Ann. rheum. Dis. (1976), **35**, 487

Effect of anti-inflammatory drugs on sulphated glycosaminoglycan synthesis in aged human articular cartilage

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McKenzie, L. S., Horsburgh, B. A., Ghosh, P., and Taylor, T. K. F. (1976). *Annals of the Rheumatic Diseases*, **35**, 487–497. **Effect of anti-inflammatory drugs on sulphated glycosaminoglycan synthesis in aged human articular cartilage.** The anti-inflammatory drugs, sodium salicylate, indomethacin, hydrocortisone, ibuprofen, and flurbiprofen, were examined for their effects on sulphated glycosaminoglycan synthesis in aged human cartilage *in vitro*. Cartilage was obtained from femoral heads removed during surgery and drug effects were found to vary significantly from one head to another. Statistical analysis of the results showed that sodium salicylate exhibits concentration-dependent inhibition of glycosaminoglycan synthesis over the concentration range used. Indomethacin, hydrocortisone, and ibuprofen, at concentrations comparable to those attained in man, caused a statistically significant depression of sulphated glycosaminoglycan synthesis in cartilage from some femoral heads but not others, reflecting the variable response of human articular cartilage to anti-inflammatory drugs. Sodium salicylate and indomethacin at higher doses produced significant ($P<0 \cdot 005$) inhibition of sulphated glycosaminoglycan synthesis in all femoral heads studied. The results for flurbiprofen were less conclusive; this compound appears not to inhibit glycosaminoglycan synthesis over the concentration range used.

An early event in osteoarthrosis is proteoglycan loss from articular cartilage (Mankin, 1974; McDevitt, 1973; Lust and Pronsky, 1972; Bjelle and others, 1972; Mankin and Lipiello, 1970, 1971; Bollet and Nance, 1966; Bollet and others, 1963; Matthews, 1953). Proteoglycans impart resilience and molecular exclusion properties to cartilage and depletion can result in fibrillation and impaired joint function (Radin and others, 1975; Bullough, Goodfellow, and O'Connor, 1973; Freeman, 1973; Kempson and others, 1970). In all but severe cases of osteoarthrosis the chondrocyte response to proteoglycan depletion results in an increase in glycosaminoglycan synthesis although the magnitude of this response is debatable (Maroudas, 1975; Mankin and others, 1971). An increase in $^{35}SO_4$ uptake by osteoarthrotic cartilage chondrocytes as compared to normal chondrocytes was observed autoradiographically as early as 1960 by Collins and McElligott. In the later stages of the disease, when severe damage to the cartilage has occurred, reduced rates of glycosaminoglycan synthesis and chondrocyte death have been reported (Mankin and others, 1971; Mankin and Lipiello, 1970; Collins and McElligott, 1960).

In animal tissues, including cartilage, anti-inflammatory drugs have been reported to inhibit glycosaminoglycan synthesis, both *in vivo* and *in vitro* (Bostrom, Bersten, and Whitehouse, 1964; Whitehouse and Bostrom, 1962). These agents have also been shown to inhibit the synthesis and release of glycosaminoglycans from cultured fibroblasts (Karzel and Domenjoz, 1969; Kalbhen, Karzel, and Domenjoz, 1967). Whitehouse (1965) noted that a close correlation exists between anti-inflammatory activity and the inhibition of glycosaminoglycan synthesis in such experimental systems.

Osteoarthrosis principally affects the older age groups. In some respects aged human cartilage differs both chemically and metabolically from young animal cartilage (Maroudas, 1975; McDevitt, 1973; Benmaman, Ludoweig, and Anderson, 1969). Therefore, we have examined the effects of some of

Accepted for publication April 25, 1976.
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the more commonly prescribed anti-inflammatory drugs on the synthesis of sulphated glycosaminoglycans in aged human cartilage, under rigidly controlled culture conditions. Particularly notable was the highly variable response to the drugs of human cartilage from different individuals, highlighting the need for exacting statistical analysis of the results.

Materials and methods

ANTI-INFLAMMATORY DRUGS

The following drugs were obtained in pure form from the manufacturers: indomethacin (Merck, Sharp and Dohme, Rahway, N.J.), sodium salicylate (Nelson Laboratories Pty., Sydney, Australia), ibuprofen and flurbiprofen (Boots, Nottingham, England), hydrocortisone sodium succinate as an injectable preparation (Upjohn Pty., Rydalmere, Australia).

