1979

Studies on prostaglandins in hemorrhage and sepsis

Roy Douglas Carlson
Yale University

STUDIES ON PROSTAGLANDINS IN HEMORRHAGE AND SEPSIS

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Roy D Carlson

Signature of Author

28 March 1979

Date

STUDIES ON PROSTAGLANDINS IN HEMORRHAGE AND SEPSIS

by

Roy Douglas Carlson
B.A. (Yale), 1975

A Thesis Submitted to
the Yale University School of Medicine
in Partial Fulfillment of the Requirement
for the degree of
Doctor of Medicine
May, 1979

ACKNOWLEDGMENTS

I would like to thank Dr. Irshad Chaudry for his effort in assisting this project at every point in its development. His patience was as essential as his scientific expertise, and made these studies pleasant as well as intellectually stimulating. Dr. A.E. Baue also contributed in a number of substantial ways, particularly by his critical comments at several points along the way, for which I am grateful. I would also like to thank those several members of the Department of Surgery whose comments at the Research in Progress meeting of 8 January have aided my thinking about this project. Drs. Hal Behrman and Burt Caldwell ably and wittily oriented me to several aspects, both technical and theoretical, of the prostaglandins. For the excellent technical assistance of Sarah Schleck, Betsy Wedemeyer, and Sandy Alberino I am also grateful.

The support of a number of friends has aided me in many ways through the interval of this thesis. In particular, Fr. William Kibitz, Tommy Fenerty, and David Brewer have helped me maintain a reasonable perspective. Finally, I would like to dedicate this thesis to my family, old and new.

R. D. C.

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ABBREVIATIONS USED

AA	Arachadonic Acid (Eicosatetraenoic Acid)
ADH	Anti-diuretic Hormone
ATP	Adenosine Triphosphate
cAMP	Adenosine 3',5'-Monophosphate
GC-MS	Gas Chromatography-Mass Spectrometry
IC_{50}	Concentration (of drug) which gives 50% inhibition
LD_X	Dose lethal to x% of treated animals
MAP	Mean Arterial Pressure
Na-K ATPase	Sodium-Potassium Dependent Adenosine Triphosphatase
PG	Prostaglandin
PGDH	15-Hydroxy-Prostaglandin Dehydrogenase
RIA	Radioimmunoassay
TLC	Thin Layer Chromatography
TX	Thromboxane

SUMMARY

1. Well established models of hemorrhage and sepsis in the rat have been used to study possible roles of prostaglandins (PGs) during these conditions.

2. Direct measurement of plasma PGE and $PGF_{2\alpha}$ levels before and during hemorrhage, by radioimmunoassay, yields large variations and reveals no patterns of change.

3. The single pass pulmonary metabolism of injected PGE_2 was dramatically decreased following 75 minutes of hemorrhagic hypotension. Associated with the decrease in pulmonary PG metabolism was an increased retention of radioactivity by the lung tissue. This is the first in vivo demonstration of altered metabolism of PGs during hemorrhage.

4. Treatment with indomethacin, a well known inhibitor of PG synthesis, caused a significant decrease in survival following 75 minutes of hemorrhagic hypotension at 40 mm Hg. These effects were not reversed by infusion of PGE_2 (0.1 μg/kg/min) alone.

5. In a model of sepsis produced by cecal ligation and puncture, indomethacin treatment during early sepsis was found to be deleterious. During late sepsis, however, indomethacin treatment did not further diminish the low survival rate of animals. The present results demonstrate, for the first time, adverse effects of indomethacin on survival following sepsis. This suggests that PGs play a protective role during sepsis.

Summary continued

6. It is concluded that PGs have a protective role in hemorrhagic hypotension and early sepsis. The mechanism of protection is not known, but probably involves sparing of tissue ischemia by vasodilation.

GENERAL INTRODUCTION

Hemorrhage and sepsis are pathophysiologic situations characterized by significant alterations in circulatory dynamics, tissue perfusion, cellular energetics and function. These alterations lead eventually to cell death, organ failure, and, all too frequently, demise of the organism. Despite the vast literature dealing with clinical and experimental observations of shock, many questions remain unanswered (20, 42, 281).

In particular, there are several major unanswered questions regarding the control of the vascular bed at several levels: the relationship of the pump function of the heart to the resistance and capacitance vessels; the relationship of organ function to organ perfusion during altered circulatory states; the interactions of the microvasculature with cell energetics and perfusion.

Prostaglandins (PGs) have been demonstrated to produce striking effects on many tissues even at low concentrations. Two of the circumstances in which PGs have been implicated are hemorrhage and sepsis (184). Yet little is known concerning the effects and role of PGs during such adverse circulatory conditions. Therefore, the studies described in this thesis were undertaken in an attempt to determine possible roles of prostaglandins in hemorrhage and sepsis.

BACKGROUND

1. Hemorrhage

Hemorrhagic hypovolemia produces circulatory compromise in a number of different ways, as many reviewers have pointed out (20, 147, 281). The fundamental problem during hemorrhage is decreased circulatory volume (239), which leads in turn to hypoperfusion, tissue hypoxia, and cell injury. The organism responds to this circulatory insult with a complex neurohumoral response (6, 58, 71, 179, 223), which is designed to direct the residual volume preferentially to vital organs (i.e., brain, heart, liver, and kidneys), even at the expense of other sites. This response is not entirely salutary in prolonged hypovolemia, however, since continued hemorrhagic hypotension is associated with several pathologic processes. The nature of these adverse processes has been recently reviewed by Zweifach and Fronek (281).

Cardiac function, for example, has been found to deteriorate during prolonged hypovolemia (103, 153), despite the fact that coronary blood flow is preferentially maintained at adequate rates. Proposed explanations of the deterioration of cardiac function include: a failure of sympathetic output in late hemorrhagic shock; alterations in cell metabolism leading to decreased pump function; and the effect of toxic factors, such as "myocardial depressant factor" (92, 149). Another possibility is that the control mechanisms in the coronary bed function less effectively during hypovolemia; this might be due to alterations in any of a number of local and humoral factors, including the PGs.

The circulatory failure resulting from hemorrhagic hypotension may produce significant alterations in renal blood flow and function. In particular the following effects are seen: the redistribution of blood flow in favor of the inner cortex and medulla (198), activation of the renin-angiotensin system (178), and alterations in renal concentrating ability, which involve anti-diuretic hormone (ADH, ref. 234). The genesis of these changes is described as a complex interaction of neuro-humoral vasopressor effects and local vasodilation (17).

Alterations in pulmonary blood flow and function during hemorrhage have been reviewed by Bryant et al. (33) and, more recently, by Sykes (249). In brief, the pattern of intrapulmonary blood flow is changed so that the lower, dependent areas receive more flow (255). These gravity favored areas have been identified as the site of subsequent changes in capillary permeability (49, 131, 233, 254) and of malfunction of surfactant-producing Type II pneumocytes (176). These changes may also contribute to decreased compliance (50) and to the increase in pulmonary vascular resistance (83). In addition, alterations in the pulmonary vascular bed and capillary endothelial membranes may lead to changes in other non-respiratory functions of the lungs, such as metabolism of circulating hormones (89).

Immunologic (8) and hematologic (158) changes have also been documented during hemorrhagic hypotension. It must be borne in mind, however, that experimental techniques, including heparinization, may contribute to these changes. Alterations in hepatic function have also been well documented during hemorrhage, and recent work has correlated functional changes with alterations in cell ultrastructure and function (87).

A recurrent theme in the above mentioned alterations in organ function is the associated changes in regional blood flow. These effects appear to be mediated by catecholamines and humoral vasoactive substances, but modulated subsequently at the level of the microvasculature (281).

The microvasculature is itself subject to diverse factors during hemorrhage, including acidosis (140, 274), catecholamines (6), and the kallikrein-kinin system(25, 220). There may be other factors which affect the microvasculature, such as prostaglandins (167) and unidentified local metabolites. The response of the microvascular bed has been observed during hemorrhage by direct microscopy. In skeletal muscle (107, 121) hemorrhage induces constriction of small arteries and veins in the early stages, followed by venous dilation at a later stage. In the enteric bed (85) no changes in arterial or venous tone have been noted, but capillary flow has been observed to decrease. These changes are consistent both with the decrease in blood flow to most vascular beds and with the degree of variation from tissue to tissue of vasoconstriction-vasodilation.

The consequences of diminished flow during hemorrhage have also been documented at the cellular level. The effects which have been observed include the production of hypoxia (196, 240), alterations in cell membrane function leading to changes intracellular fluid and electrolytes (64), and significant alterations in cellular energetics (21, 38). The defect in cell energy production may be due to many factors, among which the following are prominent: altered microcirculation which may limit substrate availability to cells; membrane alterations which include defects in membrane transport (224) and response to hormones (40); and changes in the capacity of cells to provide energy for or to control active transport. Decreases in cellular

cyclic nucleotide levels and high energy phosphate compounds have been documented during hemorrhage (39). Since many intracellular functions are energy requiring, the documented decrease in available intracellular energy stores may impair tissue function as well. Indeed, this has been documented (21).

In summary, hemorrhagic hypotension might be conceived as the stimulus for the organism to respond with measures which simultaneously vasoconstrict and vasodilate. The former is protective of critical organs and is due to the action of sympathetics, renin-angiotensin, and perhaps other humoral substances. Vasoconstriction when prolonged is, however, injurious to cells. The organism must also respond with actions to mitigate the injurious effects of vasoconstriction. It does so by vasodilation of local microvascular and small vessel beds. A key to understanding the pathophysiology would appear to lie in the interaction of neuro-humoral and local factors at the level of the local circulatory bed.

2. Sepsis

For the sake of clarity, sepsis is defined here as the severely altered pathophysiologic state resulting from infection, with or without associated septicemia. In spite of the frequent occurrance of sepsis, little is known of the complex mechanism by which such serious infections alter physiologic parameters and lead to high mortality (170, 271). A number of investigators have attempted to address the problem of the pathophysiology of sepsis and several approaches have been used. Previous work falls into three broad classes: clinical studies of septic patients; animal models using purified endotoxin; and animal models of bacteremia, usually produced either by injection of live organism, by abscess formation, or by peritonitis. The relationship of the experimental models to clinical observations is still unclear (271).

The many clinical descriptions and studies (37, 55, 108, 146, 170, 177, 189, 267, 275, 276) have been concerned, in general, with empiric observation of the clinical syndrome, although the more recent advent of clinical hemodynamic monitoring has afforded the possibility of documenting the dramatic hemodynamic and metabolic alterations which occur during sepsis. Briefly, the clinical studies document an early "warm" phase, characterized by increased cardiac output, vasodilation, hyperventilation, and hyperglycemia. This is followed by the "cold" phase, which is associated with diminished cardiac output, vasoconstriction, hypovolemia, oliguria, acidosis, hypoglycemia, and high mortality (42, 146, 267). Sepsis, therefore, is a complex and evolving phenomenon, which is capable of producing profound physiologic alterations.

Christy has written a thorough review of the pathophysiology of sepsis, although it is vintage 1971 (42). He describes several features associated with sepsis. These include: 1.) depression in cardiac, renal, and pulmonary function in the late stage of sepsis; 2.) marked increases in many humoral factors, such as acetylcholine, bradykinin, catecholamines, histamine, and serotonin; 3.) decreases in other circulating factors such as coagulation factors and complement; 4.) coagulopathy; 5.) a major immunologic-inflammatory reaction; and 6.) significant changes in the microcirculation. The microcirculatory changes are characterized pathologically (169) as a generalized Shwartzman reaction. The underlying mechanisms for the phenomenologically observed alterations, however, are not known.

The prominence of immunologic features in sepsis has prompted investigation and mechanistic speculation concerning the role of endotoxin in sepsis.

Endotoxin is a lipopolysaccharide present in the cell wall of most gram-negative organisms (60), which, when injected into animals, produces hemodynamic alterations similar to those described in late sepsis (260), as well as high mortality. Because of these observations, many investigators have studied endotoxin shock as a model of sepsis, assuming that their observations of endotoxin effects will contribute to the understanding of human sepsis and help to formulate treatment programs for clinical use. This is not necessarily true(271). Some features of sepsis, such as activation of immunologic host defense systems, may be ascribed appropriately to endotoxin (175), but other features, such as hemodynamic effects, cannot be so readily assumed to be due to endotoxin. Furthermore, study of endotoxin shock has been plagued by other problems, such as: 1.) variations in endotoxin preparations which may lead to vastly different effects (175); 2.) confusion over amount of endotoxin to be administered, for it is likely that large doses (LD_{100}, with 2 - 20 mg/kg) correspond more nearly with anaphylaxis than with bacteremia; and 3.) species variations which further complicate efforts to translate conclusions based on endotoxin studies to human sepsis. Finally, there is confusion over the actual role of endotoxin in clinical sepsis, since quantification has detected concentrations of endotoxin in the range of picograms per milliliter; this differs from experimentally used quantities by a factor of 10^6 (209, 271).

Animal models of bacteremia, as recently reviewed by Wichterman and associates (271), have been intended to reproduce more accurately human sepsis. The major difficulty with these models has been reliability and reproducibility of the bacteremic insult. A recently described model of cecal

ligation and puncture in the rat has been found to cause bacteremia in all animals within six hours and to produce a high mortality rate (272). In addition, this model is notable for its imitation of the hemodynamic and metabolic alterations of human sepsis, in particular for an early phase of hyperdynamic circulation and hyperglycemia (272) and a later phase of depressed hemodynamics and hypoglycemia. Similar hemodynamic findings have been documented in a canine model (143). It is hoped that these models will allow inquiry into the mechanism of septic injury, without the complicating concerns of applicability, as has been noted for endotoxin models.

In summary, the clinical features of sepsis have been observed and reported by many authors, but the roles and significance of the various factors are still largely unknown. Concern over the appropriateness of various animal models has made the pathophysiology of sepsis difficult to understand in a controlled manner.

3. Prostaglandins

Prostaglandins (PGs) are a set of ubiquitous compounds which have been the subject of extensive research during the past decade and the subject of an equally extensive literature. Because this literature is so immense, it will not be possible to delineate all of the PG effects in this section. The major effects of PGs are, however, listed below, and a more detailed description is provided in the Appendix (p.73).

The PGs are unsaturated fatty acid derived compounds synthesized by virtually every type of mammalian tissue yet studied. The biosynthesis requires the release of arachadonic acid (AA) from cell membrane phospholipids, which occurs following a number of physical, chemical, and physiological

alterations. AA is then transformed by a series of membrane bound enzymes, as shown in Figure 1, into the PGs: PGE, PGF, PGI, and thromboxane A (TXA). These are the compounds of greatest physiologic significance. The plasma half-lives of these PGs are short, however, ranging from 30 seconds (TXA_2) to a few minutes (PGs E, F, and I_2). The primary PGs (PGE and PGF) are inactivated in the pulmonary vascular bed, primarily by the action of the intracellular enzyme 15-hydroxy-PG dehydrogenase. PGI and TXA are broken down primarily by hydrolysis in plasma. The pulmonary metabolism of the primary PGs assures, in general, that the effects of these two PGs (E and F) are primarily local tissue effects.

