Host Resistance in Hemorrhagic Shock. IX. Demonstration of Circulating Lethal Toxin in Hemorrhagic Shock.* (23316)

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In previous publications of this series evidence was given that hemorrhagic shock inflicts severe damage on the host's antibacterial defense mechanisms(1-6). Impairment of these mechanisms carries a threat to the host if bacteria are already in the tissues, or if they or their toxic products gain access to the circulation during shock. Except for clostridia in the tissues of the dog,† most of the tissues and the blood of the dog, rabbit and rat during hemorrhagic shock do not as a rule yield any bacteria by conventional cultural technics. Nevertheless there are data strongly indicating that bacteria or their toxins are operating in hemorrhagic shock, and, if the shock is allowed to persist long enough, that they render the peripheral vessels refractory to transfusion(7-9). In the absence of evidence from cultural data of the presence of bacteria within the blood or tissues, we proceeded to search for a toxin in the circulating blood during shock. A leucotoxin in the plasma of advanced hemorrhagic shock was identified by an *in vitro* comparison of the effect of plasma from normal and shocked animals on phagocytic and bacteriostatic activity of phagocytes(7). To establish the thesis that the refractory state of shock resulting in death is due to bacterial activity, a toxin of bacterial origin, which induces a state of irreversibility to transfusion, must be identified. The data which follow demonstrate the presence of a lethal toxin in the blood of the dog and rabbit in prolonged hemorrhagic shock, and indicate that intestinal bacteria are the source of the toxin.

Method. Dogs were put into hemorrhagic shock by the elevated reservoir technic(10) and allowed to remain in shock until they became irreversible to transfusion. One or 2 hours after transfusion was shown to be ineffective, *i.e.* when the blood pressure had again fallen below 60 mm of Hg, they were considered preterminal and were exsanguinated. This occurred in nearly every case within 6 hours after inducing shock. The blood so removed, or its plasma, was immediately transfused into a recipient, or stored overnight at 4°C and infused into a recipient the next day. The donor blood or plasma was cultured in every case. With few exceptions which yielded contaminants,‡ the cultures were found to be sterile. For control data blood or plasma from healthy dogs was treated and used in the same way. The recipients were either healthy dogs, or dogs exposed to severe hemorrhagic shock for 2 hours. They served as test animals for the presence of a toxin in the blood of irreversible shock. Normal dogs can be expected to detoxify a considerable amount of toxin. But dogs in shock for 2 hours, which recover when transfused with normal blood or with their own shed blood at 2 hours, might not recover if transfused at

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† Clostridia in the dog's tissues are considered not to be of primary importance because (1), polyvalent clostridial antitoxin in large doses is of no prophylactic or therapeutic value; (2), neomycin was among the most effective antibiotics which prevented development of irreversibility to transfusion(8), and (3), as will appear below, non-absorbable antibiotics given orally in advance of producing shock prevent appearance of a lethal toxin in the blood of dogs in advanced shock.

‡ Three of 12 donor bloods yielded a coagulase negative, non-hemolytic *Staphylococcus albus* in 2 *Pseudomonas fluorescens* n. 1. in one, and *B. subtilis* in one. The same bacteria were found in the skin surrounding the groin incision made for cannulating the femoral artery. Toxicity of these bacteria was tested by intravenous injection of 100 million bacteria into dogs during shock. These dogs were transfused after 2 hours in shock. Their blood cultures were sterile the next day and they recovered with no apparent injury.

