Influence of Antibiotics and Hemorrhage upon Serum Properdin Titers.* (26706)

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During the past decade, Fine and his collaborators have adduced increasing experimental evidence which suggests that bacterial endotoxins play a significant role in hemorrhagic shock(1). They have demonstrated that an increase in survival rate resulted from pre-treatment with antibiotics (2). On the other hand, when the reticuloendothelial system (RES) is blocked, antibiotics given by gavage may have a lethal effect(3). They have postulated that, under these conditions, antibiotics liberate increased quantities of endotoxin from the Gram-negative intestinal flora.

The present study was undertaken to test this hypothesis. It has been demonstrated that, after a certain interval of time, injections of endotoxin increase serum properdin titers(4). In a series of experiments, the effect of orally administered antibiotics upon serum properdin titers was ascertained, to observe whether serum properdin titers would also rise under these conditions. In addition, these animals were subjected to hemorrhagic hypotension to determine the effects of this antibiotic regimen upon survival rate and upon changes in properdin titre associated with hemorrhage.

Method. Thirty mongrel dogs weighing from 9-16 kg were selected and randomly divided into 3 equal groups. Group 1 received no treatment prior to hemorrhagic hypotension. Group II was given 2 oz. milk of magnesia orally for 5 days, starting 7 days before hemorrhage. Group III also received milk of magnesia for 5 days, but in addition was treated with 6 g of sulfasuxidine and 4 g of neomycin sulfate orally for the 7 days prior to hemorrhage.

Estimations of the viable Gram-positive organisms in the fecal flora were made on enriched nutrient agar containing 0.2 g of sodium azide per liter. For the Gram-negative organisms, Levine's E.M.B. agar (Difco) was used. A loop 1 cm in diameter was used to streak serial 10-fold dilutions of the feces on the surface of the agar plates. Observations were made of the highest dilution showing growth and whether or not the metallic sheen typical of *E. coli* was present on the E.M.B. plates. Streaks were made prior to initiation of treatment and before induction of hypotension.

The dogs were subjected to hemorrhagic hypotension in random sequence from Groups I, II and III. All animals were deprived of food but not water for 15 hours prior to experiment. Sodium pentobarbital (30 mg/kg) was given intravenously. All apparatus was sterile and incisions were made using aseptic technic. A femoral artery and vein were cannulated, and heparin‡ (2 mg/kg) was administered. The artery was connected by means of a T-tube to a mercury manometer and a collecting reservoir. The zero level of the manometer was set at the hydrostatic level of the femoral artery. The dog was allowed to bleed until its arterial pressure fell to 35 mm Hg, which was established by the height of the reservoir. After 3 hours of hypotension, the blood remaining in the reservoir was returned to the dog, and the incision was repaired.

Blood for properdin determinations was drawn before treatment and periodically throughout the experiment. Plasma was stored at −30°C in sealed vials. Properdin titers were determined by the phage neutralization method(5) and are expressed in terms of per cent of control value, as mea-

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sured in 50% neutralization units/ml of undiluted plasma.§

Results. The 2 control groups—I, untreated, and II treated with milk of magnesia —had 4 and 5 survivors, respectively, for a total of 9 survivors out of 20. In Group III, treated with milk of magnesia plus antibiotics, 8 out of 10 survived, giving a statistically significant increase in survival rate ($P = 0.05$) as compared to the 2 control groups.

Examination of the fecal streaks made before treatment revealed Gram-positive and Gram-negative bacteria. Typical *E. coli* were present in the feces in all animals. Milk of magnesia alone reduced the growth by approximately 10 to 100-fold, but failed to eliminate *E. coli* in any of the animals in Group II. Group III, which received antibiotics in addition, showed a considerable diminution in fecal flora. An average of 10^3 to 10^4-fold reduction in both Gram-positive and Gram-negative growth was noted. *E. coli* were absent in all but one animal, and in this dog, the Gram-negative organisms had been reduced from growth in the 10^{-7} dilution before treatment to the 10^{-2} dilution after antibiotics.

