Effect of homologous synovial membrane on adult human articular cartilage in organ culture, and failure to influence it with D-penicillamine

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SUMMARY Adult human articular cartilage has been maintained in organ culture for 8 days, and the culture medium, which was changed on alternate days, was pooled. Normal and rheumatoid cartilage was obtained from patients and 4 types of culture were prepared: (1) cartilage alone; (2) cartilage + D-penicillamine; (3) cartilage + homologous synovium; (4) cartilage, synovium, and D-penicillamine. The hexosamines and hexuronic acid were measured in the cartilage explants and in the medium. The quantity released was divided by the amount measured in the original cartilage explant and the different culture variables were compared. D-penicillamine did not alter the release of cartilage proteoglycan, but the addition of synovium did. The rheumatoid cartilage released significantly more proteoglycan than normal cartilage whether or not homologous synovium was present.

It has been shown that D-penicillamine is an effective drug in the treatment of patients with rheumatoid arthritis (Multicentre Trial, 1973) and that joint erosions are less likely to increase in patients on this drug than in control patients. The experiments described in this paper were designed to show, on cartilage explants with adjacent synovial membrane, whether human cartilage proteoglycan catabolism could be modified by the addition of D-penicillamine. Experiments performed on cartilage explants from pigs have shown that adjacent synovial tissue depletes the metachromatic proteoglycan from the cartilage matrix in organ culture (Fell and Barratt, 1973).

Material and methods

Adult human cartilage was removed from the non-weight-bearing surfaces of the femoral condyles of 5 patients undergoing meniscectomy, and from the unaffected areas of patellar cartilage of 6 patients with chondromalacia patellae. Diseased rheumatoid cartilage from 5 patients having total knee replacement operations was also obtained. Cartilage

explants were prepared and cultured after the method of Dingle *et al.* (1966), and synovial membrane from the same joints was also maintained in 2 of the 4 cultures prepared from each patient. Dish A=cartilage alone, dish B=cartilage + D-penicillamine, dish C=cartilage + synovium, dish D=cartilage + synovium + D-penicillamine. The tissue culture medium BGJ5 (Reynolds, 1972) was used in these experiments with 5% fetal calf serum. D-penicillamine was added to the medium in dishes B and D at a concentration of 0.08 mg/ml, which is the peak plasma concentration that could be expected in a patient after taking a 250 mg capsule. The explanted tissues were maintained in culture for a period of 8 days, the medium being changed every 48 hours and the gas mixture (20% oxygen, 5% carbon dioxide, 75% nitrogen at atmospheric pressure) renewed at the same time.

The culture medium from each dish was stored at –20°C, and at the end of the culture period the medium from each explant was pooled. The subsequent analyses refer to the cartilage explants alone and do not include the synovial membrane, which was discarded at the end of the culture period. The cartilage explants were dried to a constant weight over silica gel and digested in 2 ml of buffered papain (Jacoby and Jayson, 1975).

The uronic acid content of the cartilage digest and

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the medium was estimated on 0.2 ml of liquid by acid carbazole method (Bitter and Muir, 1962) after precipitation with cetyl pyridinium chloride (Jacoby, 1976). The cartilage digest and the medium were prepared for hexosamine analysis by a gas-liquid chromatography method which has been described in a previous paper (Jacoby, 1976).

Results

Uronic acid and hexosamine analysis of the blank medium (unexposed to cartilage and synovium) failed to reveal significant quantities of these materials. An aliquot of uncultured medium from each experiment was used as a blank.

Histological sections of 2 explants were prepared after 8 days in culture. These specimens showed loss

of metachromasia of the cartilage matrix when maintained in culture with synovium. The control cultures showed no loss of metachromasia if cultured without synovium.

The hexosamine and hexuronic acid shed by the explanted tissues have been expressed as a fraction of the quantity present in the cartilage explants at the end of the culture period (Tables 1-3).

EFFECT OF SYNOVIUM ON THE CARTILAGE PROTEOGLYCANS

The addition of synovium to the cartilage explants caused a significant increase in the galactosamine and uronic acid (from chondroitin sulphate) shed into the medium (Table 4). The quantity of glucosamine (from keratan sulphate) shed into the medium was not influenced by additional synovium.

