

# Monitoring of experimental arthritis in rabbits

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**SUMMARY** The effect of ambient temperature and air flow on the radiometric measurement of experimental arthritis in rabbit knee joints has been studied. Temperature changes due to inflammation in such joints could be detected up to 70 days after induction of the arthritis by the use of radiometry. The method has been used to quantitate the anti-inflammatory activity of intra-articularly injected cortisol acetate and orally administered aspirin. It is suggested that this method of noninvasive monitoring has a number of advantages over other procedures.

Animal models of human arthritis are of use in the screening of potential anti-inflammatory compounds and in the determination of the mechanisms involved in inflammatory reactions and their subsequent pathogenesis (Willoughby, 1975; Imrie, 1976). The assessment of changes occurring within experimental inflammatory sites such as articular joints has largely depended on invasive sampling techniques and subsequent analysis of the site contents (Davis, 1971; Deshmukh and Hemrick, 1976; Goldlust *et al.*, 1977). The use of such an approach leads necessarily to the use of large numbers of animals because of the wide variation encountered both in response to the induced arthritis, and to the effects of therapy. Recent advances in radiometric and thermographic techniques can provide useful objective clinical data on the thermal emission changes which are related to the state of inflammation. Such analysis of inflammatory sites in man and animals has enabled the monitoring and assessment of inflammation to be carried out by noninvasive techniques.

Thermographic assessment of joint changes in human arthritis has shown that alterations in skin temperature over areas of chronic inflammation properly reflect changes in other chemical and cellular parameters of inflammation (Collins and Cosh, 1970). The activity of anti-inflammatory compounds in reducing joint inflammation in experimentally arthritic rats has been quantitated by the use of radiometry (Collins and Ring, 1972), and in arthritic patients by the use of radiometry (Ring, 1975) and of thermography (Huskisson *et al.*, 1973; Collins *et al.*, 1974; Ring *et al.*, 1974). We wish to

report the use of radiometric measurements for the monitoring of an experimental inflammatory arthritis in rabbits and for the assessment of the activity of anti-inflammatory compounds.

## Materials and methods

### INDUCTION OF INFLAMMATION IN RABBIT ARTICULAR JOINTS

An experimental inflammation of the rabbit knee was induced by the intra-articular injection of a complex formed from poly-D-lysine and hyaluronic acid. It has previously been shown that such an injection causes an acute proliferative synovitis, followed by granuloma formation and subsequent scarring of the synovial membrane (Page-Thomas, 1977). Male or female Old English Rabbits weighing 1.8 to 2.4 kg were used for the study. Before induction of the inflammation hair was removed from both knee joints by the use of a commercial depilatory cream. The inflammation was then induced by the intra-articular injection of a preformed insoluble complex of poly-D-lysine (7.5 mg, Sigma, MW 150 000) and hyaluronic acid (7.5 mg, Miles, human umbilical cord) in 1.0 ml sterile, pyrogen-free saline.

### DETERMINATION OF JOINT TEMPERATURE

Radiometric determination of the joint surface temperature was carried out by means of a Heimann KT 41 radiation thermometer. The rabbit was placed in a supine position with the knees held at an angle of 45°, and the temperature of an area of skin 0.6 cm in diameter situated on the lateral side of the knee over the joint space was determined. All radiometric measurements were carried out under carefully controlled temperature and air-flow conditions.

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#### DETERMINATION OF JOINT DIAMETER

The diameter of both knees was measured in the coronal plane at the articular space by means of a precision micrometer, the knee joint being in flexion as described above. Care was taken not to compress oedematous tissue when measuring the diameter.

