Prostaglandin-Stimulated Bone Resorption by Rheumatoid Synovia

A POSSIBLE MECHANISM FOR BONE DESTRUCTION IN RHEUMATOID ARTHRITIS

DWIGHT R. ROBINSON, ARMEN H. TASHJIAN, JR., and LAWRENCE LEVINE

From the Department of Medicine (Arthritis Unit), Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114; the Laboratory of Pharmacology, Harvard School of Dental Medicine and the Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115; and the Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154

ABSTRACT Synovial tissue from patients with rheumatoid arthritis was maintained in organ culture for 3-14 days. Conditioned media from these synovial cultures contained bone resorption-stimulating activity, measured in vitro by using calcium release from mouse calvaria as the assay system. The synovial cultures also produce prostaglandin E_2 (PGE_2) as measured by serologic methods. The production of both the bone resorption-stimulating activity and PGE_2 was inhibited by more than 90% by treatment of the synovial cultures with indomethacin (5 μg/ml). In contrast, treatment of the synovial cultures with colchicine (0.1 μg/ml) caused a marked and parallel increase in the concentration of both bone resorption-stimulating activity and PGE_2 in the conditioned media. The bone resorption-stimulating activity was quantitatively extracted into diethyl ether. Within the limits of experimental error, all of the bone resorption-stimulating activity in medium was accounted for by its content of PGE_2, itself a potent osteolytic factor. We conclude that the bone resorption-stimulating activity produced by rheumatoid synovia in culture is PGE_2.

INTRODUCTION

Resorption of bone is an important component of the joint destruction and deformity regularly seen in chronic rheumatoid arthritis. Release of lysosomal enzymes by cells participating in the rheumatoid inflammatory reaction has been proposed to account for destruction of cartilage and other soft tissue components of articular structures. On the other hand, the mechanisms involved in bone resorption in rheumatoid arthritis are not well understood. It has previously been shown that prostaglandins (PG)[1] of the E series are potent stimulators of bone resorption in vitro (1). Recently, it has also been shown that the production of large amounts of PGE_2 by a murine fibrosarcoma (2) and rabbit carcinoma (3) can account for both the hypercalcemia observed in tumor-bearing animals and the bone resorption-stimulating (BRS) activity extracted from these tumors and secreted by the tumor cells in culture. In the experiments described in this report we have found BRS activity in medium from rheumatoid synovial organ cultures, and evidence is presented that leads us to conclude that this activity can be accounted for by PGE_2 produced by the synovial tissue.

METHODS

Synovial organ cultures. Synovial organ cultures were prepared from surgical specimens from patients with rheumatoid arthritis by methods described previously by others (4). All patients had classical rheumatoid arthritis according to the accepted criteria (5), and active synovitis was present by clinical and histologic examination. The synovial membrane was dissected from underlying connective tissue and cut into explants approximately 2 × 2 mm. Approxi-

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[1] *Abbreviations used in this paper:* BRS, bone resorption-stimulating; PG, prostaglandin.

mately eight fragments were incubated in 60-mm Falcon plastic dishes (Falcon Plastics, Division of BioQuest, Oxnard, Calif.) with 2.0 ml of culture medium. The medium was minimal essential medium (Eagle's) with Earle's salts (No. F-15, Grand Island Biological Co., Grand Island, N. Y.), containing glutamine 2 mM, penicillin 250 U/ml, streptomycin 250 μg/ml, and 10% fetal calf serum. Cultures were incubated at 37°C in an atmosphere of 95% air and 5% CO_2. Medium was changed at 3–5 day intervals, and the conditioned medium was stored at −20°C until assay of PGE_2 and BRS activity. Each culture dish contained 30–40 mg wet wt of synovial tissue.

Prostaglandin assays. Culture media were assayed without extraction for PGB by a radioimmunoassay procedure and antisera described in detail elsewhere (6). Before assay, all samples were heated at 100°C for 5 min in 0.02 N NaOH, a procedure that converts PGE and PGA quantitatively to PGB (7). Therefore, measurement of PGB after alkali treatment determines concentrations of PGE + PGA + PGB.

