EVIDENCE FOR A LETHAL ENDOTOXEMIA AS THE FUNDAMENTAL FEATURE OF IRREVERSIBILITY IN THREE TYPES OF TRAUMATIC SHOCK*

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Several lines of evidence indicate that dogs and rabbits develop a lethal endotoxemia in severe and prolonged hemorrhagic shock as a result of damage to the detoxifying potential of the RE system (1). The evidence also indicates that the endotoxin is derived primarily from the Gram-negative flora in the intestine (2). If this is a correct hypothesis, a lethal endotoxemia should be demonstrable in all types of severe and prolonged traumatic shock, because the hemodynamic disorder in most respects is the same in all of them. Studies were undertaken to see if this is so in two other types of shock, *i.e.*, hypovolemic septic shock and tourniquet shock. This report presents evidence confirming the hypothesis that a lethal endotoxemia develops in other types of shock as well as in hemorrhagic shock, and for the same reasons.

Hypovolemic Septic Shock

Method.—

Dogs were used exclusively because rabbits do not lend themselves to the production of septic shock. Hypovolemic septic shock was induced by a method described in a previous publication (3). Briefly summarized, the method is as follows: An exudative peritonitis is produced in dogs fasted for 12 hours by an intraperitoneal injection of 15 ml. of a heavy suspension of normal dog feces in saline, filtered through gauze. Profound shock develops within 1 to 3 hours. The peritoneal cavity accumulates a considerable volume of hemorrhagic exudate with a resulting severe hypovolemia. All the hemodynamic features of profound peripheral vascular collapse are present, including hemoconcentration, which is also characteristic of burn shock and of tourniquet shock. At postmortem examination of the untreated dog, which dies in 3 to 9 hours, the peritoneal cavity shows a violent inflammatory reaction with some 350 ml. or more of bloody purulent fluid.

Plasma, given in large quantities repeatedly or continuously to correct the hypovolemia, escapes almost immediately into the peritoneal cavity, and so does not alleviate the shock or prevent death. Nor do antibiotics given intravenously at the time of injection of the fecal suspension, or afterward. But antibiotics given *prior* to the injection produce recovery in spite

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of the denial of plasma volume therapy for the hypovolemia. After some 12 hours of severe shock, the pulse slows, urine flow begins, cardiac output rises, the peritoneal fluid begins to be resorbed, and is fully resorbed within 36 hours (3).

The foregoing type of hypovolemic septic shock was induced in a series of 13 adult dogs. They received no treatment, and were exsanguinated into bottles containing 2 ml. of heparin after the blood pressure had fallen to 60 mm. Hg.[1] Immediately thereafter the peritoneal exudate was removed as completely as possible, and also put into heparinized flasks. There was a rough inverse correlation between the volume of exudate and the volume of blood harvested from each dog. Although the plasma was always sterile on culture, it was put through a Seitz filter and held at −30°C. until used. The peritoneal exudate, which was heavily laden with bacteria, was also Seitz-filtered, and held at −30°C. until used. After sterility of both filtrates was assured, the plasma or exudate from each dog was tested for the presence of endotoxin by intravenous injection into test recipients.[2]

After 2 hours of hemorrhagic shock thirteen test recipients received plasma in volumes ranging from 50 to 120 ml. Thirteen additional test recipients received exudate in volumes ranging from 50 to 97 ml. At the same time, all test recipients also received all of their own shed blood, minus an amount of plasma equal to the volume of test plasma or exudate injected.

Results.—Of thirteen recipients of plasma twelve promptly developed a bloody diarrhea, remained prostrate and in shock, and died before the next morning or within 36 hours. The thirteenth dog behaved like the others except that it recovered slowly. In all twelve dogs the postmortem findings were exactly like those in recipients of irreversible hemorrhagic shock blood.

Of thirteen recipients of exudate eleven died in 18 to 36 hours, with the same manifestations as the recipients of plasma. The two survivors behaved similarly, but recovered slowly.

Of four normal dogs which received plasma or exudate in double the volume administered to test recipients in shock, none were ill and all survived without incident.

Comment.—Thus it is plain that the plasma and the peritoneal exudate in hypovolemic septic shock contain a toxin not unlike the endotoxin in the blood of severe and prolonged hemorrhagic shock. Since less than 100 ml. of either plasma or exudate was nearly always lethal, whereas less than 100 ml. of hemorrhagic shock blood or plasma was only occasionally fatal, the concentration of toxin appears to be greater in the plasma or exudate of septic shock blood.

