The Intestinal Factor in Irreversible Endotoxin Shock *

RICHARD C. LILLEHEI, M.D.,** LLOYD D. MACLEAN, M.D.†

From the Department of Surgery, University of Minnesota Medical School and the Ancker Hospital Surgical Research Laboratory, St. Paul, Minnesota

THE PRINCIPAL autopsy finding in dogs dying of either irreversible hemorrhagic or endotoxin shock is hemorrhagic necrosis of the mucosa of the bowel. In irreversible hemorrhagic shock, the bowel necrosis is apparently a result of critical ischemia due to vasoconstriction and oligemia and is responsible before death for a progressive loss of plasma volume and an increasing hematocrit.[17] Plasma hemoglobin also increases at the same time because of the reabsorption of hemolyzed blood into the systemic circulation from the congested necrotic bowel.[18] If the hemorrhagic necrosis of the bowel is prevented by pretreating animals before hemorrhage with adrenergic blocking agents,[12, 24, 27] or if the ischemic intestine is perfused with donor arterial blood during the period of hemorrhage,[17] irreversible shock following retransfusion does not occur. Moreover, the omens of the irreversible state, decreasing plasma volume, increasing hematocrit and an increasing plasma hemoglobin do not appear.

There is increasing evidence that endotoxins also produce their deleterious effect by their ability to incite severe and lasting vasospasm in the small arteries and veins.[2, 4, 34] As in hemorrhagic shock, the effects of this vasoconstriction are particularly severe in the bowel and also as in hemorrhagic shock, we have measured in endotoxin shock a progressive decrease in plasma volume, increase in hematocrit, and an increase in plasma hemoglobin which foretell the hemorrhage necrosis of the bowel seen at autopsy.

A number of drugs (chlorpromazine,[1, 23] Dibenzyline,[23] metaraminol,[31] norepinephrine,[32] and hydrocortisone [30]) and antibiotics [28] have been used to alter the response of animals to endotoxin as well as to prolonged hemorrhage. In addition, enterectomy,[14] hypothermia,[33] and fluid replacement [7] for lost plasma have been employed to alter the response of animals to hemorrhagic shock. In the present study, we have recorded the effectiveness of all these procedures in altering the responses to endotoxin which we believe are characteristic of the irreversible state in the dog.

Methods and Procedures

The experiments were done in an air-conditioned room using adult mongrel dogs of both sexes. One hour before the experiment the dogs were sedated with morphine sulfate, 2 mg./Kg., and placed on an experimental table where they laid quietly throughout the course of the experiment. Using local procaine anesthesia, a plastic cannula was advanced into the aorta via a femoral artery and attached to a mercury manometer to monitor blood pressure during the experiment. Both femoral veins were also cannulated for the giving of medications and the taking of blood samples. Plasma volumes and hematocrits were

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** U.S.P.H.S. Postdoctoral Research Fellow in Arthritis and Metabolic Diseases.

† Markle Scholar in the Medical Sciences.

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measured on all dogs before the endotoxin was given and at 90 minute intervals thereafter for periods up to eight hours. These measurements were repeated if the dog was alive on the day following an experiment. The plasma volumes were measured with T-1824, the injected dye being weighed and the plasma analyzed in microcuvettes in the Beckman DU Spectrophotometer.[29] Plasma hemoglobin determinations were also made at 90 minute intervals using the method of Flink and Watson.[5] Early in the study it was noted that a clotting deficit often occurred with the development of irreversible endotoxin shock. Accordingly Lee-White clotting times were also done at frequent intervals, along with platelet and white blood cell counts.[29] Normal clotting time in the dog using the Lee-White method was five to seven minutes. In a number of dogs blood cultures were drawn eight hours following the administration of the endotoxin and again 24 hours later if the dog was still alive.

All dogs dying during the course of the experiments were autopsied and tissue sections saved for microscopic study. All surviving dogs were sacrificed at varying intervals following the experiment and autopsied; tissue section was saved for microscopic study.

Following the preliminary measurements, the dogs were given an intravenous lethal dose of crude endotoxin prepared as previously described from killed Escherica coli bacteria.[21] Each new batch of crude endotoxin used was assayed on a new group of control dogs to establish the dose lethal for 95 per cent of adult dogs. The dose of endotoxin used in these experiments averaged 7.5 mg./Kg. body weight.

Ten groups of dogs were studied in the manner outlined above with the following modifications in the protocol:

Group I—Controls: These dogs were treated exactly as outlined above.

Group II—Sterilization of the bowel: Eleven dogs were given 6 Gm. of Sulfasuxidine,* 4 Gm. of neomycin and 2 ounces of milk of magnesia daily for five days. All medications were given in large gelatin veterinary capsules which were placed by hand in the esophagus of the dog to make sure each dose was received. On the sixth day, after preliminary measurements were made and a stool culture taken, a lethal dose of endotoxin was given intravenously. The dogs were then followed until death occurred or until the experiment was over.

