THE ROLE OF WEAR PARTICLES IN PROSTHESIS LOOSENING

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SUMMARY

Loosening of total joint prostheses is often associated with the accumulation of prosthesis wear particles in the surrounding tissues, a macrophage and multinucleate giant cell (MNGC) response, and bone resorption. The studies described in this thesis were undertaken to determine the effects of wear particles, released from the articulating surfaces of prostheses, on cells and tissues, and to investigate the role of wear particles in bone resorption and prosthesis loosening. The investigation was divided into four main sections:

1. Initial studies were performed to determine the type and size of wear particles, and the associated cellular response in human tissues around uninfected total hip arthroplasties. The periprosthetic tissues were examined by light microscopy, transmission electron microscopy (TEM), and energy dispersive X-ray (EDX) microanalysis. The type of cellular response seemed to be related to the number, type, and size of wear particles. A macrophage and MNGC response was common in the presence of large numbers of wear particles. The accumulation of macrophages, which contained large numbers of cytolysosomes, was seen in the presence of particles. Lymphocyte aggregates occasionally were seen in association with metal particles. Polymorphonuclear leucocytes (PMN) were sparse.

2. To determine the effect of wear particles on tissues, an animal model was developed using the intra-articular injection of particles. Light microscopy and TEM examination demonstrated that wear particles similar in size to those seen in human periprosthetic tissues produced a similar tissue response in the rat knee. Cobalt-chrome alloy particles

induced a predominant macrophage infiltrate, necrosis of macrophages, and a transient lymphocytic infiltrate. Aluminium oxide particles and small polyethylene particles also induced a macrophage infiltrate, but little necrosis or lymphocytic infiltrate. Large particles of polyethylene produced aggregation of macrophages and an MNGC infiltrate.

3. Further studies, using a semi-quantitative method of assessment of the tissue response to particles, demonstrated that the number of particles and the extent of the associated macrophage infiltrate changed very little from two weeks to one year following the injection of particles. Further, a greater macrophage infiltrate was seen following injection of a high dose suspension of cobalt-chrome alloy particles compared with a low dose suspension, and a significantly greater macrophage response was seen to cobalt-chrome alloy than to aluminium oxide particles. Thus, the tissue response to particles is related to the persistence of particles in the tissues, the type and amount of particulate material, and, possibly, the degree of cell necrosis induced by particles.

4. The relationship between the tissue response to wear particles and bone resorption was studied using an animal model which involved the injection of wear particles into a rat knee joint adjacent to an acrylic cement plug inserted into the distal femur. In the absence of infection or mechanical causes for loosening, the formation of a connective tissue layer and bone resorption between the cement plug and bone occurred following multiple injections of polyethylene particles.

The results of these investigations indicate that prosthesis wear particles are responsible for a macrophage and MNGC response in the periprosthetic tissues and play a major role in bone resorption and loosening of prostheses.

DECLARATION

This thesis contains no material which has been accepted or published for the award of any other degree or diploma in any University. Furthermore, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of this thesis.

I hereby give consent for this thesis to be made available for photocopying and loan.

Signed.

Donald W. Howie,

30th May, 1987

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LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Materials
cement	acrylic cement, polymethylmethacrylate
EDX	energy dispersive X-ray
HE	hematoxylin and eosin
mg	milligram(s)
ml	millilitre(s)
mm	millimetre(s)
MNGC	multinucleate giant cell(s)
PMMA	polymethylmethacrylate
TEM	transmission electron microscopy
PMN	polymorphonuclear leucocyte(s)
UHMWP	ultra-high molecular weight polyethylene
VK	Von Kossa

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Total joint arthroplasty has an established place in the management of patients with arthritis and, in particular, arthritis of the hip and related conditions, but the variety of prostheses available testifies to the problem of joint replacement failure.

The commonest cause of failure of total hip and other arthroplasties is loosening of the prostheses. Long-term reviews of the results of total hip arthroplasties report revision rates due to loosening during the first five to ten years following arthroplasty of approximately one percent per year, and an increase in loosening with time (Charnley and Cupic, 1973; Smith and Turner, 1973; Amstutz et al, 1976; Beckenbaugh and Ilstrup, 1978; Cupic, 1979; Dobbs, 1980; Stauffer, 1982; Sutherland et al, 1982; Poss et al, 1984). Loosening is also the commonest cause of failure of arthroplasties of other joints (Crachiolo et al, 1979). The loosening rate has been reported to be higher in younger patients (Dorr et al, 1983; Collis, 1984) but this has not been a consistent finding (Thomas et al, 1986).

The causes of loosening may be classified into mechanical (Amstutz et al, 1976; Pellicci et al, 1979; Gruen et al, 1979) and biological (Fitzgerald and Kelly, 1979). Mechanical loosening may be due to inadequate bone, inappropriate materials and prosthetic design, failure to achieve or maintain fixation at the bone-implant interface, and excessive stress or stress shielding (Huiskes, 1984). Improvements in

design and improved methods of achieving interlock of the implant in bone have decreased the loosening rate of total hip arthroplasties (Lee et al, 1973; Ling, 1979; Salvati et al, 1981; Harris et al, 1982; Harris, 1984; Paterson et al, 1986) and future developments are likely to further decrease the incidence of mechanical loosening.

Despite the decrease in the incidence of loosening due to mechanical causes, late loosening associated with extensive bone resorption remains a problem. It is likely that biological causes of loosening significantly contribute to late loosening. Of the biological causes, infection is the most worrying (Charnley and Eftekhar, 1969; Wilson et al, 1973; Coventry, 1975; Salvati, 1976; Fitzgerald et al, 1977; Hunter and Dandy, 1977; Buchholtz et al, 1979), but infection has become a less common complication of hip (Kamme and Lindberg, 1981; Gristina and Kolkin, 1983; Poss et al, 1984) and other arthroplasties (Grogan et al, 1986) in the last decade. There is substantial evidence to implicate the adverse tissue response to prostheses wear particles as the other important biological cause of loosening (Vernon-Roberts and Freeman, 1976; Revell et al, 1982).

The common prosthesis materials used over the past twenty years have been cobalt-chrome alloys, stainless steel, ultra-high molecular weight polyethylene (UHMWP), and aluminium oxide ceramics. Recently titanium alloys have gained popularity. Polymers of other types have been used less commonly.

The use of these materials as the articulating surfaces of prostheses results in liberation of variable numbers of wear particles (Mears et al, 1978a; Clarke, 1982; Mirra et al, 1982) into the surrounding tissues. The use of cobalt-chrome alloy articulating against itself results in moderate wear (Walker et al, 1974) and the accumulation of

large numbers of metal wear particles in the tissues (Winter, 1974; Vernon-Roberts and Freeman, 1977). Initial experience with various types of polymers also resulted in severe wear (Newman and Scales, 1951; Heck and Chandler, 1954; Scales, 1958; Charnley, 1963; Heilmann et al, 1975) and UHMWP remains prone to wear if used as a convex bearing surface (Dahl, 1976; Revell et al, 1978). Aluminium oxide ceramic may be more resistant to wear (Boutin and Blanquaert, 1981).

While an adverse tissue response to wear particles has been frequently reported, the mechanism whereby wear particles provoke loosening has remained obscure. Therefore, the role of wear particles in loosening, and the possibility of other factors being involved requires further investigation.

This thesis describes animal studies aimed at providing a better understanding of the relationship between wear particles and prosthesis loosening. The animal studies have concentrated on the tissue response to particles of materials commonly used as the articulating surface of prostheses: cobalt-chrome alloy, UHMWP, and aluminium oxide ceramic. In addition, human studies were performed to determine the types of wear particles and the tissue response around failed hip arthroplasties, and to relate these findings to the animal studies.

1.2 HISTORICAL REVIEW OF HIP ARTHROPLASTY

Hip arthroplasty began in the nineteenth century with simple excisional arthroplasty, which was followed by the development of various interposition arthroplasties. Materials used for interposition were those of either biological origin, such as fascia and muscle, or those of non-biological origin. In general, interposition of biological materials gave poor results which led to a search for better interpositional materials (Black and Sholtes, 1982).

The most encouraging results of early prosthetic hip arthroplasty were reported by Smith-Petersen. Initially a glass mould arthroplasty was used but, due to problems with glass breakage, a more successful cobalt-chrome alloy arthroplasty was developed (Smith-Petersen et al, 1947; Smith-Petersen, 1948). Despite the relative success of this arthroplasty, the results were often unpredictable. Stiffness, displacement of the cup, and shortening of the neck of the femur were common problems (Law, 1948; Aufranc, 1957).

Total femoral head replacement seemed a logical development of interposition arthroplasty and was popularized by Judet and Judet (1950), who developed an acrylic hemi-arthroplasty. But loosening and fracture of the component became problems (Merle-d'Aubigne and Postel, 1954). Further developments of this principle led to stemmed cobalt-chrome alloy femoral hemi-arthroplasties. Good results were achieved in post-traumatic cases (Barr et al, 1964), and fair results have been achieved in arthritic hips (Salvati and Wilson, 1973).

Charnley (1960), McKee and Chen (1973), and Muller (McKee, 1982) are credited with the development of successful total hip prostheses which utilized an acetabular component of polyethylene or metal and a metal

femoral component inserted into the metaphysis of the femur. Acrylic cement (polymethylmethacrylate, PMMA) was developed to fix both components in bone (Haboush, 1953) and its use was popularized by Charnley (1960). Development of modern day prostheses has largely been based on this concept. But an increasing loosening rate with time, and problems associated with acrylic cement failure, have caused renewed interest in cementless fixation of prostheses.

Cementless fixation of metal or polyethylene acetabular components and metal femoral stem components has been used successfully for many years by Judet (Judet et al, 1978), Ring (Ring, 1974, 1978) and others (Groher, 1983). A number of prostheses have also been developed which use cementless microporous fixation of metal components (Cameron and Pilliar, 1974).

Aluminium oxide ceramic total hip prostheses were developed as it was claimed the ceramic, as an articulating surface, had a number of advantages over metal and polyethylene (Boutin, 1972; Griss et al, 1975; Heimke et al, 1979). Ceramics have been reported to be more biocompatible in solid form than stainless steel or cobalt-chrome alloys, and to have a very low coefficient of friction and a low wear rate (Boutin and Blanquaert, 1981). The clinical results of ceramic prostheses in the short term have been reported to be as good as the results of prostheses of metal and polyethylene (Boutin, 1972; Zweymuller, 1979; Stock et al, 1980; Griss and Heimke, 1981; Rampoldi, 1984), but a high incidence of femoral loosening of uncemented ceramic prostheses has been reported recently (Trepte et al, 1985; O'Leary and Mallory, 1986).

At the same time as these developments in total hip replacement, attempts were made to improve upon the results of the mould arthroplasty

by preserving the femoral head. This led to the development of double cup resurfacing arthroplasties. The early results of cemented and cementless resurfacing arthroplasties were encouraging (Trentani and Vaccarino, 1978, Tanaka, 1978; Wagner, 1978; Amstutz et al, 1977, 1978, 1981; Bierbaum and Sweet, 1982). Inappropriate design and choice of materials led to problems with some types of double cup resurfacing arthroplasties using polyethylene femoral head components (Freeman et al, 1978; Revell et al, 1978; Furuya et al, 1978).

Amstutz et al (1984) reported satisfactory clinical results with resurfacing hip arthroplasty compared to total hip arthroplasty in a similar group of patients, but a much higher incidence of acetabular bone-cement radiolucency was seen in the resurfacing group. Ritter and Gioe (1986) reported a thirteen percent failure rate of resurfacing hip arthroplasties compared to a two percent failure rate of total hip arthroplasties of the opposite hip in patients followed for up to seven years. Loosening was the major cause of failure.

With longer follow-up, the failure rate of resurfacing hip arthroplasties has increased significantly. Failures have been attributed to acetabular component loosening (Freeman and Bradley, 1982), fracture of the femoral neck (Capello et al, 1978; Freeman et al, 1978), femoral component loosening (Trentani and Vaccarino, 1978), excessive wear of components (Revell et al, 1978), osteonecrosis of the femoral head (Trentani and Vaccarino, 1982; Nishio et al, 1982; Head, 1981), and an adverse response to wear particles (Revell et al, 1978; Bell et al, 1985).

The question remains as to whether the high incidence of failure, and particularly late loosening of resurfacing arthroplasties, implies inherent problems with this technique alone, or whether there are

problems with all types of arthroplasties which will lead to late loosening. One of these problems may be the adverse effect of wear particles on tissues. If wear particles are an important cause of loosening of arthroplasties loosening might be decreased by appropriate choice of materials for articulating surfaces and improved design of arthroplasties to minimise wear. Loosening might also be decreased by development of chemotherapeutic agents which modify the effects of particles on tissues or decrease bone resorption. Clearly an understanding of the relationship between wear particles and prosthesis loosening is important.

1.3 THE CAPSULE AND BONE-IMPLANT INTERFACE AROUND STABLE PROSTHESES

1.3.1 The capsule

The joint capsule around articulating metal on metal or metal on polyethylene prostheses has been studied extensively by a number of authors (Charosky et al, 1973; Evans et al, 1974; Winter, 1976; Vernon-Roberts and Freeman, 1976; Mirra et al, 1976; Willert and Semlitsch, 1976). The joint is lined by a synovial like membrane and capsule. The subsynovium and capsule show varying degrees of fibrosis and are infiltrated by macrophages and multinucleate giant cells (MNGC), and there is accumulation of wear particles and corrosion products. Occasional lymphocytes, plasma cells and eosinophils are seen. Polymorphonuclear leucocytes (PMN) are not usually seen in significant numbers unless there is infection (Charosky et al, 1973; Mirra et al, 1976).

Similar appearances are seen in the joint capsule surrounding aluminium oxide ceramic on ceramic prostheses (Harms and Mausle, 1979; Stock et al, 1980), but it has been suggested the number of cells and wear particles is considerably less than around metal on metal or metal on polyethylene prostheses (Griss and Heimke, 1981).

1.3.2 The bone-implant interface

The appearance of the tissue at the bone-implant interface prior to loosening has been delineated by studies of stable prostheses revised for reasons such as dislocation and by post-mortem studies of the tissues around stable prostheses (Charnley et al, 1968; Willert et al, 1974; Vernon-Roberts and Freeman, 1977). This knowledge has been complemented by studies in animals (Radin et al, 1982; Paul and Bargar, 1986).

The response of bone to the implantation of acrylic cement may be divided into three phases and the response to cementless implants is similar. There is some variability in the duration of these phases, and some overlap between phases, but this arbitrary division is useful as it explains the pathological features seen around stable prostheses at different periods after implantation.

The initial phase, lasting some weeks, is that of bone death. Immediately after surgery the major part of the marrow and bone are structurally normal and in animal studies there is no evidence of thermal necrosis. During this initial phase it becomes evident that death of bone and bone marrow has occurred a number of millimetres from the interface. At the junction between living and dead bone there is vascular dilatation, a mild PMN infiltrate, and the appearance of fibroblasts, osteoblasts and osteoclasts. Variable numbers of cement particles, are seen.

The next stage is that of repair. The dead bone is progressively invaded by granulation tissue and fibrous tissue. Appositional new bone is formed on necrotic bone. A very thin, acellular fibrinous layer separates cement from bone in some areas but there is usually a cellular, and later a fibrous tissue layer up to half a millimetre thick between cement and bone. Occasional macrophages and MNGC are seen.

The third phase, that of stabilization of the prostheses, continues for up to two years after implantation in humans. By this time most necrotic bone has been replaced by woven and lamellar bone. Necrotic

bone persists longer on the femoral than on the acetabular side. The bone trabeculae are often realigned parallel to the bone-cement interface and cortical remodelling continues. Normal marrow is replaced by fibrous tissue. Some areas around the femoral stem may show evidence of osteoporosis.

The bone-prosthesis interface around cementless implants shows similar features to those at the cement-bone interface. During the time to stabilization, there is necrosis of bone and repair. The result is a thin fibrinous layer and a fibrous layer of variable thickness between bone and smooth prostheses. The interface between solidly fixed smooth cobalt-chrome alloy hip hemi-arthroplasties and bone is occupied by a dense connective tissue layer up to three millimetres in width but macrophages and MNGC are rare in the presence of small numbers of metal particles. Fibro-cartilage may be seen in areas carrying compressive loads (Kozinn et al, 1986).

There may be a difference between the interface between bone and either acrylic cement or metal. Freeman et al (1982) described a highly cellular connective tissue layer at the interface between the bone and cement but not between the bone and the cobalt-chrome alloy screws of total knee replacement tibial components.

A thin fibrous connective tissue layer is also found separating viable bone from the polyethylene peg of stable acetabular components (Bertin et al, 1985). The bone-prosthesis interface around stable polyethylene tibial flanges shows a thin fibrous tissue layer between the polyethylene and bone and little evidence of a macrophage or MNGC infiltrate (Blaha et al, 1982).

The fibrous tissue layer around porous metal implants may be less than around smooth implants. Bone growth into the deeper layers of a porous coated cementless total hip prosthesis has been reported at four months following insertion (Brooker and Collier, 1984), but fibrous tissue and numerous MNGC have also been seen at the interface of a porous patellar component revised two months following insertion (Cook et al, (1986).

1.3.3 The cement-prosthesis interface

The prosthesis-cement interface has received little attention as it does not seem to be the major problem in prosthesis loosening. A thin fibrous tissue layer less than one hundred micrometers is found at the stable prosthesis-cement interface of smooth femoral stems and the gap is thought to be due to differential shrinkage of the stem and cement, and blood accumulation between the prosthesis and cement during insertion of the prosthesis (Fornasier and Cameron, 1976).

1.4 THE EFFECTS OF WEAR PARTICLES ON PERIPROSTHETIC TISSUES AND THEIR RELATIONSHIP TO LOOSENING

The wear particles in the capsule and at the bone-implant interface may come from two sources, wear of the articulating surfaces of the prosthesis components, and abrasion particles from the bone-implant interface.

Particles range in size from less than one to over two hundred micrometers. Small particles of metal, polyethylene and ceramic appear to cause a predominantly macrophage response and larger particles of polyethylene and cement are usually associated with a macrophage and MNGC response (Vernon-Roberts and Freeman, 1977).

The finding of wear particles in the lymph nodes draining these joints (Walker and Bullough, 1973; Vernon-Roberts and Freeman, 1977), lends support to the concept that there is continuous clearance of particles from the tissues around prostheses. It has been proposed that around stable prostheses an equilibrium is reached in which production of wear debris is matched by removal of debris from the tissues (Willert and Semlitsch, 1977). Continuous production of wear particles over many years, or excessive production over a shorter period, may result in loss of this equilibrium, and accumulation of large numbers of wear particles in the tissues (Vernon-Roberts and Freeman, 1977; Willert and Semlitsch, 1977).

Histochemical studies suggest wear particles are responsible for increased lysosomal and proteolytic activity within macrophages and MNGC at the bone-cement interface but this activity is not generalized. Localized areas of macrophages with high cellular activity in response

to wear particles are seen adjacent to areas containing abundant macrophages which are quiescent (Eftekhar et al, 1985).

1.4.1 Identification of wear particles in the periprosthetic tissues

The types of wear particles in the periprosthetic tissues can usually be identified by examination under direct and polarized light, but there has been some disagreement about the appearance of PMMA particles.

Metal particles are visible under ordinary light. They are dark irregular rods or granules up to three micrometers in diameter. These particles have a light scattering effect when viewed under polarized light. The particles may be extracellular or within macrophages.

Particles of polyethylene are invisible under ordinary light but are highly birefringent when viewed under polarized light. These particles are of two types, either large strands, shreds, splinters, or ovoids up to two hundred micrometers long, and smaller irregular particles five micrometers and smaller. The large particles are usually contained in or surrounded by MNGC while the smaller particles are often seen within macrophages or are extracellular.

There has been disagreement as to the appearance of acrylic cement particles in the tissues. These particles are soluble to a varying degree in the commonly used clearing agents such as xylene and chloroform (Mirra et al, 1976) so that they will dissolve to a varying degree during routine tissue processing. The particles are birefringent in frozen section but appear as voids surrounded by MNGC in processed sections (Willert et al, 1974). These voids, known as acrylic pearls, are usually oval or circular and are approximately eighty micrometers in diameter, but larger particles up to a millimetre in diameter may be seen. The voids often contain residual recrystallized acrylic, and may

have residual radio-opaque marker around the periphery (Mirra et al, 1976).

Acrylic particles can be identified by more complicated techniques. Acrylic can be distinguished from polyethylene in frozen section by observing the change in birefringence of the particles when they are heated to their glass transition temperatures and then cooled (Crugnola et al, 1977). The acrylic can also be identified by examination by electron microscope microanalysis techniques to detect the radio-opaque markers, barium and zirconium, which are found in acrylic cement (Willert et al, 1974).

Sudan-III staining has been suggested as useful in distinguishing various polymer particles in frozen sections but polyester, polyethylene and PMMA particles all showed sudanophilia, and small particles of these materials in macrophages cannot be distinguished from each other by this staining technique (Lintner et al, 1982).

For the routine microscopic assessment of tissues around prostheses, the description of acrylic particles as acrylic voids or "pearls" seems adequate (Willert et al, 1974; Mirra et al, 1976; Vernon-Roberts and Freeman, 1977).

Aluminium oxide ceramic particles appear as fine partially transparent crystals up to three micrometer in diameter which are found within macrophages or extracellularly. To distinguish these particles from other very small particles such as polyethylene may require sophisticated techniques including a combination of scanning electron microscopy with microprobe analysis and cathodiluminescence (Roschger et al, 1980).

1.4.2 Wear particles from articulating surfaces

Metal wear particles have been identified in the synovial fluid around joint prostheses (Mears et al, 1978b) by ferrography (Seifert and Westcott, 1972), and it has been suggested the number and morphology of metal and polymer wear particles correlates with the amount of wear of prosthesis components and the synovial response to these particles (Mears et al, 1978a).

Winter (1974) related the macrophage infiltrate to the number of metal particles in the tissues around metal on metal prostheses and Vernon-Roberts and Freeman (1977) found cell necrosis was associated with large numbers of metal and polyethylene particles at the bone-cement interface.

PMN and lymphocytes are not commonly seen in these tissues in the absence of infection, although Evans et al (1974) suggested large numbers of lymphocytes may indicate metal hypersensitivity and silicon elastomer fragments and particles from toe implants have been reported to cause a lymphocytic infiltrate (McCarthy et al, 1986). Mirra et al (1982) also reported lymphocyte aggregates around total joint arthroplasties in patients with rheumatoid arthritis.

Problems of severe wear have occurred when polymers were used in early joint replacement prostheses. Newman and Scales (1951) reported severe wear of polyethylene when used as a cup hemi-arthroplasty of the hip. The tissues around the prostheses consisted of highly vascular granulation tissue containing abundant MNGC, and extensive fibrosis was seen in association with large amounts of polyethylene wear particles. Similar tissue was found between the prosthesis components and bone. Similar appearances have been reported following wear of nylon hemi-

arthroplasties of the Judet design (Heck and Chandler, 1954) and nylon cup hemi-arthroplasties (Scales, 1958). Newman and Scales (1951) suggested the difference in the cellular response to polymer particles was probably a reflection of the different size of the particles.

Charnley (Charnley et al, 1963) initially used teflon as the acetabular component of total joint prostheses because of its low coefficient of friction and excellent biocompatibility in solid form. Unfortunately, severe wear resulted in a macrophage and MNGC response to particles and tissue necrosis, and this was associated with loosening of the prostheses. Wear of polyester components of total joint arthroplasties caused similar problems (Heilmann et al, 1975; Willert and Semlitsch, 1977), as did wear of proplast and plastipore prostheses (Kerr, 1981).

High density polyethylene acetabular components of total hip prostheses produce fewer wear particles and less tissue response than either polyester (Heilmann et al, 1975), or teflon (Charnley, 1963), but when large numbers of polyethylene wear particles are produced they provoke a severe macrophage and MNGC response (Vernon-Roberts and Freeman, 1977; Willert and Semlitsch, 1977).

A high rate of aseptic loosening, in association with a prolific macrophage and MNGC response and osteolysis, has been reported following severe wear of hemi and total hip arthroplasties using high density polyethylene as the femoral head component (Dahl and Mikhelson, 1976; Wroblewski, 1979; Mossing and Erin-Madsen, 1980; Webb et al, 1980; Austin and Stoney, 1982; Hybbinette, 1985). Brown and Ring (1985) attributed severe bone resorption in the proximal femur around porous coated femoral stems to stress shielding. However, almost all these components had polyethylene femoral heads which are known to wear excessively.

Revell et al (1978) reported severe wear of high density polyethylene when used as the femoral component of hip resurfacings, the convex component of a total ankle prosthesis, and the tibial component of total knee prostheses. Wear was attributed to cold flow of the polyethylene and to cement particle interposition between the articulating surfaces. Using semi-quantitative methods of assessment of the tissues around failed metal on polyethylene prostheses, Revell et al (1978) demonstrated a correlation between the amount of polyethylene debris, and the macrophage and MNGC response and the degree of tissue necrosis.

There is a bimodal distribution of polyethylene wear particles released from joint simulators (Rose et al, 1978). Particles are predominantly either greater than one hundred micrometers or less than ten micrometers in diameter and the larger particles are associated with high wear rates while the smaller particles are associated with low wear rates (Rose, 1979). The number of polyethylene particles in tissues has been found to correlate with the time to failure of total hip arthroplasties (Mirra et al, 1982).

A macrophage and MNGC response, and varying degrees of osteolysis, have been reported in response to a number of polymers including: polyethylene and cement particles around hip arthroplasties (Bell et al, 1983), carbon impregnated polyethylene particles around human knee arthroplasties (Dannenmaier et al, 1985), and silicon and teflon particles in the temporomandibular joints of rabbits (Timmis et al, 1986). There is disagreement as to whether wear of carbon reinforced polyethylene prostheses provokes a more severe tissue response than polyethylene (Groth and Shilling, 1983; Dannenmaier et al, 1985).

Evidence that wear particles from the articulating surfaces of prostheses can migrate significant distances is provided by Pazzaglia

and Byers (1984) who described a pathological fracture through a large osteolytic lesion in the mid-shaft of the femur below a cemented total hip prosthesis. It is unclear whether the prosthesis was loose. As well as cement and metal particles, polyethylene particles were found in the diaphysis of the femur, a long distance from the hip joint and well below the distal tip of the femoral component.

An interesting finding was the relatively benign tissue appearance reported by Jaffe et al (1985) around a prosthesis with an acrylic head which showed little wear after thirty years service.

Wear of aluminium oxide ceramic on ceramic prostheses occurs to some degree and ceramic particles are seen around animal and human prostheses. Harms and Mausle (1979) found particles two to 0.1 micrometers in macrophages in the tissue around these prostheses. These particles have been seen as soon as six months after implantation (Stock et al, 1980) and provoke a predominantly macrophage infiltrate.

Heimke and Griss (1981) reported slight wear of ceramic on ceramic prostheses retrieved at revision, and severe wear was seen in a small number of prostheses which had been incorrectly inserted, resulting in subluxation and abnormally severe wear. Aluminium oxide particles two micrometers or less in diameter, but occasionally up to twenty micrometers, were a regular finding around failed ceramic prostheses (Griss and Heimke, 1981).

Thus ceramic particles in large amounts may provoke a macrophage infiltrate in the periprosthetic tissues and therefore a knowledge of the relative toxicity of ceramic wear particles compared to other materials is important.

1.4.3 Abrasion particles from the bone-prosthesis interface

Acrylic particles are a common finding in the synovium and at the bone-cement interface around loose cemented prostheses (Vernon-Roberts and Freeman, 1976; Willert and Semlitsch, 1977; Green and Anderson, 1980). At the site of bone resorption at the bone-cement interface around three fractured femoral stems Charnley (1975) found a MNGC and macrophage response to loose particles of acrylic cement. Highly birefringent particles, which were probably polyethylene, were also present but were not thought to be significant.

Charosky et al (1973) examined the tissues around infected and non-infected total joint replacements and concluded that acrylic particles were not a major cause of an adverse tissue response. Acrylic particles were described as highly birefingement and no mention was made of the possibility that the acrylic particles had dissolved during tissue processing and that the birefringent particles were polyethylene.

Mirra et al (1976) used a semi-quantitative histological method to assess the tissue response around failed prostheses and found the number of acrylic particles correlated with the stage of loosening. Polyethylene in large amounts were found to provoke a severe macrophage and MNGC response, and metal particles were seen in small amounts around metal on polyethylene prostheses and in larger numbers around metal on metal prostheses. In a larger study the numbers of both polyethylene and cement particles correlated with loosening (Mirra et al, 1982).

Harris et al (1976) described the radiological appearance of extensive bone resorption around stable cemented femoral components with no evidence of infection. The membrane around the cement revealed sheets of macrophages, some MNGC, and multiple fragments of birefringent

material which were interpreted as cement particles. The authors commented that these particles were not dissolved in acetone and it therefore seems likely many of these particles were not acrylic but were polyethylene.

Massive osteolysis around cemented metal on metal and metal on polyethylene prostheses in association with a macrophage and MNGC response has been attributed to cement particles by Scott et al (1985). All small defects in the tissues were thought to be cement particles but no mention was made of the birefringence of particles seen in the tissues. The conclusions that cement particles were the cause for the tissue response, and that polyethylene could not be implicated, do not seem justified by the histopathological findings.

A papillary synovial like layer of connective tissue has been described around loose cemented prostheses by Goldring et al (1983), and cement particles in association with MNGC were seen in this tissue.

Extensive localized bone resorption adjacent to four rigidly fixed non-infected cemented components of metal on polyethylene total hip replacements was reported by Jasty et al (1986a). The tissue at the sites of bone resorption consisted of sheets of macrophages and MNGC containing abundant cement particles but no polyethylene particles were described. The authors supported the hypothesis originally suggested by Willert and Semlitsch (1976) that the cement particles accumulate at the interface, either due to incomplete polymerization of acrylic on the surface of the cement, or due to abrasion of particles due to micromotion at the interface.

Metal particles may be produced by abrasion of cementless prostheses on bone. The bone-implant interface around smooth cobalt-chrome cementless

femoral components consists predominantly of mature connective tissue. A few macrophages are seen in the presence of small numbers of metal particles (Kozinn et al, 1986). There is some evidence that porous coated metal implants may produce large numbers of abrasion particles. Large numbers of metal abrasion particles have recently been reported at the bone-implant interface of loose porous cementless femoral stems and these particles were associated with a severe macrophage infiltrate (Buchert et al, 1986).

Remagen and Morscher (1984) reported the finding of polyethylene particles at the interface between bone and solidly fixed cementless polyethylene acetabular prostheses, suggesting abrasion of cementless polyethylene components may contribute to wear particle accumulation at the interface prior to obvious loosening. However, it was not possible to exclude migration of wear particles from the articulating surfaces of the prostheses.

Clearly abrasion particles accumulate in the periprosthetic tissues around loose prostheses and these particles may contribute to the adverse appearances seen at the bone-implant interface. There is also evidence that abrasion particles may be produced at the bone-implant interface prior to macroscopic evidence of loosening.

1.5 OTHER FACTORS DETERMINING THE APPEARANCE OF THE PERIPROSTHETIC TISSUES AND THE STABILITY OF THE BONE-IMPLANT INTERFACE

A number of factors other than the presence of wear particles will determine the appearance of the periprosthetic tissues and the type of bone-implant interface which develops following insertion of an implant, and these must be considered when interpreting the appearances of the tissues around stable and loose prostheses. These factors include: the technique of preparation of the bone, the technique of insertion of the implant, the biocompatibility of the implant material in solid form, the implant design, the amount, site, direction and timing of loading of the implant, and the possibility of infection.

1.5.1 Bone preparation and insertion techniques

The degree of trauma to bone due to cutting and reaming will influence the amount of bone necrosis and repair. Bone necrosis can be minimized by sufficiently delicate techniques (Linder and Lundskog, 1975), but these techniques are not always attainable when performing joint replacement surgery. Reaming of bone causes interruption of the endosteal blood supply (Rhinelander et al, 1979) and is a major cause of the bone necrosis seen around recently implanted prostheses. The degree of impairment of blood supply due to reaming, and the presence of acrylic cement will therefore determine the appearances at the bone-implant interface for up to two years following insertion of a cemented implant (Sorensen et al, 1979).

During insertion of an implant the accuracy of fit of the prostheses in bone may influence the thickness of the fibrous tissue layer between bone and implant. Acrylic cement should ideally fill any gap between bone and cement, but any gaps between bone and an uncemented prosthesis

must initially be filled by granulation tissue and then by new bone. Lack of close fit between porous cementless implants has been shown to delay bone ingrowth (Harris and Jasty, 1985).

Ling (1979) has suggested improved interlock between bone and cement can be achieved by meticulous cleaning of debris from the bone, the use of low viscosity cement, and pressurization of cement into the bone. While these techniques may not influence the development of a thin fibrous membrane between bone and cement, they will fill large gaps and improve fixation, so decreasing micromovement between cement and bone. Micro-movement may cause micro-fractures of dead bone, which has been implicated in the development of a thick fibrous membrane between bone and cement (Vernon-Roberts and Freeman, 1977). Prevention of micromovement may also prevent repeated trauma to an established membrane at the bone-cement interface, a suggested cause of progressive resorption of bone (Willert and Semlitsch, 1976). Micromovement will cause the same problems around cementless components.

In summary, accurate preparation of the bone bed, minimal trauma, accurate fit of cementless components, adequate cleaning of debris, and advances in cementing techniques can be expected to decrease the degree of bone necrosis and improve early stability at the bone-cement and cementless bone-prosthesis interface, so preventing development of a thick fibrous membrane.

1.5.2 The effect of implant materials

The likely effects on tissues of different materials has been assessed by examination of the effects on tissues of different materials implanted in soft tissues, and implanted in bone in unloaded and loaded situations.

1.5.2.1 Metals and corrosion

The commonly used metals in prosthesis manufacture are cobalt-chrome alloys, stainless steel, and titanium alloys. These metals have been developed because they largely fulfill the mechanical and biocompatibility requirements of joint replacement prostheses.

Release of metal ions will occur to some degree despite passivation of the surface of the implant and careful control of surface finish. The effect of these metal ions on the tissues will be determined by the amount of corrosion of a metal and the relative toxicity of the particular metal ions.

Corrosion of stainless steel internal fixation plates increases with time but, interestingly, the tissue response decreases with time (Harding et al, 1986). Williams (1971) concluded that cobalt-chrome alloys were more resistant to corrosion than stainless steel, and in vitro studies demonstrate titanium is more resistant to corrosion than cobalt-chrome alloy. Titanium ions are corroded from implants in animals without visible corrosion of the implant (Ferguson et al, 1962), and Meachim and Williams (1973) found titanium ions in tissues around human implants, but no untoward tissue response compared to the response to cobalt-chrome alloy and stainless steel.

Ferguson et al (1960) reported that ions of the constituent elements of metal implants can be detected in muscle adjacent to implants and that this is true even if there is no macroscopic evidence of corrosion of the implant. Laing et al (1967) studied the tissue response in rabbits to intramuscular implantation of metal discs of pure metals and metal alloys commonly used in prosthesis manufacture. The cellular response and thickness of the surrounding membrane was compared with the

concentration of metal ions in the tissues. Many pure metals used as constituents in prosthesis manufacture induced a severe response, but the commonly used implant materials such as 316L stainless steel, cobalt-chrome-molybdenum and cobalt-chrome-nickel alloys, and the titanium alloys caused only a mild response. Stainless steel and cobalt-chrome alloys produced a tissue response which usually was proportional to the amount of metal released, but similar amounts of titanium ions as cobalt and chromium ions appeared to cause less tissue damage.

After release of metal ions from implants, the ions are then transported from the local site. It seems there is preferential clearance of different ions from the site of the implant to other organs. Cobalt may be more rapidly corroded from cobalt-chrome alloys and more rapidly cleared from the tissues than chromium (Ferguson et al, 1962; Laing et al, 1967; Vernon-Roberts, 1978).

In the tissues in the vicinity of cemented metal hip prostheses the high levels of constituent metal ions correlates with the degree of corrosion of the constituent metals; nickel being the highest, then cobalt, iron, molybdenum and chromium (Michel et al, 1984). The level of corrosion products in the tissues will be determined by the corrosion process and the biochemical properties of metal alloys. Clearance of the metal ions from these tissues may further alter the concentration of metal ions in the tissues and may explain reports of very high chromium levels around loose prostheses (Dielert et al, 1983).

Metal ions from titanium alloy animal implants were found in the serum and urine and most organs in variable proportions by Woodman et al (1984), but these ions were not found up to one year following insertion

of loaded porous coated mechanically stable titanium alloy hemiarthroplasties in animals (Watson et al, 1986).

Couple corrosion due to the use of implants of different metals results in accelerated corrosion of some metals. Stainless steel is vulnerable to couple corrosion, cobalt-chrome alloys are resistant, and titanium alloys show some accelerated corrosion (Rostoker et al, 1974). The tissue response around cobalt-chrome alloys has been reported to be minimal compared to a thicker and slightly more cellular response to titanium in couple corrosion studies (Rostoker et al, 1978a).

There has been concern that the introduction of porous metal implants might cause an increased tissue response due to the increased surface available to corrosion and the possibility of promoting crevice corrosion. These potential problems appear to have been overcome by scintering wire or balls onto the prosthesis surface, a process which produces a protective oxidation layer over the whole surface of the prosthesis. Bundy et al (1986) demonstrated that the corrosion of porous coated titanium alloy may increase if the alloy is stressed compared to the unstressed state. The release of metal ions from unstressed titanium porous coated alloy and stressed and unstressed porous cobalt-chrome alloys was proportional to the increased surface area of these porous implants compared to smooth implants of the same material. Implantation in soft tissues of cobalt-chrome alloy prostheses with a scintered surface caused no increase in tissue reactivity (Cameron and Pilliar, 1974).

It seems that in the unloaded situation corrosion from cobalt-chrome alloy prostheses with either a smooth or a porous surface will not prevent bone from growing up to the prosthesis or possibly into the pores. But bone is usually separated from the prosthesis by a very thin

connective tissue layer, which may be due to the toxic effects on bone due to the release of small amounts of metal ions from the implant. A difference in the interface between bone and between 316L stainless steel or pure titanium coated implants has been reported by Albrektsson and Hanssen (1986). The interface between stainless steel and bone was one to two cell layers thick. The interface between titanium and bone had no cells and consisted of a proteoglycan layer one to two hundred angstrom thick. Similar findings at the bone-titanium interface have been reported by others (Linder et al, 1983).

The problem of corrosion can be expected to increase if the surface area of metal exposed to the tissue is increased, as would occur with the release of metal wear particles. These particles are known to dissolve in serum (Swanson et al, 1973), and there is evidence that high levels of metal ions are found around prostheses in which metal articulates directly with metal, resulting in the production of large numbers of metal wear particles. Elevated cobalt and chromium levels are found in the blood of patients with all metal prostheses (Coleman et al, 1973), and in the hair of patients with all metal prostheses compared to patients with metal on polyethylene prostheses (Owen et al, 1976). Elevated serum chromium levels have been reported following cemented cobalt-chrome on polyethylene prostheses (Bartolozzi and Black, 1985), but nickel levels were not elevated if stainless steel on polyethylene cemented implants remained stable (Linden et al, 1985). Elevated levels of cobalt, chromium and nickel are found in the synovial fluid from patients with total knee prostheses, and in particular metal hinged prostheses (Crachiolo and Revell, 1982).

The origin of metal particles and granules seen around prostheses has been extensively investigated by Winter (1974, 1976) and by Vernon-

Roberts and Freeman (1976), and both have concluded that the metal ions present are due to a combination of wear and corrosion products around both stainless steel and cobalt-chrome prostheses. These studies suggest that elevated levels of the constituent ions are often seen where there are large numbers of wear particles in the tissues, either due to wear at the articulating surfaces of prostheses or abrasion of particles at the prosthesis-cement or bone-prosthesis interfaces during loosening.

1.5.2.2 Sensitivity to metals and other materials

A delayed hypersensitivity reaction to metal ions released from joint replacement prostheses has been implicated as a cause of prosthesis loosening (Evans et al, 1974; Vernon-Roberts, 1978). It was suggested by Evans et al (1974) that the hypersensitivity to metals, seen in patients with loose prostheses, causes a severe inflammatory response in the periprosthetic tissues. This response was characterized by lymphocytic invasion, vascular occlusion, tissue necrosis and subsequent prosthesis loosening. The hypersensitivity was not common in patients with stable prostheses.

Brown et al (1977) reported no evidence of metal sensitivity in twenty patients who had failed cobalt-chrome metal on metal prostheses. Neither fibrinoid necrosis nor vascular occlusion were seen in the tissues around these prostheses, and the tissue necrosis and lymphocytic infiltrate were felt to be consistent with a foreign body response to wear particles and not necessarily due to hypersensitivity.

Others (Elves et al, 1975; Elves, 1977a; Deutman et al, 1977; Waterman and Schrik, 1985) have found skin sensitivity develops in some patients following metal on metal and metal on polyethylene

arthroplasties, but no definite relationship between hypersensitivity and prosthesis loosening has been established. Metal sensitivity has been found not to alter following metal on polyethylene prostheses which remain solidly fixed (Rooker and Wilkinson, 1980; Benson et al, 1975).

Histological sections of the tissues around failed metal on metal prostheses often show evidence of necrosis of soft tissue and bone, a macrophage infiltrate, and lesser numbers of lymphocytes and plasma cells. This chronic inflammatory response is commonly seen around failed metal on metal prostheses and should not be confused with a cell-mediated delayed hypersensitivity response of the type suggested by Evans et al (1974).

The lymphocyte transformation test (Elves, 1977b), and leucocyte migration inhibition test (Merritt et al, 1980) detect a high incidence of nickel sensitivity following total hip arthroplasty, but no attempt has been made to correlate these tests with prosthesis loosening.

From these studies it seems metal sensitivity may occur following implantation of metal prostheses and may be more common following release of large amounts of metal ions due to wear of metal on metal prostheses. A hypersensitivity reaction to these metal ions has been suggested as a cause of prosthesis loosening but there is disagreement as to incidence of sensitivity and its importance in prosthesis loosening. Lewin et al (1982) found no compromise in the fixation of stainless steel and cobalt-chrome metal screws in guinea pigs previously sensitized to nickel and cobalt and no adverse histological appearance at six weeks following implantation.

If metal wear particles are not produced in large amounts, as would be expected when metal on polyethylene prosthesis are used and the

prostheses do not produce metal abrasion particles, then metal sensitivity is unlikely to be a major problem. As hypersensitivity to polyethylene has not been reported and only a few cases of sensitivity to acrylic have been reported (Pegum and Medhurst, 1971; Waterman and Schrik 1985), problems of hypersensitivity may not be a problem with the current designs of cemented prostheses. However, recent evidence that loosening of current designs of porous coated metal implants results in the accumulation of large numbers of metal particles at the bone-implant interface (Buchert et al, 1986) suggests the possible role of hypersensitivity in exacerbating loosening may need to be re-examined.

1.5.2.3 Acrylic polymers

Early animal studies suggested acrylic cement was relatively well tolerated in bone (Henrichsen et al, 1952). Briggs et al (1979) found unloaded acrylic cement implants in bone provoke a 0.1 to 0.5 millimetre investing layer of fibrous tissue. After eight years there was no evidence of breakdown of acrylic and no significant histological evidence of chronic inflammation. On the other hand, vitallium implants did not demonstrate this investing fibrous layer.

Initially, Charnley (1970), who was largely responsible for introducing the use of acrylic cement into orthopaedic surgery, concluded that the clinical success of cement and the histopathology findings of a relatively benign bone-cement interface negated previous concerns regarding the use of cement in humans. Ten years later Charnley (1979) felt that the presence of macrophages at the bone-cement interface may represent an untoward tissue response which could not be ignored.

There are two major criticisms of the use of a self curing acrylic cement as an endoprostheses material. These are the heat liberated during polymerization, and the possible toxic effects of released monomer.

The effect of heat of polymerization of acrylic cement on the implant bed remains controversial. Initially there was reluctance to recommend the use of acrylic cement because of worries that the heat of polymerization may have adverse effects on surrounding tissues (Wiltse and Hall, 1957; Homsy et al, 1972), but Lundskog (1972), in a study of the threshold temperature for thermal damage, could find no evidence of significant necrosis of bone due to heat of polymerization. Berman et al (1984) felt that temperature may be an important cause of tissue necrosis if a thick cement bolus was used, resulting in temperatures above seventy degrees centigrade. From direct measurement at the bone-cement interface during total hip replacement, it seems heat has little effect, as heat is dissipated from the bone-cement interface by the local circulation (Jefferies et al, 1975; Reckling and Dillon, 1977).

Monomer toxicity has been suggested as a cause of an adverse tissue response to cement but no monomer, or very low levels of monomer, have been found in the cancellous bone adjacent to cemented implants in animals immediately, and some months after insertion (Petty, 1980; Radin et al, 1980). The very low levels detected are far below the toxicity level of monomer as assessed by in vitro toxicity studies. But it is possible that monomer levels very close to the cement may be higher than the levels obtained from the large biopsies of bone in these studies.

Studies of possible tissue toxicity due to PMMA monomer, by implantation of both pre-polymerized cement and polymerizing dough, demonstrate that

the early release of monomer from polymerizing cement does not significantly affect the bone any more than the effects of surgical trauma (Linder, 1977).

The possibility that continued presence of PMMA monomer may have a detrimental effect on tissues was studied by Garcia et al (1981) using bone forming matrix cylinders of demineralized bone. PMMA was found to inhibit bone formation by thirty-eight percent compared to twelve percent inhibition by vitallium, and neither material caused accelerated resorption. When varying monomer content of the acrylic was used a dose response relationship was seen which suggested the bone inhibition was due to residual monomer. Whether this effect significantly influences the short term and long term stability at the bone-cement interface is unknown.

Freeman et al (1982) noted the universal finding of a radiographic lucent line at the bone-cement interface but not at the interface between bone and cobalt-chrome alloy screws of total knee prostheses. As there was no differential movement between the cement and the screw it was concluded that the cause for the development of the connective tissue layer responsible for the lucent line was not mechanical, but represented an adverse tissue response at the interface, possibly due to the PMMA . The membrane at the bone-cement interface was examined in one patient. Macrophages, MNGC, and polyethylene and acrylic debris were seen at the bone-cement interface, but not at the bone-metal interface. It was concluded that the presence of macrophages may be due to the PMMA, but the possibility of production of acrylic abrasion particles at this interface due to micromotion was not discussed, nor was the possible effect of polyethylene particles considered.

Linder and Hansson (1983) studied the bone-cement interface by light and electron microscopy in three specimens of isolated areas of macroscopically solid contact between cement and bone around total hip prostheses. Light microscopy identified areas of close contact between bone and cement and areas where cement was separated from bone by either a thin connective tissue layer or, more commonly, by areas of soft tissue containing fibroblasts, macrophages and MNGC resembling osteoclasts. Electron microscopy of the areas of close contact between cement and bone identified a layer of uncalcified proteoglycan varying in thickness from 0.3 to seven micrometers, but typically one to three micrometers, which separated the cement from the collagen and calcium of bone.

Another problem with acrylic cement is shrinkage of the cement after polymerization. Shrinkage is usually in the order of one to two percent of the size of the cement but may be as high as five percent in some studies (Haas et al, 1975). Shrinkage is of some importance as the gap created must be replaced by bone to achieve optimal fixation.

1.5.2.4 Non-Acrylic polymers

The types of tissue response to solid polymers commonly used up to twenty years ago has been extensively investigated and the results were summarized by Calnan (1963). The tissue response to solid blocks of polymer is predominantly a mononuclear cell response, followed by fibrosis. At three months a fibrous envelope surrounds the implant. Different polymers incite different degrees of inflammatory response. Thus teflon and polyethylene (Bing, 1955) cause less inflammatory cell infiltrate and a thinner fibrous tissue capsule than nylon.

Further studies of high density polyethylene and, in particular UHMWP used in total joint replacement, have shown that the material in solid form is well tolerated in the paravertebral muscles of rabbits (Escalas et al, 1976). It is important that these findings are not extrapolated to the material in any form other than solid polyethylene, as it has been well shown in tests of the biocompatibility and carcinogenesis of polymers that the response to an implant material is greatly determined by the size and shape of the material (Bischoff and Bryson, 1964). This difference in response has also been shown with UHMWP (Escalas et al, 1976).

Bone ingrowth into the pores of teflon (Howe et al, 1974) and high density polyethylene (Spector et al, 1976) has been shown to provide useful anchorage of the femoral components of prostheses in dogs (Sauer et al, 1976). Blaha et al (1982) have demonstrated bone ingrowth between flanges on the pegs of the tibial components of human total knee replacements. This has allowed successful anchorage of the prostheses without cement. Histopathological examination of the bone-prosthesis interface thirty-six months after implantation demonstrates a thin fibrous membrane between the polyethylene and bone but no evidence of a macrophage or MNGC infiltrate, thus confirming the biocompatibility of solid polyethylene in humans.

1.5.2.5 Aluminium oxide ceramic

Early studies by Griss et al (1973) and Harms and Mausle (1979) suggest aluminium oxide ceramic in solid form is very biocompatible and, when implanted in bone, produces less fibrous tissue response and quicker incorporation in bone than stainless steel (Heimke et al, 1979).

Richardson et al (1975) implanted discs of alumina ceramic, cobalt-chrome alloy and ultra high molecular weight polyethylene intramuscularly, and found similar biocompatibility on histopathological grounds. Fibrous adhesion to polyethylene and cobalt-chrome was superior than adhesion to ceramic, as judged by scanning electron microscope examination, but whether this implies superior biocompatibility of one or other of the materials is unknown.

Griss et al (1975) found a fibrous membrane always present between aluminium oxide ceramic and bone in loaded sheep implants. This may be due to differences in modulus of elasticity between ceramic and bone and possibly due to micromovement at the interface. Aluminium oxide ceramic allows early growth of bone up to a prosthesis (Griss et al, 1976), and bone deposition immediately adjacent to ceramic human dental implants has been reported (Busing et al, 1983). Whether ceramic offers advantages over cobalt-chrome alloys or titanium alloys is unknown.

1.5.3 The effect of weight bearing and implant design on the tissues at the bone-implant interface

Whether slight differences in the biocompatibility of the commonly used prosthesis materials in solid form is important must be judged in the light of studies of the tissue response to these materials when the interface between the material and bone is loaded. Miller et al (1979) reviewed their experience with loaded and unloaded implants in the femur and tibia of animals and concluded that there was no difference in the tendency to form a connective tissue layer at the bone-implant interface around stainless steel, carbon, acrylic cement and polyethylene. The use of various shapes such as ridges and threads did not avert the development of this layer. The layer was thicker around weight bearing implants and appeared to increase with the duration of implantation. If

interlock with bone at intervals of five hundred micrometers was achieved, then the connective tissue layer remained very thin.

Early loading of a prostheses inserted in bone is likely to contribute to development of a fibrous tissue layer if micromovement occurs. Some micromovement will occur even if rigid interlock is initially achieved as any difference in the modulus of elasticity of the prosthesis material and bone will cause interface movement. Therefore, cyclical loading will result in differential movement at the interface. At the site of the femoral stem of a prosthesis this is likely to be ten micrometers (Swanson, 1977). Early cyclical loading and micromovement have been shown to inhibit osteogenesis (Schatzker et al, 1975) and to prevent bone ingrowth into prosthesis with porous surfaces (Ducheyne et al, 1977). If interlock is not achieved at the time of implantation, the effects of cyclical loading will be even more severe and a thick fibrous envelope will develop (Miller et al, 1979). Movement causes recurrent trauma to granulation tissue at the interface and prevents capillary ingrowth which is necessary for bone deposition, resulting in a fibrous tissue layer (Cameron et al, 1973).

While a soft tissue layer at the bone-implant interface may be useful, as it acts to dampen the effects of micromovement due to unequal moduli of elasticity at the interface, if the movement exceeds the plasticity of the membrane further tissue damage and further fibrous scarring will occur (Willert and Semlitsch, 1976). This sets the scene for progressive loosening.

The design characteristics of the surface of an implant adjacent to bone will also influence the appearance of the periprosthetic tissues. To achieve and maintain fixation between a prosthesis and bone the shear forces created by transfer of stress from the prosthesis to the bone

must be overcome by adequate interlock (Swanson, 1977). Interlock may be macro-interlock achieved by using grooves, recesses or cement irregularities some millimetres in size, or micro-interlock, where the irregularities are measured in micrometers. Micro-interlock can be achieved by porous or scintered implants or by achieving very fine interdigitation of cement between bone trabeculae. The use of porous or scintered implants relies on bone ingrowth to achieve the initial micro-interlock while interlock can be achieved immediately using cement. Both methods achieve superior fixation than smooth prosthesis.

The optimal pore size to encourage bone ingrowth and so achieve micro-interlock will probably vary according to the site of insertion, prosthesis design, type of material and timing and degree of loading. Therefore, these factors will affect the type of tissue seen immediately adjacent to the implant. A pore size of two hundred to five hundred micrometers appears to be ideal for bone ingrowth in humans (Vernon-Roberts and Freeman, 1977) and this is also true in both unloaded (Weinstein et al, 1979) and loaded (Harris and Jasty, 1985) experiments in animals. Bone ingrowth occurs within weeks in unloaded (Galante et al, 1971), and rigidly fixed loaded implants (Harris and Jasty, 1985), and unloaded porous implants become more solidly fixed in cortical than cancellous bone (Weinstein et al, 1979). Bone ingrowth into loaded porous implants increases with time and by twenty-six weeks in animals is well established, with no fibrous tissue between titanium fibre mesh and bone (Ronningen et al, 1980).

The tissue seen at the interface around porous implants varies from one site to another. The extent of bone ingrowth may involve only a very small amount of the porous layer and the extent varies at different sites on a prosthesis (Jasty et al, 1986b), and between different types

of porous coatings (Sumner et al, 1986), and appears to increase with load bearing (Barth et al, 1986).

While excessive load leading to micromovement is undesirable, it appears some load is necessary to encourage bone-ingrowth. Ronningen et al (1980) have achieved solid fixation and bone ingrowth into cementless scintered titanium femoral components of total hip arthroplasties in dogs when the arthroplasties were loaded to full weight bearing within a week of implantation. Inferior bone ingrowth and remodelling, and increased thickness of a fibrous tissue layer at the bone-porous titanium interface, was reported by Kim et al (1986) in unloaded acetabular components of fibermesh titanium implants in dogs compared to loaded implants in the opposite hip, at approximately two months following implantation.

The modulus of elasticity of the implant material and surface coatings of the material may also influence the appearances at the bone-implant interface. In studies of dental implants in baboons, Cook et al (1983) demonstrated that biocompatibility is not the only determinant of implant success. A material of low modulus of elasticity caused less fibrous tissue at the interface than the same material with a high modulus. To optimise fixation attempts have been made to enhance bone formation to fill gaps between implants and bone with hydroxyapatite, with some success (Liebrecht et al, 1986). However, Russotti et al (1986) reported dense particulate hydroxyapatite/tricalcium phosphate crystals did not increase the rate of bone formation compared to unfilled voids around porous femoral components, despite reports that hydroxyapatite on the surface of some porous implants may enhance bone ingrowth (Ducheyne et al, 1986).

In summary, the appearances at the bone-implant interface are related to a number of mechanical and design factors. These will determine the amount of bone formation at the interface during the repair and remodelling stages of stabilization of an interface. If a thick layer of fibrous tissue forms during these stages, then the scene is set for progressive loosening. These factors need to be considered when assessing the tissue response to an implant.

