Clin. exp. Immunol. (1979) 36, 449–455.

The spleen is required for the suppression of experimental allergic encephalomyelitis by prostaglandin precursors

J. MERTIN & A. STACKPOOLE *Clinical Research Centre, Harrow, Middlesex*

(*Accepted for publication* 22 *December* 1978)

SUMMARY

In this paper we report a study of the effects of splenectomy on the immunosuppressive action of essential fatty acids (EFA) which is thought to be mediated through prostaglandins (PG) produced in the spleen. Experimental allergic encephalomyelitis (EAE) was induced in normal, splenectomized and sham splenectomized Lewis rats. EFA were administered orally, the animals in the control groups being treated with liquid paraffin. Treatment with EFA significantly suppressed clinical disease in those animals in which EAE was induced by the inoculation of central nervous system material of guinea-pigs or by passive transfer by Con A-stimulated spleen cells. Splenectomy abrogated the suppressive effect of EFA. This observation, together with previous results showing the abrogation of EFA immunosuppression by an inhibitor of the biosynthesis of PG from EFA, led us to postulate a close relationship between EFA, PG and a splenic factor suppressing immunopathological mechanisms in EAE.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is a widely accepted model of organ-specific cell-mediated autoimmune disease. Inoculation into animals of susceptible strains of either central nervous system (CNS) tissue or CNS myelin basic protein, emulsified in an adjuvant, causes well-defined histopathological changes in the CNS that are responsible for a range of characteristic clinical symptoms (Bornstein, 1977; Paterson, 1977). Induction of the disease is T lymphocyte-dependent, and its passive transfer from diseased animals to histocompatible normal recipients can only be achieved with the help of lymphocytes, but not by serum (Paterson, 1977). Acute EAE is a monophasic disease resembling the acute attacks of a human CNS disease, multiple sclerosis (MS). In the mechanisms underlying the remission of acute EAE attacks, suppressor cells from the thymus, migrating to spleen and lymph nodes (Adda, Beraud & Depieds, 1977), seem to be of crucial importance and it has been argued that such suppressor activity may also determine the clinical course of MS (Arnason & Antel, 1978). The relapsing variant of EAE, in which clinical relapses and remissions as well as plaques of demyelination in the CNS are observed, has made it possible to draw the parallel between EAE and MS somewhat closer (McFarlin, Blank & Kibler, 1974; Wisniewski & Keith, 1977; Raine & Traugott, 1978). First observations of fluctuations in lymphocyte subpopulations in relation to the clinical course of this EAE variant have recently been reported (Traugott, Stone & Raine, 1978).

Essential fatty acids (EFA) of the ω-6 family, i.e. linoleic acid and its derivatives, are precursors of prostaglandins whose importance for various regulatory systems, including immunoregulation, has become increasingly evident (Pelus & Strausser, 1977). EFA have been shown to influence cell-mediated immune reactions (Mertin & Hughes, 1975; Mihas, Gibson & Hirschowitz, 1975; Mertin, 1976; Mertin & Hunt, 1976; Weyman *et al.*, 1977; Santiago-Delpin & Szepsenwol, 1977; Meade & Mertin, 1978). In studies on EAE, fewer guinea-pigs and rats in the EFA-treated groups showed clinical signs of disease.

Correspondence: Dr J. Mertin, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ.

