Host Resistance to Hemorrhagic Shock. XII. Mechanism of Protective Action of Dibenamine.* (23813)

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Protection by dibenamine against the development of irreversibility to transfusion in hemorrhagic shock was first demonstrated by Wiggers *et al.* in dogs(1). This finding was confirmed by Remington *et al.*(2), and later in rats by Shorr *et al.*, who also observed protection against drum shock by dibenamine (3). Jacob *et al.*(4) found that the protective effect of dibenamine given prior to induction of hemorrhagic shock in dogs was difficult to assess, since the hypotensive effect of the drug altered the framework of the standard experiment which they used to produce hemorrhagic shock. The considerably lower bleeding volume in their dibenaminized dogs suggested a less severe degree of shock; for when they were bled a volume equal to that required in untreated dogs to lower the blood pressure to a minimum level (30 mm Hg), they died. This finding was similar to that of Wiggers *et al.*(1), who found that if dibenamine was given to a dog after it had bled out to an arterial pressure of 30 mm Hg, infusion of a considerable fraction of the shed blood was required to prevent a further and lethal decline in arterial pressure. Shorr (personal communication), however, found that dibenaminized rats tolerate as great a bleeding volume as is required in the untreated rat to lower the blood pressure to a minimum level (50 mm Hg).

With the discovery of an endotoxemia in the dog and rabbit in hemorrhagic shock(5), the possible relationship between the protective action of dibenamine and the endotoxin came under consideration. The same question arose when it was shown that dibenamine prevents death from an otherwise fatal type of shock which develops following release of a one hour occlusion of the superior mesenteric artery, and which also appears to be due to endotoxemia(6). With this suggestive evidence that dibenamine might be acting as an anti-endotoxic agent, experiments were undertaken to see if dibenamine in hemorrhagic shock protects by preventing the appearance of the toxin, or by blocking its capacity to damage the peripheral vessels. In this communication evidence is presented that dibenamine protects against the development of irreversibility to transfusion in hemorrhagic shock by blocking the activity of the toxin which kills a non-dibenaminized animal(5).

Method. Adult white rabbits (Av. wt. 2.3 kg) were employed for this study. *Exp. 1. Effect of Dibenamine on the Course of Hemorrhagic Shock.* This experiment was performed in order to see if the protective effect of dibenamine in hemorrhagic shock as observed in dogs and rats is applicable to the rabbit. Hemorrhagic shock was produced as described elsewhere(7). In this experiment the rabbits bleed out into an elevated reservoir so as to lower the blood pressure to 50 mm Hg, where it remains until a transfusion is given. The volume of shed blood in the reservoir reaches a maximum within one hour (Fig. 1), after which it begins to return to the circulation. By or before the 6th hour all or most of the blood has returned spontaneously, and death occurs soon thereafter. If all the blood remaining in the reservoir is rapidly infused at any time after the fourth hour, the pressor response is poor, the rabbit remains prostrate, and death follows in a few hours. All animals show intramural focal hemorrhages in the intestine, a feature which is attributable to the action of endotoxin(5). The effect of dibenamine on the course of these events was determined as follows: In one group of rabbits dibenamine, in a dose of 20 mg/kg, was given intravenously three

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hours before inducing hemorrhagic shock.‡ In another group twice this dose was given 18 hours before inducing shock in order to avoid the influence of the toxic effects of the drug (*e.g.* convulsive tremors), which develop occasionally shortly after its administration, on the results of the experiment. The shock was continued in these two groups as in non-dibenaminized rabbits for six hours, after which all the shed blood remaining in the elevated reservoir was transfused. The pressor response to transfusion, the presence or absence of the hemorrhagic lesion in the gut,§ and the survival time were noted, and the results compared with those from non-dibenaminized rabbits.

Results. (*20 experiments with dibenamine and 15 without dibenamine*). The dibenaminized rabbits differed from the controls in many respects.‖ In the dibenaminized rabbits the maximum volume of hemorrhage required to lower the blood pressure to 50 mm Hg, and to keep it at this level, usually was 70% of that in untreated controls which were run simultaneously. The time taken to achieve maximum bleed-out in the dibenaminized rabbits was 2 hours, whereas the maximum bleed-out in the controls occurred within one hour. In the former there was almost complete absence of spontaneous return of blood from the elevated reservoir during the 6 hours of hypotension, and the pressor response to transfusion was significantly greater (72 ± 5 mm Hg) than in the controls (52 ± 4 mm Hg). Restoration to the pre-shock level was nearly complete (−10 mm ± 5 Hg) in the dibenaminized group, as compared to a deficiency of 50 mm ± 5 Hg in the control group. The controls responded poorly to the transfusion, showed the hemorrhagic lesion in the gut wall, and all but one died shortly after transfusion. In spite of a good pressor response the dibenaminized animals continued to be sick, and were unable to drink or eat on return to their cages. All but one developed severe sepsis in the groin wounds and died within 24-48 hours. Postmortem examination disclosed no injury to the gut, which is a characteristic finding in the irreversibly shocked animal.

