

Synthesis of the active metabolite of vitamin D, 1,25(OH)₂D₃, by synovial fluid macrophages in arthritic diseases

M E HAYES,¹ J DENTON,² A J FREEMONT,² AND E B MAWER¹

From the Departments of ¹Medicine and ²Rheumatology, Manchester University Medical School, Manchester

SUMMARY Synthesis of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) has been shown in cells from knee joint synovial fluid of 20 patients with inflammatory rheumatoid disease, reactive or psoriatic arthritis, or gout, all of which had high synovial fluid cell counts, and by cells from a patient with aseptic necrosis of a femoral condyle after short term (<24 hours) or long term (seven days) primary culture. Cells from 18 patients with inflammatory arthritis, five of which had low synovial fluid cell counts and cells from six patients with osteoarthritis were unable to synthesise this metabolite from 25-hydroxyvitamin D₃ (25(OH)D₃). Macrophages are believed to be the cells responsible for synthesising 1,25(OH)₂D₃ because these were significantly more numerous in samples that formed 1,25(OH)₂D₃; they were also the predominant cell type present in the aseptic necrosis sample and the only cell type present in preparations maintained for one week in monolayer culture.

Vitamin D is metabolised by sequential hydroxylation steps, first to 25-hydroxyvitamin D₃ (25(OH)D₃) in the liver and then to either 24,25-dihydroxyvitamin D₃ (24,25(OH)₂D₃) or the active hormonal form, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), in the kidney. The last metabolite then exerts calcium homeostatic actions in the intestine, bone, and renal tubule after binding to specific intracellular receptors.¹

Peripheral blood lymphocytes and synovial tissue derived fibroblasts from patients with rheumatoid arthritis have also been shown to possess specific receptors for 1,25(OH)₂D₃,^{2,3} but the role of the metabolite in these cells is not known. Furthermore, little is known of the actions of vitamin D and its metabolites in arthritis, though they are clearly involved in normal bone and calcium metabolism, and deficiencies are associated with the development of rickets or osteomalacia.

Fairney *et al* have recently shown that knee joint synovial fluid from patients with arthritic disorders contains 25(OH)D₃, 24,25(OH)₂D₃, and the vitamin D binding protein at about half serum concentra-

tions.⁴ Similarly, 1,25(OH)₂D₃ is also present in synovial fluid at 50–100% serum concentrations (Mawer, unpublished observations). These metabolites and their binding protein are probably present because synovial fluid is formed as a plasma dialysate supplemented with mucins such as hyaluronic acid and lubricating glycoproteins.

Studies of the extrarenal metabolism of 25(OH)D₃ have now shown that 1,25(OH)₂D₃ is synthesised *in vitro* by normal human macrophages activated with interferon gamma⁵ or bacterial lipopolysaccharides⁶; by macrophages activated by peritonitis from the peritoneal cavity of patients with renal failure receiving continuous ambulatory peritoneal dialysis⁷; and by alveolar macrophages from patients with granulomatous conditions such as sarcoidosis.⁸ In sarcoidosis this synthesis appears to depend on the substrate concentration and is not homeostatically controlled, in contrast with the normal well regulated synthesis of 1,25(OH)₂D₃ in the renal tubule. Possibly, activated macrophages in one or more of the many forms of arthritis can also synthesise 1,25(OH)₂D₃ within the affected joints.

This study was undertaken to establish whether cells, including macrophages, present in synovial fluid from patients with various forms of arthritis or gout are able to synthesise 1,25(OH)₂D₃.

Accepted for publication 23 January 1989.
Correspondence to Dr M E Hayes, Department of Medicine, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT.

Patients and methods

Samples of synovial fluid were obtained from 45 patients (details in Table 1) with knee joint effusions attending the rheumatology outpatient clinics of various local hospitals. Patients were assigned to well recognised diagnostic groups on the basis of clinical criteria supplemented by appropriate immunological, radiological, and crystallographic investigations. Six patients with symmetrical polyarthritis or oligoarthritis could not be assigned to a specific diagnostic group and were classified together as inflammatory arthritis not otherwise specified.

