the disease in the bowel and in the joints. Enteropathic arthritis is also seen in Whipple's disease which is a chronic wasting disease, mainly of the male, characterised by weight loss, diarrhoea, polyarthritis and abdominal pain (Haslock 1978; Maizel, Ruffin and Dobbins 1970; Whipple 1907). In addition, several forms of gastrointestinal infection in man can be complicated by joint disease e.g. salmonellosis (Berglof 1963; Vartiainen and Hurri 1964) and yersinia infections (Haslock 1978). Huxtable and Davis (1976) did report subacute catarrhal duodenitis in two of their 17 dogs with polyarthritis although the significance of this observation is uncertain.

Pedersen et al (1976b) reported an inflammatory arthropathy in 2 dogs associated with pancreatitis. It is interesting that case ID 36 had laboratory evidence of pancreatitis (grossly elevated serum amylase level (see Section V) although no clinical signs referrable to it. Barrett(1977) mentions arthritis in the dog associated with neoplastic disease elsewhere in the body. Some of the dogs in the present study did show neoplastic lesions in addition to the arthritis e.g. heart base tumour, tonsillar carcinoma, lymphoma, atrial neoplasm, testicular neoplasms(see Section IX). In man, carcinoma of the pancreas, prostate, breast and lung have all been reported in association with a polyarthritis (Calabro

1967; Haslock 1978; Virshup and Sliwinski 1973). Leukaemia (Fink, Windmiller and Sartain 1972) and lymphosarcoma (Martin, Bennett, Hughes and Holt 1973) have also been associated with the development of synovitis.

Huxtable and Davis (1976) described tonsillar enlargement in 4 of their polyarthritic dogs; four of the dogs in the present study showed a marked tonsillitis.

Ocular disease in the rheumatic diseases of man is not uncommon and includes conjunctivitis, keratoconjunctivitis sicca, scleritis, uveitis and retinal vasculitis (Boyle and Buchanan 1971; Hazleman 1978). Five of the dogs in this study had evidence of retinal disease and one had lesions consistent with uveitis. Disease of the skin and mucous membranes is also regularly encountered in the human rheumatic patient (Boyle and Buchanan 1971; Gilkes 1978) and was again seen in some of the dogs in the present study. Glomerulonephritis associated with proteinuria and weight loss was another complication seen in some of the dogs (see Sections V & IX) and although renal disease is not uncommon in several of the human rheumatic diseases (Boyle and Buchanan 1971) it is classically seen in systemic lupus erythematosus (Dubois and Tuffanelli 1964; Freedman and Markowitz 1962a,b; Miyasato, Pollak and Barcelo 1966; Muehrcke, Kark, Pirani and Pollak 1957).

There are several reports of unclassified inflammatory arthropathies in the human patient and it has been suggested that some of these at least could be related to a viral disease (O'Sullivan and Cathcart 1972; Sauter and Utsinger 1978; Schumacher and Kitridou 1972; Steere, Malawista, Hardin, Ruddy, Askenase and Andiman 1977). In addition, there are confirmed cases

of viral arthritis usually accompanied by fever, malaise, anorexia/ inappetence, skin rashes, nephritis, neurological disturbances, cardiac abnormalities and other symptoms. The viruses have included arboviruses (Sauter and Utsinger 1978), rubella (Smith and Guzowska 1970), hepatitis B (Alarcon and Townes 1973), adenoviruses (Panush 1974; Utsinger 1977), mumps (Caranasos and Felker 1967), variola and vaccinia (Cockshott and MacGregor 1958), infectious mononucleosis (Adebonojo 1972) and chicken pox (Mulhern, Friday and Perri 1971). Although only limited viral studies were done in this group of dogs, no positive results were recorded (see Section VIII).

Further attempts at classifying this group of dogs are discussed in the sections on radiography, haematology and biochemistry and pathology.

SECTION III

RADIOGRAPHICAL ASSESSMENT OF DOGS WITH INFLAMMATORY JOINT DISEASE

Veni, Sancte Spiritus,
Et emitte coelitus
Lucis tuae radium.

Archbishop Stephen Langton 1228.

METHODS

All dogs in this study except for 3 (Rh 8, BE 6 and BE 7) were subjected to a detailed radiographical examination. It was normal to radiograph all clinically affected joints and in many of the polyathritic dogs, clinically non-affected joints were also radiographed. Standard views of the limb joints were taken; the commonest were lateral projections except for the carpus and hips where anteroposterior and ventrodorsal views respectively were used. Additional views of the joints were sometimes employed, particularly if obvious abnormalities were seen on the initial film. Lateral and sometimes dorsoventral radiographs were also taken of the abdomen and thorax as routine. Radiography was performed in conscious, sedated and anaesthetised animals.

All radiographs were taken using the Siemens Heliophos 4S X-ray machine with the 3DM overhead tube support with motor drive. High definition screens (Siemens Rubin High Definition Screens) were used for the elbow, carpus, stifle, hock and digital joints with Kodak X-Omat H film (Kodak House, Station Road, Hemel Hempstead, Herts.) although occasionally non-screen film was used (X-Omat MA film). For radiography of the shoulder and hip joints, thorax and abdomen, Kodak X-Omatic Casette Regular intensifying Screens were used with Kodak X-Omat H film and using the catapult Bucky with a Lysholm grid (Elema-Schonander, Stockholm, Sweden). All radiographs were processed using the automatic Kodak RP X-Omat processor, Model 101.

Occasionally, the technique of macroradiography was used to provide enlarged images of joints, to aid in the radiological interpretation.

This technique involved increasing the subject/film distance so that it was the same as the tube/subject distance. Although the radiographic image was magnified, there was a loss of clarity. However, visualisation of certain lesions such as minor articular erosions in the small digital joints, was often facilitated by this technique.

RESULTS

GROUP I (RHEUMATOID) (TABLE 10)

Of the 20 dogs in this group which were radiographed, all but 2 showed loss of bone from the epiphyses of at least one,and more often of several joints. This is to be expected, however, since this was one of the diagnostic criteria used for the recognition of canine rheumatoid arthritis. This loss of bone was characterised by the following:- (i) a diffuse,generalised loss of mineral (ii) discrete radiolucent foci,and (iii) an irregular joint margin. The diffuse loss of bone density was seen in the epiphyses of the joint or in the individual bones comprising the joint and was a fairly consistent feature. Similarly, radiolucent focal areas occurred in the epiphyses or individual bones of, for example, the carpus and tarsus and also in the patella and fabellae of the stifle and the volar sesamoid bones of the metacarpo/metatarso-phalangeal joints. There were usually several of these focal areas in any one joint and these often had the appearance of small "punched-out" defects confined to the subchondral area and in some cases these lesions appeared confluent with the articular margin giving the latter an irregular outline. However, in some cases these lesions occurred well within the epiphyses and were sometimes very extensive. The joints showing these radiographic features were,in decreasing order of frequency, the carpus (11 cases), the stifle (6 cases),the metacarpo-phalangeal(6 cases), the metatarso-phalangeal (4 cases) and the hock (3 cases). The elbow (2 cases), inter-phalangeal (2 cases), shoulder (1 case) and hip (1 case) were less commonly affected. In most cases similar radiographic changes occurred in the same joint on either side of the body.

Fifteen dogs also showed bony proliferation at the joints in the form of typical osteophyte formation or as a more extensive periosteal new bone deposition. Often the osteophyte development was less extensive than might be expected in cases of osteoarthritis of comparable temporal standing and it was usual to have both proliferative change and destructive change in the same joint. In some cases there was an obvious explanation for the osteophyte development e.g. cases Rh 2 and 11 were complicated by anterior cruciate rupture, case Rh 6 had obvious instability of the metacarpo-phalangeal and metatarso-phalangeal joints and case Rh 19 had bilateral radiocarpal and proximal intertarsal subluxations; all these lesions were visible on the X-ray films. Three cases (Rh 13, 14 and 18) also showed patellar luxation on the radiographs and this again could have explained secondary osteoarthritic changes.

Soft tissue swelling around affected joints and/or synovial fluid effusion within joints was regularly recorded. Alteration of the joint space was thought to be present in 5 cases, 3 showing a decrease and 2 an increase. One dog had calcification of the periarticular soft tissues of both hock joints. Sclerosis of the subchondral bone was only seen in three joints from 2 dogs. The presence of hip dysplasia and spondylosis was regarded as co-incidental.

It is obvious from Table 10 that several joints showed no obvious radiographical abnormalities despite their clinical involvement in many instances. However, the joints showing most obvious clinical involvement were those usually exhibiting the typical radiographic abnormalities.

The thoracic radiographs revealed an increase in bronchovascular markings in 9 dogs although the significance of this was uncertain. Lung changes consistent with pneumonia, were present in cases Rh 2, 7 and 10. Three dogs showed enlargement of the spleen on the abdominal radiographs, three had some evidence of hepatomegaly and three had slight prostatic enlargement. Case Rh 17 had an enlarged uterus containing the ossified skeletons of three foetuses.

GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS) (TABLE 11)

None of the joints of the four dogs in this group showed any evidence of bone destruction. In fact, most of the joints exhibited no obvious abnormalities. Synovial effusion was present in both stifle joints of dogs SLE 1, 2 and 3 and in addition case SLE 3 had slight soft tissue swelling of both carpi. Case SLE 1 had bilateral medial patellar luxation and case SLE 3 showed osteophyte formation in a single metacarpophalangeal joint of the left foreleg.

Case SLE 3 also had an increase in the bronchovascular pattern as well as hepatomegaly and SLE 4 exhibited a marked enlargement of the spleen on the abdominal radiograph.

GROUP III (INFECTIOUS)(TABLE 11)

As already discussed, most dogs in this group showed involvement of a single joint only. Radiolucent foci were noted in one or both epiphyses of the affected joint in cases INF 2, 3, 7, 11 and 12 and a generalised loss of mineralisation from the epiphyses was seen in cases

INF 6, 7 and 9. These destructive changes were in all cases, far less severe than those seen in the rheumatoid group and the incidence of joints affected was certainly different (two hip joints, two stifle joints, two shoulder joints and one carpus). Seven dogs showed osteophyte development or periosteal reaction at the infected joint. A sclerotic reaction of the subchondral bone was present in four cases. Soft tissue swelling was apparent in the majority of cases and synovial fluid effusion was seen in the three infected stifle joints. Three dogs (INF 3, 4 and 11) showed calcification of the soft tissues around the infected joint.

Case INF 1 had increased bronchovascular markings and prostatic enlargement, case INF 3 had slight enlargement of the prostate and case INF 4 had slight hepatomegaly. The radiographic examination of case INF 7 also revealed fractures of the left ischium and right ilium with an extensive periosteal reaction along the right ilial shaft consistent with an osteomyelitis. Several pieces of lead shot were scattered throughout the trunk and limbs of case INF 8 and one piece was localised to the infected elbow joint. An obvious sequestrum in the proximal end of the fifth metacarpal bone of the left foreleg of INF 11 was seen. Extensive osteomyelitis affecting the proximal humeral shaft (right) was also apparent on the radiographs of case INF 12.

GROUP IV (BACTERIAL ENDOCARDITIS) (TABLE 12)

Cases BE 6 and 7 died before they could be radiographed. Two of the remaining five cases showed radiolucent areas in the epiphyseal bone of affected joints and/or a generalised loss of epiphyseal mineral-

isation. These changes were similar to those of Group III and far less severe than those seen in the Group I dogs. In addition, case BE 2 showed partial collapse of the right hip joint with a decrease of the joint space and a possible pathological, impacted fracture of the femoral neck. Soft tissue swelling and/or synovial effusion was seen in 4 of the cases and 2 dogs had new bone deposits in affected joints. Some clinically affected joints showed no radiographical abnormalities.

Three dogs had coincidental spondylosis of the lumbar vertebrae. Cases BE 3, 4 and 5 all had evidence of pneumonia on the thoracic radiographs and the other two cases had obvious bronchovascular markings. Case BE 4 also showed renal calcification on the abdominal radiographs.

GROUP V(IDIOPATHIC) (TABLE 13)

The majority of joints which were radiographed in this group of dogs showed no obvious abnormalities despite being clinically affected. Five dogs did have evidence of mild destructive changes in the form of erosions and loss of mineralisation. Just under half the dogs had osteophyte development and/or periosteal reaction in an occasional joint. Periarticular swelling of one or more joints was present in fourteen cases and synovial effusion was apparent, mainly in the stifle joints, of 16 dogs. Cases ID 20 and 27 showed soft tissue calcification around the right elbow and right hock joints respectively. In the latter, the calcification involved the Achilles tendon. Dog ID 4 exhibited a decreased joint space of both elbow joints and dog ID 20 showed a decrease in the left elbow; dog ID 31 had an increased joint space in the left proximal intertarsal joint. Evidence for an anterior cruciate rupture was present in case ID 14 and there was complete luxation of the distal

interphalangeal joint of digit 5, right foreleg in dog ID 29.

Fourteen dogs had increased bronchovascular markings and five dogs showed obvious pneumonia on the thoracic radiographs. Another two dogs exhibited free pleural fluid and one of these (ID 17) had thickening of the parietal pleura. These features were consistent with the presence of a pleuritis. As well as showing bronchopneumonia, case ID 37 also had enlargement of the anterior sternal lymph nodes and possible enlargement of the thymus. Another two dogs (ID 22 and 30) showed extensive pulmonary oedema and cardiac enlargement associated with heart failure. A third dog (ID 35) also had cardiac enlargement.

The abdominal radiographs of 9 dogs revealed splenomegaly; eight dogs had some enlargement of the liver and prostatic enlargement was recorded in three dogs. Other abnormalities seen on the abdominal radiographs included bladder and urethral calculi (ID 18), ascites (ID 30), and extensive accumulations of intestinal gas (ID 17, 23, 25 and 33). Incidental features included hip dysplasia (14 dogs), spondylosis (7 dogs), disc disease (3 dogs) and evidence of an old fractured distal end radius and ulna in a single dog.

TABLE 10

SUMMARISING THE MAIN RADIOGRAPHICAL FEATURES OF THE GROUP I (RHEUMATOID ARTHRITIS) DOGS.

L - left

R - right

F - foreleg

H - hindleg

ACL- anterior cruciate ligament rupture

DOG IDENTIFICATION		JOINTS														
		N.A.D.	Bony prolif. Osteoph. Periost. react.	Radiolu. foci. Irreg. jt. margins Gen. loss mineral.	Sclerosis	Sublux. Instabil.	Soft tissue swelling	Effusion	Alterat. joint space	Calcific. periartic. soft tissue	Hip dysplas.	Spondylosis	Thorax	Abdomen	Others
	Rh 1	R st. LR carp. + dig. L elb. L.Reh	L.hoc. Lst. L carp.	LR carp.			L carp.					Thor.	Sl.incr. b-v mark.	Splenomeg.	Old fract. tib./fib. I.M. pin
	Rh 2		LR st. LR carp.	LR carp. LR st.	LR st.	L st. (ACL)	LR carp. LR st.	LR st.			+		Incr.b-v mark. Pneumonia		
	Rh 3	L sh. LR hoc.	LR elb. LR st. LR carp.	R st.			LR carp.				+				
	Rh 4	LR st.	LR carp.	LR carp. MCP LR LR hoc.MTP LR			LR carp. LR hoc.		Decrease LR inter-carp.		+		Incr.b-v mark.	Prost.sl. enlarg. Hepatomeg.	Calcif. i-v discs
	Rh 5	Lsh.LR elb. R carp.Lst. LR hoc.		LR hip			L carp.	R st.			+		Incr.b-v mark.	Prost.sl. enlarg. Sl.hepato-meg.	
	Rh 6	LR elb. LR hoc.	LRst.MCP LR.MTP LR. Prox.I-P LR FH	Lst.MCP LR.MTP LR	L st.	MCP LR MTP LR	L st. LR carp.	L st.	Increase MCP LR MTP LR						
	Rh 7	LR carp.	R st. Prox.I-P RF Dist.I-P RF	LR st. Prox.I-P LF Dist.I-P LF L elb.			L st.	LR st.					Incr.b-v mark. Pneumonia	Prost.sl. enlarg. Hepatomeg.	
	Rh 8														
	Rh 9	LR hoc. LR elb. L carp.		R carp.			R carp.	LR st.					Incr.b-v mark.		
	Rh 10	L elb. L carp.	R st. R hoc.				LR carp.	LR st.					Incr.b-v mark. Pneumonia		
	Rh 11	R hoc.MCP LR Prox. Dist.I-P LF RF. Lst.		LR carp. MCP LR Dist.I-P RF		L st. (ACL)	R elb. (triceps tend) R hoc (Achill. tend)				+			Splenomeg.	

TABLE 10 (CONTINUED)

		JOINTS														
		N.A.D.	Bony prolif. Osteoph. Periost react.	Radioluc. foci. Irreg. jt. margins. Gen.loss mineral.	Sclerosis	Sublux. Instabil.	Soft tissue swelling	Effusion	Altérat. joint space	Calcific. periartic soft tissue	Hip dysplas.	Spondylosis	Thorax	Abdomen	Others
DOG IDENTIFICATION	Rh 12	L elb.R st. Dig.jts.LF	L sh.R.hoc.	L carp.			L carp.								
	Rh 13	Dig.jts.LR H.I-P LR F L carp.	LR st. MCP L LR sh. LR elb.	LR elb. LR st. L hoc. MCP LR		Bilat.pat. (med)	LR st.	LR st.	Decrease R carp.		+	Lumber		Incr. intest. gas	
	Rh 14	LR hip	LR st.	LR st.		Bilat.pat. (med)		L st.							
	Rh 15	LR hoc.LR sh.LR elb. LR st.	MCP LR MTP LR LR carp.	MCP LR MTP LR LR carp.											
	Rh 16			LR carp.			LR carp.								
	Rh 17	L sh. LR carp.		R sh.				LR st.						Pregnant (3 foetuses)	
	Rh 18	L st. LR hoc. R st.	LR sh. LR elb. LR hip L st.			Bilat.pat. (med)	LR carp. LR elb.	L st.	Decrease LR elb.						
	Rh 19		LR carp. LR hoc.	LR carp.		Radio-carp. LR Prox.I-tar. LR	LR carp. LR hoc. Achill. tend. LR			LR hoc.					
	Rh 20	LR st.	MTP R MCP R Prox.I-P LH L carp.	LR carp. MTP R L hoc.			L carp.		Increase prox I-tar. L				Incr.b-v mark.	Incr. intest. gas	
	Rh 21	LR hip	LR carp.MCP LR.MTP LR Prox I-P LR FH.LR hoc.	R carp. MCP LR MTP LR			LR carp. LR elb. MCP LR MTP LR Prox.I-P LR FH	LR st.					Incr.b-v mark.	Splenomeg.	

TABLE 11

SUMMARISING THE MAIN RADIOGRAPHICAL FEATURES OF THE GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS) AND GROUP III (INFECTIOUS) DOGS.

Key as for Table 10.

DOG IDENTIFICATION		JOINTS									Hip dysplas.	Spondylosis	Thorax	Abdomen	Others
		N.A.D.	Bony prolif. Osteoph. Periost. React.	Radiolu. foci. Irreg. Jt. margins Gen loss mineral.	Sclerosis	Sublux. Instabil.	Soft tissue swelling	Effusion	Alterat. joint space	Calcific. periartic soft tissue					
	SLE 1	LR elb. LR hoc.				Bilat.pat. (med)		LR st.							
	SLE 2	LR elb. LR carp. LR hoc.						LR st.			+				
	SLE 3	LR sh. LR hoc.	MCP L				LR carp.	LR st.			+		Incr.b-v mark.	Hepatomeg.	
	SLE 4	LR hip Lumbar spine												Splenomeg.	
	INF 1	L st. R hoc.	R st. L hoc.				R st. L hoc.	R st.			+		Incr.b-v mark.	Prost. enlarg.	
	INF 2		L hip	L hip	L hip						+				
	INF 3	R elb.	R sh. L st.	L st.			L st.	L st.		R sh.				Prost. enlarg. Hepatomeg.	
	INF 4		L carp.				L carp.			L carp.	+				
	INF 5						R carp.								
	INF 6			L st.			L st.	L st.							
	INF 7		LR hip	R hip	R hip						+				Recent frac.L isch. + R ilium Osteomy. R ilium

TABLE 11(CONTINUED)

DOG IDENTIFICATION		JOINTS														
		N.A.D.	Bony prolif. Osteoph. Periost. react.	Radiolus. foci. Irreg. jt. margins Gen. loss mineral.	Sclerosis	Sublux. Instabil.	Soft tissue swelling	Effusion	Alterat. joint space	Calcific. periartic. soft tissue	Hip dysplas.	Spondylosis	Thorax	Abdomen	Others
	INF 8						L elb.								Numerous gun-shot, one within L elb.
	INF 9	L elb.		L sh.											
	INF 10						L elb.								
	INF 11		L carp.	L carp.	L carp.					L carp.	+	Thoracic			Sequest. prox.MC 5 LF
	INF 12		R sh.	R sh.	R sh.										Osteomy. prox. R humerus

TABLE 12

SUMMARISING THE MAIN RADIOGRAPHICAL FEATURES OF THE GROUP IV (BACTERIAL ENDOCARDITIS) DOGS

Key as for Table 10.

DOG IDENTIFICATION		JOINTS														
		N.A.D.	Bony prolif. Osteoph. Periost. react.	Radioluc. foci. Irreg.jt. margins Gen. loss mineral.	Sclerosis	Sublux. instabil.	Soft tissue swelling	Effusion	Alterat. joint space	Calcific. periartic. soft tissue	Hip dysplas.	Spondylosis	Thorax	Abdomen	Others
	BE 1	LR sh. LR elb. LR hoc. Lumbar spine		L st.			LR carp. R st.	LR st.			+		Incr.b-v mark.		
	BE 2	LR carp. + dig.jts. LR elb. R st. R hoc.	L st. LR sh.	R hip L st.			L st.	L st.	Decrease R hip		+	Thorecic Lumbar	Incr.b-v mark.		Collapse R fem. head
	BE 3	LR hoc.						LR st.				Thoracic Lumbar	Incr.b-v mark. Pneumonia		
	BE 4	LR elb. LR st. LR hoc.	LR hip LR sh.									Thorecic Lumbar	Broncho-pneumonia		Renal calcific.
	BE 5	L elb.					LR hoc. Achill. tend.LR	R st.					Pneumonia		
	BE 6														
	BE 7														

TABLE 13

SUMMARISING THE MAIN RADIOGRAPHICAL FEATURES OF THE GROUP V (IDIOPATHIC) DOGS

Key as for Table 10.

		JOINTS														
		N.A.D.	Bony Prolif. Osteoph. Periost. react.	Radioluc. foci. Irreg. jt. margins Gen loss mineral.	Sclerosis	Sublux. Instabil.	Soft tissue swelling	Effusion	Alterat. joint space	Calcific. periartic. soft tissue	Hip dysplas.	Spondylosis	Thorax	Abdomen	Others
DOG IDENTIFICATION	ID 1	Cerv.spine L st. R elb. Dig.jts.LRFH					LR carp. LR hoc.				+		Cardiomeg. Incr.b-v mark.	Splenomeg.	
	ID 2	LR elb. LR hoc.						LR st.							
	ID 3		LR elb. R sh.	R st.							+				
	ID 4	LR sh.LR carp.LR st.	MCP L						Decrease LR elb.		+		Incr.b-v mark.		
	ID 5	L carp.						L st.			+		Incr.b-v mark.	Prost.sl. enlarg. Sl. hepatomeg.	
	ID 6	LR carp.Dig. jts. LRF R sh.R elb.												Prost. enlarg. Splenomeg.	
	ID 7	R elb.Dig. jts.LRF L hoc. L st. LR hip	R carp.				LR carp.	R st.					Incr.b-v mark.	Splenomeg. Sl. hepatomeg.	
	ID 8	LR sh. R elb.						L elb.			+				
	ID 9	L carp. Dig.jts.LRF					R carp.	R st.							
	ID 10	Lcarp.Dig. jts.LRF R elb.	R sh.				R carp.				+				

TABLE 13(CONTINUED)

DOG IDENTIFICATION		JOINTS														
		N.A.D.	Bony prolif. Osteoph. Periost. react.	Radioluc. foci. Irreg. jt. margins Gen. loss mineral.	Sclerosis	Sublux. Instabil.	Soft tissue swelling	Effusion	Alterat. joint space	Calcific. periartic. soft tissue	Hip dysplas.	Spondylosis	Thorax	Abdomen	Others
	ID 11	LR sh. LR hip						LR st.							
	ID 12	LR elb.						LR st.			+				
	ID 13	LR carp.Dig. jts.LRF LR hip.LR st. L hoc.	R sh. R hoc.												
	ID 14	LR elb. L st.	MCP LR Prox.I-P LRF L sh. R st.	R st.		R st. (ACL)	LR carp. R st.	R st.			+				
	ID 15		L sh. L elb.					LR st.							
	ID 16	LR carp. Dig.jts. LRF.LR elb. LR hoc.					R st.	LR st.					Incr. b-v mark.		
	ID 17	LR carp.Dig. jts.LRF LR elb. R st.	LR hoc.					L st.			+		Incr. b-v mark. Pleur. thick. Pleur. fluid	Prost.sl. enlarg. Sl. hepatomeg. Splenomeg. Incr.intest. gas	
	ID 18	Cerv.spine L carp.Dig. jts LF.L elb. LR hip	LR sh.									Coccygeal	Incr. b-v mark.	Blad/ureth. calculi Hepatomeg.	
	ID 19	LR carp.Dig. jts.RF.L sh. LR elb.LR st. I-P jts.LF	MCP L								+				C2/C3 i-v space decreased
	ID 20	Dig.jts.LRF	LR sh. LR carp.	LR carp.			L carp.		Decrease L elb.	R elb.	+	Lumber	Incr. b-v mark.	Splenomeg. Sl.hepa-tomeg.	L2/L3 disc protr.

TABLE 13 (CONTINUED)

DOG IDENTIFICATION		JOINTS													
		N.A.D.	Bony prolif. Osteoph. Periost. react.	Radioluc. foci. Irreg. jt. margins Gen. loss mineral.	Sclerosis	Sublux. Instabil.	Soft tissue swelling	Effusion	Alterat. joint space	Calcific. periartic. soft tissue	Hip dysplas.	Spondylosis	Thorax	Abdomen	Others
	ID 21	R elb. LR hoc.	L carp.				LR carp. LR st.	LR st.					Incr. b-v mark. Pneumonia	Hepatomeg.	Old frac. rad./ulna
	ID 22	LR carp.Dig. jts. LRF LR st.LRelb. L hoc.	LR sh.										Cardiomeg. Pulm.oed.		C6/C7 i-v disc calcif.
	ID 23	Dig.jts.LRF L elb.L hoc. L st.					LR carp.	R st.					Pneumonia	Incr. intest. gas	
	ID 24	LR carp.Dig. jts.RF.I-P LF	L st. L sh. MCP L								+	Thoracic	Incr. b-v mark. Pleur. fluid	Splenomeg.	
	ID 25	L carp.Dig. jts. LF LR hoc.						R st.						Splenomeg. Inc. intest. gas	
	ID 26	LR carp. Dig.jts.LRF LR st.													
	ID 27	R st.	R hoc.				R hoc.			R hoc. (Achill. tend.)		Thoracic Lumbar	Incr. b-v mark.	Splenomeg.	
	ID 28	Cerv.spine Thor./lumb. spine LR hip.L st.													
	ID 29	LR carp. L elb.	MCP R L sh.			P2/P3 dig. 5 RF	Dig.jts. LRF					Thoracic Lumbar	Incr. b-v mark.	Prost. enlarg.	
	ID 30	L elb. L sh.											Cardiomeg. Pulm.oed.	Splenomeg. Hepatomeg. Ascites	

TABLE 13 (CONTINUED)

		JOINTS													
		N.A.D.	Bony prolif. Osteoph. Periost. react.	Radioluc. foci. Irreg. jt. margins Gen. loss mineral.	Sclerosis	Sublux. Instabil.	Soft tissue swelling	Effusion	Alterat. joint space	Calcific. periartic. soft tissue	Hip dysplas.	Spondylosis	Thorax	Abdomen	Others
DOG IDENTIFICATION	ID 31	LR st. LR hoc.									+				
	ID 32	LR carp. L st. R hoc.	LR sh. MCP LR Prox I-P LRF. L hoc. C3/C4 centr.	MCP LR C3/C4 centr. Prox I-P LRF	C3/C4 centr.		MCP LR L hoc.		Increase Prox.I-tar. L hoc.			Thorecic		Hepatomeg.	
	ID 33	Dig.jts. LR FH.Lst. R elb.					LR carp. L hoc. R st.	R st.					Incr. b-v mark.	Incr. intest. gas	
	ID 34	LR carp.Dig. jts. LRF LR elb.	LR sh. LR st.	LR sh.				R st.				Thoracic	Incr. b-v mark. Pneumonia	Sl. hepatomeg.	
	ID 35	LR hip.LR elb. R sh. L st.L hoc.											Cardiomeg. Pneumonia		
	ID 36	L carp.Dig jts.LRF.LR sh.LR elb. LR st.					R carp.								
	ID 37	LR carp.Dig jts. LRF R elb.R st.											Broncho-pneum. Enlarg. ant. stern.LN Enlarg. thym.	Prost.sl. enlarg. Sl. hepatomeg.	
	ID 38	LR carp. LR hoc. Dig.jts. LR FH. R sh.													

FIGURE 2

CASE Rh 6. RHEUMATOID ARTHRITIS. ANTERO-POSTERIOR RADIOGRAPH OF THE METACARPOPHALANGEAL JOINTS, RIGHT FORELEG.

Note the irregular joint margins caused by subchondral bone resorption. A large juxta-articular erosion is visible in the metacarpal bone of digit 5 (arrow). Proliferative changes are also present (p) and around the metacarpophalangeal joint of digit 2, calcified bodies appear separate from the metacarpal and phalangeal bones. The metacarpophalangeal joints of digits 2 and 5 show subluxation.

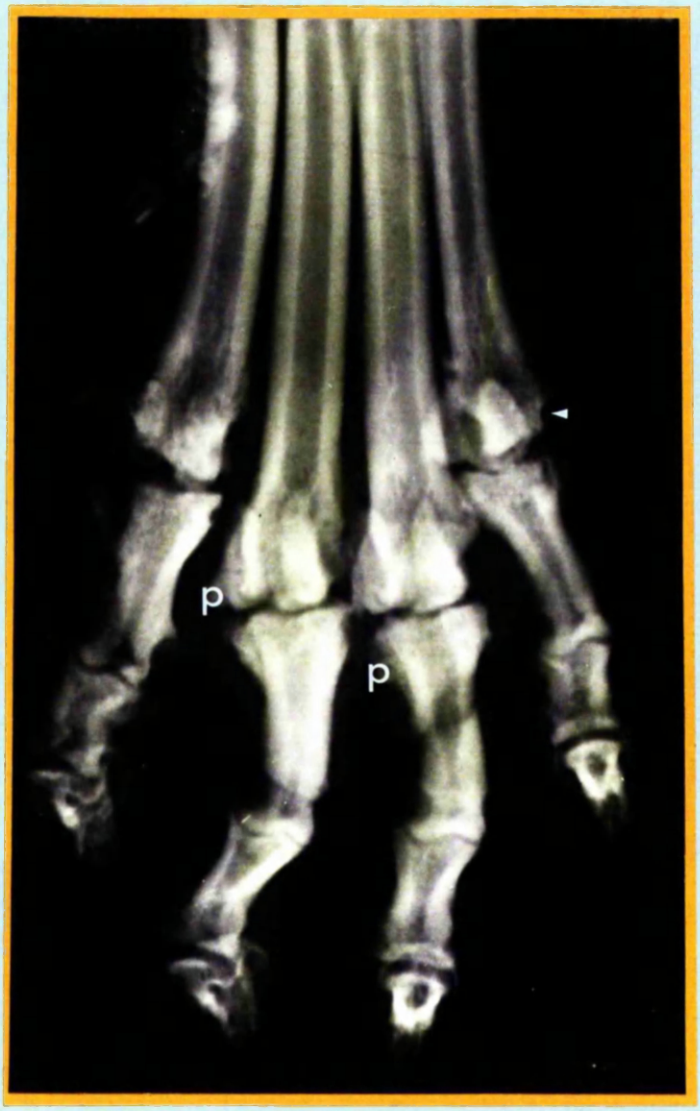

p
p

FIGURE 3

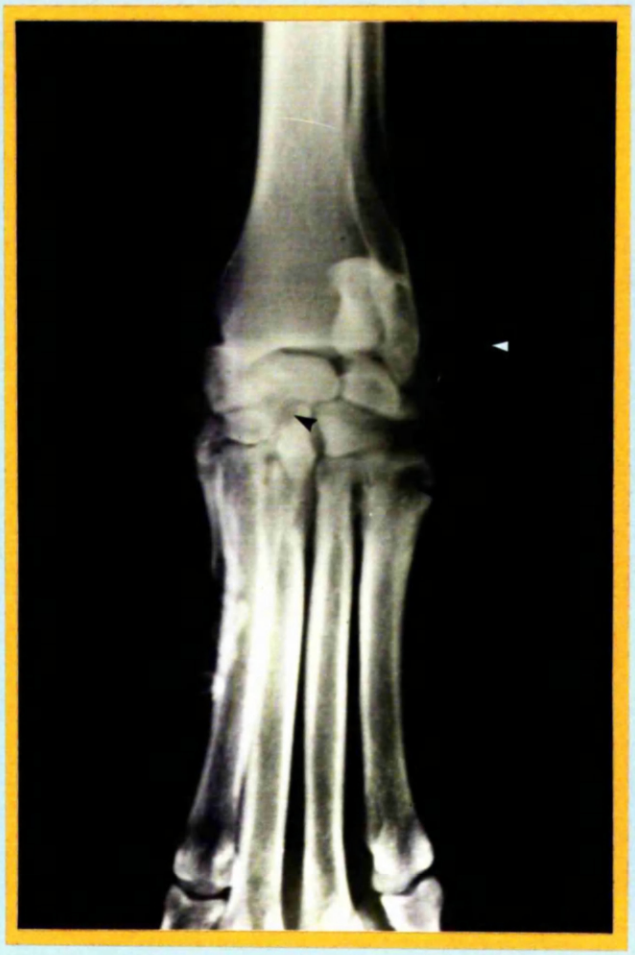
CASE Rh 2. RHEUMATOID ARTHRITIS.
ANTEROPOSTERIOR RADIOGRAPH OF THE
RIGHT CARPUS.

There is marked soft tissue swelling (white arrow)and radiolucent defects within the carpal bones and the proximal 5th metacarpal bone(black arrows).

FIGURE 4

CASE Rh 19. RHEUMATOID ARTHRITIS.
LATERAL RADIOGRAPH OF THE LEFT CARPUS.

There is marked soft tissue swelling (small arrows) and subluxation of the radiocarpal joint (large arrow).



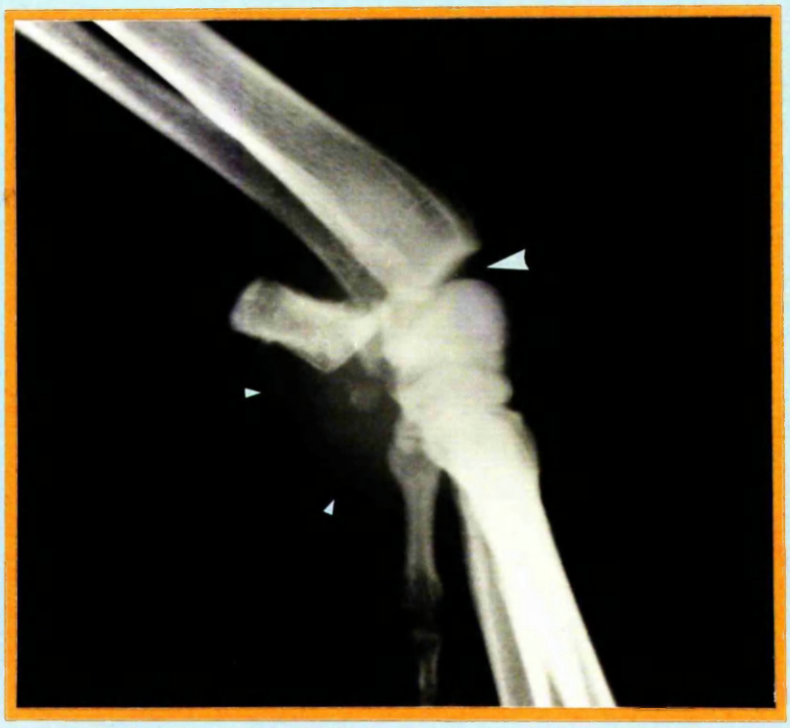


FIGURE 5

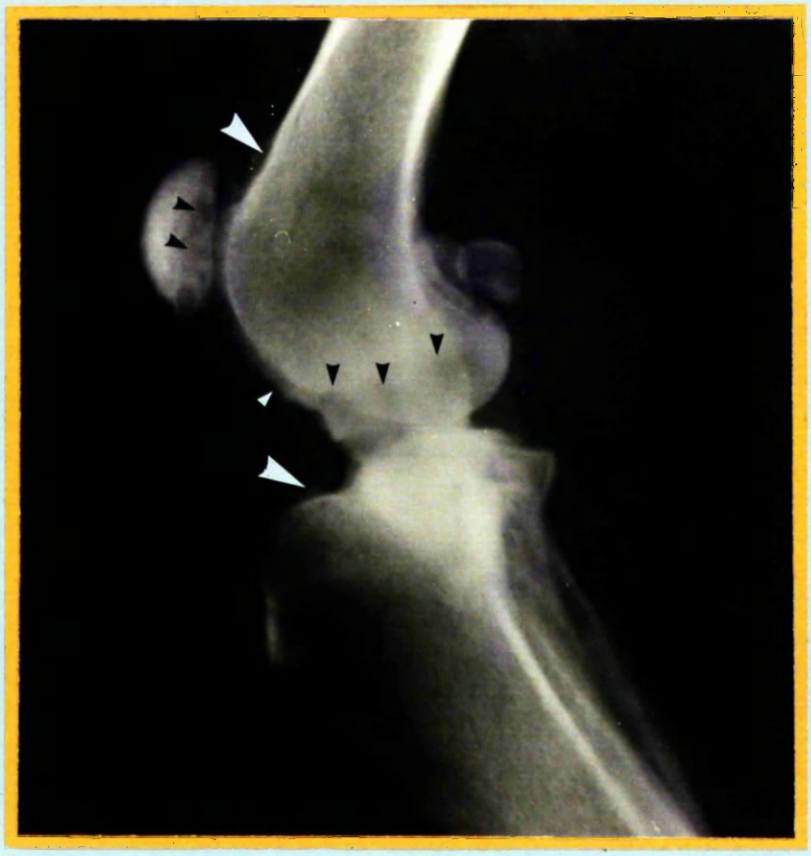
CASE Rh 2. RHEUMATOID ARTHRITIS. LATERAL RADIOGRAPH OF THE LEFT STIFLE JOINT.

There are several radiolucent areas within the patella and distal end of the femur (black arrows). Irregularity of the articular surface of the femur, due to bone loss is also visible (small white arrow). Increased soft tissue density within the joint space is apparent. Bony proliferative changes are also present (large white arrows), probably the result of an osteoarthritis secondary to a rupture of the anterior cruciate ligament which occurred in this dog some time after the rheumatoid arthritis had appeared as a clinical problem.

FIGURE 6

CASE Rh 13. RHEUMATOID ARTHRITIS. OBLIQUE LATERAL RADIOGRAPH OF THE LEFT ELBOW JOINT.

A very large erosion is present in the head of the radius (white arrow). Bone resorption is also obvious within the distal end of the humerus (black arrows).



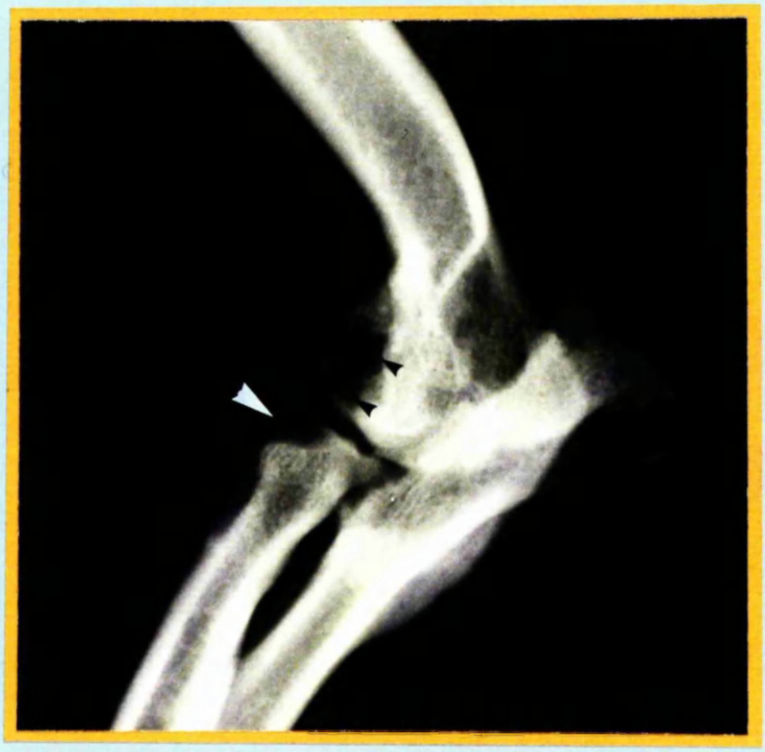


FIGURE 7

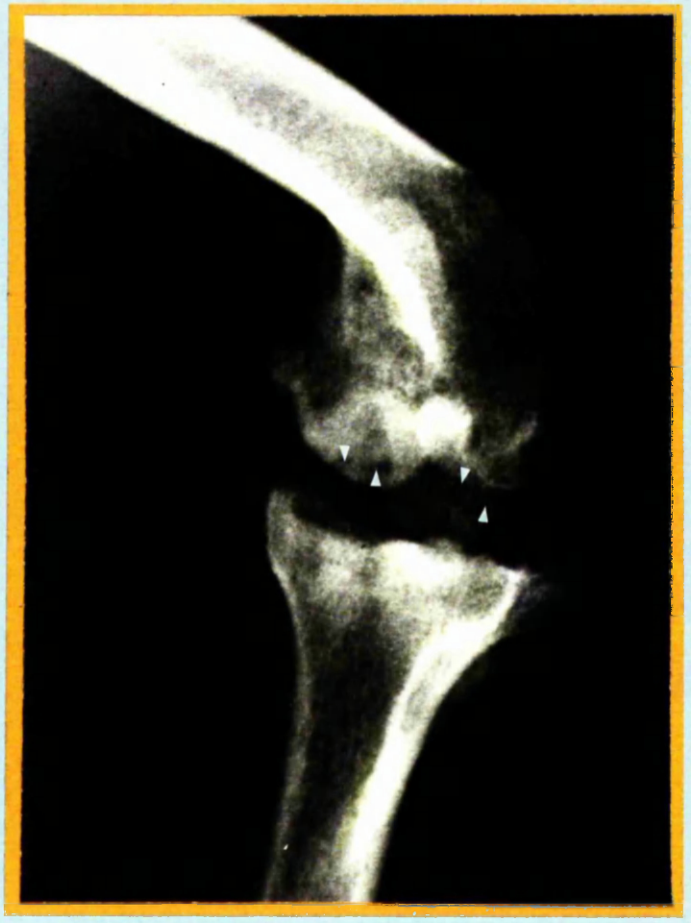
CASE Rh 14. RHEUMATOID ARTHRITIS. ANTERO-POSTERIOR RADIOGRAPH OF THE LEFT STIFLE JOINT.

Several radiolucent areas within the femoral condyles are present (arrows). In addition, there is an overall loss of mineralisation of the distal end of the femur. Proliferative changes, particularly affecting the proximal tibia are also apparent.

FIGURE 8

CASE Rh 4. RHEUMATOID ARTHRITIS. ANTERO-POSTERIOR RADIOGRAPH OF THE RIGHT CARPUS.

There is an overall loss of mineralisation of the distal end radius, carpal bones and proximal metacarpal bones. There is also a loss of joint space within the carpal joint. Periarticular soft tissue swelling is also evident.



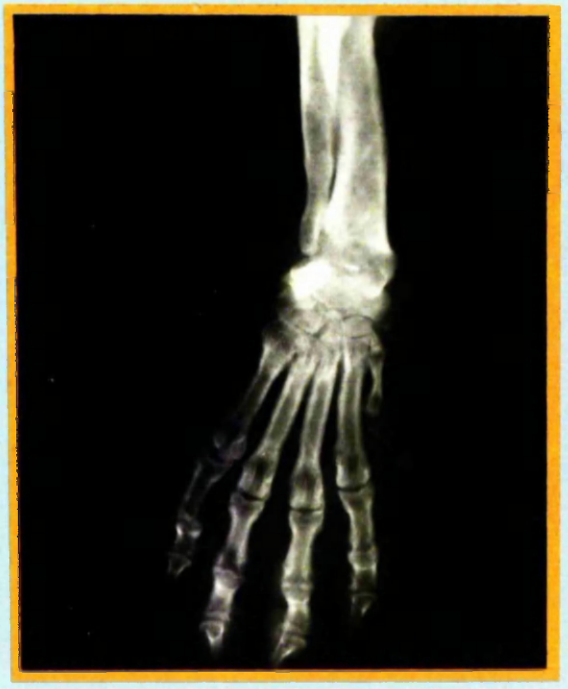


FIGURE 9

CASE INF 7. INFECTIOUS ARTHRITIS. VENTRO-DORSAL RADIOGRAPH OF THE PELVIS.

The right femoral head is mis-shapen and there are several focal areas of radiolucency within the femoral head (small black arrows). Periosteal new bone associated with the proximal femur is evident (small white arrows). Sclerosis of the acetabulum is also visible (s). Extensive periosteal new bone is present along the right ilium (large white arrows) associated with an osteomyelitis and there is a fracture line across the right ilium (large black arrow). The left hip joint shows dysplastic changes with secondary osteoarthritis (osteophyte development).

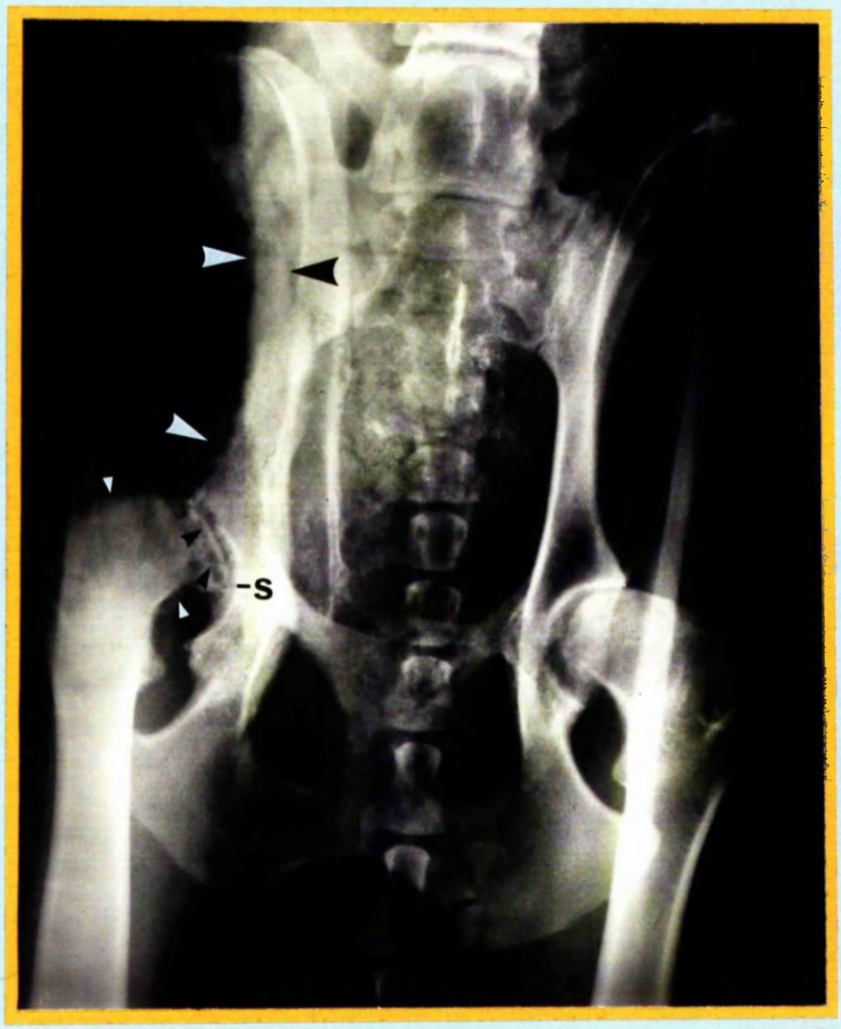

S

FIGURE 10

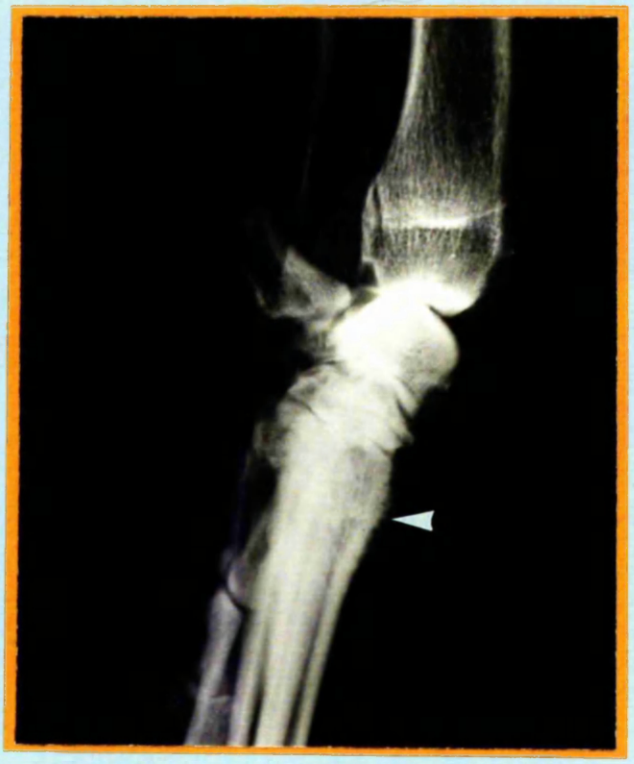
CASE INF 11. INFECTIOUS ARTHRITIS. LATERAL RADIOGRAPH OF THE LEFT CARPUS.

