

Lysosomal enzymes in synovial membrane in rheumatoid arthritis

Relationship to joint damage

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There is considerable experimental evidence linking lysosomal enzymes with tissue damage in joint disease (*e.g.* Weissmann, 1966; Page Thomas, 1969). In particular the release of lysosomal hydrolases during cell injury or excessive phagocytosis may play a role in damage to articular cartilage (Ziff, Gribetz, and Lospalluto, 1960; Dingle, 1962; Weissmann and Spilberg, 1968; Hamerman, Janis, and Smith 1967). Biochemical studies have shown increased enzyme activity in synovial fluid in rheumatoid arthritis and related inflammatory disorders. Acid phosphatase (Smith and Hamerman, 1962; Beckman, Beckman, and Lemperg, 1971), β -glucuronidase (Jacox and Feldmann, 1955), and β -acetyl glucosaminidase (Caygill and Pitkeathly, 1966) have been identified. The source of these enzymes is presumed to be either synovial fluid leucocytes or lining cells of the synovial membrane.

The activity of acid phosphatase and cathepsin has been studied in the synovial tissue by Luscombe (1963). Higher activity was found in rheumatoid synovial membrane when compared with tissue from patients undergoing meniscectomy. These results were confirmed by Wegelius, Klockars, and Vainio (1968), and are in keeping with electron microscope and histochemical studies showing enlarged lysosomes in the type A surface cell in rheumatoid arthritis (Barland, Novikoff, and Hamerman, 1964; Norton and Ziff, 1966; Ghadially and Roy, 1967).

Despite the possible importance of lysosomes in the pathogenesis of rheumatoid arthritis, few studies have been reported comparing enzyme levels in the joint with clinical features. Hendry and Carr (1963) showed that high enzyme activity in the synovial fluid tended to be associated with clinical acuteness as judged by heat and swelling of the joint. Ziff, Scull, Ford, McEwen, and Bunim (1952) showed that a decrease in concentration of aminotripeptidase (an enzyme of the cathepsin group) after the intra-

articular injection of hydrocortisone was associated with improvement in the clinical status of joints and with a rise in the viscosity of the synovial fluid. Our present study was designed to correlate lysosomal enzyme concentrations in homogenates of synovial membrane removed at synovectomy with the surgeon's estimate of cartilage and joint damage. The histology of tissues adjacent to the homogenized segments was assessed and also synovial fluid characteristics when fluid was available at the time of surgery.

Material and methods

Synovial tissue was obtained at synovectomy from fourteen joints in twelve patients with rheumatoid arthritis (7 female, 5 male). All satisfied standard diagnostic criteria for 'classical' or 'definite' disease. The mean age of the patients was 48 years (range 16 to 63) and the duration of disease in the operated joint varied from 9 months to 10 years. Material came from one shoulder and thirteen knee joints.

At the time of operation the surgeon assessed the joint damage in four grades:

1. Synovitis only; cartilage and bone appeared normal.
2. Early pitted erosions along bone-cartilage junction; cartilage appeared normal.
3. Extensive erosions; thinned cartilage.
4. Extensive erosions and cartilage damage; joint structures considered to be permanently damaged.

The synovial membrane was immediately dissected free of fascia and cut into segments each weighing approximately 1 g. Adjacent segments were kept for histology and for enzyme estimations. The material for histology was fixed in neutral phosphate-buffered 10 per cent. formalin and the sections were stained with haematoxylin and eosin. The sections were examined without knowledge of the patients' identity or grade of joint damage. The degree of synovial lining cell proliferation and the infiltration of inflammatory cells were each graded 1 to 4 depending on an assessment of increasing numbers of cells (Muirden and Mills, 1971).

The other synovial tissue were placed in 0.25M sucrose and frozen and thawed twice before homogenization. The latter was accomplished mechanically in a ground glass homogenizer and the homogenate spun at 36,000 G. (17,500 r.p.m.) for 55 mins. The supernatant was used for the enzyme assays and the sediment to estimate the DNA concentration of the sample as an index of cellularity.

