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CHEMOTHERAPY OF ARTHRITIS INDUCED IN RATS BY MYCOBACTERIAL ADJUVANT

BY

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Arthritis induced in rats by mycobacterial adjuvant has been used for the study of compounds of known value in the treatment of rheumatoid arthritis in man. The development of the arthritic syndrome in treated and control rats was followed by measuring the changes in foot thickness of both hind-feet with a micrometer. This method allowed the effect of anti-inflammatory compounds to be expressed quantitatively. Anti-inflammatory activity was readily observed in certain steroids, pyrazolidines, salicylates and sodium aurothiomalate. Chloroquine and hydroxychloroquine were inactive. The inhibition obtained by daily treatment with the steroid paramethasone disappeared when treatment was withdrawn.

Inadequate knowledge of the aetiology and pathogenesis of rheumatoid arthritis in man has seriously impaired the development of laboratory tests for compounds with specific anti-arthritic activity. The value of many tests commonly used for the laboratory study of arthritis has been critically discussed in an excellent review by Gardner (1960). Most of them attempt to produce, in laboratory animals, terminal aspects of the human condition: for example, inflammation or deposition of granulation tissue. Such tests have limited value because they have no more than a superficial and partial relationship to rheumatoid arthritis in man and are thus unlikely to detect compounds that modify the arthritic process at a stage before or after the development of inflammation. Moreover, many are so insensitive that they are incapable of detecting anti-inflammatory activity in clinically useful compounds of some classes. Clearly, better methods are desirable.

The work described in this paper follows an observation first described by Stoerk, Bielinski & Budzilovich (1954) and later elaborated by Pearson (1956, 1959). These authors reported that, in rats, an intradermal injection of an emulsion containing various tissues incorporated in Freund's adjuvant (a mixture of dead *Mycobacteria* with liquid paraffin) produced inflamed lesions in areas of the body remote from the injection site after a delay of 10 to 15 days. Subsequent work showed that inclusion of animal tissues was unnecessary, that the foot-pad route of injection was superior to any other (Pearson & Wood, 1959) and that the arthritis could be produced by suspending the organisms in either mineral or vegetable oils (Ward & Jones, 1962). A variety of acid-fast bacilli has been used to produce the arthritis, which has also been claimed to result from injection of mixtures of liquid paraffin

and Wax Fraction D of the Canetti or Brevannes strains of tubercle bacilli (Pearson & Wood, 1959). Unsuccessful attempts to produce the arthritis in rats by injecting pure wax fraction from *Mycobacterium butyricum* in mineral oil have been reported (Ward & Jones, 1962). The inhibiting effects of steroids on the development of arthritis induced by adjuvant were described by Pearson & Wood (1959) and by Houssay & Frangione (1961).

Extensive studies have been made in these laboratories on the pathology, biochemistry and aetiology of this condition and they will form the basis of further communications. The object of this paper is to describe the effects of known anti-inflammatory compounds on the syndrome which follows the intradermal injection of mycobacterial adjuvant into the foot-pad of rats.

METHODS

Male, specific pathogen-free albino rats, Alderley Park strain I, were used. They belonged to a colony-bred strain of rats of Wistar origin, derived by caesarean section, and weighed approximately 200 g.

The arthritic syndrome was induced by an intradermal injection of 0.05 ml. of a fine suspension of dead tubercle bacilli in liquid paraffin B.P. (concentration 5 mg/ml.), through a No. 20 needle into the plantar surface of the right hind-foot (Fig. 1A). The tubercle bacilli were derived from human strains PN, DT and C which were grown for 8 weeks, killed by steam and dried in a vacuum oven.

Swellings in the hind-feet were measured with a micrometer across a sagittal section (Fig. 1B). Compounds undergoing test were administered orally for 14 days in aqueous suspensions, unless otherwise stated.

RESULTS

Development of arthritis induced by adjuvant in rats

The injection of dead tubercle bacilli in liquid paraffin into the right hind-foot produced an inflamed swelling which reached its maximum size during the first 3 days (Fig. 2E). Thereafter, the swelling slowly subsided until the 8th day when the foot began to swell again. Ten days after injection, inflamed lesions (which we call secondary lesions) were detected on the left hind-foot, which began to increase in thickness (Fig. 2E), and in the fore-paws, ears and tail (Fig. 3). Little further swelling of the feet or joints occurred after the 13th day, and by the 30th day the inflammation had started to subside leaving pale granulomatous swellings around the joints. Ear lesions, which were first seen on the 10th day as small patches of dilated capillaries, had developed into inflamed red nodules up to 3 mm in diameter by the 13th day and had begun to subside by the 30th day. Numerous inflamed lesions on the tail, which were also first detected on the 10th day, had also started to subside by the 30th day. By this time the tail had become noticeably thicker and in some rats spondylitis had developed.

