Behaviour of synovial complement C3 and C4 components in inflammatory and degenerative joint diseases, before and after synoviotherapy

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Many authors have described a rise in total haemolytic complement in the synovial fluid (SF) of articular inflammatory processes, with the exception of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (Pekin and Zvaifler, 1964; Peltier, Coste, and Delbarre, 1966; Hedberg, 1967; Sonozaki and Torisu, 1970; Kourilsky, Peltier, and de Séze, 1972). Other investigators have shown an inverse correlation between the fluid complement activity and the titre of the latex test for rheumatoid factor in the SF and serum of RA patients (Hedberg, 1967; Vaughan, Barnett, Sobel, and Jacox, 1968; Lundh, Hedberg, and Laurell, 1970; Townes and Sowa, 1970; Hedberg, 1971; Hedberg and Laurell, 1972). It has also been shown that the decrease in components C2 and C4 was more marked than that of C3 in the SF from RA and SLE patients (Peltier and others, 1966; Ruddy, Britton, Schur, and Austen, 1969; Sonozaki and Torisu, 1970; Peltier and de Séze, 1971; Ruddy, Matsuura, Stillmann, and Austen, 1971).

The first objective of our study was to determine the concentrations of the components C3 ($\beta_1A$) and C4 ($\beta_1E$), expressed as the ratio of their SF concentration to serum concentration in the following three entities: (i) seropositive rheumatoid arthritis, RA+; (ii) seronegative rheumatoid arthritis, RA--; (iii) osteoarthritis, OA.

We also investigated the relationship between SF protein concentrations, the SF latex levels, on one hand, and the SF/serum ratio of $\beta_1A$ and $\beta_1E$, on the other.

Another of our objectives was the investigation of possible changes in our initial data that appear during a relapse, after an unsuccessful synoviotherapy with...
osmic acid. For this purpose C3, C4, and their relationship to different SF parameters mentioned above were analysed.

Material and methods

We studied 31 samples of SF in RA+ (from 25 knees) before synoviorthesis, and 24 samples (from 15 knees) after unsuccessful synoviorthesis (carried out by intraarticular injections of 100–200 mg osmic acid) (Boussina, Kuzmanovic, Esselinckx, and Fallet, 1974; Micheli, Boussina, and Fallet, 1975). The same study was repeated on 5 samples of RA− (5 knees) before and 4 samples (3 knees) after synoviorthesis.

These determinations were also carried out on 15 samples from 13 OA knees before synoviorthesis and 10 samples collected at the time of repeated relapses after synoviorthesis from 3 knees of OA patients.

The β₁A (C3) and the β₁E (C4) were measured by the radial immunodiffusion method of Mancini, Carbonara, and Heremans (1965) by using the specific antisera of Dutch Red Cross anti-β₁A (KH 41-10-P1) and anti-β₁E (KH 12-6-P1). The SF protein concentration was determined by the biuret method and the rheumatoid factor sought by the quantitative latex fixation test according to Rheins, McCoy, Burrel, and Buehler, (1957).

The SF concentration is expressed as the ratio of C3 (β₁A) and C4 (β₁E) SF concentration to serum concentration measured simultaneously (β₁A*/β₁A* and β₁E*/β₁E*). For simplification, these ratios will be expressed, in this study, as β₁A and β₁E.

Results

A The mean concentrations and standard deviations of β₁A and β₁E in RA+, RA−, and OA, before and after synoviorthesis, are shown in Table I. This table clearly shows a lower level of β₁E in RA+ before and after synoviorthesis compared to the RA− and OA fluids. This difference, which is significant in RA+ and OA before synoviorthesis, may be expressed as the ratio β₁A/β₁E indicated in Table I, II. Before synoviorthesis, the three conditions can be differentiated in a significant manner by this ratio. These differences persist after synoviorthesis but according to a statistical analysis, they are significant only between RA+ and OA.

B Fig. 1 illustrates the relationship between the level of SF protein concentration and the levels of β₁A and β₁E in RA+, RA−, and OA. In OA the concentration of β₁A and β₁E increases as the concentration of the fluid protein rises. However, this correlation does not seem to exist in RA+ nor in RA−.

