Combination of theophylline and prostaglandin E_1 as inhibitors of the adjuvant-induced arthritis syndrome of rats

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SUMMARY   The effects of daily subcutaneous administration of prostaglandin E_1 (PGE_1), theophylline, and of both drugs together were studied on the Freund's adjuvant-induced inflammatory and arthritic syndrome in rats. In the doses used, neither drug affected the acute inflammatory response in the adjuvant-treated paw, but together they caused marked inhibition. Chronic inflammation in the contralateral (nontreated) hind paws was slightly inhibited by each drug and combined treatment resulted in marked inhibition. The drugs also counteracted splenomegaly in adjuvant-diseased rats and their effects on spleen weight paralleled the inhibition of chronic inflammation. Arthritic lesions, as judged by x-rays of tibiotarsal joint destruction in the nontreated paws, were partially prevented by PGE_1 alone, but not by theophylline. The combined treatment entirely prevented these joint lesions. PGE_1 did not cause an increase in adrenal weight, but enhanced the effect of theophylline on adrenal weight. Only PGE_1 improved gait in arthritic rats, simultaneous theophylline treatment having little additional effect.

Other workers have found that PGE_1 increases intracellular cAMP and that this effect is enhanced by the phosphodiesterase inhibitor, theophylline. We propose that the anti-inflammatory and anti-arthritic effects of combined drug treatment involve cAMP changes in phagocytic cells at the site of tissue injury and in systemic lymphocytes.

Exogenously administered E-type prostaglandins (PGE) in high doses have been shown to suppress adjuvant-induced arthritis in rats (Aspinall and Cammarata, 1969; Zurier and Quagliata, 1971; Glenn and Rohloff, 1972; DiPasquale *et al.*, 1973; Zurier *et al.*, 1973). *In vitro*, PGE increased the level of cyclic 3′, 5′-adenosine monophosphate (cAMP) in leucocytes and this effect was associated with inhibited release of lysosomal enzymes from phagocytic cells (Scott, 1970; Weissmann, 1972; Weissmann *et al.*, 1976; Lichtenstein *et al.*, 1972). It has been suggested that such a cAMP-mediated effect is one of the mechanisms underlying the anti-inflammatory effect of PGE on adjuvant arthritis (Zurier *et al.*, 1973). Evidence that PGE-induced elevation of leucocytic cAMP *in vitro* is enhanced by the simultaneous addition of the phosphodiesterase inhibitor, theophylline (Bourne *et al.*, 1972, 1974), supports this. We therefore decided to examine the influence of

PGE$_1$ and theophylline *in vivo* on various parameters of adjuvant arthritis in rats. Some of our findings have been published (Bonta *et al.*, 1977c).

Methods

ANIMALS AND INDUCTION OF ADJUVANT SYNDROME

Male Lewis rats (weight 220–280 g), from the Central Animal Breeding Institute, Hanover, W. Germany, were used. The animals were caged in groups of 5–7 and allowed water and laboratory food (Hope Farms) *ad libitum*. Each animal was injected with 0·1 ml Freund's complete adjuvant (5 mg/ml killed *Mycobacterium butyricum* (Difco, Detroit) in liquid paraffin) into the left hind paw. Body weight was recorded at the start of the experiment and thereafter at weekly intervals.

EVALUATION OF INFLAMMATION

Hind paw volumes were determined by a differential

volume meter (Ugo Basile, Milan). Acute inflammation was measured by the increase in volume of the treated paw 6 hours after administration of the adjuvant. Chronic inflammation was evaluated by the increase in volume of the untreated paw 8 days after adjuvant injection. The following formula was used to calculate this increase:

$$\frac{\text{Volume on day 28} - \text{Volume before adjuvant injection}}{\text{Volume before adjuvant injection}} \times 100.$$

The value thus obtained was corrected for 100 g body weight.