During the development of the arthritic syndrome the rats invariably lost weight. The heavier the rat at the time of injection, the greater was the weight loss in absolute terms.

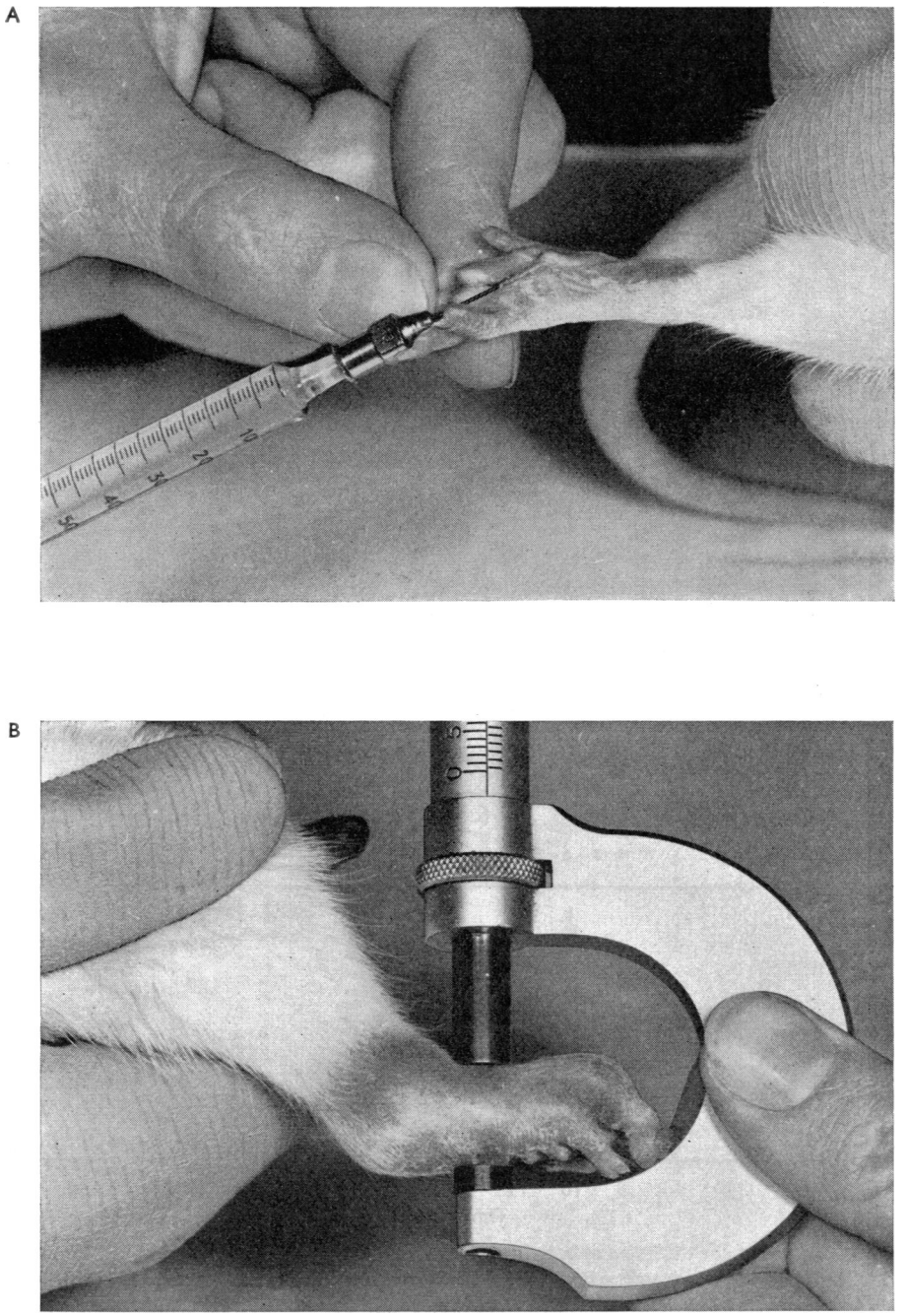

Fig. 1. A: Injection of mycobacterial adjuvant into the right hind-foot; B: measurement of foot thickness with a micrometer.

