CRYSTAL-INDUCED INFLAMMATION IN CANINE JOINTS

II. IMPORTANCE OF POLYMORPHONUCLEAR LEUKOCYTES*

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PLATE 9

Urate crystals have been identified in virtually all gouty synovial fluids by compensated polarized light microscopy (1, 2). Phagocytosis of crystals by polymorphonuclear, and to a lesser degree, by mononuclear phagocytes is a constant feature of the acute attack (3). Microcrystalline calcium pyrophosphate and adrenocorticosteroid esters are phagocytosed during acute pseudogout (4) and during the "postinjection flare" (5) respectively. Injection of microcrystalline sodium urate (6, 7), sodium orotate (6), calcium oxalate (7), calcium pyrophosphate (4), and corticosteroid esters (5) all induce an acute inflammatory reaction. Phlogistic responses to crystals of different chemical composition are identical, leading to the use of the phrase "crystal-induced synovitis" as a generic term to emphasize the lack of specificity of the response (4).

The response is also nonspecific with respect to host species (7–9). Therefore, an experimental model of crystal-induced inflammation was developed in the dog (10). The purpose of this work was to investigate the role of the polymorphonuclear leukocyte in crystal synovitis using this model.

Method

In a previous paper, we described a reproducible method for quantitating the intra-articular pressure, pH, and leukocyte (WBC) response to intrasynovial injection of monosodium urate crystals (10). In this study, 10 male mongrel dogs weighing between 12 and 25 kg were given 0.2 to 0.3 mg vinblastine[1] (VLB) per kg intravenously. Total and differential peripheral blood leukocyte counts were performed daily; crystals were injected 3 to 4 days later when the animals were leukopenic.

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[1] Velban (Eli Lilly & Co, Indianapolis).

Control experiments using the same crystal dose were performed either before administration of VLB or after complete recovery. Most of the dogs remained in good health throughout the experiments although a few developed diarrhea and several died of infection during the leukopenic phase. Two survivors of the VLB experiment died before a control experiment could be performed. Intra-articular pressures were recorded continuously in mm Hg. The values presented herein represent the recorded pressure from which the half-hour reading was subtracted. This procedure was followed for reasons outlined previously (10).

In 1 dog a control experiment was performed prior to giving the dog VLB. The effects of crystal injection in the ensuing leukopenic state were followed for $4\frac{1}{2}$ hr and then the test
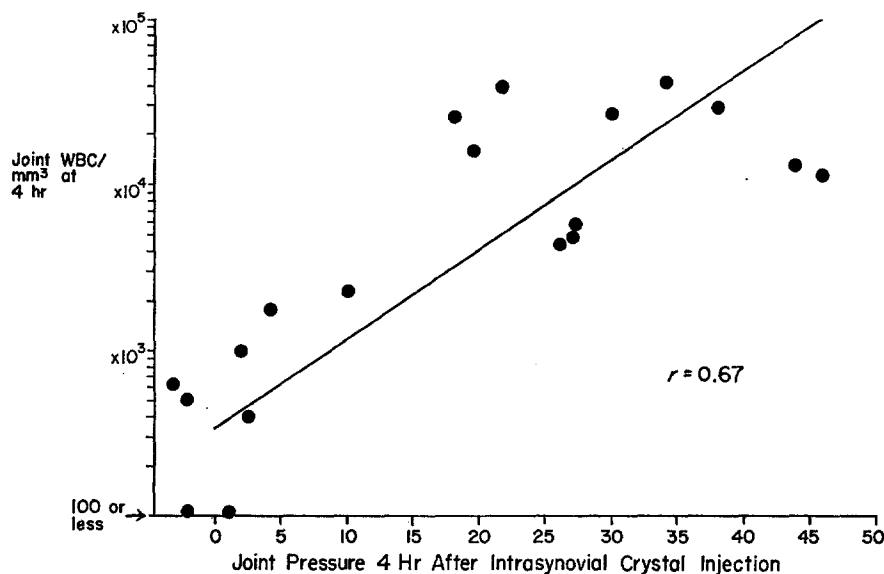
TEXT-FIG. 1 The intra-articular pressure at 4 hr (minus the intra-articular pressure at $\frac{1}{2}$ hr) plotted against the synovial fluid leukocyte concentration/mm^3 at 4 hr. Control and leukopenic trials are combined.

limb was perfused with blood from a normal dog. The femoral artery and vein of the leukopenic dog were exposed surgically as were the carotid artery and jugular vein of a normal donor dog. The carotid artery of the donor was then anastomosed to the femoral artery of the leukopenic dog whose femoral vein was anastomosed to the donor's jugular vein. The pressure, pH, and leukocyte count was measured for a further 4 hr after opening the anastomosis. The donor dog was not given anticoagulants.

