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Research Article

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Inorganic Pyrophosphate Pool Size and Turnover Rate in Arthritic Joints

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ABSTRACT Recent studies have shown elevated inorganic pyrophosphate (PPi) levels in most knee joint fluid supernates from patients with pseudogout (PG) or osteoarthritis (OA) and more modestly elevated levels in some supernates from patients with gout or rheumatoid arthritis (RA) relative to PPi levels found in the venous blood plasma of normal or arthritic subjects. We measured the intraarticular PPi pool and its rate of turnover to better understand the significance of the joint fluid–plasma PPi gradient. Preliminary studies in rabbits showed that [^{32}P]PPi passed from joint space to blood and vice versa without detectable hydrolysis. Incubation of natural or synthetic calcium pyrophosphate dihydrate (CPPD) microcrystals with synovial fluid in vitro in the presence of [^{32}P]-PPi tracer showed no change in PPi specific activity in the supernate over a 19-h period so that exchange of PPi in solution with that in CPPD microcrystals could be ignored. Clearance rates of [^{32}P]PPi and of [^{33}P]Pi, as determined by serially sampling the catheterized knee joints of volunteers with various types of arthritis over a 3-h period, were nearly identical. The [^{32}P]PPi/[^{32}P]Pi was determined in each sample. A mixture of a large excess of cold PPi did not influence the clearance rate of either nuclide. The quantity of PPi turned over per hour was calculated from the pool size as determined by isotope dilution and the turnover rate. The residual joint fluid nuclide was shown to be [^{32}P]PPi. The PPi pool was generally smaller and the rate of turnover was greater in clinically inflamed joints. The mean±SEM pool size (μ-moles) and turnover rate (percent/hour) in PG knees was 0.23±0.07 and 117±11.9, hydrolysis rate (%/h) to Pi was 27.7±13.2; in OA knees: 0.45±0.26 and 72 ±9.2, hydrolysis 6.9±0.9; in gouty knees: 0.8±0.41 and 50±11.6, hydrolysis 9.8±2.8; and in RA knees: 0.14 ±0.14 and 114±35.8, hydrolysis 236±116. PPi turnover (μmoles/hour) correlated with the degree of OA change present in the joint as graded by radiologic criteria irrespective of the clinical diagnosis. Mean PPi turnover in joints with advanced OA was greater than in those with mild or moderate changes ($P < 0.001$), but the mild and moderate groups showed no significant difference. We conclude that high synovial PPi turnover and elevated PPi fluid concentrations are not specific for PG patients, and that these factors alone cannot be the only determinants of CPPD crystal deposition.

INTRODUCTION

The deposition of calcium pyrophosphate dihydrate (CPPD) [1] microcrystals in articular structures and joint fluid has been called pseudogout (PG) by analogy with sodium urate crystal deposition disease (classic

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[1] *Abbreviations used in this paper:* CPPD, calcium pyrophosphate dihydrate; G, gout; [^{32}P]PPi, radioactive inorganic pyrophosphate; [^{33}P]Pi, radioactive inorganic orthophosphate; Pi, inorganic orthophosphate; PG, pseudogout; PPi, plasma inorganic pyrophosphate; RA, rheumatoid arthritis; SA, specific activity.

gout) (1, 2). This condition was originally called "articular chondrocalcinosis" by Zitnan and Sitaj, who used the characteristic radiologic appearance as the definitive diagnostic feature (3). The mechanism leading to formation of this peculiar mineral phase in articular tissues is unknown, but several groups of investigators recently have measured synovial fluid and plasma inorganic pyrophosphate (PPi).

Russell et al. first reported that joint fluid PPi in PG averaged eight times the mean levels of plasma or of control joint fluids (4). The mean and range of PPi in these control fluids and in control and PG plasma were essentially that of normal plasma. All values in PG joint fluid exceeded the highest control value. McCarty, Solomon, Warnock, and Paloyan extended these observations in reporting that the mean joint fluid PPi levels in PG were about five times that of control fluids, but overlap of values between PG and controls were found (5). All of the above data were obtained using radioisotopic dilution and anion exchange column chromatographic separation of PPi.

Newer analytical methods have been applied to further study of joint fluid PPi levels. Altman, Muniz, Pita, and Howell (6), using an adaptation of the uridine diphosphoglucose pyrophosphorylase method of Johnson, Shanoff, Bass, Boezi, and Hansen (7), found elevated PPi levels in osteoarthritis (OA) and gouty synovial fluids and even higher levels in fluids from patients with PG, with respect to fluids from patients with inflammatory types of arthritis and normal or patient plasma. These newer findings have been corroborated by those of Silcox and McCarty using isotopic dilution and yeast inorganic pyrophosphatase (8, 9). They found comparable PPi elevations in PG and OA synovial fluids and elevated levels in some patients with gout (G) and rheumatoid arthritis (RA), as compared to PPi values in normal or patient plasma. PPi levels were found to be significantly higher in chronically symptomatic PG joints than in actively inflamed joints, and in OA the PPi levels were correlated with the degree of joint degeneration as determined by X-ray examination.

