proteins such as plasma-albumin? The acidic non-steroidal anti-inflammatory drugs displace a number of compounds that are bound to albumin, so this factor might complicate the whole issue. I wonder if you have any evidence in relation to this?

DR. BERRY McCarty, Polcyn, Collins, and Gottschalk (1970) found that it was albumin-bound, but we were unable to prove this ourselves. There was no evidence that drugs displaced technetium from protein binding.

PROF. J. J. R. DUTHIE (Edinburgh) It would not be inappropriate to say that many of our young men have devoted a vast amount of energy and expertise to acquire information which might be obtained more readily by asking the patient a simple question, namely 'Do you feel better?'

DR. HUSKISSON It is because of the unreliability of this technique in the past that we are spending so much time and energy to find a better method.

A. SPEAKER We have been studying the metacarpal-phalangeal joints using technetium, and have found in some patients, who have been on large doses of salicylates, that, although there has been a clinical improvement, the technetium count rate has in fact increased. This may reflect displacement of technetium from its binding sites by the salicylates.

References

- Collins, A. J., and Ring, E. F. J. (1972) *Brit. J. Pharmacol.*, **44**, 145
 Jayson, M. I. V., and Barks, J. S. (1971) *Brit. med. J.*, **2**, 555
 Marks, J., Birkett, D. A., and Shuster, S. (1972) *Ibid.*, **1**, 782
 McCarty, D. J., Polcyn, R. E., Collins, P. A., and Gottschalk, A. (1970) *Arthr. and Rheum.*, **13**, 11

Search for Mycoplasma in Synovial Fluids from Patients with Rheumatoid Arthritis. By G. D. WINDSOR, A. NICHOLLS, R. N. MAINI, R. LEMCKE, D. G. EDWARD, and D. C. DUMONDE (*Kennedy Institute of Rheumatology, Wellcome Research Laboratories, and Lister Institute of Preventive Medicine, London*)

This communication describes attempts to isolate mycoplasmas from the joints of patients with rheumatoid arthritis. Reports of culture isolations have included different strains of mycoplasmas (Bartholomew, 1965; Jansson and Wager, 1967; Williams, 1968) and tiny colonies suspected to be a 'mycoplasma' (Jansson, Mäkisara, Vainio, Vainio, Snellmann, and Tuuri, 1971). One interpretation of the lack of uniformity of observations has been that the organisms were tissue culture contaminants or commensals of doubtful clinical significance. However, the findings of Williams and his colleagues of a particular species, *Mycoplasma fermentans*, in 40 per cent. of synovial fluids from patients with rheumatoid arthritis using sucrose gradient separation techniques, accompanied by an apparent specific *in vitro* cellular immunological reaction to the organism has led to speculation as to its possible aetiological significance (Williams, 1970; Williams, Brostoff, and Roitt, 1970).

73 samples of synovial fluids and nine membranes were obtained from patients with rheumatoid disease (including those with arthritis of recent onset) and with other conditions causing joint effusions. The samples were investigated for the possible presence of mycoplasmas:

(a) By direct culture in solid and liquid media. Some of the specimens were cultured in two independent laboratories.

(b) A sucrose gradient of a similar composition to that described by Williams (1968) was used to separate synovial fluid into visible bands by ultra-centrifugation, and 0.2 ml. volume of material from each band was cultured in liquid and solid medium.

No mycoplasmas were isolated by the direct technique from fourteen synovial fluids and homogenates from nine synovial membranes. The results of examining 69 synovial fluids on sucrose gradients are summarized below:

Fluids	RA	Non-RA
Number synovial fluids	64	5
Visible bands cultured	141	7
Mycoplasma isolations	0	0
Achromobacter isolations	13	0

A colour change to an acid pH was observed in the liquid medium of cultures of sixteen bands which on subculture yielded an organism belonging to the *Achromobacter* species. This was regarded as a contaminant as further isolation of these bacteria was prevented by suitable precautions. Mycoplasmas were not isolated from any of the samples examined.

