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Host Resistance to Hemorrhagic Shock X. Induction of Resistance by Shock Plasma and by Endotoxins.* (23539)

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The blood and tissues of an animal (dog, rabbit) in prolonged hemorrhagic shock contains a toxin which is the product of bacterial activity(1). It accumulates in consequence of injury to the antibacterial defense mechanisms and produces irreversibility to transfusion and death. If the integrity of the antibacterial mechanisms could be sustained, so that these toxins would be neutralized, or their production suppressed, the shock, even if severe and prolonged, should remain reversible to transfusion.

Accordingly, experiments were designed to explore the possibility of inducing tolerance to hemorrhagic shock, *i.e.* preventing development of irreversibility to transfusion, by prior exposure to known bacterial endotoxins. Should tolerance to shock be so induced, the same result might also be obtained by prior exposure to plasma from irreversibly shocked animals, if the toxic factor in this plasma which produces irreversibility is in fact a bacterial endotoxin. The data in this communication demonstrate that protection against irreversibility to transfusion and death from severe and prolonged hemorrhagic shock can be secured by prior treatment with plasma from severely shocked animals, as well as by prior treatment with known endotoxins.

Method. Rabbits were selected as the most suitable species in which to produce tolerance to endotoxin, and subsequently to gauge the effect of such tolerance on the course of hemorrhagic shock. The MLD/100 of Difco *E. coli* Endotoxin (Batch No. 0127-B8) was found to be 2 mg/kg body weight in normal rabbits. The MSD/100 (maximal surviving dose, *i.e.* the highest dose that can be given with a zero mortality rate) was 0.03 mg/kg. Seven groups of adult albino rabbits (average weight 2-3 kg) were trained for 3 days so that spontaneous fever would not occur in response to the required manipulations(2). Each rabbit then received a daily dose (1 MSD/100 or less—see Table I) of endotoxin intravenously for 7 successive days. In every instance observations were made on the fever response, and on the total and differential leucocyte count during the subsequent 6 hours. The fever response was characteristic for endotoxin and showed the anticipated daily decline, so that by the seventh day the curve was characteristic of induced tolerance to the endotoxin (Fig. 1). On the eighth day hemorrhagic shock was produced by a standard method described elsewhere(3), and tolerance to shock observed, especially with regard to rate of "take-up" (*i.e.* spontaneous return of the shed blood to the animal from the elevated reservoir), pressor response to transfusion, survival time and survival rate. One of the 7

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TABLE I. Survival of Rabbits Subjected to 6 Hours of Hemorrhagic Shock following Pretreatment with 7 Daily Injections of Endotoxin* or Shock Plasma.

Exp.	Daily dose, mg/kg	Total No. Animals	Survivors
1	.03 endotoxin	6	6
2	.015 "	6	5
3	.0075 "	6	5
4†	.002 "	7	6†
5	.001 "	6	5
6	.0005 "	10	3
7	13 ml shock plasma	10	8

* MLD = 2 mg/kg/b.w.

† 3 of these 6 animals recovered from shock but died of sepsis after 48 hr. Gastrointestinal hemorrhage, characteristic of shock, was not found.

groups of rabbits was exsanguinated 30 minutes after the transfusion, and the blood so removed was tested, as described elsewhere (1),‡ for the presence of the toxin causing irreversibility. The foregoing experiments were repeated in another series of rabbits, except that, in place of endotoxin, a daily intravenous injection of 13 ml§ of irreversible shock plasma, known to contain the lethal toxin, was given for 7 successive days. If the toxin in this plasma is an endotoxin, one should expect the plasma to act like known endotoxin in respect of the induction of tolerance. Accordingly, the fever and leucocyte responses were obtained following each daily injection. Some of these rabbits were then tested for tolerance to prolonged shock. Others were put into shock for 1½ hours and compared with unprepared (*i.e.* normal) rabbits in shock for 1½ hours for their susceptibility to the toxin in shock plasma causing irreversibility.‡

Results. I. *Fever Response.* The upper 2 curves in Fig. 1 depict the characteristic course of the fever following intravenous injection of one MSD/100 or less of endotoxin on the first and on the seventh days. The lower 2 curves in Fig. 1 show the corresponding fever curves in response to shock plasma. Except for the occasional absence of the typical notch in the first day curve at about 1½ hours, they are virtually the same.