SAMPLING OF ARTICULAR CARTILAGE

Human articular cartilage was obtained from femoral heads removed surgically after subcapital fracture. The ages of the patients ranged from 63 to 83 years. A special tool, holding four parallel razor blades spaced 1 mm apart, was used to cut sections of cartilage 1 mm wide perpendicular to the articular surface. Great care was taken to sample the full depth of articular cartilage since glycosaminoglycan distribution and metabolism is reported to vary with distance from the articular surface (Jones and Lemperg, 1975; Maroudas, 1975; Stockwell and Scott, 1967). For each femoral head, cartilage was sampled from the superior surface which is generally considered to be a weight-bearing area (Maroudas, Evans, and Almeida, 1973). Each section of cartilage was divided into full-depth slices, 2–3 mm long, for culturing.

CULTURE METHODS

Explants were set up in culture within 2 hours of surgical removal and were maintained in organ culture using a modification of the method of Trowell (1959). Explants were supported on stainless steel grids, bathed in culture medium 37°, in a humidified atmosphere of 5% CO_2 in air. The culture medium used was Dulbecco Modified Eagle's Medium supplemented with 10% fetal calf serum (Commonwealth Serum Laboratories). The serum was found to be essential for maintaining a high level of viability for several days. Under these conditions incorporation of ^{3}H-uridine into RNA, and $^{35}SO_4$ into glycosaminoglycans (Curran and Gibson, 1956) showed no reduction for at least 3 days, but all experiments were performed within 2 days. The culture methodology will be fully described elsewhere.

MEASUREMENT OF RADIOSULPHATE UPTAKE INTO SULPHATED GLYCOSAMINOGLYCANS

Synthesis of sulphated glycosaminoglycans was measured by the specific incorporation of $^{35}SO_4$ (Radiochemical Centre, Amersham, England) into the glycosaminoglycan fractions of articular cartilage, rather than uptake into whole tissue. When cartilage slices were incubated with $^{35}SO_4$, considerable nonspecific binding could be measured even in controls incubated at 0°. The majority of unbound $^{35}SO_4$ was removed by washing each slice for 40 minutes in four 5 ml changes of ice-cold physiological saline containing nonradioactive sulphate as a chaser. Each slice was dried in acetone and alcohol, then under vacuum at 40°, and weighed. Dry weights were found to be more accurate and reproducible than wet weight determinations. Slices were solubilized by overnight digestion at 65° with crystalline papain (Merck) (1·5 mg/ml in 0·1 mol/l acetate buffer pH 5·6, containing 0·05 mol/l EDTA and 0·005 mol/l cysteine). Papain digests were dialysed overnight against water and analysed for uronic acid by the method of Bitter and Muir (1962), and for radioactivity using standard scintillation counting techniques. Incorporation of $^{35}SO_4$ into glycosaminoglycans was expressed as cpm/μg uronic acid.

ANTI-INFLAMMATORY DRUG STUDIES

After cartilage slices had been allowed to stabilize in culture for 1 day, the effects of anti-inflammatory drugs on sulphate incorporation into glycosaminoglycans were studied by incubating cartilage slices in medium containing the drug and $^{35}SO_4$ at 40 μCi/ml. Each drug was studied at three concentrations whose effects on sulphate incorporation were compared to controls with no added drug. The concentrations used covered the range of therapeutic blood plasma levels reported for the drugs examined (Mills and others, 1973; Chalmers and others, 1972; Domenjoz, 1971; Karzel and Domenjoz, 1969; Hucker and others, 1966; Holt and Hawkins, 1965). However, indomethacin was also examined at higher concentrations. The effect of the drugs was measured over 6 hours, triplicate slices of cartilage being removed from culture at hourly intervals for each drug concentration and the control, making a total of 72 slices for each drug studied. For these experiments, cartilage slices were removed from the stainless steel grids to Falcon Microtest II tissue culture plates (Falcon Plastics, Los Angeles, Calif.), which consist of 96 identical wells. One cartilage slice was incubated in each well in 0·25 ml medium containing $^{35}SO_4$ and the anti-inflammatory drug. Incubations were terminated by transferring slices into 5 ml ice-cold saline. All drugs dissolved freely in culture medium except indomethacin, which, however, was soluble in medium at approximately pH 9·0, and remained in solution when the pH was readjusted to neutrality.