Acid phosphatase was estimated by the method of Caygill and Pitkeathly (1966) after Torriani (1960), using *p*-nitrophenyl phosphate disodium salt (Koch-Light Laboratories Ltd., Colnbrook, Bucks, U.K.) and including 0.1 per cent. Triton X-100 (0.5 ml) in the incubation mixture (total volume 3.0 ml.). The enzyme β -acetyl glucosaminidase was estimated by the method of Woollen, Heyworth, and Walker (1961), using *p*-nitrophenol-2-acetyl amino-2-deoxy- β -D-glucopyranoside (Koch-Light Laboratories) and including 0.5 ml. 0.2 per cent. Triton X-100 in a total incubation volume of 2.5 ml. The sediment was dissolved in perchloric acid and DNA estimated by the indole method (Paul, 1965).

Synovial fluid was available from ten joints. Total white cell counts and differential counts were performed and the remainder of the sample spun at 36,000 G. for 20 mins to remove cells and particle bound enzyme. The fluid was diluted with distilled water for enzyme assays.

The two lysosomal enzymes were estimated in synovial membranes removed from five non-rheumatoid patients undergoing medial meniscectomy. Macroscopically the synovium appeared normal and this material was considered to represent a noninflammatory 'control'.

Results

The clinical features, histological grading, surgeon's estimate of the grade of joint damage, and biochemical results are shown in Table I. The synovial membrane enzyme concentrations are the means of results of between two and five segments of tissue homogenized from the one joint. In the 'control' (medial meniscectomy) cases only one sample was generally available. The mean acid phosphatase

(AP) concentration per g. tissue for the fourteen rheumatoid synovectomies was 38.93 μ moles P.N.P. and β -acetyl glucosaminidase (AGA) 42.98 μ moles. Corresponding values for the controls (5 cases) were 3.29 and 4.62 μ moles. These differences are significant at the 1 per cent. level. The values proportional to DNA concentration in the sediment were 0.132 and 0.145 for the rheumatoids and 0.021 and 0.027 for the controls ($P < .02$).

Fig. 1 shows the comparison between the joint damage and the enzyme levels in synovial membrane recorded as the mean of AP and AGA per g. tissue in each rheumatoid synovectomy.

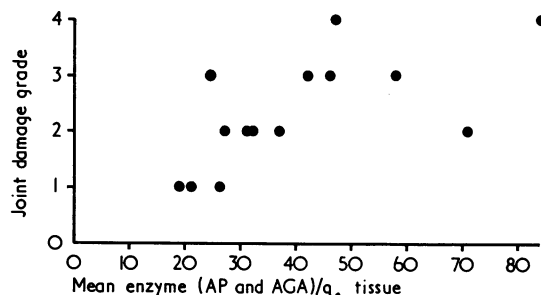


FIG. 1 Comparison of joint damage with mean concentration of two enzymes in μ moles, PNP per g. synovial tissue

A similar comparison using enzyme figures per μ g DNA is shown in Fig. 2 (opposite). A direct correlation is apparent; for the former $r = 0.65$ ($P < 0.01$); for the latter $r = 0.62$ ($P < 0.05$). Similar results were obtained when the enzymes were considered individually. The ratios of the two enzyme activities were remarkably constant; for the fourteen rheumatoid joints $r = 0.798$ ($P < 0.001$).

Fig. 3 shows that the enzyme concentrations followed very closely histological assessment of the

Table I Clinical, histological, and biochemical results

Clinical details					Synovial membrane				Synovial fluid					
Case No.	Sex Age (yrs)	Joint	Duration disease in joint (yrs)	Latex test	Grade of joint damage	Histology (grade)		Lysosomal enzymes		Leucocyte count/mm. ³	Lysosomal enzymes			
						Lining cells	Cell infiltrate	AP/g.	AGA/g.		AP/DNA	AGA/DNA	AP/ml.	AGA/ml.
1	M 40	Knee	4	Pos	4	4	2	91.51	77.75	0.386	0.336	NA	NA	NA
2	F 63	Knee	3	NA	3	2	4	25.05	23.84	0.057	0.059	NA	NA	NA
3	M 61	Shoulder	9 mths	Pos	4	4	1	35.71	59.76	0.121	0.206	NA	NA	NA
		Knee	1		3	3	2	32.80	60.92	0.109	0.202	18,000	1.96	2.40
4	M 51	Knee	10	Neg	1	1	4	16.58	25.29	0.045	0.068	NA	NA	NA
5	F 60	Knee	1	Pos	2	2	3	38.70	24.37	0.148	0.093	11,900	1.58	0.90
6	F 41	Knee	3	Pos	2	3	2	35.66	26.75	0.121	0.099	NA	0.98	2.26
7	F 65	Knee	3	Pos	3	3	2	43.80	40.32	0.136	0.124	15,500	4.31	2.501
8	F 43	Knee	5	Pos	2	3	3	23.05	31.25	0.069	0.094	15,900	2.38	1.52
9	F 46	Knee	9	Pos	2	3	3	68.20	75.74	0.238	0.258	13,450	1.14	2.80
10	F 40	Knee	1	Pos	2	2	3	38.39	36.68	0.118	0.110	10,800	0.36	0.88
11	M 16	Knee (L)	1	Neg*	1	1	3	17.71	21.53	0.064	0.070	20,850	0.58	1.44
		Knee (R)	18 mths		1	2	3	18.99	32.11	0.056	0.010	6,350	NA	0.58
12	M 54	Knee	1	Pos	3	3	3	49.44	67.93	0.144	0.198	13,600	2.60	2.24

