The Distribution of Prostaglandins in Afferent and Efferent Lymph From Inflammatory Sites

Miles G. Johnston, PhD, John B. Hay, PhD, and
Henry Z. Movat, MD, PhD

Arachidonic acid labeled with ^{14}C was injected directly into lymph nodes that had been stimulated at various times with *Escherichia coli*. The efferent lymph was collected, and labeled catabolites were extracted and analyzed chromatographically. The primary conversion product recovered was prostaglandin E_2 (PGE_2), with the lesser products thromboxane, prostacyclin and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) also detected. When the efferent lymph was analyzed by radioimmunoassay after subcutaneous injection of *E coli* into the hock, PGE and PGF levels rapidly increased, reached the highest levels in the first 10 hours, and then returned to normal by 24 hours. When the afferent lymph plasma draining inflammatory sites was compared directly with efferent lymph, PGF levels were similar, but the PGE level was always several times higher in the afferent lymph. To examine the catabolism of PG, either ^3H-$PGF_{2\alpha}$ or ^3H-PGE_2 was injected into the node, and the efferent lymph plasma was analyzed. No conversion of $PGF_{2\alpha}$ to other products was found. In contrast, catabolic products of PGE_2 were detected. With the use of equilibrium dialysis techniques, the binding of PGE_2 and $PGF_{2\alpha}$ to proteins in lymph and to bovine serum albumin (BSA), human serum albumin (HSA), and BSA stripped of its fatty acids was established. The binding to lymph proteins correlated with the albumin concentrations in the lymph. This albumin binding probably facilitated the retention and transport of PG in the lymph. PG appears in the lymph at a time corresponding to the uptake and processing of antigen by the node and near the time when lymphokines are detected in lymph and could modulate several steps in the immune response. The PGE detectable in the lymph draining an inflammatory site may play a role in the modulation of blood flow. (Am J Pathol 1980, 99:695-714)

PRODUCTS OF ARACHIDONIC ACID METABOLISM have been shown by many investigators to be important mediators or modulators of inflammation and hemostasis.[1,2] These findings implicate prostaglandins (PGs) with alterations in vascular permeability, blood flow changes, and modulation of inflammatory cell function. Many of these actions are a consequence of effects on cyclic nucleotides.[2] Research of this type has been extended to chronic inflammation and the immune system, where

From the Division of Experimental Pathology, Department of Pathology, University of Toronto, Toronto, Ontario, Canada.

Supported by the Medical Research Council of Canada (Grant MT-4056 [J.B.H.]) and the Ontario Heart Foundation (Grant T-1-15 [H.Z.M.]). Presented in part in the Minisymposium "Mediators of Inflammation," FASEB, Dallas, Texas, April 14, 1979.

These studies were performed while Dr. Johnston was holding a Studentship from the Medical Research Council of Canada. His present address is ACR Institute of Animal Physiology, Babraham, Cambridge, England.

Dr. Movat is a Career Investigator of the Medical Research Council of Canada.

Accepted for publication January 24, 1980.

Address reprint requests to Henry Z. Movat, MD, Division of Experimental Pathology, Department of Pathology, Medical Sciences Building, University of Toronto, Toronto, Canada M5S 1A8.

0002-9440/80/0609-0695$01.00

PGs (particularly of the E-type) have been shown to inhibit activities of immune cells *in vitro*, including lymphokine release,[3] antibody formation,[4] cytotoxicity,[5,6] mitogenesis,[7,8] and others (reviewed [9,10]). Other studies have shown that indomethacin, a PG synthetase inhibitor, enhances antibody formation [11,12] and delayed hypersensitivity skin reactions.[13]

Since PGs have the potential to modulate complex immune and inflammatory processes it is relevant to consider the concentrations of these highly active molecules in pathophysiologic situations, the kinetics of their appearance in relation to other events, and the distribution of PGs in the fluids that constitute the natural milieu of immune and inflammatory reactions. The lymph draining the reaction site either before or after it enters the regional lymph node is a convenient site in which to monitor these events.[14] The kinetics of PG appearance in lymph from sheep following a variety of insults has been described.[15] The studies reported here outline some differences between afferent and efferent lymph, demonstrate the significance of the interaction of PG with lymph plasma proteins, and define the selective catabolism of PGE_2 by lymph nodes.

Materials and Methods

Animals

Twenty-five- to forty-kilogram sheep of both sexes were used in all experiments. The animals were kept unrestrained and allowed free access to food and water.

Surgical Procedures and Lymph Collection

Lymphatic vessels afferent to and efferent from the popliteal lymph node were cannulated with polyethylene catheters by the methods described by Hall and Morris.[14] Plastic bottle holders were sutured to the animal's skin, and lymph was collected at room temperature into sterile bottles. Each collection bottle contained 250 units USP heparin (Organon, Canada, Ltd.) and 500 IU each of penicillin and streptomycin (Grand Island Biologicals, Grand Island, NY). The animals were placed in metabolism cages and allowed to recover from the anesthetic (Nembutal Sodium, Abbott Laboratories, North Chicago, Ill) for 1 day before the experiments were performed. In some experiments, labeled PGs and arachidonic acid were injected directly into the nodes, and the lymph was collected on the operating table. Cells were counted with a Model B Coulter Counter. Lymph samples were centrifuged at 600g for 5 minutes, and the supernatants were frozen and stored at −20 C for no more than 1 week before the prostaglandins were assayed. The cell pellets were smeared and stained with Leishman's stain for differential counts.

Measurements of Prostaglandins

Prostaglandin E and F equivalents were measured with a radioimmunoassay developed in this laboratory.[15] Briefly, an antibody was raised in rabbits to $PGF_{2\alpha}$. This antibody cross-reacted with $PGF_{1\alpha}$ 75% and with PGE_2, PGE_1, PGA_2, and $PGB_2 < 0.01\%$. PGE levels were assayed by reduction to the corresponding PGF compounds with sodium borohydride according to the method of Levine.[16] The anti-$PGF_{2\alpha}$ antibody did not distinguish the degree of unsaturation; therefore, the results were expressed as PGE and PGF equiva-

lents. Since substances that interfere with the binding of the PG to the antibody are present in extracts of biological fluids, a suitable amount of extract from control samples was added to the standard curve tubes. As a consequence, the results were expressed as picograms or nanograms above the control lymph or blood samples. Only in this way can the radioimmunoassay function properly. If the extract is omitted from the standard curve, more displacement of the PG from the antibody occurs in the samples than in the standard curve, with the result that measurements are too high. The labeled PGs, metabolites, and arachindonic acid were purchased from Amersham (Oakville, Ontario, Canada) (^{3}H-PGE$_2$ and ^{3}H-PGF$_{2\alpha}$, 120–170 Ci/mmole; ^{3}H-13, 14-dihydro-15-keto PGE$_2$, 60–100 Ci/mmole; ^{14}C-arachidonic acid, 55.5 mCi/mmole).

