Does the Bacteremia Observed in Hemorrhagic Shock Have Clinical Significance?

A Study in Germ-Free Animals

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We have recently reported the rapid appearance of bacteria and endotoxin in the blood of rats and of trauma patients in the course of 30 minutes to 2 hours of hemorrhagic shock. The current study was designed to determine the effect of this bacteremia and endotoxemia on survival. Thirty-three conventional (C:group 1) and 36 germ-free (GF:group 2) Sprague Dawley rats were subjected to our previously described model of treated hemorrhagic shock. Survival in the GF group was significantly better than the C group at 24, 48, and 72 hours after shock. Endotoxin levels were elevated in 88% of C group during shock and in 28% of GF group. The gut of the GF animal contains endotoxin (26 ng/gm of stool) as does the sterile food supply (393 ng/gm of rat chow).

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BERG,[1,2] DEITCH[3–8] AND GUZMAN-STEIN[9] have reported translocation of bacteria to mesenteric lymph nodes and other organs following malnutrition, starvation, burns, immune suppression, abdominal radiation, and exposure to endotoxin. With the discovery that the multiple organ failure syndrome occurring in the post-traumatic or postoperative patient is related to the presence of infection[10,11] and that often no specific focus of infection can be identified,[12] the concept of bacterial translocation has attracted increasing attention. In most of this work,[2,13] bacterial translocation was considered a subacute or chronic problem with bacteria appearing in organs one to several days after the initial stress. We have found that translocation occurs in hemorrhagic shock and happens acutely within two to four hours during the period of shock with an increase in incidence, as judged by positive blood cultures, over the next several days.[14] We have also found that the alimentary tract in rats is a source for bacterial translocation.[15] Moreover, positive blood cultures are found within two hours of trauma and shock in a majority of seriously injured patients.[16]

Much of the current excitement concerning translocation of bacteria replicates similar work on the role of absorbed endotoxin in the generation of irreversible shock that appeared mainly from the laboratories of J. Fine of Boston in the 1950s and early 1960s.[17,18] Fine's work was robbed of much of its luster by a report by Zweifach that the deaths after shock in germ-free animals after hemorrhagic shock was the same as for conventional animals.[19,20] This was confirmed by others.[21,22] Fine protested that the food and gut of germ-free animals contained endotoxins that could be absorbed and play a role in the shock syndrome, just as in conventional animals.

For those interested in the role of bacterial translocation in various stress syndromes, Zweifach's results remain a puzzle that must be solved before the role of translocation in experimental and clinical stress states can gain full credibility. In this study, we propose to repeat Zweifach's study comparing shock survival in conventional and germ-free rats using our standard model of treated hemorrhagic shock. We have also examined some of Fine's claims by determining the concentration of endotoxin in the food and gut of the germ-free rat and determining whether endotoxin is found in the blood of such animals at varying periods during and after hemorrhagic shock.

Materials and Methods

Germ-free Sprague Dawley rats weighing 350 to 450 g were obtained from Taconic Farms (Germantown, NY).

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They were shipped in a sterile flexible film isolator with extra water, food, and bedding. The animals were then transferred to a flexible film isolator designed to our specifications by Standard Safety Equipment Company (Pallatine, IL), to allow passage of tubes for infusion, transducer cords, and temperature probes, as well as to provide adequate room for the equipment and supplies needed to conduct these experiments. The germ-free rats were maintained using standard gnotobiotic techniques.[23,24] The chemical sterilant used was Alcide (Alcide Corporation, Norwalk, CT).

Thirty-six germ-free rats and 33 conventional rats were subjected to our unanesthetized, unrestrained treated model of hemorrhagic shock, which has been previously described.[14] Briefly, the rats were operated on in the isolator under ketamine anesthesia (100 mg/kg intraperitoneal injection) and the femoral artery was cannulated with polyethylene 50 plastic tubing. This was led under the skin to the subscapular area where a harness and swivel were attached. Twenty-four hours after cannulation the rats were bled to a mean systemic systolic arterial pressure (MBP) of 30 torr. This pressure was maintained by removing or replacing blood until 80% of the maximal shed blood was returned. At this point all remaining blood was returned and a bolus of Ringer's lactate solution was infused to raise the MBP to 80 torr or equal to the volume of the maximal shed blood. Thereafter the animals were maintained on a constant infusion of a solution containing Ringer's lactate and 20% glucose equal to twice the normal daily requirement. Both conventional and germ-free isolators were kept in the same room, exposing them to identical ranges of temperature and humidity, as well as the same diurnal cycle. All rats were followed for survival up to three days after the initial shock.

TABLE 1. *Conventional* Versus *Germ-Free Rats: Initial Comparison**

	Conventional	Germ-Free
Weight	379 ± 47	406 ± 50
Temp. (degrees C)		
Postcannula	35 ± 1.2	36 ± 0.3
End shock	29 ± 1.3	29 ± 0.7
24 hrs. post	30 ± 2.7	32 ± 3.2
Max. bled out (cc/100 gms)	4.3 ± 0.7	4.1 ± 0.6
BP preshock	108 ± 7	103 ± 8
BP postshock	63 ± 20.4	75 ± 18.9

* p = NS.

