# SUPPRESSION OF ACUTE AND CHRONIC INFLAMMATION BY ORALLY ADMINISTERED PROSTAGLANDINS

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**Oral administration of a stable analog of prostaglandin $E_1$ ($PGE_1$), 15-(S)-15-methyl-prostaglandin $E_1$, can suppress both chronic adjuvant-induced polyarthritis and acute immune complex-induced vasculitis in a dose dependent manner. Histopathologic studies of tibiotarsal joints from rats with adjuvant disease showed suppression of arthritis in animals treated with the $PGE_1$ analog from time of adjuvant challenge. This study represents the first demonstration of suppressed experimental polyarthritis by an orally administered prostaglandin. Suppression of the acute immune complex-induced vasculitis was demonstrated using 15-methyl-$PGE_1$ administered orally 12 hours prior to antigen-antibody challenge. Diminution of tissue injury resulting from immune complex-induced vasculitis is reflected by a decrease in vaso-permeability, indicating suppressed vascular damage in animals treated with prostaglandin. These studies demonstrate the potential use of orally active prostaglandins as an antiinflammatory agent.**

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It is becoming increasingly evident that prostaglandins (PG) play an integral role in regulating the functions of cells involved in immune and inflammatory reactions (1). Clearly not only are PG produced by inflammatory cells and able to influence the outcome of inflammation (2), but they can also suppress diverse effector systems in inflammatory reactions. Thus, PGE compounds, which increase levels of cyclic AMP in leukocytes, reduce chemotactic responses of polymorphonuclear leukocytes (PMN) (3), reduce selective extrusion of lysosomal enzymes from PMN (4), prevent IgE-induced histamine release from lung fragments and basophils (5), and inhibit lymphocyte mediated cytotoxicity (6). Pharmacologic doses of $PGE_1$ and $PGE_2$ administered subcutaneously suppress both acute and chronic inflammation in several experimental models (7). Furthermore, PGE have been shown to modulate the effects of vasoactive compounds (8), which are responsible for many early changes in both acute and chronic immune reactions.

Prostaglandin E compounds appear to have a regulatory effect on the character and intensity of immune responses in vivo (9). Long-term administration of $PGE_1$ has proved feasible and has been shown to markedly prolong survival of NZB/NZW (lupus) mice, even when treatment is begun after nephritis has developed (10). However, under these conditions, the dose of $PGE_1$ required is substantial (200 $\mu$g subcutaneously, twice daily). A derivative of $PGE_1$—15-(S)-15-methyl $PGE_1$(15-M-$PGE_1$)—has been developed and is more resistant than $PGE_1$ to the activity of 15-hydroxy-prostaglandin dehydrogenase (11). Previously, we have reported (12) that a very small dose of 15-M-$PGE_1$ (4 $\mu$g given subcutaneously, twice daily) prolongs survival of NZB/NZW mice in which there is established disease

at the time treatment is begun. In addition, we have reported (13) that immune complex-induced vascular damage can be markedly suppressed by treatment of rats with 15-M-$PGE_1$.

We now show that *oral* administration of 15-M-$PGE_1$ in a dose dependent manner suppresses acute immune complex-induced vasculitis and adjuvant-induced chronic polyarthritis in rats. The reversed passive Arthus reaction (RPA) is induced by the intravenous administration of antigen and the local (intradermal) injection of antibody and is a good model for immunologically induced acute vasculitis. Adjuvant arthritis appears in rats 10–14 days after a single intradermal injection of Freund's complete adjuvant and is a good model for chronic inflammation that is dependent on cell mediated immunity; thus, adjuvant arthritis provides a convenient experimental model for in vivo evaluation of antiinflammatory and/or immunosuppressive agents (14). This work to be reported here represents one of the first efforts to regulate both acute and chronic inflammatory reactions in animal models by oral treatment with PG.

## MATERIALS AND METHODS

**Animals.** Adult male Lewis rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 200–250 gm were used throughout these studies.

