CRYSTAL-INDUCED INFLAMMATION IN CANINE JOINTS

I. An Experimental Model with Quantification of the Host Response*

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(Received for publication 20 January 1966)

The nearly constant finding of sodium urate crystals in aspirated gouty synovial fluid (1) led to the induction of acute arthritis in both human (2, 3) and canine (3) subjects by intrasynovial crystal injection. Calcium pyrophosphate crystals, identified in the synovial fluid from patients with a goutlike syndrome ("pseudogout"), and crystals of adrenocorticosteroid esters are also irritants when injected (4, 5). Crystal-induced inflammation is completely reversible, dose related, and nonspecific with regard to host or to the chemical composition of the injected crystal. These observations confirmed and extended findings recorded by His and Freudweiler at the end of the nineteenth century (6–8).

An experimental model of crystal synovitis in the dog and several methods for its quantification are described herein. Applications of these methods to the study of the host response mechanism are described in the accompanying paper (9) and in reference 10.

Methods

General Considerations.—The knee (stifle) joints of mongrel dogs weighing 12 to 25 kg were used. Light anesthesia was induced with intravenous sodium pentobarbital (25 to 35 mg/kg); additional small doses were given as needed for maintenance. One experiment was performed using the "physiologic" anesthetic chlorolose (50 mg/kg intravenously). Body temperature, monitored by rectal thermometer, was controlled with an electric blanket within a range of 2°C. In some experiments, the trachea was intubated and breathing was maintained with a me-

* Supported by Grants No. AM 06611 and No. AM 5201-04, United States Public Health Service, Bethesda, Maryland.

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chanical respirator. In these experiments, hyperventilation was deliberately attained to eliminate the spontaneous respiration of the dog; systemic alkalosis invariably accompanied these experiments. Blood pH in nonventilated dogs was normal or slightly acidotic.

Joint Catheterization.—The dog was positioned either on its side or back and the hind leg fixed with adhesive tape so that the angle between the femur and tibia was 90°. The skin over the joint was shaved and prepared by scrubbing with soap and water and then with 70%

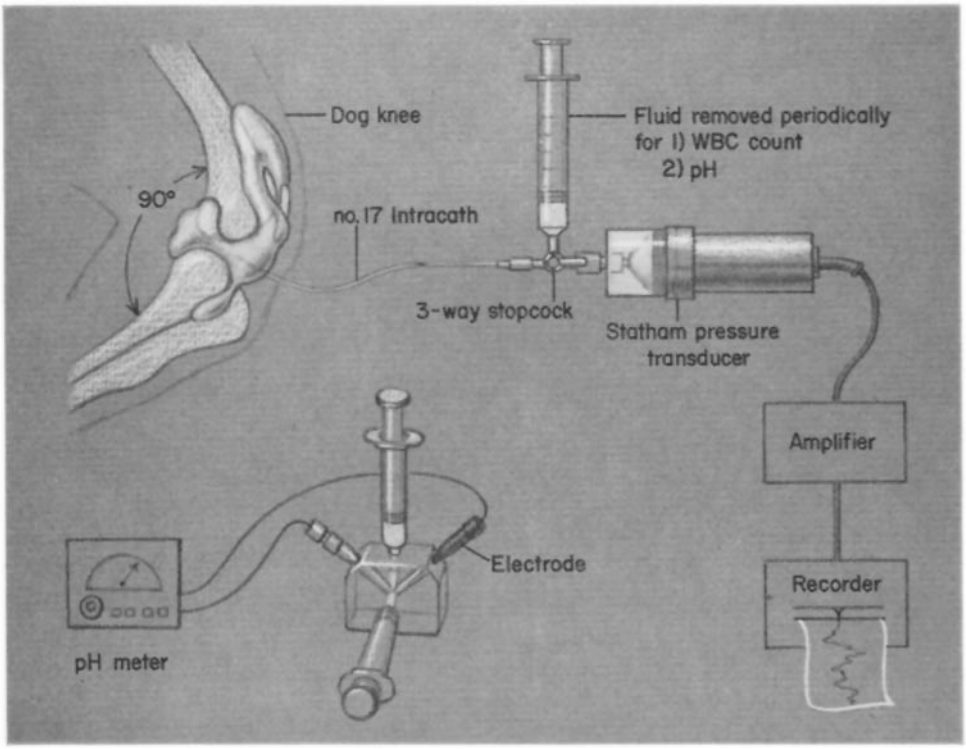

FIG. 1 Experimental model of crystal synovitis in catheterized canine stifle joint. Continuous measurement of intra-articular pressure was assured except during injection or aspiration. Later pH measurements were made with a micro-pH assembly instead of the specially constructed lucite block shown here.

alcohol. A No. 17 Intracath® needle[1] was inserted through the patellar tendon into the joint. Often 0.1 to 0.5 ml of highly viscous fluid was aspirated. 5 to 10 ml of sterile, isotonic, pyrogen-free sodium chloride solution (P.S.S.) were injected and a sterile polyethylene catheter was inserted through the needle into the joint. The needle was then withdrawn from the catheter, which was pulled tight at its proximal end and connected to a sterile 3-way plastic stopcock (Fig. 1). The tip of the catheter was carefully positioned so that a free exchange of fluid could be effected; the joint was then aspirated as completely as possible.

