PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 173, 181–189 (1983)

Phagocytosis of Hydroxyapatite or Calcium Pyrophosphate Dihydrate Crystals by Rabbit Articular Chondrocytes Stimulates Release of Collagenase, Neutral Protease, and Prostaglandins E_2 and $F_{2\alpha}$ (41628)

HERMAN S. CHEUNG, PAUL B. HALVERSON, AND DANIEL J. McCARTY

Rheumatology Section, Department of Medicine, The Medical College of Wisconsin, 8700 W. Wisconsin Avenue, Milwaukee, Wisconsin 53226

Abstract. Synthetic hydroxyapatite (HA) and calcium pyrophosphate dihydrate (CPPD) microcrystals are phagocytosed by rabbit articular-cartilage chondrocytes in primary culture. The ingestion of crystals greatly stimulated the release of collagenase, neutral protease, and prostaglandins E_2 and $F_{2\alpha}$ into the ambient medium. Lactate dehydrogenase was not released by either crystal despite electron microscopic evidence of cell damage by HA crystals (partial loss of phagolysosomal membrane and increased myelin figures). HA, but not CPPD crystals, stimulated release of β-glucuronidase. HA crystal concentrations from 50 to 200 μg ml^{-1} induced a dose-dependent release of collagenase and of extracellular protein. Both phagocytosis and collagenase release were greatly attenuated when HA crystals were added to the chondrocyte monolayers in the absence of serum. As HA and CPPD crystals have been identified in human articular cartilage in association with degenerative changes, it is possible that the cell–crystal interaction described here may be pathogenetically important.

Acute attacks of arthritis associated with monosodium urate (MSU) or calcium pyrophosphate dihydrate (CPPD) crystals are associated with phagocytosis by polymorphonuclear leukocytes (1). But perhaps the more significant clinical association of CPPD crystal deposition disease is with a destructive arthropathy rather than with the more dramatic acute arthritis (2). Intrasynovial hydroxyapatite (HA) and CPPD crystals have been correlated with radiologic evidence of joint destruction (3). These observations have focused our interest on the role of synovial cells in the pathogenesis of the devolutionary joint changes induced by crystals. Recently, we reported that phagocytosis of hydroxyapatite (HA) or calcium pyrophosphate dihydrate (CPPD) crystals stimulated the release of collagenase and neutral protease, and, in the case of HA crystals, prostaglandins (PG) E_2 and $F_{2\alpha}$ from human rheumatoid synovial cells in monolayer culture (4). An augmentation of release of collagenase, neutral protease, and PGE_2 after phagocytosis of MSU or latex beads by cultured synovial fibroblasts has also been described by a number of investigators (5–7).

However, there is increasing evidence to suggest that articular chondrocytes may be active participants in joint destruction in human osteoarthritis (8, 9). Factors isolated from monocytes (10, 11) and synovium (12) are known to stimulate chondrocytes to secrete proteases capable of matrix macromolecular degradation.

Since HA and CPPD crystals are found in cartilaginous tissue in both chondrocalcinosis (13) and osteoarthritis (14, 15) we have postulated that articular chondrocytes may play an active role in the matrix destruction associated with these crystal deposition diseases. We show here that both HA and CPPD crystals are phagocytized by rabbit articular chondrocytes in primary culture and that this is associated with augmented secretion of collagenase, neutral protease, and PGE_2 and $PGF_{2\alpha}$.

Materials and Methods. All culture medium such as Dulbecco modified Eagle medium (DMEM) with high glucose (4.5 g/liter w/v), fetal calf serum, and penicillin–streptomycin–fungizone mixture were obtained from M.A. Bioproducts (Walkersville, Md.). Trypsin, soybean trypsin inhibitor, lactate dehydrogenase, and β-glucuronidase colorimetric diagnostic kits were purchased from Sigma Company (St. Louis, Mo.). Highly characterized hydroxyapatite (HA) crystals were a gift from Dr. M. D. Francis of the Proctor and

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Gamble Company (Cincinnati, Ohio). [^{3}H]Glycine (20 Ci/mmole) was obtained from Amersham–Searle (Arlington Heights, Ill.).

Tissue culture. Cells were released from articular-cartilage slices taken from the knee and hip joints of immature New Zealand white rabbits by a modification (16) of the method of Green (17). The chondrocytes were plated at a density of approximately 1.5×10^6 cells per 100-mm-diameter Falcon plate in DMEM supplemented with 10% fetal calf serum and a 1% solution of penicillin–streptomycin–fungizone. Only confluent primary cell cultures were used in these experiments. The number of cells per confluent plate varied from 5.0 to 5.5×10^6 cells.

Cells were harvested at the end of each experiment using 0.25% trypsin and counted by hemocytometer.