These findings together suggest that elevated PPi levels may occur in joints afflicted with different types of arthritis and that this elevation, if related to the formation of CPPD crystals, may account for their occurrence in some patients with RA (10, 11), G (2, 10, 12) or OA (1, 2, 13). In any event, the weight of evidence suggests a local articular, rather than a systemic metabolic disturbance of PPi metabolism. The occurrence of CPPD crystal deposits in individuals with perfectly normal joints on clinical and radiological examination and the well-defined hereditary forms of CPPD deposition disease (14–16) may be taken as evidence that a "primary" form of this condition exists.

The present studies were done to determine the intra-articular pool size and turnover rate of PPi in patients with PG or with other types of joint disease, assuming that the calculated PPi turnover would serve as an index of local joint PPi production.

METHODS

Materials. Disposable plastic ware or acid washed glassware was used throughout; all solutions were prepared in doubly-distilled deionized water. Radioactive inorganic pyrophosphate ([^{32}P]PPi) with specific activities ranging from 795 to 5,520 μCi/μmol and radioactive inorganic orthophosphate ([^{32}P]Pi) with specific activity of 50 μCi/μmol were obtained from New England Nuclear, Boston, Mass. Calcium pyrophosphate dihydrate crystals prepared by the method of Brown, Lehr, Smith, and Frazier (17) were rendered pyrogen-free by heating to 200°C for 2 h. New Zealand white rabbits were used for the qualitative experiments described below. All radioisotopic counting was done in a Packard Tricarb Liquid Scintillation Counter Model 3320, Packard Instrument Co., Inc., Downers Grove, Ill. Reagents included: bovine testicular hyaluronidase, type IV, Sigma Chemical Co., St. Louis, Missouri, and heparin-liquaemin sodium "100", Organon Inc., West Orange, N. J.

Preliminary experiments. (*a*) Rate of exchange of PPi in solution with mineral phase PPi in CPPD microcrystals. The following experiment was performed to determine whether exchange occurred rapidly enough to influence the apparent clearance rate of intrasynovially injected [^{32}P]PPi: the CPPD crystals in 20 ml of synovial fluid obtained from a patient with definite PG were pelleted by centrifugation at 38°C (20,000 *g* for 30 min). This procedure will pellet more than 99% of synthetic [^{45}Ca]CPPD microcrystals (9). The pH of the fluid had been adjusted to 7.4 with 1 N HCl after prolonged stirring at 38°C. A few crystals of thymol were added for bacteriostasis. 15 ml of crystal-free supernate was transferred to a 50-ml Erlenmeyer flask containing a 10-mm magnetic stirring bar, and 20 mg of synthetic CPPD crystals were added. The remaining fluid, containing all the natural CPPD crystals, was transferred to a second flask. Both flasks were covered with parafilm, placed in an incubator at 38°C, and stirred at a constant rate for 20 min. 10 μl of stock [^{32}P]PPi containing 0.025 μmol of PPi was then added to each flask. 3-ml samples were removed from each flask at varying times up to 19 h later, and after the addition of 16 U of hyaluronidase (in 5 μl), incubated for 15 min at 38°C. This enzyme was used here and later to reduce the viscosity of joint fluid, thereby increasing the efficiency of centrifugation. The crystals in each sample were then separated at 38°C by centrifugation at 20,000 *g* for 30 min. All procedures after separation were performed at 4°C or in crushed ice. 2 ml of supernate was diluted to 5 ml and assayed for PPi by isotopic dilution (8); the percentage of nuclide that had hydrolyzed to [^{32}P]Pi was determined by isobutanol extraction as described by Hall (18) and modified by Russell, Bisaz, Donath, Morgan, and Fleisch (19). The pellet was resuspended briefly in ice-cold water and centrifuged again at 20,000 *g* for 30 min, dissolved instantaneously in 0.3 ml of concentrated HCl, immediately diluted to 5 ml with sufficient NaOH to achieve a pH of 11–12, and analyzed for PPi. A 200-μl sample was

