Discussion

Dr. Robert S. Rhodes (Jackson, Mississippi): Dr. Rush and his colleagues are particularly to be commended for a very high survival rate despite the fact that their animals were maintained hypotensive and unresuscitated until 80% of their shed blood had been spontaneously returned. This is remarkable in view of our own experience as well as that at the University of Pennsylvania where return of shed blood was accomplished at 40%. There was still an almost 100% mortality rate.

By applying newer concepts of resuscitation they have challenged the older concepts of the role of intestinal flora in hemorrhagic shock. Thus they have done more than simply fill in a piece of the puzzle; they have really re-examined the whole puzzle.

In a study that Dr. Ralph DePalma and I did a number of years ago, we noted in a similar, but not germ-free, model that the shed blood contained significant amounts of endotoxin. We were very concerned that refusion of this blood during the resuscitation gave the animal an additional bolus of endotoxin that either directly or indirectly added to the insult. I am curious as to whether Dr. Rush and his colleagues examined their shed blood for endotoxin or whether they would consider using some endotoxin-free type of volume expanders to carry out their resuscitation.

I am also anxious to know if they examined the gastrointestinal mucosa either grossly or histologically in their animals. We looked at the role of barrier function using horseradish peroxidase as a tracer. (Slide) In the top panel is the control intestinal mucosa and the dark activity simply represents endogenous peroxidase activity in red blood cells. The lower panel represents the shock group. Exogenously administered peroxidase can be seen passing through the intestinal mucosa between the cells. When we temporarily correlated this loss of barrier function to peroxidase with endotoxemia, we could only detect endotoxemia when there had been gross ulceration of the small bowel. Thus I would like to know if Dr. Rush and his colleagues examined the small bowel of the animals that died in their experiment.

Finally I would like to hear more of your thoughts about the role of endotoxin in hemorrhagic shock. Did the germ-free animals with endotoxemia have a poorer prognosis than those without endotoxemia? You imply this from the data, but it is not mentioned specifically. Similarly, did any of the 12% of control animals that did not develop endotoxemia seem to fare better than the animals that had endotoxemia? Again I could not determine this from the manuscript.

Dr. Jonathan Meakins (Montreal, Canada): This is but the latest in a long series of very stimulating and significant studies from Dr. Rush's group in which he has been able to link important clinical observations with studies in the laboratory.

I would like to follow up on Dr. Rhodes' question with respect to the small bowel, as it has been our belief for some time that the small bowel may be more important as a target organ in these processes than the colon, even though traditionally the large volume of bacteria is, indeed, in the colon. As I read the data in the abstract, the deaths in the germ-free group seem to have lagged behind those in the controls by either 24 or 48 hours. The difference in mortality rates are similar when one compares the control group at two hours and the germ-free group at 24 hours or the control group at 24 hours and the germ-free group at 72 hours. This makes me wonder if the process that initiates the mortality rate isn't similar, but perhaps the mechanisms, or for some other reason the timing, is different. In light of that and the fact that bacteria and endotoxin have been brought up, the business of activated mediators, which was touched on by Dr. Rush in his presentation, must be introduced. Do you have any information on the differences between your control and germ-free groups with respect to any of the mediators of end organ damage that we might expect in this situation?

Last, could you comment on the role of antibiotics either against gram-positive or gram-negative aerobic or anaerobic organisms, but most particularly against gram-negative aerobes, to separate the role of viable bacteria as they might cross the gut mucosal barrier and present as septicemia, or at least bacteremia, and the importance of endotoxin, which in the absence of viable bacteria in the blood may be as important a mediator or initiator of the morbidity and mortality seen in this situation.

Dr. Edwin A. Deitch (Shreveport, Louisiana): I, too, would like to compliment Dr Rush and his coworkers on an excellent piece of work. The basic question addressed in this study is whether the gut microflora or their products play an important role in modulating survival after a major episode of shock. Based on the results presented it appears that long-term survival after hemorrhagic shock is better in germ-free than conventional rats, with 25% of the germ-free and 6% of the conventional rats surviving the shock insult. Based on these results, Dr. Rush justifiably concludes that the absence of bacteria reduces but does not prevent the incidence of shock-induced deaths. As Dr. Rush suggests, the fact that so many of the germ-free rats died after the shock insult may be related to the fact that endotoxin is present in significant levels in the intestines of the germ-free as well as the convention rats.

