TECHNICAL NOTES

"Technical Notes" in BPR originated when certain Progress Reports went beyond the usual scope to present a wealth of detail about some single aspect of the research, typically a matter of instrumentation or materials or procedure.

With the establishment of the category, authors have been finding the department a useful channel of communication. Material available has tended to grow in intellectual and technical stature. BPR's editorial posture has changed in response, so that the earlier informal verification by staff and occasional external review has tended to give way to a more formal procedure which involves reviewers from the publication's Editorial board, ad hoc reviewers, or both, as may be appropriate to deal with complex or wide-ranging subject matter. This "double-anonymous" review effort frequently equals that given to all regular feature articles in this publication.

Therefore, while these more ambitious Technical Notes may lack the number of subjects, weight of data, and discovery of or replication of new knowledge required for a traditional scientific article, they have been handled with the greatest possible respect for the authors' devotion and the readers' requirements.

The Wear Particles of Synovial Fluid:
Their Ferrographic Analysis
and Pathophysiological Significance*

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Abstract—This article reviews recent progress in our investigations into the wear particles which accumulate in prosthetic and natural diarthrodial joints. Ferrographic analysis of these particles is providing new insight into the manner in which joints undergo wear in situ. It also has the potential to serve as a diagnostic and prognostic technique, with special application to monitoring very early changes in the wear status of joints and distinguishing the various arthritides. Wear particles interact with periarticular cells and tissues, provoking the release of lytic enzymes and eliciting other biochemical changes, which exacerbate the destruction of the joint.

Introduction

Recently a novel analytical tool, ferrography, has enabled us to undertake a non-invasive study of the very early deterioration that accompanies various arthritides when applied to an aspirated sample of synovial fluid. The method permits a critical separation of diverse types of biological wear particles generated from the bearing surfaces of an articular joint.

Ferrography is a technique devised originally for the analysis of wear particles in machines. Ferrous wear particles are retrieved from a sample of the lubricating oil under the influence of an external magnetic field. Microscopic examination of the particles thus retrieved permits accurate diagnosis of the wear status of the machine (q.v.).

Given the success of the ferrographic technique with machines, it was suggested that ferrography could be profitably applied to the analysis of wear particles contained in the synovial fluid of natural and artificial joints. For prosthetic joint replacements, this would allow a non-surgical; sophisticated analysis of the rate and type of wear of the implant and prognostication of its future performance. With natural joints, there is the enticing prospect of vastly improving the differential diagnosis of arthritis. Particularly important is the potential of ferrography to facilitate an early diagnosis, before the disease has progressed to conspicuous radiological manifestations, by which time potentially prophylactic treatments may not longer suffice and surgical procedures remain the only solution.

Wear particles in joints are of additional significance. By influencing the metabolism of cells in the surrounding tissues, particles eroded from prosthetic devices could contribute to various of the adverse reactions which result, for example, in loosening of the implant, or infection. In natural joints, the wear particles may be mediators of tissue destruction during arthritis, in which case their investigation should provide insights into the disease process itself.

In this article, we review progress made here and at Foxboro Analytical, Burlington, MA, in the application of ferrography to the study of wear particles derived from human and prosthetic joints, and in establishing their possible role in the initiation and progression of arthritis.

The Analysis of Wear by Ferrography

Ferrography is a technique of proven worth in evaluating tribological processes by studying the wear particles they produce. Developed by Vernon Westcott of Foxboro Analytical, Burlington, Massachusetts, ferrography is based upon the magnetic retrieval and separation of wear particles
The ferrograph analyser. The photograph at the top of the page shows a "dual" analyser capable of processing two samples simultaneously. The diagram illustrates the interaction of the suspension of wear particles with the magnetic field during the ferrographic analysis.

A ferrogram. This ferrogram has been deliberately overloaded with cartilaginous wear particles and stained to illustrate the technique. The substrate is 60 mm long. The entry point is at the left of the photograph, and contains the highest concentration of particles.

Ferrographic analysis of prosthetic joint replacements—Ferrography was first applied to the examination of synovial fluids and washings obtained from replacement joints. Such metal-on-metal or metal-on-plastic arthroplasties constitute a type of machine confined within the body, and provide the from lubricating fluids (1). It was originally designed to monitor wear in machines, where the particles are typically ferrous and thus intrinsically susceptible to an external magnetic field. Most biological materials are, of course, diamagnetic, so, as described later, special techniques have had to be developed to enable their magnetic separation.

With machines, a small volume (usually 1–3 ml) of lubricating oil is slowly pumped along a thin glass microscope slide (the substrate) which lies in a high-gradient magnetic field (Fig. 1). The substrate is raised slightly at the point where it first comes into contact with the sample (the entry point), so that particles encounter a perpendicular magnetic force which is weakest at the entry point and which increases progressively down the length of the substrate. Consequently, for particles of the same unit volume susceptibility, the largest particles deposit nearest the entry point, with a grading of particles according to size down the length of the substrate. A washing solution is pumped over the particles on the substrate, which is then allowed to air dry. The resultant ferrogram (Fig. 2) can then be examined by various optical and electron microscopic techniques.

Particles are classified on the basis of their size, shape and other morphological features, to indicate the operation of certain wear modes in the machine from which the sample was drawn. Once the "running-in" period of the machine is over, it is normal to find a moderate number of elongated particles (Fig. 3) indicative of rubbing wear. Abnormal conditions are indicated by increased numbers of particles of greater size and of distinctive morphology indicative of cutting wear, fatigue, or other disturbances (Fig. 3). These particles appear on ferrograms before adverse symptoms affect the machine (2): As different components of engines are made from different alloys, identification of the constitution of the wear particles permits the site of excessive or abnormal wear to be located.