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[1] The bleed-out volume becomes too little if bleeding is attempted at lower pressures.

[2] In a previous paper (4) we showed that dogs in hemorrhagic shock for 2 hours recover if transfused with their own shed blood, but die if part of the transfusion is blood or plasma from a dog dying of irreversible hemorrhagic shock. Those transfused only with their own blood, if killed 12 to 24 hours later, show no disorder. But recipients of irreversibly shocked donor blood show the same pathology as the donor dogs; *i.e.*, strongly hemorrhagic fluid in the gut, a varying amount of necrotic mucosa in the colon, and jejuno-ileum, and inconstant foci of hemorrhage in the mesenteric nodes, lung, and elsewhere. The conversion of reversible hemorrhagic shock to irreversible hemorrhagic shock in these test animals is taken as evidence of the presence of a toxin in the donor blood. This toxin, as stated above, has been extracted and shown to be an endotoxin (5).

The presence of toxin in septic shock plasma would appear to be as expected; *i.e.*, it accords with the assumption that if a septic peritonitis due to Gram-negative bacteria exists, endotoxin generated in the peritoneal cavity by proliferating bacteria can get into the circulation and produce endotoxemic shock. Moreover, when there is endotoxemia as well as hypovolemia one would not expect the shock to yield to plasma volume therapy alone. This is in accord with experience. But if it is true that the intraperitoneal bacteria are the source of the endotoxemia, then antibiotics given parenterally at the time of the injection of the fecal suspension or soon thereafter should be helpful, because they can be expected to inhibit in some degree the proliferation of the injected bacteria; whereas the same antibiotics given considerably in advance of this injection should not be as helpful, because they cannot be expected to be as effective in halting proliferation of the bacteria injected some hours later. The observed facts, however, were quite the reverse, as we have already stated. Since the administration of non-absorbable antibiotic given orally in advance of the injection was far superior to the parenteral administration of antibiotic with or after the injection, the intraintestinal flora must be regarded as the main site of action of antibiotic given in advance. Thus the intraintestinal flora appear to be a more important factor in the pathogenesis of the *refractory* state of shock than the bacteria injected to produce the shock. Other indirect evidence that bacterial proliferation in the peritoneal cavity is only a minor source of the circulating endotoxin is a previously reported observation that a boiled fecal suspension (proved sterile on culture) is no less lethal than the same preparation unboiled. Therefore the main source of the circulating endotoxin must be the intestinal flora, since this is the only other pool from which endotoxin can be drawn.

Direct evidence that the intestinal flora and not bacteria in the peritoneal cavity is the main source of the endotoxemia, and the cause of the refractory state of shock, was obtained from the following experiments: Three dogs were treated orally with 300 mg. of polymyxin and three with 300 mg. of neomycin daily for 3 days.[3] The powdered antibiotic was mixed with fresh ground meat and fed by hand to assure intake. A final dose of 500 mg. was given 3 hours before injection of the fecal suspension. Stool specimens taken at this time showed absence of *E. coli* and coliform bacilli, and presence of *Clostridia*, *Bacillus subtilis*, a few *Bacillus proteus* or *Pseudomonas*. The six dogs were exsanguinated after 6 hours in shock. The exudate was collected. Both plasma and exudate were Seitz-filtered and tested in vulnerable recipients, using an amount of plasma varying from 120 to 300 ml. and of exudate varying from 80 to 175 ml.

Results.—All of the twelve dogs tested survived even though the volume of

[3] Since only a tiny fraction of neomycin and none of polymyxin are absorbed from the gut, their action may be considered to have been exerted on the intraintestinal flora exclusively.

test material administered was considerably larger than in the previous experiments. The plasma from the 3 neomycin-pretreated dogs produced no sign of any disturbance. The plasma from the 3 polymyxin-pretreated dogs made 2 of the 3 recipients ill with a moderate non-bloody diarrhea and mild weakness, from which they recovered rapidly. The peritoneal exudate caused slight to moderate illness with some bloody diarrhea and marked weakness in all six recipients, but these recovered completely after several days. Thus, although the peritoneal exudate in these experiments was more toxic than the plasma, as expected, the major source of the circulating endotoxin is not the toxin in the exudate, but the pool of endotoxin in the gut, just as is the case in hemorrhagic shock.