Table 1 Details of patients studied

	Number of patients	Comments
Rheumatoid arthritis	14	Three taking drugs*
Reactive arthritis	7	
Psoriatic arthritis	5	Three taking drugs†
Unspecified inflammatory arthritis	6	
Lymphocytic arthritis	1	
Gout	5	
Aseptic necrosis of a femoral condyle	1	
Osteoarthritis	6	

*One each taking gold, penicillamine, and methotrexate.

†Taking methotrexate.

Cells were harvested from the synovial fluid samples by centrifugation at 2000 g for 10 minutes, resuspended in RPMI 1640 medium containing 10% fetal bovine serum, 2 mmol/l glutamine, and 50 µg/ml streptomycin (Flow), and cultured overnight in atmosphere of 95% air/5% CO₂ at 37°C. Cells from nine samples were also maintained in monolayer culture for one week to select for adherent macrophages which were positive for non-specific esterase. The non-adherent lymphocytes, polymorphs, and macrophages were removed by exchanging the culture media 24–48 hours after plating the cells and also when the culture media were replaced with incubation media after seven days.

To assess 1,25(OH)₂D₃ synthesis the cells were incubated with 100 pg of [³H]-25(OH)D₃ (10⁵ dpm/6.5 TBq/mmol, Amersham, UK) for six hours in 1 ml of serum free RPMI 1640 medium supplemented with 1.5 mg/ml of bovine serum albumin (Immuno Diagnostika) to stabilise the vitamin D metabolites in aqueous solution. The incubations were terminated and extracted by addition of 5 ml of chloroform:methanol (1:1). For each sample the chloroform extract was analysed by normal phase high performance liquid chromatography (HPLC) using a Zorbax-Sil column (4.6 mm×25 cm, DuPont) developed with a mobile phase of n-hexane:propan-2-ol:methanol, 110:6:4 by volume at 1 ml/min. For each analysis eluent fractions were collected and the radioactivity associated with the substrate and 1,25(OH)₂D₃ was estimated by

Table 2 Total and differential synovial fluid cell counts and 1,25-hydroxyvitamin D₃ synthesis. Values are given as mean (SEM)

	Number of patients	Cells/l ($\times 10^6$)				1,25(OH) ₂ D ₃ synthesis
		Total	Polymorphs	Lymphocytes	Macrophages	(fg/h/10 ⁶ macrophages)
<i>1,25(OH)₂D₃ formers†</i>						
Rheumatoid arthritis	8	7.87 (2.01)*	5.24 (2.01)*	1.68 (0.33)	0.94 (0.14)***	394 (84)
Reactive arthritis	7	12.03 (2.40)**	8.28 (2.01)**	1.91 (0.48)	1.53 (0.24)***	373 (89)
Gout	2a	18.8	16.17	0.38	2.26	350
	b	9.22	5.90	2.12	1.20	861
Non-specific inflammatory arthritis	2a	18.24	13.68	4.01	0.57	667
	b	3.68	3.34	0.04	0.29	80
Psoriatic arthritis	1	8.94	6.17	2.06	0.38	142
Aseptic necrosis	1	2.0	0	0.05	1.95	580
<i>Non-formers†</i>						
High cell count	13	12.73 (1.44)**	9.93 (1.69)**	2.33 (0.54)	0.40 (0.11)	0
Low cell count	5	2.00 (0.16)	0.28 (0.07)	1.33 (0.26)	0.01–1.4 (range) 0.1 (median)	0
Osteoarthritis	6	0.31 (0.13)		Predominantly synoviocytes		0

*p<0.05, **p<0.01 compared with low cell count non-formers; ***p<0.01 compared with high cell count non-formers.

†Results from patients were divided into 1,25(OH)₂D₃ formers and non-formers. The formers were subdivided into types of arthritis of gout and the non-formers into low and high cell count samples.

liquid scintillation counting. Standard 25(OH)D₃, 24,25(OH)₂D₃, and 1,25(OH)₂D₃ (donated by Dr M R Uskokovic, Hoffmann-LaRoche Inc, New Jersey, USA) were also chromatographed, and the retention times of these markers were determined by ultraviolet absorption at 265 nm. Further analyses of selected chloroform extracts were carried out on the normal phase (Zorbax-Sil) and on reverse phase (Zorbax-ODS, 4.6 mm×25 cm) HPLC systems, developed with mobile phases of 2.5% methanol in dichloromethane (2 ml/min) and 15% water in methanol (2 ml/min) respectively, to confirm the identity of the metabolic product. For these analyses [³H]-1,25(OH)₂D₃ was used as a marker.