Comment. Normal plasma or plasma from normal blood standing in a reservoir for 6 hours at room temperature (to simulate the conditions of the shock experiment, in which

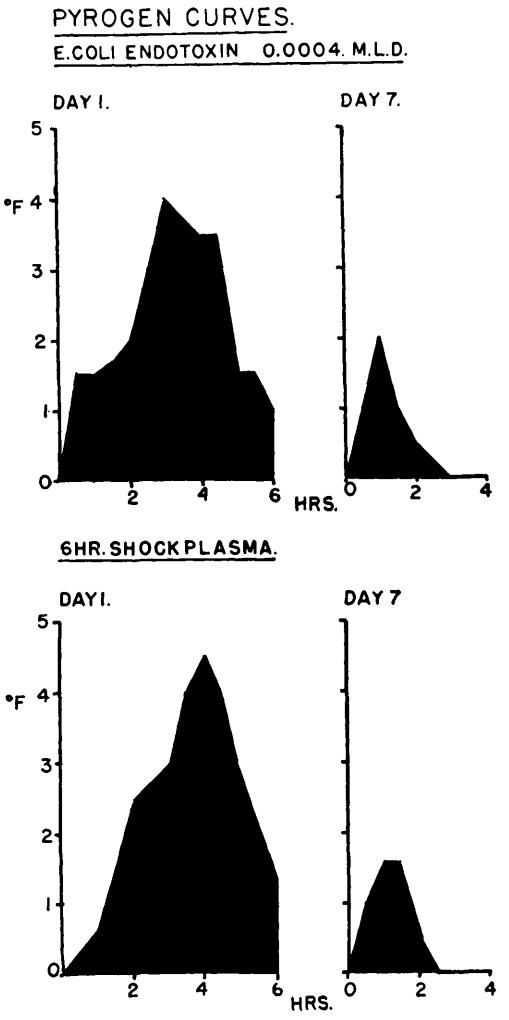

FIG. 1. Upper left curve depicts characteristic fever response of normal rabbit during 6 hr following first intrav. inj. of a sublethal dose of endotoxin. Upper right curve shows fever response on the seventh of seven successive daily inj. of the same dose of endotoxin. Lower curves depict corresponding fever response curves to 13 ml irreversible shock plasma given in place of the endotoxin.

‡ A Normal rabbit transfused after 1½ hours of hemorrhagic shock with its own blood or plasma recovers promptly and survives without damage to the gut. If transfused with toxic blood or plasma, instead of its own shed blood, the response to transfusion is poor, the gut is hemorrhagic and death follows.

§ Previous experience had shown that as little as 13 ml of such plasma could kill a 1½ hour shock recipient.

shed blood, which is later returned to the animal, might become contaminated with pyrogens in the reservoir) were uniformly non-pyrogenic (six experiments).

II. *Leucocyte Response* (Total and Differential Counts). A decline in the total and granulocyte count begins almost immediately following injection of endotoxin. The granulocytopenia reaches its lowest level within 60 to 120 minutes. Recovery of the normal total and differential counts begins after this time and is back to normal after 3 or more hours, depending on dose of endotoxin given. The injection of shock plasma produces a similar response. The acquisition of tolerance to endotoxin is reflected in the declining fever response to repeated daily injections of endotoxin or shock plasma. It is not reflected in the granulocytopenic response, which is quantitatively the same from day to day. Hemorrhagic shock also produces a granulocytopenic response in both tolerant and non-tolerant rabbits. But the drop in the count is less in the tolerant animal, and there is a rapid return to a normal or above normal count during the shock period (Fig. 2B). In the non-tolerant animal the count remains at its lowest level until death (Fig. 2A).

III. *Course of Hemorrhagic Shock.* The maximum bleeding volume, *i.e.* maximum amount of blood lost into the reservoir (some 60-80 ml), is the same in rabbits tolerant to endotoxin or shock plasma as in normal rabbits. As described elsewhere(3), little or none of the shed blood in the elevated reservoir connected to the femoral artery returns spontaneously during the first 1½-2 hours of shock in the normal, *i.e.* the non-tolerant animal. The same is true for the tolerant animal. But if the shock is allowed to continue, the course of events differs markedly between these 2 preparations. In the non-tolerant animal, blood returns spontaneously at a variable rate, and when 40-50% of the total has returned, forced infusion of the remainder is followed by a transient pressor response and death. Spontaneous return of 40-50% of the blood usually occurs within 4-5 hours. Even if less than this volume has returned by this time, forced infusion of the remainder is not curative. The spontaneous return of blood ("take-up") is evidence of failing vasoconstrictor compensation. As a rule, the more rapid the take-up, the shorter the survival after normovolemia has been restored. In most instances death occurs within 12 hours. Only a few survive up to 24 hours. None survive beyond the sixth hour if none of the shed blood is force-infused. In the rabbit which is tolerant to endotoxin or to shock plasma no blood from the reservoir returns to the animal during the period of exposure to shock (6 hours). Transfusion after 6 hours of the entire content of the reservoir produces an immediate and sustained pressor response, with prompt and full recovery in 70% of rabbits tolerant to endotoxin, and 80% of