1.5.4 The effect of infection on the periprosthetic tissues

During the early development of total joint prostheses infection rates of nine percent (Charnley and Eftekhar, 1969) and eleven percent (Wilson et al, 1973) following various forms of hip arthroplasty, and seven percent following hinged total knee arthroplasty (Deburge, 1976), were reported. The use of improved operative techniques, clean air theatres, personal isolators, prophylactic antibiotics, and antibiotic cement have decreased the infection rate to two percent or less (Petty et al, 1975; Fitzgerald et al, 1977; Andrews et al, 1981; Hill et al, 1981; Poss et al, 1984; Grogan et al, 1986).

Infection may be divided into superficial and deep to the deep fascia, and into either acute, delayed or haematogenous (Wilson et al, 1973; Coventry, 1975). Infection is most commonly due to Staphylococcus aureus, Staphylococcus epidermidis, Streptococci, gram negative bacilli, and anaerobes (Charnley and Eftekhar, 1969; Kamme et al, 1974; Fitzgerald et al, 1977; Bourgault et al, 1980).

Although infection following total joint arthroplasty remains a major problem, its importance as a cause of loosening may have been over-estimated in the past. Fitzgerald and Kelly (1979) reviewed the biological causes of joint arthroplasty failure and concluded infection

was the major problem. Scant attention was given to the possibility that wear particles of metal, polyethylene and cement might contribute to a chronic inflammatory response in the periprosthetic tissues. The tissue response to wear particles was dismissed as relatively unimportant in the new era of total hip prostheses in which metal articulates with polyethylene. Previously reported infection rates were discussed in some detail, but the authors did not address the problem that, in most studies till that time, the criteria used to diagnose infection were either omitted from reports or, when reported, the criteria could be considered questionable.

Diagnosis of acute infection is not difficult. Diagnosis of chronic low-grade infection, and distinguishing infection from contamination, may be extremely difficult. Many arthroplasty infections are due to bacteria which have relatively low pathogenicity, are common skin, oral, nasopharyngeal, gastro-intestinal and vaginal flora, and require culture techniques which must include proper transport of specimens and anaerobic culture.

Given operative contamination rates as high as thirty percent (Dillon et al, 1969; Fitzgerald et al, 1973; Murray, 1973; Kamme and Lindberg, 1981) and laboratory contamination rates above five percent (Murray, 1973), it seems that prolonged highly sensitive culture techniques and frequent sub-cultures, as recommended by Buckholtz et al (1979, 1981) run considerable risk of over-diagnosing infection in failed arthroplasties.

Kamme and Lindberg (1981) investigated the clinical significance of a positive culture of bacteria at the time of surgery by taking five biopsies for culture from the areas adjacent to cement or prosthesis at

the time of revision arthroplasty, and from control hips undergoing initial arthroplasty.

A positive culture was obtained in a third of the control hips but there were never more than two positive cultures in the five biopsies, and this was considered as evidence of contamination. The majority of bacteria isolated were Propionebacterium acnes. Occasionally, Staphylococcus epidermidis, Streptococci and Staphylococcus aureus were also isolated. In the revised hips the five biopsies often gave a positive culture. Staphylococcus aureus, Streptococcus pneumoniae and Enterobacteriae were common in the early and haematogenous infections and Staphylococcus epidermidis, Peptococci, Propionebacterium acnes and Fusobacteriae were common in the delayed infections. It was suggested that multiple biopsies were the best method of detecting infection and it was concluded that growth in one or two biopsy samples indicated contamination, while growth in four or five indicated infection.

Histopathology examination of the tissues also is useful in detecting infection. Mirra et al (1976, 1982) found more than five PMN per high power field was evidence for infection. Large numbers of lymphocytes were thought to be suggestive of infection.

Clearly, infection is an important cause of an acute and chronic inflammatory response in the periprosthetic tissues and a strict protocol for collection of specimens and culture is required to exclude its presence. Conclusions as to the possible effects of wear particles must be made only after chronic low-grade infection has been excluded.

1.6. CAUSES OF WEAR OF THE ARTICULATING SURFACES OF JOINT PROSTHESES

Wear of joint prostheses may be due to either adhesive, abrasive, corrosive or fatigue types of wear. Adhesive wear is common and occurs due to shearing of adhesively fixed junctions between two materials. Abrasive wear is also common and may be due to surface roughness of components, or two-body or three-body wear due to interposition of particles of the same or other materials. Adhesive and abrasive wear are proportional to the coefficient of friction, load and sliding distance and are indirectly proportional to the hardness of the material (Weightman, 1977).

Three-body wear of polyethylene may be particularly severe due to interposition of cement particles between metal and polyethylene (Rostoker et al, 1978b; Revell et al, 1978; Ungethlum et al, 1983), and also occurs with titanium implants (McKellop et al, 1979a, 1980). Corrosive wear may be of more importance when metal components are used. Fatigue wear and deformation have been implicated as a late cause of wear of polyethylene acetabular components (Dowling et al, 1980).

The diameter of the cup of a total hip replacement will determine the amount of wear as it influences the sliding distance. The amount of volumetric wear is increased with increasing diameter, so that a smaller diameter would seem appropriate. But smaller diameters are associated with increased speed of penetration of the cup (Wroblewski, 1985). A compromise is necessary and the ideal diameter selected will vary according to the material hardness.

Charnley et al (1969) suggested, from studies of teflon cups of varying diameter, that twenty-two millimeters was the optimal size for teflon and polyethylene. There seems little difference in wear of polyethylene

up to a diameter of thirty-two millimeters but above this increased volumetric wear may occur (Gold and Walker, 1974; Weightman, 1977).

While the co-efficient of friction is important, other factors such as material hardness, cup diameter and load must be considered. Weightman (1977) felt that, because of the differences in hardness of polyethylene and metals, metal on polyethylene combinations may actually produce more volumetric wear than metal on metal combinations, even though the metal on metal combinations have a much higher co-efficient of friction. Clarke (1982a) has emphasized the importance of adequate test protocols and the type of experimental machines used to determine wear rates.

Polymer wear needs to be differentiated from penetration due to plastic creep or cold flow, a property of plastics under pressure. Creep has been implicated as a cause of increased wear of polyethylene in pin or disc tests (Rostoker and Galante, 1979; Wright et al, 1982), and as an important early cause of penetration of Muller acetabular prostheses (Buckhorn et al, 1984).

Creep makes interpretation of actual wear rates from radiographs difficult. Early studies of the radiographic measurement of the amount of acetabular cup wear (Charnley, 1972; Walker et al, 1974) need to be reassessed as these techniques measure a combination of volumetric wear and creep, and volumetric wear will contribute no more than a third to the amount of penetration seen in even the most severely worn cups (Afifi and Jacob, 1981). Clarke et al (1976) found uni-radiography and duo-radiograph was too inaccurate to measure small amounts of wear of prostheses and supported this contention by laboratory radiographic studies which demonstrated that the wear measurement error was at least half a millimetre (Clarke et al, 1979). Others have suggested different

techniques of duo-radiography were reasonably accurate (Griffith et al, 1978; Afifi and Jacob, 1981).

Stern et al (1986) suggested that carbon fibres may limit the amount of plastic flow of polyethylene and felt that this explains the finding of increased production of small particles, less than twenty micrometers, and decreased production of large particles, greater than one hundred micrometers, in wear simulator tests where carbon reinforced UHMWP was used instead of ordinary UHMWP.

Trainor and Haward (1980) demonstrated the temperature and pressure of moulding polyethylene and the amount of added antioxidant also affected wear. Implantation in the body may affect the wear rate of some polymers (Amstutz and Lodwig, 1976), but polyethylene seems relatively resistant to wear despite crystallinity changes which occur after long periods of implantation (Grood et al, 1982). Rose et al (1980, 1982) emphasized the enormous variability in the wear of different prostheses and the need for better control of manufacture as differences in molecular weight of UHMWP significantly affect the rate of wear.

Severe wear and fracture of polyethylene acetabular components of Muller total hip replacements has been reported by a number of authors (Harley and Boston, 1985; Heller et al, 1986).

Aluminium oxide ceramic on ceramic, or ceramic on polyethylene prostheses have been reported to have superior wear characteristics than metal on polyethylene prostheses when tested either dry (Dowson and Linnet, 1980) or when lubricated (Boutin, 1972; Semlitsch et al, 1977; Dorre and Dawihl, 1980). But McKellop et al (1979b) did not find any differences in wear between either metal or aluminium oxide articulating against UHMWP and suggested testing conditions need to be more

rigorously controlled to make valid comparisons between materials. Wear of polyethylene against other materials is clearly related to surface finish. If the surface finish of stainless steel and ceramic are similar, then the wear rate of polyethylene is similar (McKellop et al, 1978; Weightman and Light, 1986).

A slightly elevated co-efficient of friction is seen in the early "wearing in" period of ceramics but this decreases with time as opposed to the co-efficient of friction between metal on polyethylene prostheses, which increases with time (Dorre and Dawihl, 1980). The low co-efficient of friction between ceramic on ceramic prostheses can be expected to cause low wear rates, but wear rates of ceramic on ceramic prosthesis components of up to five times that seen in ideal situations have been reported if there is even minor incongruity due to lack of sphericity during production (Hinterberger et al, 1980).

Abrasive wear of polyethylene articulating against ceramics may be decreased due to the low co-efficient of friction, but wear due to creep and deformation of polyethylene (Dowling et al, 1980), and possibly due to acrylic cement interposition, will not be prevented by the use of ceramic on polyethylene prostheses. Whether ceramic on ceramic prostheses will wear significantly if acrylic cement particles become trapped between the components is unknown.

In summary, wear of the currently used prostheses will occur to varying degrees. The amount of wear debris produced by prostheses will depend on a number of factors, some of which may be outside the control of the prosthesis designers or surgeon. A compromise in selection of materials is often necessary to achieve certain design criteria. As there is no

ideal bearing material which currently fulfills all the requirements of arthroplasty design, some degree of wear particle accumulation in the periprosthetic tissues can be expected.

1.7 THE EFFECTS OF WEAR PARTICLES ON CELLS AND TISSUES

1.7.1 In vitro effects of wear particles

The advantages of in vitro tissue culture techniques are that they are a quick, sensitive, quantitative test of the response to materials by living cells and, in particular, human cells. A disadvantage with in vitro testing which makes extrapolation of the results to the clinical situation difficult is that the techniques preclude complex tissue interactions because the cell culture is stagnant and, therefore, is separated from a blood supply and from possible neural, hormonal and metabolic control mechanisms (Rae, 1980).

Rae (1980) summarized the usual techniques of tissue culture assessment of biocompatibility of implant materials and emphasized the possible influence of the following factors: the physical form of the material, the size and shape of particles, the presence of soluble products, the method of assessment of toxicity, and the choice of cell and tissue type.

Studies of the haemolytic affect on cells, and the amount and type of enzyme release from cell cultures induced by solutions and particles of different metals suggest differences in response to pure metals and alloys (Heath et al, 1969; Rae, 1978). The effect of particles seems related to the type of metal, the size of the particle (Rae, 1978), and the presence of serum (Heath et al, 1969). Clearly, there is a threshold level for cell toxicity (Heath, 1954). Very small cobalt-chrome alloy particles have been reported to be more toxic than stainless steel (Mital and Cohen, 1968), and both materials inhibit cell replication (Pappas and Cohen, 1968).

Rae (1975) reported that cobalt, nickel, and cobalt-chrome alloy particles caused significant damage to macrophages whereas titanium, chromium and molybdenum particles were well tolerated. It is important to note the toxic particles, cobalt, nickel and cobalt-chrome alloy were all one micrometer or smaller, whereas the other particles were five micrometers or larger.

Based on the effect of soluble metal salts and particulate metals on human synovial fibroblasts, Rae (1981) concluded that cobalt and vanadium were the toxic components of the commonly used alloys. Very small nickel particles were not phagocytosed and were less toxic to fibroblasts whereas macrophages had previously been shown to phagocytose these particles and to be adversely affected (Rae, 1975). The toxicity of cobalt and vanadium was explained by their high solubility rate and nickel was found to be moderately soluble. There was significant variation in the size of particles used in these studies. Rae (1976) has suggested that monocytes may be more sensitive to the effects of particulate cobalt-chrome alloy than connective tissue and cartilage.

The phagocytic function and viability of macrophages is decreased by cobalt-chrome alloy particles, and the "rounding off" of macrophages after phagocytosis of metal particles suggests a change in membrane function of the macrophage due to metal particles (Garrett et al, 1983). Whether these effects are due to the particles alone or the soluble metal ion, or both, is unknown. Certainly, similar concentrations of cobalt chloride are capable of causing death of rat fibroblasts (Daniel et al, 1963).

Titanium and titanium-aluminium-vanadium alloy particles have little effect on human synovial fibroblasts and mouse peritoneal macrophages,

but release of enzymes by the macrophages suggests both these metals may have mild inflammatory potential (Rae, 1986a).

Evans and Thomas (1986) found cobalt-chrome alloy was less toxic than cobalt and nickel but emphasized that the patterns of toxicity varied for each metal, suggesting difficulty in using any single tissue culture toxicity test to assess the biocompatibility of implant materials.

Particles of titanium were reported by Plenk (1980) not to significantly inhibit the growth rate of human fibroblasts whereas aluminium oxide particles were mildly inhibitory and stainless steel produced even more inhibition. But comparisons of the effects of these particles on cell cultures are difficult because different sizes of particles of each material were used and the larger particles, above ten micrometers, were not phagocytosed. Harms and Mausle (1979) suggested aluminium oxide particles less than five micrometers in diameter have little effect on macrophage viability.

PMMA particles have been found to be moderately toxic whereas nylon and teflon were only mildly toxic to fibroblasts (Rice et al, 1978). PMMA inhibits human lymphocyte function (Panush and Petty, 1978) and inhibits the complement system (Petty and Caldwell, 1977), and PMMA particles inhibit macrophage protein synthesis (Horowitz et al, 1986). Barium sulphate in the concentration contained in acrylic cement is not toxic to mouse peritoneal macrophages (Rae, 1977).

In summary, the degree of particle toxicity will be determined by a number of factors which include: the inherent toxicity of the material to cells, the solubility of the particle in body fluids, the size of particles, the degree of phagocytosis of particles by different types of cells, and the solubility of the particle within cells. As well as

causing cell necrosis, particles adversely effect the functional capability of cells, and induce lysosomal enzyme release by phagocytic cells.

1.7.2 In vivo studies of the biocompatibility of particles

Wear particle biocompatibility has been studied in vivo by subcutaneous (Cohen, 1959; Paiement et al, 1986; Goldring et al, 1986), intramuscular (Heath, 1976; Griss et al, 1974), intra-peritoneal (Helbing et al, 1980), intravenous (Harms and Mausle, 1979), and intra-articular (Stinson, 1965; Wagner et al, 1976; Gourlay et al, 1978; Rushton and Rae, 1982, 1984; Meachim and Brooke, 1983; Uchida, 1985; Rae, 1986b) implantation of particles. There is evidence that particles of most materials will provoke a similar pattern of tissue response, but that the response will vary in severity and duration according to the type of material, size and number of particles, techniques of preparation and insertion of particles, and methods of assessment of the tissue response (Cohen, 1959; Stinson, 1965; Escalas et al, 1976).

1.7.2.1 Metals

Cohen (1959) injected particulate cobalt-chrome alloys, stainless steel, mild steel and silica subcutaneously in rats and examined the tissue response. Initially there was a PMN infiltrate and moderate oedema which resolved by one week. Mononuclear cells gradually increased in number to peak at two weeks but decreased throughout the rest of the study. The tissues were scarred and remained coloured grey due to the presence of particles up to one year after injection. The type of material and size of the particles influenced the severity of the tissue response. Cobalt-chrome alloy was the least toxic, stainless steel more toxic, and mild steel and silica the most toxic. Small particles less

than 0.5 micrometers were more toxic than coarse particles larger than ten micrometers. Sterility of the injected suspensions was checked but the culture methods were not described, and in particular, no mention was made of attempts to exclude low grade aerobic or anaerobic infection at the site of injection.

Escalas et al (1976) studied the long term effects of twenty-six particulates and solids by implantation in the paravertebral muscles of rabbits. Particles were prepared by a number of different methods. The metals were prepared according to A S T M recommendations while the other particles were washed in detergent, rinsed and dried. No attempt was made to prevent aggregation of particles during sizing. Similar volumes of each material but not necessarily similar numbers of particles were implanted. Particles of different sizes were sterilized and inserted dry by emptying a capsule containing the particles into the paravertebral muscles. At six months the severity of cellular infiltrate was graded from zero to three using stainless steel as a control. The reaction to cobalt-chrome alloy, stainless steel and aluminium oxide particles greater than eight micrometers, and to these materials in solid form, was described as mild.

The response to intra-articular injection of stainless steel particles 0.1 to ten micrometers was studied by Wagner et al (1976). Two hours following injection most particles were seen lying free within the joint, and no inflammatory reaction was seen. At one week a subacute inflammatory response characterized by macrophages, lymphocytes, occasional lymphoid follicles, and PMN was present. A more severe reaction occurred to a larger dose of particles. Studies of the effects of intra-articular injection of cobalt-chrome alloy particles demonstrate the particles are widely distributed several cell layers

from the surface of the synovium and the particles provoke a predominantly macrophage response (Meachim and Brooke, 1983).

Recently Rae (1986b) reported that intra-articular injection in mice knee joints of titanium, and titanium-aluminium-vanadium alloy particles of five micrometers and less, showed the materials were well tolerated, there being no necrosis and a predominantly macrophage response. At sixteen weeks following injection the tissue response was considered minimal and the response was the same at one year.

1.7.2.2 Polymers

Stinson (1965) implanted particulate low density polythene, PMMA and nylon in the muscle and knees of guinea pigs. The particle size ranged from less than one to seventy-six micrometers. Particles were sterilized by gamma irradiation and implanted in gelatin capsules. These polymers caused an initial macrophage and MNGC response gradually giving way to fibrosis. No mention was made of acute inflammatory cells. Interestingly, lymphocytes were not seen in large numbers until a few months after implantation of polyethylene, and not until twelve months after implantation of PMMA. The tissue response was reported as similar for all three materials. In reviewing the literature, Stinson (1965) found most reports agreed with these findings, but some acrylic polymers had been reported to produce a more intense reaction including necrosis and sterile abscess formation. Large high density polyethylene shreds are reported to produce a severe MNGC response in muscle as severe as that to teflon particles, even though both these materials are very biocompatible in solid form (Escalas et al, 1976). Using a modified scoring system developed by Sewell et al (1955) for assessing the short term biocompatibility of stitch materials by intramuscular implantation, Gourlay et al (1978) found that following intramuscular

implantation particles of PMMA were as toxic as teflon and rated two in a toxicity score ranging from zero to three.

Paiement et al (1986) studied the response in a rabbit wound chamber model to various types of particles including: pure titanium and cobalt-chrome alloy particles less than ten micrometers in diameter, particulate commercially available PMMA twenty to thirty micrometers in diameter, and UHMWP particles approximately thirty micrometers in diameter. The metal particles produced little tissue response but PMMA and polyethylene produced a macrophage and MNGC response. Using the same animal model, Goldring et al (1986) demonstrated a macrophage and MNGC response to PMMA and UHMWP particles but a bland fibrovascular response to solid implants of the same material. Intra-articular injection into mice knee joints of polyethylene particles prepared in a joint simulator produced a similar macrophage and MNGC response to two sizes of particles injected, five to one hundred micrometers and seventy to five hundred micrometers (Rushton and Rae, 1982).

Tetik et al (1974) injected particles of polyethylene and polyethylene-graphite composite over seventy micrometers, and particles of graphite less than ten micrometers, into the knees of a small number of animals and observed a relatively benign response with minimimal cellular infiltrate. Rushton and Rae (1984) reported no difference in the response to intra-articular injection in mice of carbon fibre reinforced polyethylene and polyethylene. Others have reported a benign response to carbon particles (Helbing et al, 1980). Uchida (1985) examined the tissue response to intra-articular injection in rat knees of powders of cobalt-chrome alloy, stainless steel, alumina ceramic, high density polyethylene and PMMA and included saline injected and uninjected control animals. Rats were sacrificed at regular periods from one week

up to twenty-five weeks. Ceramic powder was reported to cause the most significant tissue response, followed by PMMA, high density polyethylene, stainless steel and cobalt-chrome alloy powders. The length of time to sacrifice did not seem to effect the tissue response but no quantitative method of assessment of the tissue response was used.

1.7.2.3 Aluminium oxide ceramic

Griss et al (1974) studied the biocompatibility of aluminium oxide particles 0.5 to five micrometers in diameter by subcutaneous injection, injection into the foot pads of mice, intravenous injection into rats, and injection into the knees of mice. Animals were sacrificed at periods of up to one hundred and fifty days following injection. Following subcutaneous injection, a PMN response was seen which peaked at one week and was followed by a macrophage and fibrocyte response. It was concluded that ceramic particles were well tolerated as there was no progressive fibrosis and particles were trapped in macrophages with no evidence of a persisting inflammatory reaction. But other authors have suggested a persisting PMN response at one week may be evidence for a moderately toxic response to a material (ANSI Doc 41,1979; FDI Doc 198, 1980). Following intra-articular injection a massive proliferation of the synovium and a subsynovial cellular infiltrate occurred within three days, followed by an intense phagocytic response and then fibrosis with scarring of the subsynovium. The criteria for assessing fibrosis and distinguishing fibrosis from the normal subsynovial fibrous tissue were not described and no quantitative assessment of cell numbers was made. Particles were also seen in the local lymph nodes and in the liver and spleen.

Harms and Mausle (1979) examined the effects of aluminium oxide particles two to five and five to ten micrometers in diameter by intramuscular and intra-peritoneal implantation in animals. It was concluded that the local tissue response was good evidence of the biocompatibility of aluminium oxide particles because the acute inflammatory response had changed to a macrophage response by four weeks. The continuing presence of lymphocytes in association with a macrophage response was described. This also has been interpreted by others as a moderately toxic response (ANSI Doc 41, 1979; FDI Doc 198, 1980).

It has been concluded from these studies that aluminium oxide ceramic particles have little effect on tissues. Problems in both these studies were that particles were injected in saline, which will not prevent clumping of particles, no method was used to exclude infection, no control was included and no quantitative assessment of the tissue response was made. The PMN peak at one week, and the persisting lymphocyte response is cause for concern.

In summary, there are a number of criticisms of many of the previous in vivo studies of the biocompatibility of particles. These include: the wide variation in size and shape of particles, aggregation of particles in milling solutions, the use of different types of milling solutions, the lack of sterility control, the use of arthrotomy to implant particles, variable sites of biopsy for histological examination, the lack of inclusion of control materials, and the lack of quantitative methods of assessment of the tissue response.

1.7.3 In vivo studies of wear particle carcinogenesis

Carcinogenesis due to intramuscular and subcutaneous injection of metal and polymer particles has been demonstrated in rodents but the incidence of neoplasia may depend upon a variety of other factors, including the site of implantation, the species and strain of animal used, the physical form of the material and additives to milling fluids.

Heath (1976) reviewed the results of extensive studies involving intramuscular injection in rats of particles of pure metals. The initial inflammatory response was followed by a repair process which resulted in three possible outcomes. The particles were either dispersed or dissolved leaving varying degrees of scarring, or the particles were surrounded by a fibrous envelope, or, quite commonly, a malignant transformation of the repair process occurred. The high incidence of sarcomas in this study confirmed previous reports of the carcinogenic effects of solid and particulate pure metals implanted subcutaneously (Oppenheimer et al, 1956). A similar response was seen following intramuscular injection of cobalt-chrome alloy wear debris prepared in an artificial joint simulator (Heath et al, 1971).

Pauli et al (1986) studied the carcinogenic risk of implantation of solid and powdered metals in rats. Solid implants and particles of titanium alloys and cobalt-chrome alloys were implanted in rat femurs. Only three of eight hundred rats developed tumours at the site implantation, suggesting these materials were not carcinogenic. Meachim et al (1982) found no malignant neoplasms in two species of rats and one species of guinea pig at two years following intramuscular injection of cobalt-chrome alloy particles.

The evidence is that a number of factors influence carcinogenesis following polymer implantation. An important influence is the physical form of the implant. Oppenheimer et al (1961) found the carcinogenic properties of polymers was greatly reduced by reducing the size of implants to particles. It was suggested that sarcomas did not arise because particles can be phagocytosed, whereas sarcomas are thought to arise due to transformation in the membrane which develops in an attempt to isolate a large foreign body (Oppenheimer et al, 1958). A similar reduction in carcinogenesis was not seen when shredded polymer was implanted (Carter and Roe, 1969), presumably because the shreds were large enough to act as a solid material.

In a study of the intra-articular effects of particles of polyethylene, PMMA and nylon, Stinson (1965) found no evidence of malignant transformation in guinea pigs followed for thirty-six months after injection. Tumours have not been reported following intra-articular injection of cobalt-chrome alloy particles in guinea pigs (Meachim and Brooke, 1983), polyethylene in mice followed for up to twenty-three weeks (Rushton and Rae, 1984), carbon reinforced polyethylene followed for one year (Rushton and Rae, 1984) and titanium and titanium alloy particles in mice followed for two years (Rae, 1986b).

In summarizing the effects of solid surfaces on tissues, Bischoff and Bryson (1964) concluded that any large solid implant provokes a prolonged low-grade inflammatory response which might result in malignant transformation. It might be hypothesized that a bolus of metal particles implanted intramuscular or subcutaneously acts like a solid implant if the particles are not phagocytosed and cleared from the site of implantation. This may contribute to the formation of tumours at the site of implantation. Whereas the lack of tumours following

intra-articular injection of particles may suggest this is a better method of detecting the carcinogenicity of particles of different materials.

1.8 SUMMARY OF CURRENT SITUATION

It is clear there is a volume of literature addressing the problem of prosthesis loosening. While in recent years a number of laboratories have undertaken research attempting to understand the biology of the loosening process, it is evident from the continuing activity in this field of research that many aspects of this important clinical problem remain unexplained. In particular, the relationship between the tissue response to prosthesis wear particles and loosening requires further investigation. Of particular interest is the effect of particles on cells in the absence of infection or mechanical causes for damage to tissues. Also the possible role of particles in the stimulation of bone resorption needs to be determined.

CHAPTER TWO

THE PATHOLOGICAL FINDINGS IN THE TISSUES AROUND FAILED HIP ARTHROPLASTIES

2.1 AIMS

This study was undertaken to examine the tissues around failed hip arthroplasties retrieved at revision surgery by light microscopy, transmission electron microscopy (TEM) and energy dispersive X-ray (EDX) microanalysis to review the type of cellular response, and the morphology of wear particles originating from the articulating surfaces of the prostheses.

2.2 INTRODUCTION

The histological appearances of the tissues around joint arthroplasties varies considerably (Vernon-Roberts and Freeman, 1977). The connective tissue layer at the bone-implant interface prior to and following loosening of joint arthroplasties may consist of fibrocartilage (Charnley et al, 1968), fibrous tissue (Vernon-Roberts and Freeman, 1977), or highly cellular tissue described by some as a "synovial like membrane" (Goldring et al, 1983). Direct contact between bone and cement without an intervening tissue layer has also been reported (Lindner and Hansson, 1983).

The tissue appearance is likely to depend on a number of factors including the presence or absence of infection (Mirra et al, 1976), the

degree of loosening, the type of prosthesis material, the duration of the implant (Vernon-Roberts, 1985) and the amount and type of wear debris (Mirra et al, 1982).

This study was undertaken to determine the tissue appearance around a variety of cementless and cemented total hip arthroplasties with articulating surfaces composed of various materials, thus allowing comparison of these appearances with subsequent animal studies of the in-vivo response to wear particles of the same materials. Strict criteria were used to exclude infection which might have caused an inflammatory response in the tissues.

2.3 MATERIALS AND METHODS

2.3.1 Arthroplasty revision surgery protocol

Fifty-four hip arthroplasties in fifty-four patients were revised because of pain, usually in association with radiographic evidence of loosening of the prostheses. Seven arthroplasties were excluded from this study because of infection, leaving forty-seven arthroplasties in forty-seven patients available for study. There were twenty-six males and twenty-one females. The original diagnosis was osteoarthritis in thirty-nine, rheumatoid arthritis in seven and post traumatic avascular necrosis in one. The age range was twenty-four to eighty-seven years, with a median of seventy-one years. The duration of implantation of the arthroplasties prior to revision ranged from one to fourteen years, with a median of nine years. The arthroplasties were divided into cementless and cemented prostheses, and according to the type of articulation, there being metal on bone, metal on metal, ceramic on ceramic, or metal on polyethylene. The arthroplasties were further divided into the three

categories, hemi-arthroplasty, resurfacing arthroplasty and total hip arthroplasty.

The following protocol was followed at the time of revision surgery. The degree of loosening of components was recorded. For histopathology examination a biopsy was taken of the joint capsule and the connective tissue layer between the implants and acetabular and femoral bone. Where loosening was absent or slight between the implant and bone, a block of bone and the interface tissue was removed if this did not jeopardize revision surgery. The whole of the femoral head was retrieved at revision of femoral resurfacing components. The biopsies were fixed in ten percent formal saline. For TEM examination and EDX microanalysis selected biopsies of capsule and interface tissues were taken and fixed in glutaraldehyde.

The following specimens were taken for microbiological examination using separate unused instruments for each biopsy: a hip aspirate, three specimens of capsule, and one specimen of both acetabular and femoral interface connective tissue. The specimens were transported to the laboratory in Stuarts media. If present, pus was aspirated in a syringe and sent for culture.

The degree of loosening was recorded prior to removal of the components. The interface between the acetabular and femoral components and bone around cementless arthroplasties, or between the prostheses and cement and cement and bone around cemented arthroplasties was exposed with bone nibblers and pushed and pulled using a clamp. The degree of loosening was divided into the following categories: (a) no loosening, there being no movement at the interface; (b) possible loosening, there being fluid movement at the interface but no definite movement of either the prosthesis or cement; (c) slight loosening, evidenced by slight movement

at the interface but removal of the prosthesis or cement required hammering or firm leverage; and (d) gross loosening, where obvious movement at the interface was present and removal of the prosthesis or cement was easily accomplished by hand or gentle leverage.

2.3.2 Tissue processing for histopathology and electron microscopy

The soft tissue biopsies were fixed in ten percent buffered formal saline for three days and then dehydrated through graded alcohol, cleared in chloroform and embedded in paraffin wax. Six micrometer sections were cut using a hand operated LKB 2259 multirange microtome and stained with hematoxylin and eosin.

The bone biopsies and femoral heads were fixed in formal saline for a minimum of seven days. Two sections of each specimen were cut using a band saw in a plane at right angles to the interface in the case of femoral shaft biopsies, and in the coronal plane of the femoral heads. One of the sections was decalcified using a commercial decalcifying solution (Decal, Omega Chemical Corporation, N.Y.). The extent of decalcification was controlled by daily radiographs (Kodak Min-R film, Hewlett Packard Series 43805N Faxitron Cabinet X-ray Machine). After neutralization in five percent silver sodium sulphate, the specimen was processed and embedded in wax and eight micrometer sections were cut which were stained with haematoxylin and eosin. The other specimen which was not decalcified was dehydrated in ethanol, cleared in acetone and impregnated with araldite. Eight micrometer thick sections were cut using a Jung motorised microtome. The araldite was removed with potassium hydroxide and the sections stained with hematoxylin and eosin and Von Kossa (VK) stains. All sections were examined under transmitted light and polarized light.

Soft tissue biopsies for electron microscopic examination were fixed in 2.5% glutaraldehyde in 0.05 M cocodylate buffer. Each biopsy was then divided in two. One specimen, for future morphological examination, was post-fixed in 2% osmium tetroxide in 0.05 M cocodylate buffer for one hour and then dehydrated in graded alcohol. The other specimen, for future metal analysis, was passed directly into graded alcohol. The specimens were passed through two changes of propylene oxide and then through increasing concentrations of propylene oxide and araldyte and epoxy embedding resin, until final embedding in araldyte and epoxy embedding resin at sixty degrees centigrade for twelve hours.

Semi-thin sections of osmium and non-osmium fixed samples, approximately half to one micrometer thick, were cut and stained with toluidene blue and examined by light microscopy. Representative sections were selected and ultra-thin sections were cut using an L.K.B. ultra-microtome.

Sections for morphological examination were stained with aqueous uranyl acetate and lead citrate and examined in a JEOL 100 CX TEM SCAN analytical electron microscope fitted with an EDAX 707 energy dispersive X-ray analyser.

Sections for EDX microanalysis were prepared from tissues processed without osmium tetroxide post-fixation and were examined without staining with either uranyl acetate or lead citrate.

Intracellular and extracellular inclusions were analysed by condensing the electron beam so as to illuminate only the inclusion (target) under study and then recording the spectra for a live time of 200 or 400 seconds. For each target spectrum a background spectrum was obtained in an adjacent area under the same operating conditions which was then subtracted from the target according to the EDIT TEM programme.

2.3.3 Microbiology Techniques

The biopsies for microbiological examination were removed from Stuarts transport media and, using a stomacher, were homogenised in half a millilitre of glucose cooked meat medium. In an anaerobic chamber the homogenates and joint aspirates were inoculated on to a supplemented blood agar (B.A.Y.H.) plate, an anaerobic blood culture broth, and a glucose cooked meat broth, and these were incubated anaerobically. Samples were also inoculated into a chocolate agar plate and incubated in carbon dioxide. Plate cultures were continued for four days and broth cultures for fourteen days. A subculture was taken if any broth became turbid. Organism identification was according to standard laboratory techniques (Cowan, 1975; Holdeman et al, 1977).

The criteria for diagnosis of infection were the presence of pus and a positive culture or, in the absence of pus, a positive culture in four or five of five biopsies of the periprosthetic tissues (Kamme and Lindberg, 1981).

2.4 RESULTS

2.4.1 Cementless metal on bone, metal on metal, and ceramic on ceramic arthroplasties

Five cobalt-chrome alloy Smith-Petersen cup resurfacing arthroplasties, six cobalt-chrome alloy Austin Moore stemmed hemi-arthroplasties, three cobalt-chrome alloy Ring total hip arthroplasties, and two Mittelmier aluminium oxide ceramic on ceramic total hip arthroplasties were revised.

The Smith-Petersen cups were all grossly loose and three had subsided into varus. The femoral heads were shortened and the superior trabeculae were thickened. A connective tissue layer was seen on the inferior non-weight bearing surface of the femoral heads (Fig. 2.1). Two Austin Moore hemi-arthroplasties were slightly loose and two were grossly loose. Histological examination revealed that the capsule around these prostheses and the connective tissue beneath the cups and around the femoral stems consisted mainly of mature fibrous tissue. Occasional macrophages were present in association with small numbers of metal particles. Neither MNGC, lymphocytes nor PMN were present in significant numbers.

The acetabular components and two of the three femoral components of the cementless Ring total hip arthroplasties were slightly loose. One femoral component was solidly fixed (Fig. 2.2). The capsule and the connective tissue layer at the bone-prosthesis interface were stained grey on naked eye examination of the fresh tissues. Histological examination revealed that the capsule consisted mainly of mature fibrous tissues. In some areas a macrophage infiltrate in association with metal wear particles was seen (Fig. 2.3). Occasional lymphocytes and lymphocytic aggregates were seen and there were occasional areas of necrosis. A biopsy of the bone-prosthesis interface around the solidly fixed femoral component showed accumulation of macrophages and metal particles in the tissue adjacent to bone (Fig. 2.4). Particles were found in cells immediately adjacent to bone (Fig. 2.5). These appearances were also seen in the tissue around the other loose Ring prostheses. The connective tissue at the acetabular bone-prosthesis interface was highly cellular and metal particles were often seen in cells (Fig. 2.6).

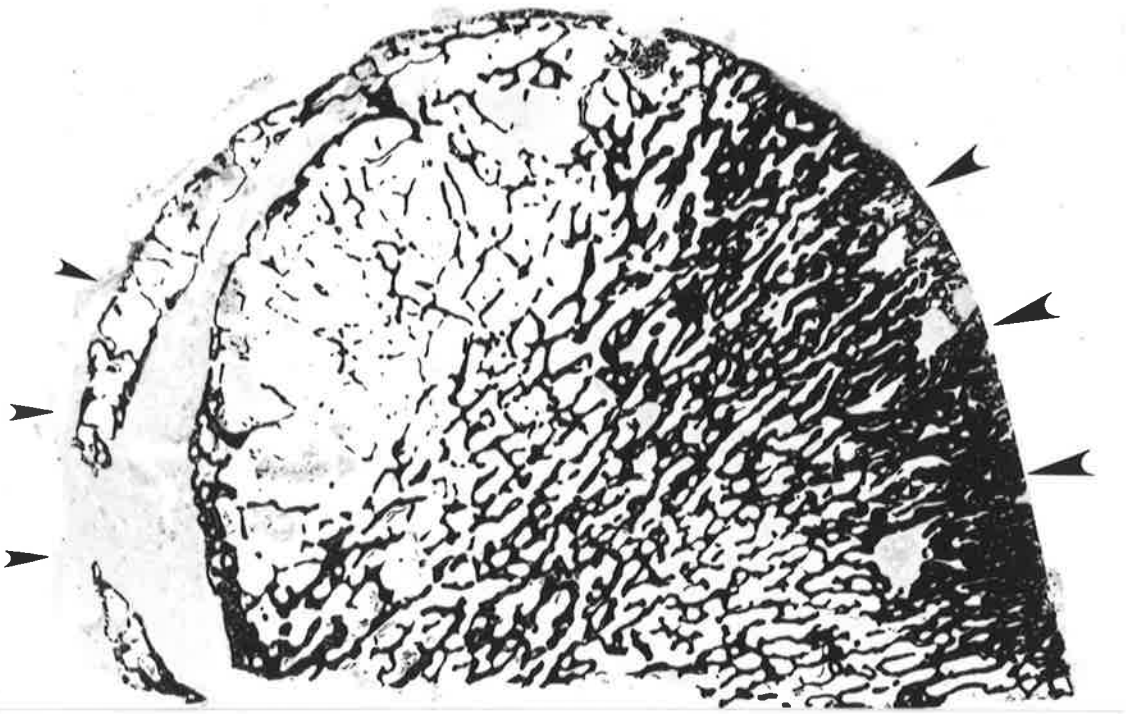

Fig. 2.1. An undecalcified coronal section of a femoral head beneath a cementless metal resurfacing arthroplasty seven years following insertion. The calcified bone is stained black by the Von Kossa technique. The superior weight bearing area (large arrows) is eburnated and trabecular thickening is evident. On the inferior non-weight bearing surface (small arrows) mature connective tissue is seen. Few metal particles or macrophages were seen in this tissue. HE VK x 3

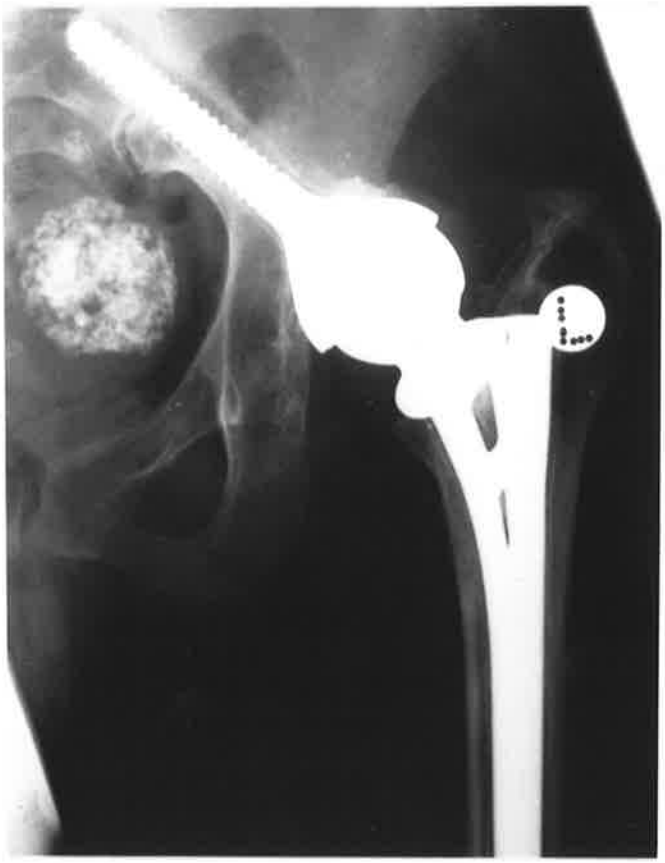

Fig. 2.2. Radiograph of a Ring cementless metal on metal total hip prosthesis eight years following insertion. The acetabular component was slightly loose and the femoral component was solidly fixed. The periprosthetic tissues were stained grey.

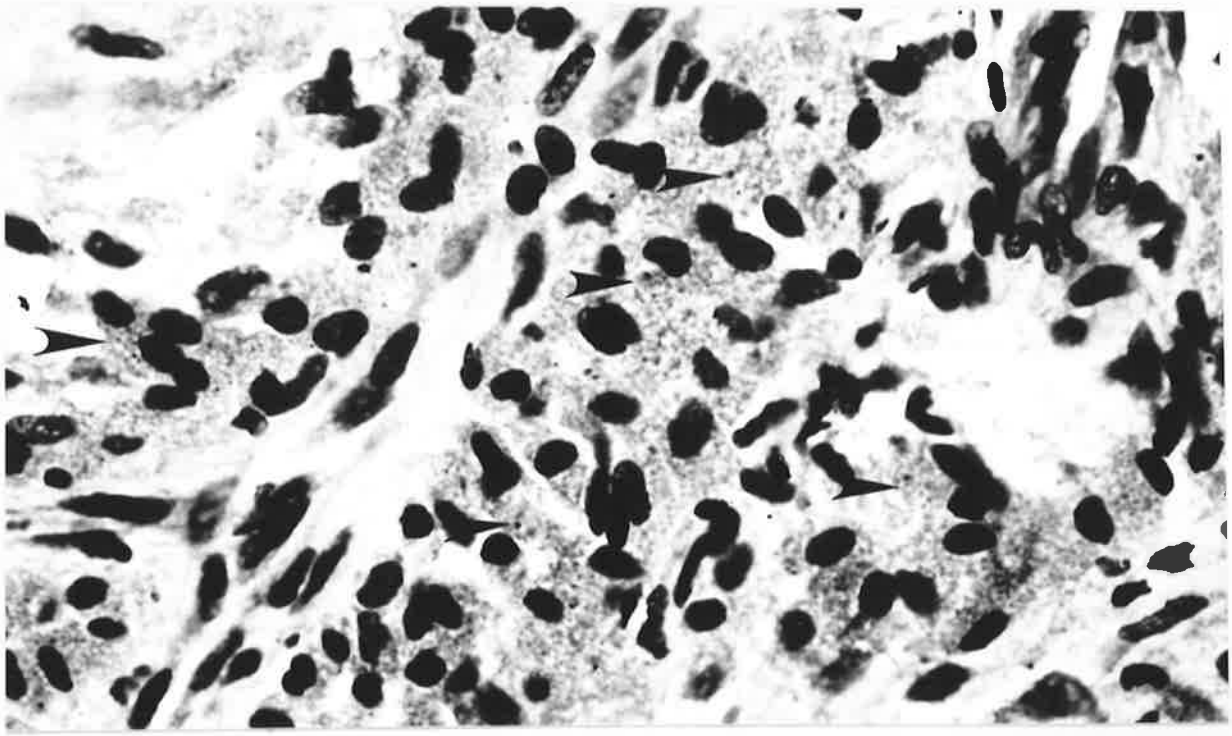

Fig. 2.3. Photomicrograph of the capsule surrounding the Ring prosthesis seen in Figure 2.2. It shows accumulation of macrophages containing small black metal particles (arrows). HE x 400

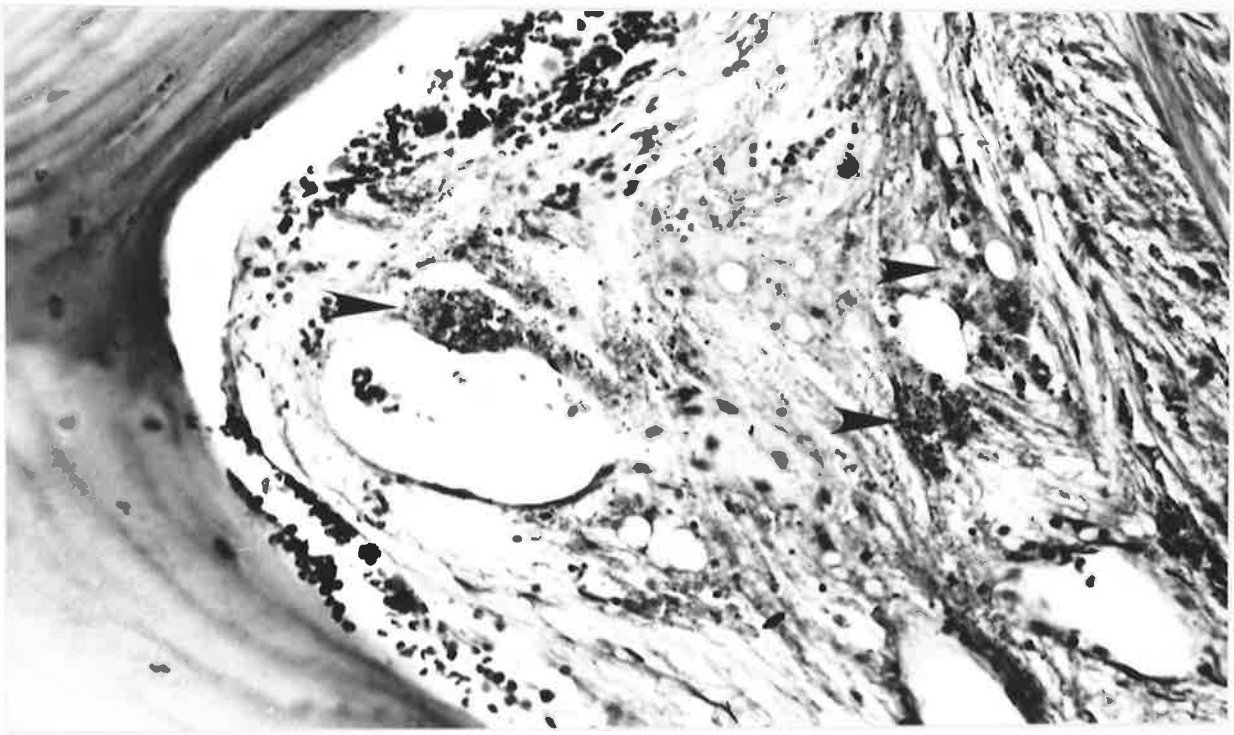

Fig. 2.4. Photomicrograph of the connective tissue layer at the proximal bone-prosthesis interface of the solidly fixed femoral component of the Ring prosthesis seen in Figure 2.2. It shows small groups of macrophages containing metal wear particles (arrows) in the tissue adjacent to bone. HE x 160

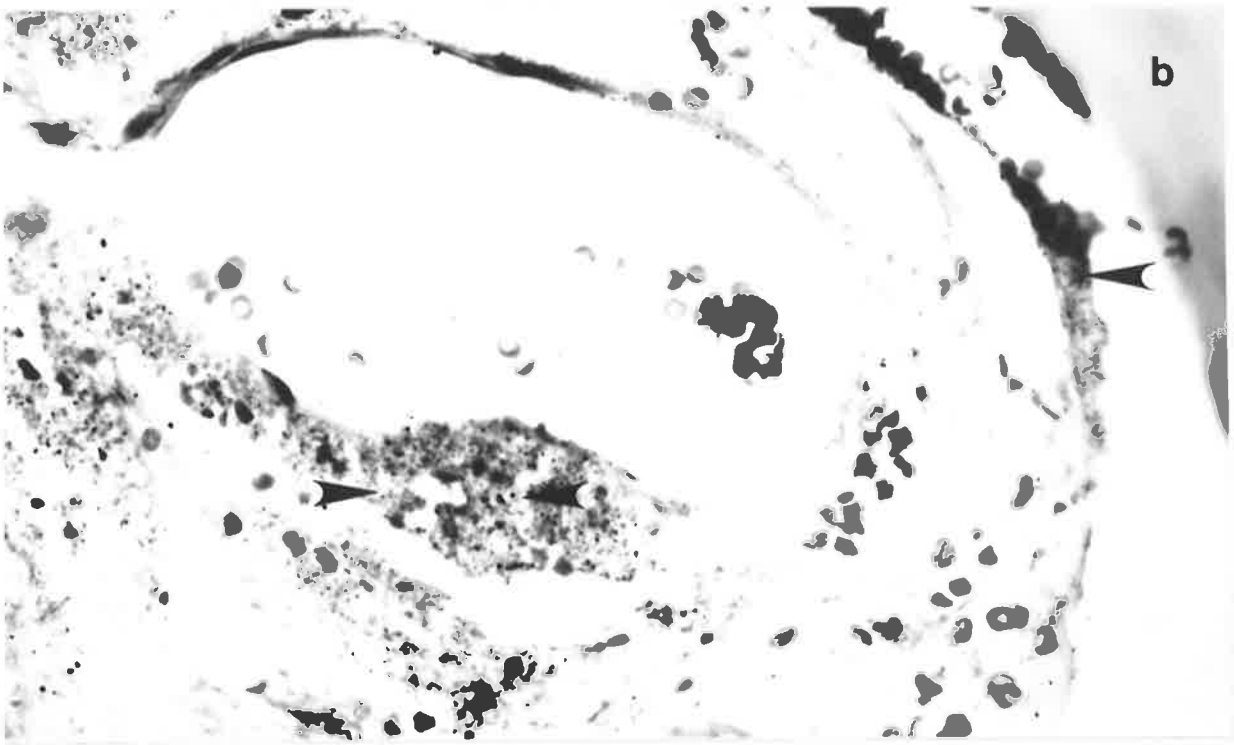

Fig. 2.5. High power photomicrograph of the connective tissue at the bone prosthesis interface seen in Figure 2.4. It shows numerous metal particles (arrows) within macrophages adjoining a dilated vascular channel adjacent to the bone (b). The gap between cells and bone is an artefact which occurred during processing of the specimen. HE x 400

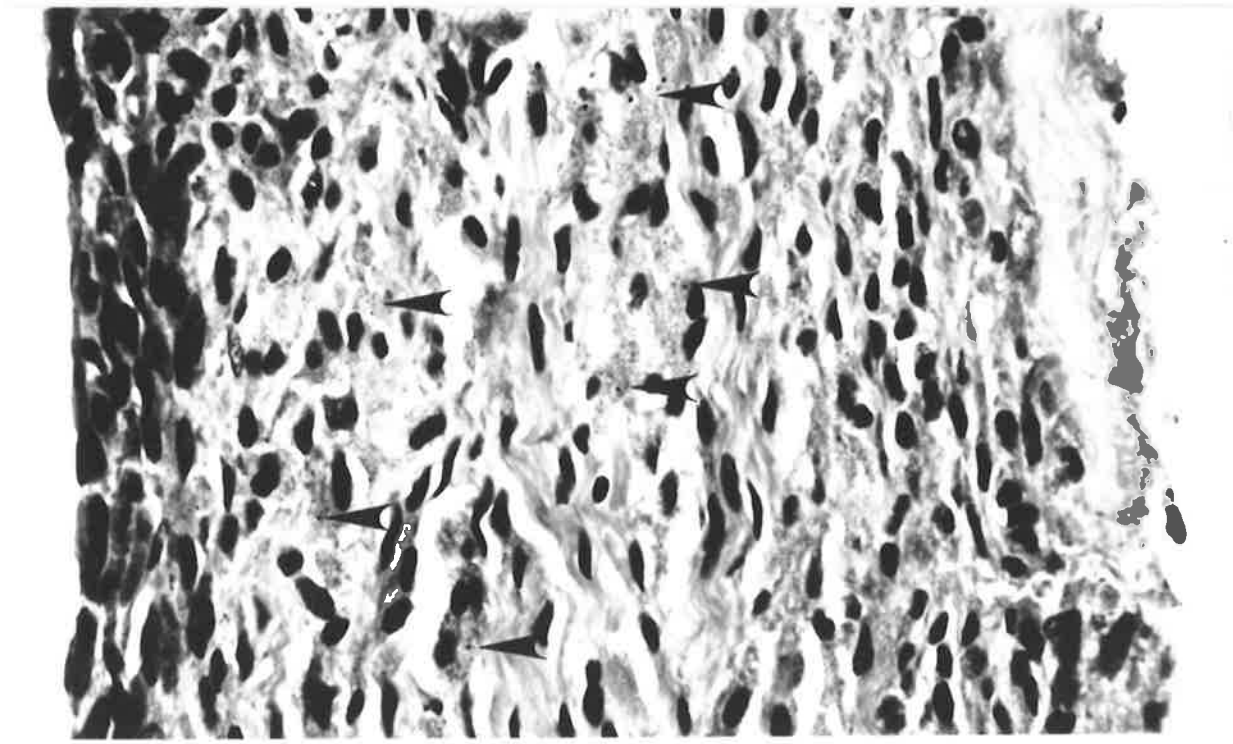

Fig. 2.6. Photomicrograph of the connective tissue at the acetabular bone-prosthesis interface of the Ring prosthesis seen in Figure 2.2. The surface on the left of the figure was adjacent to the prosthesis and the opposite surface was attached to bone. The tissue contains numerous macrophages which have phagocytosed small metal particles (arrows). HE x 400

Electron microscopy examination of the tissue around the cementless metal on metal arthroplasties showed accumulation of electron-dense particles in macrophages in the capsule (Fig. 2.7), and in the connective tissue at the interface between bone and the acetabular (Fig. 2.8) and femoral components (Fig. 2.9). Some macrophages which had phagocytosed particles showed extensive accumulation of cytolysosomes (Fig. 2.7). Other macrophages showed total loss of cell membrane and clumping of nuclear chromatin, suggesting degeneration (Fig. 2.9). EDX microanalysis confirmed that the electron-dense particles seen in macrophages were composed of cobalt-chrome alloy (Figs. 2.10 and 2.11).

The acetabular and femoral components of both cementless ceramic on ceramic prostheses were slightly loose. Both prostheses were removed less than two years after insertion. The articulating surfaces showed no macroscopic evidence of wear (Fig. 2.12). The capsule and interface tissues were composed of mature connective tissue. Occasional macrophages were observed and no aluminium oxide particles were seen. Electron microscopy showed that the surface lining of the capsule was composed of macrophages and occasional macrophages were seen in the connective tissue at the acetabular bone-prosthesis interface, but no particles were seen in these cells (Figs. 2.13 and 2.14).

