Depolymerisation products of hyaluronic acid after exposure to oxygen-derived free radicals

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SUMMARY Preparative chromatographic fractions of human umbilical cord hyaluronic acid (HA) of a molecular weight of 10^6 were subjected to graded oxygen-derived free radical (oxy radical) fluxes produced by: (a) the autoxidation of ferrous ions; (b) the action of xanthine oxidase (XO) on hypoxanthine (HX); and (c) by peripheral blood polymorphonuclear leucocytes that had been stimulated by phorbol myristate acetate (PMA). Analysis by gel chromatography of the products obtained with each of the oxy radical generating systems showed polydispersity in size. The smallest molecules detected had a molecular weight of 10^4 . This limiting size was not reduced further by exposure to a second oxy radical flux. The relative proportions of large, medium, and small degradation products were established for various levels of oxy radical flux. Consistently a relatively rapid transition from large to small material was seen on Sepharose 2B chromatography, suggesting an ordered element to the breakdown process. Although the decrease in molecular weight after oxy radical exposure was confirmed by analytical ultracentrifugation, the procedure showed that those samples of lowest viscosity did not have the lowest sedimentation values, possibly reflecting oxy radical-induced repolymerisation. If the size and possibly the conformational characteristics of HA are altered, oxy radical exposure might be expected to alter its biological properties.

Key words: molecular modifications.

Analyses of the hyaluronic acid (HA) content of synovial fluids from inflamed joints have shown not only decreased HA concentration but also the presence of some hyaluronate of reduced molecular weight and reduced specific viscosity.¹ In addition, the viscosities of solutions of HA and of synovial fluid can be lowered by exposure to an oxygen-derived free radical (oxy radical) flux.² Activated polymorphonuclear leucocytes generate oxy radicals, and these cells predominate in synovial effusions, even in chronic forms of inflammatory arthritis, e.g., rheumatoid arthritis. Moreover, the presence of enzymes that are able to degrade HA has not been convincingly shown in inflamed joint tissues or synovial fluid.³ Oxy radical-induced HA depolymerisation may therefore be responsible for

the presence of lowered molecular weight HA in inflammatory synovial fluids.

HA consists of repeating disaccharide units of D-glucuronic acid and N-acetylglucosamine.⁴ These form long unbranched polymers that can adopt a random coil conformation in solution. Despite this simple structure, HA has a number of important physiological functions which are dependent upon its molecular weight. An example of this was demonstrated by Forrester and Balazs,⁵ who studied macrophage phagocytosis in the presence of HA. They showed a biphasic effect dependent on molecular weight, with inhibition of phagocytosis by HA of $1-2 \times 10^6$ molecular weight and stimulation by HA of 10^5 or lower molecular weight for solutions with concentrations between 0.2 and 0.5 g/l. The extent to which exposure to oxidants alters the size of HA and possibly its structure is therefore of considerable importance in joint pathophysiology.

Initial work in this area was done by Greenwald

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and Moy³ who showed the generation of a polydisperse population of breakdown products included on Sepharose 2B chromatography with oxy radicals generated both enzymatically and by stimulated polymorphonuclear leucocytes. We have sought to extend their observations by using maximal and repeated fluxes and a number of chromatographic gels to determine the extent of oxy radical-induced depolymerisation of HA. In addition, we have determined the molecular weights of depolymerised HA samples by analytical ultracentrifugation. The collection of a large series of spectra of sizes of the products of oxy radical-induced HA depolymerisation can then be analysed in order to investigate the mechanism of depolymerisation.

Materials and methods

Hyaluronic acid (grade III from human umbilical cord) and xanthine oxidase (grade III from butter-milk) were obtained from the Sigma Chemical Co., St Louis, Missouri. Sepharose CL-2B and CL-4B, Sephadex G100, and Sephacryl S400 were obtained from Pharmacia, Uppsala, Sweden. All other chemicals were of the highest purity available from Sigma or from Ajax Chemicals, Sydney, Australia.

