Carrageenin-induced arthritis. VI. Alterations in amino acid transport by articular cartilage in acute inflammatory arthritis

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SUMMARY The mechanism of transport of alanine and aminoisobutyric acid into chondrocytes in rabbit articular cartilage was shown to be mediated by transport systems similar to that described for other eukaryotic cells namely the A, ASC, and L systems. Three days after the initiation of an acute inflammatory arthritis by the intra-articular injection of carrageenin into one knee joint the rate of transport of both these amino acids was decreased. Although all three transport systems were depressed, it appeared that the A and ASC systems were partially susceptible to damage by the induced inflammation. The rate of amino acid transport by the affected cartilage had recovered by 28 days after carrageenin treatment. This depression in amino acid transport is discussed in relation to a decrease in general metabolic processes in chondrocytes as a consequence of inflammation.

Key words: chondrocyte, membrane, inflammation, proteoglycan, glycosaminoglycan, protein synthesis.

A single intra-articular injection of carrageenin results in an inflammatory response within the joint; this is characterised by an influx of polymorphonuclear leucocytes into the synovial fluid and accompanied by the proliferation of the synovial membrane. Concomitant with this inflammatory response a decrease in both the ability of the treated cartilage to synthesise proteoglycan and the amount of proteoglycan present in the extracellular matrix is observed.¹⁻⁴ The rate of synthesis of proteoglycan is at a minimum three to seven days after carrageenin treatment and then slowly returns to a normal level four weeks after treatment. It also appears that protein synthesis by articular cartilage is depressed seven days after a single injection of carrageenin.⁵ It is not known how this depression in proteoglycan or protein synthesis is brought about or whether this inhibition reflects the general response in the metabolism of this tissue to an inflammatory situation.

Neutral amino acids have been shown to be

transported across cellular membranes by specific transport systems. These systems, which appear to be ubiquitous in a wide range of mammalian and avian cells, are termed systems A, ASC, and L.⁶⁻⁹ System A is a sodium-dependent, pH sensitive system, which preferentially transports amino acids with short polar or linear side chains.¹⁰ It will tolerate N-monomethylation of its substrate amino acids and this has led to the development of a model substrate, α -methylaminoisobutyric acid (MeAIB), which is transported solely by system A.¹¹ Transport by system ASC appears to be restricted to a narrow range of three- and four-carbon amino acids. Uptake by this system is also sodium dependent and pH sensitive. However, it will not tolerate N-monomethylation of its substrate amino acids, and this feature is generally used to distinguish system ASC from system A.¹² System L has a greater affinity for amino acids with branches or rings on their side chain, and a bicyclic molecule, 2-amino-2-norbornane carboxylic acid (BCH), is the most commonly used model substrate of this system.¹³ Unlike systems A and ASC system L is independent of sodium ion concentration, and transport is also maintained when the extracellular pH is lowered.¹⁰

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In general, neutral amino acids are transported by a combination of these three systems. However, the transport by an individual system can be determined by taking advantage of the factors listed above which can affect one system but not the others.

In this paper the characteristics of the manner in which alanine and aminoisobutyric acid are transported by articular cartilage from normal rabbit joints and from animals after a single intra-articular injection of carrageenin is reported. It was found that the rates of transport of both alanine and aminoisobutyric acid were depressed in the treated cartilage but recovered with time – a response similar to that observed for proteoglycan and protein synthesis by this tissue.

Materials and methods

L-[3-³H]Alanine (sp. act. 82.7 Ci/mmol), α-[1-¹⁴C]aminoisobutyric acid (sp. act. 53.2 Ci/mol), and [¹⁴C-(U)]sucrose (sp. act. 3.6 Ci/mol) were purchased from New England Nuclear (Boston, USA). [³⁵S]Sulphate (sp. act. 5 mCi/μg) was supplied by Amersham International (Amersham, UK). L-Alanine, amino-oxyacetic acid, cycloheximide, α-methylaminoisobutyric acid, and α-aminoisobutyric acid were purchased from Sigma Chemical Company (St Louis, USA). PD-10 columns (Sephadex G-25) were purchased from Pharmacia (Uppsala, Sweden). The λ-carrageenin fraction was prepared from *Chondrus crispus* (Bonnington Co., Sydney) by ethanol precipitation as previously described;¹⁴ this fraction was excluded from Sepharose 2B.