GAIT TEST

Chronic inflammation and/or arthritis reduces the use of the untreated paw in adjuvant-diseased rats. On day 30 each rat was placed on a table top and allowed to move freely. The impaired function of the adjuvant-treated paw was disregarded because in several rats this paw was not only swollen but also abscessed and few of the animals were able to use this limb. Two observers, unaware of the drug treatments and of each other's assessment, scored the use of the untreated (right) paw: no use of the paw 0, passive use to support the body 1, or active use 2. Owing to gross damage of the treated paw, animals with a score of 0 displayed a 'creeping' behaviour (moving on the 2 fore legs, dragging the 2 hind paws). The scores of the two observers were added together to give a combined score for each rat. The scores for each rat were also added together to obtain a group score. In addition to the above evaluation, the gait of a few selected rats was recorded with a cine-camera, and the validity of the scoring was retrospectively verified by viewing the films.

CHRONIC ARTHRITIS OF UNTREATED PAW

On day 35 after adjuvant injection the rats were anaesthetised with ether and x-rays taken in a lateral-medial direction of the untreated hind paw. An experienced x-ray technician (J. F. Fasotte, Department of Experimental Surgery, Medical Faculty), unaware of the different drug treatments, scored the condition of tibiotarsal joints. These scores (destroyed or intact joint) were used as a quantal test for bone necrosis.

SPLEEN AND ADRENAL WEIGHTS

The rats were then killed with ether, the spleen and adrenals removed, and the wet weights of the organs recorded. Both adrenal glands were weighed together and all the organ weights were corrected for 100 g body weight.

DRUG TREATMENT

Four groups of 6 rats treated with complete adjuvant were used. The first (control) group received a daily subcutaneous injection of 0·9% (w/v) saline (1 ml/kg). The second group was treated with PGE₁ in a daily dose of 0·75 mg/kg subcutaneously. A third group was injected daily with theophylline 75 mg/kg subcutaneously. The fourth group received subcutaneous injections of PGE₁ and theophylline* combined in the given doses, in one experiment lasting 28 days and in another until day 35. The drug solutions were freshly prepared once a week in 0·9% (w/v) saline, stored at 4°C and, warmed to room temperature before injection.

STATISTICS

Although in one experiment treatment lasted only 28 days, and since chronic inflammation in both experiments was evaluated on this day, all data obtained on day 28 were pooled. Significance of differences from saline-treated controls was calculated using the one-tailed Student's t test. All other data were derived from the experiment in which treatment lasted 35 days. The one-tailed Student's t test was used to calculate the significance of differences in organ weights, and significance of differences in joint destruction and in gait was derived from χ^2 tests.

Results

PGE₁ caused diarrhoea in several rats and approximately one hour after treatment some animals were slightly sedated. In the theophylline-treated animals piloerection and a somewhat increased respiration rate were observed. These effects were also noticeable in the animals receiving the combined treatment, but in every case were transient and the next day before receiving the drugs they displayed normal behaviour. Except for these general observations, no attempts were made to quantify the behaviour effects.

The 6-hour acute inflammation was not affected by either drug administered separately, but in the group receiving the combined drugs the increase in volume of the treated paw was markedly inhibited (Table 1). Chronic inflammation of the untreated paw (increase in volume on day 28) was slightly inhibited by each of the drugs, combined treatment resulting in further inhibition (Table 1). This marked inhibitory effect on the chronic inflammatory swelling following combined treatment is illustrated in Fig. 1.

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*PGE₁ purchased from Unilever, Vlaardingen, The Netherlands; theophylline from Merck AG, Darmstadt.

Table 1 *Effects of PGE₁ and theophylline on acute and chronic inflammation in adjuvant-diseased rats*

	Acute inflammation at 6 h (treated paw, % volume increase)*	Chronic inflammation on day 28 (untreated paw, % volume increase)*
Adjuvant control	109 ± 4 (12)	63 ± 10 (12)
Theophylline 75 mg/kg per day subcutaneously	110 ± 10 (12)	42 ± 2† (12)
Prostaglandin E₁ 0·75 mg/kg per day subcutaneously	117 ± 5 (12)	40 ± 7† (12)
Prostaglandin E₁ plus theophylline	70 ± 7§ (12)	30 ± 7‡ (12)

Note: Values are means ± SEM. Number of observations are given in parentheses. In the theophylline group one rat died on the 24th day. *Paw volumes are corrected for 100 g body weight. Significance of differences between injected *vs* uninjected paws was calculated using the one-tailed Student's *t* test: † P<0·05; ‡ P<0·01; § P<0·001.