The ferrographic analysis of engine oil has been extensively reviewed (2,3).

Ferrographic Analysis of Human Joints

With the foregoing background, ferrography seemed of potential value in studying wear in human artificial and natural joints. Wear particles accumulate in synovial fluid in much the same way as they do in engine oil. Non-invasive, objective, diagnostic and prognostic, this technique yields important information pertaining to the rates of wear, types of wear and reasons for failure of prosthetic joint replacements. This information may greatly aid the development of improved implants, and would permit convenient, non-invasive, serial assessment of the progress of implanted prostheses with minimum discomfort to the patient. Ferrography also has potential application to studies of osteoarthritis, where mechanical wear of the joint surfaces occurs.

Ferrographic analysis of prosthetic joint replacements—Ferrography was first applied to the examination of synovial fluids and washings obtained from replacement joints. Such metal-on-metal or metal-on-plastic arthroplasties constitute a type of machine confined within the body, and provide the
opportunity for a logical extension of the original ferrographic technique.

Synovial fluids, or saline washings, from these sources can be processed by a method analogous to that used to make ferrograms from engine oil. This is so because the tiny metallic particles become embedded in the surrounding materials, thereby imparting a magnetic susceptibility which is sufficiently high for normally diamagnetic wear particles to be retrieved under the influence of the magnet of the ferrograph. Under these conditions, wear particles of cartilage, bone, plastic, polymethylmethacrylate and synovium can be analyzed without the need for artificial means of magnetization (4).

Synovial fluid is obtained by sterile needle aspiration from joints after arthroplasty. If synovial fluid is unobtainable, the joint can be flushed with sterile saline. About 2 ml of the synovial fluid are pumped over the substrate as described below.

Following washing and drying, the ferrogram is first examined by optical microscopy. A useful instrument in this context is the bichromatic microscope, providing both reflected and transmitted illumination (1). Using the appropriate filters, the transmitted light is green and the reflected light red. Under the bichromatic microscope, metallic wear particles appear red, due to their attenuation of the green transmitted light and reflection of the red, direct light. Non-metallic materials appear green, yellow or pink, depending upon their density or thickness. Polarized light is advantageous when examining particles of bone, cartilage, methylmethacrylate and plastic.

Following optical inspection, particles can be examined with greater morphological precision using the scanning electron microscope (SEM) and their elemental composition can be determined by energy dispersion X-ray analysis.

Ferrographic analysis of saline washings of failed prostheses (Fig. 4) reveals the presence of metallic, plastic and polymethylmethacrylate particles in synovial fluid (Fig. 5). The relative proportions vary with the type of prosthesis and the severity and type of wear which has occurred. Thus, larger numbers of metallic particles are found when both articular surfaces are composed of metal. With metal rubbing on polyethylene, most of the particles are plastic, and range from 1–10 μm in diameter up to shredded fibres several hundred micrometers long. Metallic particles range in size from under 0.25 μm where wear is relatively small, to 1 mm in length where fatigue or abrasive wear has occurred. Polymethylmethacrylate particles are generally cuboid with large variations in size from 1 μm to 1 mm or more. Sometimes, these particles adhere to metallic fragments. Particles of bone, cartilage and synovial tissue are also found. Histological examination of synovium demonstrates the presence of embedded wear particles.

FIGURE 3
Wear particles from engine oil. (A) Normal rubbing wear particles, mag. 450×. (B) Cutting wear particles, scanning electron microscopy, 225×. (C) Fatigue wear particles, mag. 450×. (D) Laminar wear particle, mag. 1000×.
FIGURE 4
Macroscopic appearances of wear in failed prosthetic joint replacements. At top of page, erosion of the polyethylene surface of a failed total knee replacement is seen; the depressions are filled with polymethylmethacrylate. Above, a failed total hip replacement. Again, there is pitting of the polyethylene surface with polymethylmethacrylate infilling. Note that the metallic component is less severely eroded.

FIGURE 5
Wear particles from prosthetic joints, recovered by ferrography. At top, metallic wear particles, mag. 1000x. Center, polyethylene wear particle under polarized light, mag. 400x. Immediately above, polymethylmethacrylate particles under polarized light, mag. 400x.
while phagocytic cells containing endocytosed wear debris can be retrieved from the synovial fluid of affected joints (Fig. 6).

X-ray elemental analysis of the metallic particles confirms that they originate from both the arthroplasty and the surgical instruments, most especially haemostats, scissors, and osteotomes (4).

This study revealed interesting and encouraging similarities to the ferrographic analysis of the lubricating oil of machines. In both cases, excessive wear is signalled by an increase in the numbers and sizes of particles, and the presence of wear particles representing altered and abnormal modes of wear. Thus, ferrography appears to offer the same advantages to studying wear in prosthetic joints as it presently does to studying wear in machines. The presence of wear debris embedded in the synovium (Fig. 6) indicates how rapid prosthetic wear could provoke a painful,

FIGURE 6
Interaction of wear particles with surrounding cells and tissues. Above, right: a polarized light micrograph of synovium shows embedded wear particles. Mag. 100×. At right, a transmission electron micrograph of a polymorphonuclear leukocyte retrieved from the synovial fluid. Engulfed particles occur in intracellular vesicles. Mag. 38,300×.
proliferative synovitis. Such information has stimulated further modification of implant design, and it has indicated one other pathogenesis of a painful joint replacement.