INDUCTION OF EXPERIMENTAL ARTHRITIS

Adult rabbits aged six to nine months (mixed breeds) were anaesthetised by intravenous injection of pentobarbitone according to the method outlined by Lowther and Gillard.⁵ 0.3 ml of 1.0% carrageenin (λ fraction) was injected intra-articularly into the right hind knee joint, while the contralateral joint received 0.3 ml of pyrogen-free saline.

MEASUREMENT OF AMINO ACID UPTAKE BY CARTILAGE SLICES

At a predetermined time after the administration of carrageenin the rabbits were killed by being placed in an atmosphere of CO₂ and the articular cartilage removed. The control cartilages from all rabbits were pooled, as were the treated cartilages, and cut into small pieces less than 2 mm². The tissue was washed in amino acid-free medium consisting of 1.8 mM CaCl₂, 5.4 mM KCl, 0.8 mM MgSO₄, 109.5 mM NaCl, 2.8 mM NaH₂PO₄, 5.5 mM glucose, 3.7 mM NaHCO₃, 14.0 mM HEPES, (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulphonic acid), and 0.1

mM cycloheximide pH 7.2. Approximately 40 mg of cartilage was preincubated for 30 min at 37°C in 2 ml of the above medium containing 0.2 mM amino-oxyacetate (AOA), which inhibited the metabolism of intracellular alanine.¹⁵ In experiments investigating the transport of aminoisobutyric acid no inhibitor was included in the preincubation medium. After preincubation the tissue was washed in amino acid-free medium to remove all traces of AOA and then placed in 2 ml of the above medium containing unlabelled alanine. The incubation was started by the addition of 0.2 μCi of [³H]alanine or [¹⁴C]aminoisobutyric acid. After a predetermined period the cartilage was removed, blotted dry on Whatman 54 filter paper, and its wet weight determined before extraction with 0.5 ml of 0.5 M NaOH for several hours at room temperature. The extract was neutralised with 0.5 ml of 0.5 M HCl and assayed for radioactivity. An aliquot of the incubation medium was also taken and weighed before determining its radioactivity.

DETERMINATION OF EXTRACELLULAR SPACE AND TISSUE WATER SPACE

It was necessary to make a correction for the amount of labelled alanine present in the extracellular space when calculating the amino acid uptake (see below), and this was achieved by measuring the uptake of [¹⁴C]sucrose into cartilage, since sucrose is not taken up by the chondrocytes. Cartilage was incubated as described above but with 0.2 μCi of [¹⁴C]sucrose for one hour at 37°C. Experiments showed that equilibration of [¹⁴C]sucrose was achieved within 10 min. The extracellular space was calculated from the amount of radioactivity found in a known mass of tissue and expressed as a percentage of the amount of radioactivity observed in the same mass of incubation medium. The extracellular space of normal rabbit articular cartilage was found to be 69.5±3.9% of the wet weight (mean±SD) where n=48, comprising values from 23 different experiments. There was no significant difference between this value and that measured in cartilage from rabbits treated with an intra-articular injection of carrageenin.

The percentage of the tissue accessible to water was determined by incubating the tissue as described for sucrose but with 0.2 μCi of [³H]water for one hour at 37°C. The percentage of the wet weight of tissue which was accessible to water was found for normal rabbit tissue to be 77.3±1.9% (mean±SD) where n=6 from three experiments. The cellular water space was calculated from the difference between tissue water accessible space and the sucrose accessible space, and for normal cartilage it was approximately 7.8% of the wet weight. This

value was used when calculating the degree to which the amino acids were concentrated.

CALCULATION OF AMINO ACID UPTAKE BY CARTILAGE SLICES

The amount of alanine or aminoisobutyric acid within the chondrocyte was determined from the radioactivity within the cartilage slices after this had been corrected for the amount of amino acid in the extracellular space. A sample calculation is set out below.