Discussion

DR. D. TAYLOR-ROBINSON (London) What I have to say confirms what has just been said. I shall briefly summarize about 7 years' work; my colleagues and I have been looking for mycoplasmas in joints since 1965. We started in Salisbury obtaining specimens from a variety of places and after moving to Northwick Park Hospital in 1970 we continued the studies. Synovial membranes and synovial fluids have been examined, and although there were a range of diagnoses, rheumatoid arthritis comprised 109 of 146 cases. Most of these were acute exacerbations of chronic disease, and very few of them early cases. From one case of rheumatoid arthritis, we isolated a glucose-fermenting mycoplasma which was not identified further. In addition, we isolated T-mycoplasmas from the joints of two patients having polyarthritis and psoriasis. This was work done in collaboration with Dr. E. M. C. Dunlop and Prof. Barrie Jones. It is interesting that about 6 months ago Dr. P. Gill (McGill University) told me that he had isolated a T-mycoplasma from the joint of a boy suffering from Still's disease. In collaboration with clinicians at Taplow (Prof. Bywaters, Dr. Ansell, Dr. Gumpel, and Dr. Swannell) specimens have been obtained and divided, a portion being examined by us and a portion by Miss Auriol Hill at the Laboratory Animals Centre, Carshalton. The isolation of a mycoplasma from one patient was of particular interest, because there was a 16-year history of agammaglobulinaemia. Three years before the synovial membrane specimen was taken, a diagnosis of nodular rheumatoid arthritis had been made. At the time of admission to hospital the patient had a respiratory infection and an acute exacerbation of arthritis. The mycoplasma was identified as *Mycoplasma pneumoniae*. In view of the history of respiratory infection, the identification of the mycoplasma and its isolation in two separate laboratories, it is extremely unlikely to be a laboratory contaminant. Finally, my colleague Andrew Campbell has

examined specimens not only by conventional mycoplasma isolation methods but also by the sucrose density gradient technique. In no case was a mycoplasma isolated. In summary, it seems to me that it is possible very occasionally to isolate a mycoplasma from a joint. This does not seem unreasonable in a patient who has an immunity defect such as agammaglobulinaemia. On the basis of these studies I cannot believe that *Mycoplasma fermentans* is present in a large proportion of joints. I think, however, it is worth remembering that, in mycoplasma-induced arthritis in animals and birds, the organisms disappear although the manifestations of the disease continue. Although my enthusiasm for this sort of study is waning, I firmly believe the continued studies should be directed at looking at very early cases of rheumatoid arthritis where these can be obtained and certainly not at the chronic disease.

DR. K. N. LLOYD (*Cardiff*) I am a clinician and worked with Dr. Williams for some years. I think that there may be a transport problem here. Our specimens came from the patient and were straightaway stored at -70°C . and examined at leisure, can I ask at what temperature your specimens were transported by post?

DR. WINDSOR As the synovial fluid has to be centrifuged for 2 hours at 4°C . we thought it reasonable to transport them on ice. Transport time was about 2 hours. There were some synovial fluids that had been stored at -70°C . but the later ones were cultured direct.

DR. K. N. LLOYD (*Cardiff*) Concerning the band found on the sucrose density gradient, have you looked at it under the electron microscope to see whether there are any mycoplasma-like bodies?

DR. WINDSOR I should think that cultural techniques are more sensitive than electron microscopy; we can put *Mycoplasma fermentans* into synovial fluids and then recover one or two particles per ml. were the isolation techniques good enough.

DR. K. N. LLOYD (*Cardiff*) Dr. Taylor-Robinson and Dr. Mervyn Williams have stressed that the high isolation rate came from the early case. In only ten of your patients was disease duration 1 year or less. Finally, what was the clinical activity of these patients and in particular were they on gold therapy, because we know that gold is mycoplasmicidal?

DR. MAINI All the patients had active rheumatoid arthritis when the synovial fluid specimens were obtained, and they included ten whose arthritis was of very recent onset (a few months to one year). None of the patients was receiving drugs known to suppress mycoplasma growth, such as gold, chloroquine, or antibiotics.