**Ferrographic analysis of wear in natural joints**—Before the biological wear particles present in the synovial fluid of natural joints can be analyzed effectively by ferrography, it is necessary to make them susceptible to external magnetic fields. Research conducted at Foxboro Analytical has produced methods of achieving this (5). The ‘magnetisation’ process is based on the sorption of paramagnetic cations of the rare earth element erbium (III) to particles of bone and cartilage (6). Because Er³⁺ interacts strongly with many other substances in synovial fluid, producing troublesome precipitates, the particles are first collected by centrifuging and washed three times by resuspension and recentrifuging in saline (0.9% w/v). Even this may be insufficient to adequately remove hyaluronic acid, a sticky macromolecule which may pose problems by tenaciously coating the particles. For this reason, the washed particles are treated mildly with a highly specific hyaluronidase before analysis. (That step is usually unnecessary for particles obtained through saline washings of joints.)

**Bloody samples** are difficult to process. If the degree of contamination is slight, the erythrocytes can be lysed in distilled water prior to ferrography. This leaves a residue of cellular debris which, in modest quantities, is not a problem. Samples of synovial fluid quickly form gelatinous precipitates. For this reason, all such specimens must be centrifuged, washed and resuspended in saline before storage. Sodium azide (final concentration 0.02% w/v) should be added to stored saline suspensions of wear particles to prevent microbial contamination.

**Peculiarities of “biological” ferrography**—Although biological particles achieve considerable positive magnetic susceptibilities on treatment with magnetising solutions containing Er³⁺, the induced susceptibilities are many times less than those of corresponding metallic particles. For this reason, it is necessary to position the substrate flat against the magnet (Fig. 1) to ensure adequate recovery of the biological material. Unfortunately, this arrangement reduces the longitudinal gradient in magnetic force that is exerted on the particles as they flow along the substrate, thereby interfering with the size-grading of particles along the ferrogram. This is exacerbated by the chemical heterogeneity among the wear particles themselves which influences their uptake of Er³⁺ and thus their individual magnetic susceptibilities. Such heterogeneity results from intrinsic variations in chemistry at different locations within the cartilage, from different mechanisms of wear, and from alterations produced by the disease. (Eventually, the resultant change in ferrographic behaviour of such particles may prove of diagnostic value.)

**Dust-free work area**—When making and examining ferrograms, it is necessary to work in an environment which is free from dust. Particles of dust are often optically active and in many cases resemble cartilaginous wear particles. Extraneous particulates can be prevented by situating the ferrograph analyser and the bichromatic microscope in a laminar flow hood.

**Notes on Optical Analysis**—Examination of the ferrograms is first undertaken with the bichromatic microscope, as described in the previous section. Ferrograms made from synovial fluid aspirates reveal a variety of deposited materials, not all of which are yet completely understood.

As bone and cartilage are both optically active, polarized light microscopy is especially useful. Whereas most cartilaginous and osseous wear particles are conspicuous under polarized light, soft tissue components such as synovium or other cellular material are not. Osseous particles usually have high optical activity and a compact, chunky appearance; much of this is due to the scattering of light when it encounters the polycrystalline mineral of bone. Consequently, osseous particles remain bright as the direction of prolongation is changed while maintaining crossed polars. On the other hand, materials exhibiting birefringence, in which the molecular structure of the particle is organized over a period of several micrometers, will exhibit a change in light intensity as a function of the direction of prolongation. Cartilaginous particles vary greatly in their optical activity—some resemble bone in having high optical activity, while others are barely visible under crossed polars. This may reflect their degree of mineralization, the extent to which the collagen fibres are oriented along a single axis, and the thickness of the particles.

Occasionally, especially in the larger cartilaginous particles, one can observe lacunae which, in life, contain the chondrocytes. Phase contrast microscopy facilitates such observations.

**Histological and chemical notes**—Various histological techniques have been brought to bear on the complex problem of particle identification. It would seem, at first sight, that these various types of particle could be elucidated by standard histological techniques, many of which have been specifically devised for differentially staining the tissues of the joint. This, however, has not proved as straightforward as it appears.

The reasons for this are several. The chemistry of the wear particles probably differs from that of the intact tissue. Specifically, metachromatic staining of articular cartilage is markedly reduced in early osteoarthritis, reflecting a loss of the proteoglycans which are responsible for many of the staining properties of cartilage. Also, once released into the synovial fluid, further biochemical modifications of the minute articular particles may occur. However, some success has been obtained with a modified Movat's pentachrome stain (7).

**X-Ray analysis of materials**—Particles of bone and cartilage which have not undergone excessive degradation can be distinguished readily by energy dispersion X-ray analysis. As shown in Figure 7, cartilaginous particles give a strong sulphur emission from their sulphated glycosaminoglycans. Bone provides strong calcium and phosphorous peaks (Fig. 8), while mineralized cartilage

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Footnote:

* Procedures required to avoid staining problems, when Er³⁺ has been employed to impart magnetic susceptibility to tissue particles, are discussed in (7).
FIGURE 7—cartilaginous wear particle (arrowed), mag. 200×, with (at right) its energy dispersion X-ray analysis spectrum. The analysis shows the presence of sulphur, from the sulphated glycosaminoglycans of cartilage, and erbium, which is used to magnetize the particles. The sulphur peak is irregular due to subtraction of the background spectrum produced by the glass substrate.

contains both of these and sulphur. Figure 8 compares part of the elemental spectrum of bone (background) with soft tissue (foreground). The bone gives a major peak of calcium, while the soft tissue has only a small calcium peak, which is dwarfed by that of potassium. A difference in their relative amounts of chlorine is also seen.