Cytocentrifuge preparations were made with 40 000 cells per slide for each sample. Cells were

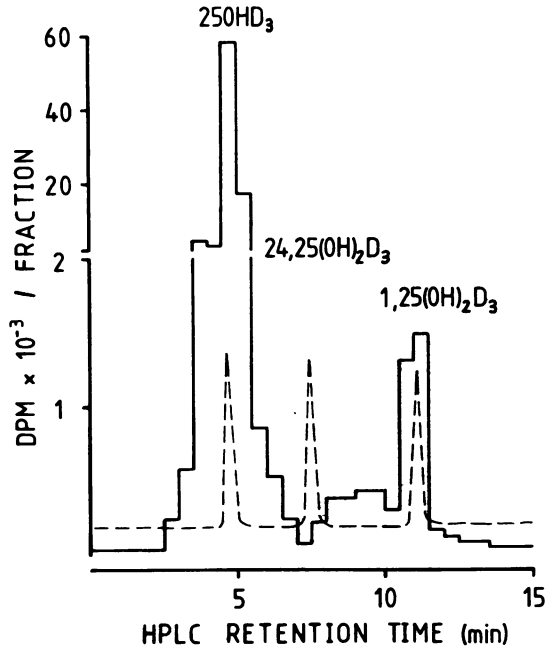


Fig. 1 *Metabolism of [³H]-25(OH)D₃ in a six hour in vitro incubation system by synovial fluid cells harvested from patients with reactive arthritis, rheumatoid arthritis, or gout (Fig. 1A), and by cells (95% macrophages) harvested from a patient with aseptic necrosis of a femoral condyle (Fig. 1B). Bars represent the amount of tritiated material in eluent fractions obtained by high performance liquid chromatography (HPLC) analysis of cell extracts on a Zorbax-Sil column developed with n-hexane:propan-2-ol:methanol (110:6:4). Standard 25(OH)D₃, 24,25(OH)₂D₃, and 1,25(OH)₂D₃ were used as markers, and detected by ultraviolet absorbance at 265 nm (---).*

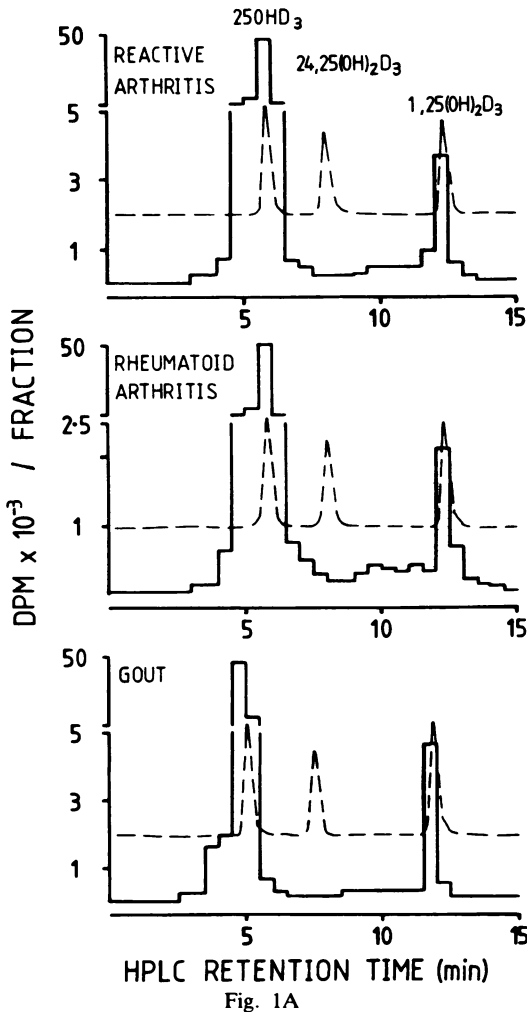


Fig. 1A

identified and differential cell numbers determined on the basis of their morphology in Giemsa stained preparations and their reaction for non-specific esterase with α-naphthyl propionate as substrate.⁹