**Adjuvant-induced chronic arthritis.** Polyarthritis was induced in the Lewis rat by the intradermal injection of 500 μg heat-killed *Mycobacterium tuberculosis* organisms (Difco Laboratories, Detroit, MI) in 0.1 ml of mineral oil into the left hind paw. Adjuvant-induced arthritis appeared in all positive control rats at approximately day 14. The severity of the polyarthritis was evaluated in the 3 uninjected paws by using both an arbitrary point system and direct measurement with a caliper. The uninjected paws were scored in the following manner: 1 point was assigned for each of the inflamed tibiotarsal joints, 5 points for each of the inflamed tarsal joints, and 5 points for arthritic involvement of the tail. Each of 4 groups containing 10 rats each were injected with the *M tuberculosis* in oil and were separated into Group 1, which received 200 μg of 15-M-$PGE_1$ once daily from the day of adjuvant challenge; Group 2, which received 100 μg of 15-M-$PGE_1$ by mouth from the day of adjuvant injection; Group 3, which received 50 μg of 15-M-$PGE_1$ once daily again from the time of adjuvant injection; and Group 4, which was given 0.5 ml of fluid. All of the above experiments were conducted in triplicate on three different occasions. The progress of the polyarthritic joint disease was also followed in the noninjected paws by measuring each of the joints with a caliper at 2-day intervals.

**Immune complex-induced acute vasculitis.** The reversed passive Arthus reaction using rabbit IgG antibody to bovine serum albumin (BSA) was used as the antibody to elicit the immune complex vasculitis, as previously described (15). Approximately 150 μl of anti-BSA IgG containing 150 μg *N* antibody was injected intradermally, followed by the intravenous injection of 10 mg BSA and 1 μCi of $^{125}I$ rat serum albumin. The radiolabeled rat serum albumin served as a permeability marker and as an index for the degree of vascular damage (16). Negative control sites were injected with saline or antibody from which the intravenous injection of antigen was omitted. Three hours after antigen–antibody challenge, the animals were killed. At the site of each intradermal injection, a circular punch of skin 1 cm in diameter was excised. In addition, 1 ml of blood was removed from the left ventricle of the heart; both skin and blood samples were measured for gamma emissions. The ratio of radioactivity in each skin site/1 ml of blood was computed and used as the permeability index.

**Prostaglandins.** Prostaglandins were a gift of Dr. John E. Pike, Upjohn Co., Kalamazoo, Michigan. Stock solutions of $PGI_2$, 15-M-$PGE_1$, and 16,16 dimethyl $PGE_1$ were prepared in absolute ethanol and diluted to 10 mg/ml in sterile phosphate buffered saline (PBS), pH 7.2. Prior to use, the PG were diluted to the desired concentration with PBS.

**Morphologic analysis.** Skin samples from the various inflammatory and control sites were fixed in phosphate buffered (pH 7.0) 10% formaldehyde and were then prepared for routine light microscopy. After killing the animals with ether anesthesia, the legs of all those challenged with the *Mycobacterium* in mineral oil were removed and fixed in buffered 10% formaldehyde and then decalcified with acetic acid. Sections of each fixed and decalcified tibiotarsal joint were stained with hematoxylin and eosin.

**Statistical analysis.** The unpaired Student's *t*-test was used to analyze the data.

## RESULTS

**Suppression of polyarthritis by oral prostaglandin treatment.** A severe polyarthritic response was grossly visible in all non-PG treated control rats. Using oral treatment with 15-M-$PGE_1$, a dose dependent amelioration of the polyarthritis was observed. Little or no gross change was observed in the joints of animals given 200 μg of the 15-M-$PGE_1$ daily from the time of adjuvant administration. The oral administration of 100 μg and 50 μg of 15-M-$PGE_1$ resulted in a significant gross attenuation of the polyarthritis as compared to the control animals. Prostaglandin treatment of rats with 200 μg 15-M-$PGE_1$ led to diarrhea and somnolence which lasted approximately 1 hour, while the lower doses produced only transient diarrhea and the animals remained active.

As shown in Figure 1, oral administration of 15-M-$PGE_1$ markedly suppressed the polyarthritic response in a dose-dependent manner. The oral administration of 200 μg 15-M-$PGE_1$ resulted in a 90% reduction in the mean arthritic score of the joints in the non-adjuvant treated paws by day 20. It is interesting to note that not only was the degree of swelling of the soft tissue around the joints markedly diminished in the ani-