Thin Layer Chromatography (TLC)

To 1 ml lymph was added 1 ml PBS and 2 ml absolute ethanol. This was extracted with petroleum ether (boiling point 40–60 C) to remove neutral lipids and the ether phase discarded. The pH was adjusted to 3.5 with formic acid, and the lymph was extracted twice with 4 ml chloroform. The chloroform was evaporated under a stream of air, and the extract was redissolved in absolute ethanol or acetone. Prostaglandin standards (10 μg in ethanol) or 10 μl lymph extract were applied to silica gel G plates (BDH, 20 cm × 20 cm, Toronto, Ontario), and the plates were placed in a glass tank for development. Prostaglandin standards were visualized with iodine vapor, and the positions of labeled PGs were determined by scraping 5-mm segments of the silica gel into plastic scintillation vials (New England Nuclear, Lachine, Quebec), which contained 10 ml of ACS scintillation cocktail (Amersham). The solvent systems used were: 1) chloroform, methanol, acetic acid, water, 90:9:1:0.65; 2) chloroform, methanol, acetic acid, 95:1:5; and 3) ethyl acetate, formic acid, 80:1.

In order to compare plates more easily, the total radioactivity on the plate was determined by adding each point (0.5-mm scraping). Each point was expressed as a percentage of the total radioactivity applied to the plate.

Equilibrium Dialysis

The binding of PGs to lymph, blood, and various proteins was studied using equilibrium dialysis techniques similar to those of Raz.[17,18] Briefly, ^{3}H-PG and unlabeled PG was added to heparinized (5 U/ml) sheep lymph and blood plasma and to different protein solutions so that the final concentration of cold PG was 10 ng/ml (lymph draining inflammatory lesions often contains this amount of PG). Dialysis bags were prepared from dialysis tubing (cellulose 1 cm flat diameter) (Fisher Scientific, Pittsburgh, Pa) previously washed 5 times in boiling deionized water.

Two milliliters of the PG-protein solution were pipetted into the dialysis bags and the bags placed in plastic test tubes (14 mm × 100 mm) containing 5 ml 0.02 M phosphate-buffered saline (PBS) with 5.0×10^{-4} M ethylene diaminetetracetate (EDTA). The tubes were rotated gently for 20 hours at room temperature. After dialysis 0.1 ml of the solutions inside and outside the bag were counted for radioactivity with the use of an Intertechnique Scintillation Counter (SL30). The percent of PGs bound to protein in the inside solution after dialysis was calculated as

$$\frac{\text{cpm (0.1 ml inside solution)} - \text{cpm (0.1 ml outside solution)}}{\text{cpm (0.1 ml inside solution)}}$$

The recovery of radioactivity in the inside and outside solutions averaged 90%, reflecting some binding of the labeled PG to the dialysis membranes and plastic tubes.

Experimental Procedures

Experiments With Arachidonic Acid

Prefemoral or mesenteric lymph nodes were cannulated and drained on the operating table. ^{14}C-arachidonic acid (2.5×10^6 dpm) was injected simultaneously and 1, 2, and 3 hours after the injection directly into the node of 5.0×10^9 heat-killed *E coli* (Strain X, Medical Teaching Laboratories, University of Toronto, Toronto, Ontario). In this manner the various PG types formed from the precursor arachidonate could be determined in a sequential fashion after stimulation. Evans blue dye was included at each injection and its appearance in efferent lymph confirmed the positioning of the lymphatic catheter. The lymph was collected on the operating table into plastic test tubes containing 10 units heparin. The samples were spun and frozen prior to extraction and TLC analysis. In another group of experiments ^{14}C-arachidonic acid was injected into the hock of the animal 3 hours after 2.0×10^{11} of *E coli* were injected into the hock. Lymph from draining afferent lymphatics was collected and processed in the manner described.

Studies With Bacteria

Afferent lymphatic vessels entering the popliteal node and efferent lymphatic vessels leaving the node were cannulated. Approximately 2.0×10^{11} heat-killed *E coli* organisms in 2 ml PBS containing sterile 2% Evans blue dye were injected in 2-ml volumes into 3 sites in the hock of the animal. Evans blue appeared in the afferent and/or efferent lymph within a few minutes after subcutaneous injection into the hock in all preparations used. In another group of studies *E coli* organisms were infused directly into the node via an indwelling cannula placed in an afferent vessel.

Lymph Collections for Equilibrium Dialysis

Lymph was collected from efferent and afferent lymphatic vessels for periods of up to 1 week. The collections were spun and frozen as previously described and before use were thawed, pooled, and stored at 4 C. Blood samples from the jugular vein were drawn into heparin (5 U/ml blood), spun at 600g for 10 minutes, and the plasma was frozen at −20 C until use.

Metabolism Experiments With Labeled PGE_2 and $PGF_{2\alpha}$

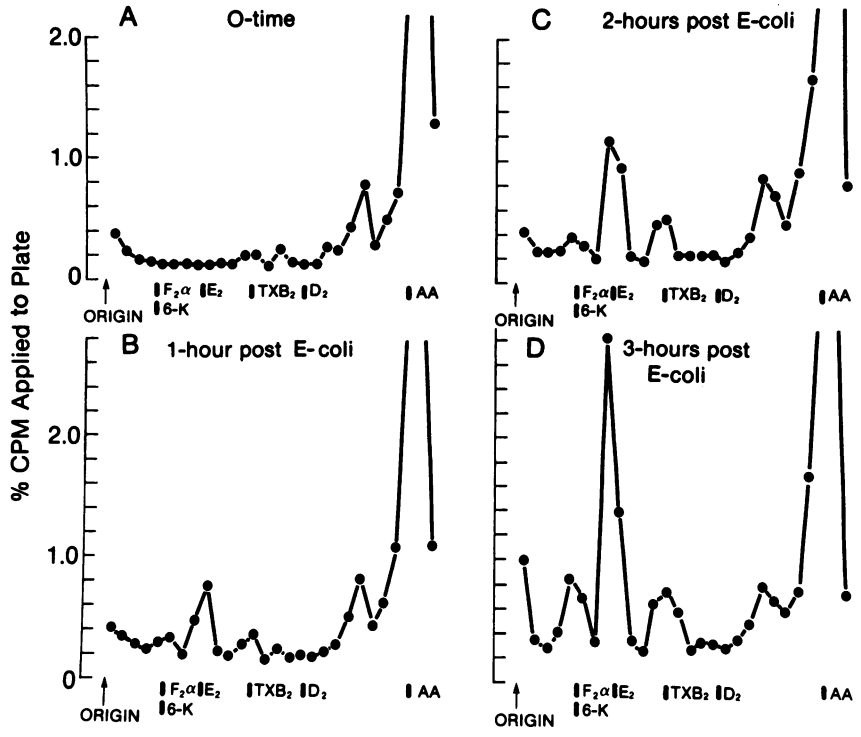
Popliteal or mesenteric lymph nodes were exposed on the operating table, and their corresponding efferent lymphatics were cannulated. *E coli* (1.0×10^9) organisms were injected directly into the nodes. Before, with, or 1 hour after *E coli* challenge ^{3}H-PGE_2 or ^{3}H-$F_{2\alpha}$ (1.2×10^7 dpm) were injected directly into the node. Some PG was injected into nodes draining 6-week-old granulomatous lesions induced with the subcutaneous injection of 1.0 ml complete Freund adjuvant into the hock. The lymph was collected on the operating table into plastic test tubes containing 10 units of heparin. The samples were spun and frozen prior to extraction and TLC analysis. In this manner any catabolism of PGE_2 or $PGF_{2\alpha}$ could be determined. PGE_2 or $PGF_{2\alpha}$ (1.2×10^7 dpm) were also incubated in lymph for 1 hour at 37 C and extracted and processed in a similar manner to determine whether any catabolism occurred in lymph.