The adjuvant control group showed gross malfunction of the untreated paw, which was apparent from the very low group score for gait of 6 (on the basis of the zero-hypothesis a similar nondiseased group of rats might have achieved a score of 24). Nearly maximal improvement in gait was seen in the rats treated with PGE₁ only, whereas theophylline produced no significant improvement. Combined treatment resulted in virtually the same effect as PGE₁ alone. These results are shown in Table 2. Evaluation of x-rays of the tibiotarsal joints is also presented in Table 2. Gross destruction of the joints of the untreated paws was observed in 5 out of 6 rats in the adjuvant control group. Theophylline alone failed to produce any significant improvement.
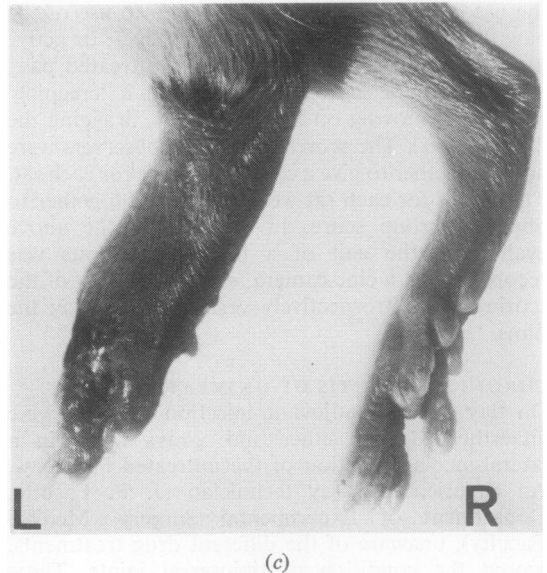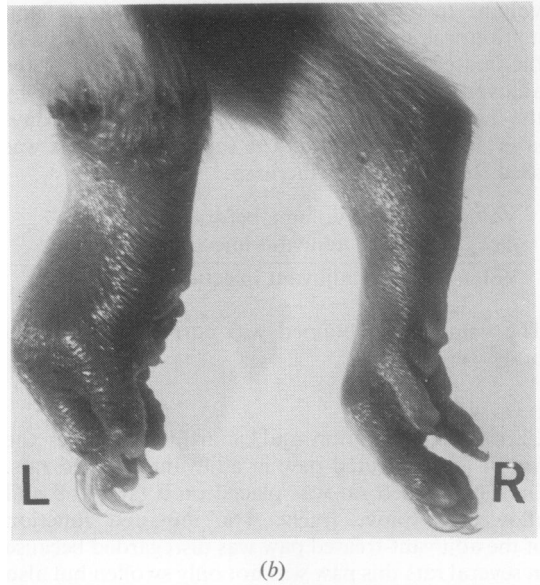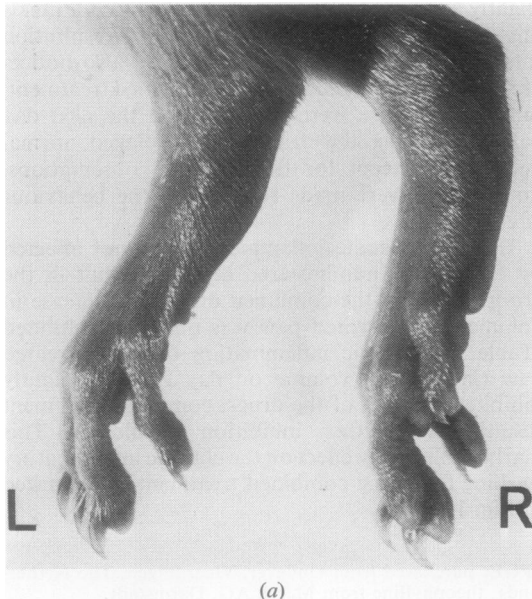

