*Annals of the Rheumatic Diseases*, 1980, **39**, 207–216

# Synovial fluid $\beta_2$ microglobulin and hydroxyproline fractions in rheumatoid arthritis and nonautoimmune arthropathies

D. MANICOURT,[1] H. BRAUMAN,[2] AND S. ORLOFF[1]

*From the [1]Department of Rheumatology and the [2]Department of Biochemistry, University Hospital Brugmann (ULB–VUB), Brussels*

SUMMARY Fourteen patients with classical and definite seropositive rheumatoid arthritis (RA), 5 patients with microcrystalline arthritis, and 7 patients with osteoarthrosis were studied with respect to markers of newly synthesised collagen (synovial NDOH pro levels); markers of connective tissue resorption (synovial DOH pro and NBH levels); markers of lymphoid tissue activity (synovial and plasma $\beta_2$m levels). Higher amounts of NDOH pro in RA synovial fluid are compatible with the hypothesis of a local connective tissue production as suggested by Uitto *et al.* on basis of a higher protocollagen proline hydroxylase activity in RA synovial tissue. DOH pro and NBH do not differ significantly in synovial fluid from RA or gouty patients, but the correlations between these forms of OH pro and, respectively, synovial lymphocytes and polymorphonuclear leucocytes are indicative of different processes of connective tissue remodelling in the 2 conditions. Synovial $\beta_2$m levels are a direct function of total synovial lymphocyte counts independently of the type of arthropathy being explored. The ratio of synovial to plasma $\beta_2$m is systematically above unity in RA patients only.

Recent work on $\beta_2$ microglobulin ($\beta_2$m) led to the discovery of its immunogenetic aspect, though its exact function in this respect is still unknown (Marx, 1974). This 11 700 molecular weight (MW) protein has a close homology with the constant domains of the heavy and light chains of immunoglobulins, especially with the CH3 domain (Berggard and Bearn, 1968; Peterson *et al.*, 1972; Smithies and Poulik, 1972; Cunningham *et al.*, 1973).

$\beta_2$m is normally present in low concentrations in serum and generally speaking in all biological fluids (Evrin *et al.*, 1971; Evrin and Wibell, 1972). The circulating $\beta_2$m, however, does not derive from a splitting of immunoglobulins, and its production does not depend only on B cell function (Cejka *et al.*, 1973; Hutteroth *et al.*, 1973; Nilson *et al.*, 1973). $\beta_2$m is a part of the major histocompatibility cell-membrane-bound antigenic complexes (Cresswell *et al.*, 1973; Grey *et al.*, 1973; Nakamuro *et al.*, 1973; Cresswell *et al.*, 1974; Peterson *et al.*, 1974; Tanigaki and Pressman, 1974) and is produced by lymphocytes (T and B) and cell lines of tumoral or nontumoral lymphoid tissues (Bernier and Fanger, 1972; Hutteroth *et al.*, 1973; Nilson *et al.*, 1973; Poulik and Bloom, 1973; Kithier *et al.*, 1974; Nilson *et al.*, 1974).

Except for cases of renal insufficiency or pregnancy, increased serum levels of $\beta_2$m were reported in patients with advanced malignant disease and in some inflammatory autoimmune disorders such as ulcerative colitis, regional enteritis, systemic lupus erythematosus (SLE) dysglobulinaemic purpura, and rheumatoid arthritis (RA) (Evrin and Wibell, 1973; Brauman *et al.*, 1977). Talal *et al.* (1975) reported elevated concentrations of $\beta_2$m in saliva and synovial fluid from patients with Sjögren's syndrome. In patients with RA we described increased circulating $\beta_2$m paralleling the lymphocytosis and reflecting the severity and extension of the joint involvement as well as the onset of extra-articular involvement (Manicourt *et al.*, 1978). An intense subsynovial lymphocytic and inflammatory cell infiltration characterises the RA joint cavity (Talal, 1972), and an increased collagenase production by this hypercellular rheumatoid synovial tissue may be an

Accepted for publication 23 June 1978

Correspondence to Dr S. Orloff, Hôpital Universitaire Brugmann, 4 Place Arth. Van Gehuchten, 1020 Bruxelles, Belgium.

important cause of joint destruction (Evanson *et al.*, 1968; Harris *et al.*, 1969; Wegelius *et al.*, 1970).

Hydroxyproline (OH pro) was described as a metabolic marker of the collagen whose biosynthesis is increased in RA synovial tissue (Uitto *et al.*, 1972). Among the different OH pro fractions dialysable OH pro (DOH pro) and mainly nonprotein bound OH pro (NBH) appear to be relevant markers of bone collagen disorders and especially of bone resorption (Kivirikko, 1970; Krane *et al.*, 1970; Bishop and