STATISTICAL ANALYSIS OF RESULTS

The results of each drug experiment, using cartilage from a single femoral head, were analysed by a two-factor (fixed model) analysis of variance (Snedecor and Cochran, 1967). This type of analysis determines the significance of the effect of the drug in question on the synthesis of sulphated glycosaminoglycans over the time range studied at different drug concentrations. Since each drug was tested using cartilage from more than one femoral head, the significance of the differences between the responses of different femoral heads was determined using a three-factor (mixed value) analysis of variance.

Bartlett's test showed significant heterogeneity of variances ($P<0·05$) and all data were transformed to natural logarithms to stabilize variances. The results obtained using the statistical methods described are shown in Figs. 1–6 and summarized in Tables I–VI.

Results

INDOMETHACIN

The effect of indomethacin over a concentration range of 0·02–0·005 mmol/l was studied using cartilage from two femoral heads (FH1 and FH2). The effect of the drug on sulphated glycosaminoglycan synthesis differed for the two specimens. One (FH1) showed a significant inhibition of glycosaminoglycan synthesis ($P<0·005$) with increasing indomethacin concentration. The other (FH2) showed no significant response to the drug (Fig. 1; Table I). When the results for the two femoral heads were analysed together the difference between them was significant ($P<0·05$). At the higher concentration range (1·0–0·1 mmol/l), indomethacin was examined for its effect on glycosaminoglycan synthesis using cartilage sampled from three femoral heads (FH3, FH4, and FH5). In each instance a significant depression of sulphated glycosaminoglycan synthesis ($P<0·005$) was observed with increasing indomethacin concentration (Fig. 2; Table II). When the results for the three femoral heads were analysed together the difference between the individual femoral heads was highly significant ($P<0·005$).

SODIUM SALICYLATE

The effect of sodium salicylate was examined using cartilage from three femoral heads (FH3, FH4, and FH5) and in each case a significant depression of $^{35}SO_4$ incorporation ($P<0·005$) was observed with increasing sodium salicylate concentration up to 5·0 mmol/l (Fig. 3; Table III). As for indomethacin, the difference between individual femoral heads was highly significant ($P<0·005$).

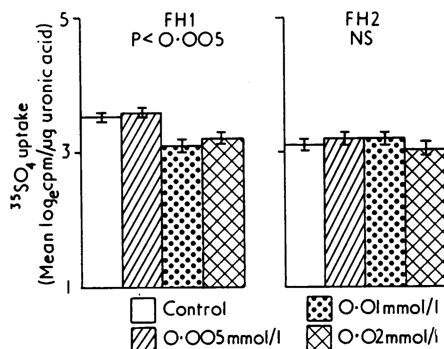

FIG. 1 *Inhibition by indomethacin of $^{35}SO_4$ uptake into sulphated glycosaminoglycans of human cartilage from 2 femoral heads (FH1 and FH2) at concentrations of 0·005–0·02 mmol/l. Increased drug concentration caused a highly significant ($P<0·005$) decrease in $^{35}SO_4$ uptake for FH1. The drug had no significant effect for FH2*

Table I *Analysis of variance for the effect of indomethacin (0·005–0·02 mmol/l) on sulphated glycosaminoglycan synthesis in cartilage from 2 femoral heads*

Source of variation	*Sum of squares*	*df*	*Mean square*	*F*
FH1				
Drug concentration (D)	1·6818	3	0·5606	8·96*
Incubation time (T)	24·0000	5	4·8000	76·68*
Interaction between D and T	0·9543	15	0·0636	1·02NS
Residual	3·0075	48	0·0626	
Total	29·6436	71		
FH2				
Drug concentration (D)	0·2283	3	0·0761	1·55NS
Incubation time (T)	20·2574	5	4·0515	82·35*
Interaction between D and T	1·9671	15	0·1311	2·66†
Residual	2·3623	48	0·0492	
Total	24·8151	71		
Pooled FH1 and FH2				
Drug concentration (D)	1·0678	3	0·3559	1·27NS
Incubation time (T)	44·0868	5	8·8174	67·05*
Femoral head (F)	1·6346	1	1·6346	29·24*
Interaction between D and T	1·2175	15	0·0812	1·00NS
Interaction between D and F	0·8423	3	0·2808	5·02†
Interaction between T and F	0·6574	5	0·1315	2·35‡
Interaction between D and T and F	1·2171	15	0·0811	1·45NS
Residual	5.3698	96	0.0559	
Total	56·0933	143		