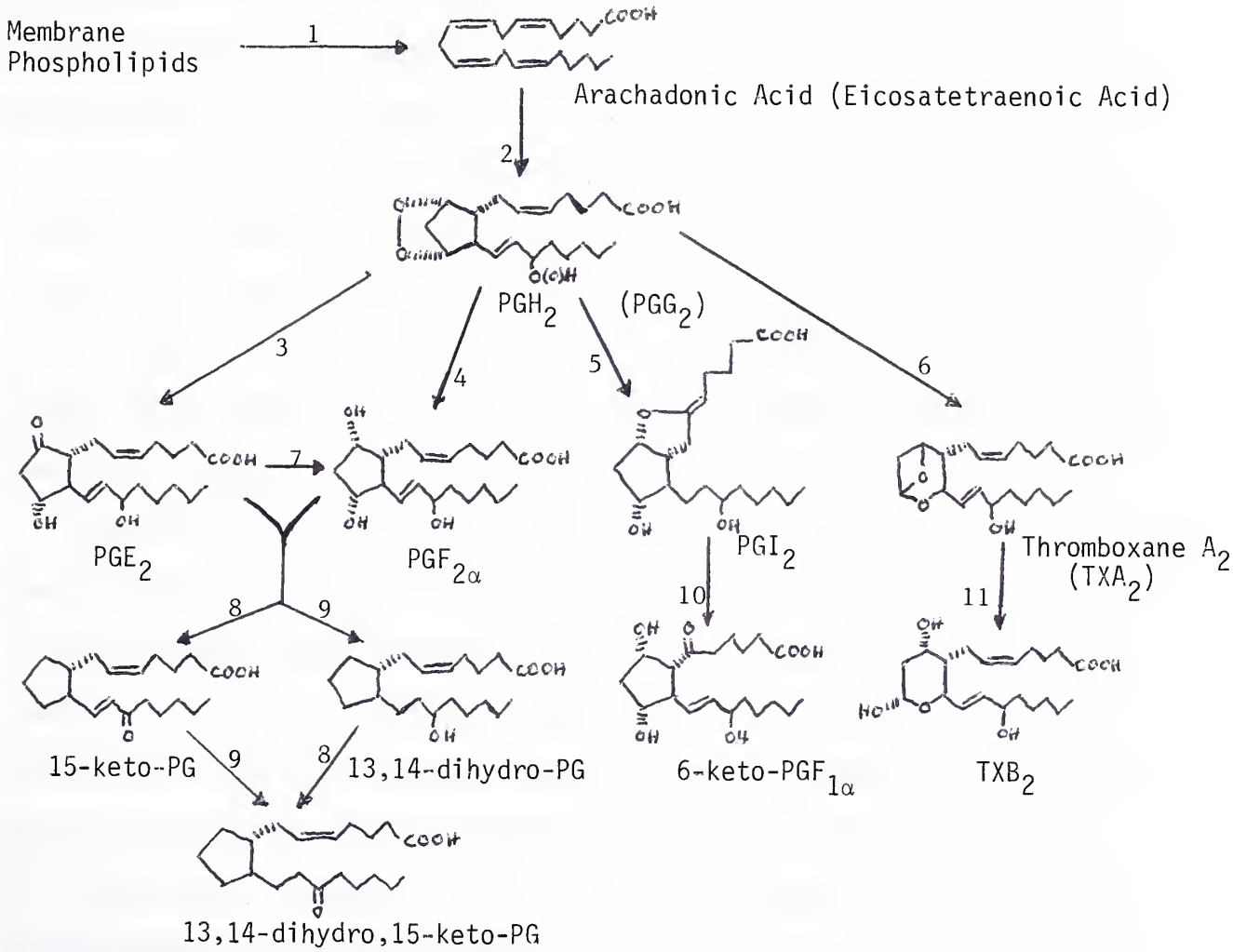
The variety and magnitude of effects which PGs evoke are striking. For the most part, the vascular effects of PGs may be summarized by the statement that PGE_2 and PGI_2 are vasodilators and $PGF_{2\alpha}$ and TXA_2 are vasoconstrictors. This has been noted in many vascular beds in a number of species, although there are exceptions. For example, the rat renal bed has been found to vasoconstrict in response to PGE_2.

The PGs have a number of renal effects as well. They are thought to constitute a major part of the renal vascular autoregulatory response. In addition, they are thought to contribute to the control of systemic blood pressure, to the control of renin release, to adjustment of fluid and electrolyte balance, and to mediation of erythropoietin synthesis stimulation.

In the lungs PGs have bronchial smooth muscle effects analogous to those in vascular smooth muscle. Thus, PGE_2 is bronchodilatory and $PGF_{2\alpha}$ is bronchoconstrictive (PGI_2 and TXA_2 are largely unstudied, since they are relative newcomers to the field). The PGs are thought also to contribute to the pulmonary response to hypoxia and anaphylaxis.

FIGURE 1

BIOCHEMISTRY OF THE PROSTAGLANDINS (PGs)



LEGEND:

Enzyme

1: Phospholipase A_2
2: Cyclooxygenase
3: PG Endoperoxide E Isomerase
4: PG Endoperoxide Reductase
5: Prostacyclin Synthetase
6: Thromboxane Synthetase
7: 9-keto Reductase
8: 15-hydroxy-PG Dehydrogenase
9: PG Δ^{13} Reductase
10: non-enzymatic hydrolysis
11: non-enzymatic hydrolysis

1: Corticosteroids (?)
2: Nonsteroid anti-inflammatory agents (aspirin, indomethacin, etc.)
5: 15-peroxy-arachadonic acid
6: Imidazole, benzydamine
8: Indomethacin, ?furosemide

The gastrointestinal effects of PGs include smooth muscle activity (PGE_2 modulates motility) and protective effects against ulceration. The endocrine effects are prominent in the reproductive system. Recent investigations have implicated PGs and cAMP in the control of hormone release.

In the nervous system both central and autonomic effects have been described. Mediation of fever and neurotransmission effects have been ascribed to PGs in the central nervous system. More important are the autonomic effects: PGE_2 inhibits adrenergic release prejunctionally and enhances cholinergic release; PGE_2 also, in general, modulates effects of sympathetic stimulation of vascular tissue by vasodilation.

Control of local circulation seems to be a major function of the PGs. Not only do vasodilatory PGs oppose the vasopressor effects of adrenergic stimulation, but they also appear to modulate or mediate the effects of a number of circulating vasoactive factors as well, such as angiotensin and bradykinin. In general, the PGs seem to function as homeostatic regulators of local circulatory beds in response to stimuli noxious to tissues.

Recent work indicates that PGI_2 and TXA_2 play major and opposing roles in the control of platelet aggregation. PGI_2 inhibits aggregation, whereas TXA_2 is a potent stimulus for platelet aggregation.

The central mechanism of PG action, as has been learned principally from the study of platelets, is modulation of intracellular cAMP. The PGs have also been implicated in the control of intracellular calcium movements and stores. The precise details, however, of the interactions of the PGs, cAMP, and intracellular Ca^{2+} are still unknown.

It may be taken as a general hypothesis that the PGs subserve the function of membrane messengers, i.e., the release of PGs from altered or injured membranes leads to stimulation of cells. Whether the cell(s) which is influenced is the same cell, nearby, or distant is still a matter of investigation, although most investigation suggests that PGs are primarily local hormones, affecting nearby cells.

Study of PGs and PG effects have benefited greatly from the use of inhibitors of PG synthesis, particularly the nonsteroidal anti-inflammatory drugs, such as aspirin and indomethacin (see Figure 1, step 2). The direct measurement of PGs, on the other hand, has been problematic and the significance of plasma PG levels has also recently been questioned. Moreover, the PGs are present in tissues in small concentration and are effective in equally small concentrations (i.e., 10^{-11} to 10^{-9} M). Thus, ordinary biochemical measurement techniques are not useful. Instead, very sensitive assays must be used. At present, radioimmunoassay and gas chromatography-mass spectrometry are the best methods of measurement; the latter is currently believed to be the more dependable.

REVIEW OF RELEVANT LITERATURE

There are a number of clinical and experimental studies which indicate that alterations in PG synthesis may be involved in the pathogenesis or manifestations of a number of clinical situations. Associated with increased PG synthesis and concentrations are: trauma, burns, shock, sepsis, malignant tumors, fever, subarachnoid hemorrhage, pulmonary hypertension, and perhaps others (184). On the other hand, patent ductus arteriosus, peptic ulcer, renal ischemia, and others have been associated with decreased PG synthesis (184). These, in conjunction with the striking effects observed at low PG concentrations, suggest that they may play a role in the altered circulatory dynamics of hemorrhage and sepsis. Several previous investigators have examined some aspects of this question.

1. Hemorrhage and Prostaglandins

The circulatory insult of hemorrhagic hypotension is precisely the circumstance in which one might expect to find increased PG synthesis. This is due to the fact that the microcirculation is affected during hemorrhage and cells are subjected to hypoxia, which is a potent stimulus of PG synthesis (161). The decreased blood flow to organs and tissues, which leads to altered cell functions throughout the organism, might lead specifically to a depression of cell membrane transport and metabolism of PGs by the endothelium in the lung. This, in turn, might subject the organism to further increased PG levels. In attempting to understand the role of PGs during hemorrhage, a number of investigators have used several different approaches. These have included: 1.) direct measurement of plasma PG levels during

hemorrhage; 2.) assessment of metabolic capabilities of organs and cells to inactivate PGs; and 3.) use of pharmacologic inhibition of PG synthesis or of exogenous PGs to alter survival or physiologic parameters.

The possibility of direct measurement of plasma PG levels has attracted several investigators. Most have measured PGE levels, since PGE has significant vasodilatory actions, which might either ameliorate local blood flow or exacerbate systemic hypotension. PGE also has effects related to a number of other vasoactive stimuli, i.e., sympathetic stimulation, angiotensin, and bradykinin, so that alterations in PGE levels may affect the vasomotor activity of these entities. $PGF_{2\alpha}$ has been studied rarely, perhaps because its vascular activity is not believed to be as significant physiologically. Studies concerning the newer PG compounds, PGI_2 and TXA_2, have not yet been reported.

The first report of elevated PGE during hemorrhage was that of Flynn and associates (80). They subjected the anesthetized dogs to hemorrhagic hypotension to 60 mm Hg and obtained arterial blood for determination of plasma PG levels by RIA. They found an increase in plasma PGE from 0.34 to 1.3 ng/ml after 90 minutes of hypotension. They also reported increased $PGF_{2\alpha}$ levels, from 0.62 ng/ml baseline to 1.25 ng/ml after 60 minutes. Jakschik and co-workers (129) subjected anesthetized dogs to 45-50 mm Hg hemorrhagic hypotension and used bioassay to determine arterial PGE levels. These investigators found an increase from control levels which were immeasurably low to levels of 2.6 ng/ml after 30 minutes; they associated the above time with maximal compensation. Johnston and Selkurt (132) subjected animals to hemorrhage to achieve 60 mm Hg hypotension for 90 minutes, followed by further bleeding to 35-40 mm Hg. They observed an increase in PGE levels, as

measured by RIA, from 450 pg/ml to 740 pg/ml in arterial samples. These investigators (132) also measured renal output of PGE by renal vein sampling and found that PGE output did not change during the period of hemorrhage. When shed blood was reinfused, however, they noted a striking increase in renal output of PGE from 8 ng/min baseline and during hemorrhage to 109 ng/min. Most recently, Frölich (89) has reported increased PGE_2 levels in aortic blood, but not in right atrial blood, during an undescribed hemorrhagic insult in both anesthetized and unanesthetized dogs. Using gas chromatography-mass spectrometry (GC-MS) he found a tremendous increase in PGE_2 from basal values of less than 50 pg/ml to values greater than 10000 pg/ml after 30 minutes of hemorrhage. Thus, it appears on the basis of these studies (80, 84, 129, 132) that PGE_2 levels increase during hemorrhage in dogs.

The possibility has been raised that part of the elevated arterial PGE_2 levels might be the result of diminished pulmonary metabolism of circulating PGE. Hissen et al. (117) presented data in 1969 that in retrospect suggests decreased metabolism. They found that PGE_1 injected intravenously during hemorrhage produced increased arterial levels as assessed by increased inhibition of platelet aggregation. Jakschik and associates (128) lent support to the hypothesis of decreased pulmonary metabolism. Using bioassay, they found an increase in the ratio of arterial to venous PGE. Blasingham and Selkurt (30) and Fletcher and Ramwell (74) reported similar findings using RIA. They documented a decrease in extraction ratio of PGs using mixed venous and arterial blood. (1-(A/V)). The findings of these three groups (30, 74, 128) are consistent with those of Frölich (84) using GC-MS. These four investigations, however, could not distinguish decreased metabolism from increased pulmonary synthesis and release of PGE during hemorrhage. Indeed, Frölich's data support

the latter as the more important factor. On the other hand, Flynn and Lefer (81) found no decrease in the ability of rabbit liver, kidney, or lung homogenates to metabolize PGE or PGF following systemic hypotension. Whether or not the altered metabolism seen *in vivo* by previous workers (30, 74, 117, 128) was reversed by isolation and handling is not clear, however, so that caution must be used in accepting the data of Flynn and Lefer (81) as evidence of intact metabolic capacity. Nevertheless, the different *in vivo* and *in vitro* findings do create confusion concerning the issue of alterations in pulmonary metabolism of PGs during hemorrhage.

The experimental use of PG synthesis blockade both as therapeutic intervention and as pharmacologic tool has attracted many investigators. Jakschik and co-workers (129) noted an increase in mean arterial pressure (MAP) and an increased blood loss following a moderate dose of indomethacin (1-2 mg/kg). Johnston and Selkurt (132) reported that the same dosage of indomethacin did not affect the small increase in arterial PGE they had observed, but did block the large increase in renal release of PGE during reinfusion. The thorough study of Leffler and Passmore (150) showed that indomethacin increased systemic blood pressure by increasing systemic vascular resistance in dogs subjected to 3.5 hours of severe hypotension. These authors hemorrhaged animals to 30 mm Hg and gave indomethacin once the MAP began to fall after reinfusion. In particular, their investigation demonstrated increased vascular resistance in the heart, brain, gut, and kidney, as well as a 57% diminution in renal blood flow. This study (150), therefore, suggests a protective role for PGs in late hemorrhagic shock.

A number of other reports have documented (by PG synthesis blockade) the importance of PGs in the renal bed during hemorrhagic hypotension. Bell et al. (23) showed that indomethacin inhibits the recovery of renal blood flow following hemorrhage to 30 mm Hg. Using microspheres, Data and co-workers (47) demonstrated decreased renal blood flow in animals treated with 8 mg/kg indomethacin prior to a 33% reduction in MAP. They noted that the decrease in blood flow was particularly evident in juxtamedullary nephrons and in the cortex. Tyssebotn and Kirkebø (259) found similar results using platinum electrode measurement of renal blood flow in dogs bled to 50 mm Hg. They suggested that PGs play a role in dilating vessels throughout the kidney. The studies of Henrich and associates (110, 111) have shown that PG blockade with indomethacin or with RO20-5720, a peripheral blocker of PG action, leads to diminished blood flow and glomerular filtration rate. They also showed that the combined effects of denervation and angiotensin II blockade returned blood flow to rates found in animals not treated with PG blockade. These studies strongly suggest that PG synthesis is increased in the renal bed, perhaps to counteract the effects of the renal sympathetics and of circulating angiotensin II, thereby maintaining renal blood flow. Thus, it appears that renal PGs exert a protective vasodilatory effect during hemorrhagic hypotension. This notion is consistent with studies on acute renal failure induced by renal artery clamping (127) and by glycerol administration (258, 270).