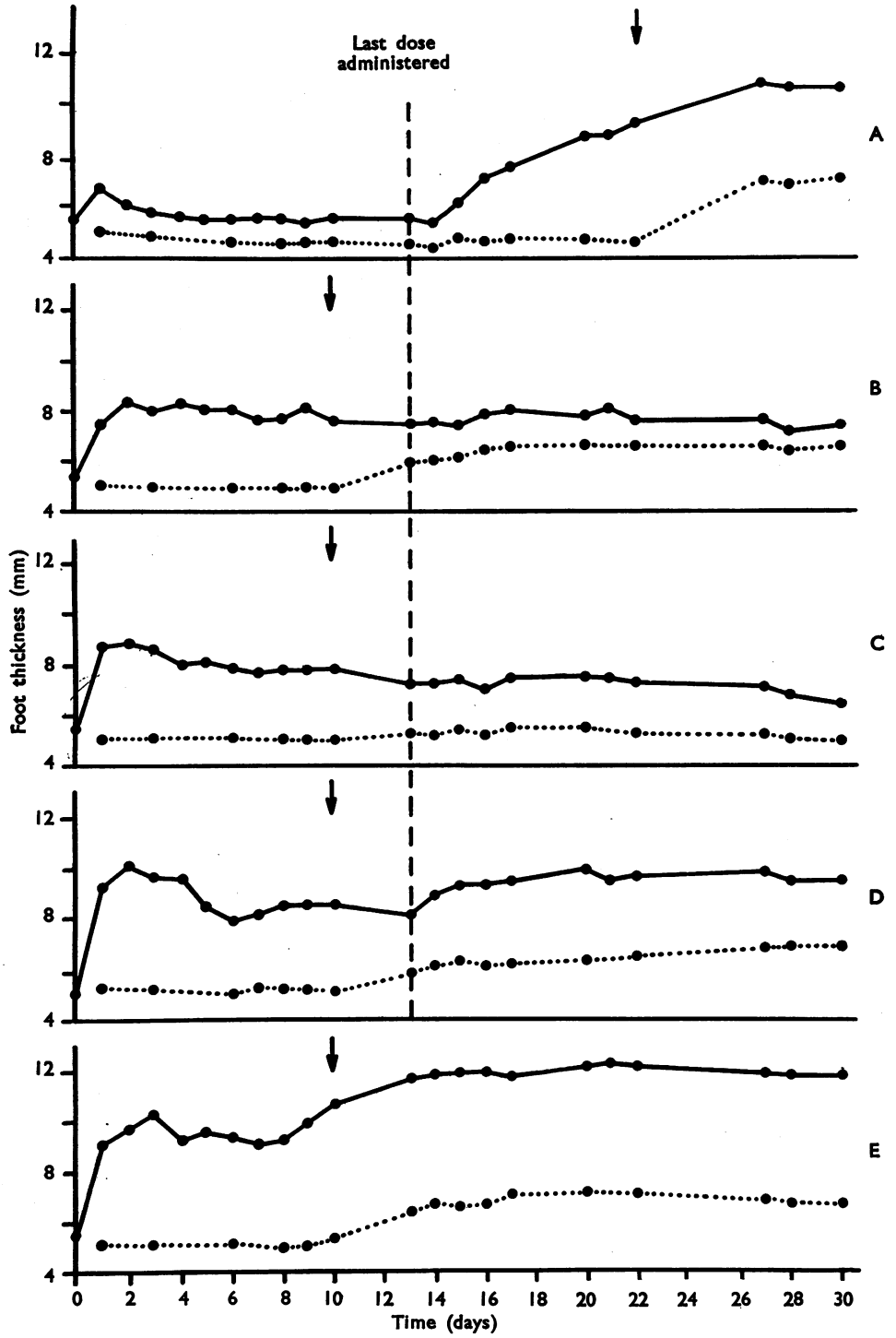

Fig. 2. Changes in the thickness of both hind-feet of treated and control rats during the development of the arthritic syndrome. Each point represents the mean thickness for three rats. Rats were given daily doses for 14 days (starting 1 day before injection into the foot-pad) of: A: paramethasone (0.5 mg/kg orally); B: phenylbutazone (100 mg/kg orally); C: acetylsalicylic acid (200 mg/kg orally); D: sodium aurothiomalate (25 mg/kg intramuscularly); and E: untreated controls. ● —— ●, thickness of the injected foot; ● - - - ●, thickness of the other hind-foot; ↓ =day on which secondary lesions were first detected.

