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Hyaluronic acid production in vitro by synovial lining cells from normal and rheumatoid joints

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SUMMARY Organ cultures and primary cell cultures were established from synovial tissue collected from patients with rheumatoid arthritis. Hyaluronic acid measured by the incorporation of [^{3}H]glucosamine into the polysaccharide was found to be synthesised in the cultures immediately after transfer from in-vivo to in-vitro conditions. This was in contrast to the primary cultures established from cells isolated from normal joints. The latter cells did not synthesise any detectable hyaluronate. 90–100% of the cells in primary culture were found to be esterase positive, indicating their macrophage nature. The molecular weight of the hyaluronate produced by the pathological cells was low (~50 000) compared with the molecular weight of hyaluronate found in joint fluid from normal or rheumatoid joints. Cell lines of fibroblasts established from rheumatoid joints and studied after four or seven passages also produced hyaluronate of low molecular weight. It is known that similar cell lines from normal joints produce a high molecular weight polymer.

Key words: biosynthesis, synovial cells, rheumatoid arthritis, in vitro.

Rheumatoid arthritis is characterised among other things by a hypertrophy of the cell layer lining the synovial cavity, an accumulation of polymorphonuclear cells, lymphocytes, plasma cells, and macrophages in the synovial tissue, and an increased volume of synovial fluid and thereby an increased amount of hyaluronic acid (sodium hyaluronate) in the joint.

The synovial lining cells exhibit different forms, which have been designated A, B, and C cells.[1] The A cells show the morphology of macrophages and they are active in phagocytosis; the B cells exhibit an extensive Golgi apparatus, indicating a secreting function, and they have been assumed to produce hyaluronic acid; and the C cells have an intermediate appearance. When the synovial cells have been established as cell lines in vitro they take on a fibroblast-like appearance[2] even if certain of their previous characteristics can be induced by various compounds.[3–5]

Many authors have grown synovial cells, both normal and rheumatoid, in culture and studied the production of hyaluronic acid and the effects of drugs on this activity. However, these studies have been performed mainly with well established cultures when the cells exhibit a fibroblast-like appearance (see e.g., references 3, 6–12). It can be argued that the results do not reflect in-vivo hyaluronic acid metabolism. Some studies have, however, been performed on organ cultures where the synovial membrane or villi from this membrane were used.[13] In the present investigation we have attempted to isolate synovial lining cells from normal and rheumatoid joints and to study their hyaluronate production immediately after transfer to culture when they still possess a morphology resembling that shown in vivo. We have found that the normal lining cells under these conditions do not produce any appreciable amount of hyaluronate, whereas the rheumatoid cells produce the polysaccharide but with an unexpectedly low molecular weight.

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Patients and methods

PATIENTS

Synovial tissue was obtained during therapeutic synovectomies from 10 women and four men (Table 1)

Table 1 *Patients with classical or definite rheumatoid arthritis subjected to surgical synovectomy*

Patient No	*Age (years)*	*Sex*	*Rose-Waaler (titre)*	*ESR (mm/h)*	*Source of synovial tissue*	*Cellular infiltration of synovium*	*Bony destruction (x-ray)*	*Medical treatment*
1	74	F	40	31	Knee	Severe	Moderate	Chloroquine, Indomethacin
2	64	F	80	30	Knee	Mild	Moderate	Penicillamine
3	21	M	<20	35	Ankle	Severe	None	Naproxen
4	52	M	2000	93	Knee	NE*	Severe	Indomethacin
5	47	M	320	10	Wrist	Severe	Moderate	None
6	58	F	40	55	Knee	Mild	Severe	Penicillamine, Ibuprofen
7	60	F	<20	49	Knee	Moderate	Severe	Prednisolone
8	57	F	<20	20	Knee	Moderate	Severe	Prednisolone, Hydroxychloroquine
9	70	F	4000	39	Knee	Moderate	Severe	Prednisolone, Hydroxychloroquine
10	61	F	NE	36	MTP*	NE	Moderate	Prednisolone, Cyclophosphamide
11	23	F	<20	145	Hip	Severe	Severe	Prednisolone, Azathioprine
12	62	F	40	65	Wrist	Moderate	Severe	Penicillamine, Piroxicam
13	48	M	4000	44	MCP*	Moderate	Severe	Myocrisin, Naproxen
14	76	F	<20	23	Knee	Mild	Moderate	Indomethacin

*NE=not examined; MTP=metatarsophalangeal joint; MCP=metacarpophalangeal joint.

with definite or classical rheumatoid arthritis according to the criteria of the American Rheumatism Association.[14] Histology of the removed synovium was performed in 12 cases and was consistent with the diagnosis active rheumatoid arthritis.

