# Kinetics of Prostaglandin Production in Various Inflammatory Lesions, Measured in Draining Lymph

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**Efferent lymph was collected over long periods via catheters surgically placed in popliteal and prefemoral lymph nodes of sheep. Prostaglandin (PG) E and F equivalents were measured with a radioimmunoassay. After stimulation with heat-killed *Escherichia coli*, PG levels rose dramatically in the efferent lymph but were undetectable in the contralateral control lymphatics or in the systemic circulation. When *E coli* were infused directly into a lymph node, the PG levels in the effluent lymph were inhibited with indomethacin. Carrageenan, delayed hypersensitivity, and lymphocyte transfer reactions were also studied. In the classic acute inflammations (caused by *E coli* and carrageenan) the PG levels rose early in the response (first 4 to 6 hours) compared with delayed production in the immune reactions. With PPD, PG levels peaked between 10 and 20 hours after injection, while PG rose 127 hours after allogeneic lymphocytes were injected. These results are discussed in relation to the role of PG in inflammation, and the use of the sheep lymphatic model in PG research is emphasized. (Am J Pathol 95:225–238, 1979)**

In 1971 attention was drawn to the role of prostaglandins (PG) in the inflammatory response with the discovery that the non-steroidal anti-inflammatory drugs inhibit PG synthesis.[1-3] Many animal models have evolved to study inflammatory mediators, the most productive utilizing the rat pleural and peritoneal cavities,[4-6] the rat hind paw,[7,8] and subcutaneous air blebs.[9,10] We investigated the possibility of using a model developed in the laboratory of Morris [11] to study PG levels in lymph draining inflammatory lesions. Plastic catheters were surgically inserted into lymphatics of the sheep and externalized. Lymph was drained for long periods from inflammatory sites and studied for its content of mediators and cells. This technique evolved in conjunction with studies of lymphocyte migration, before and after challenge with antigen, and the various immune parameters associated with these events.[12-14] However, the model is also suited to deal with more acute forms of injury since all the classic signs of an inflammatory reaction are reflected in changes in the draining lymph.[15,16] Several acute mediators have been investigated in the

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lymph of dogs, including histamine, kinin,[17] and PGS.[18,19] Physiologic collections of lymph are difficult in dogs, which must be kept under anesthesia during the procedure. Sheep, on the other hand, tolerate catheters well and allow collections of lymph for periods up to months while standing in their pens free of anesthetic effects. In this paper the kinetics of PG appearance in lymph-draining inflammatory lesions is discussed. The usefulness of the experimental model to study acute inflammatory reactions in general and of PG research in particular is emphasized.

## Materials and Methods

### Animals

Twenty-five- to forty-kilogram sheep of either sex from commercial flocks were used in all experiments.

### Surgery and Lymph Collection

While the sheep were under anesthesia (Nembutal Sodium, Abbott Laboratories, North Chicago, Ill), lymphatic vessels efferent to the popliteal and prefemoral lymph nodes were cannulated with polyethylene catheters using the methods described by Hall and Morris.[20] Plastic bottle holders were sutured to the animal's skin and the lymph was collected in sterile bottles at room temperature. Each collection bottle contained 250 units USP heparin (Organon, Canada Ltd.) and 500 IU each of penicillin and streptomycin (Grand Island Biol. Co., Grand Island, NY). Animals were placed in metabolism cages and allowed to recover for 1 day before any experiments were performed. They had unlimited access to food and water. Cells were counted in a Coulter counter model B (Coulter Electronics, St. Hialeah, Fla). Lymph samples were centrifuged at 600 *g* for 5 minutes and the supernatants were frozen and stored at −70 C until assayed for PG. A portion of the cell pellet was smeared and stained with Leishman's stain for differential counts.

### Injections of Inflammatory Substances

#### Heat-Killed *Escherichia coli*

A culture of *E coli* (Strain X, Medical Teaching Laboratories, University of Toronto, Toronto) in Difco nutrient broth (Difco Laboratories, Detroit) was incubated overnight at 37 C in a shaker bath. The culture was autoclaved at 15 lb for 30 minutes at 120 C and the bacteria were spun down and resuspended in phosphate-buffered saline (PBS). Approximately $2.0 \times 10^{11}$ bacteria in 2 ml PBS were injected into the hock of the sheep in two sites.

#### Carrageenan

Two milliliters of a 1% carrageenan (Viscarin Carrageenan, Springfield, NJ) in PBS solution was injected in three sites into the prefemoral drainage area.

#### Delayed Hypersensitivity Reactions

Animals were immunized with 2.5 human doses of BCG (Connaught Laboratories, Toronto, Canada) given by intramuscular injection 1 to 2 months prior to challenge with 200 μg PPD (Connaught Laboratories, Toronto, Canada). The PPD was injected subcutaneously in multiple sites in the prefemoral drainage area.

### Normal Lymphocyte Transfer Reactions (NLT)

Allogeneic lymphocytes ($1.0 \times 10^9$) in 1 ml of PBS were infused via an afferent lymphatic into a node using efferent lymph cells from an allogeneic sheep.

