# Antiarthritic and Antiinflammatory Effects of Certain Prostaglandins (36128)

E. Myles Glenn and Norman Rohloff
*The Upjohn Company, Kalamazoo, Michigan 49001*

Adjuvant-induced arthritis is thought to be a disease of the delayed hypersensitivity type (1, 2). It is characterized by a variety of clinical features including joint pathology, splenomegaly, iritis, urethritis, and many other features (3). Various workers (4–9) have shown inhibition of the disease with anti-inflammatory steroids, nonsteroidal anti-inflammatory drugs and immunosuppressants. One can differentiate these three types of drugs in this disease. Recent reports that $PGE_1$ and $PGE_2$ inhibit arthritis of rats (10, 11), along with other reports that these agents are pro-inflammatory (12–14), and that their synthesis is inhibited in several tissues by the anti-inflammatory drugs, aspirin and indomethacin (15–17), prompted our investigation of the relative merits of these seemingly paradoxical pathologic–pharmacologic observations. This report summarizes work concerning the antiarthritic and anti-inflammatory effects of prostaglandins. Another summary of their pro-inflammatory effects is in preparation.

*Methods.* Adjuvant arthritis of the rat is produced by the inoculation of 0.5 mg of *Mycobacterium butyricum* (Difco) in 0.1 ml of heavy mineral oil into the tail. Most references to this disease are appended to another report (18). Prostaglandins are made up in 0.5 ml of sterile saline each day and injected subcutaneously twice each day for 14–15 days. Steroidal or nonsteroidal drug-treated, nontreated, and vehicle-treated controls are done simultaneously. Ten animals per dosage group are used routinely, except in those instances, shown here, in which normal, nonarthritic drug-treated animals are assessed simultaneously; in which case, five animals per group are used.

When therapy is terminated after 2 weeks, animals are scored visually for arthritis; heparinized bloods are removed from the dorsal aortas of anesthetized rats and plasma inflammation units (19) and fibrinogen (20) are determined. White cell counts are made on H and E stains by routine methods. Various organs are then removed, blotted on filter paper, and weighed on a torsion balance.

Acute inflammation is produced essentially by the method of Winter *et al.* (21). Drugs are given orally or subcutaneously 1 hr prior to inoculation of 0.1 ml of carrageenan (0.5% in saline) into the right hindpaw. Five hours later, animals are killed with chloroform and both hindpaws are removed with a Harvard guillotine, weighed, and the differences are expressed as milligrams of edema/100 g of body weight. Rats (250 g) are fasted overnight for 16 hr prior to treatment with drugs and throughout the experimental interval. Standard drugs, usually aspirin at multidoses, and vehicle-treated controls are assessed simultaneously in all acute assays of this type and 10 animals/dosage group are employed routinely. Results in both *in vivo* systems are expressed as the mean, along with the standard error of the mean or the standard deviation from the mean.

Prostaglandins were obtained from Dr. John Pike of The Upjohn Company; and all other drugs were from other reliable commercial sources.

*Results.* Therapy of both normal and arthritic rats with $PGE_1$ and $PGE_2$ at relatively high pharmacologic dosages inhibits developing arthritis, produces adrenal hyperplasia (especially in normal rats), and reduces the weights of the spleens and thymuses (Table I). The underlying plasma inflammation units is only slightly affected. Routine assays with effective steroidal and nonsteroidal drugs are always accompanied by significant reductions in this parameter. Given to rats with established disease (therapy

TABLE I. Effect of $PGE_1$ and $PGE_2$ in the Preventive Therapy of Adjuvant-Induced Polyarthritis.[a]