More detailed characterization of the prostaglandins produced by one of the synovial tissues (R-42) has been carried out; the details of these methods will be published elsewhere (8). In brief, measurements of PGF concentrations in tissue culture media were performed by serologic methods (radioimmunoassay) before and after $NaBH_4$ treatment. This procedure is known quantitatively to convert PGE, but not PGB or PGA, to PGF (7). The concentrations of PGE in medium samples were therefore calculated from the increment in PGF concentrations after $NaBH_4$ treatment. It was also determined that the PGF formed by $NaBH_4$ was $PGF_{2\alpha}$, establishing that the form of PGE present in culture media was predominantly PGE_2. These results indicate that the prostaglandin measured as PGB after alkali treatment was accounted for entirely by PGE_2, within experimental errors, and therefore any PGB or PGA in the media was present in low or undetectable concentrations (less than 20% of PGE). We assume that these findings are valid for culture medium from synovial tissues other than R-42. Thus, all our results are expressed as PGE_2 equivalents. It was shown experimentally that neither colchicine, indomethacin, nor fresh culture medium interfered significantly with the radioimmunoassay method used.

Ether extraction of synovial culture media. Volumes of 1.5 ml of each medium sample to be extracted were diluted to 10 ml with Dulbecco's phosphate-buffered saline and the pH was adjusted to 3.5 with HCl. Each sample was then extracted three times with 2.5–3 vol of anhydrous diethyl ether. The combined ether extracts were then dried by evaporation under nitrogen, and the residue was suspended in 1.5 ml of phosphate-buffered saline.

Assay for BRS activity. The method used was essentially that described by Tashjian and his colleagues (2, 3), which is based on the procedure of Goldhaber (9). In the experiments described in this report the only significant modifications of previous methods were: (*a*) The culture medium used was Dulbecco's modified Eagle's medium (Microbiological Associates, Bethesda, Md.), supplemented with bovine serum albumin (5 mg/ml); and (*b*) The donor mice were not injected with ^{45}Ca. Instead, the release of ^{40}Ca into the bone culture medium was measured as described below. After a 24-h equilibration period in culture, medium was removed and fresh medium containing control, standard, and test substances was added to groups of four bones (2, 3). 24 h later a portion of the medium was removed from each culture for analysis of total calcium concentration. The experiment was terminated at 48 h (after 2 days of treatment) and the concentration of calcium in the medium was again determined. Total calcium in medium was measured either by the method of Copp (10) or by automatic fluormetric titration with the Corning Model 940 calcium analyzer (Corning Scientific Instruments, Medfield, Mass.). The data in each experiment were subjected to an analysis of variance. The standard errors (SE) were calculated from the residual error term of each analysis. This method of determining BRS activity by measuring ^{40}Ca in the bone culture medium gives essentially the same quantitative results as the ^{45}Ca method reported by us previously (2); however, the precision of the ^{40}Ca method is greater.

RESULTS

Calcium released from mouse calvaria in response to increasing amounts of PGE_2 is shown in Fig. 1. The maximum response to PGE_2 occurs at about 20 ng/ml and the concentration of PGE_2 required to give a half-maximum response is about 5 ng/ml or 1.4×10^{-8} M. There is some variation in the calcium concentration in medium from control bones between experiments; however, the dose-response characteristics of the PGE_2 effect indicate that the maximum response is usually an elevation of medium calcium concentration of approximately 4.0 mg/dl above that observed in control cultures without added PGE_2.

The results in Table I show the BRS activity in conditioned medium from synovial organ cultures from two patients with rheumatoid arthritis. Incubation of replicate synovial cultures from each case with indomethacin almost completely prevented the appearance of BRS activity in the conditioned medium. Indomethacin alone has been shown previously to have no detect-

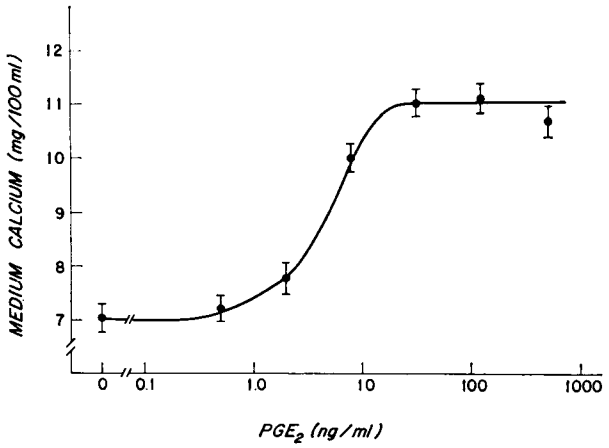

FIGURE 1 The relationship between medium calcium concentration and added PGE_2 in the mouse calvaria assay system. Each point gives the mean value of four bones, and the bars give the standard errors. The data shown were obtained at the end of a 48-h period of culture without or with PGE_2.