Table 1 Uronic acid content of medium $\div$ cartilage digest, $\mu\text{g}/\text{mg}$ dry weight of cartilage digest

Source of cartilage	Cartilage control culture	Cartilage + D-p	Cartilage + synovium	Cartilage + D-p + synovium
F N	$\frac{0.4}{5.4} = 0.0727$	$\frac{1.4}{2.0} = 0.700$	$\frac{2.7}{2.3} = 1.1739$	$\frac{1.8}{1.3} = 1.3846$
F N	$\frac{0.3}{3.3} = 0.0909$	$\frac{0.9}{0.9} = 1.000$	$\frac{0.8}{0.9} = 0.8889$	$\frac{7.4}{5.9} = 1.2542$
F N	$\frac{9.2}{5.0} = 0.0400$	$\frac{0.8}{0.5} = 1.6000$	$\frac{2.2}{1.9} = 1.1579$	$\frac{2.4}{1.3} = 1.8462$
F N	$\frac{1.6}{1.0} = 1.600$	$\frac{0.6}{3.9} = 0.1935$	$\frac{2.1}{1.6} = 1.3125$	$\frac{1.9}{1.7} = 1.1176$
F N	$\frac{1.8}{2.3} = 0.7826$	$\frac{3.2}{2.0} = 1.6000$	$\frac{2.8}{1.5} = 1.8667$	$\frac{3.6}{1.5} = 2.4000$
P N	$\frac{0.7}{1.4} = 0.500$	$\frac{0.8}{2.9} = 0.2759$	$\frac{1.9}{1.2} = 1.5833$	$\frac{2.5}{3.6} = 0.6944$
P N	$\frac{1.2}{1.0} = 1.200$	$\frac{0.9}{5.0} = 1.8000$	$\frac{2.1}{1.3} = 1.6156$	$\frac{2.6}{1.9} = 1.3684$
P N	$\frac{0.6}{4.0} = 1.500$	$\frac{0.8}{3.6} = 0.2222$	$\frac{3.5}{3.1} = 1.1290$	$\frac{2.9}{3.0} = 0.9667$
P N	$\frac{0.7}{5.2} = 0.1346$	$\frac{1.1}{2.8} = 0.3929$	$\frac{3.6}{3.1} = 1.1613$	$\frac{3.3}{2.1} = 1.5714$
P N	$\frac{0.6}{0.9} = 0.6667$	$\frac{1.8}{3.1} = 0.5806$	$\frac{2.2}{1.5} = 1.4667$	$\frac{2.4}{2.0} = 1.2000$
P N	$\frac{1.5}{2.8} = 0.5357$	$\frac{1.5}{2.3} = 0.6522$	$\frac{2.9}{1.8} = 1.6111$	$\frac{2.1}{2.0} = 1.0500$
Mean and standard deviation	$\frac{0.6476}{0.6757}$	$\frac{0.8198}{0.7034}$	$\frac{1.3606}{0.4936}$	$\frac{1.3503}{0.5751}$
F RA	$\frac{6.4}{1.4} = 4.5714$	$\frac{3.5}{0.4} = 8.7500$	$\frac{2.9}{0.9} = 3.2222$	$\frac{3.2}{0.9} = 3.5556$
F RA	$\frac{8.0}{2.1} = 3.8095$	$\frac{9.2}{2.0} = 4.6000$	$\frac{10.3}{1.5} = 6.8667$	$\frac{9.0}{0.9} = 10.000$
F RA	$\frac{4.0}{1.2} = 3.3333$	$\frac{5.5}{1.7} = 3.2353$	$\frac{3.6}{1.5} = 2.4000$	$\frac{5.0}{1.8} = 2.7778$
F RA	$\frac{4.1}{1.1} = 3.7273$	$\frac{6.1}{1.2} = 5.0833$	$\frac{12.1}{1.9} = 6.3684$	$\frac{9.3}{2.1} = 4.4286$
F RA	$\frac{29.1}{6.0} = 4.8500$	$\frac{2.7}{0.6} = 4.5000$	$\frac{8.1}{2.3} = 3.5000$	$\frac{1.9}{0.9} = 2.1111$
Mean and standard deviation	$\frac{4.0583}{0.7224}$	$\frac{5.2337}{1.1860}$	$\frac{4.4715}{1.3103}$	$\frac{4.5746}{1.4731}$

F = Femoral condyle, P = Patella, N = Normal, RA = Rheumatoid arthritis, D-p = D-penicillamine.