While this technique is rather tedious for routine use, it serves to resolve the identity of enigmatic particles and to cross-check the various other methods tested in the search for improved identification techniques.

Comments on an Analysis of 50 Synovial Samples

Having developed a technique for the ferrographic analysis of synovial fluid aspirates, its applicability was investigated by an analysis of about 50 synovial samples. This survey yielded encouraging results (7). A variety of different particles deposited on the ferrograms, indicating that discrete wear particles do indeed exist in human synovial fluid, and that they are susceptible to ferrographic analysis. It was also encouraging to observe that wear particles were not pieces of random detritus, but that many of them fell into one of several different, identifiable morphological categories.

On the basis of that preliminary work, we are beginning to appreciate the different types of wear particle in joints, the wear conditions that produce them, and their significance. In the work outlined below, the ferrographic analysis was compared to radiographic, arthroscopic, and physical

FIGURE 8—Osseous particles under (A) unpolarized and (B) polarized light, mag. 200×, with (C) their energy dispersion X-ray analysis. The background spectrum is that of bone showing a marked calcium peak. In the foreground is, for comparison, non-osseous tissue which has only a little calcium.
examination of the joint from which the sample was obtained.

Ferrograms made from the wear debris of joints with even modest degrees of articular erosion often contain a sizable and varied population of wear particles. Some of these are indicated in Figures 9 (a–h) and 10 (a–e). The array shown in Figure 9a is the entry deposit, viewed under polarized light at 100x magnification, of a ferrogram made from the synovial fluid of a patient with chondromalacia patella. Closer examination of samples such as these enables many of the particles to be categorized.

**FIGURE 9**
Types of cartilaginous wear particles are shown in the photographs at right and below.

(a) Entry deposit on ferrogram from chondromalacia patella, polarized light, mag. 100x.
(b) Thin, angular lamellae of superficial cartilage, unpolarized light, mag. 200x.
(c) Cartilaginous wear particles from a moderately eroded knee joint. Mag. 200x, polarized light.
(d) Fibrous cartilaginous wear particle, mag. 200x, polarized light.
(e) Spherical cartilaginous particles. Mag. 400x, unpolarized light.
(f) Triangular fragment of cartilage and cartilaginous sphere, polarized light, mag. 400x.
(g) Cartilaginous wear particles retrieved from osteoarthritic joint, mag. 200x, polarized light.
(h) Large cartilaginous wear particle from osteoarthritic joint, mag. 100x, unpolarized light.
Benign forms of low-grade wear in joints appear to generate small (< 40 μm) flakes of thin, angular lamellae of cartilage with weak optical activity. These occur on most ferrograms, even where damage is light, and they are likely to originate from the superficial layers of the cartilage. An SEM micrograph of one such particle is shown in Figure 9b.

More severe damage—When damage to the articulating surfaces is more severe, the lamellae are larger (> 80 μm), more numerous, and sometimes give the impression of being laminate. More severe wear also produces larger particles of higher optical activity and a more compact appearance, as shown in Figure 9c under polarized light. Possibly, these are derived from the deeper zones of the cartilage or are produced by different wear mechanisms, or both. Other cartilaginous wear particles are elongate rods, some of which have a coarse fibrous nature (Fig. 9d). (We suspect that many of the latter are derived from the meniscus.)

Spherical particles—Under certain conditions, spherical cartilaginous particles occur. The ones shown in Figure 9e are about 5 μm in diameter and were retrieved from the knee of a patient with chondromalacia patella. The sphere, shown by SEM in 9f is larger (~ 25 μm diameter) and of stronger optical activity.

The mechanism of production of cartilaginous spheres remains to be determined. In machines, spherical wear particles are shaped by their rolling in minute cracks in the surface of one of the components of the machine. Spherical particles in synovial fluid may be formed from non-spherical precursor particles in an analogous manner, or they may have been spherical to begin with. In the latter case, an unusually precise wear process must have carved them out of the cartilage, or, more probably, pre-existing spheres within the cartilage might have been released by the wear process. In several examples, there was evidence of some degree of mineralization associated with the spheres (7). This tentative suggestion may bear some relationship to the observations of Pautard (8) on the morphology of crystallized material within bone. In one instance, a high concentration of mineralized spheres of diameter 25–30 μm was seen on a ferrogram made from the synovial fluid of a patient with bursitis and primary osteoarthritis (Fig. 10e). McCarty has observed slightly smaller spheres of hydroxyapatite in the shoulder joint of certain patients (9).

FIGURE 10
Photographs (a) through (e) show examples of other types of deposits encountered on ferrograms.

(a) Optically inactive membrane, mag. 100x, unpolarized light.
(b) Amorphous debris, mag. 100x, unpolarized light.
(c) As (b), polarized light.
(d) Crystals, mag. 400x.
(e) Crystalline spheres, mag. 200x, polarized light. (Inset shows SEM of one sphere, mag. 1000x.)
Other evidence of crystal deposition in osteoarthritic joints is growing. This would provide an ideal mechanism for the generation of the "cutting wear particles" of cartilage we have observed on ferrograms made from the fluid of osteoarthritic joints. (Wear particles could conceivably serve as foci for the deposition of minerals.)