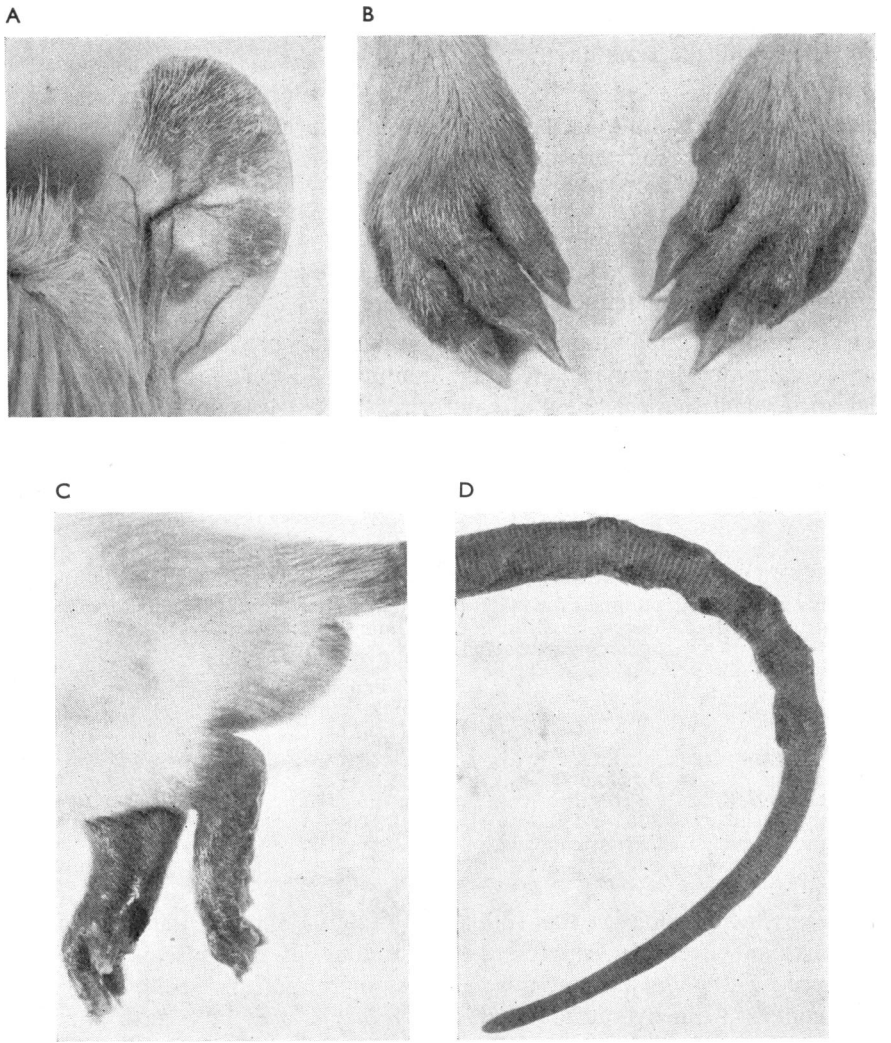

Fig. 3. Lesions in a rat 13 days after the injection of adjuvant into the right hind-foot. A: Ear; B: fore-paws; C: hind-paws; and D: tail.

Chemotherapy

Each compound was given daily for 14 days, the first dose being given 1 day before injection of adjuvant into the foot-pad.

Frequent measurements of the thickness of the hind-feet showed that treatment with paramethasone (Metilar, I.C.I.), phenylbutazone, acetylsalicylic acid and sodium aurothiomalate suppressed to various degrees the swellings in these feet during the period of dosing (Figs. 2A, B, C and D). Secondary lesions appeared on the 10th day in control rats and in those treated with phenylbutazone, acetylsalicylic acid and sodium aurothiomalate. In rats treated with paramethasone there was no appreciable

swelling in the injected foot until dosing was stopped, and secondary lesions did not develop until the 22nd day.