(b)

(c)

(a)

Fig. 1 *Hind paws of Lewis rats. (a) Untreated rat. (b) and (c) 22nd day after adjuvant injection into the left paw. The rat in (b) received subcutaneous saline daily. The adjuvant-injected paw was swollen throughout while in the contralateral paw the lesion is localised in the ankle joint. (c) Showing the paws of a rat which received daily subcutaneous prostaglandin 0.75 mg/kg, plus theophylline 75 mg/kg. The adjuvant-treated paw is swollen, but the contralateral paw is apparently normal.*

However, only 2 out of 6 PGE₁-treated rats had noticeably damaged joints. The protective action of PGE₁ was further increased by simultaneous administration of theophylline. In this latter group, in fact, no tibiotarsal joint lesions were detectable.

Table 2 *Effects of PGE₁ and theophylline on gait and arthritic lesions in the untreated paw of adjuvant-diseased rats*

	Gait* (total score in group)	Tibiotarsal joint† (no, of intact/ observed)
Adjuvant control	6 (6)	1/6
Prostaglandin E₁ (75 mg/kg)	22‡ (6)	4/6‡
Theophylline (75 mg/kg)	13 (7)	2/6
Prostaglandin E₁ plus theophylline	25‡ (7)	5/5 §

Note: The scoring system for gait and the method of evaluation of the joint lesions are described in 'Methods'. *Day 30. Number of rats tested in parentheses. †X-rays on day 35. Significance of differences between injected *vs* uninjected paws was determined by the χ^2 test. ‡P<0·05; §P<0·001.
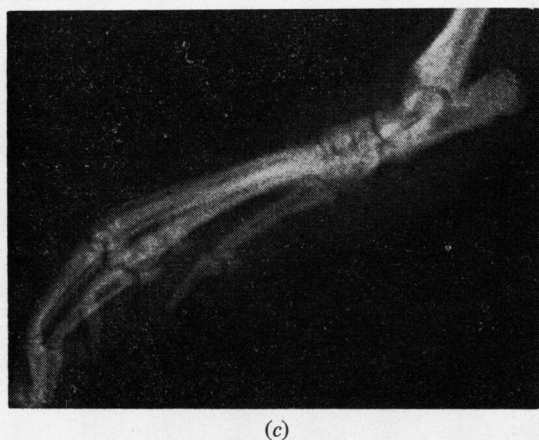

(a)

(b)

(c)

Fig. 2 X-ray of right hind paws of Lewis rats. (a) Untreated rat. (b) and (c) 35th day after adjuvant injection into the left paw. (b) This rat received daily treatment with subcutaneous saline. Note the bone resorption and destruction at the tibiotarsal and metatarsal joints. (c) This rat received daily subcutaneous PGE₁ and theophylline. Joints are apparently intact.

Fig. 2 shows an example of the arthritic destruction caused by the adjuvant disease and the marked protective effect of the combined PGE₁ and theophylline treatment.

Body weights and various organ weights are given in Table 3. Theophylline alone caused slight loss of body weight, but the initial and 35-day weights were not significantly different from the adjuvant controls in any of the other drug-treated groups. PGE₁ alone did not increase adrenal weight, but markedly enhanced the slight increase caused by theophylline. Splenomegaly was also apparent. In a separate experiment (data not included), the mean spleen weight of 6 rats treated with incomplete adjuvant, of the same age and weight, was 162 ± 2 mg. In contrast, the mean spleen weight of the adjuvant control rats was 247 ± 16 mg (Table 3). The increase in the weights of the spleens from adjuvant-diseased rats was counteracted equally by both drugs given separately and a further significant reduction in spleen weight occurred in animals receiving the drugs combined.