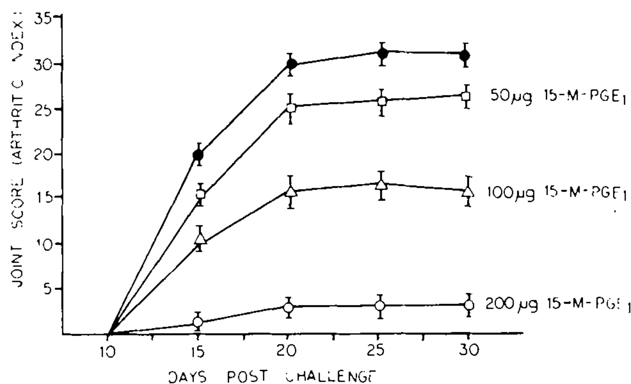


**Figure 1.** Effect of orally administered 15-(S)-15-methyl-prostaglandin $E_1$ on adjuvant polyarthritis. ●——● = positive test; □——□ = 50 µg 15-M-$PGE_1$; △——△ = 100 µg 15-M-$PGE_1$; ○——○ = 200 µg 15-M-$PGE_1$. Each point represents the mean arthritic score for the 3 uninjected paws (10 rats per group treated from the day of adjuvant challenge). Error bars represent ± standard error of the mean.

mals receiving this dose of 15-M-$PGE_1$, but there was also a significant delay in the onset of disease.

Although not as dramatic as with the higher dose, the daily oral administration of 100 µg and 50 µg of 15-M-$PGE_1$ suppressed the polyarthritis by 50% and 20%, respectively (Figure 1). In both PG treated and nontreated animals, the peak arthritic response occurred at approximately day 21. As compared to nontreated rats, the mean paw thickness in all the 15-M-$PGE_1$ treated animals was significantly reduced, indicative of a suppression of soft tissue swelling in each of the joints. As shown in Table 1, the mean paw thickness measured at day 20 following adjuvant injection was suppressed in a dose-dependent manner. There was little statistical difference between the mean paw thickness in the 200 µg 15-M-$PGE_1$ treated animals (injected with adjuvant) and normal (non-adjuvant treated) control animals. Although not as dramatic as with the 200 µg dose, the oral administration of 100 µg and 50 µg attenuated the joint swelling in each of the noninjected paws. It is also of interest that the swelling of soft tissue in the adjuvant-injected left hind paw was suppressed in a dose-dependent fashion.

Histology of tibiotarsal joints from treated and untreated rats 20 days after adjuvant injection was compared with joints from normal rats. A histologic section from a joint of an animal with polyarthritis is shown in Figure 2. The histologic changes evident in polyarthritic rats demonstrated significant encroachment of the joint space by an inflamed synovium and advancement of pannus to the center of the cartilage. The inflammatory response associated with erosion of articular cartilage was characteristic of fully developed adjuvant arthritis (Figure 2). Arthritis failed to develop in joints of rats treated (from day 1) with 200 µg of 15-M-$PGE_1$; histologically, these joints appeared normal. Joints from animals treated with 100 µg 15-M-$PGE_1$ demonstrated extension of pannus only over the periphery of cartilage. Also, invasion of cartilage and bone by pannus was seen in joints of animals treated with 50 µg/day 15-M-$PGE_1$, but the joint space was preserved (Figure 3), demonstrating the dose response of PGE treatment. These results indicate that the arthritic reaction in animals treated orally with 15-M-$PGE_1$ are either attenuated (100 or 50 µg) or totally suppressed (200 µg) in a manner that is dose-dependent.

**Suppression of acute vasculitis.** Oral administration of 15-M-$PGE_1$ suppressed acute immune complex–induced vasculitis in a dose-dependent manner (Figure 4). In this particular study, animals were treated 12 hours previously with the 15-M-$PGE_1$ and then injected with antigen and antibody. Changes in the vascular permeability indicative of the intensity of the inflammatory reaction and tissue injury were always related to values found in saline injected sites. Animals serving as positive controls received the intradermal injections of antibody and intravenous injection of BSA plus labeled rat albumin, but they were not treated with PG. For each