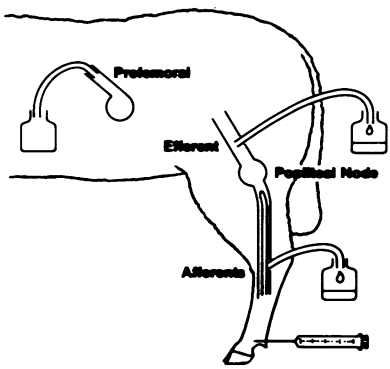
### Experimental Procedures

The general anatomy of the lymphatics used in this study is outlined in Text-figure 1. Phlogistic substances were injected mainly into the hock, allowing chemical mediators to enter the afferent lymphatics, pass through the node, and ultimately collect in the efferent vessel from which the lymph was collected. Occasionally, the antigens were infused directly into the node via an indwelling cannula placed in an afferent vessel. To be sure that the injection site was in the drainage area of the node, sterile 2% Evans blue was always included in the injection solution. The dye binds to albumin and can be visualized in the efferent lymph. To sample blood, catheters were often inserted in the jugular vein for systemic collections and in the femoral vein for local samples if the popliteal node was being used. In any one experiment, contralateral lymphatics were catheterized to serve as controls. In this way one could obtain a complete picture of the production and fate of the PG as they made their way through the various fluid compartments.

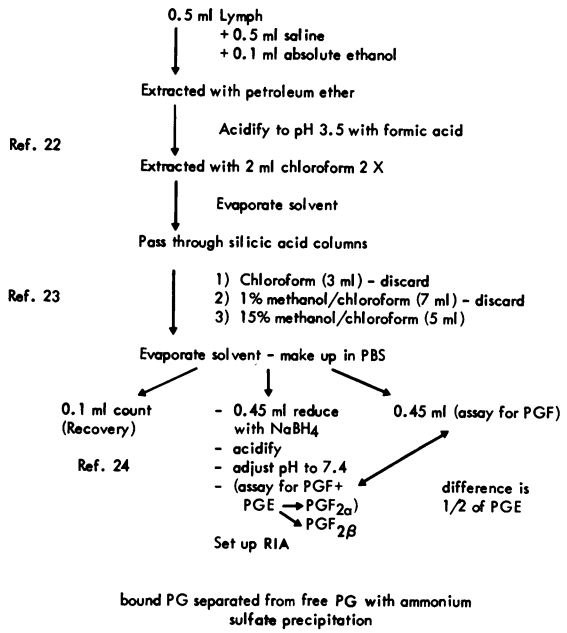
### Measurement of Prostaglandins

A radioimmunoassay (RIA) was used for the measurement of PGE and PGF equivalents. An antibody was produced by immunizing New Zealand white rabbits with a conjugate of $PGF_{2\alpha}$ (Dr. J. Pike, UpJohn Co., Kalamazoo, Mich), and bovine serum albumin (BSA) (Pentex, Kankakee, Ill) was prepared with the carbodiimide method.[21] The procedure for the extraction of PG from the lymph and blood and the passage through silicic acid columns is shown in Text-figure 2. PGE and PGF are not separated in this procedure. The column chromatography step is inserted because it tends to stabilize the assay results. The antibody to $F_{2\alpha}$ crossreacts 75% with $F_{1\alpha}$ and less than 0.01% with $PGE_2$, $PGE_1$, $PGA_2$, and $PGB_2$. PGE levels were estimated with the anti-F antibody by reducing them to the corresponding PGF compounds with sodium borohydride according to the method of Levine.[24] The anti-F antibody does not distinguish the degree of unsaturation; therefore, results are expressed as PGE and PGF equivalents. The sensitivity of the assay was 200 pg/ml PGF or PGE equivalents.

In our experience, lymph and blood plasma contain substances that inhibit the binding of the PG to the antibody. This interference is not removed by the extraction or the column step. The assay can be validated only if a suitable amount of control extract is processed in the same manner as the unknown samples and is added to the standard curve tubes. This

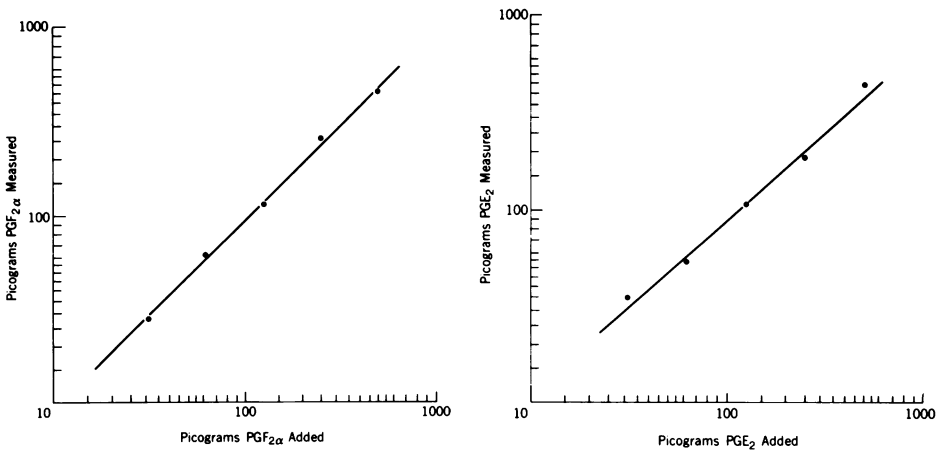



TEXT-FIGURE 1—Schematic outline of sheep, showing lymphatics and their drainage.