HYALURONIC ACID PURIFICATION

HA was further purified by passage through a Sephacryl S400 preparative column (volume 280 ml) with 0.5 M sodium acetate as the buffer. Fractions with a K_{av} between 0.1 and 0.25 were pooled. This represented a hydrodynamic size of between 2×10^6 and 5×10^5 . Protein contamination was reduced to less than 1% w/v by predigestion with pronase, a procedure shown by Bartold *et al.*⁶ to have no effect on the hydrodynamic size of HA.

OXYGEN RADICAL GENERATION

Ferrous ion autoxidation

The reaction mixture comprised: (a) HA 1 g/l; (b) potassium phosphate buffer 50 mM pH 7.4; (c) ferrous sulphate at concentrations varying between 5 and 1000 μ mol/l; (d) ethylenediaminetetra-acetic acid (EDTA) in a 1:1 ratio with ferrous ions.

Stock solutions of HA were prepared at an initial concentration of 4 g/l in sterile distilled water. This slightly cloudy solution was cleared by passage through a 1.2 μ m Millipore filter and was then stored either frozen or at 4°C until required. Before use a solution of 2 g/l HA in 50 mM phosphate buffer was prepared by the addition of an equal volume of 100 mM potassium phosphate buffer to the initial 4 g/l HA stock solution. Stock solutions of 10 mM EDTA were prepared in phosphate buffer, and stock solutions of 10 mM and 1 mM ferrous

sulphate were prepared in water. The reaction volume in each case was 1.7 ml. Under these conditions and in the presence of atmospheric oxygen autoxidation of ferrous ions proceeds with the production of the hydroxyl radical (OH $\cdot$), the agent reported to be directly effecting HA depolymerisation.^{7,8} The reaction proceeds to completion in less than five minutes.⁷

Xanthine oxidase/hypoxanthine (XO/HX)

For the enzymatic system the reaction mixture comprised: (a) HA 1 g/l; (b) potassium phosphate buffer 50 mM pH 7.4; (c) HX 6 mM; (d) XO at concentrations varying between 5×10^{-3} and 1 U/ml.

The action of XO on HX under aerobic conditions in vitro leads to the production of superoxide anion and hydrogen peroxide in addition to xanthine and subsequently uric acid. Trace quantities of iron present in the phosphate buffer used allow further reactions leading to the production of hydroxyl radicals.⁷ The reaction was allowed to proceed to completion (greater than 20 minutes).⁷

Stimulated polymorphonuclear leucocytes

For the phorbol myristate acetate (PMA) stimulated polymorphonuclear leucocyte system the reaction mixture comprised: (a) HA 1 g/l; (b) Dulbecco's phosphate-buffered saline pH 7.4; (c) EDTA 60 μ M; (d) ferric chloride 10 μ M; (e) PMA 200 μ g/l.

Blood was obtained by venepuncture from healthy laboratory personnel and was anticoagulated with EDTA. Polymorphonuclear leucocytes were separated by low speed centrifugation over Ficoll-Hypaque in a single step procedure as outlined by Ferrante and Thong.⁹ The cells were then washed three times in Dulbecco's phosphate-buffered saline. Immediately before the experiment viability was assessed by trypan blue exclusion. Only cell preparations showing greater than 95% exclusion were used. PMA 200 μ g/l was used as the stimulant, and superoxide production was confirmed by observing the reduction of nitroblue tetrazolium.¹⁰ Graded fluxes were obtained by varying the number of cells added to the reaction mixture between 0.6×10^9 and 12×10^9 /l. The cells were incubated at 37°C for three hours and then sedimented by centrifugation for 10 minutes at 1000 g. The viscosity of the supernatant was then measured.