Results

Synovial fluid samples were divided into two groups according to whether or not the cells were able to form 1,25(OH)₂D₃. Cells from 21 patients synthesised [³H]-1,25(OH)₂D₃ after overnight culture followed by a six hour incubation with [³H]-25(OH)D₃. The cells with this ability were found in eight out of 14 samples from patients with rheumatoid arthritis, all seven of those with reactive arthritis, two out of five with gout, and three out of 12 with other types of arthritis (two with non-specific inflammation and one with psoriatic arthritis)

and in the patient with aseptic necrosis of a femoral condyle (Table 2). Figure 1A shows typical chromatograms from HPLC analyses from the first three groups; peaks of radioactivity corresponding to the precursor [^3H]-25(OH) D_3 and a product cochromatographing with 1,25(OH) $_2\text{D}_3$ are clearly shown, but there was no radioactive peak corresponding to 24,25(OH) $_2\text{D}_3$. Synthesis of [^3H]-1,25(OH) $_2\text{D}_3$ was similarly shown in cells from the patient with aseptic necrosis (Fig. 1B); by all morphological and histochemical criteria more than 95% of these cells were macrophages (Fig. 2). To confirm that macrophages were responsible for 1,25(OH) $_2\text{D}_3$ synthesis cells were placed in culture for one week before incubation with [^3H]-25(OH) D_3 for six hours. Figure 3A shows [^3H]-1,25(OH) $_2\text{D}_3$ synthesis by macrophages from a psoriatic arthritis sample maintained for one week in culture. The identity of the metabolite formed by these cells was confirmed by cochromatography with authentic [^3H]-1,25(OH) $_2\text{D}_3$ on two further HPLC systems (Figs 3B and C)

The non-formers included all six of the patients with osteoarthritis, three patients with gout, six with rheumatoid arthritis, four with psoriatic arthritis, four with unspecified arthritis, and a patient with lymphocytic arthritis.

In general terms, therefore, it appears that cells from the synovial fluid of patients with non-inflammatory arthropathies did not form 1,25(OH) $_2\text{D}_3$, whereas those fluids in which 1,25(OH) $_2\text{D}_3$ synthesis occurred were from patients with inflammatory arthropathies. This is clearly not a complete explanation of the results, however, as synovial fluid cells from 18 of the 38 patients with inflammatory arthropathies did not synthesise 1,25(OH) $_2\text{D}_3$, whereas those from the patient with non-inflammatory aseptic necrosis did. We have examined all the available data on these patients to establish if there are any obvious variables that might distinguish formers from non-formers.

Non-formers differed from formers in one of two ways. Either they had high total cell counts like the formers but with a low proportion of macrophages (high cell count non-formers) or they had total cell counts below 2.5×10^9 cells/l (low cell count non-formers). The first group contained six patients with rheumatoid arthritis (including three taking drugs), four with psoriatic arthritis (including three taking methotrexate), one with unspecified inflammatory arthritis, and two with gout. The second group consisted of three patients with unspecified inflammatory arthritis, one patient with lymphocytic arthritis, and one with gout. Interestingly, one exception to this generalisation was the sample from the patient with aseptic necrosis, which was a non-inflammatory arthropathy characterised by a low

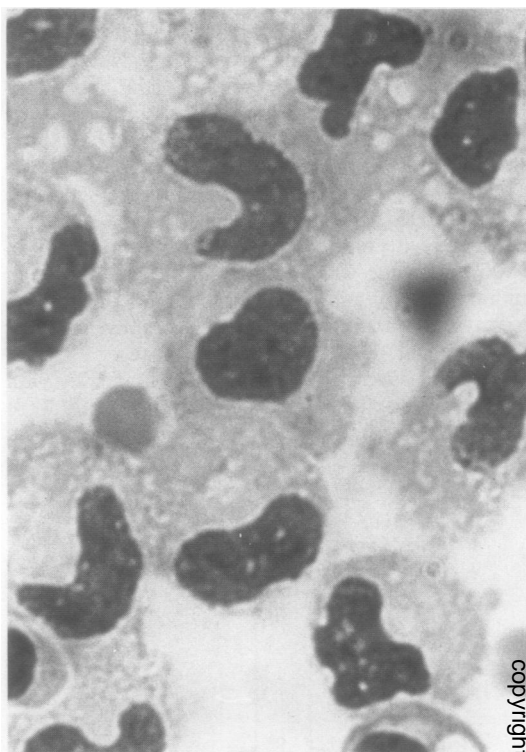


Fig. 2 Synovial fluid macrophages from the patient with aseptic necrosis of a femoral condyle stained with Giemsa.