[1] C. R. Bard, Inc., Summit, N. J.

Intra-Articular Pressure (IAP).—One end of the stopcock was connected directly to a Statham P23AA pressure transducer which was positioned at the same level as the knee joint. This instrument had been cold sterilized with a 1,1000 solution of dimethylbenzylammonium chloride (Detergicide®),[2] flushed thoroughly and then filled with sterile, pyrogen-free P.S.S. After amplification, the signal was transmitted to a Mosely 680 linear recorder. The record was continuous except for brief intervals when material was either being injected or aspirated from the joint. A satisfactorily positioned catheter always showed minute rhythmical changes in IAP due to variations in the cardiac cycle, rising with systole and falling with diastole (Fig. 2 *a*). More marked changes in pressure coincided with changes in the respiratory cycle, pressure decreasing with inspiration and rising with expiration. These changes became greater as the mean IAP increased. If these excursions disappeared, the catheter was either totally blocked or not in the joint space; if they become attenuated at the same mean IAP, the catheter was partially blocked.

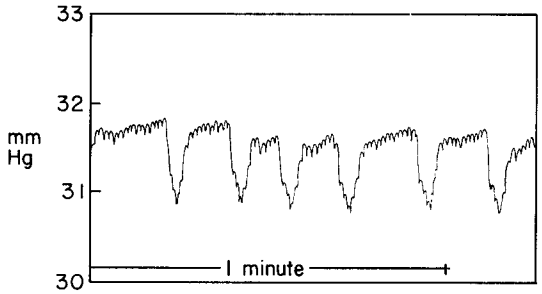

FIG. 2 *a*. Rhythmical changes in intra-articular pressure due to the cardiac and respiratory cycles were invariably present. The pressure increased with systole and expiration and decreased during diastole and inspiration. These changes were more pronounced as the baseline pressure rose and served as a reliable indicator of the location of the catheter tip.

Injection of radiopaque dye Hypaque®[3] at the end of several early experiments confirmed the intra-articular position of the catheter tip and its relationship to the observed rhythmical cardiac and respiratory pressure changes (Fig. 2 *b*). All IAP changes recorded herein are mean pressures.

Synovial Fluid Bulk pH.—In early experiments joint fluid pH was measured by aspiration of fluid into a 0.4 ml chamber in a specially constructed lucite block holding Beckman electrodes (Fig. 3). The temperature of the fluid was determined with a thermistor probe inserted into the chamber through an aperture drilled into the block; the pH reading was then corrected for temperature. This apparatus is strikingly similar to that devised by Cummings and Nordby for pH measurements in human joints (11). In later experiments all pH measurements were performed in triplicate on samples of approximately 20 lambda in either a Beckman or Radiometer micro pH assembly with a constant temperature water bath at 38°C. The joint fluid was mixed by gentle barbitage with the plastic syringe through the stopcock prior to sampling. Both methods yielded comparable results, although the Radiometer electrode proved most satisfactory. Simultaneous venous blood pH measurements were made to detect changes that might accompany changes in ventilation secondary to variation in depth of anesthesia. Although very little change in venous blood pH was actually detected during most

[2] C. R. Bard, Inc.

[3] Diatrizoate sodium, Winthrop Laboratories, New York.

experiments, the difference between synovial fluid pH and venous blood pH (ΔpH) is recorded here.

Concentration of leukocytes and crystals.—Total leukocyte counts were performed in duplicate from samples collected in 2 pipettes (4 counts) obtained periodically during an experiment; 0.3% sodium chloride solution was used as diluent. A differential count was obtained on

FIG. 2 *b*. The complex anatomical structure of the canine stifle joint is shown in this laterally exposed roentgenogram obtained after injection of radiopaque dye through the catheter. Reduction, one-half.

Wright's stained smears of fluid obtained at the end of most experiments. Counts of the relative number of intra- and extracellular crystals were done in over 50 experiments but because of the lack of correlation with the other parameters and the lack of precision, this technique was discontinued. When counts were performed using compensated polarized light under high dry magnification in a counting chamber (2), only the larger crystals were seen. Counts of crystals per 200 leukocytes may be performed on a "wet" preparation visualized under oil (5); however, small clumps of crystals, twinned and very small crystals, and the statistical hazards of generalizing from a small sample combine to make "crystal counting" an unreliable procedure, at least in our hands.

Materials Injected.—Most test injections were of a sterile suspension of sodium urate crystals, prepared as previously described (13). These were stored in dry form in 100-mg vials and were suspended in sterile, pyrogen-free P.S.S. just prior to use. Injections of P.S.S. alone were used as controls. In 2 of the P.S.S. trials, the needle had been inserted through a small skin incision; retraction of the edges of the wound insured passage of the needle through sterile subcutaneous tissue. Several experiments were performed without injecting anything through the catheter. The synovial reaction was usually monitored for 4 to 6 hr after injection; one experiment was followed for 12 hr.

