The Initial Stage of Canine Endotoxin Shock as an Expression of Anaphylactic Shock: Studies on Complement Titers and Plasma Histamine Concentrations *

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Within 30 to 60 seconds after endotoxin is injected into adult mongrel dogs there is a decline in systemic blood pressure, a rise in portal vein pressure, a reduction in the venous return of blood to the heart, and a decrease in renal blood flow (1, 2). Endotoxin shock is comparable in many ways to acute anaphylactic shock in the dog (3). Several observations have suggested that an immune mechanism causes the initial vascular changes. It was demonstrated that the action of endotoxin on blood vessels was mediated through a heat labile factor in plasma or serum (4, 5). Gilbert and Braude (6) in studies on Escherichia coli endotoxin shock in rabbits reported that doses of endotoxin greater than the LD₅₀ caused a decline in the titer of complement and a decrease in the serum concentration of E. coli antibody. Spink and Potter (7) also observed a prompt decrease in plasma complement values in canine endotoxin shock.

The immediate effect of endotoxin on smaller vessels suggested that a vasoactive substance or substances were also implicated. If an immune mechanism were involved, histamine could be one of the substances that was liberated by an antigen antibody mechanism, and studies do point to histamine as a factor causing the altered vascular activity (3, 8–10).

This report concerns the study of complement titers, plasma histamine concentrations, and blood pressure changes in a series of dogs given a lethal dose of endotoxin. In addition, since epsilon-aminocaproic acid and cortisol protect dogs against endotoxin, we were interested in ascertaining the effect of these two agents upon complement and histamine values. The data support the concept that the initial hemodynamic phase of canine endotoxin shock is related to an immune mechanism of the immediate anaphylactic type.

Methods

Animals. A total of 85 adult mongrel dogs was used in a series of 10 experiments.

Endotoxin. The same lot of E. coli endotoxin was used throughout and was prepared as described elsewhere (11). The LD₅₀ of the endotoxin was first established in mice and then in dogs. The LD₅₀ for the latter species was 0.55 mg per kg.

Complement assay. Titers were expressed as 50% hemolytic units, employing the method outlined by Kabat and Mayer (12). Blood was collected before the injection of endotoxin and then 10 minutes, 1, 3, and 6 hours later. After the blood had clotted at room temperature, serum was removed by centrifugation and stored at −20° C overnight. Control studies showed that this temporary storage did not result in significant deterioration of complement.

Histamine assay. Serial plasma histamine concentrations were determined by a modification (13) of the fluorometric assay described by Shore, Burkhalter, and Cohn (14). The reliability of this method was determined by bioassay on duplicate samples through the courtesy of Dr. Charles Code of the Mayo Foundation for Medical Research. A good correlation of the two techniques was found.

Epsilon-aminocaproic acid (EACA). One g contained in sterile vials was infused intravenously in 100 ml of 5% dextrose and distilled water over a period of 15 minutes before the administration of endotoxin. Complement and histamine assays were carried out before and after the injection of EACA, and after endotoxin had been given.

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1 Supplied by Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.
Cortisol. One hundred mg contained in sterile vials was infused intravenously in 100 ml of 5% dextrose and water over a period of 15 minutes before the administration of endotoxin. Complement and histamine assays were likewise obtained after this infusion, as well as after endotoxin.

EACA plus cortisol. Quantities of these two agents as given above were infused simultaneously into animals, and complement and histamine values were obtained during the same periods before and after the injection of endotoxin.

Observations on shock. The animals, anesthetized with sodium pentobarbital, were studied in a manner previously described (1). Continuous femoral arterial blood pressure determinations and urinary output were recorded up until 6 hours after endotoxin, or until death.

Control animals. To assess the role of anesthesia, the loss of blood necessary for the tests, trauma due to femoral catheterization, and the effect of intravenous injections of physiologic solutions, the following studies were carried out: 1) complement titters on three dogs anesthetized and given 100 ml of 5% dextrose and water (this was done to control the effects on complement following the infusion of EACA and cortisol); 2) serial histamine assays on six dogs to determine the effects of anesthesia, the trauma of catheterization, and the injection of 2 ml of saline, which was the amount of solution required for the administration of endotoxin; 3) complement titters in six dogs after a lethal dose of the snake venom, Crotalus terrificus, since the initial hemodynamic alterations in the dog could be produced by substances other than endotoxin, such as snake venom.

