Source and significance of 5-nucleotidase in synovial fluid

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The levels of 5-nucleotidase (5-NT) activity are raised in the serum of patients with rheumatoid disease and this is thought to indicate hepatic dysfunction (Kendall, Cockel, Becker, and Hawkins, 1970). Much higher levels have been found in the synovial fluid from rheumatoid patients (Kendall, Farr, Bold, and Hawkins, 1971), but there is no correlation between the enzyme activities in the two fluids. This suggests that the joints do not contribute significantly to serum 5-NT levels, and that synovial 5-NT is not derived from the blood.

The present study was designed to find out the diagnostic value, relevance, and source of 5-NT in the joint fluid. The diagnostic value of this estimation has been assessed by comparing the results in a rheumatoid group with those in patients with other arthropathies. The relevance of the synovial 5-NT level has been studied by comparing those with high and low values and by correlating the 5-NT results with the activity of the disease. Since synovial 5-NT appears to be produced locally, the possible sources include the cells in the fluid (red cells, leucocytes, and synovial cells) and the synovial membrane itself. These possibilities have therefore been investigated.

Patients and methods

PATIENTS

(1) *Diagnostic survey*

5-NT levels were estimated on synovial fluid obtained from 124 unselected patients with various arthropathies on their first visit. Seventy had rheumatoid disease and by the revised ARA criteria (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959) forty were classical, 23 definite, and seven probable. There were 44 females. The ages ranged between 25 and 77 years (mean 51.1). 32 patients had osteoarthritis and 22 had other types of joint disease, namely gout, ankylosing spondylitis, psoriatic arthropathy, hypertrophic pulmonary osteoarthropathy, Reiter's syndrome, traumatic conditions, other connective tissue disorders, such as dermatomyositis and erythema nodosum, and arthritis associated with alimentary tract disorders such as Crohn's disease, ulcerative colitis, and Whipple's disease.

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(2) *Significance of synovial 5-NT*

75 rheumatoid patients were assessed clinically, radiologically, haematologically, and in addition the following biochemical estimations were made on their serum and synovial fluid: albumin, globulin, alkaline phosphatase, acid phosphatase, 5-NT, glucose, and glutamic oxaloacetic transaminase (SGOT). These patients were then divided into two groups, 49 with a synovial 5-NT greater than 30 i.u./litre, and 26 with lower values, so that the features of patients with a high synovial 5-NT could be compared with those showing lower readings.

(3) *Correlation of synovial 5-NT and disease activity*

The degree of disease activity was determined using an arbitrary scale of three grades, + to +++, before the 5-NT results were known. The final grade was based on a semi-quantitative assessment of pain, stiffness, swelling, heat, redness and tenderness of each joint.

METHODS

(a) *5-NT*

This was estimated by the method of Persijn, van der Slik, Kramer, and de Ruitjer (1968) modified by the inclusion of a blank to measure residual adenosine in adenosine monophosphate. The synovial fluid was centrifuged at 5,000 r.p.m. to remove any cells which we have found will interfere with this method. The normal range for serum in this hospital is 0–10 i.u./litre.

5-NT activity was also measured in the synovial fluid sediment and in the buffy coats and red cells of blood from controls and rheumatoid patients. Since cellular material interferes with the Persijn technique, the nickel inhibition method was used for these estimations (Campbell, 1962).

(b) *Cytology*

Total and differential cell counts were carried out on each specimen of synovial fluid. Smears were also made and were stained for 5-NT using the lead method (Naidoo and Pratt, 1954). Normal and rheumatoid buffy coats, normal, and rheumatoid red cells and the sediment from synovial fluid were studied.

(c) *Histochemistry*

Specimens of synovial membrane from rheumatoid and osteoarthritic patients obtained during surgery were prepared by Chayen's method (Chayen, Bitensky, Butcher, and Poulter, 1969) and stained for 5-NT by the lead method.

Table I Comparison of rheumatoid patients with high and lower synovial 5-NT levels

Estimation	5-NT under 30 (Mean $\pm$ SD)	5-NT over 30 (Mean $\pm$ SD)	Significance of difference
Haemoglobin (g./100 ml.)	12.17 $\pm$ 1.97	11.76 $\pm$ 1.38	NS
E.S.R. (mm./1st hr)	46.77 $\pm$ 37.4	55.71 $\pm$ 24.5	NS
Serum Albumin (g./100 ml.)	3.70 $\pm$ 0.42	3.48 $\pm$ 0.34	S (P < 0.05)
Alkaline phosphatase (KA units per cent.)	13.37 $\pm$ 6.4	10.78 $\pm$ 3.1	NS
SGOT (units per cent.)	5.91 $\pm$ 2.16	11.10 $\pm$ 8.96	HS (P < 0.005)
Synovial fluid Acid phosphatase	2.88 $\pm$ 1.61	5.35 $\pm$ 3.5	S (P < 0.05)
Glucose (mg./100 ml.)	92.6 $\pm$ 32.8	67.4 $\pm$ 22.6	S (P < 0.05)
WBC ($\times 10^3$ per mm.)	4.312 $\pm$ 1.7	16.333 $\pm$ 8.8	HS (P < 0.005)

Results

(1) Diagnostic value of synovial 5-NT

The results for the rheumatoid group and the other arthropathies are shown in Fig. 1. The mean value

for the rheumatoids was 44.5 i.u./litre (range 3-115 i.u./litre).

(2) Type of rheumatoid patient with a raised synovial 5-NT

Comparing the group with 5-NT above 30 i.u./litre with those below, there was no difference in age, sex, duration, and treatment of this disease. In addition, the serum globulin and the synovial fluid protein, GOT, and alkaline phosphatase levels were similar in the two groups. Points of difference are given in Table I, the main ones being serum albumin and SGOT, the synovial acid phosphatase, glucose and white cell count.

