SCAT titre that those were the patients who benefited most. May I also briefly mention one additional side effect. One of our patients who developed loss of taste was a man of 66, he also developed a remarkable sustained state of priaprism of which he was extremely proud. He told a friend of mine, who rang me up urgently in the middle of lunch one day and said, 'What is this wonderful drug that you have given with this truly remarkable effect which also apparently relieves the symptoms of arthritis and can you spare some for me'?

DR. HUSKISSON We had a patient who illustrated another interesting new measurement for rheumatoid arthritis; when she started to get better with penicillamine she asked for the contraceptive pill. Our patients did not have vasculitis as far as we could tell. We did not have any patients with vasculitic skin lesions in this group. The latex test had no relevance to response, and there was no particular tendency for patients who had large falls in latex test to get better. We have not seen any patients with the side effect that you mention.

References

Aptekar, R. G., Atkinson, J. P., Decker, J. L., Wolff, S. M., and Chu, E. W. (1973) *Arthr. and Rheum.*, 16, 461
Multicentre Trial Group (1973) *Lancet*, 1, 275

Influence of Tubercle Aggregate Size on the Severity of Adjuvant Arthritis in the Rat. By S. P. LIYANAGE, H. L. F. CURREY, and B. VERNON-ROBERTS. (*The London Hospital and the London Hospital Medical College*)

It is known that adequate grinding of the dead tubercle aggregates before incorporation into Freund's complete adjuvant (FCA) is essential for producing polyarthritis in the rat, suggesting that the actual size of aggregate is critical. Our data support the conclusion that smaller aggregates are arthritogenic by virtue of their influence on the development of cellular immunity.

A series of metal sieves was used to separate ground tubercle aggregates into different sized ranges. Aggregates smaller than 90 μm proved essential to produce arthritis, larger aggregates failing to induce arthritis in any rats. Between 90 and 45 μm, there was no evidence that smaller aggregates produced more intense arthritis. Severe arthritis could be induced by the injection of volumes of FCA as small as 0·1 ml if the FCA contained small aggregates at a concentration of 6 mg/ml.

Cell mediated immune responses to tuberculous antigens studied by the delayed skin test, production of macrophage migration inhibition factor, and PHA-induced blast transformation showed that the smaller, arthritogenic aggregates clearly induced delayed hypersensitivity, while with the larger nonarthritogenic aggregates (180–250 μm) this effect was much less marked and variable. In contrast, small as well as large aggregates produced equal titres of antimycobacterial antibodies. Smaller aggregates also produced a significant reduction of peripheral blood lymphocytes bearing surface membrane immunoglobulin (B-cells) but no significant change in cells without surface immunoglobulin.

Incorporation of human serum albumin (HSA) in FCA revealed that tubercle aggregates of either size augmented the humeral antibody and the delayed skin responses (conventional adjuvanticity) to HSA to an equal extent.

Discussion

DR. P. A. BACON (*Bath*) It is an interesting piece of work, but I wonder if you have taken it far enough. Pearson, Koga, Narita, and Kotani (1973) in Los Angeles, have been looking at soluble fractions obtained from mycobacteria which can induce adjuvant arthritis. They, moreover, dissociated the arthritogenic fraction from the adjuvant fraction. If you go to a soluble fraction then you cancel out the effect of aggregate size.

DR. LIYANAGE Although we have not been able to split the aggregate into separate components, our results show that the arthritogenic effect is in fact different to the adjuvant effect, thereby confirming the findings you mention.

DR. I. C. M. MACLENNAN (*Oxford*) Is there any difference in antigenicity of the two fractions or are you splitting different types of antigens? That is, do small particles contain one type of antigen, large particles another type of antigen? Can you grind down your 180–250 μm sized particles and get the 45–63 effect?

DR. LIYANAGE I use the word aggregate rather than particle to point out that one aggregate is a collection of bacilli.

DR. H. BERRY (*London*) It is a very interesting piece of work and I enjoyed very much the way you presented it. I wonder, though, whether this isn't just a reflection of particle size related dissemination. Have you had a chance to look at radioactive-labelled bacillae to see whether you were not just dealing with altered dissemination?

DR. LIYANAGE We have not done radioactive studies. We have looked at the lymph nodes histologically, and even on day + 1 the abdominal lymph nodes of animals injected with large aggregate adjuvant show tubercle bacilli, albeit within the adjuvant droplets. Further, the equal titres of antimycobacterial antibody to the small as well as the large aggregates signifies that aggregates of both sizes are being transported. The adjuvanticity experiments showed that there was no difference between the small and the large aggregates in augmentation of humoral antibody response and delayed skin response, again signifying that the large aggregates also are getting through.

DR. I. M. ROITT (*London*) I wonder to what extent you are considering studying the two possible phases of production of arthritis by inducing low sensitivity to the mycobacteria in a different way, say with BCG or by passive transfer or sensitized cells in an inbred strain and then against this background of induced delayed sensitivity injecting different types of antigen microbacterial preparations.

DR. LIYANAGE We haven't considered that line yet.

References

Pearson, C. M., Koga, T., Narita, T., and Kotani, S. (1973) *Arthr. and Rheum.*, 16, 563