Profile of Circulating Vasoactive Substances in Hemorrhagic Shock and Their Pharmacologic Manipulation

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A B S T R A C T (a) Hemorrhage in dogs (to 45–50 mm Hg) was associated with a 10-fold increase in plasma renin activity (PRA) which remained elevated throughout the time-course of shock including the irreversible (decompensation) stage. The presence of angiotensin II (AII) in arterial blood was demonstrated by the blood-bathed organ technique and confirmed by blockade with specific AII antagonists (cysteine^8-AII or isoleucine^8-AII). The contribution of AII to systemic peripheral resistance during hemorrhage shock in dogs was established by administering AII antagonists which immediately cause a further fall in blood pressure.

(b) Plasma catecholamines (CA) steadily increased during hemorrhage and peaked during compensation (a 100-fold increase). The CA decreased progressively during decompensation.

(c) Prostaglandin (PG) E-like material was observed in arterial blood for 15–60 min (after hemorrhage); the peak arterial concentration was 2.6 ng/ml blood. Indomethacin (i.v., before 80% of maximum bleedout): (i) confirmed the presence of PGE, (ii) increased blood pressure, and (iii) increased blood loss.

(d) Thus: peripheral resistance during hemorrhagic shock seems temporally correlated with blood CA levels (and not PRA), and the renin-AII system contributes to the maintenance of vascular resistance and may markedly decrease perfusion of organs, such as kidney; the administration of the proper combination of specific antagonists of vasoconstrictor humoral substances may radically improve organ perfusion and could contribute to ultimate recovery from hemorrhagic shock.

INTRODUCTION

Hemorrhagic shock is associated with three main stages: (a) hemorrhage, (b) compensation, and (c) decompensation. When the reservoir technique is used, compensation is characterized by equilibrium of the blood in the reservoir with the blood in the animal, and decompensation is characterized by reuptake of blood. The decompensatory (irreversible) phase has been closely correlated with mortality (1–3). Although a number of investigators have evaluated catecholamine (CA)[1] and renin changes in the initial phase of hemorrhagic hypovolemia, there has been little study of hormonal and cardiovascular interrelationships throughout the progression of the hypovolemia to irreversible shock.

Superfusion with a stream of blood over selected isolated smooth muscle preparations permits immediate, continuous, and direct bioassay of changes in concentration of hormonal substances present in the circulation (4). This method permits a temporal correlation between blood levels of vasoactive substances (CA, prostaglandins [PG], angiotensin) with changes in cardiovascular parameters (peripheral resistance, blood pressure, heart rate) during the time-course of hypovolemia and all stages of shock. In addition, the availability of new pharmacologic tools (angiotensin antagonists and inhibitors of PG synthetase) make it possible to confirm the presence of these vasoactive substances in the blood and permits manipulation of the circulatory and hormonal status of dogs in hemorrhage.

In this study, the time-course of CA, angiotensin II (AII), and PG after hemorrhage is investigated in carotid artery blood of the dog. Carotid artery blood was chosen because it should contain the highest AII con-

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[1] *Abbreviations used in this paper:* AI and AII, angiotensin I and II; CA, catecholamines; PG, prostaglandins.

centration, since converting enzyme activity (conversion of angiotensin I [AI] to AII) is highest in the lung (4). However, PGE or PGF are reported to be metabolized by one passage through the lung (5, 6). Therefore, very low levels of PGE and PGF would be expected in arterial blood. Surprisingly, PG-like activity was detected in carotid artery blood, and therefore its time-course was determined.

METHODS

Anesthesia was induced with thiopental in dogs (10–20 kg) and maintained with chloralose (40 mg/kg), supplemented with 20 mg/kg when necessary. The dogs were intubated with a cuffed endotracheal tube. Cannulae were inserted into both femoral arteries and veins, one carotid and one jugular vein. Blood pressure was measured with a P-1000 A linear core pressure transducer (Physiograph, Narco Bio-Systems, Inc., Houston, Tex.). The cannula from one of the femoral arteries was connected to a plastic bag for bleeding. The plastic bag was suspended from a myograph (F-1000, Physiograph, Narco Bio-Systems), and therefore, the bleedout was recorded as blood weight. A simplified schematic overview of the experimental setup is given in Fig. 1. The dogs were bled to a mean arterial pressure of 45–50 mm Hg, according to a modification of the Wiggers technique (7). Bleeding was performed by opening a stopcock between the cannula and the bag which allowed the blood to flow into the bag. The height of the bag was adjusted to maintain the pressure at 45–50 mm Hg until all of the blood is taken up by the dog. The dogs were heparinized with 1,000 U/kg.

Bioassay technique. The presence of CA, AII, and PG was investigated in carotid artery blood by a continuous bioassay, the blood-bathed organ technique of Vane (4), and superfusion with a dialysate against blood as developed by Collier (8). Blood was taken from the carotid artery and pumped by a peristalic pump (Harvard Apparatus Co., Inc., Millis, Mass.) through a bifurcation. One blood supply (11 ml/min) went over one set of assay organs and was returned by gravity to a vein. The other blood supply (25 ml/min) went through a plate dialyzer (Meltec Ltd., Buckinghamshire, England) with Cuprophan membranes, 150 PM, (Cobe Laboratories, Inc., Lakewood, Colo.) and was dialyzed counter-current against warm (37°C) oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution (7 ml/min). The blood returned to another vein, and the dialysate cascaded over a second set of assay organs. The tubing containing dialysate and the blood to the organs was surrounded by a warming jacket to maintain the temperature at 37°C.

The assay organs were selected for their sensitivity to the substances analyzed. Three organs were used in each set: rat stomach strip (4, 9), rat colon (4, 10), and rat aorta (11) in the blood-superfused set, and chicken rectum (5), rat stomach strip, and rat colon in the dialysate-superfused set. The combination of rat stomach, rat colon, and chick rectum is particularly suitable for assaying PG-like activity (5). Simultaneous contraction of the chick rectum and rat stomach demonstrates the presence of PGE-like material, while contraction of the rat colon with little or no contraction of the rat stomach or chick rectum would indicate the presence of PGF$_{2\alpha}$. The correlation of the above pattern of response to the presence of PGE or PGF$_{2\alpha}$ has been verified by chromatographic, chemical, and immunologic methods (12–16). Therefore, the qualitative identification
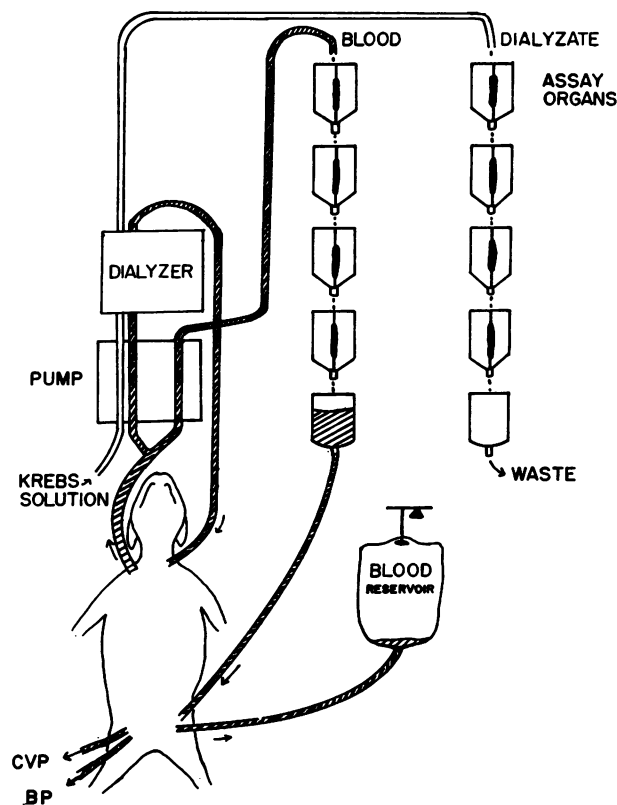

FIGURE 1 Schematic diagram of experimental setup. BP is blood pressure; CVP is central venous pressure.

of PG is based on the specificity of the bioassay system (5, 12) but is reinforced by the disappearance of activity after indomethacin, a potent and specific inhibitor of PG biosynthesis (17–19).