The basic question remains—are patients who die after hemorrhagic shock, especially those with distant organ failure, dying from or with endotoxin and bacteria that escaped from the gut? That is, is gut barrier failure the trigger that initiates a cascade of events resulting in multiple organ failure or sepsis, or, is the gut just one more organ that fails? Its failure of limited bacterial translocation would suggest that gut barrier failure is important in the evolution of lethal sepsis in a number of models including hemorrhagic shock. How can the results of our experimental studies be reconciled with the excellent work presented today? The answer to this question lies in the differences between our and Dr Rush's shock models. In Dr. Rush's shock model, the period of shock lasts from four to five hours and as he showed us today is associated with a 94% mortality rate after 72 hours, despite treatment. Thus in many ways this is an irreversible shock model, even though the majority of animals survive the shock insult. We know from clinical experience that patients sustaining prolonged periods of shock frequently do not survive, even though the cause of shock has been corrected. Because most of the patients we treat in clinical practice have limited periods of hypotension, for example due to blood loss in the operating room or after mechanical trauma, we evaluated gut barrier function in rats subjected to limited periods of hypotension. These studies documented that gut barrier failure and bacterial translocation would occur after a hypotensive episode as short as 30 minutes. The long-term survival rate in this 30-minute shock model has consistantly exceeded 90%.

My interpretation of all these data is that the degree of shock-induced intestinal injury is related to the magnitude and duration of the shock insult; and that after limited periods of shock, when recovery is possible, the development of gut barrier failure leading to systemic endotoxemia and bacteremia is an important variable that affects outcome. However a point is reached after which the presence of an intestinal injury leading to the escape of intestinal bacteria and endotoxin into the portal and systemic circulation is no longer of importance. That is, the injury is irreversible. Along that line I would like to ask Dr. Rush whether he plans to repeat the work presented today in animals subjected to a less severe shock injury in which the presence of intestinal bacteria may be of more importance.

Dr. Andrew M. Munster (Baltimore, Maryland): We are now working with a new tool, the chromogenic assay, which is extremely accurate in measuring endotoxin, something that Dr. Fine did not have 30 years ago.

The discrepancy in the data with our own data seems to be that 25 picograms per mL, at least in humans, in our experience, represents a very modest insult. A multitrauma patient ranges between 30 to 50 pg/mL within the first few hours.

A major burn can get up to 60 to 80 micograms per mL within the first hour or two. A typical patient with acute cholecystitis on admission will be about 100 pg/mL and in the very septic patient we have seen endotoxin levels up to 300 to 400 pg/mL, and I would be interested in Dr. Rush's comments on that.

My other question, to extend Dr. Meakin's question a little further, is that of intervention. Clinical intervention against endotoxemia is just around the corner. We have now treated about 60 burn patients with Polymyxin B. I would be curious to know if you have applied monoclonal antibodies or Polymyxin to this model to see if the effect of endotoxemia can, in fact, be negated.

Dr. Stanley M. Levenson (Bronx, New York): Dr. Rush mentioned some of the work of the late Dr. Jacob Fine, Professor of Surgery at the Harvard Medical School and the Beth Israel and Boston City Hospitals. I want to add that Dr. Fine described bacteremia after severe hemorrhagic

shock. He worked with dogs primarily. He also described the impaired ability of the shocked animal to clear bacteria when injected intravenously. Many of us will recall that central to Dr. Fine's thesis of the basis for "irreversibility" after prolonged, severe shock was the influx of endotoxin into the circulation from the gut and the impairment of reticuloendothelial (RES) function so that the endotoxin was not cleared. Fine believed that endotoxin was likely absorbed from the GI tract normally, but rapidly cleared by the RES, but that after shock absorption of endotoxin was increased and, very importantly, because of the impaired RES function due to shock, the endotoxin was not cleared and the serious pathophysiologic sequelae due to endotoxin followed.

Dr. Fine also demonstrated that an animal subjected to even mild shock was exquisitely sensitive to exogenous injected endotoxin.

Dr. Rush mentioned that perhaps the reason why Dr. Zweifach's findings of no difference in survival after hemorhagic shock of germ-free and conventional rats was different from Dr. Rush's finding that germ-free rats were significantly more resistant was because in Dr. Zweifach's study, the experimental shock model was highly lethal, much more so than in Dr. Rush's study. That was the case in some of Dr. Zweifach's experiments, but not all. In one experiment the mortality rate was comparable to that in Dr. Rush's study, albeit the number of rats was small, and the germ-free rats were not more resistant.

