Synthesis of arachidonate cyclo-oxygenase products by rheumatoid and nonrheumatoid synovial lining in nonproliferative organ culture

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SUMMARY  Specimens of human rheumatoid and nonrheumatoid synovial lining were maintained in nonproliferative organ culture for 20 hours. The culture fluids were then assayed for prostaglandin E2 (PGE2), thromboxane B2 (TXB2), and 6-keto-prostaglandin F1α (6-keto-PGF1α) by specific radioimmunoassay. The presence of each of these substances was confirmed by gas chromatography and mass spectrometry. Rheumatoid tissue produced significantly more of each cyclo-oxygenase product than nonrheumatoid tissue.

The initial step in the biosynthesis of prostaglandins from the unsaturated fatty acid precursor (for example, arachidonic acid) is the formation of unstable cyclic endoperoxides (PGG2 and PGH2) by the enzyme fatty acid cyclo-oxygenase. The endoperoxides can be converted to thromboxane A2 (TXA2), prostacyclin (PGI2), or the ‘primary’ prostaglandins (PGE2, PGD2, and PGF2α) (review1).

It is now generally accepted that ‘primary’ prostaglandins, in particular PGE2, are important inflammatory mediators (review2). These prostaglandins have been detected in human synovial fluids collected from inflamed joints, and concentrations are reduced after treatment with nonsteroid anti-inflammatory drugs. Furthermore, tissue cultures of rheumatoid synovial lining produce prostaglandins. More recently thromboxane B2 and 6-keto-PGF1α (the stable hydrolysis products of thromboxane A2 and prostacyclin respectively) have been detected in inflammatory exudates from experimental animals. Also, synovial effusions from rheumatoid joints have been found to contain thromboxane B2 and 6-keto-PGF1α in addition to the ‘primary’ prostaglandins.

In the present study we have investigated the capacity of human synovial lining tissue to generate cyclo-oxygenase products. Synovial explants have been maintained in nonproliferative organ culture, and tissue from rheumatoid and nonrheumatoid patients has been compared. Some of these results have been reported to the British Pharmacological Society and to the Heberden Society.11 12

Materials and methods

TISSUE CULTURE  Biopsy specimens of human synovial lining were taken either at arthroscopy for internal derangement or at synovectomy. The nonrheumatoid specimens were taken from the knee, either from quiescent joints or from otherwise normal joints after recent mechanical trauma. The rheumatoid specimens were taken mainly from the knee during synovectomy from patients who had ‘definite’ or ‘classical’ disease according to the diagnostic criteria of the American Rheumatism Association.13

All specimens were removed in a bloodless field within 10 min of the application of the tourniquet and were transferred to the laboratory in a sterile container on a gauze moistened with Trowell’s T-8 culture medium (Gibco). The tissue was cut into segments, each of approximately 4 x 4 mm planar surface and of the thickness of the membrane (not exceeding 4 mm). These were maintained individually in Trowell’s nonproliferative adult organ maintenance culture at the relevant pH for the tissue. For some segments the culture medium contained indomethacin (Wellcome) at 10-5 M. Explants were maintained under an atmosphere of 95% oxygen and 5% carbon dioxide at 37°C for 20 hours.

Accepted for publication 23 December 1981.
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The medium was then withdrawn, snap-frozen to 
−70°C and stored at −20°C. After removal of excess water each specimen was weighed, then chilled by precipitate immersion in n-hexane (BDH ‘low in aromatic hydrocarbons’ grade, boiling range 67–70°C) at −70°C, and stored at −70°C in a corked dry glass tube. Histological confirmation of the pathology was obtained from suitably stained cryostat sections of the explants, prepared as described by Chayen et al. 15

**RADIOIMMUNOASSAY**
PGE₂, 6-keto-PGF₁₀, and TXB₂ in diluted culture media were assayed directly by radioimmunoassays. These assays are relatively specific, 16 though PGE₂ does cross-react significantly (approximately 5%) in the assay for 6-keto-PGF₁₀. Therefore the cyclo-
oxigenase products in several samples were extracted and separated by thin-layer chromatography prior to radioimmunoassay 7 to confirm data obtained by direct assay. The amount of each cyclo-
oxigenase product generated in the culture period was expressed as pg/mg wet weight of tissue.