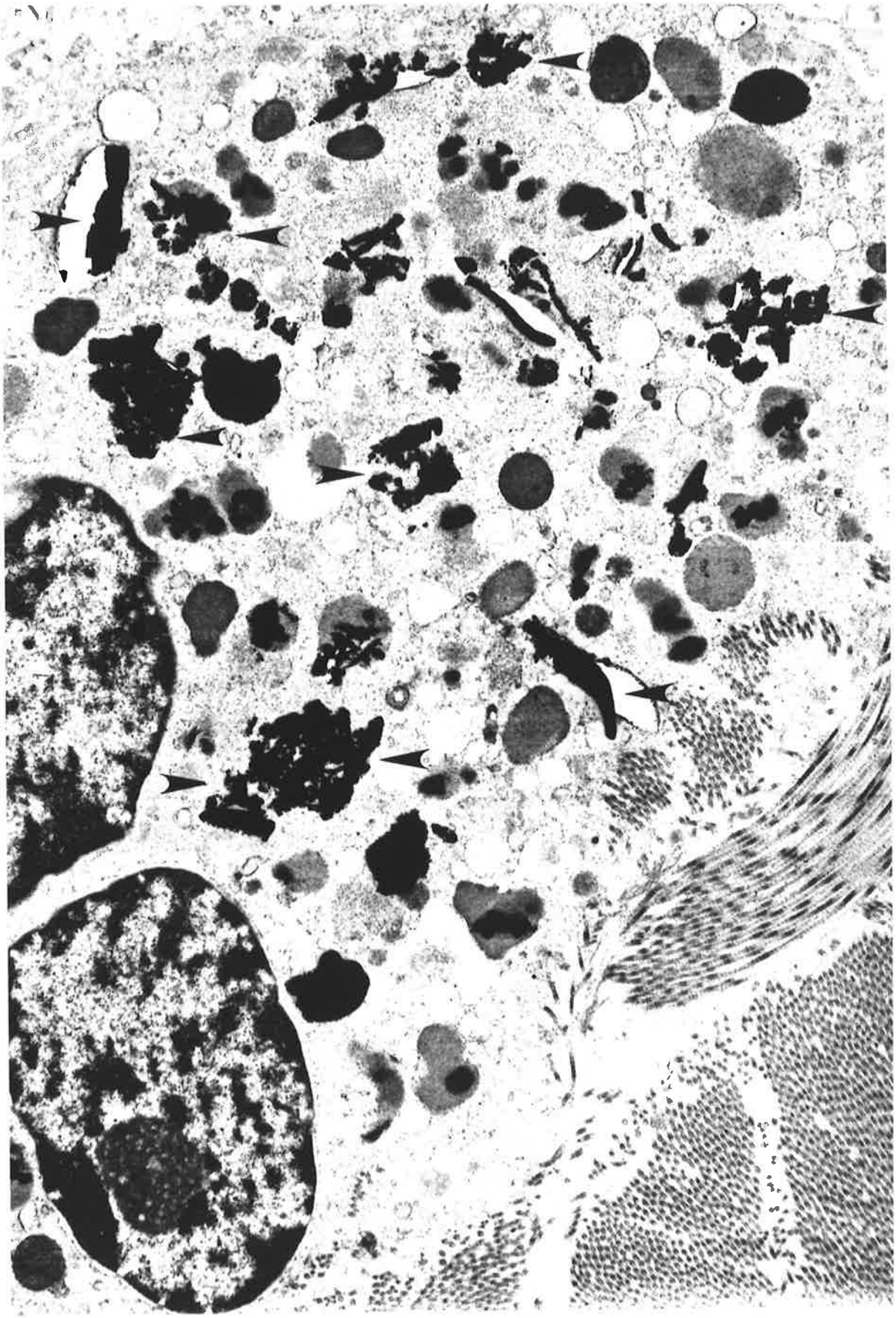

Fig. 2.7. Electron micrograph of the capsule around a cementless metal on metal prosthesis. The macrophage shows extensive accumulation of electron-dense particles (arrows) and large numbers of cytolysosomes. x 13,000

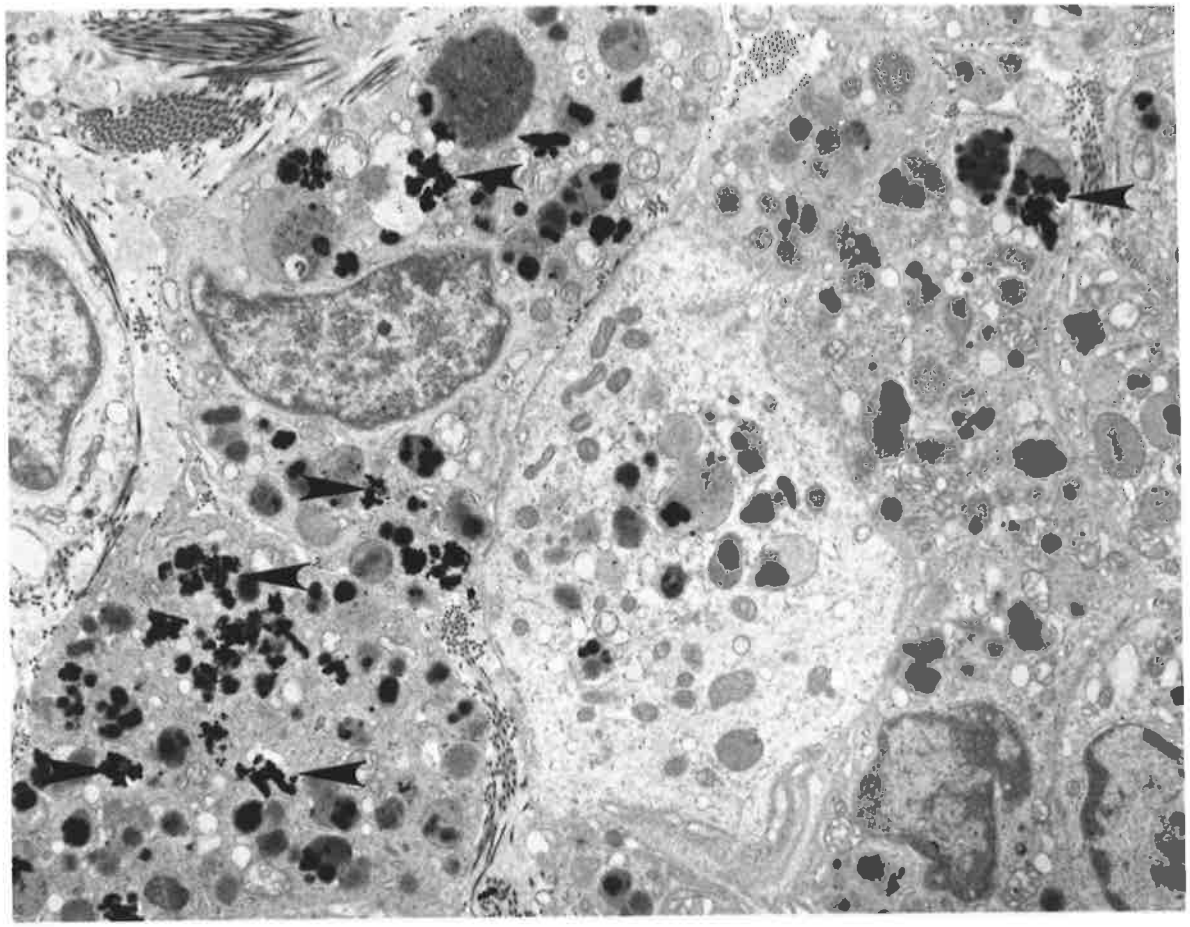

Fig. 2.8. Electron micrograph of the acetabular connective tissue adjacent to a cementless metal on metal prosthesis. Macrophages contain electron-dense particles (arrows) and numerous cytolysosomes. x 7,000

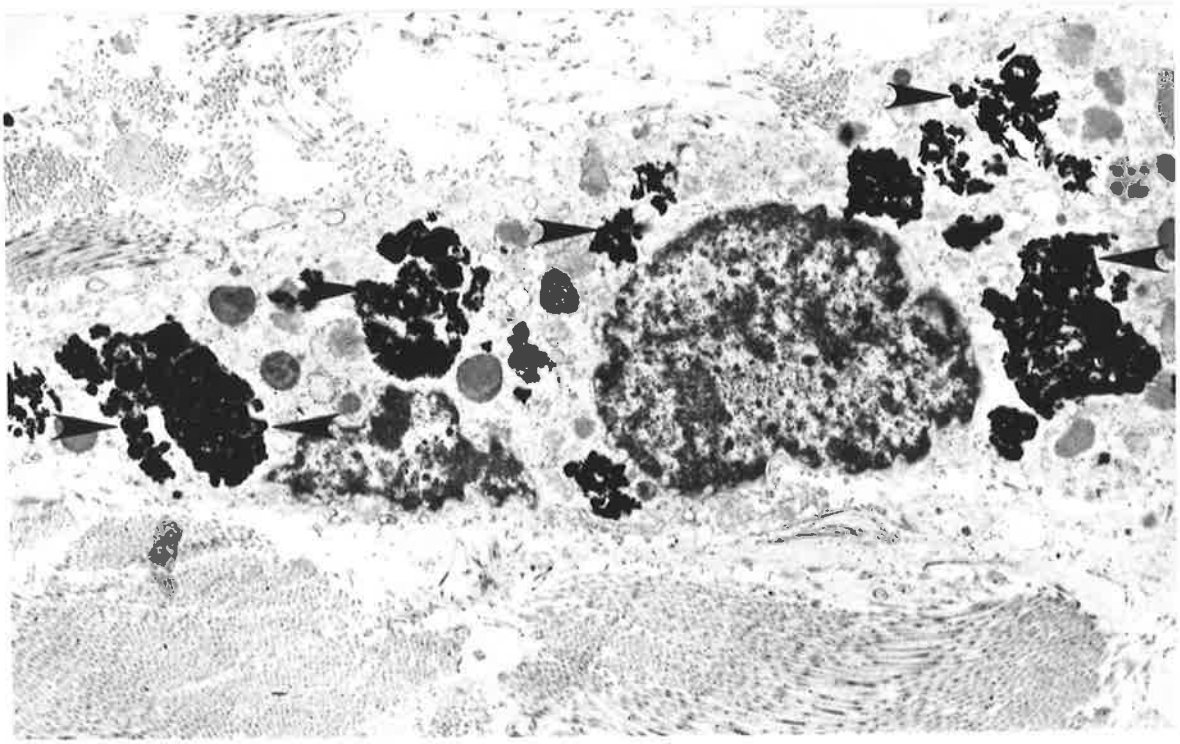

Fig. 2.9. Electron micrograph of the femoral connective tissue adjacent to a cementless metal on metal prosthesis. A macrophage containing electron-dense particles (arrows) shows loss of its cell membrane and some clumping of nuclear chromatin, suggestive of degeneration. x 7,000

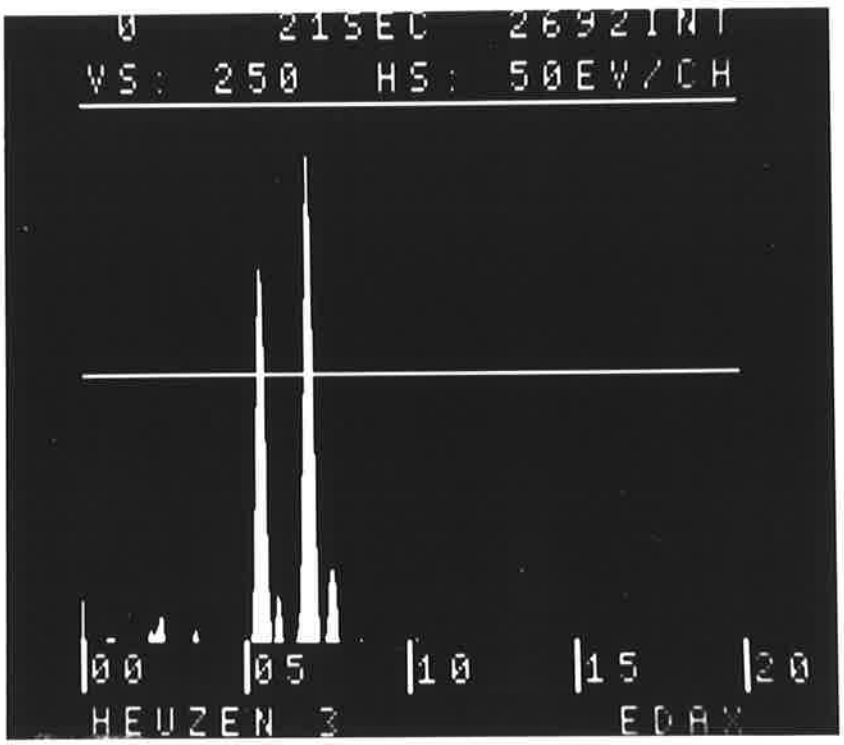

Fig. 2.10. EDX microanalysis of the electron-dense particles seen in Figure 2.7. It shows peaks for chromium at 5.4 KeV and 5.9 KeV and for cobalt at 6.9 KeV and 7.6 KeV.

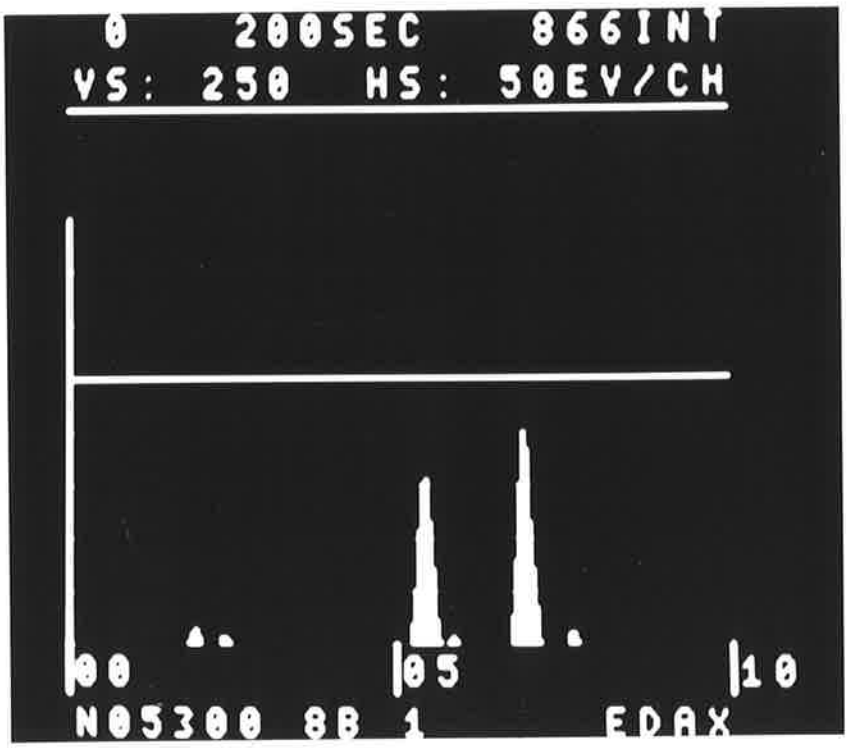

Fig. 2.11. EDX microanalysis of the electron-dense particles seen in Figure 2.8. It shows major peaks for chromium at 5.4 KeV and for cobalt at 6.9 KeV.

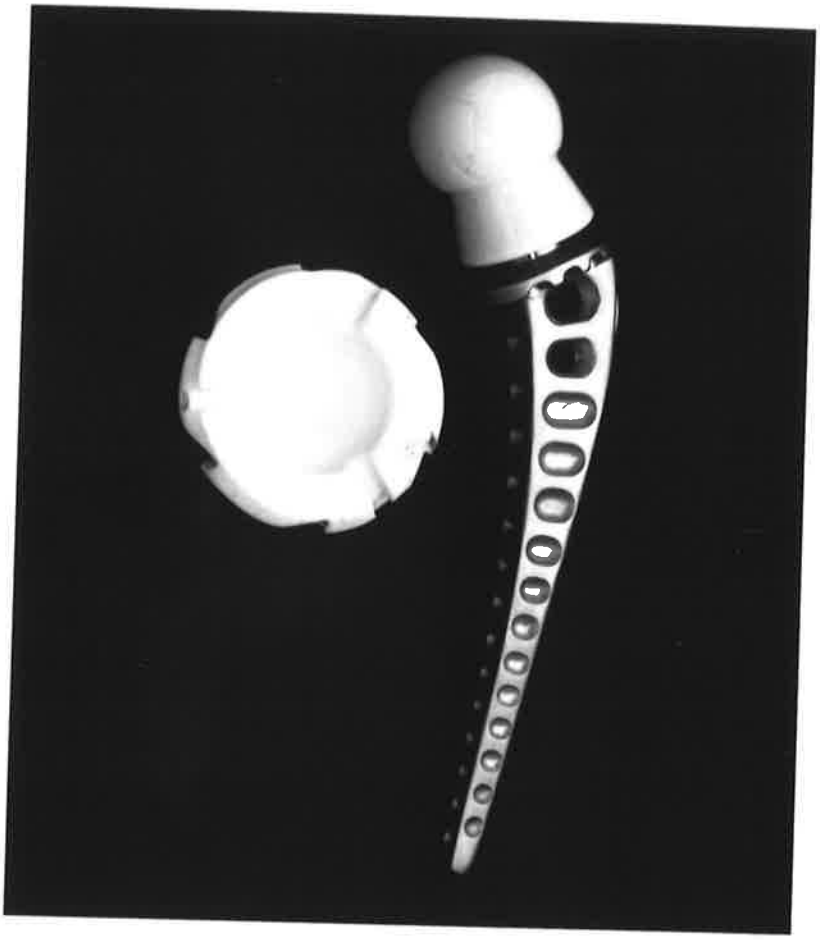

Fig. 2.12. Photograph of a cementless aluminium oxide ceramic on ceramic prosthesis revised after fourteen months. It shows no macroscopically obvious wear of the articulating surfaces.

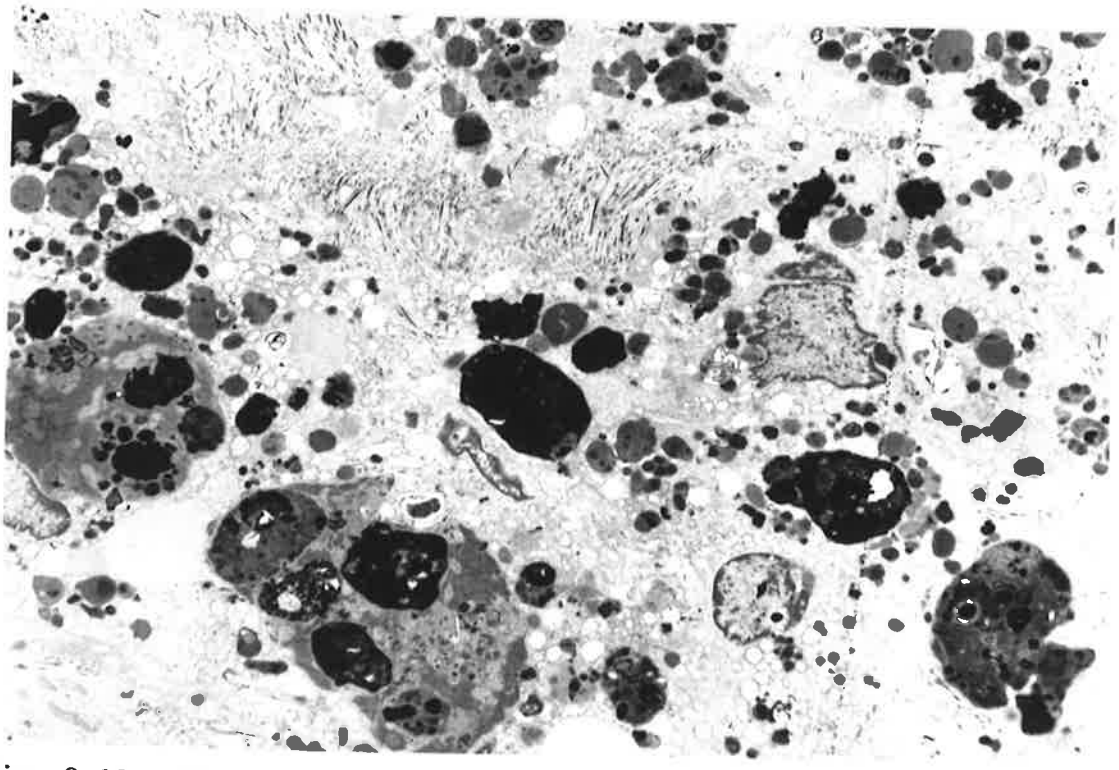

Fig. 2.13. Electron micrograph of the acetabular connective tissue adjoining a cementless aluminium oxide ceramic on ceramic prosthesis. Macrophages are present but no particulate material is seen. x 3,000

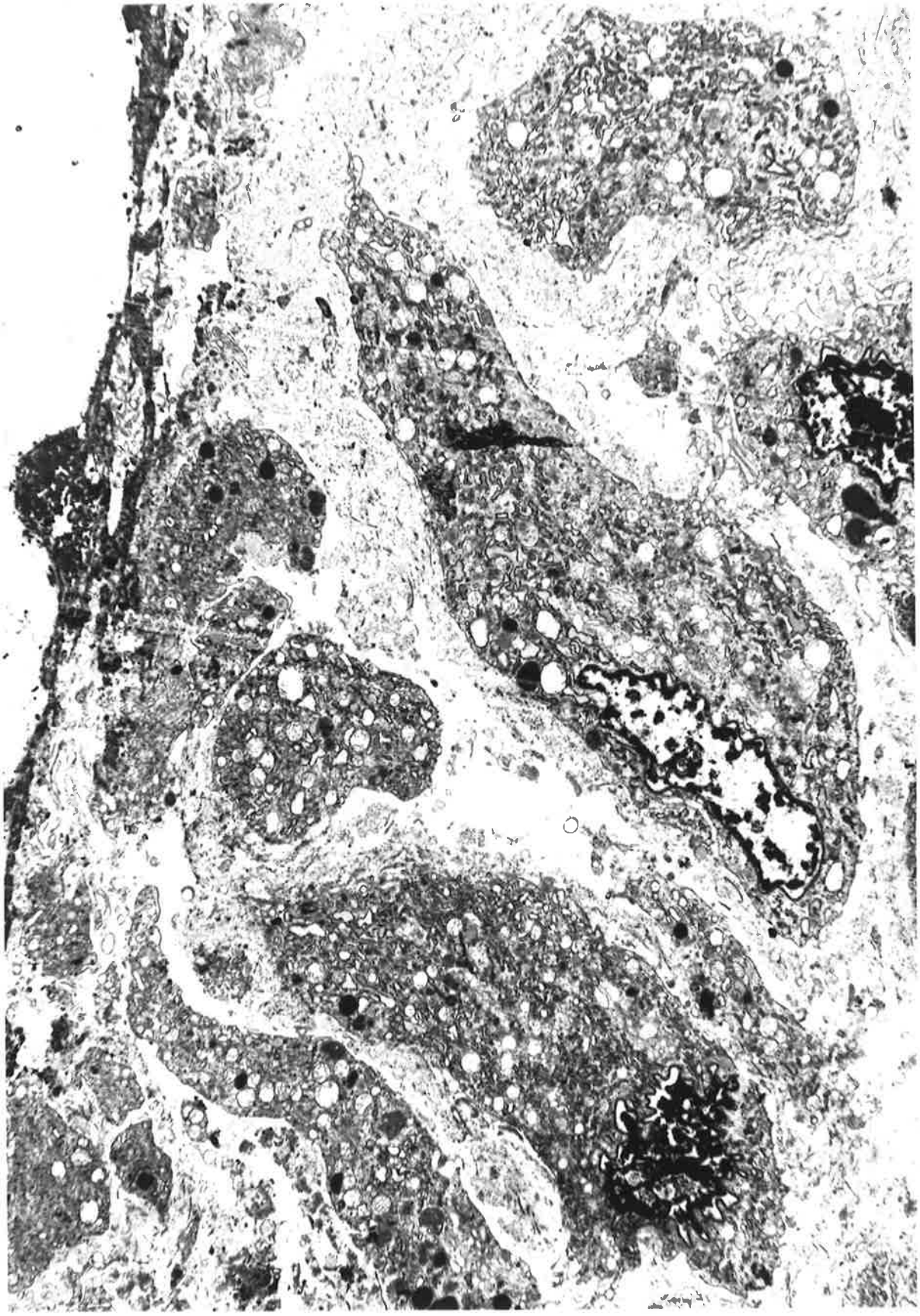

Fig. 2.14. Electron micrograph of the capsule around the cementless ceramic on ceramic prosthesis seen in Figure 2.12. While the macrophages near the synovial surface contain numerous organelles, no particulate material is visible. x 5,500

2.4.2 Cemented metal on metal, and metal on polyethylene arthroplasties

Five McKee all cobalt-chrome metal on metal total hip arthroplasties, two THARIES metal on polyethylene resurfacing hip arthroplasties and twenty-four metal on polyethylene stemmed Charnley and Muller total hip arthroplasties were revised.

All the acetabular components and four of the femoral components of the McKee prostheses were grossly loose at the bone-cement interface. One femoral stem was solidly fixed in cement and the cement was solidly fixed in bone distally. Loosening of these prostheses was often associated with severe bone loss (Fig. 2.15) but bone loss was also seen in the proximal femur around the solidly fixed femoral stem.

The universal histological feature was the presence of a macrophage infiltrate in the capsule in association with very small intracellular metal wear particles (Fig. 2.16). Areas of necrosis occasionally were seen. MNGC occasionally were present in association with cement particles and lymphocytic aggregates sometimes were seen. Similar findings were seen in the connective tissue at the acetabular and femoral bone-cement interfaces (Fig. 2.17). At these interfaces, connective tissue containing macrophages and metal particles was seen adjacent to and extending between bony trabeculae (Fig. 2.18).

All components of the resurfacing arthroplasties were slightly loose. The degree of loosening at the bone-cement interface of the acetabular components of the metal on polyethylene stemmed total hip arthroplasties was: three with possible loosening, eight slightly loose, and thirteen grossly loose. The acetabular prostheses were solidly fixed in cement except in seven cases in which the components were grossly loose in the cement mantle. Two of these loose acetabular

components were completely worn through (Fig. 2.19) and another component was severely worn and had fractured. Wear of the articulating surfaces of the components was evident as early as fourteen months following insertion (Fig. 2.20).

The degree of loosening at the bone-cement interface of the femoral components was: three not loose, two slightly loose, and nineteen grossly loose. The degree of loosening at the prosthesis-cement interface was, three solidly fixed, five slightly loose, and sixteen grossly loose. Loosening of these prostheses was often associated with severe bone loss and one femoral shaft fracture occurred (Fig. 2.21). Two fractured Charnley femoral component stems were revised.

Histological examination of the tissues around these prostheses revealed a common pattern of findings. The capsule and synovium were infiltrated to a varying degree by macrophages and MNGC. Occasional areas of necrosis were present. Lymphocytic aggregates and PMN were rare. The tissues contained varying numbers of highly birefringent polyethylene particles and occasional voids due to acrylic particles dissolved during tissue processing. Metal particles occasionally were present. Small particles of polyethylene were contained within macrophages and larger particles of polyethylene and acrylic were contained within MNGC (Figs. 2.22 and 2.23).

The appearance of the interface connective tissue ranged from mature relatively acellular fibrous tissue to highly cellular tissue. When loosening of the bone-cement interface was absent the surface of the interface tissue adjacent to the cement was lined with flat mononuclear cells. When loosening was established this surface often was abraded, while in other areas the tissue was lined with polygonal cells. The tissues contained varying numbers of polyethylene, acrylic, bone, and

metal particles. The polyethylene particles ranged in size from large fragments more than a hundred micrometers in maximum dimension to very fine particles, barely perceptible by light microscopy (Figs. 2.24 and 2.25). At the junction between the interface connective tissue and bone, various numbers of osteoclasts often were observed (Figs. 2.26 and 2.27).

An important finding was the tissue response seen at the distal aspect of the cement mantle in three cases where the femoral stem remained firmly fixed in the femur. Tissue from the bone-cement interface in these areas showed accumulation of very fine polyethylene particles. In addition, the tissue in one of these contained large numbers of acrylic particles which may have originated from unrecognized micromotion at the bone-cement interface. The interface tissue contained large numbers of macrophages and MNGC.

Sections of the femoral heads beneath the resurfacing hip arthroplasties provided an overall picture of the interface. The cement was separated from bone by a connective tissue layer one to five millimetres thick except on the superior aspect where bone was abraded by the cement (Fig. 2.28). The tissue contained variable numbers of macrophages and MNGC. Fine polyethylene particles were seen in the superior region of the interface and larger numbers of polyethylene particles and acrylic particles were seen in the basal regions of the interface.

Electron microscopy of the connective tissue at the bone-cement interface showed accumulation of particles in macrophages (Fig. 2.29). These particles were assumed to be polyethylene as no metal was detected on EDX analysis of the particles.

Electron microscopy also showed macrophages adjacent to cement particles (Figs. 2.30). Some macrophages showed loss of cellular membrane and clumping of nuclear chromatin suggesting degeneration (Fig. 2.31). Particles at the edge of cement (Fig. 2.31) were identified by EDX microanalysis as radio-opaque barium particles mixed with the cement.

The appearance of the periprosthetic tissues and the morphological and analytical characteristics of the tissues around the forty-seven arthroplasties are summarized in Tables 2.1 and 2.2.

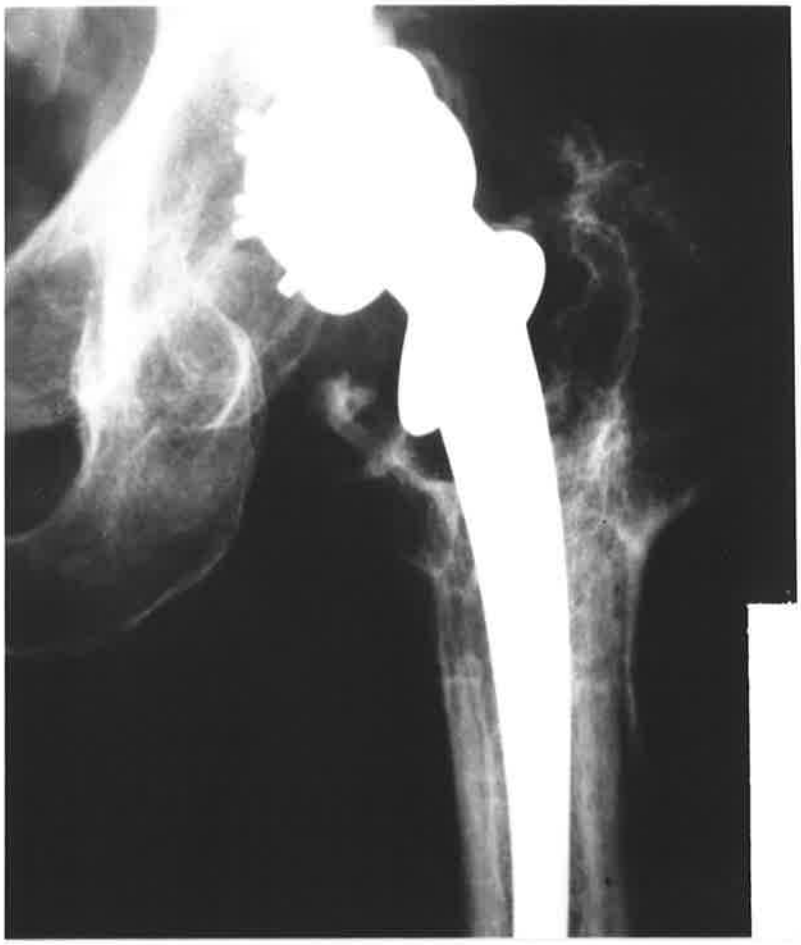

Fig. 2.15. Radiograph of a loose McKee cemented metal on metal prosthesis showing bone loss around the acetabular component and in the proximal femur. The cement is radio-opaque.

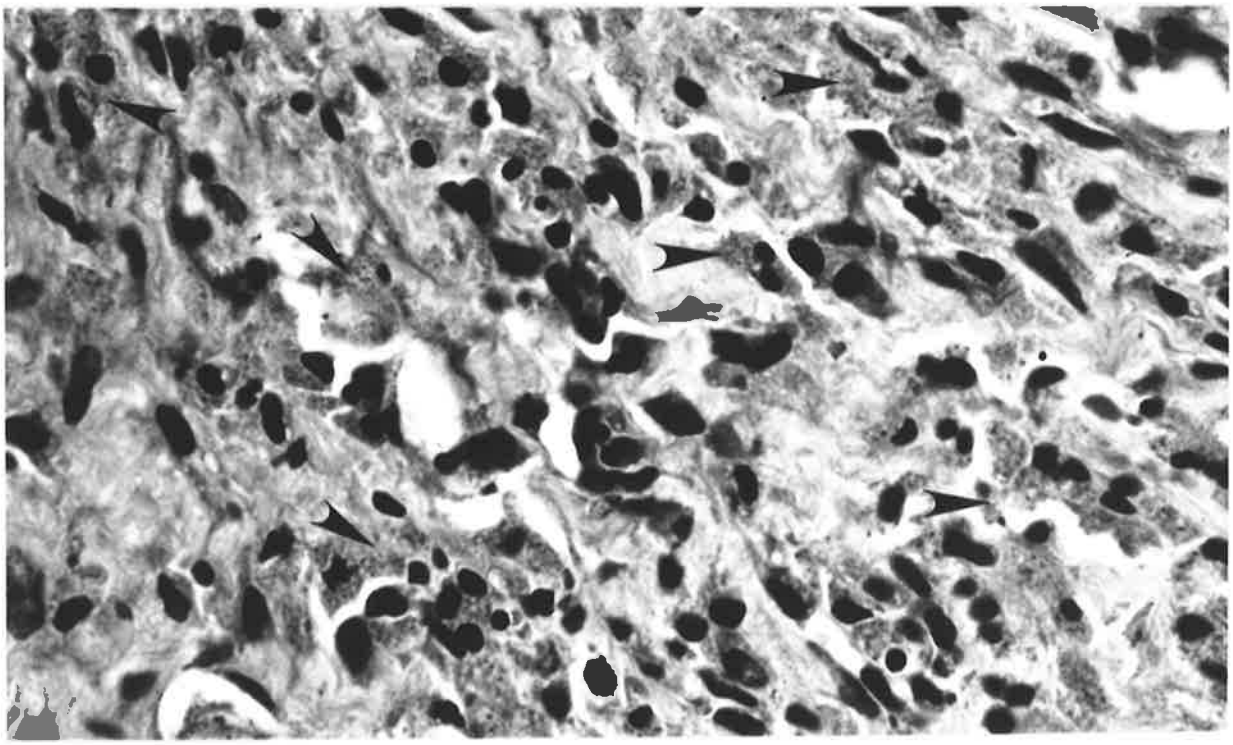

Fig. 2.16. Photomicrograph of the capsule around a cemented metal on metal prosthesis showing an infiltrate composed of macrophages which have dark granular cytoplasm (arrows) due to their content of metal particles. HE x 400

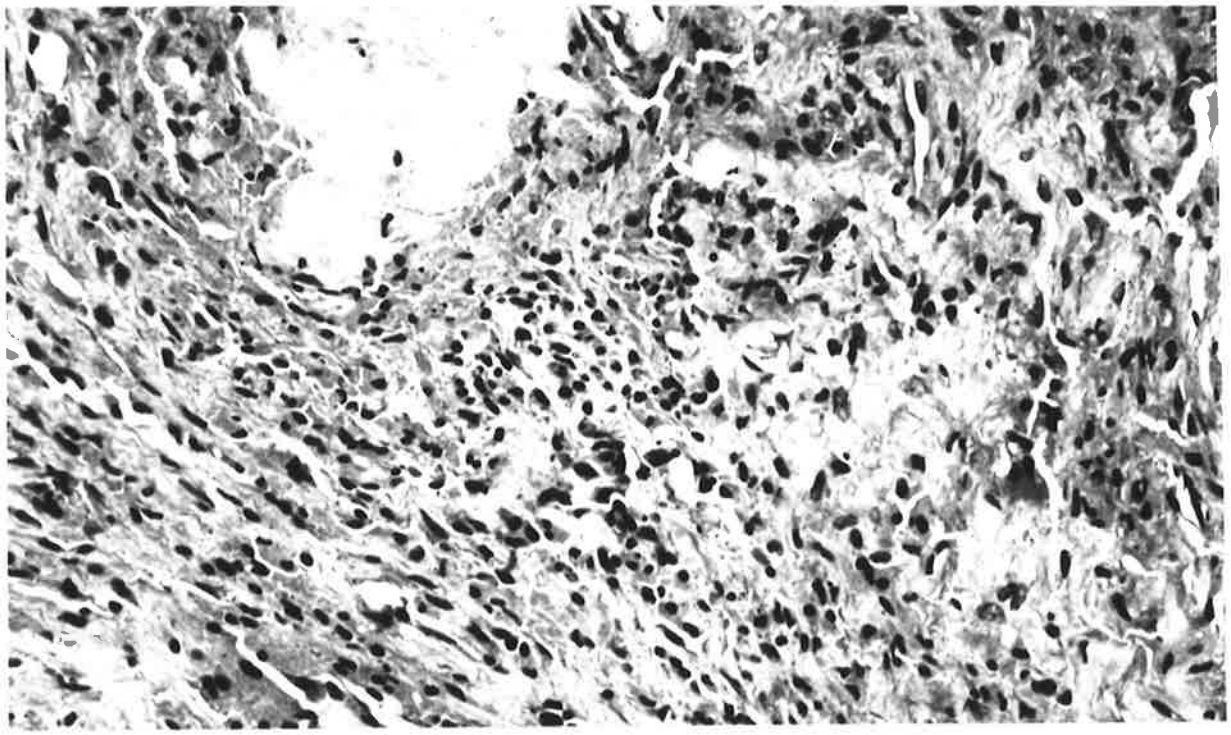

Fig. 2.17. Photomicrograph of the connective tissue at the acetabular bone-cement interface of a loose cemented metal on metal prosthesis. It shows sheets of macrophages which, when viewed under high power magnification, contain very small metal particles. HE x 160

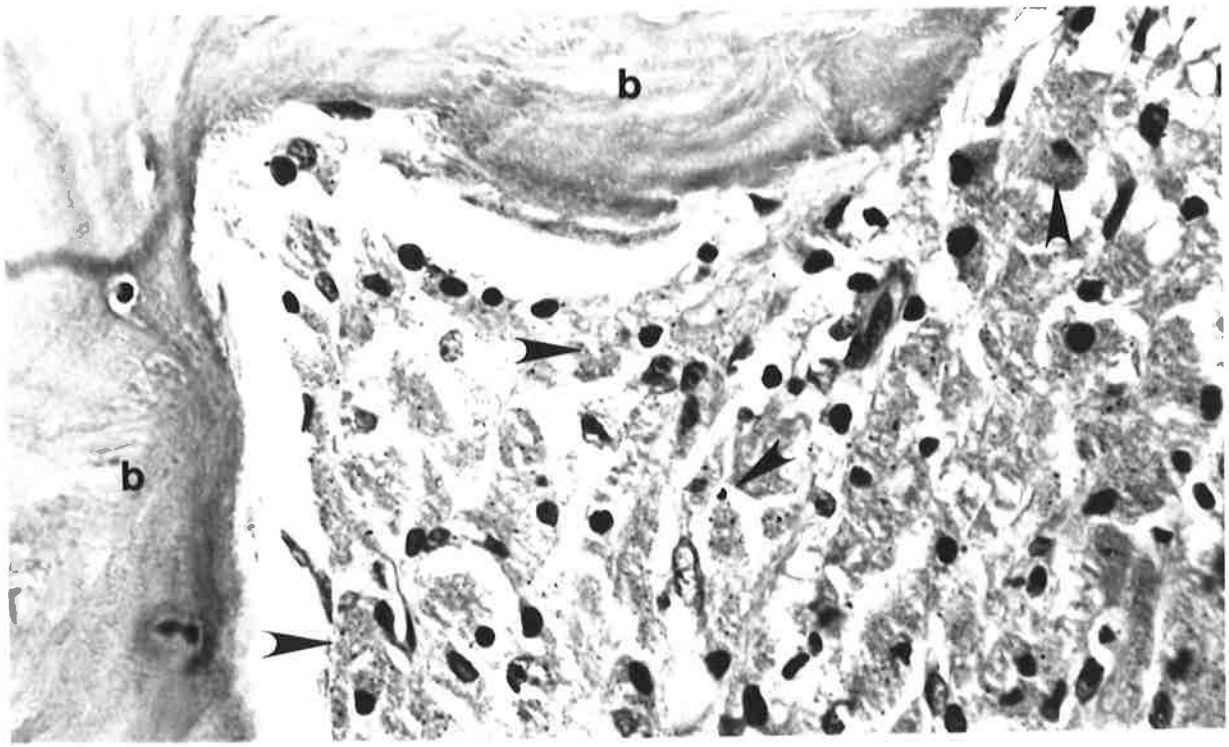

Fig. 2.18. Photomicrograph of the connective tissue at the bone-cement interface around a solidly fixed femoral stem of a cemented metal on metal prosthesis. The tissue adjoining the bone (b) consists of macrophages containing abundant fine metal wear particles (arrows). The gap between cells and bone is an artefact which occurred during tissue processing. HE x 400

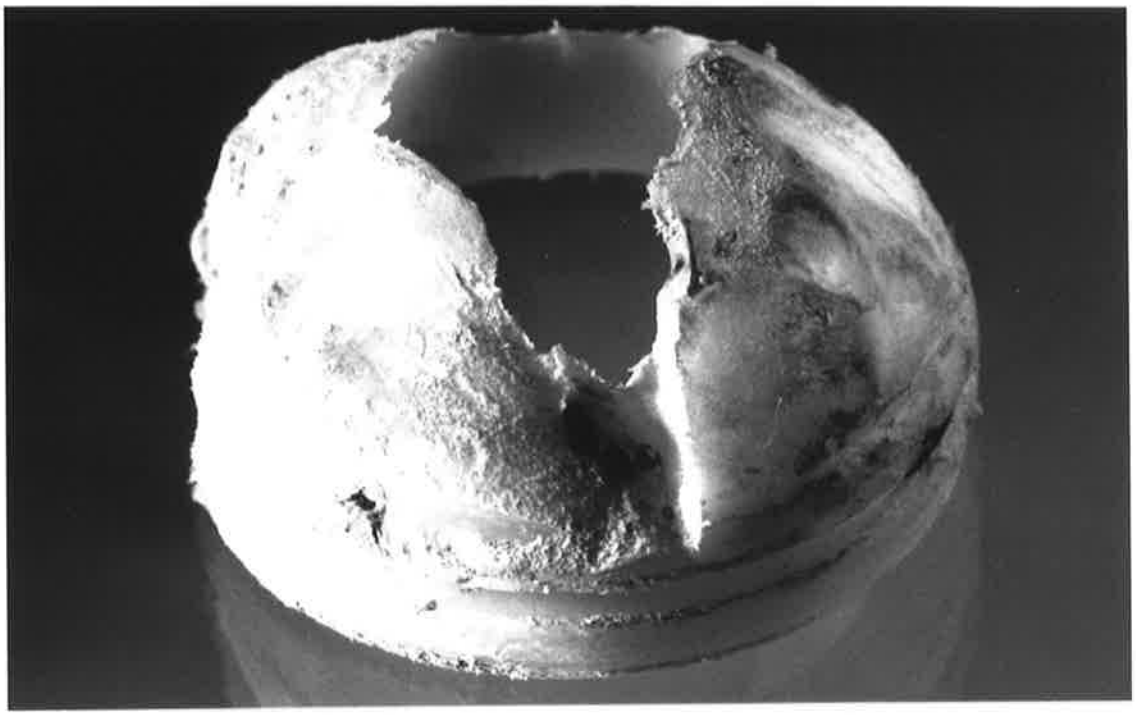

Fig. 2.19. Photograph of polyethylene Muller acetabular component retrieved eight years following insertion. There has been complete penetration of the component which had become loose in the cement mantle. Large cement particles were seen on the articulating surface of the component and these probably produced three-body wear. x 2

Fig. 2.20. Photograph of a carbon reinforced polyethylene Muller acetabular component retrieved fourteen months following insertion. Abrasive wear of the articulating surface is obvious. x 2

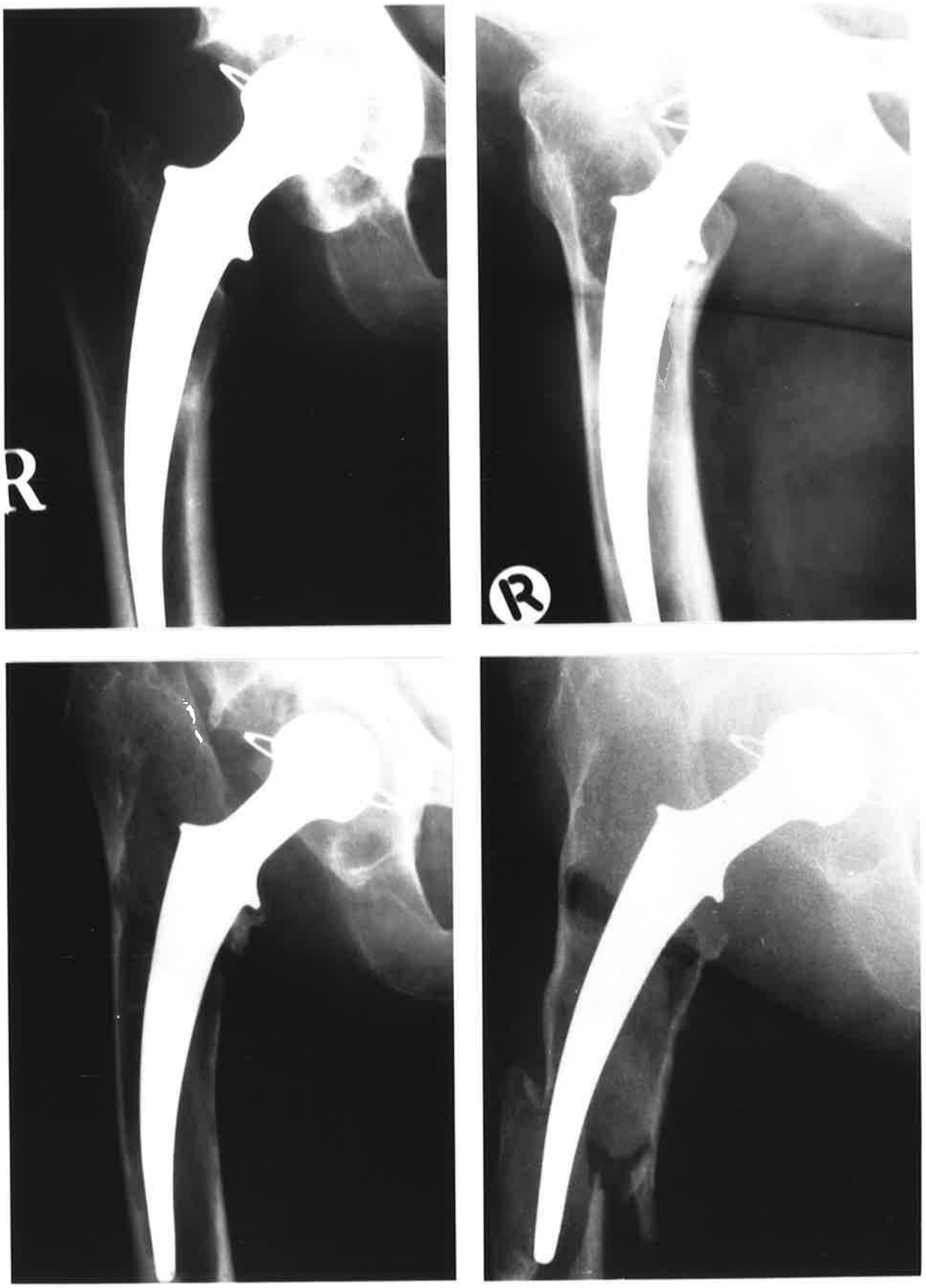

Fig. 2.21. Serial radiographs of a cemented Muller metal on polyethylene arthroplasty at approximately two yearly intervals following insertion. The cement is radio-lucent. Progressive bone loss is seen in association with loosening of the prosthesis, culminating in fracture of the femoral shaft. The scalloped appearance of the bone is typical of bone resorption due to the response to particulate material.