0099-9104/79/0060-0449$02.00

There were also less extensive histopathological changes in the CNS of the animals of these groups when compared with animals from liquid paraffin-treated control groups (Meade *et al.*, 1978; Mertin & Stackpoole, 1978; Mertin, 1979). Furthermore, lymph node cells from EFA-treated guinea-pigs showed significantly less sensitization to myelin basic protein than their controls, as measured by the macrophage gold uptake test (Meade *et al.*, 1978). This suppression of EAE was observed when the animals were treated with EFA from the seventh to the twenty-first day after antigen inoculation, whereas animals treated at the time of sensitization showed only slight suppression. Therefore, it appeared that treatment with EFA might preferably interfere with the effector phase rather than with the process of induction of the immune response in EAE. To examine this question further we have now studied the effect of EFA treatment on EAE induced by passive transfer with Con A-stimulated spleen cells. Recent experiments have indicated that EFA-derived prostaglandins (PG) are responsible for the immunosuppressive effects of EFA in EAE (Mertin & Stackpoole, 1978). Indomethacin is known to inhibit the biosynthesis of PG from EFA (Vane, 1976) and treatment with this drug completely abrogated suppression of EAE by EFA. As the spleen is considered to be a major site for the production and release of immunologically active PG (Osheroff, Webb & Paulsrud, 1975; Webb & Osheroff, 1976), we have also investigated the effect of splenectomy on EAE and its suppression by EFA.

MATERIALS AND METHODS

Animals. Lewis rats were obtained from our own breeding colony. In all experiments the animals in control and treated groups were age- and sex-matched at the time of antigen inoculation. This was usually performed when the animals were 11 weeks of age. The rats received a standard diet for rodents (Spratts Laboratory Diet 1, Spillers Limited) and water *ad libitum.*

Induction of EAE. Brain stem and spinal cord were removed from adult Duncan–Hartley guinea-pigs, homogenized in an equal amount (w/v) of a balanced salt solution and lyophilized. The dry material was stored at −20°C. For the sensitization, one part of lyophilised material, reconstituted with three parts water, was emulsified in an equal volume of Freunds' complete adjuvant (Difco) and this emulsion was injected into all four foot pads of the animals. The total inoculum for each animal was about 200 mg/kg body weight of CNS material. Under this regimen of sensitization all control animals developed clinical signs of EAE, but deaths attributable to EAE were very rare.

Passive transfer of EAE. Passive transfer of EAE was performed according to the modification by Panitch & McFarlin (1977). Spleen cells from diseased animals were stimulated by Con A and, after a culture period of 72 hr, $20–30 \times 10^6$ viable cells were injected intravenously into normal Lewis rats.

Splenectomy. Rats were splenectomised (Sx) at 4 weeks of age. The animals were anaesthestised with a barbiturate and the spleen was withdrawn through a dorso-lateral incision and removed by cauterization. The control animals were sham operated (sham Sx). The incisions were closed by autoclips. CNS antigen was inoculated when these animals were 11 weeks old.

Treatment. EFA were administered orally (with the help of a pipette) in form of a plant oil containing 73% linoleic acid and about 8% of the linoleic acid derivative γ-linolenic acid (Naudicelle, BIO Oils, Nantwich). The mean daily EFA dose was 500 mg/kg body weight and the period of treatment was from day 7 to day 21 after antigen inoculation, unless otherwise stated. This treatment regimen has previously been shown to significantly suppress EAE in Lewis rats both clinically and in the extent of histopathological changes in the CNS (Mertin, 1979). Animals in the control groups underwent the same handling but liquid paraffin was substituted for EFA. In the cell transfer experiments treatment was commenced on the day of cell injection and continued for 14 days.

Assessment of clinical EAE. The severity of the disease was assessed by a clinical scoring system (Mertin & Stackpoole, 1978) ranging from score 0 (= no clinical signs of EAE) to score 8 (= death due to EAE). The animals were weighed and clinically scored each day (at the same time), starting from the day of antigen inoculation. Loss of body weight due to EAE can be considered a crude but objective measure of disease activity and correlated in our experiments well with the clinical scores (Fig. 1). In order to demonstrate the clinical course of the disease, the daily clinical scores of individual animals in a group were summed.

Statistical analysis of the results. The differences between the number of animals in the groups showing signs of disease (score 1 and above) were analysed by the χ^2 test. Differences in the clinical scores were determined with a non-parametric test (Wilcoxon sum of rank test) and all animals, including those with score 0, were ranked.

