

Temperature and biochemical studies of joint inflammation

A preliminary investigation

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Clinical studies of the temperature of inflamed joints have led us to seek a relationship between temperature and biochemical changes in arthritis. This report describes a preliminary investigation into the presence of kinins and acid phosphatase in synovial fluid during the course of inflammatory arthritis, with parallel measurement of surface temperature.

JOINT TEMPERATURE

The surface pattern of raised skin temperature due to vasodilation over an inflamed joint is well shown by thermography (Cosh, 1966; Cosh and Ring, 1967), but this technique does not easily permit quantitative measurement. In this study, therefore, we have preferred the simpler method of radiometry, which measures the temperature of an area of skin by detection of infra-red radiation. The skin temperature over the patella is an approximate guide to the intra-articular knee temperature, although 2 to 3°C. lower (Lloyd-Williams, 1969). We have shown, for example, that the skin temperature over the patella may fall as much as 3°C. after injection of steroid into an inflamed knee joint (Lloyd-Williams, Ring, and Cosh, 1968) and it is such observations that have prompted us to look for biochemical changes in the synovial fluid associated with the rise or fall of temperature in inflammatory arthritis.

KININS

A number of biologically active agents are known to produce changes resembling those of inflammation *e.g.* histamine, 5 hydroxytryptamine, and kinins, and of these we have selected kinins for study as they are known to be potent inducers of pain, vasodilation, and increased vascular permeability (Armstrong, Jepson, Keele, and Stewart, 1957). Kinin has been found in synovial fluid in rheumatoid arthritis (Melchiorri, 1963) and appeared to correlate well with the severity of the inflammation in selected

patients (Melmon, Webster, Goldfinger, and Seegmiller, 1967). Jasani, Katori, and Lewis (1969) measured the kinin-forming potential in synovial fluid from different types of arthritis and thought it unlikely that kinin release played a major part in joint inflammation; the presence of kininase too in synovial fluid suggests that, if kinins are formed in the synovial tissue or fluid, their presence must be transient. However, all of these reports are based on single measurements made in individual patients, so that an attempt to measure kinin levels in synovial fluid in serial fashion seemed justified.

LYSOSOMAL ENZYMES

A relationship between lysosomal enzymes and kinins has often been suggested in the genesis of inflammation. Both kinins and lysosomal enzymes may be involved independently, or they may act through one another. Some lysosomal hydrolases are capable of forming kinin from the inactive precursor (Greenbaum, Yamafuji, and Kim, 1966) and conversely other lysosomal enzymes, the carboxypeptidases, are capable of destroying kinins (Greenbaum and Yamafuji, 1966). The relationship between kinin formation and destruction and lysosomal enzymes is complex, the result being decided by such factors as the pH of the immediate surroundings. Lysosomal enzymes may also bring about release of histamine from mast cells (Seegers and Janoff, 1966; Janoff and Schaefer, 1967) which may contribute to increased capillary permeability.

Reports linking raised synovial fluid levels of lysosomal enzymes with activity of inflammation in rheumatoid arthritis have come from Jacox and Feldmahn (1955) (β -glucuronidase), Weissmann (1966), and Caygill and Pitkeathly (1966) (β -acetylglucosaminase and acid phosphatase). We have confirmed that the average level of free lysosomal enzyme in joint fluid from cases of rheumatoid arthritis is higher than that from cases of osteoarthritis, but we found considerable overlap between

the ranges of levels in the two conditions, as have previous authors. A factor that has not been studied is the individual patient's usual range of lysosomal enzyme concentration in the synovial fluid. We have found this to differ widely between patients, but to be relatively stable for the individual patient.

The levels of lysosomal enzyme activity cited by previous authors are based almost entirely on analysis of single samples of synovial fluid from individual patients, and only occasionally have enzyme estimations been repeated. The figures obtained must therefore be influenced both by the individual patient's usual level (undetermined) and by any variation in this level associated with fluctuations in the disease. We have shown that certain individuals have higher lysosomal enzyme levels during a non-inflammatory period than others during an inflammatory episode. For the readings to be meaningful, a patient's usual range of enzyme levels must, therefore, be determined by estimates from serial samples of synovial fluid.