Group III—Enterectomy: After preliminary preparations and measurements, ten dogs were anesthetized with pentobarbital and the intestine from the second portion of the duodenum to the distal rectum quickly excised. The remaining duodenum was brought out through a stab wound as a duodenostomy and the distal rectum closed and allowed to retract into the pelvis. In two of the dogs, the stomach, proximal duodenum, pancreas, and spleen were also excised along with the intestine, the common bile duct and esophagus being simply tied off. The arterial blood supply to the liver was carefully preserved in all dogs. When the dogs were awakening about two hours following surgery, endotoxin was administered and the animals followed.

Group IV—Pretreatment with Chlorpromazine: Thirty dogs were given chlorpromazine in amounts from 2.5 to 50 mg./Kg. intramuscularly 30 minutes before the endotoxin was given intravenously and then followed.

Group V—Pretreatment with Dibenzyline: Eight dogs were given Dibenzyline, 0.5 mg./Kg. intravenously dissolved in 100 ml. of normal saline one hour before the administration of endotoxin. Two dogs were given Dibenzyline 2.5 mg./Kg. intravenously in 100 ml. of normal saline 24 hours before the endotoxin was given.

Group VI—Hypothermia: After preliminary preparations, five dogs were lightly

TABLE 1*

Group	No. Dogs	No. Permanent Survivors†	Duration Survival, Hours	Hematocrit Increase, %	Plasma Loss, %	Maximum Increase Plasma Hemoglobin in mg. %
I. Controls	90	6	10.3 ±5.5	36.1 ±14	34.8 ±13	91.0 ±49
II. Intestinal antibiotics	11	1	12.3 ±6.0	37.2 ±19	—	84.7 ±44
III. Enterectomy	10	1	21.8 ±5.1	—	—	—
IV. Chlorpromazine 2.5–50 mg./Kg.	30	11	22.1 ±5.1	14 ±10	11.6 ±7.2	37.6** ±12
V. Dibenzyline	10	9	—	6.70 ±3.8	6.72 ±8.2	28.2 ±15
VI. Hydrocortisone	10	9	—	7.91 ±3.5	4.06 ±3.7	33.2 ±13
VII. Hypothermia	10	1	10.5 ±5.6	28.9 ±10	33.4 ±9.4	82.6 ±16
VIII. Aramine	10	0	5.10 ±2.8	31.5 ±9.1	40.4 ±8.5	193 ±83
IX. Levophed	10	0	10.2 ±5.2	29.7 ±12	34.7 ±14	144 ±58
X. Blood or Dextran	10	0	12.2 ±5.5	—	—	—

* Mean values with standard deviations.

** 20 hours later the plasma hemoglobin had increased to 74.8 ± 35 mg. % in those dogs subsequently dying but had decreased to 18.4 ± 16 mg. % in those dogs that survived permanently.

† Survived 72 hours or more.

anesthetized with pentobarbital and placed in an ice water bath. The dogs were removed from the bath when their rectal temperature had fallen to 30 to 31° C. and placed on the experimental table where their rectal temperature usually drifted to 26 to 28° C. They were then given endotoxin and followed. Six to eight hours later they were rewarmed to normal body temperature if still alive. In five other dogs endotoxin was administered 20 minutes before they were cooled in the manner described. These dogs were rewarmed if alive six to eight hours later.

Group VII—Pretreatment with Hydrocortisone: Ten dogs were given hydrocortisone 15 mg./Kg. intramuscularly daily for four days. On the fifth day they were prepared as described and given endotoxin.

Group VIII—Support of Blood Pressure with Metaraminol (Aramine®): Ten dogs were given ½ to 1 mg. metaraminol intravenously simultaneously with the administration of endotoxin. When the blood pressure fell below 80 mm. Hg, ½ to 1 mg. intravenous doses of metaraminol were repeated. This was continued as long as the

dog remanied responsive to the effects of the metaraminol.

Group IX—Support of Blood Pressure with Norepinephrine (Levophed®): In ten dogs a slow intravenous drip of norepinephrine was started simultaneously with the administration of endotoxin. This drip contained 16 to 32 mg. of Levophed base in 250 ml. of normal saline and was used to support the blood pressure of the dogs given endotoxin at normal levels for six to ten hours or until death occurred.

Group X—Fluid Therapy: Ten dogs were prepared in the usual manner and given endotoxin. Plasma volumes were determined at hourly intervals and all plasma losses immediately replaced with whole blood or Dextran®. This was continued for eight hours.

Results

Table 1 is a summary of results in these experiments. The mean values given for hematocrit, plasma volume and plasma hemoglobin are maximums measured in the first eight hours following the endotoxin injection. Figures 1, 2, and 3 show results in typical experiments. Control dogs given endotoxin usually followed a characteristic course. Immediately following

