Vitamin B₁₂ Metabolism in the Synovial Fluid in the Patients with Joint Diseases

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Vitamin B₁₂ levels and its binding capacity in the synovial fluid of the patients with joint diseases have been investigated, and the presence of vitamin B₁₂ and its binding capacity in the synovial fluid have been demonstrated.

Synovial B₁₂ levels in the patients with rheumatoid arthritis were much lower than those in osteoarthritis, whereas B₁₂ binding capacity in the synovial fluid of rheumatoid arthritis was higher than that in osteoarthritis.

These results suggest that B₁₂ metabolism in the synovia is related to the clinical features seen in rheumatoid arthritis.

Since vitamin B₁₂ was recognized to have a remarkable effect on pernicious anemia (1), its characteristics have been investigated in many respects from the biochemical and clinical aspects, but much less information is available concerning the presence of vitamin B₁₂ in the synovial fluid of human subjects.

In the present study, the authors attempted, for the first time, to determine the presence of vitamin B₁₂ and its binding capacity in the synovial fluid of the patients with joint diseases.

MATERIALS AND METHODS

For this study, patients with classical rheumatoid arthritis were chosen who would fulfill the criteria of the American Rheumatism Association. Synovial fluid was taken from the patient with osteoarthritis and rheumatoid arthritis.

Vitamin B₁₂ concentration in the synovial fluid was determined according to the methods of Ford (2), Kamikubo (3) and Subcommittee on B₁₂ Determination of the Vitamin B₁₂ Research Committee of Japan (4).

_Lactobacillus leichmanii_ ATCC 7830 and the stock culture, preculture and assay media of Nissan were used. (Nissui Pharmaceutical Co., Ltd. Tokyo, Japan).

Determination of B₁₂ binding power of the synovial fluid was carried out in the following way.

One ml of synovial fluid was mixed with an equal volume of radiocyanocobalamin (⁵⁷Co-CN) solution (60 μg/ml) with a specific activity of 1.1 μCi/μg purchased from Radio Chemical Centre, England.

The mixture as well as controls were incubated at 37° for 60 minutes, and then dialyzed in Visking cellophane tubes against running tap water at 4° for 48 hours.

The content inside the tube was then transferred quantitatively into a plastic container and its radioactivity was measured with a well type scintillation counter (Aloka TDC-6 PS-9 Tokyo, Japan).

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Vitamin B$_{12}$ binding capacity of synovial fluid was calculated in terms of undialyzable radioactivity, and nitrogen concentrations of synovial fluid were determined according to Lowry et al.

Synovial B$_{12}$ binding: N ratio was represented by \( \frac{1}{X} \cdot 10^{-3} \times \left( \frac{\text{Nitrogen content}}{\text{Binding capacity of B}_{12}} \right) \).

**RESULTS AND DISCUSSION**

Vitamin B$_{12}$ levels in the synovial fluid of the patients with osteoarthritis and rheumatoid arthritis were 0.56±0.15 μg/ml and 0.33±0.06 μg/ml, respectively.

B$_{12}$ binding: N ratio of synovial fluid in the patients with rheumatoid arthritis and osteoarthritis was 1/217.3±14.7 and 1/324.6±53.0, respectively.

In the present study, we have verified, for the first time, the presence of vitamin B$_{12}$ in the synovial fluid and its binding capacity and that there are significant differences in synovial B$_{12}$ levels between rheumatoid arthritis and osteoarthritis (\( P<0.01 \)). These results indicate that low levels of B$_{12}$ in the synovial fluid are characteristic findings in rheumatoid arthritis and that B$_{12}$ probably participates in the appearance of clinical features of rheumatoid arthritis. However, synovial B$_{12}$ binding capacity in the rheumatoid arthritis was higher than that in osteoarthritis (\( P<0.01 \)). It is presumed that synovial B$_{12}$ levels in the rheumatoid arthritis are insufficient and, subsequently, B$_{12}$ binding capacity is increased compensationally to normalize the synovial metabolism.

**TABLE 1**

<table>
<thead>
<tr>
<th>Vitamin B$<em>{12}$ levels and vitamin B$</em>{12}$ binding: N-ratio in the synovial fluid of patients with joint diseases</th>
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<tbody>
<tr>
<td>No. of case</td>
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<tr>
<td>Synovial B$_{12}$ level (μg/ml)</td>
</tr>
<tr>
<td>Synovial B$_{12}$ binding: N-ratio (×10⁻³)</td>
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</tbody>
</table>

* in t-test of Fisher
** standard error of the mean

No specific vitamin deficiencies have been found to be a cause or even a constant part of rheumatoid arthritis, but there are some reports on the characteristic changes in vitamin metabolism in this disease.

Sugiyama (5) reported a decrease of riboflavin contents in the synovial fluid and blood in the patients with rheumatoid arthritis as compared with those in osteoarthritides of healthy subjects, and suggested that lowering of phosphorylation of riboflavin in the blood and synovial fluid in the rheumatoid arthritis resulted from disorder of the pituitary adrenal axis.

Gough et al. (6) reported that patients with rheumatoid arthritis may develop megaloblastic anemia due to a deficiency of or greater need for folic acid.

Fukui (7) recently reported that there are close relationships between B$_{12}$ metabolism and rheumatoid arthritis from the changes in $^{57}$Co-cyanocobalamin distribution in the organs and the B$_{12}$ binding capacity of rat stomach receiving the treatment of oral administration of synovial fluid of rheumatoid arthritis.

Furthermore, we (8) reported that blood B$_{12}$ levels in a few patients with rheumatoid arthritis were low as compared with those in osteoarthritis and suggested that it may play some role in the development of rheumatoid arthritis.

**REFERENCES**