The work of Gerkes and Shand (87) on hepatic artery autoregulation is analogous to the studies on the renal bed described above. These investigators found that indomethacin increased hepatic artery resistance during 33%

hypotension as compared with untreated animals. This observation suggests that PGs play a role in vasodilation in the hepatic bed.

Studies have also been conducted to determine whether exogenous PG would ameliorate the effects of hemorrhagic hypotension. Glenn (91) reported that intravenous infusion of $PGF_{2\alpha}$ led to prolonged survival time in cats subjected to a lethal hemorrhage protocol. Priano et al. (211) reported increased survival time and improved cardiopulmonary function in anesthetized dogs bled to 40 mm Hg and given an intraventricular infusion of PGE_1 at a rate of 0.4 μg/kg/min. Machiedo and associates (154, 155) reported prolonged survival and improved cardiac and respiratory function in anesthetized dogs bled to 30 mm Hg and maintained until they received 25% of shed blood, at which time the remaining shed blood was returned and intravenous PGE_1 was infused at a rate of 1 μg/kg/min. Finally, in a more recent paper, Flynn and Lefer (82) found that infusion of arachadonic acid intravenously (120μg/kg/min) had a beneficial effect on lysosomal enzymes, but not on hemodynamic function. A slightly beneficial effect on survival was observed (82).

Thus, it appears from the studies of infusion of PGs E and F and of the precursor AA during hemorrhage that each of these may have a salutory effect. All infusions were capable of improving survival in lethal hemorrhage models. On the other hand, the small number of animals used in these studies of PG infusions raises serious doubt concerning the validity of the conclusions drawn from these experiments. Moreover, it should be noted that infusions were given intravenously in most of these studies. Since PGs E and F are rapidly inactivated by the pulmonary vascular bed in normal circumstances, there is cause for concern that infused PG would be largely metabolized

in one passage through the lung. On the other hand, as was noted above, the metabolism of primary PGs is apparently diminished during hemorrhage. The amount of PG reaching the tissues in these studies (91, 154, 155) remains, therefore, unknown. The salutory effects of intraventricular PGE_1 (211) are comparatively minor and based on small numbers of animals. AA, however, is not affected adversely by passage through the pulmonary vascular bed (122) and infusion of this compound during hemorrhage has been shown to be beneficial (82). This study cannot, however, distinguish which PG, whether a primary PG (PGE or PGF) or a different PG, such as PGI_2 or TXA_2, is beneficial during hemorrhage. The above study is also supported by the finding that total inhibition of PG synthesis by indomethacin is detrimental to organ function, such as kidney function (110, 111).

The studies described above indicate that arterial PGE_2 levels are elevated during hemorrhage and that associated with this increase was an equivocal decrease in the ability of the pulmonary bed to inactivate circulating PGE. The effect of PG synthesis blockade appears to be deleterious to renal autoregulation, but no convincing effect on survival following hemorrhage has been documented. Finally, PG infusion during hemorrhage appears to be beneficial, although little data is available regarding this aspect.

2. Sepsis, Endotoxin Shock, and Prostaglandins

A large number of studies have been conducted to determine possible roles for PGs in sepsis and sepsis-like states. Of these many studies, only one has used an animal model of bacteremia. The remainder have all used endotoxin, generally derived from Escherichia coli, although not necessarily from the same bacterial strain. The endotoxin has, however, generally been obtained from a common commmercial source.

The single study of PGs in bacteremia was reported by Culp and co-workers (46). These investigators injected an LD_{100} dose of E. coli, 4×10^9 organisms/kg of the enteropathic Dunwald strain, into anesthetized dogs and observed a slow decrease in MAP to 45% of baseline values 1 hour after injection. This effect persisted for the remaining three hours during which the animals were observed and was associated with an increase in portal vein pressure. Pretreatment with indomethacin (20 mg/kg) or with other anti-inflammatory drugs (flufenamate, aminopyrine, and phenylbutazone) was found to reduce the decrease in MAP and to improve survival. The increased survival reported, however, was not statistically significant in this study.

Prostaglandin levels during endotoxin shock have been the subject of a number of reported studies. Anderson et al. (12, 14), using RIA, measured plasma levels of PGE and PGF 60 to 90 minutes after administration of endotoxin. They found PGE levels to increase from baseline 0.27 ng/ml to 6.1 ng/ml after 60 minutes and PGF concentrations to increase from 1 to 5 ng/ml during endotoxemia. Collier and co-workers (43) also found increased PGE_2 levels in renal vein plasma during endotoxemia, as measured by bioassay (to 2-8 ng/ml). Kessler et al. (138) also found a vasodilating substance in plasma following injection of endotoxin, which they inferred to be a PG. Herman and Vane (113), using bioassay, reported increased renal vein PGE_2 and $PGF_{2\alpha}$ following endotoxin administration, but found no increases in arterial, inferior vena cava, or femoral vein samples. Isakson and associates (124) obtained similar results. Most recently, Fletcher and Ramwell demonstrated increased $PGF_{2\alpha}$ (by RIA) in arterial and venous plasma of dogs (73) and baboons (76) during endotoxemia. They obtained $PGF_{2\alpha}$ levels in the

range of 100 pg/ml which increased to 400 pg/ml following E. coli endotoxin injection. Administration of endotoxin to calves has been demonstrated to produce increased pulmonary synthesis of $PGF_{2\alpha}$, associated with pulmonary hypertension (13, 15). This finding is strikingly similar to anaphylaxis in the calf (5). Thus, a number of investigations have documented increase in PGE levels (12, 14, 43, 113, 124) or in $PGF_{2\alpha}$ levels (12-15, 73, 76, 113, 124) following endotoxin administration. There is, however, disagreement about both the site of synthesis and the absolute levels of PGE. Moreover, these results have not been substantiated using GC-MS.

There has been only one study reported concerning in vivo metabolism of PGs during endotoxemia. Fletcher and Ramwell (74) demonstrated increased PG extraction ratios in baboon subjected to LD_{100} endotoxin. Two groups of investigators have described in vitro data. Nakano and Prancan (187) noted a marked diminution of PG metabolism by cell free systems derived from rat liver and kidney following an LD_{80} dose of endotoxin. Subsequently, they have reported (210) a decreased activity of PGDH in rat lung homogenates following administration of a large dose of endotoxin (10 mg/kg). These findings, however, are contrary to those reported by Flynn and Lefer (81). These investigators found no decrease in PG metabolic capabilities of rabbit lung, liver, and kidney homogenates following endotoxin administration. Thus, the question whether pulmonary metabolism of PGs during endotoxemia is altered remains unanswered.

The question of the role of PGs during endotoxemia has interested a number of investigators. The approach used by many has been the use of pharmacologic blockade of PG synthesis using indomethacin or aspirin.

Erdös and colleagues (63, 116) found that both indomethacin and aspirin blocked both the acute (2 minutes after injection) systemic hypotension and pulmonary hypertension and also the delayed hemodynamic and metabolic alterations of endotoxemia in dogs. The hemodynamic parameters which were observed were systemic pressure, cardiac output, and portal vein pressure; the metabolic parameter was arterial pH. These early studies in dogs have been subsequently confirmed by Hall et al. (104), Herman and Vane (113), Hilton and Wells (115), and the group of Fletcher et al. (72, 73). In addition, the latter two groups reported increased survival, as assessed by survival time (115) and by increase in number of survivors from 46% to 100% following treatment with indomethacin or aspirin (72).

The existence of marked species variations in response to aspirin has been documented. Hall et al. (104) showed that both cats and sheep had more striking acute hemodynamic alterations, which were aspirin sensitive. These investigators also found, however, that the delayed effects of hypotension and acidosis were both of smaller magnitude and less responsive to aspirin in cats and sheep than in dogs. Greenway and Murphy (66) also described marked acute physiologic alterations in cats given Salmonella enteriditis endotoxin, which they found to be eradicated by aspirin pretreatment. Parratt and Sturgess demonstrated amelioration of acute changes in endotoxemic cats using indomethacin, meclofenamate, and flubiprofen (203-206). They also reported improved cardiovascular function and diminished acidosis following treatment with these three structurally different PG synthesis inhibitors, but did not obtain statistically significant increases in survival. Reichgott and Engelman (214) found that indomethacin did not alter survival

in rats subjected to endotoxin shock. Studies in baboons have revealed that indomethacin or aspirin improved acute hemodynamics in animals given a massive dose of endotoxin (LD_{100}, 20 mg/kg, ref. 76). Baboons subjected to a less severe endotoxemia (LD_{50}) showed no benefit of indomethacin on delayed hemodynamic parameters, but did show an increase in survival rate following indomethacin treatment (75).

The effects of PG synthesis blockade on organ function in endotoxin shock has been studied only by Isakson and co-workers (124). They found improvement in systemic blood pressure and decreased renin output following indomethacin pretreatment of canine endotoxemia. These authors did not, however, comment whether renal function or blood flow was altered.

From these studies of PG synthesis blockade in endotoxin shock, three conclusions may be drawn: 1.) administration of large doses of endotoxin causes a species specific anaphylactic reaction, the hemodynamic element of which is partly dependent on PG synthesis; 2.) the delayed effects of endotoxin, i.e., hypotension, may be improved by treatment with PG synthesis blockers, but effects on survival are minimal; 3.) species variations confound efforts to construct a unified pathophysiologic theory of sepsis.

Data concerning the therapeutic use of PG infusion during endotoxin shock have been similarly inconclusive. Raflo and associates (93, 212) reported improved hemodynamics and increased survival of dogs which received intravenous infusions of PGE_1 or $PGF_{2\alpha}$ following endotoxin administration. Sorrells et al. (243) noted hemodynamic improvements in dogs pretreated with 9.5 µg PGE_1 infused over 10 minutes prior to endotoxin injection. The numbers of animals used in these studies, however, were small and it is therefore, unclear whether or not these results may be regarded as significant.

Moveover, other investigators have failed to observe improvements in survival or in physiologic parameters following PG infusion during endotoxin shock. They have used several different infusion schedules: PGE_1 (1 μg/kg/min for 2 hours) in dogs given an LD_{80} injection of endotoxin (165); using PGE_1 (2 μg/kg/min for 4 hours) in dogs (235); or using PGA_2, PGE_2, or $PGF_{2\alpha}$ (1 μg/kg/min) following LD_{50} endotoxin administration to rabbits (79). Of note, however, is Flynn's observation (79) that arachadonic acid infusion (15 μg/kg/min) did improve survival following endotoxin shock. This suggests that a different PG, such as an endoperoxide, PGI_2, or TXA_2, may play a protective role during endotoxemia. Alternatively, this finding might suggest that the availability of AA for PG synthesis is a limiting factor, so that infusion of AA allows tissues to synthesize PGs as required.

Thus, endotoxin shock studies reveal a confusing picture in relation to PGs. The body of evidence suggests that levels of PGE and $PGF_{2\alpha}$ are altered during endotoxemia; yet whether this represents increased synthesis or decreased degradation is not known. Most studies using PG synthesis inhibitors have implied that PGs play a role in the acute hypersensitivity reaction to endotoxin administration; the effects of PGs during endotoxic shock are, however, unclear. Studies intended to define a role of PGs by infusion of different PGs have yielded contradictory information, although the most recent of these studies implies that PGs play a protective role in an indeterminate manner.

Finally, as has been noted earlier, the significance of endotoxin shock in understanding the pathophysiology of clinical sepsis is unclear. Certainly the acute hemodynamics of endotoxin injection in laboratory animals,

using overwhelming doses of endotoxin, has little to do with the clinical observations. Thus, the role of PGs in sepsis remains unknown.

AIM OF THE PRESENT INVESTIGATION

Despite the large number of investigations into the possible roles of prostaglandins in hemorrhage and endotoxemia, there is still much debate on that subject. Studies on PGs during hemorrhage and endotoxin shock have not provided consistent results, as has been described in the previous sections, and frequently results have been questioned on the basis of the small number of animals used. For example, although arterial PGE_2 has been reported by many authors to be elevated during hemorrhage, there is still no clear indication whether altered pulmonary metabolism of circulating PGs contributes to this increase in arterial concentrations. There is little agreement on the effect of PG synthesis inhibition or of PG infusion during hemorrhage. Studies in endotoxin shock have shown repeatedly that PG synthesis inhibition abolishes the acute effects of endotoxin administration; the effect of this intervention (PG blockade) is unclear in the later stages of endotoxin shock.

One of the sources of the disagreements concerning PG roles in the altered circulatory states of hemorrhage and sepsis may be that previous studies have usually used large animals, particularly dogs. Use of large animal models, however, may introduce variables, such as species differences, uncertain diets prior to studies, and so on. The use of small animals, such as rats, may allow more control of these variables, since genetically identical animals, of the same age and sex, and on the same diet, are available in large numbers without undue expense. Thus, a number of biological variables are minimized, and numbers for adequate statistical analysis are available. Therefore, it would be constructive to carry out investigation concerning the role of PGs during hemorrhage and sepsis using a small rodent, such as

the rat, for which reliable models are well established. The following questions might be then addressed:

1.) Are prostaglandin levels altered during hemorrhage, as assessed by readily available methods, such as RIA?

2.) Is the capacity of the pulmonary vascular bed to metabolize PGs altered during hemorrhage, and how might this affect circulating levels of PGs?

3.) Does PG synthesis inhibition by indomethacin affect the outcome following hemorrhage?

4.) Does infusion of PGE_2 following inhibition of endogenous PG synthesis by indomethacin benefit survival following hemorrhage?

5.) Does PG synthesis inhibition affect the outcome following various stages of sepsis, and if so, does this direct to further studies?

MATERIALS AND METHODS

Animal Models

A. Hemorrhage Model

1. For Measurement of Plasma Prostaglandin Levels

Albino male rats of the Holtzman strain (Charles River, Wilmington, Mass.) weighing 300 to 375 gm were fasted for 16 hours prior to experiments, but allowed water ad libitum. Under light ether anesthesia, cannulae were inserted into the right femoral artery, right femoral vein, and right subclavian artery, using PE-50 polyethylene tubing. For studies of plasma PG levels only, the renal vein was also cannulated. Using electrocautery, a 2 cm midline abdominal incision was made and the peritoneal cavity opened. The right iliofemoral vein was isolated and a silastic cannula (O.D., 0.037") was inserted and directed so that its tip lay in the left renal vein approximately 6 mm from the hilum. The silastic cannula was brought out through the skin via a stab wound in the flank and the abdomen was closed. Each of the above cannulae was flushed with 250 U sodium heparin. The femoral artery cannula was connected to a 1.75 m length of PE-50 tubing previously calibrated in mm Hg to allow constant monitoring of mean arterial pressure (MAP). The animals were restrained in a supine position and allowed to awaken. Ether was used only during cannulation, thus avoiding any metabolic or hemorrhagic alterations which might be incurred by circulating anesthetic.