TABLE I

BRS Activity in Conditioned Media from Rheumatoid Synovia Cultured without or with Indomethacin (5 μg/ml)

Synovial culture medium*	Day	Volume‡	Medium calcium	P§
		μl/ml	mg/dl	
R-36				
No addition		0	4.6	
Control	3	100	8.0	<0.001
Control	3	100	8.5	<0.001
Indomethacin	3	100	6.5	<0.05
Indomethacin	3	100	5.3	NS
Control	6	100	8.8	<0.001
Control	6	100	9.7	<0.001
Indomethacin	6	100	5.7	NS
Indomethacin	6	100	5.0	NS
R-33				
No addition		0	6.1	
Control	14	55	11.1	<0.001
Control	14	62	8.8	<0.01
Indomethacin	14	65	5.9	NS
Indomethacin	14	40	6.3	NS

* Synovial tissues from two patients were cultured without or with indomethacin (R-36) and (R-33). 3-day conditioned medium samples were obtained on days 3 and 6 from tissue R-36 and 8-day conditioned medium was obtained on day 14 of culture from R-33. The "no addition" entries refer to a set of control calvaria incubated for 48 h without added synovial culture media. Indomethacin, at the concentration used, does not interfere with the response of the bones to a variety of BRS agents, including prostaglandins (2).

‡ Volume of synovial culture medium added to bone culture medium. There are 2 ml of culture medium in each bone culture.

§ The statistical significance is indicated for differences between the experimental values indicated and values for control bones cultured without synovial medium added. NS, $P \geq 0.05$.

able effect on either basal or stimulated bone resorption in this assay system (2, 11).

All of the culture media assayed for BRS activity were also assayed for prostaglandins by radioimmunoassay. In every case, concentrations of PGE_2 in media from indomethacin-treated cultures were less than 1% of the corresponding untreated controls.

The factor in conditioned medium responsible for the BRS activity was characterized further in experiments with tissue sample R-37. Results obtained with media from the first 3 days of synovial culture are summarized in Fig. 2. Synovia 1 and 2 were cultured in medium alone and samples 3 and 4 were cultured with indomethacin. In this experiment, samples 3 and 4 contained undetectable PGE_2 (less than 1% of the amount measured in samples 1 and 2). Portions of medium from 1 and 2 were extracted with ether and reconstituted to the original volume of the media extracted. As shown in Fig. 2, both the original medium and ether extracts were examined for activity at four different dose levels. In the original medium as well as in the ether extract, maximum BRS activity was observed at a dose of 4 μl/ml of bone culture medium. There was no significant difference between the activity of the original synovial culture media and equal volumes of the reconstituted ether extract, indicating essentially quantitative transfer of the BRS activity into ether. In contrast to the results with untreated synovial cultures, indomethacin-treated cultures produced no detectable activity even at the highest concentration tested (20 μl/ml of bone culture medium).

The same synovial cultures were incubated for an additional 3-day period, after a complete change of medium on day 3. The second 3-day collections (day 6) were analyzed similarly to those from day 3, and the results are given in Table II. The BRS activity was again transferred in good ($\geq 80\%$) yield into ether, and

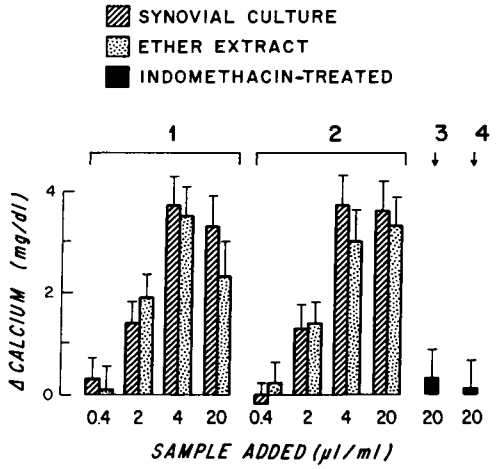

FIGURE 2 BRS activity in rheumatoid synovial organ culture media (R-37, day 3). Samples 1 and 2 were untreated controls, and 3 and 4 were media from synovial fragments cultured with indomethacin (5 μg/ml). Media from samples 1 and 2 were extracted with ether as described in the Methods, and the reconstituted ether extracts were also assayed. The bars give the mean differences in medium calcium concentrations between control bone cultures and bones treated with synovial culture media. The brackets give the SE of the mean difference. The abscissa gives the volume of synovial culture media (or its equivalent) added to the bone culture medium.