* Synovial fluid positive

AP = acid phosphatase

AGA = β -acetyl glucosaminidase

NA = not available

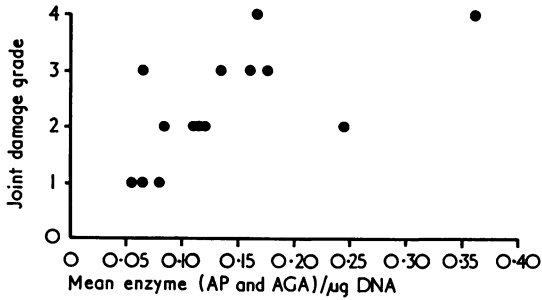


FIG. 2 Comparison of joint damage with the mean of the enzyme concentration per µg: DNA in synovial tissue

grade of lining cell proliferation ($r = -0.74$; $P < 0.01$). Comparison between enzyme levels and grade of infiltration of inflammatory cells failed to reach statistical significance ($r = 0.44$).

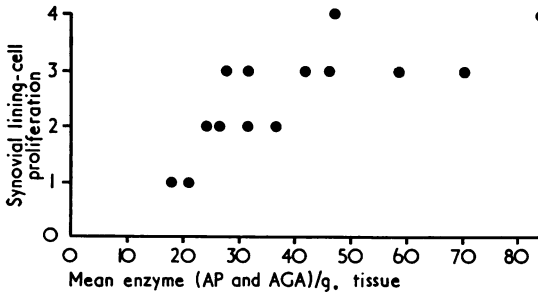


FIG. 3 Comparison of lining cell proliferation and mean enzyme concentration per g. synovial tissue

Fig. 4 is a section of the synovial membrane in Case 1; the dominant feature is a massive proliferation of synovial lining cells (Grade 4) whilst there is a little stromal infiltration of inflammatory cells. Enzyme levels were high and the joint was severely damaged. The reverse type of synovial picture is seen in Fig. 5 taken from Case 11. Infiltration with lymphocytes predominates over lining cell proliferation, enzyme levels were low and intra-articular cartilage remained intact. The histological picture appeared not to be related to disease duration (for further details of the histology and its relation to joint destruction, see Muirden and Mills, 1971).

Synovial fluid results

Comparisons between synovial fluid enzyme values, cell counts, joint damage, and enzyme concentrations in synovial membrane are tabulated in Table II. The number of samples is small, but significant values are found for the correlation between synovial fluid enzymes and joint damage. There is a significant correlation between AGA (the more stable enzyme of the two) in synovial fluid and synovial tissue (Fig. 6) but not between fluid AGA and fluid white cell count (Fig. 7). In a larger series of 28 synovial fluids from cases of classical or definite rheumatoid arthritis, there was also no correlation between enzyme concentration in fluid and with either total white cell count or neutrophil count ($r = 0.21$ and 0.19 respectively). These results suggest that synovial lining