TEXT-FIGURE 2—Flow sheet showing radioimmunoassay procedure.

interference is remarkably consistent from one experiment to another and, if dealt with in the above manner, allows the RIA to quantitate picograms of PGE and PGF equivalents above control lymph or blood. The close relationship between the amount of $PGF_{2\alpha}$ or $PGE_2$ added to lymph and the amount the assay measures is demonstrated in Text-figure 3. The crystalline PG standards were the gifts of Dr. John Pike from UpJohn Co. (Kalamazoo, Mich). The labeled PG were purchased from Amersham ($PGE_2$ and $PGF_{2\alpha}$, 120 to 170 Ci/mmole).




TEXT-FIGURE 3—Known amounts of PG were added to lymph and processed in the manner outlined in Text-figure 2. The validity of the assay was tested by plotting the amount added vs the amount measured.

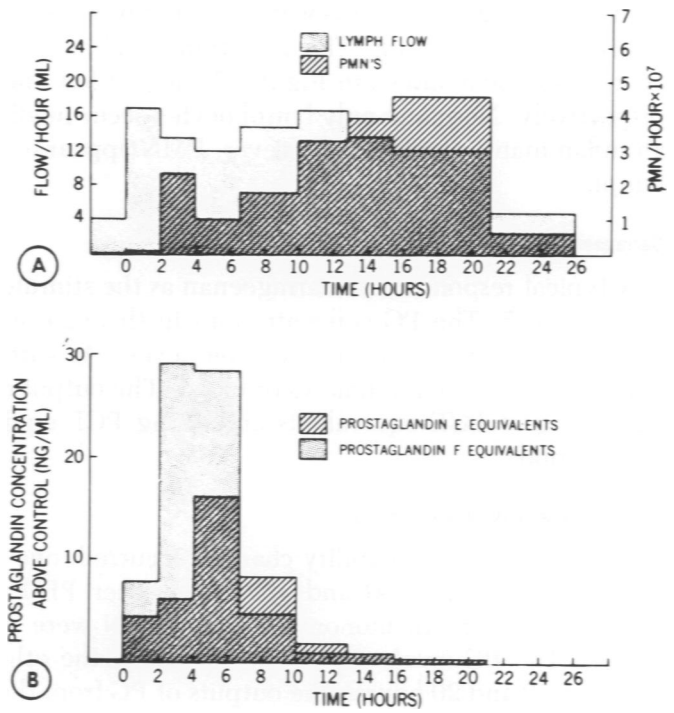
### Indomethacin Infusion

Indomethacin (Sigma Chemicals Co., St. Louis) was dissolved in Tris-buffer (0.05 M) at pH 9. Immediately before infusion, the pH was adjusted to 8 with 0.01 M HCl, and 0.9% NaCl was added. For the duration of the indomethacin infusion (4.5 hr), Tris saline (pH 8.0) was infused into the control node, after which PBS (pH 7.4) was substituted for infusion into both nodes.

## Results

### *E coli* Response

One of the most dramatic responses obtained with bacteria is outlined in Text-figure 4. After the injection of *E coli* the lymph flow rate increased fourfold, indicating marked permeability changes in the terminal vascular bed. The output of polymorphonuclear leukocytes (PMN) increased steadily to approximately $4.5 \times 10^7$ per hour in the efferent lymph. Both of these parameters had subsided by 21 hours. During these events, PGF equivalents, ie, $PGF_{1\alpha}$ and $PGF_{2\alpha}$, rose to almost 30 ng/ml above control and the PGE equivalents rose to approximately 16 ng/ml above control. The PG appeared very early in the response, peaking between 2 and 7 hours. By 10 hours the PG levels had fallen, although some residual




TEXT-FIGURE 4—*E coli* response. Heat-killed *E coli* ($2.0 \times 10^{11}$) were injected into the hock of the sheep at 0 time. *A*—Histogram monitoring lymph flow and PMN output. *B*—Radioimmunoassay measurements of PGE and PGF equivalents above control.

production continued up to 21 hours. The contralateral control popliteal and a prefemoral control draining on the same side as the stimulated popliteal contained no detectable PG. Similarly, no PG was found in the systemic venous circulation. In more recent experiments, blood from the femoral vein has been sampled during *E coli* challenge, and in these cases as well PG were not detected. In view of the fact that PG are metabolized very quickly in the systemic circulation, it is not surprising that PG levels would be low in venous blood. However, the blood close to the lesion was expected to contain some PG, since low-molecular-weight substances would be expected to equilibrate rapidly between blood and lymph. It would appear that at least a substantial portion of the PG draining from the lesion is confined to the lymphatics.