Discussion

Administration of Freund's complete adjuvant to the rat induces a complex inflammatory syndrome (Pearson, 1964): acute inflammation in the injected paw and chronic inflammation and arthritic lesions in the uninjected paw, between 10 and 14 days after

Table 3   *Effects of PGE₁ and theophylline on body weight, spleen and adrenal weights in adjuvant-diseased rats (mean ±SEM)*

| | Body weight (g) | | Spleen weight (mg) | Adrenal weight (mg) |
	Initial	35 day		
Adjuvant control	233±11	242±11	247±16	20±4 (5)
Prostaglandin E₁ (75 mg/kg)	243±4	249±7	188±9†	21±5 (6)
Theophylline (75 mg/kg)	241±4	226±3	189±9†	31±4* (7)
Prostaglandin E₁ plus theophylline	253±6	257±8	152±9‡	57±8† (6)

Note: Necropsy was performed on day 35. The organ weights refer to wet weight and are corrected for 100 g body weight. The number of animals are given in parentheses. Significance of differences between injected *vs* uninjected paws was determined by the one-tailed Student's *t* test: *P<0·05; †P<0·01; ‡P<0·001.

the adjuvant injection. The chronic phase is accompanied by splenomegaly and lymphocyte-mediated events, akin to delayed hypersensitivity (Van Arman, 1976). The present results are consistent with this oversimplified concept of the acute and chronic phases of the adjuvant-induced syndrome.

Zurier *et al.* (1973) suggested that the anti-inflammatory effects of PGE₁ on acute inflammatory responses are mediated by inhibition of lysosomal enzyme release, possibly through raised cAMP in phagocytic cells, as shown *in vitro* (Weissmann *et al.*, 1976). Furthermore, PGE₁ and the phosphodiesterase inhibitor, theophylline, act synergistically in raising intracellular cAMP and inhibiting lysosomal enzyme release from immune-sensitised cells *in vitro* (Bourne *et al.*, 1972, 1974; Lichtenstein *et al.*, 1972). In these studies the doses of PGE₁ were so large that the levels may have far exceeded those which probably occur under pathological conditions. Qualitatively, however, the synergism observed in this study between PGE₁ and theophylline in inhibiting acute adjuvant-induced inflammation (injected paw), may be explained on the basis of cAMP-mediated inhibition of lysosomal enzyme release.

It has been suggested that PGE₁ inhibits the chronic phase of adjuvant disease either by suppressing lymphocyte activation or by reducing the number of circulating lymphocytes (Zurier *et al.*, 1973). Recently, *in vitro* studies have shown that PGE-induced inhibition of T lymphocyte activation is correlated with increased intracellular cAMP levels (Henney *et al.*, 1972; Bourne *et al.*, 1974; Morley, 1974; Gordon *et al.*, 1976). We have now found that theophylline and PGE₁ each exerts a moderate suppressive effect on the chronic component of the adjuvant-syndrome. When combined, however, they exhibit an enhanced inhibitory effect, paralleled by inhibition of splenomegaly. It may be that *in vivo* PGE₁ and theophylline each enhances the chronic anti-inflammatory effect of the other by increasing cAMP levels in splenic and/or lymph node lymphocytes which might then result in reduced immunological activation and subsequent inhibition of the infiltration of circulating lymphocytes into the synovium. Parnham *et al.* (1977a) have shown that changes in cAMP levels in perfusates of noninjected arthritic rat paws correlate with the early, but not the later, stages of the chronic response. Thus, the major part of the mutual inhibitory activity of the two drugs on the chronic response was probably mediated by cAMP changes remote from the inflamed paw. As early as 1966, Ryzweski observed that cholinergic agents potentiated, whereas adrenergic agents inhibited, the chronic phase of adjuvant arthritis. Although no satisfactory explanation of these effects was possible at the time, it now appears, from *in vitro* studies, that cholinergic agents stimulate intracellular cGMP and adrenergic agents stimulate cAMP and that these cyclic nucleotides exert antagonistic effects on cellular function (Bourne *et al.*, 1974; Weissmann *et al.*, 1976).