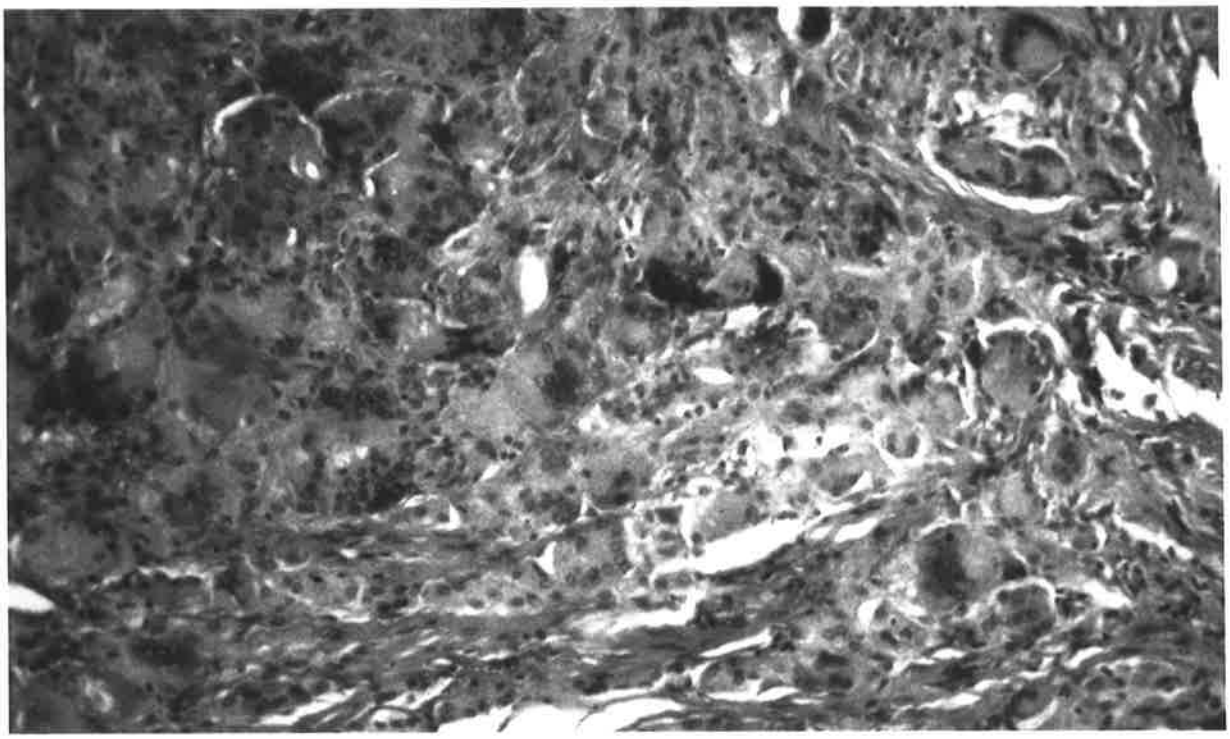

Fig. 2.22. Photomicrograph of the capsule surrounding the severely worn polyethylene acetabular component shown in Figure 2.19. It shows the presence of macrophages and many MNGC. HE x 200

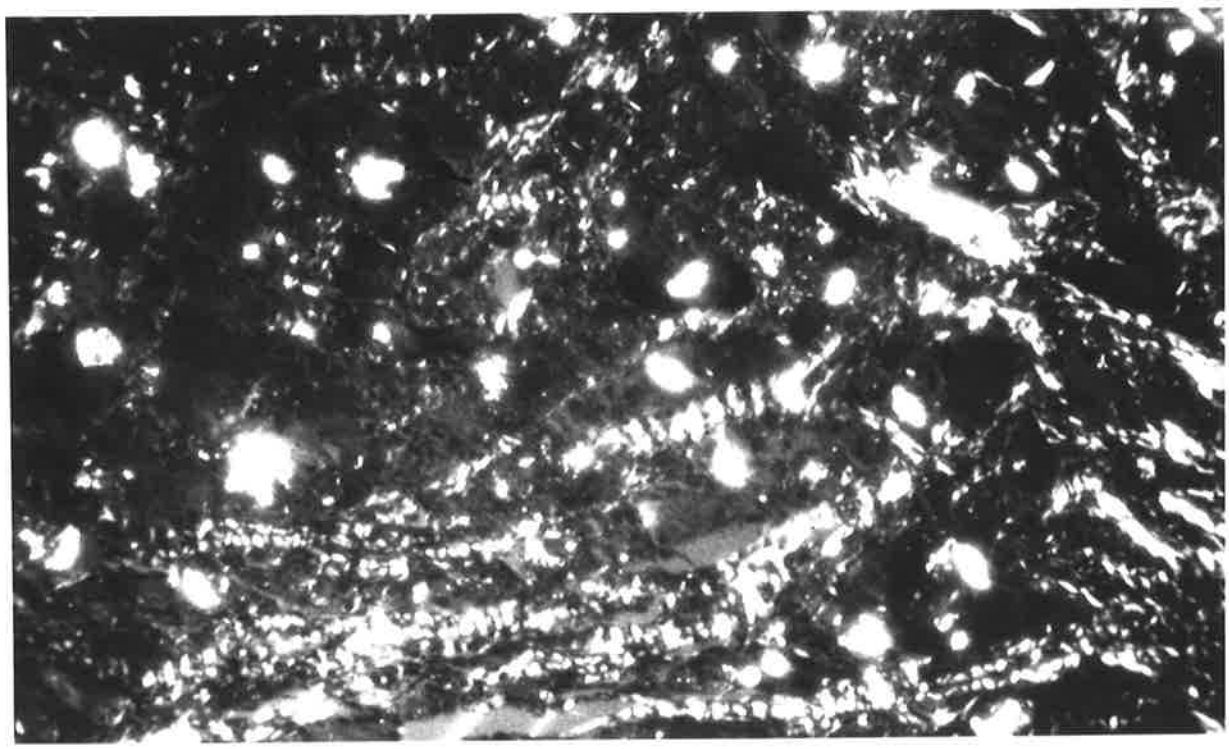

Fig. 2.23. Photomicrograph of the same section as Figure 2.22 viewed by polarised light. It shows large numbers of highly birefringent polyethylene particles. The small particles are contained within macrophages, and the large particles are surrounded by or contained within MNGC. The banded material is collagen. HE x 200

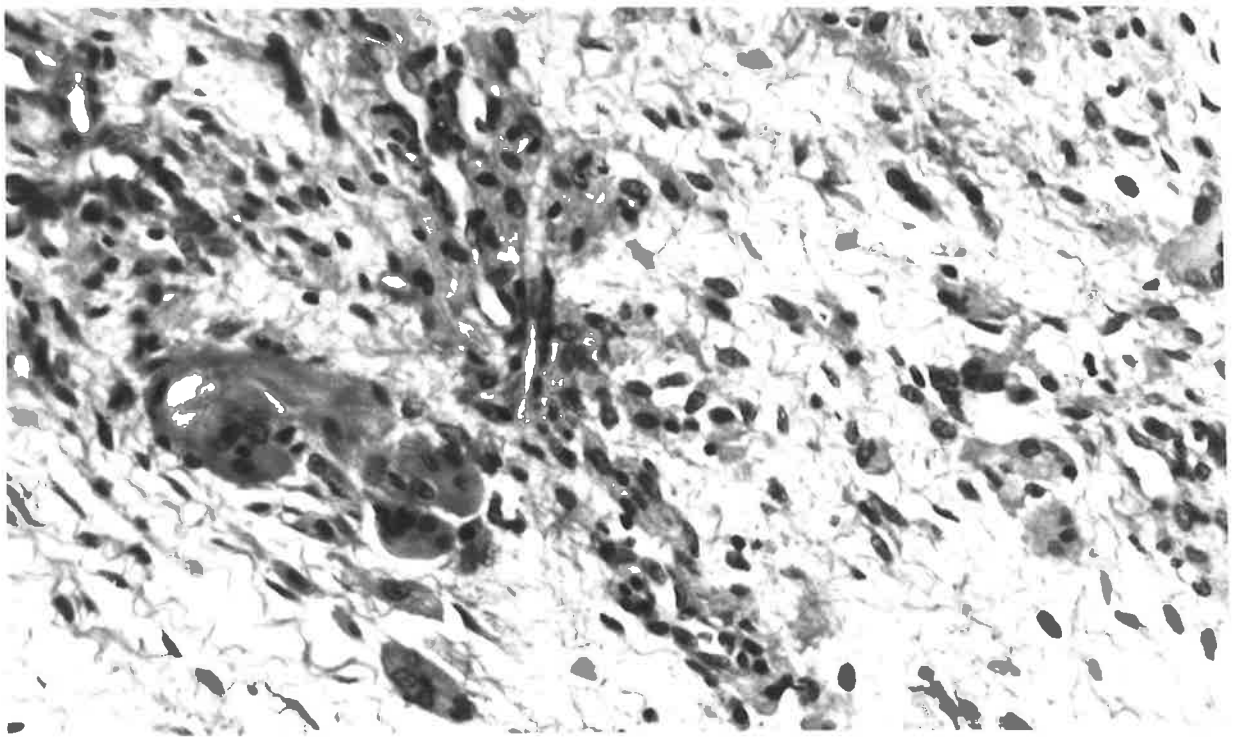

Fig. 2.24. Photomicrograph of the acetabular connective tissue layer at the bone-cement interface of a slightly loose cemented metal on polyethylene arthroplasty. It shows a group of macrophages and occasional MNGC in a collagenous matrix. HE x 400

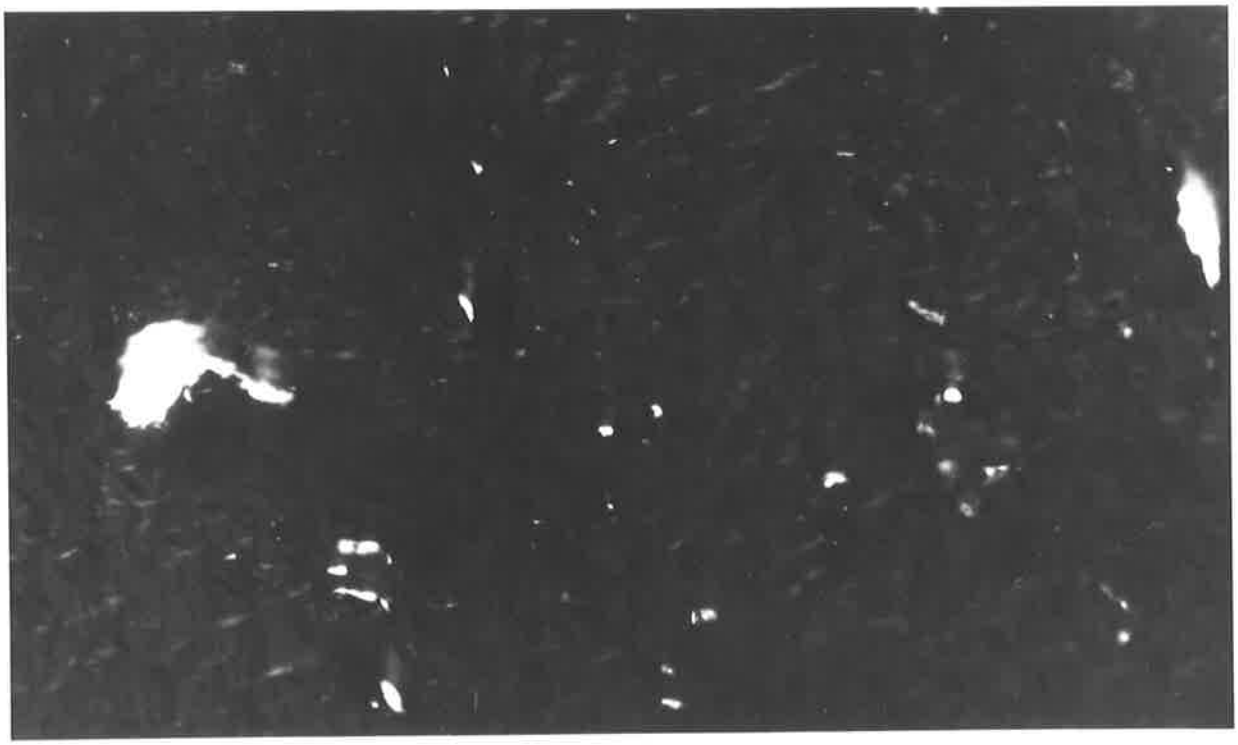

Fig. 2.25. Photomicrograph of the same section shown in Figure 2.24 viewed by polarised light. It shows small highly birefringent particles in macrophages, and large particles in MNGC. HE x 400

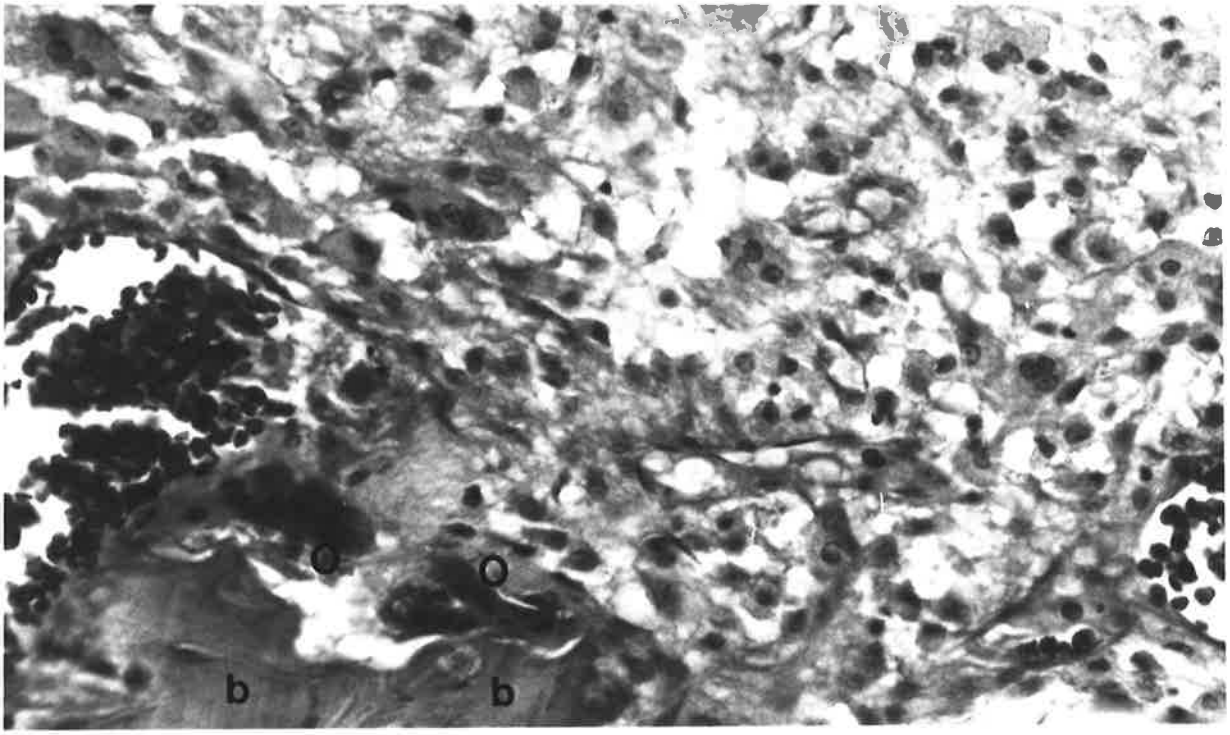

Fig. 2.26. Photomicrograph of the connective tissue layer at the femoral bone-cement interface beneath a loose cemented metal on polyethylene resurfacing hip arthroplasty. The tissue adjacent to bone contains large numbers of macrophages. Osteoclasts (O) are seen on the surface of bone (b). HE x 400

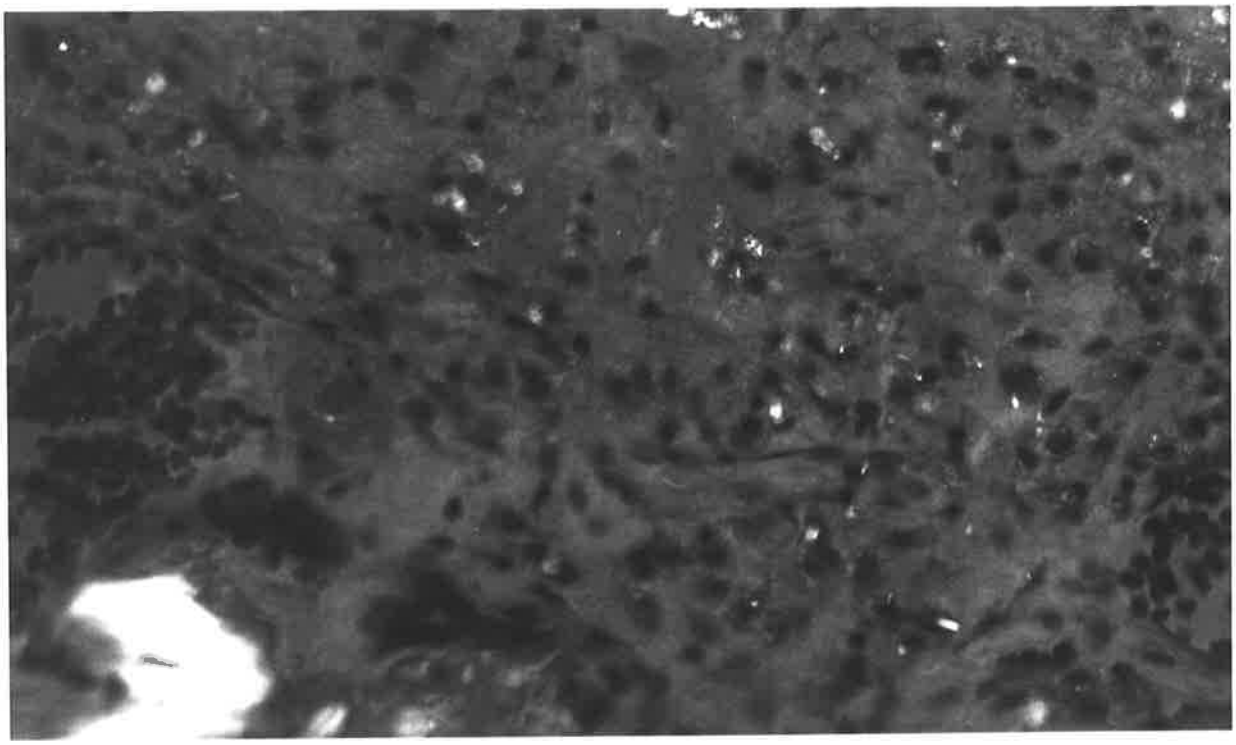

Fig. 2.27. Photomicrograph of the same field shown in Figure 2.26 viewed by polarised light. It shows abundant small highly birefringement polyethylene particles within macrophages. HE x 400

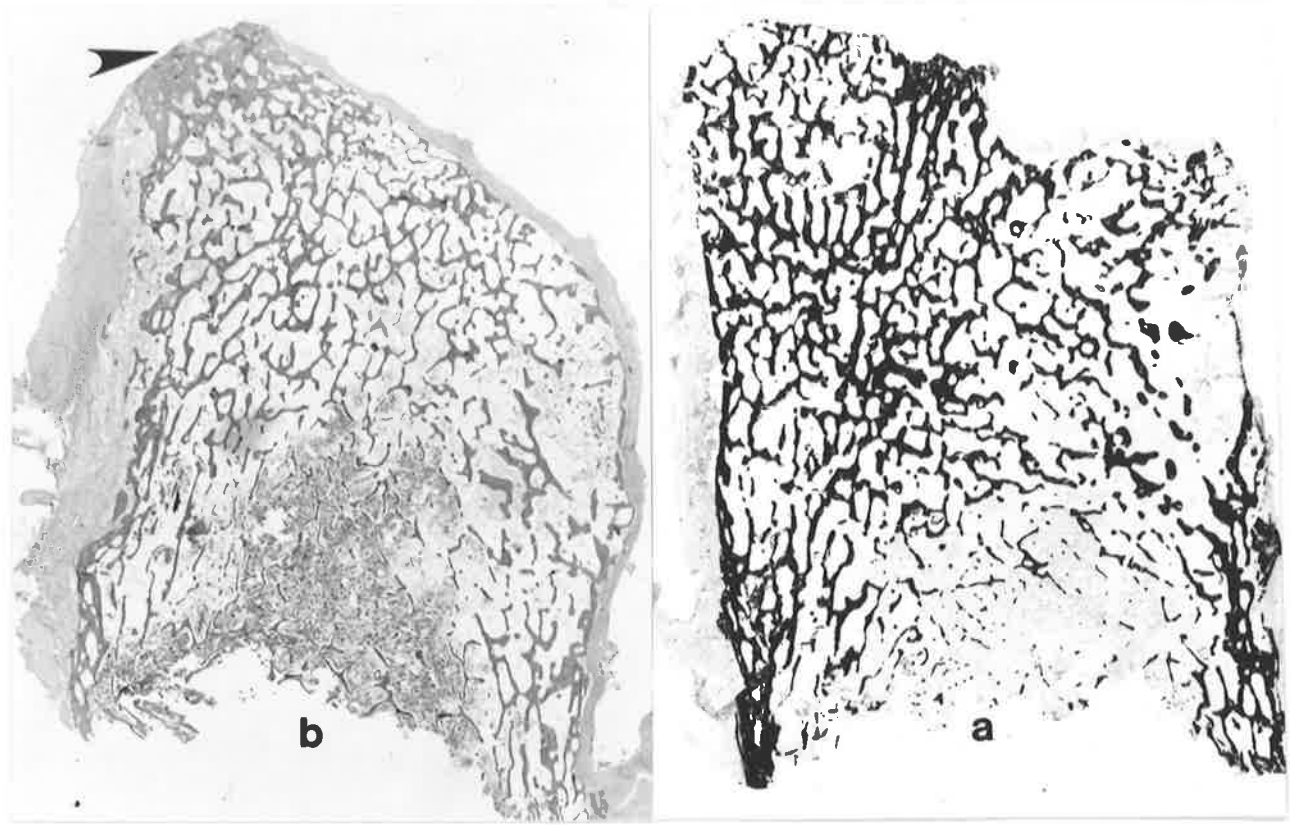

Fig. 2.28. Undecalcified (a) and decalcified (b) coronal sections of a femoral head beneath a slightly loose femoral component of a cemented metal on polyethylene resurfacing arthroplasty. It shows an area on the superior surface of the head (arrow) where bone has been abraded by cement. A connective tissue layer of variable thickness is present at the bone-cement interface. (a) HE VK, (b) HE x 2

Fig. 2.29. High power electron micrograph of part of a macrophage in the capsule around a cemented metal on polyethylene resurfacing arthroplasty. The cytoplasm of a macrophage contains numerous voids (arrows) where particles have been dislodged during cutting of the section. EDX micro-analysis of the particles showed no metal. x 17,500

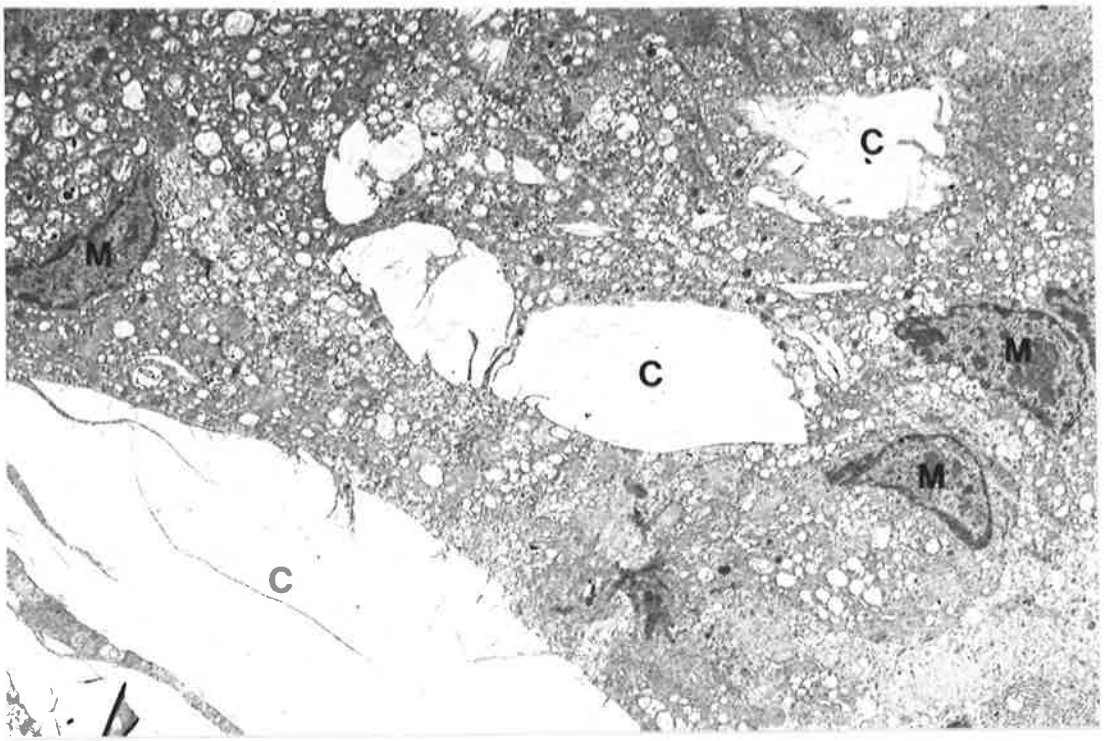

Fig. 2.30. Electron micrograph of the connective tissue at the acetabular bone-cement interface of a loose cemented metal on polyethylene prosthesis. It shows large and small cement particles (C) and adjacent macrophages (M) extending delicate cytoplasmic processes into the cement. x 4,250

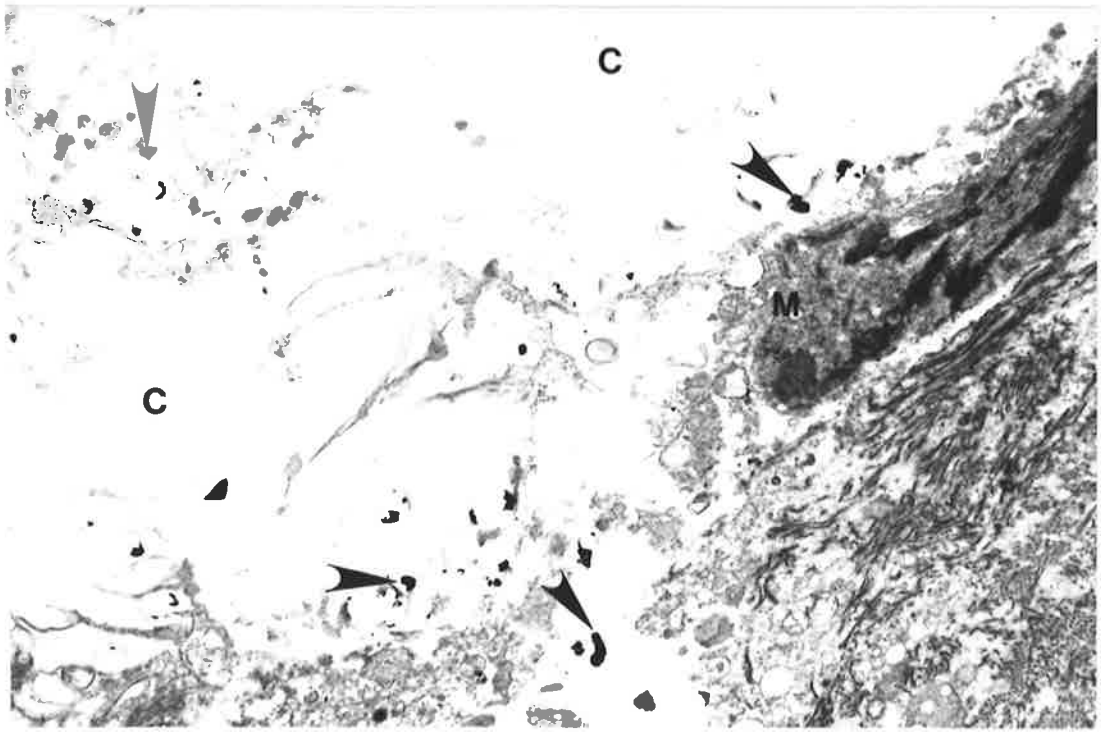

Fig. 2.31. High power electron micrograph of the connective tissue at the acetabular bone-cement interface of a loose cemented metal on polyethylene prosthesis. It shows large numbers of electron-dense particles (arrows) at the junction between the cement (C) and soft tissue, and a degenerate cell (M). EDX microanalysis shows these particles to be barium. x 8,000

Prosthesis Type (number)	Tissue Morphology	Particle Type
Metal on bone, cementless. (11)	Mature connective tissue, occasional macrophages.	Occasional metal particles.
Metal on metal, cementless. (3)	Abundant macrophages, rare MNGC, rare lymphocytes.	Abundant intracellular and extracellular metal particles.
Ceramic on ceramic, cementless. (2)	Mature connective tissue, occasional macrophages.	None seen.
Metal on metal, cemented. (5)	Abundant macrophages occasional MNGC, rare lymphocytes.	Abundant metal particles and occasional cement particles.
Metal on polyethylene, cemented. (26)	Abundant macrophages and MNGC, rare lymphocytes.	Abundant polyethylene particles, occasional cement particles, rare metal particles.

Table 2.1 The appearances of the periprosthetic tissues around forty-seven arthroplasties.

Particle type	Light Microscopy	Electron Microscopy
Metal (cobalt-chrome alloy).	Black granules, rods and needles. Light scattering effect by polarized light. Less than 3.0 micrometers.	Electron-dense particles. EDX = cobalt, chromium.
Metal (stainless steel).		Electron-dense particles. EDX = nickel, chromium.
Ceramic (aluminium oxide, only two prostheses of short duration).	None seen.	None seen.
Polyethylene	Invisible by direct light. Highly birefringent large fragments over 200 micrometers, and small particles at limit of resolution.	Voids in cells. EDX = no metal.
Cement (PMMA with and without barium marker).	Oval voids after tissue processing. Weakly birefringent if incompletely dissolved. Approximately 80 micrometers and less.	Voids less than 10 micrometers with peripheral electron-dense particles. EDX = barium (when mixed with cement).

Table 2.2. The morphological and analytical characteristics of wear particles in the tissues around forty-seven arthroplasties.

2.5 DISCUSSION

The tissues around failed hip arthroplasties showed a variety of appearances. In the absence of large numbers of wear particles, as seen in the tissues around the Smith-Petersen cup hemi-arthroplasties and the Austin-Moore hemi-arthroplasties, the capsule and interface tissue consisted predominantly of mature connective tissue. Occasional metal wear particles were seen in association with macrophages. These findings agree with the reports of others of the tissue appearance around cementless metal hemi-arthroplasties (Kozinn et al, 1986).

The small numbers of particles seen in the tissues may have arisen from the articulation between prostheses and articular cartilage or bone, and from abrasion at the bone-prosthesis interface. The tissue appearance is quite different from that reported around prostheses of similar design but using different materials. Examples include nylon cup hemi-arthroplasties (Newman and Scales, 1951; Scales, 1958) and polyethylene heads on stemmed hemi-arthroplasties (Dahl and Mikkelsen, 1976; Wroblewski, 1979), which suffer severe wear and are associated with a chronic inflammatory response and extensive bone resorption.

The tissues around the Mittelmier ceramic arthroplasties contained few macrophages and MNGC and no particles were seen. These prostheses had only been implanted for less than two years. Others (Heimke and Griss, 1981) have reported the presence of small numbers of particles around well-positioned ceramic on ceramic prostheses, but large numbers were present if there was severe wear of the prostheses due to malposition.

Electron microscopic examination of the periprosthetic tissues showed a macrophage response in the capsule and occasional macrophages in the interface tissues, but no particles were visible in the areas selected

for biopsy. Definite conclusions cannot be made from examination of the tissues around the two arthroplasties in this study as they were revised after less than two years.

The tissue around the cementless Ring metal on metal prostheses frequently contained large numbers of macrophages and metal wear particles, but MNGC were not common. MNGC were occasionally seen in the tissue around cemented McKee metal on metal prostheses. The highly cellular tissue containing abundant metal wear particles was found extending between bone trabeculae, even prior to loosening of components. The amount of bone destruction was variable, but the subjective impression was gained that bone destruction was not as severe around the cementless compared to the cemented metal on metal prostheses. Thus, while cement particles possibly may contribute to the tissue response around cemented implants, the findings of highly cellular tissue containing wear particles around loose cementless implants emphasizes the importance of the wear particles generated from the articulating surfaces of prostheses.

TEM examination demonstrated that endocytosed particles, confirmed to be cobalt-chrome alloy by EDX microanalysis, were not only associated with degeneration of some macrophages, but also with increased cytolysosome content of macrophages. This evidence of increased lysosomal activity supports previous findings of increased lysosomal enzyme levels in the tissues around loose prostheses (Eftekhar et al, 1985) and confirms the role of wear particles in inducing a chronic inflammatory response in the periprosthetic tissues.

The tissues around the metal on polyethylene prostheses showed the most striking changes. A highly vascular and often very friable connective tissue layer was often found at the bone-cement interface and extensive

bone resorption was present. Large polyethylene particles, due to shredding and severe wear, and very fine particles, due to lower rates of wear over long periods (Rose et al, 1978) were seen. The larger particles appeared to be more common when cement interposition between articulating surfaces of the components had resulted in three-body wear, when there was impingement of the neck of the femoral prosthesis on the edge of the acetabular component, or when the acetabular component became loose in the cement mantle. Electron microscopic examination demonstrated voids, attributed to polyethylene particles, in cells in the periprosthetic tissues. Importantly, many of these particles were too small to be seen on light microscopic examination, emphasizing that routine histological examination of these tissues may not give a true estimate of the amount of wear debris in the tissues.

The findings of polyethylene wear particles at the distal aspect of the femoral bone-cement interface provides support for the concept that wear particles may migrate along an apparently stable bone-cement interface. Migration of polyethylene and cement particles distal to the tip of loose stems has been reported (Pazzaglia and Byers, 1984; Harris et al, 1976) and acrylic particles have also been seen in these areas around stable components (Jasty et al, 1986a).

Occasional metal particles were seen in the tissues around metal on polyethylene prostheses. These particles may have arisen from the articulating surface of the femoral components, particularly if there was three-body wear, and also from abrasion of the femoral stems in the cement mantle following loosening, with or without fracture of the femoral stem.

Cement particles appeared to be more common at the sites of grossly loose components, and Mirra et al (1976) found a correlation between the

numbers of these particles and loosening. The problem of the identification of acrylic particles (which dissolve during normal tissue processing) makes it difficult to correlate the numbers of cement particles with the tissue response, because only the larger particles can be reliably identified by light microscopy of processed tissues, whereas smaller particles may go unrecognized.

Electron microscopic examination of the interface between cement particles and tissue demonstrated the accumulation of large numbers of barium particles. The barium is used to render the cement radio-opaque. These barium particles were not seen in adjacent macrophages in the biopsies examined. Degenerate macrophages were seen adjacent to the cement while in other areas the macrophages were normal. The degenerative changes may be due to the acrylic or barium. Rae (1977) found that the barium sulphate in the concentration seen in acrylic cement was not toxic to mouse peritoneal macrophages in vitro, while it has been reported that cement particles cause the inhibition of DNA synthesis in macrophages in vitro (Horowitz et al, 1986).

Lymphocytic aggregates were seen occasionally in the tissues around the cementless Ring and the cemented McKee metal on metal prostheses, and rarely around the metal on polyethylene prostheses. These aggregates were seen in patients with rheumatoid arthritis and osteoarthritis, whereas Mirra et al (1982) claimed that large numbers of lymphocytes were restricted to infected hips and rheumatoid patients. The presence of lymphocytes has been suggested as indicating hypersensitivity to cobalt by Evans et al (1974) but, apart from necrosis, the other features described by these authors, and attributed to metal hypersensitivity, were not seen in this study. Clearly, necrosis could be due to a number of causes other than hypersensitivity. PMN rarely

were present, and this agrees with other reports that these cells only are present in significant numbers in the presence of infection (Mirra et al, 1976).

2.6 CONCLUSIONS

In conclusion, the human studies have confirmed the presence of a wide range of types and sizes of wear particles in the periprosthetic tissues, and have revealed marked differences in the appearances of tissues around different prostheses. The extent of the macrophage and MNGC infiltrate in the periprosthetic tissue seems to be related to the number and size of wear particles in the tissues. It remains unclear whether there are significant detectable differences in the tissue responses to particles composed of different materials, or whether the response is solely or largely dependant on the number and size of particles. However, because previous joint pathology, mechanical loosening, and excess stress or stress shielding, may influence the tissue appearance around prostheses and cause bone resorption, possible differences in the biocompatibility of wear particles of different materials and the role of particles in loosening cannot be determined from studies of periprosthetic tissues alone. Further, there are difficulties in isolating the effects of wear particles from the articulating surfaces of implants from the effects of abrasive particles arising from the bone-cement interface. For these reasons, in vivo animal studies are required to examine the relative effects of wear particles on cells and tissues and to determine the role of wear particles in bone resorption around implants.

CHAPTER THREE

THE SYNOVIAL RESPONSE TO INTRA-ARTICULAR COBALT-CHROME WEAR PARTICLES

3.1 AIMS

The aims of this study were to prepare particles in a manner which allowed the injection of constant amounts of non-aggregated particles of the size and shape of wear particles seen in human periprosthetic tissues, and to attempt to simulate the human tissue response in small animals using techniques suitable for future biocompatibility studies. Because of the emphasis on aerobic and anaerobic infection as a possible cause of low grade inflammation around failed total joint prostheses (Bourgault et al, 1980; Buckholtz et al, 1981), particular attention was also directed at excluding possible infection as a cause of some of the tissue changes observed.

3.2 INTRODUCTION

Microscopic examination of the tissues around failed total joint replacement prostheses of the hip and knee usually shows a cellular response dominated by macrophages and MNGC, with occasional lymphoid cells. Often associated with this response are varying degrees of cell necrosis and fibrosis (Charosky et al, 1973; Evans et al, 1974; Winter, 1974; Mirra et al, 1976; Vernon-Roberts and Freeman, 1977; Revell et al, 1978). Only in the presence of infection are PMN present (Mirra et al, 1976).

Metal and polymer wear particles from the articulating surfaces of joint prostheses are detectable within the macrophages and MNGC in the tissues around prostheses, and it appears that increasing numbers of wear particles are associated with greater degrees of macrophage and MNGC response and necrosis (Vernon-Roberts and Freeman, 1977; Revell et al, 1978). These particles have been implicated as a cause of pain and late prosthesis loosening (Vernon-Roberts and Freeman, 1977; Willert and Semlitsch, 1977; Revell et al, 1978). A macrophage and MNGC response to large numbers of cobalt-chrome particles has recently been reported in the tissues around loose cementless porous coated femoral components of total hip prostheses (Buchert et al, 1986). Whether wear particles or their corrosive products alone cause the characteristic cellular response and necrosis is difficult to determine from histological study of the tissue around failed human and animal prostheses, as other factors such as trauma from the original surgery, low grade infection, or mechanical loosening may contribute to these changes.

This study was undertaken, therefore, to try to reproduce the tissue response seen in the articular tissues around failed prostheses by the intra-articular injection of wear particles in animals. Intra-articular particle injection was used to eliminate the complicating effects of open surgery and prosthesis movement, and to allow the possible presence of infection to be assessed by bacteriological examination of the injected joint. This model was also developed for possible use in determining the relative toxicity of wear particles of different materials.

The large majority of previous animal studies of the effects of wear particles on tissues usually have utilized subcutaneous or intramuscular implantation (Cohen, 1959; Swanson et al, 1973; Griss et al, 1974;

Escalas et al, 1976; Heath, 1976; Gourley et al, 1978). A major drawback of these studies is that the implanted bolus can only have an effect at the periphery of the bolus where it interfaces with surrounding solid tissue, and this tissue response may bear little relationship to the effects on synovium due to continuous shedding of wear particles and their dispersal by the synovial fluid.

Studies of the intra-articular injection of particulate polymers (Stinson, 1965), stainless steel (Wagner et al, 1976), and graphite (Tetik et al, 1974) in large laboratory animals have used particles suspended in water or saline, which causes clumping and rapid sedimentation of aggregates of particles (Garrett et al, 1983), and consequently results in uneven distribution of particles. Clumping, and its effects on particle distribution, must be taken into account when considering the findings in studies of the effects of implanted plugs of tamped particles in the knee joints of mice (Rushton and Rae, 1984; Rae 1986b). By contrast, pilot studies which preceded the findings reported here showed that milling in the presence of two percent homologous rat serum in normal saline, and subsequent resuspension of particles of random sizes in the same fluid, maintained the particles in a dispersed state suitable for injection into rat knee joints (Vernon-Roberts, 1985).

3.3 MATERIAL AND METHODS

3.3.1 Preparation of cobalt-chrome particles

Wear particles of cobalt-chrome alloy (Vitallium, Howmedica Inc.), were prepared in a shaker made of two Smith-Petersen cups (Fig. 3.1) as previously described (Garrett et al, 1983) with the following

modifications. Instead of using a milling fluid constituted from 20% foetal calf serum added to RPMI 1640 medium, the fluid comprised homologous serum prepared from J.C. Lewis rats which was diluted one in fifty in sterile normal saline (sodium chloride 0.9% W/V). Prior to milling the Vitallium screws and chamber cups were washed and acid neutralized according to A.S.T.M. standards for biocompatibility testing of metals (A.S.T.M. F 361-80, F 86-76), and sterilized by autoclaving.

The size distribution of particles was determined by differential sedimentation (Garrett et al, 1983) and particles three micrometers and less were utilized for injection. The concentration of metal and proportion of cobalt and chromium in the particle suspension were assessed by atomic absorption spectroscopy (Varian Techtron Model 1200) (Gatehouse and Willis, 1961) after dissolving particles by heating in concentrated nitric acid, and then in hydrochloric acid (McPherson et al, 1963). The content of metal was measured against cobalt and chromium salt standards and against dry prepared weighed samples of cobalt-chrome alloy (A.S.T.M. D 3558-77, D 1687-77). The metal particle suspension prepared in the shaker was found to have a concentration of 1.11 mg per ml of cobalt and 0.55 mg per ml of chromium, thus showing a cobalt:chromium ratio of 2:1. Since cobalt comprises 60 per cent of the Vitallium alloy, the total metal content of the particle suspension was calculated to be 1.85 mg per ml. This particle suspension was diluted to 0.74 mg per ml in serum/saline prior to injection.

3.3.2 Technique of injection and sacrifice of animals

Twenty male J.C. Lewis rats eight to sixteen weeks old, and weighing 180 to 300 gm, were injected with metal particles suspended in serum/saline in one knee and with serum/saline solution in the other. They were then allocated randomly to four groups to be sacrificed at one day, three

days, one week, and three months post-injection. Three control rats, which had the skin prepared but received no injection in either knee, were included in each group. Injection and sacrifice was performed under anaesthetic using 3% halothane in 50% nitrous oxide in oxygen.

Prior to injection and sacrifice, the legs were shaved and cleaned with one per cent alcoholic chlorhexidine. Injection, using a 0.5 ml syringe (B-D Plastipak 0.5 ml Insulin syringe) was performed through the patellar tendon while the knee was held at thirty degrees of flexion. Distension of the suprapatellar pouch was taken to indicate a successful injection. The amount injected was 0.01 ml per 25 gm body weight, as this was found in pilot studies to be the amount necessary to distend the knees of variously sized rats to an equal extent. The average injection volume was 0.09 ml. Animals recovered within one hour and did not limp. Animals were housed in metal cages and fed a routine diet.

At the time of sacrifice the animals were inspected externally for swelling of the knee or development of tumours. Under anaesthesia, using aseptic techniques including sterile instruments for each knee, an arthrotomy was performed and a synovial biopsy was taken (Fig. 3.2). The biopsy was placed in Stuart's transport medium for microbiological culture. Selected synovial biopsies were also taken for electron microscopic examination and EDX microanalysis and immediately placed in glutaraldhyde. After sacrifice, the whole leg was resected and fixed in 10% formal saline. The knee was held flat on a glass slide to allow consistent sectioning of the knee for histopathology (Fig. 3.3).

Finally, a post-mortem examination was performed and lumbar para-aortic lymph nodes, liver, spleen, kidneys and lungs were removed and placed in 10% formal saline.

Fig. 3.1. The cobalt-chrome alloy cups and cobalt-chrome alloy screws used to produce particles.

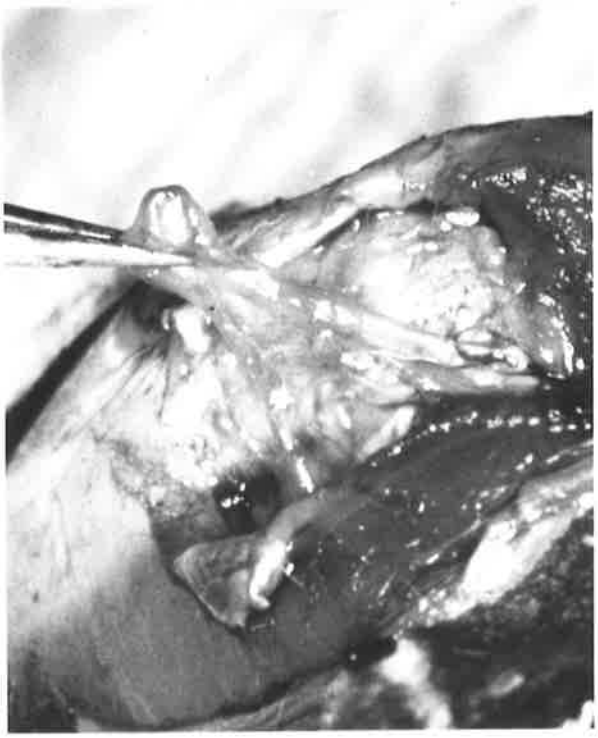

Fig. 3.2. Photograph showing the site of arthrotomy and synovial biopsy of a rat knee joint.

Fig. 3.3. Photograph showing an excised rat knee joint held flat on a slide to prevent distortion during fixation.

3.3.3 Tissue processing for histopathology

After fixation in 10% buffered formal saline for three days, the rat knees were decalcified using a commercial decalcifying solution (Decal, Omega Chemical Corporation, N.Y.). The extent of decalcification was controlled by daily radiographs (Kodak Min-R film, Hewlett Packard Series 43805N Faxitron Cabinet X-ray machine). After neutralization in five per cent silver sodium sulphate, the central portion of the knee was removed by sagittal section either side of the patella (Fig. 3.4) to provide a block four millimetres thick. The block was then dehydrated through graded alcohol, cleared in chloroform, and embedded in paraffin wax. After trimming, beginning at the side of the arthrotomy, six micrometer sections were cut using a hand operated LKB 2259 Multirange microtome (Fig. 3.5). Sections were stained with hematoxylin and eosin and examined by transmitted light and by polarized light microscopy, since very small metal particles can be readily discerned because of their light-scattering effect (Vernon-Roberts and Freeman, 1977). The pathological features were quantified subjectively and independently by the author and his supervisor, using an agreed scale. The grading system used was 1+, present occasionally but in less than one in ten high power fields of synovium examined, 2+, present in up to half the synovium, and 3+, present in more than half the synovium of the knee.

3.3.4 Microbiology techniques to exclude infection

Aerobic and anaerobic cultures were performed on all rat synovial biopsies, and on suspensions of metal particles and serum/saline prior to, and after injection.

The synovial biopsies were removed from Stuart's transport medium and, using a stomacher, were homogenised in half a millilitre of glucose

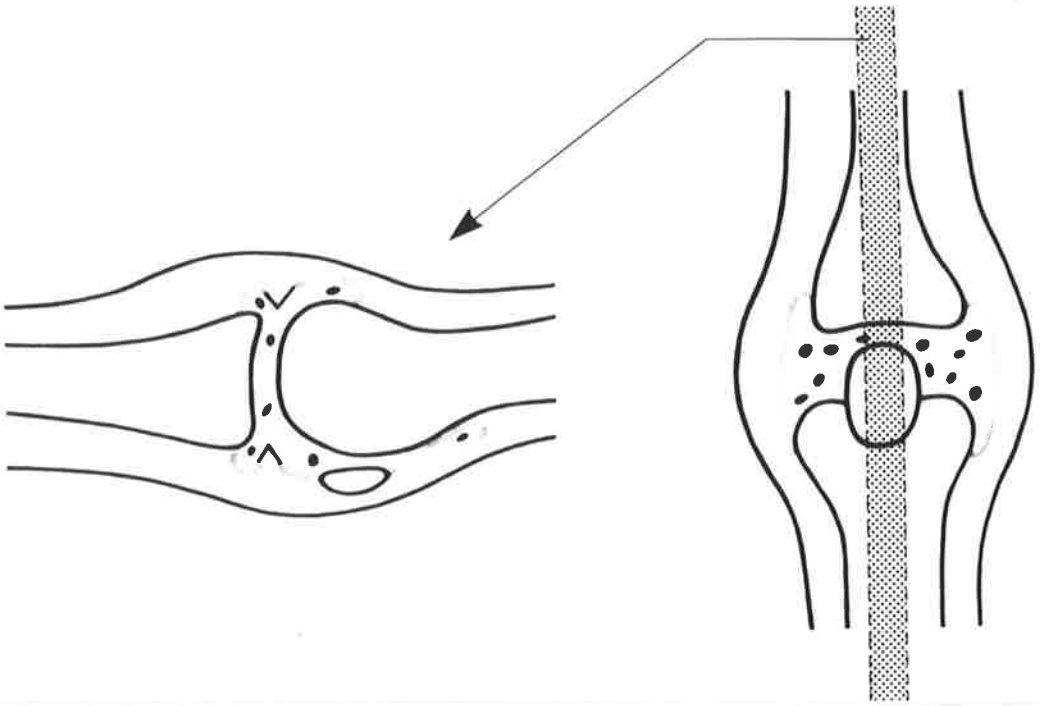

Fig. 3.4. Diagram showing the shaded portion of each rat knee joint excised following fixation, thereby obtaining a sagittal section of a similar area from each knee.

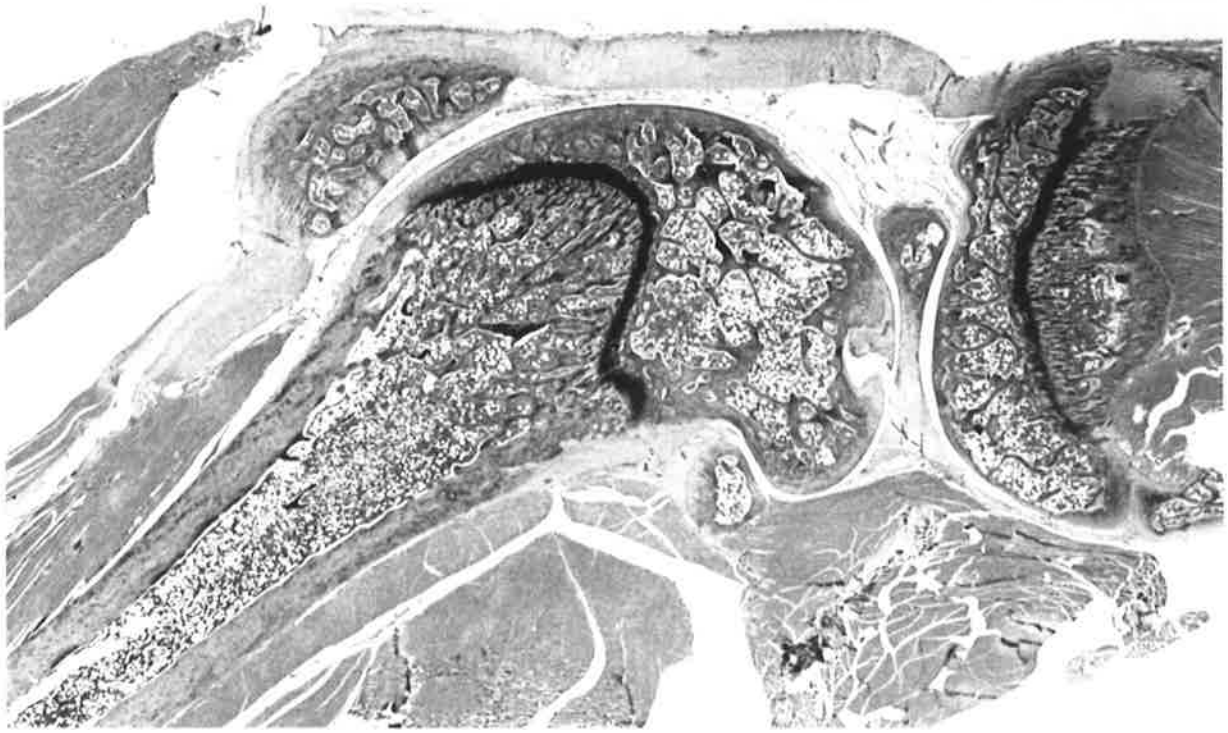

Fig. 3.5. A typical sagittal section of a rat knee joint showing the synovial recesses of the joint.

cooked meat medium. In an anaerobic chamber, the homogenate and the samples of injected materials were inoculated on to a supplemented blood agar (B.A.Y.H.) plate, an anaerobic blood culture broth, and a glucose cooked meat broth, and these were incubated anaerobically. The sample was also inoculated on to a chocolate agar plate and incubated in carbon dioxide. Plate cultures were continued for four days and broth cultures for fourteen days. A subculture was taken if any broth became turbid.

The uninjected knees were used as negative controls. Two types of positive controls were used. To test the adequacy of the technique of collecting a synovial biopsy in detecting possible infection, septic arthritis was induced by injection of 0.1 ml of pure coagulase-positive Staphylococcus aureus or coagulase-negative Staphylococcus epidermidis into the knees of four rats. After three days a synovial biopsy was taken in the same manner as that described for rats injected with metal particles. To test the adequacy of the transport media used and the culture technique, 0.05 ml of pure cultures of the same two strains of Staphylococci, Propionebacterium acne, and Escherichia coli were inoculated on to an autoclave sterilized biopsy of rat synovium and this was placed into Stuart's transport medium. All bacterial suspensions were in a concentration of one million bacteria per ml as determined by serial dilution and pour plate techniques (Rotheram, 1975). Organism identification was according to standard laboratory techniques (Cowan, 1975; Holdeman et al, 1977).

3.4 RESULTS

3.4.1 Histopathology of rat knees

One day after injection of metal particles, a joint effusion containing many PMN, macrophages and necrotic cell debris was present. The synovium showed patchy surface ulceration, and the subsynovium was extensively infiltrated to a moderate degree by macrophages. A few PMN also were present. While some metallic particles were present within the lining synoviocytes, the particles in the subsynovium were predominantly extracellular. In a few areas of the subsynovium, aggregates of metal particles were present and were associated with necrosis of cells (Fig. 3.6). In the control knees injected with serum/saline, there was a small effusion and subsynovial cellular infiltrate of macrophages and a few PMN , but synovial ulceration and necrosis were absent.

Three days after injection, the effusion had largely disappeared from test and control joints. The appearances of the synovium and subsynovium were indistinguishable from those seen one day after injection.

One week after injection of particles, there were randomly distributed aggregates of metal particles within the focally hyperplastic synoviocyte layer and within macrophages in the subsynovium. The greater amounts of particles appeared to be present in the tissues of the synovial recesses of the joints. Focal aggregation of large numbers of metal particles was associated with an intense macrophage infiltrate and necrosis of these cells (Figs. 3.7 and 3.8). Lymphocytes commonly were present in association with the macrophage aggregates and necrosis (Figs. 3.7 and 3.9).

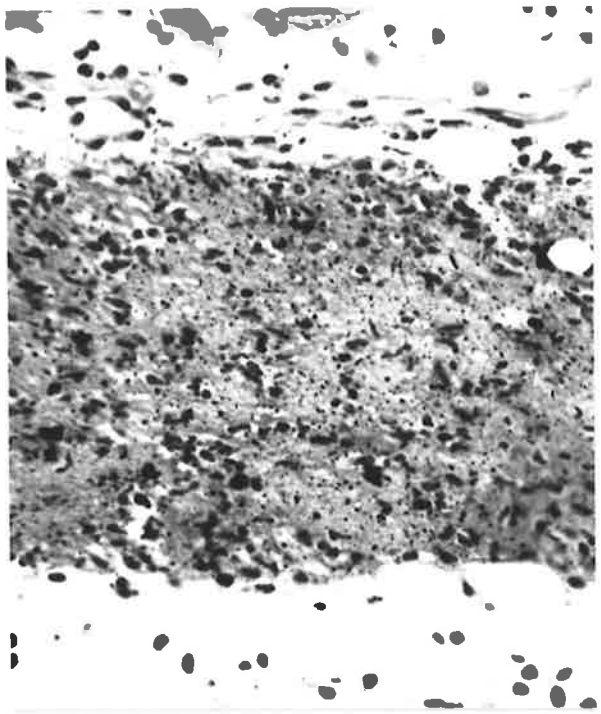

Fig. 3.6. The subsynovium three days following cobalt-chrome injection shows a collection of particles and cell debris. HE x 200

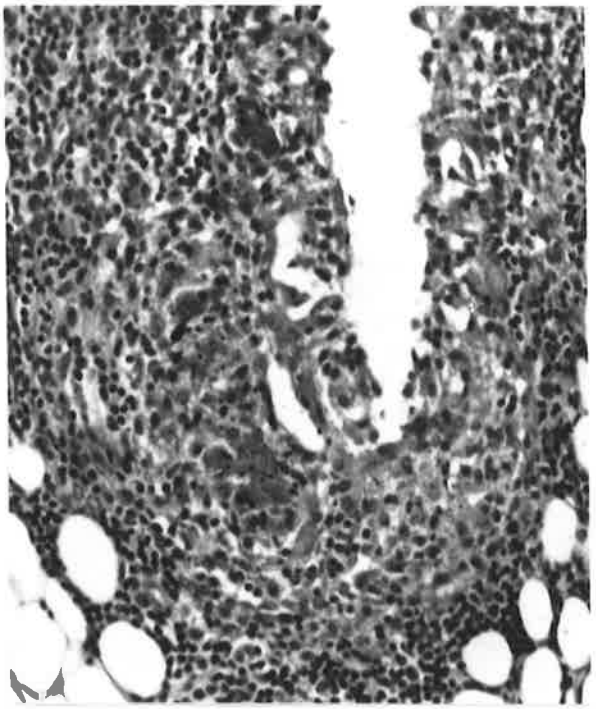

Fig. 3.7. One week following cobalt-chrome particle injection the synoviocyte layer is ulcerated. There is a macrophage response with surrounding lymphocytic infiltration. HE x 160

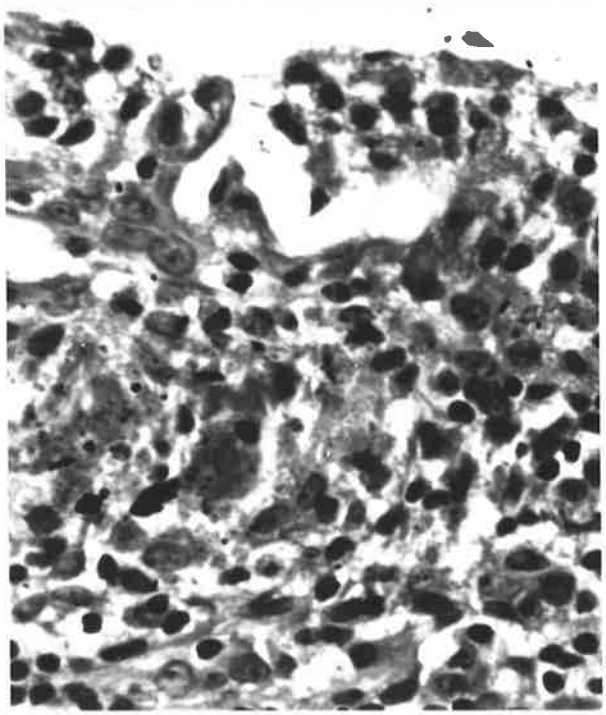

Fig. 3.8. One week following cobalt-chrome particle injection the particles are located intra-cellularly within macrophages and extracellularly. Cell necrosis is associated with focally large numbers of particles. HE x 500

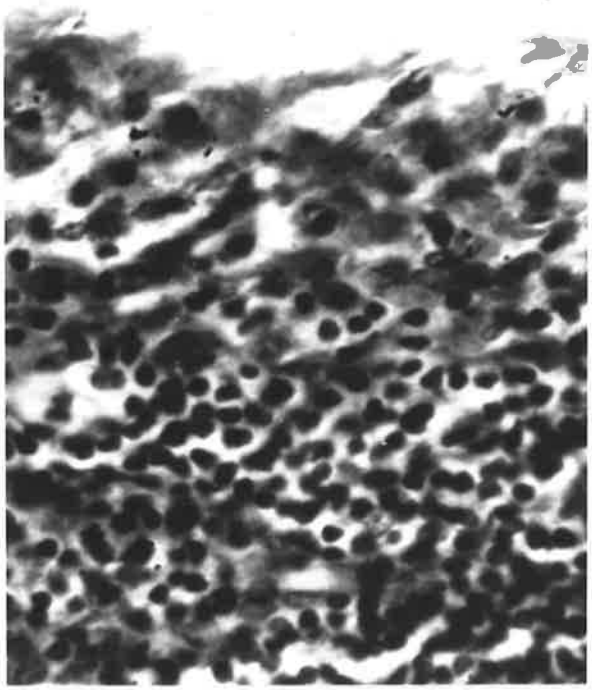

Fig. 3.9. The synovium one week following cobalt-chrome particle injection, showing synovial ulceration, occasional macrophages containing particles, and a dense infiltrate of small lymphocytes. HE x 500

In some of the control knees there was a mild macrophage infiltrate alone and occasional lymphocytes were present, but in general the knees showed very little change (Fig. 3.10).

Three months after injection, metal particles were present in small numbers in synoviocytes and as prominent aggregates within macrophages in the subsynovium (Fig. 3.11). However, while the number of aggregates had increased, the number of diffuse macrophages and amounts of particulate material clearly were reduced when compared with the reaction observed at one week. The aggregates of particles frequently were associated with necrosis. Fibrosis and necrosis of the subsynovium were seen in some areas, and extracellular particles were present in such fibrous and necrotic tissue (Figs. 3.11 and 3.12). Lymphocytes and PMN were absent. The control knees were completely normal in appearance. No MNGC were observed at any stage.

The histological features in the particle and control injected knees were assessed according to the extent of the knee synovium involved and the results were plotted against the time till sacrifice (Fig. 3.13).