Crystal preparation and characterization. Clumps of hydroxyapatite (HA) crystals were sieved to less than 20 μm in greatest diameter (Endicott test sieves, London, G.B.). The HA crystals showed a remarkable tendency to clump—the individual crystals were less than 1 μm in length by transmission electron microscopy. These crystals showed only the interplanar spacings of hydroxyapatite by X-ray diffraction. Their molar Ca/P was 1.66 by X-ray energy-dispersive analysis and by direct chemical analysis for calcium and phosphorus. Triclinic CPPD crystals were synthesized as recorded earlier (18). These crystals showed only the interplanar spacings of triclinic CPPD by X-ray diffraction and a molar Ca/P of 1.0 by both direct chemical analysis and by X-ray energy-dispersive analysis. They were crushed and sieved to between 20 and 50 μm in greatest diameter before use.

Enzyme analyses. Collagenase activity was determined by measuring the release of soluble radioactive product from ^{14}C-labeled reconstituted fibrils as described by Evanson *et al.* (19), except that the radioactive neutral-salt-soluble rabbit-skin collagen substrate was prepared according to the method of Johnson-Wint (20). One unit of collagenase activity = solubilization of 1 μg of reconstituted collagen fibrils/min at 37°C. Incubation of 100 μl of tissue culture fluid with 100 μg collagen gels was carried out for 12 to 24 hr. All assays were performed in duplicate and included a control gel incubated with trypsin 20 μg/ml (w/v). Results were discarded if release of radioactivity by trypsin was greater than 15% above control gels incubated with buffer alone. A trypsin activation curve was determined on conditioned medium obtained on Days 2 and 7 after addition of HA crystals using 10, 20, 50, and 200 μg trypsin/ml. These samples were incubated at room temperature for 30 min and the trypsin was then inactivated by addition of a fourfold excess of soybean trypsin inhibitor. Trypsin activation of collagenase was performed as outlined by Dayer *et al.* (21).

We determined the rabbit articular-cartilage collagen degradation products using a modified method of Malemud *et al.* (22). Conditioned media from chondrocytes was pooled for 1 week after HA crystals had been added as described below; 0.5 ml of medium was incubated with 0.5 ml of pepsin soluble type II rabbit cartilage collagen in 50 m*M* Tris–Cl, pH 7.5, 3 m*M* $CaCl_2$, 200 m*M* NaCl. The reaction was carried out at 27°C for 48 hr. Collagenase digestion products were examined by $NaDodSO_4$–polyacrylamide gel electrophoresis (23).

Neutral protease activity was estimated in duplicate in each conditioned medium as outlined previously (24), except that ^{125}I-human gamma globulin (Cohn fraction II) (specific activity = 1.5×10^5 cpm/mg) was substituted for ^{125}I-bovine serum albumin. One unit of activity = 1 μg of protein digested/min at 37°C.

Assay for lactate dehydrogenase (LDH) was performed daily on each conditioned medium as described elsewhere (25). The LDH contents of cells was determined at the end of each experiment by addition of Triton X-100, 0.2% (v/v) final concentration, and sonication. Results were expressed as the mean ± SEM percentage release of total enzyme m/(cellular LDH + m) × 100; m = conditioned medium Days 1–7. β-Glucuronidase levels were determined daily in each conditioned medium using a colorimetric assay (Sigma kit).

Prostaglandins (PG) E_2 and $F_{2\alpha}$ were measured by radioimmunoassay as outlined by Dunn *et al.* (26). From 0.3 to 3 ml of conditioned medium were extracted for each determination depending on the PG concentration. Levels of both PGE_2 and $PGF_{2\alpha}$ were

determined on the conditioned medium from the first 5 days of the experiment. Day-one medium contained 10% fetal calf serum and the subsequent medium contained 0.2% LH.

Experiments. Crystals were sterilized and rendered pyrogen-free by heating (180°C × 2 hr) and suspended in DMEM containing 10% fetal calf serum and 1% penicillin–streptomycin–fungizone mixture. HA or CPPD crystals were added to chondrocyte monolayers in final concentration of 1 mg/10 ml of culture medium. After 24 hr, the medium was replaced by DMEM containing 0.2% lactoalbumin hydrolysate (LH), then replaced daily with the same for 7 days. Collagenase activity was determined in each conditioned medium after dialysis against 50 m*M* Tris–HCl buffer, pH 7.5, containing 200 m*M* NaCl and 3 m*M* calcium chloride. β-Glucuronidase and LDH levels were estimated daily in each conditioned medium. Prostaglandin levels were determined on the conditioned medium for the first 5 days only.

At the end of the initial 24-h incubation with crystals, all cell cultures were examined by phase-contrast microscopy. Scanning and transmission electron microscopy (EM) were performed on one plate at this time and again at the end of the experiment, i.e., 7 days after the crystals were added. The cultures were fixed *in situ* in Karnovsky's solution. The bottom of the plastic culture dish was scored and broken. Scanning EM was performed as outlined before (4). The tissues were postfixed in 1% osmium tetroxide and embedded in Spurr's low-viscosity epoxy resin for transmission EM.