Note the periosteal reaction, particularly along the proximal metacarpus (arrow).

FIGURE 11

CASE INF 4. INFECTIOUS ARTHRITIS. LATERAL RADIOGRAPH OF THE LEFT CARPUS.

Note the area of periarticular soft tissue calcification (arrows).



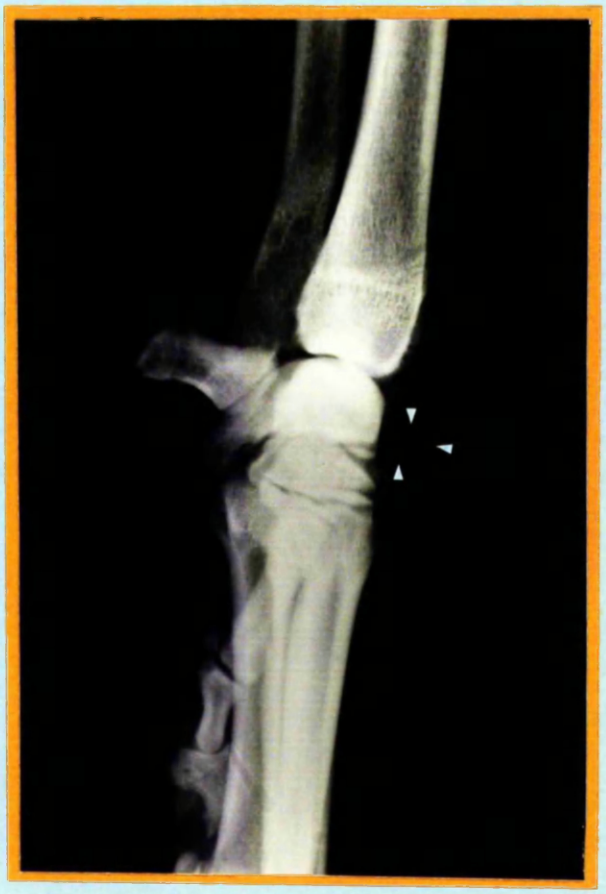


FIGURE 12

CASE ID 3. IDIOPATHIC INFLAMMATORY ARTHROPATHY.

LATERAL RADIOGRAPH OF THE RIGHT ELBOW JOINT.

Osteophyte development on the anterior aspect of the distal humerus is seen (arrow). The osteo-arthritic changes are coincidental, being present long before the inflammatory joint problem developed.

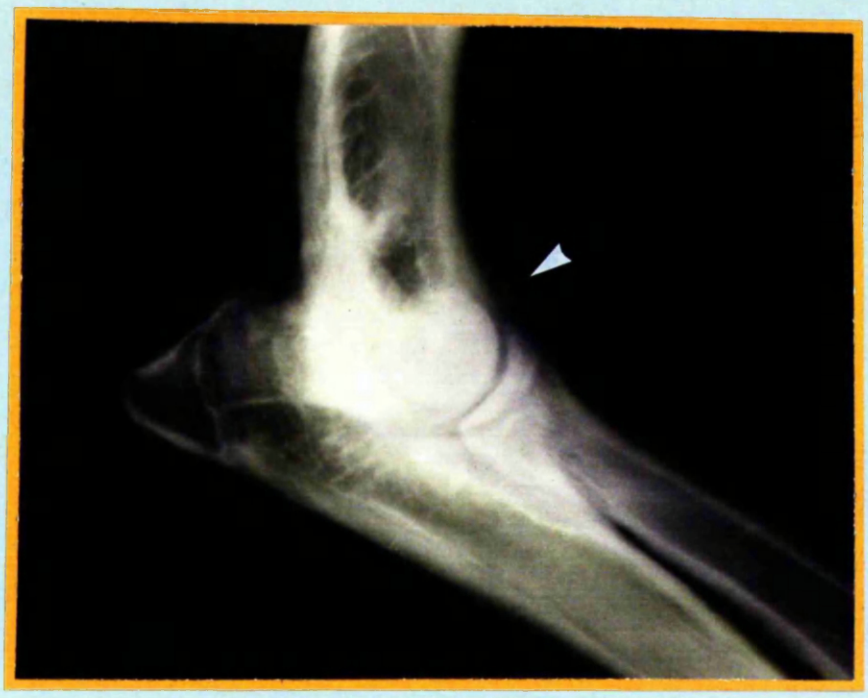
Joints with idiopathic inflammatory arthropathy often show no obvious radiographical abnormalities.

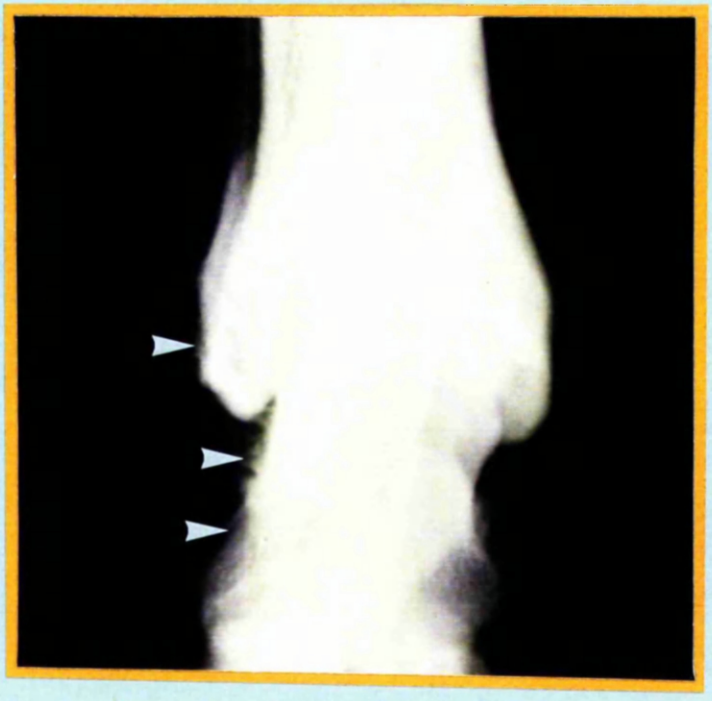
FIGURE 13

CASE ID 17. IDIOPATHIC INFLAMMATORY ARTHROPATHY.

ANTEROPOSTERIOR RADIOGRAPH OF THE RIGHT HOCK JOINT.

An extensive periosteal reaction is seen along the lateral aspect of the distal fibula and of the tarsal bones (arrows).





DISCUSSION

GROUP I (RHEUMATOID)

The most obvious radiographical feature in these dogs was the presence of bony erosions and/or loss of mineralisation affecting the epiphyses or individual bones of the joints. The bone erosions may occur in the subchondral bone or juxta-articular bone; they may have well-defined borders ("punched-out" appearance) or be poorly demarcated and they vary in size. Erosions are often seen at the attachment of ligaments and joint capsule to the epiphyseal bone.

These lesions are classically seen in human rheumatoid patients (Boyle and Buchanan 1971; Buchanan 1978). Sharply demarcated erosions have been called "bone cysts" or "geodes" and have been attributed to the inflamed, destructive synovium (pannus), typical of this disease, being forced into the porous cancellous bone by increases in the intra-articular pressure (Jayson, Rubenstein and Dixon 1970) although erosions can seemingly arise in association with local osteoclastic activity without any granulation tissue in the proximity (Muirden 1975).

The erosions may be confluent with the articular margin or appear set-back from the joint surface and the time taken for them to develop varies considerably and is probably related to disease severity and chronicity (Kennedy and Lindsay 1977). Once formed, an erosion will persist with very limited repair being possible (Fletcher and Rowley 1952; Soila 1958).

In addition to erosive bone disease, considerable local destruction of bone occurs close to the site of inflamed joints without evidence of disruption of the bone cortex (Kennedy and Lindsay 1977). Such bone loss is likely to be directly related to the degree of local inflammation within the joint.

A generalised rarefaction of bone can also be seen in rheumatoid patients, associated with osteoclastic resorption (Jaffe 1972). This may represent some form of bone atrophy due to disuse although a calcium imbalance may exist in these patients (Kennedy, Allam, Boyle, Nuki, Rooney and Buchanan 1975). Many rheumatoid patients are hypoalbuminaemic and if corrections are made for this, a hypothetical hypercalcaemia can be shown which has been related to a hyperparathyroid syndrome (see Section V).

Little (1973) has hypothesised that the status of bone depends on the balance between anabolic and catabolic conditions and any chronic inflammatory process within the body, such as rheumatoid arthritis,where there is excessive catabolic activity, can be expected to promote bone loss. Corticosteroids, used in treatment, may increase loss of mineral from the skeleton and other hormonal factors may also play a part in bone loss in rheumatoid arthritis, e.g. post-menopausal osteoporosis in female patients (Kennedy and Lindsay 1977).

Radiographic lesions similar to those reported in this study have been described by other authors in the canine rheumatoid patient(Alexander et al 1976; Biery and Newton 1975; Halliwell et al 1972; Newton et al 1976; Owens and Ackerman 1978; Pedersen et al 1976a; Liu et al 1969; Wentink 1973). In addition to subchondral erosions and

loss of mineralisation, Biery and Newton (1975) reported enlargement of the supracondylar foramen of the humerus in diseased elbow joints. This feature was seen in case Rh 13 of the present study.

Changes in joint space may be apparent in canine rheumatoid arthritis (Biery and Newton 1975; Newton et al 1976; Pedersen et al 1976a). Initially a joint effusion may widen the joint space but as the disease progresses with destruction of cartilage and bone by pannus formation and proteolytic enzyme release, a decreased joint space may result. However, the radiographic interpretation of joint space width is difficult in the dog because of the problems in exact positioning and setting the X-ray beam exactly perpendicular to the joint, and also trying to avoid traction on the limb during restraint for positioning, particularly in the conscious patient. It is more relevant to radiograph an affected joint during weight bearing for the assessment of joint space. However, this is technically very difficult in the small animal patient and no attempts were made to do this in the present study. Changes in joint space are regularly used as a diagnostic sign for human rheumatoid arthritis (Weissman and Sosman 1975). Five dogs in this study were thought to show an alteration in joint space.

Proliferative lesions in the form of osteophyte production or periosteal reaction were recorded in about 75% of the dogs and in 2 of these there was, in addition, sclerosis of the subchondral bone. Osteophyte development and sclerosis have been described elsewhere in canine rheumatoid arthritis (Alexander et al 1976; Biery and Newton 1975; Newton et al 1976). These changes may occur secondary to the inflammatory process especially where this has led to ligament damage and joint

instability (Alexander et al 1976). However, it is also possible that the osteoarthritic change was present as a primary disease or secondary to some other abnormality such as trauma or dysplasia, before the rheumatoid, inflammatory disease developed (Biery and Newton 1975). The periosteal type of reaction noted in this study has only been described in the dog by Halliwell et al (1972)but is certainly present in some of the illustrations published by several other authorities. This change, which does not represent osteophyte development, is regularly seen in human rheumatoid patients (Boyle and Buchanan 1971) and is thought to result from elevation of the periosteum by inflammatory oedema affecting the periosteum adjacent to the inflamed joint. The elevated periosteum leads to calcification outside the original cortex; this subperiosteal bone eventually fuses with the cortical bone and may in the later stages become resorbed. Soft tissue calcification around an affected joint, sometimes involving the joint capsule itself, may also be seen (Biery and Newton 1975; Newton and Lipowitz 1975; Owens and Ackerman 1978).

In four of the dogs showing involvement of the metacarpo/metatarso-phalangeal joints, the proximal ends of the phalangeal bones at these joints showed a "mushrooming" or "flared" appearance. This was thought to be due to new bone deposits at the articular margins and has been described also by Liu et al (1969) but is visible in several illustrations published by other authors. Similar changes were described by Pedersen et al (1976a) in the proximal metacarpal and metatarsal bones at the carpo/tarso-metacarpal/metatarsal joints and these authors suggested it was the result of a collapsing of the subchondral bone.

Soft tissue swelling around affected joints and synovial effusion within joints was seen in several dogs. It is obvious that radiographic appreciation of soft tissue swelling and synovial effusion is more likely with some joints than with others. For example, the carpal and tarsal joints readily show soft tissue swelling and synovial effusion is easily recognised in the stifle joints by reduction or obliteration of the infra-patellar fat shadow on the lateral projection. The latter is in fact due to synovial effusion, soft tissue thickening and to oedema of the fat pad. Fat pad oedema can result in its total obliteration or the appearance of coarse septe within it or in a haziness of the joint space/fat pad interface (Namey and Halla 1978). Soft tissue swelling has been recognised in canine rheumatoid patients by other workers (Newton et al 1976) and has been described as an early radiographic change (Biery and Newton 1975) although it is also present in the later stages. Soft tissue swelling is a reflection of the inflammatory process involving the joint structures and extending into the periarticular tissues although it may also represent a healing fibrosis particularly where a joint has become unstable. Synovial effusion reflects the increased production of synovial fluid by the inflamed synovium.

Radiographic changes in the apophyseal joints of the cervical spine have been described in one dog (Pedersen et al 1976a) although other unpublished cases have been seen in the U.S.A. Three dogs in the present study had some evidence of spinal pain but no spinal radiographs were taken although parts of the vertebral column were seen on the thoracic and abdominal radiographs. The cervical spine is commonly involved in human patients (Bland 1967; 1974) and can show

a wide variety of radiological abnormalities (Boyle and Buchanan 1971; Buchanan 1978).

"Punched-out" defects in the olecranon at the attachment of the tendon of the triceps muscle has been described in the dog (Liu et al 1969). This possibly resulted from an involvement of the olecranon bursa in the inflammatory process. A similar phenomenon has been described in the os calcis of human rheumatoid patients associated with Achilles tendon bursitis (Bywaters 1954).

The absence of radiographic abnormalities in some joints of some rheumatoid dogs has also been reported by other authors (Pedersen et al 1976a), especially in the early stages of the disease and this is again a feature of human rheumatoid arthritis (Smiley 1975).

Luxation and subluxation of affected joints was seen in some cases in the present study and has been reported elsewhere (Alexander et al 1976; Newton et al 1976). Joint luxation and subluxation are also features of human rheumatoid arthritis (Buchanan 1978). Ankylosis may also be seen but is rare in the dog and no case was recorded in the present study.

Three dogs had radiographical evidence of pneumonia, a feature which has not been reported in canine rheumatoid arthritis by other workers. Pneumonia is not one of the most common pulmonary diseases seen in human rheumatoid patients but does occur with increased frequency in such patients compared to the general population (Aronoff, Bywaters and Fearnley 1955; Kay 1967; Short, Bauer and Reynolds 1957; Talbott

and Calkins 1964; Walker 1978; Walker and Wright 1968). The commonest thoracic lesions which are recognised in man on radiography, are pleurisy with or without effusion, rheumatoid pneumoconiosis (Caplan's syndrome), diffuse interstitial pulmonary fibrosis (fibrosing alveolitis), non-pneumoconiotic intrapulmonary rheumatoid nodules and pulmonary artery disease (Boyle and Buchanan 1971; Buchanan 1978; Walker and Wright 1968). The increased bronchovascular markings noted in some of the dogs could have represented pulmonary fibrosis but this abnormality is regularly seen in older dogs and its exact significance remains uncertain.

Three dogs also had radiographic evidence of splenomegaly. The latter occurs in human patients (Boyle and Buchanan 1971) and is more likely to be seen in the juvenile form of the disease(Still's disease) or in the adult with accompanying neutropaenia (Felty's syndrome) (Felty 1924). One of the dogs did show a persistently low white blood cell count (see Section VII); all three dogs were adult. Enlargement of the liver was noted in three dogs. Hepatomegaly may occur in human rheumatoid arthritis associated with secondary amyloidosis (Boyle and Buchanan 1971).

GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS)

Most of the joints from the 4 dogs in this group showed no obvious abnormality except for synovial effusion;this is similar to the findings of Pedersen et al(1976b)in 29 suspected cases and of Alexander et al (1975) in one dog. Pedersen and his colleagues(1976b)reported mild narrowing of the carpal joint spaces in 2 long-standing cases and slight periosteal reaction in the area of the joint capsule attachments. These authors suggest the use of contrast arthrography which is also recommended by Owens and Ackerman (1978). This is reputed to show joint distension with irregularity and poor

definition of the synovial margins. No reported cases of canine systemic lupus erythematosus have shown destructive joint changes on radiography. In the human patient, radiography of affected joints may reveal no abnormalities other than soft tissue swelling although juxta-articular osteoporosis and occasionally loss of joint space and bony erosions may be seen (Boyle and Buchanan 1971). However, it is generally believed that the absence of erosions is an important factor in the differentiation of systemic lupus erythematosus from rheumatoid arthritis (Hughes 1978a).

Radiographic abnormalities within the chest and abdomen were not recorded in any of the four dogs although various abnormalities have been reported in human lupus patients. For example, pericarditis (Shearn 1959), heart failure secondary to myocarditis (Hejtmancik, Wright,Quint and Jennings 1964) or hypertension (Bridgen, Bywaters, Lessof and Ross 1960), pleurisy or pneumonitis (Haffbrand and Beck 1965), splenomegaly (Harvey, Shulman, Tumulty, Conley and Schoenrich 1954) and hepatomegaly (Anderson, Buchanan and Goudie 1967). Pleuritis is mentioned in several review articles as a manifestation of canine systemic lupus erythematosus although no detailed case reports describing this lesion have been published.

GROUP III (INFECTIOUS)

In most cases there was some evidence of bone loss and soft tissue swelling around the infected joint and in three cases there was peri-articular soft tissue calcification. Owens and Ackerman (1978) report that the shoulder, stifle and hip are the most commonly affected joints

in the dog which is in agreement with the present study and these are also the joints most commonly involved in the human patient (Dalinka, Lally, Koniver and Coren 1975; Goldenberg, Brandt, Cathcart and Cohen 1974; Kelly, Martin and Coventry 1970). Owens and Ackerman (1978) report the earliest changes as thickening of the soft tissues, distension of the joint capsule and widening of the joint spaces. With progression of the disease,signs of joint destruction may become apparent e.g. periarticular rarefaction, irregular joint surfaces and ankylosis. Similar features have been described by Morgan (1972) and by Putnam and Archibald (1968). Destruction of articular cartilage and bone can be associated with the release of enzymes during the inflammatory process, by the production of pannus tissue and by the extension of the infective process into the subchondral bone (Morgan 1972).

Seven of the dogs in the present study showed bony proliferation in affected joints in the form of osteophytes probably due to an osteo-arthritis secondary to the inflammatory disease, and periosteal reaction probably resulting from inflammatory oedema of the periosteum. Similar features have been reported by Morgan (1972).

Radiographical features of infected joints in the human patient are not dissimilar to those described for the dog (Ansell 1978; Boyle and Buchanan 1971). However, changes of joint destruction do not usually appear until the third or fourth week of the untreated infection and by this time severe long-term functional impairment is likely (Clark 1963). Early radiographs may show no abnormality or soft tissue swelling and/or synovial effusion only (Namey and Halla 1978). Periarticular calcification is reported as an occasional phenomenon in human septic joints and is

most often associated with pneumococcal disease; it is usually seen in the chronic case and is thought to be caused by extension of the infection into the surrounding soft tissues due to capsular rupture (Shawker and Dennis 1971).

GROUP IV (BACTERIAL ENDOCARDITIS)

The joint changes in this group were,as might be expected, not unlike those of the previous group. The case reported by Lewis and Hathaway (1967) showed most of the features recorded in this study such as marked soft tissue swelling around affected joints, narrowing and deformity of joint spaces, diffuse osteoporosis, loss of subchondral bone and ankylosis.

Three dogs showed pulmonary changes on the thoracic radiographs, which could have been consistent with pulmonary thrombosis,haemorrhage or pneumonitis. One case also had calcification within the kidneys,probably of old renal infarcts.

GROUP V (IDIOPATHIC)

Destructive joint changes were only rarely seen in this group of dogs and when present were only mild and generally only involving 1 or 2 joints. Just under half the dogs had proliferative changes but again these were never extensive and were only present in 1 or 2 joints. Synovial effusion and/or periarticular swelling was apparent in some of the dogs. The most characteristic feature of these dogs was in fact the absence of radiographical abnormalities in the majority of affected joints. These findings are similar to those reported elsewhere(Pedersen et al 1976b). The radiographic features

of the cases reported by Huxtable and Davis (1976) were not recorded although the pathological descriptions mentioned destructive changes of articular cartilage and subchondral bone but these were not severe.

Similar types of human arthritis have been associated with comparable radiographical features. In the various types of enteropathic arthritis, for example, it is usual to see no obvious abnormalities although soft tissue swelling, osteoporosis and even slight erosive damage can occur (Boyle and Buchanan 1971; Haslock 1978). Arthritis associated with non-articular neoplastic disease is again often characterised by minimal radiographical change (Barnes 1978) although lymphosarcomatous arthritis can be associated with multiple radiolucent areas adjacent to joints (Martin, Bennett, Hughes and Holt 1973). Suspected and confirmed cases of viral arthritis in the human patient are usually characterised by a synovitis only and destructive changes are not generally seen on the radiographs.

Other radiographic abnormalities were reported in the dogs e.g. pneumonia, pleuritis, pulmonary oedema, cardiomegaly, splenomegaly and hepatomegaly. Some of these features as reported in the rheumatic diseases of man have already been discussed. It is possible that some of these could have been co-incidental findings.

SECTION IV

SYNOVIAL FLUID ANALYSES OF DOGS WITH INFLAMMATORY JOINT DISEASE

The most neglected differential diagnostic test in arthritis.

J. L. Hollander 1960.

INTRODUCTION

Many reports on the analysis of synovial fluid samples from human arthritic patients have been published (Broderick, Corvese, Pierik, Pike and Mariorenzi 1976; Coggeshall, Warren and Bauer 1940; Hollander 1960; Hollander, Jessar and McCarty 1961; Hollander, Reginato and Torralba 1963; Kling 1938; Naib 1973; Ropes and Bauer 1953; Schmid and Ogata 1965). The most consistent feature in these reports as regards the inflammatory group of joint disorders is the presence of large numbers of leucocytes and in most cases the majority of these are polymorphonuclear cells. Synovial fluid evaluation is certainly useful in distinguishing the inflammatory arthropathies from other types of joint disease. Similar but less detailed reports of synovial fluid analysis in the dog have been made (Barrett 1977; Hardy and Wallace 1974; Miller, Osborne, Hardy and Wallace 1975; Miller, Perman, Osborne, Hammer and Gambordella 1974; Perman and Cornelius 1971; Sawyer 1962; 1963).

METHODS

1. COLLECTION OF SYNOVIAL FLUID SAMPLES

Samples were collected from most dogs in the five groups of inflammatory joint disease. In addition several samples were taken from dogs suffering other types of joint disease as listed below:-

(a) Osteoarthritis. Most of these were cases of ruptured anterior cruciate ligament of the stifle joint, of at least three weeks duration;-

(b) Traumatic arthritis. These were all cases of less than one week's duration and comprised dogs with acute "sprains" often with ligament damage and dogs which had sustained fractures of the articular surface(s) of the joint.

(c) Osteochondritis dissecans. These included samples from diseased shoulder and elbow joints.

Many normal joints were also sampled, mainly from dogs which were being destroyed for other reasons. All the joints which were sampled whether normal or diseased, were carefully assessed by clinical, radiographical and where possible pathological examinations.

A strict aseptic technique was always used when samples were aspirated. The hair was closely clipped from the skin around the joint and the skin cleaned with an antiseptic detergent (generally chlorhexidine gluconate, Hibitane concentrated solution, ICI Ltd., Alderley Park, Macclesfield, Cheshire) followed by the application of a skin antiseptic (generally povidone-iodine, Pevidine antiseptic solution, Berk Pharmaceuticals Ltd., Catteshall Lane, Godalming, Surrey). Most samples were taken with the animal under sedation with acepromazine maleate (0.25ml per 5Kg body weight intravenously; Acetylpromazine,C-Vet Ltd. Minster House, Western Way, Bury St. Edmunds, Suffolk) or with a mixture of acepromazine maleate and pentazocine (Fortral, Winthrop Laboratories, Surbiton-upon-Thames, Surrey: 1-3ml i.e. 30-90mg depending on size of the dog mixed with an equal volume of acepromazine and given intravenously). Occasionally, the samples were taken whilst the animal was under general anaesthesia and this was always found necessary when aspirating the shoulder and hip joints. The usual system of general anaesthesia was

sedation with intramuscular or subcutaneous acepromazine maleate, induction with intravenously administered thiopentone sodium (Intraval Sodium,May and Baker Ltd., Dagenham, Essex, approximately 15mg per kg body weight using a 2% or 5% solution), intubation and maintenance with halothane (Fluothane, ICI Ltd., Alderley Park, Macclesfield, Cheshire) and oxygen. Figure 14 illustrates the anatomical approaches used to aspirate various joints. Sterile, disposable 2ml or 5ml syringes fitted with 20 gauge 1/1½ inch needles were used.

2. GROSS APPEARANCE, VISCOSITY, MUCIN CLOT TEST

The colour and clarity or turbidity of each sample was assessed with the naked eye and the volume noted. The viscosity of the sample was determined by agitation of the syringe in which the sample had been collected and by two simple "string tests". One of the latter involved allowing drops of the fluid to fall from the end of the needle and assessing the length of the "string" formed by each drop before it separated from the end of the needle. The alternative test involved placing a drop of synovial fluid between the finger and thumb and evaluating the "string" formation when the finger and thumb were separated.

The mucin clot test, which gives an assessment of the quality and quantity of the mucin (hyaluronic acid)in synovial fluid was carried out in only a few cases. The test was performed by adding a couple of drops of synovial fluid to a few millilitres of 5% acetic acid in a beaker and allowing this to stand for 1 minute and then assessing the character of the clot which formed on shaking the beaker. If the mucin content of the synovial fluid is normal, a firm clot will form but if mucin has been degraded, e.g. by enzymes released during inflammatory disease,the

clot is poor and friable or may even not form at all (Hollander et al 1963). Because both viscosity and clot are dependent on the character of the mucin, it is usual for fluids with poor viscosity to also form poor mucin clots. The main exception to this is in effusions from recent inflammation where viscosity has been decreased by dilution with plasma dialysate but where no enzyme degradation has occurred - in such cases the quality of the clot may remain normal. The mucin clot test should always be performed on fresh synovial fluid samples which have not been mixed with the anti-coagulant ethylene diaminetetra-acetic acid since this substance can cause a decrease in synovial fluid viscosity by degradation of the hyaluronic acid component of mucin (Ogston and Sherman 1959). The results of the mucin clot test are not recorded in this thesis since the number of dogs tested was only small.

3. TOTAL CELL COUNTS

These were carried out using thoma diluting pipettes and a haemocytometer with two chambers and the improved Neubauer ruling. Generally, red cells and white cells were counted together and the thoma white cell diluting pipette was used togive a synovial fluid dilution of 1:20. Isotonic saline was used as the diluting fluid and allowed red and white cells to be counted in the single preparation. It is important not to use glacial acetic acid which is a white cell diluting fluid used for blood, since the acetic acid may cause the synovial fluid to clot yielding inaccurate counts (Hollander 1961; 1966; Sawyer 1963). The counts were made by placing the haemocytometer under the microscope (Zeiss Standard 14 light microscope) and using

the high-dry power. Each sample was counted twice.

4. EXFOLIATIVE CYTOLOGY

Either a direct smear of the synovial fluid sample or a smear of the sediment collected after centrifugation of the sample was made and stained by the Papanicolaou method. To collect the sediment, the sample was spun for approximately 20 minutes at 2000rpm in the BTL Bench centrifuge. The supernatant was discarded and the sediment transferred onto microscope slides. In all cases, smears were made by drawing one microscope slide across another and generally several smears from the one sample were prepared. Attempts were always made to prepare thin smears since this allowed easier cytological interpretation.

The smears were fixed in methanol and stained by the following method:-

(i) Smears transferred into 95% alcohol and then through 80%, 70% and 50% alcohols to distilled water (10 dips);

(ii) Smears stained in Harris haematoxylin for 5½ minutes;

(iii) Smears rinsed in tap water until clear;

(iv) Smears rinsed in 0.5% hydrochloric acid (3 quick dips);

(v) Smears rinsed in tap water until clear;

(vi) Smears placed in saturated (distilled water)solution of lithium carbonate for 2 minutes;

(vii) Smears rinsed in distilled water and run through 50%,70%, 80% and 95% alcohols (10 dips);

(viii) Smears stained in Papanicolaou stain OG 6 (BDH Chemicals Ltd., Poole) for 1 minute;

(ix) Smears rinsed in two changes of 95% alcohol (10 dips);

(x) Smears stained in Papanicolaou stain EA 36 (EA 50) (BDH Chemicals Ltd., Poole) for 5½ minutes;

(xi) Smears rinsed in three changes of 95% alcohol (10 dips);

(xii) Smears dehydrated and cleared by passage through absolute alcohol (two changes, 3 minutes each), a mixture of equal parts of absolute alcohol and xylol (10 dips) and xylol (two changes, 2 minutes each);

(xiii) Smears mounted with Harleco.

The smears were examined using the Zeiss Standard 14 light microscope.

5. MICROBIOLOGY

Most synovial fluid samples taken from dogs with inflammatory joint disease were examined for the presence of bacteria and mycoplasma. The microbiological investigations are described in Section VIII.

It was not possible with every dog showing inflammatory joint disease to perform all the investigations listed above since in some cases only small volumes of synovial fluid were collected. This was true even of joints where an obvious effusion could be palpated and it may have been that the needle became blocked by fibrin deposits or by inflamed, hypertrophied synovial membrane. The microbiological assessment was regarded as the most important and it was usually also possible to prepare a smear of the synovial fluid for cytological examination.

FIGURE 14

DIAGRAMMATIC REPRESENTATIONS OF THE APPROACHES USED TO ASPIRATE SYNOVIAL FLUID SAMPLES FROM THE MAIN LIMB JOINTS OF THE DOG.

A - shoulder

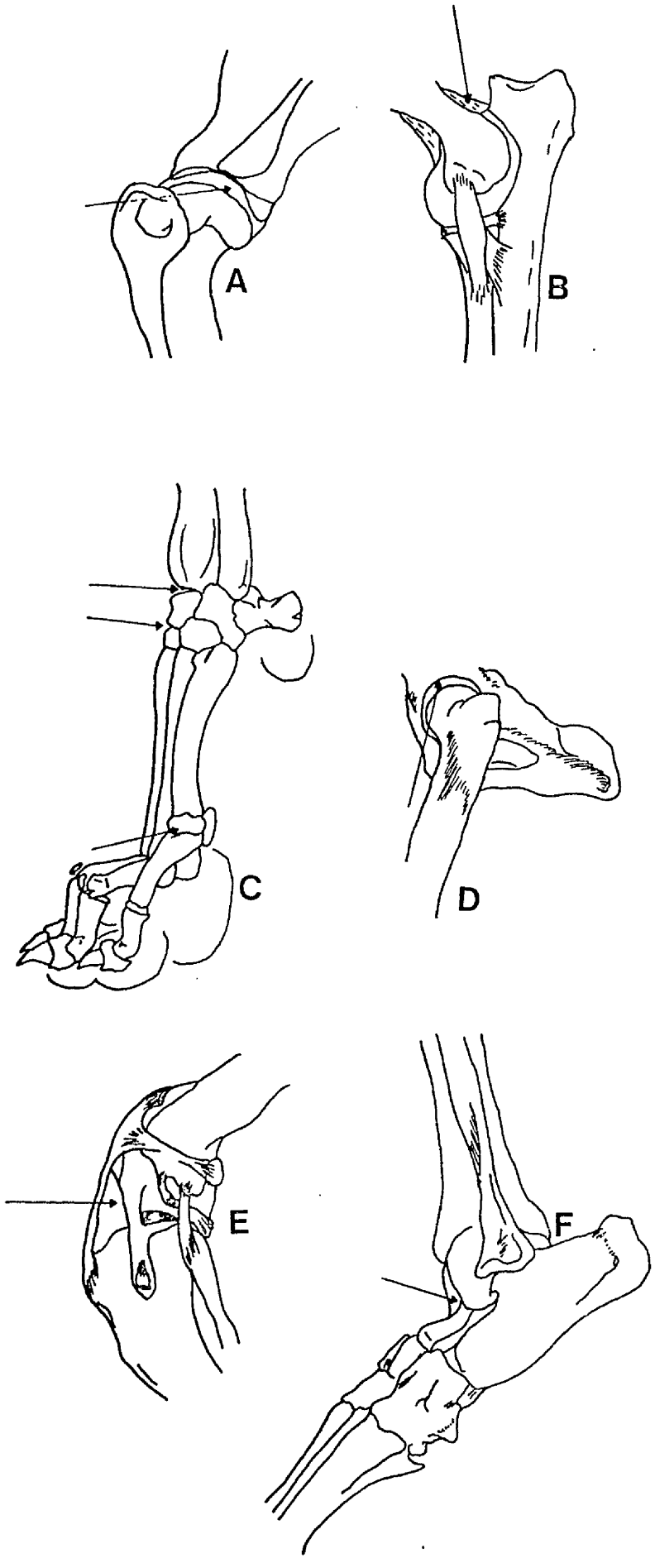
B - elbow

C - radio-carpal
inter-carpal
metacarpo-phalangeal

D - hip

E - stifle

F - hock


A
B
C
D
E
F

RESULTS AND DISCUSSION

The results of the total white and red cell counts, together with the differential white counts are given in Table 14 and Figure 15. The average figures for the cell counts are shown, together with the ranges obtained and the number of joints sampled in each group of dogs. The average percentages of polymorphonuclear leucocytes recorded in each group is also shown and the range of the percentages is indicated. Compared to some of the human studies, the number of samples examined is only small but some conclusions are justified.

The numbers of white cells in the inflammatory groups of joint disease were generally much higher than those in the other types of joint disease. Another consistent feature was the high proportion of polymorphonuclear cells in the inflammatory types of arthropathy. Mononuclear cells usually predominated in the osteoarthritic, osteochondritic and traumatic types of joint disorder. There was however, as indicated by the ranges recorded in Table 14 an overlap between the "inflammatory" and "non-inflammatory" types which must be realised when interpreting the results and reaching a diagnosis. However, the highest cell count recorded in the "non-inflammatory" groups was 7000 cells/cmm and the majority of the "inflammatory" samples were well above this. It should also be noted that the "non-inflammatory" joints sampled for this study were selected as straight forward cases to avoid any confusion which might have arisen, for example, from a crystal-induced synovitis superimposed

on an osteoarthritis. The average white cell count for normal joints was 1125 cells/cmm although a count of 2400/cmm was recorded on one occasion. A much larger number of normal dogs (55) were examined by Sawyer (1963) who reported an average of 430 cells/cmm with a range up to 2900/cmm. The majority of cells in the normal samples were of the mononuclear type.

There were no great differences in cell counts and percentages of polymorphonuclear cells between the various groups of inflammatory arthropathy, except perhaps for the higher numbers of white cells in the bacterial endocarditis cases. It is interesting that in one of these dogs (BE 1) the synovial fluid from some of the joints sampled showed only low numbers of white cells (e.g. left shoulder 1200/cmm, right elbow 1200/cmm and right carpus 1900/cmm) but the predominant cell in each case was the polymorphonuclear cell (95%). Histopathological evidence of synovitis was present in these joints although bacterial cultural examinations were negative. These features of low cell counts in sterile pathologically affected joints could be consistent with for example, an immune complex synovitis rather than the embolic spread of infection from the heart lesion to the joint. Immune complex hypersensitivity reactions have been suspected in human patients with bacterial endocarditis (Gutman et al 1972). Similarly, inflammatory changes in the synovial membrane and fluid associated with other generalised infections of the body but where the joint appears sterile have been reported in human patients (Beck and Lauber 1929; Coggeshall, Bennett, Warren and Bauer 1941; Labor and van Babogh 1919; Ropes and Bauer 1953). A further discussion of this phenomenon is given in Section VIII.

Table 14 also shows the ranges and numbers of red cells counted in each group of dogs. The presence of red cells in synovial fluid is not of any real diagnostic value although they tended to occur in higher numbers in the septic joints of Groups III and IV.

Total and differential white cell counts similar to those recorded in the present study have been given by other investigators (Alexander et al 1976; Barrett 1977; Brown 1978; Hardy and Wallace 1974; Miller et al 1974; 1975; Newton et al 1976; Pedersen 1978; Pedersen et al 1976a,b). Most authorities agree that the highest white cell counts are seen in cases of septic arthritis and that infectious arthritis, rheumatoid arthritis, lupus arthritis and idiopathic arthritis are all characterised by elevated white cell counts and a predominance of polymorphonuclear leucocytes. Hardy and Wallace (1974) regard septic arthritis and degenerative joint disease as two extremes and state that synovial fluid samples from other types of inflammatory joint disease can show characteristics of both these types, i.e. early samples may indicate severe inflammatory disease but the cytology may return to normal as the disease process progresses. The average number of white cells in the septic arthritic cases of the present study are lower than those of other workers and this is probably accounted for by several cases of chronic, low grade infectious arthritis (see Section VIII). The cases of septic arthritis associated with bacterial endocarditis had higher white cell counts.

Synovial fluid samples from the inflammatory groups of dog were generally yellow in colour and often turbid and in some septic cases resembled pus. Normal synovial fluid was always colourless or slightly straw-coloured. Fluid from the "non-inflammatory" joint diseases was

generally colourless or pale yellow and invariably clear. The volume of synovia was usually increased in pathological joints although sometimes it was difficult to aspirate large volumes probably because the needle became blocked by hypertrophied synovium and/or fibrin strands. The volume of synovial fluid aspirated from normal canine joints varies with particular joints and the size of the dog but is rarely more than 0.75ml. The viscosity and mucin content of normal and "non-inflammatory" synovial fluids were generally high and those of the inflammatory groups usually low. Some samples from the "inflammatory" joints also tended to clot on exposure to air whereas this was never encountered with either normal or "non-inflammatory fluids, although these could be so viscous that they behaved as a "jelly". Mucopolysaccharide material could be identified in stained smears of "degenerative" synovial fluids (Fig.17)but rarely in"inflammatory" fluids.

Although it was easy to differentiate polymorphonuclear and mononuclear cells in the stained smears,it was more difficult to classify the mononuclear cells into their various types. The latter included lymphocytes,monocytes, clasmatocytes and "other phagocytic cells"(Ropes and Bauer 1953). The classification of the various mononuclear cells is not particularly useful for diagnostic purposes and is not dealt with here in detail, Other cells were also seen in some cases,e.g. synovial cells, cartilage cells,osteoblasts and osteoclasts. It was not uncommon in any pathological synovial fluid smear to find "reactive", swollen phagocytic cells with cytoplasmic inclusions (Fig.17). Also,in the "inflammatory" fluids degenerative polymorphonuclear cells with fragmented and pyknotic nuclei were often seen (Fig.16). LE-cells have been described in the synovial fluid from dogs with inflammatory joint disease (Brown 1978; Miller et al 1974; Pedersen 1978). No such cells were seen in this study, although free nuclear (Feulgen-positive) material was identified in several preparations from all five groups of inflammatory joint disease (Fig. 16). Occasionally, poly-

morphonuclear cells were seen to contain cytoplasmic inclusions although these were seldom seen in large numbers and again tended to occur in all groups. None were seen in the fluids taken from normal joints, or joints with degenerative disease. Such cells have been reported in human rheumatoid arthritis and have been labelled "rheumatoid arthritis cells" (Hollander, Jessar and McCarty 1961) or ragocytes (Delbarre, Kahan, Amar and Krassinine 1964) and the cytoplasmic granules have been identified as complexes of gamma globulin with rheumatoid factor (Rawson, Abelson and Hollander 1965). It is certain that some cells contain other types of inclusion,e.g. engulfed nuclear material or deoxyribonucleic acid and some of these have also been described as ragocytes (Hollander 1966). Inclusions of IgG and complement in synovial fluid leucocytes have been identified in dogs, particularly in cases of rheumatoid arthritis (Barrett 1977; Pedersen 1978).

In some smears, cellular detritus, unidentified solid material and irregular amorphous particles were observed but these occurred in all types of fluid, including normal samples. Small shreds of fibrin were apparent in many of the "inflammatory" fluids.

Many of the basic observations reported here in the dog are similar to those recorded in man (Broderick et al 1976; Coggeshall et al 1940; Hollander et al 1963; Kling 1938; Naib 1973; Ropes and Bauer 1953; Schmid and Ogata 1965). There is however, some disagreement over the characteristics of synovial fluid from patients with systemic lupus erythematosus. A possible explanation for this may be the difficulty experienced in accurately diagnosing this disease. The stage of the disease at which the sample is collected may also influence the cytological characteristics

of the joint fluid. Some authoritis have reported only a mild increase in white cells with a predominance of mononuclear cells (Hollander et al 1963; Ropes and Bauer 1953). Another report however, suggests that the findings in lupus are similar to those of rheumatoid arthritis and other inflammatory joint conditions, although fewer cases are considered (Schmid and Ogata 1965). Unfortunately, synovial fluid from only two cases of canine systemic lupus erythematosus was examined in this study; in both cases the white cells were mainly polymorphs. Other reports in the dog also indicate a predominance of polymorphs,usually with moderately high total cell counts (Barrett 1977; Miller et al 1974) although Brown (1978) gives a range of polymorphs from 10-65%.

Many different types of inflammatory joint disease seen in man can be included in the idiopathic group as defined for the dog. Viral arthritis, for example, can be associated with elevated white cell counts, sometimes with a predominance of polymorphonuclear cells, sometimes of mononuclear cells (Alarcon and Townes 1973; Duffy, Lidsky, Sharp, Davis, Person, Hollinger and Min 1976; McCarty and Ormiste 1973; Onion, Crumpacker and Gilliland 1971; Panush 1974; Schumacher and Gall 1974; Shumaker, Goldfinger, Alpert and Isselbacher 1974; Steere, Malawista, Hardin,Ruddy, Askenase and Andiman 1977; Utsinger 1977). Enteropathic arthritis in man is characterised by synovial fluid with a white cell count in the range 4000-40000/cmm, up to 98% being polymorphs (Haslock 1978).

TABLE 14

SUMMARISING THE MAIN FEATURES OF SYNOVIAL FLUID CYTOLOGY IN NORMAL DOGS, IN DOGS WITH DEGENERATIVE TYPES OF JOINT DISEASE AND IN DOGS WITH INFLAMMATORY TYPES OF JOINT DISEASE.

TYPE OF JOINT DISEASE	AVERAGE TOTAL WBC COUNT/ CUMM.	RANGE TOTAL WBC COUNT/CUMM.	AVERAGE TOTAL RBC COUNT/CUMM.	RANGE TOTAL RBC COUNT/CUMM.	AVERAGE % POLYMORPHO-NUCLEAR CELLS	RANGE % POLYMORPHO-NUCLEAR CELLS	NO. OF JOINTS ASSESSED
NORMAL	1125	500-2400	0.21m	0.01-0.85m	4.0	0-16	30
ACL RUPT.>3WK. OSTEOARTHRITIS	2750	400-7000	0.35m	0.07-0.39m	6.0	0-54	45
OSTEOCHONDRITIS DISSECANS	2700	1500-4700	0.17m	0.10-0.38m	14	0-40	10
TRAUMATIC ARTHRITIS	2050	1100-4200	0.35m	0.16-0.71m	19	0-65	10
GROUP I (RHEUMATOID)	29700	6200-87200	0.17m	0.06-0.46m	74	17-95	41
GROUP II (SLE)	13350	3200-23500	0.85m	0.23-1.47m	50	40-60	2
GROUP III (INFECTIOUS)	26800	2300-91900	0.50m	0.07-1.58m	80	50-98	12
GROUP IV (BACTERIAL ENDOCARDITIS)	72450	1200-192000	0.43m	0.15-1.01m	85	16-98	17
GROUP V (IDIOPATHIC)	32100	3200-106300	0.32m	0.05-1.47m	79	40-99.5	37

FIGURE 15

HISTOGRAM SUMMARISING THE NUMBERS OF LEUCOCYTES AND THE PROPORTION OF POLYMORPHS IN SYNOVIAL FLUID SAMPLES FROM NORMAL DOGS AND FROM DOGS WITH DEGENERATIVE TYPES OF JOINT DISEASE AND INFLAMMATORY TYPES OF JOINT DISEASE.

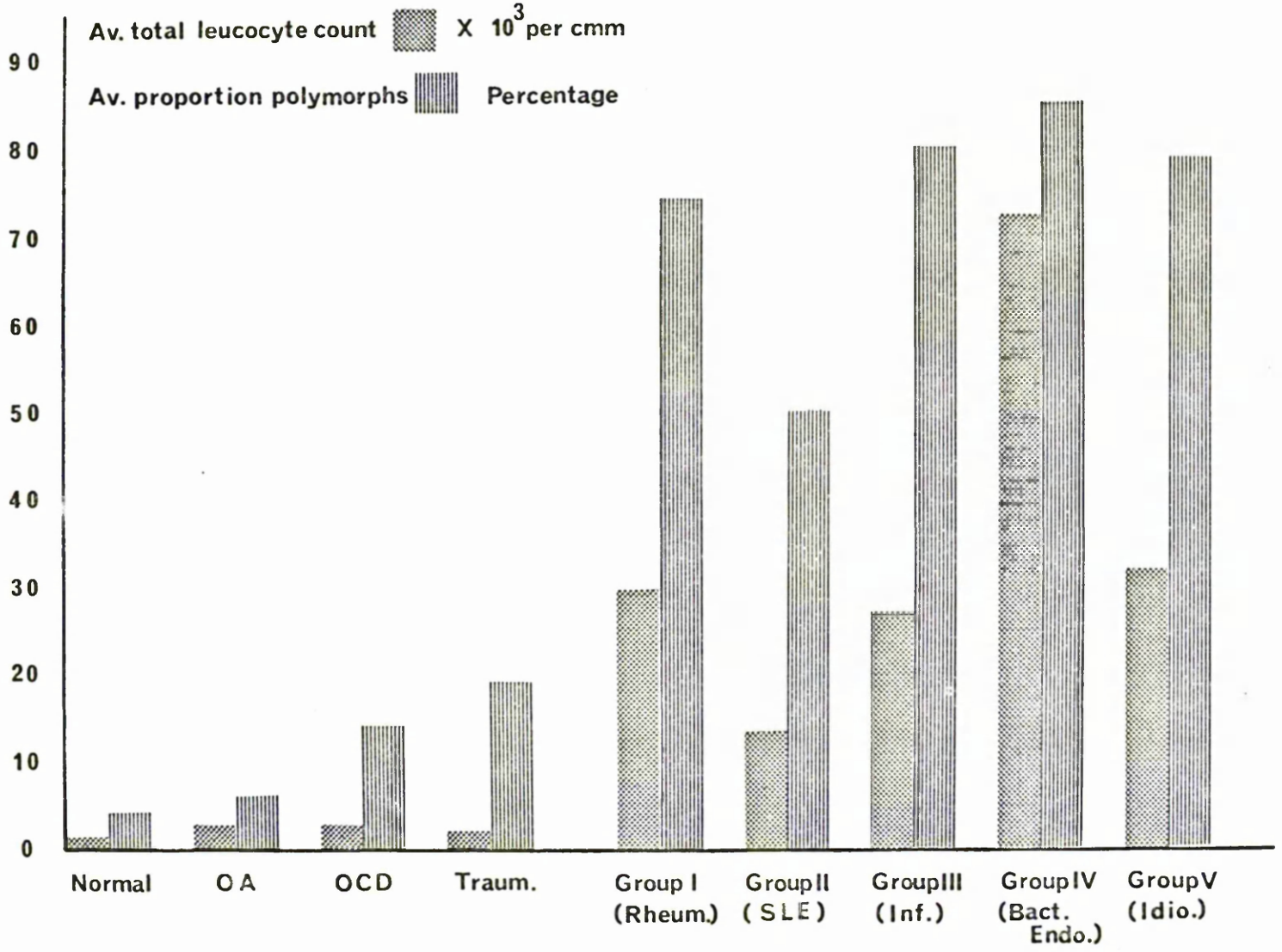

Av. total leucocyte count X 10^3 per cmm
Av. proportion polymorphs Percentage
90
80
70
60
50
40
30
20
10
0
Normal
OA
OCD
Traum.
Group I (Rheum.)
Group II (SLE)
Group III (Inf.)
Group IV (Bact. Endo.)
Group V (Idio.)

FIGURE 16

PHOTOMICROGRAPH OF A SYNOVIAL FLUID SMEAR

FROM A DOG WITH RHEUMATOID ARTHRITIS.

There are several polymorphonuclear leucocytes (p), some with pyknotic nuclei (n). There are several masses of free nuclear material (f). A few mononuclear cells are also seen (m). Several red blood cells are also present (r). There is no mucopolysaccharide staining.

