J. Pharm. Pharmac., 1968, **20**, *Suppl.*, 126S–129S Received May 1. 1968

SHORT COMMUNICATION

The effect of alloxan diabetes upon adjuvant-induced arthritis in the rat

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THE relation between inflammation and carbohydrate metabolism has been the subject of several investigations using acute inflammatory models such as the anaphylactoid reaction in the rat (Adamkiewicz & Adamkiewicz, 1959; 1960) and true anaphylaxis in the rat and mouse (Kraus, 1964; Gulbenkian, Yanell Grasso & Tabachnick, 1967; Dhar, Sanyal & West, 1967). These acute inflammatory reactions are depressed by the hyperglycaemia produced by alloxan (Adamkiewicz & Adamkiewicz, 1959; 1960), by 2-deoxyglucose (Goth, 1959), by glucose loading (Dhar & others, 1967) and by glucagon (Lefebvre & Van Cauwenberge, 1962).

Using the model of "adjuvant arthritis" in the rat, produced by the injection of Freund's adjuvant (Pearson, 1956), Kellett (1965) showed that this chronic arthritic condition was also suppressed when alloxan diabetes was induced in rats before an injection of adjuvant. The syndrome of adjuvant arthritis occurs in two phases, firstly an acute inflammatory oedema at the injection site which is followed after a latent period of about ten days by a secondary, chronic polyarthritis involving all limbs and the tail.

Because of the biphasic course of the inflammation, and the possible dependence of the chronic phase on the prior appearance of an adequate initial phase, we decided to investigate the effect of alloxan diabetes induced at various times during the development of the adjuvant arthritic syndrome.

EXPERIMENTAL

Groups of seven male Wistar rats (Scientific Products Farm Ltd.), weight 150–200 g were used. Arthritis was produced by the intradermal injection of 0·03 ml of a 5 mg/ml suspension of dead tubercle bacilli in liquid paraffin, into the plantar surface of the left hind paw. The volume of both hind paws and the animals' body weights were determined at intervals during the next 28 days.

Paw volume was measured by immersion in a mercury manometer, the rise in the level of mercury being detected by a Devices pressure transducer and recorded using a calibrated Devices DC 2c amplifier and pen recorder. The paw volume is expressed as ml/kg body weight: standard errors of the means were calculated, and significance of difference between groups calculated using Student's *t*-test.

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To produce diabetes, rats were starved for 24 hr and, under halothane anaesthesia, given 35 mg/kg of alloxan in 0·9% saline into the right femoral vein. All animals so treated had a glucosuria of at least 0·5% within 48 hr of the injection. Non-diabetic control animals were similarly treated receiving intravenously only the appropriate volume of saline.

Urine from both diabetic animals and non-diabetic controls was examined daily for glucose using "Clinistix" (Ames and Co.). The diabetic rats consistently showed a glucosuria in excess of 0·5%. In contrast, glucose was never detected in the urine of the control animals of this strain.

RESULTS AND DISCUSSION

The course of the arthritis was followed in control animals and animals made diabetic at three different stages: (*a*) alloxan was administered four days before the injection of adjuvant; (*b*) alternatively, alloxan was given on the fourth day *after* the adjuvant injection, i.e. after the acute inflammation had become maximal, but before the onset of the secondary phase; (*c*) alloxan was administered on the fifteenth day after adjuvant injection, i.e. when the second chronic arthritic phase had been established.

In the first experiment, when diabetes was produced before the injection of adjuvant, our results agree with those obtained by Kellett (1965) in a similar experiment; the diabetes significantly attenuated both the acute and secondary chronic reactions to the adjuvant (Fig. 1A). This effect of diabetes on the initial acute local response can be related to other work on acute inflammation and hyperglycaemia in the rat (Adamkiewicz & Adamkiewicz, 1959; Goth, 1959). It has been pointed out (Goth, 1959) that dextran and egg-white both cause inflammation and both contain a carbohydrate moiety. Lack of insulin may inhibit the participation of these carbohydrate-containing molecules in inflammatory reactions. A similar situation may arise with Freund's adjuvant since it has been shown (Tanaka, Ishibashi & Sugiyama, 1967) that the antigenic component of Wax D from *Mycobacterium tuberculosis* also contains carbohydrate. With respect to the secondary reaction, Waksman, Pearson & Sharp (1960) maintain that the chronic reaction to adjuvant in rats is probably a delayed hypersensitivity to the tubercular antigen. Consequently the diabetic state may interfere with the antibody formation fundamental to the development of the chronic response.