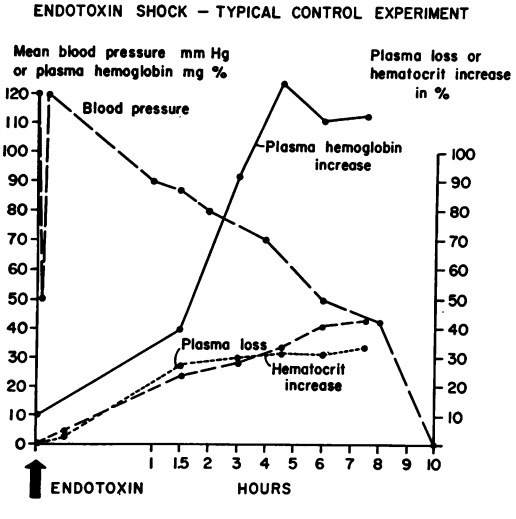

FIGURE 1

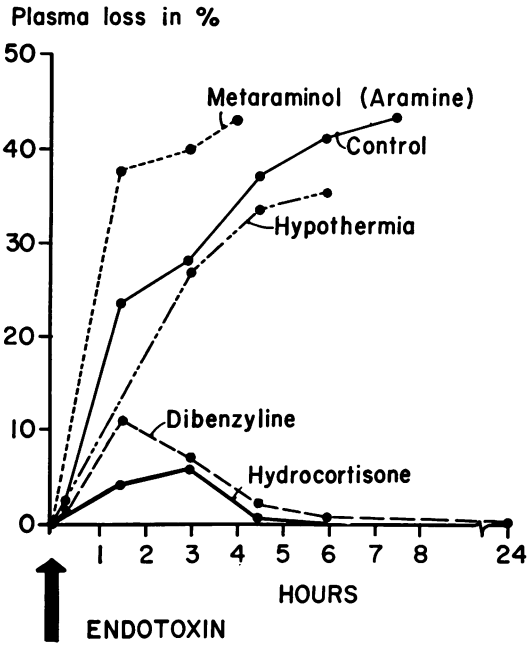

FIGURE 2

the endotoxin there was a sudden drop in mean blood pressure to 40 to 50 mm. Hg followed by a return to normal or above normal levels in three to five minutes. A normal blood pressure was then maintained for 60 to 90 minutes at which time it began to fall progressively until death occurred in about ten hours. Coincident with the blood pressure fall at 60 to 90 minutes, a significant plasma loss was measured and this loss increased with successive measurements until death occurred. Hematocrit increases closely paralleled plasma losses. Bloody diarrhea often started in one to three hours after the endotoxin was given and continued until death. Plasma hemoglobin rose significantly although a plateau was often reached before death.

A number of control dogs followed a somewhat different course. After the immediate blood pressure drop and return to

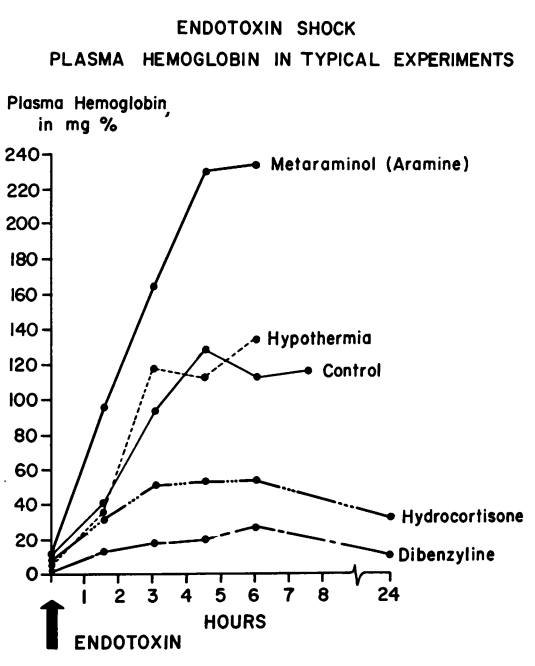

FIGURE 3

normal characteristic of other controls, these dogs were able to maintain a normal pressure for several hours despite measurable plasma losses and hematocrit increases. At the time of death, blood pressure in these dogs suddenly fell from normal levels to zero, and they died in about the same average time as the other control dogs. Plasma hemoglobin levels also increased in these dogs in a manner similar to other controls.

At autopsy all dogs had varying degrees of hemorrhagic necrosis in the mucosa of the small and large bowel. Liver and kidney congestion were also present. In most control dogs the lungs were normal or only moderately congested unless the dog had lived beyond the average survival time of ten hours. In these dogs the lung findings were often more severe in proportion to less extensive mucosal necrosis in the bowel. Subendothelial hemorrhages in the left ventricle of the heart were also a common finding in all controls at autopsy. The brain, pancreas, and adrenal glands usually appeared normal.

Sterilization of Intestinal Contents. The course before death and the autopsy findings of dogs receiving intestinal antibiotics in no way differed from that seen in the control dogs. Stool cultures taken before the experiment were sterile for aerobic and anaerobic organisms in eight of the 11 dogs. There was extensive yeast growing in the stool of 2 dogs and Gram-positive cocci were cultured from the stool of a third dog.

Enterectomy. In these dogs there was the usual immediate fall in blood presssure following the administration of the endotoxin with a rapid return to normal levels. The blood pressure of these dogs then remained at near normal levels for an extended period if blood loss was not great. But, because of the alteration in clotting time and an obvious increase in bleeding time which resulted from the endotoxin, these dogs bled profusely from all cut surfaces into the peritoneal cavity. This necessitated giving transfusions of whole blood or dextran to make up for blood loss which could be measured by aspirating blood from the peritoneal cavity with a plastic catheter placed there at the time of surgery. Indeed, blood aspirated from the peritoneal cavity was often returned to the systemic circulation via the femoral vein catheter because of the extensive blood loss.