In a second set of experiments, the endotoxin concentration in autoclaved rat chow and the cecal contents of conventional and germ- free rats were measured. The rat chow and stool samples were collected and processed using sterile instruments and glassware that were depyrogenated by dry heat incubation at 200 C for a minimum of 24 hours. Endotoxin in the plasma of germ-free rats was measured during shock and after shock at 2, 24, 48, and 72 hours. Plasma samples of cannulated but unshocked germ-free rats were assayed for endotoxin levels at similar time periods. Endotoxin was measured by a quantitative

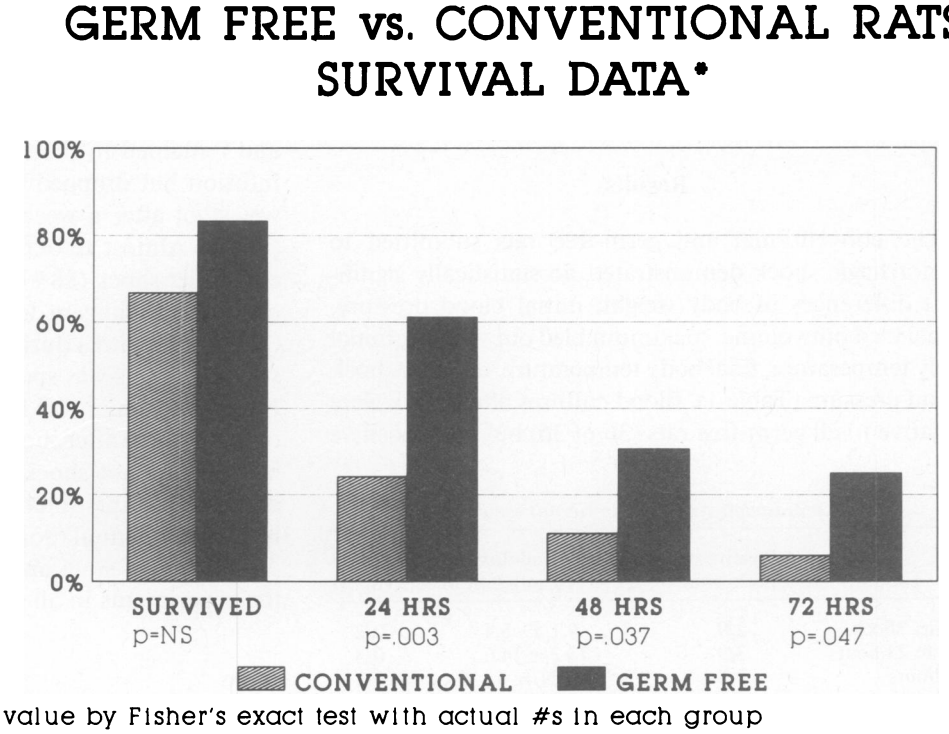

FIG. 1. This represents the survival of germ-free *versus* conventional rats immediately after shock, 24, 48, and 72 hours, respectively. The p values using Fisher's exact test are labeled on the graph.

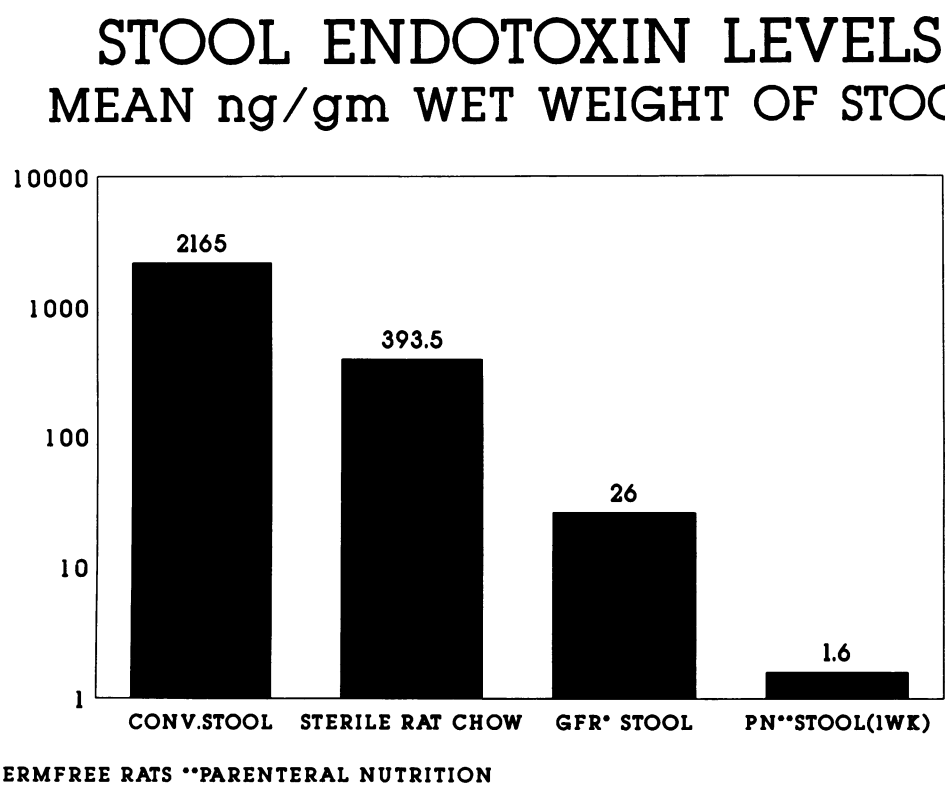

FIG. 2. This demonstrates the mean levels of endotoxin in ng/g wet weight of stool in our conventional rats, sterile rat chow, germ-free rat stool (without any surgical procedure performed) and the endotoxin content of germ-free rat stool after 1 week of total parenteral nutrition and no food by mouth.