TABLE I

Clinical Data Relevant to Patients Studied

Study no.	Patient	Age	Sex	Diagnoses	Joint	Clinical state*	X-ray grade (23)
		y					
1	W. S.	68	M	PG	LK	C	4
2	W. S.	68	M	PG	RK	C	2
3	W. S.	68	M	PG	RK	C	2
4	H. B.	69	F	PG	LK	C	5
5	H. B.	69	F	PG	LK	C	5
6	H. L.	61	F	PG	LK	C	3
7	H. L.	61	F	PG	LK	C	3
8	O. J.	64	M	PG	LK	C	2
9	O. J.	64	M	PG	LK	C	2
10	L. P.	63	F	PG	RK	C	2
11	H. F.	79	M	PG	RK	C	1
12	I. B.	76	F	PG	RK	C	2
13	D. S.	42	M	PG	LK	SA	0
14	M. W.	42	F	PG	LK	SA	3
15	H. M.	65	F	PG	RK	SA	2
16	H. M.	65	F	PG	LK	A	3
17	W. B.	70	M	PG	RK	SA	3
18	A. B.	61	F	G	LK	C	1
19	J. W.	72	M	G	RK	C	4
20	J. W.	72	M	G	RK	C	4
21	N. B.	48	M	G	LK	SA	0
22	S. F.	40	M	G	RK	SA	4
23	JMcD.	40	M	G	RK	A	0
24	B. H.	55	F	OA	RK	C	1
25	L. S.	74	F	OA	RK	C	4
26	MV. D.	65	F	OA	LK	C	1
27	A. B.	53	F	OA	LK	C	1
28	P. D.	54	M	OA	LK	C	1
29	E. E.	66	M	OA	LK	C	0
30	S. C.	73	F	OA	RK	C	4
31	O. R.	51	M	OA	RK	C	2
32	L. J.	35	F	OA	RK	C	1
33	S. B.	57	F	OA	RK	C	3
34	A. C.	77	M	RA	LK	SA	3
35	R. G.	54	F	RA	RK	SA	2
36	M. R.	64	F	RA	LK	SA	3

* C, chronic; SA, subacute; A, acute.

taken for the determination of the extent of PPi hydrolysis after the protein had been precipitated with TCA (8).

(*b*) Qualitative assessment of [^{32}P]PPi diffusion into joint fluid from blood and vice versa. Approximately 1 μCi of [^{32}P]PPi was injected into the stifle (knee) joint of a normal rabbit with minimal trauma through a no. 25 gauge needle. Blood was collected from a femoral vein catheter that had been inserted before isotope injection. The reverse experiment was performed in another animal, with nuclide injected through a catheter into the femoral artery and radioactivity determined in 2 ml of 0.9 NaCl that had been injected previously into the joint space. The percentage of nuclide present as [^{32}P]Pi was determined in each instance on deproteinized samples using the isobutanol extraction method (18).

Patients. 36 studies were performed in one or both knee joints of 29 volunteers, all patients were from the arthritis clinic or in-patient service at the University of Chicago. 17 studies were done in all patients with definite PG by previously published criteria (20). 10 patients with chronically symptomatic OA, 5 patients with G proven by specific identification of monosodium urate crystals (21), and 3 patients with definite RA by the criteria of the American Rheumatism Association (22) were studied. "Acute" or "subacute" is used to designate joints that were tender with detectable increased warmth of the overlying skin and with a synovial fluid leukocyte concentration above 5,000/cm. "Chronic" is used to refer to joints in patients with G or PG that were indistinguishable chemically from symptomatic OA joints. The grade of degenerative change in each knee

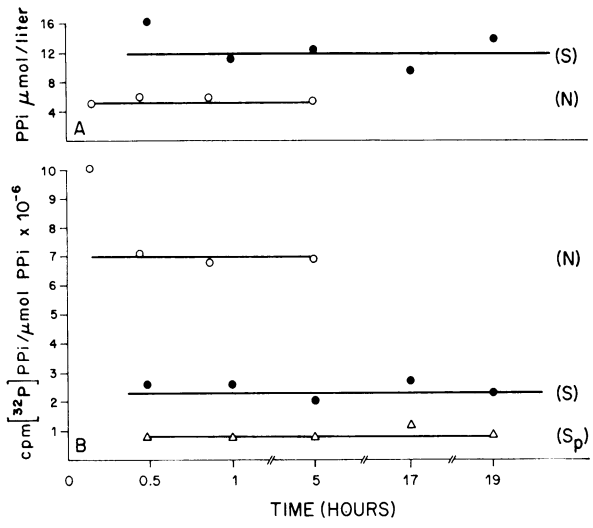

FIGURE 1 (A) The concentration of supernatant PPi in synovial fluid incubated with natural (N) or synthetic (S) CPPD crystals is plotted against time. There was essentially no change despite the presence of crystals in both samples. (B) The specific activity of supernatant PPi in synovial fluid incubated with natural or synthetic CPPD crystals is plotted against time. Again essentially no change is apparent, even with incubation up to 19 h. Sufficient synthetic crystals were present in all pellets to permit analysis; the specific activities here were quite low and were multiplied by 10^4 to permit graphing. The [^{32}P]PPi in the pellets may have been trapped supernate. See text for details.

was determined using the roentgenographic criteria of Kellgren and Lawrence (23). The procedure was explained and informed consent obtained in each instance. Most studies were performed in the out-patient department, but some were performed in the General Clinical Research Center. A summary of clinical data relating to the patients studied is given in Table I.