The dialysate-perfused organs were made more specific by infusing hyoscine hydrobromide (1 μg/min, anticholinergic), methysergide maleate (2 μg/min, antiserotonin), phenoxybenzamine hydrochloride (1 μg/min, α-adrenergic blocking agent), propranolol hydrochloride (20 μg/min, β-adrenergic blocker), diphenhydramine hydrochloride (1 μg/min, antihistaminic) (12), and indomethacin (10 μg/min, inhibits PG biosynthesis) (20).

Although CA, PG, and other substances appear in the dialysate, the presence of adrenergic (α and β) blockers inhibit the CA response (and similarly histamine, acetylcholine, and serotonin are inhibited). Therefore, the contraction caused by PG is not opposed by CA relaxation. The indomethacin was perfused directly over the assay organs to eliminate the possibility that the assay organs might release PG and also to enhance the sensitivity of the assay organs to exogenous PG (20).

Angiotensin has been shown to dialyze poorly from heparinized blood into the Krebs solution (8). This was confirmed in our own experiments. Thus, direct superfusion of assay organs with blood was employed for estimation of circulating angiotensin. Blood-superfused organs were readily standardized by infusion of AII solutions. However, this standardization could not be applied for computation of AII blood levels in shock because of the opposing relaxation

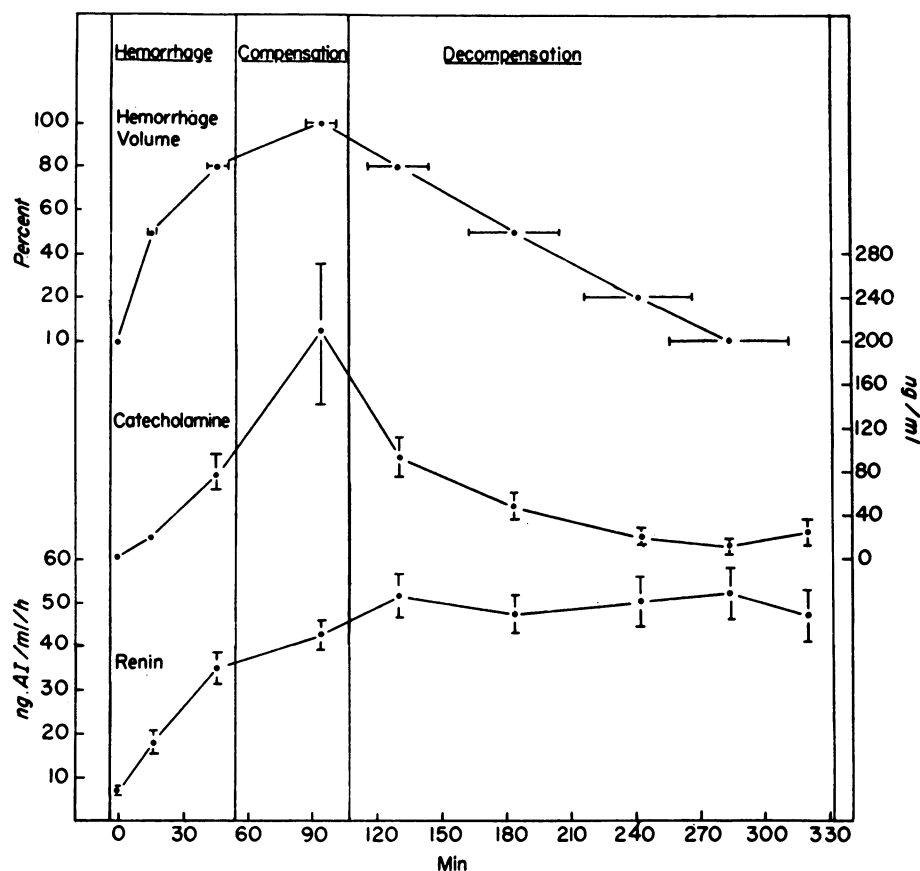*Endocrine Profile in Hemorrhagic Shock* 843

FIGURE 2 Time-course of plasma renin activity and plasma CA in relation to hemorrhage volume. Plasma renin activity ($n = 18$) was determined by radioimmunoassay and plasma CA levels ($n = 12$) by the trihydroxyindole method. The renin and CA values plotted are at control, at 50, 80, and 100% of maximum bleedout volume, at 20, 50, 80, and 100% reuptake of blood and approximately 10 min before death. The time (min) for these points was averaged from 18 experiments. The vertical or horizontal bars indicate SE.

produced by the high CA levels. Specific competitive antagonists were used to qualitatively confirm the presence of AII, but quantitation of the contribution of the renin-AII system was measured by plasma renin activity determinations.

The efficiency of the dialyzer was determined in separate experiments by infusing solutions of PGE$_2$ standards into the blood stream before the dialyzer or into the Krebs solution after the dialyzer. An amount of PGE$_2$ standard (nanograms per minute), which gave a submaximal contraction when infused before the dialyzer, was matched with that amount causing an equivalent contraction of the assay organs when infused after the dialyzer. From these amounts (nanograms per minute), the efficiency of the dialyzer was calculated to be 12%. Collier (8) investigated hormone binding to plasma proteins with this dialysis membrane and machine. Dialysis with Krebs solution on both sides of the membrane was compared with dialysis from blood to Krebs solution. In these experiments, protein binding did not influence the quantity of PGE$_2$ or PGF$_{2\alpha}$ dialyzed.

The dead space for the blood in the dialysis system is 30 ml, and the tubing dead space for the direct blood super-

fusion is 15 ml. The changes in smooth muscle tension were measured isotonically with myograph transducers (Harvard Apparatus Co., Inc.).

Renin assay (radioimmunoassay). Blood samples, 0.5 ml, were taken from a catheter placed in the vena cava above the renal vein. The blood was collected in chilled tubes containing 4 mg of EDTA and immediately centrifuged to separate the plasma. The plasma was frozen until the assay was performed. The radioimmunoassay for AI (E. R. Squibb and Sons, Princeton, N. J.) was used according to the method described by Lubran, Fu, and Sundeen (21).

