Host Resistance to Hemorrhagic Shock. XI. Role of Deficient Flow Through Intestine in Development of Irreversibility.* (23747)

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There is substantial evidence that bacterial endotoxins derived from the intestinal flora are the cause of irreversibility to transfusion and death in prolonged hemorrhagic shock(1-3). The accumulation of these toxins in the blood and tissues is in a large part owing to the failure of the systemic antibacterial and detoxifying defenses(4-10). Since the intestinal flora are the source of the endotoxins it is possible that the deficient flow through the intestine with resulting injury to the local defenses may also play a significant role in this phenomenon. Consequently, the effect of deficient flow through the intestine on the systemic circulation was investigated.

Method. A group of healthy adult New Zealand white rabbits (av wt 2-3 kg) were submitted to laparotomy under general or local anesthesia. The superior mesenteric artery was encircled by a silk thread, both ends of which were passed through a stiff plastic tube long enough to reach from the artery to a subcutaneous pocket, where the ends were buried. Two or 3 days later the ends of the loop were recovered under local anesthesia and the artery occluded by tightening them against the plastic tube. The artery was released after one hour, and the thread and tube removed without entering the abdomen. On several occasions the abdomen was opened to allow observation of the result of the occlusion, and it was noted that the occlusion produced spasm and pallor of the entire small intestine and the proximal half of the colon. The observations made included pulse rate, blood pressure (via a catheter in the femoral artery), rectal temperature, total and differential white blood count, hematocrit, plasma volume (by the radioactive iodinated albumin method, taking a single sample at 15-20 minutes), blood and peritoneal cultures, survival time and gross pathology at post mortem examination. The experiment was repeated in 2 additional groups of rabbits, one of which (Group II) was pretreated with antibiotics, the other (Group III) with dibenamine. The antibiotics were 100 mg of Neomycin and 10,000 units of Bacitracin given in 10 ml saline by gavage for 4 days, the last dose 2-3 hours before occlusion of the artery. Dibenamine was given intravenously in a dose of 20 mg/kg. In the later experiments with this drug it was given 18 hours before occlusion, because in earlier experiments, in which the drug was given 3 hours before occlusion, nystagmus, ataxia and convulsive seizures occurred in several animals which died during occlusion or soon after release of the occlusion. By the use of the longer interval it was possible to exclude these complicating effects of the drug, which develop soon after its administration. In one additional group of rabbits the occlusion was not released, and no treatment was given. In this group only the gross response and survival time were observed.

Results. Group I: Occlusion without pretreatment. The course of events was consistently as follows: During the hour of occlusion there was no significant change in general appearance, alertness, or vital signs. Following release of the occlusion the animal was quiet and responded little when prodded. Respirations were rapid and shallow. Of 37 rabbits in this group 33 (89%) died within 40 minutes to seven hours (average 2 hours) after release of the occlusion. In 12 animals the blood pressure was observed continuously throughout the experiments. The mean systolic blood pressure before occlusion was 100 mm Hg (range 80-110), and during occlusion it was 84 (range 78-110). Immediately after release of the occlusion it fell to 76 (range 65-105), where it remained with slight fluctuations until about one hour before death, when

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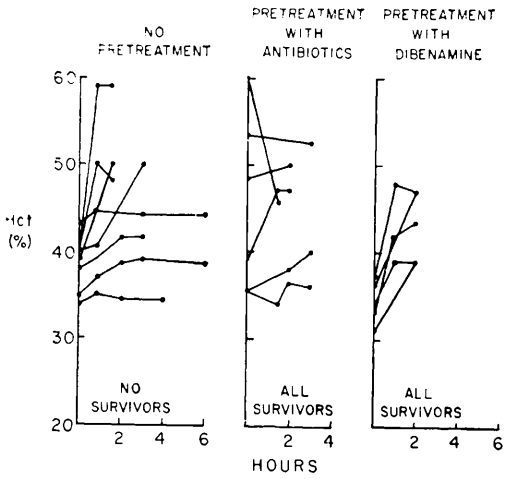