Joint catheterization. The knee joint was catheterized and the end of the catheter connected to a three-way stopcock as described previously (24).

Nuclide clearance rate. The patient remained supine for the 3-h study period. The joint fluid was aspirated as completely as possible into a large sterile plastic syringe. Approximately 2 μCi of [^{32}P]PPi and in most instances 2 μCi of [^{33}P]Pi in a total volume of 1 ml of 0.1 M Tris Cl buffer, pH 7.4, sterilized by passage through a millipore filter (Swinnex R13 filters, Millipore Corporation, Bedford, Mass.), were mixed thoroughly with the joint fluid using the three-way stopcock. The fluid was then reinjected into the joint (time zero). After 30 min, a 200–400-μl sample was obtained after three partial aspirations and reinjections were performed; 5 μl heparin was added to a 200-μl portion of the sample. Such samples were obtained at 15-min intervals for the next 2.5 h. 3 h after isotope injection, the joint was emptied as completely as possible and the volume measured. A 20-ml sample of femoral arterial blood was obtained at this time for PPi and Pi analysis as previously described (8). A 50-μl portion of each sample was counted for both ^{32}P and ^{33}P in a solution containing toluene 2,010 ml, PPO 16.5 g, POPOP 0.3 g, and 990 ml Triton X100 per 3 liters of solution. The amount of each isotope present was calculated using the channels ratio method to correct for the mutual spillover of each isotope. The correction factors were determined for each synovial fluid specimen by adding an internal standard of [^{32}P]PPi to a portion of each odd numbered sample and an internal standard of [^{32}P]Pi to a portion of each even numbered sample. Both the original samples and those containing internal standards were counted for at least 2 cycles. Stability was invariable provided sufficient water was added to bring the total volume to 1 ml.

5 μl of hyaluronidase (1 mg/ml) was added to the 200-μl heparinized portion of each sample and incubated at room temperature for 10 min. After deproteinization with 6 N $HClO_4$ (final acid normality 0.5), the percentage of [^{32}P]Pi was determined using isobutanol extraction of phosphomolybdate as described by Hall (18).

The rates of clearance of [^{32}P]PPi and [^{33}P]Pi were then calculated after plotting the natural logarithms of the cpm/unit volume of each isotope against time. The observed data were analyzed by the digital computer program of Berman, Weiss, and Shahn (25, 26) adapted to computer CDC 3,800. Steady-state conditions were assumed throughout the study period.

It was assumed also that [^{32}P]Pi produced by hydrolysis was leaving the joint at the same rate as [^{33}P]Pi, and that

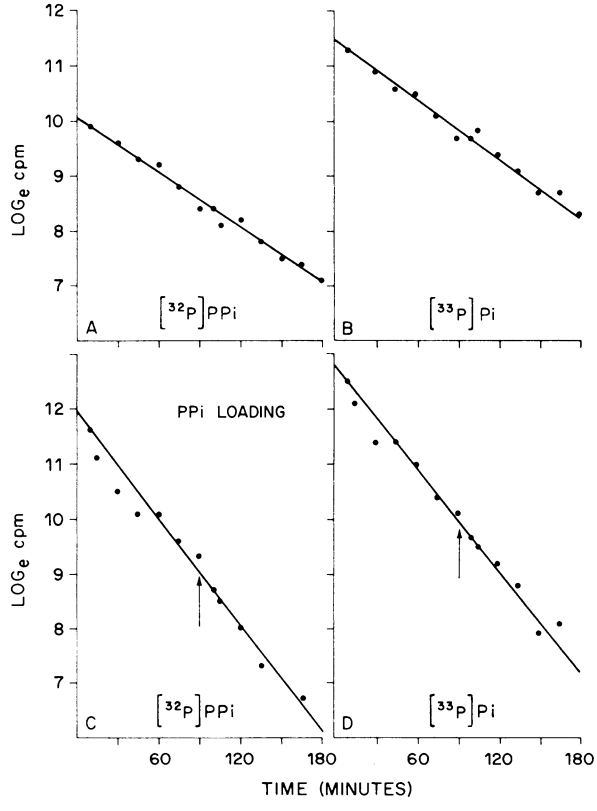

FIGURE 2 (A and B) Representative clearance curves of [^{32}P]PPi and of [^{33}P]Pi determined simultaneously are shown. The rates of clearance of the two isotopes were nearly identical. (C and D) A mixture of a relatively large quantity of cold PPi (100 μmol) during the experiment (arrows) had no effect on the rate of clearance of either nuclide, suggesting that clearance of PPi was by simple diffusion. See text for details.