Despite the bleeding problem and despite the fact that these dogs had the added factor of an anesthetic agent which we have found to potentiate somewhat the deleterious effects of endotoxin, these dogs survived for a significantly longer period of time than did the controls. Because of the bleeding problem and the necessity for transfusion, it was not possible to make accurate determinations of plasma volume, hematocrits, or plasma hemoglobins. At autopsy, we invariably found large amounts of free blood in the peritoneal cavity of these dogs. Liver congestion and subendothelial hemorrhages in the left heart ventricle were other common findings.

Pretreatment with Adrenergic Blocking Agents. Dogs given chlorpromazine could be divided into two distinct groups on the basis of their response to the endotoxin. Twenty dogs pretreated with 2.5 to 15 mg./Kg. of chlorpromazine had the usual immediate blood pressure drop with a rapid return to normal. At 60 to 90 minutes the blood pressure again dropped but rarely to less than 70 mm. Hg mean pressure. Following this the pressure gradually rose toward normal by the end of a six-hour period. There was only a moderate plasma loss, hematocrit increase and plasma hemoglobin increase in these dogs in the first eight hours. Bloody diarrhea occurred rarely. On the morning following the experiment almost all these dogs were still alive but lethargic. However, all but five were dead by 24 hours after the endotoxin was given. Just before death these dogs had a greater plasma loss than was measured during the first eight hours along with higher plasma hemoglobin levels. Respirations in these dogs were shallow and labored, moist rales could be heard by auscultation and the venous blood was quite dark. At autopsy, hemorrhagic necrosis of the bowel was present but less extensive than in controls. The lungs were mottled in appearance and edematous. The characteristic heart findings of subendothelial hemorrhages in the left ventricle were also present.

Ten dogs pretreated with larger amounts of chlorpromazine, 25 to 50 mg./Kg., also had the immediate primary blood pressure drop followed by return to normal but the secondary drop in 60 to 90 minutes was mild and the mean pressure had usually returned to normal by four hours following the endotoxin. Plasma losses and hematocrit increases were slight and only a moderate rise in plasma hemoglobin occurred. At 24 hours following the experiment there had been little change in these measurements over those measured in the first eight hours and six of these dogs survived permanently. These dogs did not have diarrhea and at sacrifice the bowel and lungs were normal although liver congestion and subendothelial hemorrhages in the left ventricle were usually present.

The four dogs in the group who did not survive permanently had a few significant pathologic changes at autopsy. The death of these dogs may well have been a result of the very large amounts of chlorpromazine used.

Dogs given Dibenzyline had the characteristic primary drop in blood pressure but the secondary fall in 60 to 90 minutes rarely went below 80 mm. Hg. By four hours following the endotoxin the mean blood pressure had returned to pre-experimental levels. There was little change in plasma volume or hematocrit. Plasma hemoglobin levels also increased only slightly. On the day following the experiment these dogs usually were quite alert in contrast to survivors pretreated with chlorpromazine and all plasma measurements were at pre-experimental levels. These dogs also did not get diarrhea and at sacrifice the bowel was normal as were the lungs. The only significant finding was liver congestion.

Hydrocortisone. Dogs pretreated with hydrocortisone differed conspicously from controls in their reaction to the endotoxin. The immediate blood pressure drop rarely carried below 85 mm. Hg and no secondary fall occurred in 60 to 90 minutes. There was no significant plasma loss or hematocrit increase in these dogs. Plasma hemoglobin increased slightly in the first eight hours but had returned toward normal by 24 hours. At sacrifice the only significant finding was a congested liver. The liver congestion was more pronounced in those dogs sacrificed one to two weeks following an experiment than in those sacrificed one to two days following an experiment.

Hypothermia. It made little difference in these dogs whether they were cooled just before or just after the endotoxin was given. The results almost uniformly paral-

leled those in the control group. Although hematocrit increase and plasma volume loss were somewhat less than controls the differences were not significant. Plasma hemoglobin increases and bloody diarrhea were a common occurrence before death. At autopsy the prominent findings were hemorrhagic necrosis of the mucosa of the small and large bowel and moderate liver congestion. The lungs usually appeared normal.

Treatment with Vasopressor Drugs. Dogs given metaraminol (Aramine®) to support blood pressure simultaneously with the injection of endotoxin followed an accelerated course to death. After three or four hours, these dogs were unresponsive to metaraminol and almost all died in five hours. Plasma losses were large and hematocrits almost doubled. Plasma hemoglobins rose precipitously to the highest levels recorded for any dogs in the entire study. At autopsy there was extensive hemorrhagic necrosis involving the entire mucosa of the small and large bowel. The bowel and liver were edematous. The lungs appeared normal.

Dogs receiving norepinephrine (Levophed®) to support blood pressure had a survival time almost identical to that of controls although systemic blood pressure was supported at normal levels throughout the course of the experiment. These dogs remained responsive to norepinephrine almost to the time of death. Plasma losses were large despite the fact that these dogs all received at least 250 ml. normal saline during the course of the norepinephrine drip. Plasma hemoglobin rose in these dogs almost to the levels seen in dogs treated with metaraminol. At autopsy the bowel was edematous and necrotic. Ischemic infarcts in the liver could be seen grossly. The lungs appeared normal.

