ON THE RELATION OF THE SIZE OF THE INTRAINTESTINAL POOL OF ENDOTOXIN TO THE DEVELOPMENT OF IRREVERSIBILITY IN HEMORRHAGIC SHOCK*

By T. WIZNITZER,‡ M.D., F. B. SCHWEINBURG, M.D., N. ATKINS, M.D., AND J. FINE, M.D.

(From the Yamins and Kirstein Laboratories for Surgical Research, Beth Israel Hospital, and the Department of Surgery, Harvard Medical School, Boston)

(Received for publication, July 28, 1960)

In any substantial series of animals exposed to severe and prolonged hemorrhagic shock some 80 per cent will die in spite of restoration of normal blood volume (1). Since circulating endotoxin has been found to be the cause of irreversibility and death (2), the survival of the remainder can be explained in terms of a greater than normal resistance to endotoxin. For survival following severe and prolonged hemorrhagic shock can be regularly obtained by prior induction of increased resistance to endotoxin (3, 4). Another equally valid explanation for survival is a greatly reduced pool of endotoxin in the intestine, from which the circulating endotoxin in shock is absorbed (5, 6). Indirect evidence for this hypothesis was obtained from experiments showing a large reduction in mortality from hemorrhagic shock by prior oral administration of *non-absorbable* antibiotics effective against Gram-negative bacteria in the gut (2, 7). In the summer of 1958 we unexpectedly encountered rabbits which were regularly tolerant of prolonged hemorrhagic shock without having been treated to increase their resistance to endotoxin (5). These rabbits were found to have an abnormal intestinal flora. Endoplate cultures of rectal and intestinal mucosa yielded *B. proteus* and *Pseudomonas*, but no coliform bacteria, except for a rare instance when a single colony of *E. coli* was found.[1] A study was undertaken to see whether the tolerance of these rabbits to hemorrhagic shock was related to the absence of the coliform bacteria from the intestinal flora.

Ten rabbits with a normal intestinal flora, and ten rabbits with a coliform-free flora were exposed to 6 hours of severe hemorrhagic shock by the technic we customarily employ (8). As expected, all ten with a normal flora died in spite of restoring normal blood volume at 6 hours, whereas nine of the coli-

* Aided by a grant from the National Institutes of Health, Bethesda, Maryland, and by a contract with the Office of the Surgeon General, United States Army.

‡ National Institutes of Health Research Fellow in Surgery, Instructor in Surgery, Hadassah University Hospital, and Hebrew University, Jerusalem, Israel.

[1] These changes from the normal pattern of the intestinal flora were the result of introducing an antibiotic into the diet by the breeders several months before.

form-free animals recovered in response to the same treatment. With this clear cut difference fully reaffirmed, we repeated the experiment with modifications in two sets of ten rabbits each, all with coliform-free flora. One set of ten received 100 ml. of an 18 hour culture of a strain of *E. coli* $0111B_4$ by gavage some 3 hours before inducing the shock. The other set of ten were fed the same bacteria in their food and drinking water every day for 7 or more days before inducing the shock.

Of the first set of ten rabbits, six survived exposure to shock and four died. Of the second ten, two survived and eight died. The dead in all four groups of experiments showed the lesions usually seen in rabbits dead from irreversible shock; *i.e.*, small intramural foci of hemorrhage in the gut wall and lung. Thus, the ability to withstand 6 hours of hemorrhagic shock was abolished in most of these rabbits as a consequence of introducing *E. coli* into the gut.

That the response of the various groups of rabbits was a function of the size of the intraintestinal pool of endotoxin was suggested by the following bacteriologic data: Healthy rabbits on the antibiotic diet since birth were killed in order to study the bacteriological status of the gastrointestinal tract. As stated, they yielded no coliform bacteria, or in a rare instance a scanty growth of *E. coli*. The same rabbits, given a single dose of 100 ml. of an 18 hour culture of *E. coli* $0111B_4$ by gavage, and killed 24 hours later, showed positive *E. coli* cultures of the intestine, but if killed 3 days later, these bacteria were no longer present. On the other hand, if rabbits were fed *E. coli* daily for many days, the intestinal cultures remained positive for *E. coli* for longer, and this organism became an established species only after 21 days.