Purpose of present study

To make serial measurements of the level of lysosomal enzyme activity, as indicated by acid phosphatase, and of kinins in synovial fluid from patients with inflammatory arthritis, in parallel with temperature measurements of the affected joint.

Material and methods

The subjects of this pilot study were five patients with persistent knee effusions undergoing in-patient treatment at this hospital. Two had rheumatoid arthritis and they had five joint aspirations each; two had psoriatic arthropathy (eight and four aspirations respectively); one had chondrocalcinosis with a possible but unproven crystal synovitis (five aspirations).

The following estimations were made serially on the affected knee joints and correlated with joint temperature in four of the five patients:

Synovial fluid volume
Protein content
Acid phosphatase (E.C.3.1.3.2.) activity
Kinin content

Joint temperature

The temperature of the skin over the centre of the patella was measured with the radiometer described by Ring and Cosh (1968). The radiometer is sensitive to infra-red radiation in the wave length 0.25 μ . It was positioned 10 cm. above the centre of the patella, and received radiation from an area of skin 1 cm. in diameter. The temperature of the skin was read on an attached meter to the nearest 0.1°C. The joint under investigation was exposed to the temperature of the laboratory (20°C.) for at least 10 minutes before the temperature was taken. No direct sunlight or intense artificial light was allowed to fall on the area of skin whilst the skin temperature was being taken.

Aspiration of joint fluid

Synovial fluid was aspirated under aseptic conditions into sterile plastic syringes. For kinin estimation, 2 or 4 ml. synovial fluid were withdrawn and then ejected as quickly as possible into a polythene tube containing approximately three times this volume of a mixture of ethanol/0.002 M oxalic acid 8 : 2 (Greenbaum, Yamafuji, and Hosoda, 1965) cooled to -10°C . The synovial fluid was mixed for 5 minutes by inversion and shaking. The precipitate was spun down, and the supernatant poured into a plastic Petri dish, and evaporated to dryness in a flow of cold air. The residue was stored at -20°C . for not more than 3 days before being assayed for kinin.

Fluid for other biochemical estimations was withdrawn immediately after the fluid for kinin estimation. It was cooled to 4°C . and centrifuged at 20,000 G. for 30 minutes at 4°C . The cell-free fluid was then stored at -20°C . until required (usually within 3 days). The joint was always aspirated to dryness and the volume recorded.

Estimation of acid phosphatase

Acid phosphatase activity was measured by the method of Huggins and Talalay (1945). Cell-free fluid was diluted with three times its volume of distilled water. Incubations were carried out at 37°C . in a shaking water bath. Each reaction tube contained the following mixture: 0.5 ml. p-nitrophenyl phosphate (disodium tetrahydrate) 4 mg./ml., 0.5 ml. 0.1 M citric acid buffer pH 4.8, and 0.5 ml. diluted synovial fluid. Incubation was carried on for 1 hour, when the reaction was stopped by the addition of 2.5 ml. 0.1 N sodium hydroxide. The p-nitrophenol released in the reaction was read at 410 μm . It was necessary to run reagent and synovial fluid blanks with the reaction. The enzyme activity was expressed as μ M of p-nitrophenol, released by 1 ml. synovial fluid per hour.

Protein

This was estimated by the method of Lowry, Rosebrough, Farr, and Randall (1951). The results were compared with a standard solution of bovine albumin, and expressed as g. protein per 100 ml. synovial fluid.

Kinin

This was estimated by its effect on the isolated rat uterus. Female Wistar rats of 250-300 g. were injected intramuscularly with 100 μg . stilboestrol in arachis oil 12 to 24 hours before use. One or both horns of the uterus were suspended in Ringer Locke solution at 29°C . in a 5-ml. bath. The residue from the alcoholic extract of the synovial fluid was reconstituted with a convenient volume of Ringer Locke, and contractions evoked by doses of this solution were compared with contractions produced by a solution of standard bradykinin (Sandoz). Using this preparation, concentrations of kinin down to 0.05 ng./ml. of bath fluid could be detected.