**When articular erosion is severe**, larger particles are produced which do not fall into distinct morphological categories such as the lamellae, spheres, and rods mentioned earlier. The triangular particle shown in Figure 9f is one such example. Others are shown in Figures 9g and 9h. Lacunae are visible in the particle shown in Figure 9g. Part of another large cartilaginous particle is shown in Figure 9h under polarized light; again, lacunae are evident. In some cases, cartilaginous wear particles have a blue-grey sheen under polarized light (7).

Ferrograms contain other types of wear particle in addition to the cartilaginous ones described above. Thin, amorphous membranes without optical activity (Fig. 10a) probably represent fragments of synovium. In contrast, amorphous debris of the type shown in Figure 10b has appreciable optical activity (Fig. 10c). Preliminary energy dispersion X-ray analysis suggests an appreciable level of associated sulphur, indicating the presence of cartilage proteoglycans. These deposits may thus constitute a degraded form of cartilage, possibly that subjected to enzymic attack. In agreement with this interpretation, the few samples of rheumatoid synovial fluid which we have analysed by ferrography, and where the enzymic mode of degradation of cartilage would be expected to be operative, have high amounts of this material (7).

Minerals in the form of crystals (Fig. 10d) or spheres (Fig. 10e) also occur on ferrograms. Those containing phosphate become especially magnetic (6) as Er\(^{3+}\) has a high affinity for phosphate groups.

The sizes of the synovial wear particles are generally much larger than their metallic counterparts. Even in joints where the damage is light, wear particles reached 20–30 µm in size, with fibres being larger than this. As with machines, increased severity of wear produced particles of increased size, particles up to 2 mm in length often appearing on ferrograms. Even larger fragments occur in severely arthritic joints. (The upper size limit for detection by ferrography is set by the gauge of the hypodermic needle used to aspirate the sample, and the bore of the turret tube used in the ferrographic analysis (Fig. 1).) Along with the increased size which accompanies more advanced degeneration, there is increased morphological variety among the particles, which thus become difficult to classify.

**Studies of Selected Patient Groups**

The preliminary survey already described has confirmed the applicability of ferrography to synovial fluid analysis: the wear particles fell into a number of unique categories which varied from one patient to another; the sizes and morphologies of the particles altered with the severity of wear. However, no clear-cut differences could be found between the various groups of diseases studied (chondromalacia patella, torn meniscus, osteoarthritis, rheumatoid arthritis) (7). From these findings, it became clear that realising the diagnostic and predictive potentials of ferrography would require close scrutiny of selected groups of patients whose medical histories and arthritic conditions were minutely detailed.

To develop that phase of the project, in collaboration with Dr. Carl Stanitski of the University of Pittsburgh Health Center, we are presently limiting ourselves to ferrographic analysis of saline washings of knee joints recovered during arthroscopic examination of the knee. This eliminates many variables—we are dealing with only one joint (the knee) and with a limited number of disorders. Most patients suffer from a torn meniscus, chondromalacia patella, or early osteoarthritis. On occasion, arthroscopic examination will reveal no joint abnormalities, thus providing valuable "normal control" samples, which are otherwise difficult to obtain. Arthroscopy also has the advantage of permitting close visual examination of the articulating surfaces and synovium, thus providing the detailed information needed in a study of this kind. Ancillary historical data, physical examinations, and radiological assessments can be correlated with the ferrographic analyses and arthroscopies.

The most interesting group of patients in this study have been those whose arthroscopic examinations revealed essentially no damage to the cartilaginous surfaces of the joint. One illuminating subset contained three patients of equivalent age (11, 12, 14 years). The initial arthroscopic examination classified each of these as normal. However, ferrographic analysis revealed marked differences between one of these patients, whose knee contained very little wear debris, and the two others, whose ferrograms contained evidence of cartilaginous damage. On arthroscopic re-evaluation, one of the latter two patients was found to have a possible slight softening of the patella, and the other a barely detectable softening of the femoral condyle. Thus, ferrography seems much more sensitive than arthroscopy to subtle changes in the integrity of the cartilage.

Examination of five patients with torn anterior cruciate ligaments reinforced that conclusion. Ferrograms made from saline washings of the affected knees contained an appreciable amount of wear debris. Although the number of wear particles was elevated, their size remained small; some were rounded particles only 1–5 µm in diameter. In each case, arthroscopy failed to detect this "micro-damage" to the cartilage. The suggestion has been made that such damage may arise from the alteration in biomechanical forces within the joint as a result of the ligamentous injury. With ligamentous laxity, sliding of the articular surfaces may supplement the normal rolling motion and greatly alter the wear pattern. In the presence of peripheral ligamentous injury (or with abnormal joint congruity perturbed by traumatic, congenital, or arthritic change) the local sites of linear or point contact on the bearing surfaces might result in excessive forces on those bearing surfaces, so that abnormal wear modes ensue. This suggestion finds support in the analysis of the wear particles in the knees of two patients in which the torn cruciate ligamentous injury was superimposed on another defect; in one of these this was softening of the articular cartilage and in the other it was a slightly "ragged" meniscus. In both cases, the numbers and types of wear particle revealed damage in excess of that caused by torn ligaments or the other injuries alone.
Patients with articular damage of sufficient severity to be detected by arthroscopy yielded elevated numbers of wear particles of large size and varied morphology. These studies illustrate the extreme sensitivity of ferrography to articular damage. Its superiority over arthroscopy has two aspects. First, there is the magnification factor; ferrograms are routinely examined under the optical microscope under magnifications of 100x–400x. With the electron microscope, much higher magnifications are possible. Arthroscopy, however, scans for articular damage at magnifications of only 3x–5x. A second aspect is the greater resolution permitted by the means used for examining the wear particles, in contrast to that used to view the bulk material, when searching for evidence of damage. It appears, then, that even grossly normal knee joints do contain some wear particles. This observation raises questions about normality. Preliminary analysis of a few samples seems to indicate that the “background” wear debris of asymptomatic joints increases with age. This ties in with the observation that most, if not all, joints degenerate with age, even though only a fraction suffer from clinically overt arthritis. We are presently trying to analyse ferrographically a sufficient number of samples from asymptomatic knee joints to permit an accurate assessment of this matter.