total cell count. This sample contained a high proportion of macrophages but few lymphocytes and no polymorphs and was able to form large amounts of 1,25(OH) $_2\text{D}_3$ (Table 2). It would seem therefore, that a major difference between formers and non-formers is the absolute number of macrophages in the joint; however, samples from two patients with low total cell counts had macrophage counts of 0.84 and 1.38×10^9 /l but failed to synthesise 1,25(OH) $_2\text{D}_3$.

Synthesis of 1,25(OH) $_2\text{D}_3$ was shown by cells from high cell count samples that had been maintained in cell culture for one week to select for adherent macrophages. Cells from six out of nine of these samples synthesised 1,25(OH) $_2\text{D}_3$ during a six hour incubation period (50 – 2970 fg/h/incubation). Of the six samples that formed 1,25(OH) $_2\text{D}_3$ after seven days in cell culture, three did not initially form 1,25(OH) $_2\text{D}_3$; of these, one was from a patient taking methotrexate and two had low macrophage numbers. In the three samples in which 1,25(OH) $_2\text{D}_3$ synthesis could not be shown the numbers of adherent cells were low after seven days in culture

Discussion

These results show for the first time that synovial fluid cells are capable of synthesising the active vitamin D metabolite, $1,25(\text{OH})_2\text{D}_3$, when maintained in either short term (<24 hours) or long term (seven days) primary culture. In contrast, no synthesis of $24,25(\text{OH})_2\text{D}_3$ could be shown, though other workers have previously reported synthesis of this metabolite in normal human macrophages *in vitro*. In one such study macrophages exposed to interferon gamma initially formed $1,25(\text{OH})_2\text{D}_3$, but after prolonged exposure they began to synthesise $24,25(\text{OH})_2\text{D}_3$.¹⁰ Synthesis of $24,25(\text{OH})_2\text{D}_3$ has also been observed in articular cartilage and by chondrocytes cultured *in vitro*.¹¹

Although synovial fluid contains a heterogeneous population of cells, with particularly high numbers of polymorphs in high cell count inflammatory arthritis, it appears to be the macrophages that synthesise $1,25(\text{OH})_2\text{D}_3$. Macrophages were significantly more numerous in samples that formed $1,25(\text{OH})_2\text{D}_3$ and were also the predominant cell type present in the aseptic necrosis sample and the only cell type present in preparations maintained for one week in cell culture. Macrophages from most forms of high cell count inflammatory arthritis and gout appeared to synthesise $1,25(\text{OH})_2\text{D}_3$. In our study 13 cell samples did not metabolise $1,25(\text{OH})_2\text{D}_3$ but six of these patients were receiving either the antimetabolite methotrexate (one with rheumatoid arthritis, three with psoriasis), gold, or penicillamine (both rheumatoid arthritis), which may have influenced cellular functions in macrophages, including the activity of the $25(\text{OH})\text{D}$ -1-hydroxylase enzyme.

To form $1,25(\text{OH})_2\text{D}_3$ the macrophages must presumably be in an activated state (as in sarcoidosis and peritonitis), but it is uncertain whether this occurs before or after they enter the synovial fluid. In gout the cells are activated by uric acid crystals within the joint, whereas in reactive arthritis, which develops secondary to systemic inflammatory conditions, the monocytes are probably activated before they enter the joint. The need for cells to be activated may explain why two of the patients with low cell count non-specific inflammatory arthritis did not form $1,25(\text{OH})_2\text{D}_3$ despite having high numbers of macrophages. In the aseptic necrosis sample the cells were probably activated by particles of necrotic bone and thus actively synthesised $1,25(\text{OH})_2\text{D}_3$.