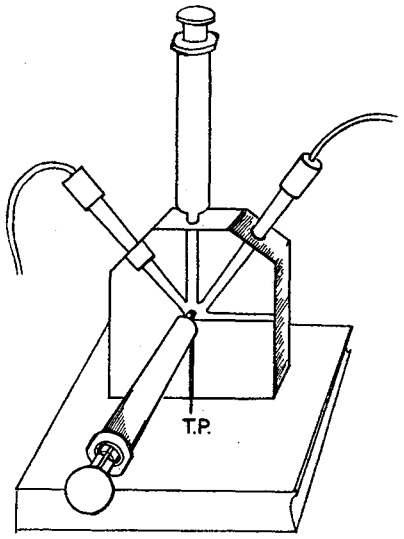

FIG. 3. A specially constructed chamber (0.4 ml) drilled from a solid block of lucite was used to measure pH in earlier experiments. Beckman electrodes were used and the temperature of the chamber contents was measured by a thermistor probe (T.P.).

RESULTS

Intra-articular Pressure (IAP).—IAP measured through a needle attached to a 3 way stopcock and inserted into a stifle joint flexed to 90° is approximately the same as atmospheric. A noninflamed joint aspirated to dryness through a catheter also showed atmospheric (or slightly subatmospheric) pressure. After injection of P.S.S. by itself or with suspended crystals, IAP rose; the magnitude of the rise was directly proportional to the volume injected. The pressure-volume relationship was quite constant for the 2 stifle joints of a given dog (Fig. 4 *a*) but the increase in pressure per unit volume injected varied considerably between dogs.

When a urate crystal suspension was injected, IAP fell for approximately 30 min coincident with resorption of P.S.S. from the joint (determined by serial aspiration of the joint to zero pressure with direct measurement of the volume).

After injection of P.S.S. alone, IAP usually fell gradually to zero (atmospheric) or lower over a period of several hours but sometimes increased slightly. After crystal injection however, IAP began to rise after 30 min (sometimes delayed to

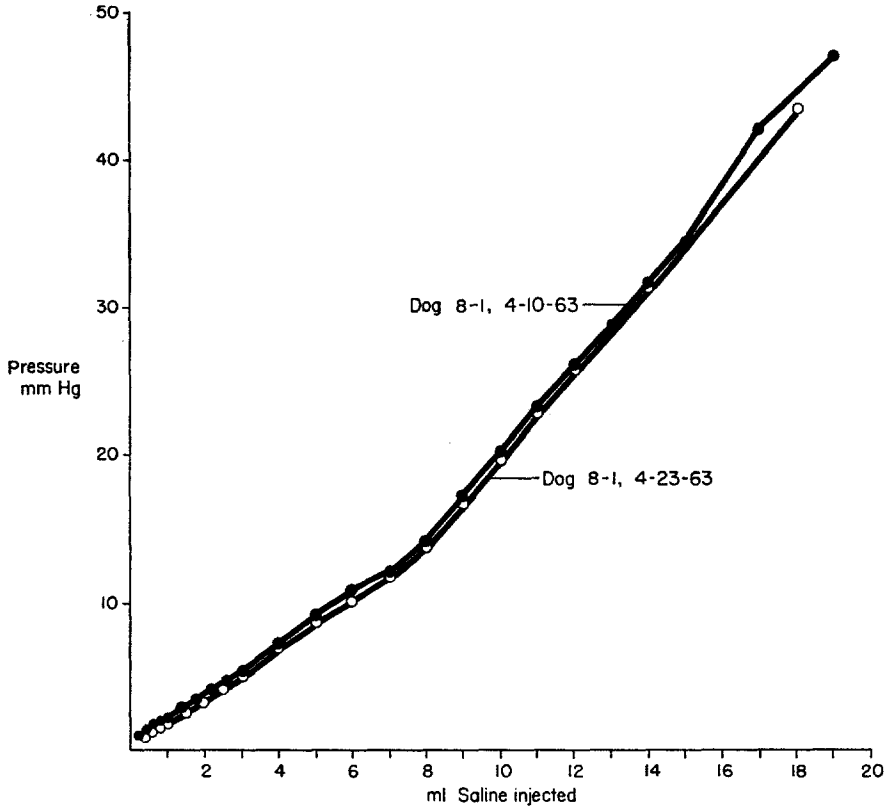

FIG. 4 *a*. Intra-articular pressure increased after injection of isotonic sodium chloride; the magnitude of the increase was proportional to the volume injected and was quite constant in the 2 joints of a given animal. The pressure-volume relationship is shown here for a stifle joint of 1 dog catheterized on 2 different days. Small aliquots of saline were injected sequentially. 90% of the total volume injected could be recovered through the catheter. The curves of "articular compliance" are nearly identical.