| Drug | mg/kg b.i.d. sc ×15 days | Body wt gain (g/rat) | | Thymus (mg) | | Spleen (mg) | | Adrenal (mg) | | Plasma inflammation units | | Score | | PCV[b] (mm) | | ESR[c] (mm/60 min) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Norm. | Arth. | Norm. | Arth. | Norm. | Arth. | Norm. | Arth. | Norm. | Arth. | Norm. | Arth. | Norm. | Arth. | Norm. | Arth. |
| 1. Vehicle | — | 72 | 18 | 724 ± 54 | 354 ± 61 | 932 ± 52 | 1078 ±134 | 50 ± 4 | 56 ± 2 | 19.5 ± 6.4 | 146.0 ± 20.0 | — | 3.0 ± 2.1 | 47.0 | 45.0 | 1.8 ± 0.4 | 12.1 ± 3.9 |
| 2. $PGE_1$ | 0.5 | 34 | 16 | 478 ± 25 | 290 ± 35 | 712 ± 36 | 908 ±111 | 66 ± 6 | 70 ± 4 | 11.7 ± 2.3 | 135.3 ± 7.9 | — | 3.4 ± 1.7 | 48.6 | 43.2 | 1.6 ± 0.2 | 12.4 ± 1.6 |
| 3. $PGE_1$ | 1.0 | 35 | 19 | 324 ± 28 | 312 ± 40 | 718 ± 23 | 856 ± 92 | 52 ± 6 | 70 ± 6 | 23.6 ± 6.0 | 125.6 ± 6.9 | — | 3.4 ± 2.7 | 49.0 | 45.4 | 3.0 ± 1.5 | 12.5 ± 2.5 |
| 4. $PGE_1$[d] | 2.0 | 27 | 10 | 212 ± 44 | 158 ± 27 | 592 ± 46 | 663 ± 68 | 74 ± 4 | 73 ± 7 | 44.6 ± 8.5 | 118.6 ± 4.6 | — | 2.5 ± 1.9 | 48.2 | 47.8 | 3.6 ± 1.0 | 6.0 ± 1.8 |
| | | | (1/5 dead) | | | | | | | | | | | | | | |
| 5. $PGE_2$ | 0.5 | 38 | 34 | 374 ± 67 | 468 ± 82 | 714 ± 65 | 976 ± 67 | 62 ± 6 | 70 ± 6 | 24.5 ± 3.6 | 132.0 ± 12.4 | — | 3.0 ± 1.7 | 47.8 | 44.4 | 3.0 ± 1.0 | 12.4 ± 2.7 |
| 6. $PGE_2$ | 1.0 | 37 | 23 | 342 ± 17 | 308 ± 42 | 646 ± 40 | 794 ± 84 | 58 ± 4 | 66 ± 2 | 34.6 ± 8.7 | 108.4 ± 8.7 | — | 0 | 48.6 | 44.2 | 2.1 ± 0.6 | 9.7 ± 2.0 |
| 7. $PGE_2$ | 2.0 | 40 | 41 | 311 ± 43 | 258 ± 58 | 612 ± 44 | 913 ± 54 | 78 ± 6 | 80 ± 5 | 22.6 ± 6.4 | 117.5 ± 10.5 | — | 2.0 ± 2.2 | 48.2 | 43.2 | 2.8 ± 1.2 | 13.8 ± 4.2 |
| 8. No treatment (no arthritis) | | 71 | | 590 ± 75 | | 914 ± 36 | | 66 ± 6 | | 13.6 ± 6.4 | | — | — | 47.2 | | 1.9 ± 1.0 | |

[a] Normal and arthritic rats obtained at same time and had same starting weights.
[b] Packed red cell volume.
[c] Erythrocyte sedimentation rate.
[d] Rats on higher doses of $PGE_1$ and $PGE_2$ were prostrate for several minutes after injections and always had transient diarrhea.

started twice each day for 2 weeks on day 16 after inoculation of *M. butyricum* into the tail), neither $PGE_1$ nor $PGE_2$ has antiarthritic effects (Table II).

$PGE_1$ and $PGE_2$, at effective antiarthritic dosages (2.0 mg/kg, b.i.d. × 15 days), reduce the elevated granulocyte counts of arthritic rats ($12.5 \times 10^3 \pm 250$ WBC/$mm^3$) to $2.5 \times 10^3$ WBC $mm^3$. Neither drug has inhibitory effects on the granulocyte count of normal rats. Both agents reduce lymphocyte counts slightly in normal and arthritic rats. These latter effects are dose related. The total white cell count of normal rats is $13.5 \times 10^3 \pm 227/mm^3$, while that of arthritic rats, on day 15 after inoculation of *M. butyricum*, is $25.5 \times 10^3 \pm 1350$ WBC/$mm^3$. Treatment with the two prostaglandins normalizes the elevated white cell counts in arthritic rats but does not reduce them below those of normal rats.