Fluid Therapy. Replacing plasma losses in these dogs with whole blood or dextran maintained a normal systemic blood pressure in the early stages of the experiment but had little final value since survival time was not influenced. Bloody diarrhea was especially severe in those dogs receiving blood rather than dextran for replacement. Autopsy findings in these dogs were identical with those seen in the controls.

White Blood Cell and Platelet Counts. White counts fell precipitously in all dogs in all groups within 90 minutes after the endotoxin was given. The white cells then returned toward pre-experimental levels and if the dogs were alive the day following an experiment, a leukocytosis occurred. Platelets also fell with the white blood cells but not below 50,000. There were no significant differences between survivors and non-survivors in these findings.

Clotting Times. Although almost all dogs in the control group had an increase in clotting time of from 10 to 30 minutes, the period during the experiment when this increase occurred was variable. Some dogs had a progressive increase in clotting time until death while others had an initial increase in the first 90 minutes and then a return to normal by the time of death. Surviving dogs usually had only a moderate elevation in clotting time to eight or nine minutes in the first 90 minutes or else no change at all.

Bacteriology. Blood cultures taken from control dogs eight hours after endotoxin was given usually were negative or else occasionally contained Gram-positive organisms such as *Bacillus subtilis* which were considered contaminants. In contrast, a few cultures taken from chlorpromazine treated dogs, both survivors and non-survivors, 22 to 24 hours following the giving of endotoxin contained Pseudomonas aeruginosa. For this reason, a number of chlorpromazine treated dogs and an equal number of controls were given neomycin 0.25 Gm. intramuscularly before the administration of endotoxin and again 6 to 8 hours later when the dogs were returned to their cages. While this eliminated the occasional positive blood culture in chlorpromazine treated dogs, it did not alter survival time. The

control dogs given systemic neomycin also did not differ in survival time from untreated controls.

Discussion

Endotoxins of Gram-negative bacteria probably cause their lethal effects in the experimental animal by means of their ability to act as potent sympathomimetic agents or by sensitizing the animal to levels of endogenous sympathomimetic agents which are ordinarily not toxic. Following the application of endotoxin to the rabbit ear or rat mesoappendix, Zweifach [3,4] and others [2] observed an intense vasospasm, alternating with brief periods of vasodilation, which persisted for several hours. Similarly in the dog, we have observed the bowel and its mesentery following the intravenous injection of endotoxin. Initially the bowel contracted into a tight narrow cord and this was followed by violent peristaltic rushes. Simultaneously the vessels in the mesentery constricted until they were scarcely visible. This constriction was maintained for several hours and was accompanied by the appearance of punctate hemorrhages in the mesentery. The mucosa of the bowel which was at first remarkably pale gradually became congested until fluid, first clear and later bloody, leaked into the lumen of the bowel. Correlating with these changes there was a marked increase in resistance to flow in the radicals of the superior mesenteric artery. Using a Sigmamotor pump, we delivered a normal flow of blood, 10 ml./Kg./min., at a constant rate into the superior mesenteric artery of an anesthetized dog at a mean pressure of 100 mm. Hg. Following the intravenous injection of endotoxin the mean perfusion pressure increased to 300 mm. Hg with this same flow. We did not find the same increase in resistance to flow when similar amounts of blood were pumped into the head via a carotid artery after the giving of endotoxin.

It is evident that the sympathomimetic action of endotoxin on the bowel and its mesentery can create a profound degree of bowel ischemia leading to an increase in vessel permeability, plasma loss, and eventually irreversible shock due to bowel necrosis, a picture not unlike that seeen in chronic epinephrine shock.[9] The protection afforded by chlorpromazine or Dibenzyline is apparently a result of their ability to block the sympathomimetic effect of endotoxin. Although both drugs are also effective in blocking serotonin and histamine, these effects do not seem to be of importance in these studies since we have not found that substances which specifically block serotonin and histamine action are effective in preventing endotoxin shock. The central nervous system depression caused by chlorpromazine also does not seem to be of importance in the protection afforded by this drug because Dibenzyline, which is even more effective against endotoxin than chlorpromazine, has an excitatory effect on the central nervous system.[11] A greater degree of protection could possibly be obtained with chlorpromazine if it was administered in multiple small doses throughout the course of the experiment rather than in a single large dose before the endotoxin injection. Abernathy and Spink [1] have shown that this manner of giving chlorpromazine is effective in protecting mice against a lethal dose of endotoxin.

Further evidence for the importance of the bowel in the development of irreversible endotoxin shock is obtained from dogs in Group III. The removal of the major portion of the intestine increased survival time even though the bleeding diathesis resulting from the endotoxin denied us the opportunity to observe whether this procedure would also prevent the death of the dogs. The cause of the increased bleeding and clotting times in dogs given endotoxin is unexplained and this phase of the problem is currently being explored. A

plausible explanation of the bleeding problem might be the thrombocytopenia which occurs as a result of endotoxin administration. But, in our studies we did not observe platelet counts to reach levels which ordinarily are associated with a bleeding tendency.

The mode of action of hydrocortisone in these experiments is also uncertain although indirect evidence indicates that it also in some manner protects the animal against the pernicious effects of prolonged vasoconstriction or perhaps decreases the intensity or the duration of the vasospasm. In support of this belief are the results of recent experiments in this laboratory in which pretreatment with hydrocortisone protected dogs against the lethal effect of high concentrations of epinephrine given intravenously.

