The effect of sunflower seed oil on Freund's adjuvant-induced arthritis in the rat

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Linoleic acid has been shown to reduce the responsiveness of T lymphocytes to a number of antigenic stimuli. It has an inhibitory action on lymphocyte responsiveness to phytohaemagglutinin (PHA) and a purified protein derivative of *Mycobacterium tuberculosis* (Offner & Clausen, 1974); it increases skin graft survival in mice (Mertin, 1974) and given orally, may enhance renal allograft survival times in man (Uldall, Wilkinson & others, 1974). In addition, in multiple sclerosis, it has been shown to reduce the reactivity to thyroid antigen in the macrophage electrophoretic mobility test (Field, Shenton & Joyce, 1974). In view of these reports of its immunosuppressive action and because of the potential benefit to be gained from therapy with polyunsaturated fatty acids, we have made quantitative assessment of its action in an animal model of a T-cell mediated disease namely polyarthritis induced in rats by injection of micobacterial adjuvant (Newbould, 1963, which is generally recognized as being an example of a T-cell mediated reaction (Waksman, Pearson & Sharp, 1960; Waksman & Wennersten, 1963; Pearson & Wood, 1969; Kourounakis & Kapusta, 1974).

We have therefore measured the effect of linoleic acid (in the form of sunflower seed oil) on the progress of the adjuvant-induced arthritis in rats. In addition, we have measured two parameters of T-cell function, PHA responsiveness, and the ability of the rat lymphocyte to recognize a specific antigen.

Polyarthritis was induced in male, Wistar rats, 200–250 g, by subcutaneous injection into the left hind foot pad of 0·1 ml of Freund's incomplete adjuvant, containing 5·0 mg of *M. tuberculosis* in a light liquid paraffin water emulsion (9:1). The arthritis was usually allowed to develop for up to three weeks.

Rats were randomized into groups of 16 animals. Seven days before the injection of Freund's adjuvant, and daily throughout the development of the arthritis, sunflower seed oil (Evans Medical) was given orally at 4 ml kg^{-1} or at 8 ml kg^{-1} to two groups of animals while control groups were given orally saline or light liquid paraffin (B.P.C.) at 4 ml kg^{-1}.

The overt course of the inflammatory reaction was followed by measurement of the ankle circumference of both hind feet of the animals, and by measurement of total body weight, throughout the procedure.

To assess the lymphocyte response to mitogenic and specific antigenic challenge, the animals were lightly anaesthetized with ether and blood was withdrawn from the aorta into heparinized syringes. Blood samples, within groups, were pooled, and the samples so obtained used to test for either lymphocyte transformation in response to PHA or by migration inhibition reaction in the presence of *M. tuberculosis*, as described below.

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Lymphocyte transformation test. The blood was layered over a mixture of Ficoll (6·5%) and Triosil (11·7%) and centrifuged at 1000 *g* for 25 min at 20°. The lymphocyte band was removed and washed in phosphate-buffered saline, pH 7·4 before being suspended in tissue culture medium (see below) to a concentration of 10^6 ml^{-1}. Cells were cultured in replicates of six. Half of the cells were cultured in the presence of 1:20 PHA (PHA-m, Difco) and half in its absence. Cells were cultured for 68 h at 37° in a humidified incubator in a medium consisting of TC 199 (Wellcome, TC 20) with Earle's salts buffered with 20 m M Hepes buffer, supplemented with penicillin (100 U ml^{-1}), streptomycin (100 U ml^{-1}), 20% autologous plasma and 2 mM L-glutamine. After this time, a 4 h pulse of 1·0μCi thymidine—[methyl-^{3}H] (5·0 Ci $mmol^{-1}$, Amersham) was given. Cultures were terminated by cooling to 0° and the cells were harvested onto glass fibre filter pads (GF/C, Whatman). Material remaining in the culture tube was washed onto the pad with 40 ml ice-cold saline and acid insoluble material precipitated with 20 ml ice cold 5% trichloroacetic acid. The filter pads were finally washed with 20 ml methanol, placed precipitate side up in scintillation vials and dried at 120° for 4 h. 10 ml scintillation fluid (0·4% PPO, 0·05% POPOP in toluene) was added and the samples counted in a Phillips scintillation counter, corrected for quench. The results were expressed as a transformation index which was calculated as: thymidine incorporation in PHA stimulated cultures/thymidine incorporation in non PHA stimulated cultures.