#### TREATMENT WITH ANTI-INFLAMMATORY COMPOUNDS

The response of the experimental arthritis to 2 anti-inflammatory agents, cortisol acetate and acetylsalicylic acid, was determined. Cortisol acetate (2 mg, Sigma) in 0.5 ml sterile, pyrogen-free saline was injected intra-articularly into 1 knee joint 4 days after the induction of the inflammation. Joint temperature and diameter were monitored for 3 days after treatment. Acetylsalicylic acid, as soluble aspirin (Nicholas Ltd.), was introduced into the drinking water for 2 days 19 days after induction of the inflammation. The average dose per rabbit was 50 mg on day 1 and 42.5 mg on day 2. Joint temperature and diameter were monitored for 3 days after beginning treatment.

#### Results

##### EFFECT OF AMBIENT TEMPERATURE ON JOINT TEMPERATURE

The optimum conditions for joint temperature measurements were determined by allowing the exposed knees to equilibrate to constant temperature at controlled ambient temperatures and under still-air conditions. The equilibration rate, expressed as the rate of change in degrees per minute, and the equilibrated temperature were determined in the ambient range 17.5 to 27.5°C. The equilibrated temperatures in both normal and inflamed joints were linearly related to the ambient temperature (Fig. 1A). The maximum temperature difference between normal and inflamed joints was seen at 20°C, and all subsequent measurements of joint temperature were taken at this ambient temperature. Below 20°C the temperature of the inflamed joint fell sharply to be within normal temperature range. The sharp decrease in heat radiation from the inflamed joints may have been due to a reduction in blood flow resulting from shivering reflex, which occurred below 18°C.

The initial rate of equilibration (Fig. 1B) was also linearly related to the ambient temperature. In inflamed joints the rate of equilibration was very slow at the higher temperatures, whereas the rate in normal joints appeared to be only slightly affected by ambient temperature.

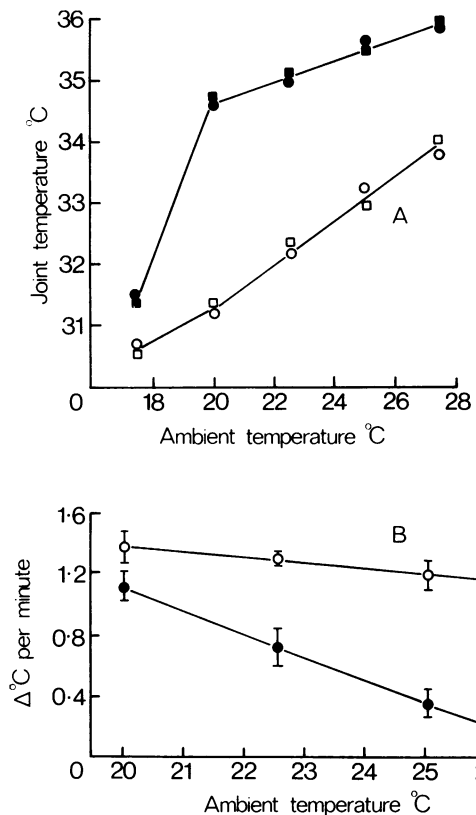


Fig. 1 Effect of ambient temperature on joint temperature. A. Equilibrated joint temperature as a function of ambient temperature. ■, ●, Left and right knees of a rabbit 10 days post-induction; □, ○, left and right knees of a normal rabbit. B. The rate of joint temperature change as a function of ambient temperature. ●, Rabbit knees 10 days post-induction; ○, normal rabbit knees (mean ± SEM, n = 3).

##### EFFECT OF FORCED COOLING ON JOINT TEMPERATURE

Because of the length of time required for the inflamed and normal joints to equilibrate with the ambient temperature the effect of forced cooling on the time taken for equilibration was determined. Inflamed arthritic joints were placed in a controlled fanned airstream at 20°C for periods of up to 40 minutes (Fig. 2), and the time taken for the joints to reach the unforced equilibrated temperature was measured. Fig. 3 shows the results of forced cooling on the equilibration time for an inflamed joint. Unassisted equilibration required a period of 9 to 10 minutes before a constant joint temperature was reached. Forced cooling of the joint resulted in