$PGA_2$ and $PGF_{2\alpha}$ given at four multidoses (0.5–4.0 mg/kg, b.i.d. sc × 14 days) to 10 rats/dosage group, fail to elicit antiarthritic effects in rats with developing and established disease. $PGE_1$, $PGE_2$, and $PGE_{2\alpha}$ given orally at 2 mg/kg, po, b.i.d. × 14 days are ineffective as prophylactic or therapeutic agents for the treatment of adjuvant arthritis. Arachidonic acid is ineffective when given orally or subcutaneously.

When compared with cortisol, both $PGE_1$ and $PGE_2$ inhibit the hindpaw edema elicited by carrageenan (Table III). These acute antiinflammatory effects are dose related. Histamine and 5-hydroxytryptamine, two other vasoactive drugs and "mediators" of inflammation, are effective inhibitors also of acute inflammation (Table IV). Digitonin, Filipin, and saponin, all of which are "pro-inflammatory" (22) produce dose-related inhibition of acute inflammation when given intraperitoneally (Table V).

Since certain of the prostaglandins ($PGE_1$ and $PGE_2$) are vasoactive (23), the acute anti-inflammatory and chronic antiarthritic effects of another well-known vasoactive drug, alcohol, have been examined. For these studies, ethyl alcohol is given orally in varying amounts as a 100-proof solution (50%) in water. At 4 and 10 ml/kg, b.i.d. for 14 days,

TABLE II. Effect of $PGE_1$ and $PGE_2$ on Established Arthritis.

| Treatment | Dose (mg/kg, sc b.i.d. × 15 days) | Body wt gains (g/rat) | Arthritic score (units) | Arthritic score % Inh. | PIU[a] (units) | PIU[a] % Inh. |
|---|---|---|---|---|---|---|
| Vehicle | — | 15 | 15.8 ± 0.2 | — | 95.8 ± 8.0 | |
| $PGE_1$ | 2.3 | 32 | 14.6 ± 0.6 | 8 | 125.3 ± 7.0 | 0 |
| $PGE_2$ | 2.2 | 33 | 15.0 ± 1.1 | 0 | 130.2 ± 7.6 | 0 |
| No treatment | — | 3 | 15.9 ± 0.1 | — | 130.0 ± 8.0 | 0 |
| Normal nonarthritic | | 50 | — | — | 5.8 ± 2.1 | — |

[a] Plasma inflammation units (19); 10 rats/group.

TABLE III. Effect of $PGE_1$ and $PGE_2$ on Hindpaw Edema of the Rat.[a]

| Compound | Dose (mg/kg, sc) | Edema[b] | SD[c] | Percentage inhib. | Potency |
|---|---|---|---|---|---|
| Saline vehicle | 0.0 | 0.302 | 0.014 | 0 | |
| Hydrocortisone | 0.433 | 0.235 | 0.018 | 22 | |
| | 0.825 | 0.125 | 0.005 | 59 | |
| | 1.674 | 0.092 | 0.013 | 70 | 1.0 |
| | 3.468 | 0.079 | 0.007 | 74 | |
| | 6.894 | 0.089 | 0.013 | 70 | |
| $PGE_1$ | 0.415 | 0.262 | 0.011 | 13 | |
| | 0.832 | 0.230 | 0.013 | 24 | 0.42 |
| | 1.658 | 0.187 | 0.014 | 38 | $(0.31–0.56)_{95\%}$ |
| | 3.320 | 0.155 | 0.016 | 49 | |
| | 6.754 | 0.060 | 0.014 | 80 | |
| $PGE_2$ | 0.416 | 0.260 | 0.011 | 14 | |
| | 0.862 | 0.226 | 0.011 | 25 | 0.43 |
| | 1.735 | 0.186 | 0.015 | 38 | $(0.32–0.57)_{95\%}$ |
| | 3.420 | 0.135 | 0.018 | 55 | |
| | 6.867 | 0.069 | 0.013 | 77 | |

[a] Diarrhea and sedation at last 3 dosages of both drugs.
[b] Milligrams/100 g of body weight; 10 rats/dosage group.
[c] Standard deviation.

