only the stimulus to a pathogenetic mechanism which is partly immune complex and partly cell-mediated hypersensitivity.

DR. MAINI Of course, we were fully aware of your results and we were glad to have chosen gouty patients as one of our control groups. The gouty patients in this study had often had recurrent attacks of arthritis, but there is nothing to suggest that they had anything other than gout; certainly they did not have rheumatoid arthritis. Our results show very clearly that, if one compares rheumatoid with non-rheumatoid subjects, using any of the three mycoplasma strains, there is a statistically significant difference in hypersensitivity *in vitro*. If one compares rheumatoid arthritics with healthy individuals, the difference is very striking—it is only with gouty controls that the difference is not so impressive and there is this apparent anomaly. We shall have to look into this further.

The second point was the interesting finding with *M. gallisepticum* which is not found in man and is a chicken mycoplasma.

Thirdly, I did not say that delayed hypersensitivity to diphtheroids and mycoplasma was suppressed in rheumatoid disease. If anything, these organisms may act as adjuvants for some autoimmune responses. If there is a 'rheumatoid virus', its persistence may indeed represent a selective failure of immune surveillance, whereas adjuvant and inflammatory responses to other organisms, such as mycoplasma or diphtheroids, may underline the results we get. Of course viruses are known to induce immunodepression, particularly a selective suppression of some cellular immune responses. We have recently studied patients with shingles, whose lymphocytes transform to PHA and PPD but not to the varicella virus antigen.

DR. P. J. L. HOLT (*Hammersmith*) Many circulating peripheral blood lymphocytes in active rheumatoid disease are already stimulated and the spontaneous DNA synthesis (*e.g.* during the first 2 hours) is often very high. Thus it may be that, instead of testing a response to mycoplasma or other known antigen *in vitro*, the response you are measuring is at least in part the result of previous activation *in vitro* and has nothing to do with whether you add mycoplasma, etc., or not. Thus, without eliminating this spontaneous activity, it is impossible to ascribe these results to mycoplasma, etc. The relevant controls are difficult to design, but you might find similar results in, say, Hodgkin's disease where spontaneous activity can be high.

DR. MAINI We also have considerable experience in lymphocyte transformation in rheumatoid arthritis. We find the lymphocyte transformation test very difficult to standardize, and that the patients studied must also be carefully controlled, so that they have not received, *e.g.*, steroids or gold, which might be immunosuppressants. We do not agree with your findings. We find that the spontaneous lymphocyte transformation in rheumatoid arthritis is, if anything, suppressed.

DR. D. A. RAJAPAKSE (*Hammersmith and Taplow*) We have obtained entirely different results using the macrophage migration inhibition test. We tested rheumatoid arthritics to assess cellular hypersensitivity to *Mycoplasma fermentans* membrane preparations. With guinea-pig macrophages and human lymphocytes, no cellular immunity as demonstrated. We tested six patients, and only one showed migration inhibition. The absence of cellular immunity was also supported by a lymphocyte transformation test using the same antigen. Therefore these two tests seemed to show no evidence of cellular immunity to *Mycoplasma fermentans* membrane antigen in rheumatoid arthritics. The macrophage migration inhibition method used by us was first tried on tubercular patients and subsequently in the study of rheumatic fever using streptococcal cell wall antigen. We have screened fifty different antigens successfully by this method. Besides, neither the macrophage migration inhibition test nor the lymphocyte transformation method measures adjuvant activity—they measure cellular immunity.

DR. MAINI I cannot comment on your findings or your interpretations. Your technique, of course, is quite different and perhaps the dose of antigen you used was not the right one.

Plasma Lipid Levels and Platelet Adhesiveness in Gout. By L. G. DARLINGTON, S. SHAW, and J. T. SCOTT (*Charing Cross Hospital and Kennedy Institute of Rheumatology*) Plasma lipid levels were investigated in a group of 27 adult patients with gout and in 27 closely matched controls. No subject had any known predisposing cause for hyperlipidaemia.

No significant difference in plasma cholesterol levels was found between the two groups, but significant increases in both phospholipid and glyceride values and a highly significant increase in unesterified fatty acid levels were observed in the gouty patients.

The results were analysed to find whether factors, such as obesity, smoking, heavy alcohol intake, hypertension, drug treatment, social class, occupation, or blood group, affected the values obtained. The only significant effect on the lipids was a possible marginal one of obesity on plasma unesterified fatty acids.

The complex inter-relationships between the lipid results were investigated in detail.

In addition, correlation was sought between plasma lipids, and plasma and urinary uric acid values. No significant correlation was found in the gouty patients between plasma uric acid and plasma lipid levels, or between urinary uric acid and plasma cholesterol or unesterified fatty acid levels, but between urinary uric acid and plasma glycerides a marginally significant correlation was noted and between urinary uric acid and plasma phospholipids a highly significant correlation was observed.

A full analysis was made of relationships between haemoglobin values and plasma and urinary uric acid and plasma lipid levels.

Blood group distribution was investigated in both gouty patients and controls and, while gouty subjects showed ABO and Rhesus distributions, the controls showed an unexpected relative deficit of Rhesus-negative subjects.

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