Table 1 *Group A patients with rheumatoid arthritis*

| | *Plasma* $\beta_2m$ *mg/l* | *Synovial* $\beta_2m$ *mg/l* | *Synovial OH pro* (μg/ml) | | | | *Synovial leucocytes count* $10^3/mm^3$ | | *JC* | *ESR mm/1h* | */LF* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *TOH pro* | *DOH pro* | *NDOH pro* | *NBH* | *Neutrophil polymorphs* | *Lympho-cytes* | | | |
| 1 | 1·07 | 2·02 | 15·5 | 8·7 | 6·8 | 5·8 | 10·50 | 4·11 | 1 | 24 | 40 |
| 2 | 2·48 | 4·15 | 15·0 | 8·4 | 6·6 | 6·0 | 13·20 | 4·03 | 2 | 60 | 80 |
| 3 | 1·74 | 6·74 | 17·0 | 10·4 | 6·6 | 8·0 | 14·10 | 3·34 | 2 | 85 | 640 |
| | | 6·60 | 14·0 | — | — | — | — | — | 2 | 85 | 640 |
| | 1·60 | 5·24 | — | — | — | — | — | — | 1 | 80 | 320 |
| 4 | 1·92 | 3·39 | 18·5 | — | — | — | 12·80 | 1·51 | 3 | 20 | 40 |
| 5 | 1·92 | 3·62 | 16·0 | 9·3 | 6·7 | 6·9 | 9·40 | 2·18 | 2 | 35 | 160 |
| 6 | 4·59 | 9·68 | 19·0 | 12·0 | 7·0 | 10·1 | 19·11 | 6·20 | 9 | 115 | 1280 |
| | 2·57 | — | — | — | — | — | — | — | 3 | 83 | — |
| 7 | 1·18 | 2·44 | 14·5 | 7·2 | 7·3 | 4·7 | 10·00 | 1·50 | 2 | 20 | 40 |
| | | 3·00 | 15·5 | 9·1 | 6·4 | 7·4 | 9·50 | 2·32 | 2 | 20 | 40 |
| 8 | 1·72 | 2·83 | 15·0 | 8·0 | 7·0 | 5·4 | 8·10 | 2·21 | 2 | 30 | 80 |
| 9 | 1·91 | 4·90 | 17·0 | 9·9 | 7·1 | 7·1 | 7·33 | 2·94 | 2 | 37 | 80 |
| | 2·13 | — | — | — | — | — | — | — | 2 | 110 | — |
| | 4·98 | 11·67 | 23·5 | — | — | — | 15·01 | 7·23 | 10 | 120 | 2560 |
| | 4·10 | — | — | — | — | — | — | — | 7 | 100 | 2560 |
| | 4·98 | 12·98 | — | — | — | — | 14·06 | 8·64 | 10 | 110 | 2560 |
| | 4·00 | — | — | — | — | — | — | — | 8 | 120 | — |
| 10 | 1·58 | 3·23 | 16·0 | 9·8 | 6·2 | 7·5 | 16·90 | 2·10 | 2 | 70 | 160 |
| | | 1·96 | 12·0 | 7·4 | 4·6 | 5·8 | 9·74 | 1·32 | 2 | 70 | 160 |
| | 2·00 | 3·60 | 16·0 | — | — | — | — | — | 3 | 55 | 320 |
| | | 5·20 | 19·0 | 11·5 | 7·5 | 8·7 | 12·70 | 6·25 | 3 | 55 | 320 |
| 11 | 2·82 | 5·26 | 16·0 | 9·2 | 6·8 | 7·0 | 8·90 | 3·47 | 4 | 100 | 640 |
| 12 | 1·20 | 2·40 | — | — | — | — | 10·50 | 2·20 | 2 | 30 | 160 |
| | | 3·60 | — | — | — | — | 11·50 | 2·90 | 2 | 30 | 160 |
| 13 | 2·20 | 3·90 | 18·5 | 9·0 | 9·5 | 7·4 | 9·26 | 4·22 | 2 | 60 | 640 |
| 14 | 1·24 | 3·42 | — | — | — | — | 8·91 | 0·53 | 1 | 53 | 320 |
| M | 2·45 | 4·86 | 16·6 | 9·3 | 6·9 | 7·0 | 11·6 | 3·5 | 3·4 | 66 | 583 |
| ± SD | 1·25 | 2·95 | 2·5 | 1·4 | 1·0 | 1·4 | 3·1 | 2·1 | 2·8 | 34 | 816 |

Table 2 *Patients with microcrystalline osteoarthritis (Group B) and osteoarthrosis*

| | *Plasma* $\beta_2m$ *mg/l* | *Synovial* $\beta_2m$ *mg/l* | *Synovial OH pro* (μg/ml) | | | | *Synovial leucocytes count* $10^3/mm^3$ | | *ESR* |
|---|---|---|---|---|---|---|---|---|---|
| | | | *TOH pro* | *DOH pro* | *NDOH pro* | *NBH* | *Lymphocytes count* | *Neutrophil polymorphs* | |
| 1 | 2·70 | 1·94 | 15·5 | 11·0 | 4·5 | 9 | 2·2 | 7·80 | 55 |
| | | 1·55 | 16·4 | 13·7 | 2·7 | 11 | 1·4 | 9·40 | 55 |
| 2 | 1·99 | 1·30 | 17·5 | 14·0 | 3·5 | 12·5 | 1·0 | 11·90 | 80 |
| 3 | 1·94 | 1·58 | 12·0 | 7·5 | 4·5 | 5 | 1·30 | 5·80 | 30 |
| 4 | 2·66 | 2·22 | 13·5 | 11·2 | 2·3 | 8·7 | 2·74 | 7·00 | 60 |
| | | 2·10 | 16·0 | 11·0 | 5·0 | 9·3 | 1·20 | 9·50 | 60 |
| 5 | 3·10 | 2·40 | 12·5 | 9·5 | 3·0 | 10 | 2·00 | 7·90 | 45 |
| | | 2·55 | 13·0 | 8·0 | 5·0 | 6 | 2·20 | 6·90 | 45 |
| M | 2·5 | 2·0 | 14·6 | 10·7 | 3·8 | 8·9 | 1·8 | 8·3 | 54 |
| ± SD | 0·5 | 0·4 | 2·0 | 2·4 | 1·1 | 2·5 | 0·6 | 1·9 | 15 |
| *Group C: Osteoarthrosis* | | | | | | | | | |
| 1 | 1·92 | 1·57 | 9·0 | — | — | — | — | — | 18 |
| 2 | 1·20 | 0·83 | 10·0 | — | — | — | 0·67 | 0·31 | 25 |
| 3 | 1·63 | 1·07 | 7·5 | — | — | — | 0·82 | 0·18 | 30 |
| 4 | 1·90 | 1·30 | 8·5 | — | — | — | 1·10 | 0·63 | 37 |
| 5 | 1·40 | 1·00 | 9·2 | — | — | — | 0·76 | 0·57 | 18 |
| 6 | 1·70 | 0·93 | 8·0 | — | — | — | 0·75 | 0·12 | 24 |
| 7 | 2·53 | 1·66 | 8·7 | — | — | — | 1·00 | 0·02 | 20 |
| M | 1·7 | 1·2 | 8·7 | | | | 0·8 | 0·3 | 25 |
| ± SD | 0·4 | 0·3 | 0·8 | | | | 0·2 | 0·2 | 7 |

Smith, 1971; Kaye, 1971; Sagar *et al.*, 1971; Goulding *et al.*, 1974; Varghese *et al.*, 1973), whereas the non-dialysable OH pro (NDOH pro) are derived either from collagen newly synthesised and rapidly degraded or from fragments recently synthesised but not incorporated into tropocollagen (Krane *et al.*, 1967, 1970; Haddad *et al.*, 1970).

As $\beta_2$m production is associated with lymphocytes it was of interest to study the levels of $\beta_2$m in the synovial fluid of patients suffering from RA and to correlate these data with the synovial cytology, the synovial total and different OH pro fractions, and the degree of disease activity as expressed by the joint count (Hollander and McCarty, 1972), the erythrocyte sedimentation rate (ESR), the serum latex fixation test (LF), and the plasma $\beta_2$m levels (Manicourt *et al.*, 1978). Furthermore it was interesting to compare the data observed in RA with the values observed in degenerative or inflammatory nonautoimmune conditions.