TABLE IV. Effect of Histamine and 5-Hydroxytryptamine on Hindpaw Edema of the Rat.[a]

| Compound | Dose (mg/kg, sc) | Edema | SD | Percentage inhib. |
|---|---|---|---|---|
| Saline vehicle | 0.0 | 0.314 | 0.016 | 0 |
| 5-Hydroxytryptamine | 4.0 | 0.159 | 0.016 | 38 |
| | 9.0 | 0.159 | 0.005 | 49 |
| | 20.0 | 0.085 | 0.015 | 73 |
| Histamine | 4.0 | 0.246 | 0.013 | 22 |
| | 10.0 | 0.257 | 0.016 | 18 |
| | 20.0 | 0.187 | 0.016 | 40 |
| Medrol | 5.0 | 0.136 | 0.011 | 57 |

[a] Conditions and description same in Table III.

TABLE V. Effect of Lytic Drugs on Carrageenan-Induced Inflammation.

| Drug | Dose (mg/kg, ip) | Edema[a] | SEM | Percentage inhib. |
|---|---|---|---|---|
| None | — | 381 | 23 | — |
| Digitonin | 4.0 | 297 | 28 | 22 |
| | 8.0 | 292 | 16 | 23 |
| | 16.0 | 247 | 26 | 35 |
| | 32.0 | 182 | 45 | 52 |
| Filipin | 4.0 | 296 | 22 | 22 |
| | 8.0 | 275 | 18 | 28 |
| | 16.0 | 245 | 10 | 36 |
| | 32.0 | 175 | 11 | 54 |
| Saponin | 4.0 | 315 | 20 | 17 |
| | 8.0 | 300 | 11 | 22 |
| | 16.0 | 275 | 20 | 28 |
| | 32.0 | 225 | 10 | 41 |

[a] Expressed as milligrams of edema/100 g of final body weight.

alcohol inhibits developing arthritis (Table VI). Compared to aspirin simultaneously, it is an effective inhibitor of acute inflammation (Table VII). It lowers temperatures of yeast-fevered rats (Table VIII). There are many other studies with still another vasoactive drug, isoproterenol. Results are similar to those obtained with $PGE_1$. That is, this drug also produces antiarthritic and acute anti-inflammatory effects while eliciting signs

TABLE VI. Effect of 100-Proof Alcohol on Adjuvant-Induced Polyarthritis of Rats.[a]

| Drug | Dose (ml/kg, po, b.i.d. × 14 days) | Final body wt. | Arthritic score/rat | Percentage inhib. |
|---|---|---|---|---|
| Vehicle (water) | — | 214 ± 5.0 | 11.2 ± 1.2 | — |
| Ethyl alcohol, 50% | 1.0 | 204 ± 5.1 | 10.1 ± 1.6 | — |
| | 2.0 | 205 ± 4.9 | 9.9 ± 1.6 | — |
| | 4.0 | 208 ± 12.2 | 7.8 ± 1.5 | — |
| | 10.0 | 206 ± 6.1 | 3.7 ± 1.6 | 66 |

[a] Ten rats/group; all rats weighed 190 g at beginning of experiments.

TABLE VII. Effect of 100-Proof Alcohol on Hindpaw Edema of the Rat.[a]

| Drug | Dose (mg or ml/kg) | Edema | SD | Percentage inhib. |
|---|---|---|---|---|
| Water | 0.0 | 0.330 | 0.018 | 0 |
| Ethyl alcohol, 50% | 0.6 | 0.337 | 0.032 | —2 |
| | 1.2 | 0.278 | 0.007 | 16 |
| | 2.4 | 0.269 | 0.021 | 18 |
| | 4.8 | 0.229 | 0.014 | 31 |
| | 9.0 | 0.220 | 0.012 | 33 |
| Aspirin | 66 | 0.231 | 0.010 | 30 |
| | 132 | 0.222 | 0.028 | 33 |
| | 262 | 0.092 | 0.011 | 72 |

[a] All drugs given orally to 10 rats/group 1 hr prior to carrageenan inoculations into right hindpaw. Paws were removed 5 hr after carrageenan; 6 hr after drug.

of "stress" or adrenal stimulation at high dosages. Isoproterenol produces intense inflammatory reactions at injection sites.

*Summary and Conclusions.* $PGE_1$ and $PGE_2$ produce antiarthritic and acute anti-inflammatory effects when given at high dosages to rats. $PGA_2$ and $PGF_{2a}$ are inactive in these tests. All of the prostaglandins examined here are inactive when given orally to rats with acute or chronic inflammation. Other vasoactive drugs (ethyl alochol and isoproterenol) inhibit acute and chronic inflammation. Paradoxically, perhaps, it has been reported that prostaglandins are pro-inflammatory (12–14). Other pro-inflammatory drugs (digitonin, saponin, and Filipin) also elicit nonspecific acute anti-inflammatory effects when given intraperitoneally. The high pharmacologic doses of prostaglandins required to elicit antiarthritic and anti-inflammatory effects in animals cause adrenal hyperplasia, prostration, and diarrhea. It is conceivable that their secondary antiarthritic and anti-inflammatory effects may be related to these primary phenomena.