Results

TLC Analysis of PG Types Formed After *E coli* Challenge

Arachidonic acid can be metabolized to classic PGs, ie, PGE_2, $PGF_{2\alpha}$, and PGD_2, as well as thromboxanes, prostacyclin, and other products.

Many of these compounds have interesting biological actions, and a number of products have been identified in our studies by their comigration with authentic standards. Text-figure 1 shows the radiochromatogram of extracts of lymph collected from a prefemoral lymph node at various times after *E coli* challenge. At the time the bacteria were injected no discernible product of arachidonic acid metabolism was present, but as the lesion progressed up to 3 hours, 3 identifiable peaks became visible. The largest migrated with authentic PGE_2. Some label corresponded to thromboxane B_2, the stable metabolite of thromboxane A_2. The peak of radioactivity close to the region could be either $PGF_{2\alpha}$ or the metabolite of prostacyclin, 6-keto $F_{1\alpha}$, because this solvent system (ethyl acetate: formic acid, 80: 1) does not resolve these two compounds. However, in recent

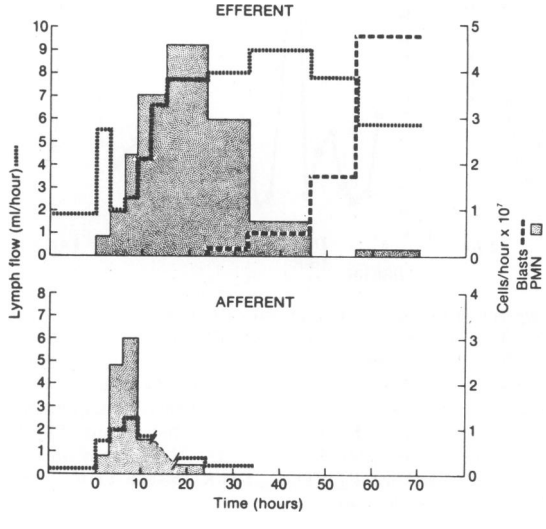


TEXT-FIGURE 1—Thin layer radiochromatograms of extracts of lymph after the injection of ^{14}C-arachidonate into *E coli*-stimulated nodes. Bands (0.5 mm) were scraped into scintillation vials for counting. The plates were developed twice with ethyl acetate: formic acid (80: 1). Each point is expressed as a percentage of the total cpm applied to the plates. The positions of authentic PG standards outlined with iodine vapor are depicted at the bottom of each plot. A—Metabolism of arachidonate injected at the same time as the *E coli*. B—Metabolism of arachidonate injected 1 hour after *E coli*. C—Metabolism of arachidonate injected 2 hours after *E coli*. D—Metabolism of arachidonate injected 3 hours after *E coli*. Abbreviations: $F_{2\alpha}$ ($PGF_{2\alpha}$), 6-K (6-keto $PGF_{1\alpha}$), E_2 (PGE_2), TXB_2 (Thromboxane B_2), D_2 (PGD_2), AA (arachidonate).

studies with another solvent system, isooctane:ethyl acetate:acetic acid: water 5:11:2:10 (organic phase), a mixture of both of these compounds was found. No PGD_2 was detected. In studies of this kind it is common procedure to incorporate labeled precursor arachidonic acid into the phospholipids of the tissues before experimentation and quantitate its endogenous release and conversion. In the studies reported here the arachidonic acid was injected and collected immediately, thereby providing exogenous substrate for the cyclooxygenase enzyme system that generates the various PG types. The conversion of arachidonic acid to PGs (mainly PGE_2) rose steadily for the first 3 hours after *E coli* challenge (after which point no samples were taken). A similar pattern emerged in afferent lymph after *E coli* and ^{14}C-arachidonate were injected into the hock. Because PGE_2 has been implicated as a modulator of immune and inflammatory reactions, we were interested in determining the kinetics of PG production relative to the physiologic and cellular events that take place after stimulation by *E coli*.

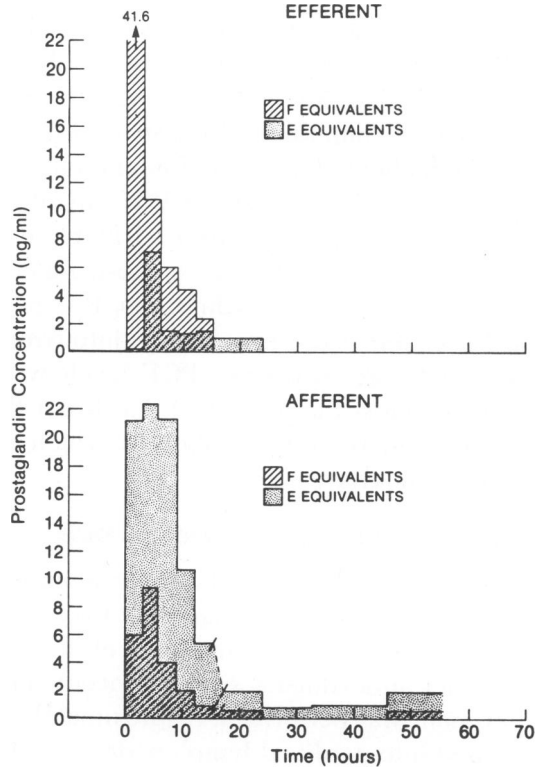
Pattern of PGE and PGF in Afferent and Efferent Lymph Following *E coli* Injection Into the Hock

Text-figure 2 (top) outlines a typical response to *E coli* in efferent and afferent lymph. Increased vascular permeability is reflected by an enhancement of lymph flow rate, rising in the efferent lymph from a control level of 1.9 ml/hr to a peak of 9 ml/hr at 33 hours. This represented a 5-fold increase from normal values. The usual cell population in efferent lymph is entirely lymphocytes, but during the inflammatory response large numbers of neutrophils (PMNs) entered the lymph, rising to 4.6 ×



TEXT-FIGURE 2—The response in lymph following the subcutaneous injection of *E coli* into the hock of a sheep. *Top*, lymph flow and cellular changes in efferent lymph. *Bottom*, the same change in afferent lymph.