RESULTS

The leukocyte concentration in the joint fluid at 4 hr correlated roughly with the pressure rise at 4 hr. Text-fig. 1 shows the 4 hr values for all experiments in all dogs, both control and following vinblastine ($r = 0.67$; $P < 0.01$).

The data from these dogs can be divided into three groups (Text-fig.2). In the control experiments (Table I A) the peak pressures varied between 18 and

51 mm Hg with a mean of 32.4 mm Hg. The WBC concentration ranged between 11,750 and 43,000 per mm³, with a mean of 25,860. The average differential leukocyte count showed 3 bands, 83 polymorphs, 5 monos, 8 lymphs, and 1 disintegrated cell.

After VLB treatment 4 dogs were able to react partially (Table I B). At 4
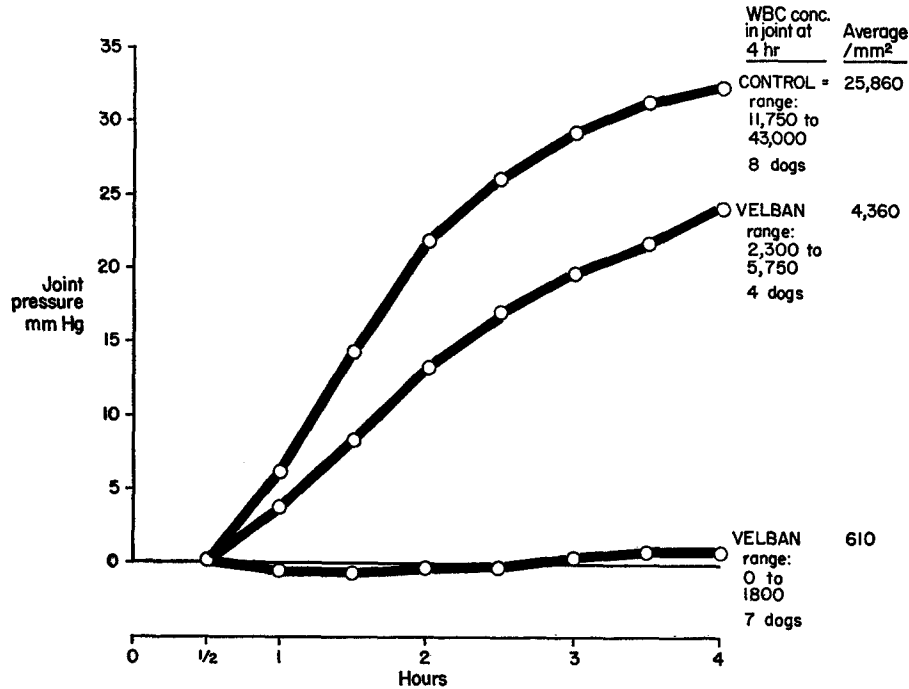
TEXT-FIG. 2 The rise in intra-articular pressure is plotted against time. The top curve represents the average of 8 control experiments, the middle curve the pressure rise in 4 dogs still capable of some leukocytic response, and the bottom curve the pressure in 7 dogs with virtually no cellular response. Dog 4-4 appears in all three groups.

hr there was an average of 4360 cells/mm³ in the joint and an average pressure rise of 24.2 mm Hg. The pressure and leukocyte concentration at 4 hr was lower in every instance than the corresponding level reached during the control experiment in the same animal.

The final group (Table II) had virtually no leukocytic response in the joint, averaging only 610 cells/mm³, and had an average pressure rise of only 0.8 mm Hg at 4 hr. Once again, every value during the leukopenic state was lower than in the corresponding control experiment.