60 to 90 min). The pressures recorded in this paper represent the observed values minus the value recorded ½ hr after injection. This time was chosen as it nearly always coincided with the low point in the IAP after injection of the crystal suspension. Mean pressure changes observed after injection of urate crystals and of saline in opposite joints of 3 dogs are shown in Fig. 4 *b*. A period of rapid increase in IAP over the next 2 to 3 hr then followed. A gradual further

rise was sometimes observed; IAP began to fall gradually in one experiment followed for 12 hr (Fig. 4 *c*). Table I summarizes the IAP changes recorded 4 hr after injection. This time was selected as it invariably included the phase of rapid pressure rise.

The sequence of events described above were quite predictable and have been reproduced in over 100 dogs. The absolute value of the rise varied considerably

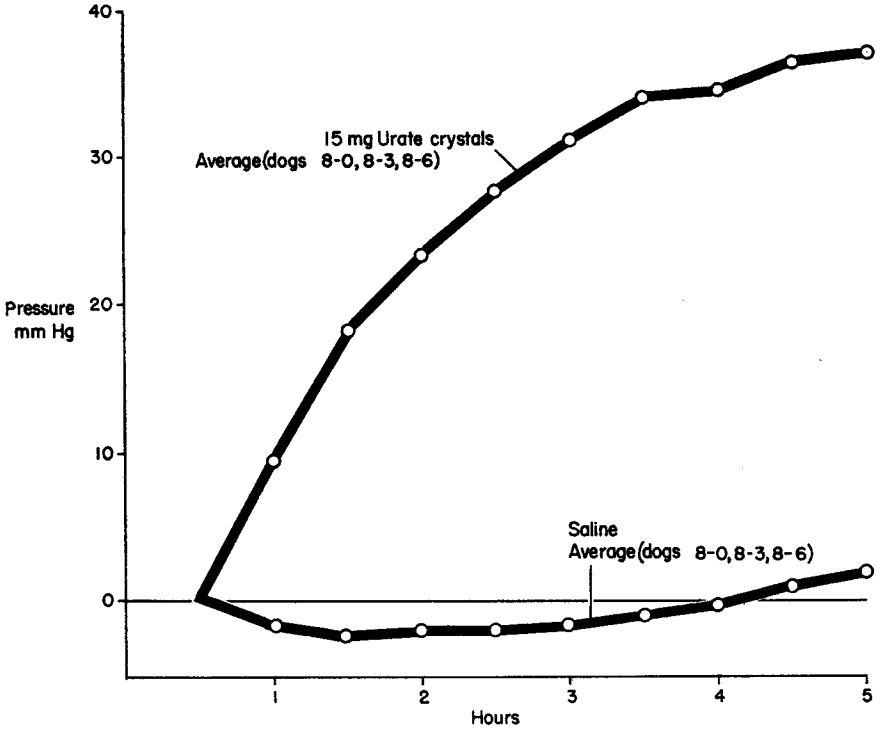

FIG. 4 *b*. Mean changes in pressure in the joints of 3 dogs following injection of urate crystals are compared to pressure changes after injection of isotonic saline into the opposite joints. The pressures are recorded as the observed pressure minus that observed ½ hr after injection.

between animals. In general, the pressure change varied with the dose of crystals injected as shown in Fig. 5 *a*, although overlap was noted (Table I). Variation in phlogistic potency between crystals synthesized at different times was noted; the reason for this is not understood but might be related to changes in size and to the number of crystals per milligram of crystal weight. The size of the joint injected also influenced the pressure rise, smaller rises usually occurred when the joints of large dogs were used. For these reasons it was not felt necessary or advisable to calculate the mean rise and standard deviation for the pressure

increases observed. This parameter is valid only when the animal serves as his own control. When both knees were injected either at the same or at different times, the IAP changes were nearly identical (Fig. 4 *c*). It should be pointed out however, that 2 dogs were encountered that had experienced previous trauma to 1 leg. The traumatized side showed a decreased inflammatory response as compared with the normal side. About 1 dog in 20 failed to react to