**GAS-LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY**
Cyclo-oxygenase products from 3 culture fluids were determined by gas liquid chromatography—mass spectrometry. Approximately 500 ng of each of the following internal standards were added to each fluid; 3,3,4,4-D₄-6-keto-PGF₁₀a, 5,6,8,11,12,14, 15-D₆-PGE₂, and 5,6,8,9,11,12,14,15-D₈-TXB₂. The fluids were mixed with 2 volumes of ice-cold acetone to precipitate protein, and then the supernatant was washed with 2 volumes of n-hexane to remove neutral lipids. The remaining aqueous-acetone phase was acidified to pH 4 with citric acid and extracted twice with 2 volumes of chloroform. The combined extracts were evaporated to dryness and the residues were subjected to silicic acid column chromatography with mixtures of chloroform and methanol to elute the cyclo-oxygenase products. 17 Fraction II, which contains PGE₂, TXB₂, and 6-keto-PGF₁₀a, was concentrated to dryness and then reacted successively with methoxamine hydrochloride (Pierce Chemical Co.) diazomethane and N₂O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS; Pierce Chemical Co.) as previously described. 15 Aliquots of the derivatised samples and standards were injected into a Hewlett-Packard Model 5730 A GLC combined with a VG Micromass 16F mass spectrometer; a 1% OV1 column was employed at 230°C. The following ions were monitored: m/z 301-20 (TXB₂), 305-23 (D₆-TXB₂), 508-33 (PGE₂ and 6-keto-PGF₁₀a), 512-35 (D₄-6-keto-

**RESULTS**
The results presented in Tables 1 and 2 and Fig. 1 are derived from radioimmunoassay of culture media directly. Culture fluids from samples 2207, 2208 (Table 1), and 2209 (Table 2) were extracted and purified before radioimmunoassay, and these experiments both the qualitative and quantitative results obtained by direct assay. The presence of PGE₂, 6-keto-PGF₁₀a, and TXB₂ in extracts of culture fluids from sample 2342 (Table 2) was positively confirmed by gas-liquid chromatography and mass spectrometry.

All the culture fluids tested contained detectable concentrations of PGE₂ and 6-keto-PGF₁₀a, and in each case, with the exception of sample 2207 (Table 1), PGE₂ was the predominant cyclo-oxygenase product. All the rheumatoid tissues produced significant amounts of TXB₂, but in most cases it was the minor
product (Table 2). In 2 out of 6 cultures of nonrheumatoid tissues TXB₂ was not detected (Table 1). The mean production of each cyclo-oxygenase product by rheumatoid tissue was significantly higher than mean values for the nonrheumatoid group (Fig. 1). Indomethacin (10⁻³ to 10⁻² M) caused a dose-dependent inhibition of the generation of each cyclo-oxygenase product and in tissues from 7 different patients 10⁻⁴ M indomethacin suppressed cyclo-oxygenase activity by at least 85%.

Discussion

The results presented in this paper confirm the observations that synovial lining explants produce PGE₂, ⁵ ⁶ and support the findings that rheumatoid synovium has a greater prostaglandin synthetase capacity than nonrheumatoid synovium. ¹⁸ ¹⁹ They also show that these tissues are capable of generating thromboxanes and prostacyclin, though PGE₂ is the predominant product in both groups. It is possible therefore that the TXB₂ and 6-keto-PGF₁α detected in synovial fluids* originate from synovial tissue.

Prostacyclin, like PGE₂, is a potent vasodilator and hyperalgesic agent, ³ and generation of prostacyclin as well as PGE₂ by inflamed tissues could contribute to inflammatory symptoms such as erythema, oedema, and pain. The role of thromboxanes in inflammation is less clear. Thromboxane A₂ is a potent vasoconstrictor and aggregator of platelets,¹ and these properties may reduce haemorrhage at an inflamed site. Also, thromboxane B₂ has been reported to have chemotactic activity for leucocytes. ²⁰ There are, however, no reports as yet which indicate that thromboxanes are important inflammatory mediators. The increased cyclo-oxygenase activity in rheumatoid tissue may be fundamental to the rheumatoid process, or it may be a less specific consequence of the pathology of these joints.

Generation of cyclo-oxygenase products by synovial tissue in culture appears to be independent of prior drug treatment. Tissues from patients receiving nonsteroid anti-inflammatory drugs such as indomethacin, flurbiprofen, or diclofenac, which are known to inhibit prostaglandin synthesis, ² do not have significantly less cyclo-oxygenase activity than tissues from patients receiving other drugs (Table 2). These observations are in agreement with the findings that preparations of human synovial microsomes from patients receiving indomethacin, naproxen, or ibuprofen did not have decreased cyclo-oxygenase activity. ³¹ These authors reported, however, that tissues from patients taking aspirin did not generate prostaglandins in vitro, and they attributed this to an irreversible effect of aspirin which was not shared by other nonsteroid anti-inflammatory drugs.

