factors, although experimental models suggest that cellular immunity is important in the induction of chronicity. We have investigated cell mediated immunity (CMI) to synovial-antigens in patients with rheumatoid arthritis and compared them to controls with gout or osteoarthritis.

CMI was tested in vitro, using the leucocyte migration test with microcapillary tubes, which diminish the variability in the test system. Synovial antigens were prepared from tissue extracted at synovectomy in two ways—either as a crude homogenate of tissue, after careful removal of fat and connective tissue, or as partially purified elution fractions, designed to extract in a step-wise fashion cell membrane lipoproteins or immune complexes.

The results, using ten rheumatoid synovial homogenates, showed that five of seven patients reacted to their own autologous synovium, while twenty of twenty-four other rheumatoid patients also reacted to these homogenates, but healthy controls and osteoarthritic subjects were negative. Further results with a larger specimen showed that rheumatoid subjects reacted to homologous rheumatoid synovial homogenate at a dose which did not inhibit the healthy controls. A similar homogenate from a psoriatic arthritis did not produce inhibition in the rheumatoid subjects or controls. Preliminary testing with synovial membrane elutions suggested that the activity is related to cell membrane fractions and not to immune complexes. An elution fraction producing inhibition of leucocyte migration was obtained from rheumatoid synovial membrane but not from psoriatic synovial membrane.

These results show that patients with rheumatoid arthritis have cell mediated immunity to an antigenic fraction of synovial membrane which is present in rheumatoid synovial membrane but not in the osteoarthritic or psoriatic synovial membranes tested.

**Behaviour of synovial complement C3 and C4 components**

<table>
<thead>
<tr>
<th>Native test sera</th>
<th>Native test sera + fresh human serum*</th>
<th>Inactivated test sera + fresh human serum*</th>
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<tr>
<td>Titer</td>
<td>Control R.A. SLE Scleroderma</td>
<td>Control R.A. SLE Scleroderma</td>
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R.A. = Rheumatoid Arthritis
SLE = Systemic Lupus Erythematosus
* as source of complement

**Figure** Binding of C3 (β1,c|β1,A) component of complement (indirect immunofluorescent technique)

A simple immunofluorescence method for differentiation of ANF between RA and healthy persons and SLE and scleroderma patients. By B. Stojan (Basle, Switzerland)

The indirect immunofluorescent staining technique to show the antinuclear factor (ANF) is very sensitive but the differential diagnostic value of this test is not ideal (MacSween and others, 1968; Müller-Eckhardt and others, 1968; Svec and Veit, 1967; Vischer and Ziff, 1969; Zitnan and Cebecauer, 1968). We believe that the properties of ANF to fix complement may improve the differentiation of ANF between RA and healthy persons and SLE and scleroderma patients.

The indirect immunofluorescence method was used to show the ANF and C3 binding. Three variations of test sera were employed, namely native test sera, native test sera with fresh human serum (as source of complement), and inactivated test sera with fresh human serum. The antisera used were Anti-Human Gamma Globulin (BBL) and Anti IgG (Behring-Werke) both marked with FITC. Anti β1,c|β1,A (C3) marked with FITC or Rhodamin was used for the mixed staining technique. (The monospecific sera were controlled by immunoelectrophoresis and the Ouchterlony technique.) A total of 86 ANF-positive sera were tested: 14 from healthy persons, 16 from RA patients who fulfilled the ARA criteria of classical or definite RA, 43 from SLE, and 13 from scleroderma patients. The diagnoses of the two latter groups were made by typical clinical pictures, biopsies, and in three cases confirmed with post-mortem examination.

**Results** (1) ANF-C3 complexes were found most frequently in SLE and scleroderma patients. (2) No ANF-C3 complexes were found in healthy persons with positive inflammatory and degenerative joint diseases, before and after synoviotomy. By R. Garay, A. Micheli, and G. H. Fallet (Department of Medicine, Division of Rheumatology, University of Geneva, Switzerland). Published in full in the Annals, 1975, 34, 166.
ANF, (3) From 16 patients with RA, only one showed a C3 titre of 1:80. (4) Among the 56 native test sera from SLE and scleroderma patients, 52 had a titre of 1:10 or more and 4 were negative. (5) ANF-C3 binding cannot be shown with sera that have been inactivated at 56°C for 30 min. (6) No correlation could be found between ANF titre and ANF-C3 binding. (7) The demonstration of C3 binding in native test sera had the highest differential diagnostic value (see Figure). The method is simple and the reproducibility very good.