this time with donor blood or plasma containing a toxin, because their defenses are down. In some cases the entire volume of donor blood obtained from a single dog was given to a single recipient. When the recipient was a normal dog, a volume of blood equal to the volume of blood or plasma to be transfused was removed just prior to the transfusion. When the recipient was a 2 hour shock dog, the volume of donor blood given never exceeded the blood volume deficiency of the recipient. When the volume of donor blood given was below the blood volume deficiency of the recipient, the latter received enough of his own shed blood to restore the normal blood volume. In some experiments 3 or 4 two-hour shock recipients were tested simultaneously with different fractional volumes of the blood from one donor, in order to determine its lethal potency. In these, as in the other experiments, the recipient received the total volume of blood in the reservoir, *i.e.* his own shed blood, minus the volume of donor blood administered at the same time. When plasma instead of whole blood was infused, the recipient received his own shed red cells instead of his own shed whole blood as a supplement. In some of these instances, therefore, the normal blood volume was not fully restored, but the deficiency was not sufficient to prevent full recovery. The foregoing experiments were also performed on rabbits, except that only whole blood was used as the test substance, and, in the case of the shock recipient, the donor blood was given after 1½ instead of 2 hours of shock. Additional experiments were performed with donor blood from dogs which received non-absorbable antibiotics orally and by rectum prior to inducing shock. The antibiotic therapy was as follows: bacitracin (200,000 U) and polymyxin B (200 mg) were given daily for 3 days. They were buried in food, with care to see that the food was totally consumed. On the next (fourth) day, 3 hours before inducing shock, 200,000 U of bacitracin and 600 mg of polymyxin B in 200 ml of saline were given by gavage, and 200,000 U of bacitracin and 100 mg of polymyxin B were given in normal saline by proctoclysis. We have elsewhere (8) noted that dogs receiving prophylactic antibiotic therapy often display greater than normal resistance to prolonged severe hypotension. In the present series of experiments many of the dogs pretreated with antibiotics were still compensating, *i.e.* they were not taking blood back from the reservoir even after 6 hours in shock. All these dogs were transfused at this time. Their response to transfusion was in most cases good, and they were exsanguinated 15 to 20 minutes after transfusion. The blood from these dogs was tested for toxicity by infusion into 2 hour shock recipients.

The above experiment was repeated in 3 groups of 5 rabbits each, except that only one antibiotic was administered to each animal and none was given by rectum. All 15 rabbits receiving bacitracin (10,000 U daily for 4 days), polymyxin B (100 mg for 3 days and 200 mg on fourth day) or neomycin (100 mg for 3 days and 200 mg on fourth day) were kept in shock for 6 hours. In our experience rabbits not pretreated with antibiotics almost never tolerate hemorrhagic shock for this length of time. The pretreated rabbits remained fully compensated throughout this period, *i.e.* none took back more than a few ml of blood from the reservoir. Transfusion was then performed, and after some thirty minutes they were exsanguinated. Rabbits in shock for two hours were transfused with these bloods instead of their own shed bloods.

Results. (Table I). I. Five normal dogs received whole blood from irreversibly shocked dogs immediately after removal of an equal volume of their own blood (500 to 1000 ml). They survived without noticeable harm.

II. Fifty dogs were transfused with their own shed blood after 2 hours in severe hemorrhagic shock. These dogs were able to stand and take fluids within an hour or 2 after the transfusion. Two died and 48 (96%) recovered rapidly and uneventfully.

III. Twenty-six dogs were transfused after 2 hours in severe hemorrhagic shock with blood from dogs dying of irreversible hemorrhagic shock. The volume of donor blood varied from 50 to 1078 ml. Eight (31%) survived. Of the 8 survivors 6 were very sick, exhausted, took little food or fluid, and

TABLE I. Effect of Whole Blood or Plasma from Animals in Advanced Shock on Survival Rate of Animals in Reversible Shock.

Amt and type of test substance	No. exp.	Survival rate (%) Normal recipients	Survival rate (%) 2 hr shock recipients
Dogs			
Whole normal blood	20		90
Whole blood (R)*	50		96
" " (I.S.)†	26		31
" " (I.S.)	5	100	
Plasma (I.S.)	10		20
Rabbits			
Whole blood (R)	50		90
" " (I.S.)	12		8
" " (I.S.)	4	100	
Pretreatment of donors with non-absorbable antibiotics			
Whole blood (P.S.)‡	16 (dogs)		63
" " (P.S.)	15 (rabbits)		93

* R = Recipients' own shed blood standing in reservoir for 2 hr.

† I.S. = Irreversible shock. Donor blood obtained by exsanguination following ineffective transfusion for shock of 4 or 5 hr duration.