On the 13th day secondary lesions were well developed in control rats and subsequently there was little further swelling in their injected feet. This day was therefore chosen to compare the following effects in rats of treated and control groups:

(a) Weight change from initial to 13th day.

(b) The severity of secondary lesions. These were assessed by classifying them as nil, mild, moderate, moderately-severe or severe.

(c) The percentage inhibition of increase in thickness of the injected foot, obtained from the following formula: percentage inhibition $= 100\ [1 - (a - x)/(b - y)]$, where y = mean injected foot thickness of control rats before injection; b = mean injected foot thickness of control rats on the 13th day; x = mean injected foot thickness of treated rats before injection; and a = mean injected foot thickness of treated rats on the 13th day.

TABLE 1

THE EFFECTS OF DAILY TREATMENT WITH FOUR ANTI-INFLAMMATORY AGENTS

The agents were given intradermally for 14 days starting 1 day before the right hind-foot was injected with a mixture of dead tubercle bacilli in liquid paraffin. Each result represents the mean with approximate standard errors for three rats

Compound	Dose (mg/kg/day)	% inhibition of thickening of the injected foot	Change in weight (g)	Secondary lesions
Paramethasone	0·5 (orally)	92 (±2·0)	−56 (±7·5)	Nil
Phenylbutazone	100 (orally)	64 (±7·5)	−15 (±7·5)	Mild
Acetylsalicylic acid	200 (orally)	70 (±5·6)	−12 (±7·5)	Mild
Sodium aurothiomalate	25 (intramuscularly)	38 (±1·3)	−23 (±7·5)	Moderate
Controls	—	—	−50 (±7·5)	Severe

The assessment made on the 13th day (Table 1) showed that treatment with paramethasone effectively suppressed the swelling in the injected foot and the appearance of secondary lesions: weight loss of rats in this group was similar to that in controls. Phenylbutazone and acetylsalicylic acid both gave good control of the swelling in the injected foot, and weight loss was less than in the control rats. Secondary lesions, though less severe at the 13th day, appeared in rats of these two groups at the same time as in controls. Sodium aurothiomalate was the least impressive of the four compounds listed in Table 1; nevertheless some control of the injected foot thickness was obtained, secondary lesions were only moderate and weight loss was less than in the control rats. The appearance of the hind-feet of one rat from each group on the 13th day is shown in Fig. 4.

From many results assessed on the 13th day the following simplified test for anti-inflammatory activity was designed:

Groups of three rats were weighed and given the compounds to be investigated, one untreated control group being included for every five groups treated. One day later, the right hind-foot was measured for thickness and injected with adjuvant. Daily treatment was continued until the 13th day when the weight of each rat was