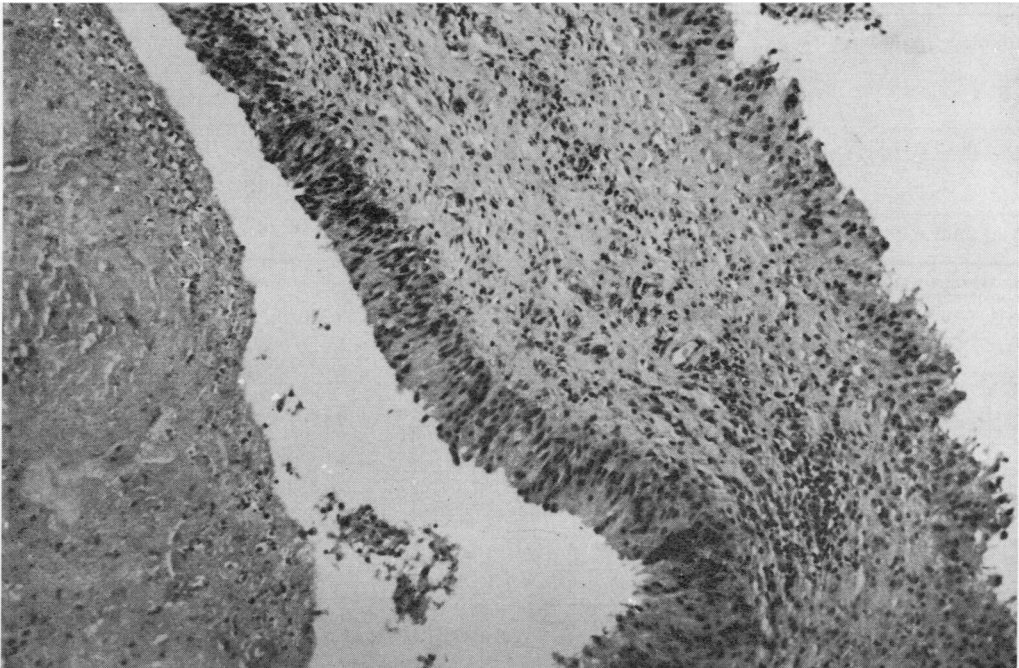


FIG. 4 (Case 1) Synovial tissue showing a massive of palisade lining cells covering a synovial stroma which contains few infiltrating cells. Haematoxylin and eosin stain. $\times 90$

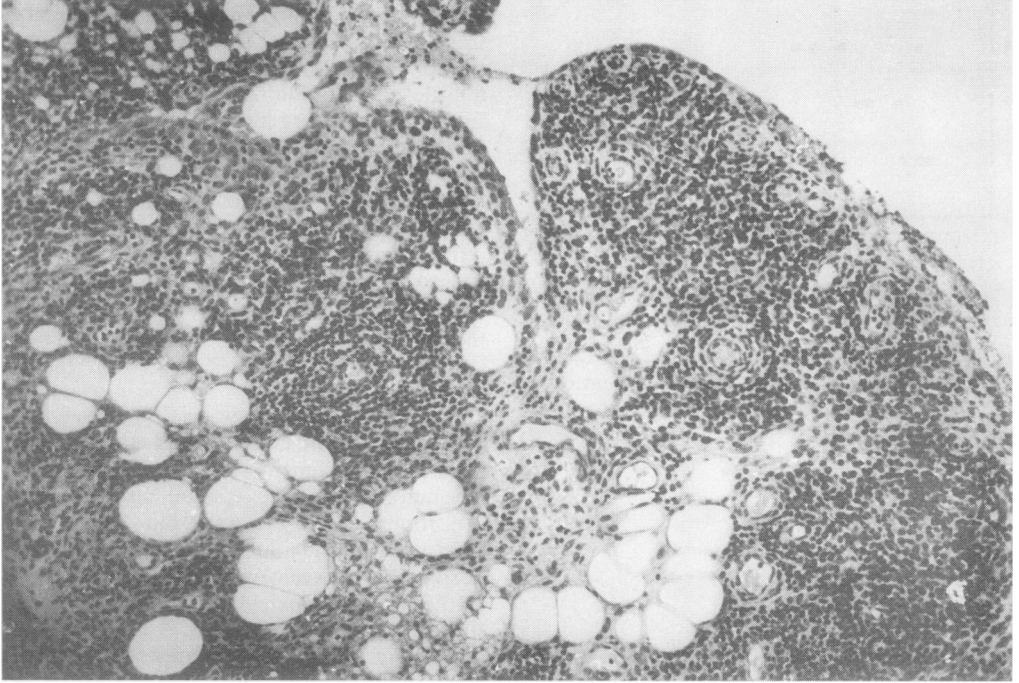


FIG. 5 (Case 2) The synovial surface is composed of a thin layer of horizontally arranged lining cells. The stroma is heavily infiltrated with lymphocytes and plasma cells. Haematoxylin and eosin stain. $\times 90$