10^7 PMNs/hr between 15 and 24 hours. The total cell output rose to 1.6×10^8 cells at 70 hours, at which point the catheter clotted. The appearance of blast cells in the lymph was noted and, in other responses, these cells reached a maximum 3–4 days after antigenic challenge. The lower half of the illustration outlines the flow and cell characteristics in the afferent lymph of this response. The flow rate increased almost 8-fold, and the PMNs were maximal at 3.0×10^7/hr between 6 and 9 hours. PMNs represented approximately 95% of the total cells, the remainder being lymphocytes and macrophages. Text-figure 3 outlines the concentration of PGE and PGF equivalents above control lymph in efferent and afferent lymph, respectively. In efferent lymph leaving the node PGF levels rose dramatically in the first 3 hours and declined rapidly to control levels by the end of a day. PGE levels, on the other hand, were maximal between 3 and 6 hours and were much lower than PGF levels. In the afferent lymph (Text-figure 3, bottom) PGE levels were much higher than PGF levels, almost completely reversing the pattern noted in the efferent lymph. By the end of a day both PGF and PGE levels had returned to near normal values.



TEXT-FIGURE 3—Radioimmunoassay measurements of PGE and PGF equivalents (ng/ml) above control lymph in efferent and afferent lymph.

This dramatic shift in the PG pattern across the node was a consistent finding. In all experiments PGE levels in afferent lymph were higher than in efferent lymph. In contrast, PGF concentrations were similar.

There are a number of explanations that could account for these differences. Since PGs are very small molecules, they should diffuse readily from lymph to blood. In order for us to explain these results by diffusion, the E-type PG would have to diffuse to a greater extent than the F-type PG. However, their molecular sizes are similar, and PGE_2 binds slightly more avidly to albumin; therefore it should diffuse less.

In some experiments systemic venous blood and local venous blood were sampled close to the site where the nodal vein enters the femoral vein, and in these cases PGs have never been detected in the blood. The radioimmunoassay has a sensitivity of 200 pg/ml PGE and 200 pg/ml PGF equivalents. This may indicate that very little PG enters the blood, compared with the lymph, or that the dilution factor and rate of blood flow overcome the sensitivity of the assay. High levels in lymph with perhaps minimal diffusion could be facilitated by protein binding. Once albumin leaves the vascular system, it does not diffuse back, but enters lymphatics to eventually reach the circulation via the thoracic duct or other major lymphatics. Furthermore, radiolabeled albumin infused into an afferent lymphatic vessel is almost totally recovered in the efferent lymph, indicating that any molecule bound to albumin would be confined to the lymphatics (unpublished observations). Another explanation to account for the higher values of PGE in afferent, compared with efferent, lymph is that selective catabolism of PGE might occur in the node. Bolla et al [19] demonstrated the conversion of PGE_2 to $PGF_{2\alpha}$ by fetal and adult sheep blood presumably through a prostaglandin 9-ketoreductase enzyme that was confined to the erythrocytes. It is possible that this enzyme is active in our system, since hemorrhage into lymphatics is not uncommon after *E coli* challenge. However, PGF levels would have to be consistently increased across the node, and that is not the case. PGE may be converted to one or more of its metabolites, which would not be detected by the radioimmunoassay.

Infusions of *E coli* Organisms Directly Into Nodes

PGs infused into the lung are almost completely catabolized on one passage [20] in spite of the fact that they can be synthesized and released uncatabolized during stimulation of this organ.[21] In order to determine whether an analogous situation occurs in lymph nodes, the following experiment was performed. Approximately 1.0×10^{10} *E coli* organisms were infused into popliteal lymph nodes via afferent catheters, and the efferent

Table 1—PG in Efferent Lymph Following the Infusion of *E coli* Directly Into a Popliteal Lymph Node

Time (hrs)	$PGF_{2\alpha}$ (pg/ml)	PGE_2 (pg/ml)
0–1	2080	10,760
1–2	1040	2,520
2–3	900	1,320
3–4	440	2,800
4–6	440	1,760
6–8	580	1,840
8–15	440	1,720
15–23	—	1,800
23–29	260	1,360

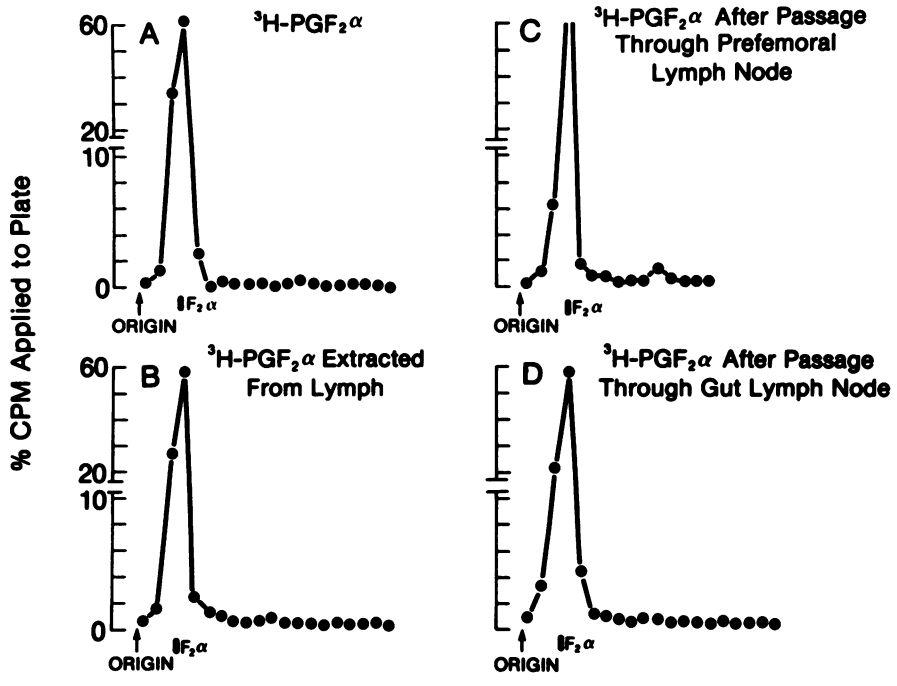
PGF and PGE equivalents were measured with a radioimmunoassay, and the results are expressed as picograms of PG equivalents above those of the control sample.

lymph was analyzed for PGs. Table 1 shows a typical result obtained from one such trial. The concentration of PG is expressed as picograms above that of the control sample. *E coli* bacteria were infused at zero-time. Under these circumstances the efferent samples contained much higher levels of PGE equivalents than PGF equivalents. This result was a consistent finding whenever bacteria were infused directly into the node. If degradation occurred, it would appear that PG formed in the node escaped catabolizing enzymes.