CELL AND ORGAN CULTURES FROM RHEUMATOID TISSUE

The specimens of proliferated and inflamed synovium (50×50 mm) intended for organ cultures were immediately incubated at 37°C in 10 ml RPMI medium 1640 (Flow Laboratories, Irvine, Scotland) containing 20% new-born calf serum (Flow Laboratories) and 12·5 mCi/l of [^{3}H]glucosamine (Amersham International, Amersham, UK) for 10, 15, 60, and 90 min. The incorporation was terminated by addition of a large excess of non-labelled glucosamine and the medium analysed for labelled hyaluronate.

The synovial specimens intended for cell cultures were transferred to RPMI medium prewarmed to 37°C and taken to the laboratory within 15 min. Single cell suspensions were prepared as described by Abrahamsen *et al.*[15] and Klareskog *et al.*[16] The proliferated synovium was carefully dissected under the microscope from fat and fibrous material and cut into small pieces. These were suspended in a balanced salt solution containing 20 mM HEPES pH 7·4, 0·5 g/l of collagenase (Worthington, New Jersey, USA), 0·15 g/l of deoxyribonuclease (Sigma, St Louis, Missouri, USA) and 10 g/l of human serum albumin (AB KABI, Stockholm, Sweden). The mixture was stirred carefully (100 rotations per min) on a New Brunswick incubator shaker for about 2 h at 37°C. Large pieces remaining in the digest were removed by filtration through a sterile Dacron filter (mesh 80). The resulting single cell suspension was cleared from dead cells and subcellular material by centrifugation at room temperature through a cushion of 22% Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) containing 0·15 g/l of deoxyribonuclease.[16] In three patients the cells were separated according to density on a Percoll gradient. Fractions with densities of 1·043–1·055 g/ml and 1·055–1·080 g/ml were collected.[17]

After washing with RPMI medium containing 20% human serum, 50 IU/ml of penicillin, and 50 mg/l of streptomycin the cells were counted in the presence of trypan blue and showed a viability of more than 95%. The cells were seeded on Petri dishes (diameter 60 mm, Costar, Cambridge, Massachusetts, USA) in the same medium in an amount of $1·5\times10^6$ cells in 5 ml. To this was added [^{3}H]glucosamine (10 mCi/l). After incubation for 30 min to 48 h at 37°C in 5% CO_2 the cultures were washed free of non-adherent cells, and the medium was analysed for labelled hyaluronate. The adherent cells (about 40–80% of the original cells in suspension: 20 000–30 000 cells/cm^2) were incubated with fresh medium containing the same additions as above, including [^{3}H]glucosamine, and grown for 15 to 84 h. Aphidicolin (kindly donated by Dr Hans Krokan, Tromsø) at a concentration of 4 mg/l was added in three cases to prevent cell division.[18] After termination the medium was analysed for labelled hyaluronate. In some experiments the above cell cultures were treated with 0·05% trypsin for 3 min to remove possible fibroblasts (very few). After addition of fresh medium and serum the subsequent synthesis of hyaluronic acid was recorded.

CULTURES OF NORMAL SYNOVIAL LINING CELLS

Lining cells were isolated within 15–120 minutes after death from three persons aged 63, 71, and 83 years respectively, who had died in heart infarction. They had no history of joint disease or postmortem signs of joint affections. The procedure described by Fraser and McCall was employed.[2] Briefly, the knee joints were rinsed twice with medium warmed to 37°C through a needle inserted beneath the patella. Then a balanced salt solution containing 0·25% trypsin (SBL, Stockholm, Sweden) was deposited in the joint. After 5 to 10 min of gentle massage the cell/trypsin mixture was withdrawn, serum was added to inactivate the trypsin, and the cells were filtered through a Dacron filter and collected by centrifugation at 400 *g* for 5 min. The cells were resuspended in RPMI medium containing 10 mCi/l of [^{3}H]glucosamine and 20% fetal calf serum or human serum. The cells were cultured as described above for rheumatoid cells. The trypan blue exclusion test showed a viability of between 95 and 98%.

The cells prepared by this technique have been well characterised[19] and represent a subpopulation of normal synovial lining cells. A large majority are esterase positive, carry Fc receptors, and express HLA-DR antigens like the cells obtained from rheumatoid joints. In our experiments both isolated cells from rheumatoid tissue and normal synovial lining cells were examined for non-esterase activity by the method of Yam.[20] All the cell preparations appeared to contain 90–100% esterase positive cells.