Although combined treatment with PGE₁ and theophylline resulted in a marked increase in adrenal size, it is unlikely that release of endogenous corticosteroids accounted for the inhibitory effects observed. Firstly, PGE₁ has been shown to suppress adjuvant disease in adrenalectomised animals (Zurier *et al.*, 1973). Secondly, in our investigation, the increase in adrenal weight did not appear to be correlated with other changes seen during chronic inflammation in rats treated with both PGE₁ and theophylline. Thirdly, the anti-inflammatory, modulating role of endogenous corticosteroids during chronic inflammation in rats is, in fact, questionable (Parnham, 1977). Fourthly, adjuvant disease itself is accompanied by a very high output of adrenal corticosteroids (Weissmann, 1972) and it is unlikely that any further PGE₁- and theophylline-induced increases would produce significant effects.

Inhibition of chronic inflammation was also accompanied by suppression of the arthritic lesions observed in the noninjected paw. The x-rays showed that PGE₁ alone, and more particularly when administered with theophylline, almost completely counteracted the joint destruction. Although *in vitro* studies on cultured rat bone cells have shown that PGEs stimulate bone resorption (Klein and Raisz, 1970), Morley (1974) has proposed that lymphokines (LKs) from sensitised lymphocytes stimulate macrophages to release PGs, which then inhibit further LK release and thus curtail the maintenance of the inflammatory stimulus. The large amounts of exogenous PGE₁ in the present experiments may have potentiated this negative feedback effect, preventing LK production and the subsequent inflammatory effects, including the release of endogenous

bone-resorbing PGs. Since PGE appears to inhibit lymphocyte activation through increasing intracellular cAMP (Bourne *et al.*, 1974), this probably accounts for the further prevention of the bone lesions when theophylline was administered with the PGE_1.

Although pharmacological amounts of PGE_1 are required to suppress adjuvant arthritis, Zurier *et al.* (1973) suggested that in chronic disorders endogenous PGEs may build up to levels high enough to retard inflammation. While this is only a suggestion, we have indirect evidence that in the chronic phase of local tissue damage the anti-inflammatory activity of endogenous PGE prevails over its pro-inflammatory activity. Thus, PGE-precursor deprivation results in enhancement of the chronic phase of the adjuvant syndrome (Bonta *et al.*, 1977a) and increased tissue production in granulomata induced in rats (Bonta *et al.*, 1977b), an effect which is accompanied by increased collagen synthesis (Parnham *et al.*, 1977b).

Although our results suggest that theophylline enhances the inhibitory effect of PGE_1 through a cAMP-mediated mechanism, the details have yet to be elucidated. Because of the short biological half-life of exogenously administered PGs the possibility of PG-metabolite involvement has to be considered in interpreting our results. Thus, irrespective of whether given alone or in combination with theophylline, exogenous PGE_1 may not have remained unmetabolised long enough to affect cellular infiltration and perhaps the ultimate effect was caused by a metabolite rather than by PGE_1.

References

Aspinall, R. L., and Cammarata, P. S. (1969). Effect of prostaglandin E_1 on adjuvant arthritis. *Nature*, **224**, 1320–1321.

Bonta, I. L., Parnham, M. J., Adolfs, M. J. P., and Van Vliet, L. (1977a). Dual function of E-type prostaglandins in models of chronic inflammation. *Perspectives in Inflammation; Future Trends and Developments*, pp. 265–275. Ed. by D. A. Willoughby, J. P. Giroud, and G. P. Velo. MTP Press, Lancaster.

Bonta, I. L., Parnham, M. J., and Adolfs, M. J. P. (1977b). Reduced exudation and increased tissue proliferation during chronic inflammation in rats deprived of endogenous prostaglandin precursors. *Prostaglandins*, **14**, 295–307.

Bonta, I. L., Parnham, M. J., Van Vliet, L., and Vincent, J. E. (1977c). Mutual enhancement of the effects of prostaglandin E_1 and theophylline on the Freund adjuvant-induced arthritis syndrome of rats. *British Journal of Pharmacology*, **59**, 438P–439P.

Bourne, H. R., Lichtenstein, L. M., and Melmon, K. L. (1972). Pharmacologic control of allergic histamine release in vitro; evidence for an inhibitory role of cyclic 3′, 5′-adenosine monophosphate in human leucocyte. *Journal of Immunology*, **108**, 695–705.