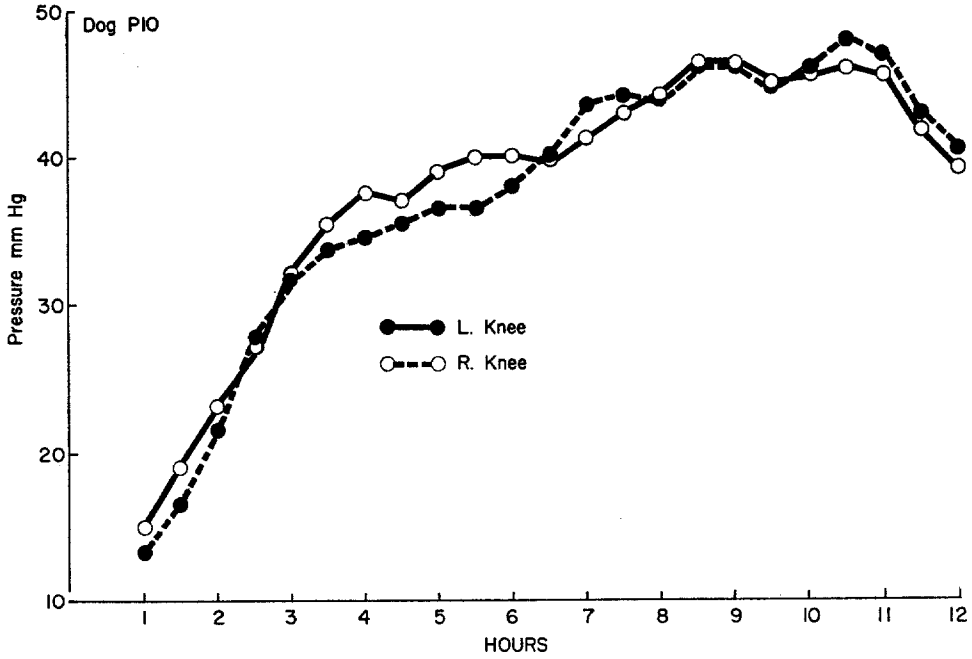

FIG. 4 *c*. Observed pressure changes over a 12 hr period in 2 stifle joints of 1 dog after each had been injected with 15 mg of sodium urate crystals.

intra-articular crystals at all, despite repeated attempts. The reason for this failure is not known at the present time.

Meaning of IAP Increase—Although a number of factors (e.g. blood flow to the limb, resistance of soft tissues about the joint etc.) may contribute to the IAP, the critical factor is volume change within the joint. In several experiments, the joint cavity was successfully aspirated to zero IAP periodically after crystals had been injected; the rise in IAP correlated very well with increasing volume of exudate. An example of this relationship is shown in Fig. 5 *b*. A more refined technique for intra-articular volume determination is needed as direct measurement is often not possible and is unphysiologic. The volume-pressure relationship was also demonstrated by the sequential addition of

TABLE I

Changes in Intraarticular Pressure, Leukocyte Concentration, and pH 4 hr after Injection of Isotonic Saline and Suspensions of Microcrystalline Sodium Urate and Calcium Pyrophosphate

Injected material	Volume	No. trials	ΔIAP*		WBC		WBC differential‡				ΔpH	
			Mean	Range	Mean	Range	N	L	M	Other	Mean	Range
	ml		*mm Hg*	*mm Hg*	*cells/mm³ × 10³*	*cells/mm³ × 10³*						
Nothing (catheter only)		2	0.5	0–1.0	No fluid obtained	—	—	—	—	—	—	—
0.9% NaCl	2–9	14	2.4	−6.5–7.0	2.4	0.3–4.1	78	7	4	11	±0.1	—
0.9% NaCl§	2	2	0.75	0–1.25	0.55	0.2–0.9	Not obtained				0	—
MSU‖-5 mg	2.5	2	12.3	11–13.5	19.1	9.5–20.2	96	3	1	0	0.27	—
MSU 10 mg	2–3	5	16.1	11.5–19.5	22.5	6.4–42.0	92	5	3	0	—	—
MSU 13 mg	2–3	11	17.3	11.0–34.0	27.1	7.3–54.0	90	6	3	1	—	—
MSU 15 mg	2–3	13	21.5	13.0–41.0	23.4	2.4–49.5	92	2	3	3	0.19	0.11–0.23
MSU 20 mg	2–3	14	30.0	12.0–62.0	22.3	6.6–62.0	92	1	4	3	0.17	0.04–0.53
MSU¶ 13 mg	2–3	2	15.8	15.3–16.3	32.6	27–38.2	94	0	2	4		
CPP** 15 mg	2–3	3	21.5	20.5–23.0	21.2	19–24	91	5	4	0	0.12	0.10–0.13

* Difference between observed values at 30 min and at 4 hr.

‡ Per cent based on 200 cell count of Wrights-stained smear: N, neutrophil; L, lymphocyte; and M, monocyte.

‖ Microcrystalline sodium urate. § Catheter inserted through skin incision.

¶ Chlorolose anesthesia.

** Calcium pyrophosphate dihydrate.

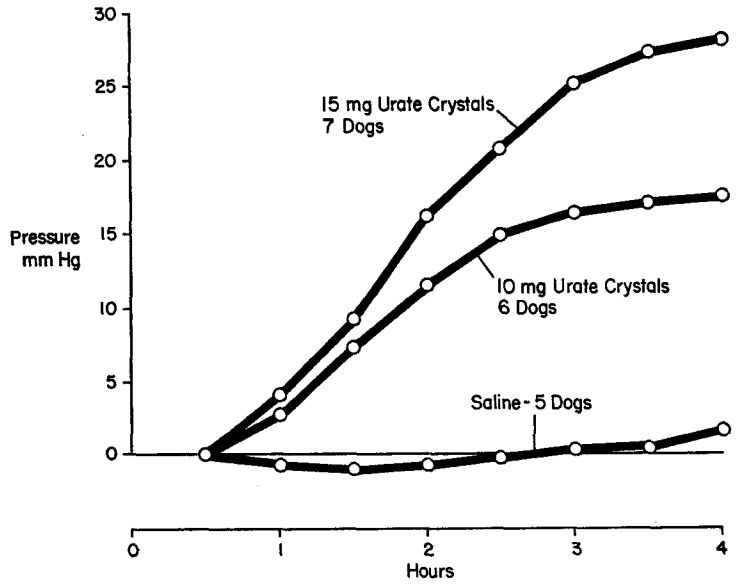

FIG. 5 *a*. Mean intra-articular pressure changes after injection of saline and 2 doses of urate crystals.

small volumes of P.S.S. to the joint over a short period of time. This curve is virtually identical in the same dog in both knees or in the same knee at different times (Fig. 4 *a*). If this procedure is carried out in an inflamed joint, the curve is displaced to the left, i.e. a greater pressure rise occurs per unit volume of P.S.S. added.