Catecholamine assay. Blood samples, 10 ml, were taken from the vena cava, collected in chilled tubes, and immediately centrifuged. 4 ml of plasma was used for the assay. The CA extraction and fluorometric determination was performed according to the method reported by Anton and Sayre (22). Since epinephrine plasma levels are much higher than norepinephrine levels during hemorrhagic shock, the standard curves were prepared with epinephrine standards.

Materials. Methysergide was kindly supplied by Sandoz Pharmaceuticals, East Hanover, N. J., propranolol by

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PRESSURE mm Hg

100

0

BLOOD WEIGHT

5
|←min→|

RAT AORTA

RAT STOMACH

RAT COLON

HEMORRHAGE

CYSTEINE^8-AII 20μg/min
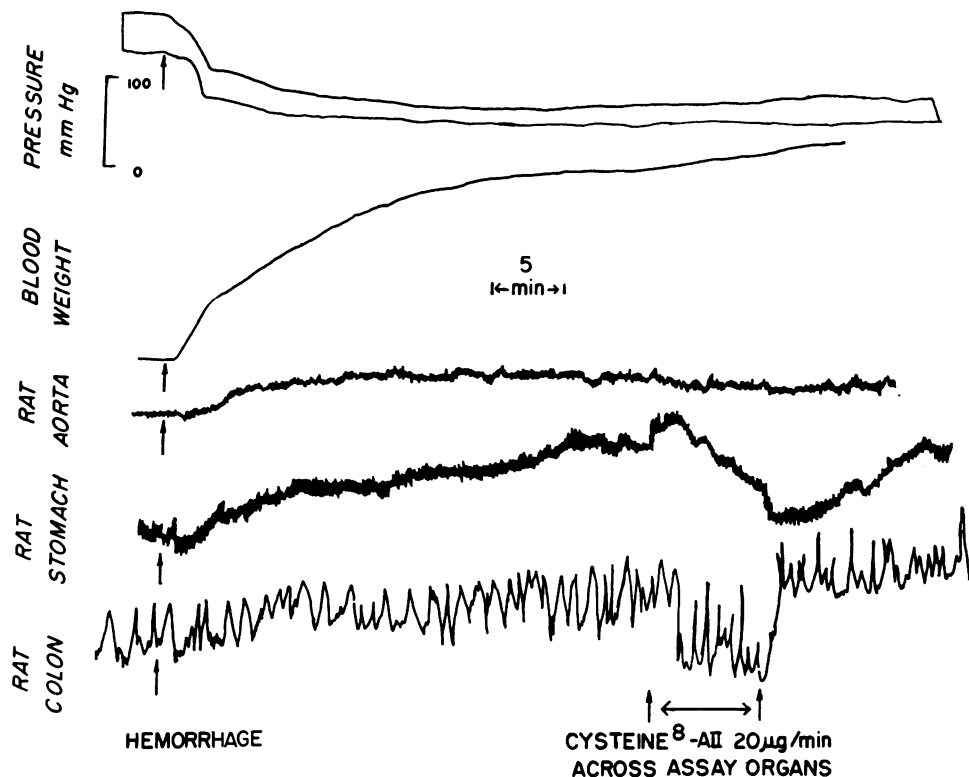ACROSS ASSAY ORGANS

FIGURE 3 The dog is hemorrhaged to 45–50 mm Hg mean blood pressure. The pressure shows systolic and diastolic pressure. The blood weight indicates the bleedout. The dog is bled into a plastic bag suspended from a myograph. The assay organs are superfused with carotid artery blood. The contraction of the rat aorta suggests increased CA levels in the blood, and the contraction of the rat stomach strip and rat colon suggests a rise in angiotensin levels. Infusion of cysteine^8-AII into the blood superfusing the assay organs causes a relaxation of the rat stomach strip and rat colon, confirming the presence of angiotensin.

Ayerst Laboratories, New York, phenoxybenzamine by Smith, Kline and French Laboratories, Philadephia, Pa., prostaglandins and heparin sodium by the Upjohn Company, Kalamazoo, Mich., indomethacin by Merck, Sharp and Dohme, West Point, Pa., and SQ-20881 by E. R. Squibb and Sons. The AII employed was Asn', Val^5-AII (Hypertensin) kindly supplied by Dr. Werner Rittel of Ciba-Geigy, Ltd., Basal, Switzerland.

RESULTS

Time-course of renin and catecholamine levels. Fig. 2 gives the time-course of renin and CA levels in relation to alterations in the hemorrhage volume. Both CA and renin levels rose quickly, about 100-fold and 8-fold, respectively, after the start of hemorrhage and were maintained at high levels. However, as blood was taken up by the dog during decompensation, CA levels fell but remained well above control (10-fold), whereas renin levels were maintained throughout. The same profile was observed in every dog ($n = 12$).

The CA levels in these experiments are higher than most of those reported in the literature for hemorrhagic shock (23–25). However, many of these investigators used pentobarbital anesthesia, which was shown by Walker et al. (26, 27) to diminish greatly adrenal medullary secretion. The CA levels in hemorrhagic shock in unanesthetized dogs reported by Walker et al. (27) are comparable to those reported in this study.

Presence and role of AII during hemorrhage. The presence of AII in carotid artery blood was investigated by continuous bioassay (blood-bathed organ technique). Fig. 3 gives a representative experiment of the events at hemorrhage. The contraction of the rat colon and rat stomach strip suggests the presence of AII. In 15 (out of 19) experiments, the assay organs sensitive to AII contracted. The presence of AII was confirmed in nine experiments by infusing the specific AII antagonist, cysteine^8-AII or isoleucine^8-AII (28) (20 μg/ml), across the assay organs. In all instances, the assay organs sensitive to AII relaxed (Fig. 3). Angiotensin antagonists have been shown to be absolutely specific and do not block the action of biological substances other than angiotensin (29).
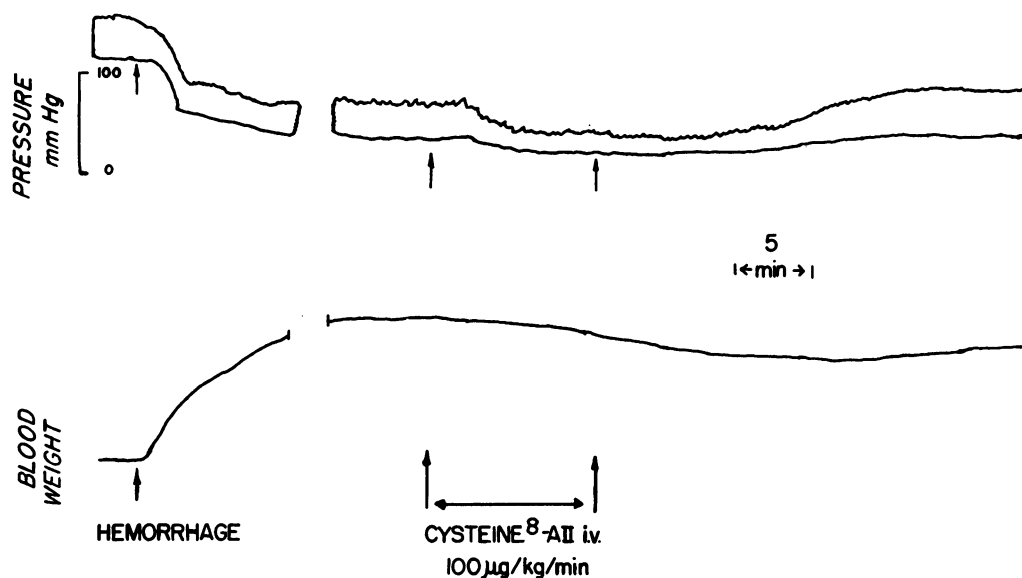
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FIGURE 4 The dog is hemorrhaged to 45–50 mm Hg mean blood pressure and the pressure maintained for approximately 30 min. i.v. infusion of cysteine[8]-AII causes a fall in blood pressure which returns to preinfusion level after the infusion is discontinued. Blood is returned to the dog indicating vasodilation (decrease in blood weight). During the time of drug treatment, the height of the blood reservoir is not changed.