Table II Synovial membrane, synovial fluid, and joint damage correlations

Joint damage (grade)	Synovial fluid AP/ml.	$r = 0.73$ (9*)	$P < .05$
	Fluid AGA/ml.	$= 0.64$ (10)	$P < .05$
	Fluid total white cell count	$= 0.23$ (9)	NS
	Fluid neutrophil count	$= 0.08$ (9)	NS
Synovial fluid AGA/ml.	Fluid total white cell count	$= 0.31$ (9)	NS
	Fluid neutrophil count	$= 0.36$ (9)	NS
	Synovial membrane AGA/g.	$= 0.67$ (10)	$P < .05$
Synovial fluid AP/ml.	Synovial membrane AP/g.	$= 0.14$ (9)	NS

* These figures are the number of comparisons
 NS = not significant
 AP = acid phosphatase; AGA = β -acetyl glucosaminidase

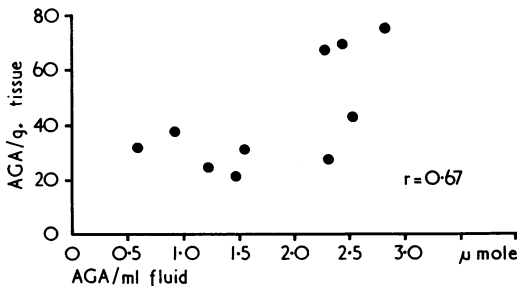


FIG. 6 β -acetyl glucosaminidase per g. synovial tissue compared with activity per ml. synovial fluid

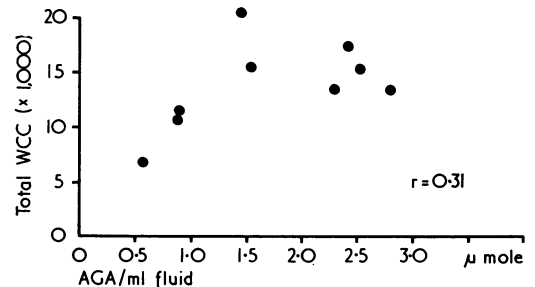


FIG. 7 Synovial fluid white cell count per mm.^3 compared with AGA activity per ml. synovial fluid

cells are a more important source for synovial fluid enzymes than synovial fluid leucocytes.

Results in relation to therapy

Most patients had received intra-articular injections of corticosteroids in the past but not within a month of operation. Five patients were receiving aspirin alone and two were taking no drugs at the time of surgery. Three patients were on low-dose oral corticosteroids (less than 10 mg./day prednisolone or its equivalent). There was no discernible relation between enzyme levels and current drug therapy.

Discussion

Despite much speculation as to an infective cause of rheumatoid arthritis, proof that micro-organisms provoke the disease remains elusive. More headway has been made with studies of processes which sustain the inflammation once it has been initiated (Glynn, 1968). Immunofluorescent studies have revealed rheumatoid factor, other gamma globulins, and complement in rheumatoid synovia, and there is evidence that immune complexes form in the synovial cavity (Hollander and Rawson, 1968). These complexes are thought to perpetuate local inflammation, possibly through a release of lysosomal enzymes, and to explain the low levels of complement in the synovial fluid (Zvaifler, 1965). A defect in the fibrinolytic system may favour the persistence of fibrin in the joint and cause chemotaxis of leucocytes, thus maintaining inflammation (Riddle, Blum, and Barnhart, 1965). Activation of the kinin system has also been mentioned as a contributory factor in synovial inflammation (Melmon, Webster, Goldfinger, and Seegmiller, 1967).

Evidence which links lysosomal enzymes with tissue damage and sustained inflammation in joint disease has already been noted. High enzyme concentrations are found in rheumatoid synovial fluid and we have confirmed the work of Luscombe (1963) in showing a marked increase in synovial tissue enzymes by comparison with synovia removed at the time of arthroscopy for meniscus injuries. Differences are significant whether values are compared per g. synovium or in proportion to the DNA content of the tissue as a measure of its cellularity. These observations have been carried further to consider the extent of joint damage noted by the surgeon at the time of synovectomy. For some time it has been known from experiments *in vitro* that certain lysosomal enzymes have the capacity to degrade articular cartilage (Ziff and others, 1960; Dingle, 1962; Hamerman and others, 1967). The enzyme mentioned as being responsible is cathepsin D. Ali (1964) has implicated cathepsin B but the recent experiments of Dingle (1971), using immunoinhibition, indicate that

cathepsin D is the principal and probably exclusive agent of cartilage matrix degradation in organ culture work. Another enzyme of the cathepsin group is leucineaminopeptidase and this has been demonstrated in synovial lining cells, in pannus, and in cartilage erosions in rheumatoid arthritis (Vainio, 1970). Although we have not estimated cathepsins in this study, a correlation has been shown between the concentration of the enzymes acid phosphatase and β -acetyl glucosaminidase and cartilage and erosive bone damage within the joint cavity. Furthermore, high levels of enzymes arise from synovial tissue showing marked lining cell proliferation, the swollen surface cells forming a palisade of vertically arranged cells. Electron microscopical and histochemical studies noted above and confirmed in our own laboratory indicate that a majority of these lining cells contain enlarged and numerous lysosomes in this disease.