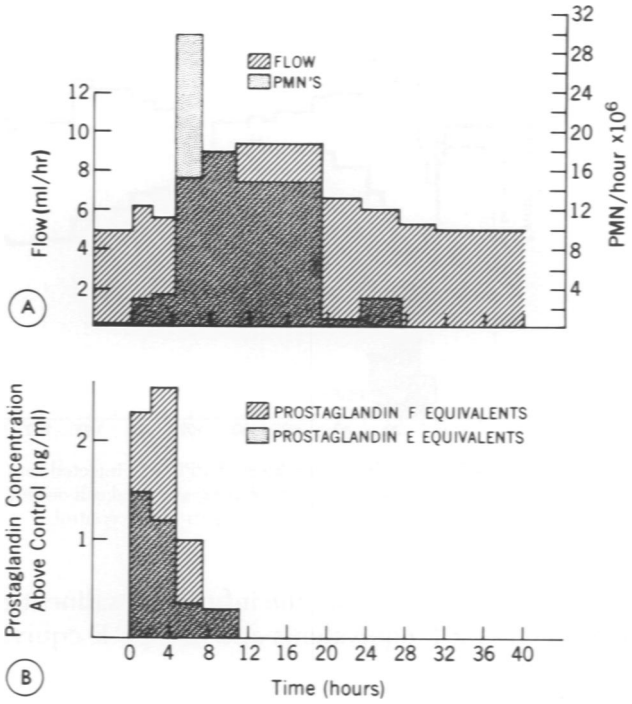
Figures 1 through 6 are representative histologic sections taken from intradermal injections of the same strain of *E coli* in sheep skin. These sections allow one to correlate events taking place in the skin with the cellular changes in the lymph. The lymphatics demonstrate more clearly the dynamic nature of the inflammatory events and allow quantitations that are not possible with most other techniques. For example, the output of PG from the lesion can be calculated from the flow rates and times of collection. In this response the total output of PGE equivalents during the 21 hours over which the PG were detected was 1.3 $\mu$g and the output PGF equivalents was 2.3 $\mu$g. The appearance of the lymph smear during an acute response is shown in Figures 7 and 8 at low and high magnification, respectively. Normally only lymphocytes occur in efferent lymph. During an inflammatory response, however, PMN appear in large numbers in the lymph.

#### Carrageenan Response

A typical response with carrageenan as the stimulus is demonstrated in Text-figure 5. The PG concentrations in this case were lower and by 11 hours had decreased to undetectable levels. As with *E coli*, no PG were detected in control lymphatics or blood. The output of PG from the lesion was 51 ng of PGE equivalents and 92 ng PGF equivalents over the 11-hour period.

#### Delayed Hypersensitivity Reactions

Two peaks of permeability changes occurred: one at 10 to 50 hours and the other between 100 and 150 hours after PPD (Text-figure 6). The lymph cells were all mononuclear; no PMN were visible in the efferent lymph. The PG levels peaked later than in the other two responses, ie, between 10 and 20 hours. The outputs of PG from the lesions were 377 ng PGE equivalents and 90 ng PGF equivalents.



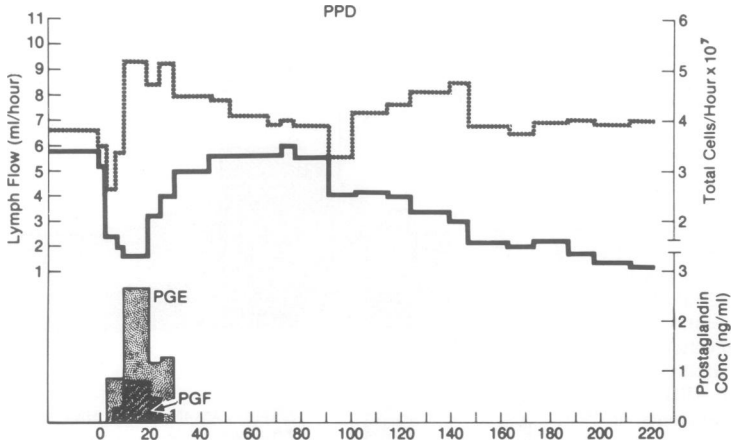

TEXT-FIGURE 5—Carrageenan response. *A*—Lymph flow rates and PMN levels in response to 2 ml of a 1% carrageenan solution given in multiple injections into the prefemoral drainage area at 0 time. *B*—Radioimmunoassay measurements of PGE and PGF equivalents above control lymph.