TABLE II

Pi and PPi Pool Size and Turnover Rate in Patients with PG

Study no.	PPi		Pi		Volume distribution		Observed JF volume	Pool size		Turnover rate/hour		Hydrolysis rate
	P	JF	P	JF	[^{32}P]PPi	[^{33}P]Pi		PPi	Pi	PPi	Pi	
	μM		mM		ml		ml	μmol		%		%/h
A. Chronic inflammation												
1	4.3	10.4	1.23		116			1.20		91		
2	1.0	8.5	0.81	0.79	43		40.0	0.35		50		
3	1.6	9.4	0.99	0.92	19	27	12.0	0.17	24.8	105	108	7.5
6	2.4	6.4	0.92	1.05	10	12	2.0	0.07	12.0	130	107	0.3
7	2.2	10.6	1.05	1.06	9	7	7.0	0.09	7.5	65	115	11.5
8	1.4	3.4	1.12	0.96	156	84	110.0	0.39	94.0	88	135	10.6
9	1.2	2.3	0.77	0.88	128	68	55.0	0.28	59.5	87	59	142
10	1.6	5.1	0.98	0.98	5	9	9.4	0.04	4.6	164	188	10.1
11	0.4	4.3	0.89	0.88	16	8	9.2	0.03	13.9	142	135	23.5
12	1.7	5.8	1.08	1.08	86	63	8.0	0.47	67.8	78	96	8.8
$\bar{x}$	1.78	6.6	0.98	0.96	58.6	34.6	28.1	0.31	35.5	100	118	26.8
±SE	0.31	0.88	0.04	0.03	17.0	10.5	11.2	0.10	11.2	11.2	13.1	16.6
B. Acute and subacute inflammation												
13	1.6	2.9	0.88	0.86	26	18	25	0.07	16.1	98	97	0.2
14	1.8	3.5	1.28	1.25	69	81	15	0.23	101.0	198	173	10.7
15	2.0	4.3	1.05		20		36	0.08		171		
16	1.8	1.6	1.38	1.42	12		9	0.02		95		
17	0.7	2.4	1.05	1.08	13	9	5	0.03	9.3	190	164	79.1
$\bar{x}$	1.6	2.9	1.13	1.15	27.9	25.8	18	0.09	42.1	150	145	30.0
±SE	0.21	0.42	0.08	0.10	9.6	18.9	5	0.03	24.5	22.4	24.0	24.7
C. All studies												
$\bar{x}$	1.67	5.39	1.03	1.02	48.4	35.0	24.5	0.23	37.3	117	125	27.7
±SE	0.24	0.75	0.04	0.05	12.4	9.2	7.5	0.07	10.5	11.9	11.6	13.2

JF, joint fluid.

the [^{32}P]Pi was distributed in the same compartment as [^{32}P]PPi. The hydrolysis rate (λh) was calculated directly by fitting the observed appearance of [^{32}P]Pi to the best curve with the computer and then introducing the rate constants of disappearance of [^{32}P]PPi (λpp) and of [^{33}P]-Pi (λp). The model being therefore:

$$[^{32}P]PPi \xrightarrow{\lambda h} [^{32}P]Pi \longrightarrow \lambda p$$
$$\lambda pp - \lambda h$$

Effect of PPi concentration on clearance rate. The right knee of patient W. S. was restudied. After 90 min, 1 ml of a sterile, freshly prepared solution of sodium pyrophosphate in water containing 100 mmol per liter was mixed thoroughly with the joint fluid aspirate and reinjected. The effect on the clearance rate was noted. The pH of the joint fluid, collected under oil, was determined before and after mixing with the concentrated PPi, using a Radiometer pH Meter Model 27 (Radiometer Copenhagen) with a microelectrode.

Identity of nuclide not extracted with isobutanol. 12 samples of synovial fluid (6 PG, 2 G, and 4 OA) were studied to ascertain that the ^{32}P not extracted with isobutanol was indeed [^{32}P]PPi rather than nuclide transferred to glucose or similar phosphate acceptor. In each case 5 × 1-ml samples of fluid were incubated at room temperature with hyaluronidase, 2.5 mg/ml of fluid. One sample contained 200 U of yeast inorganic pyrophosphatase, $MgCl_2$ in a final concentration of 6 mM, and EDTA in a final concentration of 2.5 mM. Another contained EDTA and Mg in the same concentrations without enzyme, and the others contained either EDTA or Mg without enzyme. An additional sample was incubated with 0.9% NaCl (wt/vol) only. All samples were brought to the same volume with 0.9% NaCl. After a 3-h incubation, the percent hydrolysis to Pi was determined by isobutanol extraction (18).