Thin Layer Chromatographic Analysis of ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ After Passage Through Lymph Nodes

In order to test whether PGE_2 and $PGF_{2\alpha}$ were catabolized, they were injected into lymph nodes at various times after *E coli* deposition in the node. Text-figure 4 shows the results of experiments with $PGF_{2\alpha}$. Labeled $PGF_{2\alpha}$ when tested on a silica gel G plate in a solvent system comprising ethyl acetate : formic acid (80 : 1) ran as a single peak (Text-figure 4A). After incubation of ^{3}H-$PGF_{2\alpha}$ in lymph for 1 hour at 37 C and extraction, the pattern was unchanged (Text-figure 4B). After passage through prefemoral lymph nodes (Text-figure 4C) or mesenteric lymph nodes (Text-figure 4D) before, at the same time, or 1 hour after *E coli* challenge, no other product was formed. Data obtained 1 hour after stimulation are shown in this figure.

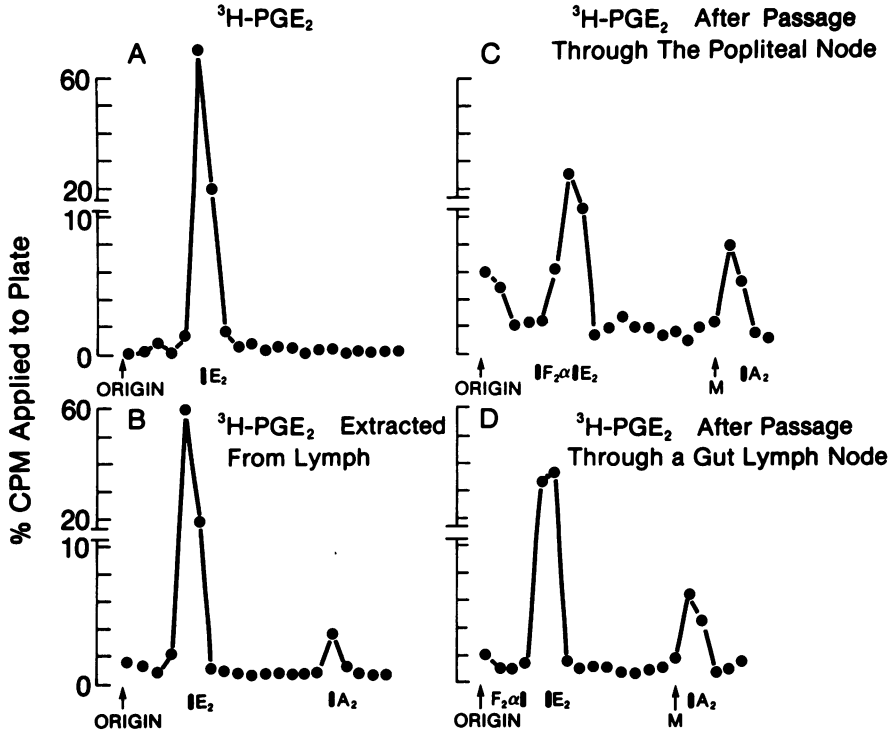
After purification on TLC plates with the solvent system chloroform : methanol : acetic acid : water (90 : 9 : 1 : 0.65), ^{3}H-PGE_2 migrated as a single peak (Text-figure 5A). After incubation in lymph and extraction and development in ethyl acetate : formic acid (80 : 1) a second peak emerged, one corresponding to PGA_2 as an *in vitro* artifact. After passage through stimulated popliteal or mesenteric lymph nodes (Text-figure 5C and D)



TEXT-FIGURE 4—Radiochromatograms demonstrating the fate of ^{3}H-PGF$_{2\alpha}$ after extraction from lymph or passage through lymph nodes. A—^{3}H-PGF$_{2\alpha}$ applied directly to plate. B—^{3}H-PGF$_{2\alpha}$ incubated in lymph for 1 hour at 37 C. C—^{3}H-PGF$_{2\alpha}$ after passage through an *E coli*-stimulated prefemoral lymph node. D—^{3}H-PGF$_{2\alpha}$ after passage through an *E coli*-stimulated mesenteric lymph node. The solvent system employed was ethyl acetate : formic acid (80 : 1). Abbreviation: F$_{2\alpha}$ (PGF$_{2\alpha}$).

two peaks were again visible, the second peak considerably larger than after extraction from lymph alone. The time of PGE$_2$ injection relative to *E coli* challenge made no difference. The data shown were obtained by injecting PGE$_2$ one hour after the bacteria. The peak seemed to migrate very close to PGA$_2$. Therefore, other solvent systems were tried to determine if another product was present. Chloroform : methanol : acetic acid (95 : 1 : 5) achieved resolution of this material into three distinct peaks (Text-figure 6A): the first migrated with authentic PGE$_2$ standard, the second was tentatively identified with 13,14-dihydro-15-keto-PGE$_2$ metabolite, and the third was identified with authentic PGA$_2$ standard. No conversion of PGE$_2$ to PGF$_{2\alpha}$ could be identified in these studies, indicating that the PG 9-ketoreductase enzyme probably plays no role in the lymph node.

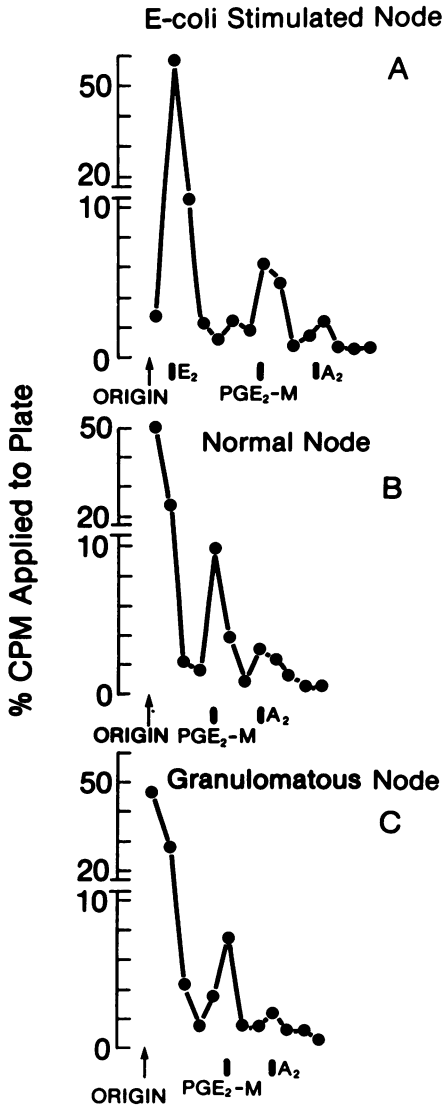
It is evident that the lymph node itself is responsible for the conversion of PGE$_2$ to some other form. Extraction from lymph alone and develop-