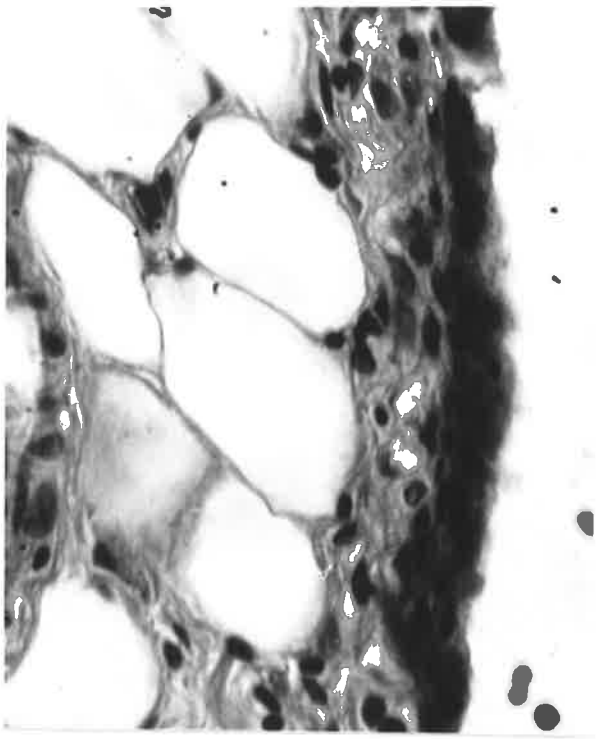

Fig. 3. 10. The synovium one week following injection of control solution showing synovial hyperplasia and a few lymphocytes in the subsynovium. HE x 500

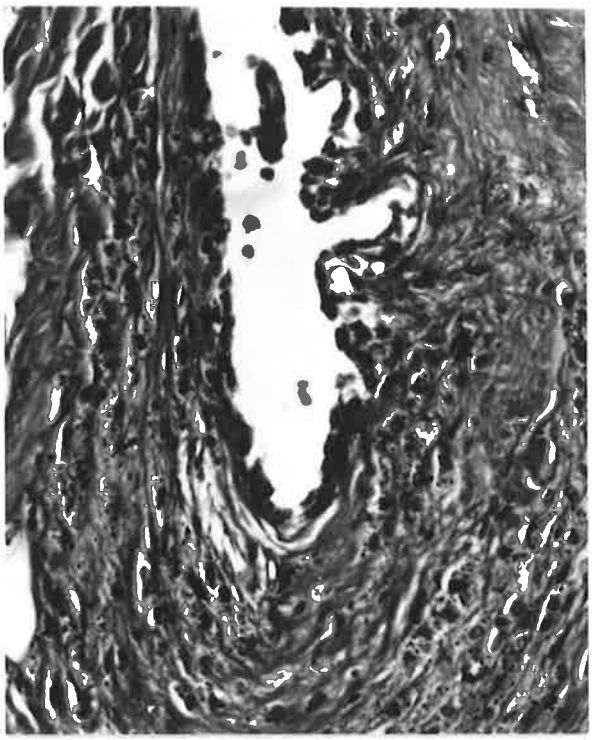

Fig. 3.11. A synovial recess three months following injection of cobalt-chrome particles. Particles are within macrophages and in fibrous tissue. HE x 200

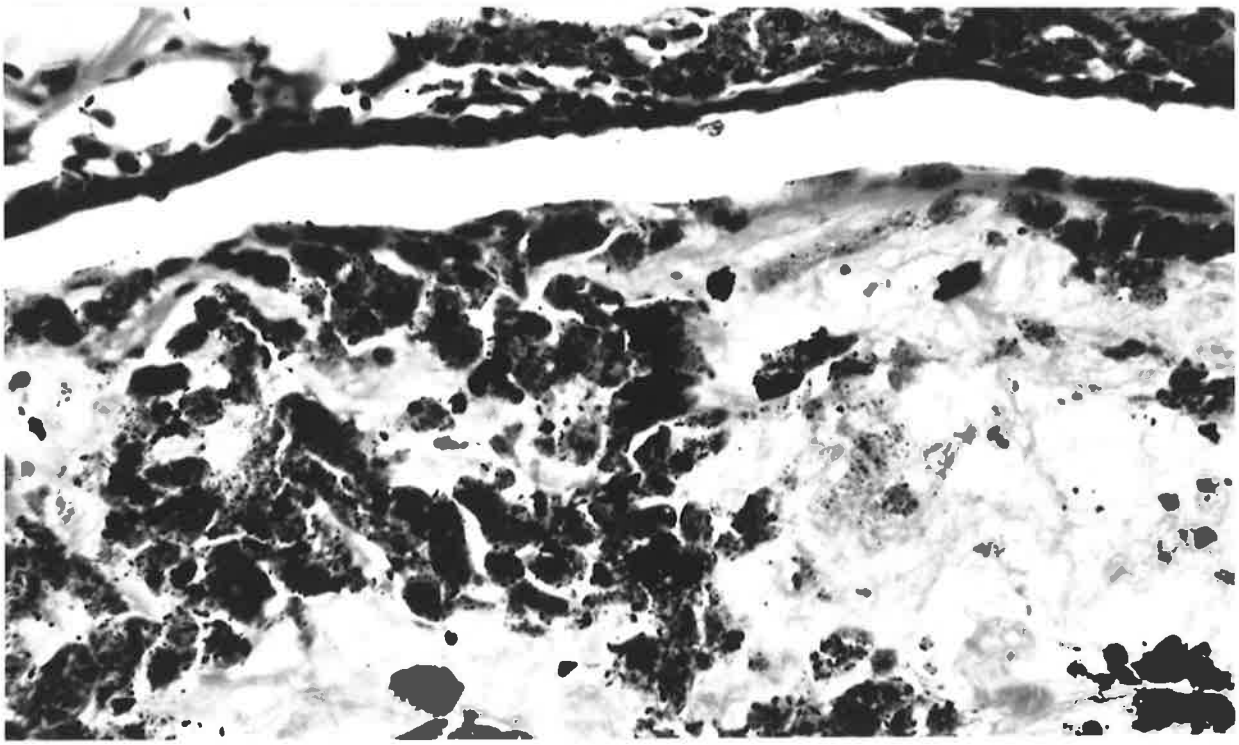

Fig. 3.12. The synovium three months following cobalt-chrome particle injection showing a surface synoviocyte layer one cell thick, macrophages showing many intracytoplasmic particles, and extracellular particles also lying in the fibrous and degenerate stroma which has replaced the normal subsynovium. HE x 500

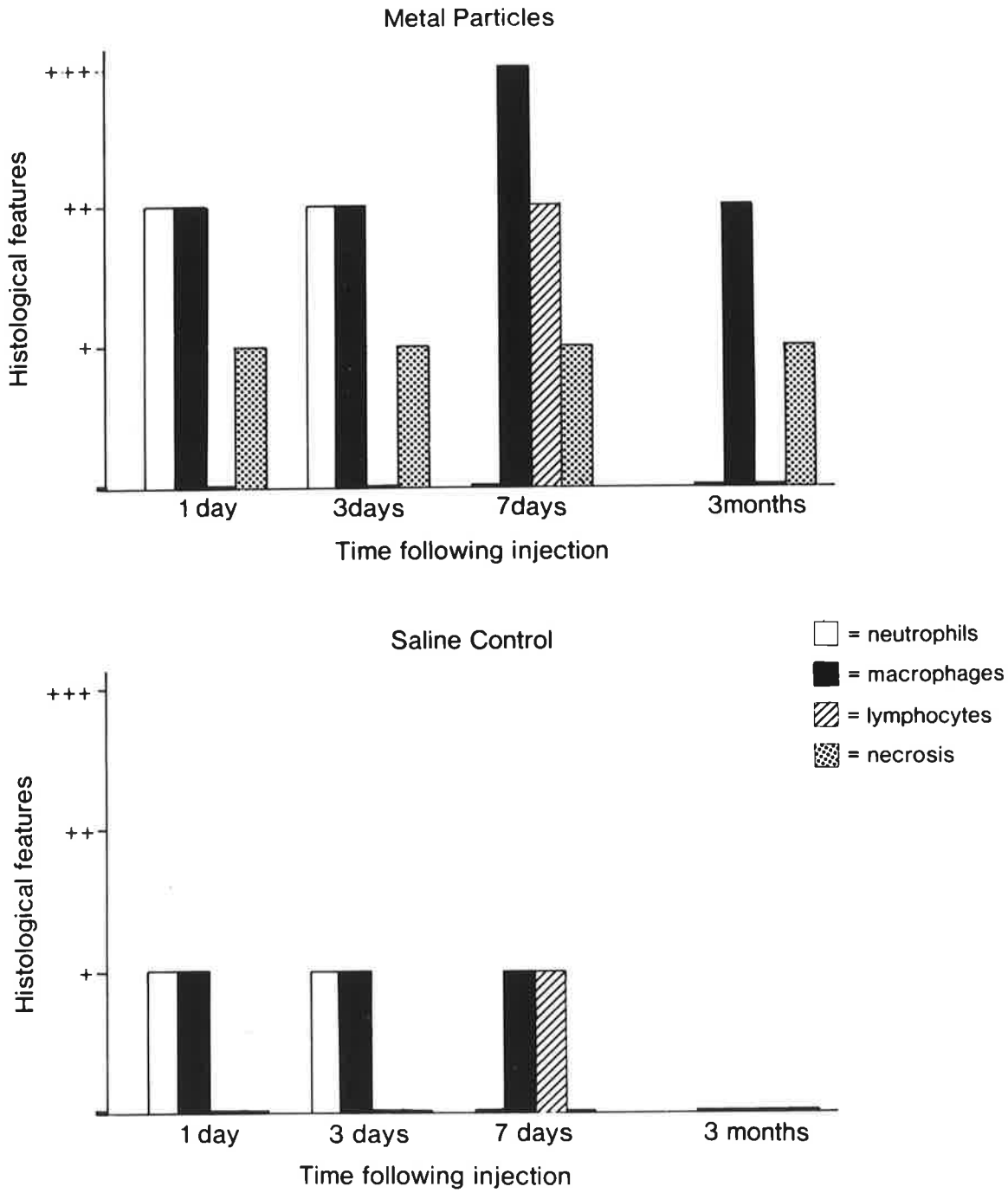

Fig. 3.13. Histogram showing the histological features in cobalt-chrome particle and control solution injected knees at various time periods following injection. Features were graded as 1+, occasionally present, 2+, present in up to half the knee, 3+, present in more than half the knee.

3.4.2 Post-mortem findings

Histopathological examination of all organs showed no evidence of tumour or other abnormalities and no metal particles were seen in extra-articular tissues.

3.4.3 Microbiology culture results

Cultures of the metal particle suspensions and serum/saline control solutions were sterile. One metal injected rat knee grew a Staphylococcus epidermidis and one uninjected control rat knee grew a Propionebacterium acne. Both positive cultures were only on broth culture and not on the plates, and were assumed to be contaminants. A positive culture was obtained from all rat knees with induced septic arthritis due to Staphylococcus aureus or Staphylococcus epidermidis. The cultures from all synovial specimens which had been inoculated with known bacteria were positive and demonstrated the sensitivity of the culture techniques to detect aerobic and anaerobic bacteria.

3.5 DISCUSSION

Cobalt-chrome alloy particles similar in size to the wear particles seen microscopically in the tissues adjoining metal on metal total joint prostheses and, to a lesser degree, metal on polyethylene joint prostheses have been shown in this study to provoke the accumulation of macrophages containing metal particles. This macrophage response occurred in the absence of infection. The particles also caused ulceration of the synovium and, when present in focally high concentrations, produced necrosis of the subsynovium. The macrophage response and necrosis seen in response to the injection of particles

into rat knees are similar to the appearances seen in the synovial tissues around loose human total hip prostheses. This study demonstrates that a macrophage response to wear particles will occur in the absence of infection or mechanical causes for prosthesis loosening (Vernon-Roberts and Freeman, 1977).

The possibility that the tissue reactions observed in humans could be due to infection, a view which has been put forward on several occasions, has been excluded by extensive microbiological tests to exclude aerobic and anaerobic infection of the knees injected with particles. In addition, positive controls were used to establish the sensitivity of the method used to obtain a synovial biopsy in detecting infection.

The appearance of the rat knee joints injected with particles changed with time. Initially there was ulceration of the synovium and necrosis of macrophages at the site of large concentrations of particles in the subsynovium. Particles were endocytosed by macrophages. The number of particles and the number of macrophages in the subsynovium appeared to decrease with time but the extent of the particle and macrophage distribution throughout the knees did not appear to change. This may support the concept that joints possess the ability to dispose of wear particles (Willert and Semlitsch, 1977). When wear is severe, the ability of the joint to clear large numbers of particles may be exceeded, and may be further impaired by the fibrosis and necrosis which accompanies the reaction to wear particles.

The amount of metal wear particles produced at the articulating surfaces of all metal prostheses is high. Large numbers of metal particles have also been reported in the tissues around porous metal implants. Small numbers of metal wear particles are seen in the tissues around metal on

polyethylene prostheses in which the predominant wear particle produced is polyethylene. It has been shown previously that high concentrations of metal particles are toxic to murine macrophages in vitro (Garrett et al, 1983). The mechanism of this toxicity remains speculative, but it could account for the necrosis associated with focally high concentrations of particles as aggregates in the injected joints. By contrast, fibroblasts may be more resistant to the toxic effects of cobalt (Daniel et al, 1963), and this may explain the persistence of metal particles in fibrous tissue. Alternatively, fibrous tissue formation in the subsynovium could represent a repair reaction following focal necrosis of the tissues.

Clearly, the effects of wear particles of different alloys have important implications for the tissue response around implants. It is suggested that the testing of the biocompatibility of particles of materials proposed for use in joint prosthesis manufacture may be performed in a similar manner as in the animal model developed in this study.

3.6 CONCLUSIONS

Intra-articular injection of cobalt-chrome alloy wear particles similar in size to those seen in the tissues around human prostheses produces synovial ulceration, focal degeneration of synovial tissue and proliferation of macrophages in the absence of infection. These appearances in the knee joints of rats are similar to those seen in the tissues around human prostheses.

CHAPTER FOUR

THE LONG TERM EFFECTS OF INTRA-ARTICULAR INJECTION OF COBALT-CHROME WEAR PARTICLES IN RATS

4.1 AIM

The aim of this study was to investigate the long term effects of the intra-articular injection of cobalt-chrome alloy wear particles on the synovium and subsynovium of the rat knee joint assessed semi-quantitatively.

4.2 INTRODUCTION

Despite previous long-term in vivo studies of the biocompatibility of various prosthesis materials in particulate form, it remains difficult to quantify the tissue response to different materials. Previous studies suggest there is a common pattern of tissue response to such particles. Following subcutaneous and intramuscular injection, a predominantly macrophage response occurs initially and is followed at a later stage by fibrosis (Cohen, 1959; Escalas et al, 1976). A similar response occurs following intra-articular injection, but the degree of fibrosis is variable (Stinson, 1965; Tetik et al, 1974; Rushton and Rae, 1984; Rae, 1986b). There are, however, considerable variations between reports of the degree of tissue response to the same and different metals (Cohen, 1959; Escalas et al, 1976) and polymers (Stinson, 1965; Escalas et al, 1976; Gourlay et al, 1978; Uchida,

1985). These variations probably are due largely to differences in methods of particle preparation, injection sites, and methods of interpretation of results. The interpretation of the results of future studies would be simplified, therefore, by the use of standard methods of particle preparation and injection, the inclusion of standard control materials, and the use of objective methods of assessment of the tissue response.

4.3 MATERIAL AND METHODS

4.3.1 Injection and sacrifice of animals

Forty male and forty female J.C. Lewis rats eight to sixteen weeks of age and weighing between 180 and 300 grams, were injected with cobalt-chrome particles in one knee and dilute serum/saline in the opposite knee. The rats were allocated randomly into eight groups with five male and five female rats in each group. Five male control rats, which had their skin prepared but received no injections, were included in each group. The groups were sacrificed at one, two, four, eight, thirteen, twenty-six, fifty-two and one hundred and four weeks following injection.

The methods for the preparation of particles, injection and sacrifice of animals, preparation of tissue for histopathology, and microbiological procedures to exclude infection, were performed according to previously described studies of the short-term effects of particles in Chapter Three.

Microbiological cultures of synovial biopsies were performed on rats sacrificed up to and including four weeks following injection. Selected

synovial biopsies were taken for electron microscopic examination and EDX microanalysis and were fixed in glutaraldehyde. A post-mortem examination was performed on all but one rat.

4.3.2 Histopathology grading

Histological examination was performed on all sections. In addition, a semi-quantitative assessment of the tissue response was performed on sections of knees of all animals sacrificed up to one year following injection. The animals sacrificed two years after injection were not included in this assessment due to the death of some animals.

The semi-quantitative scoring method used was based on the presence or absence of a particular feature in each successive high power field of synovium and subsynovium of the section examined microscopically. The cell and particle scores were based on a modification of those used by Mirra et al (1976) to assess the tissue response to wear particles in humans. A positive macrophage score was more than thirty macrophages per high power field (x 400, field area 4.7 sq.mm.), and a positive particle score was more than twenty particles per high power field. Neither necrosis, fibrosis, presence of other cell types, nor grading of macrophages or particles scores, was attempted since pilot studies had shown that quantitation of these features was unreliable. The macrophage and particle scores were derived as a percentage of the number of high powered fields of synovium in which these features were present in each section of the rat knee relative to the total number of fields containing synovium in the section as a whole.

4.4 RESULTS

4.4.1 Histopathology of rat knees

The uninjected control knees remained normal throughout the study. The knees injected with serum/saline showed a light infiltrate of macrophages and occasional lymphocytes at one week following injection. At one week following injection of particles, the particles were located intracellularly within macrophages and extracellularly. There was a predominantly macrophage infiltrate of the subsynovium and occasional aggregates of lymphocytes were present (Fig. 4.1). Patchy synovial ulceration was seen. At the sites of accumulation of large numbers of particles, necrosis of macrophages was present (Fig. 4.2). PMN were rare.

At two weeks the serum/saline injected knees were no different for the uninjected control knees and remained so. From two weeks to thirteen weeks, the numbers of extracellular particles decreased. Synovial ulceration was not observed after four weeks. The macrophage infiltrate remained the predominant finding throughout this period, while lymphocytes decreased in number and were not seen after four weeks. PMN were absent. Areas of necrosis remained but appeared to be reduced in number and extent (Fig. 4.3). At thirteen weeks most particles were contained within macrophages (Fig 4.4). There appeared to be a slight increase in fibrous tissue formation in the subsynovium at the sites of particle accumulation, but an increase in fibrous tissue could not be reliably distinguished from the normal subsynovial fibrous tissue until at least thirteen weeks.

Between thirteen weeks and two years the large majority of particles were contained within macrophages. However, occasional areas were seen

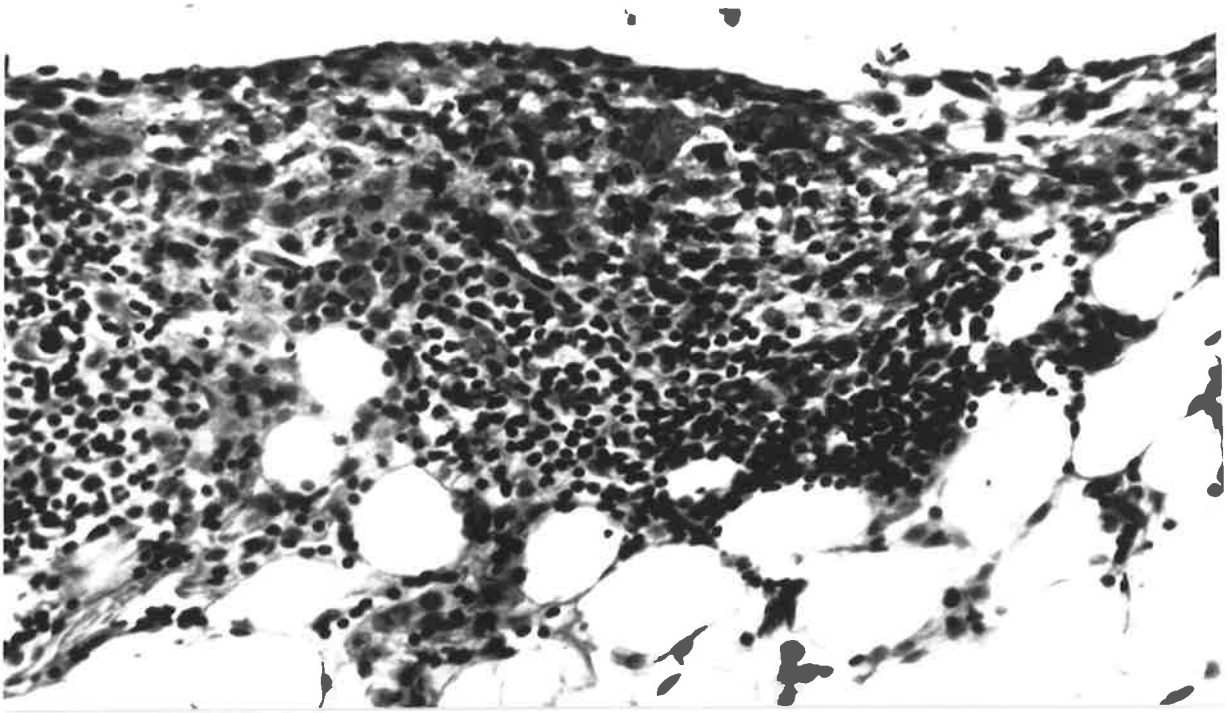

Fig. 4.1. The synovium one week following injection of cobalt-chrome particles shows aggregates of macrophages and infiltrated small lymphocytes. HE x 200

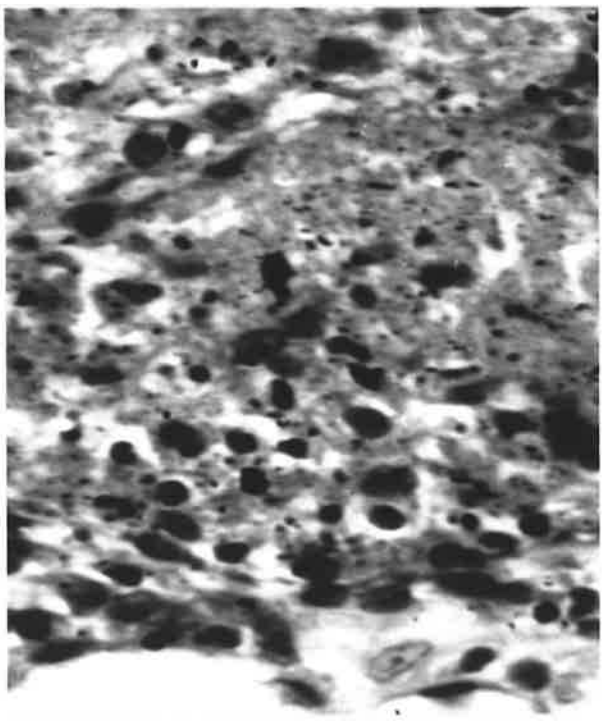

Fig. 4.2. The synovium one week following injection of cobalt-chrome particles shows necrosis of some macrophages and a lymphocytic infiltrate in the adjacent tissue. HE x 500

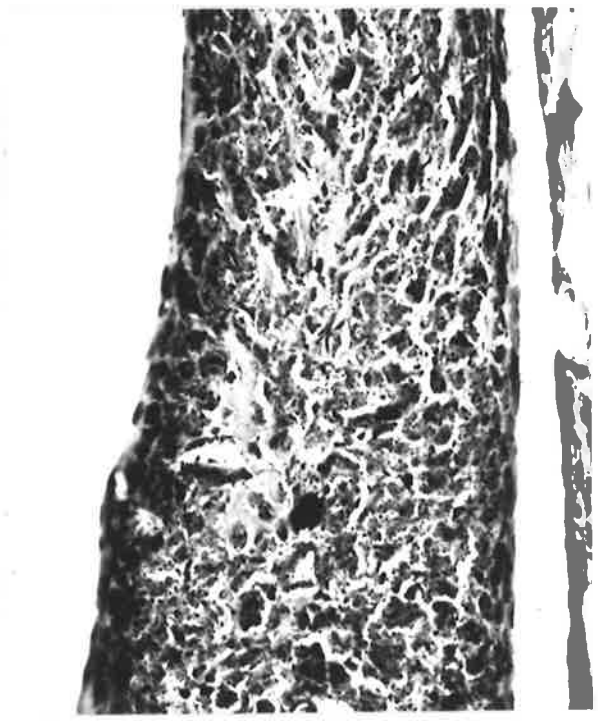

Fig. 4.3. A synovial villus eight weeks following injection of cobalt-chrome particles shows numerous intracellular and extracellular particles and necrosis of macrophages. HE x 160

where particles persisted in acellular amorphous eosinophilic material which had replaced zones of normal subsynovium in degenerate villi (Fig. 4.5 and 4.6). Macrophages containing abundant intracellular particles were also found in the subsynovium of degenerate villi (Figs. 4.7 and 4.8), and interspersed with bands of mature fibrous tissue (Fig. 4.9).

4.4.2 Particle and macrophage scores

The particle scores for injected knees at various intervals following injection are shown in Figure 4.10. It can be seen that, while there was substantial variation during the first two weeks, the median particle score did not change significantly between one and fifty-two weeks following injection.

The macrophage scores for injected knees at various times following injection are shown in Figure 4.11. The median macrophage score decreased after the first week and thereafter remained at the same level for up to fifty-two weeks.

Subjectively, the total numbers of both particles and macrophages appeared to decrease during the study, and the persisting macrophages appeared to contain larger numbers of particles. However, the scoring system used did not allow the total number of particles and macrophages to be assessed and only indicated the percentage area of knee synovium having sufficient particles present to achieve a positive score. Nevertheless, the findings showed that the extent of these areas remained relatively constant at fifteen to twenty per cent of the synovium during the first year of the study.

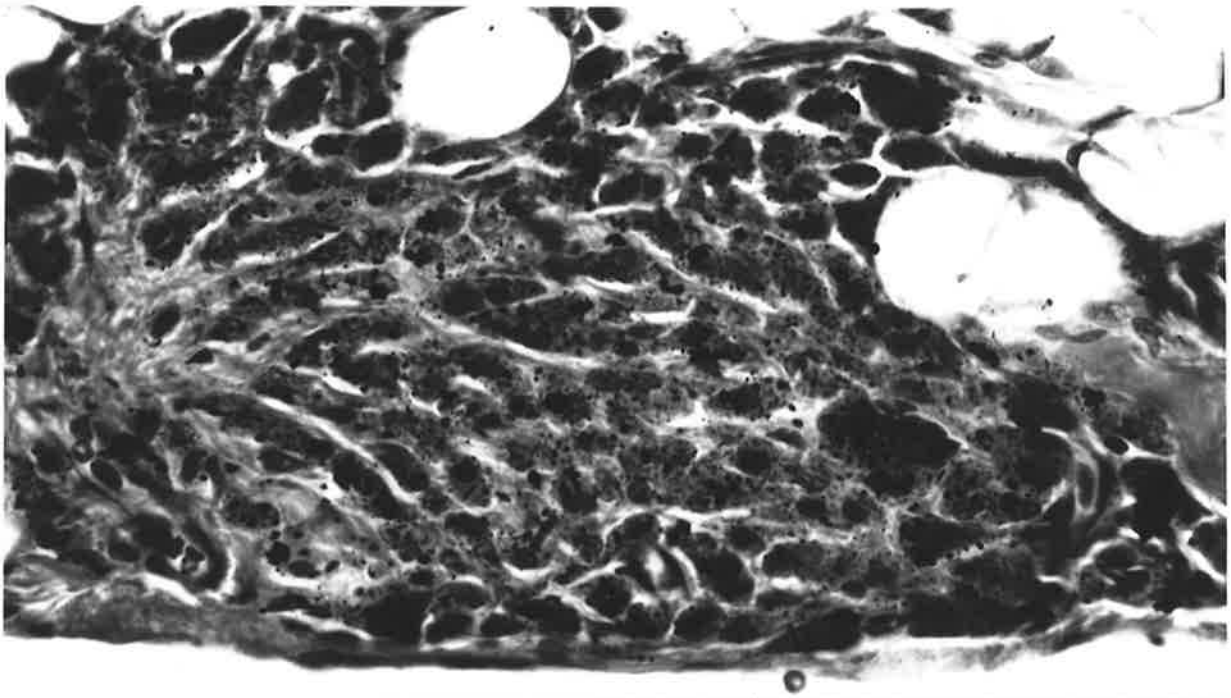

Fig. 4.4. The synovium thirteen weeks following injection of cobalt-chrome particles shows an aggregate of macrophages containing numerous particles, and some particles which are extracellular. Lymphocytes are absent. HE x 500

Fig. 4.5. A synovial villus twenty-six weeks following injection of cobalt-chrome particles shows extensive necrosis of the subsynovium and its replacement by esinophilic material containing particles. HE x 320

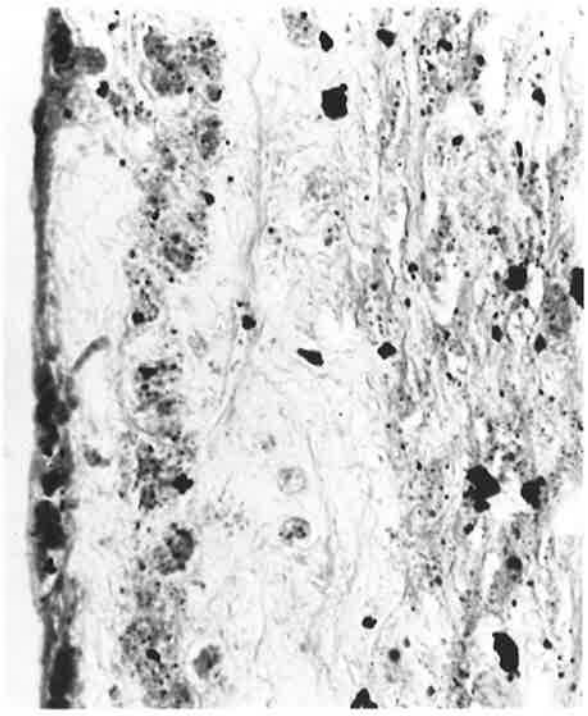

Fig. 4.6. A part of the synovial villus seen in Figure 4.5. shows discrete and clumped particles lying in the acellular subsynovium, and focal loss of the synovial cell lining. HE x 320

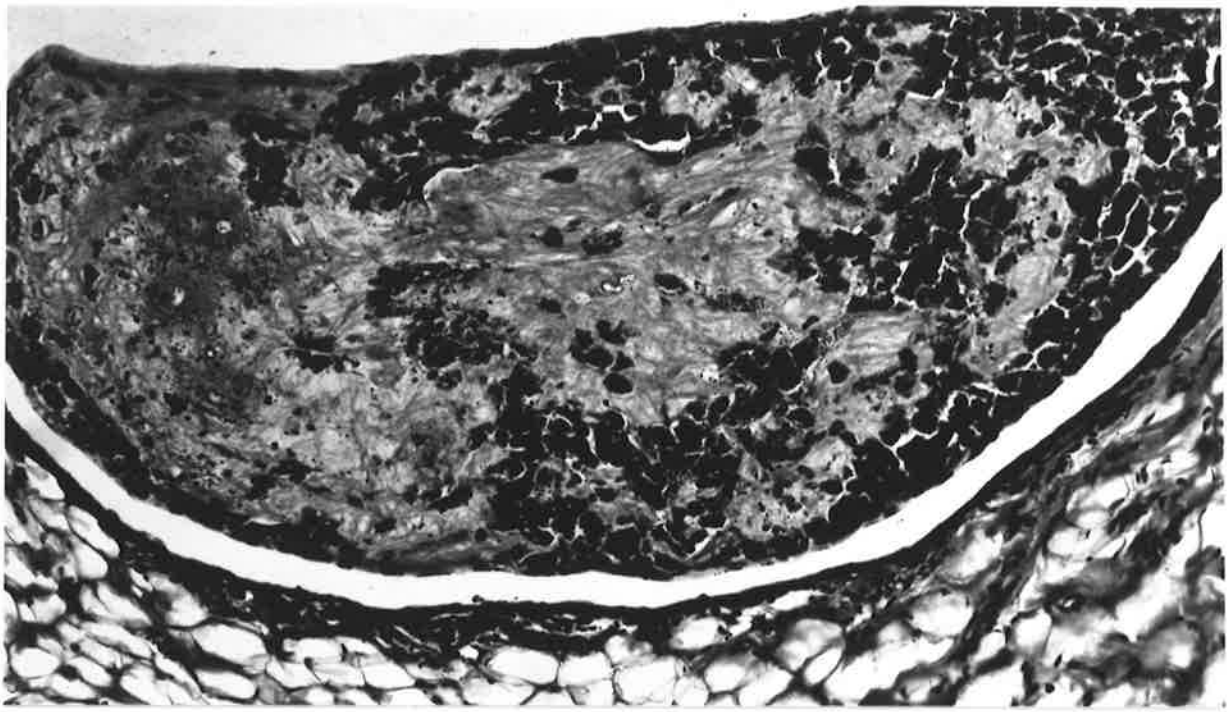

Fig. 4.7. A synovial villus one year following injection of cobalt-chrome particles shows macrophages containing abundant particles such that their cytoplasm appears black. They are readily distinguished from the focally degenerate and collagenized subsynovial connective tissue in which relatively few scattered particles are present. HE x 160

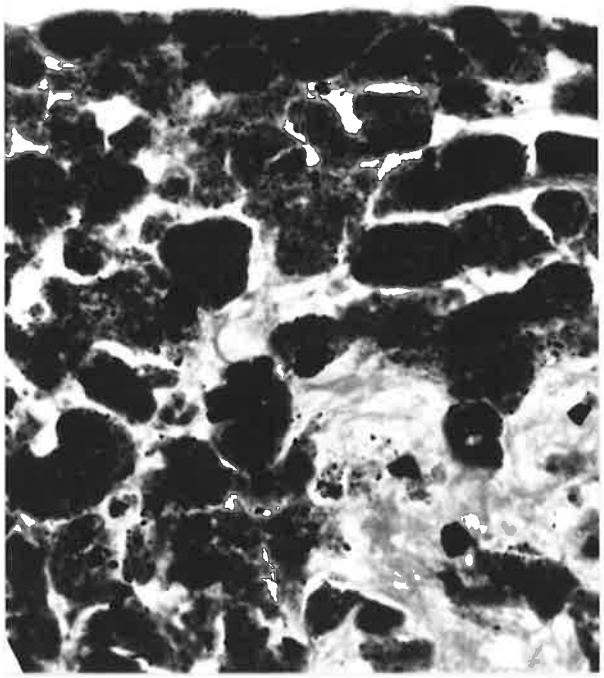

Fig. 4.8. High power photomicrograph of the synovial villus in Figure 4.7 shows large numbers of particles predominantly contained within macrophages. HE x 500

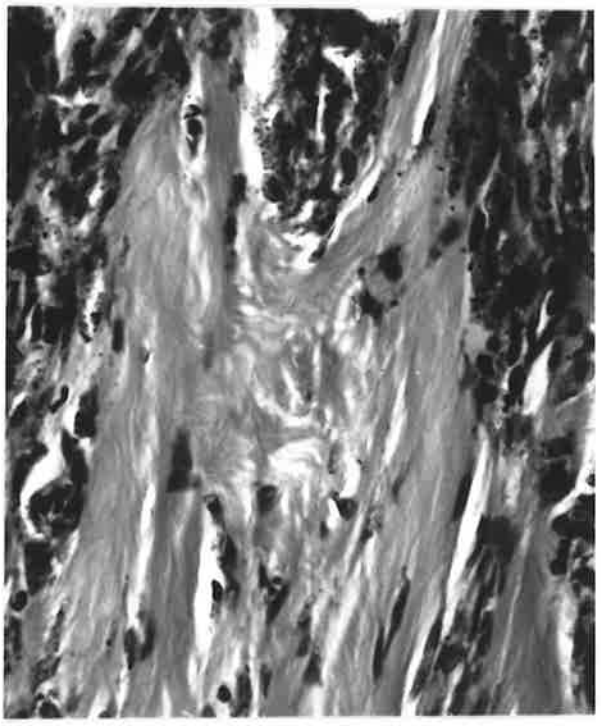

Fig. 4.9. The subsynovium two years following injection of cobalt-chrome particles shows clusters of macrophages containing many particles separated by heavily collagenized fibrous tissue. HE x 400

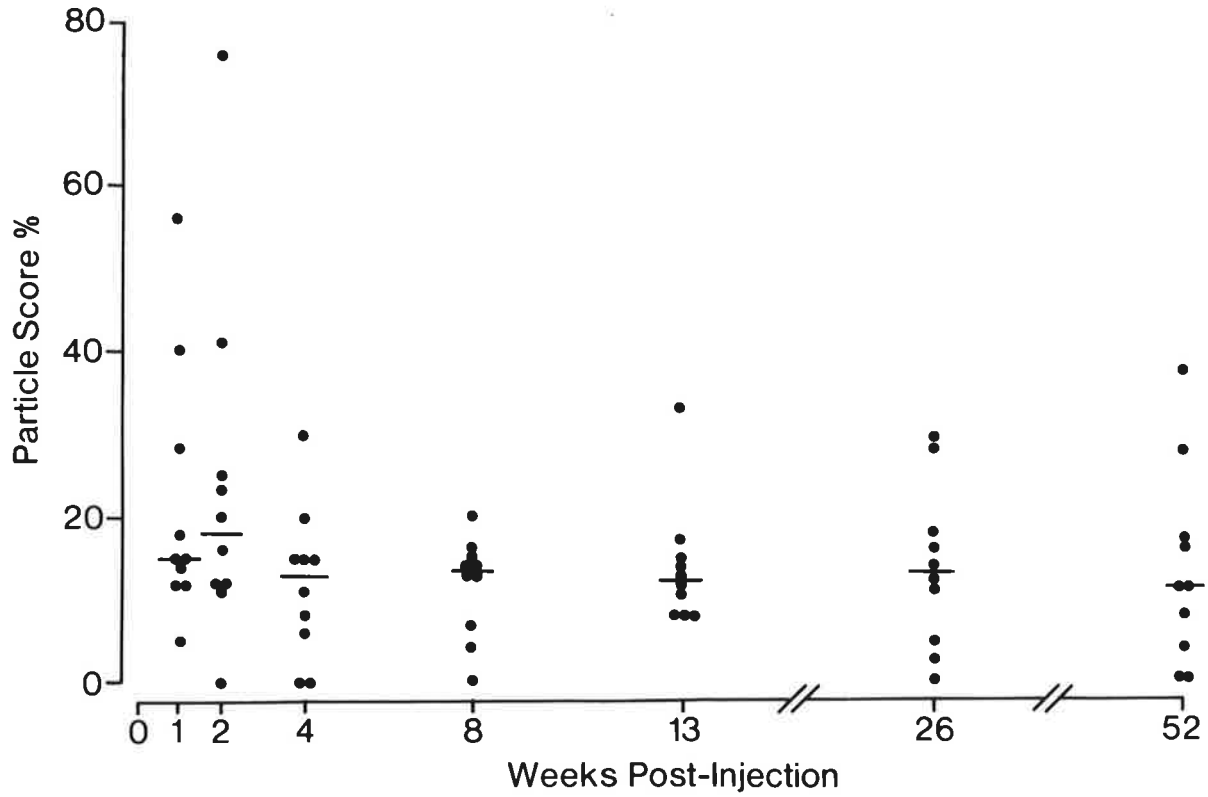

Fig. 4.10. Median and individual particle scores of knees at various time intervals following injection of cobalt-chrome particles.

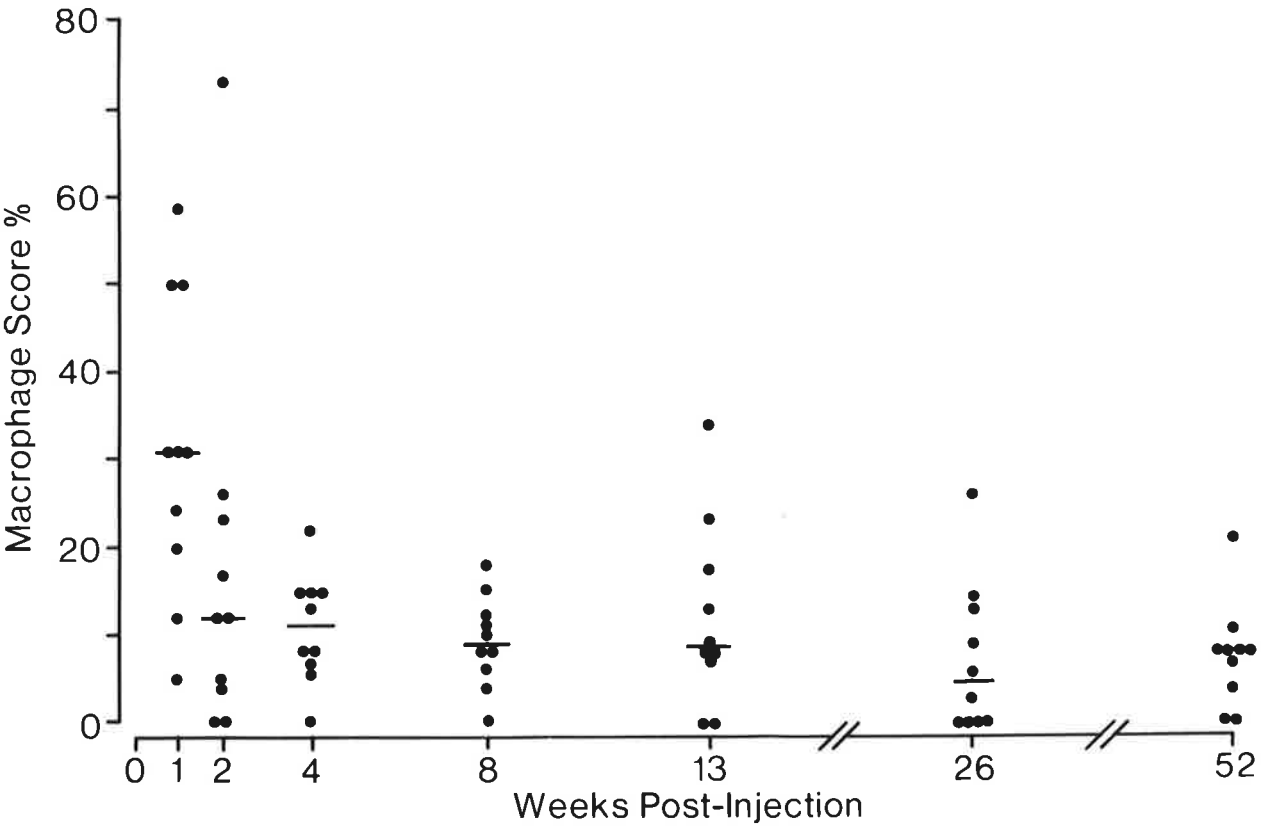

Fig. 4.11. Median and individual macrophage scores of knees at various time intervals following injection of cobalt-chrome particles.

4.4.3 Death of animals, post-mortem findings, and microbiology results

No deaths occurred in the first seventy injected and thirty-five control rats sacrificed up to one year after intra-articular injection. Between one and two years following injection, there were three deaths in the group of ten injected rats and two deaths in the group of five control rats; two injected rats and two control rats were sacrificed between one and two years because they developed respiratory infections; one apparently healthy injected rat died overnight during this period and was eaten by fellow rats, such that no post mortem was possible. No tumours were present in any of the animals in the study. Post-mortem examination did not reveal particles or abnormal findings in the extra-articular tissues. Two synovial biopsies from knees injected with serum/saline control solution grew a Propionebacterium acne on broth cultures alone. This was assumed to be a contaminant as the plate cultures were negative and histology revealed no evidence of acute inflammation.

4.5 DISCUSSION

Following intra-articular injection of cobalt-chrome alloy wear particles, the distribution of particles and macrophages within the synovium did not alter from two weeks to one year following injection. Other studies have also shown the persistence of particles and macrophages for long periods following intra-articular injection of particles. Thus, Stinson (1965) studied the tissue response to PMMA, polythene and nylon injected into the knee joints of guinea pigs. The response to these polymers varied slightly but consistent features were an early macrophage and MNGC response followed by fibrosis, but with

the persistence of some macrophages and MNGC for up to three years. Reviewing the literature, Stinson (1965) discussed the wide variability of the tissue response to various polymer particles of various shapes and sizes, and noted the previous reports of persistence of particles and a cellular response for long periods of time.

Recently, Rae (1986b) reported the results of a study of the tissue response in the knee joints of mice to the intra-articular injection of titanium and titanium-aluminium-vanadium alloy particles five micrometers and less in size. The tissue response was assessed at intervals between two and fifty-two weeks. There was a predominantly macrophage response without necrosis, and it was concluded that the materials were well tolerated.

Studies of the biocompatibility of particles of numerous materials, including polyethylene (Rushton and Rae, 1982), polyethylene, graphite, and graphite reinforced polyethylene (Tetik et al, 1974), and carbon reinforced polyethylene (Rushton and Rae, 1984), have shown a mild tissue response to each of these materials when injected intra-articularly. Short-term and long-term studies of the intramuscular, subcutaneous and intra-peritoneal injections of aluminium oxide ceramic particles also have shown a mild response when assessed subjectively (Griss et al, 1974; Harms and Mausle, 1979).

In the present study, none of the animals developed tumours up to two years following injection. These findings are in agreement with previous studies involving intra-articular injection of PMMA, polythene and nylon (Stinson, 1965), polyethylene (Rushton and Rae, 1984), cobalt-chrome alloy (Meachim and Brooke, 1983) and titanium and its alloys (Rae, 1986b). In contrast, a high incidence of tumours has been reported following the intramuscular injection of pure metals (Heath,

1976) and metal alloys (Heath et al, 1971), and following the subcutaneous injection of metals (Oppenheimer et al, 1956). The incidence of tumours probably is related to the tissue response of different species of animals, the physical form of the implant (Oppenheimer et al, 1961), the methods of preparation of the particles, and the site of implantation.

The findings of this study, and of other studies of intra-articular injection of particles, agrees with the extremely low incidence of tumours around human joint prostheses (Hamblyn and Carter, 1984), some of which produce large numbers of wear particles over many years, and suggest intra-articular injection is a more appropriate method of assessment of the biocompatibility and tumour-inducing properties of particles.

The results of this study suggest that if the scoring system used is applied to biocompatibility studies, useful information as to the degree of the cellular response to particles can be obtained within two weeks following the intra-articular injection of particles. This and other studies have shown that the cellular response is unlikely to increase after this interval, and no further information of value will be gained regarding the degree of cellular response by sacrificing groups after two weeks, although evidence of previous necrosis of the subsynovium is well demonstrated at three to six months.

In the present study, the particle score did not alter throughout the study period. This implies that enough particles remained at the sites of initial accumulation to achieve a positive score, although the total numbers may have decreased. Clearly, particles in significant numbers remained in the subsynovium of the knee joint up to one year following injection, and the associated macrophage response also persisted. It

can be expected that continual shedding of particles from joint prostheses will cause accumulation of particles in the surrounding tissues if the volume of particles produced exceeds the volume cleared from the joint (Vernon-Roberts and Freeman, 1977). Whether the particles continue to cause an adverse effect on the tissues is not known. In the present study there was no evidence for an increase in the macrophage response after two weeks, suggesting that a stable relationship between the number of particles and macrophages had been reached. It is possible the composition of the particles may have altered during the period of implantation, leading to less toxic or stimulating effects on macrophages. This differs somewhat from the human situation where particles are continuously released into the tissues.

The persistence of a macrophage response to cobalt-chrome particles for up to two years following injection has particular relevance in light of recent reports of large numbers of these particles, and a macrophage response at the bone-implant interface around loose cementless porous coated femoral components of recently designed total hip prostheses (Buchert et al, 1986). It is reasonable to assume that these particles contribute to a chronic inflammatory response in the periprosthetic tissues. In vitro studies have demonstrated that cobalt-chrome particles induce lysosomal enzyme release by macrophages (Rae, 1986a), inhibit the phagocytic activity of macrophages (Rae, 1975; Garrett et al, 1983), and cause cell necrosis (Mital and Cohen, 1968; Rae, 1975). Also, in the tissues around loose prostheses, increased lysosomal and proteolytic enzyme activity is seen in macrophages in the presence of wear particles (Eftekhar et al, 1985). Macrophages may also play an indirect role in bone resorption (Chambers, 1985). Macrophages are known to release prostaglandins (Klein and Raisz, 1970; Galasko and

Bennett, 1976) and monokines which stimulate bone resorption (Gowen et al, 1983). While this study has shown that cobalt-chrome particles persists within macrophages for at least two years following intra-articular injection, it has not yet been ascertained whether the macrophages present two years following particle injection continue to release products which are capable of stimulating bone resorption.

4.6 CONCLUSIONS

Cobalt-chrome alloy wear particles persist in the tissues for two years following intra-articular injection in rats. Semi-quantitative assessment of the tissues demonstrated that the numbers of particles and macrophages remained relatively constant after two weeks. Necrosis of the subsynovium was followed by fibrosis. No tumours developed during the course of the study.

CHAPTER FIVE

A COMPARISON OF THE SYNOVIAL RESPONSE TO ALUMINIUM OXIDE CERAMIC AND COBALT-CHROME ALLOY WEAR PARTICLES IN RATS

5.1 AIMS

The aims of this study were: to compare the tissue response to the intra-articular injection of aluminium oxide ceramic particles and cobalt-chrome alloy particles using a semi-quantitative method of assessment, to compare the tissue response to different concentrations of cobalt-chrome particles, and to compare the tissue response at different time periods following the injection of particles.

5.2 INTRODUCTION

Aluminium oxide ceramic total hip prostheses have been developed as the ceramic has a number of claimed advantages which include: better biocompatibility than stainless steel and cobalt-chrome alloys (Griss et al, 1973; 1976; Heimke et al, 1979; Busing et al, 1983), a very low coefficient of friction (Dorre and Dawihl, 1980; Dowson and Linnett, 1980), a low wear rate (Boutin, 1972; Semlitsch et al, 1977; Boutin and Blanquaert, 1981), less wear particle production, and a lower toxicity of ceramic wear particles to tissues compared with particles of other materials (Harms and Mausle, 1979; Stock et al, 1980).

The ceramic prostheses currently used may have either a ceramic on ceramic, or ceramic on UHMWP articulation, usually have a metal femoral stem and may be implanted with or without the use of acrylic cement. The clinical results of aluminium oxide ceramic prostheses, in the short term, have been reported to be as good as the results using prostheses made of conventional materials (Boutin, 1972; Zweymuller, 1979; Stock et al, 1980; Griss and Heimke, 1981; Rampoldi, 1984). However, Trepte et al (1985) reported a high incidence of femoral loosening of uncemented components of ceramic total hip arthroplasties at a short-term follow up. Moreover, O'Leary and Mallory (1986) reported poor results at two year follow up of sixty-six Mittelmier ceramic total hip prostheses, with a failure rate requiring revision of nineteen percent, and few patients were free of pain. The major cause for failure was component loosening.

Whether ceramic prostheses are likely to be superior to prostheses made of other materials, depends on whether ceramics are more biocompatible than metals when these materials are implanted in bone under weight-bearing conditions, and whether ceramics cause fewer problems due to friction, wear and wear particle release.

Severe wear of ceramic prostheses has been reported following malposition of components (Griss and Heimke, 1981), and wear rates of ceramic on ceramic prostheses components of up to five times that seen in ideal situations have been reported if there is minor incongruity between articulating components (Hinterberger et al, 1980).

In vitro and in vivo studies suggest aluminium oxide ceramic particles have little effect on cells and tissues (Griss et al, 1974; Harms and Mausle, 1979; Plenk, 1980). Problems in these studies were that particles were injected in saline which will not prevent clumping of

particles, no method to exclude infection was mentioned, and no quantitative assessment of the tissue response was made. Some of the tissue responses described in these studies might be interpreted as evidence of mild to moderate toxicity, but are difficult to interpret as no controls were included.

5.3 MATERIALS AND METHODS

5.3.1 Preparation of cobalt-chrome particles

Cobalt-chrome (Vitallium, Howmedica Inc.) particles, three micrometers and less, were prepared in a shaker in homologous rat serum diluted in saline. The methods of preparation of particles and grading of particle size by differential sedimentation (Garrett et al, 1983) have been described previously in Chapter Three. Two concentrations of particles were used, 0.74 and 0.30 mgm per ml, and these suspensions were termed high dose and low dose cobalt-chrome suspensions.

5.3.2 Preparation of aluminium oxide particles

Aluminium oxide particles were produced by shaking irregularly shaped pieces of aluminium oxide ceramic (Biolox, Richards Medical), approximately two to five millimeters in diameter, in a shaker which consisted of two unused Biolox aluminium oxide ceramic hollow femoral head components of the Mittelmier total hip prosthesis which were clamped together. The particles were milled in a solution of homologous J.C. Lewis rat serum diluted one in fifty in normal saline.

Prior to milling, the aluminium oxide shaker and pieces were washed in a warm ultrasound bath in a solution of Sparkleen diluted one in ten in distilled water. Sparkleen (Fisher Co.) is an organic solvent used to

wash total joint replacement prostheses. After five rinses in distilled water, the particles were washed in dilute nitric acid to remove possible metal contaminants, rinsed five times in distilled water, washed in an ultrasound bath in boiling distilled water and then autoclaved. These procedures fulfilled the requirement that materials be prepared in a manner similar to their preparation for human implantation (A.S.T.M. F 603-78, F 361-80, F 86-76).

Particles above three micrometer in diameter were separated from the prepared suspension by the method of differential sedimentation (Garrett et al, 1983). These particles were discarded. The weight of the aluminium oxide particles in the remaining suspension was calculated by weighing oven dried samples of the suspension. The particles were prepared as a suspension in dilute serum to a concentration of 0.74 milligram of aluminium oxide per millilitre, which was the same concentration of metal as in the high dose cobalt-chrome suspension. The aluminium oxide suspension was diluted by the ratio of the specific gravity of aluminium oxide to cobalt-chrome to obtain approximately equivalent numbers of ceramic and cobalt-chrome particles in the respective suspensions. The particles were sterilized by autoclaving prior to injection.

5.3.3 Preliminary study

A preliminary study of the effects of aluminium oxide particles was performed by injection of a sterile sample of the particle suspension prior to grading of particle size and separation of the smaller particles to be used in the definitive study. Five rats received intra-injection of particles in both knees and, following sacrifice at one week, the tissues were processed in the same manner as in the definitive study. Histological examination of these knees (Figs. 5.1 and 5.2)

demonstrated macrophage proliferation and phagocytosis of aluminium oxide particles.

5.3.4 Technique of injection and sacrifice of animals

Sixty male J.C. Lewis rats eight to sixteen weeks of age and weighing 180 to 300 grams, were injected with the high dose cobalt-chrome particle suspension in one knee and the aluminium oxide particle suspension in the other knee. Another sixty similar rats were injected with low dose cobalt-chrome particle suspension in one knee and dilute serum/saline control solution in the other knee. The rats were randomly allocated to sacrifice groups so that there were twenty high dose metal and ceramic injected rats and twenty low dose metal and saline injected rats in each group. The groups were sacrificed at one, four and thirteen weeks following injection.

Injection and sacrifice was performed as described previously in Chapter Three. A biopsy of each knee was taken for microbiological examination. Selected synovial biopsies were taken for electron microscopic examination and EDX microanalysis and were fixed in glutaraldehyde. A post-mortem examination was performed on all rats. The histopathological scoring system described in Chapter Four was used to assess the tissue response to particles.

5.3.5 Statistical methods

The correlation between the particle scores and the macrophage scores for each suspension, and the significance of the correlation, was assessed by Kendall's Rank Correlation method modified for tied rank (Swinscow, 1978). The significance of any differences between the particle and macrophage scores of each suspension was calculated by the Wilcoxon Rank Sum Test for unpaired data (Swinscow, 1978). The

macrophage score was plotted against the particle score for the suspensions, and the significance of differences between correlation coefficients was assessed by co-variant analysis (Snedecor and Cochrane, 1967). Because a number of tests for significance of differences and correlations were performed, thereby increasing the likelihood of finding differences, a significance level of $P \leq 0.01$ was accepted.