Following equilibration of blood pressure (MAP$\geq$100 mm Hg) hemorrhage was initiated. Initial (control) blood samples (1 to 1.5 cc) were taken from the subclavian artery, renal vein, and femoral vein. The subclavian

artery cannula was then connected to a heparinized 10cc glass syringe which served as a blood reservoir and blood withdrawn so that MAP fell to 40 mm Hg within 800 seconds. Animals were maintained at this level of hypotension by furtherwithdrawal or return of shed blood. Thirty or 60 minutes following initiation of hemorrhage a second set of samples (0.8 to 1.5 cc) was withdrawn from the above cannulae.

2. For Studies of Pulmonary Metabolism of Prostaglandins

In another set of experiments designed to assess pulmonary metabolism of PGE_2 during hemorrhage the above procedure was slightly modified. Rats were used in groups of four and PE-50 tubing cannulae were inserted into the right femoral artery and vein and into the right subclavian artery. The subclavian cannula was lodged as close as possible to the aortic arch. Nine animals were subjected to hemorrhagic hypotension for 75 minutes as described above. The control group were prepared in exactly the same manner, i.e., cannulated and allowed to awaken, but were not bled. Labeled PGE_2 (5, 6, 8, 11, 12, 14, 15-^{3}H-PGE_2, specific activity 130.0 mCi/μmol), which had been rechromatographed on silicic acid columns and dissolved on 100% ethanol, was injected into the femoral vein and flushed with 0.5 cc saline. The amount of labeled PGE_2 used was approximately 1.25 μCi in 0.2 cc ethanol. Immediate-following injection blood was withdrawn from the subclavian artery cannula for 20 seconds (1 to 2 cc) and the sample quickly injected in 8 cc ice-chilled redistilled ethyl acetate. Thereafter rapid thoracotomy was performed and a small piece of lung tissue taken from the right lower lobe using stainless steel tongs which were pre-cooled in liquid nitrogen.

3. For Studies of Survival following Pharmacologic Manipulations

The hemorrhage model was modified for a set of experiments designed to assess survival following pharmacologic manipulations of PGs during hemorrhage. Animals were cannulated (right femoral artery and vein and left femoral artery), connected to a manometer as previously described, restrained in a supine position, and allowed to awaken. Animals were studied in groups of four so that animals with and without indomethacin and/or PGE_2 treatment during hemorrhage could be run concurrently. In the first set of rats the survival following hemorrhage in animals treated with indomethacin was compared with survival in animals receiving vehicle alone. Fifteen to 30 minutes prior to bleeding animals were pretreated with indomethacin (5 mg/kg); control animals received the equivalent volume of vehicle (20 mM phosphate buffered saline, pH 7.6). In the second set of experiments survival following hemorrhage was compared between two groups: those which received indomethacin pretreatment and PGE_2 infusion; and those which received vehicle alone. One group of animals served as indomethacin-controls. These animals received indomethacin pretreatment and infusion, but were not hemorrhaged.

Fifteen to 30 minutes following pretreatment with indomethacin (5 mg/kg) or buffer, hemorrhage was initiated by bleeding the animals into a 10 cc heparinized glass syringe. Simultaneously all animals received one of the following four solutions, infused via a Harvard infusion pump at a rate of 0.01 ml/min during the entire 75 minute course of hemorrhage:

Control 1 (vehicle only)	20 mM phosphate buffered saline
Indomethacin treatment	3.5 mg/kg/hr indo. in buffered saline
Control 2 (vehicles only)	3% absolute ethanol in saline
Indo. + PGE_2 treatment	0.1 µg/kg/min PGE_2 in saline.

Aliquots of PGE_2 were made up just prior to use and the infusion solution was

replaced with freshly diluted solution after 45 minutes to assure infusion of chemically unaltered PGE_2. The corresponding control group received 3% ethanol to match the ethanol concentration given to PGE_2-infused rats.

Animals were bled to 40 mm Hg and maintained for 75 minutes at that hypotension. After 75 minutes the remaining shed blood was reinfused and the cannulae were removed. Animals were returned to their cages and allowed food and water ad libitum. Survival was assessed over a period of 48 hours. Animals which died were examined at autopsy for evidence of gastrointestinal bleeding.

4. For Studies of Blood Gases during Hemorrhage

A small number of animals were used to determine arterial blood gases during hemorrhage. Animals were prepared as for survival studies and pre-treated with indomethacin (5 mg/kg) or vehicle. Infusion of indomethacin or buffer as described above was started at the initiation of hemorrhage. An initial sample of blood (1.5 cc) was withdrawn from the femoral artery for blood gas analysis. After maintenance of 40 mm Hg hypotension for 75 minutes, a second arterial blood sample (1.5 cc) was withdrawn and analyzed for blood gases.

B. Sepsis model

Sepsis was induced by cecal ligation and puncture according to the model previously described (272). Albino male Holtzman rats (300 to 375 gm) were fasted for 24 hours prior to the study. Animals were studied in groups of four so that control and treatment data could be obtained simultaneously. Following the initiation of food deprivation, animals were given indomethacin (1 mg/kg) dissolved in olive oil or the equivalent volume of oil alone

subcutaneously twice a day for a total of three days. Twenty-four hours after the initiation of fasting and treatment, animals were given a bolus of indomethacin (4 mg/kg) or the equivalent volume of phosphate buffered saline intraperitoneally. After thirty minutes animals were anesthetized lightly and a 2 cm midline abdominal incision made. The cecum was isolated and ligated with a 5-0 silk without producing intestinal obstruction. The cecum was punctured twice with an 18 gauge needle, placed back into the peritoneal cavity, and the abdominal wound closed in layers. Animals were then given normal saline (30 cc/kg) subcutaneously and returned to their cages. Food and water was withheld for an additional 48 hours.

Ten or sixteen hours later (corresponding to early or late sepsis, respectively), animals were again anesthetized and PE-50 cannulae inserted into the right femoral artery and vein. The arterial line was connected to a manometer and only animals with MAP$\geq$90 mm Hg were used and included in the study. The abdomen was then reopened and the cecum excised. The peritoneal cavity was thoroughly lavaged with warm saline and the abdominal incision closed. Following removal of the cecum, animals were treated with indomethacin (6.7 mg/kg) or buffer intravenously and given intravenous saline (15 cc/kg) over 3 to 5 minutes. Thereafter, rats were returned to their cages. Food and water were still withheld. Over the succeeding 36 hours animals were given saline subcutaneously, as well as subcutaneous indomethacin or oil vehicle, according to the schedule shown in Figure 2. Thereafter, animals were allowed food and water ad libitum. Survival was assessed over the interval of five days following cecum removal. Animals which died were examined for evidence of gastrointestinal hemorrhage.

FIGURE 2

PROTOCOLS FOR SEPSIS SURVIVAL STUDIES

Day	Time	Early Sepsis	Late Sepsis
1	4:00 pm	----------Indo 1 mg/kg or oil SQ----------	
		----------Food withheld----------	
0	8:00 am	----------Indo 1 mg/kg or oil SQ----------	
	4:30 pm	Indo 1 mg/kg or oil SQ	Indo 4 mg/kg or vehicle IP
	5:00 pm		Cecal ligation and Puncture Saline 30 cc/kg SQ Indo 1 mg/kg or oil SQ
	10:30 pm	Indo 4 mg/kg or vehicle IP	
	11:00 pm	Cecal Ligation and Puncture Saline 30/cc/kg SQ Indo 1 mg/kg or oil SQ	
1	9:00 am	----------Monitor MAP----------	
		----------Excision of Cecum----------	
		----------Lavage of Peritoneum----------	
		----------Indo 6.7 mg/kg or vehicle IV----------	
		----------Saline 12 cc/kg IV----------	
		----------Indo 1 mg/kg or oil SQ----------	
	4:00 pm	----------Saline 15 cc/kg SQ----------	
		----------Indo 1 mg/kg or oil SQ----------	
2	8:30 am	----------Saline 22.5 cc/kg SQ----------	
		----------Indo 1 mg/kg or oil SQ----------	
6	9:00 am	----------Assess Survival----------	

Legend: Indo = Indomethacin
Vehicle = 20 mM phosphate buffered saline
SQ = subcutaneous
IP = intraperitoneally
IV = intravenously

Analytic Procedures

A. Preparation of Samples for Assay of Prostaglandins

The blood samples were placed immediately on ice and centrifuged, usually within 5 minutes, at 4° C. in a Beckman J-21B centrifuge. The plasma supernatant was removed and frozen at -80° C. Plasma samples were assayed for PGs within 10 days.

B. Extraction and Measurement of Plasma Prostaglandins

Measurement of plasma PGs was done by radioimmunoassay (RIA) according to the method of Jaffe and Behrman(126).

The frozen samples were thawed to room temperature and PGs extracted with redistilled ethyl acetate at a pH of approximately 4.5. Samples were centrifuged at 3000 rpm for 10 minutes at 4° C. and the supernatant removed and evaporated under air at room temperature. The residue was dissolved in benzene:ethyl acetate:methanol (60:40:2).

A column chromatography procedure was used to separate PGE and $PGF_{2\alpha}$. Fifteen cm glass burets containing 0.5 gm silicic acid were set up. Samples were placed onto the columns and elution of PGs carried out using benzene:ethyl acetate:methanol mixtures of increasing methanol concentration. This procedure has been shown previously to give excellent separation of PGs (35).

Radioimmunoassay was carried out using standard techniques (126). PGE and $PGF_{2\alpha}$ assays were done simultaneously, using the fractions separated by chromatography. Column eluents were evaporated at room temperature, the PGF fraction under air and the PGE fraction under nitrogen. The residues were redissolved in absolute ethanol. Aliquots of 0.05, 0.2, and 0.4 cc

were placed in test tubes and evaporated under air (PGF) or nitrogen (PGE) at room temperature. Anti-sera were obtained from goats which had been injected with PGE or PGF conjugated to bovine serum albumen by carbodiimide. Antisera and labelled PGE or $PGF_{2\alpha}$ were added to assay tubes. Known standards of each PG were run in triplicate concurrently, using concentrations of 0.1 to 20 ng/ml. Mixtures of antiserum, labelled PG, and samples/standards were allowed to equilibrate at 4° C. over a two hour interval. Antigen-antibody complexes were precipated using charcoal-coated dextran and samples centrifuged at 2500 rpm for 5 minutes at 4° C. Supernatant free PGs were decanted into scintillation vials and 10 cc Formula-963 scintillation fluid added. Samples were counted for 10 minutes on a Packard Tri-carb liquid scintillation counter. Concentrations of unlabelled PG in rat plasma samples were calculated from the curves of known standards.

C. Thin Layer Chromatographic Separation of Prostaglandin Metabolites

A number of investigators have used several thin layer chromatographic systems (TLC) to separate the primary pulmonary metabolic degradation products of the PGs (10, 99, 213, 244). For the <u>in vivo</u> pulmonary metabolism study described below, it was essential to be able to separate quantitatively the PGE_2 metabolites. To determine the best system for separation four solvent systems were tested.

Prostaglandin E_2 and its three principal pulmonary metabolites, 13,14-dihydro-PGE_2, 15-keto-PGE and 13,14-dihydro,15-keto-PGE_2, were dissolved in absolute ethanol and stored under nitrogen at -80° C. TLC of these four compounds was performed using 250 μ Silica Gel G plates. Along a line 1 cm from the lower edge of the plate 300-500 μg of each

compound was spotted. The plates were dried under room air. Four solvent systems were made up:

1. Ethyl acetate:water:2,2,4-trimethylpentane:acetic acid 110:100:20:1
2. Benzene:dioxane:acetic acid 20:10:1
3. Benzene:dioxane:acetic acid 66:33:1
4. Benzene:dioxane:acetic acid 40:10:1.

Solvents were placed in a chromatography tank to a depth of approximately 0.5 cm and the plate placed into the tank. Plates were left in the solvent until the solvent edge had run 12-13 cm, which required 70-90 minutes. Following this, the plates were removed, air dried, sprayed with 3% phosphomolybdic acid, and heated gently on a warm hot plate for 10 minutes. Spots corresponding to each compound were identified and R_f values calculated as (distance traveled by compound) / (distance traveled by solvent front).

D. Preparation of Blood and Lung Samples for Metabolism Studies.

Blood samples, which had been injected into ice-chilled ethyl acetate immediately after withdrawal, were centrifuged at 4° C. at 6000 rpm for 15 minutes in a Bedkman J-21B Centrifuge and the supernatant removed. To assess recovery of labeled PGE_2 an aliquot of 0.1 cc was taken for counting. The remainder of the sample was evaporated under nitrogen at room temperature and redissolved in 0.2 cc absolute ethanol.

Lung samples were stored at -32° C. until a sufficient number were collected within 24 hours. Samples were weighed and homogenized quickly in 1 cc trichloracetic acid (10%)-HCl (0.1 N). To each homogenate 8 cc ice-chilled redistilled ethyl acetate was added. Samples were centrifuged at 6000 rpm for 15 minutes at 4° C. and an aliquot of 0.1 cc taken to test

recovery. The remainder was evaporated under nitrogen at room temperature and redissolved in 0.2 cc absolute ethanol.

Recovery samples were evaporated under room air at room temperature and the residue dissolved in 0.1 cc absolute ethanol. To this solution 10 cc Formula-963 scintillation fluid was added and the recovery samples counted for 10 minutes on a Packard Tricarb Liquid Scintillation Counter.

E. Separation of Labeled Prostaglandins for Metabolism Studies

Ethanol dissolved residues (from extraction of plasma containing labeled PGE_2 and metabolites) were subjected to TLC, using solvent system 4 and 250 μ Silica Gel G plates. Mixtures of standards containing 350 μg of each metabolite and 350 μg of PGE_2 were spotted at two points on each plate 1 cm from the lower edge of the plate. Four samples were spotted in a volume of 0.15 cc each to run in parallel with the known standards. Spots were dried under air. Plates were run as described above, air dried at room temperature, and the two sections of the plate containing standards sprayed with 3% phosphomolybdic acid. Upon gentle heating the standards were identified and the areas on the plate containing labeled compound which corresponded with standard compounds thereby identified. These areas were removed and eluted into scintillation vials using methanol, which was evaporated under air and the residue redissolved in 0.1 cc absolute ethanol. To each vial 10 cc Formula-963 was added and samples counted on a Packard Tri-Carb Liquid Scintillation Counter for 10 minutes.

To assess the purity of the injected tritiated PGE_2, as well as to check the validity of separation achieved by TLC, a small aliquot of each lot of ^3H-PGE_2 was also subjected to TLC. Except where otherwise noted, all values

presented have been corrected for background and for initial impurity. Fractional amounts of each metabolite have been calculated as $M_i/(M_0 + M_1 + M_2 + M_3)$, where M_{0-3} represent the corrected cpm of native PGE_2, 13,14-dihydro-PGE_2, 15-keto-PGE_2, and 13,14-dihydro,15-keto-PGE_2, respectively.