The Pathological Significance of Wear Particles: Observations and Hypotheses

Specialized phagocytic cells exist for the removal of particulate matter from the body. Most active in this respect are macrophages and polymorphonuclear leucocytes, both of which are derived from the stem cells in the bone marrow. Their phagocytic activities are thought to feature in defense against invading microorganisms. Several other types of cell are able to internalize particulate materials. Of relevance to arthritis is the ability of synoviocytes to do this.

Phagocytosis has a number of biochemical and physiological consequences. One is the release of various hydrolytic enzymes, several of which degrade cartilage. A variety of particulate stimuli have been shown to elicit this response, including inert substances such as latex beads (10), asbestos (11), minerals (12), precipitated immunoglobulins (13) and collagen (14). Putting these two observations together synthesises the hypothesis that phagocytosis of wear particles provokes the release of lytic enzymes which attack the articular surfaces. This would “soften” them, thus potentiating the mechanical release of more particles. Such circumstances could alter the wear modes in operation, thus producing particles of altered morphology. These “secondary” particles could also differ chemically from bulk cartilage as a result of the enzymatic attack which had facilitated their production. Such particles might have an altered propensity to provoke the phagocytic cells into releasing enzymes. From these considerations, it is easy to appreciate the possibility of complex interactions between mechanical wear, cellular and biochemical events, and arthritis.

In initial experiments to examine these possibilities, macrophages and synovial cells were grown in tissue culture and exposed to wear particles retrieved from synovial fluid aspirates. The conditioned culture media were then examined for various proteolytic enzymes of relevance to the breakdown of cartilage (15). The results are summarized in Table 1. Both macrophages and synovial cells released proteinases in response to the wear particles. Collagenase may be important in breaking down the collagenous component of cartilage. It is interesting to note that synovial cells released more collagenase than macrophages; cultures of rheumatoid synovium are a rich source of this enzyme (16). This difference is reflected in the inability of macrophages to liberate as much hydroxyproline from cartilage as synoviocytes. Both types of cell produced equivalent activities of the other proteinases tested and were able to release quite large amounts of degraded proteoglycan from cartilage (Table 1).

In every case, mild trypsinisation of the media revealed enzyme latency (Table 1). From these data it is not possible to determine to what extent the newly synthesised enzymes are latent, as their autoactivation occurs during concentration and storage of the conditioned media.

With these initial observations, attention is being turned towards delineating which features of the wear particles are responsible for producing the cellular effects. Wear particles are very heterogeneous in size and shape. Their precise chemical make-up also varies depending on whether they are of meniscal or articular cartilage, or osseous, and to what extent they have been subject to enzymic attack.

The first of these variables to be investigated was size. Synovial fluid aspirates were not used as a source of particles for these experiments, as the yield of particles of any one size range is small. Furthermore, even particles of one size may be chemically heterogeneous. Thus, to minimize the number of variables, meniscal cartilage was used as a source of particles. This was powdered and added to cultures of cells. The response of cells exposed to cartilaginous particles of this type was compared to that elicited by latex beads of diameter 1 µm and 45 µm. From the results of such experiments (Table 2), it was concluded that internalization of particles by the cells was not a necessary condition for enzyme release. Particles which are too large for endocytosis adhere to the cell surfaces and promote the release of enzymes (17). This observation ties in with the demonstration by Harris et al. (18) that urate crystals need not be internalized by synovial cells to stimulate the production of collagenase. Thus it seems that, qualitatively, the observed tissue response is not limited to one particular size of wear particle, although there may well be qualitative differences due to variations in surface area to volume ratios, etc.

Cartilaginous particles provoked a greater release of neutral proteinases than did latex beads (19), which are assumed to be biochemically inert. It thus appears that chemical, as well as physical, stimuli are involved in the effects produced by cartilaginous wear particles on cultures of macrophages.

Chondroitin sulphate is a major component of cartilage, which appears to be one of these chemical stimuli (19). Purified chondroitin sulphate produced a marked elevation in the secretion of both lysosomal hydrolases and neutral proteinases by cultured macrophages (Table 3). The effect is
TABLE 1.
Rates of production of proteinases by cells in the presence or absence of wear particles.