Results

Case 1. Rheumatoid arthritis

A woman aged 69 had a 2-year history of sero-positive rheumatoid arthritis. When she was admitted to hospital

in the previous year the disease was already widespread, and she had an effusion and radiological changes in the left knee; she started on a regime of methyl prednisone, 8 mg. daily, and ACTH 60 units weekly.

Present admission

Her main complaint was of pain in the left knee where there was a small effusion.

Blood examination showed Hb 13 g. per cent. (88 per cent.); erythrocyte sedimentation rate 60 mm/hr (Westergren); W.B.C. 5,000/cu.mm.; Waaler-Rose test strongly positive with a titre of 1 : 2048.

The knee was aspirated five times. On the day of the first aspiration, oral methyl prednisone and ACTH were stopped and weekly depot injections of methyl prednisone were substituted (see Fig. 1). At the time of the third aspiration, after the withdrawal of fluid, 50 mg. prednisolone trimethyl acetate (TMA) was injected.

Fig. 1 shows the biochemical changes in the synovial fluid. The improvement after the injection of 50 mg. prednisolone TMA was such that there was insufficient fluid available for full analysis. A rising temperature and protein content after the withdrawal of ACTH were followed by a falling temperature and protein content after the intra-articular steroid injection. There was little change in acid phosphatase levels. Kinins were not found in any specimen.

Case 2. Rheumatoid arthritis

A woman aged 65 had an 8-year history of rheumatoid arthritis. She started oral prednisone in 1966, but later developed diabetes, and prednisone was stopped in 1968. This was followed by a relapse of the arthritis and she was increasingly disabled by lesions in the knees, wrists, and fingers.

Hospital admission in 1969

She had a persistent effusion in the left knee, which was aspirated five times.

Blood examination showed Hb 12 g. per cent. (81 per cent.); erythrocyte sedimentation rate 54 mm/hr (Westergren); W.B.C. 8,000/cu.mm.; Waaler-Rose test not carried out.

The only regular drug therapy was an analgesic, dextropropoxyphene with paracetamol. She had a transient acute febrile respiratory infection and was confined to bed for 5 days and given penicillin (Fig. 2).

Synovial fluid analyses showed some association between joint temperature and protein concentration, while acid phosphatase levels appeared unrelated to either. Kinins were not found in any specimen. A fall in synovial fluid protein was noted after the period of rest with respiratory infection. The final measurements, made on the day of discharge, showed a rise in both joint temperature and synovial fluid protein.

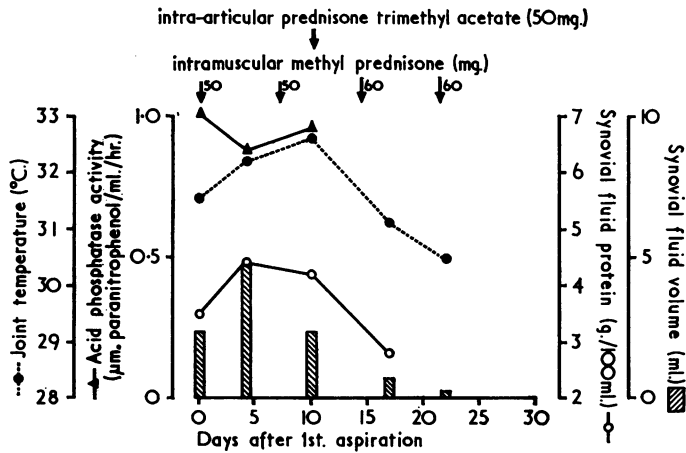


FIG. 1 Case 1. Rheumatoid arthritis. Analysis of synovial fluid from acutely inflamed left knee.

