Catalase Activity of Synovial Fluid in Joint Diseases

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Catalase activity of synovial fluid was estimated in 140 patients with various joint diseases by using a simple gasometric technique. Very high levels of the enzyme activity of synovial fluid were found in patients with inflammatory joint diseases, such as rheumatoid arthritis and infectious arthritis. With the exception of blood-mixed fluid, non-inflammatory fluids showed low levels. The results obtained by our gasometric procedure were in good accordance with those by other methods. The technique was simple, but accurate enough for practical use in the screening of joint diseases.

In recent years, a number of the enzymes in synovial fluid have been studied including aminotripeptidase,¹ β -glucuronidase,² lactic dehydrogenase,³⁻⁵ transaminase,^{4,5} acid^{6,7} and alkaline phosphatases⁷ and others.⁵ However, no study of catalase activity in arthritic synovial fluid has hitherto been reported. Various methods have been used for the estimation of catalase in biological materials; gasometry, iodometry, titrimetry using permanganate and spectrophotometry. For routine purposes, however, these methods, except for the first one, consume a great deal of time and are complicated. The present report describes a simple and sensitive method for an estimation of catalase activity in synovial fluid and its clinical application to cases with joint diseases.

MATERIALS AND METHODS

Synovial fluid was taken by sterile puncture from the knee of the patients with joint diseases. Leukocyte and erythrocyte counts were made with a hemocytometer and physiological saline solution as the diluent. Synovial fluid of which erythrocyte count was greater than leukocyte count was described as blood-mixed fluid in this report.

Diagnosis of rheumatoid arthritis was established according to the criteria of the American Rheumatism Association.

The patients with osteoarthritis had radiological evidence of hypertrophic changes but showed no evidence of local and systemic inflammatory changes. Traumatic arthritis and infectious arthritis were diagnosed on the basis of clinical history and bacteriological findings, respectively. In cases that clinical, radiological and serological informations were inadequate for a definite

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diagnosis, the disease was described as 'chronic monoarthritis'. All of these patients were submitted to serum latex agglutination tests.

Catalase test was carried out by a modification of the method of Järvinen (1958).⁸ This technique was chosen because the reagent is readily available for laboratory use, and no special apparatus other than a glass tube for testing blood sedimentation rate (Westergren) is required. The technique is as follows.

Synovial fluid was aspirated to the first mark (180) of the tube, then an equal volume (20 mm) of 3% hydrogen peroxide solution was aspirated into the tube with care not to trap any air bubbles between the fluids. Immediately after that, the tube was supported in a stand. At first, the height of the mixture is 40 mm. The time course of bubble formation in several specimens is shown in Fig. 1. After about 30 minutes their bubble formation attains the maximum. At that time, the height of the top level of bubbles (minus 40 mm) indicates gas volume. Furthermore, catalase activity was calculated in relation to the height of gas formation after 30 minutes.

Fig. 1. Catalase test and the time course of bubble formation.

The accuracy of the above test has been examined by comparing its results with those obtained by the use of two other methods for catalase determination. In 44 samples of synovial fluid, catalase activity was measured by means of iodometry using the method of Yamagata and associates.⁹ The catalase activity was expressed as gram hydrogen peroxide decomposed by 1.0 ml of synovial fluid for 30 minutes at 37°C. In 20 samples, catalase activity was measured by means of permanganate titrimetry using the modification of von Euler's method by Tarlov and Kellermeyer.¹⁰ The catalase activity was expressed in mEq hydrogen peroxide decomposed by 1.0 ml of synovial fluid for 30 seconds at room temperature.

RESULTS

Table 1 shows the results for 246 specimens of synovial fluid from 140 patients with joint diseases. In 79 samples of rheumatoid arthritis, catalase activity ranged from 30 to 260 mm, with a mean of $170.7 \pm \text{SE } 6.2$ mm. In 105 cases of osteoarthritis, with the exception of blood-mixed fluids, the activity ranged from 0 to 65 mm, with a mean of $12.7 \pm \text{SE } 1.2$ mm. The mean was markedly higher in rheumatoid arthritis than in osteoarthritis, and the difference was statistically significant ($p < 0.001$). In only two of 79 samples (2.5%) from rheumatoid subjects, the value fell below 40 mm, which are taken now as the screening level. Of 105 samples of osteoarthritis tested, only four (3.8%) showed the value above 40 mm. Provided that blood is not mixed in synovial fluid, the test is useful in differentiation of these two diseases. The mean value for the catalase activity was $81.5 \pm \text{SE } 23.5$ mm in 13 fluid samples from patients with traumatic arthritis, $73.9 \pm \text{SE } 14.8$ mm in 17 from patients with chronic monoarthritis and $178.6 \pm \text{SE } 15.6$ mm in 7 from patients with infectious arthritis.