TABLE VIII. Effect of 100-Proof Alcohol on Yeast-Induced Fevers of Rats.[a]

| | Temperature drop (°F) in yeast-fevered rats | | | |
|---|---|---|---|---|
| Drug (ml/kg, po) | 2 hr | SD | 5 hr | SD |
| No yeast, no treatment | 0.3 | 0.2 | 0.1 | 0.2 |
| Yeast (controls) | 0.2 | 0.1 | 0.5 | 0.2 |
| 50% EtOH, 1.0 | 0.5 | 0.1 | 0.6 | 0.2 |
| 2.0 | 0.0 | 0.1 | 0.5 | 0.2 |
| 4.0 | 0.4 | 0.2 | 0.4 | 0.2 |
| 8.0 | 1.0 | 0.2 | 0.7 | 0.2 |
| 12.0 | 1.5 | 0.3 | 1.0 | 0.2 |

[a] Ten yeast-fevered rats/group. All rats had starting temperatures in excess of 101.0°F; determined by ip thermistor probes.

1. Pearson, C. M., and Wood, F., J. Exp. Med. **120**, 547 (1964).

2. Waksman, B. H., and Wennersten, C., Int. Arch. Allergy Appl. Immunol. **23**, 129 (1963).

3. Pearson, C. M., *in* "Arthritis" (J. L. Hollander, ed.), p. 119. Lea and Febiger, Philadelphia (1966).

4. Rosenthale, M. E., and Nagra, C. C., Proc. Soc. Exp. Biol. Med. **125**, 149 (1967).

5. Kalliomaki, J. L., Saarimaa, H. A., and Toivanen, P., Ann. Rheum. Dis. **23**, 78 (1964).

6. Graeme, M. L., Fabry, E., and Sigg, E. B., J. Pharmacol. Exp. Ther. **153**, 373 (1966).

7. Glenn, E. M., Am. J. Vet. Res. **27**, 339 (1966).

8. Glenn, E. M., Proc. Soc. Exp. Biol. Med. **129**, 860 (1968).

9. Perper, R. J., Alvarez, B., Colombo, C., and Schroder, H., Proc. Soc. Exp. Biol. Med. **137**, 506 (1971).

10. Aspinall, R. J., and Cammarata, P. S., Nature (London) **224**, 1320 (1969).

11. Zurier, R. B., and Quigliata, F., Abstr. No. 56, Arthritis Found. Ann. Meet. Amer. Rheum. Ass., 35th, New York (1971).

13. Crunkhorn, P., and Willis, A. L., Brit. J. Pharmacol. **41**, 49 (1971).

14. Willis, A. L., J. Pharm. Pharmacol. **21**, 126 (1969).

15. Vane, J. R., Nature (London) New Biol. **231**, 232 (1971).

16. Ferreira, S. H., Moncada, S., and Vane, J. R., Nature (London) New Biol. **231**, 237 (1971).

17. Smith, J. B., and Willis, A. L., Nature (London) New Biol. **231**, 235 (1971).

18. Glenn, E. M., Bowman, B., and Koslowske, T. C., Biochem. Pharmacol. Special Suppl. **1968**, p. 27. (Mar.)

19. Glenn, E. M., and Kooyers, W., Life Sci. **5**, 619 (1966).

20. Goodwin, J. F., Amer. J. Clin. Pathol. **26**, 960 (1956).

21. Winter, C. A., Risley, E. A., and Nuss, G. W., Proc. Soc. Exp. Biol. Med. **111**, 544 (1962).

22. Glenn, E. M., Lyster, S. C., and Rohloff, N. A., Proc. Soc. Exp. Biol. Med. **130**, 110 (1969).

23. Lee, J., Kannegiesser, H., O'Toole, J., and Westura, E., Ann. N.Y. Acad. Sci. **180**, 218 (1971).

Received Sept. 20, 1971. P.S.E.B.M., 1972, Vol. 139.