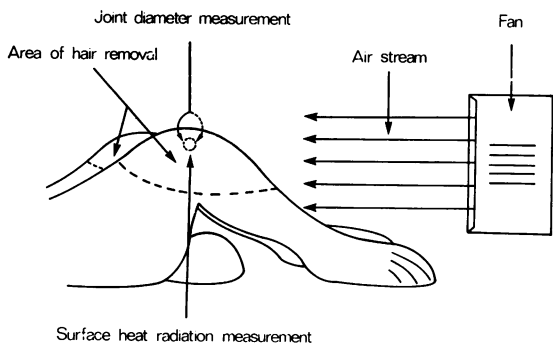


Fig. 2 Measurement of inflammation.

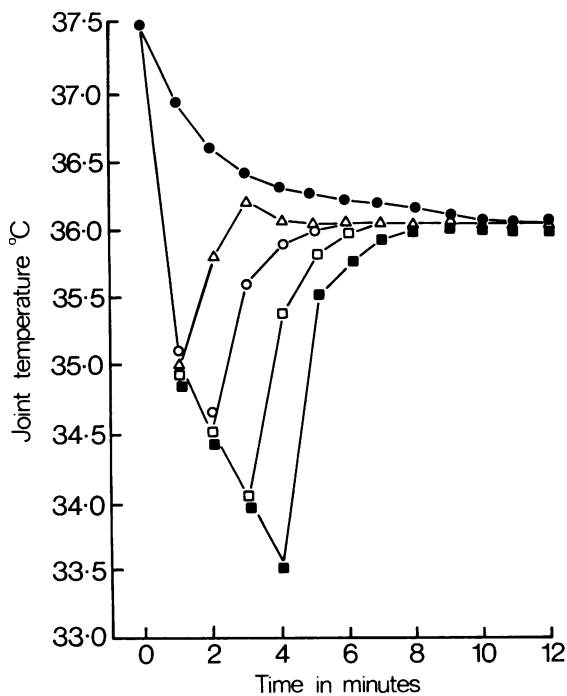


Fig. 3 Effect of forced cooling on the equilibration time. ●, No forced cooling; △, fanned for 1 minute; ○, fanned for 2 minutes; □, fanned for 3 minutes; ■, fanned for 4 minutes.

temperature over-cooling followed by a period of reheating to the constant equilibrated temperature. One minute of fan-assisted cooling was insufficient, for reheating to above the equilibrated temperature followed by a further period of cooling was observed. Similar results were obtained for normal knee joints. A period of 3 minutes' fan-assisted cooling, followed

by reheating and equilibration for a further 3 minutes in still air before temperature measurement, was found to be satisfactory and was used for all subsequent measurements.

#### CHANGES IN JOINT TEMPERATURE AND DIAMETER

Measurements of joint temperature and diameter in a group of rabbits were taken before injection of the poly-D-lysine/hyaluronic acid complex and subsequently for 70 days after induction. Inflammatory changes were apparent 3 hours after injection, and persisted for the period of the study (Fig. 4). There was a good correlation ( $r \geq 0.7$ ) between left and right joint temperatures for individual rabbits up to day 40 after induction; by day 70 the correlation had decreased ( $r < 0.2$ ) for this parameter of inflammation. The correlation between left and right joint diameters was excellent ( $r \geq 0.7$ ) for the total period studied.