Effect of Position.—The effect of postural change on IAP has not been thor-

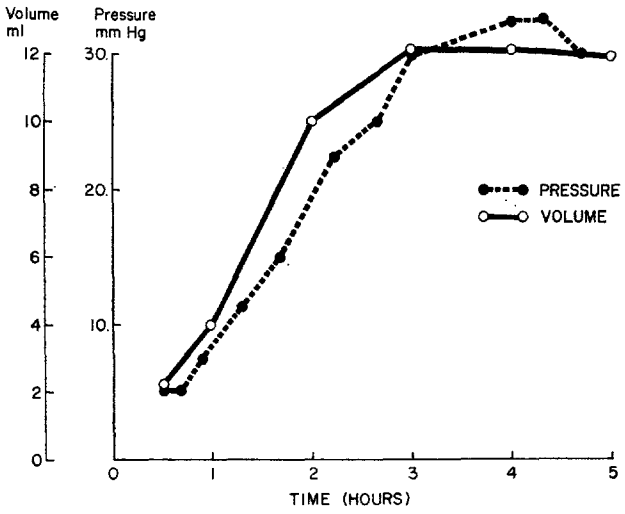

FIG. 5 *b*. The joint exudate was aspirated to zero intra-articular pressure periodically after crystal injection and was then replaced. The rise in pressure correlated with increasing volume of exudate.

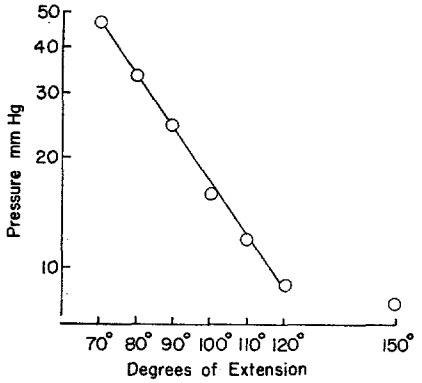

FIG. 5 *c*. Effect of change of position on intra-articular pressure. The joint was passively flexed and extended when the mean pressure at 90° was 22 mm Hg.

oughly evaluated in canine joints. As shown in Fig. 5 *c* however, the observed pressure is influenced greatly by flexion or extension of the joint. The effect of position on IAP has been shown in human joints by Eyring and Murray (14). In all experiments described herein the joint was fixed at 90° with adhesive tape.

Leukocyte (WBC) Response.—The total and differential WBC counts of normal synovial fluids aspirated during joint catheterization are shown in Table II. The WBC concentration rose progressively after crystal injection, reaching a maximum in 4 to 6 hr. The maximum occurred after the period of rapid pressure rise corresponding to the phase of rapid fluid exudation but before the

TABLE II

Leukocytes in Normal Canine Synovial Fluid

A. Total leukocytic counts per mm³ (hemocytometer)

Dog No.*	9	1–0	1-1	1-2	2-5	2-7	2-8	2-9
Right stifle	810	1025	360	875	425	1050	2375	400
Left stifle	950	1175	550	910	950	1040	2317	487

Mean of 50 other fluids, 633; overall mean§, 716; and range, 50 to 2725.

B. Differential leukocyte counts (42 fluids) (Wrights-stained smears)

	Polymorphonuclear	Lymphocytes	Monocytes	Histiocytes, synovial cells, and unidentified
Mean, %	32.4	28.7	17.7	20.2
Range, %	0–81	2–80	1–48	0–47

* Each value represents the mean of 2 to 4 counts.

§ 12,700 and 19,725 leukocytes were found in the left and right stifle joints of an otherwise healthy dog; these values are not included in the mean figure.

peak IAP had been attained. Examples of the WBC response are shown in Fig. 6 *a*. Correlation of leukocyte response and pressure rise is shown in an accompanying paper (9). In a reaction followed for 12 hr (Fig. 4 *c*), the WBC concentration peaked at 6 hr, decreasing to approximately one-half this level at 8 to 12 hr. A leukocyte response also followed P.S.S. injections but was much less than that following crystal injections (Fig. 6 *a*).