VISCOMETRY

The viscosities of solutions of undegraded and degraded hyaluronate were measured simply and reproducibly by a 1 ml tuberculin syringe and a 20 gauge needle at 20°C according to the method of

Betts and Cleland.⁷ Relative viscosities were calculated by dividing the time taken for 0.8 ml of test solution to drain from the syringe by the time taken for the same volume of buffer to drain. Specific viscosity (Nsp) was then calculated by subtracting 1 from all the relative viscosities (i.e., subtracting the contribution of the buffer). Changes in Nsp were expressed as the percentage ratio of degraded material to undergraded material. The viscosities of solutions of oxy radical exposed material were determined at two hours for solutions exposed to the ferrous ion autoxidation or XO/HX systems and at three hours for solutions exposed to the cellular system.

GEL CHROMATOGRAPHY

Aliquots of 0.25 ml of the reaction products were fractionated on Sepharose 2B or 4B and Sephadex G100 (30 × 0.7 cm). 0.7 ml fractions were eluted at 7 ml/h with 0.5 M sodium acetate at pH 5.6. Characterisation of the columns was determined with dextran blue, ³⁵S-sulphate, and glucuronic acid. In addition, in order to verify the resolution of these small columns a series of standards was run through both large (i.e., 1.5 × 90 cm) and small columns and the Kavs of the profile peaks compared.

URONIC ACID ANALYSIS

Uronic acid was estimated by the *m*-hydroxydiphenyl method of Blumenkrantz and Asboe-Hansen¹¹ or by a modification of the automated method of Rosenthal *et al.*¹²

ANALYTICAL ULTRACENTRIFUGATION

The sedimentation coefficients of intact and depolymerised HA samples were determined on a Beckman model E analytical ultracentrifuge. 0.5 ml

samples in 50 mM phosphate buffer were centrifuged at 59 780 rpm (259 000 *g* at the cell centre) in 12 mm single sector cell with plane quartz window in an An D rotor at 18°C for up to two hours. Photographs of Schlieren patterns were taken at eight-minute intervals and images measured on Nikon profile analyser with a magnification constant of 2.5. The average molecular weights of the peak material were calculated by reference to standard preparations of HA kindly provided by Dr M Mathews of Chicago and to the values derived from the data of Cleland and Wang¹³ and a standard preparation of Healon analysed by Dr P M Bartolotti *et al.*⁶

Results

VISCOSITY

A substantial decrease in Nsp was observed upon exposure of HA to oxy radicals, whether generated by the autoxidation of ferrous ions, enzymatically with XO/HX, or by incubation with PMA activated polymorphonuclear leucocytes (Table 1). The decreases in viscosity seen were proportional to the intensities of the fluxes generated within each system. After exposure to fluxes of maximal intensity the viscosity of HA approached that of the buffer alone. The decrease in Nsp with the cellular system was partly inhibited by superoxide dismutase, thus implying that superoxide ions or derived oxy radicals, or both, were at least in part, responsible.

MOLECULAR EXCLUSION GEL CHROMATOGRAPHY

Sephadex G100

Samples of HA which had been exposed to different oxy radical generating systems were applied to

Table 1 Loss of viscosity induced by each oxy radical generating system

Autoxidation of ferrous ions		Xanthine oxidase/hypoxanthine		Activated polymorphonuclear leucocytes	
Ferrous ion conc. (μmol/l)	Nsp (%)	Xanthine oxidase (U/ml)	Nsp (%)	Cell conc. (cells/l)	Nsp (%)
0	100	0	100	0	100
25	47	0.05	23	0.6×10 ⁹	63
50	31	0.1	22	6×10 ⁹	60
100	19	1.0	12	12×10 ⁹	56
200	11			12×10 ⁹ (+ SOD*)	67
500	4			12×10 ⁹ (No PMA)	65
				12×10 ⁹ (4°C)	98

In the ferrous ion autoxidation system HA 1 g/l was exposed to ferrous ions in the presence of 50 mM potassium phosphate buffer pH 7.4. EDTA was present in a 1:1 ratio with ferrous ions. In the XO/HX system HA 1 g/l was exposed to XO in the presence of 50 mM potassium phosphate buffer pH 7.4 and HX 6 mM. In the cellular system HA 1 g/l was exposed to cells in the presence of Dulbecco's phosphate buffered saline pH 7.4 to which had been added FeCl₃ 10 μM, EDTA 60 μM, and PMA 200 μg/l. SOD was used at a concentration of 60 U/ml.