**Table 1.** Effect of orally administered 15-(S)-15-methyl prostaglandin $E_1$ on soft tissue swelling in polyarthritic rats. Each number represents the mean paw thickness for either the uninjected paws or the 1 injected paw. (Ten rats per group were treated from day of adjuvant challenge)

| | Mean paw thickness (cm) 20 days post-challenge | | | |
|---|---|---|---|---|
| | Front right paw | Front left paw | Hind right paw | Hind left paw, adjuvant injected |
| Positive test | 0.61 ± 0.04 | 0.56 ± 0.02 | 0.64 ± 0.02 | 1.32 ± 0.06 |
| 50 µg 15-M-$PGE_1$ | 0.52 ± 0.02 | 0.48 ± 0.01 | 0.50 ± 0.04 | 1.22 ± 0.03 |
| 100 µg 15-M-$PGE_1$ | 0.49 ± 0.02 | 0.43 ± 0.02 | 0.47 ± 0.03 | 1.16 ± 0.06 |
| 200 µg 15-M-$PGE_1$ | 0.38 ± 0.05 | 0.40 ± 0.01 | 0.44 ± 0.03 | 0.90 ± 0.05 |

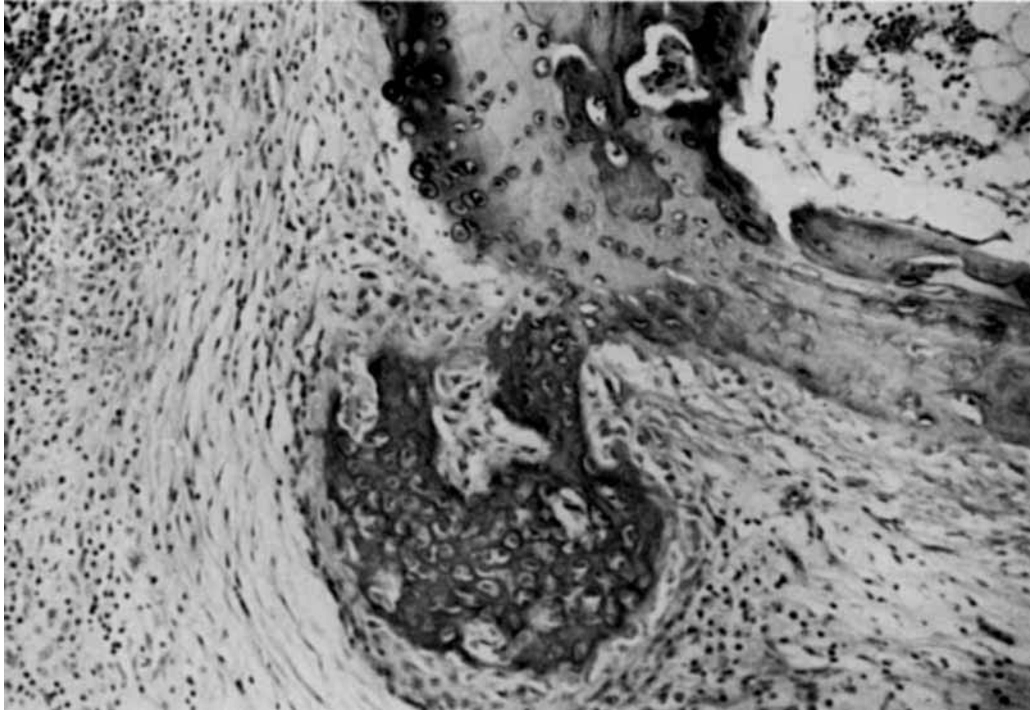

**Figure 2.** Joint from untreated rat at day 20. Invasive pannus encroaching upon the cartilage and the bone (hematoxylin and eosin; magnification × 100).

**Figure 3.** Joint from rats at day 20 treated orally with 50 $\mu$g 15-(S)-15-methyl-prostaglandin $E_1$ from day of adjuvant challenge. Synovia extends over the cartilage and bone, but the pannus has not encroached upon the joint space. Some fibrinous exudate is free in the joint space (hematoxylin and eosin; magnification × 100).