37.0 mg of tissue was incubated for 30 min in the presence of [^3H]alanine as outlined previously. The extracellular space for this tissue was determined as being 71.0%. A 100 μl sample of incubation medium was taken and found to contain 18 987 dpm. From this value and the amount of alanine in the incubation medium (0.5 mM) a new specific activity for alanine was calculated to be 0.17 $\mu\text{Ci}/\mu\text{mol}$. The radioactivity associated with [^3H]alanine in the cartilage slice was found to be 9968 dpm. If a uniform distribution of the amino acid in the extracellular space and a tissue density of unity are assumed, 37.0 mg wet weight corresponds to 37.0 μl ; 26 μl of this was determined to be extracellular space (i.e., 71.0% of 37.0 μl). Therefore the amount of radioactivity associated with the extracellular space was $18\,987 \times 26/100 = 4936$ dpm. When this value of the radioactivity associated with [^3H]alanine was subtracted from the total radioactivity in the cartilage slices, a value of the radioactivity within the chondrocyte was obtained, i.e., $9968 - 4936 = 5032$ dpm. This was equivalent to 2266 pCi and with the new specific activity determined (0.17 $\mu\text{Ci}/\text{mol}$) it corresponded to 13 330 pmol of alanine in 37.0 mg wet weight of cartilage. Therefore after 30 min the amount of alanine within the chondrocyte was determined to be 360 pmol/mg wet weight.

DETERMINATION OF APPARENT K_m AND V_{max}

At least eight or more samples (approx. 50 mg) of cartilage were preincubated as outlined previously and then incubated in medium containing 0.05, 0.1, 0.2, 0.4, or 0.5 mM amino acid. After 15 min incubation at 37°C the tissue was removed and processed as described above to determine the amount of amino acid transported. A Lineweaver-Burke plot was constructed and the intercepts ($\pm\text{SE}$) were determined by a linear regression programme. The significance of the means was tested by the Student's *t* test.

DISCRIMINATION BETWEEN THE DIFFERENT TRANSPORT SYSTEMS

The difference in sodium ion dependency^{10 12} between the A, ASC, and L amino acid transport

systems and their response to the model substance MeAIB¹¹ was the basis of the following protocol, which allowed an estimation of the contribution of the various systems to the overall transport of 0.5 mM alanine or aminoisobutyric acid over a 15 min period:

- In order to determine the transport of an amino acid or amino acid analogue attributable to system L the tissue was preincubated with a medium in which all of the sodium ions had been replaced by choline and potassium ions. Since both systems A and ASC are sodium dependent,^{10 12} the value obtained was a direct measurement of the transport via system L alone.
- Tissue was incubated in medium containing sodium ions and 10 mM MeAIB.¹¹ Under these conditions the A system will be inhibited and amino acid transport measured will be by the ASC and L systems. The transport by system ASC alone could then be determined by subtracting (a) from (b).
- Total transport by systems A, ASC, and L was measured by the incubation procedure outlined previously in 'Methods'. The percentage of transport occurring via system A was then calculated by subtracting (b) from (c).

INCORPORATION OF [^{35}S]SULPHATE INTO PROTEOGLYCAN BY CARTILAGE SLICES

Approximately 50 mg of cartilage was incubated in 2.0 ml of a modification of Dulbecco's modified Eagle's medium¹⁶ at 37°C for two hours. After this equilibrium period 50 μCi of $^{35}\text{SO}_4$ was added and the tissue incubated for another two hours in a shaking water bath. The medium was then removed and the tissue extracted in 2.0 ml 0.5 M NaOH for 48 hours at 4°C. The extracts were centrifuged. The labelled glycosaminoglycans in the supernatant were isolated on a column of Sephadex G-25 (5 $\times$ 1.8 cm, PD-10 column) previously equilibrated with 4 M guanidinium chloride, 0.1 M Na_2SO_4 , and 50 mM acetate buffer pH 6.1.¹⁷ The void volume fractions which contained the $^{35}\text{SO}_4$ labelled glycosaminoglycans were assayed for radioactivity.

MEASUREMENT OF PROTEIN SYNTHESIS

Cartilage (10 mg) was incubated in 2 ml of Dulbecco's modified Eagle's medium without serum for 30 minutes. 20 μCi of [^3H]alanine was then added; the final concentration of alanine in this medium was 0.5 mM alanine. After two hours the medium was removed and the tissue extensively washed at 4°C with 5 ml aliquots of 0.6 M trichloroacetic acid containing 20 mM alanine until the washes contained background radioactivity. The tissue was

then washed with 0.1 M sodium acetate containing 5 mM ethylenediaminetetra-acetic acid (EDTA) and 5 mM cysteine HCl, digested with 10 µg of papain in the same buffer at 60°C for 10 hours, and aliquots were assayed for radioactivity.

DETERMINATION OF RADIOACTIVITY

The radioactivity present in an aqueous sample (2 ml) was determined by adding 8 ml of scintillator (0.5% (w/v) PPO, in 50% (v/v) triton X 100, 50% toluene). The samples were then analysed for radioactivity in a Philips PW 4510/01 liquid scintillation spectrophotometer; an external standard was used.