An adequate substrate concentration is another requirement for the synthesis of $1,25(\text{OH})_2\text{D}_3$, which is likely as $25(\text{OH})\text{D}_3$ is present in synovial fluid at about half serum concentrations,⁴ and these

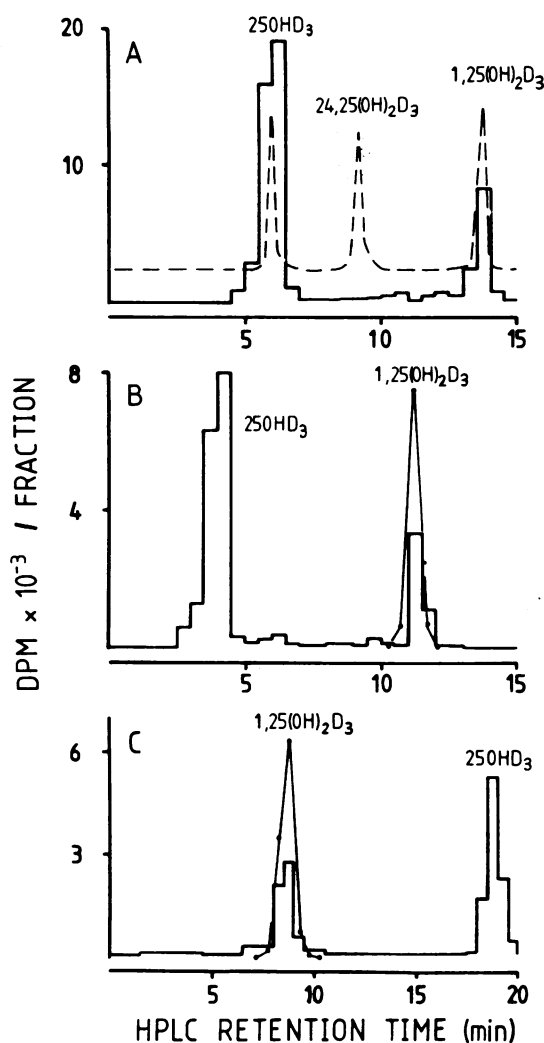


Fig. 3 Metabolism of $[^3\text{H}]-25(\text{OH})\text{D}_3$ in a six hour *in vitro* incubation system by adherent synovial fluid macrophages from a patient with psoriatic arthritis after maintenance in cell culture for seven days. Bars represent the amount of tritiated material in eluent fractions obtained by analysis of aliquots of the cell extract on three different high performance liquid chromatography (HPLC) systems: (A) Zorbax-Sil column developed with *n*-hexane:propan-2-ol:methanol (110:6:4); (B) Zorbax-Sil column developed with 2.5% methanol in dichloromethane; (C) Zorbax-ODS column (reverse phase) developed with 15% water in methanol. Standard $25(\text{OH})\text{D}_3$, $24,25(\text{OH})_2\text{D}_3$, and $1,25(\text{OH})_2\text{D}_3$ detected by ultraviolet absorbance at 265 nm (---) or $[^3\text{H}]-1,25(\text{OH})_2\text{D}_3$ (—●—●—) were used as markers.

concentrations are about 50 times greater than those presented to the cells in the incubation system used in our study.

The significance of the ability of macrophages from patients with high cell count inflammatory arthritis to synthesise $1,25(\text{OH})_2\text{D}_3$ is not clear. Evidence that the reaction may occur in vivo is provided by results showing that patients with rheumatoid arthritis respond to a single dose of $25(\text{OH})\text{D}_3$ (10 000 IU) by increasing serum $1,25(\text{OH})_2\text{D}_3$ concentrations significantly compared with controls,¹² but this rise is short lived. There is no evidence that rheumatoid patients as a group have higher than normal circulating concentrations of $1,25(\text{OH})_2\text{D}_3$ as a result of extrarenal synthesis.¹³ This may result from feedback regulation of renal $1,25(\text{OH})_2\text{D}_3$ synthesis¹ by that formed outside the kidney. Synthesis of $1,25(\text{OH})_2\text{D}_3$ mediated by macrophages may result in raised concentrations only when the $25(\text{OH})\text{D}_3$ levels are abnormally high or the mass of tissue involved is large, as in some cases of sarcoidosis¹⁴ and malignant disease.¹⁵

Measured concentrations of $1,25(\text{OH})_2\text{D}_3$ in synovial fluid are lower than in serum (Mawer, unpublished observations), probably owing to the smaller concentration of vitamin D binding protein. The importance of $1,25(\text{OH})_2\text{D}_3$ production by macrophages from arthritic patients may be greater when cells are contained within the synovium and are thus adjacent to the sites of tissue damage and bone erosion within the joint.