RESULTS

Preliminary experiments. (*a*) Rate of exchange of PPi in solution with PPi in CPPD microcrystals: There was no detectable change in specific activity (SA) of PPi in supernatant joint fluid incubated with either natural or synthetic CPPD crystals during incubation periods up to 19 h (Fig. 1). The decrease in the SA of supernatant PPi exposed to natural CPPD crystals noted between 10 and 30 min was probably due to incomplete mixing of nuclide because the concentration of PPi, as measured chemically, did not change. The SA of PPi in the washed pellets containing synthetic crystals was also constant; only the 17-h pellet of natural crystals contained sufficient PPi for analysis. It contained 0.06 μmol of PPi; unfortunately, the super-

TABLE III

Pi and PPi Pool Sizes and Turnover Rate in Patients with OA

Study no.	PPi		Pi		Volume distribution		Observed JF volume	Pool size		Turnover rate/hour		Hydrolysis rate
	P	JF	P	JF	[^{32}P]PPi	[^{32}P]Pi		PPi	Pi	PPi	Pi	
	μM		*mM*		*ml*		*ml*	*μmol*		%		*%/h*
A. Chronic inflammation												
24	2.7	6.3	1.02	1.18	28		42	0.15		60		
25	1.2	4.9	0.89	0.88	6		13	0.02		80		
26	2.7	5.9	0.73	0.86	4		4	0.00		80		
27	1.8	2.9	1.17	1.04	71		71	0.18		50		
28	2.6	5.0	1.02	1.01	64		30	0.24		57		
29	2.5	3.6	0.79	0.81	118	107	30	0.45	86.0	66	72	6.8
30	2.4	12.5	0.95	0.96	203	126	45	2.90	120.0	50	51	6.5
31	2.3	2.3	0.84	0.77	32	11	14	0.05	8.0	85	79	10.1
32	2.2	3.2	1.08	1.03	24	28	12	0.10	28.4	145	152	4.9
33	1.5	3.7	1.05	1.05	126	89	70	0.40	93.4	47	44	6.2
$\bar{x}$	2.1	5.0	0.95	0.95	67.6	72.0	33.1	0.45	67.1	72	79.6	6.9
±SE	0.15	0.87	0.04	0.03	19.0	20.5	7.1	0.26	19.1	9.2	19.2	0.9

JF, joint fluid.

nate of this sample was lost. The PPi concentration in the fluid incubated with synthetic CPPD was greater than with the natural crystals, even after only 50 min of incubation. The rapidity of this apparent dissolution was greater than we have found subsequently using ^{45}Ca-labeled synthetic CPPD crystals.[2] Despite this discrepancy, the data support the concept that the equilibrium between PPi in solution and PPi present in the mineral phase is sufficiently slow enough that for the purposes of the kinetic studies described here, it can be ignored.

[^{32}P]PPi diffusion into and from the joint space. (*b*) Approximately 1% of the dose injected into a rabbit knee appeared in the femoral vein blood collected for 23 min after nuclide injection. 0.2% of intra-arterially injected [^{32}P]PPi appeared in the 0.9% NaCl in the ipsilateral knee joint 7 min later. There was no detectable hydrolysis to Pi in either experiment. Although these data are not quantitative or definitive, they

[2] Bennett, R. M., J. R. Lehn, and D. J. McCarty. Factors affecting the solubility of calcium pyrophosphate dihydrate crystals. Submitted for publication.

TABLE IV

Pi and PPi Pool Sizes and Turnover Rate in Patients with G

Study no.	PPi		Pi		Volume distribution		Observed JF volume	Pool size		Turnover rate/hour		Hydrolysis rate
	P	JF	P	JF	[^{32}P]PPi	[^{32}P]Pi		PPi	Pi	PPi	Pi	
	μM		*mM*		*ml*		*ml*	*μmol*		%		*%/h*
A. Chronic inflammation												
18	2.7	5.1	1.37	1.50	43		25	0.20		24		
19	3.0	7.9	1.03	1.13	347	194	40	3.1	219	58	79	7.5
20	1.7	13.8	1.05	1.24	224	45	48	1.3	28.4	23	33	5.3
$\bar{x}$	2.5	8.9	1.15	1.29	205	119.5	37.7	1.5	123.7	35	56	6.4
±SE	0.33	2.1	0.09	0.09	73	53.2	5.6	0.70	68.1	11.5	23	1.1
B. Acute and subacute inflammation												
21	2.4	2.4	0.84	0.80	23		30	0.05		100		
22	2.1	5.5	1.10	1.08	37	30	30	0.20	32.5	54	53	8.5
23	2.1	2.6	0.74	0.76	41	28	26	0.10	20.9	44	48	17.8
$\bar{x}$	2.2	3.5	0.89	0.88	33	28.6	28.7	0.12	26.7	66	50.5	13.1
±SE	0.08	0.83	0.09	0.08	5	0.71	1.11	0.03	4.1	17	2.5	4.6
C. All studies												
$\bar{x}$	2.3	6.2	1.0	1.1	119	74.0	33.2	0.80	75.2	50	53	9.8
±	0.18	1.6	0.98	0.11	51	34.7	3.4	0.41	41.5	11.6	9.6	2.8

JF, joint fluid.

