

Acid Phosphatase Activity in Synovial Fluid

by

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In recent years, the problem of lysosome and acid phosphatase was subject of active discussions in the field of cytopathology. Lysosome, one of the organelles in the cytoplasm, described for the first time by DE DUVE²⁾ in 1955, contains lysosomal enzymes such as β -glucuronidase, DNA-ase, acid phosphatase, cathepsin and so on. Lysosomes are thought to play many important roles in phagocytosis or degradation of the pathological cell. Since various pathological processes such as trauma and inflammation take place in the joints and the synovial fluid contains polymorphonuclear leukocytes and macrophages which may be easily obtained by aspiration in a native state, the synovial fluid cells are very suitable materials for the study on lysosomes and lysosomal enzymes.

Concerning the acid phosphatase activity in the pathological synovial fluid, LEHMAN et al.⁷⁾, SMITH et al.²⁰⁾ and LEHMAN⁸⁾ described the acid phosphatase activity in synovial fluid but cytochemical studies have not been published.

The present report deals with the determinations of synovial fluid levels of acid phosphatase activity and cytochemical studies of the enzyme in synovial fluid cell in the pathological joint diseases.

MATERIAL AND METHODS

1. All of 105 specimens of synovial fluid were obtained from the knee of the patients with various joint diseases (Table 1). For the diagnosis of osteoarthritis, history, age, roentgenological and clinical findings were utilized. All patients with rheumatoid arthritis had either "classical" or "definite" RA as defined by the diagnostic criteria of the American Rheumatism Association. In case clinical and serological informations were not

Table 1. No of synovial fluid

Diagnosis	No. of Cases
Osteoarthritis	34
Chronic Arthritis	30
Rheumatoid Arthritis	41
Total	105

adequate for a definite diagnosis, the disease was described as "chronic arthritis" in this report.

2. Acid phosphatase activity in the synovial fluid was measured by the method of SINOWARA-JONES-REINHART¹⁹⁾. When untreated synovial fluid was used, however, deproteinization with trichloroacetic acid resulted in developing turbidity due to the precipitation of mucin, and colorimetric measurement became impossible. This was prevented by prior treatment with hyaluronidase digestion (80 V.U.M./ml of 'Sprase'). In 'Sprase' (a crude

testicular preparation), a slight contamination by acid phosphatase probably originating from the prostate was noted. Thus, the value of acid phosphatase activity had to be corrected by subtracting the amount already present in 'sprase'.

3. Acid phosphatase in synovial fluid cell was stained by the lead acetate method of TAKEUCHI²²⁾. Immediately after aspiration, the synovial fluid was smeared on a slide glass with a conventional technique. After drying with an electric fan, the slide glass was kept in a clean place at room temperature for 24 hours for drying. Air-dried smears were fixed in formalin vapor for 10 minutes. The slides were then incubated for 15 minutes at 37°C in a substrate solution. After incubation, the slides were washed sufficiently with distilled water and immersed in a 2 % ammonium sulfate solution for 5 minutes. After washing with water, the specimens were stained with hematoxylin for 2 to 3 minutes, then air dried and examined under light microscope.

4. Acid phosphatase activities in the synovialfluid cell were observed by electron microscopy.

The synovial fluid was centrifuged at 1,000~1,500 r.p.m. for 5 to 10 minutes immediately after aspiration. For preliminary fixation¹⁶⁾ the sediment was immersed in a solution of 2.5 % glutalaldehyd for 15 minutes. The specimens were then incubated in the TAKEUCHI's solution at 37°C for 15 minutes, then immersed in a solution of 2 % ammonium sulfate. For a after-fixation the specimens were fixed with CAULFIELD's solution for 1 hour. Dehydrations were then carried out with gradually increased concentration of alcohol, and the specimens were embedded in epon 812, according to the method of TAKASHIO²¹⁾. After preparation of ultra-thin slices, the specimens were observed by Hitachi Hu-11A electron microscope.