The presence of specific receptors for $1,25(\text{OH})_2\text{D}_3$ in normal human monocytes and activated lymphocytes^{16 17} and in peripheral blood lymphocytes² and synovial tissue derived fibroblasts³ from patients with rheumatoid arthritis suggests a physiological role for the metabolite in joint disease. Furthermore, many of the reported effects of $1,25(\text{OH})_2\text{D}_3$ may be relevant to arthritis. For example this metabolite appears to inhibit proliferation of both B and T activated lymphocytes and reduces interleukin 2 production by activated T lymphocytes in vitro.^{18 19} $1,25(\text{OH})_2\text{D}_3$ has also been shown to promote differentiation of monocytes into macrophages and the fusion of macrophages to multinucleated giant cells with bone resorbing activity.^{20 21} This is in addition to the known ability of $1,25(\text{OH})_2\text{D}_3$ to inhibit collagen synthesis by osteoblasts and to promote bone resorption.^{22 23} This last effect may be achieved by an indirect action of $1,25(\text{OH})_2\text{D}_3$ on osteoblasts to stimulate bone resorption by osteoclasts, which unlike osteoblasts do not appear to have receptors for $1,25(\text{OH})_2\text{D}_3$.²³ Thus within the synovial fluid and synovium $1,25(\text{OH})_2\text{D}_3$ may have complex paracrine and immunoregulatory functions which could influence

the development of the juxta-articular osteoporosis that occurs in arthritis.²⁴ If this is the case then the use of drugs that inhibit the extrarenal $25(\text{OH})\text{D}_3$ hydroxylase may play an important part in reducing the extent of tissue damage and bone erosion that occurs in arthritis.

Support for this study was provided by project grants from the Medical Research Council to Dr E B Mawer and Professor S Tomlinson, and by the Arthritis Research Council and North West Regional Research Grants Committee to Dr A J Freemont. We are also grateful to the many clinicians who collected samples to D Bayley and P Lockey for technical assistance, and to Dr M Davies for helpful discussions.

