three drugs, particularly so with sulphasalazine, and appear to reflect fairly closely the relative prophylactic value of the drugs as used clinically.

This work was kindly supported by a research grant from the Mersey Regional Health Authority.

July 8, 1980

REFERENCES

Watt, J., McLean, C., Marcus, R. (1979) J. Pharm. Pharmacol. 31: 645–646

Watt, J., Marcus, R. (1980) Proceedings. 7th Symposium of the International Committee on Laboratory Animals. Utrecht, in the press

The effect of overcrowding stress on the development of adjuvant-induced polyarthritis in the rat

R. DUANE SOFIA, *Biological Research Department, Wallace Laboratories, Cranbury, New Jersey 08512, U.S.A.*

Activation of the pituitary-adrenal axis with subsequent adrenal hypertrophy and the accompanying marked elevation of 17-hydroxy corticosteroids are long known physiological responses to stressful situations (Selye 1946, 1950; Maickel et al 1961). One such stressful situation is overcrowding. The effect of this on the development of a laboratory animal model of chronic inflammation has been examined using adjuvant-induced polyarthritis (AIP) in rats, which is a method of assessing the potential usefulness of drugs in various types of arthritis (Swingle 1974).

Male, albino Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts), 100 to 140 g at the start of the experiment, were housed in groups of five (in gang cages) for five days for acclimatization to a controlled laboratory environment of temperature (20–22 °C), humidity (35–65%) and lighting (12 light: 12 dark). Food and water were freely available. The rats were randomly assigned to the following groups (n = 10 each): non-arthritic—10 rats/cage; non-arthritic—1 rat/cage; arthritic—10 rats/cage; and arthritic 1 rat/cage. AIP was induced by injection of heat killed *Mycobacterium tuberculosis* (Newbould 1963). Each rat received 0·1 ml of a 0·5% (5 mg ml^{-1}) adjuvant suspension in heavy mineral oil injected subcutaneously into the plantar surface of the right hind paw. The rats were immediately placed in their respective housing environment which consisted of a cage 42·5 cm wide × 18·0 cm high × 25·0 cm long constructed of stainless steel and having a wire mesh floor, top and front panel and solid sides. Food and water were freely available. The volume (ml) of the injected (primary lesion) and uninjected (secondary lesion) hind paw were measured by water displacement to the lateral malleolus (ankle) just before adjuvant injection and again at weekly intervals during the 42 days of the experiment. The animals were weighed weekly. Mean values and their standard errors were evaluated using Student's *t*-test.

The response of the AIP injected paws was dramatic by day 7 when compared with control paws (Fig. 1), the rate of oedema formation was similar for both crowded and single groups during the first 21 days but by day 28 there was a clear separation of effect with crowded animals having a reduced mean volume (5·78 ml vs 6·45 ml). In addition, paw volumes in both groups reached their respective peaks by day 28 and remained at that value until the end of the experiment. Involvement of the uninjected hind paw did not become apparent until day 14 (Fig. 1), thus confirming earlier findings (Sofia et al 1975). By this time paw volumes in both groups were significantly increased compared with non-arthritic controls but the volume was significantly less in crowded rats. The rate of development of the secondary lesion in both groups of rats was parallel until day 28 when in the crowded rats a maximal mean value of 3·60 ml was reached and remained unchanged to day 42 but in the singly housed rats the volume continued to increase, reaching a mean volume of 4·78 ml by day 42.

AIP had a significant retardant effect on body weight gain (Fig. 2), rats in either housing condition gaining weight to a lesser degree than non-arthritic controls throughout the study. However, by day 28 the effect was

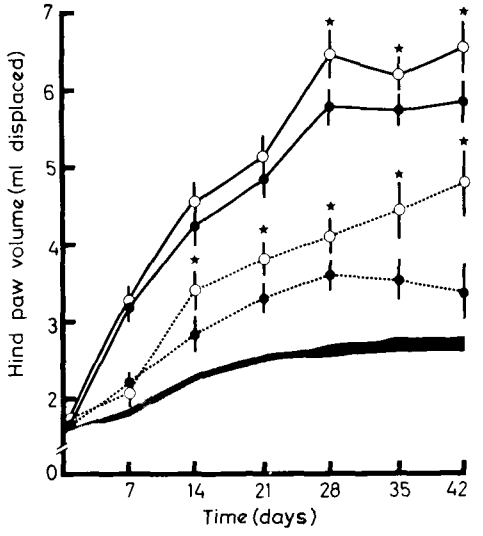

FIG. 1. Mean (±s.e.m.) volume of the injected hind paw or primary lesion (——) and uninjected hind paw or secondary lesion (- - -) of AIP in rats housed 1 per cage (○) or 10 per cage (●). Shaded area: mean range of paw volumes of each hind paw of untreated non-arthritic control rats. n = 10 for each point. $*P < 0.05$ for comparison of each housing condition.