The time-course and quantity of AII was difficult to assess with the blood-bathed organ technique because of the high levels of CA which relax the rat colon and especially the rat stomach strip. Simultaneous infusion of epinephrine and AII in separate experiments showed that the relative activity of AII in the presence of epinephrine varies from one set of smooth muscles (rat

FIGURE 5 The dog was hemorrhaged and the pressure maintained at 45–50 mm Hg for 1 h. Phenoxybenzamine (POB), 5 mg/kg, was given slowly i.v. The blood pressure and blood weight fell, indicating vasodilation. The rat aorta (blood bathed) relaxed, confirming that CA were present. During the time of drug treatment, the height of the blood reservoir is not altered.

colon and rat stomach strip) to another. CA antagonists could not be infused over the assay organs, since the blood is returned to the dog. In two experiments, the rat colon remained contracted throughout all stages of shock. In these two dogs, the CA levels were well below the calculated average (Fig. 2), and the renin levels were relatively high.

If one assumes almost complete conversion of AI to AII, the renin levels observed in these experiments (50 ng AI/ml/h) would be equivalent to 0.8 ng AII/ml/min, which is comparable with the AII levels observed after hemorrhage by Hall and Hodge (30) and Hodge, Lowe, and Vane (31).

The role of AII in circulatory compensation after hemorrhage (blood pressure maintained at 45–50 mm Hg) was tested in five experiments by i.v. infusion of cysteine[8]-AII or isoleucine[8]-AII (100 μg/kg/min or 50 μg/kg/min). The mean blood pressure fell by 20±2.5 mm Hg (Fig. 4). In four of the experiments, blood returned to the dog (decrease in blood weight) indicating peripheral vasodilation. After the infusion of the angiotensin antagonist was discontinued, the blood pressure returned to preinfusion levels or slightly higher, and blood was again forced into the reservoir. Administration of cysteine[8]-AII or isoleucine[8]-AII to unhemorrhaged dogs did not cause a fall but caused a small rise in blood pressure (data not shown). Similar results were obtained with the angiotensin-converting enzyme inhibitor, SQ-20881. I.v. infusion of 35 μg/kg/min during hemorrhagic
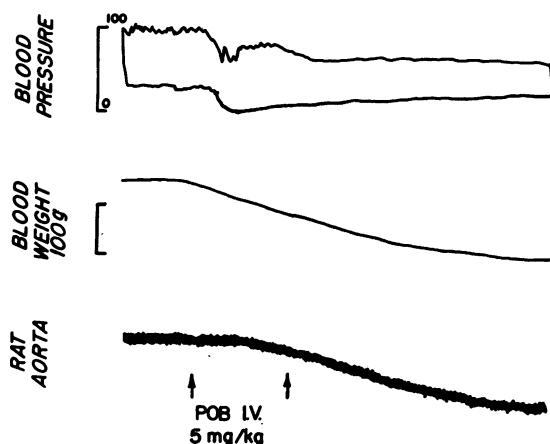
BLOOD PRESSURE mm Hg

CHICK RECTUM

RAT STOMACH

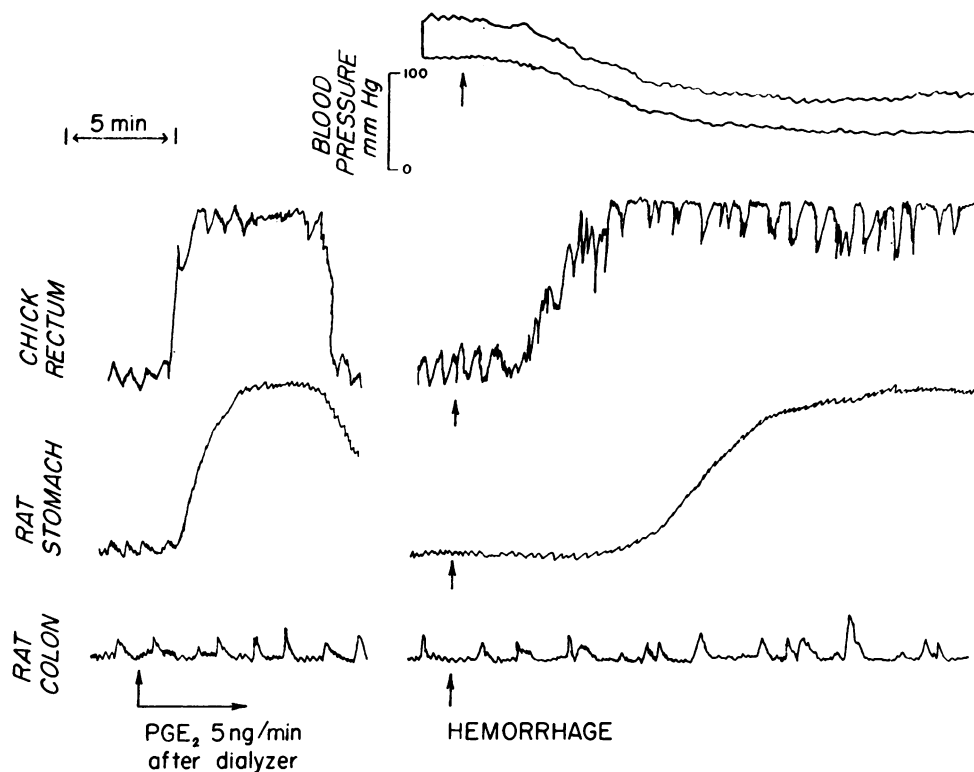RAT COLON

PGE₂ 5 ng/min
after dialyzer

HEMORRHAGE

FIGURE 6 The dog was hemorrhaged to a mean blood pressure of 45–50 mm Hg. The assay organs are superfused with dialysate (Krebs-Henseleit solution against carotid artery blood). There is a lag of 5–10 min due to the dialyzer. The assay organs were calibrated by the infusion of PGE₂ standard into the Krebs-Henseleit solution after the dialyzer (lower left of figure).

shock caused a fall in mean blood pressure of 20±4 mm Hg ($n = 5$). A similar observation has been made by Erdös, Massion, Downs, and Gecse (32) by infusing SQ-20881 into hemorrhaged dogs.

Infusion of angiotensin antagonists to dogs in decompensated shock produced comparable reductions in blood pressure as seen during compensation (not shown), thereby confirming that AII is involved in cardiovascular attempts at compensation during all phases of hemorrhagic shock.