Table 2 Glucosamine content of medium + cartilage digest, $\mu\text{g}/\text{mg}$ dry weight of cartilage

Source of cartilage	Cartilage control culture	Cartilage + D-p	Cartilage + synovium	Cartilage + D-p + synovium
F N	$\frac{0}{2.1} = 0.000$	$\frac{0.2}{0.4} = 0.500$	$\frac{0.7}{0.6} = 1.1667$	$\frac{0.8}{0.3} = 2.6667$
F N	$\frac{0}{1.2} = 0.000$	$\frac{0.2}{0.5} = 0.400$	$\frac{0.4}{0.4} = 1.000$	$\frac{0.4}{1.3} = 0.3097$
F N	$\frac{0.1}{10.8} = 0.0093$	$\frac{0}{0.3} = 0.000$	$\frac{0.3}{3.1} = 0.0968$	$\frac{0.5}{1.0} = 0.500$
F N	$\frac{0.1}{0.6} = 0.1667$	$\frac{0.9}{1.0} = 0.900$	$\frac{1.8}{0.8} = 2.250$	$\frac{1.7}{0.7} = 2.4286$
F N	$\frac{0.3}{0.4} = 0.7500$	$\frac{1.0}{0.7} = 1.4286$	$\frac{1.1}{0.7} = 1.5714$	$\frac{1.3}{0.2} = 6.500$
P N	$\frac{0.8}{1.0} = 0.800$	$\frac{0.7}{2.0} = 0.3500$	$\frac{0.8}{0.7} = 1.1429$	$\frac{2.8}{2.1} = 1.333$
P N	$\frac{0.9}{0.9} = 1.000$	$\frac{0.7}{3.3} = 0.2121$	$\frac{0.6}{0.9} = 0.6667$	$\frac{0.8}{1.1} = 0.7273$
P N	$\frac{0.9}{2.1} = 0.4286$	$\frac{0.5}{2.7} = 0.1852$	$\frac{0.6}{2.7} = 0.2222$	$\frac{0.8}{1.8} = 0.444$
P N	$\frac{0.6}{0.5} = 1.200$	$\frac{1.7}{2.4} = 0.7083$	$\frac{1.0}{1.3} = 0.7692$	$\frac{0.8}{0.8} = 1.000$
P N	$\frac{0.8}{1.1} = 0.7273$	$\frac{0.9}{1.5} = 0.600$	$\frac{0.9}{1.6} = 0.5625$	$\frac{0.8}{1.0} = 0.8000$
Mean and standard deviation	0.5082 ± 0.4473	0.5284 ± 0.4126	0.8401 ± 0.6756	1.6710 ± 1.8805
F RA	$\frac{2.5}{1.3} = 1.9231$	$\frac{1.6}{0.8} = 2.000$	$\frac{1.0}{0.2} = 5.000$	$\frac{1.6}{0.2} = 8.000$
F RA	$\frac{2.9}{1.9} = 1.5263$	$\frac{2.7}{1.1} = 2.4545$	$\frac{4.3}{1.3} = 3.3077$	$\frac{1.5}{0.9} = 1.6667$
F RA	$\frac{2.5}{0.6} = 4.1667$	$\frac{4.5}{1.0} = 4.500$	$\frac{1.6}{0.7} = 2.285$	$\frac{3.6}{0.6} = 6.000$
F RA	$\frac{3.4}{0.8} = 4.2500$	$\frac{2.5}{1.5} = 1.6667$	$\frac{4.1}{1.1} = 3.7273$	$\frac{2.1}{0.9} = 2.333$
F RA	$\frac{20.1}{4.2} = 4.7857$	$\frac{2.7}{0.9} = 3.000$	$\frac{3.4}{0.4} = 8.500$	$\frac{1.3}{0.2} = 6.500$
Mean and standard deviation	3.3304 ± 1.4915	2.7242 ± 1.1119	2.6847 ± 2.9674	4.8999 ± 2.7579

F = Femoral condyle, P = Patella, N = Normal, RA = Rheumatoid arthritis, D-p = D-penicillamine.