5.4 RESULTS

5.4.1 Histopathology of rat knees

At one week following injection, particles of cobalt-chrome (Fig.5.3) and aluminium oxide (Fig.5.4) were both extracellular and intracellular within macrophages. At four weeks and thirteen weeks, the majority of particles were intracellular. Subjectively, the number of particles seen in all injected knees appeared to decrease between one week and four weeks, and then remained constant to thirteen weeks.

The predominant cellular response to both materials was a macrophage infiltrate which subjectively was greater at one week, had decreased at four weeks, and remained unchanged at thirteen weeks.

There were differences in the response to the cobalt-chrome and aluminium oxide particles at one week. In the cobalt-chrome injected knees, prominent zones of necrosis of macrophages were seen at the sites of high particle accumulation (Fig.5.3). By contrast, in the knees injected with aluminium oxide particles in the preliminary study where the particle size was not controlled (Figs. 5.1 and 5.2), and in the definitive study of particles three micrometers and smaller (Fig. 5.4), very few macrophages showed evidence of necrosis and zones of necrosis

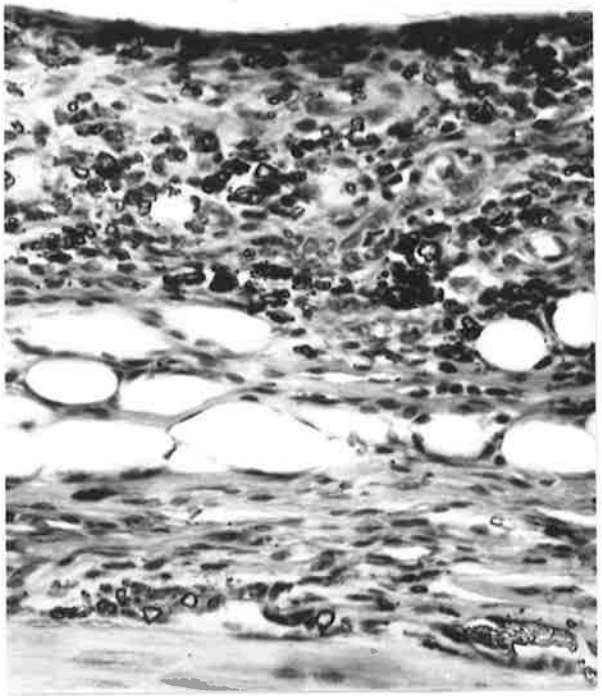

Fig. 5.1. The synovium one week following injection of unsized particles of aluminium oxide shows accumulation of macrophages associated with the presence of particles. HE x 160

Fig. 5.2. The synovium one week following injection of unsized particles of aluminium oxide. Particles predominantly are located within macrophages and necrosis is uncommon. HE x 400

Fig. 5.3. The synovium one week following cobalt-chrome particle injection shows extensive necrosis of macrophages and a lymphocytic infiltrate. HE x 160

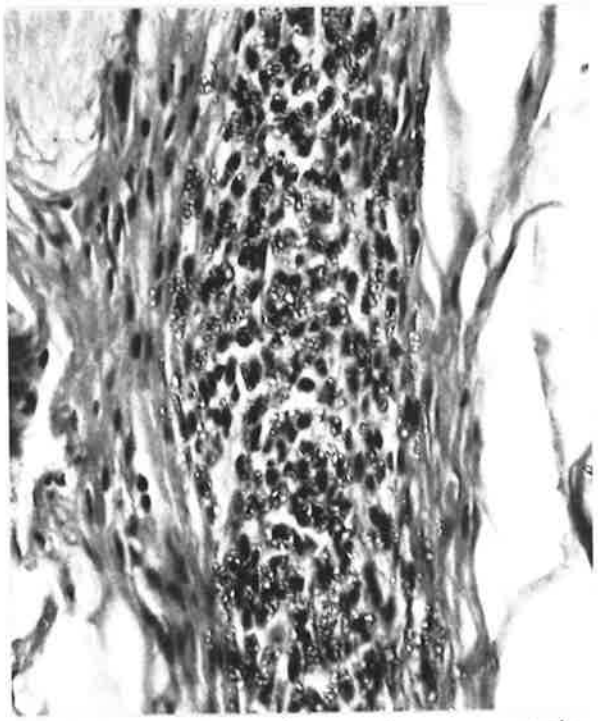

Fig. 5.4. The subsynovium one week following injection of aluminium oxide particles three micrometers and smaller shows accumulation of macrophages in response to particles. HE x 160

were absent. Moreover, while a lymphocytic infiltrate was prominent one week after the injection of cobalt-chrome particles (Fig. 5.3), lymphocytes were not a feature of knees injected with aluminium oxide at any stage (Figs. 5.1 and 5.4). At four and thirteen weeks, necrosis was confined to the reaction to cobalt-chrome particles, and lymphocytes were not observed in the reaction to either particle.

There was a difference in the response to high dose and low dose cobalt-chrome particle injections. Greater numbers of particles and macrophages were seen in the knees injected with high dose cobalt-chrome particles and necrosis was more common.

The knees injected with serum/saline control solution showed slightly increased cellularity of the subsynovium at one week and were normal thereafter.

5.4.2 Particle scores

The high dose cobalt-chrome particle scores were significantly different from the low-dose cobalt-chrome particles scores at one ($p \leq 0.001$), four ($p \leq 0.001$) and thirteen weeks ($p \leq 0.001$) following injection, demonstrating that the scoring method used detects differences in the number of particles in knees injected with different concentrations of particles of the same material.

There was no significant difference between the high dose cobalt-chrome particle scores and the aluminium oxide particle scores at one, four and thirteen weeks. There was a significant difference between the low dose cobalt-chrome particle scores and the aluminium oxide particle scores at one ($p \leq 0.001$), four ($p \leq 0.001$) and thirteen weeks ($p \leq 0.001$). These results suggest that the numbers of particles of aluminium oxide in the knees were similar to the numbers following injection of high dose

cobalt-chrome suspension, but were different following the injection of low dose cobalt-chrome suspension.

5.4.3 Macrophage scores

The high dose cobalt-chrome macrophage scores were significantly different from the aluminium oxide particle scores at one ($p \leq 0.001$), four ($p \leq 0.001$) and thirteen weeks ($p \leq 0.001$) following injection and suggest a difference in the macrophage response following injection of these particles of different materials.

The high dose cobalt-chrome macrophage scores were significantly different from the low dose cobalt-chrome macrophage scores at one ($p \leq 0.001$), four ($p \leq 0.001$) and thirteen weeks ($p \leq 0.001$) following injection, and suggest the scoring system detects differences in the macrophage response to different numbers of particles of the same material.

The low dose cobalt-chrome macrophage scores were not significantly different from the aluminium oxide macrophage scores at one, four and thirteen weeks following injection.

5.4.4 Particle and macrophage correlations

There was a high correlation between the particle score and macrophage score following injection of high dose cobalt-chrome suspension ($r= 0.92$, $p \leq 0.001$), low dose cobalt-chrome suspension ($r= 0.97$, $p \leq 0.001$) and aluminium oxide suspension ($r= 0.83$, $p \leq 0.001$).

To determine the macrophage response to the two different concentrations of particles of cobalt-chrome, the macrophage scores were plotted against the particle scores at each time interval (Fig. 5.5). The lines of best fit have similar slopes, but the low dose suspension

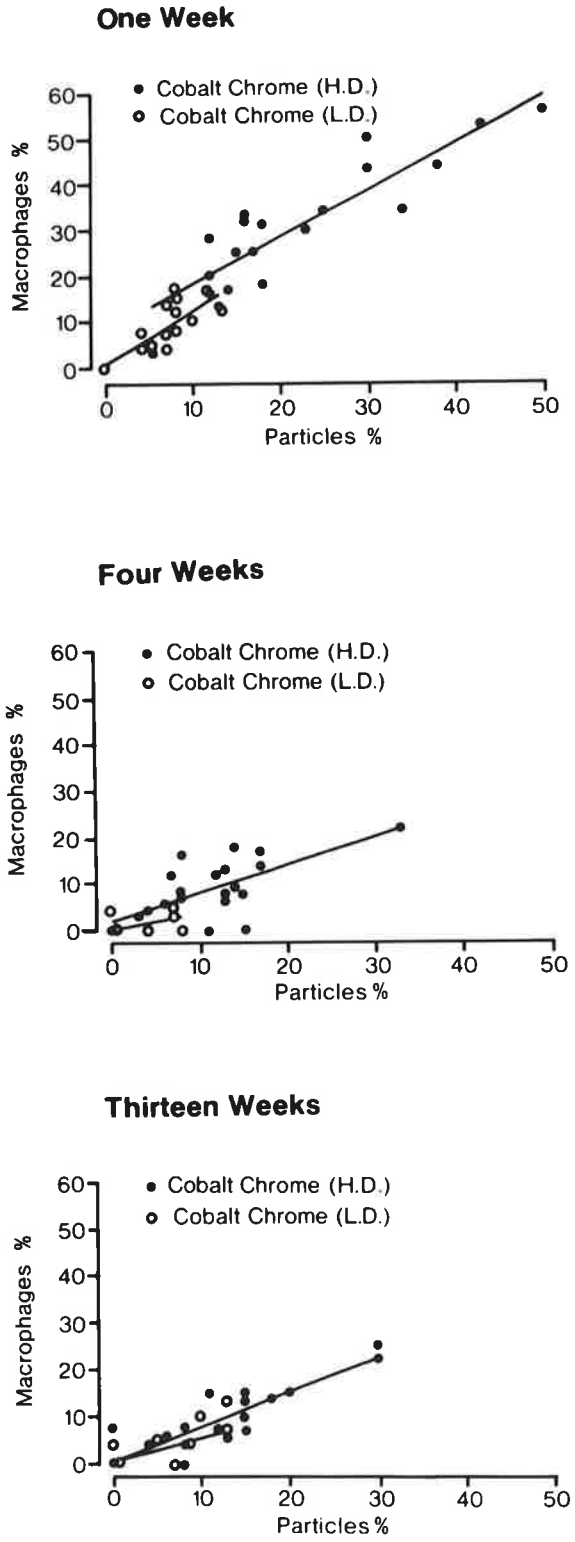

Fig. 5.5. The macrophage response at one, four and thirteen weeks following injection of high dose (H.D.) and low dose (L.D.) cobalt-chrome particle suspensions. The lines of best fit have similar shapes, indicating that the macrophage response is related to the number of particles, but is not as severe following injection of smaller numbers of particles of the same material.

consistently scores a lower particle and macrophage score, confirming the differences described previously.

The macrophage and particle scores for high dose and low dose cobalt-chrome suspensions were combined and were compared to the scores for aluminium oxide suspension (Fig. 5.6). At one week following injection, the lines of best fit between the two materials were significantly different ($p \leq 0.01$) and suggest the macrophage response to cobalt-chrome particles was approximately twice that of aluminium oxide particles. At four weeks and thirteen weeks, there were no significant differences between the slope of the lines. These results confirm a difference in the response to cobalt-chrome particles compared to aluminium oxide particles which had been suggested by the finding of significant differences between the macrophage scores for high dose cobalt-chrome and aluminium oxide. Although significant differences between macrophage scores were also detected at four and thirteen weeks, while no differences were detected for particle scores, the method of co-variant analysis did not confirm these differences.

5.4.5 Post-mortem and microbiology results

No deaths occurred and no tumours were found in any animals. Post-mortem studies did not reveal particles or abnormal appearances in the extra-articular tissues.

The cultures from the synovial biopsies of the rat knees and the human tissues were negative, except for one rat knee biopsy which grew a Staphylococcus epidermidis in one broth. This growth was assumed to be a contaminant, as the plate cultures and other broth cultures were negative and the histopathology of this knee showed no evidence for acute inflammation.

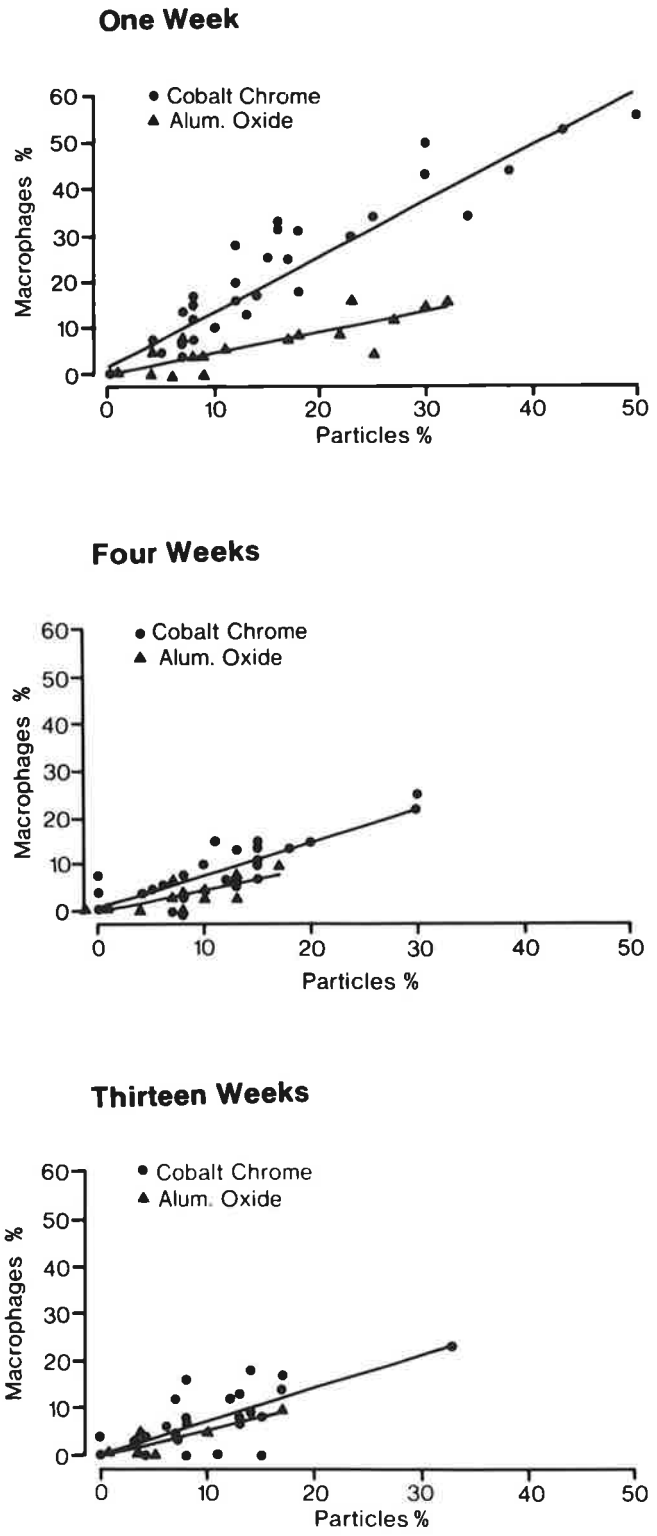

Fig. 5.6. The macrophage response at one, four, and thirteen weeks following injection of cobalt-chrome and aluminium oxide particle suspensions. The slopes of the lines of best fit are significantly different at one week ($p \leq 0.01$), but not at four and thirteen weeks following injection, indicating a more severe macrophage response to similar numbers of cobalt-chrome particles compared to aluminium oxide particles.

5.5 DISCUSSION

Intra-articular injection of cobalt-chrome alloy and aluminium oxide ceramic particles in the same size range as particles seen in the tissues around human prostheses (Winter, 1974; Vernon-Roberts and Freeman, 1977; Harms and Mausle, 1979; Stock et al, 1980; Griss and Heimke, 1981) induced a macrophage response in the rat knees which correlated with the amount of particulate deposited in the synovium at the three sacrifice times. The macrophage response to cobalt-chrome particles differed from that to aluminium oxide particles. At one week following injection, the response to cobalt-chrome particles was greater than to aluminium oxide particles, but there was no difference at four and thirteen weeks. These results suggest cobalt-chrome particles induce a greater inflammatory response shortly after injection.

Both these materials are relatively inert in solid form and Richardson et al (1975) found no difference in the response to their intramuscular implantation. In vitro comparisons have not been made between these two materials in particle form, but there is some suggestion that cobalt-chrome particles are more toxic. Cobalt-chrome particles in vitro interfere with the phagocytic activity of macrophages and are toxic to macrophages and fibroblasts (Rae, 1975, 1981; Garrett et al, 1983). Cobalt has been implicated as the most toxic of the constituent metals of this alloy and this toxicity has been related to its greater solubility (Rae, 1981). Aluminium oxide particles have been reported in vitro to mildly inhibit fibroblast growth, causing slightly less inhibition than stainless steel particles (Plenk, 1980).

In vivo studies suggest ceramic particles are relatively inert. Griss et al (1974) and Harms and Mausle (1979) concluded that the macrophage response to subcutaneous, intramuscular and intra-articular injection of particles of a similar size to those used in the current study was not of concern. These conclusions were based on subjective assessment of the tissue response, and did not include a control material nor adequate techniques to exclude infection. Some worrying features were reported in these studies. A PMN response which peaked at one week following subcutaneous injection (Griss et al, 1974), and a persisting lymphocyte response (Harms and Mausle, 1979) following intramuscular injection may be interpreted as evidence of mild to moderate toxicity (ANSI Doc 41, 1979; FDI Doc 198, 1979). These features were not observed in the current study. Uchida (1985) observed that the intra-articular injection of alumina ceramic produced a more severe tissue response than polymer and metal particles, including cobalt-chrome alloy.

The results of the current study suggest a difference in the degree of the initial macrophage response to the two types of particles, but no difference in the degree of the persisting macrophage response. Thus, it is possible that the continuing macrophage response depends on the mechanical presence of the particles rather than any continuing toxic properties which may or may not persist.

The scoring system used in this study bears further discussion. Despite attempts to inject particles of a similar size and concentration, and in approximately similar amounts, it is possible that there were large variations in the number of particles exposed to the synovia of different knees. For this reason, a scoring system was used which related the macrophage response to number of particles in the synovium. In addition, the whole of the knee was examined to take into account the

possible variations in the number of particles and the macrophage response at different sites. If the required minimum number of macrophages or particles were present in any given high power field, then a positive score was recorded. This system has limitations, as there may be far larger numbers of either macrophages or particles present in two different areas, but the score will be the same once the threshold number is reached to achieve a positive score. To assess the value of this scoring system in detecting differences in particle numbers and macrophages, two different concentrations of cobalt-chrome particles were used. The scoring system seems justified as the macrophage response correlated with the particle score in all groups, and more importantly, the scoring system detected a difference in the particle and macrophage score following injection of high dose and low dose cobalt-chrome suspensions.

The macrophage response alone was used to assess the tissue response to particles, as it could be reliably scored. Other features, such as the extent of lymphocyte and PMN infiltrate, and the degree of necrosis and fibrosis have been used by others to assess the biocompatibility of particles following intra-articular injection. Some of these features were present in the knees in this study, but could not be reproducibly scored.

5.6 CONCLUSIONS

In conclusion, the initial macrophage response to cobalt-chrome particles was different from that to aluminium oxide particles. After this initial difference, the macrophage response was related to the number of particles of each material, but not to the difference in materials.

Whether the difference in the macrophage response to the different wear particles is important in determining the deleterious effects of wear particles of different materials around human prostheses, is unknown. A material which produces wear particles which provoke less macrophage response in the surrounding tissues would seem desirable, but a difference in the macrophage response between the two materials in this study was only detectable at one week following injection.

The adverse effect of macrophages in the periprosthetic tissues and their possible role in bone resorption and prosthetic loosening, takes many years to become evident in humans. As new particles are continuously released into the periprosthetic tissues in humans, and possible toxic effects of the particles may have a cumulative effect, differences in the effect of particles seen at one week may be important. Also, in this study, the persisting macrophage response at four weeks and three months was related to the number of particles in the synovium. It is likely, therefore, that the number of particles that persist in the tissues is also an important determinant of long-term adverse effects of prosthesis wear particles in humans.

CHAPTER SIX

THE SYNOVIAL TISSUE RESPONSE TO INTRA-ARTICULAR INJECTION IN RATS OF POLYETHYLENE WEAR PARTICLES PREPARED IN A JOINT SIMULATOR

6.1 AIM

The aim of this study was to examine the tissue response to the intra-articular injection of polyethylene wear particles which were prepared in a joint simulator to obtain a range of particles similar in size and shape to those seen around human joint arthroplasties.

6.2 INTRODUCTION

Wear particles arising from polyethylene prostheses have been implicated as a cause of the tissue response in the tissues around failed joint prostheses and in loosening of joint prostheses (Willert and Semlitsch, 1976; Vernon-Roberts and Freeman, 1977). The number of polyethylene particles in the tissues around failed joint prostheses has been found to correlate with the duration of implantation of the arthroplasties (Mirra et al, 1982) and with the severity of the cellular response in the periprosthetic tissues (Revell et al, 1978).

While polyethylene particles may provoke the cellular response seen around failed prostheses, it is possible also for acrylic cement particles (Charosky et al 1973; Mirra et al, 1982; Jasty et al, 1986a), metal particles (Winter, 1974; Vernon-Roberts and Freeman,

1977), and chronic infection (Fitzgerald and Kelly, 1979), to cause similar adverse tissue responses.

Various hemi, total, and resurfacing hip arthroplasties have suffered from severe wear of polyethylene due to inappropriate design (Dahl and Mikkelsen, 1976; Wroblewski, 1979) and three-body wear (Revell et al, 1978; Bell et al, 1985). Polyethylene wear particles also are found in the capsule and in the connective tissue layer at the bone-cement interface of current designs of total hip arthroplasties (Vernon-Roberts and Freeman, 1977). While there is no dispute that polyethylene particles are present in such tissues, there appears to be some disagreement as to the relative importance of these particles in prosthetic failure. Thus, several authors (Jasty et al, 1986a; Charosky et al, 1973; Harris et al, 1976) have emphasized the importance of acrylic particles in the adverse tissue response, but have tended to ignore the importance of the polyethylene particles.

Previous in vivo studies have suggested that polythene particles provoke a macrophage and MNGC response (Stinson, 1965) which may be more severe than the response to metal particles (Paiement et al, 1986). Other studies have suggested that the cellular response to polyethylene particles is minimal (Tetik et al, 1974). Most studies have been performed using commercially available polyethylene particles which are not necessarily of the shape or size seen around failed joint arthroplasties.

6.3 MATERIALS AND METHODS

6.3.1 Preparation of polyethylene particles

High density polyethylene particles were prepared by the device illustrated in Fig.6.1. Sterile unused Wagner resurfacing hip components (Aesculap Inc.) were used to produce the particles. The femoral component was rotated under pressure in a fixed polyethylene component one size larger than the femoral component. The articular surface of the cobalt-chrome alloy femoral component was scoured with one millimetre radial grooves to increase the rate of wear of the polyethylene component.

The milling fluid used was sterile two percent homologous rat serum in normal saline. Sodium benzoate (0.05%), an anti-fungal agent, and gentamycin sulphate (1.0 mgm per 100 mls) were added to the fluid. The milling was performed for six hour periods for a total of eighty hours at room temperature. During milling, a dust cover was used to seal the components. An uncovered container of control solution of milling fluid was placed within the dust proof enclosure during milling to expose the control solution to the same environment as the milling fluid.

Between millings, the milling fluid and control solutions were stored at minus four degrees centigrade. After each milling session the resurfacing hip components were washed in Sparkleen (Fisher Co.) and rinsed a total of fifteen times in de-ionised distilled water. The control solution was also stored in glass containers washed by the same methods. Prior to milling the components were soaked in normal saline containing the same concentrations of sodium benzoate and gentamycin as the milling fluid.

Fig. 6.1. Photograph of the simplified joint simulator used to produce polyethylene particles. There are radial grooves on the metal component to increase the rate of wear.

Following preparation of the particles, the sterility of the particle and control suspension was checked by aerobic and anaerobic culture. Repeat sterility assessments were performed on each suspension prior to each injection. The particle suspension was examined microscopically under polarized light, and showed that particles varied in size from thread-like forms, two hundred micrometers in length, to particles which were just visible by light microscopy. The majority of particles were fifteen micrometers and less in maximum dimension.

Following the preparation of the particles, a sample of the particle suspension was examined by atomic absorption spectroscopy to detect the possible presence of metals. No metal was detected in the particle suspension.

6.3.2 Injection and sacrifice of rats

Five outbred Porton male rats ten weeks old, weighing 200 to 250 grams, received intra-articular injection of polyethylene particles in one knee and control solution in the opposite knee. The animals were sacrificed at one week following injection.

A further five rats fourteen weeks old, weighing 350 to 400 grams, received weekly injection of polyethylene and control suspensions for five weeks and were sacrificed one week following the last injection.

At the time of sacrifice, synovial biopsies of all knees were sent for aerobic and anaerobic culture, selected biopsies were taken for electron microscopic examination and EDX microanalysis and were fixed in glutaraldehyde. The knees were then resected and processed for histological examination by light microscopy. A post mortem examination was performed on each rat. The technique of injection, methods of

retrieval of specimens, culture methods, and tissue processing methods have been described previously in Chapter Three.

Six micrometer sections from the mid-sagittal plane of each knee were examined under direct light without knowledge of prior treatment, and then under polarized light to detect the presence of polyethylene particles.

6.4 RESULTS

6.4.1 Histopathology of rat knees

The knees injected with particles showed focal proliferation of the lining synoviocytes, and areas of accumulation of macrophages and occasional MNGC in the subsynovial connective tissues (Fig. 6.2). The synoviocyte hyperplasia and subsynovial cell infiltration was associated with the presence of polyethylene particles in the same area (Fig. 6.3). The degree and extent of these changes was greater in the animals which had received repeated injections (Figs. 6.4 and 6.5) compared with the animals which had received a single injection (Figs 6.2, and 6.3). Large particles were generally located within MNGC, while smaller particles were associated with a macrophage response (Figs. 6.6 and 6.7). While occasional degenerate macrophages and MNGC were observed (Fig. 6.6), this was not a prominent nor widespread feature. A few lymphocytes occasionally were present but were not a constant feature of the response. PMN were absent.

When viewed by polarized light, large numbers of highly birefringent polyethylene particles were seen in the synovial tissue of particle injected knees (Figs. 6.2, 6.3, 6.4, 6.5). The majority of the

particles were approximately fifteen micrometers or less in maximum dimension. Particles were located intracellularly within macrophages and MNGC. Occasional larger polyethylene particles were seen in the subsynovium and were contained within MNGC (Figs. 6.2, 6.3, 6.6, 6.7). Some mononuclear macrophages and MNGC also contained aggregates of very small particles only visible at high magnification (Figs. 6.8 and 6.9). The only difference between the knees receiving one injection of particles and multiple injections was the increased number of particles and increased macrophage and MNGC infiltrate in the subsynovium of multiple injected knees. Metal particles were not seen in the tissues. The control solution injected knees showed slightly increased cellularity of the subsynovium but were otherwise normal.

6.4.2 Post-mortem and microbiology results

No deaths occurred and no particles or abnormal appearances were seen in extra-articular tissues.

One of the eight broth cultures of the particle suspensions grew an anaerobic diptheroid between one and two weeks, but there was no growth on primary cultures. The control solution cultures were negative. One synovial biopsy of a particle injected knee grew a Bacillus species on broth culture between one and two weeks but there was no growth on primary cultures. One synovial biopsy of a control knee grew a Staphylococcus aureus on broth culture at one week but had no growth on primary cultures. All these isolates were assumed to be contaminants as they grew on broth cultures alone and not on primary culture.

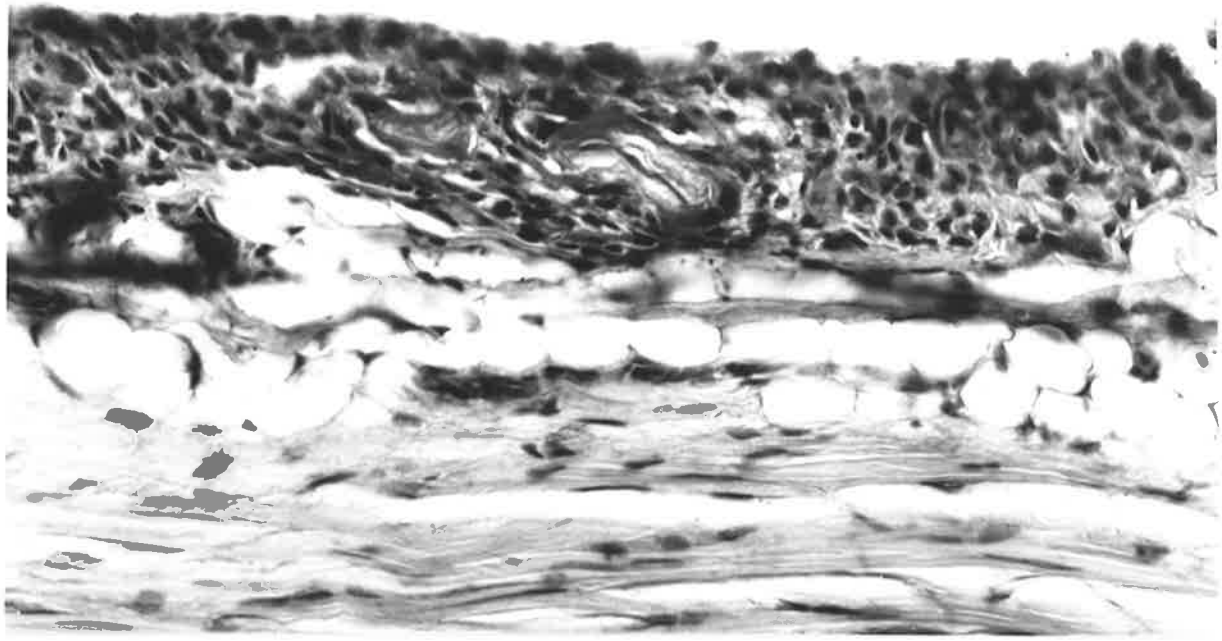

Fig. 6.2. Photomicrograph of rat synovium one week following a single injection of polyethylene particles. The synovial lining is intact, but the subsynovium has been infiltrated by macrophages and occasional MNGC. HE x 200

Fig. 6.3. The same field as Figure 6.2 viewed by polarised light. It shows macrophages contain small polyethylene particles while the MNGC have formed around large particles. HE x 200

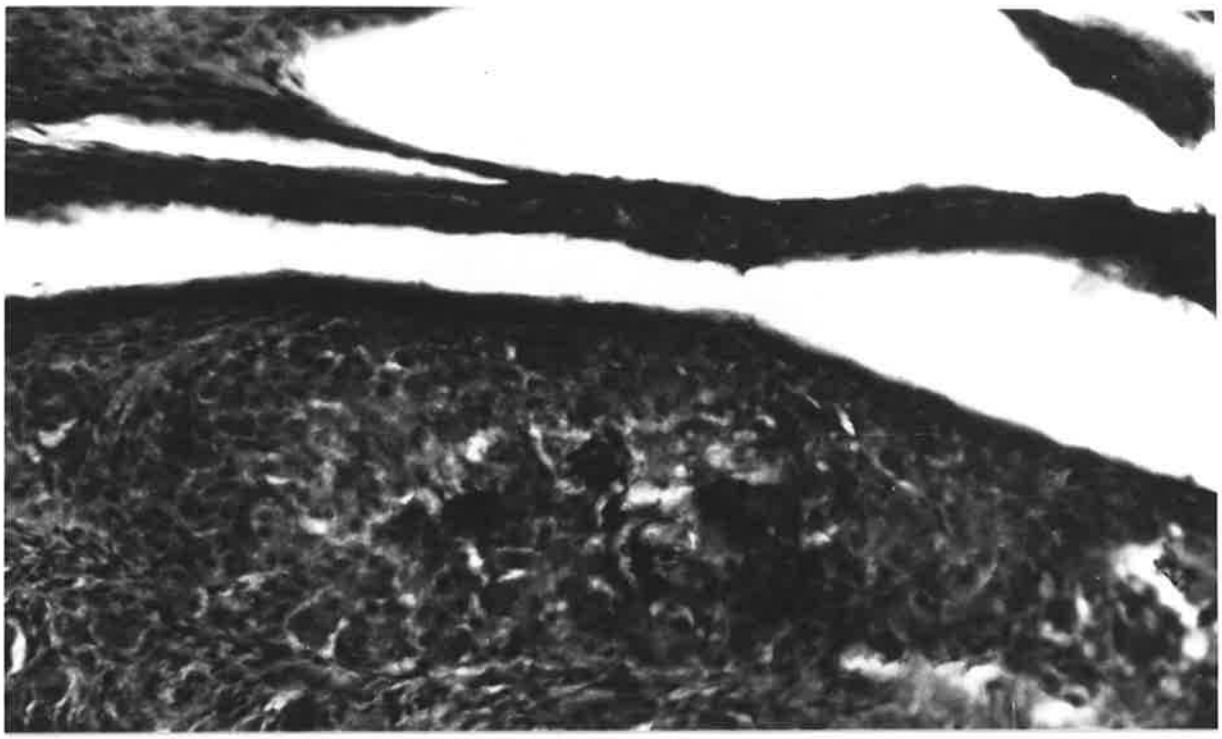

Fig. 6.4. Photomicrograph of the suprapatella pouch of a rat knee one week following five weekly injections of polyethylene particles. The synovial lining is intact but the subsynovium contains a heavy infiltrate of macrophages and occasional MNGC. HE x 160

Fig. 6.5. The same field as Figure 6.4 viewed by polarised light. It shows that the subsynovium contains numerous highly birefringent polyethylene particles of varying sizes. HE x 160

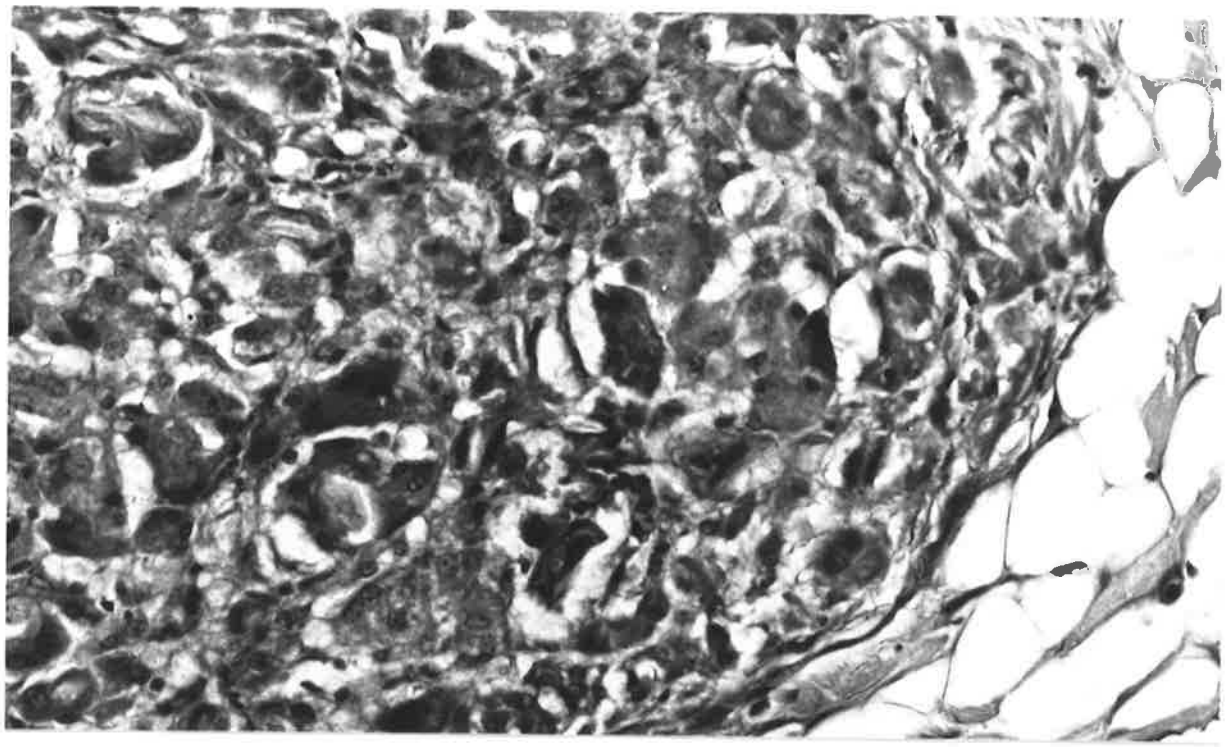

Fig. 6.6. Photomicrograph of rat subsynovium one week following five weekly injections of polyethylene particles. It shows infiltration of the subsynovium by macrophages and MNGC. Occasional macrophages show degenerative changes. HE x 320

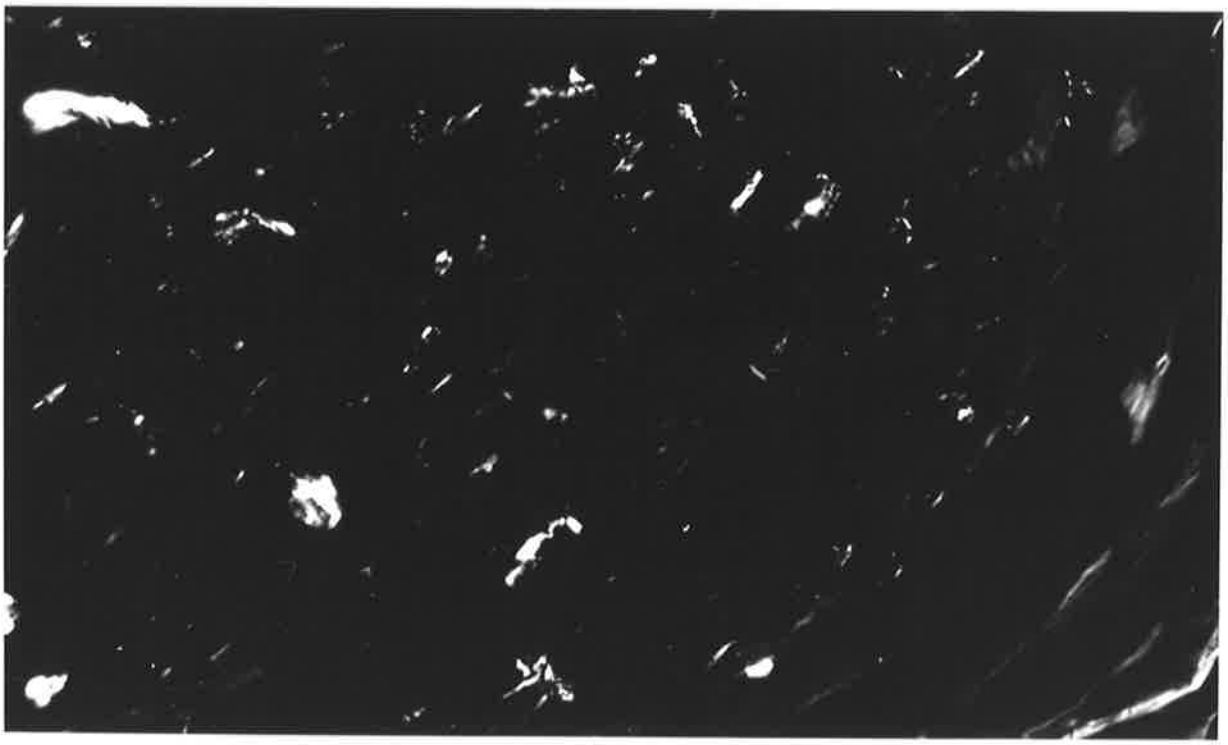

Fig. 6.7. The same field as Figure 6.6 viewed by polarised light. Comparison with Figure 6.6 shows small polyethylene partlicles are contained within macrophages, while large particles are contained within MNGC. HE x 320

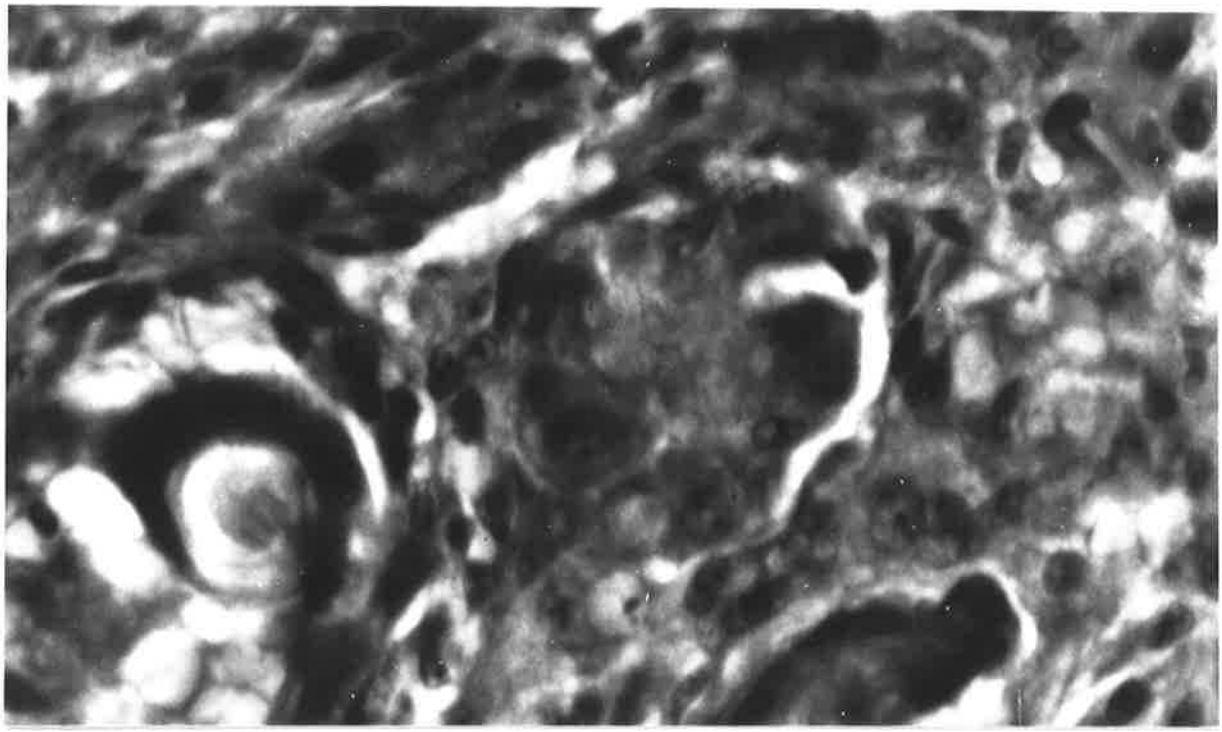

Fig. 6.8. High power photomicrograph of the rat subsynovium one week following five weekly injections of polyethylene particles. It shows infiltration by macrophages and MNGC. HE x 800

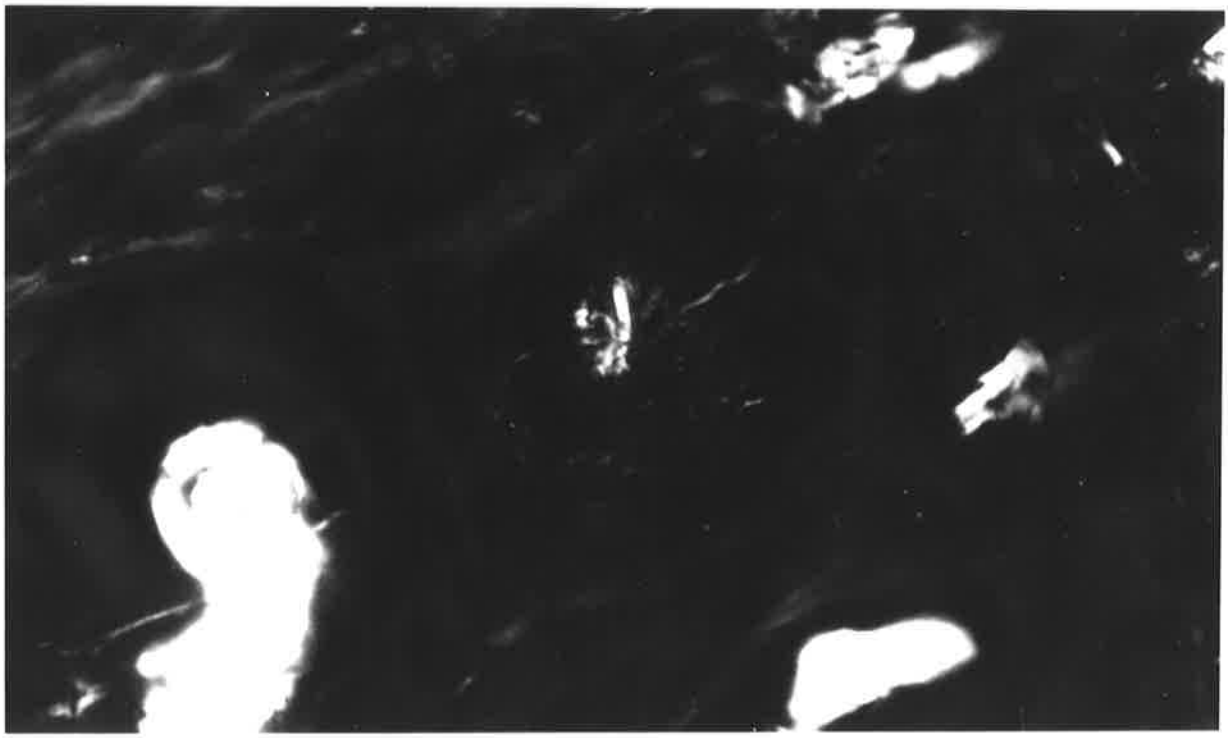

Fig. 6.9. The same field as Figure 6.8 viewed by polarised light. Comparison with Figure 6.8 shows the MNGC in the lower half of the field contain large polyethlene particles, while the central MNGC contains barely visible discrete polyethylene particles and small aggregates of particles. HE x 800

6.5 DISCUSSION

The intra-articular injection in rat knee joints of polyethylene wear particles similar in size and shape to those seen around failed human prostheses provoked a macrophage and MNGC response similar to that seen around failed human prostheses (Vernon-Roberts and Freeman 1976; Mirra et al 1982). Similar responses have been described following the intra-articular injection of similarly prepared polyethylene wear particles (Rushton and Rae, 1982), and following the injection into rat wound chambers of commercially available polyethylene particles (Paiement et al, 1986).

Essentially, particles which are larger than the size of macrophages, tend to produce a MNGC response, as do aggregates of smaller particles approximately five to fifteen micrometers in maximum dimension. Particles less than five micrometers are associated with a mononuclear macrophage response.

Of interest and possible pathological significance, was the finding of large numbers of intracellular very small polyethylene particles at the limit of resolution of light microscopy. While electron microscopy can be used to identify these particles, and sophisticated techniques which use microprobe analysis to distinguish different types of particles in tissues (Roschger et al, 1980) can be applied, these techniques are not suitable for routine assessment of the tissue response to particles since they select a very small tissue area for examination. Nevertheless, they confirm the presence of abundant finely particulate intracellular wear material in the tissues around failed human endoprostheses (Vernon-Roberts and Freeman, 1977).

Rose et al (1979) has reported that small wear particles result from low rates of wear and very small polyethylene particles, therefore, can be expected in the tissues around currently used total hip arthroplasties with small diameter femoral heads. Thus, the microscopic assessment of the tissues around implants should always include careful examination for finely particulate polyethylene material which gives a diffuse mottled birefringence when examined by polarized light (Vernon-Robert and Freeman, 1977).

Given that the macrophage may have an important role in the stimulation of bone resorption (Chambers, 1985), this study confirms that the presence of polyethylene wear particles induces the accumulation of macrophages which could play a role in prosthetic loosening by stimulation of bone resorption at the bone-implant interface.

6.6 CONCLUSIONS

The intra-articular injection of polyethylene particles of the size and shape seen in the tissues around human prostheses produced a macrophage and MNGC response in the synovium and subsynovium of rat knees. Particles ranging in size from approximately five micrometers in maximum dimension down to the limit of resolution of the light microscope were phagocytosed by mononuclear macrophages. Aggregates of these particles and larger particles were phagocytosed by MNGC. Unlike the response to cobalt-chrome particles, there was little evidence of degeneration of macrophages following phagocytosis of polyethylene particles.

CHAPTER SEVEN

ULTRASTRUCTURAL EXAMINATION OF THE CELLULAR RESPONSE TO WEAR PARTICLES IN RATS

7.1 AIMS

The aims of this study were to determine the ultrastructural appearance of cells following the intra-articular injection of cobalt-chrome alloy, aluminium oxide ceramic, and polyethylene wear particles in rat knees, to correlate these findings with the light microscopic appearances of the rat knee synovium, and to compare the appearances with those in the tissues around failed human arthroplasties.

7.2 INTRODUCTION

Light microscopy alone does not allow a comprehensive assessment of the effects of very small particles on cells because subtle ultrastructural changes are beyond the resolution of the light microscope. Thus, the ultrastructural appearances of rat knee synovium, at intervals following the intra-articular injection of cobalt-chrome alloy, aluminium oxide ceramic, and polyetheylene wear particles were examined to complement previous studies utilizing light microscopy.

7.3 MATERIALS AND METHODS

7.3.1 Tissue processing for electron microscopy examination

Synovial biopsies were obtained from rat knees one day, one week and four weeks following the injection of cobalt-chrome alloy particles and one week and four weeks following the injection of aluminium oxide and polyethylene particles. The biopsies were taken while the rats were under anaesthesia prior to sacrifice, to prevent possible post-morbid artifactual changes. The preparation of particles, techniques of injection, biopsy and sacrifice have been described previously in Chapters Three, Four, Five, and Six.

Two millimetre cubed biopsies of rat synovium were immediately fixed in 2.5% glutaraldehyde in 0.05 M cocodylate buffer for two hours. Each specimen was then divided. One half, for morphological examination, was post-fixed in 2% osmium tetroxide in 0.05M cocodylate buffer for one hour and then dehydrated in graded alcohols. The other half for analysis of metal content, was passed directly into graded alcohols. The specimens were then passed through two changes of propylene oxide, then through increasing concentrations of propylene oxide and TAAB embedding resin (araldyte and epon), until final embedding in TAAB embedding resin at sixty degrees centigrade for twelve hours.

7.3.2 TEM and EDX microanalysis

Semi-thin sections of osmium and non-osmium fixed specimens approximately half to one micrometer thick, were cut and stained with toluidene blue and examined by light microscopy. Representative sections were selected and ultra-thin sections were cut using an L.K.B. ultra-microtome. Sections for morphological examination were stained with aqueous uranyl acetate and lead citrate and examined in a JEOL 100

CX TEM SCAN analytical electron microscope fitted with an EDAX 707 energy dispersive X-ray analyser.

Sections for EDX microanalysis were prepared from tissues processed without osmium tetroxide post-fixation and were examined without staining with either uranyl acetate or lead citrate.

Intracellular and extracellular inclusions were analysed by condensing the electron beam so as to illuminate only the inclusion (target) under study and then recording the spectra for a live time of 200 or 400 seconds. For each target spectrum, a background spectrum was obtained in an adjacent area under the same operating conditions, and this was then subtracted from the target according to the EDIT 7EM programme.

7.4 RESULTS

7.4.1 Cobalt-chrome particles

Electron microscopic examination of the synovium of rat knees one day following the injection of cobalt-chrome alloy particles revealed focal ulceration of the synovial lining, evidenced by loss of synoviocytes and exposure of the subsynovium to the synovial fluid. Cobalt-chrome particles were readily recognizable as electron-dense angular or crystalline bodies, either single or clumped as aggregates. While some particles were visible within macrophages, their location at this interval was mainly extracellular, lying within fibrin and cell debris deposited on the denuded surface of the synovium (Fig.7.1).

While synovial ulceration was still present one week after injection (Fig.7.2), the majority of cobalt-chrome particles were present within macrophages located at the base of the ulcerated zones. The particles

could be seen free in the macrophage cytoplasm or within phagosomes lined by a limiting membrane. Some of the particle laden macrophages exhibited morphological appearances suggestive of stages of degeneration, in the form of focal (Fig.7.3), or extensive loss of cell membrane, swelling of the mitochondria (Fig.7.4), loss of nuclear definition, attenuation of nuclear chromatin, and absence of any recognizable cellular organelle (Fig.7.5). "Rounding off" of macrophages was also a feature at this advanced stage (Fig.7.5). These appearances contrasted markedly with those seen in the synovium of control rat knees injected with serum/saline solution, which showed no ultrastructural abnormalities one week after injection (Fig.7.6).

Four weeks after injection, the synovial surface region showed focal areas of proliferation of type B synoviocytes having extensive dilated rough endoplasmic reticulum cisternae and considerable accumulation of cytolysosomes (Fig.7.7). The type B synoviocytes did not contain cobalt-chrome particles, but were interspersed with viable and degenerating macrophages containing endocytosed particles (Fig.7.7). The subsynovium contained large numbers of macrophages exhibiting intracellular particles and cytolysosomes (Fig.7.8).

EDX microanalysis of the particles within the macrophages, as well as in the extracellular location, confirmed the presence of cobalt and chromium peaks in the particles from specimens obtained at one day, one week and four week intervals after injection, thus attesting to their origin from the injected cobalt-chrome wear particles. A representative spectrum is demonstrated in Fig.7.9.