Bourne, H. R., Lichtenstein, L. M., Melmon, K. L., Henney, C. S., Weinstein, Y., and Shearer, G. M. (1974). Modulation of inflammation and immunity by cyclic AMP. *Science*, **184**, 19–28.

DiPasquale, G., Rassaert, C., Richter, R., Welaj, P., and Tripp, L. (1973). Influence of prostaglandins (PG) E_2 and $F_{2\alpha}$ on the inflammatory process. *Prostaglandins*, **3**, 741–757.

Glenn, E. M., and Rohloff, N. (1972). Antiarthritic and anti-inflammatory effects of certain prostaglandins. *Proceedings of the Society for Experimental Biology and Medicine*, **139**, 290–294.

Gordon, D., Bray, M. A., and Morley, J. (1976). Control of lymphokine secretion by prostaglandins. *Nature*, **262**, 401–402.

Henney, C. S., Bourne, H. R., and Lichtenstein, L. M. (1972). The role of cyclic 3′, 5′-adenosine monophosphate in the specific cytolytic activity of lymphocytes. *Journal of Immunology*, **108**, 1526–1534.

Klein, D. C., and Raisz, L. G. (1970). Prostaglandins: stimulation of bone resorption in tissue culture. *Endocrinology*, **86**, 1346–1440.

Lichtenstein, L. M., Gillespie, E., Bourne, H. R., and Henney, C. S. (1972). The effects of a series of prostaglandins on in vitro models of the allergic response and cellular immunity. *Prostaglandins*, **2**, 519–528.

Morley, J. (1974). Prostaglandins and lymphokines in arthritis. *Prostaglandins*, **8**, 315–326.

Parnham, M. J. (1977). Metyrapone: a possible tool in investigating the role of endogenous corticosteroids in inflammation. *Agents and Actions*, Suppl. 2, 85–97.

Parnham, M. J., Bonta, I. L., and Adolfs, M. J. P. (1977a). Interactions between prostaglandin E and cyclic nucleotides in chronic inflammatory disease. *Perspectives in Inflammation; Future Trends and Developments*, pp. 279–288. Ed. by D. A. Willoughby, J. P. Giroud, and G. P. Velo. MTP Press, Lancaster.

Parnham, M. J., Shoshan, S., Bonta, I. L., and Neiman-Wollner, S. (1977b). Increased collagen metabolism in granulomata induced in rats deficient in endogenous prostaglandin precursors. *Prostaglandins*, **14**, 709–714.

Pearson, C. M. (1964). Experimental models in rheumatoid disease. *Arthritis and Rheumatism*, **7**, 80–86.

Ryzewski, J. (1966). The effect of stimulation and inhibition of the autonomic nervous system on the development and course of polyarthritis induced with Freund's adjuvant in rats. *Polish Medical Journal*, **5**, 1011–1018.

Scott, R. E. (1970). Effects of prostaglandins, epinephrine and NaF on human leucocyte, platelet and liver adenyl cyclase. *Blood*, **35**, 514–516.

Van Arman, C. G. (1976). Pathway to adjuvant arthritis. *Federation Proceedings*, **35**, 2442–2446.

Weissmann, G. (1972). Discussion. *Prostaglandins in Cellular Biology*, p. 169. Ed. by P. W. Ramwell and B. B. Pharriss. Plenum Press, New York.

Weissmann, G., Goldstein, I., and Hoffstein, S. (1976). Prostaglandins and the modulation of cyclic nucleotides of lysosomal enzyme release. *Advances in Prostaglandin and Thromboxane Research*, Vol. 2, pp. 803–814. Ed. by B. Samuelsson and R. Paoletti. Raven Press, New York.

Zurier, R. B., and Quagliata, F. (1971). Effect of prostaglandin, E_1 on adjuvant arthritis. *Nature*, **234**, 304–305.

Zurier, R. B., Hoffstein, S., and Weissmann, G. (1973). Prostaglandin E_1 suppression of acute and chronic inflammation in adrenalectomized rats by pharmacologic amounts of prostaglandins. *Arthritis and Rheumatism*, **16**, 606–619.