Papanicolaou method. x 900

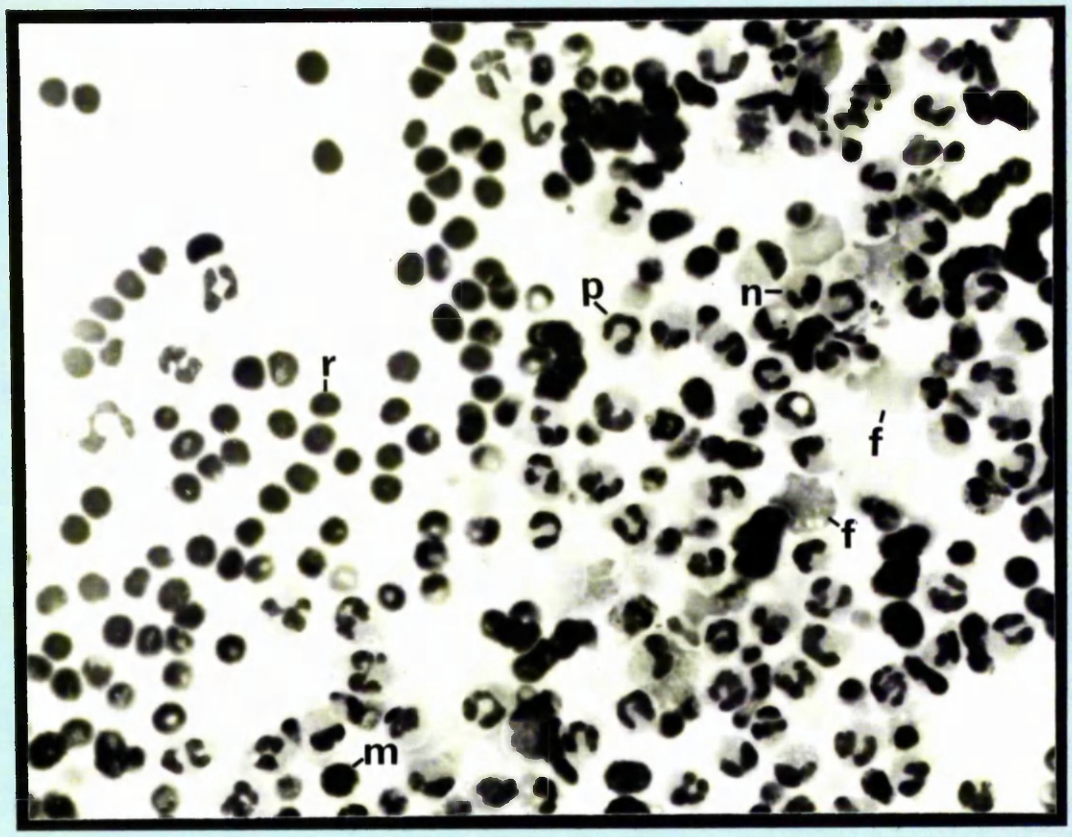
FIGURE 17

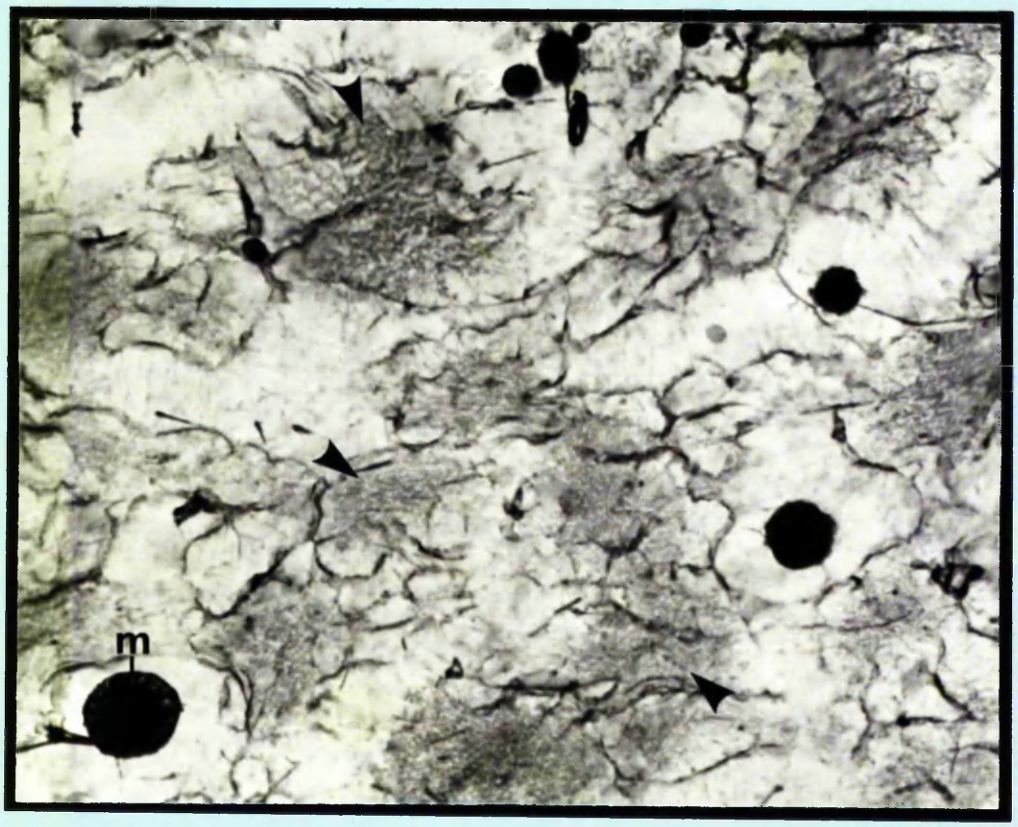
PHOTOMICROGRAPH OF A SYNOVIAL FLUID SMEAR

FROM A DOG WITH OSTEOARTHRITIS.

Only a very few mononuclear leucocytes are seen and one of these has a "foamy" cytoplasm (m). There is a great deal of mucopolysaccharide staining throughout the smear (arrows).

Papanicolaou method. x 1100


p
n
r
f
f
m


m

SECTION V

HAEMATOLOGICAL AND BIOCHEMICAL EXAMINATIONS OF DOGS WITH INFLAMMATORY JOINT DISEASE

Nothing could be more dangerous to the patients' welfare or more damaging to clinical chemistry, than attempts at either "blunderbuss" diagnosis using multitudes of tests, or attempts to "make a firm diagnosis" from a single result.

R. D. Eastham 1975

HAEMATOLOGICAL EXAMINATIONS - METHODS

The haematological examinations of the dogs in this study were carried out by the Department of Veterinary Pathology using standard techniques as outlined below.

1. TOTAL RED BLOOD CELL COUNT (x 10^{12}/L). TOTAL WHITE BLOOD CELL COUNT (x 10^{9}/L). PACKED CELL VOLUME (%). HAEMOGLOBIN CONCENTRATION (g/dL). MEAN CORPUSCULAR VOLUME (fL).

Total red and white blood cell counts, packed cell volumes and mean corpuscular volumes were determined using the Coulter Counter Model 2F 6 (Coulter Electronics Ltd., Harpenden, Herts.). The diluent used was Isoton II (Coulter Electronics Ltd.). 0.4ml of blood was mixed with 20ml of Isoton II; part of this was treated with Zapoglobin (Coulter Electronics Ltd.) for measuring haemoglobin concentration in the haemoglobinometer (Coulter Electronics Ltd.), part was used to measure the total white blood cell count after lysis of the red blood cells and 0.2ml was mixed with a further 20ml of Isoton II and the total red blood cell count measured. An automatic diluter was used for preparing the dilutions (Dial Diluter III, Coulter Electronics Ltd.).

2. PLATELET COUNT (x 10^{9}/L)

This was done on a haemocytometer using 1% ammonium oxalate solution as the diluent, after lysis of the red blood cells.

3. RETICULOCYTE COUNT (% TOTAL RED BLOOD CELL COUNT)

This was done on a blood smear stained with methylene blue (BDH Chemicals Ltd., Poole).

4. ERYTHROCYTE SEDIMENTATION RATE (mm/1hr)

This was measured by the Wintrobe method.

5. MEAN CORPUSCULAR HAEMOGLOBIN (pg)

This was calculated according to the formula:-

$$\frac{\text{Hb (g/dL)}}{\text{RBCC } (\times 10^{12}/\text{L})} \times 10$$

6. MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (g/dL)

This was calculated according to the formula:-

$$\frac{\text{Hb (g/dL)}}{\text{PCV (\%)}} \times 100$$

7. DIFFERENTIAL WHITE BLOOD CELL COUNT

This was done on a blood smear stained by Leishman's method. Leishman's stain was obtained as a 0.2% w/v solution in methanol from BDH Chemicals Ltd., Poole.

BIOCHEMICAL EXAMINATIONS - METHODS

The biochemical examinations of the dogs in this study were carried out by the Department of Veterinary Biochemistry using standard laboratory procedures as summarised below.

1. BLOOD (PLASMA) BIOCHEMISTRY

(a) Urea - *Technicon Kit AA II 01.

(b) Sodium and Potassium - flame photometry using the IL aa/ae Spectrophotometer 257.

(c) Chloride - Corning Eel chloride meter 920.

(d) Calcium and Magnesium - atomic absorption.

(e) Phosphate - method of Yee (1968).

(f) Sugar - *Technicon Kit AA II 02.

(g) Cholesterol - ±Boehringer Corporation Ltd. Kit No. 172626.

(h) Creatinine - *Technicon Kit AA II 11.

(i) Uric acid - method of Carraway (1955).

(j) Bilirubin - *Technicon Kit AA II 18.

(k) Alkaline phosphatase - *Technicon Kit AA II 06.

(l) Aspartate transaminase - ±Boehringer Corporation Ltd. Kit No. 124443 using the LKB reaction rate analyser (Ultrolab System 8600).

(m) Alanine transaminase - ±Boehringer Corporation Ltd. Kit No. 124591 using the LKB reaction rate analyser (Ultrolab System 8600).

(n) Total protein - *Technicon Kit AA II 14.

(o) Albumin:Globulin ratio - electrophoresis on cellulose acetate,

stained with Ponceau S and scanned.

(p) Amylase - $^{\emptyset}$Phadebas Amylase Test with bovine serum albumin.

2. URINARY BIOCHEMISTRY

(a) Protein - sulphosalicylic acid precipitation method.

(b) Urea - as for blood.

(c) Chloride - as for blood.

(d) Specific gravity - by a refractometer.

(e) Bence-Jones protein - by heat coagulation.

(f) Urine was also examined for albumin, glucose, ketones, bile salts, bilirubin and blood pigments using $^{\neq}$Labstix indicator strips and $^{\neq}$ Ictotest reagent tablets.

(g) Part of the urine sample was also centrifuged and the sediment examined for red blood cells, white blood cells, epithelial cells, spermatozoa, organisms, casts and crystals.

3. CEREBOSPINAL FLUID BIOCHEMISTRY

Protein was estimated as for blood.

* Technicon Auto-Analyzer, Technicon Instruments Corporation,Tarrytown, New York 10591, U.S.A.

$^{\pm}$ Boehringer Corporation (London) Ltd., Bell Lane, Lewis, East Sussex.

$^{\emptyset}$ Pharmacia Diagnostics, Uppsala, Sweden.

$^{\neq}$ Ames Company, Division of Miles Laboratories Ltd., Stoke Poges,Slough.

RESULTS

These are shown in Tables 15,16 and 17. Most of the haematological and biochemical examinations described above were performed on all the dogs although certain determinations were only done in individual cases e.g. cerebrospinal fluid protein, blood amylase and not every dog had a urine analysis. Some of the examinations which were carried out were never abnormal in any of the dogs and are not shown in the Tables - these included plasma sodium, potassium, calcium, magnesium, sugar and uric acid. A scoring system has been used to help clarify the results and Table 18 shows how the scoring system relates to each of the parameters measured. A negative score means a decrease from the normal range and a positive score means an increase. Where no score is given, the value recorded was in the normal range for that particular determination. The normal ranges are given in Table 18. In some cases, actual values are listed in the Tables e.g. the albumin:globulin ratio. The results shown in Tables 15-17 are those recorded when the animal was first presented at the Hospital.

GROUP I (RHEUMATOID) (TABLES 15 & 18)

Eleven cases showed a lowering of the number of circulating red blood cells although in most cases this was only mild. In six of these eleven dogs there was also evidence of anaemia (lowered haemoglobin concentrations) but again this was generally only slight. Three dogs had an increased packed cell volume and one had a slightly lowered packed cell volume. Only three cases demonstrated a leucocytosis and of the remainder, six had a lowered total white cell blood count. The erythrocyte sedimentation rate was elevated in eleven dogs. The platelet count was slightly lowered in two dogs.

The blood alkaline phosphatase level was increased in 11 dogs, the aspartase transaminase level in 9 dogs and the alanine transaminase in 14. Nine dogs had two or all three of these enzymes elevated. Blood urea was markedly increased in one dog. Although total blood protein was only absolutely elevated in case Rh 11, the albumin:globulin ratio in all dogs was in favour of the globulin fraction.

Urinary protein was detected in 11 dogs but in only two(Rh 6 and Rh 7)was this greater than 50mg/100 ml. Other abnormalities recorded were an elevated blood bilirubin in Rh 3, an elevated blood cholesterol in Rh 1 and an elevated blood chloride in Rh 9 and two dogs (Rh 3 and Rh 20) had lipaemic plasma.

GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS) (TABLES 16 & 18)

Three of the 4 dogs showed slightly lowered white blood cell counts; the fourth dog had a mild leucocytosis. Dogs SLE 1, 2 and 3 were anaemic with lowered haemoglobin levels and reduced red blood cell counts. One dog (SLE 4)had a slightly elevated packed cell volume and the erythrocyte sedimentation rate was markedly elevated in dog SLE 1.

The blood alkaline phosphatase level was raised in SLE 1 and 3, the aspartate transaminase in SLE 2, 3 and 4 and the alanine transaminase in SLE 3 and 4. Again, the total blood protein was largely composed of globulins and in one dog the absolute level of protein was increased and in another dog decreased compared to the normal range.

Urinary protein was only determined in one dog (SLE 2)and was

found to be high. A cerebrospinal fluid sample was taken from dog SLE 4 and the protein level was found to be within normal limits.

GROUP III (INFECTIOUS) (TABLES 16 & 18)

The red blood cell counts were lowered in 5 dogs and in 4 there was also an accompanying fall in the haemoglobin concentration. Dog INF 8 was particularly anaemic. Four dogs had slight elevation of the packed cell volume. Seven dogs showed a leucocytosis but only in one (INF 3) was this marked. Most of the other six dogs showed white cells in the range 12,000-15,000/cmm. Two dogs had white cells in the normal range, two showed a leucopaenia and in one case the haematological examination was not performed. The platelet count was lowered in 4 dogs.

Six dogs had an elevation of the blood alkaline phosphatase level, 5 had elevation of the aspartate transaminase level and 6 had elevation of the alanine transaminase level. The total blood proteins were only increased in one dog, but all dogs showed increased globulin levels in the blood. The blood urea was slightly raised in two dogs.

Urinary protein estimations were not done in the majority of dogs but two cases had a mild proteinuria.

GROUP IV (BACTERIAL ENDOCARDITIS) (TABLES 16 & 18)

Five dogs showed a marked leucocytosis. Case BE 1 showed an initial leucopaenia followed within four days of hospitalisation by a

marked elevation of the white cell count. The white blood cell count of case BE 6 was in the normal range. The red blood cell count was lowered in all cases except BE 6, but often not to a great degree and the haemoglobin concentration was decreased in three dogs,particularly BE 4. The platelet count was slightly lowered in two dogs. The erythrocyte sedimentation rate was increased in three dogs.

The biochemistry examinations were not performed in dog BE 7. The blood urea level was elevated in all but one of the six cases tested and one dog also showed elevation of blood creatinine and another of blood bilirubin. The alkaline phosphatase level was increased in four dogs, the aspartate transaminase level in 5 and the alanine transaminase in 2. Increased levels of blood globulin were again apparent. Proteinuria was present in all the five cases tested; blood was also detected in the urine of these 5 animals.

GROUP V (IDIOPATHIC) (TABLES 17 & 18)

Red blood cell counts were lowered in 21 dogs and in 8 of these, the haemoglobin concentration was also depressed. Twelve dogs had an increased packed cell volume but in only 2 was this particularly marked; five dogs showed a lowered packed cell volume and this was severe in 3, all of which were markedly anaemic. A leucocytosis was present in 23 dogs, leucopaenia in 8 and the remaining 7 dogs showed white cell counts within the normal range. Nine dogs also demonstrated a reduction in the platelet count but in no case was this very severe. The erythrocyte sedimentation rate was elevated in 17 animals.

The blood alkaline phosphatase level was elevated in 20 dogs, aspartate transaminase in 11, alanine transaminase in 17 and 16 dogs showed elevation of 2 or all 3 of these blood enzymes. The total blood protein was increased in 7 dogs and lowered in 1. In all dogs the albumin: globulin ratio indicated elevated levels of blood globulin.

Urinary protein was detectable in 20 dogs and in half these the degree of proteinuria was particularly severe. Blood in the urine was noted in 6 dogs.

Four dogs had an elevated blood urea, 2 had elevation of blood cholesterol, 2 had elevation of blood creatinine, 2 had increased blood chloride and 1 had a reduced blood phosphate level. Case ID 36 also showed high levels of blood amylase.

TABLE 15

SUMMARISING THE HAEMATOLOGICAL AND BIOCHEMICAL ABNORMALITIES RECORDED IN THE GROUP I (RHEUMATOID ARTHRITIS) DOGS.

A positive score means an increase in the measured level compared to normal, and a negative score means a decrease. The interpretation of the scoring system is shown in Table 18.

ND - not done

	DOG IDENTIFICATION																				
	Rh 1	Rh 2	Rh 3	Rh 4	Rh 5	Rh 6	Rh 7	Rh 8	Rh 9	Rh 10	Rh 11	Rh 12	Rh 13	Rh 14	Rh 15	Rh 16	Rh 17	Rh 18	Rh 19	Rh 20	Rh 21
P.C.V.	+4													+2						+1	-1
HAEMOGLOGULIN				-3		-4	-1			-2							-2			+1	-1
RBC COUNT				-1		-3	-2	-2		-3					-2	-2	-3		-2	-1	-2
WBC COUNT						-3				-2	-4	-2	-3	+3			+3			+2	-2
PLATELET COUNT																				-1	-1
E.S.R.	+2	+3		ND		+5	+6	ND		+4	ND				+3	+4	+3	+2		+3	+2
BLOOD UREA												+4									
BLOOD BILIRUBIN			+3																		
BLOOD ALK.PHOS.	+3		+4				+3	+2	+4	+3				+3	+2	+3	+3				+4
BLOOD AST	+3						+1		+3					+4	+2	+2	+6			+4	+3
BLOOD ALT	+6	+4		+1		+2	+3	+5	+3			+4	+3	+3	+3	+3	+8				+4
TOTAL PLASMA PROTEIN											+1										
A:G RATIO	0.6	0.45	0.53	0.38	0.51	0.37	0.39	0.57	0.44	0.54	0.24	0.53	0.34	0.48	0.35	0.47	0.40	0.28	0.55	0.31	0.35
BLOOD CHOLESTEROL	+2																				
BLOOD PHOSPHATE																					
BLOOD CHLORIDE									+2												+1
BLOOD CREATININE																					
URINARY PROTEIN	ND	+1	ND		+1	+2	+2	+1		+1	ND	+1	ND	ND	ND	ND	+1		+1	+1	+1
URINARY BLOOD	ND		ND								ND		ND	ND	ND	ND					
OTHER			Lipa-emia																	Lipa-emia	

TABLE 16

SUMMARISING THE HAEMATOLOGICAL AND BIOCHEMICAL ABNORMALITIES RECORDED IN THE GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS), GROUP III (INFECTIOUS) AND GROUP IV (BACTERIAL ENDOCARDITIS)DOGS.

A positive score means an increase in the measured level compared to normal, and a negative score means a decrease. The interpretation of the scoring system is shown in Table 18.

ND - not done.

	DOG IDENTIFICATION																						
	SLE 1	SLE 2	SLE 3	SLE 4	INF 1	INF 2	INF 3	INF 4	INF 5	INF 6	INF 7	INF 8	INF 9	INF 10	INF 11	INF 12	BE 1	BE 2	BE 3	BE 4	BE 5	BE 6	BE 7
P.C.V.				+1			+1	+2	+1		ND		+1		-1	-1							+1
HAEMOGLOBULIN	-2	-2	-2	+1		-1				-2	ND	-4	+2		-2	-1	-1		-1	-4			+1
RBC COUNT	-2	-2	-2		-1	-1				-2	ND	-3		-1	-3	-2	-1	-2	-2	-3	-2		-1
WBC COUNT	-1	-1	-2	+1	+2		+5	-2		+1	ND	+1	+1	+1	+3	-1	-3/+4	+4	+5	+7	+4		+3
PLATELET COUNT								-1			ND	-1	-2	-1				-1		-1	ND		ND
E.S.R.	+4			ND	+5	ND	+3			+1	ND	+1	ND	ND	+5	+1	+1	+1		+3	ND		ND
BLOOD UREA					+1							+1					+4		+4	+5	+1	+2	ND
BLOOD BILIRUBIN																			+1				ND
BLOOD ALK.PHOS.	+2		+4			+3	+7			+2	+1		+5	+5					+4	+3	+1	+1	ND
BLOOD AST		+4	+3	+3			+4				+4		+3	+5	+7		+2		+4	+5	+1	+4	ND
BLOOD ALT			+6	+6			+7	+1	+1			+1		+2	+6				+3	+6			ND
TOTAL PLASMA PROTEIN		+1		-1							+3												ND
A:G RATIO	0.64	0.31	0.44	0.71	0.31	0.52	0.31	0.73	0.47	0.30	0.28	0.51	0.53	0.43	0.25	0.28	0.38	0.31	0.38	0.26	0.33	0.32	ND
BLOOD CHOLESTEROL																							ND
BLOOD PHOSPHATE														-1		-1							ND
BLOOD CHLORIDE																							ND
BLOOD CREATININE																			+3				ND
URINARY PROTEIN	ND	+3	ND	ND	+1			+1	ND	ND	ND	ND	ND	ND	ND	ND	+2	+1	+3	+3	+6	ND	ND
URINARY BLOOD	ND		ND						ND	ND	ND	ND	ND	ND	ND	ND	+	+	+	+	+	ND	ND
OTHER				CSF Prot. 270 mg/L																			

TABLE 17

SUMMARISING THE HAEMATOLOGICAL AND BIOCHEMICAL ABNORMALITIES RECORDED IN THE GROUP V (IDIOPATHIC) DOGS.

A positive score means an increase in the measured level compared to normal, and a negative score means a decrease. The interpretation of the scoring system is shown in Table 18.

ND - not done.

	DOG IDENTIFICATION																			
	ID 1	ID 2	ID 3	ID 4	ID 5	ID 6	ID 7	ID 8	ID 9	ID 10	ID 11	ID 12	ID 13	ID 14	ID 15	ID 16	ID 17	ID 18	ID 19	ID 20
P.C.V.	+2	+3		+1		-3	+1		+2	+1				+1			+3		+1	
HAEMOGLOBULIN	+2	+3		+1		-4	+1		+2				-1		-2		+3		+1	-2
RBC COUNT					-2	-3				-1		-2	-2		-2	-2				-2
WBC COUNT	-3			+1	+2	-3	+2		+1		+2	+1	+2	+2	+2	+4	+5	+3	+3	-4
PLATELET COUNT		-2											-1				-1			
E.S.R.	+1				+1	ND	+1		ND	+3	+2	+2	+1	+3	ND				+1	+4
BLOOD UREA		+1													+3			+1		
BLOOD BILIRUBIN																				
BLOOD ALK.PHOS.	+2			+3							+2		+4	+2	+2	+4	+4	+4	+1	+4
BLOOD AST		ND		+2								+2	+3			+2	+3			
BLOOD ALT	+4	ND		+3					+3			+2	+4			+3	+4			+6
TOTAL PLASMA PROTEIN	+1	+5			+2										+3				+1	
A:G RATIO	0.33	0.26	0.35	0.55	0.29	0.44	0.59	0.68	0.70	0.37	0.36	0.48	0.40	0.36	0.31	0.42	0.38	0.35	0.32	0.43
BLOOD CHOLESTEROL																		+2		
BLOOD PHOSPHATE														-2						
BLOOD CHLORIDE																				
BLOOD CREATININE															+1					
URINARY PROTEIN	+1	ND	ND	+1	+5		+1	ND	ND	ND	ND	ND	+2	+1	+8	+1	+10	+5	+1	+4
URINATY BLOOD		ND	ND					ND	ND	ND	ND	ND					Tr.	Tr.		Tr.
OTHER																				

	DOG IDENTIFICATION																	
	ID 21	ID 22	ID 23	ID 24	ID 25	ID 26	ID 27	ID 28	ID 29	ID 30	ID 31	ID 32	ID 33	ID 34	ID 35	ID 36	ID 37	ID 38
P.C.V.				+1	-3			+2			-1	-1			-2		+1	
HAEMOGLOBULIN				+1	-6			+2	-2			-2			-5			
RBC COUNT	-1	-1	-2		-5	-1	-1	-1	-2		-2	-2	-1	-1	-3			
WBC COUNT	+4	+2	+3		-1	+4	-1	-1	+3	+1		+2	+5	+4	-3	-2		+2
PLATELET COUNT				-1		ND		-1	ND		-1		-2			-1	-1	
E.S.R.	ND	ND	+5		ND	ND			ND			+1	+2	+5	+6	+3		+2
BLOOD UREA						ND							+1					
BLOOD BILIRUBIN						ND												
BLOOD ALK. PHOS.		+2	+3			ND	+4			+5		+3	+5		+3	+5		+3
BLOOD AST			+3			ND		+2			+4		+3		+4	+4		
BLOOD ALT						ND	+5	+2		+4	+3	+5	+4		+6	+3	+1	
TOTAL PLASMA PROTEIN		+4				ND			-1						+2			
A:G RATIO	0.37	0.24	0.33	0.85	0.43	ND	0.38	0.67	0.84	0.42	0.70	0.52	0.42	0.31	0.55	0.43	0.59	0.42
BLOOD CHOLESTEROL			+2			ND												
BLOOD PHOSPHATE						ND												
BLOOD CHLORIDE				+1	+1	ND												
BLOOD CREATININE			+4			ND												
URINARY PROTEIN	+4	+2	+5			ND	+1		ND	ND	+1		+4	+5	+5			ND
URINARY BLOOD			Tr.			ND			ND	ND			Tr.		+			ND
OTHER																Blood amyl. 5650 I/L		

TABLE 18

SHOWING HOW THE SCORING SYSTEM FOR THE HAEMATOLOGICAL AND BIOCHEMICAL RESULTS IN THE FIVE GROUPS OF ARTHRITIC DOG, IS INTERPRETED.

N - normal values accepted in this study.

	SCORE FOR INCREASE												SCORE FOR DECREASE							
	N	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10		-1	-2	-3	-4	-5	-6	-7	-8
P.C.V. (%)	35-40	41-50	51-55	56-60									25-34	20-24	15-19					
HAEMOGLOBIN (g/dL)	11-14	15-16	17-18	19-20									10	9	8	7	5-6	3-4		
RBC COUNT ($x10^{12}$/L)	6-8												5-5.9	4-4.9	3-3.9	2-2.9	1-1.9			
WBC COUNT ($x10^{9}$/L)	7-12	12.1-15	15.1-20	20.1-30	30.1-40	40.1-50	50.1-60	60.1-80					6-6.9	5-5.9	4-4.9	3-3.9				
PLATELET COUNT ($x10^{9}$/L)	30 - 50												20 - 29	10 - 19	< 10					
E.S.R. (mm/hr)	0-1	2-5	6-10	11-20	21-30	31-50	>50													
BLOOD UREA (mmol/L)	0-7.5	7.6-12	12.1-15	15.1-20	20.1-30	30.1-50	>50													
BLOOD BILIRUBIN (µmol/L)	0-10	11-20	21-40	41-100	101-200															
BLOOD ALK.PHOS. (IU/L)	0-105	106-150	151-200	201-300	301-500	501-800	801-1000	1001-2000												
BLOOD AST (IU/L)	0-40	41-50	51-60	61-80	81-120	121-150	151-250	251-400	401-600											
BLOOD ALT (IU/L)	0-40	41-50	51-60	61-80	81-120	121-150	151-250	251-400	401-600											
TOTAL PLASMA PROTEIN (g/L)	50-78	79-85	86-90	91-95	96-105	106-115							45-49	40-44						
A:G RATIO	1.0																			
BLOOD CHOLESTEROL (mmol/L)	1.8-7	7.1-10	10.1-20																	
BLOOD PHOSPHATE (mmol/L)	1.3-2.8												0.9-1.2	0.6-0.8						
BLOOD CHLORIDE (mmol/L)	95-115	116-120	121-125																	
BLOOD CREATININE (µmol/L)	44-132	133-150	151-180	181-200	201-250															
URINARY PROTEIN (mg/100 ml)	0	1-50	51-100	101-200	201-300	301-500	501-700	701-900	901-1000	1001-1500	1501-2000									
URINARY BLOOD	Neg																			

DISCUSSION

When assessing the laboratory abnormalities recorded in the five groups of dogs, it was sometimes difficult to know whether the abnormality was related to the joint disease or whether it was a manifestation of a physiological variation or of some other unrelated, undiagnosed, disease process. In most cases repeat samples were taken to check any abnormal recordings. Repeat samples were also taken to monitor the various parameters during the course of the disease, during treatment and following clinical remission. Many parameters which were abnormal when the animal was first presented, became corrected after treatment and clinical remission.

RED BLOOD CELL COUNT , HAEMOGLOBIN CONCENTRATION

Lowered red blood cell counts and decreased haemoglobin concentrations were seen in dogs from all five groups.

Anaemia, usually moderate in degree, is common in human rheumatoid arthritis (Nilsson 1948) and in most cases the cause is unknown(Boyle and Buchanan 1971; Hughes 1978b) although various suggestions have been made (Ziff and Baum 1972). Several of the rheumatoid dogs in this study showed a mild degree of anaemia (mainly normocytic and hypochromic) and this has also been reported by other investigators (Alexander *et al* 1976; Halliwell *et al* 1972; Lipowitz and Newton 1975).

Anaemia also occurs in human systemic lupus erythematosus and may result from retarded erythropoiesis, thrombocytopaenia, haemorrhage, uraemia or from haemolysis (Harvey et al 1954; Shulman and Harvey 1972). Haemolysis is normally associated with red blood cell autoantibodies, detectable by the Coombs' antiglobulin test (see Section VII) and the anaemia in this instance results from an autoimmune mechanism. However, red blood cell autoantibodies may be present in lupus patients in the absence of overt haemolysis (Meacham and Weisberger 1955; Mongan et al 1967). Three of the canine systemic lupus erythematosus cases in this study were anaemic with lowered haemoglobin concentrations and red blood cell counts and the Coombs' test was positive in all three (see Section VII). Anaemia, usually associated with a positive Coombs' test, has also been described in dogs with systemic lupus erythematosus showing polyarthritis, by Pedersen and his colleagues (1976b). Halliwell (1978) reported four polyarthritic lupus dogs with positive Coombs' tests although obvious anaemia was only present in two of them. Other reports of joint disease, anaemia and red cell autoantibodies in canine lupus have been made (Lewis et al 1965; Schalm and Ling 1970).

Of the Group III dogs with infectious arthritis, five had a reduced number of circulating red cells and four of these had a corresponding lowering of the haemoglobin concentration. Anaemia has been reported as a late manifestation of septic arthritis in the human patient (Schmid 1972). No other report of anaemia with septic arthritis in dogs has been published. The fall in circulating red blood cells in these cases, may be related to a toxic effect on the red blood cells or on the bone marrow associated with the focus of infection within the joint - e.g. due to the release of bacterial haemolysins.

Most of the dogs with bacterial endocarditis also showed reduced numbers of circulating erythrocytes and some of these also had a lowered haemoglobin concentration in the blood. Although total red blood cell counts were not performed in the two dogs with polyarthritis and bacterial endocarditis reported by Caywood, Wilson and O'Leary (1977), one of these dogs did develop a low packed cell volume (26%). One of the three dogs with bacterial endocarditis discussed by Murdoch and Baker (1977) had low numbers of circulating red blood cells. Decreased red cell counts in cases of bacterial endocarditis could result from haemorrhage within internal organs or from a toxic effect directly on the red cells or on the bone marrow.

Over half the number of dogs in the idiopathic group showed reduced red blood cell counts, sometimes associated with lowered haemoglobin concentrations. In a few cases, a positive Coombs' test was recorded suggesting the presence of red cell autoantibodies; this will be further discussed in Section VII . The haematology results of the idiopathic cases described by Pedersen and colleagues (1976b)were not reported. The haemograms of the polyarthritic Greyhounds reported by Huxtable and Davies (1976) were all stated as being normal. It is not feasible to consider all the types of arthritis in the human patient which could be included in the idiopathic group as defined for the dog but reduced haemoglobin levels have, for example, been reported in cases of enteropathic arthritis in man (Ferguson 1972) and anaemia can often occur in viral arthritis of man, associated with haemolysis or haemorrhage (Sauter and Utsinger 1978).

ERYTHROCYTE SEDIMENTATION RATE

The erythrocyte sedimentation rate is an important laboratory test for inflammatory activity in the connective tissue diseases of man (Ziff and Baum 1972) although other more sophisticated tests have been introduced e.g. the direct measurement of blood viscosity. In human patients with inflammatory diseases there is an increased rate of settling of erythrocytes in the blood, although many other conditions can produce a similar effect. The sedimentation of erythrocytes is related to rouleaux formation and in general, the sedimentation rate increases in proportion to the size of the erythrocyte aggregates (Ham and Curtis 1938; Hollinger and Robinson 1953). The size of the aggregates in turn, is dependent upon the properties of the blood plasma rather than the cells themselves and the plasma components which influence the sedimentation rate are fibrinogen and the alpha and gamma globulins - when these are increased the erythrocyte sedimentation rate is raised. It is well known that there is an increase of the plasma fibrinogen as a result of increased synthesis by the liver, in the presence of inflammation somewhere in the body and the gamma globulin levels may be expected to increase in the presence of chronic inflammatory disease processes.

The blood of the normal dog generally shows no or very little settling out of the red blood cells; the erythrocyte sedimentation rate was elevated, sometimes markedly, in several of the dogs from all five groups. The erythrocyte sedimentation rate has not been widely reported in other published cases of canine arthritis although hyperfibrinogenaemia has been demonstrated in canine systemic lupus erythematosus, rheumatoid arthritis and idiopathic inflammatory joint disease (Pedersen and Pool

1978; Pedersen et al 1976a,b; Schalm and Ling 1970). Wentinck et al (1974) did report an elevated erythrocyte sedimentation rate in a single case of canine rheumatoid arthritis.

In active cases of human rheumatoid arthritis, the erythrocyte sedimentation rate is usually markedly elevated and tends to parallel the activity of the disease. Increased values were found in 85% of cases of mild severity and in 95% of cases of moderate/severe involvement (Short, Dienes and Bauer 1937). However, it is possible for clinically active cases to show normal sedimentation rates (Dawson, Sia and Boots 1930; Richardson 1957).

The erythrocyte sedimentation rate may be raised in suppurative arthritis affecting the human patient (Ansell 1978; Morrey, Bianco and Rhodes 1975; Schmid 1972) and in cases of bacterial endocarditis (Gutman et al 1972).

Human patients with systemic lupus erythematosus have been shown to have high erythrocyte sedimentation rates (Liljestrand and Olhagen 1955; Olhagen and Liljestrand 1955). Elevation of the erythrocyte sedimentation rate has also been recorded in cases of human arthritis which might fit into the idiopathic group described for the dog e.g. in enteropathic arthritis (Ferguson 1972) and viral arthritis (Sauter and Utsinger 1978).

WHITE BLOOD CELL COUNT, PLATELET COUNT

The majority of the rheumatoid dogs in this study showed normal levels of circulating white blood cells. In all cases, the differential white blood cell count was normal. In human rheumatoid arthritic patients, the white cell counts are usually in the normal range or slightly elevated (Short, Bauer and Reynolds 1957). Leucocytosis is more likely to be seen with an acute and severe onset of clinical signs and with an acute exacerbation of the disease. This association was not seen in the present study. Leucocytosis is also common in the juvenile form of human rheumatoid arthritis i.e. Still's disease (Johnson and Dodd 1955; Lindjberg 1964; Toumbis, Franklin, McEwen and Kuttner 1963). Leucopaenia is rare in human rheumatoid patients (Short, Bauer and Reynolds 1957) and when present it is more likely to be observed in the chronic stage of the disease. Rare cases of leucopaenia with arthritis and splenomegaly (Felty's syndrome) (Felty 1924) appear to be instances of rheumatoid arthritis with a selective neutropaenia due to hypersplenism. Only one of the rheumatoid dogs with leucopaenia also had splenic enlargement. Interestingly, this dog gave a positive antiglobulin consumption test for white blood cell autoantibodies (see Section VII). The differential white blood cell count in human patients is usually within normal limits (Ziff and Baum 1972) although increases in the polymorphonuclear cells have been reported (Short et al 1957).

Leucocytosis, usually of a moderate degree and mainly a neutrophilia, has been reported in the canine rheumatoid patient (Halliwell et al 1972; Lipowitz and Newton 1975; Pedersen and Pool 1978; Pedersen et al 1976a) although many rheumatoid dogs have been reported with normal

levels of circulating white cells (Alexander et al 1976; Pedersen et al 1976a). One of the dogs reported by Pedersen and his colleagues (1976a) had an absolute eosinophilia and this has also been reported in a small percentage of human rheumatoid patients (Short et al 1957). One of the rheumatoid dogs in the present study had a slightly lowered platelet count.

Three dogs with systemic lupus erythematosus had lowered white blood cell counts although not to a marked extent. Leucopaenia is encountered fairly frequently in human lupus patients (Boyle and Buchanan 1971)but is usually not severe. Many lupus cases will show white cell counts within the normal range (Shulman and Harvey 1972). Cytotoxic antibodies against autologous lymphocytes have been found in human systemic lupus erythematosus patients (Michlmayr et al 1972; Stastny and Ziff 1971) and one of the lupus dogs in the present study was positive for white blood cell autoantibodies using the antiglobulin consumption test (see Section VII).

Platelets are also often moderately reduced in human lupus cases and occasionally thrombocytopaenia may be sufficiently pronounced to produce clinical signs. Platelet agglutinins have been demonstrated in the sera of human patients (Dameshek 1958) and in dogs (Pedersen et al 1976b) with systemic lupus erythematosus. Leucocytosis in cases of human lupus usually indicates a complicating infection although it can arise as a result of corticosteroid therapy (Shulman and Harvey 1972).

In six of the seven dogs diagnosed as systemic lupus erythematosus

by Lewis and his colleagues (1965), a leucocytosis was reported. Thrombocytopaenia was also present in six of the cases. Of eighteen dogs with systemic lupus erythematosus, seen at the University of Pennsylvania, only one had leucopaenia and three thrombocytopaenia (Halliwell 1978). Only one of the 29 dogs with polyarthritis due to systemic lupus erythematosus, reported by Pedersen et al(1976b)showed leucopaenia; most of the others had a leucocytosis but with an absolute lymphopaenia. One of these dogs also had a thrombocytopaenia associated with circulating anti-platelet antibodies. Other workers have failed to demonstrate platelet autoantibodies in canine lupus patients showing thrombocytopaenia (Halliwell 1978; Wilkins, Hurvitz and Dodds-Laffin 1973). A single case reported by Alexander et al (1975) showed a lymphopaenia and two other cases of canine systemic lupus erythematosus showed lymphopaenia together with neutrophilia (Krum et al 1977; Schalm and Ling 1970).

Of the 12 cases with infectious arthritis, seven showed a leucocytosis, although this was in most cases, not marked. The platelet count was lowered in 3 dogs although again this was not severe. In human patients with septic arthritis there may be a leucocytosis but this is by no means a consistent feature (Ansell 1978; Schmid 1972). In a series of 37 children with septic arthritis, only one-third had an elevated peripheral leucocyte count and only two-thirds had a shift to the left in the differential count (Morrey, Bianco and Rhodes 1976). Leucocytosis has been reported in dogs with infected joints by Putnam and Archibald (1968).

Most cases of bacterial endocarditis with arthritis showed a

marked leucocytosis. Increased numbers of white blood cells, principally polymorphonuclear cells and"band"cells is regarded as common in canine bacterial endocarditis by Ettinger and Suter (1970). One of the two dogs reported by Caywood et al (1977) had a marked leucocytosis; the other showed a white cell count within the normal range. Both dogs had several segmented polymorphonuclear cells in the differential count. Two of the three dogs reported by Murdoch and Baker (1977) also had a leucocytosis; the third showed normal levels of white cells; all three had "band" forms in the differential smear. Leucocytosis only sometimes occurs in human patients with bacterial endocarditis (Gutman et al 1972). Ettinger and Suter (1970) reported thrombocytopaenia in some canine patients and slightly lowered platelet counts were recorded in two of the bacterial endocarditis dogs in the present study; this could possibly have resulted from haemorrhage or from a toxic effect on the bone marrow.

In the idiopathic group, 22 dogs had a leucocytosis and 8 showed leucopaenia. Nine dogs had lowered platelet counts although in most cases this was only mild. Leucocytosis in cases of canine idiopathic inflammatory joint disease has also been reported by Jones and Darke (1975), Pedersen and Pool (1978) and Schalm and Ling (1970). Normal levels of blood leucocytes were reported by Huxtable and Davis (1976) in their series of Greyhounds. In some dogs in the present study the leucocytosis may have been explained by an accompanying, even secondary infectious bacterial disease process such as dental disease or pneumonia. The explanation for the leucopaenia is not certain; unfortunately no bone marrow biopsies were taken from the affected dogs. One possibility is a toxic depression of the bone marrow and another is an immune response being

directed against circulating white cells or their precursors in the marrow as has been described in systemic lupus erythematosus and already discussed. Two leucopaenic dogs in the idiopathic group were examined for the presence of leucocyte autoantibodies by the direct antiglobulin consumption test but both were negative (see Section VII). It is interesting that the dogs with polyarthritis and leucopaenia responded well to immunosuppressive (corticosteroid) therapy and the levels of circulating white cells increased dramatically. However, corticosteroids are known to directly stimulate the release of white cells from the bone marrow into the peripheral circulation.

It is not relevant to consider all types of arthritis in the human which could be included in the idiopathic category but with enteropathic arthritis, for example, a leucocytosis is usual (Ferguson 1972).

RENAL FUNCTION

The blood urea was elevated in one of the cases of canine rheumatoid arthritis and clinically this was associated with vomiting and malaise. Urinary protein was detected in eleven dogs from Group I but in no case was this high and in 9 of the dogs it was below 50mg/100ml.

More than a faint trace of albumin was found in the urine of only 7.2% of human rheumatoid patients in a survey by Short et al(1957). Fearnley and Lackner (1955) observed persistent proteinuria in 24 of 183 patients, and in 20 this was explained by an unrelated renal disorder, and in the remaining 4 by renal amyloidosis. Similar conclusions

were made by Pollak, Pirani, Steck and Kark (1972) following renal biopsy examinations of 13 rheumatoid patients with persistent proteinuria. The biopsies disclosed normal kidneys in 3 patients, arteriosclerosis or nephrosclerosis in 4, lupus nephritis in 2 and amyloidosis in 4. It is of interest that the incidence of amyloidosis in autopsied cases of human rheumatoid arthritis is as high as 26% (Calkins and Cohen 1960). None of the dogs in the present study which were autopsied had amyloid deposits in the kidneys (see Section IX). According to Whaley and Webb (1977) sterile pyuria, microscopic haematuria and urinary casts are frequent findings in the rheumatoid patient. The blood, non-protein nitrogen was elevated in 1.7% of human rheumatoid patients according to a study by Short et al (1957). A series of Scandinavian studies have reported a small to moderate decrease in the creatinine clearance in rheumatoid patients (Allander, Bucht, Lövgren and Wehle 1963; Sørensen 1960;1961a,b). Creatinine levels were all within the normal range in the Group I dogs. Elevated blood urea and diminished creatinine clearance have been noted in human rheumatoid patients in the West of Scotland (Whaley and Webb 1977).

In most other reported cases of canine rheumatoid arthritis,the biochemical and urine analysis results are not given. In the cases reported by Newton et al (1976),urine analysis was stated as being normal in all cases although one dog developed amyloid lesions of the kidneys. Urine analysis was also normal in the single case reported by Halliwell and his colleagues (1972); the blood urea and creatinine levels were also normal. Proteinuria associated with renal amyloidosis is mentioned as a possible complication of canine rheumatoid arthritis in the review article by Barrett (1977).

One of the dogs with systemic lupus erythematosus had a significant proteinuria (200mg/100ml) but blood urea and creatinine levels were normal.

Proteinuria has been recorded fairly consistently in human systemic lupus erythematosus patients (Davis , Atkins, Josse and Hughes 1973; Dubois and Tuffanelli 1964; Trimble, Townes, Robinson, Kaplan, Chandler, Harissian and Masi 1974). In most cases this is the result of renal involvement in the lupus syndrome which can occur in up to 50% of adult patients (Dubois and Tuffanelli 1964; Estes and Christian 1971). The renal lesions include focal and diffuse proliferative and membranous glomerulonephritis associated with the deposition of immune complexes (Baldwin, Lowenstein, Rothfield, Gallo and McCluskey 1970; Estes and Christian 1971; Pollak, Pirani and Schwartz 1964). Clinical and biochemical evidence of renal failure can appear.

Renal involvement in canine systemic lupus erythematosus has been reported (Lewis et al 1965) associated with proteinuria,and in some cases,elevation of the blood urea nitrogen. Halliwell (1978) reported proteinuria in 4/18 dogs with systemic lupus erythematosus. Glomerulonephritis is regularly stated as being an important manifestation of canine lupus in several review articles on the subject. Proteinuria and elevation of the blood urea nitrogen were also reported in a single case by Alexander et al (1975) and in a few cases by Schalm and Ling (1970). Urine analyses and blood urea nitrogen values were normal in the case reported by Krum and his colleagues (1977). About 50% of the dogs reported by Pedersen et al (1976b) had a proteinuria although blood urea nitrogen values were normal in all cases.

In the infectious group of dogs, the blood urea was only slightly elevated in two and the urinary protein was slightly raised, again in two dogs. In comparison, five cases of bacterial endocarditis showed

a marked elevation of the blood urea level with a pronounced proteinuria also in five cases. Blood was present in the urine of five dogs and the blood creatinine level was markedly elevated in one dog. These features would be consistent with renal involvement in the disease process. This may arise from the spread of infected emboli from the heart valve lesion to the kidneys causing microabscessation and infarction or from the deposition of immune complexes in the glomeruli. These complexes are created by the antigens from the infecting organism and the production of antibodies against them by the host (Gutman et al 1972). Proteinuria, haematuria and elevation of the blood urea nitrogen have all been reported in human patients with bacterial endocarditis (Gutman et al 1972). Blood urea nitrogen and urine analyses were normal in one of the dogs with bacterial endocarditis reported by Caywood and his colleagues (1977) and blood urea nitrogen was normal in the other(the urine analysis results were not reported in the second case). The blood urea level was elevated in the three cases described by Murdoch and Baker (1978) although no urine analysis results are given.

Proteinuria was recorded in 20 dogs in the idiopathic group and the blood urea was elevated in four animals. Blood creatinine was raised in 2 dogs and six dogs showed haematuria but in 5 of these only a trace of blood was detected. The explanation of these features is uncertain but renal disease could have been present, e.g. an immune complex glomerulonephritis was found in some dogs at post-mortem examination (see Section IX). Detailed accounts of blood biochemistry and urine analysis in the idiopathic group of dogs have not been given by other authors.

BLOOD PROTEINS

In every dog from each of the different groups, there was always a lowered albumin:globulin ratio. Interpretation of this ratio is difficult in the dog because there is a wide variation in the normal population. A series of 25 normal dogs were examined in the present study and although the average albumin:globulin ratio was 1.0, the range was from 0.3 to 1.8. In some of the arthritis dogs, there was an absolute increase in the total plasma protein level but in most cases it was within the normal range. Unfortunately, only the total albumin and globulin were measured and no electrophoretic studies to determine the levels of individual proteins were done. Changes in the albumin: globulin ratio may be due to a decrease of the albumin level or an increase of the globulin level,or a combination of both.

In human rheumatoid patients, the total serum protein levels are often within normal limits. However, the most significant features have been a lowered serum albumin concentration and an elevation of the alpha-2 and gamma-globulins and of fibrinogen (Ogryzlo, MacLachan, Dauphinee and Fletcher 1959; Rees and Wilkinson 1959; Sunderman 1964; Sydnes 1963; Wilkinson and Jones 1962; Ziff and Baum 1972). Both the alpha-2 globulin level (Ogryzlo et al 1959) and the gamma-globulin level (Bonomo 1957) have been associated with disease activity. Lowered albumin levels may result from an increased breakdown as part of a general hypermetabolic state in rheumatoid patients (Wilkinson, Jeremy, Brooks and Hollander 1965) or from a protein leak due to renal disease.

Similar blood protein levels are seen in human lupus patients

although the gamma globulin elevation is usually more pronounced (Ziff and Baum 1972) than in patients with other connective tissue disorders (Ogryzlo et al 1959). The light chains of immunoglobulin may be increased in the blood of patients with systemic lupus erythematosus and these peptide chains constitute Bence-Jones proteins (Epstein and Tan 1966). No Bence-Jones proteins were detected in the urine of any of the arthritic dogs in the present study. Cryoglobulins may also occur in the blood of lupus patients and are often associated with active disease (Christian, Hatfield and Chase 1963; Meltzer and Franklin 1967). These proteins are detected by their precipitation under cold conditions but they occur in other disease states including other connective tissue diseases (Boyle and Buchanan 1971) and certain microbial infections including bacterial endocarditis (Dreyfuss and Librach 1952; Williams and Kunkel 1962). No attempts were made, in this study, to detect the presence of these cryoglobulins in the blood.

The cases of canine rheumatoid arthritis reported by Newton et al (1976) showed lowered serum albumin levels, lowered albumin:globulin ratios and high beta-1 and beta-2 globulin levels. Of the cases reported by Pedersen et al (1976a), the serum gamma globulin content was high in one dog, the alpha-2 globulin content in four dogs and the serum albumin level was depressed in one dog. A lowered albumin:globulin ratio was reported in the single case of Halliwell and his colleagues (1972). Details of serum proteins in the other inflammatory joint disorders affecting the dog have not been given by other authors except for fibrinogen which is increased in several of these conditions. The Australian workers, Huxtable and Davis (1976) stated that the serum proteins were normal in their idiopathic Greyhound arthritic cases.