C According to Hedberg (1967), the ‘complement activity’ in SF may be expressed by the following equation:

Complement activity = \[ \frac{C}{C^*} \times \frac{10}{\text{protein}} \times 100 \]

where C' is the SF complement concentration, C* the serum concentration measured simultaneously, and ‘protein’ is the SF protein concentration in g/100 ml.

By introducing this equation the ratios of β₁A*/β₁A* or β₁E*/β₁E*, derived from measurements by the method of Mancini and others (1965), the values indicated in Table II were obtained. In comparing RA+, RA−, and OA, it can be seen that for β₁E RA+ gives obviously lower values than OA, RA− has intermediate values. For β₁A, the differences between these various conditions are minimal. However, the comparison of components β₁A and β₁E, expressed by the values obtained from the above equation, had the same relationship as that calculated in Table I, II.

D Fig. 2 shows the relationship between the rheumatoid factor (latex test) and levels of β₁A and β₁E in RA+ before and after synoviorthesis. A significant inverse correlation exists between the titre of rheuma-

### Table I Levels of C3 and C4 and their relationship in RA+, RA−, and OA

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RA+</td>
<td>Before</td>
<td>31</td>
<td>0.41 ± 0.20</td>
<td>0.29 ± 0.20</td>
<td>P &lt; 0.001</td>
<td>1.5 ± 0.4</td>
<td>RA− P &lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>23</td>
<td>0.45 ± 0.18</td>
<td>0.36 ± 0.17</td>
<td>NS</td>
<td>1.4 ± 0.7</td>
<td>OA P &lt; 0.001</td>
</tr>
<tr>
<td>RA−</td>
<td>Before</td>
<td>5</td>
<td>0.61 ± 0.15</td>
<td>0.63 ± 0.22</td>
<td>NS</td>
<td>0.98 ± 0.05</td>
<td>See above and below</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>4</td>
<td>0.62 ± 0.18</td>
<td>0.63 ± 0.25</td>
<td>NS</td>
<td>0.99 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>OA</td>
<td>Before</td>
<td>15</td>
<td>0.39 ± 0.14</td>
<td>0.51 ± 0.16</td>
<td>P &lt; 0.001</td>
<td>0.77 ± 0.09</td>
<td>RA+ P &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>10</td>
<td>0.46 ± 0.12</td>
<td>0.56 ± 0.23</td>
<td>NS</td>
<td>0.82 ± 0.18</td>
<td>RA− P &lt; 0.0005</td>
</tr>
</tbody>
</table>

* The concentrations of synovial fluid C3 and C4 are expressed as the ratio β₁A*/β₁A* and β₁E*/β₁E*, respectively.
toid factor (latex test) and \( \beta_1 \text{A} \) and \( \beta_1 \text{E} \) values: as the titre of rheumatoid factor increases fewer complement components appear in the SF. After synoviorthesis this correlation is not significant.

A statistical analysis was made using the values of \( \beta_1 \text{A}'/\beta_1 \text{A} \) and \( \beta_1 \text{E}'/\beta_1 \text{E} \) in RA+, RA−, and OA before and after synoviorthesis, in an effort to discover any changes due to treatment. No statistically significant differences were found. In other words, almost the same values were observed when a relapse of arthritis occurred in a joint treated by synoviorthesis.

**Discussion**

Several authors have concluded that C4 decreases much more than C3 in the SF of adult and juvenile RA patients (Peltier and others, 1966; Ruddy and others, 1969; Sonozaki and Torisu, 1970; Peltier and De Sèze, 1971). We obtained the same results in RA+. Ruddy and others (1969) reported a moderate depletion of C4 in SF of RA− as compared to that of OA. Similarly, we found a slight decrease of the \( \beta_1 \text{E} \) and not of the \( \beta_1 \text{A} \); however, SF protein concentrations must be taken into consideration. It is important to note that the anti-\( \beta_1 \text{A} \) sera used in this work cannot distinguish immunologically the \( \beta_1 \text{A} \) from

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**Table II** Concentration of synovial fluid C3 and C4 corrected for serum levels, and for synovial protein concentration

<table>
<thead>
<tr>
<th>Group</th>
<th>Synoviorthesis</th>
<th>No.</th>
<th>C3*</th>
<th>C4*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA+</td>
<td>Before</td>
<td>31</td>
<td>98</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>24</td>
<td>108</td>
<td>87</td>
</tr>
<tr>
<td>RA−</td>
<td>Before</td>
<td>5</td>
<td>144</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>4</td>
<td>126</td>
<td>128</td>
</tr>
<tr>
<td>OA</td>
<td>Before</td>
<td>15</td>
<td>132</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>10</td>
<td>114</td>
<td>139</td>
</tr>
</tbody>
</table>

*Values derived from the equation \( \frac{C1}{C1 \times 10/prot} \times 100 \) (Hedberg, 1967) where \( C1 \) and \( C1' \) are the values found by Mancini test in synovial fluid (f) and serum (s), respectively; 'prot' is the concentration of protein in synovial fluid, in g/100 ml.