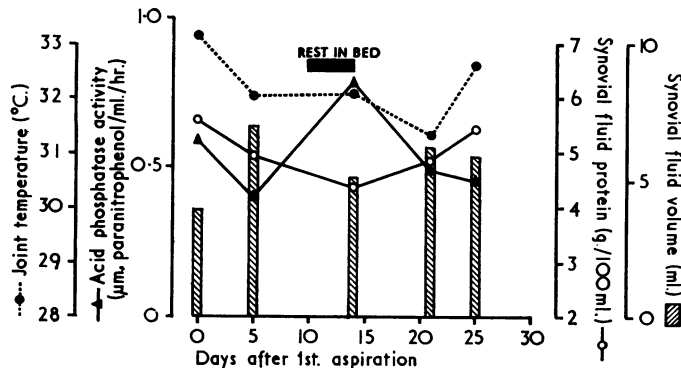


FIG. 2 Case 2. Rheumatoid arthritis. Analysis of synovial fluid from acutely inflamed left knee.

Case 3. Psoriatic arthropathy

A man aged 49 had a 5-year history of psoriasis, well controlled by topical steroid applications and occlusive dressings. He had no previous joint involvement, but 3 days before his admission to hospital, he became ill with fever, rigors, and pain and effusion in the left knee; this was followed 2 days later by similar pain and effusion in the right knee.

Hospital admission

He was still febrile (38.5°C.) with tense effusions in both knees.

Blood examination showed Hb 14.4 g. per cent. (97 per cent.); erythrocyte sedimentation rate 76 mm./hr. (Westergren); W.B.C. 5,000/cu.mm. (polymorphs 84 per cent.); serum uric acid 4.6 mg. per cent.; Waler-Rose test borderline positive at 1 : 32.

Cultures of blood and synovial fluid were sterile. No crystals were seen in the joint fluid under polarized light. There was no evidence of Reiter's disease.

The patient steadily improved on a conservative regime with plaster splints, simple analgesics, repeated aspirations, and no antibiotics or steroids. The first joint aspiration (Fig. 3) was made on the third day in hospital, and a new analgesic, 'Mervan',* was tried from the 6th to the 13th hospital day, after spontaneous improvement had already started (Fig. 3). The effusions recurred on the 17th day, without pain or fever, and again resolved with rest, analgesics, and aspirations. A second course of 'Mervan' was given on the 20th to the 27th day. He was discharged symptom-free and walking on the 33rd day, when a final aspiration of each knee was made. The most likely diagnosis was thought to be psoriatic arthropathy.

Analysis of the eight fluid aspirates from the left knee (Fig. 3) showed some similarities between the volume withdrawn, its protein content, and the temperature of the skin over the patella. The level of acid phosphatase activity in the fluid bore no relation to these other parameters. Kinins were not found in any specimen of joint fluid.

* Mervan—4 allyloxy 3 chlorophenylacetic acid, Continental Pharma, Brussels 5.

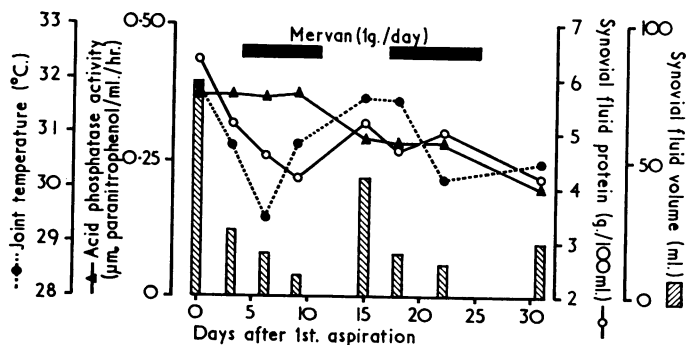


FIG. 3 Case 3. Psoriatic arthropathy. Analysis of synovial fluid from acutely inflamed right knee.

Case 4. Chondrocalcinosis and crystal synovitis

A woman aged 78, free of previous joint disease, was unwell for 2 weeks before admission, with episodes of shivering. On the day before admission the right knee became acutely painful and swollen.

Hospital admission

She was febrile (38.3°C.) and had a tense, extremely painful effusion in the right knee with a popliteal cyst. The other joints were painfree.

Blood examination showed Hb 13.7 g. per cent.; (93 per cent.); serum uric acid 4.5 mg. per cent.; Waler-Rose test borderline positive at 1 : 32.