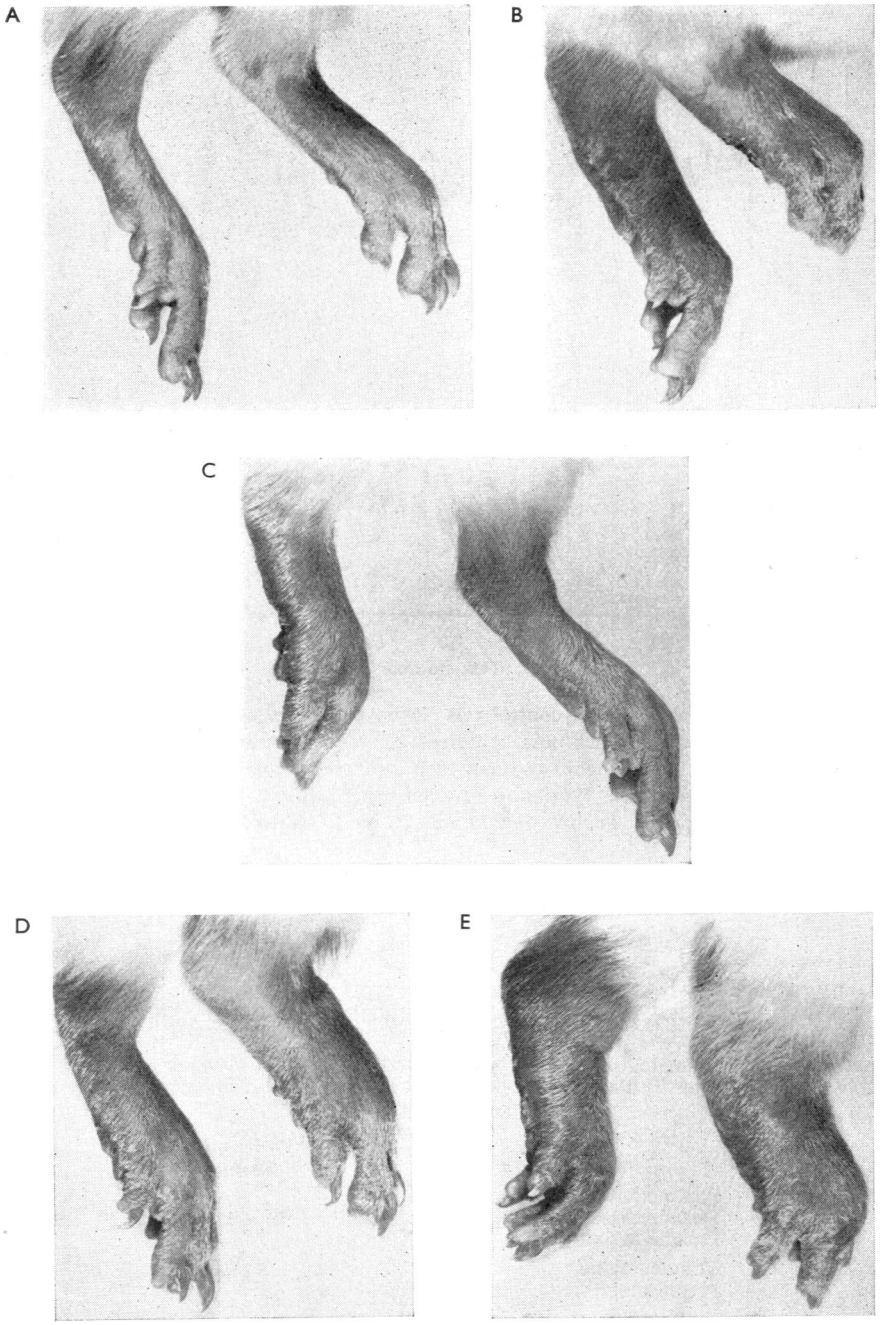

Fig. 4. Lesions in the hind-feet of rats 13 days after the injection of mycobacterial adjuvant into the foot-pad. Rats were given daily doses for 14 days (starting 1 day before injection of adjuvant into the foot-pad) of: A: paramethasone (0.5 mg/kg orally); B: phenylbutazone (100 mg/kg orally); C: acetylsalicylic acid (200 mg/kg orally); D: sodium aurothiomalate (25 mg/kg intramuscularly); and E: untreated controls.

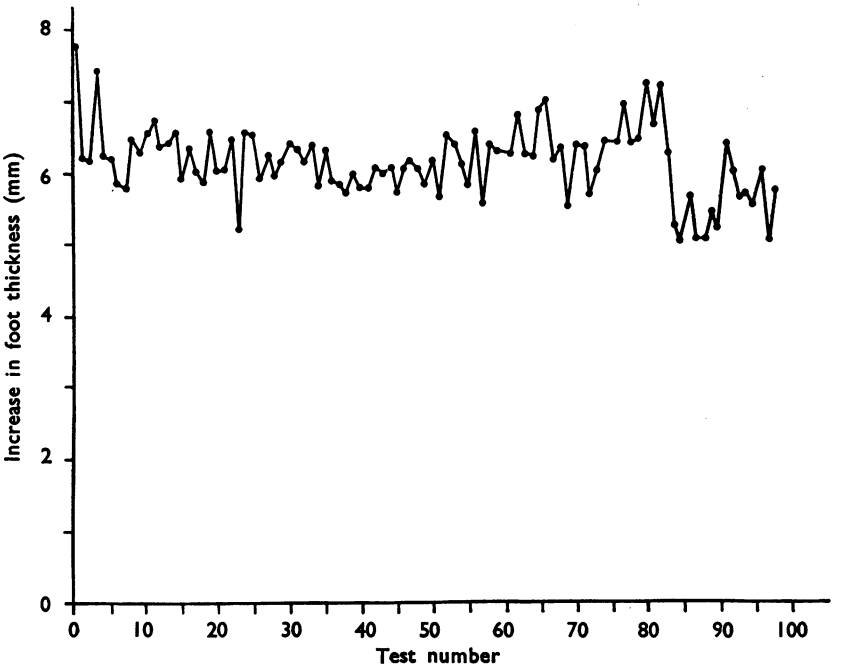

Fig. 5. Increase in foot thickness of control rats, 13 days after injection into the foot-pad, in a series of ninety-eight tests. Each point represents the mean increase in the injected foot thickness of twelve rats. The standard error of each mean is approximately ± 0.5 mm based on variation within experiments. Variation between experiments is a little larger and a slight trend with time is apparent. Such variations are very small compared with the effects of interest.