PROTEOGLYCANS SHED FROM NORMAL AND RHEUMATOID CARTILAGE

There was a highly significant increase in the hexosamine and uronic acid loss from the rheumatoid cartilage compared with normal cartilage (Table 5).

EFFECT OF D-PENICILLAMINE ON THE CARTILAGE PROTEOGLYCANS

There was no difference between the explants maintained with D-penicillamine and those explants cultured without this drug (Table 6).

Discussion

In the living joint the protein polysaccharide chains are secreted by chondrocytes into the cartilage matrix and by synovial cells into the synovial fluid. The major protein polysaccharides found in cartilage are chondroitin sulphate and keratan sulphate, whereas the synovial fluid contains hyaluronic acid, which is also present in much smaller amounts in the

articular cartilage. In tissue culture, as in vivo, these glycosaminoglycans are being synthesised and degraded throughout the culture period and shed into the medium. After papain digestion of the medium the chains are split into their hexose components, however, and it is impossible to distinguish from which tissue—that is, synovium or cartilage—the individual molecules were derived. The problem is largely overcome by measuring the hexose molecules, uronic acid, glucosamine, and galactosamine. Galactosamine is found only in chondroitin sulphate and thus cartilage tissue, whereas uronic acid is found in both cartilage chondroitin sulphate and synovial hyaluronic acid. Glucosamine on the other hand is found in both hyaluronic acid and the other cartilage glycosaminoglycan, namely, keratan sulphate. The distribution and concentration of the cartilage glycosaminoglycans are well known and have been fully reviewed elsewhere (Muir, 1973). However, suffice it to say that chondroitin sulphate is present

Table 3 Galactosamine content of medium ÷ cartilage digest, µg/mg dry weight of cartilage

Source of cartilage	Cartilage control culture	Cartilage + D-p	Cartilage + synovium	Cartilage + D-p + synovium
F N	$\frac{0.2}{4.5} = 0.0444$	$\frac{1.1}{1.4} = 0.7857$	$\frac{2.4}{2.2} = 1.0909$	$\frac{1.8}{1.5} = 1.200$
F N	$\frac{0.3}{3.1} = 0.0986$	$\frac{0.7}{1.6} = 0.4375$	$\frac{0.9}{1.1} = 0.8182$	$\frac{4.8}{5.1} = 0.9412$
F N	$\frac{0.5}{13.2} = 0.0379$	$\frac{0.5}{0.3} = 1.6667$	$\frac{2.9}{2.4} = 1.2083$	$\frac{2.8}{1.3} = 2.1538$
F N	$\frac{1.3}{1.2} = 1.0833$	$\frac{0.4}{2.5} = 0.1600$	$\frac{2.3}{1.9} = 1.2105$	$\frac{1.8}{1.7} = 1.0588$
F N	$\frac{1.1}{1.4} = 0.7857$	$\frac{3.0}{1.9} = 1.5789$	$\frac{2.9}{1.7} = 1.7059$	$\frac{3.0}{0.9} = 3.333$
F N	$\frac{0.8}{1.45} = 0.5517$	$\frac{0.8}{3.4} = 0.2353$	$\frac{1.3}{0.9} = 1.444$	$\frac{2.9}{3.0} = 0.9667$
F N	$\frac{1.9}{1.3} = 1.4615$	$\frac{1.2}{5.7} = 0.2105$	$\frac{2.8}{1.5} = 1.8667$	$\frac{2.0}{1.8} = 1.111$
F N	$\frac{0.4}{3.6} = 0.1111$	$\frac{0.6}{3.8} = 0.1579$	$\frac{3.9}{3.3} = 1.1818$	$\frac{2.9}{2.9} = 1.000$
P N	$\frac{0.7}{5.7} = 1.228$	$\frac{1.4}{3.5} = 0.4$	$\frac{3.9}{3.0} = 1.300$	$\frac{3.9}{2.1} = 1.8571$
P N	$\frac{0.9}{1.4} = 0.6429$	$\frac{1.7}{3.1} = 0.5484$	$\frac{2.8}{1.8} = 1.5556$	$\frac{2.8}{2.1} = 1.333$
P N	$\frac{1.5}{2.3} = 0.6522$	$\frac{1.0}{2.0} = 0.500$	$\frac{2.6}{1.7} = 1.5294$	$\frac{1.9}{0.9} = 2.111$
Mean and standard deviation	$\frac{0.6087}{0.5028}$	$\frac{0.6074}{0.5369}$	$\frac{1.3556}{0.2995}$	$\frac{1.5515}{0.7464}$
F RA	$\frac{6.7}{1.9} = 3.5263$	$\frac{3.8}{0.6} = 6.333$	$\frac{2.8}{0.4} = 7.000$	$\frac{3.3}{0.5} = 6.600$
F RA	$\frac{7.1}{1.9} = 3.7368$	$\frac{9.5}{2.5} = 4.5238$	$\frac{10.4}{1.4} = 7.4286$	$\frac{8.0}{1.1} = 7.2726$
F RA	$\frac{4.4}{1.2} = 3.6667$	$\frac{5.9}{1.7} = 3.4706$	$\frac{3.2}{1.1} = 2.9091$	$\frac{4.7}{0.8} = 5.875$
F RA	$\frac{4.8}{1.4} = 3.4286$	$\frac{6.1}{1.7} = 3.5882$	$\frac{12.2}{1.5} = 8.1333$	$\frac{9.1}{1.4} = 6.500$
F RA	$\frac{29.8}{6.7} = 4.4478$	$\frac{2.2}{0.9} = 3.000$	$\frac{8.8}{4.1} = 2.100$	$\frac{1.5}{0.5} = 3.000$
Mean and standard deviation	$\frac{3.762}{0.4}$	$\frac{4.1832}{1.3229}$	$\frac{9.2942}{6.8536}$	$\frac{5.8495}{1.6682}$