The greater toxicity of the blood of the dog in septic shock than the blood of hemorrhagic shock may in part be owing to a contribution of endotoxin from the peritoneal infection. It may also be owing to greater injury to the RE system in septic shock than in hemorrhagic shock.

Comment.—The cumulative evidence indicates that the primary process set in motion by the intraperitoneal injection of a fecal suspension, whether sterile or non-sterile, is the violent inflammatory reaction to a chemical irritant, leading to a rapid and huge loss of plasma, with a resulting profound degree of shock with hemoconcentration. Plasma volume therapy cannot correct the hypovolemia. With the persistence of the profound hypovolemic shock, an endotoxemia leading to irreversibility develops for the same reason that it does in prolonged severe hemorrhagic shock. But if the intraintestinal pool of endotoxin is substantially reduced by certain effective antibiotics, the endotoxemia does not develop, and the animal survives a very long exposure to severe hypovolemic shock. Thus there is no essential difference between the pathophysiology of irreversibility in septic and hemorrhagic shock. The following experiments were undertaken to see whether this can also be said of tourniquet shock.

Tourniquet Shock

We are here not concerned with the shock produced in rats by applying tourniquets for 4½ hours, for this type of shock is readily reversible at any time by giving saline solution even after many hours (6). In this study we produced tourniquet shock in dogs by tourniquets applied for 8 or more hours to both legs. This shock is regularly irreversible and fatal regardless of the therapy employed. The injury produced allows a rapid and enormous plasma loss following removal of the tourniquets. This loss can be stopped by amputation of the leg, by compression, or by reapplication of the tourniquets. But plasma infusions are futile just as they are for hypovolemic septic shock, and for the same reason (7).

Because bacteria can be cultured from the damaged tissues, and because these tissues have been so devitalized that they become infected and slough in the

surviving dog, the problem of tourniquet shock has been regarded as a complex of sepsis, hypovolemic shock, and toxic tissue products too complicated for a rewarding analysis. Recently, on reconsideration of the problem, it appeared to us that this type of shock should be comparable to hypovolemic septic shock in most particulars, except for the method of induction: There is massive plasma loss, and the hemodynamics and the general course of events are virtually the same. And because bacteria can be recovered from the injured tissues, experimental studies were set up to see whether there is also an endotoxemia.

The method employed was as follows: Elastic pneumatic cuffs were placed high on both thighs of healthy dogs under light intravenous nembutal anesthesia (5.5 ml. of a 4 per cent solution per kg. body weight with 60 mg. morphine added). The cuffs were inflated rapidly to a pressure of 350 mm. Hg and were left at this pressure for 9 to 12 hours. Nembutal or morphine or both were repeated as needed to insure the absence of distress. Just prior to removal of the cuffs one carotid artery was cannulated for blood pressure readings. Swelling of the thighs occurred rapidly following removal of the tourniquets, and blood pressure began to fall shortly thereafter. When it reached 60 mm. Hg some 2 to 3 hours later, the dogs were exsanguinated into sterile bottles containing 2 ml. heparin. The amount of blood recovered varied from 200 to 315 ml. (Exsanguination at lower blood pressures gave too poor a yield.)

Results.—Each of eight test recipients received donor blood in a volume varying from 215 to 350 ml. One dog survived after exhibiting a bloody diarrhea and prostration for 2 days. The other 7 developed copious bloody diarrhea, and remained prostrate and in shock until death, which occurred within 36 hours, but in most instances much earlier. Postmortem findings revealed a nearly complete desquamation of the mucosa from midjejunum to anus, the submucosa being hemorrhagic and strongly edematous. There were also numerous focal hemorrhages in the gut wall, lungs, and mesenteric lymph nodes. Six of the seven dead dogs showed from 10 to 70 ml. of bloody peritoneal fluid.

Two normal dogs, each injected intravenously with 300 ml. of tourniquet shock blood, were weak and had slight diarrhea for 3 days, but recovered quickly.

Comment.—The injury found at postmortem in these recipients was basically the same as that found in recipients of blood of dogs in irreversible hemorrhagic or septic shock. The pathology was much more pronounced, but this may be owing to the greater volume of toxic material administered.