TABLE II

BRS Activity in Rheumatoid Synovial Culture Media

Synovial culture medium*	Volume	Δ Calcium‡	
		Without extraction	Ether extract
	μl/ml	*mg/dl*	
Control	4	4.1	3.0
	20	3.3	2.9
Control	4	2.2	1.7
	20	3.1	2.7
Indomethacin (5 μg/ml)	20	−0.3	—
Indomethacin (5 μg/ml)	20	0.6	—

* 3-day conditioned medium samples were obtained on day 6 from case R-37. See legend of Fig. 2 for details.

‡ Mean differences between values obtained with four bone cultures incubated with and without added synovial culture medium. Ether extracts of portions of the synovial culture media were reconstituted to the original volume and assayed for activity. Each mean difference had a SE of ±0.50. Values for untreated synovial samples (control) produced highly significant ($P < 0.001$) bone resorption at a dose level of 4 μl/ml. Indomethacin-treated synovial culture media contained no significant BRS activity at 20 μl/ml.

BRS activity was absent in cultures treated with indomethacin.

A similar experiment was performed with another sample of rheumatoid synovial tissue (R-42), except that an additional pair of duplicate synovial cultures was treated with colchicine. We have shown previously that colchicine causes stimulation of prostaglandin accumulation in rheumatoid synovial culture medium (8, 12); therefore, we examined the effect of colchicine on the accumulation of BRS activity. The results in Table III show that the BRS activity present in the control synovial medium was eliminated by treatment with indomethacin. In contrast, medium from colchicine-treated cultures had slightly more BRS activity on day 3, but by day 8 there was clearly more activity in the colchicine-treated cultures. Colchicine alone, at the dose levels used, had no effect on the bone culture assay system.

The results presented thus far reveal that treatment of the synovial cultures with compounds known to increase and decrease their PGE_2 production also increases and decreases the BRS activity, respectively. If indeed a prostaglandin accounts for the BRS activity, it should be possible to show that the prostaglandin concentrations measured in the synovial culture media agree with the bioassay data obtained in the bone culture assay system. We have, therefore, calculated the concentrations of PGE_2 required to account for the BRS activity present in synovial culture media by comparing the biological effects of unknown samples with the PGE_2 standard curve shown in Fig. 1.

The differences between the mean calcium concentrations of control bone culture and bones treated with conditioned media from synovial cultures (Δ Ca concentrations) were used to quantitate the BRS activity. The Δ calcium concentrations observed were added to the base-line value of 7.0 mg/dl in the standard curve for PGE_2 in Fig. 1 to calculate the PGE_2 concentrations required to give the observed BRS activity from the PGE_2 standard curve. Only the linear portion of the PGE_2 standard curve between 2 and 15 ng/ml was used for unqualified estimates. Values falling outside this range were considered only as upper or lower limits. The estimates of PGE_2 concentrations obtained from bone culture experiments are given in Table IV, and each value is based on the results of at least two separate bone culture assays usually performed at more than one concentration of each unknown medium. Most of the values designated as upper or lower limits were derived from experiments in which the results of one of the duplicate assays fell outside the linear portion of the PGE_2 standard curve. The concentrations of PGE_2 in the same samples of medium were also determined by radioimmunoassay.

We interpret the results obtained by radioimmunoassay to be in reasonable agreement with the concentrations of PGE_2 estimated from the BRS activities of the same samples (Table IV). It is noteworthy that PGE_2 concentrations and BRS activity both varied in parallel

TABLE III

Effects of Indomethacin (5 μg/ml) and Colchicine (0.1 μg/ml) on BRS Activity in Rheumatoid Synovial Culture Medium (Tissue R-42)

Synovial culture medium	Day 3		Day 8	
	Volume	Δ calcium	Volume	Δ calcium
	μl/ml	*mg/dl*	*μl/ml*	*mg/dl*
Control	4	1.0±0.70	10	0.0
			20	1.4±0.63*
	20	3.8±0.64	50	2.9±0.39§
Indomethacin	—	—	50	0.0
Colchicine	4	2.2±0.70‡	4	2.7±0.64§
			10	2.8±0.37§
	20	4.0±0.68§	20	3.8±0.64§

Mean differences ±SE between groups of four bones incubated without and with control, indomethacin-treated, and colchicine-treated synovial culture medium. All synovial cultures were the same except for the addition of indomethacin or colchicine.