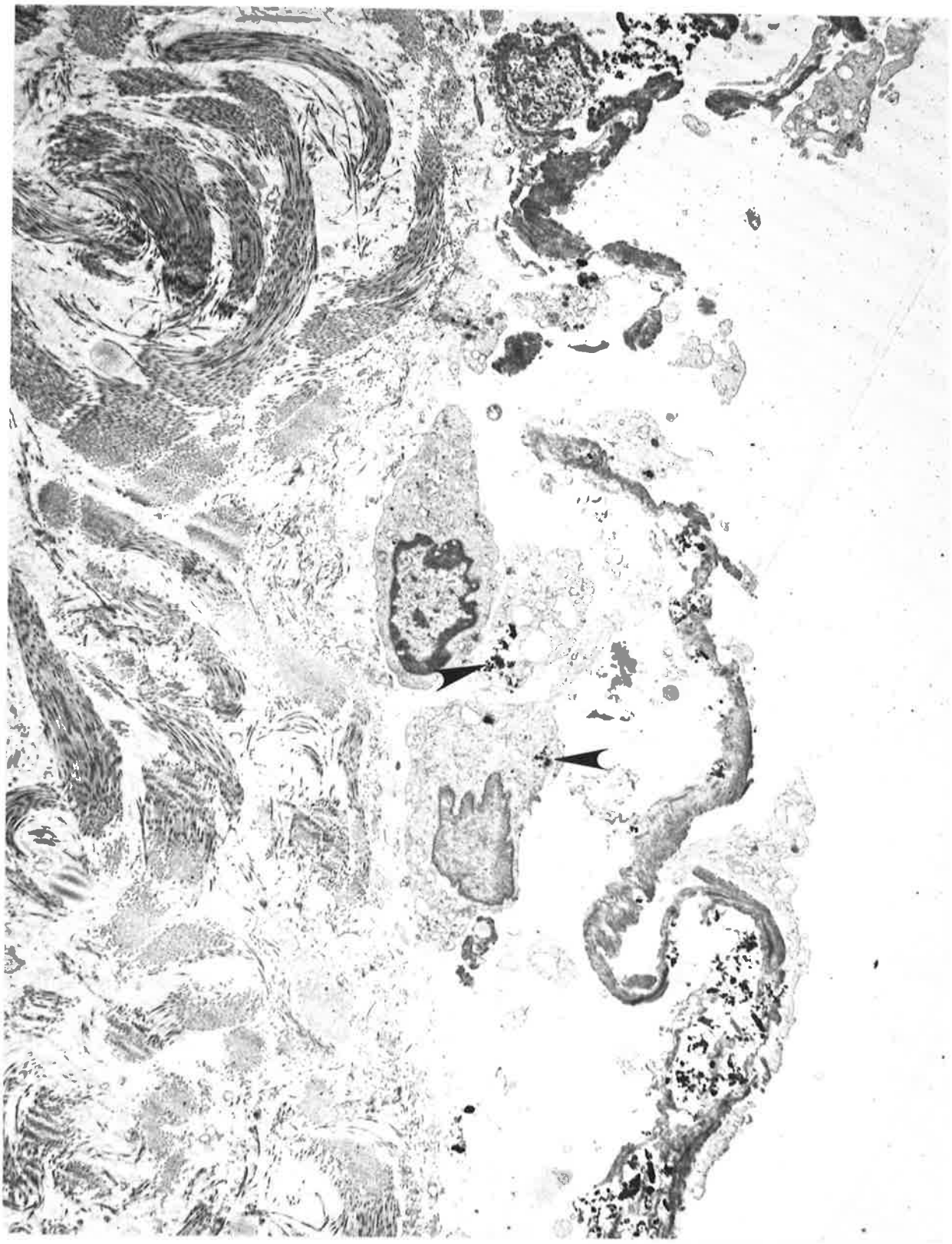

Fig. 7.1. Electron micrograph of the synovium one day following the injection of cobalt-chrome alloy particles. It shows loss of lining cells, sparse intracellular (arrow) and abundant extracellular electron-dense particles, and the surface deposition of fibrin and cell debris. x 5,000

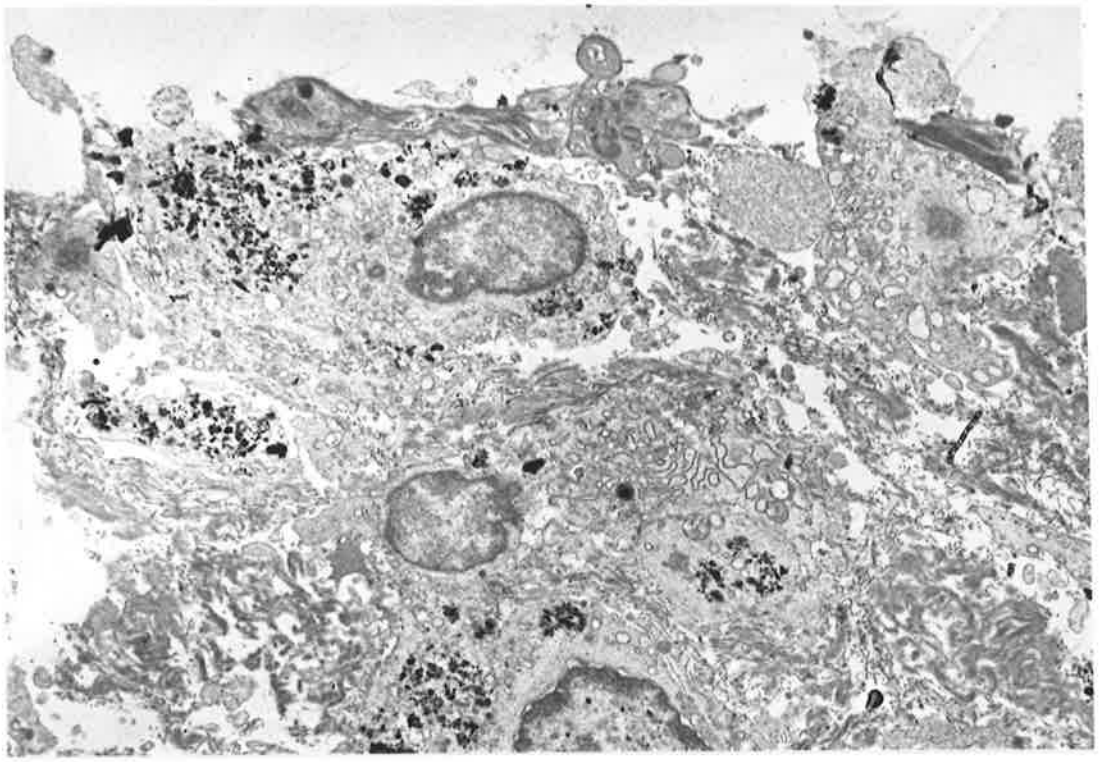

Fig. 7.2. Electron micrograph of the synovium one week following the injection of cobalt-chrome particles. It shows focal loss of the synovial lining cells and the accumulation of abundant electron-dense particles in macrophages. x 5,500

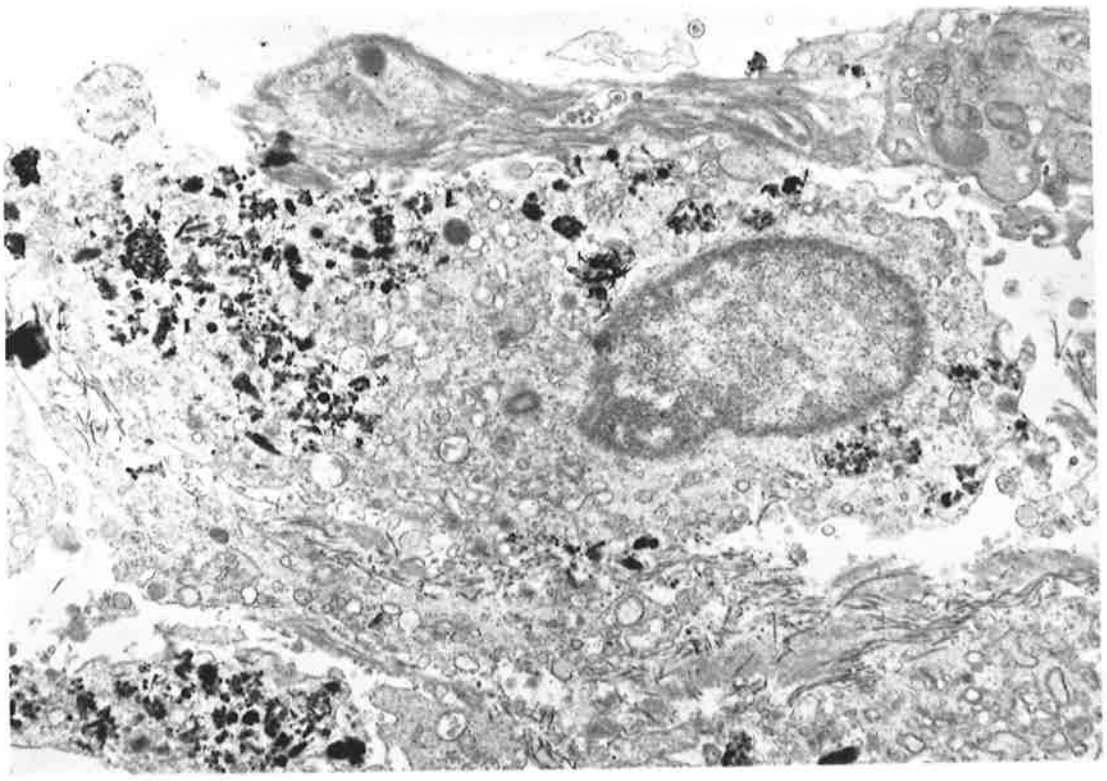

Fig. 7.3. Higher magnification of part of the synovium shown in Figure 7.2. It shows a macrophage which contains abundant electron-dense particulate material exhibiting focal loss of the cell membrane. x 10,000

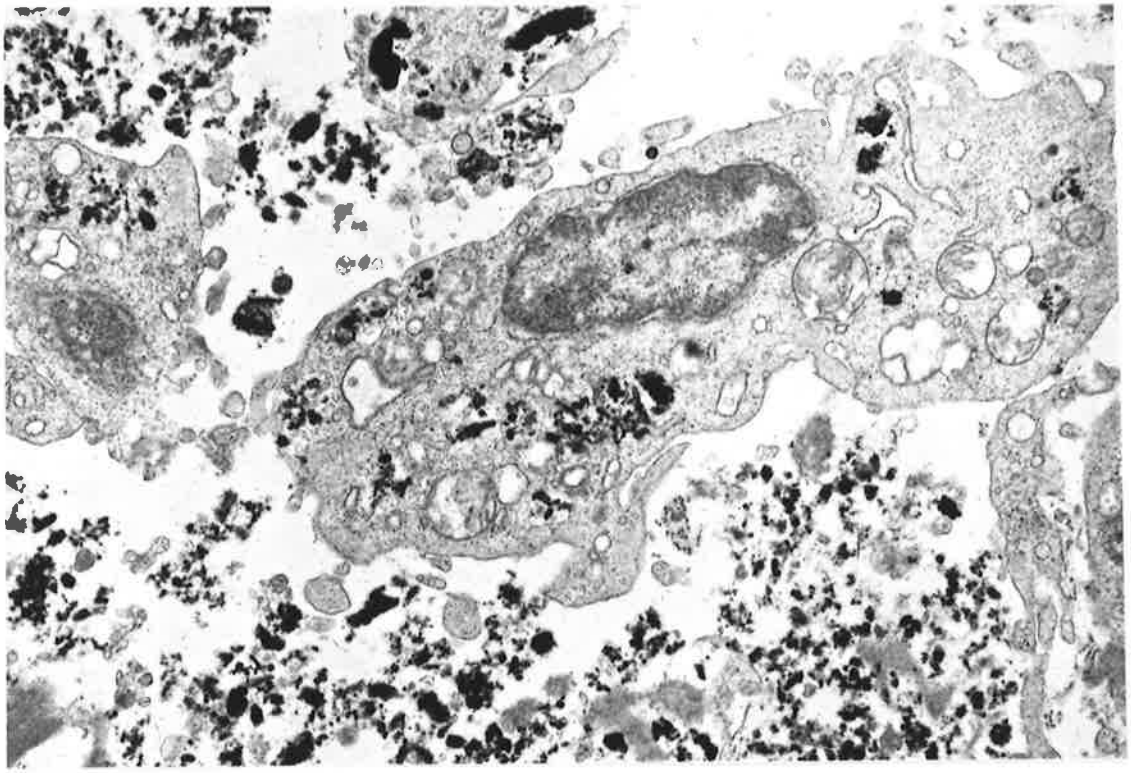

Fig. 7.4. Electron micrograph of the synovium one week following the injection of cobalt-chrome particles. A macrophage exhibits particulate material mostly within phagosomes. Dilatation of mitochondria is also observed. Abundant extracellular particles are present. x 10,000

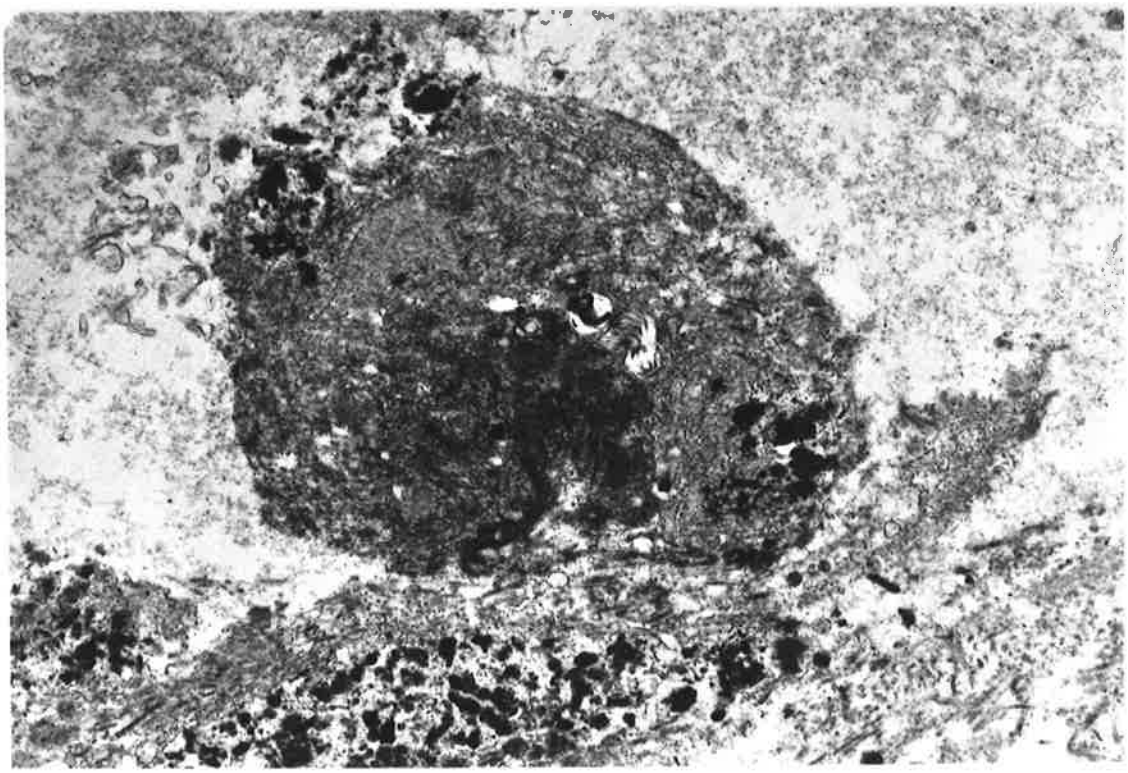

Fig. 7.5. Electron micrograph of the synovium one week following the injection of cobalt-chrome particles. A necrotic cell containing endocytosed particulate material shows a smooth rounded profile with loss of its ruffled border, loss of nuclear definition, and lack of recognizable cellular organelles. x 17,500

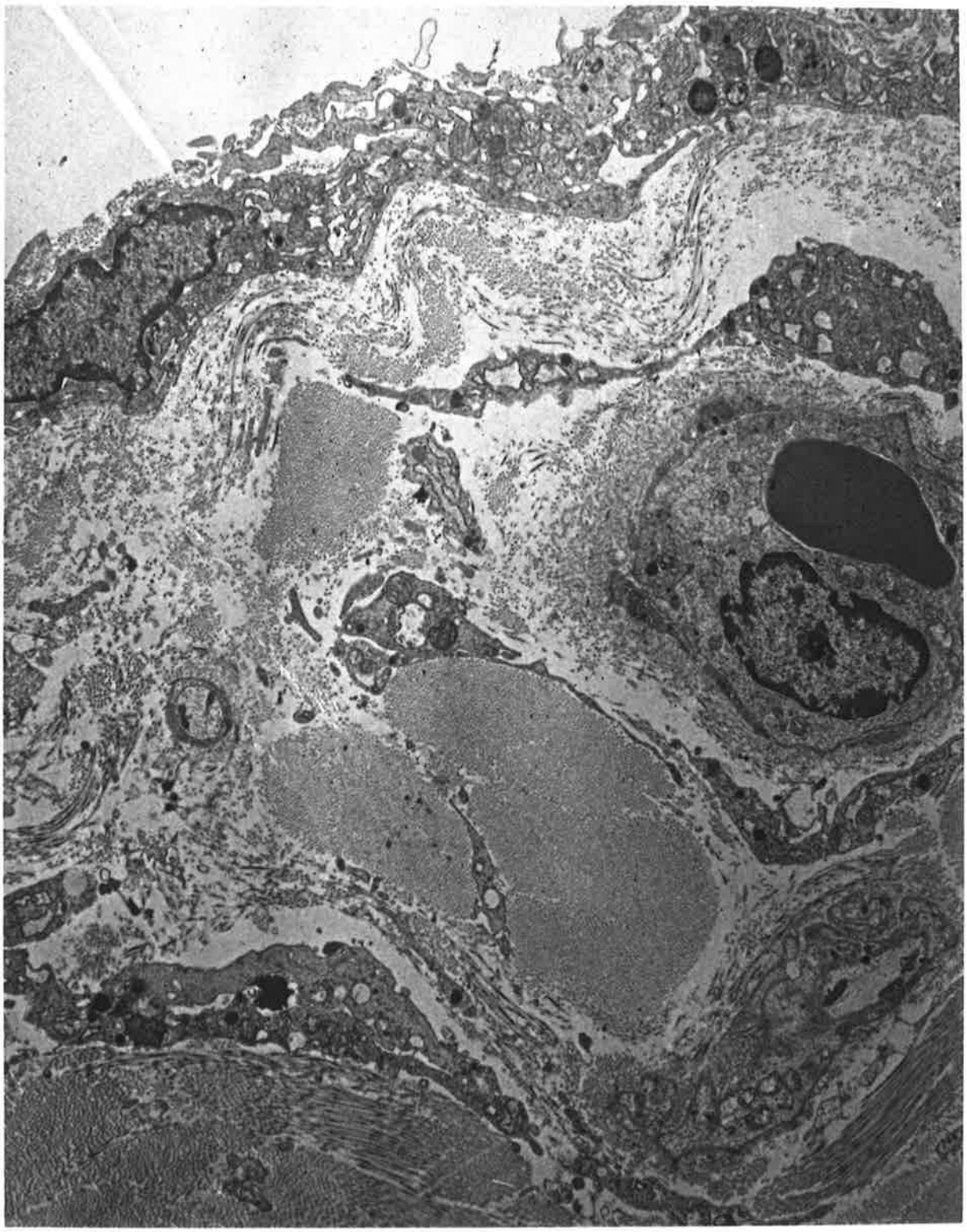

Fig. 7.6. Low-power electron micrograph of the synovium one week following the injection of serum/saline control solution. The synoviocyte lining is intact and the subsynovial fibroblasts show normal features. x 7,000

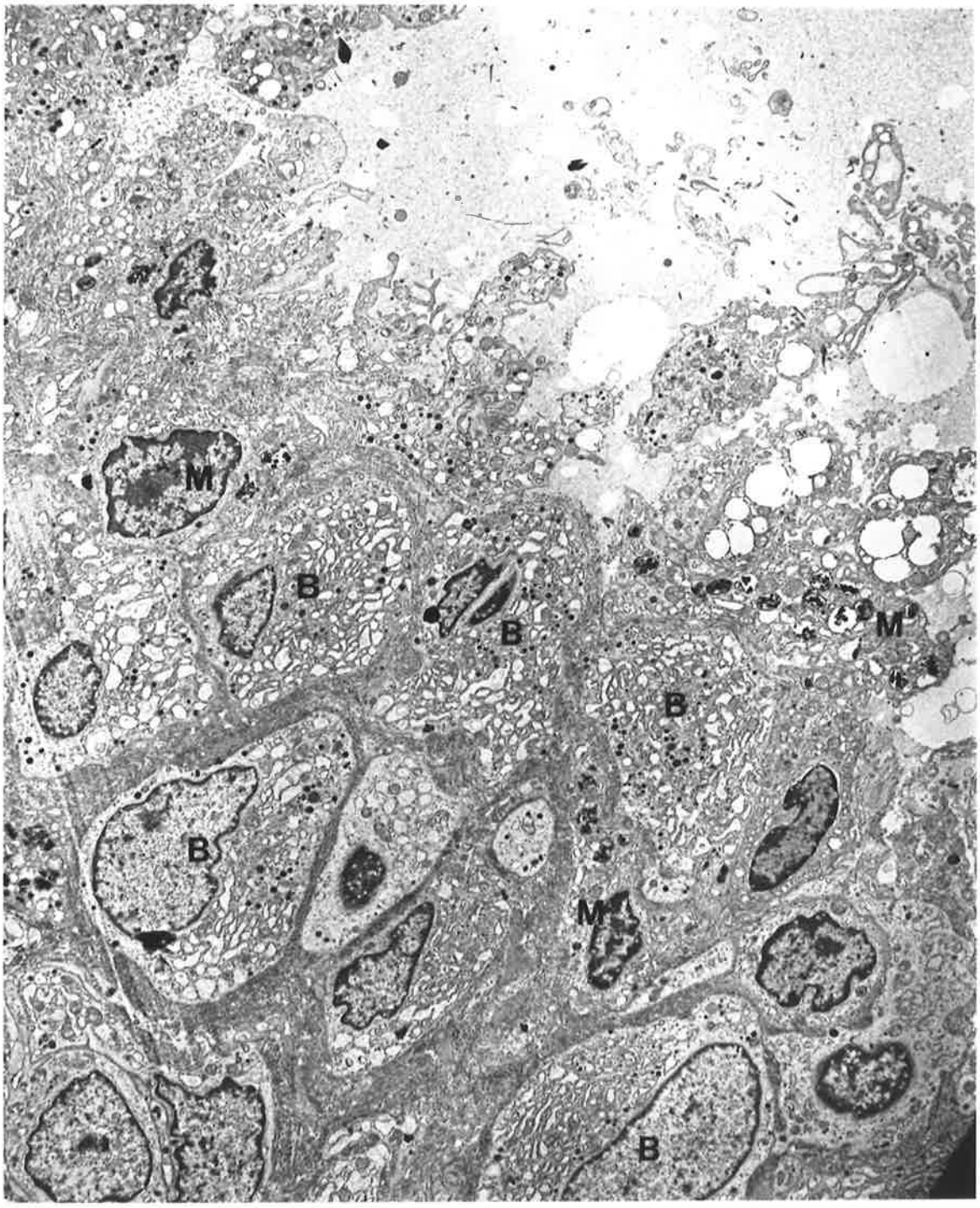

Fig. 7.7. Low-power electron micrograph of the lining zone of the synovium four weeks following the injection of cobalt-chrome alloy particles. It shows loss of cellular continuity in the synovial lining. Macrophages (M) show particulate inclusions contained within phagosomes. Many type B synoviocytes (B) containing large numbers of cytolysosomes and extensive dilated rough endoplasmic reticulum are present, but do not exhibit endocytosed particulate inclusions. x 4,000

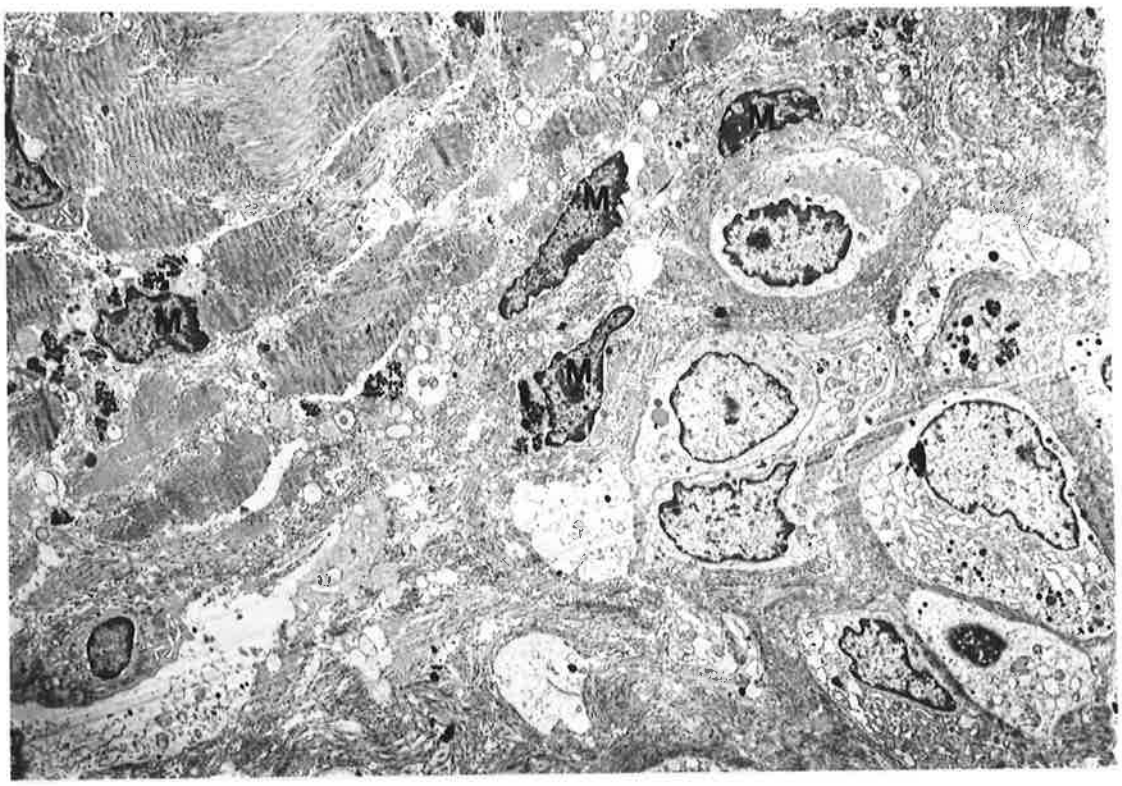

Fig. 7.8. Low-power electron micrograph of the subsynovium four weeks following the injection of cobalt-chrome particles. Elongated macrophages (M) containing endocytosed particles are surrounded by dense collagen. x 3,500

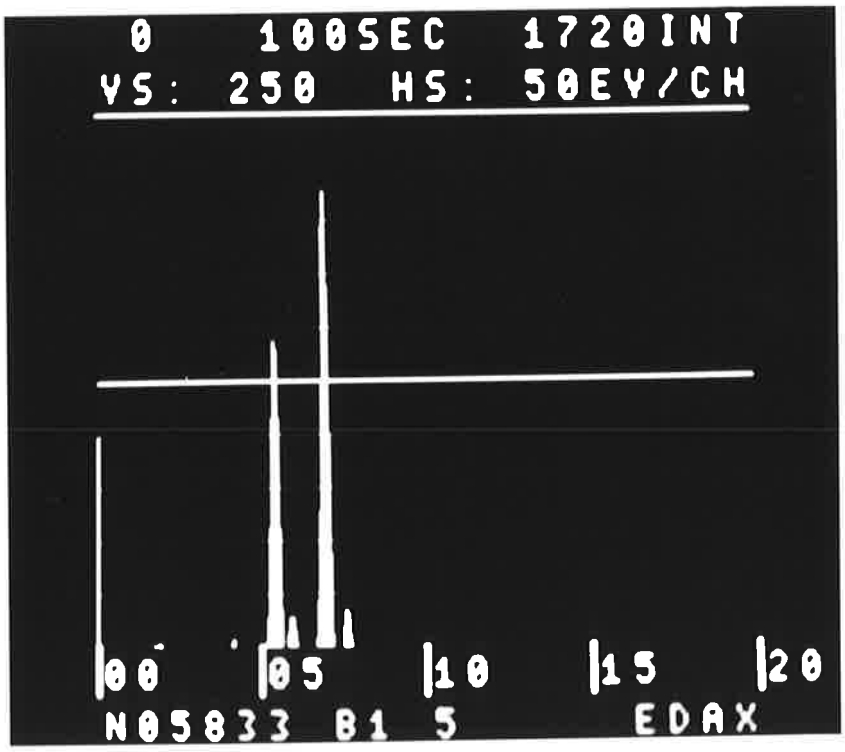

Fig. 7.9. EDX microanalysis of the phagocytosed electron-dense particles seen in macrophages following the injection of cobalt-chrome alloy particles. It shows a peak for chromium at 5.4 KeV and a peak for cobalt at 6.9 KeV.

7.4.2 Aluminium oxide particles

One week after the injection of aluminium oxide particles the synovium showed no evidence of the ulceration seen following the injection of cobalt-chrome particles. Particles were located predominantly within macrophages. While a few macrophages showed dilatation of the rough endoplasmic reticulum and perinuclear cisternae (Fig.7.10), the majority of macrophages showed no abnormal features (Fig.7.11). EDX microanalysis confirmed that the particles within the macrophages contained high peaks of aluminium, thus attesting to their origin from the injected aluminium oxide particles. A representative spectrum is seen in Fig.7.12.

At four weeks following aluminium oxide particle injection, macrophages had accumulated within the subsynovium in association with particles. Necrosis of macrophages was not a feature.

7.4.3 Polyethylene particles

One week after the injection of polyethylene particles, the synovium showed occasional areas of loss of synovial cell lining. Particles were predominantly contained within macrophages in the synovium. Occasional macrophages showed changes suggestive of degeneration in the form of focal loss of cellular membrane and loss of the normal chromatin pattern within the nucleus.

Four weeks following the injection of polyethylene particles the synovium showed focal loss of synoviocytes and, in these areas, degenerate synoviocytes lined the surface (Fig. 7.13). The subsynovium contained large numbers of macrophages, some of which contained particles (Fig. 7.13). MNGC also contained particles, and aggregates of mononuclear macrophages had formed nodular clusters around particles

(Fig. 7.14). Macrophages containing particles did not show obvious evidence of degeneration (7.15). EDX microanalysis did not demonstrate the presence of metal particles.

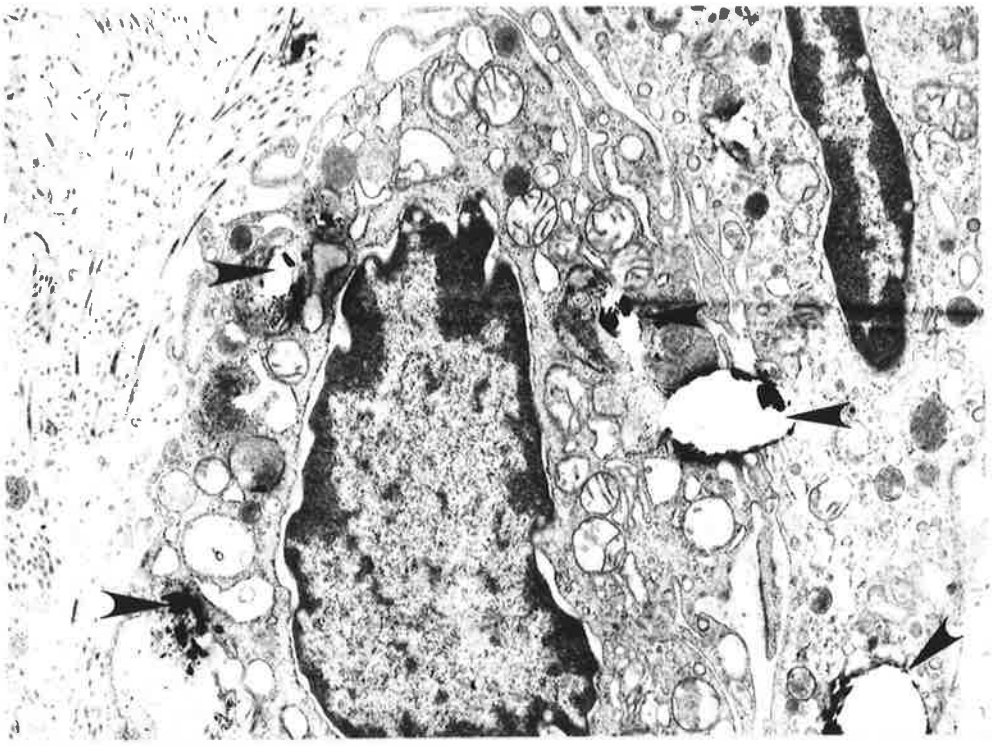

Fig. 7.10. Electron micrograph of the subsynovium one week following the injection of aluminium oxide particles. Macrophages which have endocytosed the particles (largely removed during section cutting, arrows) show some dilatation of the rough endoplasmic reticulum and of the peri-nuclear cisternae. x 13,000

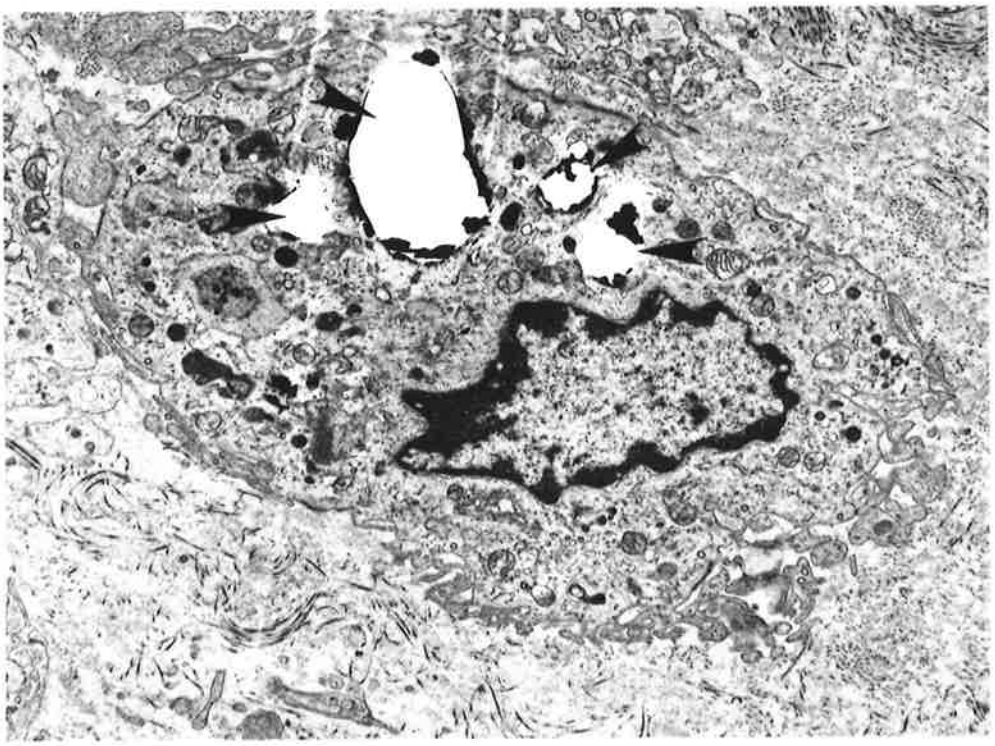

Fig. 7.11. Electron micrograph of the subsynovium one week following the injection of aluminium oxide particles. A macrophage containing endocytosed particles (arrows) shows no evidence of degeneration. x 5,500

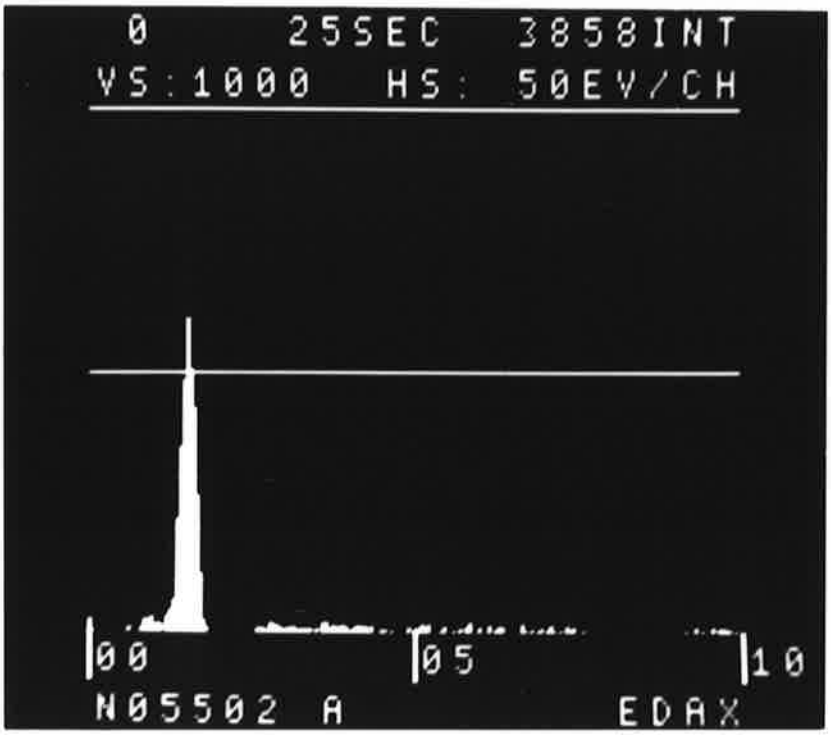

Fig. 7.12. EDX microanaylis of the phagocytosed electron-dense particles seen in macrophages following injection of aluminium oxide ceramic particles. It shows a peak for aluminium at 1.5 KeV.

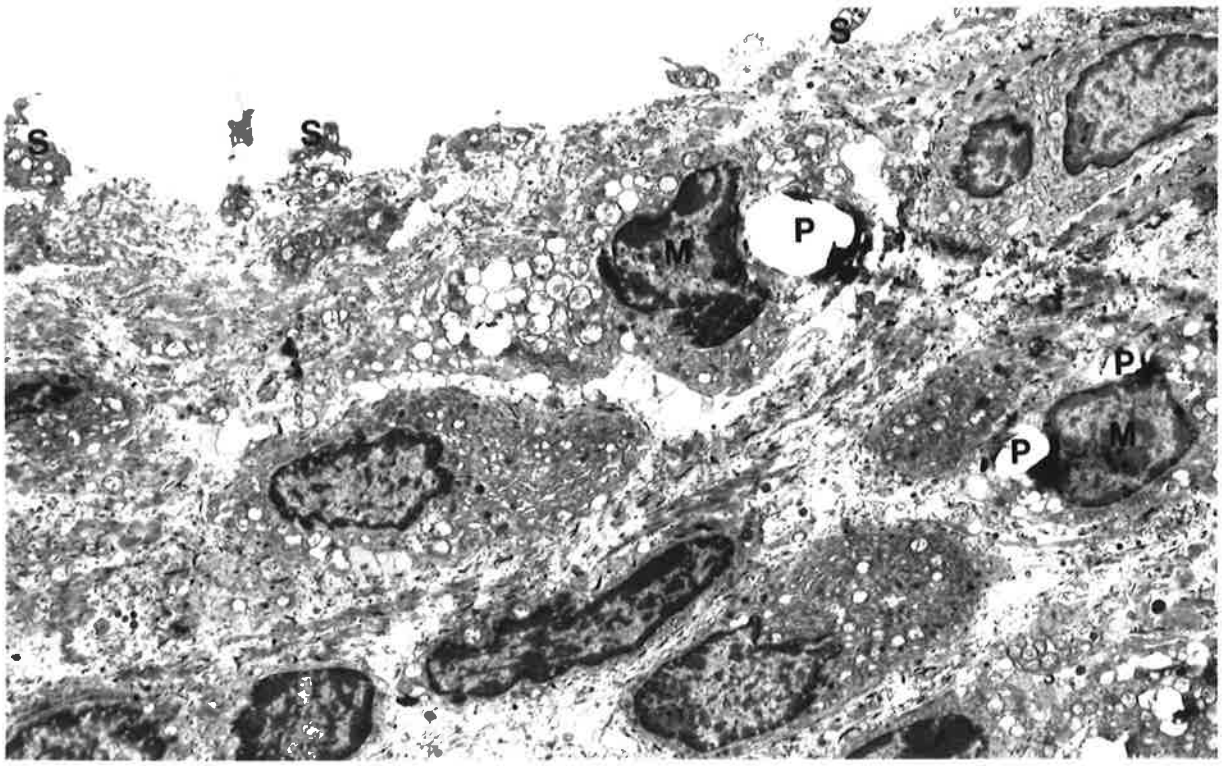

Fig. 7.13. Low power electron micrograph of the lining of the synovium four weeks following the injection of polyethylene particles. It shows remnants of synoviocytes (S) lining the surface and accumulation of macrophages in the subsynovium. Particles (P) have accumulated in macrophages (M). The particles appear as partial or complete voids within the cytoplasm of cells as the particles are dislodged during sectioning. The macrophages do not show degenerative changes. x 4,000.

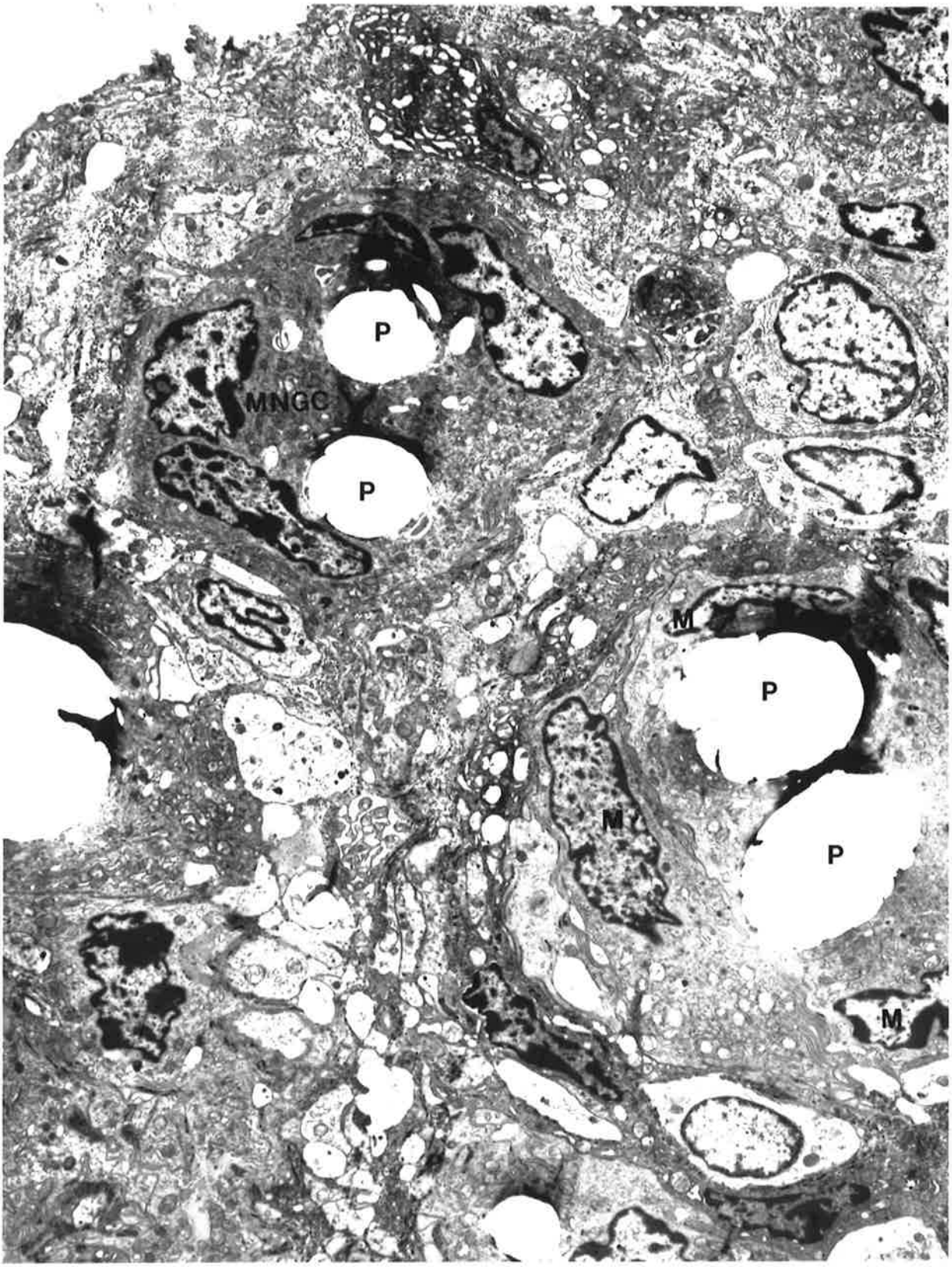

Fig. 7.14. Low power electron micrograph of the synovium and subsynovium four weeks following the injection of polyethylene particles. Within the subsynovium there are two cellular aggregates surrounding particles (P). One aggregate (MNGC) does not show the presence of cellular membranes separating the nuclei, while the other aggregate of nuclei has identifiable cellular membranes and is an aggregate of mononuclear macrophages (M) surrounding particles. x 4,000.

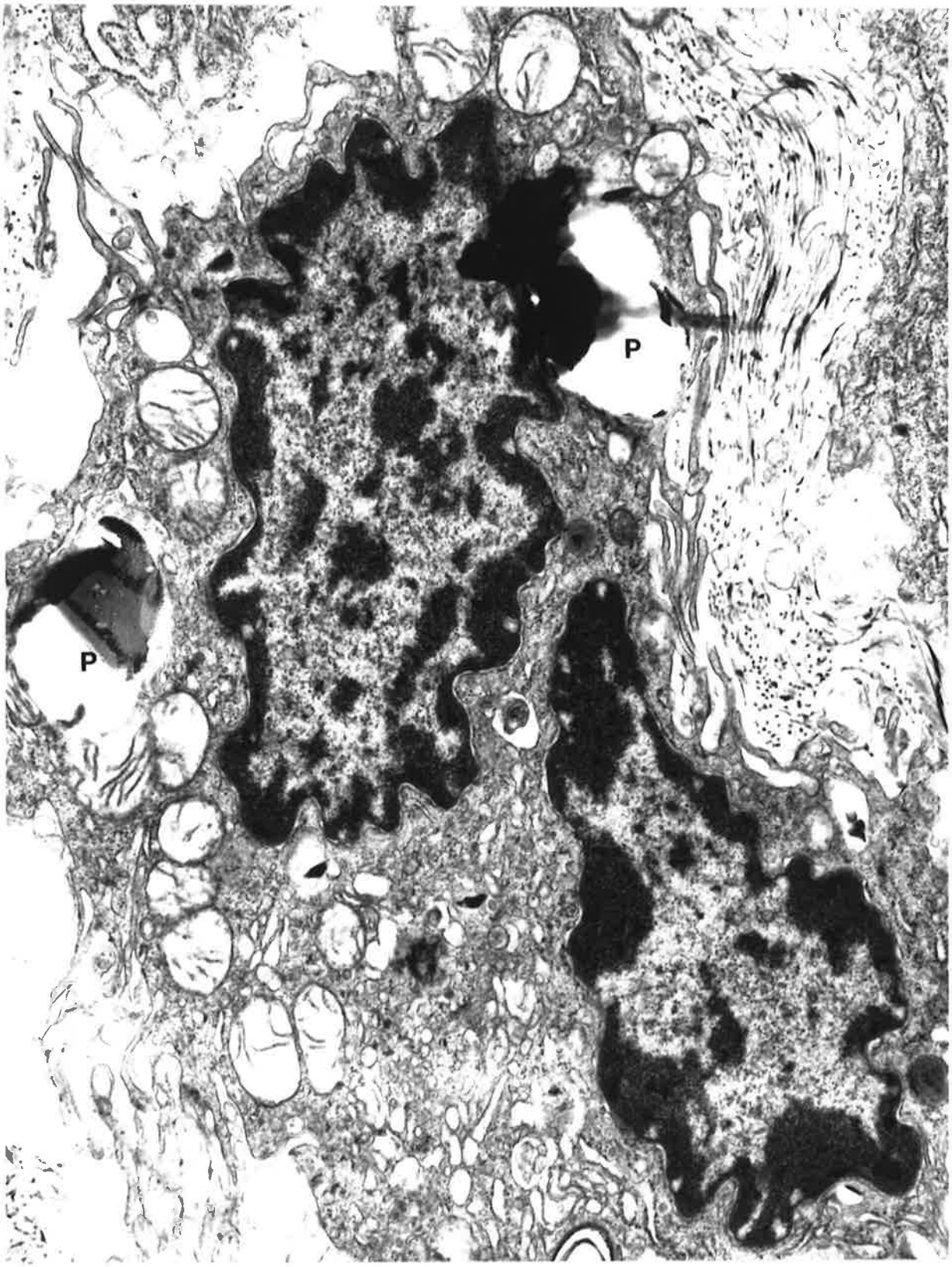

Fig. 7.15. High power electron micrograph of the subsynovium four weeks following polyethylene particle injection. It shows a macrophage containing polyethylene particles (P). The cytoplasm contains mainly mitochondria and endoplasmic reticulum. The cell surface has many filopodia. x 26,500.

7.5 DISCUSSION

Electron microscopic examination of the synovium of rat knees following the injection of cobalt-chrome alloy particles demonstrates that these particles cause varying degrees of degeneration and necrosis of synovial and subsynovial macrophages containing endocytosed particles. Appearances similar to the "rounding up" of degenerate macrophages described following the phagocytosis of cobalt-chrome particles in vitro (Garrett et al, 1983), also were seen on the surface of the synovium in this study. These findings confirm the focal ulceration and necrosis observed in light microscopic studies of rat knee synovium following the intra-articular injection of cobalt-chrome wear particles described in Chapters Three and Four, and in human tissues around cobalt-chrome prostheses described in Chapter Two.

Four weeks following the injection of cobalt-chrome particles, macrophages in the subsynovium showed evidence of increased numbers of cytolysosomes. This finding is consistent with in vitro studies which have demonstrated the release of lysosomal enzymes by macrophages in response to various metal particles (Heath et al, 1969; Rae, 1978, 1986a). Increased lysosomal enzyme activity, as indicated by acid phosphatase and naphthol esterase, also has been described in foci of macrophages and MNGC in association with various types of wear particles in the tissues around human prostheses (Eftekhar et al, 1985).

EDX microanalysis of the intra-cellular particles confirmed the presence of cobalt and chromium in the particles, similar to results obtained when macrophages around human prostheses have been analysed as described in Chapter Two.

By contrast with cobalt-chrome particles, synovial ulceration and marked macrophage degeneration did not occur following the injection of aluminium oxide particles. This is consistent with light microscopic studies showing the absence of necrosis following the intra-articular injection of aluminium oxide in rats knees as described in Chapter Five.

The findings confirm the early cytotoxic effects of cobalt-chrome particles on macrophages, followed later by the presence of apparently healthy macrophages containing endocytosed material. It is not yet clear whether the early corrosion of cobalt-chrome particles within cells, possibly liberating toxic cobalt salts within cells, causes the cytopathic effects observed early after exposure in vitro (Garrett et al, 1983) and observed in vivo in the present studies. Such corrosion could result in a progressive lessening of toxicity of the particles, thereby allowing the accumulation of abundant particles in macrophages which do not exhibit any degenerative features. By contrast, aluminium oxide which is highly resistant to corrosion, has shown minimal cytopathic effects in this study.

After polyethylene particle injection degeneration of lining synoviocytes was occasionally seen. The subsynovium was infiltrated by macrophages and MNGC which had phogocytosed particles. MNGC and nodular aggregates of mononuclear macrophages surrounded large particles. Particles less than about five micrometers in maximum dimension were contained within single macrophages. These findings confirm the light microscopic appearances but also show that aggregates of the larger particles may be surrounded by either nodular aggregates of macrophages or MNGC. Degeneration of macrophages was not a feature of the subsynovial response. The increased numbers of cytolysosomes in the cystoplasm of cells containing cobalt-chrome or aluminium oxide

particles were not observed after the injection of polyethlene particles.

7.6 CONCLUSIONS

Ultra-structural studies of the tissue response following the intra-articular injection of cobalt-chrome alloy, aluminium oxide ceramic, and polyethylene wear particles in rat knees demonstrate that cobalt-chrome particles induce initial necrosis of cells, whereas necrosis was not a feature following aluminium oxide injection. Some weeks following injection of cobalt-chrome and aluminium oxide particles proliferation of type B synoviocytes and macrophages was seen. These cells showed markedly increased numbers of cytolysosomes suggesting increased synthetic activity of cells. Proliferation of macrophages was seen following polyethylene particle injection. Particles exceeding about five micrometers in maximum dimension sometimes were contained within aggregates of macrophages or MNGC.

CHAPTER EIGHT

A RAT MODEL OF BONE RESORPTION AT THE BONE-CEMENT INTERFACE IN THE PRESENCE OF POLYETHYLENE WEAR PARTICLES

8.1 AIM

This study was undertaken to establish whether there was a direct relationship between the tissue response to wear particles and bone resorption.

8.2 INTRODUCTION

It has been widely reported that bone resorption and the presence of a connective tissue layer between bone and cement or bone and prosthesis are coincident features of loose cemented and cementless joint prostheses. Possible causes for this include excessive movement at the interface between bone and the implant, excessive repetitive load, infection, and a foreign-body response to the implant. Metallic and polyethylene wear particles produced at the articulating surfaces of the prosthesis components have been implicated as the probable cause of the macrophage and MNGC response seen in the connective tissue around many failed implants (Charosky et al, 1973; Willert et al, 1974; Mirra et al, 1976; Vernon Roberts and Freeman, 1976), and it has been suggested this tissue response leads to bone resorption around implants (Vernon-Roberts and Freeman, 1977; Willert and Semlitsch, 1977; Revell et al, 1978). A similar tissue response has been seen following severe wear of

other polymers (Charnley, 1963; Charnley et al, 1969; Dahl and Mikkelsen, 1976; Heck and Chandler, 1954).

It still remains to be proven beyond doubt that wear particles alone can cause bone resorption and connective tissue formation leading to loosening of prostheses, despite the circumstantial evidence gained by histopathological study of tissues retrieved during revision of loose prostheses. Thus, a rat model was developed in which a non-weight bearing sterile acrylic cement plug was placed in the distal femur, in continuity with the knee joint, following which polyethylene particles were injected into the knee joint.

8.3 MATERIALS AND METHODS

8.3.1 Technique of cement plug insertion

Mature male J.C. Lewis rats were used throughout. In each of ten rats, PMMA plugs were inserted into cavities drilled in both distal femurs. Under general anaesthetic, the distal articular surface of the femur was approached through a medial arthrotomy of the knee and a cavity, one centimetre long and 1.1 millimetre in diameter, was drilled into the shaft via the intercondylar notch, using a stainless steel high speed drill. The cavities were then irrigated with sterile saline. Pre-formed acrylic cement plugs were then inserted into each cavity so that the distal end of the plug lay just below the level of the articular surface of the femoral condyle (Fig. 8.1). The capsule was sutured with chromic catgut and the skin with nylon.

The PMMA plugs were made by injecting orthopaedic acrylic cement (Zimmer, U.S.A.), which was prepared sterile by mixing monomer and

polymer, into the lumen of sterile gavage feeding tubes having an internal diameter of 1.1 millimetres. When the acrylic had hardened, plugs were cut six millimetres long and the outer plastic tubing was removed. The acrylic plugs were autoclaved and stored in sterile saline.

8.3.2 Polyethylene particle injection

Polyethylene particles were prepared by dispersion of one mg of powdered UHMWP particles (Howmedica) in fifteen ml of a solution of J.C. Lewis rat serum which had been diluted one in fifty in normal saline. The solution was sterilised by autoclaving. The polyethylene particles were between fifteen and 200 micrometers in maximum dimension and irregularly shaped. Control solution was sterile serum diluted in saline. The sterility of the particle suspension and control solution was checked by aerobic and anaerobic culture.

Five of the ten animals received no injections and were sacrificed at two weeks and the knees and distal femurs were excised and processed as described below. The other five animals had polyethylene particles injected into one knee and control solution into the opposite knee (Fig. 8.2). Intra-articular injections were performed at two, four, six and eight weeks after implantation of the acrylic plug and the animals were sacrificed two weeks after the last injection (Fig. 8.3).