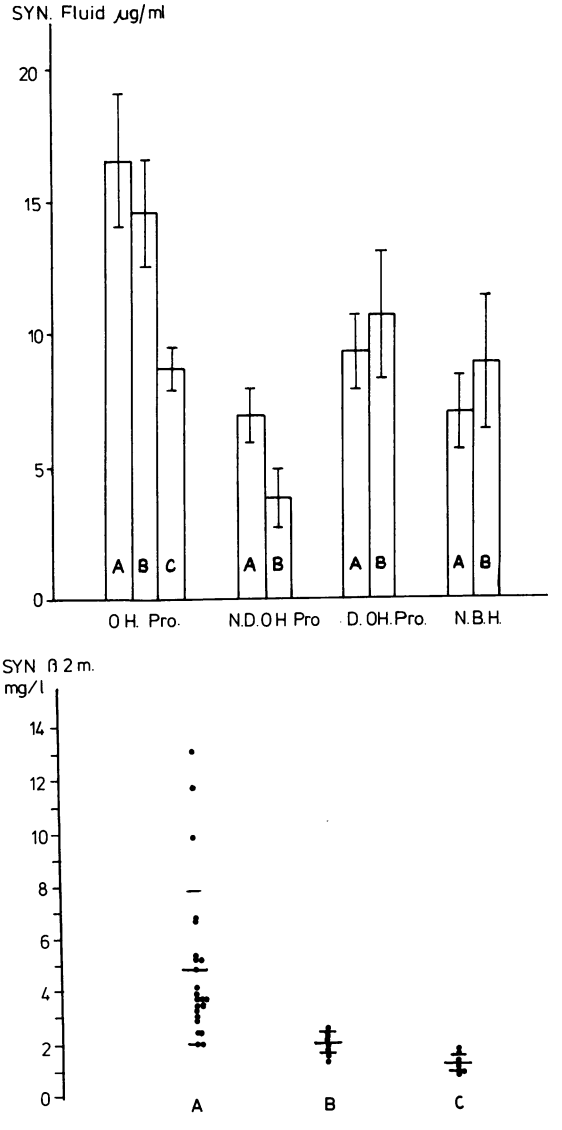


Fig. 1 (Upper) *Comparison of synovial total hydroxyproline levels (OH pro) in rheumatoid (group A), microcrystalline (group B), and osteoarthrotic patients (group C), and of synovial dialysable, nondialysable, and nonproteinbound hydroxyproline (respectively DOH pro, NDOH pro, and NBH) in rheumatoid and microcrystalline arthritides* (µg/ml). (Lower) *Comparison of synovial $\beta_2$m levels in rheumatoid arthritis (group A), microcrystalline arthritis (group B), and osteoarthrosis (group C). The traits indicate mean ± SD.*

## Patients and methods

Fourteen patients with classical and definite seropositive RA according to the criteria of the American Rheumatism Association were admitted in this study (group A), and patients with renal insufficiency amyloidosis or Sjögren's syndrome were excluded. The control group consisted of 5 patients presenting an inflammatory nonrheumatoid arthritis (gout or pseudogout, group B) and 7 patients with osteoarthrosis (group C).

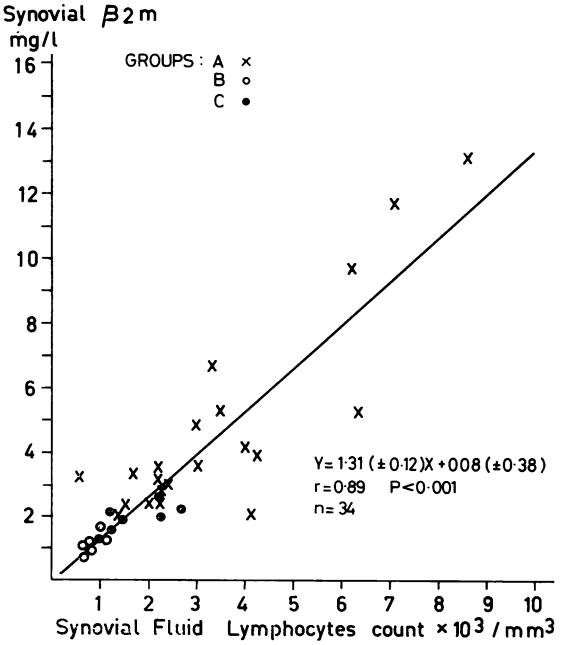


Fig. 2 *Linear correlation between synovial fluid $\beta_2$ microglobulin ($\beta_2$m) and synovial fluid lymphocyte count independently of the type of arthropathy.*

Blood and synovial fluid samples were collected simultaneously for $\beta_2$m and OH pro determinations as well as for other routine biological parameters (ESR, LF). On the same day a physical examination by a member of the staff evaluated the joint count (JC) according to standard directions (Hollander and McCarty, 1972).

$\beta_2$m was radioimmunoassayed by the Phadebas $\beta_2$m microtest (Phadebas Pharmacia, Uppsala, Sweden). The total and free OH pro (FOH pro) were measured in hydrolysed and unhydrolysed samples (Delfosse *et al.*, 1975); the nondialysable fraction (NDOH pro) was isolated (Haddad *et al.*, 1970) and the OH pro content was determined as