* $P < 0.05$ compared to controls.

‡ $P < 0.01$ compared to controls.

§ $P < 0.001$ compared to controls.

over a 20-fold range in these experiments. Although the agreement between the two independent assay methods shown in Table IV is not exact, it is well within the limits of the experimental errors for both methods.

To provide additional support for the validity of the bioassay approach used, additional measurements were performed with several culture media. In these experiments, the unknown samples and the PGE_2 standard were each tested at two or more concentrations in the same assay. The data were analyzed according to standard statistical procedures for parallel line assays (13), and the results are given in Table V. Again the bioassay and radioimmunoassay data are in reasonable agreement. In addition, this experiment confirms the good recovery of BRS activity in ether extracts of synovial culture media. Furthermore, the bone resorption stimulated by standard PGE_2 and by synovial culture media gave similar slopes based on testing at three different dose levels (Fig. 3), providing additional support for the conclusion that the BRS activity in rheumatoid synovial organ culture media is quantitatively similar to or the same as PGE_2.

TABLE IV

Comparison of PGE_2 Concentrations in Synovial Culture Media Estimated by BRS Activity and by Radioimmunoassay

Synovial culture medium	Day	PGE_2 concentration: Bioassay*	PGE_2 concentration: Radioimmunoassay‡
		μg/ml	*μg/ml*
R-33-1	14	≥0.27	0.26
2	14	0.14	0.22
R-36-2	3	1.3	0.91
R-37-1	3	≥2.8	2.4
2	3	≥2.8	2.2
R-37-1	6	≥2.2	1.1
2	6	1.1	1.2
R-42-1	3	0.55	0.49
2	3	0.75	0.42
5§	3	1.0	—
6§	3	≥1.7	1.4
R-42-1	8	≤0.14	0.08
2	8	≥0.25	0.10
5§	8	1.1	0.69
6§	8	2.1	1.4

* Calculated from the differences between mean calcium concentrations of control bone cultures and bones incubated with medium from synovial cultures. The PGE_2 concentrations are calculated from the standard curve in Fig. 1 by adding Δ[Ca] values to the unstimulated value of 7.0 mg/dl in the standard curve. Each value represents the average of two determinations, usually assayed at different dosage levels. Values expressed as limits (≤ or ≥) usually had one of the two determinations falling outside the linear portion of the PGE_2 standard curve, between 2 and 15 ng/ml.

‡ Mean values of duplicate determinations.

§ Media from cultures incubated with colchicine, 0.1 μg/ml.

TABLE V

Bioassay of Rheumatoid Synovial Culture Media for BRS Activity and Comparison with Radioimmunoassay

Sample	Volume	Medium calcium*	PGE_2 concentration: Bioassay‡	PGE_2 concentration: Radioimmunoassay
	μl/ml	*mg/dl*	*μg/ml*	*μg/ml*
PGE_2, 4 ng/ml	—	8.1		
PGE_2, 20 ng/ml	—	10.5		
R-37-1, day 3				
Medium	0.4	7.3	2.2	2.4
	2.0	8.4		
Ether extract§	0.4	7.1	2.6	—
	2.0	8.9		
R-37-2, Day 3				
Medium	0.4	6.8	1.6	2.2
	2.0	8.3		
Ether extract§	0.4	7.2	2.0	—
	2.0	8.4		

* The standard errors for all values ranged from ±0.29 to ±0.33 mg/dl, with groups of three or four bones for each determination.

‡ Concentrations of PGE_2 in the unknown samples were calculated by standard statistical procedures for parallel line biological assays (13). The slopes of plots of medium calcium concentrations as a function of log dose were shown to be not nonparallel for standard and unknown samples. A combined slope of 2.20 was utilized for the calculations. The standard errors of the potency estimates range from 1.45 to 1.49 for all four values listed.

§ Reconstituted ether extract of a portion of culture medium.