‡ P.S. = Prolonged shock. Donor blood obtained by exsanguination following transfusion for shock of 6 hr duration.

displayed a bloody diarrhea for several days. All those which died were very sick, prostrated, took no food or fluid and showed bloody diarrhea from the time of transfusion until death. Of the 26 recipients in this series, 19 dogs were divided into 5 groups, with 3 or 4 dogs per group. Each group was tested for toxicity of the whole blood from one irreversible shock donor (Table II). The volume infused into a single recipient varied from 50 to 600 ml. Of the 19 recipients only 5 survived. Three of them were in one group of 4 which had received 50, 100, 200 and 400 ml respectively of the same donor blood. Only the recipient of the 400 ml fraction died. It was the longest survivor among the 14 which died. Most of the recipients died within 24 hours.

IV. Ten dogs were transfused after 2 hours in severe hemorrhagic shock with their own shed red cells together with plasma from dogs dying of irreversible hemorrhagic shock. Two (20%) survived. Of the 10 plasmas given, 5 had been Seitz-filtered. Of these 4 were lethal in volumes varying from 150 to 200 ml. The survivor of the fifth plasma was very sick with bloody diarrhea for 3 days. Of the 5 which were not Seitz-filtered, 4 were lethal in volumes varying from 200 to 500 ml. The survivor of the fifth showed no signs of injury. The 8 recipients of plasma which died manifested the same disorder after transfusion as those which died after receiving whole blood from shocked donors.

V. Sixteen dogs were transfused after 2 hours in severe hemorrhagic shock with blood (350-800 ml) from dogs in prolonged hemorrhagic shock after pretreatment with non-adsorbable antibiotics. Ten (62.5%) survived. It is noteworthy that 8 of the survivors were free of any overt signs of illness, and recovered with the same rapidity as 2 hour recipients receiving their own shed blood. The other 2 survivors were slightly ill for 24 hours without diarrhea.

VI. Four normal rabbits received whole blood (33-54 ml) from rabbits dying of irreversible shock after removal of an equal volume of their own blood. All survived without noticeable harm.

VII. Twelve rabbits were transfused after 1½ hours in severe hemorrhagic shock with whole blood (32-60 ml) from rabbits dying of irreversible shock. One (8.6%) survived. The remainder were prostrate and showed

TABLE II. Titration of Lethal Potency of Blood from Irreversibly Shocked Dogs in 2 Hour Shock Recipients.

Exp. No.	Donor blood (ml)	Own blood (ml)	Result*
I	100	900	D 24 hr
	200	1000	D "
	600	500	D "
II	50	1090	S
	100	1280	S
	200	1000	S
	400	350	D 72 hr
III	75	1175	D 24 hr
	200	650	D "
	300	930	D "
	500	210	D "
IV	75	1025	S
	200	650	D 18 hr
	300	650	D "
	500	450	D 24 hr
V	50	1120	D 24 hr
	100	800	D "
	300	920	S
	450	550	D "

* D = Death; S = Survivor.

bloody diarrhea until they died. Death occurred within 24 hours.

VIII. Of fifteen rabbits transfused after 1½ hours in severe hemorrhagic shock with whole blood (40 ± 12 ml) from fifteen rabbits in shock for six hours, but pretreated with bacitracin and polymyxin B or neomycin, only one died. Fourteen responded promptly and recovered without any symptoms.

Comment. These results demonstrate the presence of a lethal substance in the blood of dogs and rabbits in advanced hemorrhagic shock. Since the infused blood or plasma was sterile on culture, and since Seitz-filtered plasma was as lethal as unfiltered plasma, this substance is not bacteria, but a toxin which the animal in shock cannot detoxify. Current work, to be reported later, indicates that this toxin is an endotoxin.

In a previous publication(5) a lethal substance was shown to be present in the liver of shocked dogs. Because penicillin given prophylactically prevented death, we concluded that the lethal substance was bacteria. This conclusion became dubious when subsequent data(6) showed that an antibiotic given prophylactically reduces the lethality of endotoxin. The explanation given for this was that endotoxin injures the defenses against bacteria, so that if a dose of endotoxin is itself sublethal, the unopposed action of bacteria will then produce endotoxin, which, added to the amount administered, may constitute a lethal dose.

That the toxin is present not only in blood and liver, but in other tissues and fluids of shocked animals, is evident from experiments now in progress showing that the lymph, lung and spleen also contain a toxin with properties like those of the toxin in the blood. Since the toxin is widely distributed throughout the body, it is understandable that an animal in shock cannot be detoxified by exsanguination transfusions. On the other hand, cross-circulation of the shocked animal with a healthy donor, performed so as to protect a tissue that neutralizes toxins, *e.g.* liver(11), prevents irreversibility to transfusion.