Synovial Fluid Bulk pH.—The initial pH of normal canine synovial fluid was identical to that of the blood, confirming the previous report of Joseph et al. (15). The pH of the injected crystal suspension varied between 7.0 and 7.2. Joint fluid pH rose to approximately that of the blood shortly after crystal injections during the phase of rapid exudation (16); a consistent decrease then occurred (Fig. 6 *b*). Data from reactions monitored for longer periods indicate that the maximum difference is attained at approximately 6 hr, correlating

with the peak WBC concentration. The calculated ΔpH (blood value minus fluid value) showed an excellent correlation with the WBC concentration as shown graphically in Fig. 6 *c*. The probability that this decrease was due to leukocytic metabolic activity is supported by the absence of a pH change after crystal injection into the joints of leukopenic animals (9). The mean and range

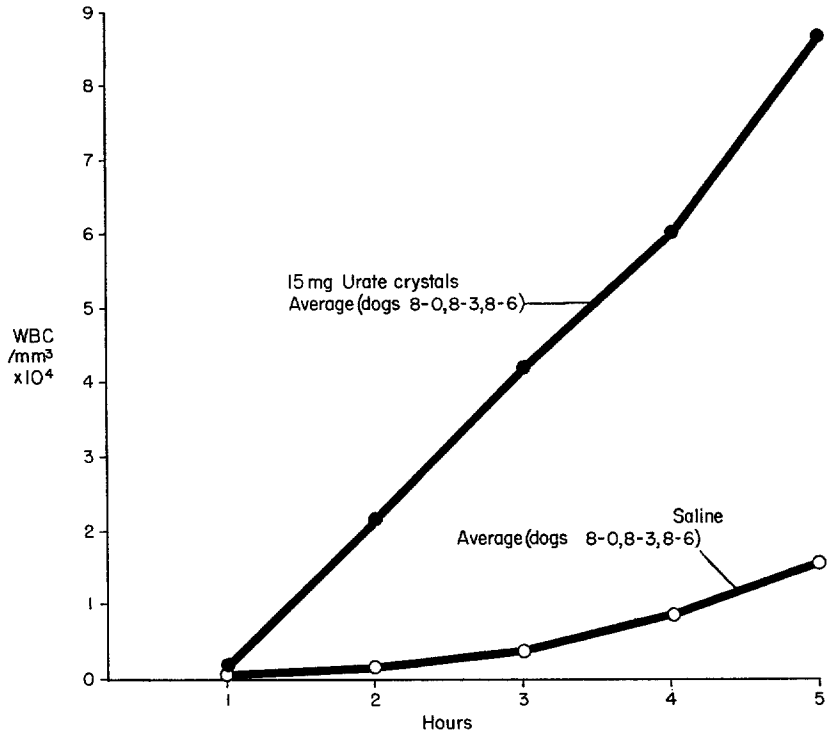

FIG. 6 *a*. Sequential leukocyte response in joint fluid exudate after injection of 15 mg sodium urate crystals; 0.9% sodium chloride solution injected into the opposite stifle joint of the same 3 dogs also attracted leukocytes but in much smaller number.

of observed change in pH 4 hr after injection of varying doses of crystals are recorded in Table I. Values as low as 6.91 were recorded.

DISCUSSION

The significance of the present work lies in the utilization of a convenient tissue space as the site of an induced inflammatory reaction. The joint cavity is considered to be a tissue space as the synovial lining cells are modified mesenchymal cells and do not constitute a true membrane (17). The stimulus is readily standarized by using a known milligram "dose" of crystals. The re-

action may be readily quantified with relatively simple equipment and the information obtained is physiologically meaningful. It has been pointed out by Moses et al. (18) that multiple parameters should always be used to quantify