In this and following tables, the significance of F tests is as follows: * very highly significant ($P<0·005$); † highly significant ($P<0·01$); ‡ significant ($P<0·05$); NS, not significant.

Table II *Analysis of variance for the effect of indomethacin (0·1–1·0 mmol/l) on sulphated glycosaminoglycan synthesis in cartilage from 3 femoral heads*

Source of variation	*Sum of squares*	*df*	*Mean square*	*F*
FH3				
Drug concentration (D)	23·8747	3	7·9582	6·00*
Incubation time (T)	5·2911	5	1·0582	0·80NS
Interaction between D and T	9·5374	15	0·6358	0·48NS
Residual	63·6164	48	1·3253	
Total	102·3196	71		
FH4				
Drug concentration (D)	3·0620	3	1·0207	7·36*
Incubation time (T)	5·5581	5	1·1116	8·01*
Interaction between D and T	12·5315	15	0·8354	6·02*
Residual	6·5548	48	0·1387	
Total	27·7064	71		
FH 5				
Drug concentration (D)	4·4176	3	1·4725	4·67†
Incubation time (T)	3·5654	5	0·7131	2·26 NS
Interaction between D and T	4·8124	15	0·3208	1·02‡
Residual	15·1177	48	0·3150	
Total	27·9131	71		
Pooled FH3, FH4, and FH5				
Drug concentration (D)	21·7049	3	7·2350	7·49*
Incubation time (T)	5·2774	5	1·0555	0·37NS
Femoral head (F)	26·8188	2	13·4094	22·64*
Interaction between D and T	0·7798	15	0·0520	11·52*
Interaction between D and F	17·2758	6	2·8793	4·86*
Interaction between T and F	9·6561	10	0·9656	1·63NS
Interaction between D and T and F	17·9681	30	0·5989	1·01NS
Residual	85·2889	144	0·5923	
Total	184·7698	215		

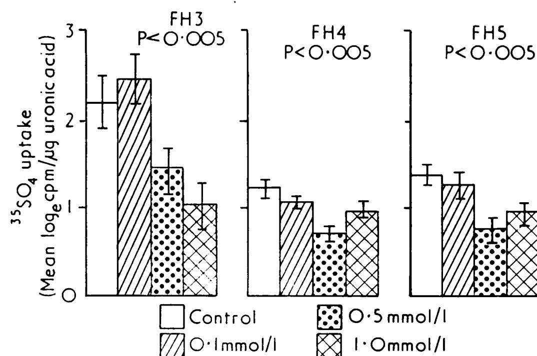

FIG. 2 *Inhibition by indomethacin of $^{35}SO_4$ uptake into sulphated glycosaminoglycans of human cartilage from 3 femoral heads (FH3, FH4, and FH5) at concentrations of 0·1–1·0 mmol/l. Increased drug concentration caused a highly significant ($P<0·005$) decrease in $^{35}SO_4$ concentration in each femoral head*

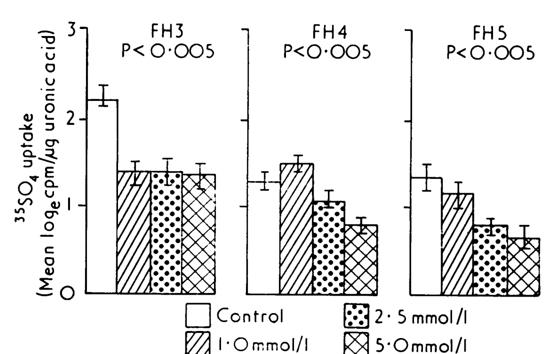

FIG. 3 *Effect of sodium salicylate on $^{35}SO_4$ uptake into sulphated glycosaminoglycans of human cartilage from 3 femoral heads (FH3, FH4, and FH5) at various concentrations. Increased drug concentration caused a highly significant ($P<0·005$) decrease in $^{35}SO_4$ uptake in each femoral head*