SYNOVIAL FIBROBLAST CULTURES

In five cases fibroblast-like cells were allowed to grow out from pieces of rheumatoid synovial membranes. During 14 days a dense monolayer was formed. The cells were then released by trypsinisation,[21] carried through four passages, and finally grown in Dulbecco's medium (Flow Laboratories) containing 20% human serum at a mean

density of 40 000 cells/cm^2. The cells were grown in plastic flasks (Costar) without coating or coated with collagen[22] or fibronectin.[23] In one flask without coating human serum was exchanged for human fetal serum. In two cases the cells were investigated after seven passages in medium containing 20% human serum or human fetal serum. The mean cell densities obtained were 250 000 cells/cm^2.

ISOLATION OF HYALURONATE

Hyaluronate was isolated from the culture medium. Only a minor part of the radioactivity incorporated into high molecular weight material was detected in the cellular fraction (20%). The media were made 6·5 M in urea and 0·05 M in trometamol (TRIS)-HCl buffer pH 6·5 and chromatographed on diethylaminoethyl (DEAE)-cellulose as described by Dahl and Cöster.[24] After application of the samples the columns were eluted stepwise with (*a*) urea/TRIS buffer; (*b*) urea/TRIS+0·15 M NaCl; (*c*) urea/TRIS+0·5 M NaCl; and (*d*) urea/TRIS+2 M NaCl. The hyaluronate was eluted entirely in the 0·5 M NaCl fraction as shown with high molecular weight standard samples of labelled hyaluronate (kindly supplied by Dr J R E Fraser). The recovery was 70–90%. This fraction was dialysed against distilled water and lyophilised.

CHARACTERISATION OF THE HYALURONATE

The molecular weight distribution of the hyaluronate was determined quantitatively by chromatography on the column of mixed agarose gels described by Laurent and Granath[25] or qualitatively by chromatography on Sephacryl S-300 and S-1000 (Pharmacia Fine Chemicals). The chromatographic details are given in the legends to the figures. To confirm the lable was incorporated into hyaluronate, the samples were dissolved in 0·1 M TRIS-HCl buffer pH 7·0 and incubated with hyaluronidase from *Streptomyces hyalurolyticus* (10 U/ml, 50°C, 12 h, Seikagaku Kogyo, Ltd, Tokyo, Japan) and rechromatographed on Sephacryl columns.

TESTS FOR HYALURONIC ACID DEGRADING ACTIVITY

Experiments were performed to check for possible hyaluronic acid degrading activity in the cultures of rheumatoid cells, in three serum batches used in cell culturing, and finally in serum and synovial fluid from patients with rheumatoid arthritis. Determination in serum was made by incubating 1 ml of serum, 4 ml of medium, and 10 μg of high molecular weight hyaluronate (Healon) for 48 h at 37°C. The mixture was then chromatographed on Sephacryl S-1000 and the eluate analysed by a sensitive assay for hyaluronic acid.[26] Experiments on cell cultures were performed with a ^{3}H-labelled hyaluronic acid of molecular weight 6×10^5 (gift from Dr J R E Fraser), which was added to cultures of rheumatoid cells or rheumatoid tissues in amounts of 240 ng per 5 ml flask. After incubation for 48 h at 37°C the medium was chromatographed as above and the eluate analysed for radioactivity. Similar experiments were performed on synovial fluid from rheumatoid patients, but in these experiments a [^{3}H]hyaluronate of molecular weight 300 000 was added in amounts of 240 ng to 0·2 or 2 ml fluid. Chromatography on Sephacryl S-1000 was also used to show that DEAE chromatography did not degrade high molecular weight radioactive hyaluronate.

Results

CELL CHARACTERISATION

Cells isolated from normal joints by trypsin treatment or cells isolated from rheumatoid synovial tissues by collagenase digestion were allowed to adhere to plastic dishes. At the light microscopic level both preparations showed similar characteristics, i.e., they showed a heterogeneous cell population with small rounded cells, non-dendritic macrophage-like cells, and dendritic cells often with swelling in the end of the extensions. A very few fibroblast-like, elongated cells were seen, and only few cells could be removed from the plastic dishes by trypsin treatment. In some experiments the cells were examined for non-esterase activity and they appeared to be highly esterase positive. Further characterisation of similar cell preparations has been published by other authors.[19 27–29] Cell lines established from rheumatoid synovial cells were studied after four and seven passages. At this stage the cells had the general appearance of fibroblasts.