*SOD=superoxide dismutase.

Sephadex G100 columns. On elution with 0.5 M sodium acetate HA from preparations with Nsps of 10% of control, or greater, were largely excluded from the gel, whereas a major proportion of the HA from lower viscosity samples had a hydrodynamic size sufficient to be included in this gel. The smallest products were seen at a K_{av} of 0.66 which corresponded to a molecular weight of 10^4 (Fig. 1).

A further experiment was performed to determine whether the exposure of HA to two sequential oxy radical fluxes would reduce the hydrodynamic

Fig. 1 Sephadex G100 chromatography of hyaluronic acid exposed to the ferrous ion autoxidation system. HA 1 g/l was exposed to ferrous ions at a concentration of 50 $\mu\text{mol/l}$ (N_{sp} =78%) and 1 mmol/l (N_{sp} =8%) in the presence of 50 mM potassium phosphate buffer pH 7.4. EDTA was present in a 1:1 ratio with ferrous ions. (mg/ml=g/l).

size yet further. HA was first exposed to the XO/HX system, and an aliquot was then chromatographed (Fig. 2). The remainder was then exposed to the ferrous ion autoxidation system, i.e., 1 mM ferrous sulphate. After this double exposure to an oxy radical flux no material was observed past a K_{av} of 0.66 on Sephadex G100, though the overall change in shape of the profile seen on Sepharose 4B with a characteristic shift towards V_t indicated that further depolymerisation had occurred.

Sepharose 4B

Fractionation of commercial HA on Sephacryl S400 provided a nominally high molecular weight starting material, however, there was still some retarded material present on Sepharose 4B chromatography. With this material a series of chromatographic profiles was obtained for graded fluxes generated by the ferrous ion autoxidation, the XO/HX system, and the stimulated polymorphonuclear leucocytes system. They are shown in Fig. 3.

The effect of an increasing oxy radical flux on these high molecular weight HA preparations in each of the oxy radical generating systems was to shift the amount of uronic acid containing material from the void volume (V_o) to the included volume, i.e., to the right. Any degree of shift to the right on chromatographic profile was reflected by a decrease in N_{sp} in each of the generating systems. The largest decrease in viscosity was seen in the ferrous ion autoxidation system where an N_{sp} of 5% of control was obtained, and at this viscosity no uronic acid containing material was seen at V_o . However, a significant amount of material was retarded by the gel (i.e. K_{av} =0.47, corresponding to a molecular weight of 2×10^5). Also, with an N_{sp} as low as 32% of control, uronic acid containing material remained present at V_o , indicating the presence of material with a hydrodynamic size of 10^6 still present in this sample.

HA exposed to the stimulated polymorphonuclear leucocytes remained predominantly excluded by the gel over the range of viscosities achieved. However, there was an increase in the amount of material seen in the included volume, and in the samples with N_{sp} s of 63% and 56% of control a small amount of material was present at a K_{av} =0.66 that was not present in the starting material.

Sepharose 2B

A large series of HA samples was exposed to the ferrous ion autoxidation system in order to resolve the initial changes in the molecular weight spectra. Small increments in ferrous ion concentration (and therefore oxy radical flux) were used, and chromatographic profiles were obtained on Sepharose 2B.

Fig. 2 Hyaluronic acid exposed to two sequential oxy radical fluxes. HA was exposed to 0.3 U/ml XO and 6 mM HX in 50 mM phosphate buffer pH 7.4. An aliquot was then fractionated on Sepharose 4B and another was fractionated on Sephadex G100. These profiles are shown in the middle panel. HA containing fractions were then pooled, dialysed against water, then dried in vacuo over phosphorus pentoxide before exposure to the ferrous ion autoxidation with a concentration of ferrous ions of 1 mmol/l. Profiles obtained after fractionation of this material on the same gels are shown in the bottom panel. (mg/ml=g/l).