F = Femoral condyle. P = Patella. N = Normal. RA = Rheumatoid arthritis. D-p = D-penicillamine.

in greater amounts than keratan sulphate, more so in young cartilage and osteoarthrotic cartilage.

The pattern of secretion into the medium certainly differs between the hexose molecules and must reflect the different protein polysaccharide chains from which they were degraded.

The results uniformly suggest that D-penicillamine does not alter the release of any of the hexose molecules from normal or rheumatoid cartilage, which implies that any beneficial effect of this drug noted in rheumatoid arthritis (Multicentre Trial Group, 1973) is not due to a cartilage sparing effect as such.

The influence of synovial tissue on articular cartilage in tissue culture would appear to be deleterious, which raises questions as to the validity of this model. Fell and Barratt (1973) have shown a similar reversible deleterious effect of synovium and subchondral bone marrow on the explanted cartilage

of pigs, and more recently Fell and Jubb (1977) have shown that minced synovial tissue alone with cartilage will disturb the matrix proteoglycans. In the undamaged joint synovium and cartilage are found together, and even in traumatised cartilage the synovium does not digest the exposed deeper layers, so why does the tissue culture model behave differently? The answer is not yet known. However, it is well known that in rheumatoid arthritis synovium can digest the cartilage matrix, and so rheumatoid arthritis may have something in common with the in-vitro model using cartilage synovium.

The chemical changes observed in the medium of explants exposed to synovium showed that galactosamine and uronic acid were substantially increased as a result. This may suggest that chondroitin sulphate degradation had occurred. The quantity of glucosamine released into the medium was not

Table 4 The effect of synovium on the cartilage. The galactosamine, uronic acid, and glucosamine shed from the cartilage exposed to synovium has been tested against the cultures maintained without synovium. The upper figure in each box is the value of Student's *t* and the lower figure indicates the significance (NS = not significant)