Comment. The degree of hypovolemia which non-dibenaminized animals display on reaching and remaining at the lowest level of blood pressure they can tolerate for several hours (50 mm Hg in the rabbit, 40-45 mm Hg in the rat, and 30 mm Hg in the dog) is a constant (± 52 ± 10 ml). Hence the severity of hemorrhagic shock in all 3 species is a function of the degree of hypovolemia as well as the degree of hypotension. Since it is not possible in most experiments in the rabbit, rat or dog(1,2,4) to produce conditions with and without dibenamine that are comparable in terms of these 2 major dependent variables, one cannot conclude from the results obtained that dibenamine protects the animal. If the results of the experiments with and without the drug are compared in terms of degree and duration of hypotension, protection appears to have been obtained. But if the experiments are performed so as to produce the same degree of hypovolemia, no protection is obtained. Thus, 6 non-dibenaminized rabbits were bled to 50 mm Hg, and immediately after reaching the maximum bleed out they were equated with dibenaminized rabbits in terms of degree of hypovolemia by returning 30% of the maximum bled volume. The blood pressure rose to 80-88 mm Hg and remained there for 6 hours, with prompt and sustained recovery in response to transfusion at that time. The foregoing experiments, therefore, leave the issue in doubt. A better judgment as to whether dibenamine protects might be reached by observing whether this drug does or does not prevent the endotoxemia of hemorrhagic shock, which accounts for the

‡ Preliminary studies confirmed in rabbits the finding of Ahlquist in the dog(8) that dibenamine in a dose of 20 mg/kg body weight produces adrenergic blockade within 15 minutes. Maximum blockade was established in 25 min. The pressor action of epinephrine was never completely blocked, for 0.2 ml of a 1/10,000 solution still produced a small initial pressor response, followed by the characteristic reversal. Duration of reversal was longer, the longer the interval between administration of dibenamine and injection of epinephrine.

§ This lesion was looked for in survivors, which were killed at 48 hours, as well as in those that died.

‖ There was no difference between the results of the 2 groups of dibenaminized rabbits.

development of irreversibility to transfusion. To this end Experiment 2 was performed.

Exp. 2. Effect of Dibenamine on Toxicity of Blood in Hemorrhagic Shock. In this experiment 30 rabbits were put into hemorrhagic shock as described above. One group of 15 rabbits received no dibenamine. The other group of 15 rabbits was given dibenamine (20 mg/kg) 3 hours before inducing shock. All 30 rabbits were exsanguinated after 6 hours of shock. Each blood was tested for toxicity by a method described previously(5). All blood from non-dibenaminized animals displayed the presence of toxin. Of the 15 bloods from dibenaminized animals 11 were free of toxin, and 4 were toxic. It is noteworthy, however, that the degree of hypovolemia was less than in the controls in all 11 dibenaminized rabbits with non-toxic blood, and the same as in the controls in all 4 dibenaminized rabbits with toxic blood.

Comment. These results are not more definitive than those of Exp. 1; for it is as valid to conclude that the toxicity of the blood is governed by the severity of the shock (degree of hypovolemia) as it is that dibenamine is acting directly to prevent toxin. To determine more definitively whether dibenamine acts directly to prevent toxicity it is necessary to test the action of dibenamine when the hypotension and hypovolemia are the same as in the non-dibenaminized animal. This was done in Exp. 3.

Exp. 3. Effect of Dibenamine (20 mg/kg) on Vulnerability of Rabbits in Reversible Shock to Toxic Blood. In this experiment 10 rabbits were bled to a blood pressure of 50 mm Hg and maintained at this pressure for 1½ hours, at which time they were transfused with toxic blood from rabbits dying of shock of 6 hours duration. Dibenamine (20 mg/kg) was given I.V. in 10 cc of saline 15 minutes before the transfusion. As already reported, non-dibenaminized rabbits transfused after 1½ hours of shock with toxic blood lie prostrate after the transfusion, and 90% die within 18 hours(5). The results of the current experiments on rabbits given dibenamine were as follows: Six rabbits recovered completely and 4 died. Of the 4 which died, one died after 24 hours with the hemorrhagic lesion in the gut wall. The other three died after 3 or 4 days with wound sepsis, and without hemorrhage in the gut wall.