TEXT-FIGURE 5—Radiochromatograms demonstrating the fate of ^{3}H-PGE$_2$ after extraction from lymph or passage through lymph nodes. A—^{3}H-PGE$_2$ applied directly to the plate. B—^{3}H-PGE$_2$ incubated in lymph for 1 hour at 37 C. C—^{3}H-PGE$_2$ after passage through an *E coli*-stimulated popliteal lymph node. D—^{3}H-PGE$_2$ after passage through an *E coli*-stimulated mesenteric lymph node. The arrow marks the position of the metabolite (M) standard. The solvent systems employed were for A chloroform:methanol:acetic acid:water (90:9:1:0.65) and B, C, D ethyl acetate:formic acid (80:1). Abbreviations: E_2 (PGE_2), $F_{2\alpha}$ ($PGF_{2\alpha}$), A_2 (PGA_2), M (13,14-dihydro-15-keto-PGE_2).

ment with chloroform:methanol:acetic acid (90:1:5) yielded only 2 peaks corresponding to PGE_2 and PGA_2 (not shown). The metabolite has not been positively identified by mass-spectrophotometric analysis, but it migrates in our TLC system with 13,14-dihydro-15-keto-PGE_2. It could possibly be 15-keto-PGE_2 metabolite, because this migrates very close to the dihydro-15-keto metabolite in many solvent systems.[22] The percentage of radioactivity in the metabolite peak in Text-figure 6 was approximately 11% (adding the percentages of the 2 points comprising the peak). A conversion of this magnitude is rather small, compared with the dramatic reduction of PGE equivalents across the node. It is possible that the experimental conditions are not optimal (for example, these experiments were carried out under anesthesia).

Normal nodes, ie, nodes that did not receive any bacterial challenge,



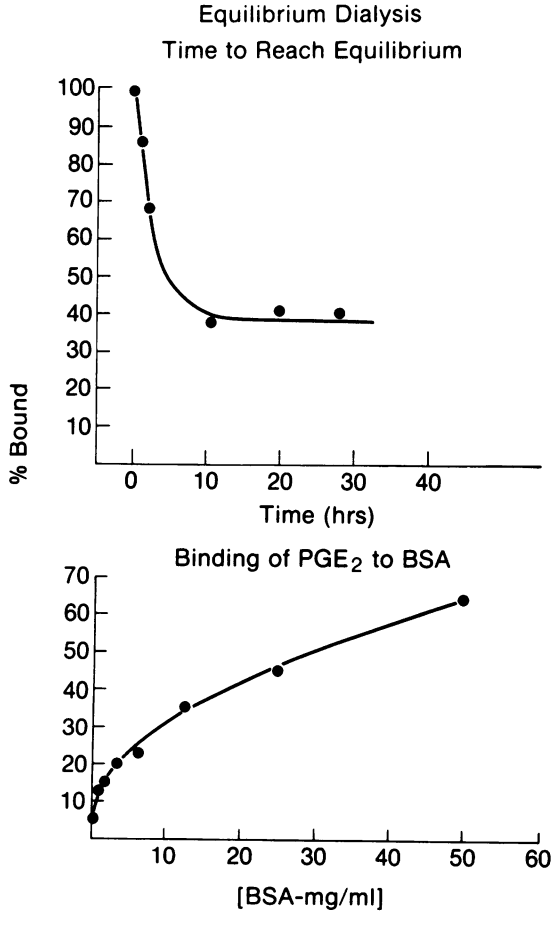
TEXT-FIGURE 6—Radiochromatograms of extracts of efferent lymph following the injection of ^{3}H-PGE_2 into A, an *E coli*-stimulated popliteal node; B, a normal popliteal node; C, a granulomatous prefemoral node. The solvent system employed was chloroform : methanol : acetic acid (95 : 1 : 5). Abbreviations: E_2 (PGE_2), PGE_2-M (13,14-dihydro-15-keto-PGE_2), A_2 (PGA_2).

and nodes draining granulomatous lesions were found to catabolize PGE_2 to the same extent (Text-figure 6B and C). Therefore, the capacity of the lymph node to catabolize PGE does not seem to be related to antigen challenge.

Binding of Prostaglandins to Lymph and Blood Proteins

The binding of prostaglandins to proteins, notably albumin, is well established.[17,18,23] A series of experiments was undertaken to determine the

degree of binding of PGE_2 and $PGF_{2\alpha}$ to lymph proteins and to estimate the variation in binding that might occur during an inflammatory response. Text-figure 7 (top) outlines the kinetics of the binding between bovine serum albumin (BSA) (15 mg/ml) and ^3H-PGE_2 with 10 ng/ml unlabeled PGE_2 added to the mixture. Twenty hours were chosen for measuring the percentage bound, since by this time equilibrium had been reached. Table 2 shows the binding of PGE_2 and $PGF_{2\alpha}$ to sheep lymph and blood and a variety of different proteins. Raz [17,18] has shown that the major binding protein is albumin. In the sheep the following values of protein concentration in lymph and blood have been reported.[24] In blood the total protein concentration averages around 7.5 g/100 ml, of which approximately 3.75 g/100 ml is albumin. In popliteal efferent lymph the total protein and albumin concentrations are approximately 3.2 and 1.6



TEXT-FIGURE 7—Studies of the binding of PGE_2 to BSA using equilibrium dialysis. *Top*, ^3H-PGE_2 and unlabeled PGE_2 (10 ng/ml) were placed in dialysis bags containing BSA (15 mg/ml). The time course of the binding was followed. *Bottom*, ^3H-PGE_2 and unlabeled PGE_2 (10 ng/ml) were placed in dialysis bags containing various concentrations of BSA. The binding was measured after 20 hours of dialysis.

g/100 ml, respectively. In popliteal afferent lymph the values are 2.8 and 1.4 g/100 ml, respectively.

As can be seen from Table 2, blood plasma bound the most PG, followed by efferent and afferent lymph, in that order. BSA at concentrations that approach the concentration of albumin in sheep blood and lymph bound roughly the same amount as the whole lymph or blood. BSA stripped of its fatty acids and human serum albumin (HSA) at the same concentrations exhibited similar binding. Gamma-globulin bound very little.

Of interest is the fact that 45% of PGE_2 bound to efferent lymph and 39% to afferent lymph and that these values paralleled very closely those of BSA and HSA at similar concentrations, supporting the contention that albumin is the major binding protein. PGE_2 represents a significant proportion of the prostaglandin, and these results suggest that the remaining PG in an *in vivo* situation is free to equilibrate with blood. Although this statement is overly simplistic, since many other factors could interfere with binding and equilibrium, this factor indicates that significant binding is possible *in vivo*. Similarly, $PGF_{2\alpha}$ binds to lymph and blood, although to a lesser extent, agreeing with the estimated association constants for the binding of these PGs shown by Raz.[17] He found the binding of PGE_2 to be slightly greater than that of $PGF_{2\alpha}$, the association constants being in the order of 2.4×10^4 for PGE_2 and 0.9×10^4 mole/l for $PGF_{2\alpha}$.