Dog 4-4 was injected on 3 different occasions (twice following VLB treat-

ment). In the first experiment, while leukopenic, a minimal WBC response occurred and the synovial fluid pressure rose to only 4.6 mm Hg. A subsequent control experiment showed good cellular and pressure responses of 43,000/mm³ and 34 mm Hg respectively. A second course of VLB induced only partial leukopenia; at 4 hr there was a moderate cellular response (5000/mm³) while the pressure rose to 27 mm Hg.

In order to show that the effects observed following VLB therapy were not due to a local tissue effect of the VLB an attempt was made to re-establish the

TABLE I

WBC Pressure Relationship in Dogs Capable of Partial Leukocyte Response

Dog No.	Peripheral blood leukocytes/mm³ day of experiment	Synovial fluid WBC/mm³ at 4 hr	Pressure, mm Hg at 4 hr
A. Control experiment			
6-9*	27,850	30,000	33.7
4-2	8,000	11,750	47
4-4	26,950	43,000	34
5-2	‡	‡	‡
B. Vinblastine experiment			
6-9	950	2,300	13
4-2	1,600	4,500	30
4-4	8,450	5,000	27
5-2	250	5,750	27

* Vinblastine experiment done before control experiment.

‡ Dog died before control experiment could be performed.

inflammatory response in a nonresponding leukopenic animal. During a control experiment in one knee the pressure and white count rose and the pH fell as expected (Text-figs. 3 a to 3 c). 3 days later the dog was given VLB and in another 3 days the absolute peripheral blood polymorphonuclear count was 250/mm³. The opposite knee was then injected with 15 mg urate crystals and in 4 hr the synovial fluid leukocyte count was only 750/mm³ (Text-fig. 3 a), the pH remained at the same level as the venous blood (Text-fig. 3 b) and the pressure did not rise (Text-fig. 3 c).

At this point the femoral artery and vein supplying the test knee were anastomosed to the carotid artery and jugular vein of a normal dog. Thus the experimental knee was suddenly perfused with normal blood. There followed a characteristic inflammatory response over the next 4 hr closely resembling the control experiment done 6 days previously. The WBC count rose (Text-fig. 3 a)

to over 40,000 mm^3, the pH fell to more than 0.5 pH unit below the venous blood of the donor dog (Text-fig. 3 b) and the joint pressure rose to almost 35 mm Hg (Text-fig. 3 c).

Histologically, the inflammatory response to crystals was evident at 5 hr. Marked tissue edema, capillary and venular dilitation, and infiltration with polymorphonuclear leukocytes are seen in Figs. 1 a and 1 b. None of these

TABLE II

WBC Pressure Relationship in Dogs Capable of Virtually No Leukocyte Response

Dog No.	Peripheral blood leukocytes/mm^3 day of experiment	Synovial fluid WBC/mm^3 at 4 hr	Pressure-mm Hg at 4 hr
A. Control experiment			
6-8	9,000	26,000	32.2
4-8	N.A.	16,250	21.0
8-1	10,300	26,500	18.0
8-9	10,875	39,975	22.0
4-4*	26,950	43,000	34.0
6-5	N.A.	13,500	51.0
6-1	‡	‡	‡
B. Vinblastine experiment			
6-8	500	600	3.2
4-8	650	500	0.5
8-1	100	1,000	2.0
8-9	725	400	0.5
4-4	1,900	1,800	4.6
6-5	450	0	1.0
6-2	850	0	3.3

N.A., data not available.

* Vinblastine experiment done before control experiment.

‡ Dog died before control experiment could be performed.

changes are present 5 hr after injection of crystals into the knee of a leukopenic animal (Fig. 1 c).

DISCUSSION

These data strongly suggest that the inflammatory response to the intra-synovial injection of sodium urate crystals requires polymorphonuclear phagocytes.