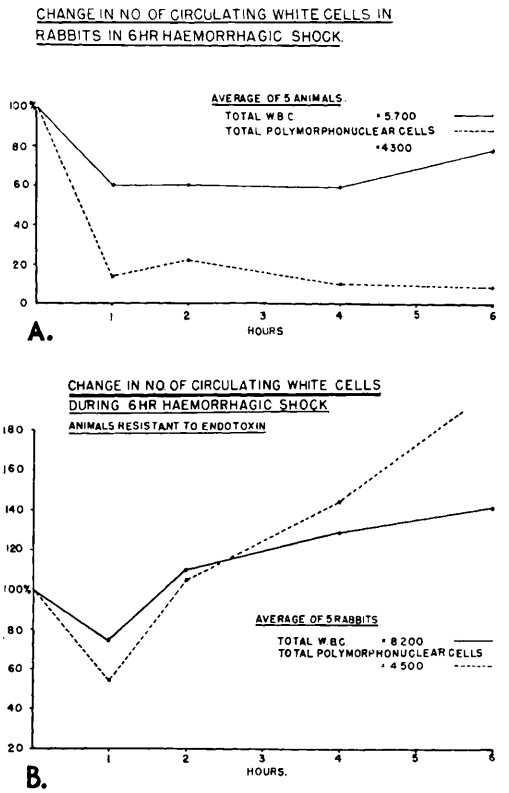

FIG. 2. A. Dotted line shows that sharp drop in granulocyte count of normal rabbits during prolonged hemorrhagic shock persists for duration of the shock. Return of the total white count to normal by the sixth hr is due to an absolute lymphocytosis. B. Rabbit resistant to endotoxin shows a much milder and very transient leucopenia, and a capacity while still in shock to respond with a granulocytosis.

rabbits tolerant to shock plasma (Table I). The non-survivors live for an average of 24 hours.

In Fig. 3 the solid line curve is typical of the rate of take-up in a normal rabbit in hemorrhagic shock at 50 mm Hg. The slope of the curve varies somewhat from one group of experiments to another, and in the warmer seasons the slope is steeper. The broken line is the curve for the rabbit resistant to endotoxin or shock plasma. Its horizontal shape signifies sustained compensation, *i.e.* no take-up for the full 6 hours.

Comment. The dose of endotoxin used to produce tolerance varied from 1/75-1/4,800 of an MLD/100. The tolerance to shock, as judged by mortality rate, was about the same for all doses down to and including 1/2400 of an MLD/100.‖ The shock plasma was as efficient as endotoxin in producing tolerance to shock. But it is difficult to say whether one can justifiably assay the endotoxic potency of the shock plasma in terms of the quantitative data on the effectiveness of endotoxin.

IV. *Toxicity of Blood of Rabbits Resistant to Shock.* The blood taken after 6 hours in shock from one group of endotoxin-tolerant rabbits, and tested for the presence of the toxin causing irreversibility, was non-toxic in seven of nine experiments.

V. *Susceptibility of Tolerant Rabbits Transfused After 1½ Hours in Shock to Toxic Plasma.* In this experiment 5 rabbits tolerant to shock plasma and, therefore, tolerant to shock, were put into shock for 1½ hours, then transfused with plasma known to be toxic to non-tolerant rabbits. Four out of the 5 survived, whereas only one of 12 non-tolerant animals survived similar treatment (1).

Comment. From the first of the latter 2 observations one may infer that the animal tolerant to shock is tolerant by virtue of its ability either to prevent production of toxin or to neutralize toxin if produced. That at least the latter is true is demonstrated by the second of these 2 observations.

Discussion. The evidence that a bacterial factor is involved in the death from hemorrhagic shock(4,5,6) is strongly supported by the foregoing observation that induction of tolerance to endotoxins induces tolerance to hemorrhagic shock. This observation also explains development of tolerance to hemorrhagic shock as well as drum shock in rats as a result of prior exposure to a sublethal amount of drum trauma(7), since there is reason to believe that bacterial invasion is facilitated by such trauma(8).