colorimetric Limulus assay (QCL-1000, M.A. Bioproducts, Walkersville, MD.), as reported previously[16] with one modification. The incubation time for plasma samples and lysate was increased to 45 minutes to increase sensitivity.[25] In all samples the diluent used was pyrogen-free water.

Significance of our means were determined by Fisher's exact test for small series. A p value of less than 0.05 was considered significant.

Results

The conventional and germ-free rats submitted to hemorrhagic shock demonstrated no statistically significant differences in body weight, initial blood pressure, initial bled out volume, maximum bled out volume, initial body temperature, final body temperature, and postshock blood pressure (Table 1). Blood cultures after shock were negative in all germ-free rats (36 of 36) but were positive in 79% of the conventional rats that had cultures (15 of 19). Figure 1 demonstrates that survival was greater in the germ-free animals during shock and at 24, 48, and 72 hours after shock. Differences were significant at 24, 48, and 72 hours. Mean endotoxin (N = 2) in the cecum of conventional rats was 2165 ng/g wet weight of stool. Sterile rat chow consumed by germ-free rats contained 393.5 ng/gm of endotoxin. Mean endotoxin content of stool in germ-free rats (N = 3) was initially 26 ng/gm of wet stool and remained in the same range at 48 and 96 hours of infusion but dropped to a mean (N = 2) of 1.6 ng/g of wet stool after a week or more of parenteral nutrition (Fig. 2). Almost all of the conventional rats had endotoxemia after shock (88%). Some of our germ-free rats demonstrated significant levels of endotoxin in their blood (7.7 to 32 pg/mL) during shock and up to 24 hours after shock, but this was sporadic, occurring in five of 18 rats. No endotoxemia was found in eight rats observed at 48 or more hours after shock. Germ-free rats cannulated and infused but not shocked (N = 18) had no measurable endotoxin in their blood at 48, 72, 96, 120, 168, and 192 hours after cannulation (Table 2).

TABLE 2. *Endotoxin Levels in Germ-Free Plasma*

Time	# Positive per Shock Time	Mean Endotoxin Level (pg/ml) ± SEM	Control No Shock
Trans. shock	2/9	9.1 ± 6.4	0/2
Up to 24 hours	3/9	25.2 ± 14.6	0/3
48 hours	0/3	N/A	0/1
72 hours	0/5	N/A	0/8
>96 hours	N/A	N/A	0/4

N/A, not applicable.

Cultures of stool and blood were taken at the end of the experiments in all germ-free rats and were negative.

Discussion

Survival was clearly superior in germ-free rats subjected to our model of hemorrhagic shock compared to the survival of conventional rats similarly treated. This obviously

is in conflict with the report of Zweifach[19,20] and others.[21,22] We believe that this is because the earlier investigators were using a "nonresuscitated" model. Because their animals were not treated, they died of the early fluid shifts that we now know take place.[26,27] Their animals did not live long enough to exhibit the differences due to either the effects of acute or subacute bacterial translocation.

Indirect evidence that bacterial translocation plays a role in the lethality of hemorrhagic shock was provided by one of our previous papers in which we showed that pretreatment of the shocked conventional rat with cefoxitin resulted in survival of about the same percentage as seen in the germ-free rat.[28]

While survival in our germ-free rats was superior to that of conventional rats, 70% of the germ-free animals died despite the absence of bacteria. Could this be caused by absorption of endotoxin as proposed by Fine? We have confirmed that sterile rat chow consumed by the germfree rats contains substantial amounts of endotoxin and that the stool of these animals contains moderate amounts of endotoxin. However, absorption of endotoxin appears to be an early and sporadic event during and after shock in the germ-free rat with only 28% showing blood levels in the first 24 hours and none thereafter. It is known that once endotoxin enters the peritoneal cavity it perpetuates the further translocation of bacteria.[7,29-31] The role of endotoxin in the death of germ-free rats thus remains conjectural. Both the bacterial and the endotoxin variables in hemorrhagic shock can be virtually eliminated by rendering the colon of the germ-free rat endotoxin "poor" by a week or more of parenteral nutrition as we have demonstrated in this investigation. We hope to determine if a lack of endotoxin, as well as bacteria in the gut, will have any further impact on survival. Our conjecture is that the excess deaths in the germ-free rat are more likely due to tissue damage from the various inflammatory mediators produced during shock.[32-36]

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