A representative sample of these is shown in Fig. 4. Examination of these profiles indicated that there were two major peaks, one toward V₀ and the other approaching V_t. As degradation progressed there was a rapid transition of material from the V₀ to V_t rather than a progression across the profile. For example in the fractionation of the sample with an Nsp of 87% of control the majority of uronic acid

containing material was excluded, whereas a profile obtained for a sample with an Nsp of 8% of control shows a peak of uronic acid containing material approaching V_t. In fact, between the relatively intact HA and the low molecular weight degradation product medium sized material does not appear as discrete peak. When the area under the curve in the first, middle, and final thirds of chromatography

Fig. 3 Sepharose 4B chromatography of hyaluronic acid exposed to each of the oxy radical generating systems. In the ferrous ion autoxidation system HA 1 g/l was exposed to ferrous ions at concentrations of 100 $\mu\text{mol/l}$ (Nsp=51%) and 1 mmol/l (Nsp=5%) in the presence of 50 mM potassium phosphate buffer pH 7.4. EDTA was present in a 1:1 ratio with ferrous ions. In the XO/HX system HA 1 g/l was exposed to XO at concentrations of 0.1 U/ml (Nsp=32%) and 1 U/ml (Nsp=20%) in the presence of 6 mM HX and 50 mM potassium phosphate buffer pH 7.4. In the cellular system HA 1 g/l was exposed to concentrations of polymorphonuclear leucocytes of 0.6×10^9 cells/l (Nsp=63%) and 12×10^9 cells/l (Nsp=56%) in the presence of Dulbecco's phosphate-buffered saline pH 7.4 to which had been added 10 μM FeCl_3 , 60 μM EDTA, and 200 $\mu\text{g/l}$ PMA. (mg/ml=g/l).

profiles from Sepharose 2B separation are plotted against percentage Nsp (Fig. 5) the area in the middle third does not increase above 33% of the total area.

With the high molecular weight fraction of HA obtained from a preparative Sephacryl S400 column and exposed to the autoxidation system a similar computation was applied to the first, middle, and final thirds of a series of Sepharose 4B fractionations. Again no more than 33% of the uronic acid appeared in the transition portion of the profiles (data not shown).

ANALYTICAL ULTRACENTRIFUGATION

Sedimentation of HA and its oxy radical-induced degradation products was achieved on the analytical ultracentrifuge. The reciprocals of the sedimentation coefficients (S_0^{-1}) and the extrapolations to derived $M_{s,D}$ values (Cleland and Wang¹³ and

Bartold *et al.*⁶) are shown in Table 2. Three samples of HA were used, each of a different starting molecular weight. They were all exposed to the ferrous ion autoxidation system. Again a lower molecular weight limit of approximately 10^4 was obtained. The calculated molecular weight values ($M_{s,D}$) followed a predictable overall drop with decreased viscosity. However, in experiment 1 the sample exposed to 20 $\mu\text{mol/l}$ ferrous ions (Nsp=87% of control) had an $M_{s,D}$ of 60 000, whereas in the sample exposed to 100 $\mu\text{mol/l}$ ferrous ions (Nsp=39% of control) the $M_{s,D}$ value was 88 000. Similarly in experiment 3, where more samples were analysed, the lowest $M_{s,D}$ value calculated was 9000 in the sample that had been exposed to 125 $\mu\text{mol/l}$ ferrous ions, whereas samples exposed to 150 and 200 $\mu\text{mol/l}$ ferrous ions showed $M_{s,D}$ values of 39 000 and 42 000 respectively before a further drop occurred.