Table III *Analysis of variance for the effect of sodium salicylate on sulphated glycosaminoglycan synthesis in cartilage from 3 femoral heads*

Source of variation	*Sum of squares*	*df*	*Mean square*	*F*
FH3				
Drug concentration (D)	8·9590	3	2·9863	8·01*
Incubation time (T)	12·4122	5	2·4824	6·66*
Interaction between D and T	9·4279	15	0·6285	1·69NS
Residual	17·8954	48	0·3728	
Total	48·6945	71		
FH4				
Drug concentration (D)	5·2153	3	1·7348	12·73*
Incubation time (T)	6·3144	5	1·2629	9·25*
Interaction between D and T	4·4988	15	0·2999	2·20‡
Residual	6·5548	48	0·1366	
Total	22·5833	71		
FH5				
Drug concentration (D)	6·6213	3	2·2071	8·10*
Incubation time (T)	5·6559	5	1·1312	4·15*
Interaction between D and T	4·3078	15	0·2872	1·05NS
Residual	13·0825	48	0·2726	
Total	29·6675	71		
Pooled FH3, FH4, and FH5				
Drug concentration (D)	15·1387	3	5·0462	5·79‡
Incubation time (T)	20·9358	5	4·1872	2·26NS
Femoral head (F)	14·7342	2	7·3671	28·27*
Interaction between D and T	7·7244	15	0·5150	1·41NS
Interaction between D and F	3·4243	10	0·3424	12·23*
Interaction between T and F	5·2294	6	0·8716	3·34†
Interaction between D and T and F	10·9675	30	0·3656	1·31NS
Residual	37·5327	144	0.2606	
Total	115·6870	215		

HYDROCORTISONE

Hydrocortisone was tested with cartilage from two femoral heads (FH3 and FH4). The effect of this steroidal anti-inflammatory drug on glycosaminoglycan synthesis differed for the two specimens. One (FH3) showed a significant inhibition of sulphate incorporation with increasing hydrocortisone concentration to 1·0 mmol/l (P<0·005), but the other (FH4) showed no significant effect of the drug on sulphated glycosaminoglycan synthesis (Fig. 4; Table IV).

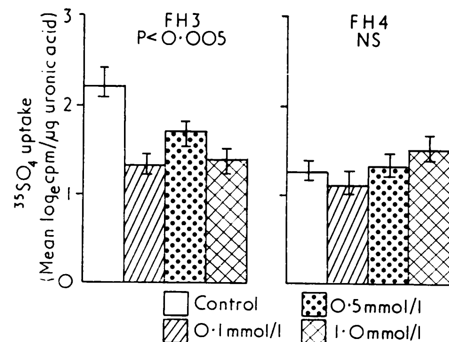

FIG. 4 *Effect of hydrocortisone on $^{35}SO_4$ uptake into sulphated glycosaminoglycans of human cartilage from 2 heads (FH3 and FH4) at various concentrations. Increased drug concentration caused a highly significant (P<0·005) decrease in $^{35}SO_4$ uptake for FH3. The drug had no significant effect for FH4*

IBUPROFEN

Articular cartilage sampled from two femoral heads (FH6 and FH7) was used to investigate the effects of ibuprofen. In one experiment (FH6), ibuprofen at concentrations up to 0·2 mmol/l had no significant effect on sulphated glycosaminoglycan synthesis. In a second experiment (FH7), a highly significant depression was caused by ibuprofen at identical concentrations (Fig. 5; Table V).