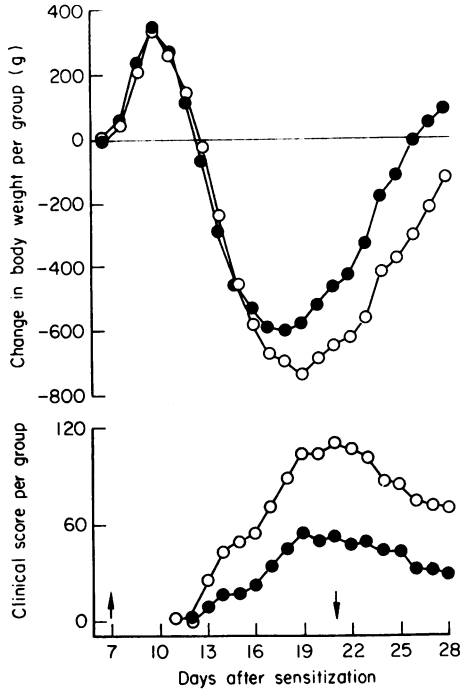

FIG. 1. Change in the body weight due to disease and clinical score per group in the course of EAE in Lewis rats. Animals were treated with essential fatty acid (EFA, ●—●, $n = 36$) or with liquid paraffin (control, ○—○, $n = 36$). The arrows indicate the start and discontinuation of treatment.

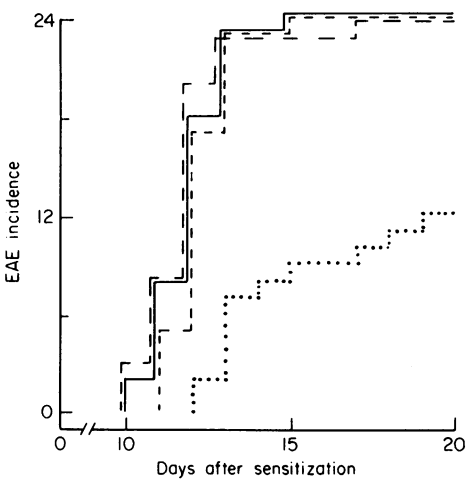

FIG. 2. The effect of treatment with essential fatty acids (EFA) on clinical EAE incidence (i.e. number of animals per group developing clinical signs) in splenectomized (Sx) and sham-splenectomized Lewis rats. The figure represents the identical, pooled results of two separate experiments. The differences between the sham EFA groups and all other groups were significant ($P < 0{\cdot}001$). (- - - - -) Sham control, $n = 24$; (. . . . .) sham/EFA, $n = 24$; (——) Sx control, $n = 24$; and (- - -) Sx/EFA, $n = 24$.

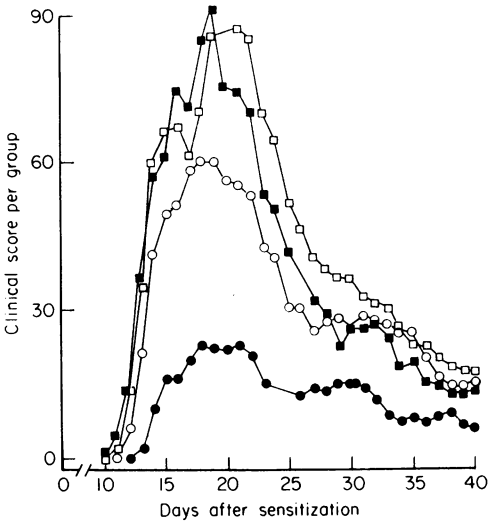

FIG. 3. The effect of treatment with essential fatty acids (EFA) on the clinical score per group during the course of EAE in splenectomized Lewis rats (Sx) and their sham-splenectomized controls. The figure represents the pooled results of two separate experiments. At the height of the disease the differences between the sham/EFA group and the other groups were significant ($P < 0{\cdot}001$). The differences between the sham control group and the Sx groups were also significant ($P < 0{\cdot}01$). (○—○) Sham control, $n = 24$; (●—●) sham/EFA, $n = 24$; (□—□) Sx control, $n = 24$; and (■—■) Sx/EFA, $n = 24$.