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A study of the incidence of articular chondrocalcinosis in Paget's disease of bone. By I. Boussina, J. C. Gerster, J. Epiney, and G. H. Fallet (Department of Medicine, Division of Rheumatology and Medical Out-Patients Clinic, University of Geneva)

Several authors have suggested a relationship between Paget's disease and articular chondrocalcinosis (ACC), but in our opinion without sufficient proof. In order to determine whether such an association does in fact exist, ACC was systematically sought in 66 patients suffering from Paget's disease. Seventy-two subjects without Paget's disease, taken at random from a population of patients hospitalized for medical or surgical conditions, constituted the control group. They are of the same race and their age and sex distributions are similar to those of the patients suffering from Paget's disease.

Among the 66 pagetoid patients, average age (median) 76 years, 9 cases of ACC have been found. This represents an incidence of 13-6% of the group. Of the 72 control subjects, average age (median) 73 years, 7 were found to have ACC. This represents an overall incidence of 9-7% of the control group but the difference is not statistically significant.

From this study we conclude that ACC does not occur more frequently in Paget's disease than in a group of control subjects with the same age distribution.

Incidence of cathepsin D agglutinators in sera, synovial fluids, and exudate cells and synovial tissue of patients with RA and other rheumatic diseases. By K. Fehr, G. Artmann, M. Velvart, and A. Boni (Universitäts-Rehumaklinik, Zurich)

Incidence and titre of cathepsin D agglutinators (e.g. antibodies reacting specifically with human Fab2 produced by cathepsin D) are significantly raised in sera of patients with seropositive RA when compared with healthy blood donors, seronegative RA, SLE, ankylosing spondylitis, osteoarthritis, and trauma (P < 0.0005). Significantly raised levels are also found in synovial fluids of seropositive RA patients when compared with seronegative RA, other forms of arthritis, and osteoarthritis (P < 0.0005 to P < 0.01). In addition, cathepsin D agglutinators were found in the tissue culture medium of incubated synovectomy specimens from 7 out of 11 seropositive RA and 2 out of 7 seronegative RA, but not in 6 incubates of patients with other rheumatic diseases. In the sera the levels of these antibodies were positively correlated with the levels of RF if the RF were determined by IgG anti-CD Ripley coated erythrocytes, but not if the RF were determined by the Waaler-Rose or latex test.

By immunofluorescence studies using FITC-labeled Fab2, binding of Fab2 to synovial exudate inclusions (phagolysosomes) occurred in 100% of seropositive RA and about 80% of seronegative RA if the exudate cells showed evidence for phagocytosis of immune complexes. Preliminary results with rheumatoid synovium of both seropositive and seronegative RA patients suggest that mononuclear cells suggestive of plasma cells can bind labeled Fab2. These findings suggest that there might be a link between the production of cathepsin D agglutinators and agglutinating RF in seropositive RA; (2) that cathepsin D agglutinators may be produced in the synovium of RA patients; and (3) that cathepsin D agglutinators take part in the formation of immune complexes in the rheumatoid synovial exudate.

Frequency of the atypical gene E3* of serum cholinesterase among patients with ankylosing spondylitis. By A. Micheli (Department of Medicine, Division of Rheumatology, University of Geneva, Switzerland)

A familiar incidence of ankylosing spondylitis (AS) has been described on several occasions. In addition, it has recently been pointed out that HL-A 27 antigen is found with a high incidence in this disease.

The present study, initiated before this relationship between HL-A 27 and AS was known, was prompted by the discovery in twin sisters, homozygotes for the atypical E3* gene of serum cholinesterase, of a bilateral sacroiliitis. The question was tentatively raised of a relationship between the E3* cholinesterase gene and AS. In a preliminary study on 10 patients with AS, three were found to have the E3* gene, representing an incidence of 30% as compared to 5% in a large control population.

Among 115 cases presently being investigated, 7-8% have the E3* cholinesterase gene. A difference is thus still apparent, although not statistically significant, if the frequency of patients bearing the E3* gene is considered. However, if the frequency of the gene E3* itself is considered, since another homozygote was found among AS patients, it raises the percentage to 9-8 and the difference compared with the control group is statistically significant (P < 0.015).

In order to confirm this apparent relationship between cholinesterase atypical gene and AS, further studies would be necessary on other groups of patients, and the coincidence of this gene with some particular criteria or features of this rheumatic disease should be looked for. This aspect is presently under study.

Liver function tests and liver biopsies in patients with rheumatoid arthritis. By R. Rau, K. Pfenninger, and A. Boni (Rheumaklinik Staatspital Triemli und Universitäts Rheumaklinik, Zurich)

In patients with rheumatoid arthritis a total of 117 liver biopsies and liver function tests were performed. Liver
Proceedings: A simple immunofluorescence method for differentiation of ANF between RA and healthy persons and SLE and scleroderma patients.
B Stojan

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