Results

ALANINE AND AMINOISOBUTYRIC ACID TRANSPORT IN ARTICULAR CARTILAGE. CHARACTERISATION OF TRANSPORT

When rabbit articular cartilage was incubated in medium containing sodium ions it was found to

Fig. 1 Time course and sodium dependency of $[^3\text{H}]$ alanine transport. Medium containing sodium ions (○—○); medium without sodium ions (●—●). Cartilage slices were incubated in medium containing a final concentration of 1 mM $[^3\text{H}]$ alanine, and at various times the uptake of the amino acid was determined as described in 'Methods'. Each point is the mean of three determinations; bars show SE.

Fig. 2 pH dependency of $[^3\text{H}]$ alanine transport. Both the preincubation and incubation medium were adjusted to the chosen pH, approximately 50 mg of cartilage was incubated with 1 mM $[^3\text{H}]$ alanine at 37°C, and after 15 min the amount of amino acid taken up by the cells was determined. Each point is the mean of three determinations; bars show SE.

transport $[^3\text{H}]$ alanine in a linear manner over the first 20 minutes of the incubation (Fig. 1). After approximately 60 min an equilibrium state was attained between intracellular and extracellular alanine; from this data and the cellular volume of rabbit articular cartilage (see 'Methods') it was calculated that this amino acid was concentrated by 3.1 times. Sodium dependency of alanine transport is shown in Fig. 1. The transport of alanine was also found to be dependent on the pH of the extracellular medium (Fig. 2). The transport of $[^{14}\text{C}]$ aminoisobutyric acid showed a similar time dependent uptake, sodium ion, and pH dependency (data not shown).

When one sample of pooled tissue was used and the protocol outlined in 'Methods' followed it was observed that 60.1% (mean, $n=3$) of the uptake of 0.5 mM alanine by rabbit articular cartilage could be attributed to the ASC system, 16.2% to the A system, and 23.5% to the L system. On the other hand 54.4% (mean, $n=3$) of the transport of 0.5 mM aminoisobutyric acid was by the A system, 20.7% by the ASC system, and 24.9% by the L system.

The apparent V_{max} and K_m values obtained for

the uptake of alanine with one sample of pooled rabbit articular cartilage were 43.5 ± 4.4 ($\pm$ SE, $n=12$) pmol/min per mg and 0.17 ± 0.03 ($n=12$) mM, respectively. For aminoisobutyric acid transport the values were 7.4 ± 1.04 ($n=10$) pmol/min per mg and 0.53 ± 0.39 ($n=10$) mM.

TRANSPORT AFTER A SINGLE INTRA-ARTICULAR INJECTION OF CARRAGEENIN
In order to compare the rate of transport of amino acids into chondrocytes the $V_{\max}$ was taken as a suitable value because it is a rate constant measured when the transport systems are saturated with substrate. This value was ascertained by measurement of the uptake of the amino acids by at least eight samples of tissue over a range of amino acid concentrations and analysis of these data with a Lineweaver-Burke plot. Fig. 3 shows such a plot obtained for cartilage three days after carrageenin treatment; it was observed that there was a 29% decrease in the $V_{\max}$ of alanine transport compared with control tissue.

The rate of alanine transport by the treated tissue remained depressed for 14 days after treatment but returned to a value greater than that measured in control tissue after 21 days (Table 2). The $V_{\max}$ values obtained for treated and control tissue

Fig. 3 Lineweaver-Burke plot for the transport of $[^3\text{H}]$ alanine by control (○—○) and treated (●—●) cartilage three days after induction of inflammation in one knee joint. Alanine transport was measured as described in 'Methods'.

Table 1 Alanine transport attributable to the A, ASC, and L systems in cartilage three days after an intra-articular injection of carrageenin*

System	$[^3\text{H}]$ Alanine transport (pmol/15 min per mg)		
	Control	Treated	(T/C $\times$ 100)‡
A	25.2 (11)†	12.3 (9)†	49
ASC	136.4 (60)	78.6 (57)	58
L	65.4 (29)	47.6 (34)	73
Total transport	227.0 $\pm$ 32.6 (n=3)	138.5 $\pm$ 12.5 (n=3)	61

*Five or more rabbits were killed three days after carrageenin treatment of the knee joint of one of the hind limbs. The articular cartilage from the treated joints was removed and pooled, as was the control tissue. The rate of transport of 0.5 mM alanine by each of the systems was measured as described in 'Methods'. Values are given as mean $\pm$ SE.
†Percentage of total transport.
‡Rate of transport by treated cartilage expressed as a percentage of control cartilage.

showed a significant difference ($p<0.01$) when subjected to the t test. There were no significant differences in the K_m values of the control and treated cartilage for alanine transport for each batch of tissue used. However, it was apparent that there was a difference in the values of K_m and $V_{\max}$ obtained for control tissue between the batches of tissue used.