RESULTS

Treatment with EFA significantly reduced clinical disease in the sham Sx animals (Figs 2, 3 & 4), confirming our previous results in Lewis rats (Mertin & Stackpoole, 1978). Only 50% of the animals in the EFA-treated sham Sx groups developed clinical signs of EAE (Fig. 2) and the sums of the clinical scores per group were correspondingly low throughout the course of the disease (Fig. 3).

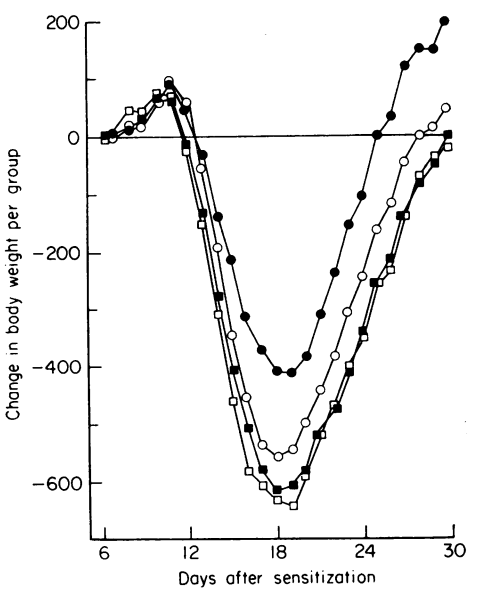

FIG. 4. The effect of treatment with essential fatty acids (EFA) on the changes in body weight in splenectomised (Sx) and sham-splenectomized Lewis rats during the clinical course of EAE. (○—○) Sham control, $n = 24$; (●—●) sham/ EFA, $n = 24$; (□—□) Sx/control, $n = 24$; and (■—■) Sx/EFA, $n = 24$.

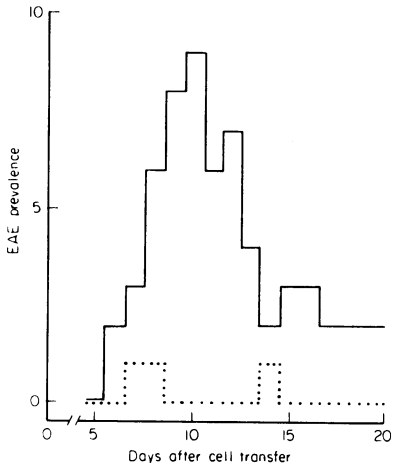

FIG. 5. The effect of treatment with essential fatty acids (EFA) on disease prevalence (i.e. number of animals showing clinical signs of EAE on any one day during the period of the experiment) in the course of EAE induced by passive transfer. Transfer of EAE was achieved by i.v. injection of Con A-stimulated spleen cells from diseased animals into normal recipients. The figure represents the pooled results of two separate experiments. In the EFA-treated rats one animal in each group showed shortlasting signs of EAE. (——) Control, $n = 10$; (. . . . .) EFA, $n = 10$.

As judged by the clinical signs of EAE, EFA-treated animals were almost completely protected against the EAE-inducing effect of Con A-stimulated spleen cells from sensitized rats (Fig. 5). In two separate experiments, only one out of six animals and one out of four animals, respectively, showed mild signs of disease, lasting only for a short period of time. In the paraffin-treated control groups, however, all animals (six out of six and four out of four, respectively) developed clinical signs which prevailed for a longer period of time, and in both experiments one of the control animals died from EAE.