F. Blood Gas Measurement

Samples of arterial blood for blood gas analysis were taken for immediate analysis on an IL Blood Gas Analyzer (Model 213). Values reported are the mean of three determinations on each sample.

Statistical Methods

All data have been analyzed according to standard statistical technique. Statistical comparison of mean values has been carried out using a two-tailed Student's t test. Survival data have been compared using a two-tailed Chi-square analysis. Differences are considered statistically significant for values of $p<0.05$.

Materials

Unlabelled prostaglandin E_2 and its three metabolites were the generous gift of Dr. John Pike (Upjohn Company, Kalamazoo, Mich.). Tritiated PGE_2 (5,6,8,11,12,14,15-^{3}H-PGE_2) was purchased from New England Nuclear (Boston, Mass.). Tritiated $PGF_{2\alpha}$ (5,6,8,11,12,14,15-^{3}H-$PGF_{2\alpha}$) for RIA was also obtained from New England Nuclear. Indomethacin was the generous gift of Merck, Sharp, and Dohme (Rahway, N.J.) and Dr. H.R. Behrman.

Tubing for cannulation consisted of PE-50 polyethylene tubing (Clay Adams, Parsippany, N.J.) and Silastic tubing (Dow Corning, Midland, Mich.).

Equipment for TLC included Silica Gel G plates, obtained from New England Nuclear, as was Formula-963 scintillation fluid. All reagents used in analyses were of analytic grade or better. Ethyl acetate was redistilled prior to its use.

RESULTS

Plasma Prostaglandin Levels during Hemorrhage

Plasma concentrations of PGE and of $PGF_{2\alpha}$ obtained from the subclavian artery, femoral vein, and from the renal vein of 10 rats both at the initiation of hemorrhage and 30 or 60 minutes after the initiation of hemorrhage are shown in Tables 1 and 2. It is apparent from these tables that PG levels vary dramatically, irrespective of source and of time obtained in relation to hemorrhage. The initial PGE levels in renal vein plasma varied from 0.59 to 26.3 ng/ml; in femoral vein from 1.5 to 61.5 ng/ml; and in subclavian artery from 0.76 to 51.3 ng/ml. Similar variations in PG levels were observed in samples taken from these sites following 30 or 60 minutes of hemorrhage. Likewise, values for $PGF_{2\alpha}$ concentration in plasma varied markedly.

Mean PGE and $PGF_{2\alpha}$ levels are presented in Table 3. It is clear from this table that there are no significant increases or decreases in either PGE or $PGF_{2\alpha}$ levels at various intervals during hemorrhage. Although mean PG levels at 60 minutes are lower than mean initial values in both subclavian artery and femoral vein samples, these decreases are misleading, as is evident from values reported in Tables 1 and 2. Moreover, the numbers of animals reported are small. The absence of significant changes is not surprising in the face of the large variations in individual values.

TABLE 1

PLASMA PROSTAGLANDIN E_2 LEVELS DURING HEMORRHAGE

		PGE (ng/ml) and relative PGE		
Animal	Source	Initial PGE	30 min.	60 min.
	SA	25.3	14.4 (0.57)	
1	FV	61.5*	15.1 (0.25)	
	RV	24.3	-	
	SA	26.6	6.5 (0.22)	
2	FV	19.2	10.8 (0.56)	
	RV	5.2	10.8 (2.01)	
	SA	51.3*	10.1 (0.20)	
3	FV	21.1	-	
	RV	22.4	-	
	SA	5.95		4.90 (0.84)
4	FV	8.48		7.50 (0.88)
	RV	5.89		11.34 (1.92)
	SA	6.68		6.74 (1.01)
5	FV	7.24		9.08 (1.25)
	RV	5.80		8.17 (1.41)
	SA	4.98		7.48 (1.57)
6	FV	5.77		6.83 (1.18)
	FV	1.55		1.66 (1.07)
7	RV	1.60		2.57 (1.61)
	SA	0.74		0.70 (0.95)
8	FV	4.13		0.94 (0.23)
	RV	10.93		4.40 (0.40)
	SA	3.44	2.02 (0.59)	
9	FV	8.68	-	
10	RV	0.59	1.43 (2.42)	

* Values determined from only one aliquot, rather than as the mean of three.

Measurement of PGE-equivalent by RIA of plasma samples obtained from subclavian artery (SA), femoral vein (FV), and renal vein (RV) at different times in relation to hemorrhage. Relative values are ratios of PGE levels during shock divided by initial values and are given in parentheses. See Methods for further details.

TABLE 2

PLASMA PROSTAGLANDIN $F_{2\alpha}$ LEVELS DURING HEMORRHAGE

		$PGF_{2\alpha}$ (ng/ml) and relative $PGF_{2\alpha}$		
Animal	Source	Initial PGF	30 min	60 min
	SA	17.6	14.9 (0.85)	
1	FV	12.0	10.6 (0.88)	
	RV	12.0	-	
	SA	13.0	11.0 (0.85)	
2	FV	8.8	6.4 (0.73)	
	RV	1.5	4.7 (3.18)	
	SA	12.5	4.6 (0.37)	
3	FV	9.8	-	
	RV	5.3	-	
	FV	0.89		0.88 (0.99)
7	RV	0.73		0.96 (1.32)
	SA	0.68		0.61 (0.90)
8	FV	0.93		0.69 (0.74)
	RV	1.26		1.73 (1.37)
	SA	0.78	1.30 (1.67)	
9	FV	1.12	1.14 (1.02)	
10	RV	0.71	0.69 (0.97)	

Plasma $PGF_{2\alpha}$ levels as measured by RIA from samples obtained from subclavian artery (SA), femoral vein (FV), and renal vein (RV) at different times in relation to hemorrhage. Relative values are ratios of $PGF_{2\alpha}$ levels during hemorrhage divided by initial values and are presented in parentheses. For details, see Methods.

TABLE 3

MEAN PLASMA PROSTAGLANDIN LEVELS DURING HEMORRHAGE

		PG in ng/ml		
PG	Source	Initial	30 min	60 min
E	SA	10.4 ± 4.1	7.3 ± 3.6	5.0 ± 1.5
	FV	11.0 ± 2.1	12.9 ± 2.1	5.2 ± 1.6
	RV	5.8 ± 1.5	6.1 ± 4.7	6.8 ± 1.9
$F_{2\alpha}$	SA	8.6 ± 3.5	10.2 ± 3.0	1.0 ± 0.4
	FV	4.6 ± 2.4	6.1 ± 2.7	0.8 ± 0.1
	RV	1.1 ± 0.2	2.7 ± 2.0	1.4 ± 0.3

Plasma prostaglandin E and $F_{2\alpha}$ levels as determined by RIA of samples obtained from subclavian artery (SA), femoral vein (FV), and renal vein (RV). Values presented are averaged data from 7 to 10 rats subjected to hemorrhage for 30 or 60 minutes. Values given are mean ± SEM.

Thin Layer Chromatography of Prostaglandin E_2 and its Pulmonary Metabolites

Four solvent systems were tested using PGE_2 and its three major pulmonary metabolites on silica gel G plates. R_f values are presented in Table 4. On the basis of this data solvent system 4 was chosen for use in studies using labeled PGE_2, since it gave complete separation of all four compounds with zones between neighboring compounds. This system, therefore, offered the possibility of minimizing erroneous identification of metabolites.

TABLE 4

THIN LAYER CHROMATOGRAPHY OF PROSTAGLANDIN E_2
AND ITS PULMONARY METABOLITES ON SILICA GEL G
USING FOUR SOLVENT SYSTEMS

	Solvent System	PGE_2	M_1	M_2	M_3
1.	Ethyl acetate:water: isooctane:acetic acid 110:100:20:10	0.82	0.76	0.78	0.84
2.	Benzene:dioxane: acetic acid 20:10:1	0.41-0.47	0.60-0.62	0.65-0.72	0.73-0.76
3.	Benzene:dioxane: acetic acid 66:33:1	0.34-0.43	0.49-0.52	0.67-0.72	0.70-0.73
4.	Benzene:dioxane: acetic acid 40:10:1	0.15-0.21	0.26-0.28	0.42-0.47	0.49-0.54

Thin layer chromatographic separation of known standard PGE_2 and its three primary metabolites on 250 μ Silica Gel G using different solvent systems. Compounds are:

PGE_2 = Prostaglandin E_2
M_1 = 13,14-dihydro-PGE_2
M_2 = 15-keto-PGE_2
M_3 = 13,14-dihydro,15-keto-PGE_2

Pulmonary Metabolism of Prostaglandin E_2 during Hemorrhage

Prior to infusion of labelled PGE_2, its purity was assessed by TLC. The first lot, which was used in 12 of the 15 experimental animals, showed 93.2% PGE_2 with the predominant impurity being M_1 (13,14-dihydro-PGE_2, 5.5%). The second lot was shown to contain 84.5% ^{3}H-PGE_2, 6.7% M_1, 3.0% M_2 (15-keto-PGE_2), and 5.8% M_3 (13,14-dihydro,15-keto-PGE_2). The impurities do not necessarily represent PGE metabolites per se, but might be impurities which simply have the same R_f as the metabolites. The results presented below, however, have been calculated with these impurities taken into account.

Studies of metabolites in arterial blood showed a significant increase in the amount of unmetabolized PGE_2 after 75 minutes of hemorrhage as compared with control animals. Fractional counts of PGE_2 and its three metabolites are given in Table 5 and mean values in arterial blood and lung tissue are given in Table 6. The increase in unmetabolized PGE_2 from 20.7% to 57.4% is statistically significant ($t=3.06$, $p<0.01$). Most of the decreased metabolism was accounted for by decreased oxidation to M_2 (from 38.9% to 17.3%, $t=2.44$, $p<0.05$) and, to a lesser degree, to M_3 (35.9% to 19.5%, $t=1.34$, $p<0.10$).

There was virtually no difference in relative amounts of metabolites in lung tissue, as is shown in Table 6. Recovery of label in the lung tissue was greater, however, in hemorrhaged animals (32.9 $\pm$ 5.6 cpm/mg lung tissue) than in controls (5.2 $\pm$ 1.2 cpm/mg lung tissue).

TABLE 5

IN VIVO METABOLISM OF PROSTAGLANDIN E_2 DURING HEMORRHAGE

	Fractional concentrations of PGE_2 and Metabolites			
	PGE_2	M_1	M_2	M_3
CONTROL	0.204	0.109	0.483	0.204
	0.162	0.060	0.748	0.030
	0.469	0.090	0.248	0.193
	0.122	0.040	0.150	0.688
	0.191	0.007	0.423	0.379
	0.093	0.041	0.263	0.602
FOLLOWING 75 MIN HEMORRHAGE				
	0.450	0.074	0.358	0.118
	0.925	0.039	0.023	0.008
	0.409	0.085	0.182	0.324
	0.314	0.076	0.428	0.182
	0.465	0.075	0.078	0.382
	0.469	0.098	0.127	0.306
	0.517	0.027	0.203	0.253
	0.663	0.025	0.143	0.169
	0.957	0.022	0.010	0.011

Relative in vivo concentrations of PGE_2 and its three primary metabolites in arterial blood 5 to 20 seconds following bolus intravenous injection of labelled PGE_2 in 9 animals subjected to hemorrhage for 75 minutes and 6 unhemorrhaged control animals. Numbers represent ratio of counts associated with each metabolite to total counts obtained, following separation of compounds by TLC. Compounds:

PGE_2 = Prostaglandin E_2
M_1 = 13,14-dihydro-PGE_2
M_2 = 15-keto-PGE_2
M_3 = 13,14-dihydro,15-keto-PGE_2

TABLE 6

AVERAGE IN VIVO METABOLISM OF PROSTAGLANDIN E_2 DURING HEMORRHAGE

	Fractional Concentration of PGE_2 and Metabolites			
	BLOOD		LUNG	
Compound	Control	Hemorrhaged	Control	Hemorrhaged
PGE_2	0.207 ± 0.055	0.574 ± 0.090*	0.267	0.284
M_1	0.058 ± 0.015	0.058 ± 0.010	0.204	0.202
M_2	0.389 ± 0.088	0.173 ± 0.043**	0.207	0.176
M_3	0.349 ± 0.121	0.195 ± 0.019	0.322	0.336

* $p < 0.01$ compared with control
** $p < 0.05$ compared with control

Average relative concentration of PGE_2 and its metabolites in arterial blood and lung tissue following injection of labelled PGE_2 into 9 rats subjected to hemorrhage for 75 minutes and 6 unhemorrhaged rats. Numbers represent ratios of counts associated with each metabolite to total counts measured, following separation of compounds by TLC. Values here represent mean ± SEM.

PGE_2 = Prostaglandin E_2
M_1 = 13,14-dihydro-PGE_2
M_2 = 15-keto-PGE_2
M_3 = 13,14-dihydro,15-keto-PGE_2

Survival following Hemorrhage

A total of 92 rats were used for this study. Of these, 72 rats are included in the calculation of survival rates following hemorrhage. An additional 16 were excluded because of technical failures, such as excessive blood loss during decannulation (n=5) or because they died during the hemorrhage procedure (n=11). All of the four unhemorrhaged indomethacin controls survived.

As shown in Table 7, the survival rate following hemorrhage in control rats (receiving buffer as pretreatment and infusion) was 68.8% (11/16). The survival rate in animals which received indomethacin pretreatment and infusion was 29.8% (5/17), which is significantly less than controls ($\chi^2=5.10$, $p<.025$). Eight animals which were receiving indomethacin died before the completion of the 75 minute hemorrhage period. These animals did not appear to be subjected to more severe hypotensive stress than other animals. Since no control animals died during the hemorrhage interval and death during hemorrhage has been extremely rare in previous studies in this laboratory, the death of 8 indomethacin treated animals reported above would appear to be related to the adverse effect of indomethacin. Indeed, the survival rate of animals treated with indomethacin, including the 8 which died during hemorrhage was 20% (5/25), which is significantly less than controls ($\chi^2=9.74$, $p<0.001$).

There were no differences in the severity of hemorrhage in the groups of animals which did or did not receive indomethacin, as shown by shed volume (10.7 vs 10.3 cc) or amount of shed blood returned during 75 minutes to maintain the animals at 40 mm Hg (29.2% vs 24.5% of total amount shed). Autopsy of all dead animals did not reveal any evidence of gastrointestinal hemorrhage.