The fact that shock plasma contains a substance which acts like endotoxin in respect of the fever response to intravenous injection, and in respect of the capacity to induce tolerance to hemorrhagic shock, is strong evidence that this substance is an endotoxin. Shear and his co-workers, however, suggest that certain tissue polysaccharides which might be released during shock as a result of tissue anoxia, might be the agents responsible for irreversibility to transfusion.** For they have isolated polysaccharides from many tissues and from plants, which, like the levans prepared by Hestrin(9) and like endotoxin, bind Properdin in the presence of complement and magnesium(10), and otherwise behave like endotoxins(11).¶ Elsewhere we have stated why such substances, if present, do not account for irreversibility(1). In particular, we find it difficult to accept this hypothesis because non-absorbable antibiotics, given orally in advance of shock, which cannot prevent severe and prolonged shock from releasing such substances, do prevent irreversibility. Most of the bloods from rabbits and dogs pretreated with such antibiotics and shocked thereafter, are as harmless to recipient test animals as is normal plasma(1). Hence the lethal substance is exogenous, and the only lethal substance that non-absorbable anti-

‖ In Exp. IV (Table I) the 3 rabbits which died at 48 hours behaved during shock and in response to transfusion as well as the others. Intestinal mucosa at death was of normal appearance. Their death was attributable to severe sepsis in the groin wounds.

** Personal communication.

¶ Evidence that the toxic properties of these compounds are not in fact due to contaminating endotoxins has not yet been provided.

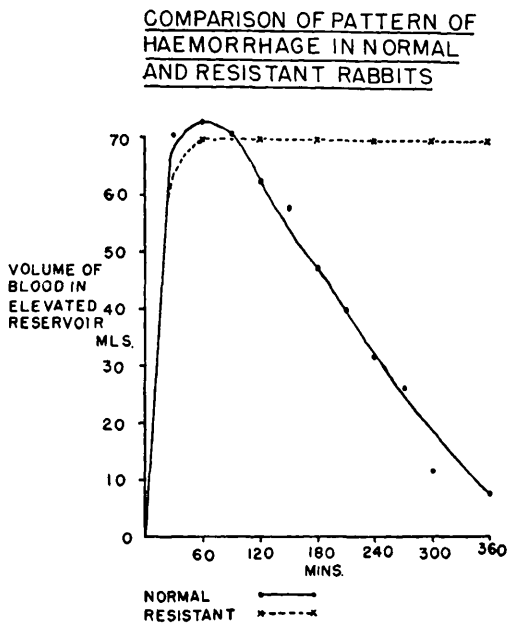

FIG. 3. Rise in the curves represents rate of bleed-out from the femoral artery of the rabbit into a reservoir elevated so as to keep the blood pressure constant at 50 mm Hg. Difference in the 2 curves thereafter represents difference in the rate at which blood returns spontaneously from reservoir to circulation.

biotics put into the gut are likely to eliminate is bacterial endotoxin.

Since shock plasma contains a transferable pyrogen which is nullified by tolerance, the pyrogen, like the toxin, is exogenous(12). Non-absorbable antibiotics eliminate the pyrogen (unpublished data) as well as the toxin from shock plasma. Therefore, it can be assumed that the pyrogen, like the toxin, is of bacterial origin, and is presumably the toxin itself.

Summary and conclusions. Rabbits made tolerant to bacterial endotoxin tolerate an otherwise lethal exposure to hemorrhagic shock. Whereas normal, *i.e.* non-tolerant, rabbits are dead after 6 hours of shock in spite of having received all the blood removed, tolerant rabbits are in satisfactory condition after 6 hours in spite of having received none of the blood removed. They respond promptly when transfused at the end of 6 hours and recover completely. The same results are secured in rabbits made tolerant to, *i.e.* pretreated with, shock plasma. Therefore, there is reason to consider the toxin in shock plasma an endotoxin. In the rabbit tolerant to endotoxin the blood taken after 6 hours in shock is free of the toxin causing irreversibility, which is in the blood of non-tolerant rabbits in shock. In the rabbit tolerant to shock the granulocytopenic response is distinctly different from that in the non-tolerant animal. The response is less intense and recovery to a normal or above normal count occurs while the rabbit is still in shock. In the non-tolerant rabbit the granulocytopenia persists throughout the shock period. The induction of tolerance to hemorrhagic shock by shock plasma as well as by endotoxin supports the view that a bacterial factor is responsible for irreversibility to transfusion in hemorrhagic shock. The fact that rabbits tolerant to hemorrhagic shock can resist a dose of shock blood or plasma that is lethal to non-tolerant rabbits, and that their bloods do not contain the toxin which develops in non-tolerant rabbits, supports the view that tolerance to hemorrhagic shock is due to the maintenance or enhancement of the normal capacity to neutralize bacterial endotoxin.

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