Aspiration of the right knee yielded 50 ml. apparently purulent fluid, and treatment with intramuscular penicillin was started immediately, but cultures showed that the synovial fluid was in fact sterile. Subsequently x rays showed chondrocalcinosis with calcification in the menisci in both knees, as well as in the joint capsules, suggesting the presence of crystal synovitis, but when the fluid was examined for crystals none was found. The patient steadily improved with rest in bed, a plaster rest splint for the knee, and repeated aspirations. Phenylbutazone was given as an analgesic.

Analyses for protein and acid phosphatase were made on five specimens of fluid from the right knee, the first on the third hospital day. The first sample of fluid collected was not included in this study. No temperature readings were possible owing to the patient's immobility through pain and frailty. A steady decrease in synovial fluid protein was seen (Fig. 4, overleaf), but there was no parallel between the volume of fluid withdrawn and its protein content. There was a similarity between the falling acid phosphatase activity and the protein concentration, which contrasts with the findings in Cases 1, 2, and 3.

Case 5. Psoriatic arthropathy

A woman aged 43 years had a history of psoriasis for 21 years and arthritis for 19 years.

Hospital admission

There was florid psoriasis on the body and limbs, and a polyarthrititis involving fingers, elbows, knees, and toes. There was an effusion in the right knee. The skin lesions

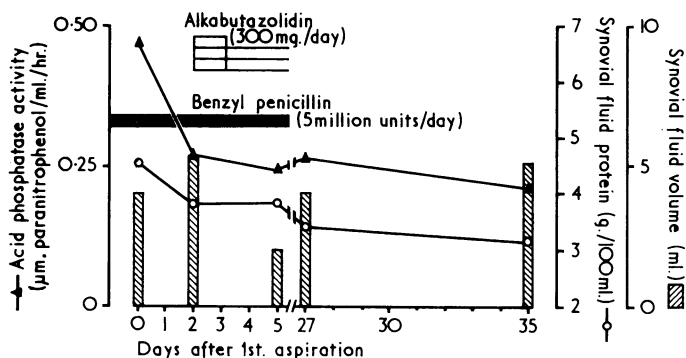


FIG. 4 Case 4. *Chondrocalcinosis.* Analysis of synovial fluid from acutely inflamed right knee.

were treated with topical steroid and Ibuprofen was given as an analgesic.

Blood examination showed Hb 10.7 g. per cent.; erythrocyte sedimentation rate 79 mm./hr (Westergren); W.B.C. 13,000/cu.mm. (polymorphs 86 per cent.); Waaler-Rose test negative.

The right knee was aspirated four times, intra-articular steroid being given on the second occasion (Fig. 5).

Analysis of the synovial fluid aspirated showed a steady fall in the volume of the effusion. This was accompanied by a fall in protein concentration, the rate of which increased after intra-articular injection of steroid. Both measurements were associated with a slow fall in joint temperature. The rate of decrease of joint temperature was not apparently affected by intra-articular steroid injection. Although acid phosphatase activity showed a continuous downward trend over 20 days, its fall did not closely follow either the joint temperature, the protein concentration of the synovial fluid, or the volume of the effusion. Injection of intra-articular steroid appeared to effect a temporary halt in the drop in acid phosphatase activity. A considerable drop in joint temperature was measured on the 27th day after the first aspiration, when the joint was found to be dry.

Discussion

A feature of the data shown in Figs 1, 2, 3, and 5 is the similarity between the timing of the changes in the levels of the joint temperature, the volume of the joint effusion, and the protein content of the synovial fluid. It is of interest, therefore, that the

acid phosphatase activity, studied as an indicator of lysosomal enzyme activity, did not apparently follow the same trends as these other parameters. On this evidence, the peak of the acute inflammatory phase, as measured by any one of the indicators, temperature, volume of effusion, or protein content of the synovial fluid, did not coincide with a peak of lysosomal enzyme activity. Inspection of the Figures shows, however, that in general the level of acid phosphatase activity did not regain its initial value during the period under study, even at times when other features of inflammation increased. Rather, there was a tendency towards a gradual fall in acid phosphatase activity, suggesting that the first recorded level represents a maximal, or even already decreasing, lysosomal enzyme activity. In Case 2 the acid phosphatase activity was raised in the sample of fluid taken at the third aspiration. This level may have been influenced by the respiratory infection contracted by the patient at this time. The fact that the activity returned to the initial level 7 days later tends to support this argument.