IDENTIFICATION AND PRODUCTION OF HYALURONATE IN SYNOVIAL CELLS

The 0·5 M NaCl fraction from DEAE-cellulose, which should contain the hyaluronic acid in the medium, was isolated as described. In order to confirm that the radioactivity of this fraction had been incorporated into hyaluronic acid the material was chromatographed on Sephacryl S-300 or S-1000 before and after treatment with hyaluronidase from *Streptomyces hyalurolyticus*. The main part of the radioctive material in this fraction produced by rheumatoid cells was susceptible to the enzyme (Figs 1A and 1B). There was, however, some material in the void fraction and a distinct peak in the middle of the chromatogram that were unaffected by hyaluronidase. The results in Table 2 concerning the primary cell cultures give both the total incorporation of radioactivity into the 0·5 M NaCl fraction

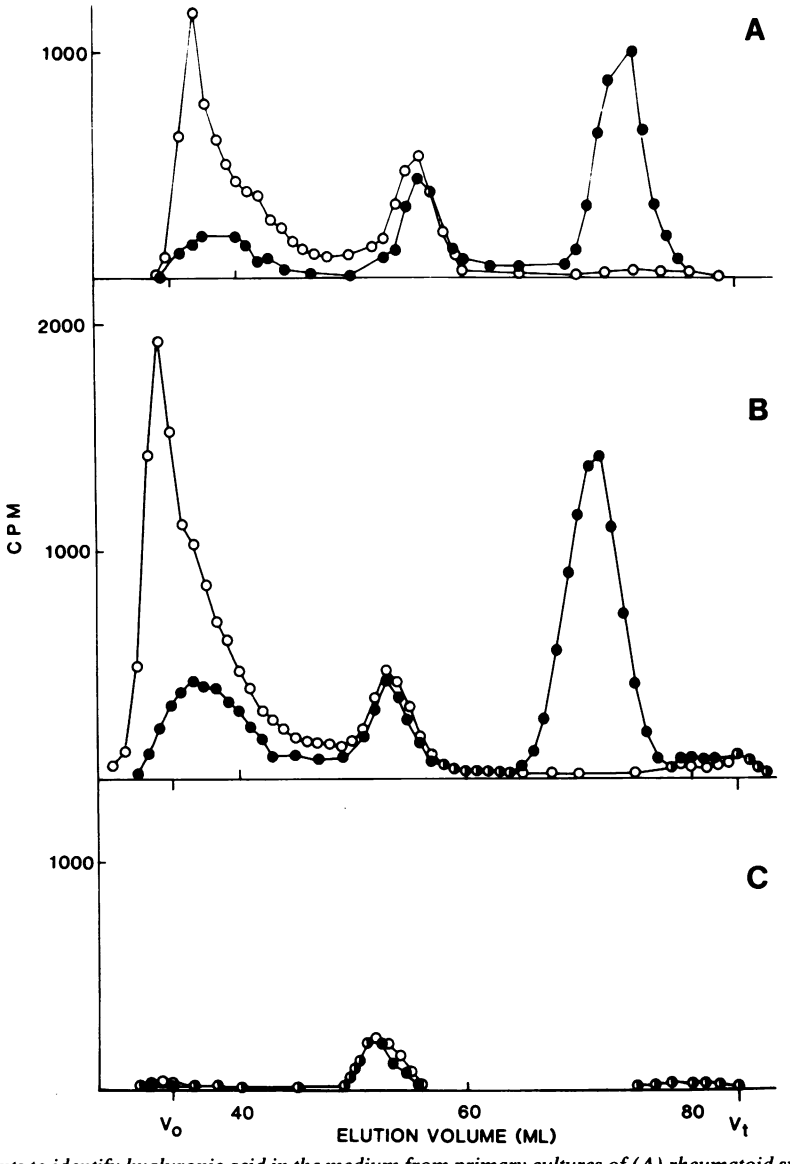

Fig. 1 *Experiments to identify hyaluronic acid in the medium from primary cultures of (A) rheumatoid synovial organ culture, (B) cell culture from rheumatoid synovium, and (C) normal synovial lining cells. (A) Specimens of proliferated and inflamed synovium were immediately incubated in medium containing [^{3}H]glucosamine for 90 min. The culture medium was chromatographed on DEAE-cellulose, the 0·5 M NaCl fraction was recovered and an aliquot applied to a 1·05 × 104 cm column of Sephacryl S-300 at room temperature. The column was eluted with phosphate-buffered saline pH 7·25, containing 0·02% sodium azide, at a flow rate of 10 ml/h, and 1 ml fractions were collected (○—○). An equal amount of material digested with hyaluronidase as described in 'Methods' was chromatographed in a subsequent run (●—●). Approximately 64% of the total radioactivity moved from the void volume to the total volume by the enzyme treatment. Some material in the void volume (17%) and a distinct peak in the middle of the chromatogram (19%) were not attacked by the enzyme. (B) Cells were established from patient No 4 and grown in [^{3}H]glucosamine. The medium collected between 5 and 27 h of culture was chromatographed on DEAE-cellulose and the 0·5 M NaCl fraction recovered. An amount of radioactivity, corresponding to the incorporation by 10^6 cells during one hour, was chromatographed on Sephacryl S-300 before (○—○) and after (●—●) hyaluronidase treatment. Approximately 61% of the total radioactivity was degraded; 15% in the middle of the chromatogram and 24% in the void volume were not attacked by the enzyme. (C) Cells were established from control No 2. They were allowed to incorporate [^{3}H]glucosamine between 5 and 27 h of culture, and radioactive material was collected and processed as above. Only trace amounts of hyaluronate could be identified, while the material in the middle of the chromatogram appeared in amounts similar to those in the rheumatoid cells.*