**Normal Lymphocyte Transfer Reaction**

The cells in the efferent lymph were made up entirely of mononuclear cells. There were two major peaks of cells, the second peak representing a cell output of over $10^8$ cells/hour. The lymph flow rate increased 127 hours after the allogeneic lymphocytes were injected, when PGE appeared in the lymph (picogram range/ml). The output of PGE equivalents was 340 ng. No PGF was found in the lymph (Text-figure 7).

**Inhibition Experiment**

Indomethacin was tested for its ability to inhibit PG synthesis in lymph nodes. Two infusions via afferent lymphatics were carried out: one into the right popliteal node and the other into the left popliteal node. The experimental protocol is shown in Text-figure 8. Saline was continuously infused for 16.5 hours into the right popliteal node at 1.6 ml/hr. Into the other node, indomethacin (360 $\mu$g) was infused over 4.5 hours and saline was infused for 12 hours at 1.6 ml/hr. Two and one-half hours after the infusions had started $1.0 \times 10^{10}$ *E coli* were infused into both nodes. As can be seen in Table 1, PG levels were consistently lower in the efferent lymph draining the node that received the drug. However, during the terminal stages of the experiment, the effects of the drug were diminish-

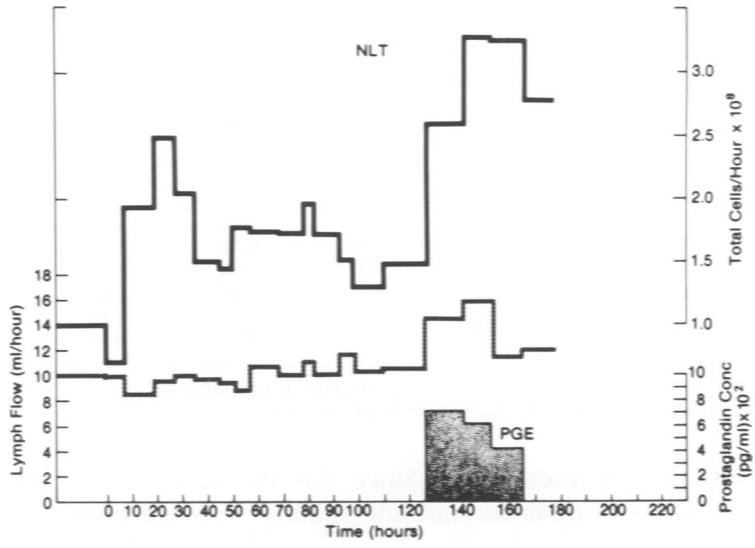



TEXT-FIGURE 6—Two hundred microliters of PPD was injected into multiple sites in the prefemoral drainage area at 0 time. Flow rate (*dotted lines*) and total cell output (*solid lines*) are shown as well as radioimmunoassay of PGE and PGF equivalents above control.

ing. It is also evident that the infusion of saline itself can liberate PG from nodes, 735 pg F equivalents and 385 pg E equivalents in this case.

## Discussion

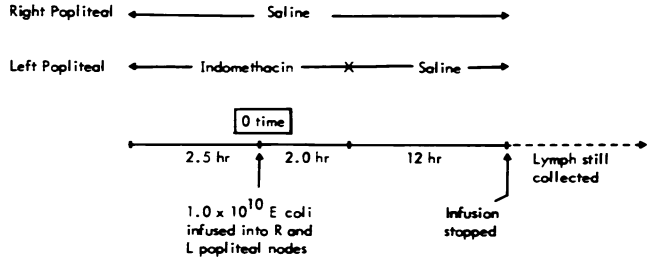
Many stimuli have been shown to liberate PG from tissues. These include mechanical trauma;[25] hormones, eg, angiotensin [26] and ACTH [27]; inflammatory mediators (eg, bradykinin [26,28]), and endotoxin.[29] Using draining lymph to capture inflammatory mediators, PG have been detected in the effluent lymph from a variety of inflammatory lesions, including those described in this paper (bacteria, carrageenan, PPD, and allogeneic lymphocytes). PG levels have also been found to increase in Arthus and Shwartzman reactions.[30] In classic acute inflammatory reactions (caused by *E coli* and carrageenan) PG emerge early in the response, the highest levels being attained in the first few hours after challenge and rarely persisting for more than 20 hours. During reactions in which the PMN is not a predominant cell (NLT and PPD) the results are more variable. In some PPD responses no PG can be detected (not shown) while in others fairly high levels are attained (Text-figure 6). The NLT is interesting since the PG appear late in the response, between 127 and 170 hours after the cells were injected. However, as with the PPD response, PG appearance in lymph is often negligible.[31] It is not known whether the absence of PGF in these lesions has any significance. Experiments are in progress to determine which inflammatory cell is more important for PG production. Certainly all inflammatory cells have been found to release PG *in vitro*, including neutrophils,[32] macrophages,[33] platelets,[34] and per-




TEXT-FIGURE 7—Allogeneic lymphocytes ($1.0 \times 10^9$) were infused into a node at 0 time. Lymph flow (*dotted lines*) and total cell changes (*solid lines*) as well as radioimmunoassay measurements of PGE equivalents above control are shown.

haps lymphocytes.[35] In the studies reported here, no definite pattern emerged correlating PG to a particular cell type. PG synthesis is a characteristic of many types of inflammatory reactions, each with different mechanisms underlying the pathogenesis. In general, however, the lesions in which PMN play an important role were consistently more impressive in terms of the quantities of PG formed. The different patterns of release and quantities released using different models offer hope that the cellular events leading to PG formation will be elucidated.