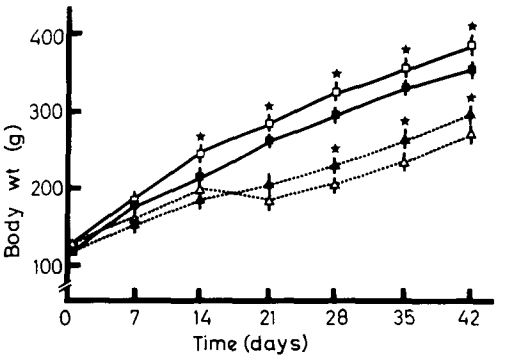

FIG. 2. Mean (± s.e.m.) body weight of non-arthritic control rats (——) and rats with AIP (- - - -) housed 1 per cage (□ △) or 10 per cage (■ ▲). n = 10 for each point. *$P < 0.05$ for comparison of each housing condition.

more pronounced in rats housed one per cage and remained so. This closely parallels the increased severity of AIP on paw volumes (Fig. 1) in similarly housed rats. By day 14 body weights of control rats were significantly higher for those housed one per cage and remained higher throughout the experiment, although the rate of gain (or slope of the body weight curve) was similar for both control groups.

The results of this experiment demonstrate that overcrowding affects the development of AIP in rats. Oedema formation for both the primary and secondary lesions of the disease and the severity of the reduction in body weight gain were less in rats housed ten rather than one per cage (of equal dimensions). In our laboratories, AIP studies are normally conducted as described above with the exception that rats are individually housed in cages affording approximately one-half the space (20·0 cm wide × 18·0 cm high × 25·0 cm long) allowed in the present study with disease development closely approximating that seen in the uncrowded rats (unpublished data).

May 28, 1980

REFERENCES

Maickel, R. P., Westerman, E. O., Brodie, B. B. (1961) J. Pharmacol. Exp. Ther. 134: 167–175

Newbould, B. B. (1963) Br. J. Pharmacol. Chemother. 21: 127–136

Selye, H. J. (1946) J. Clin. Endocrinol. Metab. 6: 117–230

Selye, H. J. (1950) The Physiology and Pathology of Exposure to Stress, Medical Publishers, Montreal, Canada, pp 52–70

Sofia, R. D., Knobloch, L. C., Vassar, H. B. (1975) J. Pharmacol. Exp. Ther. 193: 918–931

Swingle, K. F. (1974) in: Scherrer, R. A., Whitehouse, M. W. (eds) Anti-inflammatory Agents Chemistry and Pharmacology. Vol. II Academic Press, New York, pp 33–122

LETTERS TO THE EDITOR

Analgesic action of indomethacin in rats with trypsin-induced hyperalgesia

CHARLES A. WINTER, PAUL J. KLING*, *Merck Institute for Therapeutic Research, West Point, PA 19486, U.S.A.*

Vinegar et al (1976) expressed the view that some non-steroid anti-inflammatory drugs possess analgesic properties only as a consequence of the inhibition of inflammation, while others are analgesic independently of their anti-inflammatory effect, and that the two classes could be distinguished from each other by their actions on hyperalgesia induced by injection of kaolin or trypsin into the rat's paw. Only those agents possessing independent analgesic properties were said to be active in these cases, and indomethacin lacked an analgesic component independent of anti-inflammatory activity, even at the very high dose of 5 mg kg^{-1}.

If this concept is valid, it has important implications for studies on the mechanism of action of these drugs and in the search for new and improved agents. We have, therefore, made additional observations, even though Van Arman et al (1968) had demonstrated block of trypsin hyperalgesia by indomethacin.

Trypsin (Sigma Chemical Co.), 1 mg in 0·1 ml, was injected into the right hindpaw of Sprague-Dawley female rats of about 50 to 60 g. The volume of each injected paw was determined immediately after injection, and both paw volume and threshold of response to pressure were measured at various times thereafter by methods previously described (Winter et al 1963; Winter & Flataker 1965). Indomethacin was suspended in 0·5% methylcellulose in a concentration to permit administration of 2 mg kg^{-1} in a volume of 1 ml/100 g body weight. Animals not receiving drug were given methylcellulose only.

Trypsin injection induced prompt development of oedema accompanied by hyperalgesia (Fig. 1). Indomethacin did not inhibit the oedema induced by trypsin, but it was highly effective against trypsin-induced hyperalgesia, and was equally effective whether given before injection of trypsin or if treatment was delayed until after hyperalgesia was established. Comparable results have been reported for diflunisal (Winter et al 1979).

It is clear that an analgesic effect can be obtained with these compounds independently of an antiinflammatory

* Correspondence.