FIG. 1. Hematocrit values following release of one hr occlusion of superior mesenteric artery. The hematocrit curves for non-survivors pretreated with antibiotics or dibenamine were essentially the same as for survivors. In figure for dibenaminized animals values are given for 5 experiments. Terminal values were the same in 2 pairs of animals.

it fell abruptly to 30-50 mm Hg, with the manifestations of profound shock. Hematocrits were determined in 16 experiments. Measurements were made before occlusion and several times following release of the occlusion. In most instances, these did not change significantly (Fig. 1). In 8 experiments simultaneous plasma volume and hematocrit determinations were made before occlusion, and again one or more hours after release of the occlusion. In 6 instances the second plasma volume measurements were obtained while the systolic pressure was still at or above 65 mm Hg. In these the average plasma volume had fallen 18% (range 0-35%) (Fig. 2). In the remaining 2 the second plasma volume determination was made shortly before death, and the fall was 55% and 65% respectively. In most of the 8 experiments there was no correspondence between the change in hematocrit and the change in plasma volume.

During the occlusion there was a slight fall in the total white count, but no shift in the per cent of granulocytes. One hour after release of the occlusion there was a moderate leucopenia, and a severe granulocytopenia. The fall in granulocytes was from a mean control value of 67% of the total white cell count to one of 22% (range 18-25%).

The rectal temperature declined steadily until death, reaching some 10° below the initial value in the longest survivors. At post mortem examination there was no urine in the bladder. The intestine showed localized and diffuse intramural hemorrhages, most numerous in the cecum, and patches of diffuse violaceous discoloration. There was a variable but small amount of edema in the terminal ileum. There was no bloody fluid in the gut lumen, no unusual amount of peritoneal fluid, and no peritoneal inflammatory reaction. Swab cultures of the peritoneum were sterile. So were all ante-mortem and post-mortem blood cultures.

To evaluate the importance of the measured plasma volume deficit in the development of shock, four rabbits were treated with repeated infusions of plasma, given so as to prevent a rise in hematocrit (*i.e.* 10 ml every 30 minutes for 6 hours), beginning immediately after the occlusion. Although the survival time was prolonged to 12, 22, 24 and 36 hours respectively, none survived, and at post mortem all 4 rabbits showed the same findings as those not so treated.

In contrast to an average survival time of 2 hours after temporary occlusion, rabbits

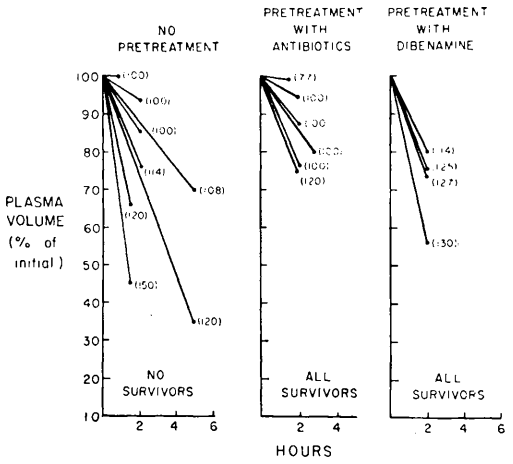

FIG. 2. Changes in plasma vol and hematocrit following release of one hr occlusion of superior mesenteric artery. Hematocrits are in parentheses adjacent to plasma vol measurements and expressed in % of preocclusion values. Values for non-survivors pretreated with antibiotics or dibenamine were essentially the same as for survivors.

with permanent occlusion remained in apparently good condition for 6 hours, and then developed severe hypotension followed by death an hour or so thereafter.

Group II: *Pretreatment with antibiotics.* Of 21 rabbits in this group 16 (76%) survived. The 5 which died lived longer than those in Group I, but the course of events and the post mortem findings were essentially the same as in Group I. The blood pressure was observed continuously in 6 experiments. The mean systolic blood pressure before occlusion was 97 (range 85-110). During occlusion it fell to 88 (range 80-100). Immediately after release of occlusion it fell to 63 (range 45-75), where it remained for the subsequent several hours of observation. In these 6 experiments plasma volume measurements were made before occlusion, and again about 2 hours after release of occlusion. The changes were about the same as those in Group I (Fig. 2). Simultaneous hematocrit determinations did not reflect these changes (Fig. 1 and 2).