In another set of experiments PGE_2 was infused during hemorrhage into animals which had previously received the PG synthesis blocker indomethacin. The purpose of this experiment was to determine whether selective replacement of PGE_2 during hemorrhage would be beneficial and whether blockade of PGE_2 synthesis was responsible for the decreased survival in animals treated with indomethacin. The control animals in this group received buffer pretreatment and a 3% ethanol infusion to correspond with the ethanol concentration in treated animals. The survival rate of these control animals was 55.6% (5/9) which is not significantly different from control animals which did not receive ethanol. Animals given indomethacin pretreatment to block de novo PG synthesis and selective infusion of PGE_2 at peak physiologic rates had a survival rate of 42.1% (8/19), which is not significantly different from controls or from animals which received indomethacin alone. Three animals which received indomethacin and PGE_2 died during the 75 minute hemorrhage period. If these animals are included in the survival data, total survival was 36.4% (18/22), which is significantly different from controls ($\chi^2=3.88$, $p<0.05$) but not from animals treated with indomethacin alone. These results indicate that infusion of PGE_2 alone following blockade of endogenous PG production does not have beneficial effects on survival following hemorrhage.

TABLE 7

SURVIVAL FOLLOWING HEMORRHAGE

Treatment	Survived	Died	% Survival	Died during Hemorrhage	Overall % Survival
Buffer pretreatment and infusion	11	5	68.8	0	68.8
Indomethacin pretreatment and infusion	5	12	29.4[a]	8	20.0[b]
Buffer pretreatment and 3% ethanol infusion	5	4	55.6	0	55.6
Indomethacin pretreatment and PGE_2 infusion	8	11	42.1	3	36.4[c]

a. $p < 0.025$ compared with control.
b. $p < 0.001$ compared with control.
c. $p < 0.05$ compared with control.

Survival data in 71 rats subjected to 75 minutes of hemorrhagic hypotension (MAP = 40 mm Hg) using different treatment protocols. Animals were pretreated with indomethacin (5 mg/kg) or buffer vehicle intravenously 15-30 minutes prior to hemorrhage and infused at 0.01 ml/min with buffer, indomethacin (3.5 mg/kg/hr), 3% ethanol in saline, or prostaglandin E_2 (100 ng/kg/min) throughout the 75 minute period of hemorrhage. Survival was assessed at 48 hours after hemorrhage. Overall percent of survival indicates rate of survival including animals which died during the hemorrhage procedure.

Blood Gas Analysis during Hemorrhage in Relation to Indomethacin

Out of the four animals used in this study, one served as a control and was subjected to hemorrhage following buffer pretreatment and with buffer infusion. The other three rats were pretreated with indomethacin and infused with indomethacin during hemorrhage. Blood gases are presented in Table 8. There are no differences between the arterial blood gas values of the two groups of animals studied.

TABLE 8

BLOOD GAS STUDIES DURING HEMORRHAGE

Animal	Treatment	Parameter	Initial	After 75 min. Hemorrhage
1	Indomethacin	P_{O_2}	128	108
		P_{CO_2}	19	16
		pH	7.54	7.28
2	Indomethacin	P_{O_2}	80	120
		P_{CO_2}	28	20
		pH	7.55	7.40
3	Indomethacin	P_{O_2}	98	120
		P_{CO_2}	36	23
		pH	7.48	7.34
Mean of above 3	Indomethacin	P_{O_2}	102	116
		P_{CO_2}	28	20
		pH	7.52	7.34
4	Buffer	P_{O_2}	85	120
		P_{CO_2}	33	20
		pH	7.55	7.26

Comparison of arterial blood gas values in 3 rats given indomethacin (5 mg/kg) pretreatment and an infusion of indomethacin (3.5 mg/kg/hr) with 1 rat given buffer in equal volumes throughout the 75 minutes of hemorrhage.

Survival Following Sepsis

Sepsis was produced by cecal ligation and puncture followed by excision of the necrotic septic focus after 10 hours (early sepsis) or 16 hours (late sepsis).

As shown in Table 9, the survival rate of late septic rats which were not treated with indomethacin was 30.8% (4/13). This is consistent with the survival rate reported previously using this model (272). Late septic rats which were treated with indomethacin had a survival rate of 23.1% (3/13). There is, thus, no significant difference in survival in late septic rats between the animals which did receive indomethacin and those which did not.

The survival rate of early septic rats not treated with indomethacin was 83.3% (10/12). Treatment with indomethacin in another group of early septic rats resulted in a survival rate of 25% (3/12), which is significantly less than the control group ($\chi^2=8.22$, $p<0.005$).

Autopsy of dead animals showed no evidence of gastrointestinal bleeding in any animals. Diarrhea, a commonly observed phenomenon in septic rats, appeared to be decreased in rats treated with indomethacin.

TABLE 9

SURVIVAL FOLLOWING SEPSIS

	Survived	Dead	% Survival
EARLY SEPSIS			
Control	10	2	83.3
Indomethacin Treatment	3	9	25.0 *
LATE SEPSIS			
Control	4	9	30.8
Indomethacin Treatment	3	10	23.1

* $p<0.005$ compared with control.

Five day survival data in 50 rats subjected to sepsis induced by cecal ligation and puncture. After 10 or 15 hours (early or late sepsis) the cecum was removed. Animals received indomethacin (1 mg/kg) or oil vehicle twice daily for 3 days beginning 24 hours prior to ligation plus indomethacin (4 mg/kg) or bugger intraperitoneally 30 minutes prior to ligation plus indomethacin (5 mg/kg) or buffer intravenously following excision of the cecum. For further details, see "Methods".

DISCUSSION

1. Hemorrhage and Prostaglandins

In attempting to assess possible roles for PGs during hemorrhage, a series of experiments were undertaken using a model of hemorrhagic hypotension in the rat (40). The specific studies which were conducted include: 1.) measurement of plasma PG levels at various time intervals after the initiation of hemorrhage; 2.) determination of *in vivo* single pass pulmonary PGE_2 metabolism after 75 minutes of hemorrhage; and 3.) assessment of survival after 75 minute hemorrhage in rats treated with indomethacin and with indomethacin-PGE_2 combination. The results of these experiments indicate that PGs probably play an important protective role during hemorrhagic hypotension.

A. Measurement of Plasma Prostaglandin Levels during Hemorrhage

In one set of experiments the rat model was adapted for measurement of plasma PGs in blood samples from the subclavian artery, femoral vein, and renal vein, using RIA. The result of this investigation was that no alterations in the levels of PGE or of $PGF_{2\alpha}$ could be demonstrated after 30 or 60 minutes of hemorrhage. From these data (presented in Tables 1-3) the following conclusions could be drawn: 1.) there was a large variation in PG levels during hemorrhage irrespective of time or site of sampling; and 2.) that there were no patterns either in absolute mean values or in the relative concentrations of the PGs during hemorrhage. Thus, it appears that no conclusions regarding the roles of PG during hemorrhage in the rat can be drawn on the basis of these data.

The large variation in both PGE (0.53 to 61.3 ng/ml) and $PGF_{2\alpha}$ (0.61 to 17.6 ng/ml) is evident from Tables 1 and 2. It is also apparent from these Tables that PG levels in the first animals studied were higher than in subsequent animals. Although data were collected from three groups of animals studied on separate occasions (i.e., animals 1-3, 4-6, and 7-10), no changes in techniques or reagents were made. Moreover, RIA of a standard plasma pool was carried out concurrently with sample assays and revealed only small variations, indicating that the variations in the levels of experimental samples were not introduced by human error. There are several other possible explanations for the large variation in PG levels which was observed. These factors include: 1.) uncontrolled and variable synthesis of PGs by plasma elements during or immediately after withdrawal; 2.) alterations induced by freezing and thawing; 3.) errors introduced by measuring small amounts of PG in very small volumes of plasma; 4.) variation inherent in the technique of RIA; 5.) spurious PG synthesis associated with surgical trauma or with animal struggling. Each of these possibilities is discussed below.

It is possible that synthesis of PGs occurred in sample blood or plasma after withdrawal, thereby altering values obtained by RIA. Platelets are known to produce PGs in large quantities (88) and might be the source of spurious PG synthesis. This might have occurred in spite of heparinization and rapid centrifugation at 4° C. Thus, this is certainly a possibility.

Freezing and thawing has been reported to cause alterations in measured PGs (126), presumably by destruction of PGs. In general, however, alterations have not been documented until 3 weeks of freezing.

In the studies of PG levels reported here, the volumes of plasma which were used for RIA were very small (0.35 to 0.80 ml), due to the limitations inherent in the use of a small animal model, particularly for hemorrhage studies. The measurement of small amounts of PG (with attendant error) when divided by small volumes (also with associated error) yielded values that may be inaccurate. Indeed, previous studies of PG levels in human plasma have suggested that for reliable RIA a minimum of 10 ml of plasma is required (53). Obviously, it is not possible to obtain such a volume from a 350 g rat before or during hemorrhage. Previous canine studies of plasma PG levels during hemorrhage, using RIA (80, 132), have not specified plasma volumes used for assay. It would have been possible, however, to use volumes of 5-10 cc in these studies.

The recent review by Granström (98) concerning RIA of PGs in plasma has indicated that this method is indeed fraught with difficulties. Erroneous values have frequently been obtained by RIA of plasma, although the technique of RIA is itself relatively reliable. The cause of the difficulties in plasma RIA is not known, and whether or not it is non-PG plasma elements which interfere with the assay has not been determined. The problems associated with RIA of plasma samples may be appreciated by noting that previous investigators studying PGE levels during hemorrhage in dogs have described baseline PGE levels as 340 and 450 pg/ml (80, 132) when measured by RIA. Other investigators using different techniques have reported baseline values one-tenth of that value (less than 20 pg/ml by bioassay, ref. 129, and less than 50 pg/ml by GC-MS, ref. 84). Thus, it is possible that values reported in this study are falsely elevated in part because of the shortcomings of RIA of plasma PGs. The review of Granström (98), however, was

published a few months after most of the plasma PG studies reported here were conducted.

Finally, the trauma of cannulation or, perhaps, withdrawal of blood might have caused release of PGs from tissues or vessels. In addition, animals in this experiment were awake at the time of the hemorrhage. This required restraining the animals in a supine position, which caused them to struggle. The animals' struggling might have led to increased PG synthesis either because of increased sympathetic output or increased muscular activity, both of which are associated with increased PG synthesis (109, 139). There are, however, two arguments against these possible explanations of spurious PG synthesis. First, these effects might be expected to be observed primarily in the initial samples, since animals struggled most at that time. The variations in PGE and $PGF_{2\alpha}$ levels were, on the other hand, also large after 30 or 60 minutes of hemorrhage, indicating that struggling or initial surgical trauma are unlikely explanations of the variations in PG levels. The second argument is that the initial samples were withdrawn from unhemorrhaged rats, in which the ability of the pulmonary bed to metabolize circulating PG levels would suggest that factors other than initial surgical trauma or awake restraint were probably responsible for the large variations in PG levels. The most likely factors, therefore, are the use of small plasma volumes, the problems associated with RIA determination of plasma PGs, and the possibility of platelet synthesis of PGs in samples after withdrawal from animals.

The absence of changes in plasma PG levels during hemorrhage stems in large part from the large variations in measured PG levels. The observed scatter of data makes conclusions based on mean values tenuous. Even using

relative PG levels (concentrations after 30 or 60 minutes of hemorrhage divided by initial values), no patterns are observable. This suggests that either alterations in rat plasma PG levels do not occur during hemorrhage or that these changes are masked by the wide range in individual values.

In summary, the attempt to ascertain whether PGs play a role in hemorrhage by direct measurement of rat plasma PGE and $PGF_{2\alpha}$ levels during hemorrhage, using RIA, did not yield meaningful results. Large variations in PG levels in samples taken before and during hemorrhage were observed, thus creating difficulty in assessing whether or not alterations in plasma PG levels occur during such conditions. The principal causes of the variations in PG levels appear to be small sample size, use of RIA on plasma samples, and possible spurious synthesis of PGs after withdrawal by blood or plasma constituents.

B. Pulmonary Metabolism of Prostaglandins during Hemorrhage

In order to study pulmonary metabolism of PGs for comparison of metabolic capability during hemorrhage with control values, the method of intravenous injection of tritiated PGE_2 and collection of arterial blood was used, similar to that used by Hammond *et al.* (106). Data from control animals indicate that rats were able to metabolize 79% of circulating PGE_2 in a single pass through the lung. This value is in agreement with values obtained in humans (106), demonstrating the reliability of this technique in the rat model.

The results presented here indicate that hemorrhagic hypotension for 75 minutes in the rat caused a marked decrease in *in vivo* single pass metabolism of PGE_2 from 79.3% to 42.6%. There was also significant decrease in

the formation of the major metabolic product, 15-keto-PGE_2 (38.9% to 17.3% of recovered radioactivity), and a smaller decrease in the formation of the metabolite 13,14-dihydro,15-keto-PGE_2 during hemorrhage, and particularly implies a diminution in the activity of 15-hydroxy-PG dehydrogenase, the intracellular enzyme responsible for oxidation of the 15-hydroxyl moiety (226).

The substantial reduction in single pass metabolism of PGE_2 during hemorrhage is in agreement with studies of several previous investigators in dogs (30, 86, 128) and in baboons (74). These four studies have determined relative arterial and venous PG concentrations and found that the ratio of arterial to venous PG concentrations increased during hemorrhage. This finding, as Frölich pointed out (86), does not exclude the possibility of pulmonary production of PGs. Indeed, Frölich's findings suggest that pulmonary production of PGs is increased markedly during hemorrhage. There has been only one report which suggests that venous PGE reaches the arterial circulation in greater amounts during hemorrhage (117). These investigators found that hemorrhage led to an increase in arterial PGE_1 levels following intravenous injection of the PG, as measured by the crude bioassay of increased platelet anti-aggregatory activity.

Flynn and Lefer (81) reported that hemorrhage does not alter the capacity of rabbit lung homogenates to metabolize PGE. It is conceivable, however, that the metabolic activity was altered during the process of isolation and homogenization, thus reconciling their *in vitro* observations with the *in vivo* studies noted above (30, 74, 86, 117, 128). Alternatively, this apparent difference might represent a species difference.

The more likely explanation of the seeming contradiction in *in vivo* and *in vitro* results is that the reportedly intact intracellular metabolic system may not come into contact with the circulating PGs. There are two possible mechanisms which could account for this: a right-to-left vascular shunt which would bypass the lung; and vascular endothelial cell membrane dysfunction.

The alterations in blood flow in the lung during hemorrhage may include the development of venous admixture, i.e., right-to-left shunting (249). Such shunts would bypass the pulmonary vascular bed, or at least the small vessels, thereby allowing circulating PGE to pass into the arterial circulation without having traversed the pulmonary bed. Thus, the PGs would not come into contact with the endothelial cells and could not be metabolized. Against this possibility is the data from blood gas studies which demonstrated the absense of hypoxemia after 75 minutes of hemorrhagic hypotension. This observation, however, does not refute the hypothesis of vascular shunting, but suggests rather that such shunting is not occurring to a major degree at that point in hemorrhage. Nonetheless, it would be instructive to determine the precise amount of shunting after 75 minutes of hemorrhage, since this would help establish the contribution of such shunting to the observed decrease in metabolism of circulating PGs during hemorrhage. This implies, then, that other possibilities must be considered as well.