RESULTS

1. Acid phosphatase activity of the synovial fluid in various diseases in shown in Table 3 and Fig. 1. The values in osteoarthritis patients were 0~1.58 unit with a mean of 0.36 ± 0.43 , most cases showing values below 0.5. Values above 1.1 was seen only in 3 cases of osteoarthritis with rather intensely inflammatory signs as in case of continued hydrops of the knee. In rheumatoid patients, the values ranged from 0.50 to 3.04 unit with a mean of 1.45 ± 0.65 , most cases showing values above 1.1, giving a significant difference in the value in osteoarthritis. In chronic arthritis, values ranging from 0.27 to 2.11 unit with a mean of 1.04 ± 0.48 were

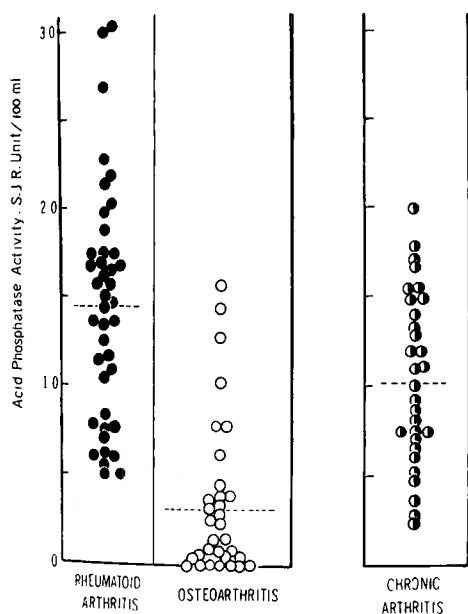


Fig. 1 Acid phosphatase activity in synovial fluid

obtained, representing an intermediate level between those of rheumatoid and osteoarthritis patients.

2. In order to elucidate the source of such acid phosphatase activity in the synovial

fluid, the activity in the supernatant, after removal of cellular components by low speed centrifugation, was determined. As shown in Table 2, in 6 cases of rheumatoid arthritis, the mean activity was 2.47 unit in the whole fluid, while the activity of the supernatant

Table 2. Acid phasphatase acitvity in supernatant

	No. of Cases	Whole Synovial Fluid Activity (a)	Supernatant (b)	Percentage (b/a × 100)
Osteoarthritis	4	0.75	0.56	74.7%
Rheumatoid Arthritis	6	2.47	0.90	36.4%