In light of the fact that the PG are efficiently metabolized in the lung [36] it was not surprising to find that PG levels in the systemic circulation were undetectable. The finding that microgram amounts of PGE and PGF could be assayed in lymph draining a lesion over a period of hours without any measurable quantities in the venous blood, close to the site of the




TEXT-FIGURE 8—Infusion schedule for inhibition experiment. (For further details see *Results*).

Table 1—Comparison of PGE and PGF From *E coli* Reactions in Lymph Nodes Receiving Indomethacin or Saline Treatment

| Time | −Ind | +Ind | % Inhibition | −Ind | +Ind | % Inhibition |
|---|---|---|---|---|---|---|
| | PGF equivalents | | | PGE equivalents | | |
| −2.5-0 | 735 | 207 | 72 | 385 | 148 | 62 |
| 0-2* | >5000 | 1612 | >68 | >5000 | 2446 | >51 |
| 2-4 | 1135 | 1090 | 4 | 1500 | 720 | 52 |
| 4-6 | 869 | 186 | 79 | 1238 | 506 | 59 |
| 6-8 | 534 | 143 | 73 | 388 | 516 | – |
| 8-10 | 275 | 137 | 50 | 645 | 280 | 57 |
| 10-18† | 0 | 335 | – | 708 | 522 | 26 |
| 18-22 | 77 | 385 | – | 632 | 589 | 7 |

* Indomethacin infusion stopped at 2.0 hours after *E coli* infusion.
† Saline infusion stopped 14 hours after *E coli* infusion.

inflammation, was unexpected. Since the PG have a small molecular weight (354) they should equilibrate rapidly with the blood. The only explanation seems to be that the PG bind to albumin, which, on entering the lymphatics, is confined to the lymphatic circulation until passage through the thoracic duct. The binding of PG to albumin is well established.[37-39] An alternative or supplementary explanation is that a portion of the PG is metabolized at the site of their production or as they pass through the node. Since the metabolite would not be recognized by the antibody in the RIA procedure, blood-borne PG would pass undetected. We have some data that suggest metabolism since the pattern of PG in lymph changes while crossing the node. In experiments in which afferent and efferent lymphatics have been drained from the same lesion, PGE levels have been found to be much higher in afferent lymph compared with efferent lymph.[40,41] This difference cannot be due to diffusion since the concentration of PGF entering the node is roughly equivalent to that leaving the node.

It is hoped that infusions of labeled prostaglandins combined with RIA measurements will allow a complete balance sheet to be prepared, outlining the production, recoveries, and metabolism of the PG produced in an inflammatory lesion. Because the sheep model has quantitative potential not found in other models, this seems a reasonable goal.

It is almost impossible to speculate on the precise role of the PG in this system. Athough the PG concentrations were inhibited in one of the nodes of a sheep (Table 1), the lymph flow rates and cell outputs were identical to those in the node from a PG synthetase inhibitor (not shown). A great many actions have been proposed for the PG based mainly on *in vitro* studies. Very few of these have been tested *in vivo*. In terms of classic inflammatory signs, blood flow changes seem to be the easiest to attribute to the prostaglandins. In earlier papers,[42,43] blood flow measurements were made in the skin, using labeled microspheres.[44] A good dose response

curve was obtained in rabbit skin between 0.25 and 4000 ng. It would seem that enough PG is produced in the inflammatory lesions to alter blood flow, even in the relatively crude assay with microspheres. Whether this is actually the case awaits further study.

