Female mice classified as immune after the first exposure to the antigen almost invariably showed a secondary response, indicating the presence of antibodies. Results in male mice were more complex, suggesting an immune recognition without antibody production.

Serum and Synovial Fluid Complement in Rheumatoid and Other Arthritides. By J. WEBB and R. G. ROBINSON (*Sutton Rheumatism Research Laboratory, The Royal North Shore Hospital of Sydney, Australia*).

Total haemolytic complement in serum and synovial fluid samples, which were paired whenever possible, was estimated by a method modified from Kabat and Mayer (1961). A normal serum range was established in 42 healthy persons of 185 to 345 C'H₅₀ units/ml (mean 265 ± 2 SD). The rank sum test was applied to the results for statistical evaluation (Table).

TABLE Mean serum and synovial fluid complement levels with standard deviations (SD) and numbers in each of the various disease groups

Disease group	Haemolytic complement levels					
	Serum			Synovial fluid		
	No.	Mean	S.D.	No.	Mean	S.D.
Normal	42	265	40			
Osteoarthritis				30	60	
Acute gout	4	400	55	7	170	45
Reiter's disease	16	390	60	16	190	60
Polyarthritis nodosa	9	405	60			
Progressive systemic sclerosis	10	290	60			
Ankylosing spondylitis	10	285	65	8	70	35
Systemic lupus erythematosus				7	10	5
Rheumatoid arthritis						
Total	107	310	85	81	65	50
(i) seronegative	25	335	75	20	90	55
(ii) seropositive	64	315	75	48	65	45
(iii) with arteritis	18	245	85	13	25	35

SERUM The mean levels in ankylosing spondylitis (285) and progressive systemic sclerosis (290) did not differ from those in normal subjects (260). There were significant elevations in gout (400), Reiter's disease (390), polyarthritis nodosa (405), and rheumatoid arthritis (310). Within the group of rheumatoid arthritides, the levels in those with complicating arteritis (245) were significantly lower than in other seropositive (315) or seronegative (335) cases.

SYNOVIAL FLUID The mean levels in inflammatory rheumatoid disease (65) and ankylosing spondylitis (70) effusion did not differ from those in non-inflammatory osteoarthritis (60). Levels were high in gout (170) and Reiter's diseases (190) and very low in systemic lupus erythematosus (10). When arteritis complicated rheumatoid arthritis (25), the levels were significantly lower than in cases of uncomplicated rheumatoid arthritis (75).

RHEUMATOID PLEURAL EFFUSION Very low complement levels were found in three cases (5, 10, and 15).

There was a significant correlation between complement levels in paired serum and synovial fluid in cases of rheumatoid arthritis (rank correlation coefficient, $R = 0.658$). However, the strength of this correlation is not great because of wide scatter and is by no means predictive. There was no correlation between complement and

rheumatoid factor levels in either serum or synovial fluid.

These findings support the evidence for local auto-immune mechanisms in rheumatoid synovitis, and provide some evidence for more general autoimmune disease when systemic arthritis complicates rheumatoid disease.

Reference

Kabat, E. A., and Mayer, M. M. (1961) 'Experimental Immunology', 2nd ed., p. 149. Thomas, Springfield, Ill.

Discussion

DR. A. G. S. HILL (*Stoke Mandeville*) Was the synovial fluid complement level lower than the serum level in all groups?

DR. WEBB Yes.

DR. A. G. S. HILL (*Stoke Mandeville*) Presumably this is unlikely to be due to consumption of complement from the joint fluid: there is a diffusion barrier?

DR. WEBB This is probably related to lower protein levels in synovial effusions. We have not correlated protein and complement levels but they do correlate in normal subjects.

Calcium Pyrophosphate Deposits in Synovial Membrane.

By E. G. L. BYWATERS (*M.R.C. Rheumatism Unit, Canadian Red Cross Memorial Hospital, Taplow*).

Crystals of calcium pyrophosphate are known to occur in chondrocalcinosis or pseudogout (Žitnan and Šit'aj, 1963), in articular cartilage, synovial fluid, and synovial membrane (McCarty and Hollander, 1961), in hypophosphatasemia (O'Duffy, 1970), in haemochromatosis in similar locations (Atkins, McIvor, Smith, Hamilton, and Williams, 1970) as well as in the intervertebral discs in that condition and in hyperparathyroidism, and in ochronotic synovial membrane (Bywaters, unpublished data).