I would like Dr. Rush to comment on the findings of one of his collaborators, Dr. James Heneghan, who reported 1 or 2 years ago no difference in the survival of germ-free and conventional rats subjected to a type of hemorrhagic shock; although not identical to Dr. Rush's model, it was one in which the conventional animals had a mortality rate in 24 hours of about 55%, which is about 20% less than that of the conventional rats in Dr. Rush's study. Although Dr. Heneghan found that the germ-free rats had a slightly lower mortality rate than the conventional, the difference was not statistically significant. It was only when germ-free and conventional rats were subjected to prior cecectomy that Dr. Heneghan found a greater resistance to hemorrhagic shock by the germ-free rats. The cecectomies were done because characteristically the cecum of germ-free rats is very large, much, much larger than in conventional rats. Although it is extremely unlikely, I wonder if in your germ-free rats, Dr. Rush, the cecum happened to be small?

DR. BENJAMIN F. RUSH (Closing discussion): I would like to thank my discussants for their very thoughtful and useful questions, and I will try to answer them in order.

Dr. Rhodes asked the question, "Did endotoxin occur in the blood from the animal?" The answer is yes. In a lot of the earlier work that was done in this area, the care with which all of the equipment was kept pyrogen-free was really less than ideal and in some of our early work we discovered that we were dealing with some contamination of our reservoirs. One must be enormously careful that the entire set-up is free of exogenous organisms as well as endogenous ones and that all equipment is pyrogen free.

We find that as the animal goes into shock, bacteria do shift out of the gut fairly rapidly; and I guess I will be getting to this with Dr. Deitch, bacteria are found, at least in our model, in the blood within two hours of shock at 30 mL of mercury. Presumably if we restored the blood at that point we would nonetheless have an example of an acute bacterial translocation having occurred at that time.

Dr. Rhodes also asked what was the state of the small bowel. We have reviewed the pathology in our animals, not in this particular series, but in other animals subjected to this model, and at least within the first 24 hours, the only ulcerations we have found have been in the small bowel. However they have not been common, so yes, it does appear that the small bowel may be a target, but thus far we have not established that this happens frequently. It may be that the injury is really microscopic rather than macroscopic.

Dr. Meakins was interested in what work we had done with mediators thus far. We are working on that as I implied in my discussion. One of the frustrating problems is that many of the tools for measuring mediators are available for mice and man, but not for rats, and because we are anxious to continue our observations in germ-free rats, we are blocked at the moment and may actually have to develop some of our own antibodies in order to work with the rat. We find a prompt activation of complement in most severely injured humans.

As to Dr. Deitch's questions of the model, the reason this model is stressed so much is that animals that are treated in this way and resuscitated do not die unless you stress them to this degree. We do, incidentally, have data available on animals stressed at 30 torr for a 60% return and at 30 torr for a 40% return. If you resuscitate your animal model, your survival rate at 30 torr for 40% return is close to 90%.

We settled on this model primarily because we knew that it was going to give us an almost 100% rate of lethality. Any time we reversed that and obtained survival, we had a significant result.

I want to thank Dr. Munster particularly for calling attention to the endotoxin method. It is true that the new techniques for analyzing endotoxin quantitatively are highly sensitive, and the usual minimal endotoxin level that will produce clinical illness is 9 picograms/mL. You can see that our germ-free animals only reached a serum concentration of 2 or 2.5 endotoxin units, which represents only a modest exposure.

Dr. Levenson, with his usual thoroughness, has reviewed the entire literature all the way back to Dr. Fine's work. Dr. Fine did, in fact, demonstrate bacteria in his dogs. Unfortunately, other investigators showed this was due to breaks in sterile technique. The reason he began to emphasize endotoxin was that after Zwiefach's paper indicating that bacteria were not important in the postshock syndrome, Dr. Fine began to suggest it was the absorbed endotoxin.

I do not think there is any question that the reticuloendothelial system is inhibited in our animals. What we are proposing is that the only way we can tell how important endotoxin is, as has already been suggested by one of our discussants, is to try to eliminate the endotoxin variable completely from this model, and if that shows us some change in mortality rate, then we could say, yes, the absorbed endotoxin did make a difference.

Because we saw endotoxin at rather low levels and saw it rather infrequently, our assumption is that perhaps this is of less importance than inflammatory mediators.

Finally the question of the cecum. I should apologize to my colleague, Carter Nance, who did publish a number of years ago with Henneghan, one of our coauthors, a paper indicating that the cecectomized germ-free rat had a survival rate after shock that was superior to the germ-free rat that was not cecectomized and to conventional germ-free rats. That particular observation left people so puzzled that it has been lost in time. In other words, the cecectomized, germ-free model didn't make sense to the average surgeon at that time. This very large cecum is a marked feature of the germ-free rat compared to the conventional animal. Perhaps by eliminating it from the germ-free animal there were certain enzymes that were eliminated. We really don't know.