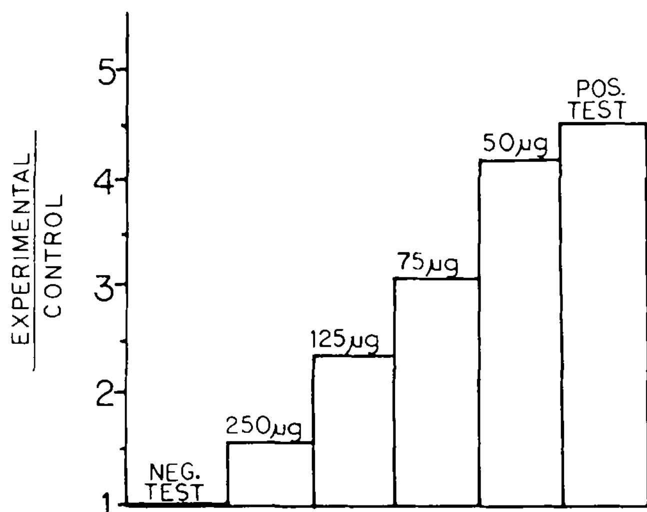


**Figure 4.** Effect of orally administered 15-(S)-15-methyl prostaglandin $E_1$ on immune complex-induced vasculitis. Animals were treated 12 hours prior to antigen-antibody challenge. The vascular permeability was computed as in Materials and Methods and expressed as experimental/control. The $P$ value for the doses of 75 µg, 125 µg, and 250 µg was $< 0.01$ and for 50 µg the $P$ value was $< 0.1$, as compared to the positive test group.

of the experiments, 6 animals were given 4 intradermal injections of saline (negative controls) and 4 intradermal injections of antibody (positive test). Thus, each animal contained both negative and positive test sites. Also there was no statistical difference in saline sites fom PG treated and nontreated animals. The 15-M-PGE₁ treatment of rats resulted in suppression of vascular damage ranging from approximately 10% at the dose of 50 µg 15-M-$PGE_1$ to 75% suppression with the 250 µg dose (Figure 4).

In an attempt to determine whether the long lasting suppression of immune complex vasculitis could only be induced by treatment with the 15-methyl derivative of $PGE_1$, both $PGI_2$, and a $PGE_1$ analog with a dimethyl at the 16 position were also studied. Oral administration of either $PGI_2$, $PGE_1$, or 16,16 dimethyl $PGE_1$ did not suppress acute vasculitis. However, since subcutaneous injections of $PGE_1$ suppress immune complex vasculitis (13), animals were treated subcutaneously with either $PGI_2$ or 16,16 dimethyl $PGE_1$, rested for 1 hour, and then challenged with antigen and antibody. The largest dose of the administered $PGI_2$ (500 µg) resulted in a reduction of vascular permeability by approximately 50%, while 250 µg $PGI_2$ caused a diminution of the vascular leakage by 10% (Figure 5). The treatment of animals with 500 µg 16,16 dimethyl $PGE_1$, in the manner as described for $PGI_2$, resulted in a suppression of the vascular leakage by 60%. When 250 µg of this $PGE_1$ analog was examined, the permeability was reduced by 35% (Figure 6).

In all positive control reactions, the skin lesions were shown to have extensive deposition of both BSA and C3 in walls of dermal venules and capillaries, as determined by immunofluorescence. In rats treated with 15-M-$PGE_1$, the deposition of BSA and C3 were confined mainly to the immediately adjacent perivascular tissue. Thus, it appeared that PG treatment did not block complement fixation or deposition of immune

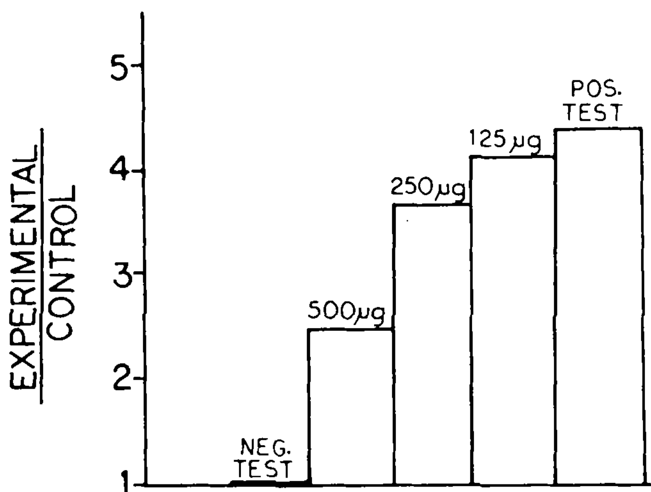


**Figure 5.** Effect of subcutaneously administered $PGI_2$ on immune complex-induced vasculitis. The vascular permeability was computed as in Materials and Methods and expressed as experimental/control. The $P$ value for the 500 µg dose was $< 0.01$, 250 µg dose $< 0.1$, and the 125 µg dose $< 0.2$.