BLOOD ENZYMES

A fairly consistent feature in all five groups of dog was an elevation of the blood alkaline phosphatase and to a lesser extent, an elevation of blood aspartate transaminase and alanine transaminase. Unfortunately, increases in the levels of these enzymes are not specific for any one pathological process. Alkaline phosphatase occurs in bone and many epithelial structures and damage to these will cause an increase in the blood level of this enzyme. In addition, it is excreted in the bile and thus blood levels will also rise in cases of liver damage, particularly of the biliary or intra-hepatic obstructive types. Normal values are also much higher in young growing animals because of bone development. Aspartate transaminase has a widespread tissue distribution but the majority is located in muscle,liver and intestine. Alanine transaminase is located mainly in the liver of dogs. Both aspartate and alanine transaminase are intracellular enzymes being located in mitochondria, cytoplasm, or both,and consequently the circulating level of these enzymes will only increase when cells are damaged or destroyed and the enzymes escape. As the enzymes are destroyed in the circulation the circulating levels will decline rapidly after the phase of acute damage has passed and so the blood enzyme levels show the greatest diagnostic deviations from normal in the early acute phase of any tissue damage (Doxey 1971). Chronic tissue damage may result in only a mild increase of these blood enzyme levels. If the alanine transaminase level is increased in excess of any aspartate transaminase rise, together with an alkaline phosphatase increase, liver damage is most likely. If the damage is primarily to the muscle, for example, the aspartate transaminase levels in the blood rise in excess of the alanine transam-

inase levels. In a few of the dogs there was an increase in alanine transaminase and alkaline phosphatase without an increase in the aspartate transaminase which would suggest some degree of liver damage. However, in the majority of cases elevation of all three enzyme levels or, alternatively, an elevation just of alkaline phosphatase, was apparent. The explanation for these blood enzyme elevations is uncertain but they could just indicate damage to the musculoskeletal system. Often, the enzyme levels became normal as the arthritic problem resolved, although an increase of these levels was often seen when the dogs were receiving corticosteroid therapy. The catabolic effect of corticosteroids might cause an increase in the level of these blood enzymes.

Mild to moderate abnormalities of liver function have been reported in human rheumatoid arthritis patients but these have been confined mainly to tests of liver function which reflect protein synthesis(Darby 1953; Lefkovits and Farrow 1955; Roy, Wigzell, Demers, Sinclair, Duthie, Atherdon and Marrian 1955). Histological changes have been found in up to 25% of biopsies of the liver but have been minimal and not correlated with the chemical abnormalities noted (Lefkovits and Farrow 1955; Movitt and Davis 1953). The histological changes have included fatty infiltration, fibrosis and rarely, infiltration of portal areas with a few mononuclear cells. Clinicial or biochemical evidence of liver disease was found in 0.7% of 997 patients with rheumatoid arthritis by Whaley, Williamson, Dick, Goudie, Nuki and Buchanan(1970). Blood alkaline phosphatase (Lehman, Kream and Brogna 1964), aspartate transaminase (Barr, Stolzer, Eisenbeis, Kurtz and Margolis 1958; Malmqvist and Reichard 1962) and alanine transaminase (Malmqvist and Reichard 1962) levels have all been recorded as normal in rheumatoid patients although

some workers have reported increased alkaline phosphatase levels(Kendall, Cockel, Becker and Hawkins 1970; Webb, Whaley, MacSween, Nuki, Dick and Buchanan 1975; Whaley and Webb 1977) and 15% of patients in one study had some elevation of blood ornithine-carbamoyl transferase (Malmqvist and Reichard 1962). The results of bromsulphalein excretion are equivocal (Ziff and Baum 1972) although abnormal retention has been reported in some patients (Whaley and Webb 1977). Levels of these blood enzymes are not discussed in the reports of canine rheumatoid arthritis made by other workers, except for the single case of Halliwell et al (1972) where the alkaline phosphatase level was stated as being within normal limits.

Hepatic damage in human systemic lupus erythematosus is rare (Pollak, Grove, Kark, Muehrcke, Pirani and Steck 1958; Read, Sherlock and Harrison 1963) although a form of the disease known as lupoid hepatitis does occur (Boyle and Buchanan 1971; Mackay and Wood 1963). The latter is associated with recurring bouts of jaundice and hepatic pain and there is usually a marked elevation of blood transaminase levels although alkaline phosphatase levels are usually normal (Mackay 1968). Increased levels of blood aspartate and alanine transaminases were recorded in two cases of canine systemic lupus erythematosus (Lewis et al 1965). In the case reported by Krum and his colleagues (1977), the levels of blood alkaline phosphatase and alanine transaminase were stated as being normal.

In two dogs with bacterial endocarditis and polyarthritis,normal levels of blood alkaline phosphatase and alanine transaminase were recorded (Caywood et al 1977). No other reports of blood enzyme levels in the various types of canine inflammatory arthropathy are available.

MISCELLANEOUS

Only a few other biochemical abnormalities were recorded in the study. One of the rheumatoid dogs and two of the idiopathic dogs, showed elevation of the blood cholesterol level; the inorganic blood phosphate level was reduced in two of the infectious arthritic dogs and one of the idiopathic dogs; blood chloride levels were elevated in two rheumatoid and two idiopathic arthritic dogs and the blood bilirubin level was increased in one of the rheumatoid dogs and in one of the dogs with bacterial endocarditis. Two dogs in the rheumatoid group also had grossly lipaemic sera and one dog in the idiopathic group had a marked elevation of the serum amylase level. Levels of sodium, potassium, calcium, magnesium and sugar were always within the normal range in all dogs tested. The cerebrospinal fluid protein of dog SLE 4 was within the normal range; this dog did show clinical signs of neurological disturbance (see Section II).

Electrolyte levels are generally normal in human arthritic patients although a small elevation in serum potassium has been claimed in some human rheumatoid patients (Kalliomäki and Kasanen 1955). Increased serum copper levels have also been described in rheumatoid patients and have been interpreted as a non-specific feature of inflammatory disease associated with a rise in the ceruloplasmin level (Jeffrey and Watson 1954) although an anti-inflammatory role of copper has been suggested (Brown and Smith 1979). Some recent work has suggested that blood zinc levels are lowered in rheumatoid patients and this can be associated with slowly healing skin ulcers. It has been shown that blood calcium levels of some rheumatoid patients are elevated after corrections are made for

any lowering of blood albumin (Kennedy, Allam, Boyle, Nuki, Rooney and Buchanan 1975). There is also a trend to hypophosphataemia in these patients. The increased blood calcium levels reflect calcium absorption from the bones (see Section III) but the explanation for this is uncertain. One suggestion has been that these patients are suffering some form of hyperparathyroidism (Kennedy and Lindsay 1977). Parathyroid over-activity might arise from a vitamin D deficiency due to a deficient intake of the vitamin or to a reduced exposure to ultra-violet light as a result of confinement. This would also explain the few patients which are hypocalcaemic rather than hypercalcaemic even after albumin correction (Kennedy et al 1975). Another possible explanation of the hypercalcaemia might be hepatic microsomal enzyme induction by anti-rheumatic drug therapy (Conney 1967) which results in an increased metabolic turnover of vitamin D. Articular erosions similar to those seen in rheumatoid arthritis have also been reported in hyperparathyroidism (Bywaters 1959) and high levels of serum gastrin which are a feature of hyperparathyroidism have also been demonstrated in rheumatoid patients (Rooney, Kennedy, Gray, Sturrock, Buchanan and Dick 1975). Unfortunately, parathyroid hormone levels in rheumatoid patients are generally reduced rather than elevated (Kennedy and Lindsay 1977) although inflammatory mediators could conceivably have parathyroid hormone-like activity (Horton, Raisz, Simmons, Oppenheim and Mergenhagen 1972). Experimental studies with foetal bone in organ culture have shown that the sera of hypercalcaemic rheumatoid patients have a bone-resorbing effect producing increased ^{45}Ca resorption and that this effect is inhibited by calcitonin (Kennedy, Lindsay, Buchanan and Allam 1976). The blood calcium levels of the dogs in this study were within the normal range but certainly most of the dogs did show a lowered albumin level although this was true of all the groups and not just the rheumatoid dogs.

No correction studies were done in the dogs because of a lack of reliable information on calcium balance and calcium levels in this species and because the technique used for the measurement of blood albumin in the dog is not entirely satisfactory (Prof. T. Douglas - personal communication).

A migratory polyarthritis has been described in human patients with a type III hyperlipoproteinaemia (Khachadurian 1968) but this disorder of lipoprotein metabolism is not associated with turbidity of the plasma. Arthralgia and arthritis have also been reported in patients with a type IV hyperlipoproteinaemia (Goldman, Glueck, Abrams, Steiner and Herman 1972) and this type of hyperlipoproteinaemia is characterised by turbidity of the plasma. Buckingham, Bole and Bassett (1975) also described a mild synovitis in association with a type IV hyperlipoproteinaemia and of their 12 patients, 6 had positive tests for rheumatoid factor. Type IV hyperlipoproteinaemia can occur in a primary, inherited (autosomal recessive) form or secondary to a number of other disease processes such as diabetes mellitus and pancreatitis (Barnes 1978). A transient, migratory inflammatory joint disease was described in patients with familial hyper-betalipoproteinaemia (a type II disorder inherited as a single autosomal dominant allele) but it was suggested that the syndrome was due to an acute periarthritis and peritendinitis rather than to a true arthritis (Rooney, Third, Madkour, Spencer and Dick 1978).

Although blood amylase levels are difficult to interpret in the dog (B. Holroyd - unpublished data), the high levels of this enzyme in the blood of case ID 36 suggested a pancreatitis; however, no clinical signs referrable to this were apparent. Interestingly, two dogs with idiopathic joint disease reported by Pedersen and his colleagues (1976b) also had a

pancreatitis although it is not clear how this was diagnosed. Another case, reported as systemic lupus erythematosus had pancreatitis together with gastroenteritis (Schalm and Ling 1970). It is possible that the pancreas may be involved in the disease process as well as the joints; pancreatitis for example, has been reported in human systemic lupus erythematosus (Paulino-Netto and Dreiling 1960). Also, pancreatic disease resulting in the release of pancreatic enzymes into the blood has been associated with arthritis in man (Virshup and Sliwinski 1973).

SECTION VI

ANTINUCLEAR ANTIBODY, RHEUMATOID FACTOR AND THEIR LABORATORY IDENTIFICATION

How to progress from the bedside to the Laboratory, what to take with one, and what to do with it when one gets there - these are the challenges to the modern diagnostician. For them to be met, the physician has to go into the laboratory, and the laboratory scientist has to come into the wards; as long as this happens it does not matter if the immunological team is one man or half a dozen.

P. G. H. Gell and R.R.A.Coombs 1975.

A. ANTINUCLEAR ANTIBODY

HISTORY

It is well known that systemic lupus erythematosus is characterised by the presence of several different autoantibodies,which are defined as antibodies capable of reacting with "self-components". The most consistent of these is antinuclear antibody or ANA (sometimes also called antinuclear factor or ANF) which is in fact a group of autoantibodies against a number of different nuclear components (Barnett 1970; Beck 1961; 1963; Boyle and Buchanan 1971; Friou 1967). However, ANA is not specific for systemic lupus erythematosus and occurs in a number of other diseases.

The lupus erythematosus (LE-)cell pnenomenon in lupus patients was initially found by accident and first described by Hargraves, Richmond and Morton(1948). It was suggested that some factor present in the serum reacted with white blood cells in vitro causing swelling and extrudation of the nuclei. These nuclei were then phagocytosed by other polymorphonuclear leucocytes to give the classic LE-cells. The serum factor responsible for this reaction was later shown to be a gamma globulin (Haserick, Lewis and Bortz 1950). The morphological appearance of LE-cells strongly suggests a reaction which directly involves the cell nucleus. Miescher and Fauconnet (1954) were the first to give direct evidence for such a reaction when they demonstrated that lupus serum could be freed of its ability to induce LE-cell formation by absorption with isolated cell nuclei. Various investigators then studied the nature of this reaction in more detail (Ceppellini, Polli and Celada 1957; Godman and Deitch 1957; Holman and Kunkel 1957; Miescher 1957; Robbins, Holman, Deicher and Kunkel 1957; Seligmann and Milgron 1957). LE-serum factor was shown to be an antibody to deoxyribonucleoprotein and immunological techniques such as complement

fixation and precipitin reactions have demonstrated that autoantibodies to various constituents of the cell nucleus and cytoplasm are a prominent feature of human systemic lupus erythematosus(Holman 1960; Doniach and Roitt 1962).

Various laboratory tests are now available to detect autoantibodies which react with different components of the cell nucleus. Antinucleoprotein antibody as mentioned above, is responsible for the LE-cell phenomenon and reacts with the deoxyribonucleic acid-protein complex which exists in normal chromosomal material (Friou 1958). Another antibody reacts with deoxyribonucleic acid which is not complexed with protein. The third antibody of importance reacts with nuclear antigen which is easily extractable in saline or isotonic buffer solutions and which does not contain deoxyribonucleic acid. This antibody occurs under a variety of circumstances whereas the first two antibodies are more often associated with systemic lupus erythematosus in the human patient (Friou 1967). There is also an antinuclear antibody which reacts only with material within the nucleoli of cells which is probably nucleolar ribonucleic acid (Beck 1961). This antibody is relatively uncommon as is also an antibody which reacts with nuclear histone (Robbins, Holman, Deicher and Kunkel 1957). It is also true that there may be further differences in specificity within each group e.g. with anti-deoxyribonucleic acid antibodies it has been demonstrated that different lupus sera may vary in their reactivity to various oligonucleotide fragments of the deoxyribonucleic acid molecule(Levine 1962). A characteristic feature of these antinuclear antibodies is their lack of species specificity or organ specificity (Holborow 1978).

PATHOLOGICAL SIGNIFICANCE OF ANTINUCLEAR ANTIBODIES

Studies on the pathological significane of ANA have largely been confined to the anti-deoxyribonucleic acid antibodies in systemic lupus erythematosus. Three main types of deoxyribonucleic acid reactive antibodies in lupus have been described (Seligmann and Arana 1968) - those reacting only with denatured deoxyribonucleic acid, those reacting only with native or double stranded deoxyribonucleic acid and those reacting with both these forms of deoxyribonucleic acid. A possibility was that these antibodies were directly cytotoxic. However, ANA fails to cross cell membrane barriers and appears incapable of reaching the location of the nuclear "antigen" within the cell and the growth of cells in tissue culture is unaffected by ANA (Ward, Cloud and Turner 1964). Newborn infants of mothers with lupus are usually positive for ANA but do not show disease (Beck and Rowell 1963; Bridge and Foley 1954) and the transfer of lupus serum to healthy recipients has not transmitted the disease (Bencze, Cserhati, Kovacs and Tiboldi 1958). Often, there can be poor correlation between disease severity and levels of ANA (Friou 1958; Townes, Stewart and Osler 1963a)and this has caused some authorities to view antinuclear antibodies as phenomena of secondary significance in systemic lupus erythematosus. It has also been suggested that immune complexes of deoxyribonucleic acid, anti-deoxyribonucleic acid and complement may be deposited in tissues e.g. glomeruli, blood vessels and produce disease by a Type III (immune complex) hypersensitivity reaction. Immunofluorescent techniques have shown deposits of gamma globulin and complement in renal glomeruli (Koffler, Agnello, Carr and Kunkel 1969; Mellors, Ortega and Holman 1957) and small vessels (Mellors et al 1957) in systemic lupus erythematosus. Deoxyribonucleic

acid has also been shown in similar locations (Browne, Hutt, Regers and Smith 1963). Gamma globulin recovered from the kidneys of patients with systemic lupus erythematosus consists largely of ANA (Freedman and Markowitz 1962; Koffler, Schur and Kunkel 1967; Krishnan and Kaplan 1967). Serum haemolytic complement is generally low in lupus patients, especially in those with renal disease (Townes, Stewart and Osler 1963a),

Antibodies reacting with double stranded ribonucleic acid have also been reported in the sera of systemic lupus erythematosus patients (Schur and Monroe 1969; Steinberg, Baron and Talal 1969), an interesting observation since double stranded ribonucleic acid is reported to occur in only trace amounts in normal mammalian tissues (Stollar and Stollar 1970) but is present in considerable amounts in tissues infected with ribonucleic acid viruses (Weissman and Ochoa 1967). Thus, antibodies to double stranded ribonucleic acid may represent a response to a ribonucleic acid virus infection. Further, since replication of many ribonucleic acid viruses involves production of a deoxyribonucleic acid - ribonucleic acid hybrid, a viral infection in systemic lupus erythematosus could also explain anti-deoxyribonucleic acid antibody production. Viral genome sequences have been identified in the deoxyribonucleic acid of systemic lupus erythematosus patients (Stollar 1976) and there is much other evidence from investigations of lupus both in the human and the canine patient which could support a viral aetiology(see Section VIII).

There is also some evidence to suggest that the formation of immune complexes involving antinuclear antibody could contribute to the immunopathology of joint disease in rheumatoid arthritis in the human patient (Zvaifler 1973). Eluates from rheumatoid synovial membrane

may contain antinuclear antibody (Munthe and Natvig 1971a)and antinuclear antibody activity in rheumatoid joint fluid may exceed that in the serum (Zvaifler and Martinez 1971).

TEST SYSTEMS FOR DEMONSTRATING ANTINUCLEAR ANTIBODY

1. LE-CELL TEST

This test involves taking a blood sample from the patient and incubating it at 37°C for 2 hours during which LE-cells are produced if the correct antinuclear antibody is present (Fig. 24). Various techniques have been described and there is some evidence to suggest a slight variation in sensitivity between individual procedures (Dubois and Strain 1973). Some authorities allow the sample to clot, others heparinise the sample and yet others defibrinate the blood. Anti-deoxyribonucleoprotein enters either a damaged or dead cell and causes swelling of the nucleus with loss of the chromatin pattern. The nucleus is then extruded as an homogeneous mass which is phagocytosed by another white cell and this phagocytosis requires complement (Aisenberg 1959). Occasionally, free homogeneous nuclear material is seen sometimes surrounded by polymorphonuclear cells. Care should be taken to distinguish the "tart" cell from the LE-cell; the former occurs when an intact extruded nucleus, not altered by antinuclear antibodies is ingested - the nucleus retains its chromatin pattern.

Between 75-80% of human systemic lupus erythematosus cases have positive LE-cell reactions although a careful laboratory technique is required to attain these figures (Hughes 1978a;Hughes and Lachmann 1975).

Positive, though generally weak reactions, are also seen in rheumatoid arthritis (Lenoch and Vojtisek 1967), Sjøgren's syndrome (Bloch,Buchanan, Wohl and Bunim 1965), "lupoid hepatitis", occasionally in allergic drug disease, systemic sclerosis (Rowell 1962), dermatomyositis (Dubois 1966), and certain other conditions (Friou 1972).

The LE-cell phenomenon is basically an in vitro process although LE-cells have been described in pleural and pericardial fluids (Hughes and Lachmann 1975) and in synovial fluid (Miller,Perman, Osborne,Hammer and Gambordella 1974).

The LE-cell phenomenon was first evaluated in the dog by Lewis (1965). This author reported 17 cases of systemic lupus erythematosus of which 16 had positive LE-cell preparations. However, no details of the diagnostic criteria used to confirm the presence of systemic lupus erythematosus were given. Six other dogs suffering diseases other than lupus also had positive LE-preparations. Twenty-five normal dogs and 77 dogs with various diseases other than lupus were all negative for LE-cells. A description of the LE-cell reaction in the dog is also given by Schalm and Ling (1970) although the association between positive reactions and clinical diagnoses is confused.

The LE-cell test is unfortunately slow and painstaking to perform and requires an experienced haematologist or pathologist for its interpretation (Boyle and Buchanan 1971).

2. INDIRECT IMMUNOFLUORESCENT TEST

Immunofluorescent tests are based on the specific reactivity of antibody with antigen and can be used to reveal the presence of antibodies in sera or other body fluids or fixed in body tissues, or to identify antigens in tissues, cells, fluids and micro-organisms. One of the reactants, usually the antibody, is labelled and can be used as a topographical tracer.

In 1930, Reiner showed how ordinary histological azo-dyes could be combined with pneumococcal antibody without affecting the ability of the antibody molecules to agglutinate pneumococci. However, the reaction of azo-dye to antibody must be restricted otherwise antibody activity is seriously destroyed and because of this the concentration of dye is insufficient for detection in thin sections under the microscope. This led to the use of fluorochromes as the marker, which are dyes that will absorb radiation e.g. ultra-violet light and become excited thereby. The excited molecules are then capable of emitting radiation (of longer wavelength than the ultra-violet light) which is easily visualised against a near black background under the microscope.

Coons and his colleagues at the Harvard Medical School were the first to combine a fluorochrome dye with an antibody (Coons,Creech and Jones 1941). They used anthracene and although this was excellent for work with bacterial organisms (pneumococci) the method was not satisfactory with tissue sections owing to the normal autofluorescence of the latter which was similar in colour to the anthracene fluorescence. Fluorescein isocyanate was then chosen as the fluorescent dye and this could be

covalently bonded to antibody globulin by the carbamide link to form a di-ureide compound (Coons, Creech, Jones and Berliner 1942; Coons and Kaplan 1950).

The original technique described by Coons and his co-workers is known as the direct immunofluorescent (or fluorescent antibody) technique. With this method, if particular antibodies are to be demonstrated in a patient's serum then the globulin fraction of this serum has to be coupled with the fluorescein dye and then reacted with the antigen. This is however, rather cumbersome since each patient's serum must be conjugated with the dye before the test can be done and with some loss of antibody activity. In 1954, Weller and Coons developed a much more versatile modification known as the indirect immunofluorescent (or fluorescent antibody)technique, also known as the sandwich technique, where the staining is done in two stages. In the first stage, the antibody-containing serum is reacted with the antigen and excess serum washed off,leaving the antibody,if present,attached to its antigen. In the second stage, a species specific antiglobulin reagent prepared in some different species and conjugated with fluorescein is reacted with the first stage antigen-antibody combination. The antiglobulin will link onto the antibody and after washing off any excess antiglobulin reagent,fluorescence will be seen under the ultra-violet microscope. Diagrammatic representations of the direct and indirect immunofluorescent tests are shown in Figures 67 and 18. It is the indirect test which is used to show ANA in the sera of patients with systemic lupus erythematosus

The fluorescein isocyanate which was used in the early studies had to be freshly prepared before use. Riggs,Seiwald, Burkhalter,

Downs and Metcalf (1958) introduced fluorescein isothiocyanate which is a more stable compound and is now produced commercially in a pure form.

The fluorescent antibody technique was first used to demonstrate ANA in human patients by Holborow, Weir and Johnson (1957). Obviously, to demonstrate this autoantibody, a substrate is necessary to provide a source of nuclear material. The most commonly used has been frozen sections of rat liver (Beck 1961; 1963; Boyle and Buchanan 1971). Other substrates have been used e.g. human thyroid, mouse liver, tissue culture cells, human white blood cells and trypanosomes. A number of different substrates have been used in the dog e.g. rat liver (Lewis, André-Schwartz, Harris, Hirsch, Black and Schwartz 1973; Lewis and Schwartz 1971), mouse liver (Hurvitz and Halliwell 1975), calf thymus (Cassan 1968), trypanosomes and white blood cells (Cassan 1968) and tissue culture cells(Halliwell 1978; Schultz 1978). Antinuclear antibody is not species specific and will combine with nuclear material from other species - this is true for the human and canine. However, different tissues,even from the same species, may show different degrees of reactivity. Fixation, age of the donor of the tissues, duration (and temperature) of storage and the type of preparation (section, imprint, suspension, layer) all influence the results in a particular antigen-antibody system.

Antinuclear antibodies are most commonly of the IgG class but IgM and IgA nuclear autoantibodies are also found in the human patient (Barnett, Condemi, Leddy and Vaughan 1964). It is usual to use an antiserum globulin reagent conjugated with fluorescein although some authorities use anti-IgG preparations. A variety of nuclear fluorescent patterns have been described (Beck 1961; 1963; Luciano and Rothfield

1972). These have included homogeneous or diffuse, speckled, nucleolar, and membranous (shaggy, peripheral, rim) patterns. Various experiments have been done to show that these different patterns of fluorescence are associated with different antibodies which react with different nuclear components or antigens. The homogeneous pattern is thought to represent antibodies against deoxyribonucleoprotein but not deoxyribonucleic acid (Beck 1961; Friou 1967). This antibody is present in virtually all cases of acute systemic lupus erythematosus, usually in high titre (Beck 1963). Speckled fluorescence is produced by a soluble saline extractable antigen which excludes deoxyribonucleic acid and nucleoprotein and is seen in approximately 25% of patients with systemic lupus erythematosus (Beck 1963). Speckled fluorescent patterns have also been associated with other nuclear constituents and have been recorded in healthy negroid subjects (Holborow 1978). Antibodies against deoxyribonucleic acid are responsible for the membranous pattern (Beck 1963; Friou 1967: Gonzalez and Rothfield 1966; Tan 1967). Antibodies to soluble nucleoprotein however can also give rise to a membranous pattern of fluorescence (Tan 1967). Anti-deoxyribonucleic acid antibodies are highly specific for active lupus (Casals, Friou and Myers 1964; Friou 1967). Nucleolar fluorescence indicates antibodies against the protein associated with nucleolar ribonucleic acid (Beck 1961; 1963; Beck, Anderson, McElhinney and Rowell 1962). If several of these autoantibodies are present together then the fluorescent pattern will only be of limited help and may vary in appearance with the dilution of the patients' serum (Friou 1967). Different classes and subclasses of immunoglobulin have also been associated with different patterns of fluorescence (Schur, Monroe and Rothfield 1972) and fluorescent patterns have also been shown to alter with different substrates (Bichel,

Barnett and Pearson 1968). Although particular fluorescent patterns have also been associated with different diseases in the human patient there is a considerable "overlap" (Holborow 1978).

Well prepared fluorescent reagents are essential if immunofluorescent techniques are to be successful. The essential requirements of a fluorochrome conjugated antiserum are:- (a) it should contain potent antibody activity of the required specificity, and (b) the level of labelling with the fluorochrome should be such that good specific staining is obtained and unwanted fluorescence avoided. Conjugated antisera can be obtained commercially or prepared in the laboratory. In both cases, the preparations should be carefully assessed to ensure that they are suitable; it is wrong to assume that commercially prepared reagents are always of good quality. The potency of the antiserum can be assessed by a capillary tube precipitin test or by a double diffusion in agar technique. The specificity of the antiserum can be analysed by double gel diffusion against purified immunoglobulin preparations and by immunoelectrophoresis.

The high fluorescence emission intensity of compounds of fluorescein and the ease with which the isothiocyanate can be coupled to protein have resulted in its adoption as the principal labelling compound for immunological tracing. The fluorescence colour is apple green and is said to be readily differentiated from the blue autofluorescence often seen in the substrate. The colour of tissue autofluorescence can, however, vary greatly, particularly with the types of microscope filter used and can appear yellow-green. Commercial samples of fluorescein isothiocyanate can vary considerably in purity as may be demonstrated by infra-

red spectrophotometry and chromatographic analysis. This variability affects the final dye-protein ratio of the conjugate as well as allowing the presence of compounds which may give rise to non-specific staining. Rhodamine is an alternative fluorochrome and gives an orange fluorescence (Nairn 1969). It is usually used only when a second fluorochrome is required, in addition to the fluorescein. Maximal excitation of rhodamine and of fluorescein occur at different wavelengths and this should be remembered during microscopy and the correct filters used for each fluorochrome.

The antibody for conjugation with the fluorochrome must be available as a high-titre, highly avid specific antiserum. Ideally, a homogeneous population of immunoglobulin molecules should be used e.g. a particular immunoglobulin fraction such as IgG obtained by chromatography on DEAE cellulose or DEAE Sephadex A50 (The and Feltkamp 1970)or by one of the more modern techniques now available. Often however, a crude globulin fraction obtained by precipitation of serum with ammonium sulphate is used. Actual preparation of the antiserum for conjugation can be done in several ways depending on the antigen used. For the indirect immunofluorescent test an antiglobulin reagent is required i.e. antibodies are taken from the species under study and form the antigen and are injected into another species where antibodies to them are produced. It is again possible to separate particular immunoglobulin fractions for immunisation or to use a crude globulin fraction which will provide antibodies against all the immunoglobulins present. The latter is generally done for ANA studies. Most of the common laboratory animals have been used for the production of antisera. Rabbits are frequently used, though for large volumes of antisera, sheep, goats

and horses are more practical. Most methods involve the repeated injection of the antigen usually, at least for the first injection, with an adjuvant such as Freund's or aluminium phosphate. The animal is bled at the peak of the circulating antibody response and the serum separated. The antibody level in the serum is then determined by, for example, the optimal proportions precipitation test, the quantitative precipitin test, the Ouchterlony gel precipitation test, the complement fixation test or by immunoelectrophoresis. Only the sera with very specific high antibody levels are suitable for preparing conjugates. The production of antisera directed against a single antigen presents many problems if the pure antigen is not readily available in adequate amounts. Antisera containing low titres of antibodies giving unwanted cross-reactions may be purified by absorption with appropriate antigens. The use of specific pure antigen-antibody precipitates from gel diffusion plates for immunisation has proved successful in producing a pure high titre antiserum (Goudie, Horne and Wilkinson 1966).

Once the antiserum has been isolated, it needs to be conjugated to the fluorochrome. Conjugation of immunoglobulin proteins with fluorescein isothiocyanate takes place readily at pH 9.0-9.5 in aqueous solution. The degree of labelling achieved i.e. the number of dye molecules attached to each protein molecule (the F:P ratio) is a function of time and temperature. For a given dye:protein ratio in the reaction mixture, the higher the protein concentration, the higher the F:P ratio in the resulting conjugate. This underlines the importance of using immunoglobulin fractions which have sufficient protein content. Various methods to conjugate proteins have been described (McKinney, Spillane and Pearce 1964; The and Feltkamp 1970; Riggs, Seiwald, Burkhalter, Downs

and Metcalf 1958).

The use of fluorescein coupled antibodies rapidly led to the discovery of the problem of "non-specific staining" (Coons and Kaplan 1950). These workers thought this was due to some heterophile antibody and hence they tried tissue powder to absorb this. This did in fact reduce the problem. Non-specific staining is now attributed to the presence of unbound dye, "over-labelled" immunoglobulin molecules or to the presence of unwanted labelled proteins e.g. if any albumin is present in the mixture it will take up large quantities of the fluorochrome,which is unnecessarily wasteful, and because albumin is an acidic, "sticky" protein it readily adheres to tissues. Gel filtration using Sephadex can be used to remove unreacted fluorochrome but for high specific activity and minimum non-specificity it is necessary to isolate optimally conjugated antibody molecules from the conjugate preparation by DEAE cellulose chromatography (Goldstein, Slizys and Chase 1961; Riggs, Loh and Eveland 1960). It is also common to help minimise non-specific staining by absorption onto homogenised tissue suspensions as used by Coons and Kaplan (1950). Unbound dye may be present not only as a result of the preparative process but also because of dissociation of the conjugate on storage.

All conjugates of antibody and fluorochrome should be evaluated in order to determine their suitability for the particular system being used. This is done in two parts:- (a) by the physicochemical characteristics which include determination of fluorochrome and antibody content, measurement of antibody content and analysis of other proteins present, (b) performance testing, to obtain information on the range of optimal

working dilutions of the conjugate in a given immunological staining system. The specificity of anti-immunoglobulin conjugates is readily demonstrated by immunoelectrophoresis. However, it is true that fluorescent staining is obtainable with amounts of antibody less than those required for the development of a visible precipitation reaction. Measurement of the total protein content of conjugates is done by conventional means. However, the assessment of fluorescein content by photometry is complicated by the change in its optical density coefficient when coupled to protein (Brighton and Johnson 1971; Goldman 1968). The simplest method is to compare the optical densities of a solution of the conjugate at 495nm and 280nm (Johnson and Holborow 1973). The presence of free dye can be detected by chromatography as mentioned above. Irrelevant protein material can be shown by electrophoresis. The specificity (performance testing) of human anti-immunoglobulin conjugates is readily carried out using bone marrow cells from cases of myeloma and macroglobulinaemia in which the serum "para" protein has been characterised by standard immunochemical procedures. This is more difficult in the dog because of the lack of availability of myeloma patients. The potency of the conjugate is also very important since many of the problems of non-specific staining are overcome simply by using diluted conjugate. In most cases, trial and error is necessary to find the best dilution for the particular immunoglobulin system under study.

Once the fluorochrome conjugated anti-immunoglobulin reagent has bound to its antigen, ultra-violet microscopy is necessary to visualise the fluorochrome and therefore the binding reaction. The fluorochrome should be excited to maximal fluorescence and to achieve this a careful

choice of filters is necessary. The excitation peak is the wavelength at which a fluorochrome absorbs light most efficiently and the emission peak is the wavelength at which the resulting fluorescence energy is maximal. For specific immunofluorescence the exciting light should be rich in energy at the excitation peak wavelength but have negligible energy at the wavelength of the emission peak, so that any colour the observer sees at the emission peak wavelength shall have originated from excitation of the fluorochrome at the object and not non-specifically from the light source itself. The primary filter selected to produce this must therefore pass light to a high degree at the excitation peak wavelength, but exclude light at or even near the emission peak wavelength. When the excitation peak wavelength is in the visible range as with both fluorescein and rhodamine a secondary or barrier filter must be used between object and eyepiece which has the converse properties i.e. passing none of the light transmitted by the primary filter but having a high power of transmission at the peak emission wavelength of the fluorochrome. Fluorescein isothiocyanate labelled antibody absorbs light most efficiently at 495nm and the peak energy of its emitted fluorescence is at 525nm, seen in the microscope as apple-green. The most commonly used lamp for providing exciting light for fluorescence is the high pressure mercury vapour arc (HBO 200). The primary filter should transmit maximally at 495nm but should not pass any colour from the lamp which could be mistaken for the green fluorescence emitted by fluorescein isothiocyanate protein i.e. it should 'cut off' to negligible transmission well short of 525nm. It has been customary to use a BG-12 (Schott and Genossen) blue glass primary filter with peak transmission at 400nm. A Wratten 12 gelatin filter or a yellow glass "cutting off" sharply below 500nm are generally used as secondary filters. These

transmit in the green region but remove any blue light. The primary and secondary filters are thus matched, the primary "cutting off" sharply just above the peak excitation wavelength of the fluorochrome, the secondary transmitting only light of wavelength above the cut-off point of the primary filter. Interference filters for use with fluorescein isothiocyanate may pass light at the red end of the visible spectrum and if this is excessive, it may be modified by inserting a BG-38(Schott and Genossen) blue filter of suitable thickness.

For much fluorescent work a darkground condenser is used with transmitted light. This is designed so that no light enters the objective lens of the microscope after passing through the condenser unless it is scattered or diverted by an object on the stage. Objects on the stage are therefore seen as bright images against a dark background. With transmitted illumination there may be absorbance of both excitation light and fluorescence in the substance of the substrate material itself. For this reason, incident light provided by means of a vertical illuminator is preferred. Dichroic mirrors (interference dividing plates) are used to reflect indirect excitation light selected, as with transmitted light, by a suitable interference filter. The reflected light is focused on the object through the objective, so that centring of the illumination and focusing of the object are simultaneous. Fluorescence from the objective (of a different wavelength to the exciting light) passes to the eyepieces through the dichroic mirror. This system is known as epi-illumination.

3. THE USE OF LATEX PARTICLES COATED WITH DEOXYRIBONUCLEOPROTEIN

As previously discussed antibodies against deoxyribonucleoprotein are thought to be responsible for the LE-cell phenomenon and for the diffuse, homogeneous patterns of fluorescence with the indirect immunofluorescent test using frozen tissue sections as the substrate. An immunofluorescent "spot" test was also introduced to identify antibodies against deoxyribonucleoprotein (Friou 1967; 1972). Yet another test used to identify these antibodies is the latex agglutination test. This test is very simple to perform and is commercially available in kit form, requiring no specialised equipment. Latex particles are coated with deoxyribonucleoprotein and when reacted with the patients' serum containing antibodies against deoxyribonucleoprotein, the particles become agglutinated and this is easily visible to the naked eye. The manufacturers (e.g. Hyland Laboratories,California) claim the test to be particularly useful in diagnosing active systemic lupus erythematosus, the test being positive in 70% of the active cases as compared to 25% of the inactive cases. In a total population of human systemic lupus erythematosus patients, the test was positive in only 35% compared to 75% using the indirect immunofluorescent test. Similar findings were reported by Dubois, Drexler and Arterberry (1961) who demonstrated a positive reaction in 31% of 59 patients who had positive LE-cell preparations and by Dubois and Strain (1973) who found the test negative in 43% of patients with LE-cells and 46% of patients with ANA on immunofluorescence. A comparison of the test with the LE-cell phenomenon was also made by Jordal and Strandberg (1964) although this study is less useful since a very diffuse population of patients was selected in which only 3 were thought to have definite lupus. The test has

been criticised by Friou (1967) who claims the results do not correlate with either the clinical diagnosis of lupus or the presence or absence of any recognisable antibody in the patients' serum.

The commercial latex agglutination test prepared for man has been used in veterinary patients as a diagnostic test for canine systemic lupus erythematosus (Jones and Darke 1975; Schalm and Ling 1970).

4. INDIRECT IMMUNOFLUORESCENT TEST USING TRYPANOSOMES

The presence of nuclear autoantibodies specifically against deoxyribonucleic acid in patients with systemic lupus erythematosus has in the past decade been exploited as a diagnostic and therapeutic aid. It is believed that these autoantibodies are more likely to be associated with systemic lupus erythematosus than any other disease process (Hughes 1975; Hughes, Cohen and Christian 1971; Hughes and Lachmann 1975; Pincus, Schur, Rose, Decker and Talal 1969). It is possible to qualify this even further and specify that the specific antibodies for lupus are against native or double stranded deoxyribonucleic acid and not single stranded deoxyribonucleic acid. The "contamination" of native deoxyribonucleic acid with single stranded portions in various test systems has proved a problem in the standardisation of test results in human medicine.

These anti-deoxyribonucleic acid antibodies were reported almost simultaneously by a number of workers (Cepellini, Polli and Celada 1957; Miescher and Strassle 1957; Robbins, Holman, Deicher and Kunkel 1957;

Seligmann 1957). The techniques used to identify these antibodies have included gel diffusion (Arana and Seligmann 1967; Cohen, Hughes,Noel and Christian 1971; Seligmann 1957; Tan, Schur, Carr and Kunkel 1966), complement fixation (Arana and Seligmann 1967; Robbins et al 1957), passive cutaneous anaphylaxis (Deicher, Holman, Kunkel and Ovary 1960), passive haemagglutination (Jokinen and Julkunen 1965), radioimmuno-electrophoresis (Bichel, Barnett and Pearson 1968), counterimmuno-electrophoresis (Davis 1971; Johnson, Edmonds and Holborow 1973), and bentonite or latex agglutination (Bozicevich, Nasou and Kayhoe 1960; Dubois and Strain 1973; Jordal and Strandberg 1964). Elevated levels of anti-deoxyribonucleic acid antibodies are more likely to be seen in cases where the disease is clinically active.

Reference has already been made to the suggestion that the membranous pattern of nuclear fluorescence in the indirect immuno-fluorescent test using frozen rat liver as the substrate, is produced by antibodies to deoxyribonucleic acid (Beck 1963; Casals, Friou and Teague 1963; Gonzalez and Rothfield 1966; Hamord, Cannat and Seligmann 1964; Tan 1967). However, this type of fluorescence is not universally accepted as being specific for anti-deoxyribonucleic acid antibodies (Bichel, Barnett and Pearson 1968) and the reading of such fluorescent patterns is hindered by the presence of other antinuclear antibodies (Tan 1967). An attempt to overcome this problem was made by Casals, Friou and Myers (1964) who used dried spots of deoxyribonucleic acid on glass slides as the substrate. However, this set-up was found to lack sensitivity and it became apparent that isolated double-stranded deoxyribonucleic acid used for the spots contained single-stranded regions or acquired them during preparation of the spots so that anti-

single-stranded deoxyribonucleic acid antibodies would give positive fluorescence (Aarden, de Groot and Feltkamp 1975). False positive results were also reported by Bichel et al(1968). Another system was developed by Thivolet, Monier, Lalain and Richard (1965) who used the protozoan organism Trypanosoma gambiense as the substrate. A similar study was reported using Crithidia luciliae (Aarden et al 1975). These organisms contain a giant mitochondrion or kinetoplast in which there is a large concentration of double-stranded deoxyribonucleic acid. It is highly unlikely that these kinetoplasts contain nuclear "antigens" other than deoxyribonucleic acid (Steinert 1965). A smear of trypanosome organisms is made on a microscope slide and treated with the patients' serum followed by the antiglobulin reagent conjugated with fluorescein isothiocyanate. Fluorescence of the kinetoplasts under the ultra-violet microscope is indicative of anti-double stranded deoxyribonucleic acid antibodies present in the serum. The indirect immunofluorescent test using trypanosomes seems to be as sensitive as the Farr assay(vide infra) for the detection of anti-deoxyribonucleic acid antibodies (Aarden et al 1975).

The indirect immunofluorescent test using trypanosomes as the substrate has been used to try and identify anti-deoxyribonucleic acid antibodies in the dog (Cassan 1968; Halliwell 1978).

5. RADIOIMMUNOASSAY TEST FOR ANTI-DEOXYRIBONUCLEIC ACID ANTIBODIES

Various methods of detecting anti-deoxyribonucleic acid antibodies have been discussed above; a more recent method has been based on a radioimmunoassay technique. In 1968, Wold and his colleagues (Wold,

Young, Tan and Farr 1968) applied the ammonium sulphate precipitation technique devised by Farr (1958) to the detection of specific anti-deoxyribonucleic acid antibodies. A year later, another group of workers published the results of deoxyribonucleic acid-binding assays in patients with systemic lupus erythematosus and other diseases(Pincus, Schur, Rose, Decker and Talal 1969). The specificity and reproducibility of the test has been confirmed (Pincus, Hughes, Pincus, Tina and Bellanti 1971). The test is based on incubating radiolabelled deoxyribonucleic acid with the patients' serum (Fig. 28). If the latter contains antibodies against deoxyribonucleic acid, then deoxyribonucleic acid/anti-deoxyribonucleic acid complexes will form and these can be precipitated with 50% ammonium sulphate leaving unbound deoxyribonucleic acid in solution. The level of anti-deoxyribonucleic acid antibody activity in the serum is then usually expressed in terms of the percentage radioactivity precipitated. Alternatively, the precipitated radioactivity can be compared with known standards and the level of antibody expressed in units/ml. The most widely used reagent is obtained by the addition of ^{14}C-thymidine to tissue culture (KB cells, Escherichia coli etc.) and subsequent phenol extraction of the labelled deoxyribonucleic acid,although a kit based on ^{125}I labelled deoxyribonucleic acid from HeLa cells is now also available. Because of the cross-reactivity of deoxyribonucleic acid antibodies with deoxyribonucleic acid from a wide variety of species, the source of the nucleic acid is, in theory, not critical. Further purification of the collected deoxyribonucleic acid by,for example, methylated albumin keiselguhr columns has not proved necessary although the presence of nucleoprotein might in theory reduce the specificity of the test.

It is possible to obtain commercially radiolabelled deoxyribonucleic acid for this test or to purchase complete assay kits which provide labelled deoxyribonucleic acid, buffers, ammonium sulphate, standards,etc. This means that many of the technical problems (Hunter 1975) can be avoided,or at least minimised. The test does require equipment for measuring radioactivity and considerable experience in pipetting techniques. It is generally accepted that the precipitates observed with this technique are due to the binding of deoxyribonucleic acid to specific autoantibodies. This may not however be universally true and other binding proteins may also react with the deoxyribonucleic acid. The small precipitates obtained with normal sera may be due to this non-specific binding.

An alternative technique to precipitating the deoxyribonucleic acid/anti-deoxyribonucleic acid complexes with ammonium sulphate is to filter the reaction mixture through a cellulose nitrate filter to remove the complexes (Ginsberg and Keiser 1973). This method has been used in dogs with apparent success (Pedersen et al 1976b). The use of glass fibre filters in place of cellulose nitrate have also been used successfully (Lewis et al 1975). Unfortunately, details of actual levels of anti-deoxyribonucleic acid antibodies in normal dogs and in clinical cases are sparse.

INDIRECT IMMUNOFLUORESCENT TEST USING RAT LIVER AS SUBSTRATE FOR THE DETECTION OF ANTINUCLEAR ANTIBODY - LABORATORY PROCEDURE

1. PREPARATION OF ANTI-GLOBULIN REAGENT CONJUGATED WITH FLUORESCEIN ISOTHIOCYANATE

(a) Commercially prepared

In most cases the anti-globulin reagent was obtained already prepared from Nordic Immunological Laboratories (60 King Street, Maidenhead, Berks.). The reagent was an antiserum against dog gamma globulin prepared in the rabbit and conjugated with fluorescein isothiocyanate. In some cases an anti-IgG preparation from the same commercial source was used and compared with the anti-gamma globulin reagent. The conjugates were provided in a freeze-dried state and reconstituted in 2ml of distilled water. Once this was done, aliquots of 0.2ml were taken and stored at -20°C until used. Each aliquot when used was allowed to thaw and to this was added 1ml of veronal buffer to give a required dilution of 1:5. The use of small aliquots avoided the repeated thawing and refreezing of the reconstituted conjugate - a procedure which can result in loss of reactivity. Each new conjugate reagent was tested with known positive and negative controls to check its suitability.

(b) Self-prepared

(i) Preparation of dog anti-globulin in the rabbit

Dog globulin was collected by precipitation with ammonium sulphate. Blood samples were collected when needed from a normal Greyhound dog

and the serum collected. Ten millilitres of the serum were placed into a centrifuge tube in an ice bath and stirred slowly. An equal volume of saturated ammonium sulphate solution was added drop-wise to the serum. The mixture was left with stirring for 1 hour at which time it was centrifuged for 10 minutes at $4^{o}C$ and 6000 rpm, using the MSE Mistral 4L refridgerator centrifuge. The supernatant was discarded and the precipitated globulin resuspended in 20ml of 50% ammonium sulphate solution prepared by diluting one part of saturated ammonium sulphate with one part of phosphate buffered saline. The mixture was stirred and centrifuged as before. After discarding the supernatant, the precipitated globulin was resuspended in 5ml of phosphate buffered saline at pH 7.2. The protein solution was then dialysed for 48 hours at $4^{o}C$ against two changes of phosphate buffered saline, to remove ammonium and sulphate ions. This was done using dialysis tubing cut to a suitable length and sealed at each end, with the globulin solution inside. The tubing was suspended in a conical flask containing 5 litres of phosphate buffered saline. The tubing was transferred into a second 5 litre flask of buffer after 24 hours.

The globulin solution was removed from the dialysis sac and centrifuged at 3000 rpm for 10 minutes. The protein content of the globulin solution was estimated using the ultra-violet spectrophotometer at 280 nm and assuming an OD of 1 was equivalent to 1mg globulin/ml. The globulin solution was diluted with phosphate buffered saline to give a concentration of approximately 5mg of protein/ml of solution. Two millilitres of this solution were mixed with an equal volume of Freund's complete adjuvant. A water-in-oil emulsion was prepared by vigorously injecting the globulin solution, a small quantity at a time, from a

syringe and needle into the adjuvant. Between each addition of the antigen the emulsion was shaken briskly and finally the emulsion was recycled several times through the syringe and needle in order to increase the dispersion of the antigen phase. The finished emulsion was tested by allowing a drop to fall onto cold water and if the emulsion was prepared correctly, the drops remained floating discretely on the surface of the water although the initial drop sometimes spread over the surface. The correctly prepared water-in-oil emulsion does not mix with water; oil-in-water emulsions mix freely with water to form a milky cloud. A simple water-in-oil emulsion (Freund's incomplete adjuvant emulsion) was also prepared in the same fashion.

One millilitre of the Freund's complete adjuvant emulsion was injected into the thigh musculature of a rabbit on day 1, followed by the same volume of the Freund's incomplete adjuvant emulsion on days 15 and 30. On day 37 the rabbit was anaesthetised with intramuscular ketamine hydrochloride (40mg/kg body weight) and 20ml of blood removed from the heart by intra-cardiac aspiration using a syringe and needle. The serum was collected and examined for the presence of anti-dog globulin antibodies by the double agar diffusion test. This involved cutting a series of patterns of 6 peripheral wells around a central well in a plate containing 0.9% Noble agar in 0.15% sodium chloride. The wells had a diameter of 2.5mm and were placed 8mm apart (centre to centre). Dilutions of antiserum in phosphate buffered saline ranging from 1:1 to 1:32 were placed in the outer wells and different dilutions of globulin solution in the central wells (ranging from approximately 0.2% to 1.8%). The agar plates were incubated overnight at room temperature and the presence of precipitin lines indicated antibodies against dog globulin.

Such antisera were then ready for conjugation with fluorescein isothiocyanate.

(ii) Conjugation of rabbit anti-dog globulin with fluorescein isothiocyanate

The globulin fraction of the dog antiserum prepared in the rabbit was collected using the method already described in (b)(i) above, and the protein content estimated using the ultra-violet spectrophotometer. Fluorescein isothiocyanate was obtained commercially (Calbiochem, Los Angeles, California) and 1.5mg was placed in a bijou and 15ml of 0.5M carbonate bicarbonate buffer added. A solution of the dog anti-globulin reagent was prepared to give a concentration of 100mg of rabbit globulin in 8.5ml of 0.15M saline. To this solution in a beaker was added the dissolved fluorescein in a dropwise fashion; a magnetic stirrer was used to achieve efficient mixing. On completion of this the pH was checked and more buffer added if necessary (the final pH was 9.0). The mixture was left overnight at $4^{o}C$ with continuous stirring, using the magnetic stirrer.