TABLE 2

DOSES OF ANTI-INFLAMMATORY AGENTS GIVEN BY MOUTH ONCE DAILY REQUIRED TO PRODUCE AT LEAST 50% INHIBITION OF INCREASE IN THICKNESS OF THE INJECTED FOOT IN ROUTINE TESTS

The doses (oral) were estimated from many repeated tests carried out over the past 3 years. The accuracy is sufficient to justify the classification of drugs shown and it is highly unlikely that any compound is wrongly classified

Compound	Dose range (mg/kg/daily)
Paramethasone Dexamethasone Betamethasone	$\leqslant 1$
Triamcinolone Prednisolone	10–20
Cortisone Phenylbutazone Hydroxyphenylbutazone	50–100
Acetylsalicylic acid Sodium salicylate	100–200
Chloroquine Hydroxychloroquine	Inactive

again recorded, the severity of the secondary lesions assessed and the thickness of the injected foot measured to enable the percentage inhibition of the increase in thickness of the injected foot to be calculated.

In a series of tests carried out in this way, at least 50% inhibition was obtained with ten known anti-inflammatory compounds administered in the doses shown in Table 2. Each of the active compounds reduced the severity of secondary lesions and only paramethasone, dexamethasone and betamethasone caused appreciable weight loss. Chloroquine and hydroxychloroquine had no effect on the swelling of the injected foot, secondary lesions were comparable in severity to those seen in control rats and weight loss was similar in rats of treated and control groups.

In a series of tests involving 1,176 control rats, the swellings of the injected feet at the 13th day fluctuated within a narrow range, showing the reproducibility of this chronic lesion (Fig. 5).

DISCUSSION

Many methods used for assessing anti-inflammatory activity in laboratory animals utilize the acute inflammation produced by the injection of materials such as formalin, 5-hydroxytryptamine or dextran into the hind-feet of rats. Unlike the inflammation associated with arthritic joints, that induced by such injections is transitory, and near-toxic doses of anti-inflammatory compounds such as phenylbutazone have to be administered to suppress partially the inflammation (Domenjoz, 1960). More recently a more sensitive method has been described which utilizes an oedema induced by carrageenin (Winter, Risley & Nuss, 1963). Other lesions caused by more sustained stimuli, such as the subcutaneous implantation of cotton-wool pellets, are sensitive to anti-inflammatory steroids but are relatively insensitive to compounds such as phenylbutazone (Winder, Wax, Scotti, Scherrer, Jones & Short, 1962).

The method described in this paper is based on a syndrome which is more akin to rheumatoid arthritis than is any other test (Pearson, Waksman & Sharp, 1961 ; Pearson & Wood, 1963 ; Lowe, unpublished). Moreover the method can detect anti-inflammatory activity in compounds of most classes which are useful in the treatment of rheumatoid arthritis in man. The only exceptions so far encountered are the antimalarial drugs related to chloroquine.

The aetiology and pathogenesis of this type of experimental arthritis is still obscure. The process involved in the development of secondary lesions does not appear to be infectious (Sharp, Waksman, Pearson & Madoff, 1961) and it has been suggested that the secondary lesions are the result of a generalized immunological response to constituents of the tubercle bacilli, which have become disseminated after injection (Pearson & Wood, 1959 ; Waksman & Sharp, 1960 ; Waksman, Pearson & Sharp, 1960).

The method of following the development of the syndrome by measuring the thickness of both hind-feet is more rapid than the scoring system used by Pearson (1959) and enables the effects of anti-inflammatory agents to be expressed quantitatively. It does not eliminate the need for a scoring system to assess the severity of secondary lesions in the fore-paws, ears and tail, but with experience these

can be adequately assessed by the simple scheme described. A record of the weight changes which occur in treated and control rats during the development of the syndrome provides useful supplementary information for assessing the activity of the compounds examined.

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