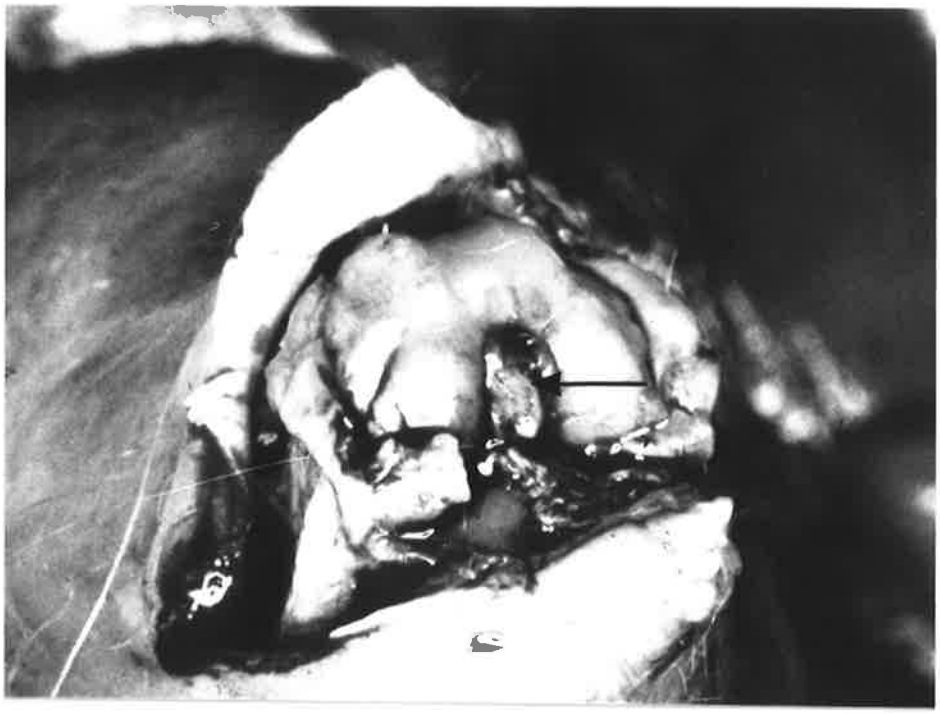

Fig. 8.1. Photograph of the rat knee joint opened to display the site of insertion of the methylmethacrylate plug in the distal femur (arrow). x 5

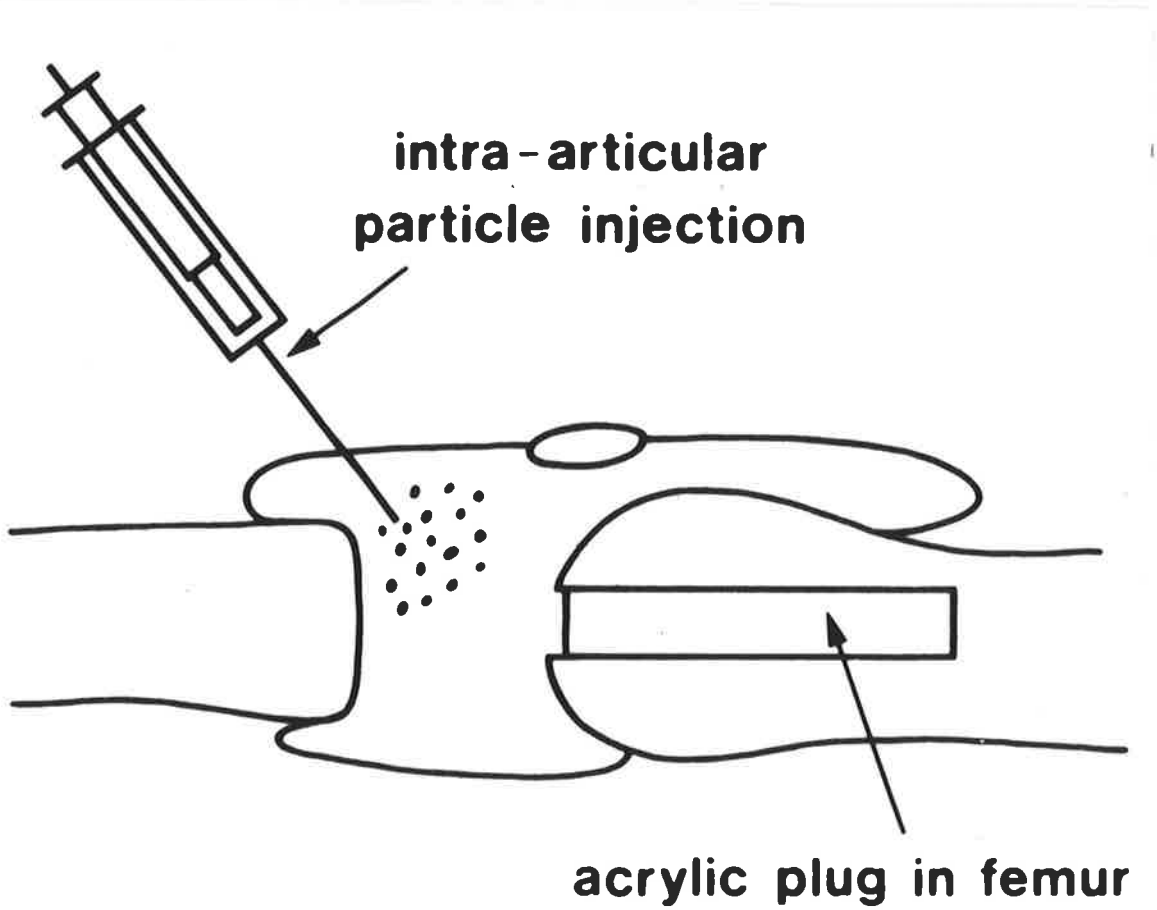

Fig. 8.2. Diagram illustrating the site of injection of particles into a knee joint adjacent to a previously inserted acrylic plug.

8.3.3 Processing of tissue for histopathology

At sacrifice the femur and knee joint were removed en bloc and fixed in formal saline for three days. During this procedure the PMMA plug dissolved at the chloroform stage of processing.

Transverse and sagittal sections of the distal femur and knee joint were cut. Within each group of animals, both femurs of three animals had sections six micrometers thick cut transversely at 100 micrometer intervals, beginning distally at the knee joint. In two animals the sections were cut in a sagittal plane across the femur. Sections were stained with hematoxylin and eosin.

Without knowledge of how the animals were treated, sections were initially examined microscopically under non-polarized light. Since polyethylene particles are not visible under direct light this ensured that interpretation of the features at the interface between the acrylic plug and the bone was not subject to bias. The sections were then examined under polarized light to study the distribution of polyethylene particles.

8.4 RESULTS

In the femurs of uninjected animals, a complete shell of new bone had formed around the acrylic plug by two weeks after implantation (Figs. 8.4, and 8.5). Between the acrylic plug and bone there was a very thin and incomplete layer of amorphous tissue (Fig. 8.6), and there was no evidence of bone resorption. Identical findings were observed in the animals which had control solution injected into their knees (Figs. 8.7 and 8.8).

In the femurs of animals injected with polyethylene particles there was active bone resorption around the implant. In proximity to the joint, at the interface between the implant and the bone, bone was replaced by cellular connective tissue composed of macrophages, MNGC and fibrous tissue (Fig. 8.9). The connective tissue contained unstained voids (Fig. 8.10) which, when examined under polarized light, were seen to contain highly birefringent polyethylene particles (Fig. 8.11 and 8.12). A highly cellular connective tissue layer was seen some distance from the knee joint and osteoclasts were present on the surface of bone (Fig. 8.13). These findings are represented diagrammatically in Figure 8.14.

In the uninjected animals and animals receiving the control solution, the synovium was normal. In the knees injected with polyethylene particles, the synovium and subsynovium were thickened and infiltrated with large numbers of macrophages and MNGC containing polyethylene particles.

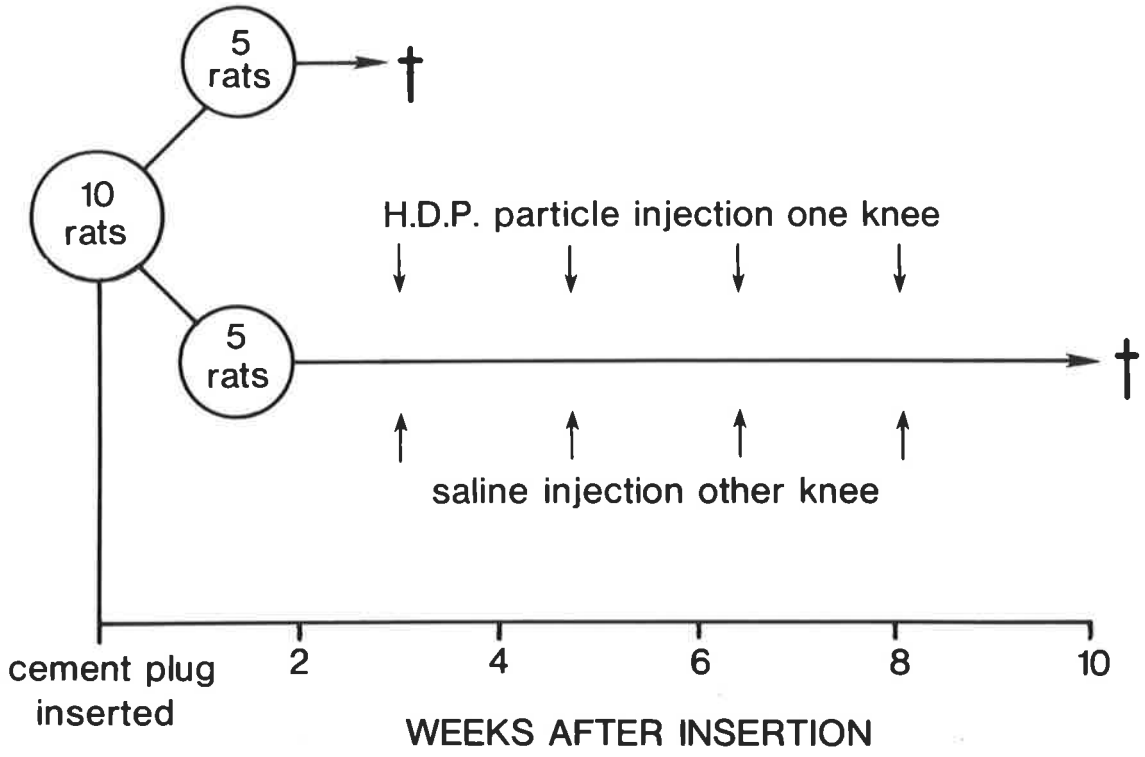

Fig. 8.3. Flow chart demonstrating sacrifice of five uninjected rats at two weeks, and sacrifice of the other five rats following injection with high density polyethylene (H.D.P.) particles and control serum/saline solution.

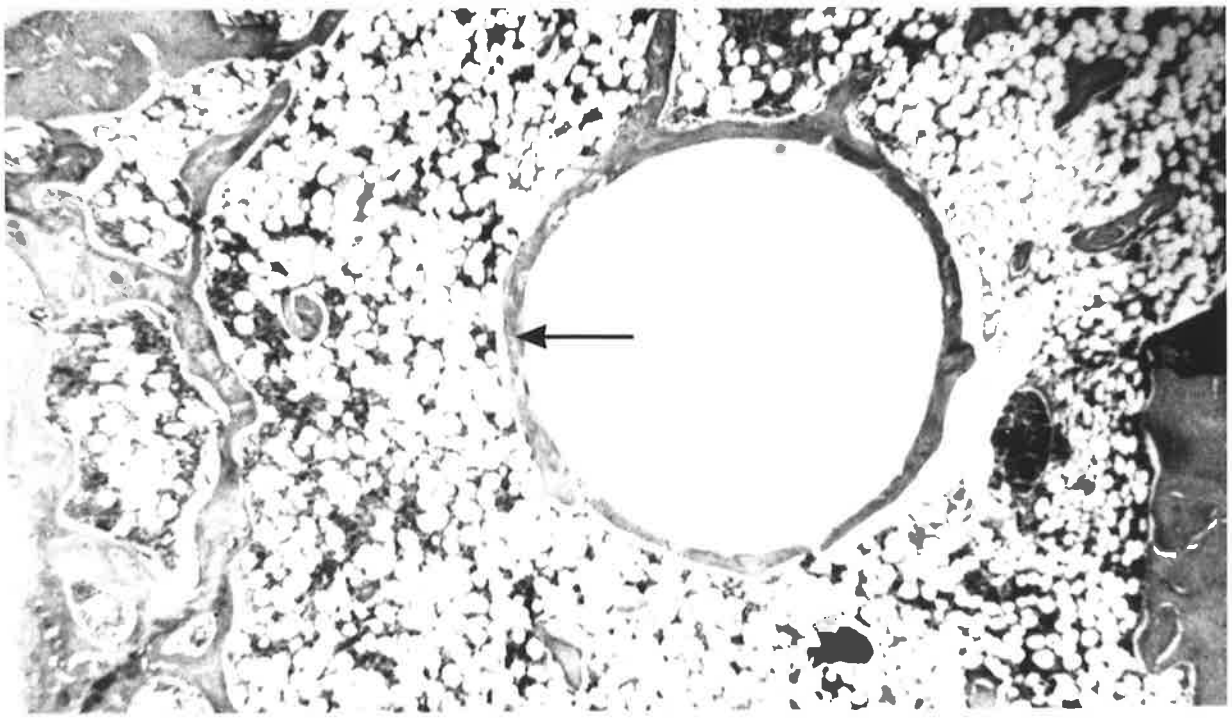

Fig. 8.4. A transverse section of the femur of an uninjected control rat two weeks after plug implantation. It shows the complete bone shell (arrow) surrounding the dissolved acrylic plug and absence of a significant connective tissue layer between the acrylic plug and bone. HE x 15

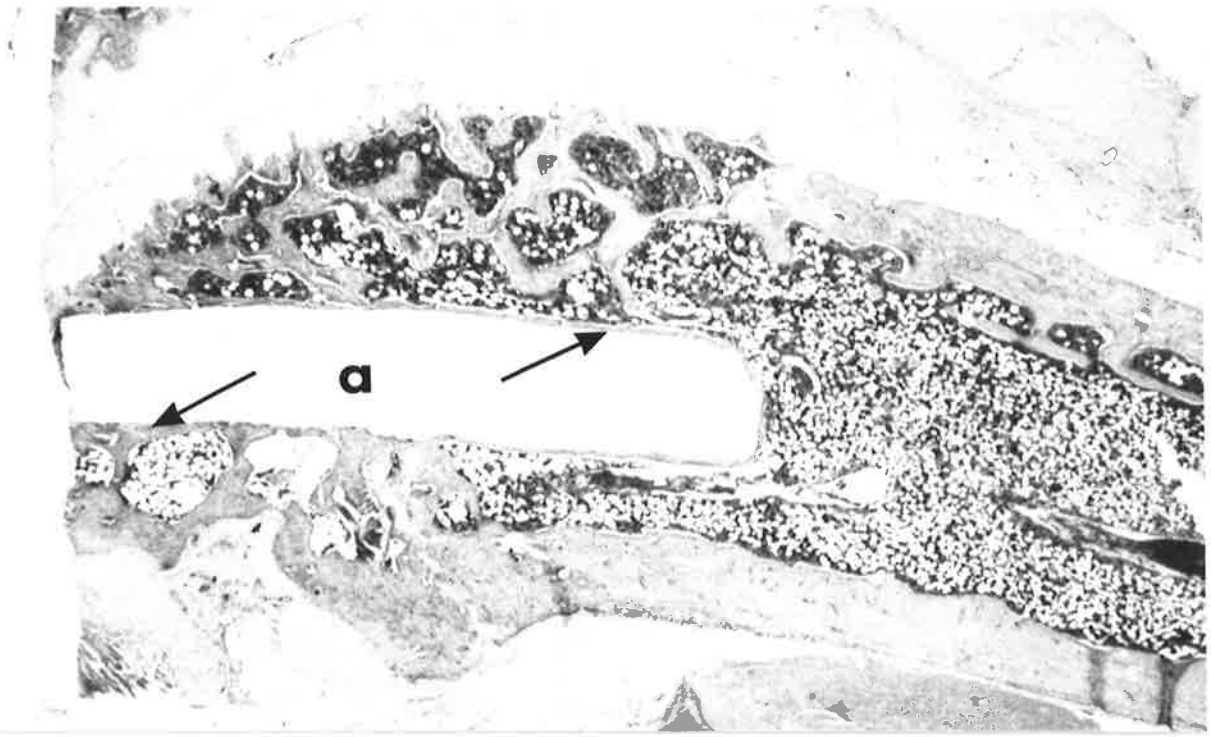

Fig. 8.5. A sagittal section of the femur showing formation of a complete bone shell (arrows) surrounding the site of the dissolved acrylic plug (a) inserted two weeks prior to sacrifice. HE x 15

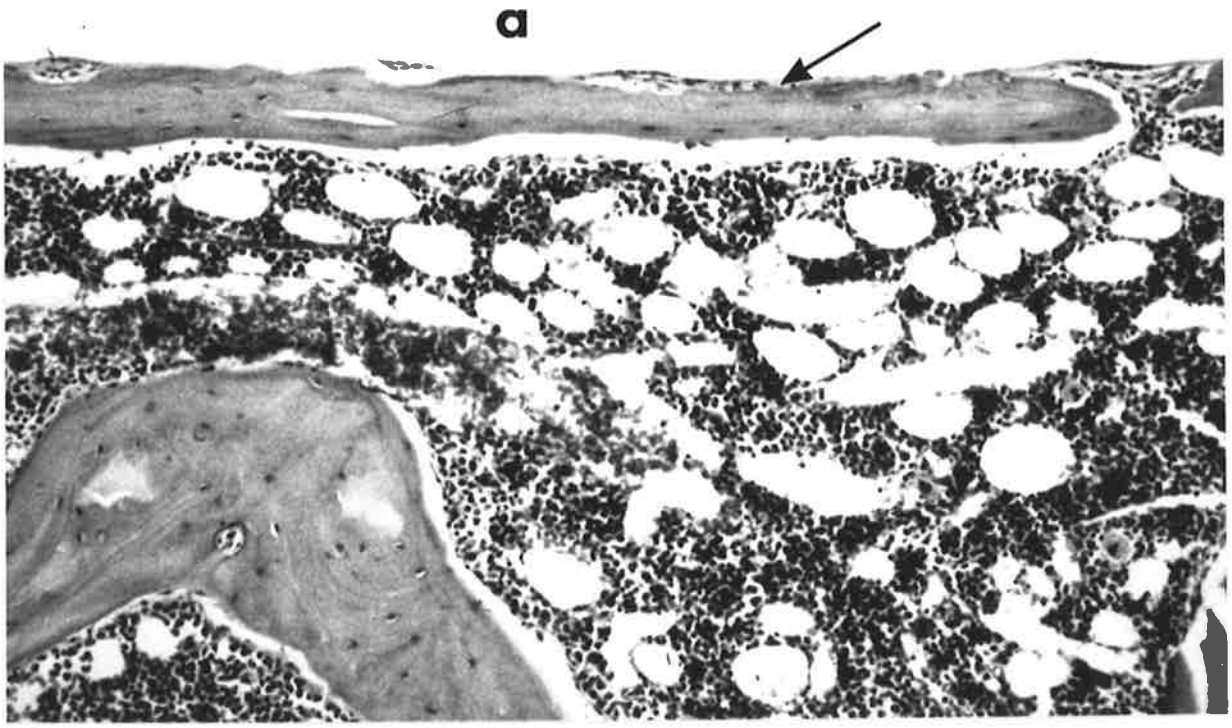

Fig. 8.6. The incomplete thin amorphous tissue layer (arrow) at the interface between the dissolved acrylic plug (a) and the bone. HE x 100

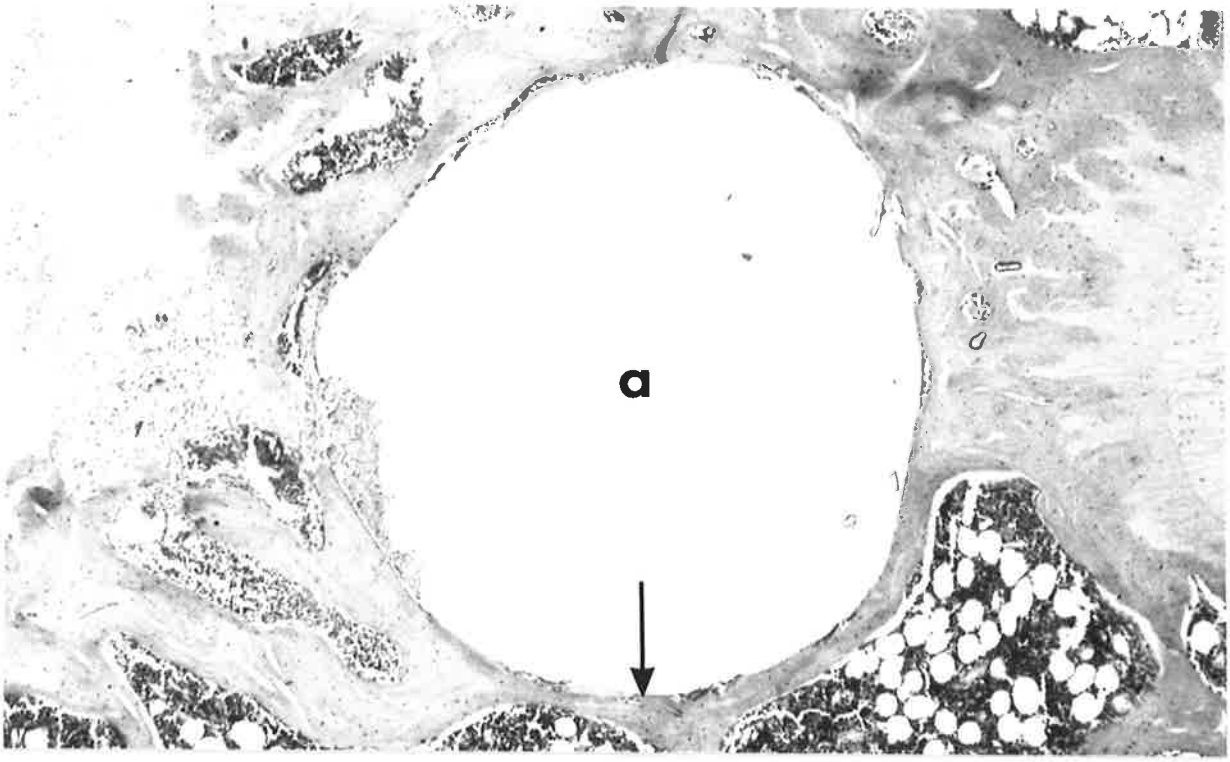

Fig. 8.7. A transverse section of a rat femur close to a knee joint injected with control solution. A complete bone shell (arrow) persists around the dissolved acrylic plug (a) ten weeks after implantation. HE x 80

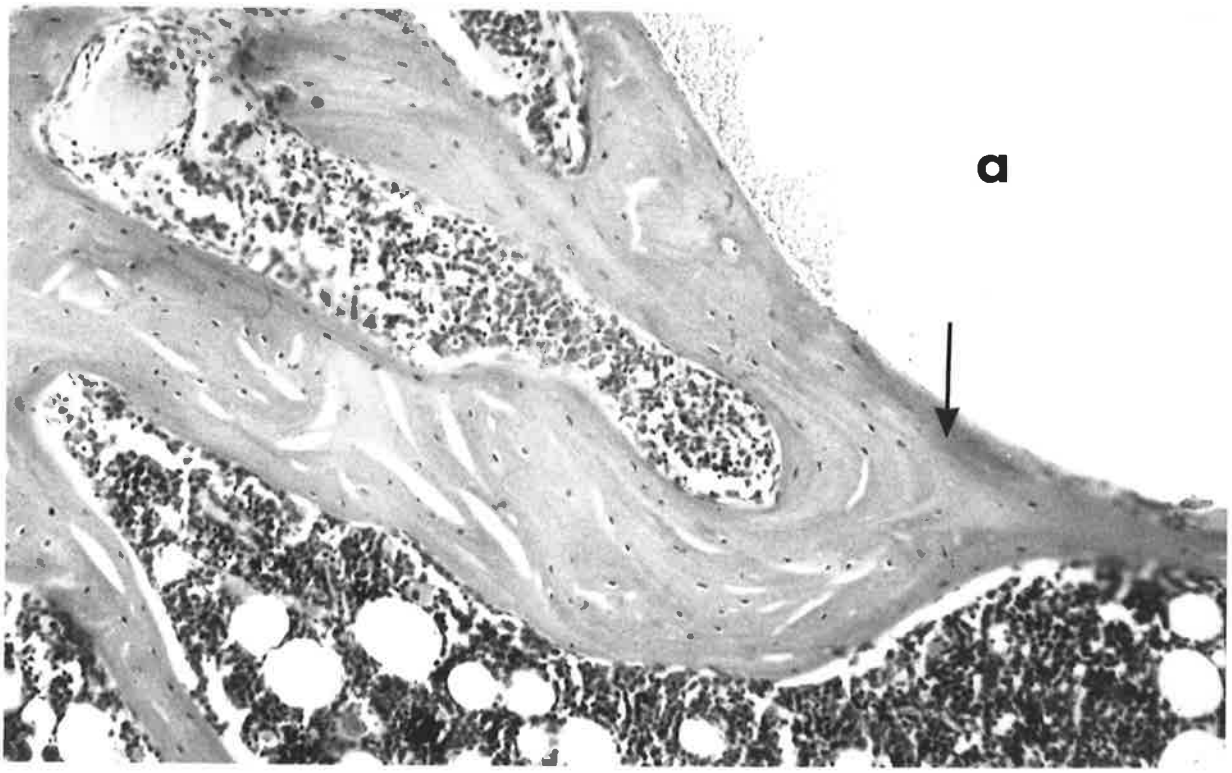

Fig. 8.8. High power photomicrograph of the same section as Figure 8.7 showing the absence of a connective tissue layer between the acrylic plug (a) and bone (arrow). HE x 160

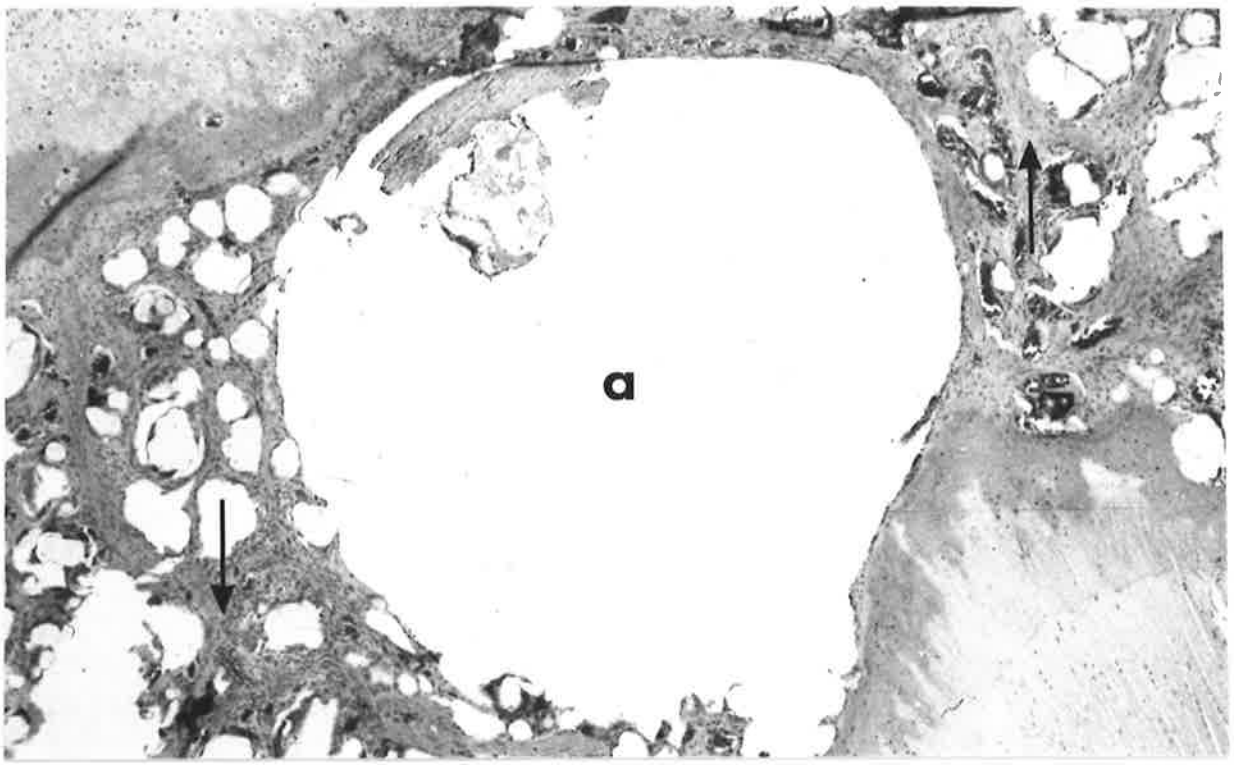

Fig. 8.9. A transverse section of a rat femur near a knee joint injected with polyethylene particles. Bone around the dissolved acrylic plug (a) has been replaced be cellular tissue (arrow). HE x 80

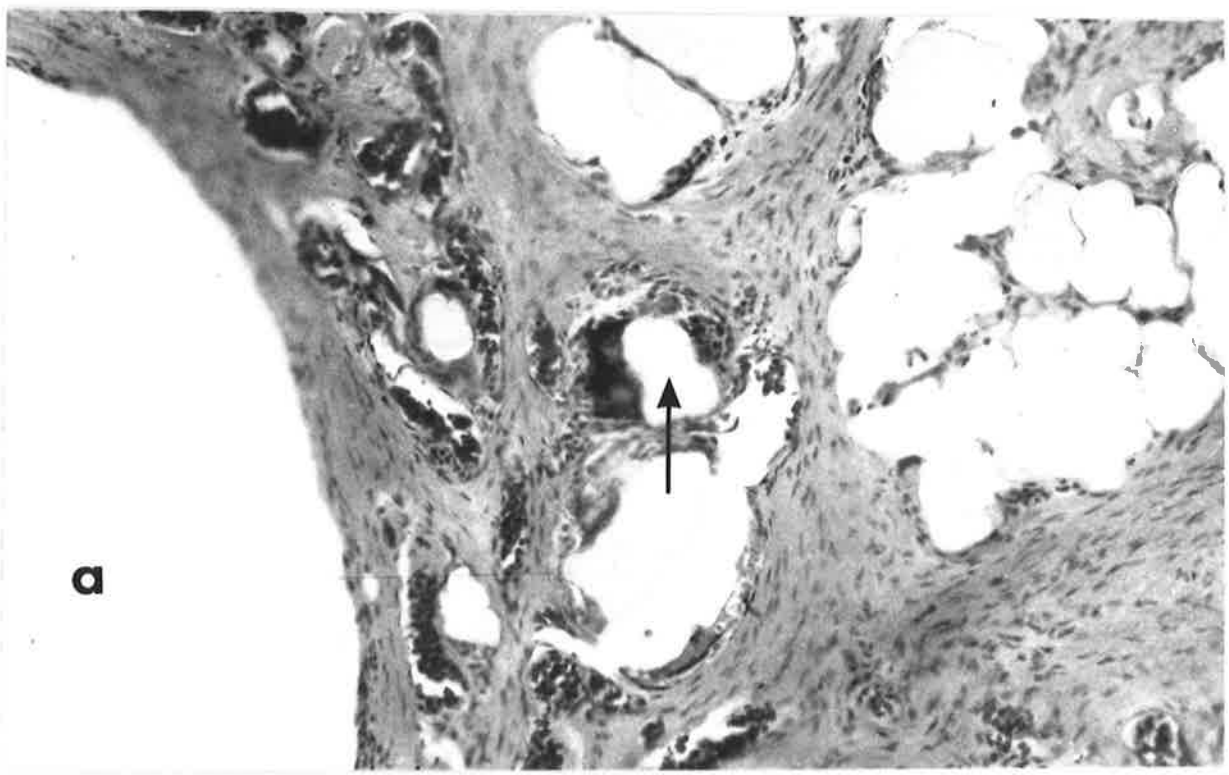

Fig. 8.10. High power photomicrograph of the same section as Figure 8.9 showing voids (arrow) in the connective tissue which has replaced bone around the acrylic plug (a). HE x 160

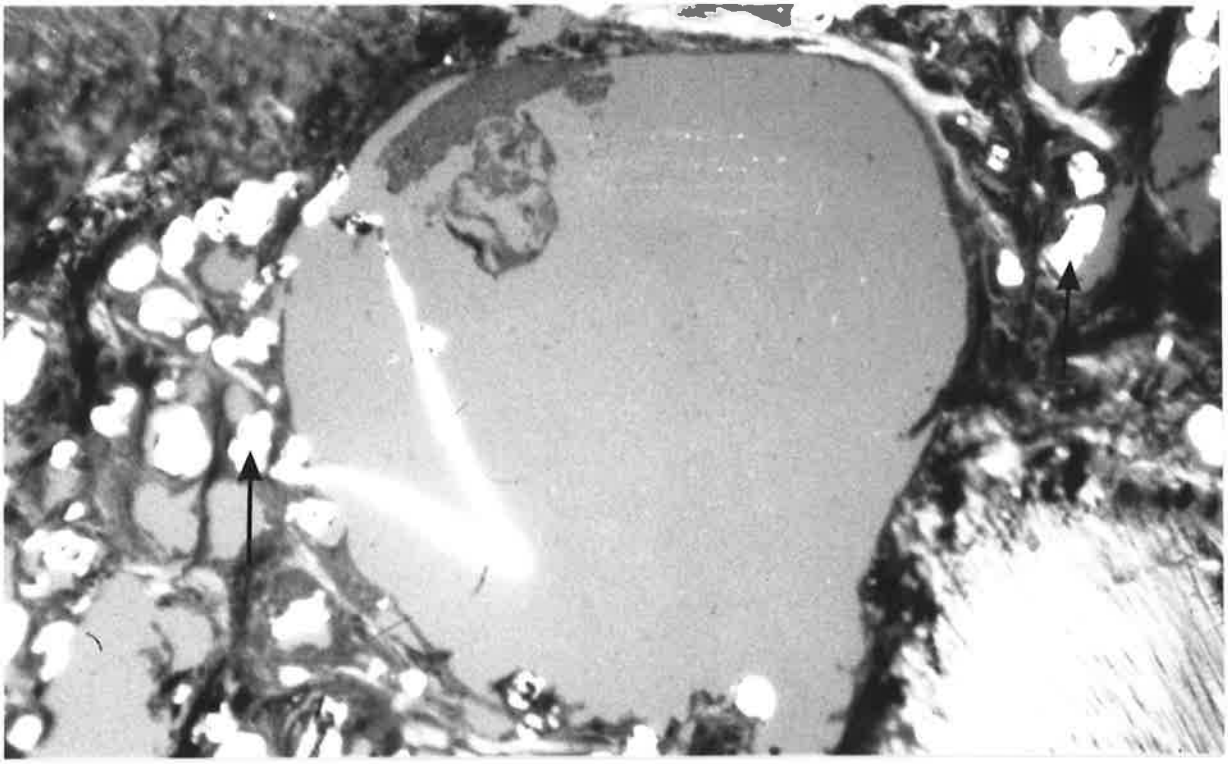

Fig. 8.11. The same section as Figure 8.10 examined under polarised light demonstrating that, in the highly cellular tissue which has replaced bone, the voids contain highly birefringent polyethylene particles (arrow). HE x 80

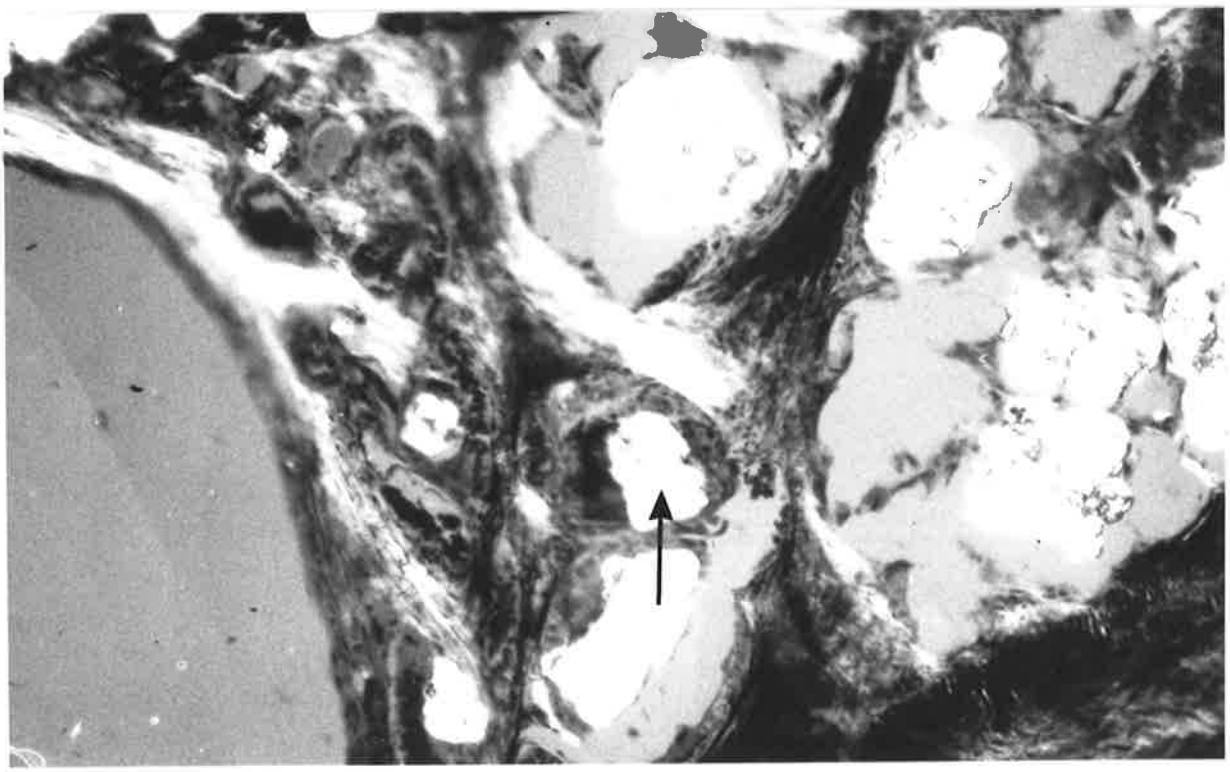

Fig. 8.12. High power photomicrograph of the same section as Figure 8.10 showing highly birefringent polyethylene particles (arrow) surrounded by giant cells. HE x 160

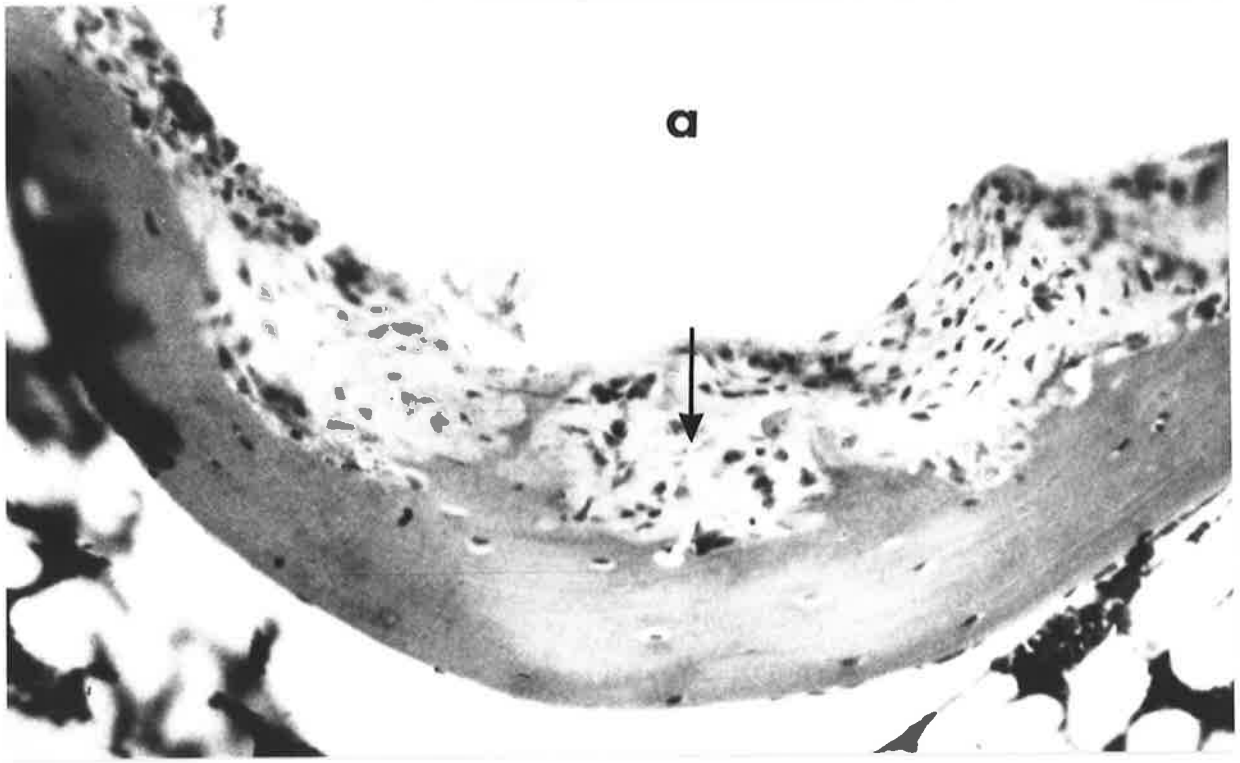

Fig. 8.13. A transverse section of a distal rat femur five millimetres from a knee joint injected with polyethylene particles. Highly cellular connective tissue (arrow) has formed between the dissolved acrylic plug (a) and the surrounding bone, and osteoclasts are present between this tissue and the bone. HE x 320

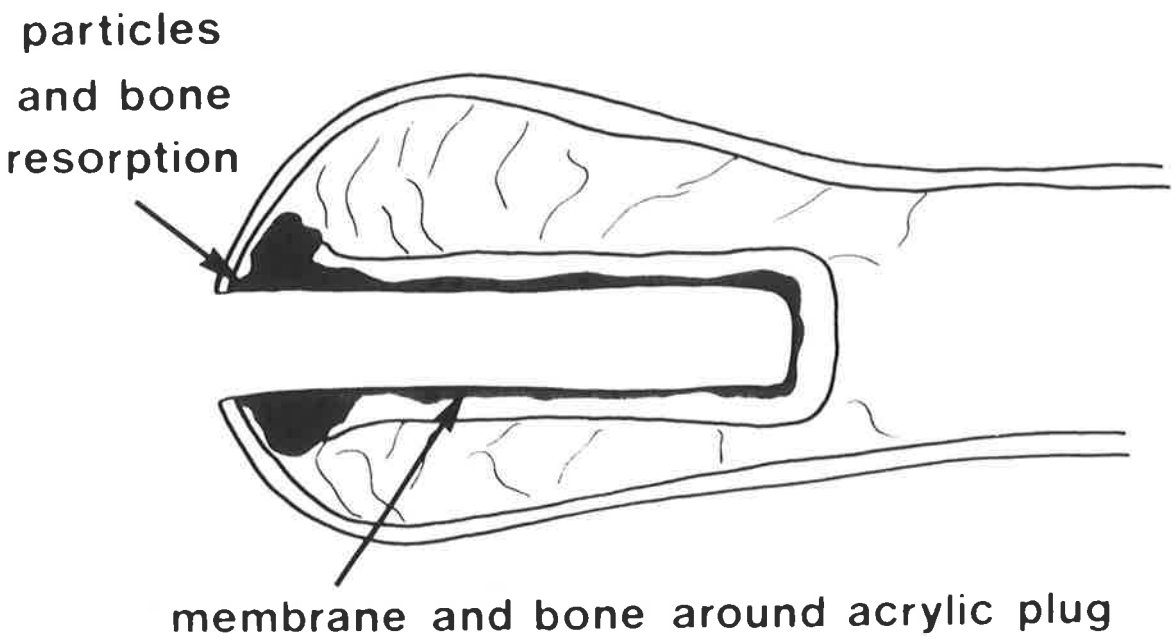

Fig. 8.14. Diagrammatic representation of a sagittal section of the rat femur showing bone resorption and formation of a connective tissue "membrane" at the interface between acrylic cement and bone in response to polyethylene particle injections into the adjacent joint.

8.5 DISCUSSION

This study demonstrates that bone resorption and the formation of connective tissue at the interface between acrylic cement and resorbing bone is associated with a macrophage and MNGC response to polyethylene particles injected into an adjacent joint.

In the initial control animals which had the acrylic plug inserted and no injections, a well developed bone envelope formed around the acrylic plug within two weeks. The more rapid development of this bone envelope than would be expected in humans presumably was due to a number of factors such as: the lack of major trauma during bone preparation, the absence of the possible adverse effects of heat of polymerization of the acrylic and initial monomer release, the lack of movement at the bone-cement interface due to the absence of loading, and rapid bone healing in the rat.

Inserting the acrylic cement plug as a preformed plug does not exactly simulate the insertion of cement in the dough form, as performed in joint replacement surgery. For the purposes of this study, preformed plugs offered an advantage as the plugs were of uniform thickness, and the possible deleterious effects on the bone due to heat of polymerization of cement and excessive monomer release were avoided. The shell of bone around the plug was established by two weeks after implantation, and as the intra-articular injection of particles was not commenced until after this time, it seems reasonable to assume that bone resorption occurred in the particle injected animals after a stable interface between bone and cement had been established.

All surgery was performed using aseptic techniques, and the acrylic plug and injected solutions were sterilized by autoclaving and the sterility

checked prior to injection. Taking into account the fact that PMN were not present in the tissue, it was concluded that bone resorption and connective tissue formation at the stable bone-cement interface was due to the injection of polyethylene particles.

The polyethylene particles used in this study were approximately fifteen to 200 micrometers in diameter, whereas the polyethylene wear particles seen in the tissues around failed prostheses are of varying size, ranging from less than one micrometer to over 200 micrometers. The size of the particles seems related to the type and severity of wear (Rose et al, 1978). Newman and Scales (1951) concluded that large polyethylene particles would provoke a predominantly MNGC response, as seen in the tissues around failed cup hemi-arthroplasties and in this study, whereas small particles, as seen in tissues around low friction arthroplasties, would provoke a predominantly macrophage response.

In this study, large numbers of polyethylene particles were injected over a short period of time. The number, size or concentration of particles necessary to cause bone resorption remains unknown. Wear of polyethylene prostheses always occurs but the severity of wear varies according to the type of prosthesis and possible three-body wear due to interposition of fragments of acrylic cement. It appears that wear particles normally are drained from the site of joint prostheses via lymphatic channels (Walker and Bullough, 1973; Vernon-Roberts and Freeman, 1977), and it is possible an equilibrium may be reached between the production of particles and their removal (Willert and Semlitsch, 1977).

That macrophages and MNGC appearing in response to wear particles participate in bone resorption around implants, directly or indirectly, requires consideration. While there is previous evidence that

macrophages may be involved in bone resorption in vitro (Mundy et al, 1977; Kahn et al, 1978; Teitelbaum, 1979), current concepts do not strongly favour a role in bone resorption in vivo for cells other than osteoclasts (Chambers, 1985). Thus, it would appear likely that macrophages and MNGC which have taken up wear particles, release soluble factors which stimulate osteoclasts. Such factors may include prostaglandins (Klein and Raisz, 1970; Galasko and Bennett, 1976), and osteoclast activating factors such as interleukin-1 (Gowen et al, 1983).

This study further emphasizes the importance of the cellular response to wear particles in bone resorption at the bone-cement interface, taking into account the fact that, while this has been suspected from the pathology studies of failed human prostheses which have undergone severe wear, it has not been demonstrated previously in a situation where mechanical causes of loosening and infection have been excluded. These conclusions have implications for the choice of materials, implant design, and techniques of implantation of prostheses, which should aim to minimize component wear.

8.6 CONCLUSIONS

The intra-articular injection of polyethylene wear particles was associated with the formation of a connective tissue layer and with bone resorption at the bone-cement interface around non-weightbearing cement plugs.

The findings complement those of previous studies which have demonstrated a macrophage response to polyethylene wear particles, and the association of this tissue response with bone resorption at the bone-cement interface.

CHAPTER NINE

CONCLUSIONS

9.1 CONCLUSIONS AND SUMMARY

The aim of this study was to determine the effect of wear particles released from the articulating surfaces of prostheses on the periprosthetic tissues, and to ascertain whether the tissue response contributed to bone resorption and prosthetic loosening.

In order to establish the types of wear particles and associated tissue response around human prostheses, the periprosthetic tissues around forty-seven total hip arthroplasties with aseptic loosening of one or more components were examined by light microscopy, TEM, and EDX microanalysis. The tissues around cementless metal on bone and ceramic on ceramic prostheses contained very few wear particles and few macrophages. The tissues around cementless and cemented metal on metal prostheses contained large numbers of metal particles and large numbers of macrophages and occasional MNGC. The tissues around cemented metal on polyethylene prostheses often contained large numbers of small and large polyethylene particles, variable numbers of cement particles, and occasional metal particles. Large numbers of macrophages and MNGC were frequently seen in these tissues. Lymphocytes were occasionally seen in association with metal particles. PMN were rarely seen.

Ultrastructural studies of these tissues confirmed the phagocytosis of submicroscopic wear particles by macrophages. Varying degrees of degenerative change in macrophages were seen in association with

phagocytosis of metal particles. Large numbers of cytolysosomes were seen in cells in association with the accumulation of wear particles.

An animal model was developed to determine the effects of wear particles on tissues in the absence of other factors which might influence the appearance of the tissues around human prostheses. Thus, the intra-articular effects of cobalt-chrome alloy wear particles similar in size to those present in the tissues around total joint prostheses were studied by injecting laboratory prepared particles into the rat knee joint. The particle size was determined by differential sedimentation and the amount of particulate injected was determined by atomic absorption spectroscopy. The particles were prepared in a solution of dilute serum/saline to prevent aggregation of particles. Particles of cobalt-chrome rapidly induced a proliferation of macrophages and focal degeneration of synovial tissues similar to the response seen in the articular tissues around loose total joint prostheses in humans. Bacteriological tests excluded infection as a cause of the tissue changes observed. These findings support the hypothesis that wear particles are responsible for the changes in the tissues around total joint prostheses. The findings also indicate that this animal model offers a method of testing the biocompatibility of materials used in the manufacture of total joint prostheses.

Further studies of the long-term effects of cobalt-chrome alloy wear particles were performed by the intra-articular injection of particles into rat knees, and the sacrifice of animals at periods from one week to two years following injection. The initial response was synovial ulceration, macrophage infiltration and necrosis. A transient lymphocyte response was present at one week. Fibrosis of the subsynovium occurred subsequently at the sites of necrosis. A semi-

quantitative assessment of the number of particles and macrophages in the synovium demonstrated that the extent of synovial particles did not alter during the period from one week to one year. The extent of the macrophage response decreased between one and two weeks and then remained constant for one year. These findings demonstrate that the extent of the cellular response to injection of particles can be determined within two weeks, and little useful information regarding the extent of the cellular response can be gained from studies which extend beyond this period. Post-mortem examination did not demonstrate the development of any tumours. This study emphasizes that wear particles and the associated macrophage response persist in the tissues for up to two years. The findings are relevant to the effects of wear particles around human prostheses which continuously release particles into the periprosthetic tissues where they may accumulate.

The effects of different amounts of wear particles of the same material and wear particles of different materials were then examined. The tissue response to intra-articular injection in rat knees of similar concentrations of cobalt-chrome alloy and aluminium oxide ceramic wear particles, and two different concentrations of cobalt-chrome particles was assessed at one, four and thirteen weeks. A semi-quantitative method of histological assessment showed a correlation between the numbers of particles and the macrophage response in the tissues, and a difference in the response to different concentrations of cobalt-chrome particles. There was a difference between the response to particles of different materials. At one week, the macrophage response to cobalt-chrome particles was significantly greater than to aluminium oxide particles, but no difference was detected at four and thirteen weeks. The difference may be due to necrosis of macrophages induced by cobalt-chrome particles.

Further studies of the effect of wear particles commonly released from the articulating surfaces of prostheses were undertaken using UHMWP particles prepared in a joint simulator. Single and multiple injections of sterile particles into rat knees caused synovial proliferation and induced a macrophage and MNGC response in the subsynovium. Of particular interest, was the macrophage response to particles ranging from fifteen micrometers in maximum dimension to the limits of resolution of the light microscope, and the MNGC response to larger particles and aggregates of small particles. These findings emphasize the importance of wear particles in causing an adverse tissue response around joint arthroplasties with polyethylene components.

Samples of synovial tissue retrieved from rat knees at periods up to four weeks after the injection of cobalt-chrome alloy, aluminium oxide, and polyethylene wear particles were examined by electron microscopy and EDX microanalysis. Cobalt-chrome particles induced early synovial ulceration and degenerative changes in macrophages containing the particles, but healthy macrophages containing abundant particles had accumulated in the subsynovium at one month. By contrast, aluminium oxide particles and polyethylene particles induced only minor degenerative changes in the macrophages. EDX microanalysis confirmed the elemental composition of the intracellular particles of cobalt-chrome and aluminium oxide. Following phagocytosis of cobalt-chrome and aluminium oxide particles, the accumulation of macrophages containing large numbers of cytolysosomes was seen. The findings suggest that these particles induce increased synthetic activity by macrophages, with lysosomal enzymes possibly having an adverse effect on the periprosthetic tissues. The accumulation of macrophages also was seen following polyethylene particle injection. Particles approximately five micrometers and less were contained within mononuclear macrophages,

whereas larger particles were surrounded by aggregates of macrophages or contained within MNGC.

Having established a direct relationship between wear particles and the tissue response seen around joint prostheses, an animal model was developed to test the hypothesis that the tissue response to wear particles contributed to prosthetic loosening. Bone resorption and formation of a connective tissue layer at the interface between bone and cement was induced by polyethylene particles similar in size to those present in the tissues surrounding human joint prostheses.

A non-weightbearing PMMA plug was inserted into the distal femur of rats via the knee joint, and rapidly became surrounded by a shell of bone. Following repeated injections of polyethylene particles into the knee joint, bone resorption occurred at this stable interface. No bone resorption occurred following injections into the opposite knee of control preparations not containing particles. The bone resorption which occurred around the plug after polyethylene particle injection into the knee took place in the absence of mechanical causes for loosening and in the absence of infection.

This study establishes a direct relationship between polyethylene wear particle accumulation and subsequent bone resorption. The clinical importance of this study is that the incidence of loosening of prostheses may be decreased by strategies which aim to minimize component wear by improvements in design of prostheses, choice of materials, and techniques of insertion of prostheses.

In summary, the studies described in this thesis establish a direct relationship between wear particles from the articulating surfaces of prostheses and the tissue response observed in the periprosthetic

tissues around human joint prostheses. The effect of wear particles on tissues is related to the extent and duration of particulate material in the tissue, and the type and size of particles. Particles of different materials induce varying degrees of necrosis of tissues, and proliferation of macrophages and increased lysosomal activity of macrophages. Large particles and aggregates of smaller particles produce a MNGC response. The presence of wear particles is associated with the formation of a connective tissue layer at the bone-implant interface and bone resorption around solidly fixed implants, the characteristic findings prior to and following loosening of joint prostheses.

9.2 DIRECTIONS FOR FUTURE STUDY

The results of this series of investigations have provided widely based morphological evidence to support the role of wear particles in causing stimulation of a macrophage response in the periprosthetic tissues, and have demonstrated a relationship between this tissue response and bone resorption at the bone-implant interface. It would be of interest to continue this line of research to examine the following issues:

(a) In vitro studies and in vivo studies, using the animal models described in this thesis, of the possible stimulatory effect of wear particles on cells to release factors which may stimulate proliferation of macrophages, and factors which may stimulate bone resorption. Of particular interest, would be the possible release by macrophages of prostaglandins and monokines, including Interleukin-1, which have been implicated in the stimulation of bone resorption in other pathological settings.

(b) Studies of the level of prostaglandins and monokines in the periprosthetic tissues and comparison with the numbers and types of wear particle in these tissues would be useful in determining whether there is a direct relationship between wear particles and release of these factors.

(c) The use of the animal models described in this thesis to determine the relative biocompatibility of wear particles from any new materials proposed for use as the articulating surfaces of joint prostheses.

(d) In vivo studies using the animal models described in this thesis to determine the effect of pharmacological agents, including anti-inflammatory agents, in modifying the macrophage response and the bone resorption seen following the injection of wear particles.

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