The conjugate preparation was then purified by a gel filtration technique. Six grams of dry Sephadex G25 powder (Pharmacia,Uppsala, Sweden) were suspended in phosphate buffered saline and stirred with a glass rod to prevent clump formation. The gel particles were allowed to sediment and the supernatant containing the excessively fine particles poured off. The suspension was left for 3 hours to allow adequate swelling of the gel granules. The suspended Sephadex was then poured into a glass tube (50cm x 1.5cm) with a capillary outlet

(Pharmacia, Uppsala, Sweden), extra phosphate buffered saline being added if necessary to give a conveniently pourable suspension. The tube was vertically mounted on a retort stand. When a layer of a few centimetres had formed, the outlet was opened to allow an even flow of fluid and as the fluid level fell, more gel slurry was added to the top. After the column had settled, it was washed through with phosphate buffered saline for 30-60 minutes using a reservoir connected to the head of the column. The fluid level was then allowed to sink to the head of the column and the fluorochrome-globulin mixture was carefully pipetted onto the top of the column. The outflow was opened and the sample allowed to enter the Sephadex bed; more phosphate buffered saline was added to fill the tube. The effluent was collected in a series of 2ml quantities and collection was started when the first yellow band approached the bottom of the Sephadex column. Sephadex G25 granules will retain small molecules up to 5000 molecular weight (which includes fluorescein) while large molecules (which includes conjugated globulin, molecular weight 150,000-180,000) will pass straight through the column. The collected material which showed adequate fluorescein-linked globulin (obvious from the yellow-green colour)was pooled. Finally, a reservoir was again attached to the head of the column and phosphate buffered saline washed through to remove at least some of the retained fluorochrome. The conjugate was stored in small aliquots at -20^{o}C.

The resultant conjugate contains some molecules which are over-labelled with fluorescein and have high negative charge. These can be removed by passage through DEAE cellulose but in this study,mouse liver powder was used to absorb these molecules. Mouse liver was homogenised in a Waring blender and repeatedly washed in isotonic

saline followed by several washings in large volumes of acetone. The supernatant was removed after centrifugation and the material spread on filter paper and dried overnight at 37°C. It was then ground in a mortar, passed through a sieve to remove coarse fibrous material and stored in air-tight containers at 4°C. One hundred milligrams of liver powder were used for every 1ml of conjugate. The powder was moistened with phosphate buffered saline and the conjugate preparation added. The mixture was allowed to stand for 2 hours at room temperature with frequent mixing and then left overnight at 4°C. The mixture was centrifuged and the supernatant collected. The latter was the conjugate ready for use. This absorption of the non-specific staining properties was performed on each aliquot of conjugate just prior to its use in immunological marking.

2. ACTUAL TEST PROCEDURE

Male hooded rats between two and six months of age were used to provide the substrate. These were killed by stunning and exsanguination and a fresh rat was killed each time the test was performed. A piece of liver approximately 3cm in size was snap frozen on a chuck in liquid nitrogen and several 5mμ sections cut on a Slee HR Mark II cryostat. Sections were stored for up to one week at -20°C.

Just prior to use, the liver sections were allowed to thaw and were placed in a moist chamber at room temperature. The patients' sera for testing were diluted 1:4 with normal saline and a drop of each of the dilutions was applied to a section of liver for 30 minutes. The sections were then rinsed with saline from a Pasteur pipette and

washed in a bath of veronal buffer agitated by a magnetic stirrer for 15 minutes. Excess buffer was shaken off and then wiped off from around the section with a tissue. The slides were returned to the moist chamber, care being taken not to allow any of the sections to dry out. The dog anti-globulin reagent conjugated with fluorescein isothiocyanate was diluted 1:5 in veronal buffer and a drop of this was applied to each of the sections for 30 minutes. After this time, the sections were rinsed with veronal buffer from a Pasteur pipette and rewashed in a bath of fresh veronal buffer for 15 minutes, again with the aid of a magnetic stirrer. The slides were dried as before and the sections mounted using 22 x 32mm cover slips and a glycerol veronal mounting medium.

Fluorescent microscopy was carried out using a Zeiss Standard 14 epi-fluorescent microscope with the IV FL epi-fluorescence condenser. A high pressure mercury lamp (HBO 50 watt super-pressure) was used as the light source. The primary filters were KP 490 and KP 500. A BG-38 (Schott and Genossen) blue filter was also used. A R fl 510 dichroic mirror was used and a LP 528 was the barrier filter. A positive result was recorded when the nuclei of the substrate (rat liver) were seen to fluoresce (Fig. 19) and the intensity of fluorescence was scored - 3+, 2+, 1+, (+) and $\pm$. No fluorescence was recorded as negative. Known positive and negative control sera were always included. Positive sera were from a case of bacterial endocarditis with polyarthritis and a case of systemic lupus erythematosus and three dilutions were used each time to show different intensities of fluorescence. Any test sera showing a 3+ or 2+ reaction at 1:4 dilution was retested at higher dilutions, viz 1:16, 1:32, 1:64, 1:128, 1:256, 1:512 and 1:1024. In

all cases showing fluorescence, the pattern of fluorescence was described as either homogeneous, speckled, peripheral/rim/membranous or nucleolar. On average, a batch of 25 sera were tested on each occasion.

The optimal dilutions of sera and reagents stated above were found by trial and error using positive and negative samples.

Photographic records of nuclear fluorescence were made using an Ikon 35mm camera attachment on the Zeiss microscope fitted with an automatic exposure. High speed Ektachrome colour film (ET 135-20, ASA 160, DIN 23, Tungsten - Eastman Kodak Company, Rochester, New York) was used and colour and black and white prints were made from the original colour slides.

INDIRECT IMMUNOFLUORESCENT TEST USING TRYPANOSOMA BRUCEI AS SUBSTRATE FOR THE DETECTION OF ANTI-DEOXYRIBONUCLEIC ACID ANTIBODY - LABORATORY PROCEDURE

1. PREPARATION OF TRYPANOSOMA BRUCEI SUBSTRATE

The trypanosomal organisms were supplied by the Department of Parasitology, University of Glasgow Veterinary School. The trypanosome antigen was derived from a stabilate of Trypanosoma brucei TREV 226 originally obtained from Dr. A. R. Gray, Edinburgh. Mice were infected with 1×10^4 trypanosomal organisms and at peak parasitaemia (usually three days after infection) the mice were exsanguinated under trichlorosthylene (ICI Ltd., Alderley Park, Macclesfield, Cheshire) anaesthesia and the trypanosomes separated on DEAE cellulose according to the method of Lanham and Godfrey (1970). The organisms were suspended in phosphate buffered saline and kept at $4^{\circ}C$ until used (maximum of 2 days). A small drop of the suspension was placed on a microscope slide and air-dried under a fan and fixed in 96% ethanol for 10 minutes at room temperature. The concentration of organisms in the smear was assessed under the Zeiss Standard 14 light microscope. An even spread of organisms on the slide was desirable and if necessary the prepared suspension was diluted by the addition of more phosphate buffered saline or concentrated by low speed centrifugation (10 minutes at 1000 rpm in the BTL Bench centrifuge) and resuspension in a smaller volume of buffer.

2. PREPARATION OF ANTI-GLOBULIN REAGENT CONJUGATED WITH FLUORESCEIN ISOTHIOCYANATE

This procedure was the same as that described above using rat liver as

the test substrate (pages 219-224).

3. ACTUAL TEST PROCEDURE

After a satisfactory suspension of the trypanosomes had been obtained, smears were prepared by air-drying and fixing in 96% ethanol. Each smear was treated with ethidium bromide (BDH Chemicals Ltd., Poole) at a concentration of 1mg/ml in phosphate buffered saline for 1 minute, followed by a 1 minute wash in phosphate buffered saline. Ethidium bromide intercalates double-stranded deoxyribonucleic acid and improves the fluorescence of the kinetoplast (ten Veen, Kuivenhoven and Feltkamp 1971). The smears were then treated with the patients' sera followed by the dog anti-globulin conjugate and the technique for this was exactly the same as that described above using liver as the substrate (pages 224-226). Fluorescent microscopic examination was carried out as already described.

A few sera from known human patients with systemic lupus erythematosus were also tested. The procedure was exactly the same as that described for the dog except that a human anti-globulin conjugate was used (supplied by Nordic Immunological Laboratories).

LE-TEST (TRAVENOL LABORATORIES LTD.)FOR THE DETECTION OF ANTI-DEOXYRIBONUCLEOPROTEIN ANTIBODY - LABORATORY PROCEDURE

This test was purchased in kit form from Travenol Laboratories Ltd., Caxton Way, Thetford, Norfolk; the test is actually manufactured in California, U.S.A. The test consists of :-

(i) LE-test latex-nucleoprotein reagent, 2ml

(ii) LE-test positive control serum (human) 0.5ml

(iii) LE-test negative control serum (human) 0.5ml

(iv) Glass slide with a designated oval area

(v) Capillary pipettes with two rubber bulbs

The test is intended for diagnosing systemic lupus erythematosus in human patients. The kit was stored in a refridgerator at $4^{o}C$ until required. The reagents were always allowed to reach room temperature before the test was carried out and the patient's serum was usually tested immediately after it had separated from the clot. Some frozen sera were tested and in such cases the sera were allowed to thaw out to room temperature.

Using a capillary pipette, one drop of the test serum was placed on the provided glass slide. The LE-test reagent (latex particles complexed with calf thymus deoxyribonucleoprotein) was mixed by gently inverting the vial and, using the dropper cap, one drop of the reagent was placed adjacent to the drop of serum. The serum and reagent were then mixed with a small wooden mixer to cover a designated area on the glass slide. The same procedure was repeated for the positive and negative control sera provided with the kit. The test was read after 2 minutes of simultaneous rocking and rotating of the slide to thoroughly mix the sera and reagent. The slide was examined with reflected light

against a black background and a positive result was indicated by macroscopic agglutination of the latex particles (Fig. 22). The latex reagent remained as a smooth or slightly granular suspension with the negative sera. If the positive and negative control sera failed to react correctly, the test was repeated.

LE-CELL TEST FOR THE DETECTION OF ANTI-DEOXYRIBONUCLEOPROTEIN ANTIBODY - LABORATORY PROCEDURE

Twenty millilitres of blood were collected from the dog in a syringe and immediately transferred into a 200ml beaker containing 30-50 glass beads. The blood was defibrinated by gentle agitation of the flask and beads for approximately 15 minutes. The blood was then strained through a "tea-strainer" to remove the beads and fibrin. The blood was incubated at 37°C in a dry oven for 2 hours. After this the blood was centrifuged at 3000 rpm for 15 minutes in a BTL Bench centrifuge, using 15ml centrifuge tubes. The buffy coat was collected and re-centrifuged (3000 rpm for 15 minutes) in Wintrobe tubes. The buffy coat was again collected and used to make thin smears on microscope slides. The smears were allowed to air-dry and were then fixed in methanol for 10 minutes. The smears were stained by two methods - using Leishman's stain and the fluorescent feulgen reaction.

LEISHMAN'S STAIN

After fixing,the smears were stained for 5 minutes with Leishman's stain followed by staining with Leishman's stain diluted with an equal volume of distilled water for another 5 minutes. Excess fluid was removed by blotting and the smears allowed to air-dry,after which they were cleared in xylol and mounted in DPX mounting fluid. The smears were examined using the Zeiss Standard 14 light microscope for a minimum of 20 minutes. A positive preparation was one in which two typical LE-cells could be found in a single smear within the 20 minute examination period. Approximately 6 smears from each blood sample were stained and examined in this manner. A positive smear is shown in Fig.25.

Leishman's stain was obtained as a 0.2% w/v solution in methanol from BDH Chemicals Ltd., Poole.

FLUORESCENT FEULGEN REACTION

This method was originally described by Culling and Vassar (1961). The staining reaction was originally used to show LE-cells by Wignall and co-workers (1962).

After the smears had fixed in methanol for 10 minutes, the following procedure was used:-

(i) the smears were hydrated;

(ii) the smears were treated with pre-heated N/1 hydrochloric acid at 60°C for 10 minutes. Unfortunately, many smears were lost at this stage - they tended to "float off" the microscope slide. This problem was minimised by using very thinly prepared smears and freshly made-up hydrochloric acid;

(iii) the smears were briefly washed in distilled water;

(iv) the smears were transferred to the fluorescent Schiff reagent for 20 minutes;

(v) the smears were washed in acid-alcohol (1% hydrochloric acid in 95% alcohol) and left for 5 minutes. This removed unreacted Schiff reagent;

(vi) the smears were transferred to fresh acid-alcohol for a further 10 minutes;

(vii) the smears were washed in absolute alcohol, with 2 or 3 changes of alcohol, to remove traces of acid;

(viii) the smears were cleared in xylol and mounted in DPX mounting fluid.

The smears were examined using the Zeiss Standard 14 epi-fluorescent microscope with the IV FL epi-fluorescence condenser. The filters etc. used were the same as those described for examining fluorescein conjugates (see page 225). Deoxyribonucleic acid fluoresces a bright golden yellow colour whereas other tissue components fluoresce green. The deoxyribonucleic acid of the LE-cell inclusion fluoresces a lighter yellow colour than the deoxyribonucleic acid of the actual LE-cell nucleus (Figs.26 & 27).Each smear was examined for 20 minutes and 6 smears were prepared from each blood sample. The criterion for a positive slide was the presence of at least two LE-cells in a single smear.

RADIOIMMUNOASSAY FOR THE DETECTION OF ANTI-DEOXYRIBONUCLEIC ACID ANTIBODIES - LABORATORY PROCEDURE

This procedure was initially carried out by the Department of Pathology, Western Infirmary, Glasgow using an ammonium sulphate precipitation technique with C^{14} labelled deoxyribonucleic acid isolated from Escherichia coli. Later in the study, the analyses were performed in the Surgery laboratory in association with the Biochemistry Department and in this instance a commercial kit was used. The latter was the Anti-DNA kit supplied by the Radiochemical Centre, Amersham, Buckinghamshire This again is an ammonium sulphate precipitation test but this time utilising I^{125} labelled deoxyribonucleic acid extracted from HeLa cells. This test is again intended for use in the diagnosis of human systemic lupus erythematosus. The kit consisted of :-

(i) Anti-deoxyribonucleic acid antibody standards i.e. 4 vials containing freeze-dried systemic lupus erythematosus serum (exact values stated on each vial)

(ii) Vial of buffer reagent A (freeze-dried)

(iii) Vial of serum buffer B (freeze-dried diluted normal serum)

(iv) Vial of I^{125} - labelled deoxyribonucleic acid (aqueous solution, not more than 2μCi I^{125})

(v) Vial of solid ammonium sulphate

(vi) Assay tubes and tube rack

(vii) 50 clear polystyrene tubes

(viii) Results plotting sheet.

The kit is used once only and will test 16 unknown serum samples in duplicate.

1. RECONSTITUTION OF THE FREEZE-DRIED REAGENTS

Using a fixed volume pipette, 200µl of distilled water were added to each of the standard vials. The two buffer preparations were each reconstituted with 10ml of distilled water. The water was added to the inside glass surface of each of the vials which were gently inverted and agitated to help dissolution.

2. PREPARATION OF SATURATED AMMONIUM SULPHATE SOLUTION

Ten millilitres of distilled water were added to the ammonium sulphate vial at the start of the assay and allowed to stand for 30 minutes in a water bath at 56°C with occasional shaking. The mixture was then stored at 2-4°C until required on the second day of the assay.

3. ASSAY PROCEDURE

The 16 test sera were diluted 1:10 by mixing 50µl of the serum with 450µl of buffer reagent A.

The assay tubes were numbered and placed in the tube rack. Fifty microlitre aliquots of each standard, the serum buffer B and each unknown serum dilution were pipetted in duplicate into the assay tubes. Care was taken to pipette the liquids to the bottom of the assay tubes. The rack of tubes was placed in the water bath at 56°C for 30 minutes, to inactivate serum complement and possibly also certain other non-specific binding proteins.

The tubes were then removed and allowed to cool to room temperature and 50µl of labelled deoxyribonucleic acid added to all the tubes which were vortex mixed without excessive turbulence. The tubes were covered with tin foil and placed in a water bath at 37°C for 60 minutes. After this time, the tubes were removed and placed in the refridgerator at 2-4°C for 20-24 hours (overnight).

4. SEPARATION OF FREE AND BOUND DEOXYRIBONUCLEIC ACID

One hundred microlitres of saturated ammonium sulphate solution were added to the first tube and vortex mixed immediately for several seconds to ensure efficient mixing. Each of the other tubes were similarly treated in turn. The tubes were centrifuged for at least 15 minutes at 3000 rpm in a BTL Bench centrifuge. The supernatant liquids were then aspirated off using a water pump. This operation was facilitated by viewing the tubes against a black background where the precipitates were more readily observed.

5. COUNTING

The I^{125} counts of the precipitates were measured in a gamma counter (Packard Auto-Gamma Scintillation Spectrometer 5230. Packard Instrument Company Inc., 2200 Warrenville Rd., Downers Grove, Illinois, U.S.A.) using a counting time of 2 minutes. The tubes containing the precipitates were placed in the counter in the same way so as to avoid variations in count-rates due to differences in positioning. If the duplicate counts varied greatly, these results were discarded. The mean of the duplicate counts was always used. A curve of I^{125} counts

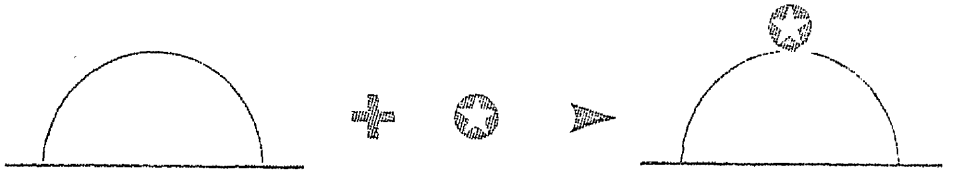
against anti-deoxyribonucleic acid antibody concentration in the provided standards was plotted on graph paper to check the results. However, because the provided standards were human sera, the results in the dog sera were not expressed in terms of anti-deoxyribonucleic acid antibody concentration. Instead, percentage binding values were calculated as follows:-

$$\frac{\text{Total counts of test serum precipitate}}{\text{Total counts of 50}\mu\text{l labelled DNA solution}} \times 100$$

The total counts of the labelled deoxyribonucleic acid solution was obtained by placing 50µl of the provided I^{125} deoxyribonucleic acid solution in duplicate assay tubes and measuring the radioactivity at the same time as the test sera and controls.

FIGURE 18 A DIAGRAMMATIC REPRESENTATION OF THE INDIRECT IMMUNOFLUORESCENT (FLUORESCENT ANTIBODY) TEST USED TO DETERMINE THE ANTINUCLEAR ANTIBODY TITRE IN DOGS' SERA.

I

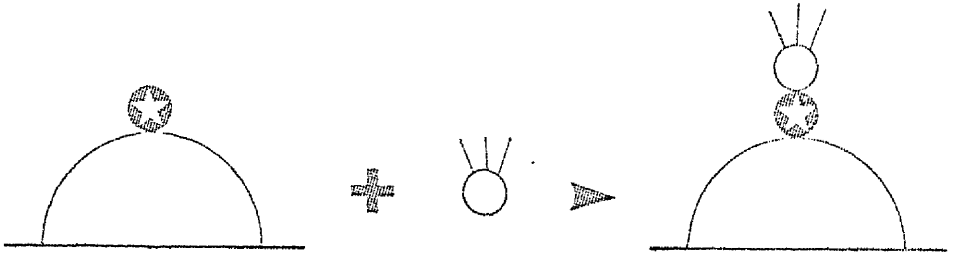


Substrate(frozen sections of rat liver).

Patient's serum containing ANA.

ANA links onto nuclei of rat liver cells.

II



Substrate + ANA

Antiglobulin reagent conjugated FITC.

Antiglobulin reagent links onto ANA. Fluorescence seen under U-V microscope.

FIGURE 19

DETERMINATION OF THE ANTINUCLEAR ANTIBODY TITRE BY THE INDIRECT IMMUNOFLUORESCENT TEST USING FROZEN SECTIONS OF RAT LIVER AS THE SUBSTRATE (POSITIVE RESULT).

Note the homogeneous pattern of fluorescence of the nuclei in this fluorescent photomicrograph.
x1300

FIGURE 20

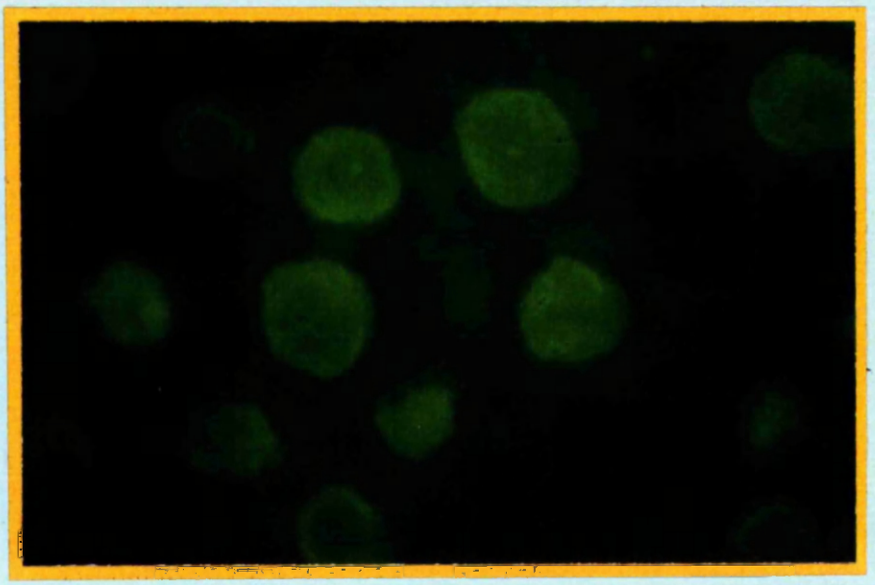
DETERMINATION OF THE ANTINUCLEAR ANTIBODY TITRE BY THE INDIRECT IMMUNOFLUORESCENT TEST USING FROZEN SECTIONS OF RAT LIVER AS THE SUBSTRATE (NEGATIVE RESULT).

There is no nuclear fluorescence.
x1300

FIGURE 21

DETERMINATION OF ANTI-DEOXYRIBONUCLEIC ACID ANTIBODY TITRES BY THE INDIRECT IMMUNOFLUORESCENT TEST USING A SMEAR OF T.BRUCEI AS THE SUBSTRATE.

There is fluorescence of the kinetoplasts(arrows) of the trypanosomes in this photomicrograph. This is a positive reaction obtained with a serum sample from a human patient with systemic lupus erythematosus.
x1300



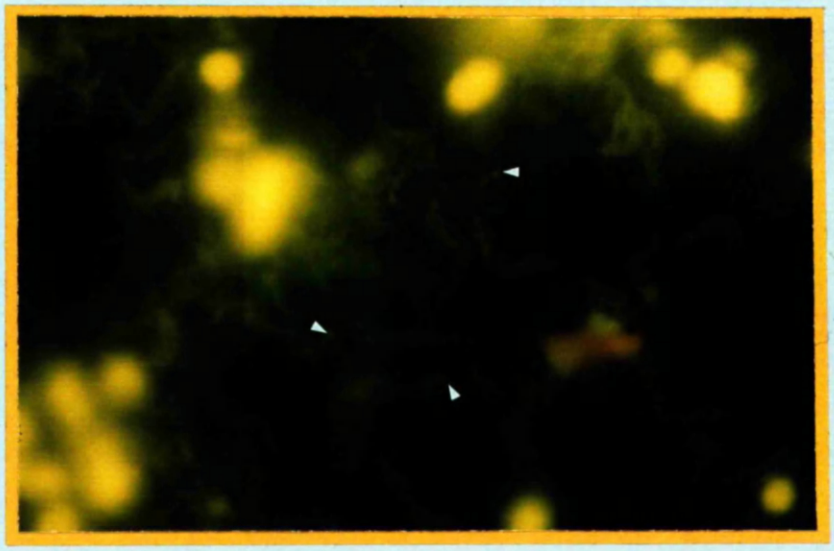


FIGURE 22

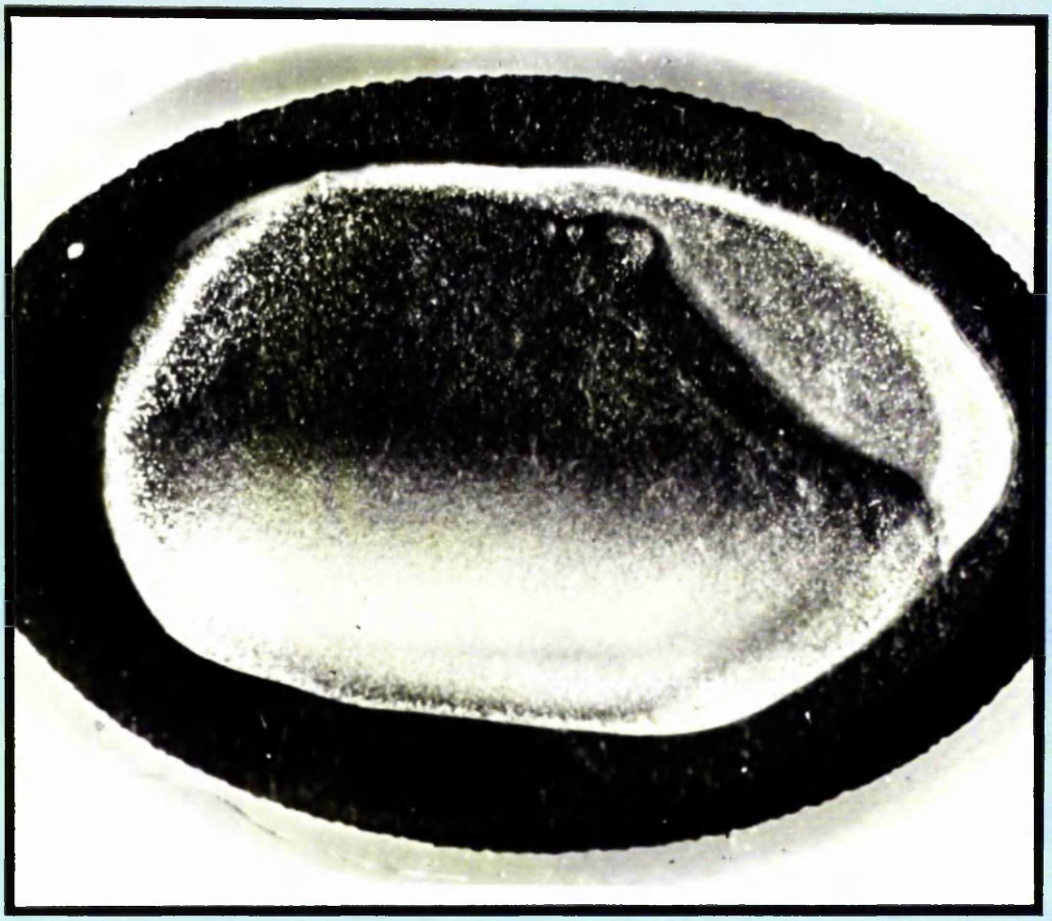
THE LE-TEST (TRAVENOL LABORATORIES LTD.)
FOR THE DEMONSTRATION OF ANTIBODIES AGAINST
DEOXYRIBONUCLEOPROTEIN (POSITIVE RESULT).

Note the granular appearance indicating the agglutination of the latex particles.

FIGURE 23

THE LE-TEST (TRAVENOL LABORATORIES LTD.)
FOR THE DEMONSTRATION OF ANTIBODIES AGAINST
DEOXYRIBONUCLEOPROTEIN (NEGATIVE RESULT).

The latex particles are remaining in suspension, forming a cloudy, milky fluid.



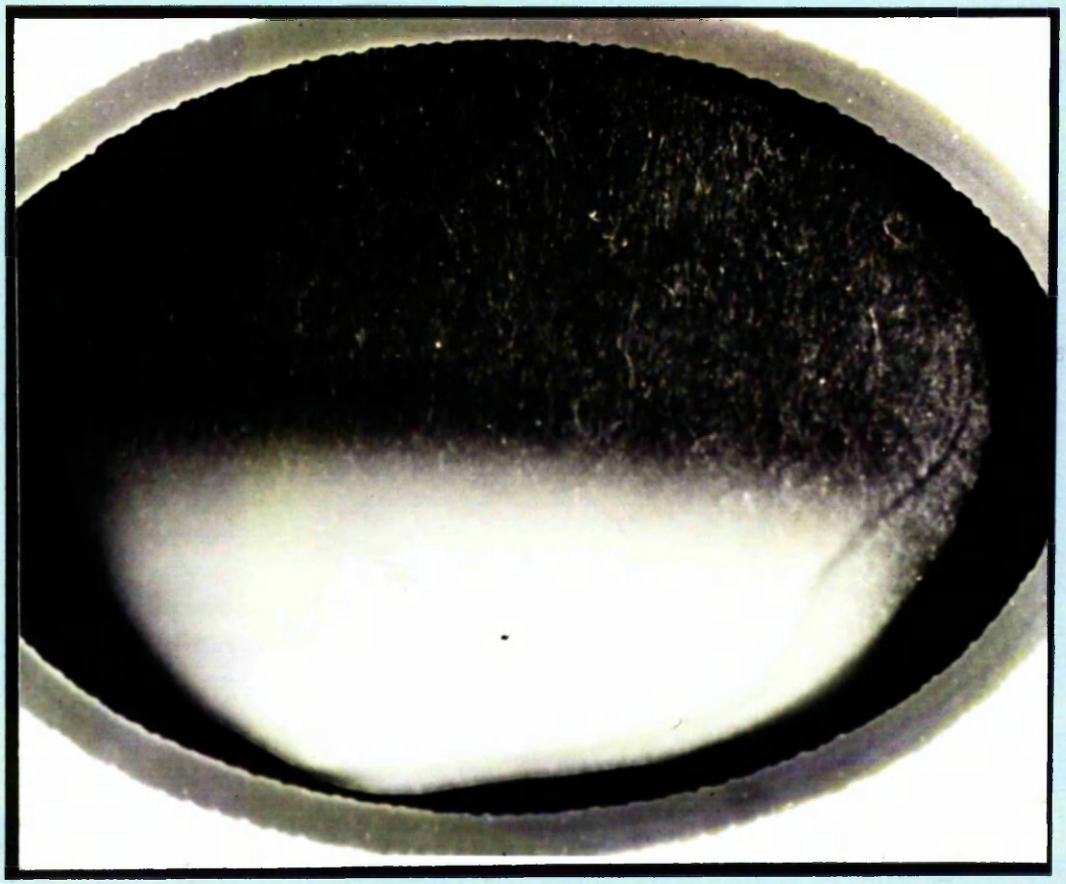
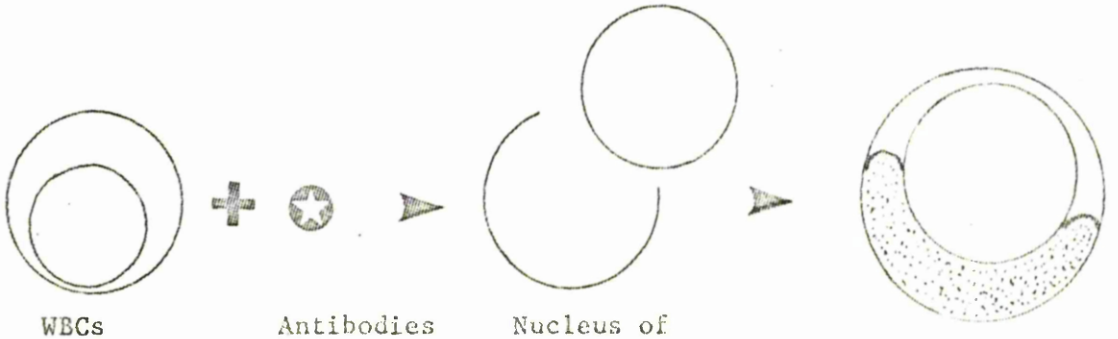


FIGURE 24 A DIAGRAMMATIC REPRESENTATION OF THE LE-CELL PHENOMENON.



WBCs

Antibodies against deoxyribo-nucleoprotein present in patient's blood.

Nucleus of WBC released as homogeneous mass.

Nuclear mass phagocytosed by another WBC to give the LE-cell.

FIGURE 25

PHOTOMICROGRAPH OF A POSITIVE LE-CELL PREPARATION FROM DOG SLE 3 (LEISHMAN'S STAIN).

A typical LE-cell is seen (arrow). There are also several white cells aggregating around extracellular masses of nuclear material in the top left of the smear.
x1000

FIGURE 26

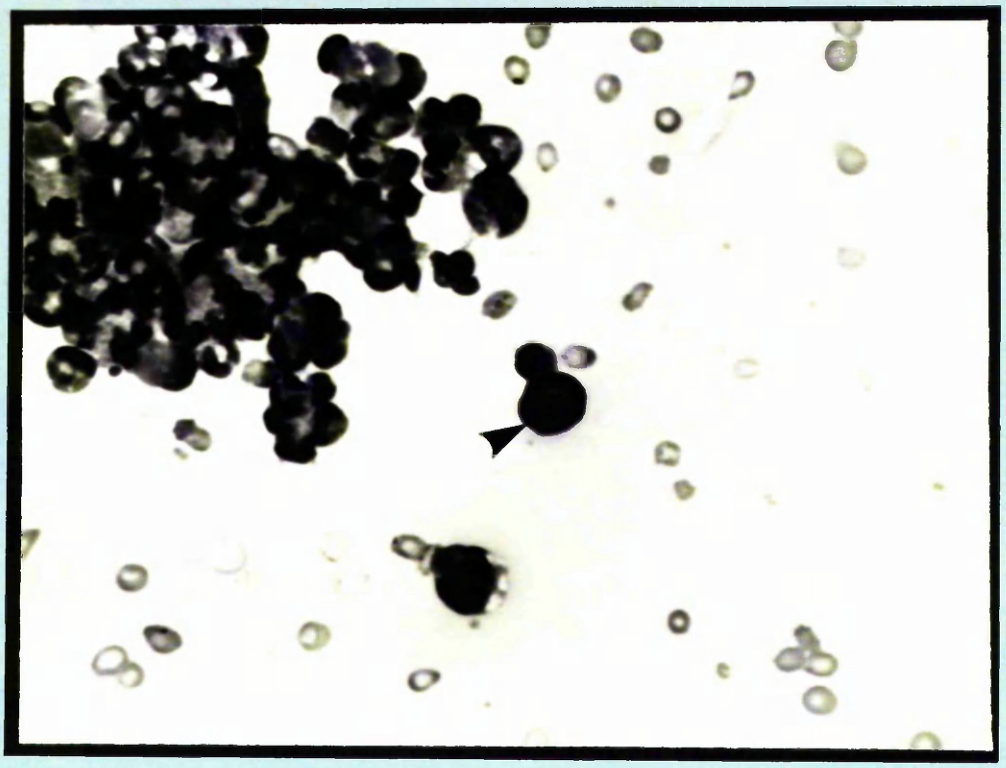
FLUORESCENT PHOTOMICROGRAPH OF A POSITIVE LE-CELL PREPARATION FROM DOG SLE 3 (FLUORESCENT-FEULGEN STAIN).

A typical LE-cell is seen (arrow).
x900

FIGURE 27

FLUORESCENT PHOTOMICROPGRAPH OF A POSITIVE LE-CELL PREPARATION FROM DOG SLE 3 (FLUORESCENT-FEULGEN STAIN).

A typical LE-cell is seen (arrow).
x900



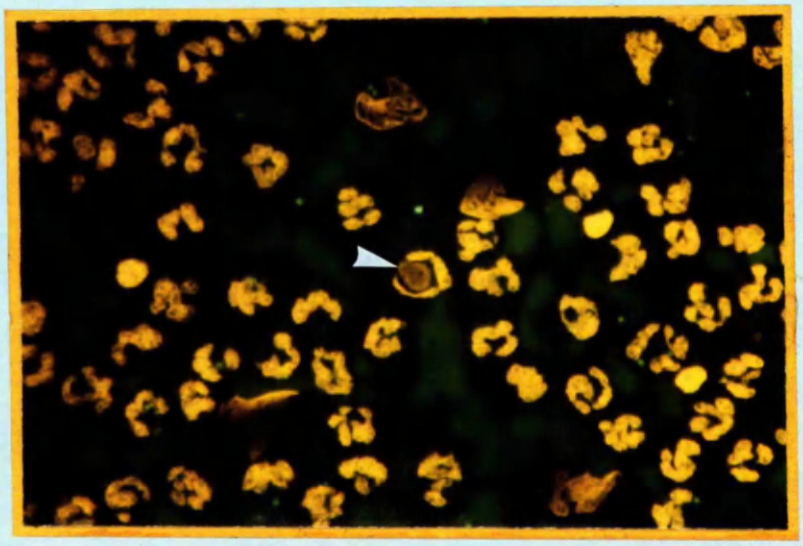


FIGURE 28 A DIAGRAMMATIC REPRESENTATION OF THE DEOXYRIBONUCLEIC ACID BINDING RADIO-IMMUNOASSAY USED TO DETECT ANTI-DEOXYRIBONUCLEIC ACID ANTIBODIES IN DOGS' SERA.

Radiolabelled DNA.

Patient's serum containing anti-DNA antibodies.

DNA and anti-DNA antibody complexes.

Complexes precipitated with ammonium sulphate.

Radioactivity of precipitate measured using gamma counter.

B. RHEUMATOID FACTOR

HISTORY

Serum from human rheumatoid arthritic patients often contains antibodies directed against gamma globulin - a paradoxical situation where antibodies are reacting with other antibodies. These antibodies against antibodies are termed rheumatoid factor or factors (RF) and are examples of autoantibodies. Their identification led to the theory that rheumatoid arthritis may be an autoimmune disease. In all of the many serological systems now available for identifying RF, the majority of rheumatoid sera react positively although the incidence of positive reactions varies with different techniques(Vaughan 1972). RF is not wholly specific for rheumatoid arthritis but its identification is an important laboratory aid to the diagnosis of this disease.

The presence of RF in the blood was initially discovered by accident during studies on the possible relationship of Streptococcus infection to the aetiology of rheumatoid arthritis (Cecil, Nicholls and Stainsby 1930). The sera from rheumatoid patients was found to agglutinate Streptococci of Group A type. However, similar agglutination of rough Pneumococci and Staphylococci by rheumatoid sera was also described (Dawson, Olmstead and Boots 1932). RF was again encountered by chance during routine work with complement fixation reactions (Waaler 1940). It was noted that the serum from a patient with rheumatoid arthritis inhibited the haemolysis of sheep red blood cells and caused marked agglutination of them. The patient's serum alone did not agglutinate the sheep blood cells but did if red blood cell antibodies were added (haemolytic amboceptor) - the agglutination occurred when only a very small dose, itself non-agglutinating, of antibody was

present. This agglutinating factor was called the "agglutination activating factor" and was found to be present in other rheumatoid patients. It was shown to be different from Group A Streptococcus agglutinins, not related to the blood groups of patients and was associated with the globulin fraction of the serum. These observations were largely forgotten until 1948 when Rose and his colleagues again demonstrated the agglutination of sheep red blood cells sensitised with rabbit antibodies,by rheumatoid sera(Rose,Ragan,Pearce and Lipman 1948).

The relationship between the streptococcal agglutination reaction and the sensitised sheep erythrocyte agglutination test was clarified by numerous studies in the 1950's (Lamont-Havers 1955; Thulin 1955; Wager 1950). Rheumatoid factor activity was separated from anti-streptococcal agglutinin activity by precipitation in distilled water. The anti-streptococcal agglutinating activity remained in the supernatant, and was generally of a low titre. The RF responsible for agglutinating sensitised sheep blood cells was in the precipitate. When the precipitate was recombined with the supernatant, the typical high streptococcal agglutination titre of the original serum was restored. The strong streptococcal agglutination reaction in the sera of patients with rheumatoid arthritis thus appeared to depend upon the presence of small (normal) amounts of anti-streptococcal antibody which reacted with the streptococcal antigen forming a "coat" of antibody on the Streptococci and thus enabling RF to react with antibody and produce widespread agglutination.

After 1948, numerous systems were developed to demonstrate the agglutinating activities in rheumatoid arthritis sera(Vaughan and

Butler 1962). All of these depended upon particles coated with IgG globulins, with which RF combines.

PHYSICO-CHEMICAL PROPERTIES OF RHEUMATOID FACTOR

In 1956, Epstein,Johnson and Ragan demonstrated that the addition of purified gamma globulin (Cohn Fraction II) to sera from rheumatoid arthritic patients produces a precipitate containing RF. The precipitation occurs only if aggregated gamma globulin is present in the added material. It has also been shown that RF activity can be differentially sedimented from some rheumatoid sera by ultracentrifugation, the factor being found in a fraction having a sedimentation coefficient of 22S (Franklin, Holman, Müller-Eberhard and Kunkel 1957). Such 22S peaks could be dissociated into 7S and 19S components when the sera were exposed to mild acid. The agglutinating activity was found to be within the 19S component and under appropriate conditions the separated 19S agglutinating factor could be made to recombine with the 7S component to reform the 22S complex. RF is an immunoglobulin mainly of the IgM class (19S) and combines with IgG molecules(7S),one molecule of RF generally being associated with 6 molecules of IgG. Complexes intermediate between coefficients 7S and 19S have also been described(Chodirker and Tomasi 1963; Kunkel,Müller-Eberhard,Fudenberg and Tomasi 1961). These too were dissociated with mild acid(or urea), this time into 7S components. This was made clear by fractionation studies in density gradient ultracentrifugation and DEAE cellulose chromatography and by the failure of the agglutinating properties of this serum to be completely abolished by treatment with 2-mercaptoethanol, a substance which completely destroys RF of the 19S variety. Schrohenloher

(1966) demonstrated that anti-IgG activity was present in the IgG molecules isolated with these intermediate complexes by utilising the capacity of peptide digestion to abolish the Fc part of the IgG molecule without affecting its antibody activity. RF activity has also been detected among IgA globulins (Abraham, Clark, Kacaki and Vaughan 1970; Allen 1966; Heimer and Levine 1964; Torrigiani and Roitt 1967). It is important to note that RF of the IgM type has immunological activity in the various in vitro test systems used to detect RF and is readily assessed, whereas IgG rheumatoid factor is not detectable in the routine test systems(Kunkel and Tan 1964).

SPECIFICITIES OF RHEUMATOID FACTOR

It was shown by Heller and colleagues (1954) that rheumatoid arthritic sera could be absorbed with sheep red blood cells coated with rabbit antibody to a point at which the sera no longer agglutinated the coated sheep cells, but still agglutinated in high titre, cells coated with human IgG. However, complete absorption with human IgG coated cells removed agglutinating activities for both types of coated cells. When rheumatoid sera are absorbed with aggregated rabbit IgG, all of the rheumatoid factor capable of agglutinating sensitised sheep blood cells is removed and with it a considerable proportion of the factor that precipitates with aggregated human IgG. Various other studies have been done (Aho, Konttinen, Rajasoline and Wager 1962; Aho,Ripatti, Saris and Wager 1962; Butler and Vaughan 1965; Harboe 1961) again suggesting that RF in the human reacts, as a whole, with aggregated or denatured human IgG while only portions react with IgG of other species like the rabbit. These findings are consistent with RF being anti-

bodies to human IgG and cross-reacting with IgG of other species. Chromatographic studies by Lospalluto and Ziff (1959) and by Williams and Kunkel (1963) have indicated that RF having reactivity with rabbit gamma globulin may have a slightly different chromatographic behaviour from RF reactive only with human gamma globulin. A slight difference in amino acid analysis for RF reacting with rabbit gamma globulin has also been reported(Heimer, Federico and Freyberg 1958). Some authorities have shown anti-rabbit gamma globulin activities in preparations exhibiting little or not anti-human gamma globulin activity. These RF of different specificities are important when considering the different test systems used to identify RF and may explain why one test is positive but another negative when performed on the same serum sample.

Most of the RF activity appears to be directed to configurations on the H-chain portion of IgG molecules, particularly the Fc fragment (Harboe 1963). Certain antigenic sites have been identified on immunoglobulins and two of these are the Gm and InV sites and like blood group determinants are genetically controlled (Aho 1961; Fudenberg and Mårtensson 1963; Grubb 1961; Steinberg 1962). There are several types of Gm and InV groups and some rheumatoid arthritic patients can be shown to have a RF which reacts specifically with the Gm or InV determinant group on their IgG. A curious feature is that some patients may possess a RF which can be shown to react specifically with a Gm determinant not apparently possessed by their own gamma globulin. It may be that some determinants are present but are "hidden" and only become available when the globulin molecule undergoes some change.

ORIGIN OF RHEUMATOID FACTOR

Several workers have used immunofluorescent techniques to identify the site of origin of RF in the body (Mellors, Heimer, Corcos and Korngold 1959; Mellors, Nowoslawski and Korngold 1961; Mellors, Nowoslawski, Korngold and Sengson 1961; McCormick 1963). These techniques allow the identification of plasma cells producing RF in various tissues taken from the rheumatoid patient. Again, some plasma cells appear to produce RF with specificity for human IgG while others produce RF which will react only with rabbit IgG, while still others appear to produce both types of RF (See also Section X).

PATHOLOGICAL SIGNIFICANCE OF RHEUMATOID FACTOR

There is much evidence to suggest that RF does not play a primary role in the aetiology of rheumatoid arthritis and this has been reviewed by Boyle and Buchanan(1971). However, it is well known that patients with high titres of RF have a more severe joint disease (Kellgren and Ball 1959; Vaughan 1959; Ziff 1957) and a poorer prognosis (Duthie, Brown, Truelove, Baragov and Lawrie 1964). Extra-articular manifestations of rheumatoid arthritis are also more frequently seen in patients with high titres of RF e.g. subcutaneous nodules (Ball 1952; Kellgren and Ball 1959), arteritis, peripheral neuropathy (Epstein and Engleman 1959) skin ulceration and Felty's syndrome where the arthritis is complicated by splenomegaly and neutropaenia (Franklin, Kunkel and Ward 1958). Some of the possible functions of RF are considered below.

1. PROTECTIVE ROLE

RF can combine with soluble antigen-antibody complexes and thereby render them less soluble and more easily cleared by the reticuloendothelial system. The patient can thus be protected against an immune complex (type III) hypersensitivity reaction. There is also evidence that RF at least *in vitro* can help prevent complement fixation by antigen-antibody complexes(Gough and Davis 1966; Heimer, Levine and Kahn 1963). On the other hand, RF can interfere with the opsonic activity of IgG antibodies and increase rheumatoid patients' susceptability to infection (Messner, Caperton, King and Williams 1969).

2. EFFECTS ON IMMUNOGLOBULIN METABOLISM

The evidence for an effect of RF on immunoglobulin metabolism is equivocal although Davis and Torrigiani (1967) noted increased breakdown of aggregated IgG when RF was present. Bluestone, Cracchiolo, Goldberg and Pearson (1970) suggested that RF of high binding avidity may be capable of reacting with IgG to produce precipitates and rapid clearance from the body fluids and Waldmann, Johnson and Talal(1971) presented evidence that RF not only accelerated the catabolism of IgG but interfered with its synthesis as well.

3. ROLE IN THE INFLAMMATORY RESPONSE

It is possible that when RF-IgG complexes are present in high concentration they become insoluble and deposited in the vascular endothelium of blood vessels and produce inflammatory disease by a

Type III (immune complex) hypersensitivity reaction. Fractions of sera rich in RF when injected into the mesenteric arteries of experimental rats can produce a vasculitis by this mechanism(Baum, Stastny and Ziff 1964). Broder, Baumal, Gordon and Bell(1969) have shown a histamine -releasing activity in the sera of patients with rheumatoid arthritis, possibly due to the presence of RF - IgG complexes. There is also evidence that the kinin system can be activated by RF (Epstein, Tan and Melmon 1969). Despite the discussion given in (1), there is also evidence that RF-IgG complexes may fix human though not guinea-pig complement (McCormick, Day, Morris and Hill 1969; Tesar and Schmid 1970; Zvaifler 1969).

There is strong support for the hypothesis that RF when present in synovial fluid may have a pathogenic role (Hollander, McCarty,Astorga and Castro-Murillo 1965; Rawson, Abelson and Hollander 1965; Restifio, Lussier, Rawson, Rockey and Hollander 1965). It is proposed that RF reacts in the synovial fluid with altered or denatured IgG and possibly complement and the resultant complexes initiate an inflammatory response - an extravascular immune complex disease(Zvaifler 1974). Although the serum complement levels are normal or slightly elevated in rheumatoid arthritis the synovial fluid complement levels are markedly depressed (Fostiropoulis, Austen and Bloch 1965; Hedberg 1964; Pekin and Zvaifler 1964; Vaughan, Barnett, Sobel and Jacox 1968). There is also much evidence (Holborow 1978) that IgG rheumatoid factor may be the most significant in promoting this depletion of synovial fluid complement and be instrumental in initiating and/or contributing to the production and maintenance of joint inflamation. The synovial membrane itself in rheumatoid arthritis is a source of active immunoglobulin synthesis

and a significant proportion of the product is IgG rheumatoid factor in both seropositive and seronegative patients. In addition, IgM rheumatoid factor is found in seropositive patients. Complexes of IgG and RF appear in the synovial tissues and fluid and are phagocytosed by synovial lining cells,macrophages or polymorphonuclear cells attracted into the joint by the chemotactic effects of complement activation. Lysosomal enzymes are released from the phagocytic cells as a result and promote the inflammatory changes(Holborow 1978). Circulating complexes are probably more important in the pathogenesis of the extra-articular lesions of rheumatoid arthritis (Theofilopoulos, Burtonboy, Lospalluto and Ziff 1974). It is not clear why IgG becomes an antigen to initiate the production of RF. A conventional view is that the stimulus arises from the formation of immune complexes of IgG antibody with an unknown antigen or antigens in the joint which could be regarded as the prime aetiological agent(s) (e.g. viruses). An alternative possibility is suggested by the observation that conformationally altered IgG molecules are present in rheumatoid sera (Johnson, Watkins, Scopes and Tracy 1974) and these alterations could stimulate antibody formation.