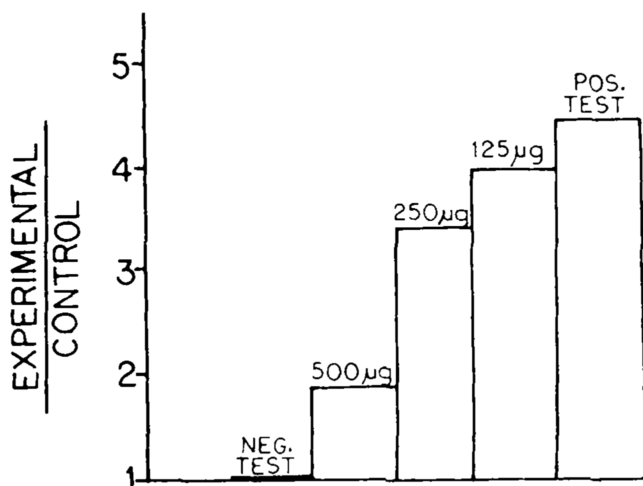


**Figure 6.** Effects of subcutaneously administered 16,16 dimethyl prostaglandin $E_1$ on immune complex-induced vasculitis. The $P$ value for the 500 µg dose was $<0.001$, 25 µg $<0.01$, and 125 µg $< 0.2$.

complexes. When vascular damage was suppressed by the prostaglandin treatment, skin reactions were visibly inhibited, with little induration, edema, or erythema. Histologically, the nontreated positive sites showed the expected diffuse intraluminal, perivascular infiltrates of polymorphonuclear leukocytes (Figure 6A). In contrast, lesions from rats treated with a suppressive dose of either PG showed a diminished infiltrate of neutrophils (Figure 6B). The histology of the PG-suppressed lesions appeared similar, and the intensity of the reactions was dose-dependent.

## DISCUSSION

The data presented in this paper indicate that oral administration of a stable derivative of $PGE_1$, 15(S)-15-methyl $PGE_1$, produces potent suppression of acute immune complex–induced inflammatory reactions, as well as suppression of the more delayed and chronic inflammatory response in adjuvant arthritis. The suppressive effect of oral 15-M-$PGE_1$ treatment appears to be partially specific since no inhibition of the inflammatory response occurred with oral administration of 16,16 dimethyl $PGE_1$ or $PGI_2$. This reflects both the enzymatic and spontaneous degradation of these two compounds, especially $PGI_2$. The ability to inhibit the arthritic response by oral treatment of therapeutic levels of 15-M-$PGE_1$ probably lies in the molecular structure of the analog, which is not susceptible to the 15-hydroxy-prostaglandin dehydrogenase (11). The activity of this enzyme is probably the reason why significant doses of the classic prostaglandins are required to achieve physiologic effects (17).

The pathogenesis of the Arthus reaction may not be dissimilar from the series of events thought to be responsible for joint tissue damage in rheumatoid arthritis (RA): formation of immune complexes in vessel walls, activation of complement and local generation of chemotactic peptides derived from the fifth component of complement, influx of polymorphonuclear leukocytes in