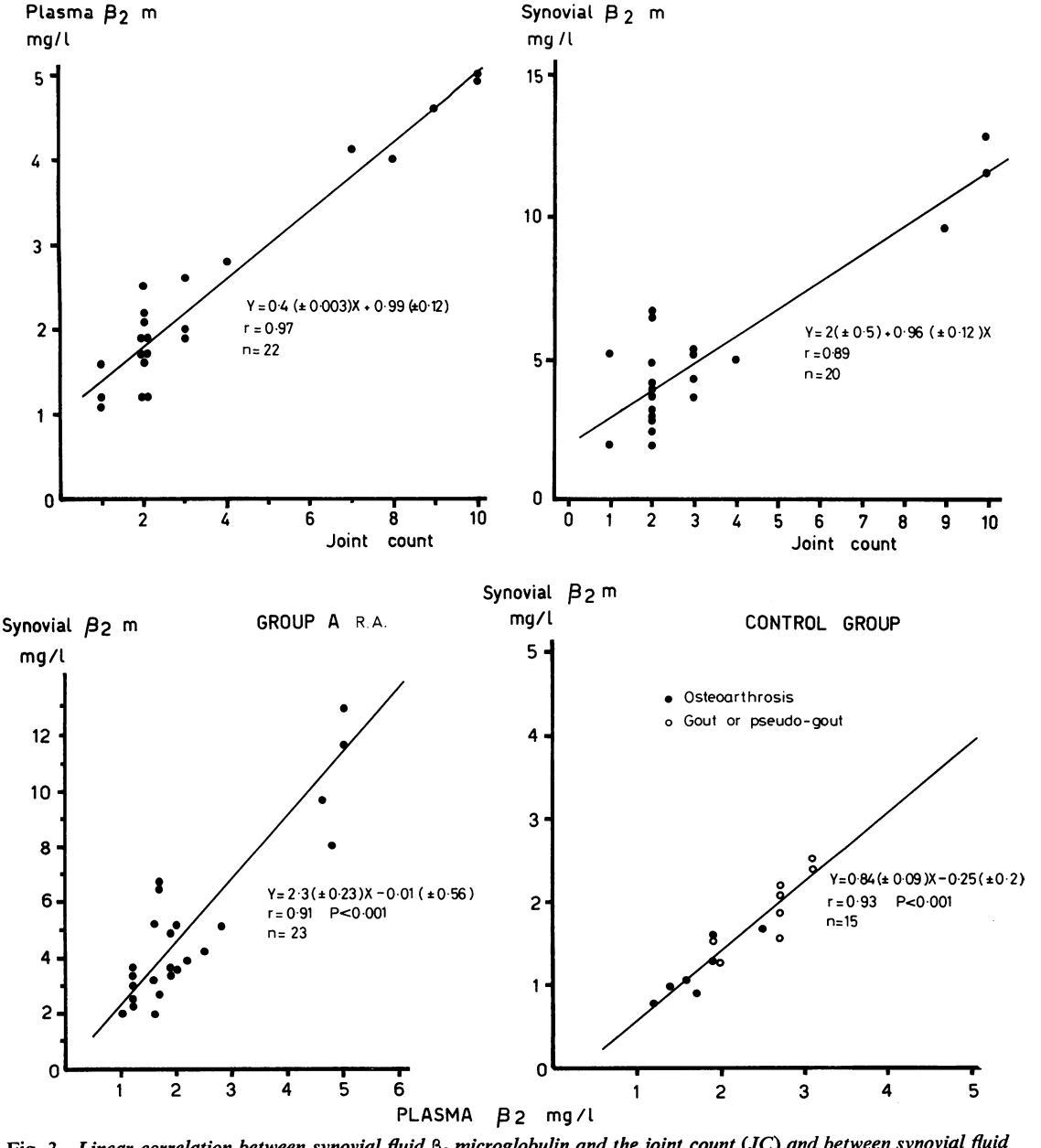


Fig. 3 *Linear correlation between synovial fluid $\beta_2$ microglobulin and the joint count (JC) and between synovial fluid $\beta_2$m and plasma $\beta_2$m in RA patients (group A) (mg/l).*

mentioned above. The dialysable fraction (DOH pro) was assessed by substracting NDOH pro from total OH pro and the NBH by substracting FOH pro from DOH pro. When several values for synovial fluid appear for the same patient, as in Tables 1 and 2, they were obtained from various joint cavities.

## Results

The relevant biological data are presented in Table 1 and 2.

Synovial $\beta_2$m levels are highest in RA patients (group A) and differ significantly between the 3 groups ($P<0{\cdot}001$) (Fig. 1).

Total OH pro levels for groups A and B (Fig. 1) are significantly higher when compared to group C ($P<0{\cdot}001$); no statistical difference is found between groups A and B ($P<0{\cdot}1$). The NDOH pro values (Fig. 1) in group A are significantly higher than those observed in group B ($P<0{\cdot}001$) whereas there is no statistical difference between the DOH pro and the NBH values in these 2 different groups (Fig. 1).

Irrespective of the type of arthropathy a signi-

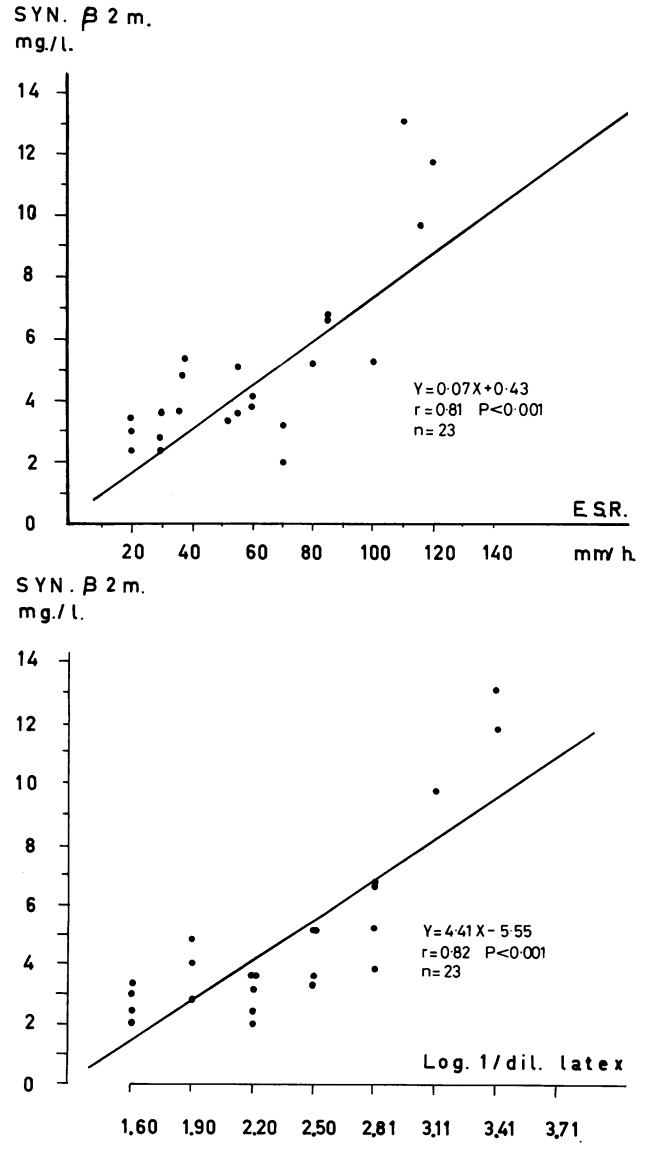


Fig. 4 (Upper) *Linear correlation between synovial fluid $\beta_2$ microglobulin ($\beta_2$m) and the erythrocyte sedimentation rate (ESR) in the RA patients* (Lower) *Linear correlation between synovial fluid $\beta_2$ microglobulin ($\beta_2$m) and the logarithm of the inverse of the latex fixation test (1/dil. latex) in RA patients (group A)*