To determine the effect of crystal dose, 0.5, 1.0, and 2.0 mg HA crystals per 10 ml DMEM–10% FCS were added to four plates each. In addition to the daily measurements of collagenase and LDH, the incorporation of [^{3}H]glycine into extracellular protein was determined. Fifty microcuries of [^{3}H]glycine was added to the original crystal suspension and to the fresh media added daily. Four 1-ml aliquots were removed from the conditioned medium obtained daily and precipitated by addition of an equal volume of 10% trichloroacetic acid. Each precipitate was redissolved in 2 ml of 1% NaCl and 50 m*M* Tris–HCl, pH 7.5, and dialyzed extensively against the same buffer at 4°C. The nondialyzable ^{3}H was measured in a Tricarb liquid scintillation counter and regarded as representing extracellular proteins synthesized by the cells.

To determine whether serum was required for crystal phagocytosis, four plates of confluent chondrocytes were fed 1 mg HA in 10 ml DMEM containing 10% FCS; four more were washed once with DMEM–0.2% LH and were then fed a similar dose of crystals. These were incubated thereafter in LH. A third set were incubated in DMEM 0.2% LH without crystals. After 24 hr, one plate from each set was removed for transmission electron microscopy. All media was replaced with DMEM with 0.2% LH and the media changed daily for 6 days. Collagenase activity was measured on each conditioned medium.

Results. Both HA and CPPD crystals were phagocytosed by chondrocytes as determined by phase-contrast microscopy, scanning EM, and transmission EM (Fig. 1a, b).

Figure 2 (A–D) summarizes the effect of phagocytosis of HA and CPPD crystals on chondrocytes compared to control cells incubated without crystals. Compared to control, HA crystals induced a 15-fold increase in collagenase, a 3-fold increase in neutral protease, and a 2.5-fold increase in β-glucuronidase released by chondrocytes. An equal mass of CPPD crystals induced an increase in collagenase secretion by 5-fold, a significant increase in neutral protease release ($P = 0.05$). Cell lysis could not account for enzyme release as LDH levels in the conditioned medium from cells incubated with crystals were nearly identical to control values. Less than 5% of total cellular LDH has been released into the medium even by the seventh day.

The cumulative release of PGE_2 and $PGF_{2\alpha}$ into the conditioned medium after feeding HA or CPPD crystals is shown in Fig. 3. Nearly all PG release occurred in the first 24 hr. CPPD was less effective per milligram. $PGF_{2\alpha}$ paralleled PGE_2 but at a lower level.

The induction of increased collagenase secretion was a function of crystal concentration (Fig. 3A). A dose-dependent relationship was also evident in the incorporation of [^{3}H]glycine into extracellular protein (Fig. 4B).

It appears that serum is necessary for the full stimulatory effect of crystals on collagenase secretion (Fig. 5). The cumulative release of collagenase by cells exposed to HA crystals