Results

1. Complement titters and histamine concentrations in dogs given a lethal dose of endotoxin.

Ten dogs, weighing from 8.1 to 12.5 kg, were anesthetized and given 0.55 mg per kg of endotoxin. Survival times varied from 2½ to 16 hours. The decline in complement is shown graphically in Figure 1. Preliminary studies on complement titters at 1, 5, 10, and 30 minutes after a lethal dose of endotoxin had shown that the average maximal decline occurred at 10 minutes. After this period there was a tendency for the complement titters to rise, especially in dogs that recovered. Although arbitrary, the time of apparent maximal decline of complement was chosen for comparison in most studies. The decline of complement in animals 10 minutes after endotoxin (Table I)

### Table 1

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<th>Pretreated with cortisol</th>
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<td>Mean ± SE</td>
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* EACA = epsilon-aminocaproic acid.
TABLE II

Mean plasma histamine concentrations (μg/ml) and systolic blood pressure (mm Hg) in adult dogs after a lethal dose of endotoxin (0.55 mg/kg)*

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma histamine</th>
<th>Control (μg/ml)</th>
<th>Plasma histamine</th>
<th>Control (μg/ml)</th>
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<td>0.04 ± 0.02</td>
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<td>0.21 ± 0.14</td>
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<tr>
<td>Post-endotoxin, 5 min</td>
<td>0.02</td>
<td>0.02 ± 0.01</td>
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</tr>
</tbody>
</table>

* EACA = epiallin-aminoacetic acid; B.P. = blood pressure.

was highly significant compared to saline control animals (p < 0.01).

Plasma histamine concentrations are presented in Table II. Many preliminary observations revealed that the maximal rise in plasma histamine occurred between 30 and 60 seconds after the injection of endotoxin. An explosive rise occurred in five dogs at the end of 60 seconds, revealing an average concentration 8 times the value obtained before endotoxin was injected. This elevation was also confirmed in selected animals by the bioassay method used by Dr. Code. The mean increase in plasma histamine was significantly greater than in saline-injected dogs 5 minutes after endotoxin (p < 0.05), but was not statistically significant after 30 and 60 seconds. In dogs that survived 4 hours, histamine values approached those of the control pre-endotoxin period.

It has been repeatedly observed in the dog that an abrupt drop in systemic pressure occurred 1 to 5 minutes after endotoxin. Within 15 to 30 minutes pressures usually approached the pre-endotoxin base line. There was a good correlation between the abrupt rise in histamine and the initial period of hypotension (Table II), although two animals failed to show an appreciable increase in histamine concentrations, and in three initial hypotension preceded a demonstrated increase in histamine concentrations.

2. Complement titers in dogs given an LD₅₀ injection of endotoxin. Six animals given an LD₅₀ injection revealed a distinct difference in the values of complement on the basis of survival or death as seen in Figure 2. All animals had an initial drop in complement. In the three animals that survived the mean titer was 77% of the control, whereas in the three that expired the mean titer was 23% of control after 3 hours. This difference is highly significant (p < 0.01).

3. Complement titers and histamine concentrations in dogs pretreated with EACA and then given a lethal dose of endotoxin. In previous studies the majority of dogs pretreated with EACA survived a lethal dose of endotoxin (15). Ten dogs were given 1 g of EACA as an infusion in 100 ml of 5% dextrose and water. At the end of this time 0.55 mg per kg of endotoxin was injected. Seven of the 10 dogs survived.
Complement titers in this group of animals were not appreciably altered by the initial infusion of 1 g of EACA in 100 ml of 5% dextrose and water, the mean decrease in complement being 19% in animals receiving dextrose and water alone, and 25% in those given EACA in dextrose and water. The mean complement titer that was present after the EACA infusion and before the injection of endotoxin was not significantly different from the values in those control animals receiving endotoxin alone. In the dogs pretreated with EACA there was no fall in complement after 10 minutes in those that survived and those that died.

Mean plasma histamine concentrations of 10 animals pretreated with EACA and then given a lethal dose of endotoxin are shown in Table II. The average values for this treated group are only slightly lower than the untreated control group. Coincident with the rise in plasma histamine there was a moderate fall in blood pressure. The infusion of EACA alone causes a rise in systemic pressure, an observation that has been confirmed by others (16).