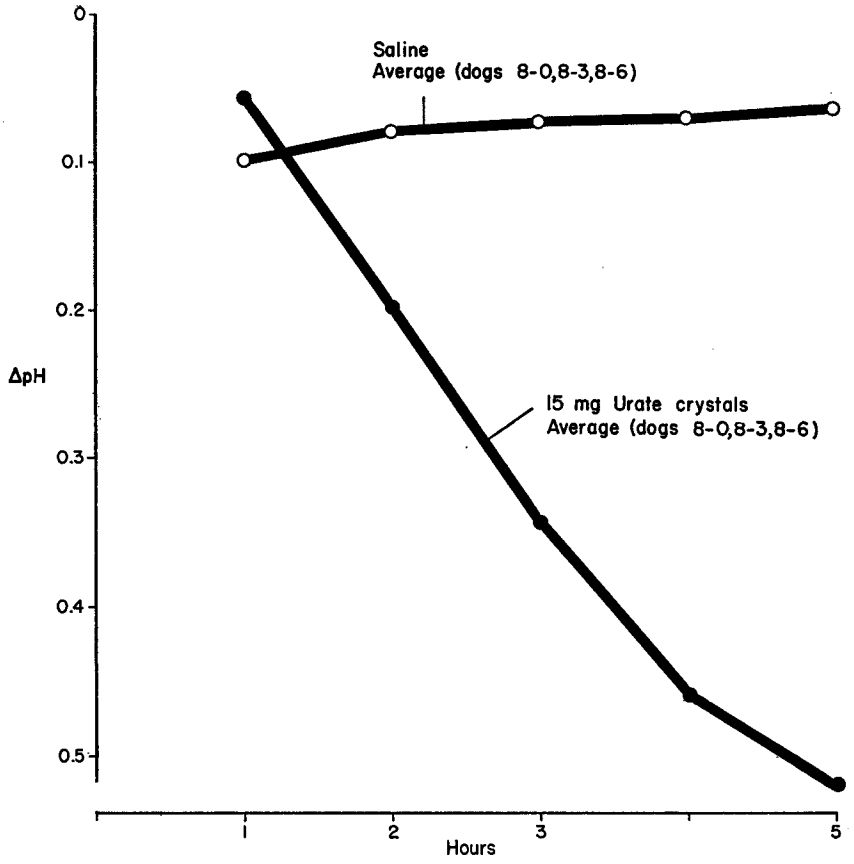

FIG. 6 *b*. Sequential change in hydrogen ion concentration (pH) in exudate from 3 joints injected with 15 mg sodium urate crystals; pH change in the opposite stifle joints in the same dogs was negligible after injection of 0.9% sodium chloride solution. The absolute pH changes were nearly identical to those shown here (which represent the difference between joint fluid pH and that of the venous blood measured simultaneously).

an inflammatory response as the cellular and noncellular exudates may not be directly related. In this model each animal served as its own control as the 2 stifle joints were, with rare exceptions, identical.

Although there is little doubt that change in joint pressure reflects volume change within the joint due to exudation of fluid from the blood, their precise

relationship awaits development of an accurate technique for serial measurement of intra-articular volume. Other factors, such as possible changes in the elastomeric properties of the surrounding soft tissues and joint capsule secondary to tissue edema and increased local blood flow, may also be important. These hypothetical "stiffness" factors probably could be measured when an accurate pressure-volume relationship is established.

The leukocytes entering the joint space after crystal injection were predominantly polymorphonuclear. The progressive increase in hydrogen ion concen-

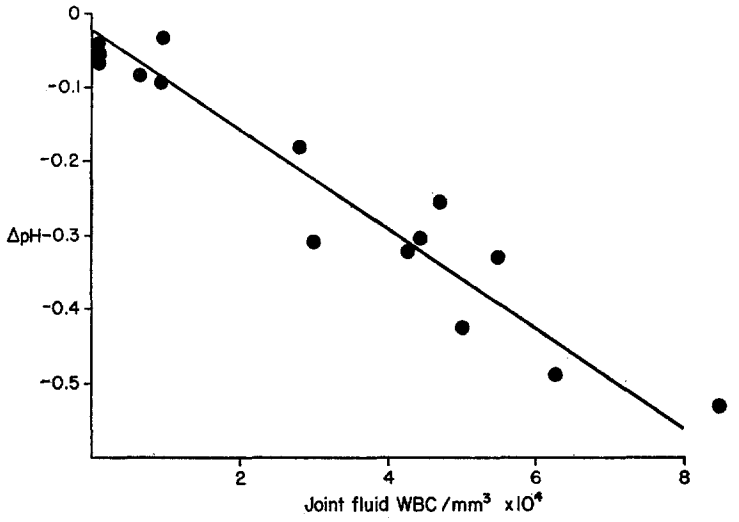

FIG. 6 *c*. The fall in pH of the exudate plotted against the leukocyte concentration showed excellent correlation.

tration with evolution of the acute reaction has been correlated with the leukocyte concentration in the joint space. No such pH change occurred after crystal injection in leukopenic animals (9). The production of lactic acid and its liberation into the surrounding medium has been described by Karnovsky (19), who also found that phagocytosis augmented lactic acid production. More recently, Seegmiller (20) noted a progressive fall in pH and an accompanying rise in lactic acid concentration in a sample of gouty joint fluid incubated in vitro. The pH fall was of the same order of magnitude as that found in the present in vivo study.

The degree of pain and tenderness can be estimated in this model by use of the unanesthetized dog (13). These parameters were measured and combined with the data obtained on the anesthetized dogs in a study designed to determine whether or not bradykinin mediates the inflammatory response to crystals (10).

Serial joint blood flow measurements have been made by periodic injection of small amounts of radioisotope into the joint through the catheter (21). Serial determination of enzymes, such as those indicative of lysosomal disruption (acid phosphatase, beta glucuronidase, and lysozyme) have also been made on the joint fluid exudate (21). These data will be reported elsewhere. In general, measurements reflecting *total* response proved more meaningful than those expressed per unit volume. For example, intra-articular pressure change reflected anti-inflammatory drug treatment more predictably than did leukocyte or pH changes. Again, a precise technique for intra-articular volume would allow estimation of *total* intra-articular leukocyte accumulation, *total* intra-articular enzyme activity etc. Applications of this model to the study of the mechanism of crystal induced inflammation are presented in the accompanying paper (9) and in reference 10.

SUMMARY

Injection of sodium urate or calcium pyrophosphate crystals into the stifle joints of anesthetized dogs almost invariably induced an acute exudative response. This response was quantified by serial measurements of intra-articular pressure, pH and leukocyte concentration. Pressure rose progressively and reflected intra-articular volume increase. The hydrogen ion concentration increased as the reaction progressed and correlated in a given exudate with the leukocyte concentration.

Analysis of sequential physiologic and biochemical changes occurring in this model of crystal-induced inflammation may provide insight into the mechanisms of acute gouty arthritis in man.

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