<table>
<thead>
<tr>
<th>Cell Types</th>
<th>Conditions</th>
<th>Productiona of Proteinases (μM/10^6 cells/day)</th>
<th>Collagenase</th>
<th>Gelatinase</th>
<th>Azocaseinase</th>
<th>Pz-Peptidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine Peritoneal Macrophages</td>
<td>without particles</td>
<td>0.73 ± 0.06</td>
<td>2.37 ± 0.18</td>
<td>1.53 ± 0.18</td>
<td>1.92 ± 0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>without particles, trypsinised</td>
<td>0.97 ± 0.10</td>
<td>2.87 ± 0.21</td>
<td>2.18 ± 0.20</td>
<td>2.41 ± 0.21</td>
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<tr>
<td></td>
<td>with particles</td>
<td>1.52 ± 0.21</td>
<td>4.84 ± 0.25</td>
<td>2.91 ± 0.23</td>
<td>3.53 ± 0.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>with particles, trypsinised</td>
<td>1.91 ± 0.52</td>
<td>6.56 ± 0.43</td>
<td>4.28 ± 0.35</td>
<td>5.08 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>Human Blood Mononuclear Phagocytes</td>
<td>without particles</td>
<td>0.33</td>
<td>2.52</td>
<td>2.05</td>
<td>1.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>without particles, trypsinised</td>
<td>0.83</td>
<td>3.15</td>
<td>3.10</td>
<td>2.42</td>
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<tr>
<td></td>
<td>with particles</td>
<td>1.61</td>
<td>4.72</td>
<td>3.22</td>
<td>3.69</td>
<td></td>
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<tr>
<td></td>
<td>with particles, trypsinised</td>
<td>1.85</td>
<td>5.98</td>
<td>4.86</td>
<td>5.11</td>
<td></td>
</tr>
<tr>
<td>Human Synovial Cells</td>
<td>without particles</td>
<td>1.31 ± 0.14</td>
<td>3.00 ± 0.21</td>
<td>1.33 ± 0.21</td>
<td>2.64 ± 0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>without particles, trypsinised</td>
<td>2.38 ± 0.21</td>
<td>5.19 ± 0.46</td>
<td>2.56 ± 0.30</td>
<td>3.82 ± 0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>with particles</td>
<td>5.52 ± 0.35</td>
<td>6.52 ± 0.53</td>
<td>2.84 ± 0.38</td>
<td>5.31 ± 0.42</td>
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</tr>
<tr>
<td></td>
<td>with particles, trypsinised</td>
<td>9.95 ± 0.89</td>
<td>11.56 ± 1.03</td>
<td>5.20 ± 0.51</td>
<td>8.18 ± 0.78</td>
<td></td>
</tr>
</tbody>
</table>

bResults shown for murine peritoneal macrophages are the means ± SEM of six replicate experiments; for synovial cells, four replicates; and for human blood phagocytes, the average of two replicate experiments.

b1 unit of collagenase, gelatinase or Pz-peptidase degrades 1μg substrate per min at 37°.
1 unit of azocaseinase degrades 1mg azocasein per hour at 37°.

reversible; within three days of changing the cultures of macrophages back to a medium which does not contain chondroitin sulphate, the production of neutral proteinases and lysosomal hydrolases returns to normal. Certain lines of evidence suggest that these effects are not limited to the artificial conditions of tissue culture. Wear particles exist in considerable amounts in many osteoarthritic joints (7). Observations such as those shown in Figure 6 demonstrate the interaction of these particles with periarticular cells and tissues, which may explain the synovitis and other signs of inflammation that often occur in osteoarthritic joints. Furthermore, the activities of several of the enzymes measured in the work described here are also elevated in tissues taken from osteoarthritic joints (20).

Animal Model: Preliminary Results—We have recently employed laboratory animals to determine, in a more direct manner, whether wear particles have arthritogenic properties (21). Intra-articular injections of particles of rabbit articular cartilage into the knees of recipient rabbits provoke an intense inflammatory arthritis. After 3-4 months of receiving 3 mg of articular cartilage per week, the recipient knees become swollen and inflamed. Histological examination of the synovium reveals a marked cellular infiltrate and the presence of cartilaginous wear particles, presumably from the injected material. Organ cultures of the synovium from knees receiving wear particles secrete much higher levels of both neutral proteinases and lysosomal hydrolases than do cultures of control synovium. The cartilage of particle-injected knees is discoloured and its metachromatic staining properties are attenuated. These changes are much more marked than those reported by Chrisman et al. (22) who conducted similar experiments with dogs. In certain rabbits, the synovium appears to have
TABLE 2.
Production of extracellular proteinases by macrophages in response to particles of cartilage and latex beads of different sizes.

<table>
<thead>
<tr>
<th>Enzyme Production (munits/10^6 cells/day)</th>
<th>Collagenase</th>
<th>Gelatinase</th>
<th>Azocaseinase</th>
<th>Pz-Peptidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Macrophages</td>
<td>0.57</td>
<td>2.1</td>
<td>1.62</td>
<td>1.85</td>
</tr>
<tr>
<td>Macrophages and Cartilaginous Particles</td>
<td>1.26</td>
<td>5.9</td>
<td>3.83</td>
<td>4.52</td>
</tr>
<tr>
<td>Macrophages and Small Latex Beads</td>
<td>0.92</td>
<td>4.3</td>
<td>3.21</td>
<td>3.93</td>
</tr>
<tr>
<td>Macrophages and Large Latex Beads</td>
<td>0.63</td>
<td>2.9</td>
<td>2.03</td>
<td>2.16</td>
</tr>
</tbody>
</table>

One unit of collagenase, gelatinase or pz-peptidase breaks down 1 μg substrate/min at 37°C. One unit of azocaseinase breaks down 1mg azocasein/hr at 37°C. Enzyme assays on conditioned media were usually incubated for 18 hr. Small latex beads were added to cultures at a concentration of 100 beads/cell; the same weight of large latex beads was added to parallel cultures. Powdered meniscus was added at a concentration of 5mg/75 cu cm culture vessel containing approx. 10^7 cells.

formed an invasive pannus, as found in human rheumatoid arthritis and in symptomatic artificial knee joints.