The presence and role of CA after hemorrhage. The presence and time-course of CA after hemorrhage can be seen from the results of the fluorometric assay (Fig. 2) and from the contraction of the rat aorta (Fig. 3) which was observed in all experiments. The presence of CA and the role in circulatory compensation after hemorrhage was supported by the i.v. administration of the α-adrenergic blocking agent, phenoxybenzamine ($n = 7$). However, phenoxybenzamine is not entirely specific for CA and could block other vasoconstrictor substances in the aorta. Phenoxybenzamine, 5 mg/kg during hemorrhagic shock, caused an additional fall in mean arterial

pressure of 26±5 mm Hg, relaxation of the rat aorta, and return of blood to the dog (decrease in blood weight). A representative experiment is given in Fig. 5.

The presence and role of PG after hemorrhage. The presence of PG in carotid artery blood was determined by continuous bioassay (superfusion with a dialysate against carotid artery blood) in 10 dogs. Humoral substances, such as PG, CA, histamine, serotonin, etc. would be dialyzed into the Krebs-Henseleit and superfused across the assay organs. However, the CA, histamine, serotonin, and acetylcholine have no effect on the Krebs-perfused organs, because the mixture of antagonists is continuously present to block the response to these substances. Therefore, a PG-produced contraction could not be influenced by the above substances. In all of these experiments, a contraction of the rat stomach strip and chicken rectum occurred shortly after hemorrhage, indicating the presence of PG-like activity. A typical experiment is given in Fig. 6. The contraction was maintained for 15–60 min. In most of these experiments, no contraction of the rat stomach or chicken rectum was observed in later stages of hemorrhagic shock. The PG

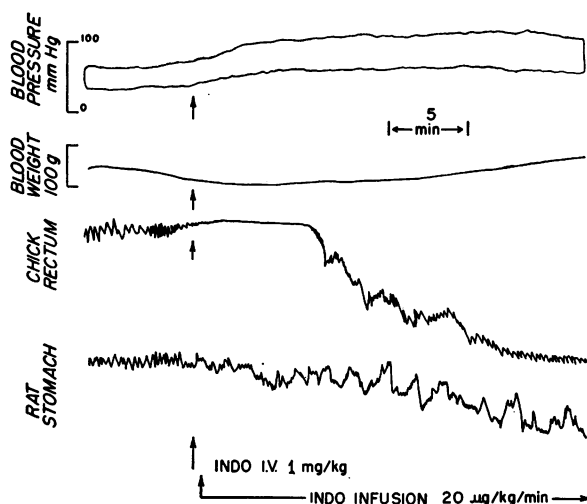*Endocrine Profile in Hemorrhagic Shock*   847

FIGURE 7 Hemorrhage of the dog to 50 mm Hg mean blood pressure caused a contraction of the chicken rectum and rat stomach strip as shown in Fig. 6. The subsequent administration of indomethacin (INDO) caused a relaxation of the chicken rectum and rat stomach strip, confirming the presence of PG-like activity. Indomethacin also caused a rise in blood pressure and increased bleedout (increase in blood weight), indicating a rise in peripheral resistance. During the time of drug treatment, the height of the blood reservoir was not changed.

observed were probably of the E type, since the rat colon, which is most sensitive to PGF$_{2\alpha}$, did not contract.

Infusions of PGE$_1$ standards into the blood passing into the dialyzer and/or into the dialysate superfusing the assay organs were performed for calibration purposes before hemorrhage in six of the above experiments (Fig. 6). From these infusions, and taking the efficiency of the dialyzer into consideration, the increase of PGE above basal levels due to hemorrhage was estimated. It amounted to 2.6±0.6 ng/ml of blood. This is only an approximation, since the sensitivity of the assay organs may change with time. Under resting conditions, arterial concentrations of PG of the E and F type are probably less than 0.02 ng/ml (33).

The presence of PG in carotid artery blood was confirmed in five experiments by the i.v. administration of indomethacin (1-2 mg/kg). This caused a relaxation of the assay organs sensitive to PGE (chicken rectum and rat stomach strip, Fig. 7). In two of these dogs, a contraction of the rat aortas was observed after indomethacin administration, indicating further CA release. The aortas in the other experiments were probably already maximally contracted at this point, and therefore, did not contract any further. This was tested in some experiments by the application of exogenous epinephrine, which did not cause any increase in contraction. However, they did relax with phenoxybenzamine, indicating

that the smooth muscle was not paralyzed. An attempt was made to quantitate the effect of indomethacin on plasma CA levels. Such determinations proved inconclusive because CA levels were rising too rapidly during compensation (the time when PG is present in the arterial blood), so that it was not possible to determine if indomethacin treatment increased blood CA levels. The influence of drug treatment on blood CA levels during compensation is further complicated by the considerable variability in levels from dog to dog. Indomethacin treatment of unhemorrhaged dogs did not change the level of contraction in the assay organs. The administration of indomethacin to hemorrhaged or unhemorrhaged dogs had no effect on plasma renin activity.

The i.v. administration of indomethacin during hemorrhage and the compensatory phase caused a rise in mean arterial pressure of 19±2.8 mm Hg ($n = 9$) which was accompanied by increased bleedout (increase in blood weight) (Fig. 7). This response was greatly attenuated when indomethacin was administered during early decompensation and completely absent during late decompensation. Indomethacin did not cause a rise in blood pressure when given before hemorrhage.

A schematic profile of the vasoactive substances examined during the different phases of hemorrhagic shock is given in Fig. 8, which summarizes the data. Hemorrhage is associated with a rise in renin, CA, and PGE-like activity. The surge of PGE-like activity is transient. CA levels rise to very high levels and then fall during decompensation, and renin remains elevated through all phases of hemorrhagic shock.