We now record calcium pyrophosphate crystal deposition in synovial membranes of three out of 43 consecutive rheumatoid arthritis patients removed during arthroplasties, and in two more since then. Each of the three patients had had chronic progressive erosive seropositive polyarthritis for over 12 years and each had shown the changes expected of rheumatoid arthritis in the tissues removed at arthroplasty. What was not expected was the presence of pyrophosphate. In one case, this was detected only microscopically: positively birefringent crystals were embedded in a basophil matrix and enclosed in giant cells, multinucleate and with eosinophil cytoplasm. In the second case, these deposits were numerous and associated with hydroxyapatite deposition in the synovial membrane. In the third case (who had had rheumatoid arthritis for 40 years, since the age of 30 with erosions, nodules, a raised erythrocyte sedimentation rate, and a positive latex test) arthroplasty showed an eroded joint covered with calcific deposits, visible grossly and also radiologically and microscopically. She had had neither steroid, gold, nor local cortisone injections. Serum calcium, phosphorus, and phosphatase were normal. Such crystal deposits occurred mainly in avascular villi and in joints with a considerable accumulation of necrotic bone fragments.

The wide spectrum of diseases with calcium pyrophosphate crystal deposition seems to imply an innate tendency for this to occur in the synovial milieu.

While in particular patients this might be due to hereditarily low phosphatase (O'Duffy, 1970), in others it

might be due to enzyme inhibition by metals such as Ca, Fe, Cu. In idiopathic chondrocalcinosis, Russell, Bisaz, Fleisch, Currey, Rubinstein, Dietz, Boussina, Micheli, and Fallet (1970) have shown that synovial fluid pyrophosphate is high, and that this is associated with low phosphatase and calcium.

Such studies might well be made in rheumatoid arthritis and particularly in those patients in whom calcific deposits are to be seen. Alternatively, such deposits, too small to be visible radiologically, may be quite common in older people and the combination with rheumatoid arthritis as recorded here may be a mere coincidence.

References

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 McCarty, D. J., and Hollander, J. L. (1961) *Ann. intern. Med.*, **54**, 452 (Identification of urate crystals in gouty synovial fluid)
 O'Duffy, J. D. (1970) *Arthr. and Rheum.*, **13**, 381 (Hypophosphatasia associated with calcium pyrophosphate dihydrate deposits in cartilage)
 Russell, R. G. G., Bisaz, S., Fleisch, H., Currey, H. L. F., Rubinstein, H. M., Dietz, A. A., Boussina, I., Micheli, A., and Fallet, G. (1970) *Lancet*, **2**, 899 (Inorganic pyrophosphate in plasma, urine, and synovial fluid of patients with pyrophosphate arthropathy (chondrocalcinosis or pseudogout))
 Zitnan, D., and Sit'aj, S. (1963) *Ann. rheum. Dis.*, **22**, 142 (Chondrocalcinosis articularis. I. Clinical and radiological study)

Discussion

DR. A. G. S. HILL (*Stoke Mandeville*) Is this commoner than the results indicate, since sections have to be processed quickly and this was a retrospective survey without precautions to avoid the disappearance of crystals during processing? Giant cells are common in rheumatoid synovium. Is this one cause of a giant cell reaction; either the main cause or a reaction to joint debris?

PROF. BYWATERS Giant cells in synovial membrane are of two types. One frequently seen is in the surface synovial layer and is not a foreign body reaction but part of the synovial hyperplasia of rheumatoid arthritis. The second is a foreign body giant cell occurring deeper in the synovial membrane which often contains bone or cartilage debris: here the cells may contain crystals.

The frequency of 3/43 is a minimal figure, but is a fair estimate for the method used, which was a detailed gross inspection of the fresh specimen. Sometimes one could recognise opaque white deposits, but we also found pyrophosphate in the microscopical sections without macroscopically visible deposit. Crystals should be confirmed where possible by examination of fluid or of teased-out synovium, but it is not absolutely necessary to process the tissue rapidly through the alcohols to find these crystals in sections. They can even be recognized in decalcified specimens, because when the crystals dissolve the surrounding matrix remains, in which imprints of the characteristic paralleloiped crystals are often seen.