FIG. 1 Scattergram of 5-NT in supernatant of synovial fluid in rheumatoid arthritis, osteoarthritis, and other forms of arthritis

AS ankylosing spondylitis;
C Crohn's disease;
D dermatomyositis;
EN erythema nodosum;
G gout;
H hypertrophic pulmonary osteoarthropathy;
Ps psoriatic arthropathy;
T trauma;
UC ulcerative colitis;
W Whipple's disease

FIG. 2 Scattergram of synovial 5-NT plotted against clinical activity of joint in patients with rheumatoid disease

(3) *Synovial 5-NT and disease activity*

Synovial 5-NT levels and the grade of activity are shown in Fig. 2 (p. 327), and the percentage polymorph count and disease activity in Fig. 3.

Figures showing the highly significant differences in both these parameters with the different grades of activity are given in Table II.

FIG. 3 Scattergram of synovial 5-NT plotted against synovial neutrophil response in patients with rheumatoid disease

Table II Correlations between clinical activity and synovial fluid 5-NT levels and percentage polymorph count

Disease activity	+	++	+++
5-NT			
No. of cases	10	18	10
Mean	11.3	24.6	47.6
S.D.	7.4	9.12	26.4
Significance	P < 0.001 HS		P < 0.005 HS
Per cent. polymorphs			
No. of cases	11	23	22
Mean	19.0	51.1	86.5
S.D.	23.5	23.4	6.1
Significance	P < 0.001 HS		P < 0.001 HS

Fig. 4 shows the correlation between the two measures of activity, 5-NT and percentage polymorph count, and once again the difference in the

FIG. 4 Scattergram of synovial neutrophil response plotted against clinical activity of joint in patients with rheumatoid disease

height of the polymorph count between those with a low and those with a high 5-NT was highly significant ($P < 0.001$).

FIG. 5 Synovial membrane in rheumatoid arthritis stained for 5-NT. $\times 750$. Synovial lining cells show marked 5-NT activity

(4) Source of synovial 5-NT

Biochemical estimations

5-NT activity was low in both the white and the red cells of the blood of normal and rheumatoid patients. The levels in the sediment from the synovial fluid were lower than the corresponding supernatant fluid presumably because of the presence of non-contributing white cells.

(5) Source of synovial 5-NT

Cytology

Leucocytes and red corpuscles in the blood of rheumatoid and control patients, and in the synovial fluid of rheumatoids, did not show increased activity of 5-NT in synovial fluid. The desquamated synovial cells however showed a raised 5-NT activity.

(6) Source of synovial 5-NT

Histochemistry

5-NT activity was demonstrated to be high in the cells of the synovial membrane of patients with rheumatoid disease (Fig. 5, p. 328). There was little activity in the synovial membrane from patients with osteoarthritis.

Discussion

Raised levels of synovial 5-NT occur in inflammatory arthropathies, whether due to rheumatoid disease, psoriasis, ulcerative colitis, Whipple's disease, or dermatomyositis. Although high levels are expected in rheumatoid disease, low levels are found when the disease is inactive; this also applies to other forms of arthropathy. A number of workers have previously observed that many synovial lysosomal enzymes also are raised in inflammatory arthropathies (Caygill and Pitkeathly, 1966).

The more active the disease, as judged by independent clinical assessment, the higher are the levels of 5-NT, and there is a correlation with the height of the synovial polymorphonuclear response. The same pattern emerges from measuring certain other biochemical changes in synovial fluid. A high white cell count, a low glucose (Ropes and Bauer, 1953), and a raised acid phosphatase (Caygill and Pitkeathly, 1966) are all pointers to active joint disease, and all of these significantly correlate with a high 5-NT.

During studies for the source of lysosomal enzymes it was noticed that, in almost all cases in which the enzyme levels were raised, the fluid contained many leucocytes, and it was inferred that the enzymes were derived from the destruction of these cells (West,

Poske, Black, Pilz, and Zimmerman, 1963). Caygill and Pitkeathly (1966) supported this with work on acid phosphatase and β -acetylglucosaminase. Smith and Hamerman (1962) found the polymorph cells of the synovial fluid to be the major source of acid phosphatase, and Lehman, Kream, and Brognan (1964) suggested lymphocytes as a further source. Schajowicz and Cabrini (1958) demonstrated acid phosphatase histochemically in chondroblasts. However, the synovial membrane has been found to be the source of lysosomal enzymes by many investigators (Hendry and Carr, 1963; Pugh and Walker, 1961; Luscombe, 1963). Although high 5-NT values were found in our patients with a raised polymorph count, the leucocytes themselves did not contain significant quantities of 5-NT, nor did the erythrocytes. The source of 5-NT was the synovial cells, for those obtained from the fluid stained positively in the smears and 5-NT activity was demonstrated histochemically to be high in the lining cells of the synovial membrane. The polymorph response was therefore considered to be an independent feature of the acute inflammatory reaction within the joint. Estimation of the 5-NT activity in the joint fluid is therefore a useful procedure because it is an indicator of the degree of activity of the inflammatory process affecting the synovial membrane.

Since synovial 5-NT has a different origin from the serum enzyme, and there is no correlation between the two levels of activity, it seems likely that synovial 5-NT is an isoenzyme of serum 5-NT. Studies are in progress to determine whether this is so.

Summary

A study has been made of the diagnostic value, relevance, and source of synovial 5-NT. The enzyme is derived from the synovial cells and the height of its activity reflects the degree of activity of the inflammatory process affecting the synovial membrane.

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