Clinically, acute attacks of gout and pseudogout (chondrocalcinosis) are invariably attended by a collection of polymorphonuclear leukocytes and crystals in the exudate from the affected joint (3, 4). Similarly, synovitis in-
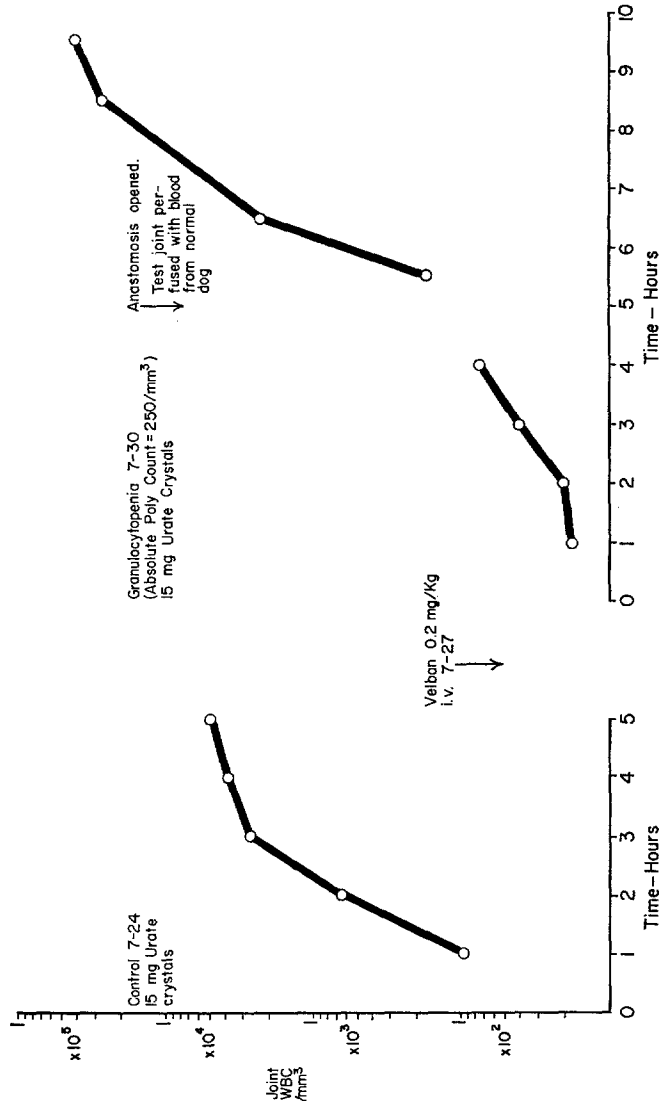
TEXT-FIG. 3 a. Leukocyte response in a leukopenic dog after crystal injection into knee. After 5 hr the test limb was perfused with normal blood. Control experiment shown at left.

TEXT-FIG. 3 b. pH response in the same leukopenic dog as in Text-fig. 3 a; the test limb was perfused with normal blood at 5 hr. The pH is expressed as the difference between synovial fluid pH and venous blood pH. After the anastomosis was opened the pH of the venous blood of the donor dog was measured. A control experiment is shown to left.