In an experiment to investigate whether the decrease in the $V_{\max}$ of alanine transport three days after carrageenin treatment could be attributed to a decrease in any one of the transport systems it was found that the greatest decrease was in the A system (51.2%) and the least decrease in the L system (27.6%) (Table 1). This suggests that the A and ASC systems may be more susceptible to damage. Indeed this observation is substantiated by the fact that transport of $[^{14}\text{C}]$ aminoisobutyric acid by cartilage three days after carrageenin treatment was depressed by 48% (Table 2), this amino acid analogue being transported mainly by the A system.

SYNTHESIS OF PROTEOGLYCANS AND PROTEIN BY ARTICULAR CARTILAGE
Glycosaminoglycan and protein syntheses by articular cartilage from different batches of rabbits treated with an intra-articular injection of carrageenin were studied. The incorporation of $[^{35}\text{S}]$ sulphate into glycosaminoglycans by cartilage from carrageenin-treated joints expressed as a percentage of the value obtained for tissue from the contralateral limb was found to be $48.7 \pm 4.7\%$ ($\pm$ SE; $n=10$) three days after carrageenin treatment; $98.0 \pm 24.6\%$ ($n=5$) at 14 days; $123.8 \pm 6.6\%$ ($n=7$) at 21 days; and

Table 2 Alanine and aminoisobutyric acid transport, proteoglycan and protein synthesis by carrageenin-treated cartilage*

Days after carrageenin treatment	3 days		14 days		21 days		28 days	
	C	T	C	T	C	T	C	T
V_{max} of [3H]alanine transport (pmol/min per mg)	28.9±0.9 n=10	20.5±1.4 (71%) n=10	30.5±2.5 n=10	20.3±2.2 (67%) n=10	20.0±1.8 n=10	33.0±3.7 (165%) n=8	44.8±0.5 n=10	42.2±4.1 (117%) n=10
K_m of [3H]alanine transport (mM)	0.18±0.02 n=10	0.18±0.03 n=10	0.18±0.04 n=10	0.16±0.05 n=10	1.25±0.75 n=10	1.0±0.39 n=8	0.68±0.07 n=10	0.67±0.44 n=10
V_{max} of [^{14}C]aminoisobutyric acid transport (pmol/min per mg)	10.5±0.3 n=5	5.0±0.5 (48%) n=5	ND	ND†	ND	ND	ND	ND
K_m of [^{14}C]aminoisobutyric acid transport (mM)	0.52±0.11 n=5	0.57±0.16 n=5	ND	ND	ND	ND	ND	ND
$^{35}SO_4$ incorporation into glycosaminoglycans (pmol/h per mg)	14.2 n=2	3.8 (27%) n=2	4.1 n=2	1.8 (44%) n=2	4.4 n=2	5.4 (123%) n=2	15.9 n=2	14.4 (91%) n=2
[3H]Alanine incorporation into protein (pmol/h per mg)	16.2±0.1 n=3	7.0±0.3 (43%) n=3	9.7 n=2	14.5 (149%) n=2	19.5 n=2	29.1 (149%) n=2	20.7 n=2	28.9 (139%) n=2

*Five or more rabbits were killed at each of the stated times after intra-articular administration of carrageenin to one knee joint. The articular cartilage from the treated (T) joints was dissected and pooled as was the control (C) tissue from the contralateral limbs. The V_{max} and K_m values were measured as described in Methods; $^{35}SO_4$ incorporation into glycosaminoglycans and [3H]alanine incorporation were also measured on the same tissue. Values are expressed as mean±SE. Values in parentheses are the treated values expressed as a percentage of the control value obtained for the tissue from the contralateral limb.
†ND=not determined.

118.9±11.8 (n=7) at 28 days. Protein synthesis, as measured by the incorporation of [³H]alanine into acid-insoluble protein, by cartilage from carrageenin untreated joints expressed as a percentage of the value measured for the contralateral limb was found to be 44.1±4.8% (n=3) at three days; 113.4±13.4% (n=3) at 14 days; 130.6±10.2% (n=4) at 21 days, and 110±14% (n=4) at 28 days. These data indicate that both glycosaminoglycans and protein synthesis by articular cartilage from treated joints are initially depressed but recover with time to control levels.