The suppressive effect of EFA was abrogated by splenectomy (Fig. 2). The first signs of EAE appeared earlier and clinical severity of the disease was significantly greater in the Sx groups than in the sham Sx groups and this was independent of whether or not the animals were receiving EFA treatment (Fig. 3). The overall loss of body weight in the groups almost mirrored the picture of the clinical course as shown by the scores (Fig. 4).

DISCUSSION

In this study we have shown that EAE can be suppressed by treatment of the animals with EFA. Our results are in agreement with earlier observations suggesting that treatment with subcutaneously or orally administered EFA can cause immunosuppression. The mechanism by which EFA affect the function of immune competent cells is unknown. Various possible explanations have been discussed in a recent review by Meade & Mertin (1978) and one of these was the suggestion that the EFA effects were mediated by PG (Mertin, 1976). In a previous publication we provided evidence supporting this hypothesis, EFA-induced suppression of EAE being abrogated in our experiments by indomethacin, an inhibitor of the biosynthesis of PG from EFA (Mertin & Stackpoole, 1978). In mice, treatment with EFA of the ω-6 family causes—besides other effects on CMI—a significant decrease in the weight of the thymus gland (Meade & Mertin, 1976). This effect can also be abrogated by indomethacin (Meade, 1979). There are also findings linking PG with the pathological process in EAE:McIlhenny *et al.* (1978) and Davison (1978) observed a deterioration of the clinical course of EAE when the animals were treated with inhibitors of the PG biosynthesis.

Immunosuppression by treatment with EFA appeared mainly to affect the effector phase of the CMI

response (Meade *et al.*, 1978). Our observation that EFA treatment greatly protected the animals from EAE induced by passive transfer provides further evidence for this assumption.

Splenectomy has been found to abolish the suppressive effect of EFA on EAE and, furthermore, to cause an increase in the clinical severity of the disease. The latter finding is consistent with the observation by Paterson & Didakow (1961) that lymph node cells from splenectomized rats have an enhanced ability to transfer EAE. The spleen is known to be a major site for the production of immunologically active PG (Osheroff *et al.*, 1975; Webb & Osheroff, 1976). Thus, our observations add further evidence in support of the above hypothesis and allow us to postulate a close relationship between EFA, PG and a splenic factor suppressing immunopathological mechanisms in EAE. Results of several investigations have implicated the spleen as being a major source of suppressor cell activity (Jacobson *et al.*, 1972; Sampson *et al.*, 1976; Coons & Goldberg, 1978; Moorhead, 1978). Adda *et al.* (1977) have demonstrated that suppressing cells migrating from the thymus to the spleen exercise protection against EAE induction in Lewis rats. Webb & Nowowiewski (1978) have shown that stimulated splenic T lymphocytes react not only by proliferation but also by releasing PG which in turn acts as a trigger for suppressor cells to release suppressor factor. Provision of PG precursors or PG itself may enhance this process, leading to a more effective suppression of the immune response. This could explain the suppressive effect of EFA treatment on various CMI reactions (Mertin, 1976; Meade & Mertin, 1978), and also observations such as the suppression of autoimmune disease in NZB/NZW mice by PG administration.

In MS, changes in suppressor cell activity may contribute to the occurrence of relapses and remissions. Supplementation of the diet with EFA has a beneficial effect in patients with a relapsing–remitting course of the disease (Mertin & Meade, 1977). In two double-blind trials amelioration of the clinical severity of relapses was observed (Millar *et al.*, 1973; Bates *et al.*, 1978). From our experiences with EFA treatment in EAE, it seems likely that this therapeutic effect in MS may be brought about by EFA-induced enhancement of suppressor activity. Here, too, PG may play a role, and the observation that clinical symptoms worsened in MS patients treated with indomethacin (Rudge, personal communication) supports this view. We are aware of the fallacies of such extrapolations but hope that our results and their possible implications might stress the necessity for further research into the role of PG and their precursors in the pathogenesis of MS.