TEST SYSTEMS FOR DEMONSTRATING RHEUMATOID FACTOR

1. USE OF RED BLOOD CELLS COATED WITH GAMMA GLOBULIN

The sensitised sheep cell system is one of the most widely used methods for demonstrating RF in man and is often referred to as the Rose-Waaler test(Rose, Ragan, Pearce and Lipman 1948; Waaler 1940). This system involves preparing antibodies against sheep red blood cells

by conventional immunising techniques in a different species, generally the rabbit. The antibodies are collected and reacted with a preparation of sheep erythrocytes to coat these cells with globulin. It has been adequately shown that the reaction in this test system is between RF and the coating material on the sheep cells (Epstein, Johnson and Ragan 1956; Heller, Jacobson, Kolodny and Kammerer 1954; Steffen and Schindler 1955a;Vaughan 1956; Vaughan, Ellis and Marshall 1958; Wager 1950). Human red cells, guinea-pig cells, ox cells, goat cells, aligator cells and probably many others can be substituted for the sheep cells, providing the erythrocytes are coated with an appropriate amount of rabbit antibody against them(Wager 1950). The antibody is used in a concentration which is itself insufficient to agglutinate the cells but which in the presence of serum from patients with rheumatoid arthritis provides strong agglutination. When the sensitised sheep cell system is used, heterophil antibody (heterogglutinins) must be absorbed from the rheumatoid serum prior to the test, to prevent agglutination by this antibody.

Red blood cells can also be coated with gamma globulin by using tannic acid. The cells are treated with tannic acid such that the cell surface is altered and able to non-specifically bind proteins to itself(Boyden 1951; Stavitsky 1954). RF will then agglutinate the cells when the latter are reacted with the patients' serum. Another system has been used for the human patient employing Rh positive human red cells coated with non-agglutinating anti-Rh antibody.

Species difference in the coating materials does influence the results obtained in the assessment of RF levels (Milgrom,Witebsky, Goldstein and Loza 1962; Vaughan 1959). For example, higher titres

are obtained with tanned cells coated with human gamma globulin than with sheep cells coated with rabbit antibody or human Rh positive cells coated with anti-Rh antibody although very rarely would one test be positive but another negative(Vaughan 1959).

The Rose-Waaler test (sheep red blood cells coated with rabbit antibody) has been used to detect RF in the dog (Alexander et al 1976; Basu 1971; Halliwell et al 1972; Newton et al 1974; Newton et al 1976; Schultz 1978). A modified Rose-Waaler test has been described for use in the dog where the sheep red blood cells are coated with dog antibodies to the sheep cells (Halliwell et al 1972; Newton et al 1974; Newton et al 1976: Pedersen et al 1976; Schultz 1978; Wentink 1973; Wentink et al 1974).

The use of tanned sheep red blood cells coated with dog gamma globulin was described by Lewis and Borel (1971) and by Pedersen et al (1976a). A method utilising a canine isoantibody against dog erythrocytes of blood type A,to coat dog erythrocytes with dog globulin has also been described(Lewis and Borel 1971).

2. USE OF LATEX PARTICLES COATED WITH GAMMA GLOBULIN

A theoretical problem with using sheep red blood cells as the "carrier" for immunoglobulin is that these cells have a complex organic structure containing many antigens which may react with components of human and animal sera (Singer and Plotz 1956). In an attempt to obviate this problem biologically inert particles (collodion,bentonite and latex) were introduced as the "carrier" (Bloch 1960; Bozicevich,

Bunim, Freund and Ward 1958; Singer and Plotz 1956). The chief value of these particle agglutination tests is the increased sensitivity they show and the ease with which they can be performed. Latex particles are the most commonly used and were first utilised in the serological identification of RF in 1956 (Plotz and Singer 1956; Singer and Plotz 1956).

When vinyl monomers are dispersed in water and polymerised by a suitable free radical forming catalyst, small spheres of polymers are produced as a colloidal suspension in water; these spheres are called latex particles. The particles are emulsified in water with the aid of a chemical emulsifier and in their dispersed phase they are negatively charged and seem to behave as lyophobic colloids. Their stability is determined by their charge and the charge of the surrounding medium, the pH of which is critical. Latex particles mixed with gamma globulin will precipitate spontaneously between pH 5.5 and 8.0 and therefore this pH range has to be avoided (Singer and Plotz 1956). A range between 8.2 and 9.0 is recommended. Latex particles with a diameter of 0.8-1.1 μm are most satisfactory and an optimum concentration of the particles has to be determined. Sodium chloride has to be added to the reaction medium to improve the agglutination reaction of the latex and the optimal concentration is between 0.31% and 1.25% (Singer and Plotz 1956). The test is generally performed as a tube agglutination test using several dilutions of each patients' serum and the particles are most often coated with human gamma globulin, sometimes the patients' own globulin (Mannik, Brine and Clark 1958; Singer and Plotz 1958). Rabbit, pig, dog, cow, horse, cat and guinea pig gamma globulin can be substituted for the human globulin (Rheins, McCoy, Buehler and Burrel 1957).

Latex particles coated with certain polysaccharides or with heparin will also agglutinate in the presence of RF (Barboriak and Forest 1958; Gofton, Thomas and Robinson 1957) although a high incidence of positive results with serum containing elevated gamma globulin levels in patients with non-rheumatoid diseases limits the practical value of the heparin latex test (Forest and Barboriak 1960). Some sera when tested with the latex agglutination reaction exhibit the prozone phenomenon (Rheins, McCoy, Burrel and Buehler 1957; Singer and Plotz 1958; Valkenberg and de Mos 1958) and this can be avoided by sufficiently diluting the serum and in some cases by heating the serum to 56°C for 30 minutes (Meiselas and Porush 1959). In fact, optimum results are obtained by incubating the latex particles, gamma globulin and test serum for 2 hours at 56°C (Singer and Plotz 1956). The order of adding the reagents when performing the test can also affect the reaction and must be standardised.

The test is more sensitive than the red cell agglutination test (Plotz and Singer 1956; Singer 1961) and there are far fewer doubtful positive reactions. As well as the tube agglutination method, a slide agglutination test has been introduced and this is particularly useful for rapid screening of possible rheumatoid patients. Several of these tests are commercially available but their performance differs and the incidence of false negative and false positive results varies greatly between individual tests (MacSween, Hughes, Breen, Kitchen, Cathcart and Buchanan 1974).

The latex agglutination test has been applied to the diagnosis of canine rheumatoid arthritis. Three separate centres have used a commercial human reagent, viz latex coated with human gamma globulin

(Halliwell et al 1972; Pedersen et al 1976a; Sikes et al 1970). Basu (1971) described the use of latex particles coated with dog globulin in both tube and slide agglutination tests for the detection of canine RF as well as the latex-heparin slide agglutination test.

3. OTHERS

It is important to note that serum RF activity in immunoglobulin classes other than IgM is not readily demonstrable by the classical sheep red cell or latex agglutination methods and other techniques have been developed for this purpose, most of them involving elution from immuno-absorbents. The principle of this method is that IgG is rendered insoluble by cross-linking (Panush, Bianco and Schur 1971; Torrigiani and Roitt 1967) or polymerised (Ilter and Turner 1973) or bound to particulate carriers (Bianco, Dobkin and Schur 1974; Florin-Christensen, Arana, Morteo, Roux and Hubscher 1974) and then exposed to rheumatoid serum. The anti-globulins are absorbed and are then eluted at acid pH and estimated by radial immunodiffusion against class specific immunoglobulin anti-sera. Such sensitive methods have shown that most human rheumatoid sera whether seropositive or seronegative by classical methods, contain varying amounts of IgG antiglobulin and often IgA antiglobulin as well. Some of the findings however, have been criticised because of technical problems and because some normal sera show significant binding (Holborow 1978). An alternative test has recently been introduced (Hay, Nineham and Roitt 1975) which measures the amount of pure radio-iodinated specific rabbit antibody to human IgG combined with the human antiglobulin factor, reacted with rabbit IgG absorbed onto the walls of plastic test tubes.

MODIFIED ROSE-WAALER TEST FOR THE DETECTION OF RHEUMATOID FACTOR - LABORATORY PROCEDURE

1. PREPARATION OF DOG ANTIBODIES AGAINST SHEEP RED BLOOD CELLS

20ml of blood from each of two sheep were collected from the jugular vein and mixed with 4ml of 3.8% sodium citrate solution. The two samples were pooled. The blood was filtered through moist filter paper and centrifuged for 10 minutes at 1000 rpm in a BTL Bench centrifuge. The plasma was poured off and the red cells washed with normal saline. The centrifugation and washing procedure was repeated three times. The final collection of sheep erythrocytes was suspended in saline and injected intravenously into a 2-year old male Greyhound. Two further blood samples were collected from the same two sheep and subjected to the same procedure. The isolated red cells were again injected into the Greyhound at 2 weeks and 4 weeks after the first inoculation.

2. DEMONSTRATION OF DOG ANTIBODIES TO SHEEP RED BLOOD CELLS

On the day of the third injection of sheep erythrocytes into the Greyhound, 10ml of blood were collected from the immunised dog and allowed to clot. The serum was collected and tested for the presence of antibodies against sheep red blood cells. For this, a fresh collection of red blood cells was made from the sheep and a 2% suspension of washed cells in normal saline (0.4ml of cells and 19.6ml of saline) was prepared. Serial dilutions of the dog's serum (sheep red blood cell antiserum) were made by double diluting using normal saline and an agglutination plate. The dilutions ranged from 1:10 to 1:2560 (0.1ml total volume in each well). 0.1ml of the 2% suspension of sheep red blood cells

was added to each dilution of antiserum and thoroughly mixed. The agglutination plate was allowed to stand at room temperature for 30 minutes, following which 0.2ml of saline was added to each well with mixing. The plate was incubated overnight at $4^{o}C$. A negative control was set up using a mixture of saline and red cells. The agglutination plate was examined for evidence of haemagglutination in each of the wells. Where agglutination had occurred, the red cells had formed a layer of cells at the base of the well with an irregular border and granular appearance. In the wells where no agglutination had occurred, the cells had settled out to form a tight compact sedimentation button with a clear sharp border. The highest dilution to show evidence of agglutination was noted and found to be 1:160. This indicated a good antibody response in the immunised dog. The dog was anaesthetised five days after the last injection of sheep cells, and the carotid artery cannulated. The dog was bled prior to destruction, the blood allowed to clot and the serum collected and stored at $-20^{o}C$ in small aliquots of 5ml until required for the actual test procedure.

3. ACTUAL TEST PROCEDURE

(a) Preparation of sensitised cells (sheep red blood cells coated with antibodies)

Each time the test was performed, the haemagglutination titre of the antiserum was determined as described in (2). Blood was always taken from one or other of the two sheep originally used as donors for the immunisation procedure. The titre always remained at 1:160.

The sheep cell antiserum was then diluted to one tenth of the

haemagglutinating titre with normal saline i.e. diluted 1:1600, such that a final volume of 10ml of diluted antiserum was prepared. To this was added an equal volume of fresh 2% washed sheep red blood cells, prepared as described in (1). After mixing, the antiserum and sheep cells were allowed to stand for 30 minutes at room temperature. This resulted in sheep red blood cells becoming coated with sub-agglutinating concentrations of red cell antibodies prepared in the dog i.e. coated with dog globulin. This was the reagent for detecting canine RF.

(b) Preparation of unknown test sera

Test sera which had been stored in the deep freeze, were allowed to thaw out at room temperature before being used. Small volumes of sera (0.4ml) were pipetted into small test tubes which were placed in racks in a water bath at 56°C for 30 minutes. This was to inactivate complement. Following this an equal volume (0.4ml) of packed, washed non-sensitised sheep red cells, prepared as described in (1), were added to each tube of serum. The tube contents were mixed and allowed to stand for 1 hour at room temperature. This was to absorb any possible heteroagglutinins i.e. any factors present in the test sera which themselves could directly agglutinate the sheep erythrocytes in the actual test. The tubes were then centrifuged at 3000 rpm in a BTL Bench centrifuge for 10 minutes and the serum reclaimed from each tube.

It was usual to test 10-16 sera together at any one time. Known positive and negative control sera were always used.

(c) Determination of rheumatoid factor titre

0.1ml of the absorbed serum prepared in(3 b) was pipetted into a tube containing 0.4ml of saline to give a dilution of 1:5. 0.1ml of this dilution was then placed in the first well of an agglutination plate. Saline dilutions of the test serum were then prepared by double dilution ranging from 1:5 to 1:2560. An equal volume (0.1ml) of sensitised sheep red blood cells was added to each well and the contents mixed by aspiration into and expulsion from the pipette (three times in each case). The plate was incubated overnight at $4^{o}C$ and examined the following day for evidence of haemagglutination as described in (2). Any evidence of agglutination was taken as a positive result and the highest dilution at which agglutination was identifiable was recorded (Fig. 30). Often there was evidence of agglutination of cells at the periphery but sedimentation in the centre of the well - these were recorded as positive results. As well as using known positive and negative sera as controls, a mixture of saline and sheep cells (0.1ml of each) and of saline and sensitised sheep cells (0.1ml of each) were also used as negative controls. If the controls were not satisfactory, the results were discarded and the test repeated. This only happened on one occasion. A diagrammatic representation of the modified Rose-Waaler test is shown in Figure 29.

With all the pipetting procedures described above, fixed volume pipettes were used.

RHEUMATON TEST(W.R.WARNER AND CO.LTD.)FOR THE DETECTION OF RHEUMATOID FACTOR - LABORATORY PROCEDURE

The Rheumaton Test is a rapid (2 minutes) slide agglutination test for detecting RF in man and is based on the Rose-Waaler test. It comes in kit form and is produced by the Denver Chemical Manufacturing Company, Stamford, Connecticut, U.S.A., and is distributed in the U.K. by W. R. Warner & Co. Ltd., Chestnut Avenue, Eastleigh, Hants. The test comprises a Rheumaton reagent which consists of a suspension of stabilised sheep erythrocytes sensitised with rabbit gamma globulin and which has to be stored at $4^{o}C$ when not being used. Negative and positive controls are also included and a glass slide is provided. Disposable stirrers and capillary tubes with rubber bulbs are also present in the kit.

Prior to performing the test,the test serum (if it had been frozen and stored) and the reagent were allowed to reach room temperature. The capillary tube was filled to the marked level with the test serum and expelled into the centre of a section of the provided slide. One drop of the Rheumaton reagent was added to the sample using the provided dropper. A disposable stirrer was used to mix the serum and reagent over the entire section. The slide was then rocked gently with a rotary motion for 2 minutes and observed for agglutination, i.e. a course granular appearance due to clumping of the red cells(Fig.31). The test was always repeated using the negative and positive controls to check the reagent and help in the recognition of agglutination.

Fresh capillary tubes and disposable stirrers were always used

for each different serum sample. The provided rubber bulb was placed over the end of the capillary tube so that the tube penetrated the thin membrane within the bulb. The capillary tube was allowed to fill,by capillary action, to the marked level. A finger placed over the hole in the bulb allowed the serum to be transferred to the slide; squeezing the bulb emptied the capillary tube. The same bulb was used each time but care was taken to prevent contamination of it by the sample being drawn up into the capillary tube. If this did happen, the bulb was discarded. Care was also taken never to touch the surface of the slide or the end of the stirrer prior to carrying out the test. After completing the test, the slide was washed with detergent, rinsed several times with tap water and finally with distilled water and then dried with alcohol or a lint-free tissue.

LATEX TUBE AGGLUTINATION TEST FOR THE DETECTION OF RHEUMATOID FACTOR - LABORATORY PROCEDURE

A 1.5% suspension of latex particles of approximately 0.81 mμ diameter were obtained from Difco Laboratories Inc.,(920 Henry Street, Detroit, Michigan, U.S.A.). Each time the test was carried out the latex suspension was passed through a Watman No. 40 Filter to remove any agglutinated particles. The test had to be determined by trial and error and while developing the test, a rabbit anti-dog globulin serum (prepared as described on pages 219-222) was used to agglutinate the latex particles coated with dog globulin, i.e. to act as a "rheumatoid factor".

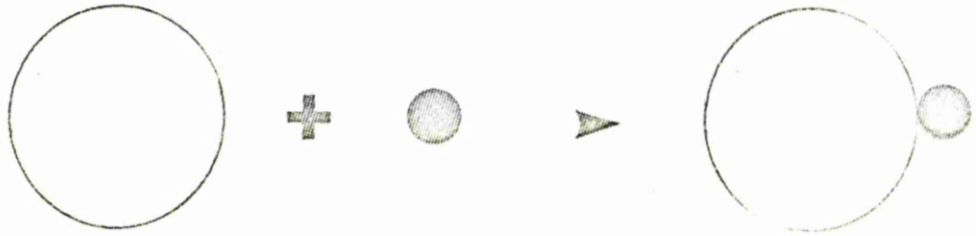
Purified dog globulin (7S fraction) was obtained in lyophilised form from Nordic Immunological Laboratories and was dissolved in glycine buffer (pH 8.45) to give a concentration of 10mg of globulin/ml of buffer. The latex reagent was made by mixing 0.1ml of the 1.5% latex suspension with 1.0ml of the globulin preparation and 18.9ml of a glycine/albumin buffer. This latter reagent was freshly prepared each time the test was carried out, by the addition of 1ml of a 3.5% solution of bovine serum albumin in distilled water to 9ml of the stock glycine buffer solution giving a final concentration of 0.35% of albumin. It was necessary to add bovine albumin to the glycine buffer when preparing the latex reagent in order to reduce the incidence of non-specific agglutination of the latex particle. It has been shown that albumin will stabilise the particles by a colloidal action - albumin adhering to the particles and giving them a net charge (Singer, Altmann, Oreskes and Plotz 1961) although the presence of albumin in high concentrations will tend to inhibit the test and reduce RF titres (Rheins, McCoy, Buehler and Burrel 1957).

Serial dilutions of each test serum were prepared from 1:5 to 1:320 using the stock glycine buffer. Each serum dilution (0.5ml volume) was placed in a 5cm x 0.7cm agglutination tube which was carefully numbered and to each was added 0.5ml of the latex reagent. Controls were also used - known positive and negative sera, glycine buffer instead of serum mixed with the latex reagent and a rabbit anti-dog globulin serum in place of the test serum. Each tube was thoroughly shaken and then placed in a water bath for 2 hours at 56°C. Following this the tubes were centrifuged for 3 minutes at 2300 rpm in a BTL Bench centrifuge. The test was then read with the aid of a light source and concave mirror held beneath the bottom of each agglutination tube. The test was again read after the tubes had been left overnight at 4°C. A positive result was indicated by agglutination of the latex particles forming a granular, dispersed layer at the bottom of the tube with a ragged, uneven edge. A tight, compact button with a clear border indicated a negative result. Shaking a positive tube dispersed the agglutinated latex particles forming a cloudy, granular suspension. Failure of the controls negated the test.

The test proved exceedingly tedious to develop and non-specific agglutination occurred with regular frequency.

FIGURE 29 A DIAGRAMMATIC REPRESENTATION OF THE MODIFIED ROSE-WAALER TEST USED TO DETERMINE THE RHEUMATOID FACTOR TITRE IN DOGS' SERA.

I

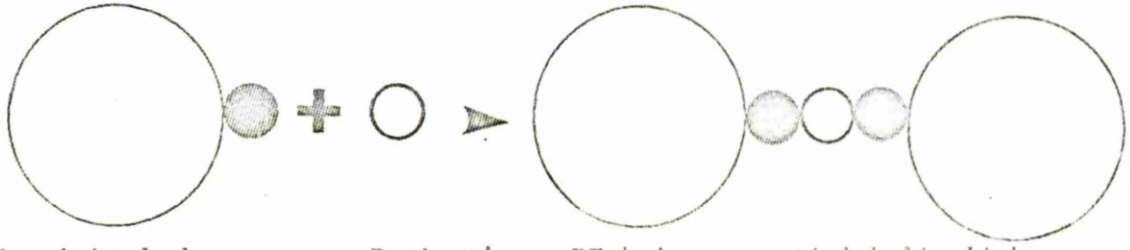


Sheep RBCs

Antibodies against sheep RBCs prepared in the dog.

Sheep RBCs coated with antibodies (sensitised sheep RBCs).

II



Sensitised sheep RBCs.

Patient's serum containing RF.

RF being an antiglobulin links onto the globulin coating the sheep RBCs and causes agglutination of the red cells.

FIGURE 30

THE MODIFIED ROSE-WAALER TEST FOR THE DETERMINATION OF THE RHEUMATOID FACTOR TITRE IN DOGS' SERA.

The top row of wells shows agglutination of red blood cells at all dilutions (positive result). Rows 3 and 4 show only the tight sedimentation button of red cells (negative result).

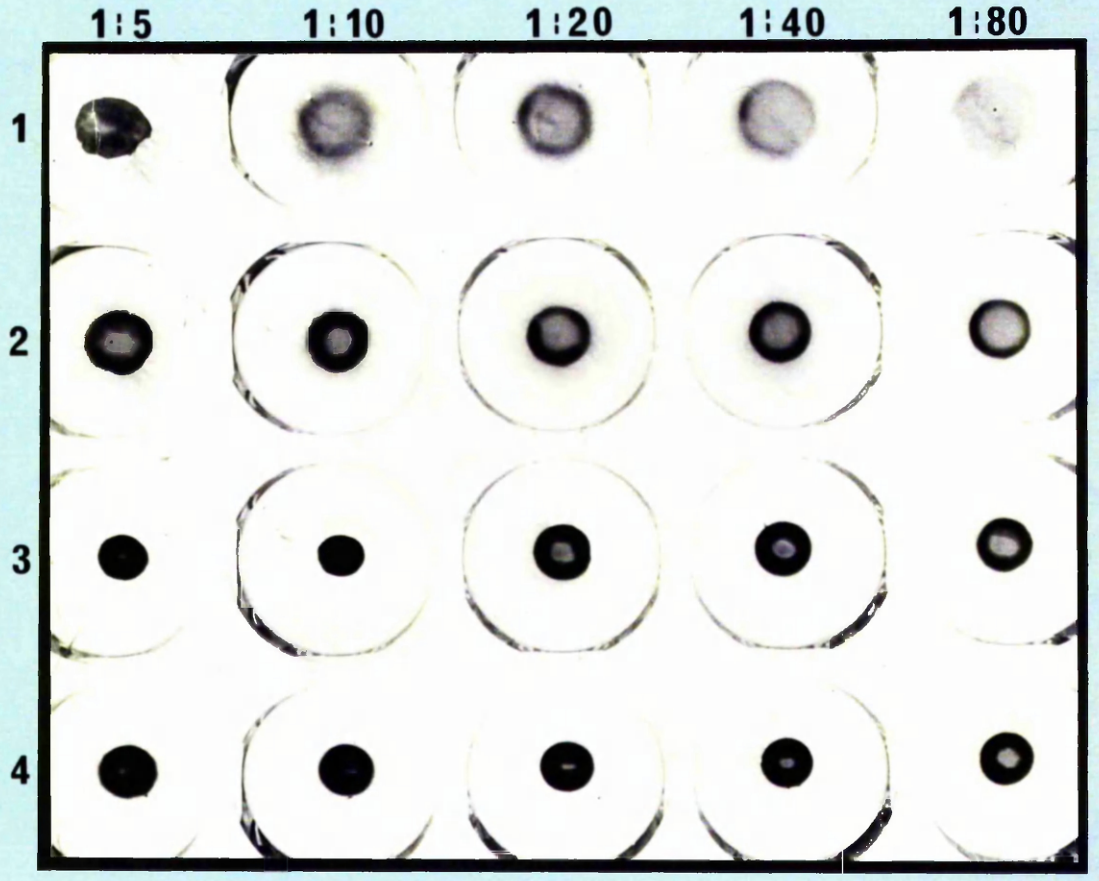

1:5
1:10
1:20
1:40
1:80
1
2
3
4

FIGURE 31

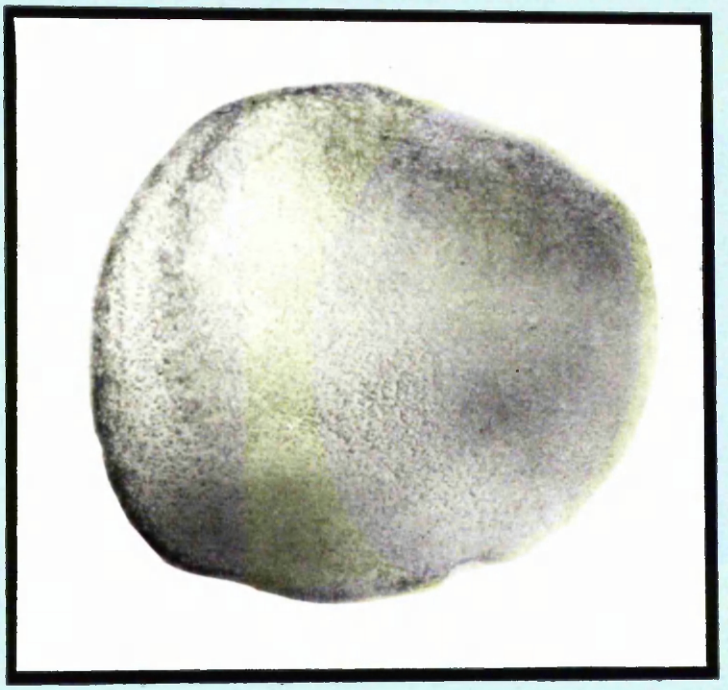
THE RHEUMATON (W.R.WARNER AND CO.LTD.)SLIDE AGGLUTINATION TEST FOR THE DEMONSTRATION OF RHEUMATOID FACTOR (POSITIVE RESULT).

Note the granular appearance indicating the agglutination of the red blood cells.

FIGURE 32

THE RHEUMATON (W.R.WARNER AND CO.LTD.) SLIDE AGGLUTINATION TEST FOR THE DEMONSTRATION OF RHEUMATOID FACTOR (NEGATIVE RESULT).

The red blood cells are remaining in suspension.



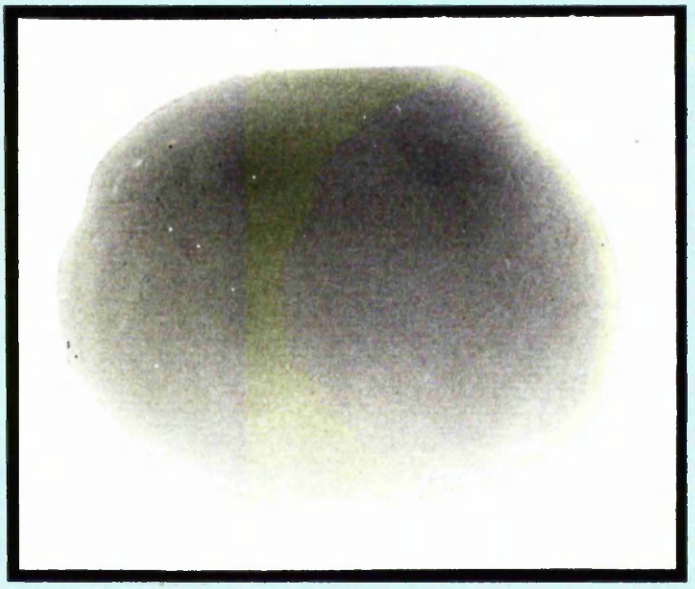
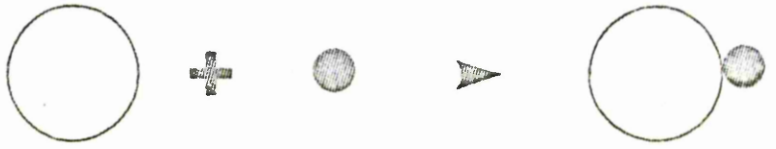


FIGURE 33 A DIAGRAMMATIC REPRESENTATION OF THE LATEX PARTICLE AGGLUTINATION TEST USED TO DETERMINE THE RHEUMATOID FACTOR TITRE IN DOGS' SERA.

I



Latex particles.

Dog globulin.

Latex particles coated with dog globulin.

II

Latex particles coated with dog globulin.

Patient's serum containing RF.

RF being an antiglobulin links onto the globulin coating the latex particles and causes agglutination of the particles.

C. IDENTIFICATION OF SERA EXAMINED FOR ANTINUCLEAR ANTIBODY AND RHEUMATOID FACTOR

COLLECTION OF SERUM

Serum was always collected on the same day that the dog was bled. Five to ten millilitres of blood were taken from either the jugular or cephalic veins and allowed to clot at room temperature in a glass container. The sample was centrifuged for 5-10 minutes at 2000 rpm in a BTL Bench centrifuge after which the serum was pipetted off using a Pasteur pipette, into a labelled bijou bottle. The serum was tested on the day of collection or alternatively stored at -20°C and tested some time later. After testing, all sera were stored indefinitely at -20°C.

INDIRECT IMMUNOFLUORESCENT TEST USING RAT LIVER (ANTINUCLEAR ANTIBODY) AND MODIFIED ROSE-WAALER TEST (RHEUMATOID FACTOR)

Serum samples from all the arthritic dogs except BE 6 and 7, were assessed for the presence of ANA and RF using these two tests. It was the usual practice to take at least two blood samples from each dog within the first 7-10 days of being hospitalised and further samples were taken when the animal was discharged and whenever it returned for re-assessment.

A large number of normal sera were also examined by the indirect immunofluorescent test and the Rose-Waaler test. These consisted of :-

(a) 30 dogs housed and used for feeding trials at Pedigree Petfoods, Melton Mowbray

(b) 20 stray dogs housed at the PDSA Kennels in Glasgow. All these animals were clinically examined prior to sampling

and blood was only taken from those animals with no evidence of disease.

(c) 41 dogs which had been admitted into the hospital after suffering road traffic accidents. Most of these dogs had sustained fractures of the limbs. Blood samples were taken on the day of admission.

(d) 50 dogs which were brought to either the Veterinary School or to the Cargill Clinic for euthanasia. All these animals were clinically examined to check for any diseases and only those animals without any signs of disease and without any history of disease were sampled.

In addition, sera from over 600 dogs with a variety of disease conditions were collected and tested. These sera were grouped according to the diagnosis:-

(a) 125 from dogs with "non-inflammatory" arthropathy, mainly osteoarthritis but also including osteochronditis dissecans and traumatic arthritis.

(b) 122 from dogs with a variety of nervous diseases (mainly distemper) and muscle diseases.

(c) 51 from dogs with different types of neoplastic lesion.

(d) 38 from dogs with anaemia.

(e) 35 from dogs with several types of cutaneous and aural lesion.

(f) 28 from dogs exhibiting pyrexia of unknown origin.

(g) 23 from dogs with kidney disease, mainly glomerulonephritis (confirmed by biopsy or post-mortem examination).

(h) 18 from dogs with various types of metabolic dysfunction, mainly liver disease.

(i) 174 from dogs with a variety of miscellaneous diagnoses (e.g. abscessation, keratitis, gastroenteritis, respiratory tract infection, poisoning) or from dogs where no diagnosis was made. This group also included a few samples from dogs whose case notes had been lost.

RHEUMATON TEST (RHEUMATOID FACTOR)

This test was carried out on 393 serum samples taken from a variety of dogs. The latter included animals in all five arthritic groups as well as normal dogs and dogs with other diseases. Examples from each of the 9 "other disease" groups defined above were included. The test was found to be unreliable in the dog and the results are not presented here in detail.

LATEX TUBE AGGLUTINATION TEST (RHEUMATOID FACTOR)

Again, this test was found to be unreliable in the dog and thus the results are not presented here in detail. Normal dogs, dogs with other diseases and dogs with various types of inflammatory joint disease were tested.

INDIRECT IMMUNOFLUORESCENT TEST USING TRYPANOSOMA BRUCEI (ANTINUCLEAR ANTIBODY)

All four dogs in Group II (systemic lupus erythematosus) were tested by this method. In addition, six dogs in Group I, three in Group III, two in Group IV and twelve in Group V were tested. Ten normal dog sera were also examined by this method.

Sera from 12 human patients with systemic lupus erythematosus were also tested. These patients had the clinical features of the disease together with high levels of ANA by the indirect rat liver immunofluorescent test, positive LE-cell preparations and high deoxyribonucleic acid binding values.

LE-TEST (HYLAND LABORATORIES) (ANTINUCLEAR ANTIBODY)

A total of 336 sera were tested. These were taken from normal dogs, dogs with diseases other than inflammatory arthropathy and from dogs exhibiting the various types of inflammatory joint disease. The test was found to be unreliable and the results are not presented here in detail.

LE-CELL PHENOMENON(ANTINUCLEAR ANTIBODY)

The animals examined by this technique included 10 normal dogs, 6 dogs from Group I, all 4 dogs from Group II, 2 dogs from Group III, 4 dogs from Group IV and 15 dogs from Group V.

RADIOIMMUNOASSAY TECHNIQUE(ANTI-DEOXYRIBONUCLEIC ACID ANTIBODY)

This test was performed on sera from 25 normal dogs, on sera from dogs Rh 3, 5, 6, 8 and 11, BE 1 and ID 1, 5, 13, 15 and 16 and on sera from 12 dogs with a variety of other diseases. The diagnoses of the 12 dogs with other diseases (see Table 36) were as follows:- muscle weakness (dog 11), renal disease (dogs 2, 5, 12), gastric ulceration and peritonitis (dog 4), pyrexia of unknown origin (dogs 6,10),

osteoarthritis (dog 7), anaemia (dogs 8, 11), spinal haemorrhage (dog 9); no diagnosis was available for dog 3 since the case notes were missing.

D. RESULTS AND DISCUSSION

INDIRECT IMMUNOFLUORESCENT TEST USING RAT LIVER(ANTINUCLEAR ANTIBODY) AND MODIFIED ROSE-WAALER TEST(RHEUMATOID FACTOR)

1. NORMAL DOGS AND DOGS SUFFERING DISEASES OTHER THAN THE INFLAMMATORY ARTHROPATHIES

As can be seen from Tables 19-29 both RF and ANA were detected in normal dogs and dogs suffering other diseases besides inflammatory joint disease. However, most normal dogs were negative for these auto-antibodies or showed only low titres - 1:5, 1:10 and 1:20 for RF and 1:4 and 1:16 for ANA. There were certainly more dogs reacting at the lower dilutions (Table 19). Three normal dogs (2.1%) did give a RF titre of 1:40 and two dogs (1.4%) an ANA titre of 1:32. No higher titres were ever recorded in normal dogs. From these observations it was decided to accept a dilution of 1:32 or greater for ANA and 1:40 or greater for RF as abnormal,i.e. as significantly high.Low titres of ANA/RF are also recorded in the normal human population(Friou 1972;Holborow 1978; Lawrence 1965; Ritchie 1967; Townes, Stewart and Osler 1963b;Turk 1969; Vaughan 1972; Zutshi, Reading, Epstein, Ansell and Holborow 1969). Several population studies have actually shown that positive reactions for RF (Ball and Lawrence 1961; Bartfield 1969; Valkenburg 1963) and ANA (Svec and Veit 1967) increase in the older age groups.

Tables 20-29 show the ANA and RF determinations in several dogs suffering diseases other than the inflammatory arthropathies. Seventy-five dogs (12.2%) were positive for RF(1:40+)and/or ANA(1:32+).Of these,22 (3.6%) were positive just for RF,3(0.5%)were positive just for ANA and 50 (8.1%) were positive for RF and ANA. Most of the positive reactions occurred in the somewhat obscure group of miscellaneous,

undiagnosed cases and also in dogs showing central nervous or muscle diseases. However, these two groups did provide a major contribution to the total number of cases assessed for these autoantibodies. Of the 125 dogs with joint disease not of the inflammatory type, only 8 (6.4%) were significantly positive for RF and all these only gave a titre of 1:40; three dogs (2.4%) were significantly positive for ANA but again only at a low titre (1:32).

Again, it is well known that RF and ANA can occur in various "non-rheumatic" diseases in the human patient. Rheumatoid factor, for example, has been noted in viral infections (especially viral hepatitis and infectious mononucleosis), certain bacterial and parasitic conditions (tuberculosis, leprosy, syphilis, malaria, schistosomiasis, trypanosomiasis and visceral larvae migrans), paraproteinaemias and some chronic fibrosing lung diseases (Bartfield 1969; Holborow 1978; Mustakallio, Lassus and Wager 1967; Shaper, Kaplan, Mody and MacIntyre 1968; Turner- Warwick and Haslam 1971; Wager, Räsänen, Hagman and Klemola 1968; Zutshi et al 1969). Similarly, ANA has been recorded in human patients with diseases such as allergies, skin disorders, hepatitis, haematological abnormalities, leprosy, infectious mononucleosis and various other infections (Bettley 1955; Bonomo, Tursi, Trimigliozzi and Dammacco 1965; Friou 1972; Holborow 1978; Marmant 1955; Meyler 1954; Pascher, Borota and Davis 1955; Wager et al 1968). Certain medications can also induce the production of ANA e.g. hydralazine, some anti-convulsants, procainamide, α-methyl dopa and chlorpromazine (Alarcon-Segovia 1969; Berglund, Gottfries, Gottfries and Stormby 1970; Blangren, Condemi and Vaughan 1972; Breckenridge, Dollery, Worlledge, Holborow and Johnson 1967; Friou 1972; Holborow 1978; Hughes 1975). Extensive

burns causing chronic tissue damage can also be associated with ANA and RF (Quismorio, Bland and Friou 1971). Human osteoarthritic patients also occasionally give positive RF reactions but usually only at low titres(Zutshi et al 1969).

In a recent study, Basu (1971) examined a routine hospital population of dogs (total 109) for the presence of RF by the Rose-Waaler test. Titres ranging from 1:8 to 1:2048 were obtained and surprisingly only a very few negative results were recorded. However, the test was particularly sensitive since a significant titre was taken as 1:128 or greater. Most of the dogs in this study were old and were sampled prior to euthanasia for reasons unrelated to the musculoskeletal system; the dogs were being used for a study of valvular endocarditis. Detailed pathological examinations were performed on 70 dogs and of these 26 showed joint disease (e.g. increased synovial fluid, osteophytes, cartilage defects) as well as circulating RF although 8 of these had low, insignificant titres. Unfortunately, detailed pathological descriptions of the joints are not given but there was certainly no suggestion of a true rheumatoid pathology in any of the joints. All but one of the 26 animals had other abnormalities besides the joint changes, e.g. interstitial nephritis, tonsillitis, hepatitis, carcinomata, eczema. The author,rather surprisingly, suggested that there could be a relationship between joint disease and interstitial nephritis since 14 dogs showed this combination. However, both interstitial nephritis and osteoarthritis are common in the older dog. Of 44 dogs without joint disorders, 27 had significant levels of RF and of these 20 had lesions demonstrable at post-mortem examination e.g. liver disease was present in 7 dogs, nephritis in 7 dogs and other lesions included

carcinoma, adenoma, fibroma, eczema, vaginitis, cystitis and pyometra.

2. GROUP I (RHEUMATOID)

Seventeen of the 21 dogs (81%) showed a RF titre of 1:40 or greater in at least one of the serum samples taken for examination. (Table 30). However 6 of these positive dogs did show an insignificant or negative titre on one or more other occasions. Four dogs (19%) failed to show a significant titre of RF in any of the blood samples tested. It should be stressed that the presence of RF in the blood of canine polyarthritic patients was one of the criteria used for the identification of the Group I dogs.

Rheumatoid factor is not always present in human rheumatoid arthritis patients (Boyle and Buchanan 1971; Dixon 1960; Holborow 1978; Svartz 1957; Ziff 1957; Vaughan 1972) - these form the so-called "sero-negative" cases of rheumatoid arthritis. There is certainly a significant variation in the percentage of seronegative cases between different test systems due to differences in test sensitivity. It is also suggested that high concentrations of IgG in the serum can block the demonstration of anti-IgG activity (Allen and Kunkel 1966; Bluestone, Goldberg and Cracchiolo 1969). De Forest and his co-workers (1958) described patients with positive RF reactions which became negative following remission of the disease and positive again when recrudescence of the disease occurred. However, other workers have shown patients under remission who have remained serologically positive for RF (Aho, Kirpila and Wager 1959). Generally, patients with advanced rheumatoid arthritis have positive RF reactions with greater frequency and show

RF at higher titres. A progressive increase in positive reactions in rheumatoid patients can also occur with age (Bywaters, Carter and Scott 1959) and patients with subcutaneous nodules usually have positive RF tests. Seropositive patients are also said to show more erosive changes on radiography and usually the disease runs a more relentless, progressive course (Sievers 1965). Mention has already been made of the fact that the conventional testing techniques only detect RF of the IgM type. It has been shown in both seropositive and seronegative human rheumatoid arthritis patients, that RF of the IgG class for example is present. There is now much evidence to suggest that IgG rheumatoid factor may be very important in forming complexes with the fixation of complement in the synovial tissues and fluid (Holborow 1978), and this could help to explain the pathogenesis of synovial inflammation in rheumatoid arthritis. No attempt was made in the present study to identify RF of types other than IgM, in the dog.

Most reports of RF determinations in the rheumatoid canine patient by the modified Rose-Waaler test have shown negative or low titres in the majority of patients. Of 10 cases described by Newton et al (1976), 3 had a titre of 1:8, one 1:16, three 1:32, one 1:64, one 1:128 and one 1:256. Similar dilutions were given by Halliwell (1978) in a total of 16 dogs. Pedersen et al (1976a) tested three dogs by the modified Rose-Waaler test and by tanned sheep red blood cells non-specifically coated with dog globulin and all were negative for RF. In the single case reported by Halliwell et al (1972), RF was detected by the Rose-Waaler test using sheep red blood cells coated with canine IgG and with rabbit IgG. The latter was found to be more sensitive giving titres of 1:64 and 1:128 in two separate serum samples compared to 1:32 and

1:64 with the canine IgG reagent. Alexander et al (1976) reported eight cases of canine rheumatoid arthritis, two of which were negative for RF, one had a titre of 1:16, three had 1:32, one had 1:64 and the other 1:160. Schultz (1978) in a review article stated that 60-75% of dogs with clinical signs of rheumatoid arthritis are positive in the Rose-Waaler test using red cells coated with dog or rabbit IgG. It is always difficult to compare one author's test system with that of another because the sensitivity of the different systems can vary greatly. For example, in the present study a significant positive titre was taken as 1:40 or greater, whereas a titre greater than 1:8 was taken as significant by Newton et al (1976) and by Halliwell (1978)and 1:16 or greater was taken as significant by Schultz (1978) although this latter value was an arbitary rather than a defined value, and 1:128 by Basu (1971). Even different test systems using the same serum sample have been shown to give widely differing values e.g. tanned sheep red blood cells coated with autologous canine 7 S globulin gave a titre of 1:1024, tanned sheep red blood cells coated with homologous 7 S globulin gave a titre of 1:256 and dog red blood cells coated with dog globulin gave a titre of only 1:16 (Lewis and Borel 1971). It is thus important to clearly define the sensitivity of the test system being used by examining an adequate number of normal dogs.

It is the belief of at least one authority (Dr. R. E. W. Halliwell - personal communication) that RF in the dog may be mainly of the IgG rather than the IgM type and hence not detectable in the routine agglutination tests. This hypothesis has not yet been confirmed.

Three rheumatoid dogs in the present study gave positive titres

for ANA although in one dog the titre was only positive on one of three occasions and in another dog the titre was only 1:32. It is well known in man that some rheumatoid patients show circulating ANA (Friou 1958; 1972; Hijmans, Kievits and Schuit 1958; Holborow 1978; Toone, Irby and Pierce 1960; Ward, Johnson and Holborow 1964). An incidence of ANA in 14% of human rheumatoid arthritic patients has been quoted (Friou 1972). A few patients have been described showing arthritis indistinguishable from rheumatoid arthritis, high titres of ANA and certain features very similar to systemic lupus erythematosus, suggesting an "overlap" between rheumatoid arthritis and systemic lupus erythematosus (Hijmans et al 1958; Kramer, Ruderman, Dubois and Friou 1970; Silver, Berkowitz, Johnston and Steinbrocker 1962). Willkens and Decker (1963) studied 14 human patients with severe deforming rheumatoid arthritis who had circulating ANA. Serum protein chromatography showed that most of the ANA was of the IgM class rather than IgG which is different to genuine systemic lupus erythematosus patients. These authors suggested that ANA in these rheumatoid patients may be produced by a chronic bacterial infection somewhere in the body. Most publications of rheumatoid arthritis in the dog report the absence of ANA and in fact some authorities deliberately exclude any dog from being a rheumatoid arthritic if ANA is present (Alexander et al 1976). In the case reported by Lewis and Borel (1971) both ANA in high titre and positive LE-cell preparations were demonstrated. However, the diagnosis of this case as rheumatoid arthritis is in doubt (see pages 23-24). One case of rheumatoid arthritis reported by Pedersen et al (1976a) showed a weakly positive reaction in the LE-cell preparation although the significance of this is not clear.

3. GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS)

Three dogs in this Group had ANA present in very high titres (greater than 1:1024); the fourth dog showed a titre of 1:64 (Table 31). It should be remembered that the presence of circulating ANA in high titre was a major criterion in the diagnosis of canine systemic lupus erythematosus. ANA in man is most closely associated with systemic lupus erythematosus, particularly when present in high titre (Holborow 1978; Holman, Deicher and Kunkel 1959). Homogeneous fluorescent patterns were recorded in dogs SLE 1, 2 and 3; dog SLE 4 demonstrated a peripheral/rim/membranous pattern.

The indirect immunofluorescent test for the detection of ANA has been well used in the canine patient (Halliwell 1978; Lewis *et al* 1973; Lewis and Schwartz 1971; Monier *et al* 1978; Schultz 1978). The main substrate used has been frozen sections of rat or mouse liver (Lewis *et al* 1973; Lewis and Schwartz 1971) although acetone-dried tissue culture slides of mammalian cell lines (e.g. monkey kidney) have recently been introduced as alternative substrates (Halliwell 1978; Monier *et al* 1978; Schultz 1978). Frozen chicken liver has also been used (Monier *et al* 1978). The sensitivity of the test systems varies greatly between different laboratories and between different substrates used, e.g. Halliwell (1978) reported 1:100 as the significant titre when using tissue culture slides and 1:20 when using mouse liver. The titre of 1:16 adopted in the present study compares favourably with the 1:20 value of Halliwell (1978). Cassan (1968) regarded 1:64 as the border-line titre. All cases of canine systemic lupus erythematosus so far reported have been positive for ANA at a significant titre using the indirect immunofluorescent test (Alexander *et al* 1975; Cassan 1968; Halliwell 1978; Krum *et al* 1977).

Three of the four dogs in Group II also showed circulating RF. In two of them only low, insignificant, titres were recorded. The third dog (SLE 1) only gave a significantly positive reaction in one of three serum samples. In human lupus patients there is a definite incidence of positive RF agglutination reactions, in some series as high as 30% (Vaughan 1972). This phenomenon is more common in those patients with lupus in which there is a rheumatoid-like arthritis (vide supra).

4. GROUP III (INFECTIOUS)

All the dogs in this group were negative for ANA or reacted only at very low titres, (Table 31). Three dogs had significant titres of RF although in all these the titre fell to an insignificant level with time and clinical improvement. The presence of RF in these cases could be a reflection of a chronic infectious disease process (vide infra).

5. GROUP IV (BACTERIAL ENDOCARDITIS)

One dog (BE 1) showed high levels of circulating ANA (greater than 1:1024) and another (BE 2) showed significant levels of RF when first presented (1:40, 1:80, 1:160)although only very low titres were demonstrated when the animal was presented a second time following a clinical relapse. The other dogs in this group were negative for these autoantibodies or showed only low titres, although dogs BE 6 and BE 7 were not tested since they died before blood samples could be taken (Table 32).

The release of autoantibodies (RF and ANA in particular) in cases of bacterial endocarditis is well documented in human patients (Bacon,

Davidson and Smith 1974; Gutman et al 1972; Messner, Laxdal, Quie and Williams 1968; Williams and Kunkel 1962). The presence of autoantibodies in bacterial endocarditis and certain other chronic diseases, could be related to extensive tissue damage for comparatively long periods of time together with a generalised "hyper-responsive" humoral immunity (Bacon et al 1974). It has, for example, been shown that the duration of the disease process in bacterial endocarditis is an important factor in producing RF (Messner et al 1968) and there are reports of human patients in which high titres of ANA disappeared completely after bacterial cure (Bacon et al 1974; Gutman et al 1972), suggesting that the rise in ANA is related to the active disease process. It is not certain whether these autoantibodies play any role in the pathogenesis of bacterial endocarditis although various suggestions have been made, including their possible significance in immune complex disease (Bacon et al 1974; Gutman et al 1972; Kaufman and McIntosh 1971) (see Section VIII). Gutman et al (1972) reported a diffuse proliferative glomerulonephritis in bacterial endocarditis patients as well as a "focal embolic" renal lesion. They detected IgG and complement deposits in the glomerular basement membrane and explained the glomerulonephritis as an immune complex phenomenon arising either from antibody/antigen reactions involving the infecting bacterial antigens as has been shown experimentally in dogs with staphylococcal endocarditis (Highman, Altland and Roshe 1959) or from similar reactions involving the patient's own antigens reacting with circulating autoantibodies as occurs in human systemic lupus erythematosus (Cochrane and Koffler 1973; Koffler 1974; Winfield, Koffler and Kunkel 1975). None of the dogs with bacterial endocarditis in this study showed immunoglobulin deposits in the kidneys. However, three dogs did show an arthritis in one or more joints where no organisms

were cultured and where the inflammatory reaction did not show a massive polymorphonuclear response similar to that seen in other joints from the same animal where organisms were present. There was a possibility that the arthritis in these cases was initiated by an immunopathological mechanism (see Section X).