Table IV *Analysis of variance for the effect of hydrocortisone on sulphated glucosaminoglycan synthesis in cartilage from 2 femoral heads*

Source of variation	*Sum of squares*	*df*	*Mean square*	*F*
FH3				
Drug concentration (D)	8·9682	3	2·9894	6·83*
Incubation time (T)	9·6262	5	1·9252	4·40*
Interaction between D and T	3·1715	15	0·2114	0·48NS
Residual	21·0014	48	0·4375	
Total	42·7673	71		
FH4				
Drug concentration (D)	1·5462	3	0·5154	2·55NS
Incubation time (T)	12·4674	5	2·4935	12·36*
Interaction between D and T	5·8657	15	0·3910	1·94‡
Residual	9·6846	48	0·2018	
Total	29·5639	71		
Pooled FH3 and FH4				
Drug concentration (D)	4·3049	3	1·4350	0·76NS
Incubation time (T)	17·1547	5	3·4309	3·73NS
Femoral head (F)	3·5348	1	3·5348	11·06*
Interaction between D and T	7·7960	15	0·5197	20·62*
Interaction between D and F	5·6503	3	1·8834	5·89*
Interaction between T and F	4·6040	5	0·9208	2·88‡
Interaction between D and T and F	0·3775	15	0·0252	12·68*
Residual	30·6860	96	0·3197	
Total	74·1082	143		

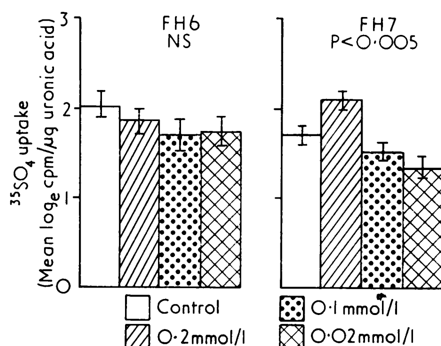

FIG. 5 *Effect of ibuprofen on $^{35}SO_4$ uptake into sulphated glycosaminoglycans of human cartilage from 2 femoral heads (FH6 and FH7) at various concentrations. Increased drug concentration caused a highly significant ($P<0·005$) decrease in $^{35}SO_4$ uptake for FH6. The drug had no significant effect for FH7*

FLURBIPROFEN

Flurbiprofen, in contrast to the other anti-inflammatory drugs, did not depress $^{35}SO_4$ incorporation over the concentration range studied (up to 0·04 mmol/l). Its effects were examined on cartilage from two femoral heads (FH6 and FH7). One (FH6) showed that the drug had a significant effect on $^{35}SO_4$ uptake ($P<0·01$) but this was not a simple depressive effect. The lowest drug concentration (0·004 mmol/l) produced an effect which was significantly lower than the control; but at the two higher concentrations (0·02 and 0·04 mmol/l) no significant differences from the control were observed (Fig. 6; Table VI). In the second femoral head (FH7), a significant rise in $^{35}SO_4$ uptake was evident at flurbiprofen concentrations of 0·02 mmol/l, but this response was not observed at the other drug concentrations (0·004 mmol/l and 0·04 mmol/l) used (Fig. 6).

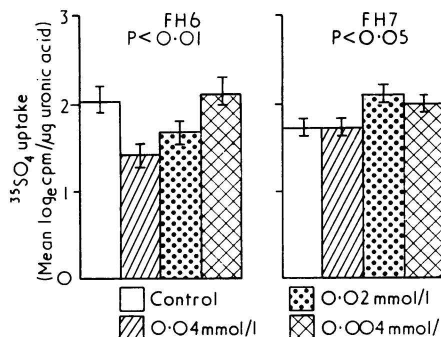

FIG. 6 *Effect of flurbiprofen on $^{35}SO_4$ uptake into sulphated glycosaminoglycans of human cartilage from 2 femoral heads (FH6 and FH7) at various concentrations. Inhibition of $^{35}SO_4$ uptake was not observed in either case up to the maximum concentration used*

Table V *Analysis of variance for the effect of ibuprofen on sulphated glycosaminoglycan synthesis in cartilage from 2 femoral heads*