DISCUSSION

CA and renin-angiotensin in cardiovascular compensation of hemorrhagic shock. The time-course of CA and renin in relation to the hemorrhage volume (Fig. 2) raises the question of relative importance of AII and CA in the compensatory and decompensatory phases of hemorrhagic shock. With the reuptake of blood, CA levels fall, while renin levels are maintained. The reason for this phenomena is not understood. However, the decrease in CA levels cannot be merely due to the dilution by the returning blood, because there is a 10-fold decrease in CA plasma levels while approximately one-half of the animal's blood was in the bag (therefore, one-half dilution). Compensation and decompensation seem to closely correlate with plasma CA levels but not with plasma renin activity. It therefore appears that the development of irreversible shock is not directly dependent on changes in circulating AII levels. A decrease in CA levels with decompensation has been observed by other investigators (23-25). A complete time-course of renin during all phases of hemorrhagic shock, according to our knowledge, has not been reported. High
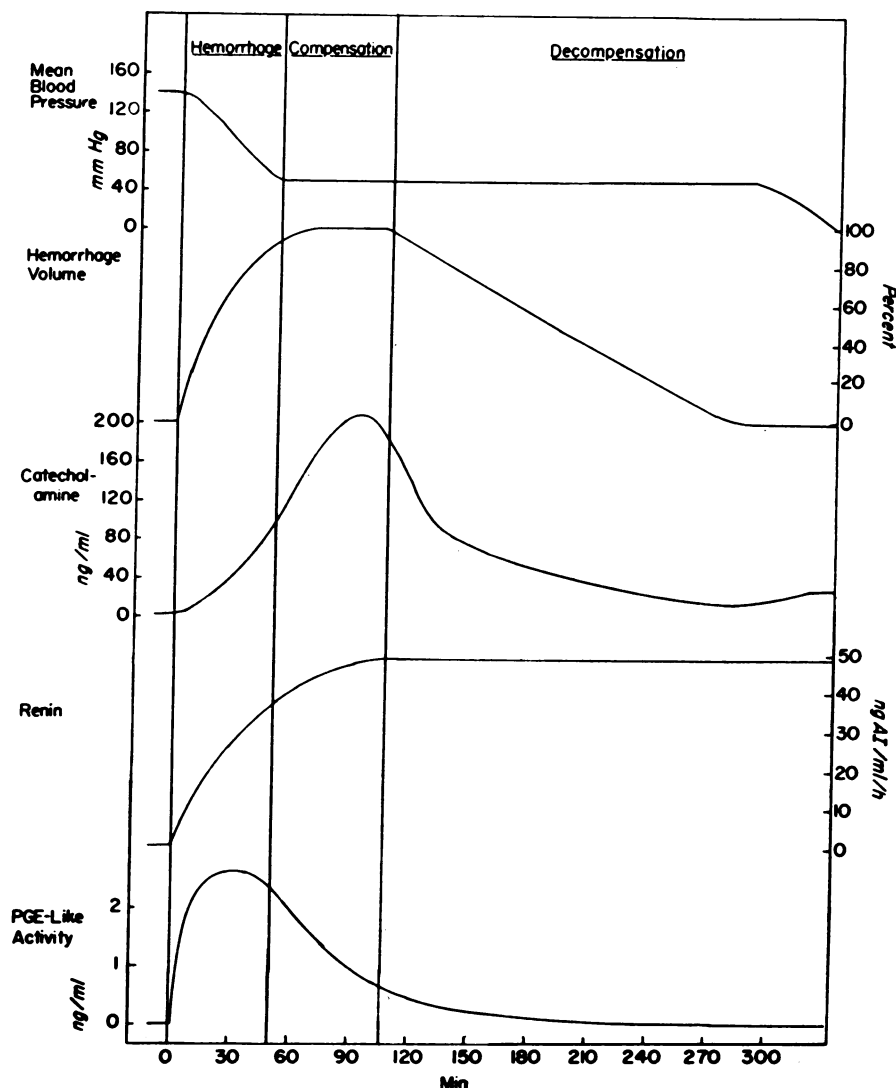
FIGURE 8 Schematic temporal profile of mean blood pressure, hemorrhage volume (percent of total), plasma CA levels, plasma renin activity, and PGE-like activity in carotid artery blood.

renin levels, however, need not necessarily imply high AII levels. There is the possibility that angiotensinogen (renin substrate) may be depleted during the late stages of hemorrhagic shock. This has been reported by Dexter, Frank, Haynes, and Altschule (34) and Collins and Hamilton (35). However, Scornik and Palidini (36) found elevated AII levels during hemorrhagic shock including the late stages. In our experiments, angiotensinogen levels were probably not depleted, at least not to such an extent as to cause a decrease in AII levels. Furthermore, plasma renin activity was measured by radioimmunoassay which utilizes endogenous angiotensinogen as substrate in generating AI which in turn reacts with the antibody.

Hollenberg, Waters, Toews, Davies, and Nickerson (37) found that continuous infusion of norepinephrine into hemorrhagic dogs accelerated the rate of decompensation, measured as a more rapid reuptake of blood from the reservoir and earlier death. Pretreatment with the α-adrenergic blocker, phenoxybenzamine, delayed the onset and reduced the rate of reuptake of blood and prolonged survival. Nevertheless, there has only been limited success in the treatment of shock with adrenergic blockers (25).

Du Charme and Beck (38) hemorrhaged dogs and determined that the potential of the renal pressor system to reduce vascular capacity during hypotension was about 60% that of the adrenergic nervous system. Thus,

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it would be anticipated that adrenergic blockade alone would not overcome the profound vasoconstriction produced in hemorrhage. Therefore, angiotensin antagonists (alone or in combination with adrenergic blockers) may have some potential value in the treatment of shock. This is exemplified by the substantial reduction in blood pressure and vascular resistance produced by the infusion of cysteine⁸-AII into hemorrhaged dogs (Fig. 4). Furthermore, Errington and Rocha e Silva (39) prolonged survival of hemorrhaged dogs by pretreatment with 1.5 mg/kg of SQ-20881 (AI-converting enzyme inhibitor). Combined use of adrenergic and angiotensin antagonists thus has the potential of restoring adequate tissue perfusion. This treatment, used in combination with other efforts to support the circulation, should help to avoid the complications which arise from the prolonged ischemia of organs, such as the kidney, where acute tubular necrosis may occur during shock.

Interaction of angiotensin, CA, and PG. Hodge, Lowe, and Vane (31), as well as Scornik and Paladini (36), observed that hemorrhage caused an increase of AII blood levels. Reduction of renal arterial pressure by balloon inflation to the same level as caused by the hemorrhage experiments did not result in comparable AII levels. Therefore, reflex nerve activity seems to be an important stimulus of renin release during hemorrhage. The sympathetic nerve is known to enter the kidney along the renal artery. Application of local anesthetics to the renal pedicle abolished the rise in AII blood levels (31) and in renin levels (40) due to hemorrhage. It therefore appears that enhanced adrenergic activity leads to renin secretion. The resultant circulating AII further amplifies systemic adrenergic tone by stimulating CA release from the adrenal medulla (41) and by enhancing the release of CA from adrenergic nerve terminals (42–44). There is some evidence that this CA-AII cycle is partially inhibited by PG of the E type, which can either (*a*) inhibit the release of CA from adrenergic nerve endings (45) or (*b*) directly cause vasodilation and thus counteract the vasoconstriction produced by CA or AII (17, 33).

A number of stimuli could be involved in the generation of PG during shock. These include ischemia (13, 46, 47), CA (47–49), adrenergic nerve stimulation (14, 47, 49), and angiotensin (15, 50, 51). The systemic effect of the circulating PG during hemorrhagic hypotension is clearly evidenced by the increase in blood pressure and vascular resistance induced by indomethacin and the associated fall in PG levels in blood (Fig. 7). Collier, Herman, and Vane (52) also noted that indomethacin administration to hemorrhaged or endotoxin-treated dogs abolishes PG in the blood and simultaneously causes a vasoconstriction. The rise in vascular resistance induced by indomethacin would appear to be the result of the deinhibition of adrenergic stimulation by removing PGE and the simultaneous removal of the direct vasodilator influence of circulating PGE. Hedqvist (45, 53) suggests that PGE is involved in a negative feedback mechanism decreasing norepinephrine outflow upon adrenergic stimulation. Inhibition of PG synthesis was found to cause an increased norepinephrine secretion from adrenergic nerve endings upon stimulation (54). During hemorrhagic hypotension, enhanced adrenergic tone exists. Therefore, blockade of PG synthesis by indomethacin might be expected to enhance the response to adrenergic stimulation during compensation, which would add to the increase in systemic blood pressure produced by the elimination of the vasodilator PGE. It is also of interest to note that during hemorrhage, CA levels are rising steeply, while PGE levels are declining after a peak (Fig. 8). This may indicate that during hemorrhage, PGE is suppressing CA release, including that from the adrenal medulla, since epinephrine levels are elevated to a greater extent in hemorrhagic hypotension than norepinephrine levels (24, 25).