TEXT-FIG. 3 c. Intra-articular pressure response in same leukopenic animal as in text-fig. 3 a; after 5 hr the test limb was perfused with normal blood. The pressure at ½ hour is used as the initial reference point for reasons described previously (10).
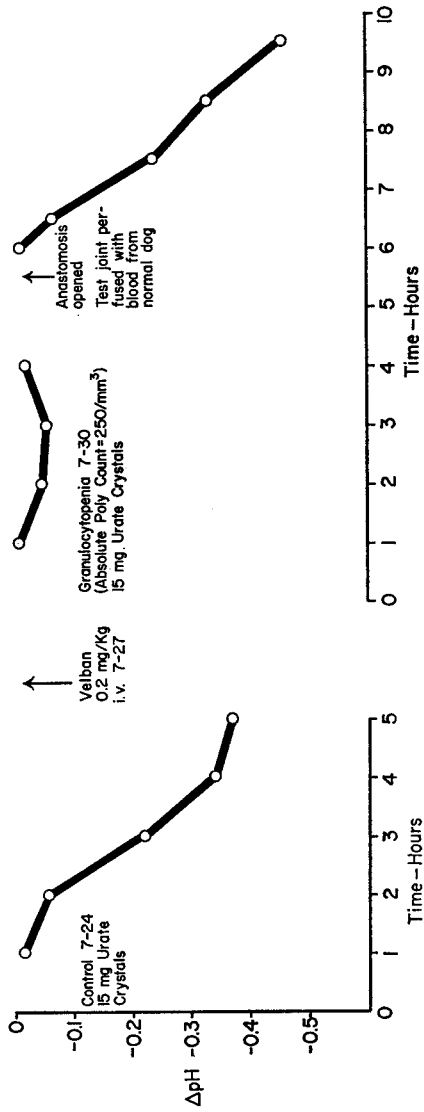
TEXT-FIG. 3 b
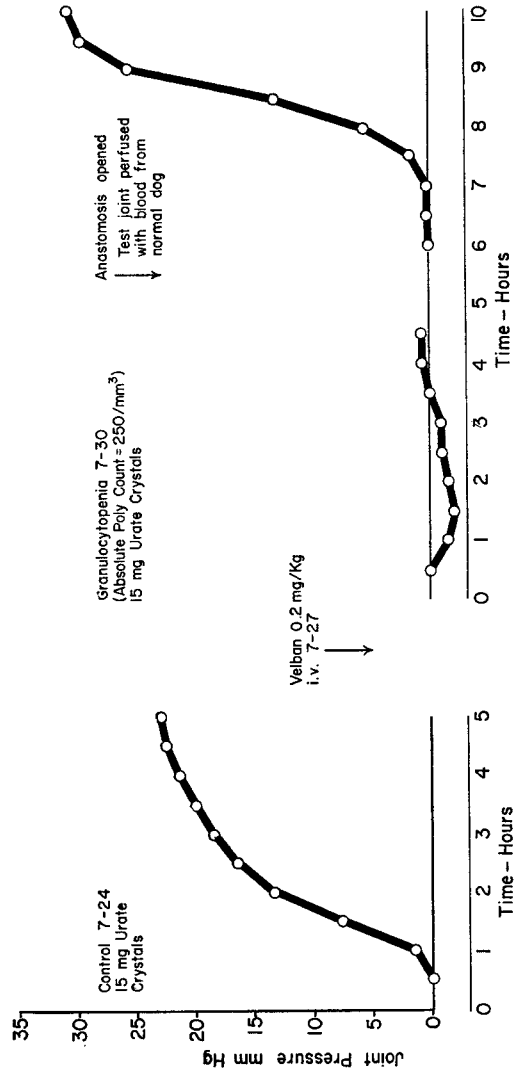
TEXT-FIG. 3 c

duced with crystals in laboratory animals shows polymorphonuclear leukocytes in the exudative reaction. Thus, coincident with acute signs and symptoms, a constant association of crystals and leukocytes exists.

The magnitude of the intra-articular pressure rise correlated roughly with the WBC concentration in the synovial exudate. When the normal cellular response was partially curtailed by the induction of leukopenia, the inflammatory response was decreased; when leukocyte depletion was profound, preventing significant phagocyte accumulation, the response was almost completely abolished. Thus the inflammatory reaction, as reflected by the intra-articular pressure response, varies concomitantly with leukocyte accumulation. The response in an animal that had previously reacted normally was virtually abolished in the leukopenic state; this response was restored when the dog was cross-transfused with normal blood.

Preliminary attempts at inducing leukopenia in dogs with nitrogen mustard or cyclophosphamide resulted in marked bleeding into the joint at the desired level of peripheral polymorphs. This was associated with profound depression of circulating platelets. On the other hand, vinblastin in doses producing granulocytopenia adequate for these studies was not associated with bleeding as the circulating platelet levels were little reduced. VLB appears to act by blocking the dividing granulocyte precursors in metaphase (11). Polymorphonuclears circulating for 3 to 4 days after VLB administration consist entirely of previously formed cells (12). The most marked effect by the 3rd to 4th day is upon the polymorphonuclear leukocyte. The drug is thought to have minimal non-hematologic effects (12).

In an extensive review of the *Vinca alkaloids*, Johnson et al. (13) found that in physiologic doses they exerted little or no effect on cellular respiration, glycolysis, protein or nucleic acid synthesis. Nor is vinblastine potent in suppressing antibody production (14).