Source of variation	*Sum of squares*	*df*	*Mean square*	*F*
FH6				
Drug concentration (D)	1·3960	3	0·4653	1·05NS
Incubation time (T)	5·1547	5	1·0309	2·33NS
Interaction between D and T	3·3153	15	1·2210	0·50NS
Residual	21·2326	48	0·4423	
Total	31·0986	71		
FH7				
Drug concentration (D)	5·8623	3	1·9541	13·13*
Incubation time (T)	6·6399	5	1·3280	8·92*
Interaction between D and T	4·0658	15	0·2711	1·82NS
Residual	7·1448	48	0·1489	
Total	23·7128	71		
Pooled FH6 and FH7				
Drug concentration (D)	4.7967	3	1·5989	4·04NS
Incubation time (T)	10·9684	5	2·1937	8·10NS
Femoral head (F)	0·08147	1	0·8147	2·76NS
Interaction between D and T	3·4538	15	0·2303	0·88NS
Interaction between D and F	0·8128	3	0·2709	0·91NS
Interaction between T and F	2·3772	5	0·3962	1·34NS
Interaction between D and T and F	3·9403	15	0·2627	0·89NS
Residual	28·3774	96	0·2956	
Total	55·5413	143		
Because all variation due to interactions is nonsignificant, it can be pooled with the residual variation, and F-tests recalculated as follows:				
Drug concentration (D)	4·7967	3	1·5989	5·45*
Incubation time (T)	10·9684	5	2·1937	7·60*
Femoral head (F)	0·8147	1	0·8147	2·82NS
Residual	38·9615	134	0·2886	
Total	55·5413	143		

Discussion

These findings highlight the variable response of human articular cartilage from different individuals to the same concentrations of anti-inflammatory drugs. Maroudas, Muir, and Wingham (1969) described differences in total hexuronic content from human femoral condyles which showed no correlation with age, and Muir, Bullough, and Maroudas (1970) observed variations in collagen content of human articular cartilage from different individuals, showing that human articular cartilage is variable in both composition and metabolism. This variability, together with individual rates of drug absorption and metabolism, would account for the present findings. Previous studies on the effects of anti-inflammatory drugs on glycosaminoglycan metabolism have been carried out using pure-bred laboratory animals or cell lines, which would be expected to show a more uniform response than human tissue. However, using either tissue, the conclusions reached were similar.

Our preliminary experiments on aged human articular cartilage showed that 98% of the radioactive sulphate taken up during incubation could be removed by simple washing procedures. Of the remaining radioactivity, a further 75% was removed by dialysis after papain digestion. This latter radioactivity may arise from the storage of $^{35}SO_4$ in various metabolic pools which may not necessarily be utilized in polymer synthesis at the same time (Lohmander, Antonopoulos, and Friberg, 1973; Rokosova and Bentley, 1973). A large part of this labelling may also arise from nonspecific binding with preformed matrix, since it occurs at 0° in tissue which has been pre-chilled at this temperature for 30 minutes before incubation with $^{35}SO_4$, in which biosynthesis would be negligible.

In the present study, $^{35}SO_4$ incorporation into sulphated glycosaminoglycans has been defined in terms of uronic acid content of the tissue after papain digestion and dialysis. The major uronic acid containing glycosaminoglycans in articular

Table VI *Analysis of variance for the effect of flurbiprofen on sulphated glycosaminoglycan synthesis in cartilage from 2 femoral heads*

Source of variation	*Sum of squares*	*df*	*Mean square*	*F*
FH6				
Drug concentration (D)	6·0994	3	2·0331	6·23‡
Incubation time (T)	11·4515	5	2·2903	7·02‡
Interaction between D and T	3·7097	15	0·2473	0·76NS
Residual	15·6682	48	0·3264	
Total	36·9288	71		
FH7				
Drug concentration (D)	2·4484	3	0·8161	4·40‡
Incubation time (T)	8·4762	5	1·6952	9·14*
Interaction between D and T	3·9436	15	0·2629	1·42NS
Residual	8·9072	48	0·1856	
Total	23·7754	71		
Pooled FH6 and FH7				
Drug concentration (D)	4·9118	3	1·6373	2·70NS
Incubation time (T)	18·9825	5	3·7965	12·05‡
Femoral head (F)	0·1991	1	0·1991	0·78NS
Interaction between D and T	5·0913	15	0·3394	0·73NS
Interaction between D and F	0·9452	3	0·3151	1·23NS
Interaction between T and F	3·6360	5	0·6060	2·37‡
Interaction between D and T and F	6·9525	15	0·4635	1·81‡
Residual	24·5754	96	0·2560	
Total	65·2938	143		