We have begun to investigate the differences between prostaglandin concentrations and BRS activity in rheumatoid and nonrheumatoid synovial cultures. Two normal synovial specimens were obtained from patients undergoing meniscectomy, and cultures were carried out as described in the Methods. The results of these experiments are shown in Table VI. Significant BRS activity is shown in these media and both the biological activity and PGE_2 concentrations were reduced to undetectable levels by indomethacin.

We have measured PGE_2 concentrations in synovial culture media from other rheumatoids and from patients without evidence of rheumatoid arthritis or other inflammatory disease, and histologically with normal synovia, by the same techniques described above. The concentrations of PGE_2 were determined by radioimmunoassay after the initial 72 h of each culture (on day 3). The mean value for rheumatoid synovia was 2.7 μg PGE_2/ml ($n = 14$, range 0.45–7.0), compared to the mean value of culture media from normal synovia of 0.23 μg PGE_2/ml ($n = 5$, range 0.064–0.41). Therefore rheumatoid synovial culture media contain approximately 10-fold higher concentrations of PGE_2 than media from normal synovial cultures. The limited

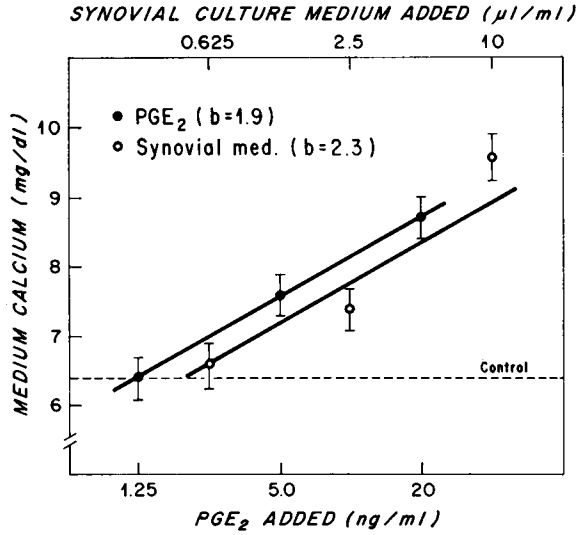

FIGURE 3 BRS activity of PGE_2 and rheumatoid synovial culture media. Each point gives the mean value ±SE of the calcium concentration of four bone cultures. The dashed horizontal line gives the calcium concentration control bone culture medium. The slopes (b) of the two lines were found to be not nonparallel by the method of Finney (13).

data available from normal synovia (Table VI) indicate that the BRS activity, although less than in rheumatoid cultures, can largely be accounted for by the concentrations of PGE_2 present in the media.

DISCUSSION

The experiments described in this report show that rheumatoid synovia in organ culture secrete into their media BRS activity. Three lines of evidence indicate that nearly all of this BRS activity can be accounted for by PGE_2: (*a*) Incubation of the synovial cultures in the presence of indomethacin reduces prostaglandin concentration to less than 1% of control cultures and also eliminates over 90% of the BRS activity. (*b*) The BRS activity is quantitatively extracted into ether. (*c*) The BRS activity is accounted for by the measured concentrations of PGE_2 in synovial culture media.

Several factors other than prostaglandins are known to possess BRS activity in vitro, either in the mouse calvaria system used by us (11) or in similar systems (14). The data presented here provide evidence against an important contribution of certain factors other than PGE_2 in the BRS activity in culture media from rheumatoid synovia. Recently, Horton and coworkers have described a protein factor that they have named osteoclast activating factor (OAF), which has BRS activity in vitro, and is released from cultured normal human peripheral blood leukocytes that have been stimulated by either antigens or mitogens (14). The inflammatory infiltrate in rheumatoid synovial membranes consists predominantly of mononuclear cells, including plasma cells, lymphocytes, macrophages, and lymphoid blast cells (15). These observations have been recently interpreted as indicating blast transformation of B and T lymphocytes in synovial tissue as a result of antigen stimulation (15). If this hypothesis is correct, it would be reasonable to expect that OAF might be produced by rheumatoid synovia. However, the finding that BRS activity is extracted quantitatively into ether is strong evidence that OAF does not make a significant contribution to the BRS activity in rheumatoid synovial culture media. Neither of the known protein factors that have potent BRS activity, OAF and parathyroid hormone, are extractable by organic solvents (16).

The vitamin D metabolite 1,25-dihydroxycholecalciferol has potent BRS activity and is soluble in organic solvents (17). However, since the site of synthesis of this compound is thought to be limited to the kidney (18), it seems unlikely that it would be released in significant concentrations from synovial tissues.