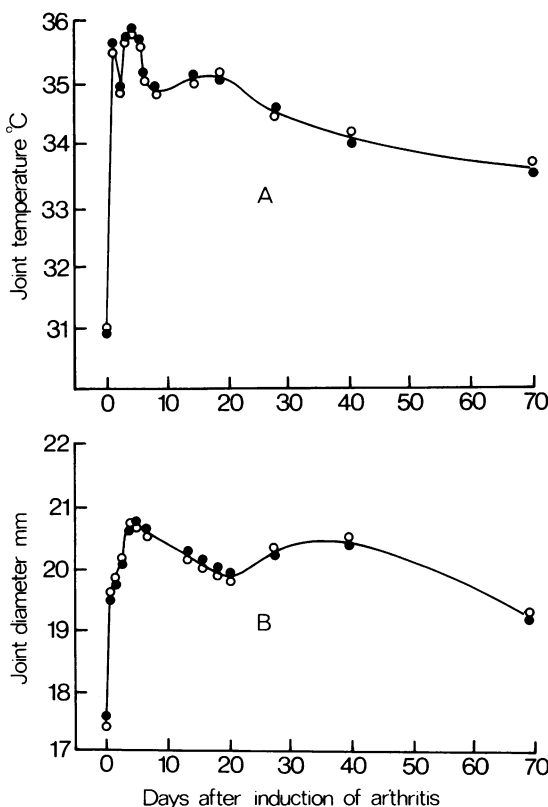


Fig. 4 Changes in parameters of inflammation. A. Changes in joint temperature. B. Changes in joint diameter. ●, Left knee joints; ○, right knee joints (mean of 10 rabbits. Standard errors omitted for clarity).

The changes in temperature with time of the injected joints were complex, with 3 main peaks being observed. The first peak occurred at 24 hours, the second between 3 and 5 days, and the third between 10 and 20 days. After the last peak there was a gradual fall in the joint temperature. There appeared to be a lag period of several days between changes in joint temperature and changes in diameter.

#### MEASUREMENT OF ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity of 2 compounds, cortisol acetate and acetylsalicylic acid, was determined by radiometry to see if the model would respond to treatment at different stages in the development of the arthritis. An arthritis was induced and the temperature and diameter of both knee joints were measured before treatment. Both treatments resulted in a significant reduction in joint temperature (Fig. 5). The intra-articular injection of cortisol acetate into one knee joint also caused a reduction in temperature of the untreated joint. The contralateral effect was presumably the result of loss of steroid from the injected joint. The anti-inflammatory activity of cortisol acetate was shown to be dose-related in this model. The reduction in diameter of the injected joint by treatment with cortisol acetate was not significantly different from that of the uninjected joint or the pretreatment values.

#### Discussion

The radiometric and thermographic monitoring and assessment of inflammatory changes depends largely on the accurate control of ambient temperature and air flow. The relationship described between ambient temperature, rate of joint temperature equilibration, and the equilibrated temperature illustrates the care that must be taken if serial measurements of a single joint or a number of joints are to be made. It has been shown that thermographic measurements of inflammation taken under uncontrolled temperature conditions are unreliable (Huskisson *et al.*, 1973) and do not correlate with other measurements such as technetium clearance. The use of forced cooling of the joint provides a means of controlling and standardising the rate of equilibration and at the same time reduces the time required for each measurement. While this may not be of paramount importance in measurements on human joints, it is essential in animal experiments, in which as short an equilibration period as possible must be used if the animal is to be held gently without serious restraint.

The changes which occur in joint temperature and diameter after induction of the arthritis persist