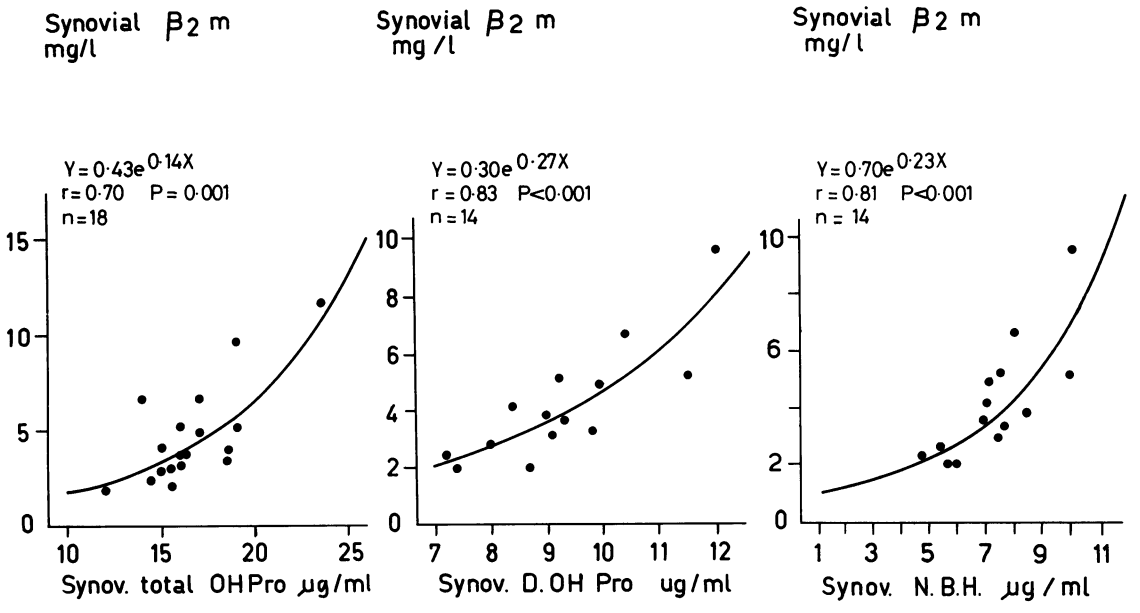


Fig. 5 *Correlation between synovial fluid $\beta_2$ microglobulin ($\beta_2$m) and synovial fluid total hydroxyproline (OH pro) dialysable hydroxyproline (D OH pro), and nonproteinbound hydroxyproline (NBH) in the RA patients (group A)*

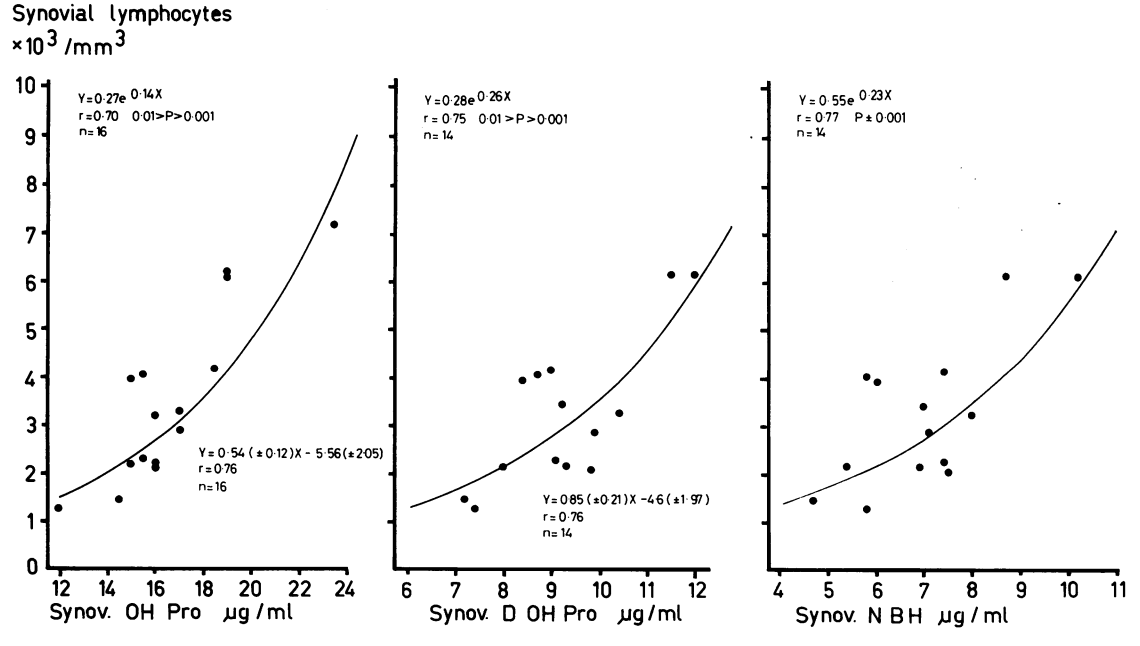


Fig. 6 *Correlation between synovial fluid lymphocyte count and synovial fluid total hydroxyproline (OH pro), dialysable hydroxyproline (D OH pro) and nonprotein-bound hydroxyproline (NBH) in RA patients (group A).*