For reasons given elsewhere(13,14), we believe that endogenous toxins that might be generated in consequence of the shock process *per se* do not account for irreversibility and death. This view is confirmed by the experiments with non-absorbable antibiotics. Since these antibiotics could not have prevented development of such endogenous toxins,§ but reduced mortality rate of vulnerable recipients (dogs and rabbits) from 81% to 24% (Table I), it follows that the toxin is derived from the activity of intestinal bacteria and is, therefore, exogenous. This conclusion is consistent with the observation that irreversibility to transfusion and death can be prevented by cross-circulation of the shocked dog with a normal donor dog *via* the superior mesenteric artery(12).

The hemorrhagic lesion in the gut of the shocked animal is due to the toxin, and not to the shock itself. This is manifest from the response of 2 hour shock recipients of blood from other animals in advanced shock. Thus, animals in shock for 2 hours recover without intestinal damage when transfused with normal blood, with their own shed blood, or with blood from donors pretreated with antibiotics. But neary all 2 hour shock recipients of blood or plasma from untreated donors in advanced shock, whether they survive or die, develop a bloody diarrhea soon after the transfusion, and show at postmortem a swollen hemorrhagic mucosa as well as intramural hemorrhage.

Since as much as a liter of blood from an animal in advanced shock does not kill a normal animal, whereas as little as 50 ml may kill an animal in shock of 2 hours duration, it follows that the major difficulty in the latter is the collapse of the detoxifying mechanisms. That this collapse occurs early in shock can be seen from the following experiment: Ten dogs were transfused after 2 hours in shock and exsanguinated after the maximum pressor response. Ten other dogs were transfused with these bloods instead of with their own shed blood after shock of 2 hours duration. Three of the latter were noticeably weak for the next 18 hours, and then rapidly

§ The same applies to exotoxins from bacteria in the tissues. Since the donor blood was in all instances free of hemolysis, clostridial exotoxins in particular, whether formed in the gut or tissues, would appear not to be involved.

recovered. The remaining seven were very sick immediately after the transfusion and displayed bloody diarrhea. Of the 7, two began to recover after 36 hours; the other 5 died during the next two days. Since their own shed blood would not have caused a hemorrhagic lesion in the gut or death, it is clear that the donor blood was toxic, whereas their own shed blood was not. (Shed blood is not toxic because it is normal blood released into a bottle in order to produce shock, and has remained out of contact with the circulation until reinfused.) This experiment identifies a toxin in the blood of animals during the first two hours of shock. Although the shock is still reversible, the detoxifying mechanisms are already failing.

Work in progress demonstrates that a toxin with the same properties as the toxin present in the blood of hemorrhagic shock is also present in the blood of animals in other types of shock.

Summary and conclusions. I. Normal dogs tolerate an infusion of blood from dogs dying of advanced hemorrhagic shock without noticeable harm. Dogs in hemorrhagic shock recover rapidly and without signs of illness if transfused after 2 hours with normal donor blood, or with their own shed blood. But about 70% of such dogs die if the transfused blood is from dogs dying of prolonged hemorrhagic shock. Most of the recipients that survive, as well as all those that die, are prostrated, unable to stand, drink or take fluid after the transfusion, and display a bloody diarrhea until recovery or death. Only 37% of the 2 hour shock recipients of blood from dogs in prolonged shock die if the latter have been vigorously pretreated with non-absorbable antibiotics given orally and by rectum. The survivors, moreover, recover rapidly and disclose no signs of illness. Hence the blood of dogs in prolonged hemorrhagic shock contains a lethal toxin, which, for the reasons given, is considered to be an exogenous toxin derived from the intestinal flora. By the same token endogenous toxins, whether present or not, are not responsible for irreversibility to transfusion or death. II. The same findings are also true for the rabbit: The mortality rate of recipients of blood from untreated donors was 91%, while the mortality rate of recipients of blood from pretreated donors was only 7%. The greater tolerance to prolonged shock of animals pretreated with non-absorbable antibiotics appears to be due to the elimination of bacterial endotoxins by the antibiotics. III. The hemorrhagic lesion in the gut of shocked animals is produced by the toxin and not by the shock itself.

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