Leucocyte migration test. The method was that of Bendixen & Søborg (1969). Leucocytes harvested from peripheral blood were washed three times in phosphate buffered saline, pH 7·4, adjusted to 50×10^6 ml^{-1} and packed into capillary tubes. Cells were then allowed

Table 1. *Mean lymphocyte transformation indices obtained from groups of 16 rats, treated orally at 4 ml kg^{-1} with saline, light liquid paraffin and sunflower seed oil.*

Experimental Group (4 ml kg^{-1})	Counts min^{-1} obtained on [^{3}H] Thymidine incorporation With PHA	Without PHA	Transformation index
Saline	23 796 ± 1076	2467 ± 389	9·8
Light liquid paraffin	26 172 ± 1983	2476 ± 206	10·2
Sunflower seed oil	24 198 ± 2061	2167 ± 498	10·4

to migrate into chambers containing Eagles medium MEM supplemented with 20% foetal calf serum. Antigen, *M. tuberculosis*, was prepared as an emulsion in this medium and added to test chambers at four protein concentrations. After 21 h incubation at 37° the areas of migration were measured and a migration index calculated as: mean area of migration in the presence of antigen/mean area of migration in the absence of antigen.

All measurements were made in quadruplicate. A migration index of less than 0·8 indicated significant inhibition of migration.

The development of adjuvant-induced arthritis followed the expected pattern. The adjuvant injected left paw showed an acute swelling and inflammation due to the injection of foreign protein, which became maximal at about day 4. The secondary, and immune induced inflammation developed in both hind limbs after about 10 days. Thereafter, there was a parallel increase in the circumference of both hind limbs until about day 20.

Measurement of ankle circumference in three groups of animals with adjuvant-induced arthritis showed that there was no significant difference between the size of the inflammatory lesion between the treatment groups. In the groups of animals dosed at 4 ml kg^{-1}, there was no difference on days 15 and 18 between animals treated with the oil and with light liquid paraffin ($P>0·1$; $P>0·1$), and between the oil and saline ($P>0·1$; $P>0·1$). Development of the secondary inflammatory response began uniformly on day 12. In the rats dosed with the oil at 8 ml kg^{-1} there was no difference in the extent of the ankle inflammation of days 15 and 21 with animals dosed with saline ($P>0·1$) or in the time to the development of the secondary inflammation.

Despite the fact that the animals used were immature, weights fell but at no time was there a statistical difference between the decline seen in the saline-treated and oil-treated groups. There was also no difference between the groups on stimulation with PHA of lymphocytes from rats treated with saline, paraffin or oil (Table 1), or in the leucocyte migration test with saline and oil. Migration inhibition was only noted at high protein concentration in both groups (Table 2).

Table 2. *The migration indices of rat mixed leucocyte population for four concentrations of* M. tuberculosis. Rats, with Freund's adjuvant induced-arthritis were treated orally either with normal saline (8 ml kg^{-1}) 1 week before, and for 20 days after induction, or sunflower seed oil at a dose of 8 ml kg^{-1}. Peripheral white cells were taken at day 20 (see text) and migrated against *M. tuberculosis*.

Treatment group (4 ml kg^{-1})	Antigen conc (ml^{-1})	Migration index
Sunflower seed oil	1·05 mg	0·77
	105 μg	0·99
	10·5 μg	1·06
	1·05 μg	0·95
Saline	1·05 mg	0·60
	105 μg	0·87
	10·5 μg	0·99
	1·05 μg	0·87

Thus measurement of the overt inflammatory lesion in rats treated with sunflower seed oil orally did not support the hypothesis that this substance affected the inflammatory phase of immune arthritis. Concurrent measurements of body weight showed that the oil was not affecting the generalized course of somatic disease when compared with control groups.

The dose which apparently enhanced allograft survival in man was approximately 0·5 ml kg^{-1} (Uldall & others, 1974). Thus, the doses used in this investigation should have been adequate to obtain a response.

Using the response to PHA as a measurement of T-cell function we were again unable to demonstrate any difference between the control and oil treated group. Similarly, no difference was found in response to specific antigen challenge.

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