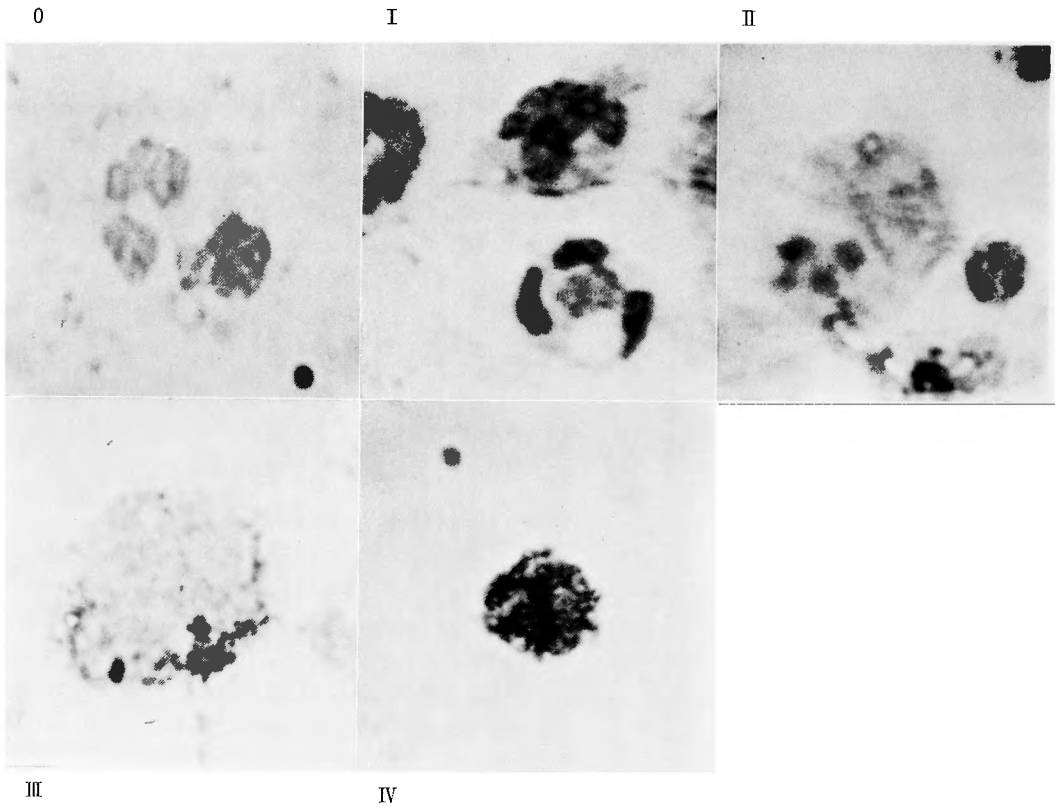


Fig. 2 Grade of synovial cells stained for acid phosphatase
Grade 0 no granules
Grade I extremely few yellow brown granules
Grade II few to moderate number of yellow brown granules
Grade III moderate to numerous granules
Grade IV cytoplasm saturated by closely approximated granules

was 0.90 unit or 36% of the whole fluid activity. On the other hand, in 4 cases of osteoarthritis, the mean activity before centrifugation was 0.75 unit, while the activity of the supernatant was 0.56 unit or 74 % of the whole fluid activity. Most of the acid phosphatase activity in the synovial fluid with an increased activity as seen in rheumatoid fluids appears to originate from the cells. In osteoarthritis, however, the difference in activity between the whole fluid and the supernatant was smaller.

3. Acid phosphatase activity in the cells of synovial fluid was expressed as follows. According to KAPLOW's⁶⁾ method, the cells were classified into 5 grade from 0 to IV, based on the intensity and appearance of the precipitated dye in the cytoplasm, as shown in Fig. 2. The calculation of the score was made by giving numbers to each of the corresponding grade of 0-IV. These numbers multiplied by the percentaga of these cells were described as "score" (index of positive). As seen in Table 3 and Fig. 3, the score ranged from 0 to 89 with a mean of 19.6 ± 6.5 in osteoarthritis. Most of the cells were showing 0-II grade. Grades above III were seen only in 3 cases. In rheumatoid arthritis, the score ranged from 11 to 328, with a mean of 160.8 ± 68.9 , giving values definitely

Table 3. Acid phosphatase activity in whole synovial fluid and synovial fluid cells

Diagnosis	No.	Acid Phosphatase Activity in Whole Synovial Fluid (S.I.R. U/dl)		Acid Phosphatase Activity in Synovial Fluid Cell (Score)	
		Range	Mean $\pm$ S. D.	Range	Mean $\pm$ S. D.
Osteoarthritis	34	0~1.58	0.36 ± 0.43	0~ 89	19.6 ± 6.5
Chronic Arthritis	30	0.27~2.11	1.04 ± 0.48	9~208	78.5 ± 55.8
Rheumatoid Arthritis	41	0.50~3.04	1.45 ± 0.65	11~328	160.8 ± 68.9