DR. D. TAYLOR-ROBINSON (*London*) Dr. Dourmashkin has looked at some of these specimens by electron microscopy. If you look at the band from a gradient by negatively staining it, then you can see anything you wish to. What you have to do is to examine sectioned material. The best approach is to take a synovial fluid, centrifuge it, and section the deposit. When we isolated *Mycoplasma pneumoniae*, there was not the slightest doubt about it. The difficult problem is looking at specimens when mycoplasma have not isolated. Occasionally you can see things that strongly resemble mycoplasmas but there is no way of knowing whether they are or not.

PROF. D. L. GARDNER (*Belfast*) It is extremely important not to dismiss the controversial work of Mervyn Williams on this one additional piece of evidence. Fraser, Shirodaria, Haire, and Middleton (1971) presented extensive evidence using immunofluorescent and serological techniques that eleven mycoplasmal strains of two species could be cultured from joint specimens. They concluded, on the basis of their immunofluorescence studies, that immunoglobulins against the mycoplasmas were not present.

DR. D. TAYLOR-ROBINSON (*London*) I had the opportunity of looking at Prof. Fraser's isolates, some of which turned out to be *Mycoplasma hyorhinis*. This, as you know, is a mycoplasma usually isolated from pigs but also from mycoplasma-contaminated tissue cultures.

PROF. D. L. GARDNER (*Belfast*) No-one has mentioned diphtheroids. Before dismissing the *Achromobacter* species as contaminants it should be remembered that they can occasionally act as human pathogens and, if you dismiss *Achromobacter*, presumably you dismiss diphtheroids.

DR. MAINI Bacteria of the *Achromobacter* species that were isolated generally occurred before rigid precautions were undertaken to prevent contamination of the vials either during transport on ice or during centrifugation in series III. The data we showed you provides circumstantial evidence that these were contaminants.

PROF. J. J. R. DUTHIE (*Edinburgh*) We were well aware of the danger of contamination in our own work on diphtheroids. On the advice of the Professor of Bacteriology, we set up a further twenty cultures of synovial membrane or fluid, taking all possible precautions to avoid contamination, and in the end we found the same rate of isolation as in our previous work, namely 30 per cent. The diphtheroid isolates are very interesting in that they are potent adjuvants, can abrogate tolerance, and in mice can induce autoreactive antibodies against red cells. I am not claiming that diphtheroid organisms are the primary basic cause of rheumatoid arthritis because I do not believe that, but in view of their interesting properties they may have some role in the pathogenesis of this disease.

References

- Bartholomew, L. E. (1965) *Arthr. and Rheum.*, 8, 376 (Isolation and characterization of mycoplasmas (PPLO) from patients with rheumatoid arthritis, systemic lupus erythematosus, and Reiter's syndrome)
- Fraser, K. B., Shirodaria, P. V., Hane, M., and Middleton, D. (1971) *J. Hyg. (Camb.)*, 69, 17
- Jansson, E., Mäkitara, P., Vainio, K., Vainio, U., Snellmann, O., and Tuuri, S. (1971) *Ann. rheum. Dis.*, 30, 506 (An 8-year study on mycoplasma in rheumatoid arthritis)
- and Wager, O. (1967) *Ann. N.Y. Acad. Sci.*, 143, p. 535
- Williams, M. H. (1968) 'Recovery of mycoplasma from rheumatoid synovial fluid' in *Rheumatic Diseases*, ed. J. J. R. Duthie and W. R. M. Alexander, p. 171. Pfizer Medical Monograph No. 3 University Press, Edinburgh
- (1970) 'An immunological response to *Mycoplasma fermentans* in rheumatoid arthritis' in 'Impaired Cell-mediated Hypersensitivity in Man', ed. J. F. Jennings and D. J. Ward, pp. 43–47. Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry
- Brostoff, J., Roitt, I. M. (1970) *Lancet*, 2, 277 (Possible role of *Mycoplasma fermentans* in pathogenesis of rheumatoid arthritis)

Familial Occurrence of Psoriatic Arthritis. By J. M. H. MOLL and V. WRIGHT (*Rheumatism Research Unit, University of Leeds*).

Published in full in the *Annals* in May, 1973 (vol. 32, no. 2, p. 181)