Lewis and Hathaway (1967) reported a case of canine polyarthritis associated with positive LE-cell preparations and ANA determinations. A diagnosis of systemic lupus erythematosus was made but at post-mortem examination a vegetative endocarditis lesion on the left atrio-ventricular valve was found, from which pure cultures of gram-negative rods were obtained. It is thus possible that the ANA found in this dog could have been associated with bacterial endocarditis rather than systemic lupus erythematosus. Another case of canine polyarthritis, reported by Lewis and Borel (1971) had at post-mortem examination a valvular endocarditis but no mention was made of any bacterial cultures from the lesion although beta-haemolytic Streptococci were obtained on culture of the left elbow joint. This dog had high levels of circulating RF (1:1024 using tanned sheep red blood cells coated with dog autologous globulin), ANA (1:1024 using the indirect immunofluorescent test), positive LE-cell preparations and high deoxyribonucleic acid binding activity (85%). There may also be other examples of polyarthritis and autoantibodies occurring with bacterial endocarditis but incomplete assessment of cases makes definite conclusions difficult (e.g. Schalm and Ling 1970). The two cases of bacterial endocarditis associated with circulating autoantibodies reported by Bennett et al (1978) are the two cases BE 1 and BE 2 included in the present study.

6. GROUP V (IDIOPATHIC)

Two dogs had a positive ANA titre but this was only at a low level (1:32) and in neither case was the titre persistent. Six other dogs gave a titre of 1:16 but again the reactions were not persistent. Seven dogs showed a significant RF titre which in three dogs was high (1:80, 1:160, 1:160). Again however, these titres were not consistently recorded in every blood sample taken from the dogs and indeed in all three of these animals many more negative reactions or reactions at insignificant titres, were obtained (Table 33). The occasional positive ANA and RF reactions in this group of dogs probably again reflects the production of autoantibodies during a chronic inflammatory disease process.

Types of human joint disease equivalent to the idiopathic group described here are difficult to identify. The viral arthridities are similar and certain types of viral arthritis in man are associated with circulating RF and ANA. For example, a positive latex fixation test for RF has been reported in human patients with rubella arthritis (Gupta and Peterson 1970; Johnson and Hall 1958) and about 15% of patients with hepatitis B viral arthritis have a positive test for RF and 10% have positive reactions for ANA (Duffy et al 1976).

The idiopathic cases of inflammatory joint disease in the dog described by Huxtable and Davis (1976) and by Pedersen et al (1976b) were negative for RF and ANA although no detailed descriptions of the laboratory examinations are given. A case of enteropathic polyarthritis in the dog was associated with positive LE-cells, ANA and RF (Quimby et al 1978) although this type of arthritis is usually negative for these autoantibodies in man.

ANTINUCLEAR ANTIBODY	RHEUMATOID FACTOR				
	Neg	1:5	1:10	1:20	1:40
Neg	49	6	8	8	2
1:4	30	11	8	6	1
1:16	9	1	-	-	-
1:32	-	2	-	-	-

TOTAL SERA TESTED 141

TABLE 19 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) - NORMAL DOGS.

ANTINUCLEAR ANTIBODY	RHEUMATOID FACTOR						
	Neg	1:5	1:10	1:20	1:40	1:80	1:160
Neg	43	20	14	12	3	3	1
1:4	24	10	11	5	9	2	-
1:16	5	2	3	3	1	-	-
1:32	-	-	-	-	-	1	-
1:64	-	1	-	-	-	-	-
1:128	-	-	-	-	-	1	-

TOTAL SERA TESTED 174

TABLE 20 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) - OTHER DISEASES. MISCELLANEOUS, UNDIAGNOSED.

ANTINUCLEAR ANTIBODY	RHEUMATOID FACTOR					
	Neg	1:5	1:10	1:20	1:40	1:80
Neg	45	7	6	5	5	-
1:4	22	7	6	4	4	3
1:16	2	2	-	-	1	1
1:32	1	-	1	-	-	-
1:64	-	-	-	-	-	-

TOTAL SERA TESTED 122

TABLE 21 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR FACTOR (INDIRECT IMMUNOFLUORESCENT TEST) - OTHER DISEASES, CNS AND MUSCLE DISEASE.

ANTINUCLEAR ANTIBODY	RHEUMATOID FACTOR					
	Neg	1:5	1:10	1:20	1:40	1:80
Neg	26	9	10	8	6	-
1:4	21	8	9	11	2	-
1:16	8	1	1	2	-	-
1:32	1	1	1	-	-	-
1:64	-	-	-	-	-	-

TOTAL SERA TESTED 125

TABLE 22 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) - OTHER DISEASES, OSTEOARTHRITIS, OSTEOCHONDRITIS AND TRAUMATIC ARTHRITIS.

ANTINUCLEAR ANTIBODY	RHEUMATOID FACTOR					
	Neg	1:5	1:10	1:20	1:40	1:80
Neg	7	3	1	1	-	-
1:4	2	2	1	1	-	1
1:16	-	1	-	-	-	1
1:32	-	-	1	-	1	-
1:64	-	-	-	-	-	-

TOTAL SERA TESTED 23

TABLE 23 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) - OTHER DISEASES, RENAL DISEASE.

ANTINUCLEAR ANTIBODY	RHEUMATOID FACTOR					
	Neg	1:5	1:10	1:20	1:40	1:80
Neg	6	1	2	-	-	-
1:4	4	4	3	3	-	-
1:16	-	1	1	1	-	-
1:32	-	-	1	-	-	-
1:64	-	-	-	1	-	-

TOTAL SERA TESTED 28

TABLE 24 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) - OTHER DISEASES, PYREXIA OF UNKNOWN ORIGIN.

ANTINUCLEAR ANTIBODY	RHEUMATOID FACTOR					
	Neg	1:5	1:10	1:20	1:40	1:80
Neg	10	6	2	3	1	-
1:4	7	3	8	3	4	-
1:16	-	-	-	-	-	-
1:32	1	-	2	1	-	-
1:64	-	-	-	-	-	-

TOTAL SERA TESTED 51

TABLE 25 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) - OTHER DISEASES, NEOPLASIA.

ANTINUCLEAR ANTIBODY	RHEUMATOID FACTOR					
	Neg	1:5	1:10	1:20	1:40	1:80
Neg	9	4	3	2	1	-
1:4	3	3	2	-	3	-
1:16	3	-	-	1	1	1
1:32	-	-	1	-	-	-
1:64	-	-	-	1	-	-

TOTAL SERA TESTED 38

TABLE 26 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) - OTHER DISEASES, ANAEMIA.

ANTINUCLEAR ANTIBODY	RHEUMATOID FACTOR					
	Neg	1:5	1:10	1:20	1:40	1:80
Neg	13	3	3	5	2	-
1:4	1	2	-	2	-	-
1:16	3	1	-	-	-	-
1:32	-	-	-	-	-	-
1:64	-	-	-	-	-	-

TOTAL SERA TESTED 35

TABLE 27 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) - OTHER DISEASES, SKIN AND EAR DISORDERS.

		RHEUMATOID FACTOR					
		Neg	1:5	1:10	1:20	1:40	1:80
ANTINUCLEAR ANTIBODY	Neg	7	2	1	-	-	-
	1:4	4	-	2	-	1	-
	1:16	1	-	-	-	-	-
	1:32	-	-	-	-	-	-
	1:64	-	-	-	-	-	-

TOTAL SERA TESTED 18

TABLE 28 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) - OTHER DISEASES, METABOLIC.

		RHEUMATOID FACTOR						
		Neg	1:5	1:10	1:20	1:40	1:80	1:160
ANTINUCLEAR ANTIBODY	Neg	166	55	42	36	18	3	1
	1:4	88	39	42	29	23	6	-
	1:16	22	8	5	7	3	3	-
	1:32	3	1	7	1	1	1	-
	1:64	-	1	-	2	-	-	-
	1:128	-	-	-	-	-	1	-

TOTAL SERA TESTED 614

TABLE 29 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) - OTHER DISEASES, TOTAL.

	DOG IDENTIFICATION																			
	Rh 1	Rh 2	Rh 3	Rh 4	Rh 5	Rh 6	Rh 7	Rh 8	Rh 9	Rh 10	Rh 11	Rh 12	Rh 13	Rh 14	Rh 15	Rh 16	Rh 17	Rh 18	Rh 19	Rh 20	Rh 21
RHEUMATOID FACTOR	1:40 1:20 1:40	1:40 1:80 1:80	1:80 1:80 1:80	1:80 1:160 1:160	1:40 1:40 1:40	1:80 1:160 1:80	1:5 1:5 1:10 Neg	1:40 1:40 1:40	Neg 1:80 1:20 1:5 Neg	1:160 1:40 1:40 1:40 1:10 1:40	1:320 1:80 1:80	1:40 1:20 1:40 1:40 1:40 1:40 1:40 1:20	1:20 1:80 1:20 Neg 1:10	Neg Neg Neg	1:160 1:160 1:160	1:80 1:80 1:80	1:80 1:40 1:40	1:80 1:80 1:80	1:20 1:10 1:10	1:40 1:20 1:40	1:10 1:5 1:10
ANTINUCLEAR ANTIBODY·	Neg Neg Neg	Neg Neg 1:4	Neg Neg Neg	Neg Neg Neg	Neg 1:256 Neg	Neg Neg Neg	1:4 1:4 Neg Neg	1:16 1:4 1:16	Neg 1:16 1:4 1:16 1:4	1:4 1:4 Neg Neg 1:4 1:4	1:32 1:4 1:16	Neg 1:4 1:4 1:4 1:4 1:4 Neg Neg	1:4 1:4 1:4 Neg Neg	Neg Neg Neg	Neg 1:4 1:4	Neg Neg Neg	1:64 1:4 1:64	1:16 1:16 1:4	1:4 1:16 1:16	1:4 Neg 1:4	Neg Neg Neg

TABLE 30 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) IN GROUP I (RHEUMATOID) DOGS

	DOG IDENTIFICATION															
	SLE 1	SLE 2	SLE 3	SLE 4	INF 1	INF 2	INF 3	INF 4	INF 5	INF 6	INF 7	INF 8	INF 9	INF 10	INF 11	INF 12
RHEUMATOID FACTOR	1:40 Neg 1:20	1:10 1:20 1:10	1:20 1:20 1:5 1:5	Neg Neg	1:5 1:10 Neg 1:5 1:10 1:20 1:20 1:20	1:40 1:20 1:20	1:5 1:5 1:10	1:5 Neg 1:10 1:5	1:40 Neg Neg	1:5 1:10 1:20 Neg 1:10	1:10 1:5 1:10	1:10 1:10 1:10	1:80 1:20 1:20	Neg Neg	Neg Neg 1:5	Neg Neg
ANTINUCLEAR ANTIBODY	1:1024+ 1:512 1:512	1:1024+ 1:512 1:1024+	1:1024+ 1:1024+ 1:1024+ 1:1024+	1:64 1:64	Neg Neg Neg Neg Neg 1:16 Neg Neg	Neg 1:4 1:4	1:4 1:4 1:4	Neg Neg 1:4 Neg	Neg Neg Neg	Neg Neg Neg 1:4 Neg	Neg Neg Neg	Neg Neg 1:4	Neg Neg Neg	Neg Neg	Neg Neg Neg	Neg Neg

TABLE 31 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) IN GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS) AND GROUP III(INFECTIOUS) DOGS.

	DOG IDENTIFICATION						
	BE 1	BE 2	BE 3	BE 4	BE 5	BE 6	BE 7
RHEUMATOID FACTOR	1:5 1:10 1:10	1:40 1:80 1:160 1:20 1:5 1:5 1:5 Neg 1:5	1:20 1:20	Neg Neg	1:10	ND	ND
ANTINUCLEAR ANTIBODY	1:1024+ 1:1024+ 1:1024+	Neg 1:4 1:16 1:16 1:16 1:4 Neg 1:4 Neg	Neg Neg	Neg Neg	Neg	ND	ND

TABLE 32 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) IN GROUP IV (BACTERIAL ENDOCARDITIS) DOGS.

	DOG IDENTIFICATION																		
	ID 1	ID 2	ID 3	ID 4	ID 5	ID 6	ID 7	ID 8	ID 9	ID 10	ID 11	ID 12	ID 13	ID 14	ID 15	ID 16	ID 17	ID 18	ID 19	ID 20
RHEUMATOID FACTOR	Neg 1:10 Neg	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	1:80 1:10 Neg	Neg Neg Neg	1:5 1:5 1:5	1:20 1:10 1:20	1:20 1:20 1:10	Neg Neg Neg	Neg Neg Neg	1:5 Neg Neg	1:40 1:5 1:5	1:160 Neg 1:10 1:5 Neg 1:20 1:20 1:10	Neg 1:10 1:5	Neg 1:5 1:5	1:160 1:5 1:5 1:5 1:20	1:20 1:20 1:10	1:20 1:10 1:5 Neg	1:10 1:10 Neg
ANTINUCLEAR ANTIBODY	Neg 1:4 Neg	Neg Neg Neg	1:16 1:4 1:4	Neg Neg Neg	Neg Neg Neg	1:4 Neg 1:4	Neg 1:4 Neg	1:4 1:4 1:4	Neg Neg Neg	1:16 1:4 1:4	Neg 1:4 Neg	1:4 Neg 1:4	Neg 1:16 1:4	1:4 Neg 1:4 Neg Neg Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	1:16 1:16 1:4 1:4 Neg	Neg Neg Neg	Neg Neg 1:16 1:4	1:4 1:4 Neg

TABLE 33 TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) IN GROUP V (IDIOPATHIC) DOGS.

	DOG IDENTIFICATION																	
	ID 21	ID 22	ID 23	ID 24	ID 25	ID 26	ID 27	ID 28	ID 29	ID 30	ID 31	ID 32	ID 33	ID 34	ID 35	ID 36	ID 37	ID 38
RHEUMATOID FACTOR	1:5 1:5 1:5	1:10 1:5 1:5	1:5 1:10 1:10	1:10 1:10 1:10	1:20 1:20 1:20	1:10 1:5 1:5	Neg Neg Neg	1:10 1:10 1:10	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	1:20 1:40 1:20	1:10 1:10 1:10 1:10	1:10 Neg Neg Neg 1:10	1:5 Neg 1:40 Neg 1:5	1:20 1:40 1:20 1:40	Neg Neg Neg	Neg 1:5 1:5
ANTINUCLEAR ANTIBODY	Neg Neg Neg	1:32 Neg 1:4	1:4 1:4 1:4	1:4 1:4 1:4	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	1:4 1:4 1:4	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	1:32 1:32 1:4 Neg	1:16 Neg Neg 1:16 1:16	Neg Neg 1:16 1:16 Neg	Neg Neg Neg 1:4	1:16 1:4 1:16	1:4 Neg 1:4

TABLE 33 (CONTINUED). TITRES OF SERUM RHEUMATOID FACTOR (MODIFIED ROSE-WAALER TEST) AND ANTINUCLEAR ANTIBODY (INDIRECT IMMUNOFLUORESCENT TEST) IN GROUP V (IDIOPATHIC) DOGS.

OTHER TEST SYSTEMS

1. RHEUMATON TEST (W.R.WARNER AND CO.LTD.)AND LATEX TUBE AGGLUTINATION TEST FOR THE DETECTION OF RHEUMATOID FACTOR.

A comparison of the Rheumaton test with the modified Rose-Waaler test is given in Table 34. As can be seen, there is a poor correlation between positive Rose-Waaler titres and positive agglutination reactions with the Rheumaton test. Of 30 sera showing a positive Rose-Waaler titre, only 6 (20%) gave positive agglutination with the slide test. Conversely, of 181 sera which were completely negative for RF with the Rose-Waaler test, 29 (16%) were positive with the Rheumaton test. Five of these 29 positive sera were from normal dogs. Only a very few of the sera showing low (insignificant) titres of RF with the Rose-Waaler test showed agglutination with the slide test (9.7%). The Rheumaton test was thus unsatisfactory for detecting RF in the dog since it gave both false positive and false negative reactions.

The Rheumaton test is based on a modification of the Rose-Waaler test described by Milgrom, Tönder and Loza (1964) overcoming the inconvenience of having to use freshly prepared sheep red blood cells. It has been shown to be over 95% accurate for the detection of RF in man and shows good correlation with the conventional Rose-Waaler procedure (Cathcart and O'Sullivan 1970; Halberstam and Szteinbok 1969; Janeff 1970). In a comparative study of several commercially available tests for detecting RF in man, the Rheumaton test only gave an 11% incidence of false negative results and an even lower incidence of false positive results (5%) (MacSween et al 1974). The test is certainly designed specifically for detecting RF in man and this may account for its unreliability in the canine species.

The latex tube agglutination test was found to be unreliable in the detection of RF in the dog since non-specific agglutination was a common problem i.e. the negative controls gave positive reactions, thus negating the test. When the test did work, RF titres similar to the modified Rose-Waaler test were obtained. Basu (1971) used the dog globulin latex tube agglutination test and also the latex heparin test to determine RF in the dog and found good correlation with his Rose-Waaler test although the titres were generally lower. A specific latex particle test for the dog was mentioned by Halliwell (1978) but no details were given. A human test (latex particles coated with human IgG) was used by Pedersen et al (1976a) to detect RF in the dog but of 7 cases tested, 5 were negative and 2 only gave a titre of 1:10. A similar test was used by Sikes et al (1970) which gave titres between 1:40 and 1:80 for one dog and 1:20 and 1:80 for another. In the single case of rheumatoid arthritis reported by Halliwell et al (1972), a human latex agglutination test was negative on one occasion and only gave a titre of 1:2 on another. It is generally stated that human latex reagents are not satisfactory for the detection of RF in the dog (Halliwell 1978; Schultz 1978). In the human, the latex agglutination test is generally more sensitive than the Rose-Waaler test giving fewer doubtful positive reactions (Plotz and Singer 1956; Singer 1961), although the test has been criticised for giving more false negative and positive results by some workers (MacSween et al 1974).

2. INDIRECT IMMUNOFLUORESCENT TEST USING TRYPANOSOMA BRUCEI FOR THE DETECTION OF ANTI-DEOXYRIBONUCLEIC ACID ANTIBODY.

None of the dogs tested gave a positive reaction with this test.

Seven of the twelve human sera taken from confirmed systemic lupus erythematosus patients did give kinetoplast fluorescence (Fig. 21). The reason for the other five cases not giving a positive reaction is not clear. However, the study by Aarden, de Groot and Feltkamp (1975) using a similar test system with Crithidia luciliae as the substrate demonstrated that several lupus patients failed to give a positive reaction. These authors did show a fairly close correlation between the results of the trypanosome fluorescent test and the Farr assay for deoxyribonucleic acid autoantibodies but in some cases a positive Farr test was accompanied by a negative fluorescent test. These authors also described weak or no fluorescence of the nucleus of the trypanosome despite fluorescence of the kinetoplast. Nuclear fluorescence of T.brucei was never seen in the present study. There is no obvious explanation for the difference in the fluorescent behaviour of the kinetoplast and nucleus but it may be due to differences in the availability of antigenic determinants or to differences in permeability of the kinetoplast and nucleus for antoantibodies. Reference to this test in the dog was made in a review article by Halliwell (1978) but no details were given although the author states that Crithidia luciliae forms an excellent and highly specific substrate for use in the test. Crithidia is probably a better substrate than T.brucei since the concentration of deoxyribonucleic acid in the kinetoplast of the former is thought to be much greater (Laurent, van Assel and Steinert 1971). The test was also used in the dog by Cassan (1968); mouse blood infected with trypanosomes was employed as the substrate. Unfortunately very few details are given. Both T. gambiense (in blood smears from infected mice) and C. lucilliae were used by Monier et al (1978) but no results were reported.

3. LE-TEST (TRAVENOL LABORATORIES LTD.) FOR THE DETECTION OF ANTI-DEOXYRIBONUCLEOPROTEIN ANTIBODY.

Table 35 shows a comparison of the LE-Test with the indirect immunofluorescent test using frozen rat liver sections as the substrate. Whereas the indirect immunofluorescent test is a quantitative test, the LE-Test can only give a positive or negative result. The obvious problem with the LE-Test was the large number of false positive reactions e.g. of 155 dogs with no ANA detectable by the fluorescent test, 51 (32.9%) reacted positive with the LE-Test. Also, in another 174 dogs which did show ANA by fluorescence but at insignificant titres, 71(40.8%) were positive by the LE-Test giving a total of 122 dogs (37.1%) which were positive by the LE-Test but negative by the indirect immunofluorescent test. Several of the normal dogs included in this comparison gave positive reactions with the LE-Test. Of 7 dogs positive by the indirect immunofluorescent test, three (42.9%) were also positive by the LE-Test.

According to some workers false positive reactions are not a problem in human patients (Jordal and Strandberg 1964). Of 100 patients with various diseases, 29 showed positive LE-cell reactions of which 3 were diagnosed as systemic lupus erythematosus. The Hyland LE-Test gave a positive reaction in all three of these patients but negative reactions in all the others. Similar conclusions were reached by Dubois and Strain (1973) who found the test quite specific for systemic lupus erythematosus but recorded a large number of false negative results, i.e. of 121 cases of lupus, only 21 gave a positive LE-Test reaction. The test was only positive in 43% of the total number of patients showing LE-cell phenomena and in 46% of those showing ANA by the indirect immunofluorescent test. However, one authority

(Friou 1967) stated that the test does not correlate with either the clinical diagnosis of systemic lupus erythematosus or the presence/absence of any recognisable autoantibody in the serum. The LE-Test is manufactured specifically for the human patient and this might explain its inapplicability to the canine species.

The test was used in the dog by Schalm and Ling (1970) although of 8 dogs reported with positive LE-cells, only 2 were positive with the latex test. No comments were made regarding the use of the test in normal sera or in sera taken from dogs with other diseases. The test was also used in the U.K. by Jones and Darke (1975). Positive reactions were described in seven dogs; six of these had autoimmune haemolytic anaemia and/or thrombocytopaenia, the other dog was diagnosed as suffering from a splenic haemangiosarcoma. Systemic lupus erythematosus was diagnosed in only one of these dogs. There are again no details of an evaluation of this test in other dogs and at least one false positive reaction can be suspected (i.e. the dog showing splenic neoplasia). Halliwell (1978) did not recommend the use of a latex agglutination test for the detection of ANA in the dog because of false positive reactions but this author does not give any detailed evaluative information; the test in this instance consisted of latex particles coated with deoxyribonucleic acid rather than deoxyribonucleoprotein. Similarly, Schultz (1978) stated that latex particles coated with deoxyribonucleic acid did not compare favourably with the presence of LE-cells or of ANA as detected by immunofluorescence, in the canine patient.

4. LE-CELL PHENOMENON FOR THE DETECTION OF ANTI-DEOXYRIBONUCLEOPROTEIN ANTIBODY.

A positive LE-cell preparation was only obtained in one of the 41 dogs tested. The positive dog was from Group II (dog SLE 3). Although most human systemic lupus erythematosus patients will develop a positive LE-cell reaction sometime during the course of their disease, it is not uncommon to record a negative result in an affected patient (Friou 1972). The incidence of positive LE-cell preparations in human lupus patients varies up to 80% (Friou 1972). Positive preparations are more likely in clinically active systemic lupus erythematosus, particularly where corticosteroid therapy is not being used. When the disease is under remission, it is conceivable that the serum factor responsible for the LE-cell phenomonen is not present in sufficient quantity. Negative LE-cell preparations in acute lupus are difficult to explain although impaired phagocytic activity has been reported (Friou 1972). Positive LE-cells have been recorded in diseases other than systemic lupus erythematosus (Bloch et al 1965; Dubois 1966; Friou 1972; Lenoch and Vojtisek 1967; Rowell 1962) and following the administration of certain drugs e.g. hydralazine (Dustan, Taylor, Corcoran and Page 1954; Miescher, Cooper and Benacerraf 1960; Perry and Schroeder 1954).

Lewis (1965) reported positive LE-cell preparations in 16 of 17 dogs which had the clinical and pathological features of canine systemic lupus erythematosus. In 83 dogs with diseases other than lupus, 6 also showed positive LE-cell preparations. Two of these had diseases of lymphoid tissue, another 3 had a chronic symmetrical polyarthritis said to be similar to rheumatoid arthritis and the remaining dog exhibited

poisoning due to dicoumoral. Six of seven cases of canine systemic lupus erythematosus reported by Lewis et al (1965) showed positive LE-cell preparations; the other dog was negative for the LE-cell test but ANA was detected in the serum by other means. These authors also demonstrated that LE-preparations from a lupus dog could be negative on one occasion and positive on another. Of 29 cases of canine systemic lupus erythematosus reported by Pedersen et al (1976b), all showing polyarthritis, only 19 showed positive LE-cell preparations. Schalm and Ling (1970) state that positive preparations made from the canine patient are likely to contain only a few LE-cells and a tedious search for them is necessary. These investigators reported 8 dogs with positive LE-cell preparations but only 3 were diagnosed as lupus. Of 15 cases of canine systemic lupus erythematosus tested by Halliwell (1978), 3 were negative for LE-cells. Although no case reports are given by Schultz (1978), it is stated that the LE-cell test is positive in about 75% of dogs with systemic lupus erythematosus.

Various methods of performing the LE-cell test have been described in the dog. The most common method in the U.S.A. is to allow the blood to clot, to remove the serum and to force the clot through a fine wire mesh. The disrupted clot is centrifuged using a Wintrobe haematocrit tube to collect the buffy coat (Lewis 1965; Schalm and Ling 1970; Schultz 1978). This procedure differs from that used in the present study where the blood was defibrinated. Another method was used by Halliwell (1978) in which heparinised blood was incubated, treated with glass beads and then re-incubated. An indirect method using serum from the patient and blood cells from a normal dog has also been discussed (Schultz 1978).

From the available evidence it does seem that the LE-cell reaction in the dog is a less consistent phenomonen than in the human. The reason for this is unclear although one suggestion has been the lowered levels of complement reported in canine systemic lupus erythematosus (Wolfe and Halliwell 1979). Complement is required for the LE-cell reaction. Also, the anti-deoxyribonucleoprotein antibody responsible for the LE-cell reaction may not be present in sufficient amounts in certain canine patients e.g. dog SLE 4 showed the peripheral pattern of immunofluorescent staining with the indirect (rat liver) immunofluorescent test indicating the presence of autoantibodies against deoxyribonucleic acid but not to nucleoprotein.

It has also been stated that positive LE-cell preparations may be seen in certain chronic infectious diseases of the dog (Barrett 1977).

5. RADIOIMMUNOASSAY FOR THE DETECTION OF ANTI-DEOXYRIBONUCLEIC ACID ANTIBODY

As can be seen from Table 36, this test, which is again standardised for use in the human, proved totally unsatisfactory for the canine species. Of 25 normal sera tested, the majority had very high binding values, the average figure being 57% with a range from 9% to 85%. With human sera it is generally reckoned that binding values over 40% are abnormal, values between 30-40% are suspicious and values less than 30% are normal (values do differ slightly between different test systems). The high values obtained with the normal dog sera makes this particular test inapplicable to this species. Certain technical factors can give high false positive values. Experience in pipetting techniques is certainly very important and two batches of sera were tested by

relatively inexperienced personnel but each serum was examined in duplicate and the values were only accepted if both results were comparable. Also, some of the sera were tested by experienced technicians at the Western Infirmary, Glasgow and these results showed equally high binding values.

Previous studies in the dog have also shown the deoxyribonucleic acid binding test to be of limited value in the assessment of anti-deoxyribonucleic acid antibodies (Thoburn, Hurvitz and Kunkel 1972). These investigators demonstrated an anionic serum protein which reacted specifically with native deoxyribonucleic acid and this was found to be present in several mammalian species including the dog, mink and guinea pig, but not in the human. This protein was not an antibody and behaved as an alpha-beta globulin and interfered with the measurement of anti-deoxyribonucleic acid antibodies in the blood, giving high false positive results. Other authorities have agreed that this test is not applicable to the dog (Halliwell 1978). Monier et al (1978) described a radioimmunoassay test for the dog based on the Farr technique but gave no results. Two groups of investigators have claimed success with the deoxyribonucleic acid binding test (Lewis et al 1973; Lewis and Borel 1971; Pedersen et al 1976a). The test used by Lewis and his colleagues involves filtering the deoxyribonucleic acid/anti-deoxyribonucleic acid solution through a glass filter to absorb the deoxyribonucleic acid molecules complexed with antibody, instead of precipitating the complexes with ammonium sulphate. A similar technique was used by Pedersen and his co-workers, cellulose nitrate filters being used to remove the complexes. Whether or not these alternative methods are applicable to the dog is not certain since at least one authority (Dr. R. E. W. Halliwell - personal communication) claims to have used these techniques and still encountered false positive reactions.

Further evaluation of these techniques in the dog is required.

The use of radioimmunoassay tests for the detection of anti-deoxyribonucleic acid antibodies has proved very useful in the diagnosis of human systemic lupus erythematosus. The majority of patients with lupus have anti-double-stranded deoxyribonucleic acid antibodies (Carr, Koffler, Agnello and Kunkel 1969; Hughes, Cohen and Christian 1971; Pincus, Schur, Rose, Decker and Talal 1969) and in some cases there is direct correlation of serum deoxyribonucleic acid binding levels with disease activity and inverse correlation with serum complement levels. A rising deoxyribonucleic acid binding level is usually associated with exacerbation of disease, especially renal disease. High deoxyribonucleic acid binding values are rare in other conditions although increased values do occur in about 33% of patients with discoid lupus erythematosus (Mandel, Carr, Weston, Sams, Harbeck and Krueger 1972), 25% of patients with Sjøgren's syndrome (Pincus et al 1969) and in a few patients with rheumatoid arthritis (Rochmis, Palefsky, Becker, Roth and Zvaifler 1974), Felty's syndrome and chronic active hepatitis. Binding of deoxyribonucleic acid in these test systems, with non-antibody proteins does occur to a minor degree with human sera (Aarden, Lakmaker, de Groot, Swaak and Feltkamp 1975) but this is not a major problem as far as interpretation of the results is concerned. The low percentage binding values obtained with normal human sera are probably explained by the non-antibody proteins reacting with the deoxyribonucleic acid.

		TITRE OF RHEUMATOID FACTOR WITH MODIFIED ROSE-WAALER TEST							
		Neg	1:5	1:10	1:20	1:40	1:80	1:160	1:320
RHEUMATON TEST	NEGATIVE	181	59	37	38	17	9	2	2
	POSITIVE	29	2	5	6	4	2	0	0

TABLE 34 COMPARISON OF THE RHEUMATON TEST* AND THE MODIFIED ROSE-WAALER TEST FOR THE DETECTION OF RHEUMATOID FACTOR.

* W.R. Warner & Co. Ltd., Chestnut Avenue, Eastleigh, Hants.

		TITRE OF ANTINUCLEAR ANTIBODY WITH INDIRECT IMMUNOFLUORESCENT TEST					
		Neg	1:4	1:16	1:32	1:64	1:1024
LE TEST	NEGATIVE	104	69	34	3	1	0
	POSITIVE	51	53	18	1	0	2

TABLE 35 COMPARISON OF THE LE-TEST* AND THE INDIRECT IMMUNOFLUORESCENT TEST FOR THE DETECTION OF ANTINUCLEAR ANTIBODY.

* Travenol Laboratories Ltd., Caxton Way, Thetford, Norfolk.

TABLE 36

SHOWING THE PERCENTAGE DEOXYRIBONUCLEIC ACID BINDING VALUES FOR 25 NORMAL DOGS, SELECTED DOGS FROM GROUPS I, IV AND V AND 12 DOGS WITH DISEASES OTHER THAN INFLAMMATORY ARTHROPATHY.

The ANA and RF titres are also shown.

	NORMAL DOGS																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
% DNA BINDING	76	60	9	18	55	57	55	47	41	57	50	61	50	57	55	55	67	58	77	85	84
ANA * TITRE	1:4	Neg	1:4	Neg	1:4	1:4	1:16	1:4	Neg	Neg	Neg	Neg	Neg	1:4	1:4	Neg	1:4	Neg	1:4	1:4	Neg
RF ≠ TITRE	1:5	Neg	1:5	1:10	1:20	Neg	Neg	1:5	Neg	Neg	Neg	Neg	1:10	1:5	1:5	Neg	Neg	Neg	Neg	Neg	Neg

	NORMAL DOGS				INFLAMMATORY ARTHROPATHIES															
	22	23	24	25	Rh3	Rh5	Rh5	Rh5	Rh6	Rh8	Rh8	Rh11	BE1	BE1	ID1	ID5	ID5	ID13	ID15	ID16
% DNA BINDING	75	64	57	64	71	64	76	34	46	66	63	62	51	64	56	64	75	56	65	25
ANA * TITRE	Neg	1:4	1:4	Neg	Neg	Neg	1:256	Neg	Neg	1:16	1:4	1:32	1:1024+	1:1024+	Neg	Neg	Neg	Neg	Neg	Neg
RF ≠ TITRE	Neg	1:10	1:5	Neg	1:80	1:40	1:40	1:40	1:80	1:40	1:40	1:320	1:5	1:10	Neg	1:80	1:10	1:40	Neg	Neg

	OTHER DISEASES											
	1	2	3	4	5	6	7	8	9	10	11	12
% DNA BINDING	0	71	64	18	71	75	73	63	54	89	9	71
ANA * TITRE	1:32	1:16	1:4	1:32	1:4	1:4	1:4	1:4	Neg	1:4	1:4	Neg
RF ≠ TITRE	1:40	1:40	Neg	1:40	1:20	1:20	Neg	1:80	1:5	1:5	Neg	Neg

NORMAL DOGS AVERAGE DNA BINDING 57% (9% - 85% RANGE)
INFLAMMATORY ARTHROPATHY DOGS AVERAGE DNA BINDING 65% (25% - 75% RANGE)
OTHER DISEASES AVERAGE DNA BINDING 55% (0% - 89% RANGE)

* ANA TITRE AS ASSESSED BY INDIRECT IMMUNOFLUORESCENT TEST USING RAT LIVER AS SUBSTRATE
≠ RF TITRE AS ASSESSED BY THE MODIFIED ROSE-WAALER TEST

SECTION VII

RED AND WHITE BLOOD CELL AUTOANTIBODIES AND THEIR LABORATORY IDENTIFICATION

In considering practically any patient whose condition presents a diagnostic problem, or is typical of a disease which, though recognised, has an as yet unknown fundamental cause, the modern physician is forced to ask himself, is there an immunlogical element?

P.G.H.Gell and R.R.A.Coombs 1975

A. RED BLOOD CELL AUTOANTIBODIES

HISTORY

Early in this century, several workers suggested the presence of antibodies in the form of haemagglutinins and haemolysins, in cases of haemolytic anaemia and haemoglobinuria. However, it was not until after the introduction of the antiglobulin test (also called the Coombs' test) that the role of autoantibodies against red blood cells in the causation of acquired haemolytic anaemia was generally accepted and the terms autoimmune or autoallergic commonly used. The principle of this test was first described in 1908 by Moreschi but was independently discovered and developed as a method for demonstrating antibodies to red cell antigens by Coombs, Mourant and Race (1945). It was Boorman and his colleagues (1946) who first demonstrated unequivocally that many patients with haemolytic anaemia had immunoglobulin on the surface of their red blood cells and that this antibody was specifically directed against those red blood cells.

SYNDROMES INVOLVING RED CELL AUTOANTIBODIES

It is well known, both in human patients (Dacie and Worlledge 1975) and in the dog (Dodds 1977; Jain 1975; Schalm 1975) that autoimmune haemolytic anaemia can exist in primary or idiopathic and in secondary or symptomatic forms and more and more it is becoming realised that cases apparently idiopathic in origin are in fact based upon some other underlying disease process. In the human patient, secondary cases of autoimmune haemolytic anaemia most commonly occur in association with malignant disease of the lymphoreticular system, chronic lymphocytic leukaemia and reticulosarcoma in particular and with other autoimmune

disorders, especially systemic lupus erythematosus. Transient haemolytic anaemia may sometimes develop after pneumonia due to mycoplasmas or possibly viruses and in association with infectious mononucleosis. Carcinomata, severe bacterial infections and inflammatory or granulomatous disease (e.g. ulcerative colitis, rheumatic fever, active and chronic liver disease and sarcoidosis) can also be associated with a positive Coombs' test (Williams, Beutler, Ersler and Rundles 1972).

Pirofsky (1969) has stressed that autoimmune haemolytic anaemia should be considered as one manifestation of "diffuse immunologic disease" and in support of this concept he exemplifies the not infrequent occurrence of autoimmune haemolytic anaemia in patients who present with or develop subsequently, signs or symptoms of other disorders thought to be autoimmune in origin, e.g. systemic lupus erythematosus, Hashimoto's thyroiditis, myasthenia gravis, rheumatoid arthritis etc. However, other authorities view such occurrences, with the exception of lupus, as relatively rare (Dacie and Worlledge 1975).

Autoimmune haemolytic anaemia in the dog can be classified into various types depending upon the clinical and immunological characteristics (Bennett 1980; Halliwell 1978). These include intra-vascular haemolysis, extra-vascular destruction, cold haemagglutinin disease, cold non-haemagglutinin disease and in-saline autoagglutinin disease. A review of the similar types of disease affecting the human patient is given by Dacie and Worlledge 1975). Drug-induced immune and autoimmune haemolytic anaemia are an important additional group in the human patient.

THE ANTIGLOBULIN TEST FOR DEMONSTRATING RED BLOOD CELL AUTOANTIBODIES

The test is used to demonstrate the presence of autoantibodies on the surface of erythrocytes (direct test) or free in the patient's serum (indirect test). In the direct test a suspension of washed red blood cells from the patient is allowed to react with a species specific anti-globulin reagent and a positive result is shown by visible agglutination of the erythrocytes. It is important to use different dilutions of the antiserum to avoid any complications with the prozone phenomenon. Ideally, antisera against IgG, IgM and complement should all be used separately and before use the antisera should have been absorbed with pooled species specific red blood cells to remove any possible antibody activity against the patient's red blood cells. The test should always be performed at both 37°C and 4°C. The indirect antiglobulin test involves reacting the patient's serum with washed erythrocytes from a normal dog or person of an identical blood group - the latter is important to obviate false positive results caused by isoagglutinins. The cells are then washed and reacted with the antiglobulin reagent; if red cell autoantibodies are present in the serum, they will coat the normal erythrocytes which will then agglutinate with the antiserum. The indirect test however, is not totally reliable in veterinary medicine because of the lack of knowledge of blood groups in the domestic animals.

A positive antiglobulin test generally indicates the presence of antibody and/or complement on the surface of red blood cells. It does not however, necessarily mean that the antibody is directed against the red cell, e.g. in human medicine there are many drugs which are absorbed onto erythrocytes and the test can actually show antibodies against the

drugs. A similar phenomenon can occur with blood parasites such as Babesia. Immune complexes can also be non-specifically absorbed onto the red cell surface and give a positive reaction with the antiglobulin test. False positive results may sometimes occur due to serum proteins bound to the red cell surface, especially if the latter has been damaged. It has also been demonstrated that blood samples containing high levels of reticulocytes, can give positive agglutination reactions of the red cells with the Coombs' reagent which are inhibited by prior absorption of the antiglobulin serum with transferrin (Jandl 1960). The agglutination in these cases was attributed to anti-transferrin antibodies in the antiglobulin reagent. Contamination of the reagent with bacteria or particulate material can lead to a non-immune clumping of the red cells.

False negative results may occur when there are very low concentrations of autoantibodies, below the sensitivity of the test, or when there is dissociation of the autoantibody from the red cells. Natural remission of the disease or treatment of the patient with corticosteroids may also lead to false negative results. About 2.4% of human patients with clinical autoimmune haemolytic anaemia have negative antiglobulin tests and some of these may be explained by antibodies of the IgA class which are missed by the routine tests (Worlledge and Blajchman 1972). The prozone phenomenon may cause false negative results (vide supra). Another cause of false negative reactions is the failure to wash the red cells adequately prior to exposing them to the antiglobulin reagent. It requires very little residual serum protein in the suspending medium to neutralise the antibodies in the antiglobulin reagent (Chaplin 1974).

COOMBS' DIRECT ANTIGLOBULIN TEST FOR THE DETECTION OF RED CELL AUTOANTIBODIES - LABORATORY PROCEDURE

In all cases tested the antiglobulin reagent used was obtained commercially (Miles Laboratories Ltd., Stoke Court, Stoke Poges, Slough, Bucks.). The reagent had activity against canine IgG and complement (C3). The antiserum was prepared by the manufacturers by immunising rabbits with canine IgG and C3. The antiserum collected from the rabbits was inactivated at 56°C for 30 minutes and absorbed with canine red blood cells. It was tested by the manufacturers to ensure its specificity.

The reagent is presented in lyophilized form and the 5ml size is adequate for 50 conventional tests although when used in the microtitre plate procedure, it is adequate for approximately 200 tests. The former method generally gives stronger reactions (Chaplin 1974) and this was used in the present study. Unfortunately the reagent used had no anti-IgM activity and both the IgG and C3 antisera had been pooled by the manufacturers and could not be used separately.

Approximately 4ml of blood was collected from each dog into a receptacle containing ethylenediamine tetra-acetic acid. The blood was centrifuged at 3000 rpm for 5 minutes in a BTL Bench centrifuge. The red cells were collected and washed in a large volume (10-20ml) of normal saline. This washing procedure was repeated 3 times. After the final centrifugation, 0.2ml of packed cells were pipetted into 9.8ml of normal saline to prepare a 2% suspension of erythrocytes.

The lyophilized antiserum was stored at 4°C until ready for use. It was reconstituted with 5ml of distilled water and after reconstitution

the antiserum was stored at -20°C in small aliquots (0.1ml) for up to 6 months. The individual aliquots were allowed to thaw just prior to being used in the Coombs' test; the reconstituted aliquots were never refrozen after thawing. 0.5ml of normal saline was pipetted into 3 difference vials marked 1:2, 1:4 and 1:8. 0.5ml of the antiglobulin reagent was mixed with the 0.5ml of saline in the 1:2 vial. Twofold dilutions were then prepared for the 1:4 and 1:8 vials. This gave 3 different dilutions of the antiglobulin reagent, viz 1:2, 1:4 and 1:8.

The test was carried out in 0.5 x 5cm test tubes. 0.1ml of the 2% suspension of the patient's cells was placed in each of 4 test tubes. The same volume of a suspension of red cells from a normal dog was placed in each of another 4 tubes. 0.1ml of the Coombs' reagent of each of the dilutions (1:2, 1:4 and 1:8) was placed in 3 of the tubes containing the patient's cells and 3 of the tubes containing the normal dog cells. 0.1ml of saline was added to the fourth tube of each dog; These were the saline controls. The contents of each tube were gently mixed and incubated at 37°C for 30 minutes. After this time the tubes were observed macroscopically for red cell agglutination, with the aid of a concave mirror held underneath the bottom of the tubes and a magnifying lens. Agglutination is seen as a granular layer of cells on the base of the test tube (Fig. 35). When agglutination does not occur, the red cells settle out as a "tight button" (Fig. 36). In some positive cases, there is some button formation with agglutinated cells around the periphery. Agglutination of the red cells was confirmed in some cases by examination under the light microscope. Obviously for the test to be valid, all the control tubes had to be free of agglutination.

	NORMAL DOG				PATIENT			
Tube No.	1	2	3	4	5	6	7	8
Negative Control Cells(ml)	0.1	0.1	0.1	0.1	-	-	-	-
Patient's cells (ml)	-	-	-	-	0.1	0.1	0.1	0.1
Saline (ml)	0.1	-	-	-	0.1	-	-	-
Antiglobulin Reagent (ml)	-	0.1 (1:2)	0.1 (1:4)	0.1 (1:8)	-	0.1 (1:2)	0.1 (1:4)	0.1 (1:8)

"Cold agglutinins" have also been recognised in the dog(Bennett 1980;Greene,Kristensen, Hoff and Wiggins 1977; Halliwell 1978) and for this the test should be carried out at $4^{o}C$. However, this was not done in the present study, principally because only the anti-IgG/C3 reagent was available and most cold agglutinins appear to be of the IgM type in the dog.

The indirect antiglobulin test was not used in this study.

Dogs Tested

The main reason for investigating erythrocyte autoantibodies in the present study was to examine their prevalence in the connective tissue disorders of the dog, particularly systemic lupus erythematosus. The test was not done as routine; it was performed on all suspected cases of systemic lupus erythematosus and on a number of selected dogs from each of the other groups (Table 37). A general population of hospital dogs, as well as a number of normal animals have been tested by the Coombs' reagent in the author's laboratory; the test is used as routine for the diagnosis of canine autoimmune haemolytic anaemia.

RESULTS AND DISCUSSION

The results are recorded in Table 37.

GROUP I(RHEUMATOID)

Only one out of twelve dogs tested gave a positive reaction to the Coombs' antiglobulin test and this dog only reacted positive on one of two occasions. Thus, despite several dogs in this group being anaemic there was no consistent evidence for an underlying autoallergic mechanism. Some human rheumatoid patients do occasionally show positive antiglobulin reactions (Mongan, Leddy, Atwater and Barnett 1967). These workers reported a positive reaction with an anti-complement but not an anti-IgG reagent in their patients.

GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS)

Three of the four dogs were positive, indicating the presence of IgG and/or C3 on their red blood cells. Positive direct antiglobulin tests are found in up to 65% of human lupus patients (Mongan et al 1967) although haemolytic anaemia occurs in only 6-15%. In human patients, the red cells are coated with either complement and IgG or complement alone (Mongan et al 1967; Worlledge 1967). The presence of red cell autoantibodies, sometimes accompanied by overt haemolysis is recognised as a common feature of canine systemic lupus erythematosus (Lewis et al 1965a; Pedersen et al 1976b). It is important to realise that positive Coombs' reactions can occur in patients not showing evidence of haemolysis or anaemia.

GROUP III (INFECTIOUS)

None of the four dogs tested gave a positive reaction.

GROUP IV (BACTERIAL ENDOCARDITIS)

Of 3 dogs tested,only one (BE 5) gave a positive result and this was only on one occasion; the dog was negative when tested a second time. Human patients with bacterial endocarditis have given positive Coombs' reactions (Williams et al 1972).

GROUP V (IDIOPATHIC)

Of 22 dogs tested, four gave a positive reaction and of the four, three were negative after remission of clinical signs with cortico-steroid treatment. Three of these four dogs were anaemic although this was only to a slight/moderate degree. The presence of red cell autoantibodies in these arthritic dogs raises the possibility of the multi-system disease systemic lupus erythematosus but all these dogs were negative for circulating antinuclear antibody. The presence of red cell autoantibodies in this group of dogs is difficult to explain; they may be part of the disease complex or they may be occurring as a secondary phenomenon as has already been discussed. None of the idiopathic cases described by Pedersen et al(1976b) were reported as having positive Coombs' reactions. Jones and Darke (1975) did describe polyarthritis in three dogs which also had red blood cell autoantibodies (detected by the papain test) and which were probably not cases of systemic lupus erythematosus. The occurrence of polyarthritis with a positive Coombs' test in the dog was also described by Schalm and Ling (1970)

although there is some confusion over the diagnosis of systemic lupus erythematosus in this publication. Eight cases were reported, all negative for circulating antinuclear body but all showing positive LE-cell preparations; the authors themselves diagnose lupus in three dogs. The haemograms of the polyarthritic Greyhounds reported by Huxtable and Davies (1976) were all stated as normal, and no positive Coombs' reactions were discussed.

OTHER DOGS

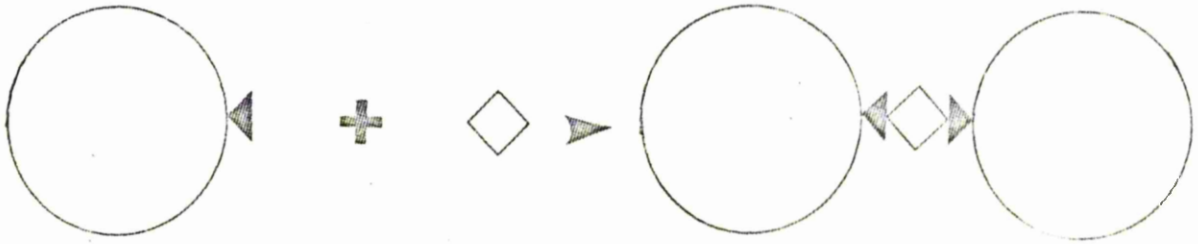
Positive results with the Coombs' test have been obtained in several dogs not showing any joint disease (Bennett, Finnett, Nash, and Kirkham 1980). Over the past 5 years, 15 cases of primary autoimmune haemolyt anaemia have been identified. These cases showed strong positive reactions. Five of these dogs were classified as the "in vivo haemolysin" type of autoimmune haemolytic anaemia and the remaining 10 as the "extra-vascular destructive" type (Bennett 1980; Halliwell 1978).

Fifty-five other dogs with a variety of diseases have also shown a positive Coombs' reaction although in many cases this was only weak, partial agglutination being seen at the 1:2 or 1:4 dilutions. Anaemia was present in 38 of these dogs although in some cases this was only mild and not suspected on clinical examination. Biochemical evidence for haemolysis was not present in these dogs. The diseases recorded in these 55 dogs included liver abnormalities, central nervous system abnormalities, gastroenteritis, pyrexia of unknown origin and various types of neoplasia (Bennett et al 1980). Similar observations have been recently published by Slappendel (1978) who demonstrated that the positive Coombs' reaction in many of these internal diseases was due

to the presence of complement but not antibody on the surface of the red cells. Such cases seldom had serious haemolysis. Slappendel (1978) recommended that the Coombs' test should always be carried out with separate reagents i.e. anti-IgG, anti-IgM and anti-complement, to help interpret the more significant reactions. Unfortunately, in the present study the anti-IgG and anti-complement reagents were combined. Obviously, care is needed in the interpretation of the Coombs' test since positive reactions not only occur in cases of primary autoimmune haemolytic anaemia and in autoimmune haemolytic anaemia associated with systemic lupus erythematosus, but also in association with a variety of other diseases. The positive reactions recorded in the dogs from Groups I, IV and V of the present study might be examples of this, where deposits of complement on the erythrocyte surface without antibody give the positive reaction. The explanation for these deposits on the red blood cells in these various disease states is uncertain.

Over 100 normal dogs have been examined by the Coombs' antiglobulin test and all have given negative reactions.

FIGURE 34 A DIAGRAMMATIC REPRESENTATION OF THE DIRECT COOMBS' (ANTIGLOBULIN) TEST USED TO DEMONSTRATE RED BLOOD CELL AUTOANTIBODIES IN THE DOG.



Patient's RBCs with attached autoantibodies.