The other end of the pathological spectrum seen in this same disease features a thin horizontal layer of lining cells enveloping a stroma packed with infiltrating cells, predominantly lymphocytes and plasma cells. When such tissue was homogenized, low enzyme concentrations resulted, and the joint concerned, in general, showed less structural damage.

Page Thomas (1967) has suggested that tissue biochemistry may be complicated by the cellular heterogeneity of the rheumatoid synovium. Our results indicate that there is less variability from place to place in the one joint than might be thought, both in enzyme concentration and in histology (Muirden and Mills, 1971). In parallel histochemical studies using the Gomori technique for acid phosphatase, marked staining was seen in many lining cells and in stromal histiocytes, whilst lymphocytes and plasma cells contributed little. Heavy lining cell staining appeared in cases showing high enzyme levels on tissue homogenization.

An unexpected result was the finding that the duration of disease in the operated joint had little bearing on the type of pathology seen and even on the joint damage. Case 3, for example, showed very high enzyme levels and joint destruction had proceeded rapidly to produce a disorganized joint within 9 months of the onset of symptoms. Case 4 (seronegative) had intact articular cartilage and bone after 10 years of almost continuous symptoms and enzyme levels were relatively low. Although this series is small it suggests that the formula for rheumatoid arthritis devised by Bayles (1966):

$$\text{Amount of inflammation} \times \text{time} = \text{joint damage}$$

is an oversimplification with the progression of disease depending on factors such as the characteristics of the joint inflammation rather than time.

Interpretation of synovial fluid enzyme results is more difficult. Sample numbers are smaller and other factors such as turnover rates and possible

inhibitors in the fluid have to be considered. In our view the synovial tissue results give a better picture of the total lysosomal enzyme potential of the joint rather than a value at one particular moment as seen in the synovial fluid enzyme levels. Even so a correlation was shown between the enzyme concentrations and joint damage and between the AGA concentration of synovial tissue and fluid. However, synovial fluid leucocyte counts, which are variable and often rendered inaccurate by the presence of particulate matter, do not regularly parallel the enzyme concentrations. Other studies showing poor correlations between leucocyte counts and enzyme concentrations include those of Bartholomew (1968), Jasani, Katori, and Lewis (1969), and Beckman and others (1971), and the evidence available suggests that enzymes in the synovial fluid originate mainly from synovial lining cells.

The findings in this study support the concept that lysosomal enzymes are involved in connective tissue breakdown important in the pathogenesis of rheumatoid arthritis. It is doubtful if the enzymes measured are themselves responsible for cartilage destruction although the effect of AGA on mucopolysaccharide including hyaluronic acid may have some relevance. We have been impressed with the very close parallel rise of the two enzymes measured in individual joints. Thus AP and AGA may be useful markers for

increased activity of connective tissue catabolic proteases such as cathepsins and collagenase. The factor (or factors) which provoke synovial lining cell proliferation and stimulate increased lysosomal enzyme activity remain obscure.

Summary

A significant correlation has been shown between the activity of two lysosomal enzymes in homogenates of synovial membrane and damage to the joint in rheumatoid arthritis. High enzyme levels were found in joints in which the histology was dominated by synovial lining cell proliferation. A correlation was also demonstrated between the concentration of β -acetyl glucosaminidase in the synovial fluid and in the synovial membrane but not between synovial fluid enzyme levels and the white cell count. These findings support the concept that lysosomal enzymes are involved in the pathogenesis of rheumatoid arthritis and in particular damage to articular cartilage

Mr. Kingsley Mills performed the surgery and recorded the operative gradings. Many of the cases were referred to Mr. Mills from the arthritis clinic of The Royal Melbourne Hospital by Dr. R. F. A. Strang. Valuable technical assistance was provided by Miss Carol Smith and Mr. Keith Rogers.

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