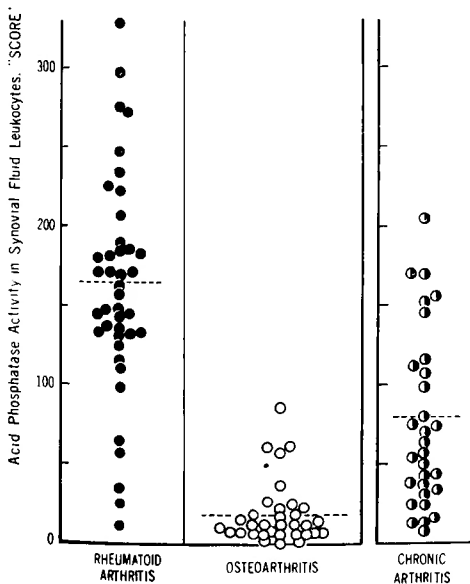


Fig. 3 Acid phosphatase activity in synovial fluid cells

higher than those in osteoarthritis. Beside the increase of cell count, a marked increase of cells with high activity above grade III was significant. In chronic arthritis, the score ranged from 9 to 208 with a mean of $78.5 \sim 55.8$, representing values between those of osteoarthritis and rheumatoid arthritis. The activity of synovial fluid cells also followed the order of rheumatoid arthritis, chronic arthritis and osteoarthritis.

Assuming that acid phosphatase originates from the cells of the synovial fluid, the difference in value between rheumatoid arthritis and osteoarthritis is due not only to the number of cells, but also to the preponderance of enzyme-rich cells. As seen in Fig. 4, considerable correlation is seen between the acid phosphatase activity in the synovial fluid and the score of cellular enzyme activity.

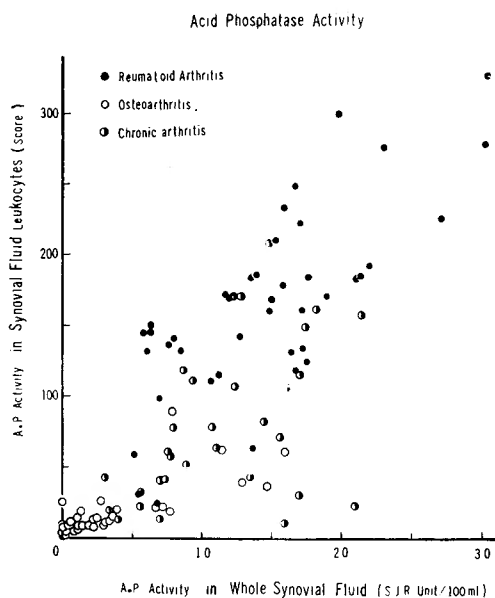


Fig. 4 Correlation between acid phosphatase activity in whole synovial fluid and synovial fluid cells

4. In the attempt to confirm the cellular localization of acid phosphatase, electron microscopic observations were carried out, and results were obtained shown in Fig. 5.

In the cytoplasm, dense granules of $0.15 \sim 1.0 \mu$ in size, representing concentrated densities, indicate acid phosphatase activity through deposition of minute particles of electron opaque lead. These granules are encapsulated by single membrane with a thickness of 4.6 to $7.2 \text{ m}\mu$. A relatively electron-dense thread granule is seen within the granule. The reaction products within the granules are seen in various form and numbers, while the degree of reaction is not homogenous in individual granules.

Despite these variations in enzyme activities, these granules are probably identical with the lysosome described by DE DUVE²⁾, NOVIKOFF¹²⁾, WOLMANS²⁶⁾ and OHKURA¹³⁾. Due to the difference in the materials used,