The use of intestinal antibiotics to protect the bowel against the deleterious effect of ischemia has become a disputed point. Fine and colleagues [13, 28] have reported favorable results in both hemorrhagic and endotoxin shock with the use of intestinal or systemic antibiotics. On the contrary we have found,[19] as have others,[10] that bowel sterilization does not prevent the development of irreversible hemorrhagic shock; nor in the present endotoxin experiments could we detect any benefit from the use of either intestinal or systemic antibiotics. While intestinal antibiotics definitely appear to protect a denuded intestinal anastomosis from ischemic breakdown,[26] it seems to us in this example that the element of intense vasospasm present in hemorrhagic or endotoxin shock is absent. The recent reports that germ-free animals are not more resistant to either hemorrhagic [35] or endotoxin shock [22] further support this impression.

Hypothermia has also been reported to protect ischemic bowel when the ischemia is a result of temporary occlusion of the superior mesenteric artery.[25] As with the use of antibiotics, we did not find that this procedure had any value in endotoxin shock whether used prior to or following the injection of endotoxin. The decrease in metabolic activity resulting from the lowered temperature was evidently not sufficient to protect the bowel from the degree of ischemia occurring during endotoxin shock since hemorrhagic necrosis in the bowel was a frequent finding in these dogs. Moreover, the contracted blood volume of hypothermic dogs [33] probably leaves them less able to compensate for the plasma losses resulting from the action of endotoxin.

If the basic pathology of endotoxin shock is based on its sympathomimetic properties it is easily seen why sympathomimetic drugs such as metaraminol and norepinephrine are no more effective in endotoxin shock than they previously were in hemorrhagic shock.[8] There is no doubt that early after endotoxin is given metaraminol and norepinephrine do increase cardiac output by increasing venous return to the heart, as reported by Weil [32] and others,[3, 6] but this is apparently done at the expense of even greater vasospasm and resultant ischemia in the viscera. The mere raising of blood pressure in the large arteries in shock is probably a specious accomplishment and bears little relationship to actual blood flow to the tissues. The fact that norepinephrine did not potentiate endotoxin shock to the degree that metaraminol did correlates with the recent reports of Thomas and Zweifach on the combined effects of endotoxin and epinephrine or norepinephrine.[34] They found that the use of epinephrine in combination with endotoxin increased the amount of skin slough in the rabbit when injected locally and also markedly increased the degree of vasospasm of the vessels in the rat mesoappendix. The enhancement of endotoxin effect was not as great when norepinephrine was substituted for the epinephrine.

It is well to recall that replacement of plasma losses was of no tangible value in

these experiments if nothing was done to alter the basic pathology causing the plasma loss. The replaced fluids merely accumulated in the already congested bowel. Similar results occur when transfusions are employed in irreversible hemorrhagic shock.[7]

In indicting the bowel as the prime organ responsible for the development of the irreversible state, changes in other organs also contributing to the development of this state can not be overlooked. Two organs in particular may be of importance. The liver has been shown by MacLean and colleagues [21] to be responsible for the initial drop in blood pressure after endotoxin administration. Using a delicate weighing device they showed that sudden hepatic vasospasm trapped large volumes of blood in the liver and portal system. This suddenly reduced venous return to the heart and lowered cardiac output. This vasospasm in the liver was short lived, however, and hepatic resistance returned toward normal in a few minutes releasing most of the trapped blood to the systemic circulation. The secondary blood pressure fall in 60 to 90 minutes was also shown by them to be correlated with an increase in intestinal weight with little change in liver weight. At autopsy we found liver congestion in almost all dogs dying of endotoxin shock but surviving dogs treated with hydrocortisone or adrenergic blocking agents also had liver congestion of a similar degree when sacrificed. On the basis of present evidence, the role of the liver in causing irreversible endotoxin shock does not appear to be crucial.

Lung changes were not prominent in the majority of dogs dying of endotoxin shock in these experiments. Nevertheless in those dogs partially protected with small doses of chlorpromazine and living for 18 to 24 hours the lung findings assumed more importance. These dogs often had obvious pulmonary edema by auscultation and at autopsy had heavy congested lungs with only moderate changes in the bowel. The same finding was observed in the occasional control dog living six to eight hours longer than the average survival time. Kuida [15] has recently reported that the basis for these findings in the lungs is a vascular response predominantely in the pulmonary venules or small veins.

Finally, what is the basis for the development of irreversible shock due to bowel necrosis if bacteria are not involved? The rising plasma hemoglobin of dogs dying of endotoxin shock indicates that there is free passage for the absorption of breakdown products from the necrotic mucosa into the systemic circulation. Moreover, Landy [16] has shown that polysaccharide complexes can be derived from almost all mammalian tissues which have "endotoxic" activity when they are released from their native state of firm combination with other cellular components. Certainly the presence of large amounts of damaged tissue in the bowel would furnish a vast reservoir for the release into the systemic circulation of such "toxic" endogenous polysaccharide complexes.