cartilage are chondroitin 4 and 6 sulphates. Dermatan sulphate, hyaluronic acid, chondroitin and heparin sulphate also contain uronic acid residues, but the levels of these in articular cartilage are very small. Therefore, incorporation of $^{35}SO_4$ expressed in terms of uronic acid largely reflects the synthesis of the chondroitin sulphates. Our procedure does not take into account the incorporation of $^{35}SO_4$ into keratan sulphate. Work by Hardingham and Muir (1972) and Davidson and Small (1963) suggests, however, that the turnover rate of this component is low. Hardingham and Muir (1972), using pig laryngeal cartilage, reported that specific radioactivity measurements based on uronic acid were only slightly modified if the contribution made by keratan sulphate was accounted for. Maroudas (1975) reported preliminary results indicating that the turnover of keratan sulphate is very much slower than that of chondroitin sulphate. Her calculations of total sulphated glycosaminoglycan turnover apply, as do ours, essentially to chondroitin sulphate. Furthermore, the levels of keratan sulphate in human articular cartilage have been reported to remain relatively constant with aging after maturity (Maroudas and others, 1969; Bollet and Nance, 1966).

The reproducible sampling of the cartilage specimens was of particular importance in the present study. Full-depth samples were taken from the surface to the subchondral bone, as glycosaminoglycan metabolism and concentration are known to vary with depth from the surface (Jones and Lemperg, 1975; Maroudas, 1975; Stockwell and Scott, 1967). Although Maroudas and others (1973) reported that there are only slight variations in glycosaminoglycan content with topographical area for normal femoral heads, Bjelle (1974) did observe variations over the surfaces of femoral condyles. Therefore, where possible, cartilage samples were removed from the same locality. Areas of obvious fibrillation or eburnation were excluded, but were uncommon in normal femoral heads removed after fracture.

In general, the results of our studies on the effects of anti-inflammatory drugs on the metabolism of glycosaminoglycans agree with the literature (Domenjoz, 1971; Whitehouse, 1965).

An *in vivo* autoradiographic study (Watson, 1976) with rabbits receiving oral doses of indomethacin showed significant suppression of $^{35}SO_4$ uptake by chondrocytes of the femoral condyles. No differences in $^{35}SO_4$ uptake between animals receiving clinical or higher doses of drug were observed. Our studies were conducted over two concentration ranges, and the response at therapeutic drug levels (Fig. 1) was less marked than at higher indomethacin doses (Fig. 2). However, in our experiments, cartilage

slices were exposed to the drug for 6 hours only, whereas Watson investigated the effects of indomethacin for periods of up to 70 days.

Roach, Tomblin, and Eyring (1975), Forney, Bentley, and Mathews (1973) and Simmons and Chrisman (1965), using morphological and histological techniques, suggested that salicylate stimulates healing of articular cartilage by some biochemical mechanism such as lysosomal stablization or enhancement of matrix formation. Salicylates and other anti-inflammatory drugs may protect cartilage by inhibiting the prostaglandin synthetase systems (Moncada, Ferreira, and Vane, 1973; Takeguchi and Sih, 1972; Tomlinson and others, 1972; Vane, 1971, 1972; Willis and others, 1972). Prostaglandins of the E group are now recognized as prime mediators of the inflammatory response (Glenn and others, 1972; Kaley, Massira, and Weiner, 1972; Willis and others, 1972; Kaley and Weiner, 1971; Arora, Lahiri, and Sanyal, 1970). Such a protective role would be of greater relevance in the therapy of rheumatoid arthritis than osteoarthrosis where the inflammatory component of the process is far less evident.

The ability of cartilage to function efficiently depends upon its mechanical properties which are an expression of matrix composition and organization. These in turn reflect the balance between synthesis and degradation of its components. The ability of drugs to directly or indirectly moderate joint inflammation and the associated degradative mechanisms accounts for their value in the treatment of rheumatoid arthritis, but not osteoarthrosis. Clearly, the place of some anti-inflammatory agents in the management of degenerative joint diseases should be re-examined in the light of their demonstrated inhibitory effects on cartilage glycosaminoglycan biosynthesis.

We gratefully acknowledge financial support for this project from the National Health and Medical Research Council, and The University of Sydney for a Fellowship to L. S. McK; and are grateful for the competent technical assistance of Linda Krick and Denise Greentree. We also express our gratitude to Dr. A. Underwood, Department of Biological Sciences, The University of Sydney, for his advice on the statistical methods used.

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