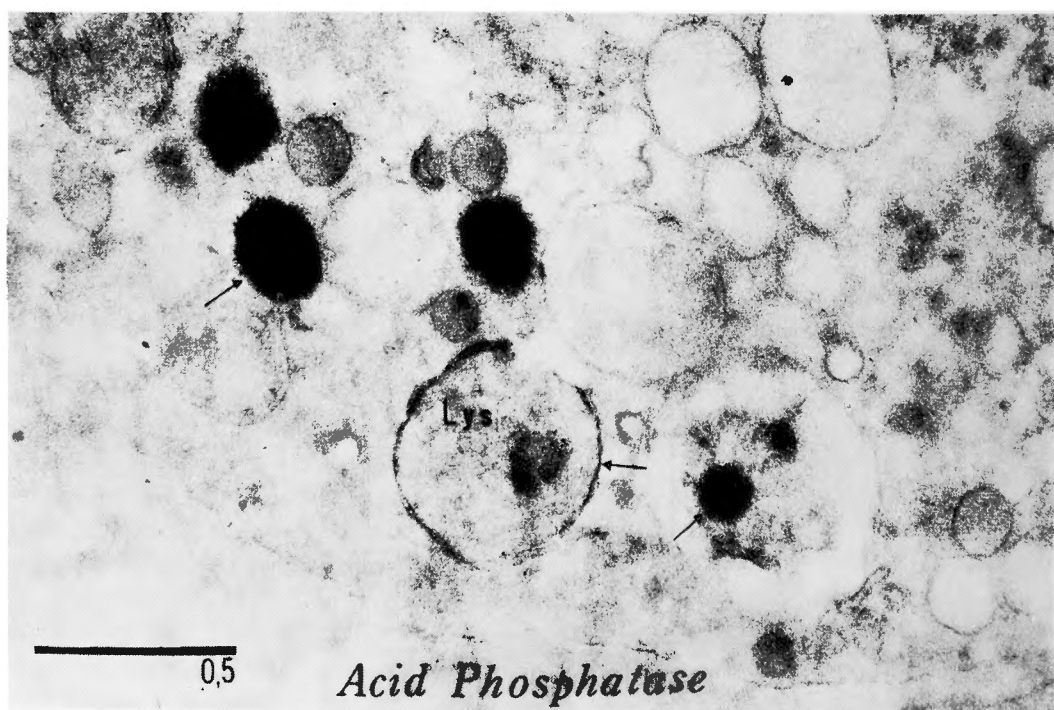


Fig. 5 The cellular localization of acid phosphatase (electron microscopic observation)

however, some variations are noted in the morphology and state of deposition of lead salt.

Various factors and pathological conditions might influence the intensity of enzymic activities as evaluated electron microscopy might explain such variations.

SHIBKO et al.¹⁸⁾ observed the mode of release of lysosomal enzymes from lysosomes by incubation and thermal stimulation. Quantitative or qualitative changes in the lysosomes may therefore be expected upon stimulation phagocytosis or degeneration, leading to changes in the activity of lysosomal enzymes. During electron microscopy, densities of various concentrations were also noted in the lysosomes.

DISCUSSION

Except for the case with prostate cancer and its metastasis, acid phosphatase activity has not been studied sufficiently in clinical aspects. In recent years, however, cytochemical studies on this enzyme in various diseases such as leukemia have been frequently reported¹⁰⁾¹¹⁾; further studies will determine the use of this enzyme in various diseases. In addition, as stated above, the role of this enzyme in inflammation is of particular interest from the cytopathological view-point. The study on the acid phosphatase in synovial fluid may therefore help to understand the biomechanism of inflammation. The phosphatase activity in the synovial fluid appears to be highest in rheumatoid arthritis, followed by chronic arthritis and finally by osteoarthritis. The increase appears to be related to the degree of inflammation of the joints. These results agree with those of LEHMAN et al.⁸⁾ and SMITH-HAMMERMANN²⁰⁾.

HIRSCH and CHORN¹⁾⁴⁾ also demonstrated the existence of granules (lysosome) with acid phosphatase activity in the cytoplasm of polymorphonuclear leukocytes from rabbit. Cells other than polymorphonuclear leukocytes are also thought to be related to the activity of this enzyme in the synovial fluid. LUSCOMBE⁹⁾ demonstrated the increased activity of acid phosphatase in the synovial membrane obtained from the patients with rheumatoid arthritis. SCHAJOUICZ and CABRINI¹⁷⁾ demonstrated histochemically the presence of the acid phosphatase activity in the osteoclasts and chondroclasts. Some part of the enzyme activity may be released from tissue-fixed cells or articular cartilage due to inflammatory changes. Viewing the cellular constituents of the inflammatory synovial fluid, however, this enzyme appears to originate mainly from the polymorphonuclear leukocytes emigrating from the blood vessels, synovial cells and macrophages. This assumption might be confirmed by the fact that acid phosphatase activity of the cell free supernatant represents only 36% of these of original fluid in inflammatory states. On the other hand, following centrifugation, the difference in the activity between supernatant and whole fluid is smaller in osteoarthritis than in rheumatoid arthritis with a value of 74.6 % of the control value; this difference is probably due to the small number of cells in the synovial fluid of noninflammatory joint and less predominant participation of the enzyme of cellular origin in the total activity in the synovial fluid. However, the total enzyme activity is still higher than in normal serum¹⁴⁾¹⁵⁾. VALENTEIN²³⁾²⁴⁾²⁵⁾ also stated that the activity of this enzyme was higher in the leukocytes than in normal serum.