Table 2 Molecular weight estimation of hyaluronic acid exposed to the ferrous ion autoxidation system with the analytical ultracentrifuge

Ferrous ion concentration (μmol/l)	Specific viscosity Nsp (%)	Reciprocal of the sedimentation coefficient at zero concentration S ₀ ⁻¹	Calculated molecular weight M _w
Experiment 1			
0	100	0.060	1 450 000
10	91	0.045	84 000
20	87	0.485	60 000
100	39	0.418	88 000
Experiment 2			
0	100	0.297	232 000
20	89	0.302	213 000
100	41	0.422	95 000
1000	10	0.583	43 000
Experiment 3			
0	100	0.428	92 000
25	86	0.417	98 000
50	72	0.495	65 000
75	56	0.518	58 000
100	52	0.897	15 000
125	46	1.106	9 000
150	35	0.610	39 000
200	24	0.589	42 000
400	13	0.906	15 000

For each experiment the reaction concentration of HA was 1 g/l, EDTA was present in a 1:1 ratio with ferrous ions, and 50 mM phosphate buffer pH 7.4 was used. The sedimentation coefficients were then determined on concentrations of HA of 1 g/l, 0.5 g/l, and 0.25 g/l, though at the latter concentration in some of the more depolymerised samples no peak was identified and therefore no value for the sedimentation constant could be used. Since the HA concentrations used were relatively low, a linear regression of the reciprocal of the sedimentation coefficient could be used to determine S₀⁻¹.¹¹

Discussion

The extent to which oxy radical-induced depolymerisation of HA occurs in the inflamed joint is unknown. Owing to its extracellular location, HA is not protected from oxy radical attack by superoxide dismutase, catalase, or the glutathione-glutathione reductase system.² Polymorphonuclear leucocytes and macrophages release superoxide anion and hydrogen peroxide when undergoing phagocytosis.¹⁴ In the presence of trace quantities of iron superoxide anion and hydrogen peroxide will react together to produce the hydroxyl radical in a

Fig. 4 A selection of Sepharose 2B chromatographic profiles after exposure of hyaluronic acid to the ferrous ion autoxidation system with small increments in ferrous ion concentration. The experimental conditions were the same as outlined in Fig. 1. The concentrations of ferrous ions used in the profiles shown were 20 μmol/l (Nsp=87%), 50 μmol/l (Nsp=73%), 100 μmol/l (Nsp=39%), 200 μmol/l (Nsp=23%) and 1000 μmol/l (Nsp=8%). (mg/ml=g/l).

Fig. 5 Area under the curve as a function of percentage Nsp for hyaluronic acid exposed to the ferrous ion autoxidation system and then fractionated on Sepharose 2B. A large series of HA samples were exposed to the ferrous ion autoxidation system and then fractionated on Sepharose 2B. In each profile obtained the area under the curve in each third was measured. This figure shows those measurements plotted as a function of percentage Nsp. The area for the first third of each profile (■.....■) therefore represents high molecular weight material. The area under the curve for the middle third of each profile (▲.....▲) represents middle molecular weight material, and the last third of the profiles (□.....□) represents low molecular weight material.

superoxide-driven Fenton reaction.^{7,8} Certainly iron is found in abundance in inflamed synovial tissue,^{15,16} and this has led Betts and Cleland⁷ and others to speculate that non-enzymatic autoxidation of ferrous ions may occur locally at sites of iron deposition in the presence of reducing agents, e.g., reduced glutathione or ascorbic acid.

The commercial HA used in this study was supplied as a lyophilisate, and Sepharose 4B chromatography shows this to be polydisperse as regards

hydrodynamic size. Attempts to obtain a high molecular weight HA preparation by fractionation on Sephacryl S400, with subsequent dialysis and lyophilisation of the fractions, reduced the amount of material retarded by Sepharose 4B chromatography but did not eliminate it entirely. This is congruent with the recent observations of Wedlock *et al.*¹⁷ who showed partial depolymerisation of HA by oxy radicals generated by the lyophilisation step.