Table 2 *Incorporation of [^{3}H]glucosamine into hyaluronic acid by dispersed rheumatoid and normal synovial cells in primary cultures**

Patient No	*Total cell population*			*Adherent cells*		
	Incorporation time (h)	*Incorporation (cpm)/ 1 h incubation of 10^6 cells*	*Degraded by hyaluronidase (%)*	*Incorporation time (h)*	*Incorporation (cpm)/ 1 h incubation of 10^6 cells*	*Degraded by hyaluronidase (%)*
1	0–½	4 400	NE†	½–48	25 900	78
2	0–3	7 100	75	3–21	11 000	83
3	0–3	12 100	95	3–15	30 800	61
4	0–5	12 900	NE	5–27	13 900	61
5	0–16	34 100	61	16–36	62 500	90
6	0–18	7 500	81	18–42	10 800	96
7	0–24	18 800	66	24–48	2 500	91
8	0–24	38 200	90	24–48	3 000	NE
9	0–48	13 300	81	48–60	14 400	89
Normal control No						
1	0–3	400		3–24	300	
				24–48	200	
2	0–5	<100		5–27	900	
3	0–12	700		12–36	600	

*The figures represent total incorporation into the fraction eluted from DEAE-cellulose by 0·5 M NaCl. The percentage of this material degradable by hyaluronidase was estimated by chromatography on Sephacryl S-300 or S-1000 and is given in separate columns.
†NE=not examined.

and the percentage of this material susceptible to hyaluronidase. Some chromatograms did not show sufficient resolution to distinguish completely between undegraded and degraded hyaluronate, and in these instances the figures give the minimum amount of degradable material.

Pieces of synovial membrane (from patients 4 and 10 in Table 1) were incubated for 15 to 90 min with [^{3}H]glucosamine. Hyaluronate production was not observed until 90 min, indicating a lag time between the addition of precursor and the appearance of the final product. The incorporation at 90 min (40 000 cpm of hyaluronidase-susceptible material per culture) was not analysed in terms of cell number in the synovial tissue and is thus only a qualitative indication for hyaluronate production in the intact organ (Fig. 1A).

Cells dispersed from rheumatoid synovium were incubated for varying times in plastic dishes, and the incorporation of isotope into hyaluronate was measured when the total cell population was present. Non-adherent cells were then removed by washing and the hyaluronate synthesis measured in the adherent cells only (Table 2). There was a significant but highly variable hyaluronate production both before and after the non-adherent cells had been removed. The accumulation of hyaluronate in the medium was observed already during the first half hour of culture and continued throughout the period of observation (up to 60 h). There was no apparent difference in the synthesis of hyaluronate in the total cell population compared with the adherent cells.

In two experiments rheumatoid cells were separated by Percoll centrifugation into a high density and a low density fraction.[17] There was no clear difference in hyaluronate production between the two cell populations (data not shown).

The normal synovial lining cells behaved very differently from the rheumatoid cells. Only trace amounts of hyaluronate were produced in the cultures (Fig. 1C, Table 2). There was, however, an incorporation into the unknown compound, which appears in the middle of the Sephacryl S-300 chromatogram (Fig. 1C).

Fibroblast-like cell lines were established from some of the patients and their hyaluronate production was studied after four and seven passages. The production of hyaluronate by these cells is shown in Table 3. On average these cells seem to synthesise less than the cells in primary cultures (Table 2), though the results are somewhat variable. The production seems to be independent of the substrate on which the cells grow (plastic, fibronectin, collagen) or the serum added to the medium (human serum, human fetal serum).

Table 3 *Incorporation of [^{3}H]glucosamine into hyaluronic acid by fibroblast-like cell lines established from rheumatoid joint tissue (cpm/h incubation of 10^6 cells)**

Patient No	*No of passages of the cell line*	*Culturing conditions*			
		In human serum	*In human fetal serum*	*On fibronectin*	*On collagen*
3	4	8 100	10 700	4 500	6 800
11	4	7 200	2 500	4 400	2 900
	7	2 000	3 000	—	—
12	4	6 100	7 300	—	—
	7	200	600	—	—
13	4	16 200	26 500	2 400	31 300
	4†	1 800	22 300	27 000	10 300
14	4	4 800	5 700	1 800	4 100

*The figures represent total incorporation into the fraction eluted from DEAE-cellulose by 0·5 M NaCl. Greater than 80% of this material was degradable with hyaluronidase.
†This cell line was established from the fibrous tissue of the joint.