Antiglobulin reagent. (Anti-IgG, anti-C3).

Antiglobulin reagent links to autoantibodies on RBCs and causes agglutination of RBCs.

FIGURE 35

THE DIRECT COOMBS' (ANTIGLOBULIN) TEST FOR THE DEMONSTRATION OF RED BLOOD CELL AUTOANTIBODIES (POSITIVE RESULT).

Note the granular appearance of the red blood cells at the bottom of the agglutination tube.

FIGURE 36

THE DIRECT COOMBS' (ANTIGLOBULIN) TEST FOR THE DEMONSTRATION OF RED BLOOD CELL AUTOANTIBODIES (NEGATIVE RESULT).

Note the "tight" sedimentation button of red blood cells at the bottom of the agglutination tube.

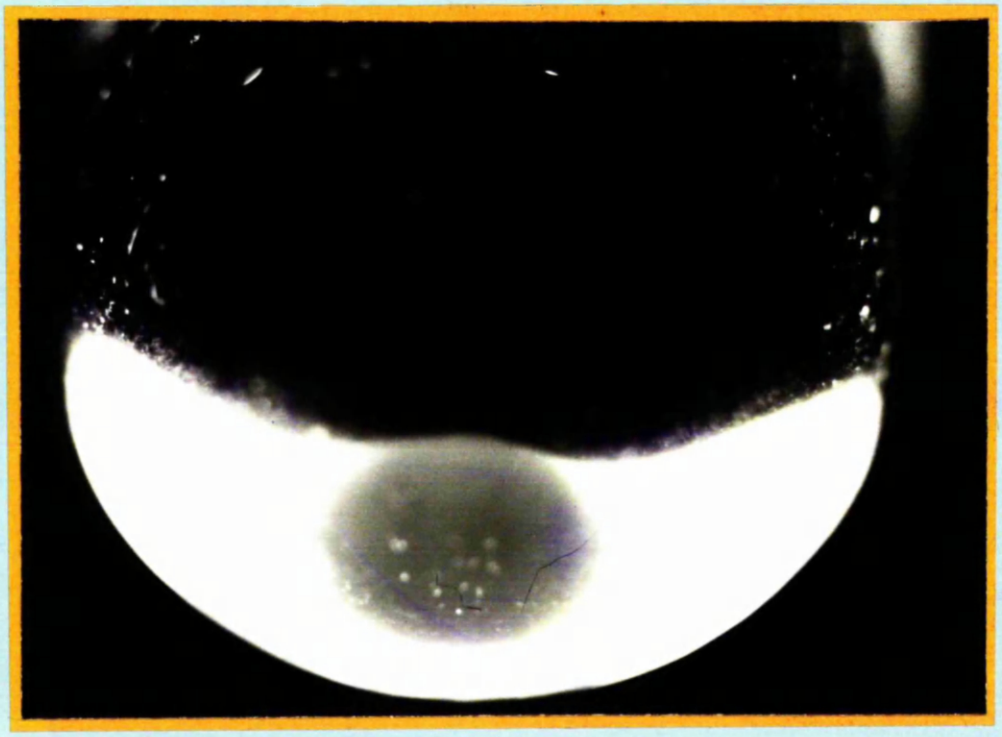


TABLE 37

SHOWING THE RESULTS OF THE RED BLOOD CELL (COOMBS' TEST) AND WHITE BLOOD CELL (DIRECT ANTIGLOBULIN CONSUMPTION TEST) AUTOANTIBODY DETERMINATIONS.

A positive score indicates the presence of autoantibodies and a negative score indicates their absence. No score means the determination was not carried out.

	Rh 1	Rh 1	Rh 6	Rh 7	Rh 8	Rh 10	Rh 10	Rh 11	Rh 12	Rh 13	Rh 17	Rh 17	Rh 19	Rh 20	Rh 21	SLE 1	SLE 2	SLE 3	SLE 4	BE 1	BE 2	BE 5	BE 5
RBC AUTOANTIBODIES	-	-	-	-	-	-	+		-	-	-	-	-	-	-	+	+	+	-	-	-	+	-
WBC AUTOANTIBODIES								-		-					+			+					
	INF 1	INF 1	INF 1	INF 3	INF 5	INF 5	INF 7	ID 1	ID 1	ID 6	ID 6	ID 6	ID 7	ID 10	ID 13	ID 14	ID 14	ID 14	ID 14	ID 14	ID 14	ID 17	ID 19
RBC AUTOANTIBODIES	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
WBC AUTOANTIBODIES										-													
	ID 19	ID 20	ID 20	ID 23	ID 24	ID 25	ID 25	ID 25	ID 26	ID 28	ID 29	ID 31	ID 31	ID 32	ID 32	ID 33	ID 33	ID 33	ID 34	ID 34	ID 35	ID 36	ID 37
RBC AUTOANTIBODIES	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-		-	-
WBC AUTOANTIBODIES		-																			-		

B. WHITE BLOOD CELL AUTOANTIBODIES

HISTORY

The study of autoantibodies against leucocytes of human patients has regained interest in recent years.

The indirect antiglobulin consumption test to detect antibodies on white blood cells was devised independently by Steffen and Schindler (1955b)and by Moulinier (1955). However, this test did not differentiate autoantibodies and iso-antibodies developing after transfusion or pregnancy. Because of this, Walford(1960)and Killman (1960)criticised many reports of leuco-agglutinins in the sera of patients suffering from blood diseases,including those associated with leucopaenia where the indirect antiglobulin test had been used.

The direct antiglobulin consumption test was devised in order to overcome some of the problems encountered with the indirect test (Dausset and Brecy 1958; 1959; Van Loghem, Hart, Hijmans and Schuit 1958). The technique is cumbersome and requires relatively large volumes of leucocytes A study using the direct antiglobulin test with the patients' own leucocytes was reported by Dausset, Colombani and Colombani(1961) and by Dausset (1965). Positive results were obtained with the leucocytes from about 40% of patients with "primary leucopaenia" and a gamma globulin could be eluted from a patient's own leucocytes and refixed to normal leucocytes. This "autoantibody" appeared to possess species specificity and to react with leucocyte cytoplasm rather than the nucleus and in particular with the ribosomal sub-fraction of the cytoplasm. However, the patients who gave positive tests did not differ clinically from those leucopaenic patients who gave negative tests.

Cytotoxic antibody tests have also been used to demonstrate white cell autoantibodies. Such antibodies were demonstrated against autologous lymphocytes by Stastny and Ziff (1971) in the sera of 12/20 patients with systemic lupus erythematosus. Michlmayr, Asamer, Huber and Huber (1972) investigated patients with systemic lupus erythematosus, rheumatoid arthritis and lymphoma and found cytotoxic antibodies against autologous lymphocytes in 11/12 patients with lupus. The antibodies acted at 37°C and were presumably effective in vivo.

The demonstration of gamma globulins associated with white blood cells may not necessarily indicate the presence of autoantibodies. It is known for example that agglutination of leucocytes can occur associated with certain drugs such as amidopyrine, sulphonamides, gold and drugs of the thiouracil groups (Dacie and Worlledge 1975; Moeschlin and Wagner 1952). The exact nature of the agglutinating mechanism in this instance is uncertain but may be related to the adherence of leucocytes to drug (antigen) - antibody complexes which circulate for only a short time after ingestion of the drug and the effective antigen is probably a drug-plasma protein complex.

DIRECT ANTIGLOBULIN CONSUMPTION TEST FOR DEMONSTRATING WHITE BLOOD CELL AUTOANTIBODIES - LABORATORY PROCEDURE

Certain dogs in the present study which exhibited leucopaenia were tested for the presence of leucocyte autoantibodies i.e. dogs Rh 11, Rh 13, Rh 21, SLE 3, ID 6, ID 20 and ID 35. Whenever a test dog was examined, two control animals with normal levels of circulating white blood cells were also assessed.

COLLECTION OF LEUCOCYTES

Fifteen millilitres of blood were collected from the jugular vein of each dog directly into a syringe containing 5-10ml of plasmagel (3% gelatin in phosphate buffered saline) with a small quantity of preservative free heparin (40 units/ml of blood) to prevent clotting. The plasmagel is heated to produce a liquid and care must be taken not to let the temperature fall if there is any delay in collecting the blood, otherwise the plasmagel will solidify. After blood collection the syringe was placed vertically in a rack inside an oven at 37^{o}C for one hour. During this time the red blood cells sediment out. A fresh needle was then placed on the end of the syringe and bent through 90^{o} and the upper layer of liquid containing the white cells was expelled into a clean, sterile universal container. The liquid was centrifuged at 3000 rpm for 10 minutes in a BTL Bench centrifuge to collect the white cells; the supernatant was poured off and the cells washed in Hank's balanced salt solution (Flow Laboratories, Irvine). The centrifugation and washing procedure was repeated three times and the cells left in Hank's solution. The volume of the latter in the final collection of cells was adjusted so that approximately

2-3mm of cells were collected in sealed capillary tubes during centrifugation in the micro-haematocrit (Hawksley, London).

The Hank's balanced salt solution was made up according to the manufacturer's instructions using de-ionised water. Sodium bicarbonate (0.35g/L) was added and the pH adjusted to achieve a final value of 7.2. The solution was sterilised by filtration under pressure to avoid loss of CO_2, through a membrane filter. Calf serum (2% solution) was added to the Hank's solution immediately before use (2ml/100ml).

ASSESSMENT OF THE END-POINT OF THE ANTIGLOBULIN REAGENT WITH SHEEP RED BLOOD CELLS COATED WITH DOG GLOBULIN

The antiglobulin reagent was obtained commercially (Miles Laboratories Ltd., Stoke Court, Stoke Poges, Slough, Bucks.); it was the same reagent as used in the direct antiglobulin (Coombs') test for the detection of red blood cell autoantibodies (vide supra).

A 2% suspension of sheep red blood cells was prepared and coated with dog antibodies against sheep erythrocytes at a sub-agglutinating titre (1:1600) using the method already described in the detection of canine RF by the modified Rose-Waaler test (Section VI).

The reconstituted antiglobulin reagent was treated in the same fashion as a test serum in the modified Rose-Waaler test. Heat inactivation at 56°C for 30 minutes was performed although this had already been done by the manufacturers in their preparation of the reagent. The reagent was also incubated with an equal volume of packed washed non-sensitised sheep red blood cells to remove heteroagglutinins (the

volumes of materials used were the same as those used in the Rose-Waaler test). Double dilutions of the antiglobulin reagent (1:8 to 1:4096) were then prepared in an agglutination plate and an equal volume (0.1ml) of sensitised sheep red blood cells added to each well. The plate was incubated overnight at 4°C and then examined for haemagglutination. Controls were also used as in the Rose-Waaler test. The highest dilution at which agglutination of the red cells was present, was taken as the end-point (1:128). For the main part of the test a dilution of the anti-globulin reagent was selected at which strong agglutination had occurred - 1:64 in this particular test system.

ADDITION OF ANTIGLOBULIN REAGENT TO THE PATIENT'S LEUCOCYTES

0.15ml of the 1:64 dilution of the antiglobulin reagent was added to the suspension of collected white blood cells in Hank's solution. Thorough mixing was ensured. The cells and antiglobulin reagent were incubated for six minutes at room temperature. The mixture was then centrifuged at 2000 rpm in the BTL Bench centrifuge and the supernatant quickly collected.

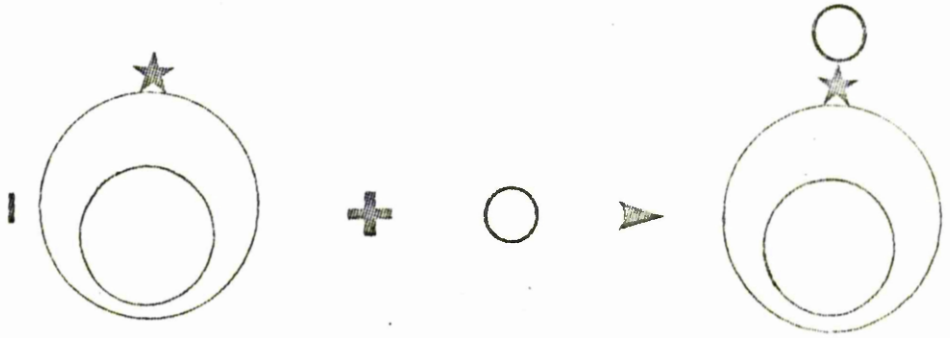
TITRATION OF THE ANTIGLOBULIN SUPERNATANT

The collected supernatant was rapidly double diluted using saline and an agglutination plate to give dilutions ranging from 1:1 to 1:512. An equal volume (0.1ml) of sensitised sheep red blood cells was added to each well and the plate incubated overnight at 4°C. The following day, the wells were checked for agglutination of red blood cells. The technical details are similar to those described for the modified Rose-

Waaler test. The highest dilution at which agglutination occurred was noted. A positive result was regarded as one where there was a reduction in the titre at which agglutination occurred, compared to the two control dogs, by at least two dilutions. A reduction in the antiglobulin reactivity meant that during the incubation stage some of the antiglobulin reagent became attached to globulin deposits (assumed to be autoantibodies) on the surface of the white blood cells.

On two occasions(with cells from Rn 21 and SLE 3)there was no evidence of agglutination of red cells by the antiglobulin supernatant of the test dog but that from the two control dogs produced agglutination at three dilutions (1:1, 1:2 and 1:4). In the other test dogs, agglutination occurred similar to the controls.

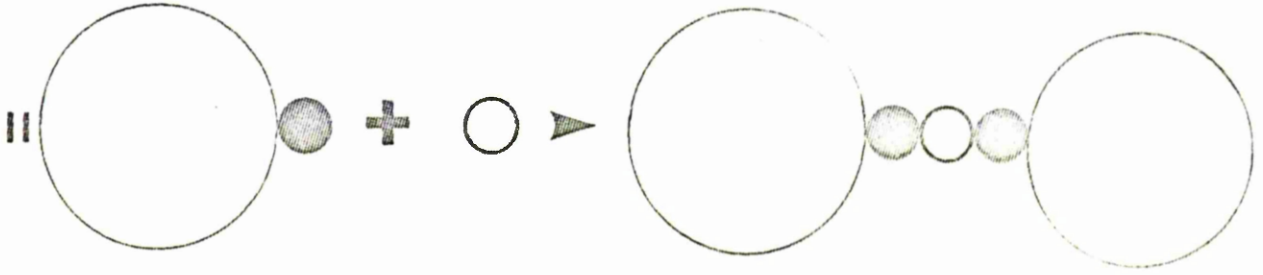
FIGURE 37 A DIAGRAMMATIC REPRESENTATION OF THE DIRECT ANTIGLOBULIN CONSUMPTION TEST USED TO DEMONSTRATE LEUCOCYTE AUTOANTIBODIES IN THE DOG.



Patient's WBCs with attached autoantibodies.

Antiglobulin reagent. (Anti-IgG, anti-C3).

Antiglobulin reagent links to autoantibodies on WBCs.



Sensitised sheep RBCs as used in modified Rose-Waaler test.

Antiserum from reaction I containing "unconsumed" antiglobulin reagent.

"Unconsumed" antiglobulin reagent links onto the globulin coating the sheep RBCs and causes agglutination of the red cells. Titre obtained and compared with normal controls.

RESULTS AND DISCUSSION

Of the 7 dogs tested only 2(Rh 21 and SLE 3) were positive(Table 37).

Dog Rh 21 showed splenomegaly as well as leucopaenia and polyarthritis and this combination of rheumatoid arthritis, enlarged spleen and lowered white blood cell count in the human patient, is generally referred to as Felty's syndrome (Felty 1924). There has been no reports of the leucopaenia in this syndrome being associated with autoantibodies. However, the leucopaenia recorded in human systemic lupus erythematosus patients has been related to white cell autoantibodies (Michlmayr et al 1972; Stastny and Ziff 1971). The positive lupus dog in this study was obviously leucopaenic; the presence of leucocyte autoantibodies has not been reported before in canine systemic lupus erythematosus.

In all the normal control dogs tested, a considerable amount of antiglobulin consumption occurred, probably reflecting bound immunoglobulin on the white blood cells. In the two positive cases (Rh 21 and SLE 3), the consumption was much greater than the controls. A possible explanation for the increased consumption in case Rh 21,other than the presence of autoantibodies on the white cells, is the binding of rheumatoid factor to the leucocytes which is known to occur in human rheumatoid patients (Maini, Lemcke, Windsor and Dumonde 1973). It is difficult to know whether a positive antiglobulin consumption test indicates the presence of cellular autoantibodies or merely indicates an increased globulin binding by white cells.

THE NATURALLY OCCURRING INFLAMMATORY ARTHROPATHIES OF THE DOG.

A Clinical, Pathological and Immunological Study, Including a Consideration of the Comparative Aspects of these Diseases in Man and the Dog.

(IN TWO VOLUMES)

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

in

THE FACULTY OF VETERINARY MEDICINE OF THE UNIVERSITY OF GLASGOW

by

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January 1980.

Dedicated to all those full-time University members of staff (and their wives) who dare to study for a post-graduate degree.

The Moving Finger writes; and having writ,
Moves on; nor all thy Piety nor Wit
Shall lure it back to cancel half a line,
Nor all thy Tears wash out a Word of it.

Omar Khayyam (11th Century).

FORMAT

VOLUME I

(Clinical, Radiographical, Laboratory)

SUMMARY

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REVIEW OF THE LITERATURE

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SECTION VI

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(Microbiological, Histopathological, Immunopathological, Treatment)

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SECTION VIII

MICROBIOLOGICAL ASSESSMENT OF DOGS WITH INFLAMMATORY JOINT DISEASE

Clearly, an infectious agent has not been isolated from any of the common connective tissue diseases, in spite of numerous attempts to isolate such agents. We must, therefore, for the time being reserve judgment - if they are infectious, they remain in spite of our best efforts, immaculate infections.

M. Ziff 1976

BACTERIAL (CULTURAL) STUDIES - METHODS

These were carried out by Dr. M. Grindlay, Dept. of Veterinary Pathology, University of Glasgow.

1. SYNOVIAL FLUID AND SYNOVIAL MEMBRANE

Synovial fluid smears were made as previously described (Section IV) and stained by the Gram's method. Impression smears were made from the synovial membrane material and similarly stained. Light microscopy was used to examine the smears for bacterial organisms.

Several different agar plates were inoculated with synovial fluid and/or synovial membrane from each case examined. With synovial fluid, a drop was placed into the centre of the plate and then spread over the surface with a sterile loop. Synovial membrane was rubbed over the surface of the agar and the deposited material was then spread with a loop. In some cases, the synovial membrane was macerated in sterile phosphate buffered saline using a sterile mortar and pestle, and some of the collected material transferred onto the agar surface and spread. The following agar plates were inoculated:-

(a) Horse blood agar (Oxoid Ltd., Wade Road, Basingstoke, Hants.), anaerobic conditions.

(b) Sheep blood agar (Oxoid Ltd.), aerobic conditions.

(c) Chocolate agar (Oxoid Ltd.), carbon dioxide enriched atmosphere.

(d) MacConkey agar (Oxoid Ltd.), aerobic conditions.

(e) Diagnostic Sensitivity Test agar (Oxoid Ltd.), for antibiotic sensitivity.

All plates were incubated at 37°C for five days and examined at intervals. If any growth occurred, subculturing was carried out for antibiotic sensitivity testing and for identification of the organism according to the methods of Cowan and Steel (1966). If a Streptococcus was isolated, subculturing into Lancefield Dextrose Broth (Oxoid Ltd.) was performed and the Lancefield Grouping determined on organisms obtained from this, using antisera to Lancefield Groups A, B, C, D, E, F and G (supplied by Wellcome Reagents Ltd., Berkhampstead, Herts.).

2. BLOOD

A Trypticase Soy Broth(a soybean casein digest medium) (Becton Dickinson & Co., Cockeysville, Maryland 21030, U.S.A.) was used. Sixty milligrams of sodium citrate (anticoagulant) were added to 50ml of the broth. To this was added 5ml of blood collected under aseptic conditions, from the cephalic or jugular veins. The broth was incubated at 37°C under aerobic conditions for five days. If any opacity developed, smears were made and stained by the Gram technique and subcultures were made onto the agar plates as described above. If after five days no growth was obvious, inoculation of the various agar plates was still done.

3. OTHERS

Occasionally, other material such as heart valve lesions,body fluid such as urine and swabs taken from infected foci were cultured and examined. Smears were made from these and stained by Gram's method and cultures were prepared on the various agar plates as in (1)above.

BACTERIAL (SEROLOGICAL) STUDIES - METHODS

These were performed by Dr. D. Taylor, Dept. of Veterinary Pathology, University of Glasgow. The following tests were carried out on serum samples taken from a selection of dogs, from each of the five different groups.

1. ERYSIPELOTHRIX INSIDIOSA ANTIBODIES

The antigen was prepared from an isolate of *E. insidiosa* of porcine origin which had been maintained on agar slopes since isolation from a clinical case of arthritis in a boar. Subculturing onto blood agar (Oxoid Ltd.) was carried out and smooth colonies of *E. insidiosa* from the blood agar were inoculated into Trypticase Soy Broth (Difco Laboratories Inc., 920 Henry Street, Detroit, Michigan, U.S.A.) and incubated for 24 hours at 37°C. Samples from the incubated broth were examined by phase contrast microscopy for the presence of clumped cells. If no clumping was observed, the cultures were mixed and formaldehyde added at the rate of one drop per 4ml (Van der Schaaf and Kramer-Zeeuw 1968). The antigen was stored at 4°C and was standardised against an antiserum of known potency (titre 1:6400). This antiserum was obtained from Difco Laboratories Inc. when their *E. insidiosa* test was available. The test was carried out in tubes with equal volumes of antigen and test serum dilution. Prepared tubes were incubated at 42°C for 4 hours and then overnight at 37°C. The method used was that described for the Bacto (Difco Laboratories Inc.) test.

2. LEPTOSPIRA ICTEROHAEMORRHAGIAE AND CANICOLA ANTIBODIES

The microscopic agglutination lysis test was used. The antigens were live L. icterohaemorrhagiae and L. canicola grown in E.M.J.H. medium (Difco Laboratories Inc.). The method was that of Schuffner as described by Wolff (1954).

3. BRUCELLA ABORTUS AND CANIS ANTIBODIES

B. abortus antibodies were detected using:

(a) the Rose Bengal Plate Test with the Rose Bengal stained antigen supplied by the Central Veterinary Laboratory,Weybridge.

(b) the Standard Agglutination Test again using antigen supplied by the Central Veterinary Laboratory

B. canis antibodies were detected using the Rose Bengal Plate Test (Canine Brucellosis Diagnostic Test, C-Vet, Pitman Moore Inc., Washington Crossing, New Jersey, U. S. A.).

B. canis antibodies were also detected using the tube agglutination test. The antigen was strain 6-66, obtained from the Central Veterinary Laboratory. It was prepared by the following method, provided by the Central Veterinary Laboratory and adapted from that described by Carmichael and Bruner (1968). One colony was inoculated into Brucella broth and incubated at 37°C for 48 hours. A chocolate agar plate streaked with the broth and incubated at 37°C for 48 hours revealed only typical B. canis colonies which on Gram staining were composed of small Gram-negative rods. Two millilitres of the 48 hour suspension were

pipetted into each of twenty, 8oz. prescription bottles with 50ml of Bacto Blood Agar Base (Difco Laboratories Inc.) layered on one side. The bottles were tilted to ensure inoculation of all the agar surface and then incubated at 37°C for 48 hours. The bacterial growth was harvested by adding 3ml of 0.1M phosphate buffered saline with 0.01% thiomensalate. Gentle shaking removed the growth from the agar surface and it was not found necessary to use glass beads. The suspension was then filtered through six layers of gauze to remove pieces of agar and centrifuged at 2000rpm for 20 minutes. The bacterial organisms were washed with phosphate buffered saline and the centrifuging repeated. The organisms were resuspended in a small amount of phosphate buffered saline and heated, in a prescription bottle, in a water bath at 60°C for one hour. This constituted the stock antigen and was stored at 5°C. A chocolate agar plate was streaked with the stock antigen and no growth was apparent after incubation at 37°C for 48 hours. A Gram stain of the stock antigen revealed only small Gram negative coccobacilli. The antigen was diluted with phosphate buffered saline to give a suspension which permitted 53% light transmission at a wavelength of 520mμ. This suspension was stored at 15°C as the working antigen.

0.5ml of the working antigen was placed into each tube with serial dilutions of the test serum and incubated at 37°C and examined at 24 and 48 hours. A titre of 1:200 or greater was considered significant.

4. STREPTOLYSIN O ANTIBODIES

Examination for antibodies against Streptolysin O was carried

out using the Wellcome Reagents for Antistreptolysin O titrations (Wellcome Reagents Ltd., Berkhampstead, Herts.). The method used was Method 1 described in the reagent test kit. An arbitrary figure of 1:200 was considered as a significant level for the dog - it was that indicating recent or active infection in man. Group G Streptococci are those most commonly found in the dog and these are known to produce Streptolysin O (Wilson and Miles 1964).

MYCOPLASMAL STUDIES - METHODS

These were carried out by Dr. E. M. Allan, Department of Veterinary Pathology, University of Glasgow. The techniques used were the same as those described by Allan (1976) and are only briefly summarised here.

Liquid and solid media were used and the following six preparations were standard:-

(a) Glucose-serum broth

(b) Glucose-serum agar

(c) U 3 broth

(d) U 3 agar

(e) Arginine broth

(f) Arginine agar.

Samples of synovial fluid or small pieces of synovial membrane were placed in each of the three broth media. The broths were incubated at 37°C for up to three weeks before being discarded as negative. Sub-culture onto solid media was carried out where growth in the broth was indicated (i.e. by a change of pH which altered the colour of the indicator in the medium), although blind passage after approximately three and seven days incubation was often done. All agar plates were incubated at 37°C in a moist atmosphere of 5% carbon dioxide in air or nitrogen. Agar plates were examined frequently, using a Watson Barnet plate microscope. Plates were discarded as negative after 14 days incubation.

Methods for the identification of mycoplasmas included indirect immunofluorescent, growth inhibition and metabolism inhibition techniques

(all employing specific anti-mycoplasma sera obtained from FAO/WHO International Mycoplasma Reference Centre, Aarhus, Denmark) and also the assessment of their biochemical properties.

VIRAL STUDIES - METHODS

These were carried out by Professor O. Jarrett, Department of Veterinary Pathology, University of Glasgow.

Pieces of synovial membrane were washed, under sterile conditions, in cell culture medium (Eagle's Minimal Essential Medium with 10% foetal bovine serum - EFB) and finely minced using sterile scissors. The fragments were placed in 5cm plastic plates (Nunclon) with 4ml of EFB and were incubated at $37^{o}C$ in an atmosphere of 5% carbon dioxide in air.

In two cases there was no growth in the primary cultures within 7 days and the plates were discarded. In the remainder, fibroblastic cells spread out from the tissue fragments and formed colonies. The medium was replaced on the third day and after 7 days the cells were removed by treatment with a trypsin-EDTA solution and were seeded into further plates, the number depending on the quantity of cells obtained. At this stage 4 of the cultures were found to be contaminated with yeasts, moulds or bacteria. The remaining 15 were cultured for a further 7 days and were examined for the occurrence of any cytopathic effect which might have been due to the presence of a virus. Four of these cultures were then subcultured again and 3×10^{5} cells were seeded into a 5cm plate. After two days when the cells were semi-confluent they were processed for electronmicroscopy. The cells were scraped off into the medium and were pelleted by centrifugation at 1000 rpm for 5 minutes. The supernatant fluid was removed and a 2% solution of paraformaldehyde:glutaraldehyde in cacodylate buffer was added. After fixation for 4½ hours the pellet was chopped up and rinsed overnight in cacodylate buffer.

The fragments were then post-fixed in 1% osmium tetroxide, dehydrated in graded alcohol and embedded in Araldite. Thin sections were prepared on a LKB Ultratome and were examined in an AEI EM 801 electronmicroscope for the presence of viral particles. The technique used was that normally followed for the detection of C-type retroviruses.

DOGS EXAMINED

Cultural examinations for bacteria and mycoplasmas were carried out on synovial fluid samples and/or synovial membrane biopsies/autopsies from all 82 dogs. In many cases synovial fluid samples were examined initially but generally a synovial membrane biopsy was also examined and this was certainly the case if the synovial fluid was negative for bacterial organisms. In those dogs with multiple joint inflammation, synovial fluid from several different joints was usually examined although it was rare for more than one synovial membrane biopsy to be taken from any one dog. Further bacteriological studies were carried out in some animals which died or were destroyed, especially in Group IV and some of these are recorded in Section IX.

Blood cultures were performed in the majority of cases showing pyrexia and bacteriological examinations of other body fluids and other tissues were performed in cases where there was some indication for so doing,e.g. cultures were made of the endocarditis lesions of all the dogs in Group IV.

The serological investigations for evidence of bacterial infections were only performed in a selected number of cases as identified in Table 41.

Viral studies were only commenced in the later stages of the study and were thus only performed in a few of the total number of dogs but did include cases from each of the five groups (8 from Group I, 1 from Group II, 2 from Group III, 1 from Group IV and 10 from Group V) (Table 42). In addition, 5 dogs with osteoarthritis were also examined.

Most of the samples for microbiological examination were taken

prior to the use of any drug treatment (including antibiotics) although several dogs when presented had received some form of antimicrobial therapy from the referring veterinary surgeon. A few days were allowed to elapse before sampling was undertaken in these cases, providing the condition of the animal allowed.

Synovial fluid samples were taken as described in Section IV. Synovial membrane biopsies were collected by open joint surgery with the dog under general anaesthesia. The latter was induced and maintained as already described (Section IV) and the skin around the joint to be sampled was clipped and cleaned as previously described (Section IV) although a larger prepared area was necessary for biopsy collection.

RESULTS

The results of the microbiological investigations are shown in Tables 38-42.

GROUP I (RHEUMATOID)

No bacterial organisms were cultured from the joints of these dogs. A Mycoplasma species was cultured from the synovial membrane of the right stifle joint of dog Rh 7. In one dog (Rh 18) Gram-positive rods were seen in a smear of the synovial fluid from the left elbow joint. Staphylococcus aureus was isolated from a nasal swab taken from dog Rh 8. A non-haemolytic Streptococcus was cultured from the vaginal discharge of case Rh 17 and Staphylococcus was isolated from the urine of two dogs (Rh 9 and Rh 12) but in both cases contamination was suspected.

Significant levels of serum antibody to the organisms listed above (Table 41) were not found in the dogs of this group except for an antibody titre of 1:80 against Erysipelothrix insidiosa in dog Rh 17.

No evidence for a virus infection of the synovial membrane was found in dogs Rh 12, 13, 15, 17, 20 and 21.

GROUP II (SYSTEMIC LUPUS ERYTHEMATOSUS)

All investigations were negative.

GROUP III (INFECTIOUS)

Bacterial organisms were found in the affected joint(s) of all 12 dogs but this was the criterion used for the identification of this group of animals.

A β-haemolytic Streptococcus of Lancefield Group G was cultured from 4 dogs (INF 1, 5, 10 and 11) and in one of these dogs (INF 1) in which two joints were found to be infected at different times, the Streptococcus was isolated in both cases although a second organism, an unidentified Gram-negative coccus, was also isolated from one of the joints. Staphylococcus aureus was isolated from 4 dogs (INF 3,4,7 and 8), Corynebacterium or diphtheroid-like organisms from 3 dogs (INF 2, 9 and 12) and Pasteurella multocida from 1 dog (INF 6). Dog INF 3 had clinical disease in two joints but only the left stifle was examined bacteriologically.

Organisms were isolated on culture of the synovial membrane from 2 dogs (INF 3 and 9)although none were demonstrated in the synovial fluid. Whenever synovial fluid and synovial membrane cultures were both positive, the same organism was always isolated in each case. Infection with a single organism only was generally the rule except for the left hock joint of INF 1 where two organisms were isolated.

Blood cultures were not made in every dog but in case INF 3 a positive culture was obtained yielding the same organism (Staphylococcus aureus) as found in the joint; the same bacterium was also isolated from the urine of this dog.

Culture of the vaginal discharge in case INF 2 yielded three different organisms (β-haemolytic Streptococcus, Staphylococcus species and mycoplasma species) to that cultured from the joint. In case INF 7, Staphylococcus aureus, as well as being found in the joint, was cultured from the right ilium and surrounding pelvic musculature and the same organism was also cultured from the periarticular tissues of case INF 10 as well as from within the joint. Culture of the vaginal discharge of case INF 8 yielded a mycoplasma and culture of the proximal right humerus of dog INF 12 demonstrated the same organism as was present in the joint (a Corynebacterium).

It is interesting that dog INF 1 developed a persistent synovitis with lameness, in the left hock joint following prolonged antibiotic therapy after the initial diagnosis. Repeated synovial fluid(3 samples) and membrane (1 specimen) cultures were negative for bacterial organisms at this time (after antibiotic therapy had been discontinued) and the inflammation was controlled by long-term corticosteroid therapy. The joint inflammation appeared to persist in the absence of viable organisms.

Serological examinations demonstrated low titres of antibodies against Streptolysin O (1:50) in two dogs (INF 1 and 11). Streptococcus was isolated from the joints of both these dogs. Neither of the other two dogs infected with Streptococci were examined for anti-Streptolysin O titres. An antibody titre of 1:160 against E.insidiosa was recorded in dog INF 11.

Virus examinations were only done in dogs INF 1 and 9 and were negative in both cases.

GROUP IV (BACTERIAL ENDOCARDITIS)

Bacterial organisms were cultured from one or more joints of all but two of these dogs. In dog BE 1 the synovial fluid from four joints was negative on culture and in dog BE 5 both synovial fluid and synovial membrane from the right hock joint were negative on culture. In addition, synovial fluid or synovial membrane samples taken at post-mortem examination were examined from other joints of all the dogs in this group and some of these were negative even though the joints were clinically and/or pathologically diseased (see Sections II and IX). It should be noted that most dogs in this group had received antibiotics prior to death which may have affected the post-mortem bacteriological investigations.

Culture of the heart lesions in these dogs always revealed bacterial organisms which matched those isolated from the joints. The organisms cultured from the heart were β-haemolytic Streptococci of Lancefield Group G (BE 3 and 5), Escherichia coli (BE 2 and 7), Erysipelothrix insidiosa (BE 6), Staphylococcus aureus (BE 4), and Pasteurella pneumotropica and multocida (BE 1). Dog BE 2 suffered two joint "attacks"; during the first a β-haemolytic Streptococcus was isolated from the joint and blood and during the second "attack" E.coli was found in the joint and blood and corresponded to the organism found on the heart lesion at post-mortem examination. Blood cultures were also positive in cases BE 2, 4 and 5 (Table 39). No examination of the blood was carried out in dogs BE 6 and 7. Urine was only cultured in one dog (BE 4) and was positive for the same organism as found in the right stifle joint and on the heart lesion; the same organism was also detected in a kidney abscess, an omental abscess and a mesenteric abscess.

Serological examinations were only performed in three dogs. One dog (BE 1) showed a titre of 1:300 against L.icterohaemorrhagiae and another (BE 2) had a very high titre of 1:30,000 against the same bacterium. Both these dogs had very low titres (1:30) against L.canicola. Case BE 2 was sampled for serological examination during its second "attack" and did not show any antibodies against Streptolysin O even though a Streptococcus was isolated during the first "attack". Case BE 3 which again suffered a Streptococcus infection, did not show antibodies against Streptolysin O.

Only one dog (BE 2) was examined for viruses and the growth of E.coli in the culture medium interfered with this examination.

GROUP V (IDIOPATHIC)

All the joints examined from the dogs in this group were negative for bacterial organisms but a Mycoplasma species was cultured from the synovial fluid of the left elbow joint of dog ID 33. Bacterial organisms were seen in Gram-stained smears of synovial fluid from two dogs (ID 4 and 21). These organisms were Gram-positive in dog ID 4 but appeared damaged and failed to take up the stain properly, and Gram-negative in dog ID 21.

Certain other tissues and fluids were also examined from these dogs. Swabs from the tonsils of dog ID 4 showed Staphylococcus aureus and many other bacteria, from those of ID 22 yielded β-haemolytic Streptococcus and Pasteurella-like organisms and those from ID 23 gave a mixed bacterial growth with no obvious pathogens. A mycoplasma was isolated from the urine of ID 6 and β-haemolytic Streptococcus (Lancefield Group G),Staphylococcus,E.coli and other coliforms were isolated from the

urine of ID 14. Sarcina was isolated from a swab taken from the prepuce of dog ID 23 and Gram-positive bacilli were demonstrated in the blood cultures of cases ID 20 and 36. These latter may have been contaminants. Haemolytic E.coli was isolated from a pyelonephritic kidney taken from dog ID 17 during post-mortem examination. The same organism was also isolated from the bladder epithelium of this dog; an obvious cystitis was present. Ziehl-Neelson positive organisms were identified in several tissues of dog ID 22. Although no cultures were made, these organisms could have represented Mycobacterium tuberculosis. Organisms were cultured from tooth root abscesses in two cases, ID 27 and 32 - E.coli and Actinomycetes in the former and Staphylococcus and diphtheroid-like organisms in the latter.

Several dogs in this group were examined serologically but no significant results were recorded.

Viral studies in six dogs (ID 14, 22, 23, 25, 32 and 36) were negative.

TABLE 38

SUMMARISING THE RESULTS OF THE BACTERIOLOGICAL CULTURES CARRIED OUT ON THE GROUP III(INFECTIOUS) DOGS.

ND - not done.

		MATERIAL CULTURED				
		SYNOVIAL FLUID	SYNOVIAL MEMBRANE	BLOOD	URINE	OTHER
DOG IDENTIFICATION	INF 1	β-haemolytic Streptococcus, Lancefield Group G and Gram negative coccus (L hock) β-haemolytic Streptococcus, Lancefield Group G (R stifle)	ND ND	Neg. Neg.	Neg. Neg.	
	INF 2	Gram positive bacillus (Diphtheroid-like) (L hip)	ND	Neg.	ND	Vaginal discharge - β-haemolytic Streptococcus, Lancefield Group G, Staphylococcus, Mycoplasma spp.
	INF 3	Neg. (L stifle)	Staphylococcus aureus (L stifle)	Staphylococcus aureus	Staphylococcus aureus	
	INF 4	Staphylococcus aureus (L carpus)	Staphylococcus aureus (L carpus)	Neg.	ND	
	INF 5	β-haemolytic Streptococcus, Lancefield Group G (R carpus)	ND	ND	ND	
	INF 6	Pasteurella multocida (L stifle)	Pasteurella multocida (L stifle)	Neg.	ND	
	INF 7	ND	Staphylococcus aureus (R hip)	Neg.	ND	R ilium + surrounding musculature- Staphylococcus aureus
	INF 8	Staphylococcus aureus (L elbow)	Staphylococcus aureus (L elbow)	Neg.	ND	Vaginal discharge - Mycoplasma spp.
	INF 9	Neg. (L shoulder)	Gram positive bacillus (Diphtheroid-like) (L shoulder)	ND	ND	
	INF 10	β-haemolytic Streptococcus, Lancefield Group G and Staphylococcus aureus (L elbow)	β-haemolytic Streptococcus, Lancefield Group G (L elbow)	ND	ND	Periarticular tissues L elbow - β-haemolytic Streptococcus, Lancefield Group G and Staphylococcus aureus
	INF 11	β-haemolytic Streptococcus Lancefield Group G and Staphylococcus aureus (L carpus)	ND	ND	ND	
	INF 12	Corynebacterium spp.	Corynebacterium spp.	ND	ND	Bone proximal R humerus - Corynebacterium spp.

TABLE 39

SUMMARISING THE RESULTS OF THE BACTERIOLOGICAL CULTURES CARRIED OUT ON THE GROUP IV(BACTERIAL ENDOCARDITIS) DOGS.

ND - not done.

See also Section IX.

		MATERIAL CULTURED				
		SYNOVIAL FLUID	SYNOVIAL MEMBRANE	BLOOD	URINE	OTHER
DOG IDENTIFICATION	BE 1	Neg. (L stifle and L shoulder R elbow, R carpus)	ND	Neg.	ND	Heart lesion - Pasteurella pneumotropica and Pasteurella multocida
	BE 2 (1st 'attack')	β-haemolytic Streptococcus, Lancefield Group G (L and R stifle)	ND	β-haemolytic Streptococcus, Lancefield Group G	ND	
	BE 2 (2nd 'attack')	Haemolytic Escherichia coli (R stifle)	Haemolytic Escherichia coli (R stifle)	Haemolytic Escherichia coli	ND	Heart lesion - Haemolytic Escherichia coli
	BE 3	β-haemolytic Streptococcus Lancefield Group G (L and R stifle)	ND	Neg.	ND	Heart lesion - β-haemolytic Streptococcus, Lancefield Group G
	BE 4	Staphylococcus aureus (R stifle)	ND	Staphylococcus aureus	Staphylococcus aureus	Heart lesion, R kidney abscess, omental abscess, mesenteric abscess - Staphylococcus aureus
	BE 5	Neg. (R hock)	Neg. (R hock)	β-haemolytic Streptococcus, Lancefield Group G	ND	Heart lesion - β-haemolytic Streptococcus Lancefield Group G
	BE 6	Erysipelothrix insidiosa (R shoulder)	ND	ND	ND	Heart lesion - Erysipelothrix insidiosa
	BE 7	ND	Haemolytic and non- haemolytic Escherichia coli (L hock, R carpus, L elbow)	ND	ND	Heart lesion - haemolytic and non-haemolytic Escherichia coli

TABLE 40

SUMMARISING THE RESULTS OF THE BACTERIOLOGICAL INVESTIGATIONS CARRIED OUT ON THE GROUP I (RHEUMATOID ARTHRITIS) AND GROUP V (IDIOPATHIC) DOGS.

Most of the investigations in these groups of dogs were negative.

A Mycoplasma species was isolated from a single joint of dog Rh 7 and from a single joint of dog ID 33.

DOG IDENTIFICATION	Rh 8	Nasal Swab - *Staphylococcus aureus*
	Rh 9	Urine - *Staphylococcus epidermidis* (? contamination)
	Rh 12	Urine - *Staphylococcus* (? contamination)
	Rh 17	Vaginal swab - mixed growth, mainly non-haemolytic *Streptococcus*
	Rh 18	Smear synovial fluid, L elbow - Gram positive rods. No growth on culture
	ID 4	R. tonsil swab - *Staphylococcus aureus* + various other bacteria Smear synovial fluid, L elbow - Gram positive rods, damaged and failed to take up stain. No growth on culture
	ID 6	Urine - *Mycoplasma spp.*
	ID 14	Urine - lactose fermenting coliforms, β-haemolytic *Streptococcus*, Lancefield Group G, non-haemolytic *Escherichia coli*, *Staphylococcus*
	ID 17	P.M.E., pyelonephritis (L kidney) + cystitis - haemolytic *Escherichia coli*, lactose fermenting coliforms.
	ID 20	Blood culture - haemolytic and non-haemolytic Gram positive bacilli (? contamination)
	ID 21	Smear synovial fluid L stifle - Gram negative rods. No growth on culture
	ID 22	L tonsil swab - β-haemolytic *Streptococcus*, Lancefield Group G, and Pasteurella - like organism P.M.E., Ziehl-Neelsen positive organisms identified in several tissues. No culture attempted
	ID 23	L and R tonsil swabs - mixed bacterial growth, no obvious pathogens Preputial swab - *Sarcina*
	ID 27	Tooth root abscesses - haemolytic *Escherichia coli* and *Actinomycetes*
	ID 32	Tooth root abscess - *Staphylococcus* and Gram positive rods (Diphtheroid-like)
	ID 36	Blood culture - Gram positive bacilli (? contamination)

TABLE 41

SUMMARISING THE RESULTS OF THE SEROLOGICAL INVESTIGATIONS FOR EVIDENCE OF BACTERIAL INFECTIONS IN DOGS WITH INFLAMMATORY JOINT DISEASE.

A negative score means no antibodies were detected. The dilutions are shown in cases where antibodies were detected.

ND - not done

RBPT - Rose Bengal Plate Test.

ANTIBODIES AGAINST	DOG IDENTIFICATION																		
	Rh 3	Rh 4	Rh 6	Rh 8	Rh 9	Rh 10	Rh 12	Rh 17	Rh 18	Rh 21	SLE 3	INF 1	INF 3	INF 4	INF 6	INF 11	BE 1	BE 2	BE 3
BRUCELLA CANIS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BRUCELLA ABORTUS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-
LEPTOSPIRA CANICOLA	-	-	ND	1:30	ND	-	ND	-	-	-	-	-	-	-	-	-	1:30	1:30	-
LEPTOSPIRA ICTEROHAEMORRHAGIAE	-	1:30	ND	-	ND	-	ND	-	1:30	-	-	-	-	-	-	-	1:300	1:30000	-
ERYSIPELOTHRIX INSIDIOSA	-	-	-	-	-	-	-	1:80	-	-	-	-	-	-	-	1:160	-	-	-
STREPTOLYSIN O	-	-	1:12	-	-	-	1:12	-	-	1:12	-	1:50	<1:12	-	<1:12	1:50	-	-	-

ANTIBODIES AGAINST	DOG IDENTIFICATION																		
	ID 1	ID 4	ID 7	ID 13	ID 14	ID 17	ID 18	ID 19	ID 20	ID 21	ID 22	ID 23	ID 25	ID 27	ID 29	ID 31	ID 32	ID 33	ID 36
BRUCELLA CANIS	-	-	-	-	-	-	-	-	-	-	-	<1:10	-	-	-	-	-	-	<1:10
BRUCELLA ABORTUS	-	-	-	-	-	-	-	-	-	-	-	<1:10	-	-	-	-	-	-	<1:10
LEPTOSPIRA CANICOLA	-	-	-	-	-	ND	ND	-	-	ND	-	-	-	-	-	-	-	-	ND
LEPTOSPIRA ICTEROHAEMORRHAGIAE	-	-	-	-	-	ND	ND	-	-	ND	-	-	-	-	-	-	-	-	ND
ERYSIPELOTHRIX INSIDIOSA	-	-	-	1:10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
STREPTOLYSIN O	-	-	-	1:12	-	-	-	1:12	-	-	-	1:12	-	-	1:12	-	-	-	-

* Inconclusive reaction with RBPT.

DOG	CULTURE	CYTOPATHIC EFFECTS	ELECTRONMICROSCOPY
Rh12	Contaminated	N/A	N/A
Rh13	Growth	None	Negative
Rh15	Growth	None	Negative
Rh17	Growth	None	Negative
Rh20	Growth	None	Negative
Rh21	Growth	None	ND
SLE 3	Growth	None	Negative
INF 1	Growth	None	ND
INF 9	Growth	None	Negative
BE 2	Contaminated	N/A	N/A
ID14	Contaminated	N/A	N/A
ID22	Growth	None	Negative
ID23	Contaminated	N/A	N/A
ID25	Growth	None	Negative
ID32	Growth	None	ND
ID36	Growth	None	ND
Osteoarthritis	No Growth	N/A	N/A
Osteoarthritis	Growth	None	ND
Osteoarthritis	Growth	None	ND
Osteoarthritis	No Growth	N/A	N/A
Osteoarthritis	Growth	None	ND
Osteoarthritis	Growth	None	ND

N/A - Not applicable ND - Not done

TABLE 42. SUMMARISING THE RESULTS OF THE VIRAL INVESTIGATIONS OF DISEASED SYNOVIUM FROM DOGS WITH INFLAMMATORY JOINT DISEASE AND OSTEO-ARTHRITIS.

DISCUSSION

BACTERIAL INFECTIONS

1. General

Most of the dogs with inflammatory joint disease were negative for bacterial infections, only those dogs in Groups III and IV were positive. However, it should be stressed that several factors can affect the ability to culture organisms from infected tissues and thus a negative culture cannot be taken as absolute proof of a non(bacterial) -infectious aetiology. A polyarthritis caused by bacterial L-forms has, for example, been identified in dogs (Pedersen and Pool 1978) and these organisms may not be isolated on conventional bacterial media. Also, the use of antibiotics prior to obtaining the specimen for examination can result in a negative finding(Sommers 1978) and the techniques used for obtaining specimens and the speed with which the specimens are examined can all affect the results.

The commonest organism isolated from infected joints in both Groups III and IV was β-haemolytic Streptococcus of Lancefield Group G. Staphylococcus aureus was the next most common and other organisms included E.coli, P.pneumotropica and multocida, E.insidiosa and diphtheroid-like organisms. Pedersen and Pool (1978) reported Staphylococcus and Streptococcus as the two most frequently isolated organisms from infected canine joints. followed by various coliform organisms and Erysipelothrix. All cases in Group III were infected with Gram-positive organisms except for one which was a Pasteurella infection. Relatively more cases were

infected with Gram-negative organisms in Group IV (3/7 dogs).

Staphylococcus and Streptococcus also account for most septic arthritis cases in man (Sommers 1978); other organisms regularly encountered include Haemophilus influenzae, Neisseria gonorrhaeae, enterobacteriaceae and Pseudomonas species. Gram-positive infections in man have always been the more important although in recent years, Gram-negative bacilli have become more significant as causes of septic arthritis (Goldenberg and Cohen 1978). One reason for this, particularly in the USA, has been the increase in the number of intravenous drug users, in particular heroin addicts.

Bacterial endocarditis in man is usually caused by Streptococci but may be caused by one of several Gram-negative organisms (Anon 1979a). S.epidermidis and diphtheroid bacilli have also been incriminated and some non-bacterial microbes can cause endocarditis, e.g. Coxiella burneti, Chlamydia psittaci and trachomatis. A viral aetiology for endocarditis in the human patient has also been postulated especially in those cases with negative blood bacterial cultures. However, there is no good evidence for this in spite of the Coxsackie group of viruses being commonly associated with myocarditis and pericarditis.

2. Methods of Tissue Damage in Bacterial Joint Infections

Several bacteria produce haemolytic and non-haemolytic exotoxins and these can have a significant role in cell and tissue destruction (Tesar and Dietz 1978). Practically all coagulase positive staphylococcal organisms produce one or more haemolysins. Many of these toxins have