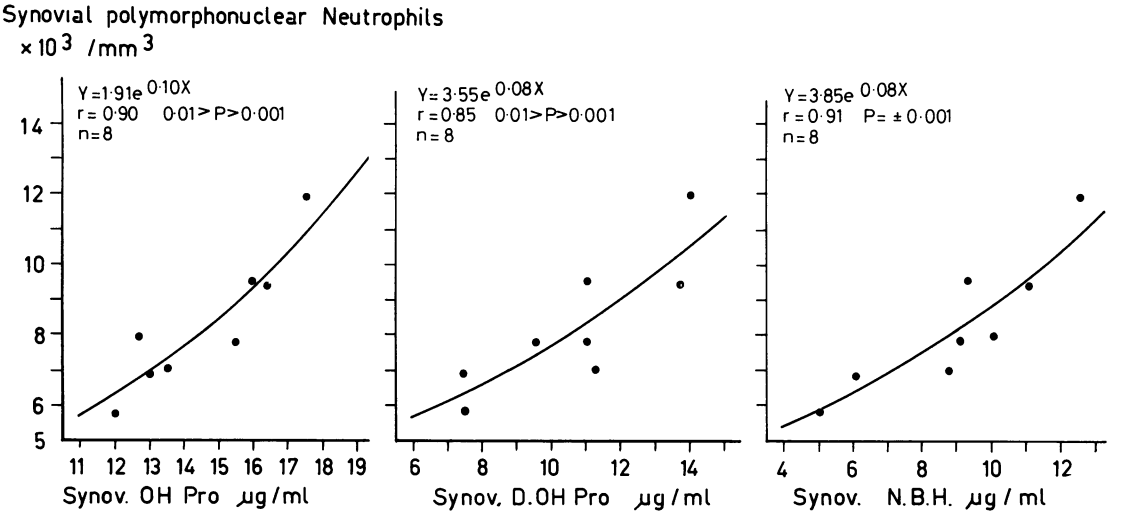


Fig. 7 *Correlation between the synovial fluid polymorphonuclear count and the synovial fluid total hydroxyproline (OH pro) dialysable OH pro (DOH pro) and nonprotein-bound OH pro (NBH) in the patients suffering from gout or pseudogout (group B).*

ficant positive linear correlation is found between the synovial $\beta_2$m levels and the synovial lymphocytosis (Fig. 2). In RA patients (group A) measurements of overall disease activity (JC, plasma $\beta_2$m levels, ESR, and LF) show a positive correlation with synovial $\beta_2$m levels (Figs. 3 and 4). Moreover, in the same patients (group A) the synovial $\beta_2$m values correlate significantly with the synovial total OH pro ($r=0\cdot70$; $P<0\cdot001$), with the synovial DOH pro ($r=0\cdot83$; $P<0\cdot001$) and with the synovial NBH ($r=0\cdot81$; $P<0\cdot001$) (Fig. 5). As might be expected from the data in Fig. 2, RA patients further show a positive correlation between synovial lymphocyte counts and synovial OH pro values, namely $r=0\cdot70$, $P<0\cdot01$, for synovial total OH pro; $r=0\cdot75$, $P<0\cdot01$ for synovial DOH pro; and $r=0\cdot77$, $P<0\cdot001$ for synovial NBH (Fig. 6). No such correlations were found in control groups.

Interestingly enough, in gouty patients (group B) the synovial OH pro fractions are correlated with the synovial polymorphonuclear neutrophil (PMN) count (Fig. 7) and not with the synovial lymphocyte count.

In none of the groups studied is there any correlation between synovial NDOH pro levels and synovial cells count (PMN or lymphocytes).

It is further noted that in each RA patient synovial $\beta_2$m levels are systematically higher than plasma $\beta_2$m levels in contrast to the 2 other groups (Tables 1 and 2).

## Discussion

From our results it emerges that synovial fluid NDOH pro is significantly higher in RA than in gouty patients and that no correlation exists between this fraction and the cellular content of the synovial fluid. Since NDOH pro is said to originate from fragments recently synthesised but not incorporated into tropocollagen (Krane *et al.*, 1967, 1970; Haddad *et al.*, 1970), a higher content of this fraction could be evidence of a higher rate of collagen biosynthesis, which agrees with a protocollagen proline hydroxylase hyperactivity in RA synovial tissue (Uitto *et al.*, 1972). In this respect RA joint connective tissue remodelling would appear to differ from microcrystalline arthritis on the grounds of the presence of an active repair process that we cannot demonstrate in microcrystalline arthritis. Nevertheless the high OH pro content of C1q (MW 40 9000; 45 residues per 1000; Reid *et al.*, 1972) may also reflect on the synovial NDOH pro levels and account for the difference seen between RA and gouty patients. We were not in a position to measure systematically the synovial complement levels in our patients, and it is thus impossible to assert to what extent synovial NDOH pro reflects connective tissue activation or increased complement levels or both. However, the usually low C1q synovial values in seropositive RA and the generally normal C1q synovial levels in gout (Hedberg, 1973; Versey *et al.*,

1973; Bunch *et al.*, 1974; Williams, 1976) suggest that NDOH pro in RA reflects rather connective tissue activation. This conclusion would hold unless it can be shown that the low levels described in synovial fluid are not only due to a high consumption rate but to a turnover that is fast enough to produce more NDOH pro in RA than in gout but also specific enough to stop at this stage, since total OH pro appears to be identical in synovial fluid of both conditions.

The amounts of synovial total OH pro, DOH pro, and NBH do not significantly differ in fluids from patients with RA or gout, but the correlation between these OH pro fractions and the 2 types of synovial fluid cells (Figs. 6 and 7) indicates that their production is related to different mechanisms involved in these different inflammatory processes rather than to different rates of connective tissue remodelling. Indeed synovial fluid PMN cells have a key role in the pathogenesis of gout or pseudogout, where it has been shown that, when leucocytes take up urate or calcium phosphate crystals, the latter interact with the lysosomal membrane, causing its rupture, with the liberation of collagenase and various proteolytic enzymes responsible for articular destruction (McCarty *et al.*, 1962; Weissmann, 1966, 1967, 1972; McCarty, 1971; Wright and Malawista, 1973).

In contrast the positive correlation between lymphocyte count and DOH pro or NBH levels in synovial fluid from RA patients (Fig. 6) stresses the primordial contribution of lymphocytes in RA joint injury and destruction. Indeed PMN do not seem to play a key role in RA joint lesions. PMN are not characteristically seen both at the invasion zones and in the synovium itself, where the infiltrate mainly comprises mononuclear cells. Collagenase not originating from PMN has been detected at the cartilage/pannus junction (Woolley *et al.*, 1977). Moreover, macrophages incubated with lymphokines can product collagenase (Wahl *et al.*, 1975), and Dayer *et al.* (1977) have recently reported that lymphocytes produce a low molecular weight factor (MW 11 500) stimulating collagenase production by rheumatoid synovial cells in culture.