The initial white blood count fell slightly during the occlusion, but the granulocyte count did not. Following release of the occlusion a granulocytopenia developed, but it was less severe than in the untreated group *i.e.* 43% (range 32-53%) of the total count as compared to 22% (range 18-25%)), and began to return toward normal by the fifth hour.

The rectal temperature fell progressively as in the untreated group. Severe shock did not develop. By the sixth hour these animals were recovering, and the next day they appeared entirely well.

Nine rabbits, not included in the series, were prepared the same way, but were killed 4 hours after release of the occlusion. Of these two showed intramural intestinal hemorrhage, 3 showed slight scattered punctate hemorrhages, and 4 showed no gross changes whatever.

Group III: *Pretreatment with dibenamine.* Fifteen (75%) of 20 rabbits pretreated with dibenamine recovered completely. (Three of the five that died exhibited nystagmus, ataxia and convulsive seizures before and during the occlusion and may, therefore, have succumbed to the toxic effects of the dibenamine.) The blood pressure was observed continuously in 5 animals. The mean systolic pressure before occlusion was 88 (range 80-100). No change occurred during occlusion. After release of the occlusion 3 animals developed a severe hypotension (30-45 mm Hg) for the subsequent 3 hour period of observation. In the other 2 the systolic pressure fell only slightly and remained unchanged at 70 and 90 mm Hg respectively. All 5 animals were among the survivors. Plasma volume measurements were made in 4 experiments. The fall 2 hours after release of the occlusion was comparable to that in the other groups (Fig. 2). The changes in hematocrit were more proportionate to the changes in plasma volume in this group than in the others.

The white blood counts showed little decline, and granulocytopenia did not develop. The rectal temperature fell as in the other groups. In several experiments not included in the series the rabbits were killed 4-6 hours after release of the occlusion. None showed intestinal hemorrhage or edema. The peritoneum was normal. In the 5 rabbits that died the findings were the same.

Discussion. Our experiments were designed on the assumption that the nature of injury to the gut inflicted by 1 hour of total ischemia should be similar to that produced by 4 or more hours of deficient flow that results in failure of animal in hemorrhagic shock to respond to transfusion. The shock which developed soon after release of occlusion was not unlike the hemorrhagic shock which is irreversible to transfusion. For vascular collapse was profound, and was not corrected by plasma volume therapy. Although this treatment prolonged life considerably, one cannot consider hypovolemia a primary factor in causation of shock, or in its lethal outcome, because hypovolemia was about equal in all 3 groups of animals, and because recovery occurred in Groups II and III without plasma volume therapy.

That death following occlusion of the superior mesenteric artery was caused by absorption of endotoxins is indicated by the fact that non-absorbable antibiotics prevented shock and death in most animals. Presumably systemic defenses were intact until occlusion was released. The rapid collapse fol-

lowing release suggests flooding of circulation with toxin sufficient to overwhelm these defenses and to destroy the integrity of peripheral vessels, in much the same way as lethal intravenous dose of endotoxin to a healthy animal produces failure of peripheral circulation. The more rapid death in rabbits in which occlusion was released than in those in which it was not released may be merely a measure of difference in rate of transfer of toxin from the gut to the systemic circulation.

Since injury resulting from intestinal ischemia in a healthy animal can produce irreversible shock, it is permissible to infer that a similar less rapidly induced intestinal injury can occur in hemorrhagic shock; and, therefore, when there is failure to respond to transfusion, it may be in consequence of toxin entering the circulation from the gut (whether or not there is any other source of toxin) in an animal whose systemic defenses against toxins are already badly damaged.

Severe hypotension which occurred in some dibenaminized animals following release of occlusion, may be due to proportionately greater hypotensive effect of a given amount of hypovolemia in the dibenaminized animal, than in the non-dibenaminized animal(11). Since hypovolemia and hypotension were as severe in these animals as in untreated ones, and did not prevent recovery, the protective effect of dibenamine must be related to some other phenomenon. Data suggesting that it acts to block endotoxins are as follows: 1. Endotoxin in blood of hemorrhagic shock is prevented from injuring a susceptible test animal if the latter is dibenaminized shortly before the test(12). 2. The hemorrhagic lesion in the gut in hemorrhagic shock is due to endotoxin(2,10). This lesion was present in the non-dibenaminized animal following release of the occluded superior mesenteric artery, and absent in the dibenaminized animal subjected to the same procedure.