The alternative explanation is that the pulmonary vascular endothelium is less capable of transporting the PG into the cell where normal amounts of PGDH are thought to be present (81). Since membrane transport is an energy requiring process (26), one might speculate that ischemic cell injury could cause a decrease in the ability of endothelial cells to provide energy

for this process. This is consistent with the alterations in other membrane mediated effects seen during hemorrhage (40). On the other hand, previous studies have indicated that lung cellular energy levels and active transport are not altered during hemorrhage (232). The precise mechanism of diminished PGE_2 metabolism by the lung remains, therefore, unclear.

In comparison with the marked decrease in metabolism of PGE_2 and in arterial blood samples following 75 minutes of hemorrhage, analysis of lung tissue samples showed no difference in relative amounts of PGE_2 and metabolites between control and hemorrhaged animals (see Table 6). The possible explanations for the differences between lung tissue and arterial blood metabolism include: 1.) that the numbers of counts recovered from lung tissue unhemorrhaged animals were very small, so that ratios of metabolites in these samples are subject to large error; 2.) the increased radioactivity which was associated with the M_1 fraction could have been due to a different PGE_2 metabolite altogether; and 3.) the delay of approximately 25 seconds between arterial blood withdrawal and removal of lung tissue makes comparison of relative metabolite concentrations between blood and tissue questionable. The total number of counts per milligram of tissue was, however, increased during hemorrhage. This increased radioactivity could have been present either intracellularly or extracellularly, although the data reported here does not allow such a distinction. Nonetheless, explanations for increased radioactivity in hemorrhaged animals can be introduced for both locations. If the counts were extracellular, this would be consistent with diminished cardiac output during hemorrhage so that clearance of PGs would be prolonged and the labelled PGs could not be entirely washed out into the systemic

circulation. If, on the other hand, the counts were retained intracellularly, this would imply that transport of metabolites out of the cell might be the rate limiting step. Which of these factors predominates during hemorrhage is not known.

From the data reported here it is clear that the rat is normally able to metabolize approximately 80% of circulating PGE_2 in a single passage through the pulmonary vascular bed. Single pass PGE_2 metabolism decreases to approximately 43% during hemorrhage, thereby doubling the arterial level of PGE_2 of venous origin. The precise cause of the decreased metabolism during hemorrhage may encompass several factors, such as: a decrease in the amount of PGDH, although *in vitro* data does not support this hypothesis (81); right-to-left vascular shunting which bypasses the pulmonary circulation; and a diminished ability of pulmonary endothelial cells to transport PGs into the intracellular site of PGDH. At present, it is not possibile to distinguish the contributions of these hypothetical factors to the decrease in *in vivo* single pass PGE_2 metabolism.

C. Survival Studies following Hemorrhage

The results presented in this study indicate that pretreatment with and infusion of indomethacin during hemorrhage caused a significant decrease in survival following 75 minutes of hemorrhage in rats. The survival rate decreased from 68.8% to 29.4% ($p < 0.05$) in animals which were treated with indomethacin in doses which would give virtually complete inhibition of endogenous PG synthesis (77, 221). Hence, these results suggest that PG synthesis plays a protective role during hemorrhage in the rat.

The mechanism of this protection is unclear. Canine studies previously reported, such as that of Jakschik et al. (29), have demonstrated that indomethacin administration causes an increase in systemic blood pressure and in blood loss in animals. In the present study, however, no alterations in blood pressure, blood loss, or reuptake were noted in indomethacin treated animals. This suggests that the effects of indomethacin were on organ perfusion or were due to local effects rather than systemic effects.

The effects of PG synthesis blockade on organ perfusion and function has been studied by a number of groups of investigators. Among these effects is the finding that indomethacin causes decreased flow to vital organs (heart, brain, gut and liver, kidney) during hemorrhage by increasing vascular resistance (150). Since indomethacin is an effective blocker of PG synthesis, this study (150) implies that PGs contribute to perfusion of vital organs during hemorrhage. Several studies on canine hemorrhagic hypotension have in fact demonstrated that indomethacin blocks the vasodilation of the renal and hepatic arteries which is induced by hemorrhage (23, 47, 87, 110, 111, 258). That PGs are the responsible agents is suggested by the finding of increased resistance in the renal vascular bed during hemorrhage when PGs were blocked with other antagonists with inhibitory mechanisms different from indomethacin. Thus, a number of organ function studies suggest that the protective effect of PGs may be on organ perfusion.

The hypothesis that the protective effects of PGs relates to vasodilatory protection of perfusion of critical organs is attractive. In the rat, however, the renal vascular effects of some of the PGs are unlike those in other species. In particular, PGE_2 is a renal vasoconstrictor and enhances the effects of the renal sympathetics (57, 109). In most other

species the PG which opposses sympathetic activity has been identified as PGE_2 (the effects of PGI_2 have not been described). Thus, unless rat renal sympathetic output leads to release of a different vasodilating PG, one might expect that PG synthesis blockade would protect renal blood flow during hemorrhage in the rat, precisely the opposite of the finding in dogs, as described above. Given the deleterious effect of indomethacin on overall survival in rats, the notion that the mechanism of this diminution in survival relates to organ blood flow is more difficult to accept. It is possible, on the other hand, that the other vital organ beds in the rat behave in a manner similar to the canine renal bed and that the balance of effects, i.e., improvement following indomethacin in the rat kidney but worsening elsewhere, leads to decreased survival. Clearly this matter requires further investigation.

In light of the hypothesized maintenance of vital organ perfusion as a mechanism of protection afforded by PGs during hemorrhage, one might consider the cause of death in the eight indomethacin treated rats which died suddenly during the 75-minute hemorrhage interval. Their deaths were clearly associated with indomethacin treatment. It is possible, for example, that these eight died because of acute failure induced by impairment of flow to a vital organ, secondary to PG synthesis inhibition by indomethacin. This is, however, purely speculative.

As a final consideration in the studies of indomethacin effect on survival following hemorrhage, it ought to be pointed out that indomethacin has other effects besides inhibition of PG synthesis (77). Although doses used would have inhibited PG synthesis completely (221), they would also have

produced drug concentrations equivalent to those which inhibit PGDH and phosphodiesterase *in vitro*. Thus, before it is possible to conclude that the deleterious effects of indomethacin treatment during hemorrhage are due to PG synthesis inhibition, other PG inhibitors ought to be used (191).

Given that PG blockade is detrimental, the next logical question is which of the PGs are the responsible protectors. Indomethacin inhibition of PG biosynthesis blocks production not only of primary PGs, but also of the endoperoxides (PGG_2 and PGH_2), thromboxanes, and prostacyclin, through its inhibitory effect on cyclooxygenase (Figure 1, step 2). In attempting to dissect out one part of this question, survival was assessed in animals treated with a combination of indomethacin (to block production of endogenous PG production) and PGE_2 (infused at peak physiologic rates, 0.1 μg/kg/min).

The selective replacement of PGE_2 was found to be of no significant benefit as assessed by survival following hemorrhage. Although PGE_2 appears to provide some benefit in that the survival rate in animals given indomethacin plus PGE_2 was greater than that in animals given indomethacin alone, this increase was not statistically significant. One possible cause of the absence of significant PGE_2 effect might be pulmonary metabolism of the intravenous PG infusate. One might expect, however, that a significant amount of the infused PGE_2 would have reached the arterial bed, on the basis of the pulmonary metabolism studies described above. In addition, indomethacin pretreatment might have limited PGDH activity, as has been reported *in vitro* (77). Thus, a significant amount of infused PGE_2 should have been able to reach the arterial circulation to be distributed to tissues. Since infused PGE_2 did not improve the survival rate, circulating PGE_2 does not appear

to be the sole responsible protective factor during hemorrhage. Other hypotheses must, therefore, be considered. Prominent among these are: 1.) that locally synthesized PGE_2 is the responsible factor; 2.) that a different PG (i.e., PGI_2) is the responsible factor; and 3.) that the combined effects of several different PGs are required for protection during hemorrhage.

In summary, indomethacin treatment during hemorrhage led to increased mortality, implying that PGs play a protective role during hemorrhagic hypotension. Circulating PGE_2 alone was not, however, the responsible factor.

D. Summary and Conclusions concerning Prostaglandins in Hemorrhage

The results and discussion above point out the fact that the studies conducted to explore possible roles of PGs in hemorrhage have answered only some questions and raised many more. The evidence that PG synthesis blockade was detrimental to survival following 75 minutes of hemorrhagic hypotension in the rat suggests that PGs do, in fact, play a significant role in hemorrhage. Where, how, and when they do is unclear.

The use of the rat model has shown some distinct advantages, notably ease of carrying out experimental protocols on large numbers of animals. The opportunity of using large numbers of animals for further survival studies suggests the possibility of dissecting the complex scheme of PGs and assessing the role of each of several factors. Thus, use of selective blockers or of infusions of PGI_2 and TXA_2 during hemorrhage might prove worthwhile.

A disadvantage of the rat model lies in the small plasma volume, limiting the accuracy of measurement of various humoral and local vasoactive substances. This is particularly true during hemorrhage. The studies reported

here have pointed out the pitfalls in measuring rat plasma PG levels by RIA during hemorrhage.

Despite the lack of success in demonstrating alterations in plasma PG levels during hemorrhage, the diminution of PG metabolism *in vivo* has been established. This finding is consistent both with pulmonary shunting and with the alterations in other cellular and organelle functions during hemorrhage.

Finally, the effect of indomethacin on survival suggests that PGs play a protective role during hemorrhage. The inability to reverse this detrimental effect with PGE_2 alone suggests several hypotheses, as discussed above, which will require further investigations. In particular, studies directed toward understanding the role various PGs play in organ perfusion during hemorrhage would appear to be essential.

2. Sepsis and Prostaglandins

The results described above indicate that indomethacin treatment caused a substantial decrease in the survival of animals following early sepsis, but not following late sepsis. The survival rate following early sepsis in the indomethacin group was 25% as opposed to 83% in animals which received vehicle alone (controls). In contrast survival following late sepsis was not significantly altered by indomethacin treatment (23% vs. 30% control). This is the first demonstration that indomethacin treatment is deleterious following experimental sepsis. These results, thus, suggest: 1.) that sepsis is a complex and evolving process; and 2.) that PGs probably play a role in this pricess, but whether this role is primarily vascular or inflammatory is not known.

The observation that sepsis is an evolving process is evidenced by the increased mortality of control animals in late sepsis as opposed to early sepsis. This data corresponds with earlier data from this laboratory which indicated that rats in the early septic phase have a hyperdynamic circulation and are hyperglycemic. In contrast, late septic animals show cardiovascular depression and hypoglycemia (41). Since the mortality rate of animals following late sepsis was significantly more than following early sepsis, this suggests that mortality varies inversely with cardiovascular status of the animal. This is precisely what has been observed in clinical settings (189).

Related to this observation is the finding that indomethacin alters survival following early sepsis but not late sepsis. These results suggest that PGs play a vital role in homeostasis during early sepsis and that factors other than PG may play a more important role during late sepsis. Certainly the profound hypoglycemia and circulatory changes seen in late sepsis would be expected to overshadow any more subtle hemodynamic alterations attributable to the PGs. Thus, PG synthesis blockade would not be expected to alter survival following late sepsis and the results presented above support this notion.

Since indomethacin, which blocks PG synthesis, affected the survival of animals following early sepsis, it is essential to ascertain the precise role PGs play during sepsis. One possible role of PGs is that they may regulate organ and local tissue perfusion analagous to their postulated role in hemorrhage. It is conceivable that indomethacin alters the hyperdynamic circulation of early sepsis and that this is detrimental. As discussed above, however, indomethacin may inhibit other enzymes (PGDH, phosphodiesterase) and

neutrophil function (77). Thus, the use of indomethacin alone does not necessarily prove that PG synthesis inhibition is the only responsible factor for increased mortality following indomethacin treatment. In addition, the PGs are believed to contribute actively to the inflammatory response (119). Thus, it is conceivable that the deleterious effects of indomethacin following early sepsis could relate more to the modification or inhibition of normal host defenses than to circulatory alterations. The mechanism of action of indomethacin could, therefore, be due to inhibition of PG related changes in host defenses (119), to prevention of the capability of bactericidal leukocytes to reach the site of infection (77), or to deleterious circulatory alteration. Clearly, this area requires further investigation.

To summarize, using a reliable model of sepsis in rats, the effects of indomethacin treatment in both early and late sepsis have been studied. The results presented indicate that indomethacin treatment following early sepsis was deleterious, but did not alter the already high mortality of late sepsis. This implies that PGs play a significant role in sepsis, but that their significance may be eclipsed by other factors as sepsis evolves. It is not clear whether the effects of PGs in early sepsis relate primarily to vascular function or to the inflammatory reaction; this requires further investigation.

Finally, the differences between sepsis and endotoxin shock are made apparent from this study. The clear demonstration of a deleterious effect of indomethacin in early sepsis is in striking contrast to observations during endotoxemia (63, 66, 72, 73, 75, 76, 104, 113, 115, 203-206, 214). Indeed, most studies using endotoxin have found benefits of PG synthesis inhibition, particularly related to the acute changes of systemic

hypotension and pulmonary hypertension (63, 66, 72, 73, 76, 104, 113, 115, 116, 203-206). Such changes are not, however, observed in sepsis. The altered state observed in late endotoxin shock appears, however, to parallel that late sepsis, at least in some respects. The circulation is depressed in both conditions, and mortality is high. Consistent with these observations is the finding that indomethacin does not produce dramatic effects on survival following either late sepsis or endotoxin shock. On account of the hemodynamic similarities between endotoxin shock and late sepsis, it is conceivable that endotoxin contributes to the conditions found in sepsis. To conclude, however, that the pathophysiology of late sepsis is identical to that of early sepsis would not appear to be justified, on the basis of the different hemodynamic patterns (45) and response to indomethacin. Sepsis seems to be a pathophysiologic process involving a number of factors. These include the host defense, circulatory, and endocrine-metabolic systems, as well as a number of hormones related to these systems, including PGs. Further studies using a sepsis model, such as has been described here, will contribute to the understanding of the complex process of sepsis.

APPENDIX

The Prostaglandins

The following pages comprise a brief compendium of the salient aspects of PG physiology as gleaned from a number of review articles which are cited and to which the reader is directed for further and fuller description. In addition there is a recent monograph (119) and three reviews of the clinical aspects of PGs (135, 183, 184).

A. Biochemistry (see ref. 11, 120, 156, 229-231, 264). The PGs are a set of 20 carbon chain fatty acids containing one to three double bonds and a five carbon ring (C_8-C_{13}). They are subdivided into three series, corresponding to the number of double bonds, and into several classes, according to the structure and substitution on the ring. Figure 1 shows the structures and synthetic pathways of the most common series, the 2 series, as well as the related thromboxanes, and the metabolic degradation products. The precursors for PG synthesis are essential unsaturated fatty acids, such as arachadonic acid (AA), which form part of the cell membrane phospholipids, from which they are released by the action of phospholipase A_2. This enzyme is affected by many factors, among which Ca^{2+} and cAMP have been implicated. The conditions which lead to release of AA and subsequent PG synthesis include mechanical stimulation, anoxia, ischemia, anaphylaxis, embolization, and probably several others (262). Free AA is then transformed to the short-lived endoperoxide PGG_2 by the enzyme cyclooxgenase (also called PG synthetase, EC 1.14.99.1), which is localized on the inner leaflet of cell membrane bilayers. PGG_2 then undergoes further transformation to a thromboxane (TXA_2), prostacyclin (PGI_2), or a primary prostaglandin (PGE_2 or $PGF_{2\alpha}$). The

enzymes responsible for these reactions are tissue-specific (e.g., platelets make thromboxanes almost exclusively, whereas vascular endothelium makes none under normal circumstances).