Newbould (1964) demonstrated that immunologically competent lymphocytes are first released from lymph nodes between the fifth and seventh days after adjuvant injection. In the second experiment (Fig. 1B) alloxan given on the fourth day after adjuvant reduced the secondary response, although the attenuation of the chronic reaction was less marked than in the first experiment. This difference could be explained on the assumption that antibody formation was markedly suppressed in the first experiment but not in the second, in which essentially normal levels of antibody would be present at the time the animal developed diabetes.

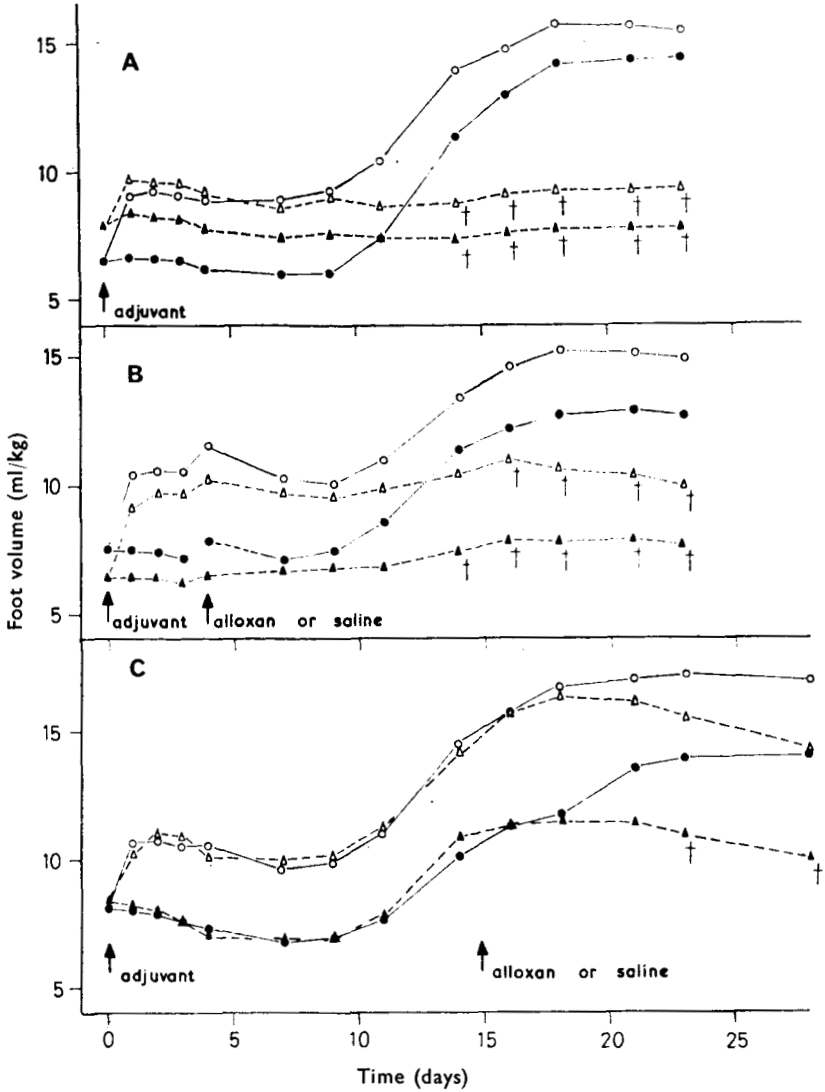

FIG. 1. Changes in hind foot volume in control rats and rats made diabetic A, 4 days before, B, 4 days, C, 15 days after the injection of adjuvant. The symbol † indicates a significant difference ($P = <0{\cdot}05$) between the corresponding feet of diabetic and control groups. Controls: ●—● right foot; ○—○ left foot. Diabetic: ▲ – – ▲ right foot; △—△ left foot.

When rats were injected with alloxan fifteen days after adjuvant injection, the hind paw oedema already present (chronic arthritic phase) slowly declined from day fifteen to day twenty-eight (Fig. 1C). Such reversal of an established chronic arthritis by diabetes may be a direct effect on the inflammatory events subsequent to antibody formation; alternatively, there might be a further reduction in the circulating antibody

necessary to maintain the inflammatory reaction. Clinically, hyperglycaemia has been shown to reduce the severity of asthmatic attacks (Abrahamson, 1941) and rheumatoid arthritis (Helmer, Kirtley & Ridolfo, 1957). Consequently, there may be a place for induced hyperglycaemia in the treatment of inflammatory disease in man.

Acknowledgements. The technique for measuring paw volume was suggested by Dr. R. T. Brittain, of Allen & Hanburys, Ware, Hertfordshire. We are also indebted to the Ministry of Agriculture Veterinary Laboratories, Weybridge, Surrey, for a supply of dead tubercle bacilli.

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