The course of properdin titers in the animals in Group I was similar to that previously reported for untreated dogs(6,7). During the oligemic period, properdin levels remained relatively unchanged. In the first 5 hours after hypotension, titers fell rapidly, and to a greater extent in the animals that died. In the survivors, titers began to rise by the 2nd day and reached a level well above control in the following 2-4 days. Later they started to fall toward control. The average control properdin titers of the 4 survivors and 6 non-survivors were vitually identical. In fact, if all the dogs included in this study are divided into survivors and non-survivors, there is no statistical relationship between control properdin levels and survival; *i.e.*, 17 survivors, 28.0 ± 14.0 (S.D.), and 13 non-survivors, 33.1 ± 9.0 $Ph.N_{50}$ units/ml of plasma.

In Group II, treated with milk of magnesia, at the time of induction of hypotension, the 5 survivors had properdin titers averaging 140 ± 12% of their pre-treatment levels. The 5 non-survivors had an average titer just prior to oligemia of 96.2 ± 8.3% of control ($P<0.001$).

Prior to oligemia, the 8 survivors of Group III (milk of magnesia plus antibiotics) showed an average increase in properdin level to 148.5% of control titer whereas the 2 non-survivors averaged 96%. The individual properdin titers at that time were 128, 80, 255, 124, 180, 133, 122, and 150% for survivors, and 112 and 80 per cent for non-survivors. The course of properdin levels in survivors of Groups II and III after hemorrhage followed a pattern similar to that described above.

Discussion. The decline of properdin levels during hemorrhagic shock was first noted by Frank *et al.*(8). Subsequently it was demonstrated(6,7) that properdin titers remain unchanged during the oligemic period but decline rapidly in the hours following transfusion, and to a greater extent in non-survivors than in survivors. After the first 24 hours, properdin titers rise to levels well in excess of control, reach a peak at the 4th to 7th day, then gradually return toward control. Injections of zymosan were shown to increase significantly properdin levels as well as survival rates(9).

In the present study, as in that reported by Schweinburg *et al.*(2), treatment with antibiotics (Group III) was successful in significantly increasing the survival rate of dogs subjected to a standard hemorrhagic hypotension. The recent work of Wiznitzer *et al.*(10), relating the size of the intraintestinal pool of endotoxin to development of irreversible hemorrhagic shock, could account in part for the survivals. Seven of the 8 survivors in this group had pronounced increase in properdin titers following antibiotic administration, again demonstrating a direct relationship between increased properdin levels and survival(9), though not necessarily a cause and effect relationship.

The augmentation of properdin levels which occurred as a result of antibiotic therapy constitutes supporting evidence for the

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hypothesis advanced by Fine and his collaborators(3). These investigators postulated that the lethal effects produced by antibiotics administered by gavage to animals in which the RES had been blocked are attributable to relase of endotoxin from the normal intestinal flora. It has been shown that injections of endotoxin induce increased properdin titers after an initial decline(4). It is not unlikely, therefore, that in the present study the antibiotics caused increased quantities of endotoxin to be released into the intestinal tract. This in turn would, after passage through the mucosa, produce the observed elevation of the properdin titer. Antibiotics probably exert effects other than their bactericidal activities, however. For example, it has been claimed that antibiotics abrogate the depressant action of endotoxin upon the RES(11). Thus, it is conceivable that the observed changes in properdin might be ascribable to some side-action of the antibiotics.

In Group II, the increase in properdin titers resulting from treatment with milk of magnesia alone is also difficult to explain. The milk of magnesia may also have released increased quantities of endotoxin, or it may have affected mucosal permeability to endotoxin, or it may have acted by some entirely different mechanism.

Conclusion. In dogs subjected to a standard hemorrhagic hypotension, survival rate was significantly increased by prior treatment with antibiotics. This regimen eliminated typical *E. coli,* and markedly reduced the Gram-negative fecal flora. Seven of 8 of these survivors had significantly increased properdin titers following antibiotic administration. In a group of dogs treated with milk of magnesia, though the survival rate was not increased, the survivors all had significantly increased properdin titers. The pre-treatment properdin titers were not significantly related to subsequent survival from standard hemorrhagic hypotension.

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Transfer of Water Across the Perfused Umbilical Cord.* (26707)

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An appreciable proportion of the water exchange of the amniotic fluid takes place through the intermedium of the fetus(1). The mechanism and site of this exchange is unknown. Since micturition and deglutition cannot account for the magnitude of this exchange, a transfer across the umbilical cord and possibly the fetal surface of the placenta has been suggested as an alternative explana-

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