We thank Miss Lorna Tilling for excellent technical assistance. Some of us (L.B., J.C., and B.H.) gratefully acknowledge general support from the Arthritis and Rheumatism Council for Research.

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### Table 1: Generation of cyclo-oxygenase products by nonrheumatoid synovial explants during 20 h nonproliferative culture

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Joint</th>
<th>Treatment</th>
<th>PGE₁</th>
<th>6-k- PGF₁α</th>
<th>TXB₂</th>
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<tr>
<td>2207</td>
<td>F</td>
<td>73</td>
<td>Torn degenerated medial cartilage</td>
<td>Knee</td>
<td>None</td>
<td>27</td>
<td>40</td>
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<td>2208</td>
<td>F</td>
<td>13</td>
<td>Chondromalacia</td>
<td>Knee</td>
<td>None</td>
<td>2174</td>
<td>856</td>
<td>0</td>
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<td>2267</td>
<td>M</td>
<td>31</td>
<td>Chondromalacia (diabetic)</td>
<td>Knee</td>
<td>Insulin</td>
<td>7047</td>
<td>1017</td>
<td>105</td>
</tr>
<tr>
<td>2270</td>
<td>M</td>
<td>43</td>
<td>Torn meniscus</td>
<td>Knee</td>
<td>None</td>
<td>936</td>
<td>690</td>
<td>17</td>
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<tr>
<td>2316</td>
<td>M</td>
<td>19</td>
<td>Torn meniscus</td>
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<td>2551</td>
<td>256</td>
<td>40</td>
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<tr>
<td>2358</td>
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<td>Chondromalacia</td>
<td>Knee</td>
<td>None</td>
<td>1188</td>
<td>607</td>
<td>31</td>
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</table>

### Table 2: Generation of cyclo-oxygenase products by rheumatoid synovial explants during 20 h nonproliferative culture

<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Joint</th>
<th>Treatment</th>
<th>PGE₁</th>
<th>6-k- PGF₁α</th>
<th>TXB₂</th>
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</thead>
<tbody>
<tr>
<td>2209</td>
<td>F</td>
<td>41</td>
<td>Rheumatoid arthritis</td>
<td>Knee</td>
<td>Gold</td>
<td>1143</td>
<td>809</td>
<td>709</td>
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<tr>
<td>2273</td>
<td>M</td>
<td>43</td>
<td>Rheumatoid arthritis</td>
<td>Knee</td>
<td>Distalgesic, Indocid, Fenoprofen, Clinoril</td>
<td>1998</td>
<td>276</td>
<td>140</td>
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<td>2311</td>
<td>F</td>
<td>53</td>
<td>Rheumatoid arthritis</td>
<td>Knee</td>
<td>Benoral, imipramine, gold</td>
<td>5131</td>
<td>974</td>
<td>29</td>
</tr>
<tr>
<td>2328</td>
<td>F</td>
<td>63</td>
<td>Rheumatoid arthritis</td>
<td>Knee</td>
<td>Indocid, Orudis</td>
<td>4725</td>
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<td>1103</td>
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<tr>
<td>2334</td>
<td>F</td>
<td>55</td>
<td>Rheumatoid arthritis</td>
<td>Knee</td>
<td>Ponstan, Depo-medrone</td>
<td>7742</td>
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<td>Knee</td>
<td>Penicillamine, Dolobid</td>
<td>12924</td>
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<tr>
<td>2353</td>
<td>M</td>
<td>22</td>
<td>Rheumatoid arthritis</td>
<td>Knee</td>
<td>Denol, nitrzaepam</td>
<td>4196</td>
<td>838</td>
<td>175</td>
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<tr>
<td>2393</td>
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<td>51</td>
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<td>Knee</td>
<td>Distalgesic, Dolobid</td>
<td>20900</td>
<td>583</td>
<td>834</td>
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<tr>
<td>2439</td>
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<td>55</td>
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<td>Wrist</td>
<td>Indocid</td>
<td>6653</td>
<td>765</td>
<td>183</td>
</tr>
</tbody>
</table>

Proprietary names: Distalgesic (dextropropoxyphene), Indocid (indomethacin), Fenoprofen (fenoprofen), Clinoril (sulindac), Froben (flurbiprofen), Benoral (benoxylate), Orudis (ketoprofen), Ponstan (mefenamic acid), Depo-medrone (methyl prednisolone), Dolobid (diflunisal).
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References


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doi: 10.1136/ard.42.1.36

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