Evidence that the introduction of Gram-negative bacteria into the gut in such coliform-free rabbits results in an amount of circulating endotoxin sufficient to kill if the rabbits are put into hemorrhagic shock several hours later has been presented elsewhere (6, 1). But a disquieting observation was made in the latter half of 1959 when an outbreak of enteritis among rabbits in this area was at its height. Most of these rabbits carried what seemed to be a normal coliform-bearing flora, but were tolerant of hemorrhagic shock. If this tolerance were the result of prior exposure to the prevailing enteric pathogens, then these rabbits might be expected to have increased tolerance of endotoxin. But direct challenge with endotoxin did not show more than the usual sensitivity to endotoxin. Was it possible that the coliforms harbored by these rabbits were relatively small in number, or that ecological factors related to the enteritis might have reduced their endotoxin content? An answer was needed because these observations were a challenge to the hypothesis of the cause of irreversibility and death in hemorrhagic shock, and because these rabbits were not suited to

our next objective, which required a supply of toxic blood from irreversibly shocked rabbits. Accordingly, some additional experiments were undertaken as follows:—

Three groups of these rabbits, all fasted for 18 hours, were used. In group 1 the rabbits were fed 200 mg. endotoxin in 30 ml. saline by gavage, after which they were returned to their cages in order to allow caudal progress of the fluid. Four hours later they were subjected to hemorrhagic shock in the usual manner. In the rabbits of group 2 the procedure was the same, except that the saline solution given by gavage contained no endotoxin. In group 3 the rabbits were given 200 mg. of endotoxin in saline but they were returned to their cages without being subjected to shock.

Of the fourteen rabbits in group 1, thirteen died within 16 to 36 hours, all showing the intramural intestinal and pulmonary hemorrhages commonly seen in death from hemorrhagic shock or endotoxin. Of the twelve animals in group 2, only five died within 48 hours, the remaining seven recovering rapidly after transfusion. These were killed after 48 hours, and postmortem showed no gross abnormalities. The same was true for the five rabbits in group 3. The seven rabbits in group 2 that survived displayed the behavior typical of animals resistant to shock; *i.e.*, the amount returning spontaneously from the elevated reservoir to the animal during the 6 hours of hypotension was much less than in the endotoxin-fed rabbits, which exhibited the rapid return of much of the shed blood—a phenomenon that is characteristic of the collapse of the compensatory mechanisms in advanced hemorrhagic shock.[2] Moreover, in the survivors of group 2, transfusion of the blood remaining in the reservoir produced nearly complete restoration of the preshock level of blood pressure, whereas in the rabbits of group 1 the maximal level of the blood pressure after transfusion of this remainder was 25 mm. Hg below the preshock level.

These data add to the increasing body of evidence for the important role that the intraintestinal coliform bacteria play in the shock syndrome. This evidence consists of the following observations: (*a*) Coliform-free rabbits tolerate severe and prolonged hemorrhagic shock (5); (*b*) protection against the development of irreversibility in normal (*i.e.* coliform-bearing) rabbits is secured by giving non-absorbable antibiotics in advance of producing shock (9); (*c*) protection against death from shock that follows a 1 hour occlusion of the superior mesenteric artery is secured by the oral administration of non-absorbable antibiotics before the occlusion (10); and (*d*) endotoxin is absorbed in about equal amounts from the gut of the normal and shocked animal; but whereas the RES in the normal animal takes up and detoxifies all the absorbed endotoxin, the RES of the shocked animal cannot take up as much endotoxin or detoxify it. Therefore endotoxemia develops (6, 11). Although the tissues of the nor-

[2] For the method employed to produce experimental hemorrhagic shock and the usual phenomena to be observed in this experimental model, see reference 8.

mal and shocked rabbit contain some intestinal bacteria which multiply during shock, the number is far too small to account for a significant fraction of the endotoxin recovered in the blood and tissues of shocked animals (12).