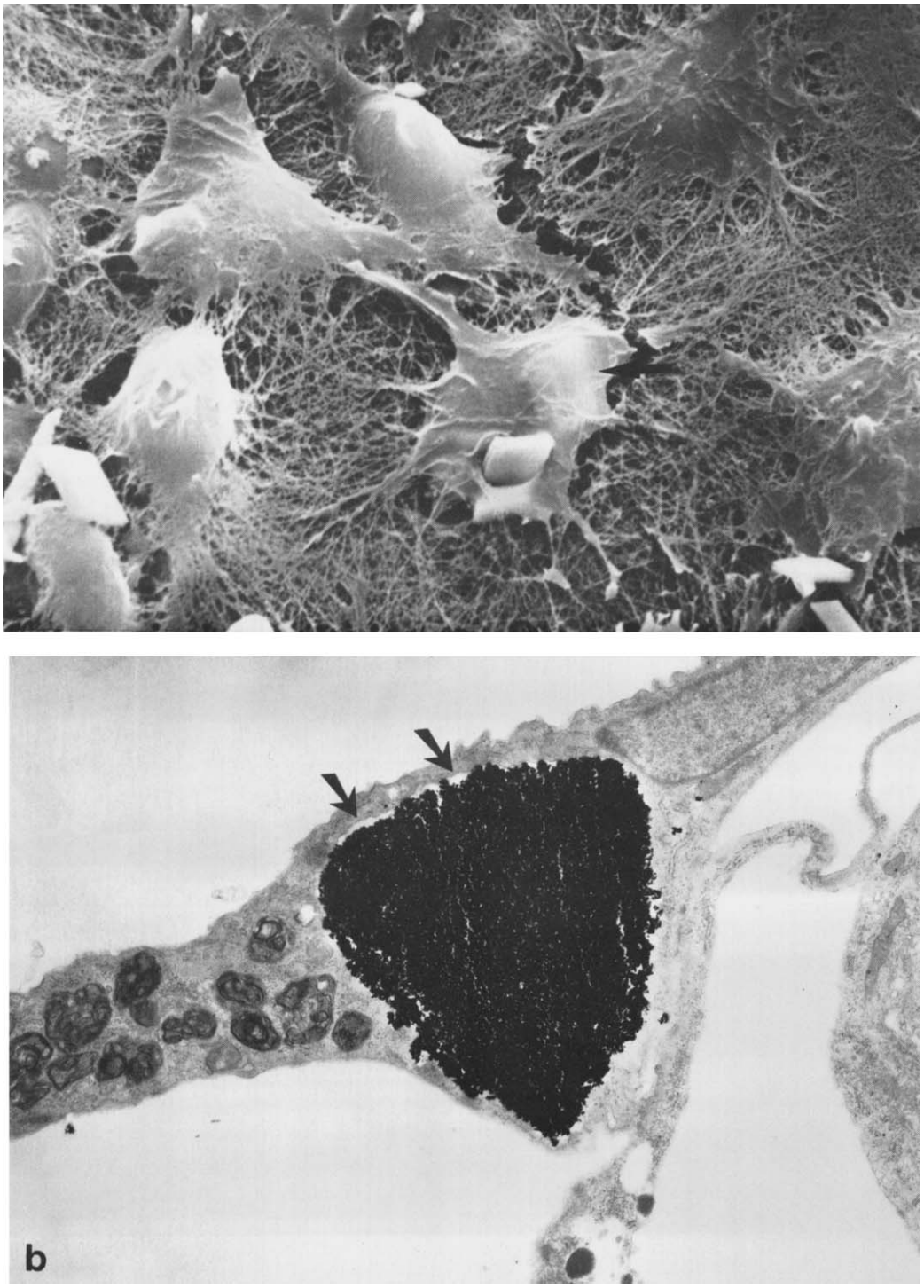

FIG. 1. (a) Scanning electron micrograph of synthetic triclinic CPPD crystals. Arrows indicate intracellular crystals. Other crystals are clearly extracellular. ×1190. (b) Transmission electron micrograph showing a mass of hydroxyapatite crystals within a chondrocyte 1 day after exposure. A limiting membrane can be seen in some (arrows) but not all areas. Extensive cellular damage has occurred as manifested by the myelin figures. Such figures were unusual in control cultures. ×10,740.

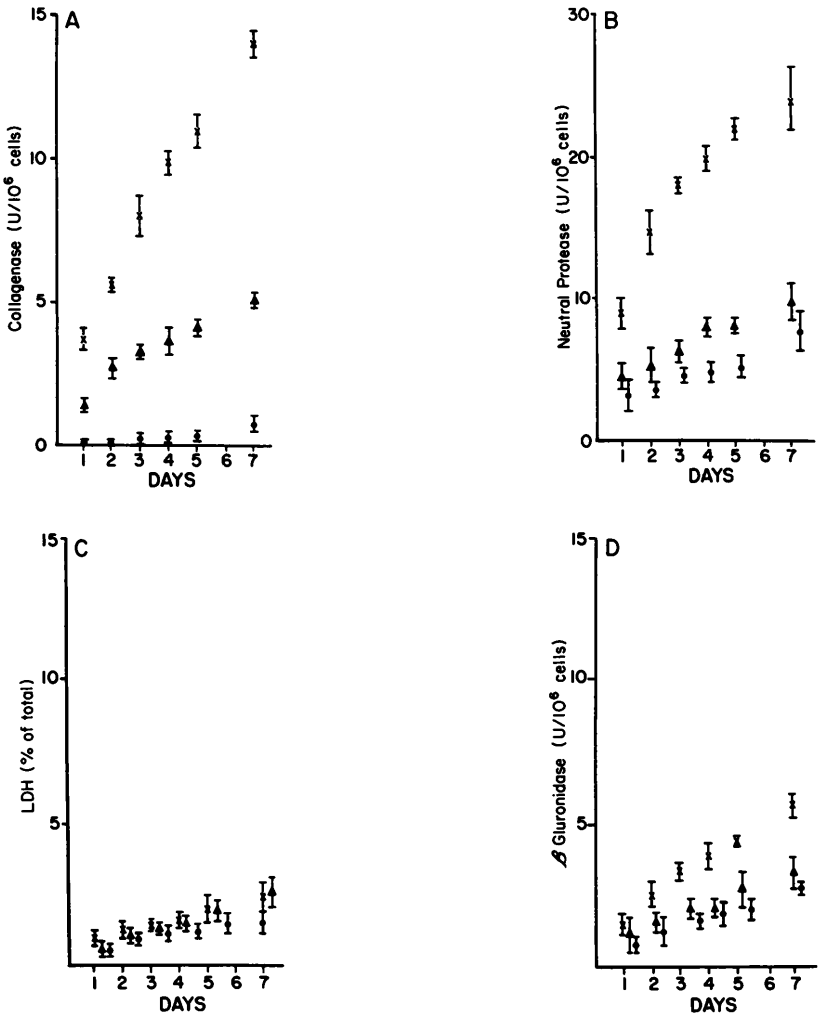

FIG. 2. (A–D) Cumulative enzyme changes induced during the week after the addition of crystals to chondrocytes in primary culture ($x \pm$ SD, $N = 4$). ×, hydroxyapatite; ▲, CPPD; ●, no crystals. See text for details.

suspended in LH was about 2.5 times that of control cells maintained in LH alone, but 5-fold less than that which followed the addition of crystals in fetal calf serum. Very few crystals were intracellular by phase-contrast, scanning, or transmission EM (data not shown).