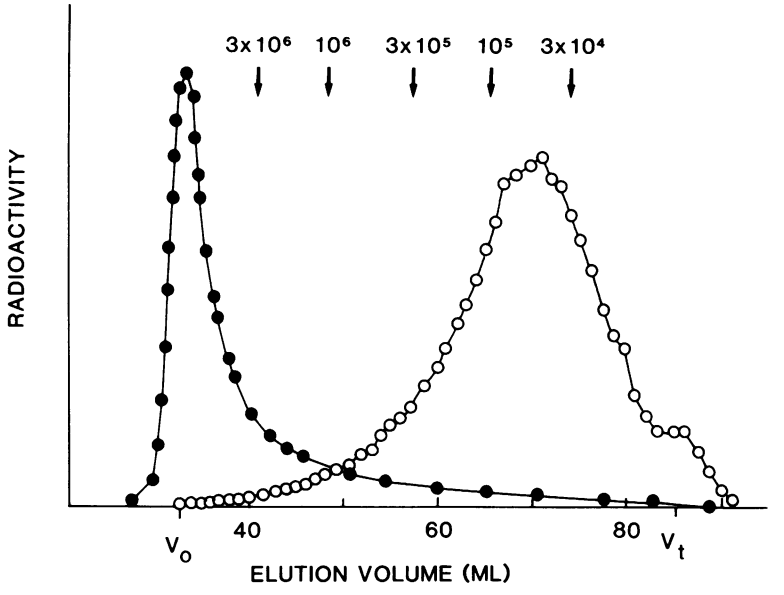

Fig. 2 *Chromatography of [^{3}H]hyaluronic acids on a mixed bed of 0·5%/4% agarose. The product synthesised by an established fibroblast-like cell line from normal synovial cells (●—●) (gift from Dr J R A Fraser) was compared with hyaluronate synthesised in a primary culture by rheumatoid synovial cells (○—○). For chromatographic conditions and calibration of the column see the paper by Laurent and Granath.[25] The sites of elution of appropriate calibration fractions are indicated at the top of the figure. The average molecular weight of the 'normal' cell product is $>3\times10^6$ and of the 'pathological' product about 5×10^4.*

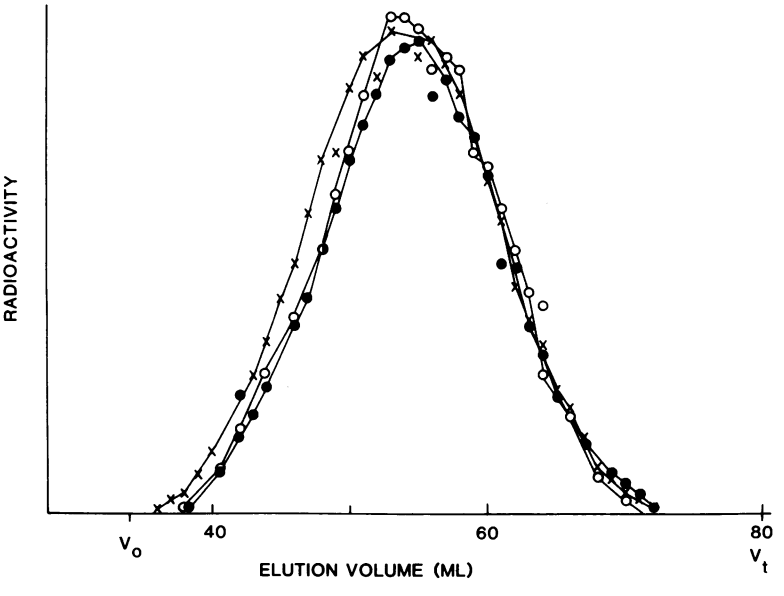

Fig. 3 *Test for hyaluronate degrading activity in cultures of rheumatoid cells or rheumatoid tissue. ^{3}H-Labelled hyaluronate with an average molecular weight of 6×10^5 was chromatographed on a column of Sephacryl S-1000 before (×—×) and after incubation for 48 h at 37°C in a primary culture of rheumatoid cells (●—●) or rheumatoid tissue (○—○). There was no significant difference in the elution patterns and thus no detectable degradation of the hyaluronate in the cultures. The chromatographic conditions were: room temperature; column size: 1·05×94 cm; solvent: 0·5 M sodium acetate pH 7·0 + 0·02% sodium azide; flow rate: 10 ml/h; fraction volume 1–2 ml.*

MOLECULAR WEIGHT OF HYALURONATE

The radioactive hyaluronates produced in organ and cell cultures were all chromatographed on a column of Sephacryl S-1000 and were all of remarkably low molecular weight and with similar elution volumes. To determine the exact molecular weight a sample from a rheumatoid cell culture (Patient No 4) was chromatographed as described by Laurent and Granath[25] on a mixed agarose bed (Fig. 2). The peak eluted in the chromatogram as a calibration fraction of molecular weight ~50 000. The elution of a sample of hyaluronate synthesised by an established cell line from normal synovial cells is shown for comparison.