DR. J. T. SCOTT (*London*) It would be interesting to know if crystals were seen in the synovial fluid of these patients, because this is a widely-used diagnostic procedure. I wonder too if there was any correlation with the previous intra-articular injection of hydrocortisone? This problem may not be related to rheumatoid arthritis alone—we have had one patient with an infective arthritis of the ankle and we recovered pyrophosphate crystals from the purulent joint fluid. We did not have an opportunity of seeing the synovium. There was no radiological calcification in this case, either at that time or subsequently.

PROF. BYWATERS We found crystals in one synovial fluid. One of the three cases previously had a bacteriologically verified septic arthritis in both knee joints, cured rapidly. Another had had a synovectomy of one of the two joints involved but the third had no known interference.

DR. C. G. BARNES (*London*) Was there any history in these patients of acute attacks of arthritis, especially mono-articular, which might have alerted one to a crystal synovitis?

I was interested to learn that there was no radiological calcification in the majority of your cases. Should one suspect more often a concomitant pyrophosphate crystal synovitis leading to an acute arthritis, which otherwise might be attributed, in the absence of calcification, to their rheumatoid arthritis?

PROF. BYWATERS The fifth patient (radiologically diagnosed by Dr. Hamilton) did not have acute attacks of arthritis although she did have an acute generalized flare of rheumatoid arthritis. Although we have little detail on the other four cases, we noted no acute attacks in the course of their rheumatoid arthritis. It is often difficult to interpret knee x rays for calcification where the joint space has disappeared.

DR. H. L. F. CURREY (*London*) We do not know the final common pathway leading to calcium pyrophosphate deposition—it is probably multifactorial. We know that it is age-related, that in areas of Chile and Czechoslovakia there is some familial influence producing early deposition, and that at least two systemic diseases produce it regularly. It may follow joint damage (perhaps combined with other factors). Joint fluids from patients with classical urate gout also may contain calcium pyrophosphate crystals. In our own and two other series of chondrocalcinosis, there was probably an increased incidence of Heberden's nodes and familial osteoarthritis. This might be another nonspecific factor leading to pyrophosphate disposition.

In examining many joint fluids from a variety of conditions over the past five years, we have been impressed that the presence of reasonably large numbers of crystals usually correlates well with clinical 'pyrophosphate arthropathy'. We have in general not found unexplained crystals in fluids from patients with rheumatoid arthritis, Reiter's disease, and psoriasis. When there is calcified cartilage and acute episodic attacks, crystals are almost always present. However, in examining joint tissue sections, our pathologist not infrequently finds occasional crystals in material from patients with rheumatoid and other arthropathies who show neither radiological nor clinical evidence of pyrophosphate arthropathy.

PROF. BYWATERS I agree that this is probably caused by many different conditions involving joint damage. Chondrocalcinosis is usually characterized as bilateral meniscal and triangular ligament calcification, but these RA deposits are more local and are probably secondary to joint damage. Finding these crystals depends upon how carefully one looks, and unless multiple sections are taken through these joints calcification will be missed.

DR. J. WEBB (*Sydney and Glasgow*) We have examined some 1,500 rheumatoid joint fluids and have found calcium pyrophosphate crystals in only one case. This was associated with generalized active polyarthritis in an otherwise well-controlled rheumatoid arthritic. In at least

twenty rheumatoid subjects with radiologically calcified synovium, we have not found crystals. This was the only instance of concurrence of the two diseases among our 87 cases of chondrocalcinosis.

DR. E. B. D. HAMILTON (London) Excluding our patients with haemochromatosis, we have seen about seventy cases of chondrocalcinosis in the last 5 years. This includes three patients with hyperparathyroidism and only one with clinical rheumatoid arthritis; although there were two others with positive tests for rheumatoid factor in low titre. More recently I have reviewed the x rays of 21 patients with hyperparathyroidism, seven of whom had chondrocalcinosis and two had co-existing rheumatoid arthritis.

Role of Cellular Immunity in the Pathogenesis of Amyloidosis. By E. S. CATHCART and A. S. COHEN (*Boston University Medical Center, Boston, Mass.*).

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Technical Difficulties in Interpreting Virus-specific IgM Antibodies in the Presence of Rheumatoid Factor. By P. V. SHIRODARIA, K. B. FRASER and C. F. STANFORD (*Belfast*).