Comment. The saline given with the dibenamine was of no therapeutic importance, for in previous experiments many non-dibenaminized rabbits receiving as much or more saline at the time of the transfusion with toxic blood did not survive. Whether dibenamine was already exerting an anti-adrenergic effect at the time of the transfusion was not certain. In seven of the 10 experiments the blood pressure did not fall after it was given, but the pressor response to transfusion was lower (92 mm Hg mean systolic blood pressure) than in 5 control experiments (98 mm Hg mean systolic pressure).¶ In any case, dibenamine appears to have acted to prevent injury to the peripheral vessels by the injected toxin.

Discussion. How does dibenamine block the action of toxin? There is strong evidence that this toxin is an endotoxin(9,10). Thomas (11) has shown that endotoxin produces injury to the peripheral vessels with hemorrhagic extravasation and necrosis by an effect upon epinephrine. Hence the protective effect of dibenamine in hemorrhagic shock, *i.e.* blockade of the effect of the endotoxin upon the peripheral vessels and prevention of the hemorrhagic lesion in the gut, appears to be attributable to its antiadrenergic property. But dibenamine may also protect in virtue of its action on other tissues which govern the peripheral circulation, *e.g.* those controlling the release of serotonin(12), and which, in its absence, might be damaged by endotoxin. Whatever its sites of action may be, dibenamine preserves peripheral flow in shock(13). Thus the integrity of the tissues concerned with the detoxification of endotoxin may be sustained, and the responsiveness of the peripheral circulation to transfusion preserved.

Summary and conclusions. Since the development of irreversibility to transfusion in hemorrhagic shock has been shown to be

¶ These control experiments were performed for the specific purpose of comparing the pressor response with and without dibenamine. The volume of the transfusion was about the same in the controls as in the dibenaminized rabbits (65 ± 2 ml).

caused by bacterial endotoxins, and dibenamine prevents the development of irreversibility, the blood of dibenaminized rabbits in hemorrhagic shock was tested for the presence of endotoxin and found to be toxin free. But because the blood loss in the dibenaminized animal in shock is less than in the non-dibenaminized animal, the absence of toxin might be due to the lesser hypovolemia rather than to the dibenamine. Accordingly, an additional experiment was performed to see if dibenamine given to the rabbit in reversible shock will permit it to survive a transfusion of toxic blood from an irreversibly shocked donor—a procedure which kills the non-dibenaminized rabbit in reversible shock. This experiment demonstrates that dibenamine blocks the action of the toxin, and thereby not only preserves the responsiveness of the circulation, but also prevents the hemorrhagic lesion in the bowel wall, which is characteristic of irreversible shock.

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Circulatory Reactions of Dogs and Rats to Polyvinylpyrrolidone or Dextran After 48/80. (23814)

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Dogs and rats injected with the potent histamine liberator 48/80 become temporarily refractory to further administration of the same dose(1-2). Temporary tachyphylaxis also occurs in dogs after repeated injections of polyvinylpyrrolidone (PVP) and in rats after dextran(3). These colloids act as histamine releasers in each species respectively(4-5). We have sought evidence of cross-refractoriness using two criteria of sensitivity. One is the appearance of bluing in animals which have been injected with Evans blue (T-1824), and indicates an increase in skin capillary permeability to the albumin-dye complex. The other is a decrease in mean arterial blood pressure, indicative of a systemic reaction.

Methods and results. Details of the experimental procedure and summary of the results appear in Table I. No experiments are included in which the blood pressure at time of challenge was not 90 mm Hg or higher. Evans blue was given usually early in the experiment and in dosages (0.6-2 mg/kg) which produce no cutaneous bluing in untreated animals. In the first experiment 6 dogs received intravenously a series of graded doses of 48/80* as used by Slomka and Goth(6). After 44-112 μg/kg, small foci of blue became visible, surrounded by erythema. Gradually the bluing extended further and the flush faded. Ten minutes after a total of about 600-700 μg/kg, the mean blood pressures measured by catheter in a femoral artery were 20-50 mm

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