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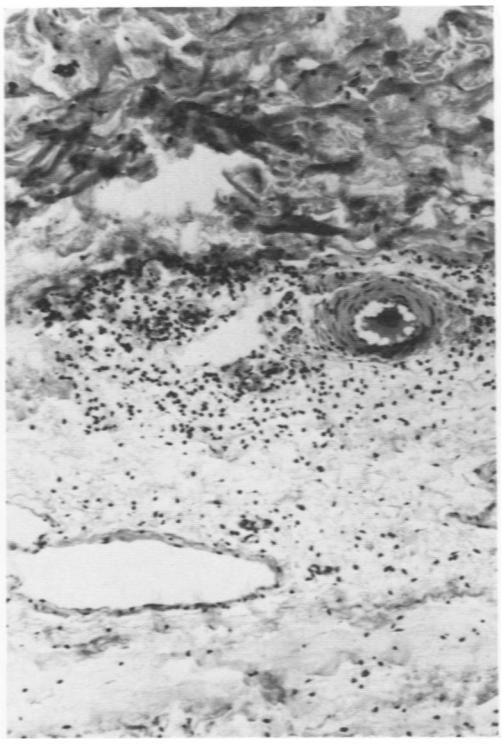
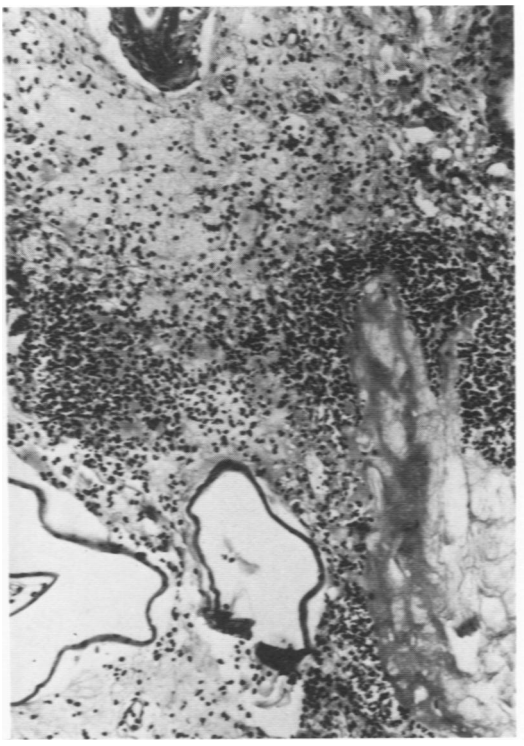
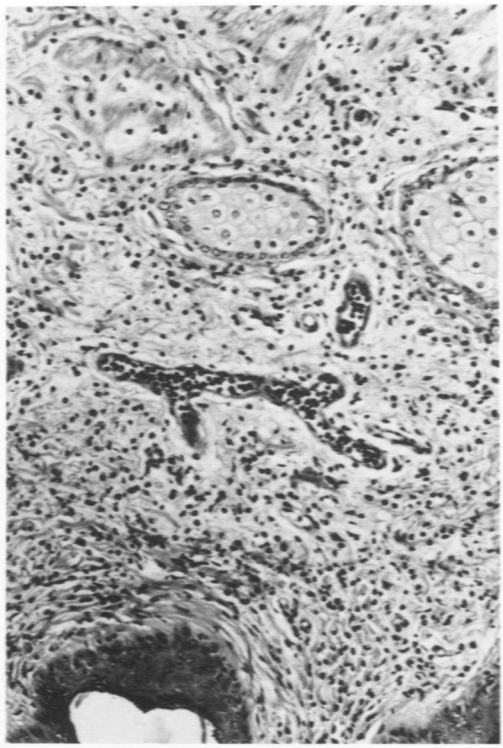






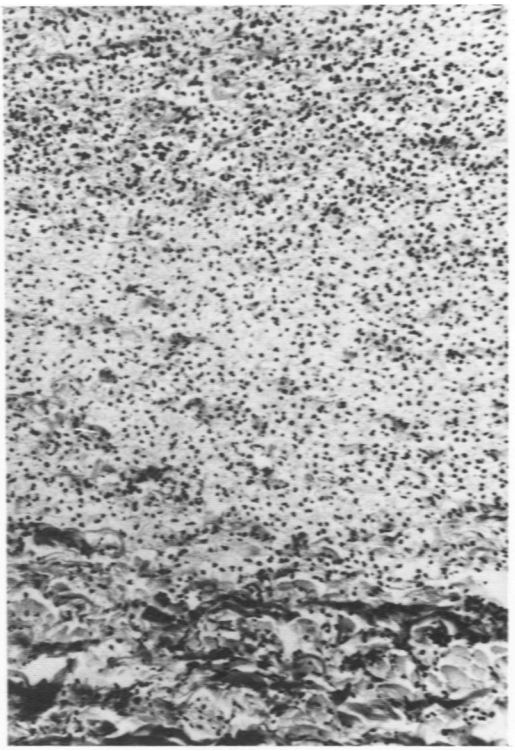
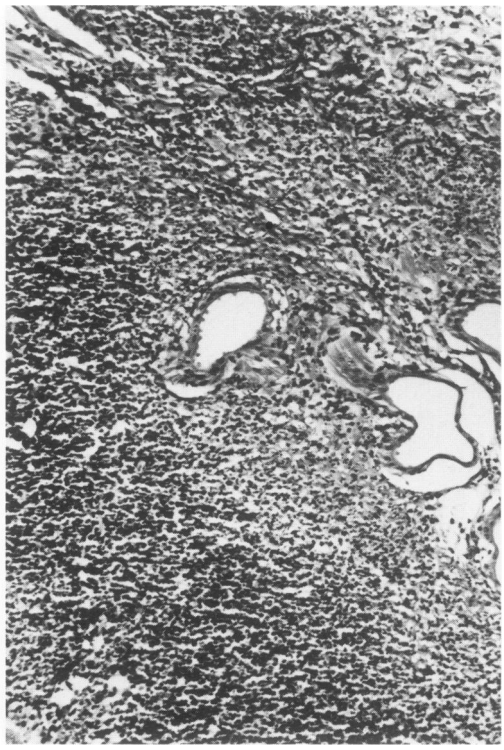