Since the concentration of albumin in the lymph changes as an inflammatory reaction progresses, it is logical to assume that the amount of PG binding changes also. The concentration of albumin in afferent lymph is roughly 14 mg/ml and seldom falls below 10 mg/ml. During a severe in-

Table 2—Percentage of Binding of PGE_2 and $PGF_{2\alpha}$ to Sheep Lymph and Plasma and a Variety of Different Proteins With Equilibrium Dialysis

	PGE_2	$PGF_{2\alpha}$
Blood plasma	61.5	53.0
Blood plasma (diluted 1/2)	50.9	30.7
Efferent lymph	45.6	33.2
Afferent lymph	39.1	32.4
BSA (15 mg/ml)	41.0	—
BSA (37.5 mg/ml)	58.2	—
SBSA (15 mg/ml)	46.3	—
SBSA (37.5 mg/ml)	57.5	—
HSA (15 mg/ml)	49.4	—
Gamma globulin (15 mg/ml)	6.2	—
Control buffer	0.0	0.0

^3H-PGE_2 and ^3H-$PGF_{2\alpha}$ along with corresponding unlabeled PG (10 ng/ml) was incubated in dialysis bags with various protein solutions and lymph. The results are expressed as the percentage of PG binding in the inside solution after dialysis for 20 hours.

flammatory response, the concentration rises to approach that of blood, around 37.5 mg/ml. Therefore, the binding of PGs is largely contained within this range. Text-figure 7 (bottom) demonstrates the effect on PG binding of various concentrations of BSA. A change in albumin concentration in the range of 10–37.5 mg/ml would represent an approximate 25% variation in binding.

Discussion

PGs have been implicated as mediators or modulators of the inflammatory reaction.[2] In order to study their possible role in the inflammatory reaction *in vivo*, we carried out studies, taking advantage of the collection and analysis of the lymph draining inflammatory lesions directly or the lymph that drains regional lymph nodes. The findings also have immunologic implications.

The PGs have been studied in lymph draining inflammatory lesions in the sheep. After *E coli* challenge PGs appear early in the lymph, reaching maximal levels during the first 10 hours and usually returning to normal levels by the end of one day. Experiments with ^{14}C-arachidonic acid demonstrated that the major product of arachidonate metabolism in nodes and in the connective tissue of the hock was PGE_2. Material comigrating on TLC plates with thromboxane, prostacyclin (both measured as their stable metabolites), and $PGF_{2\alpha}$ was also present, although in smaller amounts.

There was a fundamental difference in the profile of PGs in afferent and efferent lymph draining *E coli* lesions. Whereas PGs of the E type were dramatically elevated in afferent lymph draining the inflammatory lesion directly, the levels of PGE were considerably lower in efferent lymph after passage through the regional lymph node. TLC analysis of ^{3}H-PGE_2 incubated in lymph indicated that no conversion of PGE took place in the lymph itself. Only after passage through the node could material be identified that comigrated with the 13,14-dihydro-15-keto-PGE_2 catabolite. Therefore, selective catabolism of E-type PGs occurred within the node. In experiments with nonstimulated and granulomatous nodes, the degree of catabolism of ^{3}H-PGE_2 was similar to *E coli*-challenged nodes, indicating that the catabolism was maintained at a constant rate. Other investigators have reported selective catabolism in blood vessels. Pace-Asciak and Rangaraj [25] demonstrated preferential catabolism of PGE_1 as opposed to $PGF_{1\alpha}$ and $PGF_{2\alpha}$ in the lamb ductus arteriosus, aorta, and pulmonary artery. Our experiments with TLC did not demonstrate any conversion of PGE_2 to $PGF_{2\alpha}$. If, however, *E coli* bacteria were infused directly into the node, a considerable amount of PG formed in the node escaped the catabolizing enzymes, presumably because these enzymes are

intracellular and newly synthesized PGs are released without passing through the cytoplasm.

Considering the fact that draining efferent lymph from the node is not just a sample but rather a quantitative collection, the total output of PGE and PGF equivalents can be calculated knowing the lymph flow rates and the duration of the collection periods. It should be noted, however, that afferent collections are only samples of the lymph draining the inflammatory lesion, as up to 10 afferent vessels may enter the node, and a portion of these may drain the lesion. A series of calculations have been made comparing the theoretical delivery of PGs via the afferent vessels and the output of PGs from the node in the efferent lymphatics. Studying these responses in this way, we have estimated the loss of PGE equivalents across the node to be in the vicinity of 90%, whereas PGF equivalents change very little.

The kinetics of PG appearance in efferent lymph have been studied from a variety of inflammatory stimuli,[15] and elevated levels in lymph, but not blood, have been a consistent finding. Low levels in the venous blood were expected, because the PGs would be diluted in the large vascular space and then rapidly metabolized by the lung.[20] Albumin binding may be crucial for the transport of PGs in the lymphatics. Equilibrium dialysis studies demonstrated a relationship between albumin concentration in lymph and blood and PG binding. Sheep blood with the highest concentration of albumin bound most (> 70%), and efferent (45%) and afferent (39%) followed. $PGF_{2\alpha}$ bound less avidly than PGE_2. PGs appear to retain at least some biological activity when bound to albumin.[18] We have found that PGE_2 covalently bound to albumin for immunization purposes is still able to enhance blood flow when injected intradermally into the rabbit (unpublished observations).

The ability of PGE_2 to enhance blood flow is well established,[26] and it may be one of its important roles in the lymph node. In fact, an enhanced blood flow may account for the increased number of lymphocytes that migrate through the node after antigen challenge.[27,28] Other products of arachidonate metabolism demonstrated in this study could alter the local inflammatory response in the node. $PGF_{2\alpha}$ has been shown in some systems to elevate c-GMP and stimulate the release of leukocyte lysosomal enzymes *in vitro*.[29] Thromboxanes stimulate platelet aggregation and are vasoconstrictors,[30] while prostacyclin inhibits platelet aggregation and is a vasodilator.[31,32] Very little work has been done relating these last two products with immune functions.

Studies *in vitro* have shown that the exogenous addition of E-type PGs inhibit the expression of several immune functions, presumably through

elevations in the cyclic nucleotides. When immune and inflammatory reactions are studied in lymph, there are several events that could be influenced by PGs. Lymphokines appear in lymph near the time when PG levels are maximal.[33] Specific antigen-reactive cells disappear or are unresponsive early in many immune responses.[34] Lymph plasma taken from various stages of the immune response can modulate the synthesis of immunoglobulins by lymphoblasts. A low molecular weight lymph factor was found to suppress the number of antibody-forming cells.[35] Although the PG kinetics can vary when antigens other than *E coli* are used, in most cases the appearance of PGs is an early phenomenon.[15] Webb and Nowowiejski [4] have shown that PG synthetase inhibitors added to cultures of mouse spleen cells stimulated with SRBC enhanced the number of antibody-forming cells, whether given at the time of culture initiation or 1 to 3 days later, implying that PGs may affect both the early initiation of the immune response and its later expression. *In vivo* these effects may be mediated not only through alterations in cyclic nucleotide levels but also through blood flow changes. PGs in lymph are present in sufficient concentrations to modulate several fundamental aspects of immune reactions.