Although remote, the possibility remains that the VLB effects on inflammation are mediated by means other than by suppression of polymorphonuclear leukocytes. A local tissue effect is ruled out by the cross-transfusion experiment, but another factor might have been supplied in the blood of the donor dog. This theoretical possibility seems unlikely but cannot be entirely ruled out.

The critical role of polymorphonuclear phagocytes has been demonstrated in the Schwartzman and Arthus reactions, where polymorphs accumulate in the inflammatory lesions.

Bacterial endotoxin injected into the skin produces slight inflammation. In the Schwartzman reaction, endotoxin is injected intravenously 24 hr after an initial skin injection; severe inflammation then occurs at the site of the previous injection. In the Arthus reaction, a skin site injected with antigen becomes acutely inflamed if the host has been previously sensitized to that antigen. In the reversed passive Arthus reaction, the antibody is injected into the skin and the antigen is given intravenously.

In 1951, Stetson and Good (15) were able to abolish the acute inflammatory response seen in the Schwartzman reaction by rendering animals leukopenic with nitrogen mustard. Stetson (16) later was able to abolish the inflammatory response in the Arthus reaction in the same way. By using the reversed passive Arthus technique, Humphrey (17) confirmed Stetson's findings and correlated the volume of edema seen during the reaction with the blood level of polymorphs.

Cochrane, Weigle, and Dixon (18) using immunofluorescent staining of antigen and antibody further elucidated the role of the polymorphs in the Arthus reaction. In control experiments, antigen and antibody could be demonstrated within polymorphs taken from the inflammatory site. The antigen-antibody combination takes place as usual in sensitized leukopenic animals but there is no inflammation. Antigen and antibody has also been demonstrated in the reverse Arthus reaction to be within polymorphs (19). It would thus appear that the inflammation in the Arthus reaction results not from the antigen-antibody combination per se, but from the interaction of this complex with the polymorph.

Leukocytes have been shown to contain various inflammatory substances. Movat (20) has shown that when leukocytes obtained by intraperitoneal glycogen injection are incubated in vitro with antigen and specific antibody a substance is released that increases vascular permeability. DeDuve (21) has recently reviewed the evidence for the lysosomal theory and its possible role in the genesis of inflammation. Recently, Janoff and Zweifach (22) have separated a cationic protein substance from the lysosomal fraction of polymorphs that causes sticking and emigration of leukocytes when applied to the microcirculation of exposed rat mesentery. Further evidence that the inflammatory properties of leukocytes may reside in the granular lysosomal fraction has been presented by Thomas (23) who showed that the inflammation observed during the reversed passive Arthus reaction, abolished by making the animals leukopenic, could be restored by local injection of cell-free polymorphonuclear granules along with the antibody.

SUMMARY

In an experimental model, the polymorphonuclear leukocyte was found to be necessary to the inflammation induced by crystals.

This conclusion is based on (a) the invariable migration of polymorphonuclear leukocytes into the synovial fluid of canine joints injected with urate crystals, (b) the experimental suppression of the synovitis by the depletion of polymorphonuclear leukocytes in animals treated with vinblastine, (c) the dependence of the degree of suppression on the degree of leukocyte mobilization into the joint space, and (d) the restoration of the inflammatory response in a leukopenic animal by perfusion with normal blood.

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EXPLANATION OF PLATE 9

FIG. 1 *a*. Histologic section of synovium 5 hr after intra-articular injection of monosodium urate crystals into a normal canine knee joint; vascular dilatation, leukocytic infiltration and edema are seen. The synovial surface is on the left. Hematoxylin and eosin, × 400.

FIG. 1 *b*. Higher magnification of area shown in Fig. 1 *a* shows polymorphonuclear leukocytes in dilated venules and in edematous tissue. Hematoxylin and eosin, × 1000.