Experiments are underway to determine whether an immune response has been mounted to the injected material, or whether the effects can be accounted for by the cellular release of catabolic enzymes, and other factors, provoked by the particles. Preliminary results suggest that a systemic response to the injected particles has not occurred; the recipient rabbits give a negative skin test and attempts to demonstrate circulating IgG antibodies against the particles have failed. Furthermore, the timing and severity of symptoms is remarkably constant between animals, a feature which is not normally seen in experimental diseases that rely on an immune reaction.

Evidence that chondroitin sulphate elicits cellular responses in vivo comes from experiments in which purified chondroitin sulphate was injected into the peritoneal cavities of mice (19). After 4 days, peritoneal macrophages were harvested, counted, cultured and their release of acid and neutral lytic enzymes measured. Chondroitin sulphate elicited a concentration-dependent increase in the production of all enzymes measured, except lysozyme; this finding agrees with previous reports that the production of lysozyme by macrophages is not greatly affected by their state of activation.

It is noteworthy that this stimulation of enzyme production occurred without any increase in the number of cells per mouse. That is unusual, as most agents which activate peritoneal macrophages, such as thioglycollate, recruit additional cells, so that the yield of macrophages per mouse is greatly enhanced. Chondroitin sulphate is not antigenic (23). It may, however, have the important property of reversibly stimulating the local secretion of lytic enzymes, without triggering a full-blown inflammatory response.

Workers in Sledge's laboratory (24) have found that the ability of anionic polysaccharides, such as chondroitin sulphate, to provoke synovitis in rabbit knees increases with their molecular weight and charge density. Chondroitin sulphate was shown to produce a transient inflammation; 4 weeks after the termination of the chondroitin sulphate

TABLE 3.
Effect of chondroitin sulphate on the production of acid hydrolases and neutral proteinases by cultured macrophages.

<table>
<thead>
<tr>
<th>Concentration of Chondroitin Sulphate in culture medium (mg/ml)</th>
<th>Release from cartilage of Chondroitin Sulphate (pH 7.2)</th>
<th>Hydroxyproline (pH 7.2)</th>
<th>Azocaseinase (pH 5.1)</th>
<th>8-glucuronidase (pH 5.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>4.40</td>
<td>1.04</td>
<td>84</td>
<td>54.7</td>
</tr>
<tr>
<td>0.1</td>
<td>7.47</td>
<td>3.09</td>
<td>98</td>
<td>73.6</td>
</tr>
<tr>
<td>1.0</td>
<td>8.65</td>
<td>4.67</td>
<td>126</td>
<td>94.9</td>
</tr>
<tr>
<td>10.0</td>
<td>9.14</td>
<td>4.77</td>
<td>142</td>
<td>98.3</td>
</tr>
</tbody>
</table>

*Figures quoted are μg of substrate (azocasein, phenolphthalein-glucuronic acid) degraded, or μg of product (chondroitin sulphate, hydroxyproline) released per 10^7 macrophages per hour.
injections, the synovium continued to produce slightly elevated amounts of catabolin-like activity, but the production of lysosomal marker enzymes equalled that of controls (25).

**General Discussion**

Despite its long history and widespread debilitating effects, osteoarthritis remains a problematic disease. It is difficult to diagnose at an early stage, and it resists effective treatment. Diagnosis relies largely on patient symptoms and X-ray data supplemented, in certain instances, by techniques such as arthroscopy and arthroscopy. But the occurrence of symptoms is peculiar. Pain, for instance, is not directly related to the progression of osteoarthritis. A roentgenographical survey of Americans over the age of 75 years revealed an 85% incidence of articular degeneration (26). Yet the incidence of arthritic symptoms is much less than this. Under such circumstances, osteoarthritis is frequently diagnosed when it is too late to initiate the appropriate anti-inflammatory regime or undertake the optimal reconstructive procedures, such as osteotomies to realign the joint or to restore joint congruity. Treatment is unsatisfactory, in that symptoms rather than the underlying disease processes receive attention. Thus, an arthritic patient with advanced disease may be treated by resort to a walking aid, while the pathophysiological undertow flows unchecked. Alternatively, an unnatural replacement joint of limited anticipated functional period may be the only realistic method of treatment. This, of course, reflects an ignorance of the aetiology and mode of progression of osteoarthritis. Of the existing treatments for severe osteoarthritis, prosthetic hip replacements are outstandingly successful for a period of 5 to 10 years. Much effort is presently being directed towards extending the range of such prostheses to include knees, fingers and elbows and other types of joints. An important determinant is the biomechanical properties of the materials forming the prosthesis. In particular, it is essential that the articulating surfaces have the appropriate rheological and tribological properties. Accurate evaluation of these functions, especially when the implants are performing in situ, is needed.

From the findings reviewed in this paper, we feel that the ferrographic analysis of wear particles and studies of their biochemical and cellular properties can aid the resolution of many of these problems.

**REFERENCES**