SEARCH FOR HYALURONATE DEGRADING ACTIVITY IN RHEUMATOID FLUIDS

In view of the low molecular weight of hyaluronate produced by the rheumatoid cells experiments were performed to test whether there was any hyaluronate degrading activity present in the systems. Thus synovial fluid and blood serum from rheumatoid patients were incubated either with small amounts of unlabelled hyaluronate or ^{3}H-labelled polysaccharide, and the molecular weight of the polymer was assayed before and after incubation by chromatography on Sephacryl S-1000, without any noticeable change in elution pattern. Similarly, small amounts of radioactive hyaluronate were added to primary cell cultures or organ cultures or rheumatoid synovium and incubated for 48 h at 37°C. No degradation of the radioactive polymer was recorded (Fig. 3).

Discussion

After rheumatoid synovial tissue has been dissected free from underlying connective tissue matrix it can be treated with enzymes to disperse the cells. When cultured, the majority of the cells adhering to the substrate can be divided into two main populations: first, small cells related to the mononuclear phagocytes and secondly, larger cells with a stellate morphology, which have been shown to produce large amounts of prostaglandin E_2 and collagenase.[30] Both groups of adherent cells appear to express HLA-DR antigens.[16]

The aim of the present study was to study the production of hyaluronate in primary cultures of rheumatoid synovial cells, when they still retain the above described morphology, since this, to our knowledge, has not been done before. When cell lines are established from the cultures the cells become fibroblast like, and several authors have shown that at this stage they are producers of hyaluronate.[3 6–12]

The synovial and subsynovial layers were carefully dissected from the underlying connective tissue and exposed to collagenase for only a short period to avoid contamination with fibroblasts. In addition, aphidicolin, known to inhibit cell division,[18] was added to some of the cultures to prevent multiplication of the fibroblasts. From a morphological standpoint less than 5% of the cells had a fibroblast-like appearance 24 h after the cells had adhered. In addition, more than 90% of the total cell population were esterase positive.

After transfer to in-vitro conditions the rheumatoid synovial cells were shown to produce hyaluronate immediately in amounts equal to or larger than those produced by fibroblast cell lines established from rheumatoid joints (compare Tables 2 and 3). A pronounced variability in the synthetic rate was observed in cells from different RA patients.

In contrast to the rheumatoid cells, synovial lining cells from normal controls were not found to produce significant amounts of hyaluronate either in cell suspension or when adhered to substrate. The normal cells were obtained by intra-articular administration of trypsin. The use of a different procedure for isolation of the cells was an experimental necessity in work with human material but opens the possibility that different cell populations have been collected from the normal and pathological joints despite a similar morphology when adhered to substrate. It is unlikely that trypsin treatment of the normal cells explains the lack of hyaluronate production, since addition of similar amounts of trypsin did not affect the synthesis in adherent rheumatoid synovial cells. Hyaluronate production has also been shown in numerous cell lines established by the standard method in tissue culture of cells released with trypsin. The appearance of the unknown peak in the middle of the Sephacryl S-300 chromatogram (Fig. 1) shows that normal and rheumatoid cells have additional similarities in their biosynthetic machinery. It could also be argued that the use of collagenase in the dispersion of rheumatoid synovial cells had stimulated synthesis of hyaluronate, though this is unlikely in view of the apparent hyaluronate production in intact organ cultures of rheumatoid synovial tissue. If these objections are borne in mind the most probable explanation of our results is a pronounced increase in hyaluronate synthesis by the synovial cells in rheumatoid arthritis compared with the normal state. The high production could be a source of the increased total amounts of hyaluronate which are found in inflamed joints.

We were suprised by the observation that the rheumatoid synovial cells and short term organ cultures produced hyaluronate of molecular weight of about 50 000. Vuorio and coworkers[10 31] have previously reported on the synthesis of underdepolymerised hyaluronate by rheumatoid cells as judged chromatographically, but all previous work on the molecular weight of hyaluronic acid in joint fluid has indicated that even if there is a lower degree of polymerisation the molecular weight is still of the order of 10^6 in rheumatoid joints.[32] A recent study on the molecular weight dispersion of hyaluronate from synovial fluid in rheumatoid patients has confirmed that there is no appreciable amount of low molecular weight polymer (Engström-Laurent, Dahl, Dahl, and Granath, unpublished). This raises the question whether the low

molecular weight material is a product of the in vitro conditions.