REFERENCES

ADDA, D.H., BERAUD, E. & DEPIEDS, R. (1977) Evidence for suppressor cells in Lewis rats' experimental allergic encephalomyelitis. *Europ. J. Immunol.* **7**, 620.

ARNASON, B.G.W. & ANTEL, J. (1978) Suppressor cell function in multiple sclerosis. *Ann. Immunol. (Inst. Pasteur)*, **129C**, 159.

BATES, D., FAWCETT, P.R.W., SHAW, D. & WEIGHTMAN, D. (1978) Trial of polyunsaturated fatty acids in the treatment of acute remitting multiple sclerosis. *Brit. Med. J.* iii, 1390.

BORNSTEIN, M.B. (1977) Experimental allergic encephalomyelitis. *Scientific approaches to clinical neurology* (ed. by E. S. Goldensohn and S. H. Appel), Vol. 1, p. 325. Lea and Febiger, Philadelphia.

COONS, T.A. & GOLDBERG, E.H. (1978) Rejection of male skin grafts by splenectomised female mice. *Science*, **200**, 320.

DAVISON, A.N. (1978) Immunological studies in multiple sclerosis. *Proceedings of the European Society of Neurochemistry* (ed. by V. Neuhoff), vol. 1.p. 91. Verlag Chemie, Weinheim, New York.

JACOBSON, E.B., HERZENBERG, L.A., RIBLET, R. & HERZENBERG, L.A. (1972) Active suppression of immunoglobulin allotype synthesis. II. Transfer of suppressing factor with spleen cells. *J. exp. Med.* **135**, 1163.

MCFARLIN, D.E., BLANK, S.E. & KIBLER, R.F. (1974) Recurrent experimental allergic encephalomyelitis in the Lewis rat. *J. Immunol.* **113**, 712.

MEADE, C.J. (1979) How arachidonic acid depresses thymus weight. *Int. Arch. Allergy appl. Immunol.* (In press.)

MEADE, C.J. & MERTIN, J. (1978) Fatty acids and immunity. *Adv. Lipid Res.* **16**, 127.

MEADE, C.J., MERTIN, J., SHEENA, J. & HUNT, R. (1978) Reduction by linoleic acid of the severity of experimental allergic encephalomyelitis in the guinea pig. *J. Neurol. Sci.* **35**, 291.

MERTIN, J. (1976) Effect of polyunsaturated fatty acids (PUFA) on skin allograft survival and primary and secondary cytotoxic response in mice. *Transplantation*, **21**, 1.

MERTIN, J. (1979) Essential fatty acids and immunity. *Progress in Multiple Sclerosis Research* (ed. by H. Bauer *et al.*). (In press.)

MERTIN, J. & HUGHES, D. (1975) Specific inhibitory action of polyunsaturated fatty acids on lymphocyte transformation induced by PHA and PPD. *Int. Arch. Allergy appl. Immunol.* **48**, 203.

MERTIN, J. & HUNT, R. (1976) Influence of polyunsaturated fatty acids on survival of skin allografts and tumour incidence in mice. *Proc. Nat. Acad. Sci. (Wash.)*, **73**, 928.

MERTIN, J. & MEADE, C.J. (1977) Relevance of fatty acids in multiple sclerosis. *Brit. Med. Bull.* **33**, 67.

MERTIN, J. & STACKPOOLE, A. (1978) Suppression by

essential fatty acids of experimental allergic encephalomyelitis is abolished by indomethacin. *Prostaglandins and Medicine*, **1**, 283.

McILHENNY, H.M., LEVINE, S., WISEMAN, E.H. & SOWINSKI, R. (1978) Disposition and activity in experimental allergic encephalomyelitis of flumizole, a non-acidic, non-steroidal, anti inflammatory agent. *Exp. Neurol.* **58**, 126.

MIHAS, A.A., GIBSON, R.G. & HIRSCHOWITZ, B.I. (1975) Suppression of lymphocyte transformation by 16 (16) dimethyl prostaglandin E_2 and unsaturated fatty acids. *Proc. Soc. exp. Biol. (N.Y.)*, **149**, 1026.