**Figure 1**—Early accumulation of neutrophils in the lower part of the dermis of a sheep 1 hour after the injection of 6 × $10^9$ heat-killed *E coli.* (× 375)

**Figure 2**—Neutrophil accumulation in the dermis of a sheep 2 hours after injection of *E coli.* (× 375)

**Figures 3 and 4**—Accumulation of neutrophils in the upper (Figure 3) and lower (Figure 4) part of the sheep's dermis 4 hours after injection of *E coli.* (× 375)



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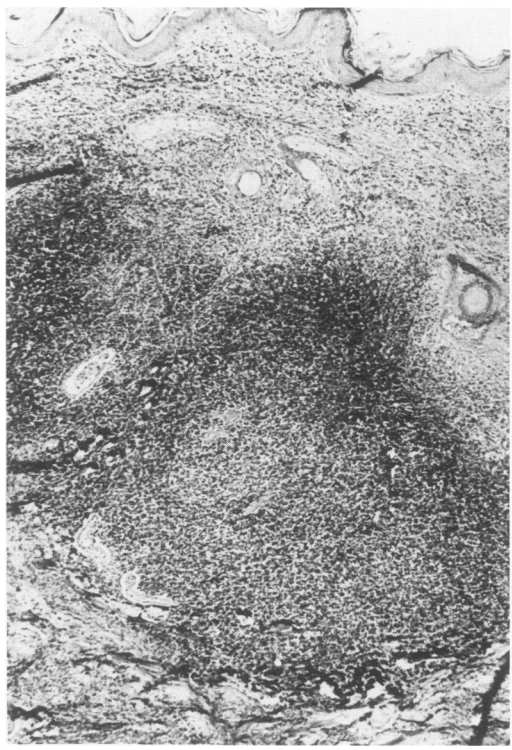

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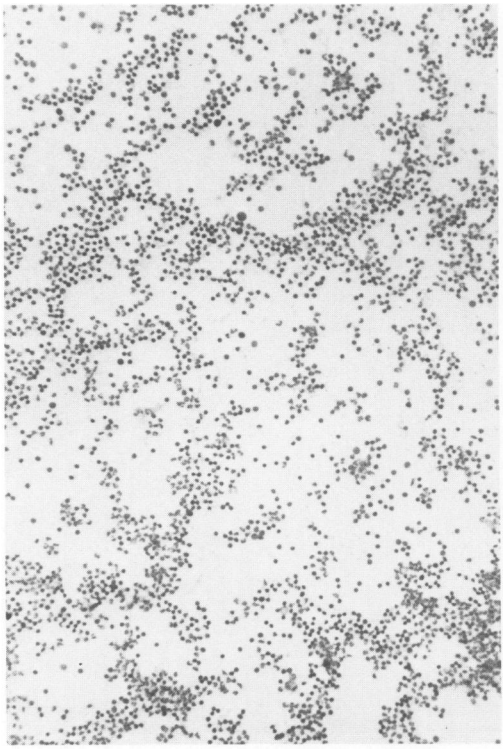

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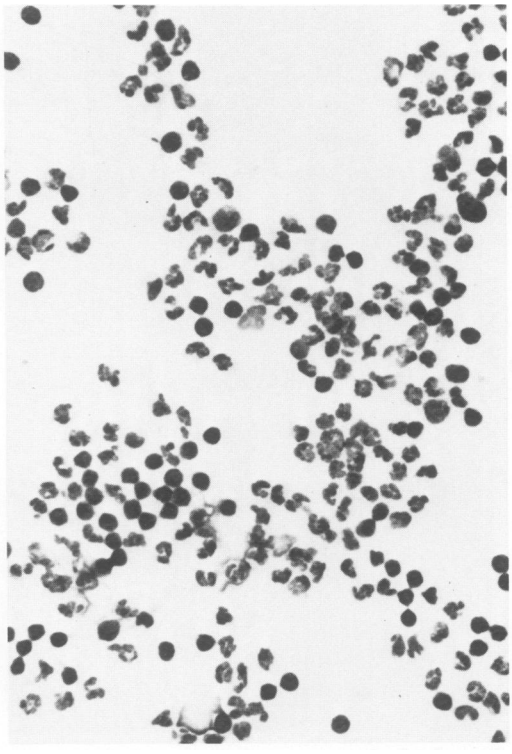

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**Figure 5**—Diffuse infiltration of a sheep's dermis by neutrophil leukocytes 8 hours after injection of *E coli.* (× 375) **Figure 6**—Abscess in the dermis of a sheep 24 hours after injection of *E coli.* (× 112) **Figures 7 and 8**—Smear of lymph from a sheep draining a 4-hour-old inflammatory focus induced by *E coli.* (**Figure 7**, × 375; **Figure 8**, × 1500)