PGE-like material in carotid artery blood after hemorrhage. The appearance of PG-like activity in carotid artery blood due to hemorrhage has not been previously reported. The evidence that the activity detected is due to PG of the E type is based on the specificity of the bioassay used (4, 5) and the blockade of the activity by indomethacin which inhibits PG synthesis (18). The origin of the PG-like activity in carotid artery blood is not known. Piper, Vane, and Wyllie (5) and McGiff, Terragno, Strand, Lee, and Lonigro (6) have shown that 90% of PG, except PGA, disappear in one passage through the lungs of a normal animal. The enzyme capacity of the lungs was exceeded at an i.v. infusion rate of 10 µg/min of PGE₂ (5). On the other hand, the lung has been demonstrated to synthesize and release PG. Piper and Vane (55) have observed that bradykinin, antigen (in sensitized lung), or mechanical stroking are all potent stimuli for the release of PG. We have shown that the adenine nucleotides, ATP and ADP, can elicit the appearance of PG in pulmonary venous effluent (56).

The initial phase of hemorrhagic hypovolemia was routinely characterized by appearance of PGE-like activity in carotid artery blood (Fig. 6). Several possibilities exist which could explain this observation: (*a*) the lungs may not destroy PG during hemorrhagic hypotension because of severe vasoconstriction or shunting of blood away from the pulmonary sites of destruction, (*b*) the enzyme capacity may be exceeded, (*c*) PG may be synthesized in the lung distal to the site of destruction. Severe pulmonary vasoconstriction during hemor-

rhagic hypovolemia has been reported by a number of workers (25, 57, 58).

Regardless of the source of PG in the carotid blood, its presence is transient while hypovolemia persists. The determinants for the duration of the PG release are unknown at this time. Autoregulatory readjustment of blood flow across the kidney (59) and the heart (60) have been associated with the appearance of PG. The functional significance of this initial PG release, its organ source, and fate are currently under investigation.

REFERENCES

1. Frank, H. A., A. M. Seligman, and J. Fine. 1945. Traumatic shock. X. The treatment of hemorrhagic shock irreversible to replacement of blood volume deficiency. *J. Clin. Invest.* 24: 435–444.
2. Lansing, A. M., and J. A. F. Stevenson. 1957. The standardization of stress in the production of hemorrhagic shock. *Can. J. Biochem. Physiol.* 35: 1085–1092.
3. Smith, J. J., and R. A. Grace. 1957. Influence of differential aortic perfusion pressure in experimental shock. *Am. J. Physiol.* 191: 135–139.
4. Vane, J. R. 1969. The release and fate of vasoactive hormones in the circulation. *Br. J. Pharmacol.* 35: 209–242.
5. Piper, P. J., J. R. Vane, and J. H. Wyllie. 1970. Inactivation of prostaglandins by the lungs. *Nature (Lond.).* 225: 600–604.
6. McGiff, J. C., N. A. Terragno, J. C. Strand, J. B. Lee, and A. J. Lonigro. 1969. Selective passage of prostaglandins across the lung. *Nature (Lond.).* 223: 742–745.
7. Werle, J. M., R. S. Cosby, and C. J. Wiggers. 1942. Observations on hemorrhagic hypotension and hemorrhagic shock. *Am. J. Physiol.* 136: 401–420.
8. Collier, J. G. 1972. New dialysis technique for continuous measurement of the concentration of vasoactive hormones. *Br. J. Pharmacol.* 44: 383P. (Abstr.)
9. Vane, J. R. 1957. A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmacol. Chemother.* 12: 344–349.
10. Regoli, D., and J. R. Vane. 1964. A sensitive method for the assay of angiotensin. *Br. J. Pharmacol.* 23: 351–359.
11. Furchgott, R. F., and S. Bhadrakom. 1953. Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. *J. Pharmacol. Exp. Ther.* 108: 129–143.
12. Gilmore, N., J. R. Vane, and J. H. Wyllie. 1968. Prostaglandins released by the spleen. *Nature (Lond.).* 218: 1135–1140.

13. McGiff, J. C., K. Crowshaw, N. A. Terragno, A. J. Lonigro, J. C. Strand, M. A. Williamson, J. B. Lee, and K. K. F. Ng. 1970. Prostaglandin-like substances appearing in canine renal venous blood during ischemia: their partial characterization by pharmacologic and chromatographic procedures. *Circ. Res.* 27: 765–782.
14. Davis, H. A., and E. W. Horton. 1972. Output of prostaglandins from the rabbit kidney, its increase on renal nerve stimulation and its inhibition by indomethacin. *Br. J. Pharmacol.* 46: 658–675.
15. Douglas, J. R., Jr., E. M. Johnson, Jr., G. R. Marshall, B. M. Jaffe, and P. Needleman. 1973. Stimulation of splenic prostaglandin release by angiotensin and specific inhibition by cysteine^8-AII. *Prostaglandins.* 3: 67–74.
16. Vane, J. R., and K. I. Williams. 1973. The contribution of prostaglandin production to contractions of the isolated uterus of the rat. *Br. J. Pharmacol.* 48: 629–639.
17. Aiken, J. W., and J. R. Vane. 1973. Intrarenal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. *J. Pharmacol. Exp. Ther.* 184: 678–687.
18. Ferreira, S. H., S. Moncada, and J. R. Vane. 1971. Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nat. New Biol.* 231: 237–239.
19. Vane, J. R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. *Nat. New Biol.* 231: 232–235.
20. Eckenfels, A., and J. R. Vane. 1972. Prostaglandins, oxygen tension and smooth muscle tone. *Br. J. Pharmacol.* 45: 451–462.
21. Lubran, M. M., P. C. Fu, and J. R. Sundeen. 1973. Estimation of plasma renin activity by radioimmunoassay of angiotensin I. *Ann. Clin. Lab. Sci.* 3: 48–52.
22. Anton, A. H., and D. F. Sayre. 1962. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmacol. Exp. Ther.* 138: 360–375.
23. Watts, D. T., and A. D. Bragg. 1967. Blood epinephrine levels and automatic reinfusion of blood during hemorrhagic shock in dogs. *Proc. Soc. Exp. Biol. Med.* 96: 609–612.
24. Lillehei, R. C., J. K. Longerbeam, J. H. Bloch, and W. G. Manax. 1964. The nature of irreversible shock: experimental and clinical observations. *Ann. Surg.* 160: 682–710.
25. Chien, S. 1967. Role of the sympathetic nervous system in hemorrhage. *Physiol. Rev.* 47: 214–288.
26. Walker, W. F., M. S. Zileli, F. W. Reutter, W. C. Shoemaker, D. Friend, and F. D. Moore. 1959. Factors influencing the resting secretion of the adrenal medulla. *Am. J. Physiol.* 197: 765–772.
27. Walker, W. F., M. S. Zileli, F. S. Reutter, W. C. Shoemaker, D. Friend, and F. D. Moore. 1959. Adrenal medullary secretion in hemorrhagic shock. *Am. J. Physiol.* 197: 773–780.
28. Needleman, P., E. M. Johnson, Jr., W. Vine, E. Flanigan, and G. R. Marshall. 1972. Pharmacology of antagonists of angiotensin I and II. *Circ. Res.* 31: 862–867.
29. Marshall, G. R., W. Vine, and P. Needleman. 1970. A specific competitive inhibitor of angiotensin II. *Proc. Natl. Acad. Sci. U. S. A.* 67: 1624–1630.
30. Hall, R. C., and R. L. Hodge. 1971. Changes in catecholamine and angiotensin levels in the cat and dog during hemorrhage. *Am. J. Physiol.* 221: 1305–1309.