TABLE 1. Results of catalase test of synovial fluids in joint diseases

	No. of patients	No. of fluids	Catalase activity (gas volume, mm)			
			Range	Mean	S.D.	S.E.M.
Rheumatoid arthritis	26	79	30-260	170.7	54.9	6.2
Osteoarthritis	70	105	0-65	12.7	11.9	1.2
—, blood-mixed fluid	20	25	13-253	88.6	63.2	12.6
Traumatic arthritis	11	13	4-260	81.5	84.9	23.5
Chronic monoarthritis	8	17	4-209	73.9	61.0	14.8
Infectious arthritis	5	7	125-235	178.6	41.2	15.6
Total	140	246				

S.D.=Standard deviation.

S.E.M.=Standard error of the mean.

Fig. 2. Relation of gas volume to catalase activity measured by other methods.

Fig. 3. Relation of catalase activity to leukocyte count in synovial fluid from patients with rheumatoid arthritis and osteoarthritis.

Forty-four parallel estimations of catalase activity were carried out on the same samples by iodometry and the present method. The results showed a correlation coefficient (r) of $+0.64$ ($p < 0.001$). Twenty comparative determinations were made of by using the present method and the permanganate titrimetry of Tarlov and Kellermeyer. The correlation coefficient obtained was $r = 0.90$ ($p < 0.001$). The data are shown in Fig. 2.

There was a significant positive correlation between the number of leukocytes in synovial fluid and the catalase activity of the fluid. This relation is illustrated in Fig. 3 for the values of gas volume ($r = +0.70$, $p < 0.001$).

DISCUSSION

A simple method for quantitatively determining the catalase activity has been based on the measurement of the amount of oxygen released by the action of the enzyme. Several methods of this type have been reported.^{8,11,12} For the estimation of catalase activity in urine a gasometry has been described by Järvinen.⁸ The technique, however, is unsuitable for the determination of synovial fluid catalase, since the large amount of bubbles cannot be measured, especially in cases of high enzyme concentrations.

The method described here for the estimation of synovial fluid catalase has the advantage of being simpler and more accurate for practical purposes. The results of the present study (Table 1) suggest that the level of the enzyme activity is a sensitive index of joint inflammation. Furthermore, our simple catalase assay may be of practical use for a screening procedure for joint diseases. Rheumatoid and osteoarthritic synovial fluid can be roughly distinguished by using the present gasometric method. However, a source of error lies in non-inflammatory fluid containing erythrocytes. Excepting such blood mixed fluid of which erythrocyte count is larger than leukocyte count, catalase activity of

synovial fluid is mainly related to the leukocyte count and indicates the degree of joint inflammation. Fig. 3 shows a significant positive correlation between them. The high levels of catalase activity found in inflammatory fluid may therefore be attributed, at least in part, to increased leukocyte count of the fluid. However, the correlation is not linear in rheumatoid arthritis. In order to explain this finding one might assume another factor necessary for increased catalase levels in the synovial fluid. When synovial fluid was examined after freezing and thawing, the increased catalase activity was noted in the supernatant fraction, suggesting that the enzyme was released from cells in synovial fluid. Furthermore, a significantly high level of catalase activity was detectable in the supernatant fraction following centrifugation of rheumatoid synovial fluid. In 10 rheumatoid patients, the activity of the supernatant fraction was 7 to 40 per cent of the value of whole fluid. These findings suggested that the increase of catalase activity in the supernatant was due to release of the enzyme from leukocytes in the synovial fluid or from synovial tissue cells on account of inflammatory reactions of varying intensities. It is also concluded that the catalase activity in whole synovial fluid may furnish information of inflammatory changes and their intensity in joint diseases.

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