SUMMARY AND CONCLUSION

Additional evidence is presented affirming the role of the intestinal pool of endotoxin in producing irreversibility in prolonged hemorrhagic shock. The fact that coliform-free rabbits tolerate exposure to a degree and duration of hemorrhagic shock which is lethal for rabbits that possess the normal flora, and that these tolerant rabbits lose their tolerance when *E. coli* are introduced into the gut several hours before inducing shock, demonstrate the critical importance of the size of the pool of endotoxin. That there is a proportionality between the size of the pool of endotoxin and the tolerance of hemorrhagic shock is suggested by the survival rate of several series of coliform-free rabbits fed *E. coli* by gavage. The rate was less the more firmly the *E. coli* were reestablished in the flora. The presence of the usual number of coliform bacteria in the intestinal flora does not mean the presence of the usual amount of endotoxin in these bacteria. The amount of endotoxin depends not only on the size of the population, but also, as our own experience demonstrates, on the particular ecological factors extant at any particular time which govern the amount of endotoxin elaborated by any given strain or strains of coliform bacteria.

BIBLIOGRAPHY

1. Frank, H. A., Seligman, A. M., and Fine, J., Traumatic shock: treatment of hemorrhagic shock irreversible to replacement of blood volume deficiency, *J. Clin. Inv.*, 1945, **24,** 435.
2. Schweinburg, F. B., Shapiro, P. B., Frank, E. D., and Fine, J., Host resistance in hemorrhagic shock. IX. Demonstration of circulating lethal toxin in hemorrhagic shock, *Proc. Soc. Exp. Biol. and Med.*, 1957, **95,** 646.
3. Smiddy, F. G., and Fine, J., Host resistance to hemorrhagic shock. X. Induction of resistance by shock plasma and by endotoxins, *Proc. Soc. Exp. Biol. and Med.*, 1957, **96,** 558.
4. Zweifach, B. W., and Thomas, L., The relationship between the vascular manifestations of shock produced by endotoxin, trauma and hemorrhage. I. Certain similarities between reactions in normal and endotoxin-tolerant rats, *J. Exp. Med.*, 1957, **106,** 385.
5. Fine, J., Rutenburg, S., and Schweinburg, F. B., The role of the reticulo-endothelial system in hemorrhagic shock, *J. Exp. Med.*, 1959, **110,** 547.
6. Ravin, H. A., Rowley, D., Jenkins, C., and Fine, J., On the absorption of bacterial endotoxin from the gastro-intestinal tract of the normal and shocked animal, *J. Exp. Med.*, 1960, **112,** 783.
7. Fine, J., Frank, E. D., Ravin, H. A., Rutenburg, S. H., and Schweinburg, F. B., The bacterial factor in traumatic shock, *New England J. Med.*, 1959, **260,** 214.

8. Fine, J., The Bacterial Factor in Traumatic Shock, Springfield, Illinois, Charles C. Thomas Company, 1954, chapter 7.
9. Freedman, E. W., Schweinburg, F. B., Jashar, J., and Fine, J., Bacterial factor in traumatic shock in the rat, *Am. J. Physiol.*, 1957, **189,** 197.
10. Shapiro, P. B., Bronsther, B., Frank, E. D., and Fine, J., Host resistance to hemorrhagic shock. XI. Role of deficient flow through intestine in development of irreversibility, *Proc. Soc. Exp. Biol. and Med.*, 1958, **97,** 372.
11. Wiznitzer, T., Better, N., Rachlin, W., Atkins, N., Frank, E. D., and Fine, J., *In vivo* detoxification of endotoxin by the reticulo-endothelial system, *J. Exp. Med.*, 1960, **112,** 1157.
12. MacDonald, J., Frank, E. D., Palmerio, C., Better, N., and Fine, J., Bacteriology of the tissues of the normal and shocked rabbit and dog, data to be published.