The activity of this enzyme is not necessarily dependent upon the number of free cells alone. When the acid phosphatase activity was expressed as scores, based on the histological findings of the synovial cells, the value was the highest in rheumatoid arthritis,

followed by chronic arthritis and finally by osteoarthritis, indicating an close parallelism between the degree of inflammation and the enzyme activity of the cells. Moreover, in the synovial fluid obtained from patients with osteoarthritis, scarcely any cells show activities above grade III in addition of being in extremely small number. On the contrary, a pronounced increase is seen in the number of cells with an intense enzyme activity in rheumatoid arthritis, indicating a relative increase of cells with an intense acid phosphatase activity. According to LOEFFER¹⁰⁾, although the appearance of cells with an intense acid phosphatase activity in some pathological conditions such as leukemia etc. in the peripheral leukocytes has been demonstrated, the biomechanism is not known at present. MITUS¹¹⁾ pointed out the usefulness of acid phosphatase determination for differentiation of cells in view of the intensely positive activity in the reticuloendothelial cells. The content of acid phosphatase activity may therefore vary according to the kinds of cells and tissues, probably in relation to the functional differentiation of cells. Even in the same kind of cells, chemical stimulus such as triiodothyronine treatment⁵⁾, experimental cell damage and viral infection etc. may increase the frequency of appearance of lysosomes, leading to the increase of acid phosphatase activity. Lysosomal enzymes appear to play a role in phagocytosis and selfcleaning of focal cytoplasmic degradation. Elevation of the activity of these enzymes might be regarded as a kind of cytopathological reaction in response to stimulus or injury.

NOVIKOFF¹²⁾ also pointed out the important role of acid phosphatase in increased lysosome in inflammatory processes. Elevation of acid phosphatase activity in the synovial fluid cells from the inflammatory joints might have resulted therefore from such inflammatory changes of cells.

In summary, the elevation of acid phosphatase activity in the synovial fluid obtained from patients with rheumatoid arthritis might be the result of the increase of inflammatory cells and the elevation of the enzyme activity within the cell. The latter appears to be related with degeneration or injury of cells. Concerning the pathological changes of cells, however, many problems apparently still remain unsolved.

CONCLUSION

In 105 cases with joint diseases such as osteoarthritis, chronic arthritis and rheumatoid arthritis, acid phosphatase activities in the synovial fluids and synovial fluid cells were determined.

1) The acid phosphatase activity of the synovial fluid measured by SINOWARA-JONES-REINHART method was 0.50~3.04 with a mean of 1.45 ± 0.65 unit in 41 cases of synovial fluid obtained from patients with rheumatoid arthritis, 0~1.58 with a mean of 0.36 ± 0.43 unit in 34 cases obtained from patients with osteoarthritis, indicating a definitely higher value in rheumatoid patients. In 30 cases obtained from patients with chronic arthritis, values of 0.27~2.11, with a mean 1.04 ± 0.48 unit were obtained, representing intermediate values between these two joint diseases. The acid phosphatase activity of the supernatant of the synovial fluid was considerably lower than in the whole fluid containing cells, suggesting that most of the acid phosphatase activity in the synovial fluid originates from the cells in the synovial fluid.

2) The smear specimens of the synovial fluid were stained by TAKEUCHI's method, and the score of the cellular enzyme activity was calculated according to KAPLOW. In 41 specimens obtained from cases of rheumatoid arthritis, the values were 11~328, with a mean of 160.8 ± 68.9 , markedly higher than the value of 0~39, with a mean of 19.6 ± 6.5 in osteoarthritis. The number of cells with high enzyme activity appears to be increased in rheumatoid arthritis.