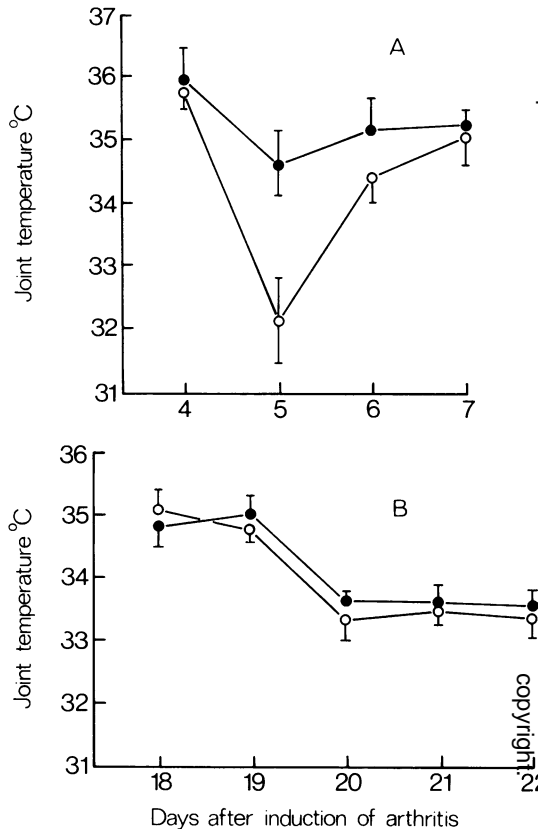


Fig. 5 Treatment of the experimental arthritis with anti-inflammatory compounds. A. Cortisol acetate. 2 mg was injected intra-articularly into one knee joint of a group of rabbits 4 days after induction of the arthritis. ●, Untreated joints; ○, treated joints (mean  $\pm$  SEM,  $n=3$ ). B. Acetylsalicylic acid in the drinking water for 2 days 19 days after induction of the arthritis. The average dose on day 1 was 50 mg, and on day 2 was 42.5 mg. ●, Left knee joints; ○, right knee joints (mean  $\pm$  SEM,  $n=4$ ).

for at least 70 days without falling to the preinduction levels. This model therefore possesses the chronicity required for comparison with human rheumatoid arthritis (Davis, 1971). Treatment in both the acute and chronic phases of the arthritis results in a reduction in the level of inflammation and thus the model may be suitable for the screening of anti-inflammatory compounds affecting one or both of these phases of development.

Some variation was observed in the level of the inflammatory response, as measured by joint temperature and diameter, between individual rabbits, but the degree of difference between the

joints in individual rabbits (as shown by the correlation coefficients) was initially very small. The decreased correlation observed for joint temperature in the latter stages of the arthritis may reflect differences between the 2 joints in the rate of regression of the inflammation.

Changes in joint temperature (Blackham *et al.*, 1974) and diameter (Davis, 1971) have been described for a monoarticular arthritis in the rabbit based on the Dumonde and Glynn (1962) allergic model. The diameter of the unchallenged joint does not appear to alter, but there is an increase in its temperature which parallels the changes occurring in the challenged joint. Such an increase has been shown to be consistent with a systemic hyperthermia (Blackham *et al.*, 1974). The contralateral rise in temperature removes the possibility of using the unchallenged joint as an internal control. The use of a bilateral arthritis, as described in this paper, overcomes this problem, and is therefore particularly useful for detecting any systemic activity of locally administered anti-inflammatory agents, the untreated knee acting as a positive control for each animal. Such an approach has the advantage of considerably reducing the number of animals required for the screening of anti-inflammatory compounds.

The monitoring of experimental arthritis by radiometry has a number of advantages over the other major objective method of assessment, technetium uptake. While it has been shown that the uptake of technetium ( $^{99m}\text{Tc}$  as the pertechnetate) in experimentally arthritic rabbit knee joints is significantly greater than the uptake in normal knee joints during the acute phase of the arthritis, the differences observed in the chronic phase are probably too small to allow the effects of drugs to be monitored (Berry *et al.*, 1973). Radiometric monitoring, however, appears to be valid for measuring the acute and the chronic phases of experimental arthritis, and for the assessment of anti-inflammatory drug activity.  $^{99m}\text{Tc}$  uptake studies are not strictly noninvasive and have other disadvantages, such as requiring the use of sedatives to ensure correct joint position during measurement of uptake, and the hazards from radiation if large numbers of animals are used. In addition preliminary results with  $^{99m}\text{Tc}$  in our experimental model indicate that the curves obtained from simultaneous knee counts do not reach a plateau for some 10–15 minutes from the time of injection. Consequently, the method is very time consuming by comparison with the measurement of joint diameter and radiometry.  $^{99m}\text{Tc}$  methylene diphosphonate,

however, may prove to be of value in the chronic stages of experimental arthritis for the study of the involvement of bone by pannus formation. We are currently evaluating its use during the development of a chronic experimental arthritis.

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