Rabbit chondrocyte collagenase was active against both type I and type II collagens, splitting the molecules into the classical fragments as shown in the $NaDodSO_4$–gel electrophoretigrams shown in Fig. 6.

Discussion. Hydroxyapatite (HA) and triclinic calcium pyrophosphate dihydrate (CPPD), two microcrystalline species sometimes found in human cartilage (14, 15), are phagocytosed by rabbit chondrocytes in primary culture (Fig. 1). As shown previously using mammalian synovial cells (4), the ingestion of crystals induces increased release of collagenase, neutral protease, and prostaglandins E_2 åd $F_{2\alpha}$ into the ambient medium (Figs. 2A,B, 3). As lactate dehydrogenase was not released from the cells (Fig. 2C), these phenomena cannot be attributed to cell lysis or cell death. HA crystals induced a more marked release of all three substances compared to an equal mass of CPPD (Figs. 2A,B, 3). HA crystals stimulated the release of β-glucuronidase,

a lysosomal enzyme, whereas CPPD crystals did not (Fig. 2D). HA concentrations from 50 to 200 $\mu g\ ml^{-1}$ induced a dose-dependent release of collagenase (Fig. 4A), and of extracellular protein (Fig. 4B). Both phagocytosis and collagenase release were greatly attenuated when HA crystals were added to chondrocyte monolayers in the absence of serum.

Acute inflammation induced by microcrystalline substances in joints is dependent on phagocytosis by polymorphonuclear leukocytes [reviewed in Ref. (1)]. Endocytosis of crystals by synovial cells has been linked to the pathogenesis of chronic destructive arthropathies such as tophaceous gout [monosodium urate monohydrate (5, 6)], the "Milwaukee Shoulder Syndrome" [hydroxyapatite (24, 27)], and the severe joint degeneration associated with CPPD crystals (2). All three of these microcrystalline species occur in articular cartilage, raising the possibility of their interaction with local chondrocytes. The data presented here show that articular chondrocytes can phagocytose crystals, release proteases capable of degrading cartilage collagen (Fig. 6), and prostaglandins which have been shown to decrease macromolecular synthesis (28). We can find no published reports iden-

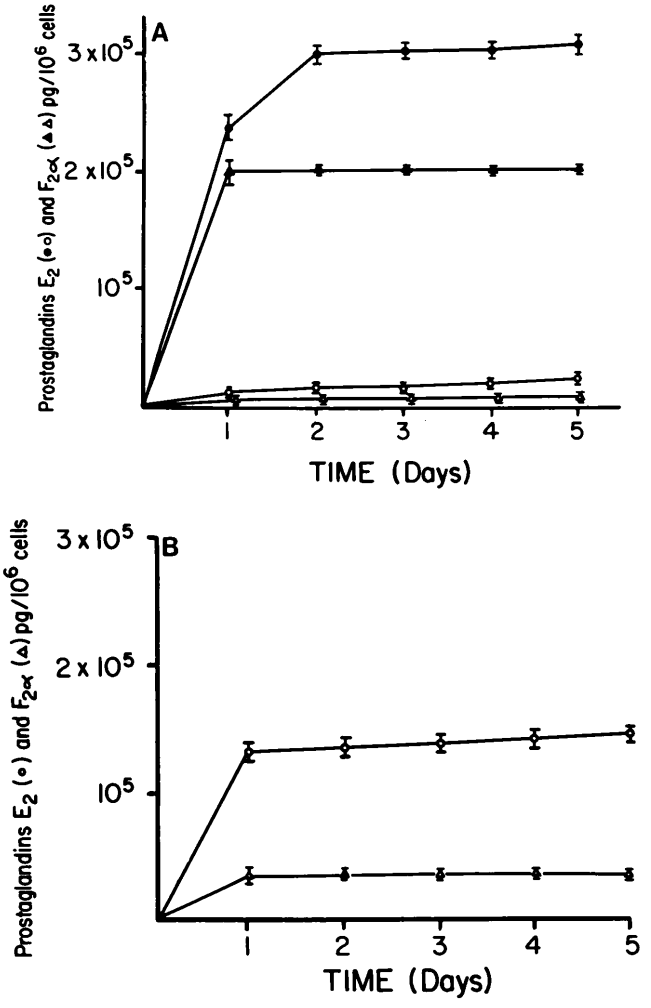

FIG. 3. (A) The cumulative release of prostaglandin PGE_2 and $PGF_{2\alpha}$ for 5 days after exposure of chondrocytes to hydroxyapatite (closed symbols) and control (open symbols). $N = 4$, $X \pm$ SD. (B) The cumulative release of prostaglandin PGE_2 and $PGF_{2\alpha}$ for 5 days after exposure of chondrocytes to calcium pyrophosphate dihydrate crystals. Control values for PGE_2 and $PGF_{2\alpha}$ are shown in (A). $N = 4$, $X \pm$ SD.