Bacteriological study of the tourniqueted muscles was made in three dogs. Biopsies of thigh muscle were taken at several loci under sterile precautions before application of the tourniquets, immediately before removal of the tourniquets, and again immediately before exsanguination in shock. The muscle tissue was flamed briefly, then ground in sterile saline, and then cultured on aerobic and anaerobic bloodplates and in aerobic and anaerobic thioglycollate. The

latter cultures were observed for 10 days, during which they were smeared and subcultured on blood plates when indicated. Gram-negative bacteria were found in only one dog, while *Clostridia* were recovered in all three. But the number of positive cultures and the number of colonies in the culture from each locus during the shock period was only moderately higher than that in the normal muscle, or in the muscle while the tourniquet was applied. This number was too small to permit the view that exotoxin in any significant quantity was produced.[4] Even if some was produced, such exotoxin in our hands has been found by guinea pig test to be of extremely low potency. Since no significant amount of endotoxin or exotoxin was produced in the leg, a study of the intestinal flora as the major source of the circulating toxin was instituted, to see whether it could account for the circulating endotoxin. This study was done as follows: Six dogs were pretreated, three with neomycin and three with polymyxin, as already described above. Tourniquet shock was induced and exsanguination performed after the pressure had fallen to 60 mm. Hg. The volume of blood varied from 150 to 300 ml. Each specimen was promptly transfused into a 2 hour shock recipient.

Results.—One of the six recipients, which received the largest volume of blood tested (350 ml.), died within 24 hours, with profuse bloody diarrhea and progressive weakness. The other 5 were all quite sick for 3 to 5 days with weakness and moderate diarrhea, and an occasional slightly bloody stool. But all five recovered.

Comment.—The results are entirely comparable to those obtained in the study of hypovolemic septic shock, and the same conclusion is warranted; *i.e.*, that most, if not all, of the circulating endotoxin is derived from the intestinal flora. This finding explains why we were unable in a previous unreported study to obtain a difference in the toxicity of venous blood from the tourniqueted extremity and blood from an uninjured region.

The death in tourniquet shock cannot be attributed solely to the endotoxemia because the uncompensated severe plasma volume deficit continues until death, which occurs within an average of 5 to 7 hours following release of the tourniquet. But because (*a*) dogs in hypovolemic septic shock tolerate such a deficit when endotoxemia has been eliminated much longer than these dogs do, (see above) and (*b*) plasma volume therapy alone does not delay death by more than a few hours, we regard the endotoxemia to be the major factor in the lethal outcome.

Although we have as yet no data on the possibility that the essential feature of fatal shock from burns is also an endotoxemia, the observations here reported indicate the likelihood of such a finding, once an appropriate experimental model for such a study is developed.

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[4] Only thioglycollate yielded slight bacterial growth. All blood agar plates remained sterile throughout.

To put the issue in more general terms, it is proper to say that when extensive tissue injury causes hypovolemic shock which becomes refractory to plasma volume therapy, it is because of the development of an endotoxemia. The pool of endotoxin in the intestine rather than the site of injury is likely to be the major source of the circulating toxin, unless perchance a severe infection by Gram-negative bacteria is present. Since there are no significant differences in the essential nature of the irreversible state in the three types of shock studied, it is appropriate to suggest that a lethal dose of endotoxin derived from the intestine is the factor responsible for irreversibility in traumatic shock, whatever the precipitating cause of the shock may be.

TABLE I

Effect of Donor Material on Test Recipient

Donor		Material tested	Result	
Type	No.		Survival	Death
Septic shock (peritonitis)	13	Plasma	1	12
" " " A.P.*	6	Plasma	6	0
Septic shock (peritonitis)	13	Peritoneal fluid	2	11
" " " A.P.	6	Peritoneal fluid	6	0
Tourniquet shock	8	Blood	1	7
" " A.P.	6	Blood	5	1

* A.P., pretreatment with antibiotics.

SUMMARY AND CONCLUSION

The data here reported (Table I) show that a toxin is present in the blood of animals with two types of irreversible hypovolemic shock. These data also show that although blood volume therapy does not correct the hypovolemia because of continuing loss of plasma at the site of injury, the major factor in the progressive decline and death is the endotoxemia rather than the hypovolemia. This is also true of severe and prolonged hemorrhagic shock that is irreversible to transfusion. The data also show that even when there is bacterial activity at the site of injury, the pool of endotoxin in the intestine is the chief source of the circulating endotoxin.

In all three types of shock, the endotoxemia develops because persisting hypovolemic shock renders the RE system unable to destroy the endotoxin.

The demonstration of an endotoxemia as the cause of irreversibility and death in three types of traumatic shock caused by three different agents suggests that a single pathophysiological mechanism accounts for the phenomenon of irreversibility in all types of traumatic shock.

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