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![Figure 1](image1.png)  
**Figure 1** Relationship between SF protein and \( \beta_1 \text{A}, \beta_1 \text{E} \) in RA+, RA−, and OA.

![Figure 2](image2.png)  
**Figure 2** Relationship between SF rheumatoid factor (latex test) and complement components \( \beta_1 \text{A}, \beta_1 \text{E} \).
βC. Therefore, by the method of Mancini and others (1965), it is not possible to evaluate the degree of activation or degradation of C3.

Compared to degenerative SF, the SF protein level and SF β1A and β1E are higher in RA−. In OA fluid a relationship exists between the value of these components and the protein level. This correlation does not appear in RA−, which suggests a certain consumption of complement in the synovial space. This impression is confirmed when ‘the complement activity’ is calculated according to Hedberg’s (1967) equation (Table II). This formula utilizes the coefficient of SF ‘protein concentration’. As shown in Table II, important differences in the β1E appear between RA+, RA−, and OA. The indicated values, obtained from mean concentrations of each group, show a low consumption of β1E in RA−, at least in some cases. Concerning β1A, the differences between these three conditions are slight, particularly between RA− and OA.

On further consideration of the β1A concentration, a phenomenon was encountered in our study which might have a certain practical or theoretical value: in RA+ fluids the β1E concentration is much more depleted than that of β1A (P < 0·001), whereas in OA this phenomenon is significantly reversed (Table I, II). If one considers that the majority of complement components pass from the blood to the synovial space, it is logical to assume that β1E depletion reflects a local consumption, which is greater than that for β1A, as we have previously shown. In OA the lower level of β1A as compared to β1E might signify a preferential permeability of the synovial membrane for C4. This probably does not depend on its molecular weight (slightly larger than that of C3), but rather on its larger glucidic moiety. In OA, C3 does not seem to be involved in a ‘by-pass’ activation process (P. H. Lambert, personal communication, 1974; Tesar, Kazmar, and George, 1973; Götze, Zvaifler and Müller-Eberhard, 1972). It is probable that C4 is produced in synovial tissue (macrophages), which might account for its relative increase in OA in the absence of consumption.

Many published reports, except that of Sonozaki and Torisu (1970), have pointed out an inverse correlation between total haemolytic complement or C3 and C4 on the one hand, and SF rheumatoid factor on the other. We found a statistically significant inverse correlation between synovial β1A, or β1E and rheumatoid factor.

Recurrence of inflammation in the treated joint causes no significant difference in the concentration of both β1A and β1E, compared to the findings before synoviorthesis, in the three conditions considered: β1E was relatively lower than β1A in RA+; the inverse being true in OA, although the difference was not statistically significant.

In conclusion, this work confirms the consumption of synovial complement in RA+, which is also observed in RA− to a lesser degree. A peculiar concentration of synovial C4 compared to C3 was found in OA, which does not appear to be caused by an ‘alternate pathway’, lowering C3, but rather by a definite relative increase of C4.

In addition, the present study suggests that in RA when local relapses occur after osmic acid synoviorthesis the initial pathogenetic process is maintained but that the immunological mechanisms are barely influenced by this treatment. In OA also a recurrence differs very little from the inflammatory process observed before synoviorthesis.

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Note added in proof

Since this paper was submitted for publication, these studies were extended to a total of 45 RA +, 8 RA −, and 19 OA patients. The values found at this stage, in each group of patients, for the ratio C3/C4 were 1-46 ± 0-44, 09-6 ± 0-10, and 0-76 ± 0-11, respectively, before synoviorthesis. These values show highly significant differences between groups at levels which confirm those indicated in Table I, II.
Behaviour of synovial complement C3 and C4 components in inflammatory and degenerative joint diseases, before and after synoviotomy.

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