The PGs, however, have short half-lives in plasma, ranging from 30 seconds for TXA_2 to several minutes for PGs E_2, $F_{2\alpha}$, and I_2. Furthermore, the primary PGs (E and F) disappear rapidly from the circulation (67); in particular, they disappear during passage through the pulmonary circulation. This has been documented in cats (48), dogs (217), guinea pigs (4), rabbits (117), rats (202), and humans during cardiac catheterization (95) and open heart surgery (106). That lungs metabolize the PGs is consistent with the observation that the pulmonary vasculature metabolizes many other vasoactive compounds (19, 89, 227, 270). Specifically, metabolism by the vascular endothelium is seen to be energy dependent (26), carrier mediated (27), and anionic (28), implying intracellular metabolism of PGs. The responsible enzymes have been identified and purified (226). The most important of these for the primary PGs is 15-hydroxy PG dehydrogenase (PGDH, EC 1.1.1.141), which reduces the 15-hydroxyl moiety to a keto group, thereby diminishing substantially the activity of the PG. Less important is PG Δ^{13} reductase which reduces the 13,14 double bond, rendering the PG inactive. The presence of these enzymes has been demonstrated in a number of tissues (2) and *in vivo* studies have been conducted to substantiate *in vitro* results (180, 181, 186, 187). More recent studies on PG metabolism have established that the turnover of PGDH is very rapid (29). PGDH can be decreased by such factors as oxygen toxicity (61) and increased by others such as pregnancy and progesterone (59).

To summarize, the PGs are synthesized intracellularly in response to a number of stimuli by well established pathways. They are inactivated rapidly

in plasma and by the pulmonary vasculature.

B. Cardiovascular Effects (see ref. 114, 148, 182, 185, 222, 265). The effects of PGs on the cardiovascular system are manifold. In general, PGE_2 and PGI_2 are vasodilators and PGF_2 and TXA_2 are vasoconstrictors. In both pairs, the latter is more effective.

The cardiac effects (190, 242, 261) include: vasodilation of the coronary bed by PGE_2 and PGI_2 (195) and constriction by $PGF_{2\alpha}$ and TXA_2 (251); negative inotropic effects by PGE_1 and E_2 (142, 199, but also see 247); arrhythmogenic potential of $PGF_{2\alpha}$ (130, 141); and opposing anti-arrhythmic activity of the PGEs (133, 135, 157, 279). The identity of the PGs primarily responsible for control of the coronary bed is still being debated, through current evidence favors PGI_2 with a minor role ascribed to PGE_2.

The pulmonary vascular bed is not only responsible for metabolism of circulating primary PGs but is also controlled by the PGs (269). PGE_1 diminishes vascular tone by relaxation of smooth muscle (134), whereas $PGF_{2\alpha}$ causes increased pulmonary artery pressure. Infusion of arachadonic acid also produces pulmonary hypertension (273), as does hypoxia and anaphylaxis; all of these may be inhibited by PG synthesis blockade. Patency of the neonatal ductus arteriosus appears to be dependent on vasodilatory PGs (245).

Similar effects of PGs E_2 and $F_{2\alpha}$ have been observed in the mesenteric (171, 238), hepatic (215), skeletal muscular (171), and cutaneous vessels (219). Thromboxane A_2 has been demonstrated to be a potent cerebral vasoconstrictor (62) and constricts peripheral vessels as well (68). These effects are not surprising in view of the demonstration that vascular smooth muscle is nearly universally relaxes by PGE_2 and contracted by $PGF_{2\alpha}$ (9).

C. Renal Effects (16, 57, 70, 147, 168, 280). PGs have been implicated in five aspects of renal function: distribution of intrarenal blood flow, regulation of systemic blood pressure, control of renin release, effects on tubular function, and mediation of erythropoietin stimulation.

Flow in intrarenal vessels, as in most of the microvasculature, is affected by PGs. PGs are synthetized in the medulla (112), but exhibit their major distributional effect in promotion of flow to the cortex (22) by vasodilation. This effect appears to be primarily reactive; i.e., they probably do not control basal resistance, but are synthesized in conditions of altered flow (200). It should be noted that PGE_2 has been found to be a vasoconstrictor in the rat kidney. However, whether PGI_2 or PGE_2 is the major dilator is not known (nor are the effects of PGI_2 in the rat renal bed).

The initial description of "medullin," a renal hypotensive lipid, promoted investigation into possible roles for PGs in opposing systemic hypertension (263). Subsequent work indicates that the PGs may participate as intermediates for other humoral substances and as intrinsic renal vasodilators rather than as circulating hormones. PGE_2 (or PGI_2) may exhibit effects through diuresis accountable to renal vasodilation (18), as well as through control of renin.

The mechanism of renin release is still unclear. As demonstrated by indomethacin blockade of PG synthesis, renin release is dependent on PGs (277), a finding supported by the demonstration that indomethacin inhibition of renin release be overcome by infusion of PGE_2. The most recent suggestion (196) is that PGs and catecholamines can independently stimulate renin release via separate receptors.

The effects of renal PGs on fluid and electrolyte balance is also the subject of continuing debate (16, 57, 166, 168, 256). PGE_2 appears to function as a natriuretic, perhaps on the basis of its vasodilatory capacity. $PGF_{2\alpha}$ has been reported to conserve sodium. One current theory (168) postulates that PGs E_2 and $F_{2\alpha}$ control sodium excretion, and they in turn are controlled through kinin effects on 9-ketoreductase (the enzyme which converts PGE_2 to $PGF_{2\alpha}$). In addition, PGE_2 is reported to be an antagonist of antidiuretic hormone (ADH), through actions on cAMP (57, 201). Thus, many theories of PG action in the kidney have been proposed, but no unifying hypothesis has been postulated as yet.

Evidence for a role of PGs in erythropoietin stimulation has been presented (101). Further substantiation, however, is required.

In summary, the most important functions of PGs in the kidney are reactive. They are synthesized in response to hemodynamic and hypoxic insults. In addition, they appear to mediate effects of some hormones (ADH, kinins).

D. Pulmonary Effects (see ref. 65, 121, 163, 164, 228, 237). As described previously, the pulmonary vascular bed both plays an important role in PG metabolism and is controlled by PGs. PGs are also capable of producing effects on airway smooth muscle analogous to effects observed on vascular smooth muscle; viz., PGE_2 is a potent bronchodilator and $PGF_{2\alpha}$ is the most potent bronchoconstrictor yet found. The lung additionally functions as an active secretor of PGs, particularly during hypoxia and anaphylaxis, suggesting that PGs may play a role in control of ventilation-perfusion matching.

E. Gastrointestinal Effects (see ref. 52, 216). The PGs are known to have striking effects on gastrointestinal smooth muscle, PGE_2 being a prominent

and potent stimulus to contraction of longitudinal smooth muscle and relaxer of circular smooth muscle. Cholera toxin diarrhea has been linked to production of PGs. PGE_2 has also been found to possess the capacity to protect against ulcer formation. Correspondingly, PG inhibitors have long been associated with ulcer production. Current theory holds that PGE_2, through cAMP, both inhibits histamine stimulated H^+ release and itself leads to secretion of the gastrointestinal polysaccharides, glycosaminoglycans.

F. Endocrine Effects. Most of the work on the endocrine effects of PGs have centered around either reproductive roles of PGs or the interactions of PGs with cAMP. Infusion of PGE_1 and PGE_2 have, in addition, been reported to cause increased plasma glucose by means of glucagon stimulation and inhibition of glucose related insulin secretion (207, 218, 225). Conversely, aspirin blockade of PG synthesis is related to diminished glucose levels, especially in diabetics, although the mechanism is unclear (173).

G. Nervous System Effects (see ref. 32, 85, 109, 159). The effects of PGs on the nervous system fall into two classes, central and autonomic. The central effects include a putative role in fever (66) and effects on neurotransmission (86). More important are the peripheral autonomic effects. PGE enhances cholinergic transmission (105). Adrenergic transmission, however, is inhibited prejunctionally by PGs, presumably by PGE_2. The postulated mechanism is interference with the movement of Ca^{2+} in stimulation-contraction coupling, but this is speculative. Rat renal sympathetic nerves differ from other species in that PGE_2 enhances sympathetic effects. In all other circumstances, however, PGs again appear to subserve the function of maintenance of local homeostasis in the face of systemic alterations.

H. Control of Local Circulation (see ref. 31, 125, 167, 172, 246, 268). In addition to negative feedback exerted on adrenergic nerve terminals and consequent modulation of adrenergic stimuli, the PGs have been found to modulate or mediate the effects of the microvasculature to a number of other vasoactive substances including antagonism of angiotensin II induced vasoconstriction (90, 192) and mediation of bradykinin induced vasodilation (1, 94, 252). They do not appear to be involved in the effects of exogenous dopamine (54). Possible roles in relation to serotonin are undetermined. PGs are believed to be responsible for human reactive and functional hyperemia (139). Thus, the PGs, in addition to a direct vasodilatory function, also appear to modulate control of the terminal bed by their interactions with other vasoactive stimuli. The general principle of PG synthesis in response to alterations in local circulatory status is thus seen to hold at all levels of the mammalian cardiovascular system.

I. Hematologic Effects (see ref. 88, 96, 162, 174). PGE_1 and, more recently, PGI_2 have been recognized as potent agents in inhibiting platelet aggregation. The mechanism is believed to be modulation of platelet cAMP (97, 251). In addition, TXA_2 is known to be a substance formed by platelets capable of stimulation of platelet aggregation. These findings have been documented in clinical settings (51, 102, 160, 248). At present, it is believed that the balance of platelet TXA_2 and endothelial PGI_2 controls *in vivo* platelet activity.

J. Cyclic Nucleotides and Prostaglandins (see ref. 88, 136, 144, 231, 236, 257). Elevation of intracellular cAMP following PGE_2, PGE_1, and PGI_2 has been noted in many other tissues besides platelets. This has been invoked as a unifying hypothesis to explain PG effects. This "middleman hypothesis"

(88), which postulates PGs as intermediates for cAMP production is supported by the observed prolongatin of PGE vascular effects by phophodiesterase inhibition (56).

It, therefore, seems unquestionable that PGs interact with cyclic nucleotides. Yet many related questions remain unanswered: 1.) it is unclear how PG synthesis is initiated; 2.) the role of Ca^{2+} in relation to PGs remains a mystery; 3.) whether PGs subserve a circulatory role is also thereby brought into controversy. As to the question of stimulus for PG synthesis, it is clear that virtually all noxious stimuli evoke PG biosynthesis. Some of these stimuli such as mechanical injury (161) might involve direct physical destruction of membranes, thereby freeing arachadonic acid for PG biosynthesis. Other stimuli, such as hypoxia, compromised perfusion, nucleotides (194), adenosine (7), Ca^{2+} (278), or other electrolyte alterations may activate phospholipase and PG synthetase as their mechanism of actions. These three stimuli of PG synthesis have in common the function of PGs as messengers from cell membrane to cytoplasm.

The interaction of PGs and cyclic nucleotides is complicated by the unclear role of cytoplasmic calcium. Indeed, PGs, cAMP, and Ca^{2+} seem to be involved in an intracellular ménage à trois. PG synthesis is increased by Ca^{2+} stimulation of phospholipase but PGs are capable of inducing calcium release from intracellular mitochondria (36). PG synthesis appears to be dependent on the oxidation-reduction capability of the cell (193), and, therefore, indirectly on nucleotides and cyclic nucleotides. At present,

it is possible only to state that PGs may serve as intracellular hormones, (291), without certain understanding of their roles and mechanisms.

The acceptance of PGs as intracellular hormones leads to the question of their circulatory role. Yet their measurable release in response to extracellular insults and the extensive capacity of vascular endothelium to metabolize PGs argues for a circulatory role. Furthermore, PG receptors have been demonstrated (143). It seems likely, therefore, that PGs serve as the messenger of the cell membrane both to the cytoplasm and to other local cell populations. The pulmonary bed serves to assure this localization, at least of the primary PGs.

K. Measurement of Prostaglandins. One of the major problems in PG research has been measurement of PGs (191). Initially, investigators used a superfusion bioassay to measure PGs, usually combined with chromatography. This method, however, presents significant problems in specificity (34, 44). Other methods involving enzymatic and receptor assays have been abandoned for lack of both sensitivity and specificity (3, 143).

Radioimmunoassay (RIA) has also been used (125) since the development of specific antisera, although problems with this technique have been persistent (98, 208). Specifically, measurements of PGs have generally resulted in plasma levels in the nanomolar range, which differs by two to three orders of magnitude from other more recent and reliable measurements (84). The cause of erroneous plasma PG measurement is not clear. The possibility of lack of antiserum specificity and of non-specific binding of antibodies by non-PG plasma elements have been proposed (98). Moreover, the plasma half-life of these compounds is very short. This observation in conjunction with

the realization that PGs are primarily local hormones, implies that measurement of levels of circulating PGs might not indicate either the rate of local synthesis of PGs or the physiologic effects due to PGs. Thus, the determination of circulatory PG levels may be meaningless. RIA does, nonetheless, hold promise for future use.

The current gold standard for measurement of PGs is the combined use of gas chromatography-mass spectrometry (84). The major drawbacks of this method are cost and time required.

The several techniques used require caution in acceptance of results of PG measurement in plasma. At present, the combination of PG synthesis blockade is generally required in conjuction with plasma measurement.

L. Pharmacologic Manipulations of the Prostaglandins (see ref. 74). Research on PGs received a great boost with the demonstration in 1971 that aspirin and several other non-steroidal anti-inflammatory drugs inhibit PG synthesis (78). Subsequently, these compounds have been of great value in delineating PG effects. The drug concentrations required for inhibition have been more difficult to establish, however. The IC_{50} of indomethacin, the most commonly used and most potent agent, has been assessed *in vitro* in guinea pig lungs to be 12 - 34 ng/ml (69). A recent *in vivo* study in rat kidneys showed a 69% reduction in PGs as assessed by bioassay and an *in vitro* inhibition of 97% (221).

Membrane stabilization and corollary inhibition of arachadonic acid release has been reported to be the mechanism of action of corticosteroid inhibition of PG synthesis (157). Other membrane active agents, particularly local anesthetics, can also inhibit *in vitro* PG synthesis (145), perhaps because of Ca^{2+} effects.

The existence of compounds which inhibit the action of PGs peripherally has also been described (24). These agents, because of competition for binding sites, may also inhibit PG degradation enzymes, such as PGDH (44).

To date, however, few inhibitors of PG metabolism have come into wide use, although diuretics have been reported to do so (250). The rapid turnover rate of PGDH renders the possibility of developing a complete inhibitor unlikely.

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