Our observation that synovial $\beta_2$m levels are a direct function of synovial lymphocyte count irrespective of the joint pathology (Fig. 2) is not surprising since the lymphoid tissue is well known to be a major source of $\beta_2$m (Bernier and Fanger, 1972). Synovial $\beta_2$m overproduction (Tables 1 and 2) and the correlation between markers of bone collagen resorption (namely, DOH pro and NBH) and synovial $\beta_2$m levels on the one hand (Fig. 5) and synovial lymphocyte count on the other (Fig. 6) are observed only in RA patients whose overall disease activity is already shown to correlate with synovial $\beta_2$m levels (Figs. 3 and 4). These data appear as an in-vivo confirmation of Dayer *et al.*'s in-vitro observation of the stimulating effect of lymphocytes on collagenase production (Dayer *et al.*, 1977). Synovial $\beta_2$m can be thus considered as a fair index of synovial lymphoid tissue overactivity in RA and of the severity of RA joint involvement and destructive potential. Since $\beta_2$m has no known function per se (either as an enzyme or as a stimulator), and in absence of further characterisation of the low molecular weight (MW 11 500) factor described by Dayer *et al.* (1977), it is at best to consider them as special evidence of lymphoid cells activity in RA. This is further substantiated by the observation that the synovial to plasma $\beta_2$m ratio is systematically raised above unity in only RA in contrast to non-autoimmune arthropathies (microcrystalline arthritis or osteoarthrosis), where it is systematically lower than unity, an observation that could eventually be useful in differentiating both conditions.

This work was supported by a grant from the National Bank of Belgium to the Rheumatology Laboratory (ULB–VUB). The technical help of Miss L. Hennes is greatly appreciated, and the authors thank Misses G. Demillecamps and J. Huyssens for secretarial work.

## References

Berggard, I., and Bearn, A. G. (1968). Isolation and properties of a low molecular weight $\beta_2$ globulin occurring in human biological fluids. *Journal of Biological Chemistry*, **243**, 4095–4103.

Bernier, G. M., and Fanger, M. W. (1972). Synthesis of $\beta_2$-microglobulin by stimulated lymphocytes. *Journal of Immunology*, **109**, 407–409.

Bishop, M. C., and Smith, R. (1971). Non-protein bound hydroxyproline in plasma in renal bone disease. *Clinica Chimica Acta*, **33**, 403–408.

Brauman, H., Manicourt, D., and Orloff, S. (1977). $\beta_2$ microglobulin in rheumatoid arthritis. *International Congress of Rheumatology–San Francisco.*

Bunch, T. W., Hurder, G. G., McDuffie, F. C., O'Brien, P. C., and Markowitz, H. (1974). Synovial fluid complement determination as a diagnostic aid in inflammatory joint disease. *Proceedings of the Mayo Clinic*, **49**, 715–720.

Cejka, J., Cohen, F., and Kithier, K. (1973). Serum $\beta_2$ microglobulin levels in normal children and sex linked agammaglobulinemia patients. *Clinica Chimica Acta*, **47**, 59–61.

Cresswell, P., Springer, T., Strominger, J. L., Turner, M. J., Grey, H. M., and Kubo, R. T. (1974). Immunological indentity of the small subunit of HLA antigens and beta-2. microglobulin and its turnover on the cell membrane-*Proceedings of the National Academy of Sciences of the USA*, **71**, 2123–2127.

Cresswell, P., Turner, M. J., and Strominger, J. L. (1973). Papain-solubilized HLA antigens from cultured human lymphocytes contain two peptide fragments. *Proceedings of the National Academy of Sicences of the USA*, **70**, 1603–1607.

Cunningham, B. A., Wang, J. L., Berggard, I., and Peterson, P. A. (1973). The complete amino acid sequence of $\beta_2$-microglobulin. *Biochemistry*, **12**, 4811–4822.

Dayer, J. M., Russel, R. G., and Krane, S. M. (1977). Collagenase production by rheumatoid synovial cells: stimulation by a human lymphocyte factor. *Science*, **195**, 181–183.

Delfosse, L., Orloff, S., Rao, V. H., Strosberg, E., and Verbruggen, L. (1975). Automated analysis of hydroxyproline in urine. *Indian Journal of Biochemistry and Biophysics*, **12**, 280–282.

Evanson, J. M., Jeffrey, J. J., and Krane, S. M. (1968). Studies on collagenase from rheumatoid synovium in tissue culture. *Journal of Clinical Investigation*, **47**, 2639–2651.

Evrin, P. E., Peterson, P. A., Wide, L., and Berggard, I. (1971). Radioimmunoassay of $\beta_2$-microglobulin in human biological fluids. *Scandinavian Journal of Clinical and Laboratory Investigation*, **28**, 439–443.

Evrin, P. E., and Wibell, L. (1972). The serum level and urinary excretion of $\beta_2$-microglobulin in apparently healthy subjects. *Scandinavian Journal of Clinical and Laboratory Investigation*, **29**, 69–74.

Evrin, P. E., and Wibell, L. (1973). Serum $\beta_2$-microglobulin in various disorders. *Clinica Chimica Acta*, **43**, 183–187.

Goulding, A., McChesney, R., and Irvine, R. O. H. (1974). Serum hydroxyproline and renal osteodystrophy in patients maintained on haemodialysis. *New Zealand Medical Journal*, **80**, 540–544.

Grey, H. M., Kubo, R. T., Colon, S. M., and Poulik, M. D. (1973). The small subunit of HLA antigens in $\beta_2$-microglobulin. *Journal of Experimental Medicine*, **138**, 1608–1612.

Haddad, J. G., Couranz, S., and Avioli, L. V. (1970). Non-dialysable urinary hydroxyproline as an index of bone collagen formation. *Journal of Clinical Endocrinology*, **30**, 282–287.

Harris, E. D., Dibona, D., and Krane, S. M. (1969). Collagenase in human synovial fluid. *Journal of Clinical Investigation*, **48**, 2104–2113.

Hedberg, H. (1963). Studies on the depressed hemolytic complement activity of synovial fluid in adult rheumatoid arthritis. *Acta Rheumatica Scandinavica*, **9**, 165–193.

Hollander, J. L., and McCarty, D. J. Jr. (1972). Methods for evaluating rheumatoid arthritis. Directions for eliciting data for the Activity Index—in Arthritis and allied conditions p. 431. Lea and Febiger: Philadelphia.

Hutteroth, T. H., Cleve, H., Litwin, S. D., and Poulik, M. D. (1973). The relationship between $\beta_2$-microglobulin and immunoglobulin in cultured human lymphoid cell lines. *Journal of Experimental Medicine*, **137**, 838–843.