Thomas has demonstrated that endotoxin potentiates local action of adrenalin, and that dibenamine blocks the effect of endotoxin by its anti-adrenergic property(13). One may therefore explain the protective effect of dibenamine in animals with occlusion of superior mesenteric artery by the hypothesis that it blocks the action of endotoxin rather than prevention of endotoxemia.

The foregoing analysis of the data is relevant to the observation that provision of an adequate flow of arterial blood to the liver (14), or the intestine of the animal(15) in severe and prolonged hemorrhagic shock by cross circulation with a healthy donor prevents development of irreversibility to transfusion and death. It is such phenomena as are under consideration here that may also be involved in the unexplained deaths following temporary interruption of flow through the lower thoracic aorta during aortic graft surgery, or shock and death that not infrequently follows surgical reduction of a strangulated hernia without resection of bowel with a marginal vascular integrity.

Summary and conclusions. 1) Transient ischemia of the gut in rabbits produced a type of shock that is similar to the state of hemorrhagic shock which is irreversible to transfusion. Since the shock was as profound and as lethal whether a substantial plasma volume loss did or did not occur, hypovolemia was not an essential feature. This is further demonstrated by the observation that plasma volume therapy delayed but did not prevent death, and by the observation that dibenaminized animals and animals given antibiotics developed an equal degree of hypovolemia, but survived without blood volume therapy. Bacterial cultures were sterile, and the only notable gross lesion at death was intramural hemorrhage in the gut wall. 2) Dibenamine given in advance of the occlusion prevented the development of irreversible shock and resulted in recovery without therapy. When killed before or after recovery from shock these rabbits did not show a visible injury to the gut. 3) Non-absorbable antibiotics given in advance achieved the same protection as dibenamine. 4) Dibenamine prevented and the antibiotics reduced the degree and duration of the granulocytopenia which occurs in untreated animals. 5) A fall in rectal temperature occurred in all rabbits exposed to the vascular occlusion of the gut whether they developed shock or not, and whether they survived or not. 6) Transient ischemia of the gut produces an endotoxemia derived from

the intraintestinal flora sufficient to overwhelm the normal detoxifying potential, and so induces irreversible shock. 7) These observations are in conformity with prior evidence that irreversibility to transfusion in hemorrhagic shock is due to endotoxins derived from the intestinal flora, and suggest further that deficient flow through the intestine accounts for the invasion of circulation by these endotoxins in sufficient quantity to produce irreversibility.

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Bactericidal Activity of Normal Serum Against Bacterial Cultures. I. Activity Against *Salmonella typhi* Strains. (23748)

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The bactericidal action of normal serum, long recognized as a factor in the total complex of "non-specific" resistance to infection, is still of uncertain significance. Smooth forms of the Gram-negative bacilli are generally relatively virulent and resistant to the bactericidal action of normal mammalian serum; rough forms are avirulent and more susceptible. On the other hand, it has been reported that *Escherichia coli* is more resistant to normal serum than pathogenic microorganisms of the Enterobacteriaceae(1). This report requires modification since it is now recognized that certain serotypes of *E. coli* may be pathogenic and that different strains of a bacterial species may show marked differences in their susceptibility to the bactericidal action of normal serum. Also, in the case of anthrax, the serum of the naturally immune dog has little effect against the anthrax bacillus, but serum of the susceptible rabbit is bactericidal(2). Moreover, different mechanisms may be involved in serum bactericidal action. Serum substances, active against some Gram-positive organisms, are non-specific and heat stable. Complement is probably not involved in the activity of these substances(3). In contrast, the action exerted upon most Gram-negative organisms is mediated by natural antibody and complement and recently the properdin system has been implicated(4). Previous investigators have concluded that bactericidal antibodies in normal serum active against Gram-negative organisms are species specific(5,6); others have considered them non-specific or of limited specificity(7,8).

The present study was undertaken to reinvestigate some aspects of the bactericidal action of normal serum with the quantitative