The controlled exposure of HA to oxy radicals is

accompanied by a predicted decrease in viscosity and the appearance of a polydisperse population of breakdown products. This phenomenon is noted whether the radicals are generated by the autoxidation of ferrous ions, by the action of XO on HX, or by stimulated peripheral blood polymorphonuclear leucocytes. Greenwald and Moy³ have reported a small series of chromatographic profiles that show adequately a generalised loss of hydrodynamic size of HA exposed to two complex radical generating systems. We have endeavoured to define the progress of oxy radical-induced degradation by increasing the number of profiles analysed. Of the oxy radical producing systems studied, the ferrous ion autoxidation system could be most readily manipulated to provide a comprehensive series of degraded HA samples. Less extensive studies with the XO/HX system produced congruent findings compatible with a similar form of hydroxyl radical attack on HA in both systems. The relatively modest degradation achieved by PMA stimulated polymorphonuclear leucocytes could be partly explained by: (a) lesser fluxes of superoxide (not quantified) or (b) less uniform production of oxy radicals by the cellular system, or both.

The observation of the effects of oxy radicals on HA by concurrently monitoring several parameters (i.e. viscosity, change in hydrodynamic profile on several gels, and M_{SD}) has indicated that change in viscosity is a sensitive indicator of early damage to HA. For example, a sample with an N_{sp} =32% of control (Fig. 3) still contains a significant amount of material that is excluded on Sepharose 4B chromatography. An explanation of this may be evident from the concept expounded by Balazs¹⁸ and others that HA in solution forms an interlocking molecular matrix. If several large chains of HA in this molecular matrix are depolymerised, the matrix would become less ordered, despite the persistence of some intact molecules. Indeed fractionation on Sepharose 4B at that stage, early in the depolymerisation process, did then show some excluded material, representing the intact chains together with some material in the included volume representing the depolymerised chains. Yet as a whole the solution was dramatically altered in viscosity. In turn these changes are likely to cause alterations of physiological properties.

In the present study the smallest degradation products obtained had a hydrodynamic size of the order of 10^4 . This lower limit was not shifted by subsequent exposure to a second oxy radical flux. Analysis of the large series of chromatographic profiles obtained with the ferrous ion autoxidation system suggested that the depolymerisation of HA by hydroxyl radicals may be at least partly ordered

with rapid progression from large to small material, namely an inevitable chain reaction.

The observed increase in sedimentation coefficient of lower viscosity HA samples on analytical ultracentrifugation may reflect some form of molecular interaction (aggregation) or conformational change due to cross linking. Balazs *et al.*¹⁸ have postulated that during pulse radiolysis of HA in addition to direct oxidative cleavage of the glycosidic linkage between glucuronic acid and N-acetylglucosamine, uronate free radicals may be formed by elimination of the hydrogen at C5. The radical would then be resonance stabilised by interaction with the carboxyl group. These radicals could interact to form cross-linked hyaluronate degradation products. Indeed the identification of these branched products in synovial fluid could be used to implicate more directly radical-induced damage to macromolecules in inflamed joints. Certainly such structural alterations could have important consequences in terms of the altered physiological properties of the hyaluronate matrix.

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Book review

Advances in Inflammation Research. Vol. 9. The Spondyloarthropathies. Eds. M Ziff and S B Cohen. Pp. 272. US\$ 70.50. Raven: New York. 1985.

This book is derived from a meeting held in Dallas in 1984. It contains updated accounts of histopathology of ankylosing spondylitis and of an assortment of specific and non-specific laboratory tests on patients' blood. Epidemiological studies show spondyloarthropathies to be related to other diseases and to require other predisposing genes besides HLA-B27. There is an excellent account of HLA-B27 and arthritis in non-Caucasians. Splits in HLA-B27 defined by monoclonal antibodies and cytotoxicity testing (but not by molecular biological techniques) are described. Hypotheses attempting to relate HLA-B27 with Gram-negative bacteria and ankylosing spondylitis receive

their usual airing and some thoughtful criticism. Perhaps the most valuable section of the book deals with fine details of the immune response to assorted organisms believed to cause arthritis. Each author seems to have found a different antigen or a different agent. Nevertheless, they leave the impression that immunologists are returning to their historic role of using antibodies to classify infections and that this approach is more likely to succeed than the current trek through nebulous concepts of autoimmunity.

This is a useful and stimulating book, strongly recommended to anyone interested in the aetiology of inflammatory arthropathies.

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