FIG. 1 *c*. Histologic section of synovial biopsy obtained 5 hr after intra-articular crystal injection of monosodium urate crystals into the knee of a leukopenic animal. Note the almost complete lack of edema, cellular exudation or vascular reaction. Hematoxylin and eosin, × 400.
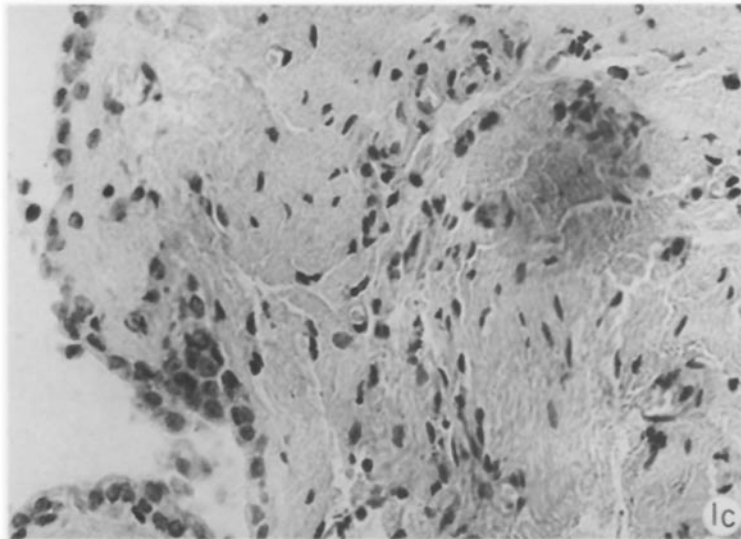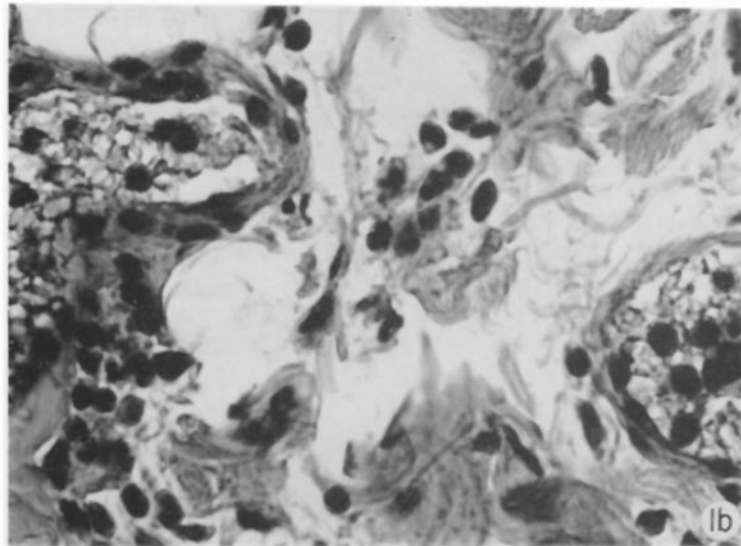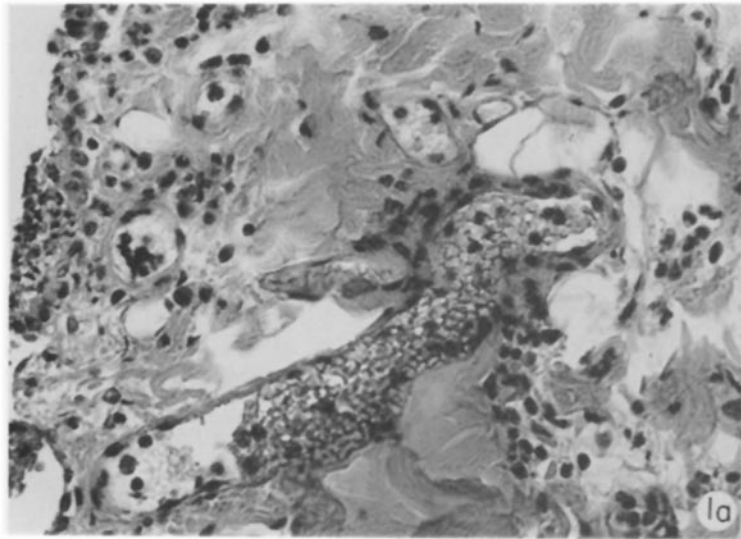
(Phelps and McCarty: Crystal-induced inflammation in canine joints. II)