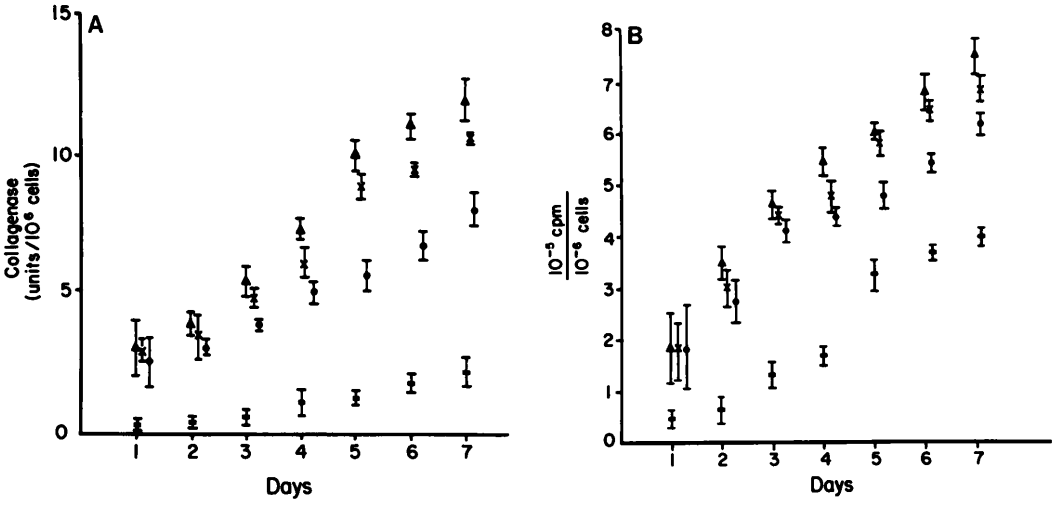

FIG. 4. (A) Cumulative collagenase secretion during the week after the addition of varying amounts of hydroxyapatite crystals to chondrocytes in primary culture ($X \pm SD$; $N = 4$). ▲, 200 μg ml^{-1}; ×, 100 μg ml^{-1}; ●, 50 μg ml^{-1}; ■, no crystals. (B) Cumulative nondialyzable ^3H in conditioned media obtained daily after the addition of varying amounts of hydroxyapatite crystals to chondrocytes in primary culture ($X \pm SD$; $N = 4$). Symbols identical to Fig. 3A; See text for details.

tifying any intracellular microcrystals in human cartilage. However, few electron microscopic studies of such material have been done.

Mammalian chondrocytes in primary culture do not behave phenotypically identical to those *in vivo* (23). Parallel experiments using articular cartilage slices in organ culture and a model of cartilage calcification by hy-

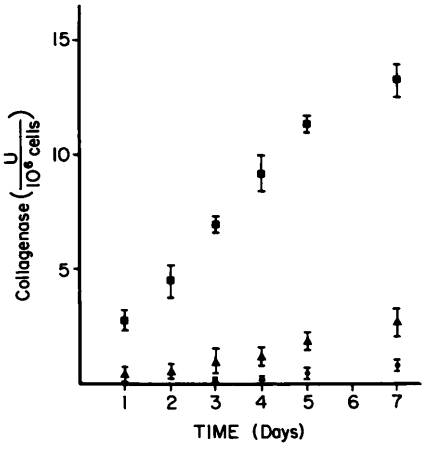

FIG. 5. Cumulative collagenase secretion by chondrocytes during the week after addition of hydroxyapatite crystals in serum (■), lactoalbumin (▲), and control (●). See text for details.

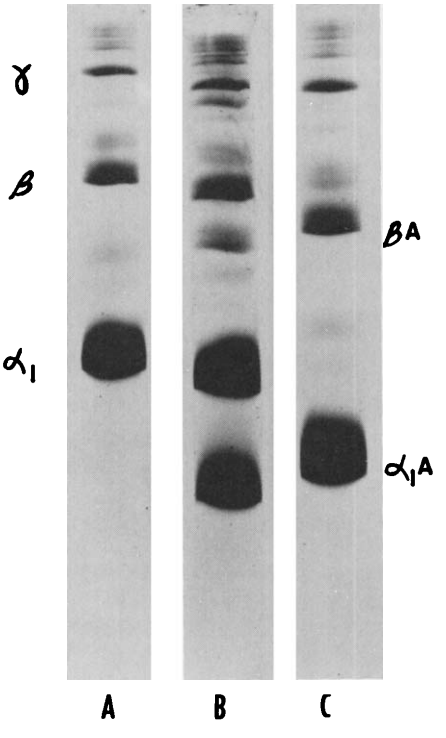

FIG. 6. NaDodSO$_4$–gel electrophoretigrams. (A) Control type II collagen; (B) partial digestion products of type II collagen after 24 hr incubation with the same pooled conditioned media; (C) complete digestion of type II collagen after 48 hr incubation.

pervitaminosis D are underway in our laboratory.