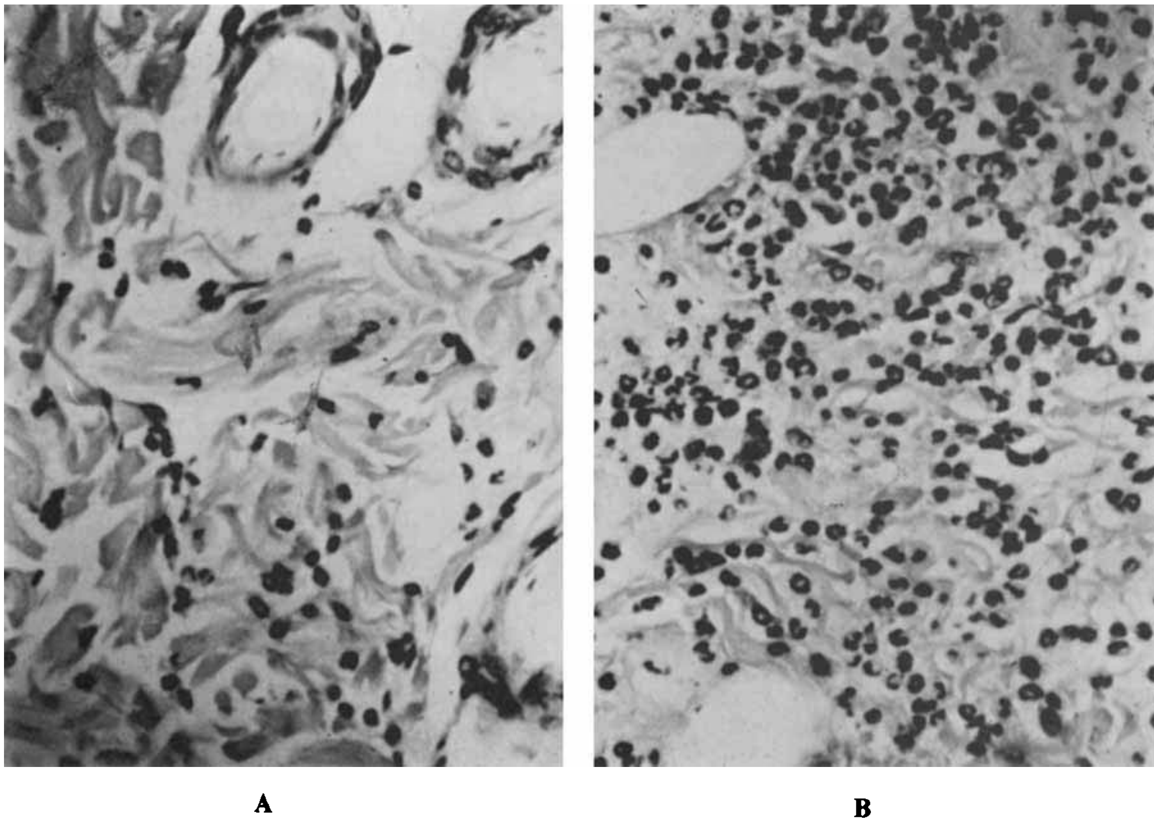


**Figure 7.** Acute immune complex–induced vasculities in **A**, prostaglandin treated and **B**, nontreated animals. The nontreated animals show an intraluminal infiltrate of neutrophils, while the infiltrate in the treated animals was significantly diminished. As stated in the text, the histology of the prostaglandin suppressed lesions appeared similar and the intensity of the reactions was dose-dependent.

response to chemotactic products, endocytosis of immune complexes, and subsequent release of lysosomal enzymes and other mediators of inflammation resulting in vascular damage (18). Previous investigations have shown that treatment of rats with 15-M-$PGE_1$ can modulate many of the above steps in the acute inflammatory response, especially the direct motion and degranulation of peripheral blood neutrophils (13).

The ability of both the classic prostaglandin $E_1$ and prostacyclin to inhibit immune complex-induced vasculitis may be due to the interaction of these particular prostaglandins with identical receptors on the neutrophil surface. Previous investigators (19) have proposed that prostaglandins of the E series and prostacyclin bind to similar granulocyte receptors to induce an increase in intracellular cyclic AMP levels, thus leading to an attenuation of inflammatory responses. The ability of $PGE_1$ and $PGI_2$ to bind identical receptors and inhibit adenosine diphosphate-induced aggregation of platelets in a number of species has also been established (20). Therefore the effects of $PGE_1$ and $PGI_2$ on neutrophil dependent, immune complex-induced vasculitis may add credence to the identical binding theory of these two prostaglandins.

Whereas acute immune complex-induced vasculitis is characterized by neutrophil infiltration, tissue proliferation is the hallmark of chronic inflammation. Therefore, appropriate control of cell proliferation is crucial to improved management of a disease like RA, for example, in which synovium develops into granulation tissue (pannus) that invades and erodes articular cartilage. Intracellular degradation of newly synthesized collagen in vitro is inhibited by PGE (21), and this action may aid in the control of collagen deposition in chronic inflammatory disorders.

The role of prostaglandins in immunologic responses and inflammatory reactions is complex, but these ubiquitous compounds do appear important in the regulation of cell function and host defenses. Studies such as those presented here do not necessarily argue for a physiologic role for prostaglandins. They do suggest that pharmacologic doses of prostaglandins, even when given by mouth, may be capable of influencing some aspects of immunity and inflammation and can prevent tissue injury. That PGE can inhibit the dermal Arthus reaction makes it not unreasonable to consider its use in treatment of cutaneous vasculitis not controlled by more traditional therapy.

The therapeutic potential of the prostaglandins appears great, and their use for a wide variety of diseases is just beginning. Whether they will prove helpful clinically as modulators of immune/inflammatory responses is not yet clear.

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