	+ synovium				
	Normal cartilage	Normal cartilage + D-p	RA cartilage	RA cartilage + D-p	
Galactosamine	4.0358 P<0.001	3.2471 P<0.005	1.6119 NS	1.5653 NS	- synovium
				Normal cartilage + D-p RA cartilage + D-p	
Uronic Acid	3.3368 P<0.001	2.3850 P<0.01	1.1440 NS	1.1115 NS	- synovium
				Normal cartilage + D-p RA cartilage + D-p	
Glucosamine	1.2953 NS	1.8768 NS	0.3888 NS	1.4633 NS	- synovium
				Normal cartilage + D-p RA cartilage + D-p	

significantly different in the synovial versus non-synovial treated explants. This evidence suggests that neither synovial hyaluronic acid nor cartilage keratan sulphate were being shed in sufficient quantities to make any difference. There is no reason to indicate that chondroitin sulphate was selectively broken down rather than keratan sulphate, so one must conclude that keratan sulphate was shed whether the synovium was present or not. It is unlikely that keratan sulphate was completely absent from

Table 5 The difference between normal cartilage and rheumatoid cartilage. The value for Student's *t* and the significance is given as in Table 4

	Normal Cartilage				
	Control	+ D-p	+ synovium	+ synovium + D-p	
Galactosamine	11.5434 P<0.001	7.1845 P<0.001	3.5860 P<0.005	6.6600 P<0.001	Rheumatoid cartilage
				Control + D-p + synovium + synovium + D-p	
Uronic Acid	9.4427 P<0.001	7.4433 P<0.001	3.5008 P<0.005	4.3776 P<0.001	Rheumatoid cartilage
				Control + D-p + synovium + synovium + D-p	
Glucosamine	5.3657 P<0.001	5.3674 P<0.001	3.5760 P<0.005	2.5538 P<0.025	Rheumatoid cartilage
				Control + D-p + synovium + synovium + D-p	

the explants, since the glucosamine that was measured in the medium can hardly have come entirely from synovial hyaluronic acid.

The number of experiments using rheumatoid cartilage is very small, and the value for Student's *t* must be interpreted with some caution. However, the differences between the diseased and normal explants were substantial in the absence of synovium suggesting a real difference. In those cultures comparing the 2 different types of cartilage all 3 hexose molecules were secreted in excess from the diseased cartilage. This evidence supports the contention that, as in osteoarthrotic cartilage, rheumatoid cartilage is also metabolically more active than normal cartilage (Jacoby and Jayson, 1976). The picture is less clear in the explants maintained with

Table 6 The difference between cartilage cultured with and without D-penicillamine. The value for Student's *t* and the significance are given as in Table 4.

+ D-penicillamine				
Normal cartilage	Normal cartilage + synovium	RA cartilage	RA cartilage + synovium	
Galactosamine	0.003 NS			D-penicillamine
		0.7703 NS		
			0.6104 NS	
			0.9767 NS	
			Normal cartilage Normal cartilage + synovium RA cartilage RA cartilage + synovium	
+ D-penicillamine				
Normal cartilage	Normal cartilage + synovium	RA cartilage	RA cartilage + synovium	
Uronic Acid	0.774 NS			D-penicillamine
		0.0566 NS		
			1.9821 NS	
			0.8754 NS	
			Normal cartilage Normal cartilage + synovium RA cartilage RA cartilage + synovium	
+ D-penicillamine				
Normal cartilage	Normal cartilage + synovium	RA cartilage	RA cartilage + synovium	
Glucosamine	0.1051 NS			D-penicillamine
		1.3150 NS		
			0.6517 NS	
			1.0936 NS	
			Normal cartilage Normal cartilage + synovium RA cartilage RA cartilage + synovium	

synovium, since the D-penicillamine-treated cultures again showed the increased secretion of all the hexose molecules, whereas those cultures maintained with synovium alone did not. The evidence points to the fact that the synovium had little or no influence

on the release of the glycosaminoglycans from the explants, and in view of the small number of experiments using rheumatoid cartilage not too much emphasis should be placed on the difference between galactosamine release and the other 2 hexose molecules. If galactosamine was truly different in the rheumatoid explants, the same should have applied to the uronic acid as the other component of chondroitin sulphate.

Conclusion

These experiments show that D-penicillamine does not appear to influence cartilage catabolism in this test system, but synovium apparently does. In rheumatoid cartilage there is a very active metabolism which does not seem to be altered by the presence or absence of homologous synovium in vitro.

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