Scarcely any cells with activities above grade III were found in osteoarthritis, whereas these cells showed an increase in rheumatoid fluid.

3) Cytochemical observations with electron microscope using a modified method of GOMORI³⁾ also revealed the development of lysosome showing acid phosphatase activity in the synovial fluid cells from rheumatoid patient.

4) From these results it was concluded that the elevation of acid phosphatase activity in the synovial fluid of rheumatoid arthritis was due to the increase of cell count and the increase of the enzyme activity within the cell on account of inflammatory processes.

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和文抄録

病的関節液の酸フォスファターゼに関する研究

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病的関節液における酸フォスファターゼの起原と、疾患による酵素活性の変動の意義を追求する目的で、関節液 105 例を病因別に、変形性関節症、関節水腫、関節リウマチの 3 群に分け観察した。

1) 比色定量による検索

Sinowara-Jones-Reinhart 法により測定した。その活性値は、変形性関節症 34 例の平均値は $0.36 \pm 0.43 \text{ u}/100 \text{ cc}$ であり、関節リウマチ 41 例の平均値は $1.45 \pm 0.65 \text{ u}/100 \text{ cc}$ で変形性関節症のそれに比し、有意の差が認められた。関節水腫 30 例では平均値 $1.04 \pm 0.48 \text{ u}/100 \text{ cc}$ で両者の中間値を示し、変形性関節症 < 関節水腫 < 関節リウマチの順にその活性値は高く、局所の炎症の進行程度すなわち関節液総細胞数の増加に併行して高値を示す傾向が観察された。

それ故、つぎに酵素活性の起原を知る目的で関節液 10 症例の上清の活性を測定検討すると、未処置の関節液のそれに比し関節リウマチ 6 例では平均 $1.57 \text{ u}/100 \text{ cc}$ 、変形性関節症 4 例では平均 $0.19 \text{ u}/100 \text{ cc}$ の低値をみた。従つて関節液の本酵素活性には細胞成分の関与が大きいものと推察した。

2) 細胞化学的検索

関節液細胞個々の酵素活性の度合を知る目的で、関節液塗抹標本について、武内法により染色し、Kaplow の方法に準じて、その活性値 Score を算定した。変形性関節症 34 例の平均値は 19.6 ± 6.5 でⅢ型以上の活性細胞は観察されず、関節リウマチ 41 例では、平均 160.8

± 68.9 で変形性関節症のそれに比し、高度の上昇を示し、特に強い活性を示す細胞の増加が注目された。また関節水腫 30 例では平均 78.5 ± 55.8 で両者の中間値を示し、定量と染色における本酵素活性の間には相関性が認められた。

3) 電子鏡的観察

本酵素の局在性を解明すべく、関節液の遠心沈澱物を用い、武内法にて染色し、高塩法にて包埋し超薄切後電子鏡下に観察するに、酸フォスファターゼ活性は一重膜に包まれた $0.15 \sim 1.0 \mu$ 大の円形顆粒像いわゆる Lysosome 内に鉛との反応生成物として密に配列している像が証明された。

以上の研究成果から、病的関節液の本酵素活性は、炎症程度に応じて増加する傾向が観察されたが、これには細胞成分の関与すなわち総細胞数と細胞自体の活性度が大なる根源をなすものであると考えられる。第 1 に細胞数に関しては、滑液膜の炎症により血管から遊出する多核白血球が主で、そのほか炎症のため滑脱遊離する滑膜細胞、軟骨破壊細胞などに由来することも考えられる。第 2 に細胞自体の含有せる本酵素活性の観察において、変形性関節症にはⅢ型以上の活性細胞が存在しないのに反し関節リウマチでは強い活性を示す細胞が多いことから、かかる炎症細胞には、傷害、変性などの変化に応じて Lysosome の出現が増量し、本酵素活性が上昇するものと考察された。