TABLE V

Pi and PPi Pool Size and Turnover Rate in Patients with RA

Study no.	PPi		Pi		Volume distribution		Observed JF volume	Pool size		Turnover rate/hour		Hydrolysis rate
	P	JF	P	JF	[^{32}P]PPi	[^{33}P]Pi		PPi	Pi	PPi	Pi	
	μM		mM		ml		ml	μmol		%		%/h
34	0.32	0.37	1.07	0.98	64	86	35	0.01	84.5	118	79	93.6
35	1.46	0.09	1.44	1.48	14	22	7	0.001	32.9	174	126	567.0
36	1.66	5.58	1.00	1.04	96	48	11	0.51	49.5	50	105	48.2
$\bar{x}$	1.15	2.01	1.17	1.17	57.7	51.8	17.7	0.14	55.6	114	103	236
±SE	0.35	1.5	0.11	0.13	19.8	15.4	7.2	0.14	12.7	35.8	13.6	166

JF, joint fluid.

suggest that PPi can pass into and from joints without being hydrolyzed in the process.

Clearance rates of [^{33}P]Pi and [^{32}P]PPi. The clearance of both isotopes was monoexponential (Fig. 2). A second exponential gave no significant improvement. It was deduced from the monoexponential disappearance that [^{32}P]PPi and [^{33}P]Pi were disappearing from a single compartment. The rate constant of disappearance was exactly equal to the exponent of the monoexponential function. The pool sizes of both [^{33}P]Pi and [^{32}P]-PPi were generally greater than the observed joint fluid volumes, as might be expected (Tables II–V). Clearance rates could not be calculated in two studies in one patient with neurotrophic joints (patient H. B.) presumably because the severe anatomical disorganization prevented adequate mixing. All patients tolerated the procedure well except one subject with OA (L. J.) who had pain for 48 h after the study. Her synovial fluid remained sterile and it was assumed that the injected solution contained bacterial pyrogen.

Infusion of 100 μmol of cold PPi during one study had no influence on the clearance rate of either isotope (Fig. 2C and D), suggesting that PPi was leaving the joint by simple diffusion rather than by active transport.

Turnover rate of PPi from the intra-articular pool. The turnover of PPi per unit time was calculated from the pool size and the turnover rate and is shown in μmoles per hour for each study in Fig. 3 irrespective of the degree of inflammation present or the clinical diagnosis. All values were separated into three groups according to the extent of radiographically evident degenerative change. The three values from the rheumatoid joints were omitted from this figure because the classification scheme of Lawrence and Kellgren cannot be applied meaningfully. The mean of the group with advanced (grade 4) degeneration was significantly greater than that in the group with mild (grade 0-1) or moderate (grade 2–3) changes ($t = 3.73$ and 3.87; $P < 0.001$), but there was no significant difference between the mild and moderate groups ($t = 1.10$, $0.2 < P < 0.4$). Duplicate studies were possible in four joints. A precision of ±16% was found.

Hydrolysis rates. The rates of hydrolysis varied considerably, being greater in inflamed joints, e.g. G

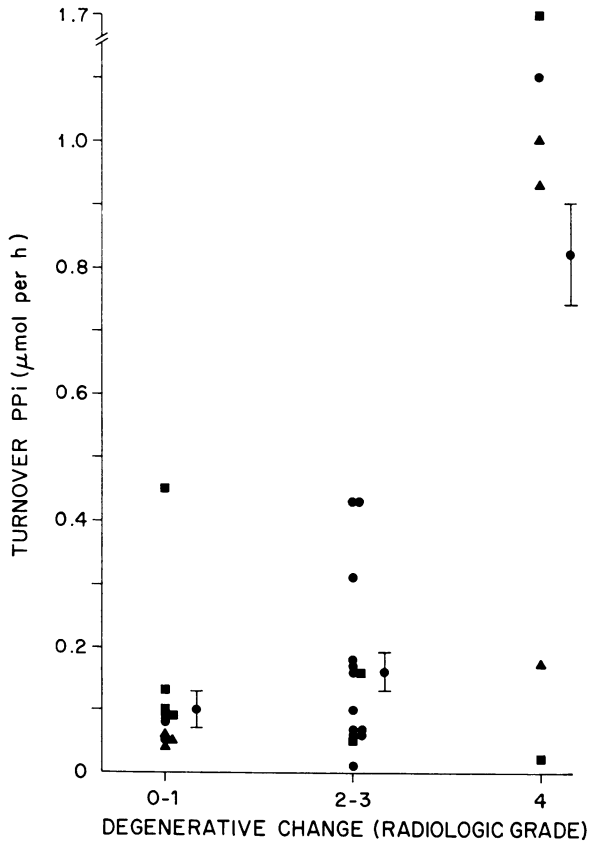

FIGURE 3 The turnover of PPi in the synovial pool, calculated from the pool size and the clearance rate, is plotted for each joint studied according to the degree of degenerative change as estimated radiographically. ▲, G; ■, OA; ●, PG. It should be emphasized that OA changes existed in many joints here labeled as "G" or "PG" on the basis of specific crystal identification.