We would therefore suggest that, if increased lysosomal levels are involved in this acute inflammatory condition, their peak values may precede the other signs of inflammation. Further, the subsequent changes in acid phosphatase levels do not appear to follow the secondary increase in inflammation detected during the course of the study

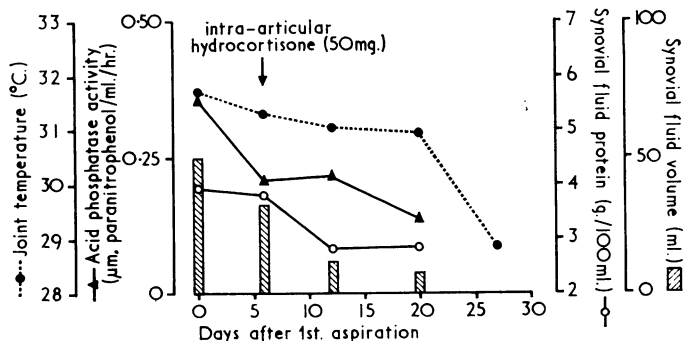


FIG. 5 Case 5. *Psoriatic arthropathy.* Analysis of synovial fluid from acutely inflamed right knee.

(Fig. 2, fifth aspiration; Fig. 3, fifth aspiration). In the human subject it is impracticable to measure the time, relative to the clinical signs of the condition, at which the lysosomal enzyme levels begin to increase, or to measure their rate of increase.

The results show that the 'between patient' variation of the acid phosphatase level is considerable, although levels in individual patients lie in a fairly narrow range. It is clear, therefore, that the changes in the levels of acid phosphatase activity in the individual must be interpreted within the range appropriate to that individual and that estimations of single values will have little meaning on an absolute scale.

In this work, no kinin-like activity was detected in any of the synovial fluids taken from five patients during periods of inflammatory activity.

In these experiments, a raised joint temperature and the presence of a joint effusion were taken as primary indicators of joint inflammation. A measure of pain was not used to evaluate the condition. On this basis, it is rash to exclude kinins altogether from involvement in some inflammatory joint conditions. Eisen (1969), using pain as a parameter, examined synovial effusions from patients with rheumatoid arthritis, and detected kinin-like activity in less than 50 per cent. of the synovial fluids examined. A few patients with severe pain had no detectable kinin activity in the synovial fluid. Our results are thus at variance with those of Melmon and others (1967), who found that the amount of free kinin present in rheumatoid synovial fluid correlated well with the severity of the symptoms in selected patients. Jasani and others (1969) found that kinin-forming activity in synovial fluid was lower than that found in plasma, but that kininase activity was as high in synovial fluid as in plasma.

Our findings thus tend to support the prediction of Jasani and others that 'In view of the high kininase activity in the synovial fluid and the synovium, it seems unlikely that free kinins will be found in the fluid even from acutely inflamed joints, as the peptides would be inactivated almost as quickly as they were formed and had exerted their pharmacological action'.

Summary

(1) Synovial fluid from the knee joints of two patients with rheumatoid arthritis, two patients with psoriatic arthropathy, and one with chondrocalcinosis was repeatedly examined while the joints were acutely inflamed.

(2) Except for the case of chondrocalcinosis, the protein concentrations of the synovial fluid and the volume of the effusion closely followed the inflammation, as measured by the temperature at the centre of the patella.

(3) The activity of lysosomal enzymes, as shown by the action of the lysosomal enzyme acid phosphatase (E.C.3.1.3.2.) did not appear to follow the course of the inflammation.

(4) No evidence of kinin-like activity was found in any of the synovial fluids examined.

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