31. Hodge, R. L., R. D. Lowe, and J. R. Vane. 1966. The effects of alteration of blood-volume on the concentration of circulating angiotensin in anesthetized dogs. *Am. J. Physiol.* **185**: 613–626.

32. Erdös, E. G., W. H. Massion, D. R. Downs, and A. Gecse. 1973. Inhibition of angiotensin I converting enzyme in shocked dogs. *Pharmacologist.* **15**: 240. (Abstr.)

33. Lonigro, A. J., H. D. Itskowitz, K. Crowshaw, and J. C. McGiff. 1973. Dependency of renal blood flow on prostaglandin synthesis in the dog. *Circ. Res.* **32**: 712–717.

34. Dexter, L., H. A. Frank, F. W. Haynes, and M. D. Altschule. 1943. Traumatic shock. VI. The effect of hemorrhagic shock on the concentration of renin and hypertensinogen in the plasma in unanesthetized dogs. *J. Clin. Invest.* **22**: 847–852.

35. Collins, D. A., and A. S. Hamilton. 1944. Changes in the renin-angiotensin system in hemorrhagic shock. *Am. J. Physiol.* **140**: 499–512.

36. Scornik, O. A., and A. C. Paladini. 1964. Angiotensin blood levels in hemorrhagic hypotension and other related conditions. *Am. J. Physiol.* **206**: 553–556.

37. Hollenberg, N. K., J. R. Waters, M. R. Toews, R. O. Davies, and M. Nickerson. 1970. Nature of cardiovascular decompensation during hemorrhagic hypotension. *Am. J. Physiol.* **219**: 1476–1482.

38. Du Charme, D. W., and L. Beck. 1971. The relative effect of the renal pressor and sympathetic nervous system on vascular capacity during hypotension. *J. Pharmacol. Exp. Ther.* **177**: 56–68.

39. Errington, M. L., and M. Rocha e Silva, Jr. 1973. The effect of a bradykinin potentiating nonapeptide (BPP$_{9a}$) on prolonged hemorrhagic shock in dogs. *J. Physiol. (Lond.).* **233**: 46P–47P.

40. Bunag, R. D., I. H. Page, and J. W. McCubbin. 1966. Neural stimulation of release of renin. *Circ. Res.* **19**: 851–858.

41. Peach, M. J., W. H. Cline, and D. J. Watts. 1966. Release of adrenal catecholamines by angiotensin II. *Circ. Res.* **19**: 571–575.

42. Peach, M. J., and G. D. Ford. 1968. The actions of angiotensin II on canine myocardial and plasma catecholamines. *J. Pharmacol. Exp. Ther.* **162**: 92–100.

43. Starke, K., U. Werner, R. Hellerforth, and H. J. Schümann. 1970. Influence of peptides on the output of noradrenaline from isolated rabbit hearts. *Eur. J. Pharmacol.* **9**: 136–140.

44. Harrison, T. S., A. Birbari, and J. F. Seaton. 1973. Reinforcement of reflex epinephrine release by angiotensin II. *Am. J. Physiol.* **224**: 31–34.

45. Hedqvist, P. 1970. Control of prostaglandin E$_2$ of sympathetic neurotransmission in the spleen. *Life Sci.* **9** (Part I) : 269–278.

46. Minkes, M. S., J. R. Douglas, Jr., and P. Needleman. 1973. Prostaglandin release by isolated perfused rabbit heart. *Prostaglandins.* **3**: 439–445.

47. Needleman, P., J. R. Douglas, Jr., E. M. Johnson, Jr., and B. Jakschik. Release of renal prostaglandins by catecholamines: relationship to renal endocrine function. 1974. *J. Pharmacol. Exp. Ther.* **188**: 453–460.

48. McGiff, J. C., K. Crowshaw, N. A. Terragno, K. U. Malik, and A. J. Lonigro. 1972. Differential effects of noradrenaline and renal nerve stimulation on vascular resistance in the dog kidney and the release of a prostaglandin E-like substance. *Clin. Sci. (Oxf.).* **42**: 223–233.

49. Dunham, E. W., and B. G. Zimmerman. 1970. Release of prostaglandin-like material from dog kidney during nerve stimulation. *Am. J. Physiol.* **219**: 1279–1285.

50. McGiff, J. C., K. Crowshaw, N. A. Terragno, and A. J. Lonigro. 1972. Release of prostaglandin-like substance into renal venous blood in response to angiotensin II. *Circ. Res.* **27** (Suppl. I) : 121–130.

51. Needleman, P., A. H. Kaufman, J. R. Douglas, Jr., E. M. Johnson, Jr., and G. R. Marshall. 1973. Specific stimulation and inhibition of renal prostaglandin release by angiotensin analogues. *Am. J. Physiol.* **224**: 1415–1419.

52. Collier, J. G., A. G. Herman, and J. R. Vane. 1973. Appearance of prostaglandins in renal venous blood of dogs in response to acute systemic hypotension produced by bleeding or endotoxin. *J. Physiol. (Lond.).* **230**: 19P–20P.

53. Hedqvist, P. 1971. Prostaglandin E compounds and sympathetic neuro-muscular transmission. *Ann. N. Y. Acad. Sci.* **180**: 410–415.

54. Hedqvist, P., L. Stjärne, and A. Wennmalm. 1971. Facilitation of sympathetic neurotransmission in the cat spleen after inhibition of prostaglandin synthesis. *Acta Physiol. Scand.* **83**: 430–432.

55. Piper, P. J., and J. R. Vane. 1969. Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature (Lond.).* **223**: 29–35.

56. Douglas, J. R., Jr., M. S. Minkes, and P. Needleman. 1973. Organ profile of prostaglandin release induced by adenine nucleotides and ischemia. *Pharmacologist.* **15**: 250. (Abstr.)

57. Shubrooks, S. J., Jr., B. Schneider, H. Dubin, and G. M. Turino. 1973. Acidosis and pulmonary hemodynamics in hemorrhagic shock. *Am. J. Physiol.* **225**: 225–229.

58. Kim, S. I., and W. C. Shoemaker. 1973. Role of the acidosis in the development of increased pulmonary vascular resistance and shock lung in experimental hemorrhagic shock. *Surgery (St. Louis).* **73**: 723–729.

59. Herbaczynska-Cedro, K., and J. R. Vane. 1973. Contribution of intrarenal generation of prostaglandin to autoregulation of renal blood flow in the dog. *Circ. Res.* **33**: 428–436.

60. Alexander, R. W., K. M. Kent, J. J. Pisano, H. R. Keiser, and T. Cooper. 1973. Regulation of canine coronary blood flow by endogenously synthetized prostaglandins. *Circulation.* **48** (Suppl. IV) : 107. (Abstr.)