Summary

Plasma loss, hematocrit increase, plasma hemoglobin increase, and hemorrhagic necrosis of the bowel, all of which characterize irreversible shock due to endotoxin, apparently result from sympathomimetic action of endotoxin on the bowel. Agents which have an adrenergic blocking effect prevent these deleterious effects of endotoxin and prevent death while the vasopressor drugs which are commonly used to treat this type of shock either are without effect or else actually potentiate the shock caused by endotoxin by increasing intestinal ischemia.

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References

1. Abernathy, R. S., F. Hallberg and W. W. Spink: Studies on the Mechanism of Chlorpromazine Protection Against Brucella Endotoxin in Mice. J. Lab. & Clin. Med., **49**:708, 1957.
2. Armin, J. and R. T. Grant: The Vasoconstriction Caused by a Pyrogen. J. Physiol., **138**: 417, 1957.
3. Collier, H. D., F. H. Meyers and G. H. Schmitt: Hemodynamic Effects of Infusions of Epinephrine and Arterenol in Normal and Shocked Dogs. Am. J. Physiol., **189**:224, 1957.
4. Delaunay, A., P. Boquet, J. Lebrun, Y. Lehoult and M. Delaunay: Le Mode d'Action des Endotoxines Bacteriennes. IV. Les Troubles Vaso-Moteurs Chez les Animaux Intoxiques et Leurs Consequences. J. Physiol. (Paris), **40**:89, 1948.
5. Flink, E. B., and C. J. Watson: A Method for the Quantitative Determination of Hemoglobin and Related Heme Pigments in Feces, Urine and Blood Plasma. J. Biol. Chem., **146**:171, 1942.
6. Fowler, N. O. and R. Franch: Mechanism of Pressor Response to l-Norepinephrine During Hemorrhagic Shock. Circulation Res., **5**:153, 1957.
7. Frank, H. A., A. M. Seligman and J. Fine: Traumatic Shock. X. The Treatment of Hemorrhagic Shock Irreversible to Replacement of Blood Volume Deficiency. J. Clin. Invest., **24**:435, 1945.
8. Frank, E. D., H. A. Frank, S. Jacob, H. A. E. Weizel, H. Korman and J. Fine: Effect of Norepinephrine on Circulation of the Dog in Hemorrhagic Shock. Am. J. Physiol., **186**: 74, 1956.
9. Freeman, N. E.: Decrease in Blood Volume After Prolonged Hyperactivity of the Sympathetic Nervous System. Am. J. Physiol., **103**:185, 1933.
10. Hardy, E. G., G. C. Morris, Jr., E. M. Yow, B. W. Haynes, Jr. and M. E. DeBakey: Studies on the Role of Bacteria in Irreversible Hemorrhagic Shock in Dogs. Ann. Surg., **139**:282, 1954.
11. Huidobro, F.: Some Pharmacological Properties of Chloro-3 (Dimethylamine-3′ Propyl) 10-Phenothiazine or 4560 R.P. (Chlorpromazine- Largactil). Arch. int. pharmacodyn., **98**:308, 1954.
12. Hershey, S. G., B. W. Zweifach and B. S. Metz: An Evaluation of the Protective Action of Autonomic Blocking Agents in Peripheral Circulatory Stress. Anesthesiology, **15**:589, 1954.
13. Jacob, S., H. Weizel, E. Gordon, H. Korman, F. Schweinburg, H. Frank and J. Fine: Bacterial Action in Development of Irreversibility to Tranfusion in Hemorrhagic Shock in the Dog. Am. J. Physiol., **179**:523, 1954.
14. Kelan, M. and B. W. Zweifach: Influence of Partial and Complete Evisceration on the Action of Drugs Protecting Against Lethal Hemorrhage. Surg., Gyn. & Obst., **99**:707, 1954.
15. Kuida, H., L. B. Hinshaw, R. P. Gilbert and M. B. Visscher: Effect of Gram-Negative Endotoxin on Pulmonary Circulation. Am. J. Physiol., **192**:335, 1958.
16. Landy, M. and M. J. Shear: Similarity of Host Responses Elicited by Polysaccharides of Animal and Plant Origin and by Bacterial Endotoxins. J. Exp. Med., **106**:77, 1957.
17. Lillehei, R. C.: The Intestinal Factor in Irreversible Hemorrhagic Shock. Surgery, **42**: 1043, 1957.
18. Lillehei, R. C.: The Relationship of the Appearance of an Abnormal Plasma Hemin Pigment to the Development of Irreversible Hemorrhagic Shock in Dogs. Circulation Res., **6**:438, 1958.
19. Lillehei, R. C.: Unpublished Data.
20. Lowry, A. H. and O. A. Bessey: The Adaptation of the Beckman Spectro-Photometer to Measurements of Minute Quantities of Biological Materials. J. Biol. Chem., **163**:633, 1946.
21. MacLean, L. D. and M. H. Weil: Hypotension (Shock) in Dogs Produced by Escherica Coli Endotoxin. Circulation Res., **4**:546, 1956.
22. McNulty, W.: Department of Experimental Biology, Walter Reed Army Institute of Research, Washington, D. C., Personal Communication, 1957.
23. Noyes, H. E., J. P. Sanford and R. M. Nelson: Effect of Chlorpromazine and Dibenzyline on Bacterial Toxins. Proc. Soc. Exp. Biol. Med., **92**:617, 1956.
24. Overton, R. C. and M. E. DeBakey: Experimental Observation on the Influence of Hypothermia and Autonomic Blocking Agents on Hemorrhagic Shock. Ann. Surg., **143**:439 1956.