Our data showing that articular chondrocytes in monolayer culture secrete small but measurable amounts of collagenase are in agreement with those of Malemud *et al.* (22) and Deshmukh-Phadke *et al.* (10). Augmentation of this basal level of enzyme secretion may occur by mechanisms other than crystal phagocytosis. "Catabolin," a substance released from synovium (12), and factors from mononuclear cells (11) have been shown to stimulate chondrocytes causing cartilage matrix degradation. Increased synthesis of both collagenase and neutral protease by chondrocytes exposed to a factor from macrophages has also been demonstrated (10). Both CPPD and HA are phagocytosed by macrophage-like synovial cells in tissue culture (4) and *in vivo* (29, 30). These cells may release factors into the synovial fluid that stimulate chondrocyte-mediated matrix degradation. Synovial cell PG release (4–6) may also contribute to cartilage damage by inhibiting macromolecular synthesis by chondrocytes (28).

Collagenase release in response to increasing doses of crystals (Fig. 4A) was stimulated out of proportion to the increase in synthesis of extracellular protein (Fig. 4B). This suggests some degree of specificity in the response to crystal phagocytosis. The recent demonstration of messenger RNA translating for rabbit synovial collagenase in a cell-free mRNA-dependent system after stimulation of rabbit synovial fibroblasts with phorbolesters or MSU crystals (31) also suggests that such stimulated cells synthesize collagenase out of proportion to other macromolecular species. Chondrocyte collagenase can degrade type II collagen readily (Fig. 6), although its kinetic properties against the various collagens have not yet been determined.

The role of chondrocytes in crystal deposition diseases is still theoretical. As hydroxyapatite, calcium pyrophosphate dihydrate, and monosodium urate crystals often occur in cartilage, and as destructive arthropathy involving articular cartilage occurs in association with all three crystals, such an interaction is anticipated and potentially very important.

We would like to thank Mrs. J. Struve, Mrs. N. White, and Mr. T. Nichols for their excellent technical assistance; and Dr. J. Garancis for electron microscopies. This work is supported by National Institutes of Health Grants AM26062, AM05621, AM18074, and AM20647; and by grants from the Wisconsin Chapter of the Arthritis Foundation and the Kroc Foundation.

1. McCarty DJ. Calcium pyrophosphate dihydrate crystal deposition disease. In: McCarty, DJ, ed. Proceedings of the Conference on Pseudogout and Pyrophosphate Metabolism. Arthritis Rheum (Suppl) **19**:275–285, 1976.
2. Menkes CJ, Simon F, Delrieo F, Forest M, Delbarre F. Destructive arthropathy in chondroicalcinosis articulosis. Arthritis Rheum **19**:329–348, 1976.
3. Halverson PG, McCarty DJ. Identification of hydroxyapatite crystals in synovial fluid. Arthritis Rheum **22**:389–395, 1979.
4. Cheung HS, Halverson PB, McCarty DJ. Release of collagenase, neutral protease and prostaglandins from cultured mammalian synovial cells by hydroxyapatite and calcium pyrophosphate dihydrate crystals. Arthritis Rheum **24**:1338–1344, 1981.
5. McMillan RM, Vater CA, Hasselbacher P, Hahn J, Harris ED. Induction of collagenase and prostaglandin synthesis in synovial fibroblasts treated with monosodium urate crystals. J Pharm Pharmacol **33**:382–383, 1981.
6. Fine I, Sannes P, Narcombe D. Human synovial fibroblasts and murine macrophages: Urate phagocytosis and prostaglandin release. Clin Res **28**:146A, 1980.
7. Werb Z, Reynolds JJ. Stimulation of endocytosis of the secretion of collagenase and neutral protease from rabbit synovial fibroblasts. J Exp Med **140**:1482–1497, 1974.
8. Erhrich MG, Mankin HJ, Jones J, Wright R, Crispen C, Vigliani C. Collagenase and collagenase inhibitors in normal human cartilage. J Clin Invest **59**:226–233, 1977.
9. Pelletier JP, Martel-Pelletier J, Woessner JF, Ghandur-Mnaymneh L, Enis J, Howell DS. Direct measurement of cartilage collagenase activity in human osteoarthritis. Arthritis Rheum **24**:S87, 1981.
10. Deshmukh-Phadke K, Lawrence M, Nanda J. Synthesis of collagenase and neutral protease by articular chondrocytes: Stimulation by a macrophage-derived factor. Biochem Biophys Res Commun **85**:490–496, 1978.
11. Jasin HE, Dingle JT. Human mononuclear cell factors mediate cartilage matrix degradation through chondrocyte activation. J Clin Invest **68**:571–581, 1981.
12. Dingle JT. The role of catabolism in arthritic damage. Sem Arthritis Rheum **11**:83–85, 1981.
13. McCarty DJ, Hogan JM, Gatter GA, Grossman M. Studies on pathological calcification in human cartilage. I. Prevalence and types of crystal deposits in

the menisci of two hundred and fifteen cadavers. J Bone Joint Surg **48**:309–325, 1966.