References

- 1 Haussler M R, McCain T A. Basic and clinical concepts related to vitamin D metabolism and action. *N Engl J Med* 1977; **297**: 974-83.
- 2 Manolagas S C, Wernitz D A, Tsoukas C D, Provvedini D M, Vaughan J H. $1,25$ -Dihydroxyvitamin D_3 receptors in lymphocytes from patients with rheumatoid arthritis. *J Lab Clin Med* 1986; **108**: 596-600.
- 3 Sambrook P N, Eisman J A, Breit S. $1,25$ -Dihydroxyvitamin D_3 receptors in synovial cells in rheumatoid arthritis [Abstract]. *Aust NZ J Med* 1987; **17**: 120.
- 4 Fairney A, Starffen A M, May C, Seifert M H. Vitamin metabolites in synovial fluid. *Ann Rheum Dis* 1987; **46**: 370-4.
- 5 Koeffler H P, Reichel H, Bishop J E, Norman A W. Gamma interferon stimulates production of $1,25$ -dihydroxyvitamin D_3 by normal human macrophages. *Biochem Biophys Res Commun* 1985; **127**: 596-603.
- 6 Reichel H, Koeffler P, Bishop J E, Norman A W. 25 -Hydroxyvitamin D_3 metabolism by lipopolysaccharide-stimulated normal human macrophages. *J Clin Endocrinol Metab* 1987; **64**: 1-9.
- 7 Hayes M E, O'Donoghue D J O, Ballardie F W, Mawer E B. Peritonitis induces the synthesis of $1\alpha, 25$ -dihydroxyvitamin D_3 in macrophages from CAPD patients. *FEBS Lett* 1987; **220**: 307-10.
- 8 Adams J S, Sharma O P, Gacad M A, Singer F R. Metabolism of 25 -hydroxyvitamin D_3 by cultured pulmonary alveolar macrophages in sarcoidosis. *J Clin Invest* 1983; **72**: 1856-60.
- 9 Freemont A J, Davies J S. Acid esterase activity in lymphocytes and other cells: a comparison of six alpha-naphthyl base substrates. *Med Lab Sci* 1982; **39**: 405-7.
- 10 Reichel H, Koeffler H P, Norman A W. Synthesis in vitro of $1,25$ -hydroxyvitamin D_3 by interferon-gamma-stimulated normal human bone marrow and alveolar macrophages. *J Biol Chem* 1987; **262**: 10931-7.
- 11 Garabedian M, Leiberherr M, N'Guyen T M, Corvol M, Bailly Du Bois M, Balsan S. The in vitro production and activity of $24,25$ -dihydroxycholecalciferol in cartilage and calvarium. *Clin Orthop* 1978; **135**: 241-8.
- 12 Palit J, Holt P J L, Davies M, Still P, Mawer E B. Abnormal vitamin D metabolism in rheumatoid arthritis [Abstract]. *Br J Rheumatol* 1988; **27** (suppl 1): 6.
- 13 Bird H A, Wright V, Hennes U, Theiss E. Comparison of serum $1,25$ -hydroxycholecalciferol concentrations in rheumatoid arthritis and osteoarthritis. *Ann Rheum Dis* 1982; **41**: 257-8.
- 14 Sandler L M, Winearis C G, Fraher L J, Clemens T L, Smith O'Riordan J L H. Studies of the hypercalcaemia of sarcoidosis: effect of steroids and exogenous vitamin D_3 on circulating concentrations of $1,25$ -dihydroxyvitamin D_3 . *Q J Med* 1988; **210**: 165-80.
- 15 Davies M, Mawer E B, Hayes M E, Lumb G A. Abnormal vitamin D metabolism in Hodgkin's lymphoma. *Lancet* 1985; **1186**-8.

- 16 Provvedini D M, Tsoukas C D, Deftos L J, Manolagas S C. 1,25-Dihydroxyvitamin D₃ receptors in human leukocytes. *Science* 1983; **221**: 1181–3.
- 17 Bhalla A K, Amento E P, Clemens T L, Holick M F, Krane S M. Specific high-affinity receptors for 1,25-hydroxyvitamin D₃ in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J Clin Endocrinol Metab* 1983; **57**: 1308–10.
- 18 Shiozawa S, Shiozawa K, Tanaka Y, Fujita T. 1α,25-Dihydroxyvitamin D₃ inhibits proliferative response of T- and B-lymphocytes in serum-free culture. *Int J Immunopharmacol* 1987; **9**: 719–23.
- 19 Tsoukas C D, Provvedini D M, Manolagas S C. 1,25-Dihydroxyvitamin D₃: a novel immunoregulatory hormone. *Science* 1984; **224**: 1438–40.
- 20 Abe E, Miyaura C, Tanaka H, *et al.* 1α,25-Dihydroxyvitamin D₃ promotes fusion of mouse alveolar macrophages both by direct mechanisms and by spleen cell-mediated indirect mechanisms. *Proc Natl Acad Sci USA* 1983; **80**: 5583–7.
- 21 Bar-Shavit Z, Teitlebaum S L, Reitsma P, *et al.* Induction of monocytic differentiation and bone resorption by 1,25-dihydroxyvitamin D₃. *Proc Natl Acad Sci USA* 1983; **80**: 5907–11.
- 22 Raisz L G, Kream B E, Smith M A, Simmons H A. Comparison of the effects of vitamin D metabolites on collagen synthesis and resorption of fetal rat bone in organ culture. *Calcif Tissue Int* 1980; **32**: 135–8.
- 23 Narbaitz R, Stumpf W E, Sar M, Huang S, DeLuca H F. Autoradiographic localisation of target cells for 1,25-dihydroxyvitamin D₃ in bones from fetal rat. *Calcif Tissue Int* 1983; **35**: 177–82.
- 24 Sambrook P N, Reeve J. Bone disease in rheumatoid arthritis. *Clin Sci* 1988; **74**: 255–30.