25. Parkins, W. M., M. Ben and H. M. Vars: Tolerance of Temporary Occlusion of the Thoracic Aorta in Normothermic and Hypothermic Dogs. Surgery, **38**:38, 1955.
26. Rabinovici, N. and J. Fine: The Effect of Aureomycin on the Intestine Subject to Vascular Injury. Ann. Surg., **135**:344, 1952.
27. Remington, J. W., W. F. Hamilton, G. H. Boyd, Jr., W. F. Hamilton, Jr. and H. M. Caddell: Role of Vasoconstriction in the Response of the Dog to Hemorrhage. Am. J. Physiol., **161**:116, 1950.
28. Schweinberg, F. B., D. Davidoff, I. H. Konen and J. Fine: Host Resistance to Bacteria in Hemorrhagic Shock. VI. Effect of Endotoxin on Antibacterial Defense. Proc. Soc. Exp. Biol. Med., **92**:662, 1956.
29. Stitt, E. R., P. W. Clough and S. E. Branham: Practical Bacteriology, Hematology, and Parasitology. Philadephia, Blakiston Co., 1948, p. 401.
30. Thomas, L. and R. T. Smith: Effect of Cortisone on Response to Endotoxin in Mature Rabbits. Proc. Soc. Exp. Biol. Med., **86**:810, 1954.
31. Weil, M. H., L. B. Hinshaw, M. B., Visscher, W. W. Spink and L. D. MacLean: Hemodynamic Effects of Vasopressor Agent (Mataraminol) on Hypotension in Dogs Produced by Endotoxin. Proc. Soc. Exp. Biol. Med., **92**:610, 1956.
32. Weil, M. H., L. D. MacLean, W. W. Spink and M. B. Visscher: Studies on the Mechanism of Shock Produced by Gram-Negative Bacteria. Trans. Ass. Am. Phy., **69**:131, 1956.
33. Wilson, J. M., S. B. Marshall, V. Beresford, V. Montgomery, D. Jenkins and H. Swan: Experimental Hemorrhage: The Deleterious Effect of Hypothermia on Survival and a Comparative Evaluation of Plasma Volume Changes. Ann. Surg., **144**:697, 1956.
34. Zweifach, B. W., A. L. Nagler and L. Thomas: The Role of Epinephrine in the Reactions Produced by the Endotoxins of Gram-Negative Bacteria. II. J. Exp. Med., **104**: 881, 1956.
35. Zweifach, B. W., H. A. Gordon, M. Wagner and J. A. Reyniers: Irreversible Hemorrhagic Shock in Germfree Rats. J. Exp. Med., **107**: 437. 1958.

Discussion

Dr. Howard A. Frank (For Dr. Jacob Fine): Dr. Fine is tremendously interested in this subject and greatly regrets his inability to be here this afternoon. He has asked me to read his comments, based on the abstract and conversation with Dr. Lillehei.

These are Dr. Fine's comments: Dr. Lillehei offers the thesis that the intestinal necrosis which is present in dogs in shock produced by injected endotoxins is responsible for the irreversibility in hemorrhagic shock, and in 1956 we isolated a toxic factor from the blood of dogs and rabbits in hemorrhagic shock which is the cause of the irreversibility and of the intestinal lesion. This toxin is a lipopolysaccharide which has since been identified as endotoxin, derived from the intraintestinal bacteria. We have shown that the toxin is also present in the blood of tourniquet shock, and of non-bacterial septic shock. In 1955, Thomas demonstrated that endotoxins potentiate epinephrine so as to produce hemorrhagic necrosis. He, together with Zweifach, then showed that when these compounds are given together, they produce a poor peripheral flow, and finally fracture and leakage from the capillaries not only in the skin, but in other areas as well. Thus the damage is widspread, although in the dog it is more obvious in the intestine than elsewhere. We have published data confirming the protective effect of dibenamine in hemorrhagic shock, first demonstrated by Harold Wiggers and later by Remington, and that this protection occurs not only in hemorrhagic shock, but also in shock induced by a one-hour occlusion of the superior mesenteric artery. In both conditions the dibenamine prevents the intestinal lesion. But whether it acts simply as an antiadrenergic agent is by no means clear. In a recent issue of the Blue Journal, we give evidence suggesting that dibenamine may in fact act directly as an antiendotoxic agent.

Dr. Lillehei's data, like our own, show that the intestinal lesion is the same in endotoxic shock and in hemorrhagic shock. This makes it difficult to escape the conclusion that there is no fundamental difference between the irreversibility of endotoxic shock and of hemorrhagic shock. When hemorrhage is the agent employed to initiate the shock state, endotoxins invading from the gut will eventually produce generalized vascular collapse. When endotoxins are the agent employed to initiate the shock state, they or the endotoxins from the gut will eventually produce generalized vascular collapse. This is because the defense against endotoxins, which lies in the reticulo-endothelial system, is lost. In the case of hemorrhage, this defense is lost because of prolonged poor flow. In the case of endotoxin, this defense is lost because