MILLAR, J.H.D., ZILKHA, K.J., LANGMAN, M.J.S., PAYLING WRIGHT, H., SMITH, A.D. BELIN, J. & THOMPSON, R.H.S. (1973) Double blind trial of linoleate supplementation of the diet in multiple sclerosis. *Brit. Med. J.*, **i**, 765.

MOORHEAD, J.W. (1978) Subpopulations of mouse T lymphocytes. II. Suppression of graft-*vs*-host reactions by naturally proliferating splenic T cells. *Europ. J. Immunol.* **8**, 163.

OSHEROFF, P.L., WEBB, D.R. & PAULSRUD, J. (1975) Induction of T-cell dependent splenic prostaglandin $F_{2\alpha}$ by T-cell dependent antigen. *Biochem. biophys. Res. Comm.* **66**, 425.

PANITCH, H.S. & MCFARLIN, D.E. (1977) Experimental allergic encephalomyelitis: enhancement of cell-mediated transfer by concanavalin A. *J. Immunol.* **119**, 1134.

PATERSON, P.Y. (1977) Autoimmune neurological disease: experimental animal systems and implications for multiple sclerosis. *Autoimmunity* (ed. by N. Talal), p. 643. Academic Press, Inc., New York, San Francisco, London.

PATERSON, P.Y. & DIDAKOW, N.C. (1961) Transfer of allergic encephalomyelitis using splenectomized albino rats. *Proc. Soc. exp. Biol. Med.* **108**, 768.

PELUS, L.M. & STRAUSSER, H.R. (1977) Prostaglandins and the immune response. *Life Sci.* **20**, 903.

RAINE, C.R. & TRAUGOTT, U. (1978) Suppression of chronic allergic encephalomyelitis. *Science*, **201**, 445.

SAMPSON, D., GROTELENESCHER, C., METZIG, J. & KAUFMAN, H.M. (1976) Suppressor activity of the human spleen and thymus. *Surgery*, **79**, 393.

SANTIAGO-DELPIN, E.A. & SZEPSENWOL, J. (1977) Prolonged survival of skin and tumour allografts in mice on high-fat diets: brief communication. *J. Nat. Cancer Inst.* **59**, 459.

TRAUGOTT, U., STONE, S.H. & RAINE, C.S. (1978) Experimental allergic encephalomyelitis—migration of early T cells from the circulation into the central nervous system. *J. Neurol. Sci.* **36**, 55.

VANE, J.R. (1976) The mode of action of aspirin and similar compounds. *J. Allergy clin. Immunol.* **58**, 691.

WEBB, D.R. & NOWOWIEJSKI, I. (1978) Mitogen-induced changes in lymphocyte prostaglandin levels: A signal for the induction of suppressor cell activity. *Cell. Immunol.* **41**, 72.

WEBB, D.R. & OSHEROFF, P.L. (1976) Antigen stimulation of prostaglandin synthesis and control of immune responses. *Proc. Natl. Acad. Sci. (Wash.)*, **73**, 1300.

WEYMAN, C., MORGAN, S.J., BELIN, J. & SMITH, A.D. (1977) Phytohaemagglutinion stimulation of human lymphocytes. Effect of fatty acids on uridine uptake and phosphoglyceride fatty acid profile. *Biochem. biophys. Acta*, **496**, 155.

WISNIEWSKI, H.M. & KEITH, A.B. (1977) Chronic relapsing experimental allergic encephalomyelitis: an experimental model of multiple sclerosis. *Ann. Neurol.* **1**, 144.

ZURIER, R.B., SAYADOFF, D.M., TORREY, S.B. & ROTHFIELD, N.F. (1977) Prostaglandin E_1 treatment of NZB/NZW mice. *Arthr. and Rheum.* **20**, 723.