TABLE VI

Effect of Yeast Inorganic Pyrophosphatase Treatment of 12 Synovial Fluids

	In vitro incubation (3 h) 25°C					
Added material	PSS only	EDTA + PSS	Mg + PSS	Mg + EDTA + PSS	Mg + EDTA + PPi + PSS	In vivo incubation (3 h)
PG	20.5±1.9	25.2±1.6	42.5±11.4	79.4±4.1	93.1±1.4	8.4±2.9
C*	17.2±1.3	36.6±6.1	37.9± 7.3	75.5±8.3	92.7±2.3	16.6±1.8

All values given as percent ^{32}P present as Pi (±SEM). See text for details.
* Control, two G and four OA fluids.

and RA, and smaller in chronically symptomatic, mildly inflamed joints, e.g. OA (Tables II–V). That the nuclide not extracted with isobutanol was [^{32}P]Pi was shown by incubation under varying conditions in vitro (Table VI). It is evident that certain cations, probably calcium, inhibit the pyrophosphatases in joint fluid, that magnesium is needed as a cofactor for some of them, that hydrolysis in most joint fluids is indeed very slow, and that hydrolysis to Pi and not phosphate transfer is the predominant biochemical reaction that occurs. Only 8% of the ^{32}P present could not be accounted for either as Pi or PPi.

DISCUSSION

The data presented here agree with recent reports that the PPi concentrations in most joint fluids exceed that of plasma, irrespective of the type of arthritis present (6, 9). This is the first comparison of PPi levels in joint fluid with platelet-poor plasma prepared from femoral arterial blood; all previous investigations have used antecubital venous blood for such comparisons. Two of the joint fluids studied here showed joint fluid PPi concentrations that were the same as (study no. 16 in a patient with acute PG) or less than (study no. 35 in a patient with RA) that in the plasma perfusing the joint. The mean turnover rates of the intra-articular PPi pool was greater in RA and clinically inflamed PG joints than it was in OA and chronically symptomatic PG joints, but the values overlapped greatly so that the differences were not as great as one might have predicted from published estimates of joint blood flow in RA and OA joints (27–29). On the other hand, even inflamed G joints showed no increase in PPi turnover as compared to OA. It would be of interest to compare the clearance rates of anions such as PPi or Pi with that of a marker cation to see if diffusion is selectively impaired. The approximately 50% increase in mean turnover rate in inflamed vs. chronically symptomatic joints probably accounts for the lower joint fluid PPi levels in the former (Table II and reference 9).

The dissolution rates of synthetic CPPD microcrystals in joint fluid are so slow that it is a matter of several days before apparent saturation is reached. As the intra-articular PPi pool turns over much more rapidly, it is apparent that CPPD crystal dissolution in vivo can contribute little to the PPi concentration of joint fluid. That physical exchange between the PPi in CPPD crystals and PPi in solution could not be detected over a period of 19 h (Fig. 1) supports this conclusion. Whether CPPD crystals would appreciably alter joint fluid PPi concentration if incubated over a much longer time is not known.

Preliminary data relative to the apparent solubility of PPi in joint fluid, obtained by incubation of synthetic CPPD labeled with ^{45}Ca, indicates that the joint fluid levels found by ourselves and others are undersaturated with respect to such crystals. If substantiated by further work, the site of nucleation and growth of CPPD crystals is clearly not in synovial fluid despite the frequency of their presence there. Cartilage and similar avascular tissues seem more likely to be the sites of primary formation of mineral phase.

The size of the intra-articular PPi pool and its rate of turnover is not specific for any disease entity. Perhaps, as in the hyperuricemia of G, local elevation of the PPi concentration is a necessary but not sufficient cause of crystal deposition. The analogy with G soon breaks down, as unlike the studies on uric acid pool and turnover, we have no control studies on normal joints. Nor do we really know that the joint space is different with respect to PPi metabolism than any other intercellular space. Deposits of CPPD have been found in the ligamenum muchae (30) and in the dura mater (31).

We can conclude that the elevated PPi concentration in joint fluid, the intra-articular PPi pool size, and PPi turnover rate are not specific for PG. We can also conclude that the rate of turnover greatly exceeds the rate of crystal dissolution and that the rate of exchange of PPi in solution with that incorporated in microcrystalline CPPD is not measurable over a period of many hours.

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