The hyaluronate in rheumatoid cell cultures could be synthesised in a low molecular weight form but could also be produced by degradation of high molecular weight material. No degradation was detected when hyaluronate was added in trace amounts to the cell or organ cultures from rheumatoid tissue or to serum or joint fluid from rheumatoid patients. This does not exclude the possibility of local degradation at the site of synthesis, e.g., before secretion from the cells. It is known that phagocytic cells produce oxygen-derived free radicals,[33] and that these radicals depolymerise hyaluronic acid.[34] The radicals have a short lifetime, and their effect should therefore be localised to the neighbourhood of the cell. Free radical formation is therefore a possible explanation for our observation of low molecular hyaluronate. Furthermore, the high oxygen tension in vitro compared with in vivo would promote free radical formation in the cell cultures.

An alternative explanation for the lack of low molecular hyaluronate in synovial fluid could be a rapid disappearance of this material by diffusion through the synovial membranes. It is interesting to note that rheumatoid patients have increased levels of hyaluronate in the general circulation and that this presumably originates from the connective tissues and is due to an increased rate of biosynthesis.[35] It is not yet known whether the hyaluronate circulating in serum of rheumatoid patients is of low molecular weight.

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Book reviews

Clinical Application of Monoclonal Antibodies. Ed. E S Lennox. Pp. 305. £12·00. Churchill Livingstone: Edinburgh. 1984.

It is indeed timely that the *Clinical Application of Monoclonal Antibodies* has been printed in the same year that the Nobel Prize for Medicine was awarded to Drs Milstein and Kohler. Their technique of fusing single antibody producing spleen cells from immunised rodents with myeloma cells capable of secreting antibodies and growing continuously in culture, has made available unlimited quantities of monoclonal antibodies (MoAbs) of exquisite specificity. This publication describes the application of some of these antibodies to a variety of different clinical fields.

Monoclonal antibodies of human origin have obvious advantages over those of rodents in their application as systemic therapeutic agents. The technical problems that still bedevil the switch to the production of human hybridomas are outlined in the opening chapter by Dr K Sikora.

Another disappointment in the study of monoclonal antibodies has been the failure in the search for HLA typing reagents as described by Julia and Walter Bodmer. The MoAbs that have been produced reacted mainly with the framework structures of class I or II molecules irrespective of their allospecificity. Although this 'monomorphic' property aided the elucidation of the protein and genomic structure of the HLA genes and their products, allospecific reagents have not become available for general use in tissue typing laboratories.

However, this publication concentrates on the advances in the clinical application of MoAbs, and I have briefly outlined below the areas covered by the various expert contributors.

Dr P Beverly describes the use of monoclonal antibodies for the analysis of T cell products that have a regulatory role in the immune system.

The combination of MoAbs and fluorescent activated cell sorter has enabled Dr Greaves and his colleagues to identify, isolate, and characterise a variety of rare cells in the early haemopoietic cell differentiation stage in leukaemias.

Dr Jannossy illustrated the use of MoAbs to reduce the graft-versus-host reaction in recipients of bone marrow transplants by the use of a cocktail of anti T cell reagents.

Drs Dongworth and McMichael have used monoclonal antibodies to inhibit in-vitro functional assays with virus infected cells that correspond to in-vivo immune mechanisms.

Dr P Stern describes the application of MoAbs to cell surface antigen expression on human teratoma cells. It is in the application of MoAbs to tumour immunology that cancer therapy may be advanced. In this respect chapters on the application of passive immunisation of MoAbs in cancer therapy (Dillman and Royston) and tumour imaging and targetry (Sikora and colleagues) are particularly exciting.

The uses of MoAbs in parasite (S Cohen), viral and bacterial (Porterfield and Talin) infection, and neural antigens (J Becker) are also described.

Finally, of specific rheumatological interest Isenberg and his colleagues use MoAbs to understand and dissect autoimmunity in systemic lupus erythematosus, myasthenia gravis, and Graves' disease.

J SACHS

Manual of Fracture Bracing. By A J Hall and R W Stenner. Pp. 61. £16·50. Churchill Livingstone: Edinburgh. 1985.

The manual of fracture bracing is a delightful little book. It contains a very brief and readable review of the conservative treatment of fractures culminating in bracing, and it then goes on in quite adequate detail to discuss the materials and the methods used in the most common fracture sites, namely tibia, femur, forearm, and humerus.

Any book on such a subject by two authors will of necessity be slightly idiosyncratic, but this detracts not at all from the efficacy of what they propound.

I find the description of the techniques very clear, and I have no doubt that this book should be to hand for the orthotist or plaster technician involved in day to day fracture bracing.

J B KING