14. Ali SY, Griffiths S. New types of calcium phosphate crystals in arthritic cartilage. Sem Arthritis Rheum **11**:124–126, 1981.
15. Schumacher HR, Gordon G, Paul H, Reginato A, Villaneuva T, Sherian V, Gibilisco P. Osteoarthritis, crystal deposition and inflammation. Sem Arthritis Rheum **11**:116–118, 1981.
16. Cheung HS, Ryan LM. A method of determining DNA and chondrocyte content of articular cartilage. Anal Biochem **116**:93–97, 1981.
17. Green WT Jr. Behavior of articular chondrocytes in cell culture. Clin Orthop Relat Res **75**:248–260, 1971.
18. McCarty DJ, Palmer DW, James C. Clearance of calcium pyrophosphate dihydrate crystals in vivo. II. Studies using triclinic crystals doubly labelled with ^{45}Ca and ^{85}Sr. Arthritis Rheum **22**:1122–1131, 1979.
19. Evanson M, Jeffrey JJ, Krane SM. Studies on collagenase from rheumatoid synovium in tissue culture. J Clin Invest **47**:2639–2651, 1968.
20. Johnson-Wint B. A quantitative collagen film collagenase assay for large number of samples. Anal Biochem **104**:175–181, 1980.
21. Dayer JM, Krane SM, Russell RGG, Robinson DR. Production of collagenase and prostaglandin by isolated adherent rheumatoid synovial cells. Proc Nat Acad Sci USA **73**:945–949. 1976.
22. Malemud CJ, Norby DP, Sapolsky AI, Matsuta K, Howell DS, Moskowitz RW. Neutral proteinases from articular chondrocytes in culture. Biochim Biophys Acta **657**:517–529, 1981.
23. Cheung HS, Harvey W, Benya PB, Nimni ME. New collagen markers of "derepression" synthesized by rabbit articular cartilage. Biochem Biophys Res Commun **68**:1371–1378, 1976.
24. Halverson PB, Cheung HS, McCarty DJ, Garancis J, Mandel N. "Milwaukee Shoulder": Association of microspheroids containing hydroxyapatite crystals, active collagenase and neutral protease with rotator cuff defects. Arthritis Rheum **24**:474–483, 1981.
25. Skosey JL, Kozin F, Ginsberg MH. Protein adsorption to monosodium urate crystals: Differential response of human peripheral blood neutrophils. J Clin Chem Clin Biochem **14**:303–306, 1976.
26. Dunn MJ, Laird JF, Dray F. Basal and stimulated rates of renal secretion and excretion of prostaglandins F_2, $F_{2\alpha}$ and 13,14-dihydro 15 beta F_2 in the dog. Kidney Int **13**:136–143, 1978.
27. McCarty DJ, Halverson PB, Carrera GF, Brewer BJ, Kozin F. "Milwaukee Shoulder": Association of microspheroids containing hydroxyapatite crystals, active collagenase and neutral protease with rotator cuff defects. I. Clinical Aspects. Arthritis Rheum **24**:464–473, 1981.
28. Lippiello L. Prostaglandins and articular cartilage metabolism: Does prostaglandin perturbation perpetuate cartilage destruction? Sem Arthritis Rheum **11**:87–88, 1981.
29. Garancis JC, Cheung HS, Halverson PB, McCarty DJ. "Milwaukee Shoulder": Association of microspheroids containing hydroxyapatite crystals, active collagenase and neutral protease with rotator cuff defects. III. Morphological and biochemical studies of an excised synovium showing chondromatosis. Arthritis Rheum **24**:484–491, 1981.
30. McCarty DJ, Palmer DW, Halverson PB. Clearance of calcium pyrophosphate dihydrate crystals *in vivo*. I. Studies using ^{169}Yb labeled triclinic crystals. Arthritis Rheum **22**:718–727, 1979.
31. Brinckerhoff CE, Nagase H, Vater CH, Gross RH, Sheldon L, Jackson RC, Harris ED Jr. Synthesis of rabbit synovial fibroblasts collagenase in a cell-free mRNA dependent system. Arthritis Rheum **24**:105(A), 1981.

Received March 9, 1982. P.S.E.B.M. 1983, Vol. 173.