Table 2 shows the rate of glycosaminoglycan synthesis measured in the same batches of tissue that were used to measure amino acid uptake. It is clear that the same trend of recovery in glycosaminoglycan synthesis was observed. Protein synthesis was also measured in the same batch of tissue. These data imply that after an initial period where protein synthesis was depressed the rate recovered to a value greater than that observed in control tissue.

Discussion

Oxender and Christensen¹⁰ were the first to define the systems in Ehrlich ascites tumour cells which transported amino acids across the cell membrane. The transport of neutral amino acids into chondrocytes of rabbit articular cartilage is achieved by the A, ASC, and L systems; there is a considerable overlap in the specificities of each system in that any one amino acid may be transported by a combination of these systems, and because of this any kinetic parameters used will reflect the contribution of the different transport systems. In the work described here it is apparent that alanine is transported into chondrocytes by all three systems, with the ASC system predominating, whereas the amino acid analogue aminoisobutyric acid is transported mainly by the A system. This is similar to data reported previously for amino acid transport by the A, ASC, and L systems in articular cartilage.¹⁸

In the present study the effect of a single intra-articular injection of carrageenin on the transport of both amino acid and an amino acid analogue was measured to ascertain the outcome of an acute inflammatory attack on a cell membrane-associated function. When alanine transport was measured there was a significant decrease in the rate of transport by carrageenin-treated tissue, which remained depressed for two weeks but returned to a value greater than that of the control after three weeks.

It is of interest that these changes in the rate of alanine transport were paralleled by similar changes in the incorporation of [³⁵S]sulphate into glycosaminoglycans by the carrageenin-treated tissue, which is

consistent with previous observations of [³H]acetate incorporation into glycosaminoglycans.⁴ Total protein synthesis was also depressed in cartilage from the treated knee joint, but it appeared to recover more quickly than amino acid transport and glycosaminoglycan synthesis. A significant variation was apparent in the values of both K_m and V_{max} in control tissue between batches of animals used, especially 21 and 28 days after induction of the inflammatory attack. It is possible that this may represent either animal variation or a time-dependent systemic response to a unilateral knee inflammation. Variation was also observed in the rates of synthesis of glycosaminoglycans and protein, again for the same reasons. It is not implied in this study that the depression of glycosaminoglycan or protein syntheses is a direct result of the suppression of amino acid transport, but a decrease in all these cellular processes suggests damage to the chondrocytes. Santer *et al.*¹⁹ have shown that one and seven days after intra-articular injection of carrageenin chondrocytes lost both the rough endoplasmic reticulum and Golgi apparatus. However, within 21 days the cellular morphology had returned to normal.

The cellular origin and nature of the factors which depress chondrocyte metabolism are not yet defined. Claims have been made that free radicals,²⁰ prostanoids,²¹ or lymphokines,²² which could originate from inflamed synovial tissue, can depress proteoglycan synthesis. However, intra-articular injection of carrageenin also resulted in an influx of cells into the synovial fluid of the joint, and the cell count remained high until seven days after treatment, the main cell type being the polymorphonuclear leucocyte.¹⁹ Increased proteolytic enzyme activity has been reported by Lowther *et al.*²³ in rabbit synovial joints after a single injection of carrageenin. Indeed, in antigen-induced arthritis in rabbits where similar changes occur Sandy *et al.*^{24, 25} have reported the presence in cartilage of a serine proteinase originating from the polymorphonuclear leucocytes. It is therefore possible that the increased proteolytic enzyme activity could be responsible for damage of chondrocyte metabolism, including amino acid transport. Evidence for a decrease in proteoglycan synthesis by cartilage slices treated with purified polymorphonuclear elastase would tend to support this idea.²⁶ It was also apparent from the work reported here that the metabolism of the chondrocytes did recover, with protein synthesis returning to control values before recovery of proteoglycan synthesis and amino acid transport; the question still remains as to the number of times the cells can be insulted before they fail to recover.

In conclusion it appears that in an acute inflammatory attack within the synovial capsule the general metabolism of chondrocytes is temporarily depressed, and it is suggested that factors produced in the inflammatory response produce intracellular structural abnormalities and altered membrane function, as indicated by the changes in the cellular uptake of amino acids.

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