The other prostaglandin with BRS activity comparable to PGE_2 is PGE_1. Although our analyses of media from one synovial tissue (R-42) revealed only PGE_2, low concentrations of PGE_1 could not be excluded entirely. Of the other prostaglandins tested, only PGA_1 and PGA_2 have detectable BRS activity, but their potency is approximately 1,000-fold less than the E prostaglandins (2). Our analyses have shown that little if any

TABLE VI
Concentrations of PGE_2 in Culture Media from Nonrheumatoid Synovia

Synovial culture medium*	PGE_2 concentration‡	
	Bioassay	Radioimmunoassay
	µg/ml	
R-39§-1 Control	0.17	0.061
2 Control	0.12	0.065
3 Indomethacin‖	<0.027	<0.004
4 Indomethacin‖	<0.027	<0.004
R-41§-1 Control	0.32	0.23
2 Control	0.22	0.35
3 Indomethacin‖	<0.050	<0.005

* All media were removed from tissues on day 3 of incubation.
‡ See footnotes to Table IV.
§ The synovium was from a patient with a torn meniscus of the knee. It was histologically normal.
‖ Indomethacin concentration was 5 µg/ml. No BRS activity was detectable.

PGA was present in synovial culture media, excluding a significant contribution from either form of PGA in these experiments.

Finally, it has been reported previously by Krane that rheumatoid synovial cultures produce a factor that promotes calcium release from embryonic rat long bones in culture (19). Since incubation of synovia with indomethacin did not regularly inhibit the bone calcium release in their experiments, it was concluded that prostaglandins did not account for the observed effects (19). We also observed small amounts of BRS activity in one synovial culture experiment that could be due to a nonprostaglandin factor. High concentration of medium (100 μl/ml) from indomethacin-treated cultures of tissue R-37 contained significant BRS activity not explainable by the measured prostaglandin concentration in the media. This concentration of medium from indomethacin-treated cultures was 25 times greater than the dose of medium from untreated cultures that caused maximum stimulation of bone resorption. Therefore, detectable BRS activity in this culture media was present and apparently due to a factor other than PGE_2. Quantitatively, the nonprostaglandin activity was small, accounting for less than 4% of the total BRS activity in control culture media.

The pathogenesis of rheumatoid arthritis is not well understood but it is widely accepted that degradative enzymes, released from cells participating in the inflammatory reaction, lead to destruction of articular cartilage and other connective tissue structures (20). Previously it has been pointed out that the destruction of juxta-articular bone cannot be accounted for by known enzymes (19). Collagenase released from rheumatoid synovia is capable of degrading bone collagen only after removal of the mineral phase (19). The results reported here indicate that PGE_2 produced by rheumatoid synovia may promote bone resorption in the absence of other major products of the rheumatoid tissue, since the activity in culture medium could be accounted for quantitatively by its PGE_2 content. It is not known which cells in rheumatoid synovial tissue are responsible for PGE_2 synthesis. It is of interest in this connection that treatment of human leukocytes by either mitogens or specific antigen has been shown to stimulate the secretion of PGE_2 into culture media (21). These observations suggest that the blastlike lymphoid cells in rheumatoid synovia described by Ziff (15) may contribute to the production of PGE_2 by this tissue.

Our preliminary results comparing prostaglandin production by rheumatoid and nonrheumatoid synovial cultures indicate that rheumatoid synovia produce approximately 10 times more PGE_2 than normal synovia. Bioassay of two normal synovial cultures for BRS activity revealed a low level of BRS activity that also can be accounted for by the PGE_2 concentrations present. This apparently increased rate of synthesis of PGE_2 by rheumatoid synovia, in addition to the marked hypertrophy of synovial tissue in rheumatoid arthritis, could result in excessive concentrations of PGE_2 around articular structures in rheumatoid arthritis.

From on our experiments, we suggest that prostaglandins (PGE_2) produced by rheumatoid synovia may contribute to the destruction of juxta-articular bone in rheumatoid arthritis. It is of interest that the pharmacologic effects of nonsteroid anti-inflammatory drugs may be at least in part related to their inhibition of prostaglandin biosynthesis (22). If our hypothesis is correct, these drugs may act to prevent or retard bone destruction in rheumatoid arthritis.

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