References

1. Moncada S, Vane JR: Unstable metabolites of arachidonic acid and their role in haemostasis and thrombosis. Br Med Bull 1978, 34:129–135
2. Johnston MG, Hay JB, Movat HZ: The role of prostaglandins in inflammation. Curr Top Pathol 1979, 68:259–287
3. Gordon D, Bray MA, Morley J: Control of lymphokine secretion by prostaglandins. Nature 1976, 262:401–402
4. Webb DR, Nowowiejski I: The role of prostaglandins in the control of the primary 19S immune response to SRBC. Cell Immunol 1977, 33:1–10
5. Lichtenstein LM, Henney CS: Prostaglandin inhibition of immediate and delayed hypersensitivity, Prostaglandins in Cellular Biology. Edited by PW Ramwell, BB Phariss. New York, Plenum Press, 1972, pp 293–308
6. Lichtenstein LM, Gillespie E, Bourne HR, Henney CS: The effects of a series of prostaglandins on in vitro models of the allergic response and cellular immunity. Prostaglandins 1972, 2:519–528
7. Stockman GD, Mumford DM: The effect of prostaglandins on the *in vitro* blastogenic response of human peripheral blood lymphocytes. Exp Hematol 1974, 2:65–72
8. Goodwin JS, Bankhurst AD, Messner RP: Suppression of human T-cell mitogenesis by prostaglandin. J Exp Med 1977, 146:1719–1734
9. Bonta IL, Parnham MJ: Prostaglandins and chronic inflammation. Biochem Pharmacol 1978, 27:1611–1623
10. Pelus LM, Strausser HR: Prostaglandins and the immune response. Life Sci 1977, 20:903–913
11. Goodwin JS, Selinger DS, Messner RP, Reed WP: Effect of indomethacin in vivo on humoral and cellular immunity in humans. Infect Immun 1978, 19:430–433
12. Webb DR, Osheroff PL: Antigen stimulation of prostaglandin synthesis and control of immune responses. Proc Natl Acad Sci (USA) 1976, 73:1300–1304
13. Muscoplat CC, Rakich PM, Thoen CO, Johnson DW: Enhancement of lymphocyte

blastogenic and delayed hypersensitivity skin responses by indomethacin. Infect Immun 1978, 20:627–631
14. Hall JG, Morris B: The output of cells in lymph from the popliteal node of sheep. Q J Exp Physiol 1962, 47:360–369
15. Johnston MG, Hay JB, Movat HZ: Kinetics of prostaglandin production in various inflammatory lesions, measured in draining lymph. Am J Pathol 1979, 95:225–238
16. Levine L: Antibodies to pharmacologically active molecules: Specificities and some applications of antiprostaglandins. Pharmacol Rev 1973, 25:293–307
17. Raz A: Interaction of prostaglandins with blood plasma proteins: Comparative binding of prostaglandins A_2, $F_{2\alpha}$ and E_2 to human plasma proteins. Biochem J 1972, 130:631–636
18. Raz A: Interaction of prostaglandins with blood plasma proteins: I. Binding of prostaglandin E_2 to human plasma proteins and its effect on the physiological activity of prostaglandin E_2 in vitro and in vivo. Biochim Biophys Acta 1972, 280:602–613
19. Bolla J, Carson GD, Challis JRG: Conversion of PGE_2 and $PGF_{2\alpha}$ by fetal sheep blood. Prostaglandins 1977, 14:873–879
20. Piper RJ, Vane JR, Wyllie JH: Inactivation of prostaglandins by the lungs. Nature 1970, 225:600–604
21. Crutchley DJ, Piper PJ, Seale JP: The nature of prostaglandin-like substances released from guinea pig lungs in anaphylaxis. Eur J Pharmacol 1977, 44:319–323
22. Crutchley DJ, Piper PJ: The behavior of the pulmonary metabolites of prostaglandins in several simple thin-layer chromatography and bioassay systems. Prostaglandins 1976, 11:987–997
23. Gueriguian JL: Prostaglandin-macromolecule interactions: I. Noncovalent binding of prostaglandins A_1, E_1, $F_{2\alpha}$ and E_2 by human and bovine serum albumins. J Pharmacol Exp Ther 1976, 197:391–401
24. Yoffey JM, Courtice FC: Lymphatics, Lymph and the Lymphomyeloid Complex. London, Academic Press, 1970
25. Pace-Asciak CR, Rangaraj G: Prostaglandin biosynthesis and catabolism in the lamb ductus arteriosus, aorta and pulmonary artery. Biochim Biophys Acta 1978, 529:13–20
26. Johnston MG, Hay JB, Movat HZ: The modulation of enhanced vascular permeability by prostaglandins through alterations in blood flow (hyperemia). Agents and Actions 1976, 6:705–711
27. Hay JB, Hobbs BB: The flow of blood to lymph nodes and its relation to lymphocyte traffic and the immune response. J Exp Med 1977, 145:31–44
28. Hay JB, Hobbs BB, Johnston MG, Movat HZ: The role of hyperemia in cellular hypersensitivity reactions. Int Arch Allergy Appl Immunol 1977, 55:324–331
29. Smith RJ: Modulation of phagocytosis by and lysosomal enzyme secretion from guinea-pig neutrophils: Effect of nonsteroid anti-inflammatory agents and prostaglandins. J Pharmacol Exp Ther 1977, 200:647–657
30. Hamberg M, Svensson J, Samuelsson B: Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci (USA) 1975, 72:2994–2998
31. Armstrong JM, Lattimer N, Moncada S, Vane JR: Comparison of the vasodepressor effects of prostacyclin and 6-oxo-prostaglandin $F_{1\alpha}$ with those of prostaglandin E_2 in rats and rabbits. Br J Pharmacol 1978, 62:125–130
32. Gerkens JF, Friesinger GC, Branch RA, Shand DG, Gerber JG: A comparison of the pulmonary, renal and hepatic extractions of PGI_2 and PGE_2-PGI_2, a potential circulating hormone. Life Sci 1978, 22:1837–1842
33. Hay JB, Lachmann PJ, Trnka Z: The appearance of migration inihibition factor and a mitogen in lymph draining tuberculin reactions. Eur J Immunol 1973, 3:127–131

34. Hay JB, Morris B: Generation and selection of specific reactive cells by antigen. Br Med Bull 1976, 32:135–140
35. English L, Morris B, Adams E: The modulating effects of lymph on the synthesis of immunoglobulins by lymphoblasts. Cell Immunol 1977, 28:90–100

Acknowledgments

The authors wish to thank Ms. W. Hunter for excellent technical assistance and Dr. J. E. Pike of the Upjohn Co., Kalamazoo, Michigan, for providing the generous supplies of prostaglandins used in these experiments. The secretarial assistance of Ms. M. Michael is gratefully acknowledged.

[*End of Article*]