Fluorescent antibody was used to detect virus-specific IgG and IgM in human sera. When four seropositive rheumatoid sera were tested, each produced IgM-specific staining of two or more virus antigens from the group measles, mumps, rubella, and herpes simplex. This effect corresponded to the presence of virus-specific IgG in the sera and was removed by the absorption of rheumatoid factor from the sera, using aggregated human IgG. Semi-purified rheumatoid factor, when added to four sera that each contained a different virus-specific IgG but no virus-specific IgM, caused IgM-specific staining of the virus to appear, but did not itself stain any virus antigen. In convalescent sera from 28 patients with virus infection (twenty patients under and eight over the age of 20 years), virus-specific IgM was found. In all but two patients this IgM staining was not removed by absorption of sera with aggregated human IgG. We conclude that there are two types of IgM staining: a *primary* staining that is caused by virus-specific IgM and a *secondary* staining that is caused by rheumatoid factor or like substances in some human sera.

Discussion

DR. P. D. FOWLER (Manchester) Did you obtain sera from children or adults following acute infections?

PROF. FRASER Mainly children.

Herpes Simplex Antibodies in Rheumatoid and Control Patients. By C. F. STANFORD, P. V. SHIRODARIA, and K. B. FRASER (*Belfast*).

A study of complement-fixing (CF) antibody in 45 rheumatoid patients and 45 age- and sex-matched controls showed that there was an inverse relationship between the titres of antibody to herpes simplex, measles, and mumps virus and the titre of rheumatoid factor. The mean antibody titres were higher in the controls than in the rheumatoid patients. After removal of rheumatoid factor with heat-aggregated human gamma globulin, the mean CF antibody titre for rheumatoid patients equalled that for

controls with mumps and became higher than that for controls with herpes simplex and measles (Stanford, C. F. *Ann. rheum. Dis.* (1972), 31, July issue).

Since IgM antibodies are frequently associated with recent infection, herpes simplex was selected, because of the above results, as the antigen to test the possibility of recent prolonged infection in rheumatoid patients. The effects of rheumatoid factor on the ability to stain virus-specific IgM by the indirect fluorescent method is discussed in the previous paper (Shirodaria, Fraser, and Stanford, submitted for publication in *Ann. rheum. Dis.*). In untreated sera, absorbed only with noninfected HEP2 cells, 37 of 45 rheumatoid patients showed strong IgM staining against herpes simplex infected HEP2 cells, while sixteen of 45 age- and sex-matched controls showed weak staining. After treatment of both rheumatoid and control sera with aggregated human gamma globulin, only seven of the rheumatoid and nine of the control sera showed IgM staining.

Thus, as might be expected from the well-known recurrence of herpes simplex in many patients, there is serological evidence of persistent or recent infection with this virus, but no great difference between rheumatoid and control patients [Further analysis of the data is to be published at a later date].

Discussion

DR. A. G. S. HILL (Stoke Mandeville) Is the final conclusion based only on herpes simplex or do you have corresponding figures for rubella?

PROF. FRASER Some years ago I did look for IgM staining with rubella virus. We got no more IgM staining with rubella virus in rheumatoid patients than in non-rheumatoid patients, but only six were involved, so that this result does not mean a great deal.

PROF. D. L. GARDNER (London) Have you any hypothesis to explain the mechanism by which this interference or blocking or removal mechanism takes effect, whether in the circulation or outside it?

DR. STANFORD I think that rheumatoid factor and complement compete for sites on the Fc fragment of the IgG molecule.

In the absence of rheumatoid factor any fluorescent staining indicates the presence of antiviral antibody in the IgG or IgM class (depending on which antiimmunoglobulin conjugates produce fluorescence). If rheumatoid factor is present it may attach to antiviral IgG and thus give the staining reaction for IgM. This is an artefact *in vitro*.

PROF. D. L. GARDNER (London) I always understood that rheumatoid factor circulated as 7S/19S (22S) complexes. If so, how can this interfere in the way suggested, and how do you account for the circulatory phenomena *in vivo*?

PROF. FRASER Our results indicate that rheumatoid factor is not bound to the antibodies that we are measuring *in vivo*, because we can separate it off from the patient's serum before we do the test, but if afterwards you add the antigen in a complement-fixation test, then it competes for attachment and interferes with the attachment of the complement. I think it is an indication that the rheumatoid factor that we are measuring is not bound to IgG *in vivo*,