To determine whether the demonstrated hyperlipidaemia (and, possibly, an increased incidence of ischaemic vascular disease in gouty patients) was related to pathological platelet function, platelet adhesion studies were performed by two different methods in gouty and control subjects. The results in gouty patients, however, were not significantly different from those in controls.

Discussion

DR D. C. DUMONDE (*London*) Is there any relationship between diabetes mellitus and gout?

DR. DARLINGTON This question has proved controversial in the past but most people do not now consider that such a relationship exists.

DR. J. A. BOYLE (*Glasgow*) I am a little unhappy about the effect of obesity on your results. Please could you explain what you did to assess this effect?

DR. DARLINGTON We decided to match gouty patients and controls for the Ponderal Index, which correlated well with the serum uric acid, and we accepted that the gouty patients were significantly heavier than the controls. We then divided both the gouty patients and the controls into two groups, *i.e.* obese and non-obese (according to whether they were more than 10 per cent. heavier than their average weight—obtained from Tables taking age and height into consideration—or not). A Student's *t*-test was applied to each group of lipid results. The only significant result was a marginal effect upon unesterified fatty acids and we were satisfied that obesity was not significantly affecting our findings.

Scanning Electron Microscopy and Replica Studies of Joint Surfaces in Experimental Arthritis. By D. L. GARDNER, G. SANDER, R. INOUE, C. SORIA-HERRERA, and P. MARRIOTT (*Division of Experimental Pathology, Kennedy Institute of Rheumatology*)

Scanning electron microscopy (SEM) has shown that mammalian articular cartilaginous surfaces are irregular. Superimposed on the broad anatomical contours are many coarse 0.4–0.5 mm. irregularities, and numerous shallow undulations 20–30 μ m. in diameter. The irregularities correspond in some species to the situation of subchondral blood vessels; the undulations appear to be formed where chondrocytes lie near the articular surface. In addition, high resolution SEM has shown lamina of collagen-rich material.

To examine the changes in these surfaces in inflammatory disease, the right knee joints of 144 rats were injected with 0.01 ml. turpentine; the left knee joints served as controls. To grade the subsequent reactions, 125 I-albumin was injected simultaneously intravenously. The accumulation of the isotope was measured in 100 animals at 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hrs, and at 3, 7, and 14 days after the administration of turpentine. Knee joints from 22 rats were fixed in buffered formalin and embedded in paraffin for light microscopy, and 22 others were fixed in glutaraldehyde and processed for SEM.

The inflammatory reaction was found to begin within 30 min. with a rapid and massive accumulation of

125 I-albumin. After 2 hrs, light microscopy revealed the margination of polymorphs in synovial venules. There was an early extravascular leucocyte immigration (2–4 hrs) and from 4 hrs recognizable intraarticular fibrin aggregates. Marginal cartilage loss (12 hrs–3 days) with early pannus formation was succeeded by granulation tissue formation in periarticular tissues (3–7 days). Scanning followed (7–14 days). The earliest 3-dimensional surface changes were demonstrable after 2–4 hrs. There was a surface accumulation of a fibrin meshwork, and a disorganization, detected at high resolution, of the cartilage surface lamina of collagen.

It is suggested that enzymatic degradation of surface collagen is one of the significant early signs of the destruction caused by this form of experimental inflammatory disease.

Discussion

DR. J. PALFREY (*London*) Have you obtained a good correlation between the sizes of the cells in the fibrin exudate by your methods? The round structures which you indicated seemed rather small to be cells—or do you think they have dried to this size during the preparations for electron microscopy? Can you say how big are the fibrils seen on the surface of the cartilage, and how these correspond with individual collagen fibrils seen by transmission electron microscopy?

DR. D. L. GARDNER Even with the light microscope preparative techniques may lead to great distortion which is due to drying. If one makes direct measurements of the diameter of a red blood cell in a formalin-fixed preparation, the diameter may be no more than 3.5–4.0 μ m, that is a 50–80 per cent. reduction in diameter. Similar changes are likely to affect what we see with the scanning microscope. To say what is a cell on the surface and what is a protuberance of underlying tissue is difficult. Some of the protuberances we have seen may be inflammatory cells like polymorphs; direct measurements show them to be as small as 3–5 μ m. in diameter.

The fibrils, shown at an original magnification of $\times 5,000$ are much bigger than individual mature collagen fibres viewed by transmission electron microscopy. They are of the order of size of the polymerized fibrin which we see at the surface in arthritis. This material is thought to be fibrin because of its similarity to earlier pictures obtained of polymerized fibrin in experimental blood clots.

DR. J. BALL (*Manchester*) One of your slides of the early stages of the lesion seemed to show empty cartilage cell spaces. Did the turpentine have a direct effect on the cartilage?

DR. D. L. GARDNER I have no evidence to show whether cartilage is directly injured by turpentine.

DR. P. J. L. HOLT (*Hammersmith*) We have been looking at human cartilage, using an etching technique, which amounts to sand-blasting the surface of the tissue and then scanning, sand-blasting, and scanning again. Although we do this very lightly we get exactly the same picture of a criss-cross pattern of fibres. Overlying this there is a thin membrane which can easily be removed by

etching. Underneath this are the fibres as you have shown them, each consisting of several fibres which one can again etch and remove the covering sheath to reveal the individual fibrils. In acute inflammation we get exactly the same picture as you but with less ground substance even at a distance from any obvious erosion. In degenerative arthrosis we get a rather different picture, the collagen bundles being more widely separated, and we feel that the gaps between them are filled with ground substance which we cannot pick up by the ordinary electron microscope technique.

DR. D. L. GARDNER I am not sure whether Dr. Holt is talking about experimental arthritis or osteoarthritis, or the natural disease in man. I think we must distinguish clearly between a controlled situation, in which you know when an injurious stimulus is applied to a joint, and the situation in man when you have no precise knowledge of the natural history of the disease.

DR. P. J. L. HOLT (*Hammersmith*) In disease one can, by this technique, follow the joint all over and at certain places find intact membrane—*i.e.* a normal-looking joint; in other parts one may find no surface membrane revealing underlying fibrils, and in other places again one may find a true erosion.

DR. D. L. GARDNER I should say that we know so little about normal surface structure that it is only recently, after 2 years or so, that one has felt capable of speculating about the disturbances that occur in controlled experimental injury. It is too early to attempt far-reaching conclusions from the study of spontaneous uncontrolled disease.

DR. G. NUKI (*Glasgow*) In preparing material for the scanning microscope, what steps do you take to distinguish precipitated hyaluronic acid and synovial fluid proteins from the appearances attributed to fibrin? Have you done any histochemical studies to correlate with these changes in fibre pattern?

DR. D. L. GARDNER We are engaged in histochemical studies using the bone cryostat demonstrated this morning (Pearse and Gardner, 1971), but histological study using unfixed undemineralised tissue is not yet at a stage where we can draw certain conclusions about abnormal material. There is such a wealth of material waiting to be studied in the normal that we have hardly approached the abnormal. With regard to identifying hyaluronate, the problem is to translate the appearances of dried, coated, scanned material into 2-dimensional histochemical sections. It is so fixed that rather poor sections are obtained when the scanned material is re-embedded in paraffin. The correlation may not be easy.

Reference

PEARSE, A. D., and GARDNER, D. L. (1971) *J. clin. Path.*, in press.