Kaye, M. (1971). Recognition of osteodystrophy in chronic uremia. *American Journal of Medicine Science*, **261**, 182–187.

Kithier, K., Cejka, J., Belamaric, J., Al-Sarray, M., Peterson, W. D., Vaitkevicius, V. K. Jr., and Poulik, M. D. (1974). $\beta_2$-microglobulin: Occurrence in fetal life and malignancy. *Clinica Chimica Acta*, **52**, 293–299.

Kivirikko, K. I. (1970). Urinary excretion of hydroxyproline in health and disease. *International Review of Connective Tissue Research*, **5**, 93–163.

Krane, S. M., Munoz, A. Z., and Harris, E. D. (1967). Collagen-like fragments: excretion in urine of patients with Paget's disease of bone. *Science*, **157**, 713–716.

Krane, S. M., Munoz, A. Z., and Harris, E. D. (1970). Urinary polypeptides related to collagen synthesis. *Journal of Clinical Investigation*, **49**, 716–729.

Manicourt, D., Brauman, H., and Orloff, S. (1978). Plasma and urinary levels of $\beta_2$-microglobulin in rheumatoid arthritis. *Annals of the Rheumatic Diseases*, **37**, 328–332.

Marx, J. L. (1974). Immunology: Role of $\beta_2$-microglobulin. *Science*, **185**, 428–429.

McCarty, D. J. (1971). Pathogenesis and treatment of the acute attack of gout. *Clinical Orthopaedics*, **71**, 28–33.

McCarty, D. J., Kohn, N. M., and Faires, J. S. (1962). Significance of calcium phosphate crystals in synovial fluid of arthritic patients: 'pseudo-gout syndrome'. I. clinical aspects. *Annals of Internal Medicine*, **56**, 711–737.

Nakamuro, K., Tanigaki, H., and Pressman, D. (1973). Multiple common properties of human $\beta_2$-microglobulin and the common portion fragment derived from H.L.A. antigen molecules. *Proceedings of the National Academy of Sciences of the USA*, **70**, 2863–2865.

Nilson, K., Evrin, P. E., Berggard, I., and Ponten, J. (1973). Involvement of lymphoid and non-lymphoid cells in the production of $\beta_2$-microglobulin: a homologue of the constant domain of IgG. *Nature New Biology*, **244**, 44–45.

Nilson, K., Evrin, P. E., and Welsh, K. I. (1974). Production of $\beta_2$-microglobulin by normal and malignant human cell lines and peripheral lymphocytes. *Transplantation Review*, **21**, 53–84.

Peterson, P. A., Cunningham, B. A., Berggard, I., and Edelman, G. M. (1972). $\beta_2$-microglobulin. A free immunoglobulin domain. *Proceedings of the National Academy of Sciences of the USA*, **69**, 1697–1701.

Peterson, P. A., Rask, L., and Lindblom, J. B. (1974). Highly purified papain-solubilised HLA antigens contain $\beta_2$-microglobulin. *Proceedings of the National Academy of Sciences of the USA*, **71**, 35–39.

Poulik, M. D., and Bloom, A. D. (1973). $\beta_2$-microglobulin production and secretion by lymphocytes in culture. *Journal of Immunology*, **110**, 1430–1433.

Reid, K. B. M., Lowe, D. M., and Porter, R. R. (1972). Isolation and characterization of C1q, a subcomponent of the first component of complement, from human and rabbit sera. *Biochemical Journal*, **130**, 749–763.

Sagar, S., Borra, S., and Kaye, M. (1971). Thermolability of alkaline phosphatase in the evaluation of renal osteodystrophy. *Nephron*, **8**, 270.

Smithies, O., and Poulik, M. D. (1972). Initiation of protein synthesis at an unusual position in an immunoglobulin gene? *Science*, **175**, 187–189.

Talal, N. (1972). In *Arthritis and Allied Conditions*, p. 849. Edited by J. L. Hollander and D. J. McCarty. Lea and Fibiger: Philadelphia.

Talal, N., Grey, H. M., Zvaifler, N., Michalski, J. P., and Daniels, T. E. (1975). Elevated salivary and synovial fluid $\beta_2$-microglobulin in Sjogren's syndrome and rheumatoid arthritis. *Science*, **188**, 1196–1198.

Tanigaki, N., and Pressman, D. (1974). The basic structure and the antigenic characteristics of HLA antigens. *Transplantation Review*, **21**, 15–34.

Uitto, J., Lindy, S., Turto, H., and Vaino, K. (1972). Collagen biosynthesis in rheumatoid synovial tissue. *Journal of Laboratory and Clinical Medicine*, **79**, 960–971.

Varghese, A., Moorhead, J. F., and Wills, M. R. (1973). Plasma calcium and magnesium fractions in chronic renal failure patients on maintenance haemodialysis. *Lancet*, **2**, 985–988.

Versey, J. M. B., Hobbs, J. R., and Holt, P. J. L. (1973). Complement metabolism in Rheumatoid Arthritis. *Annals of the Rheumatic Diseases*, **32**, 557–564.

Wegelius, O., Klockars, M., and Vaino, K. (1970). Collagenolytic activity in synovial fluid cells in rheumatoid arthritis. *Annals of Clinical Research*, **2**, 171–174.

Wahl, L. M., Wahl, S. M., Mergenhagen, S. E., and Martin, G. R. (1975). Collagenase production by lymphokine—activated macrophages *Science*, **187**, 261–263.

Weissman, G. (1966). Lysosomes and joint diseases. *Arthritis and Rheumatism*, **9**, 834–840.

Weissman, G. (1967). The role of lysosomes in inflammation and disease. *Annual Review of Medicine*, **18**, 97–112.

Weissman, G. (1972). Lysosomal mechanisms of tissue injury in arthritis. *New England Journal of Medicine*, **286**, 141–147.

Williams, D. G. (1976). Complement and the rheumatic diseases. *Modern Topics in Rheumatology*, p. 114–120. Edited by G. R. V. Hughes. Heinemann Medical Books: London.

Woolley, D. E., Crossley, M. J., and Evanson, J. M. (1977). Collagenase at sites of cartilage erosion in the rheumatoid joint. *Arthritis and Rheumatism*, **20**, 1231–1239.

Wright, D. G., and Malawista, S. E. (1973). Mobilisation and extracellular release of granular enzymes from human leucocytes during phagocytosis; inhibition by colchicine and cortisol but no by salicylate. *Arthritis and Rheumatism*, **16**, 749–768.