This experiment showed that pretreatment of dogs with EACA significantly reduced the mortality rate from endotoxin and also reduced the fall in complement when compared to animals receiving endotoxin alone (p < 0.02), as noted in Table I. In EACA-treated animals plasma histamine concentrations rose significantly 60 seconds after endotoxin (p < 0.05).

4. Complement titers and histamine concentrations in dogs pretreated with cortisol and then given a lethal dose of endotoxin. Pretreatment with cortisol protects dogs against a lethal dose of endotoxin. Ten dogs were given 100 mg of cortisol intravenously in 100 ml of 5% dextrose and saline. Seven of the 10 dogs survived. As seen in Table I this amount of cortisol did not significantly alter the initial decline in complement titers 10 minutes after endotoxin, as compared to the titers in control endotoxin animals (p = 0.2). The mean pre-endotoxin titer after cortisol infusion (66 U) was lower than in endotoxin-treated dogs (p < 0.01).

Cortisol has a pronounced effect in suppressing the rise in plasma histamine concentrations resulting from endotoxin (Table II). The control animals receiving endotoxin alone had an eightfold rise, whereas animals pretreated with cortisol had slightly less than a fourfold elevation. In animals pretreated with cortisol and given endotoxin the plasma histamine concentrations were no greater than in those animals given saline solution alone. In addition, the initial fall in systemic pressure was somewhat less in cortisol-treated animals than in the control group. Sixty seconds after injection of endotoxin, when the maximal average level of 0.19 μg per ml of histamine was reached in the plasma, the average systolic blood pressure was 136 mm Hg. Comparable values in the control endotoxin group were 0.40 μg per ml of histamine and a pressure of 91 mm Hg. Although the differences in plasma histamine were not statistically significant after 30 and 60 seconds, 5 minutes after endotoxin plasma histamine in the cortisol-treated group was 0.09 μg per ml, and in the endotoxin controls, 0.27; this difference was significant (p < 0.02). The respective mean systolic blood pressures were 123 and 95 (Table II).

5. Complement titers and histamine concentrations in dogs pretreated with both EACA and cortisol and then given a lethal dose of endotoxin. Studies reported elsewhere (15, 17) indicated that different mechanisms were involved for EACA and for cortisol in the protection afforded dogs against endotoxin. For this reason 10 dogs were
### TABLE III

Plasma histamine concentrations (µg/ml) and systolic blood pressure (mm Hg) after second lethal dose of endotoxin in dogs that survived initial injection of endotoxin*

<table>
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<th>2 F, 10.2</th>
<th>3 F, 16.6</th>
<th>4 M, 12.1</th>
<th>5 M, 11.0</th>
<th>6 M, 8.0</th>
<th>7 F, 8.0</th>
<th>8 F, 16.8</th>
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* B.P. = blood pressure; EACA = epsilon-aminocaproic acid.
infused simultaneously with 1 g EACA and 100 mg cortisol contained in 100 ml 5% dextrose and water. Seven of 10 animals survived 0.55 mg per kg of endotoxin, which was the same outcome in an equal number of animals pretreated with EACA or cortisol alone.

This combination alone had no effect on the complement titer compared to dextrose-infused dogs. The mean control titer after EACA and cortisol (87 U) did not differ significantly from the endotoxin control group (93 U). Ten minutes after the injection of endotoxin the average decrease in titer was less than that observed in control endotoxin animals (p < 0.01) or in those pretreated with either EACA or cortisol (Table I).

The average concentration of plasma histamine in this group did not exceed 0.09 µg per ml (Table II). Five minutes after endotoxin the mean plasma histamine concentration was less (0.08) than in animals given only endotoxin (0.27); the difference was significant (p < 0.02). It was again observed that the systemic pressure rose after the infusion of EACA and cortisol, and the average level in systolic pressure after endotoxin in this group did not decline below 100 mm Hg.

6. Complement titers and histamine concentrations in dogs given a second lethal dose of endotoxin. A group of dogs treated previously by a variety of methods had survived a lethal dose of endotoxin (Table III). After a period of survival varying between a few days to over 3 months, a second lethal dose of 0.55 mg per kg of endotoxin was given to each of these animals. Although there was an immediate and severe reaction following this second injection, eight of the ten animals survived. There was a marked decline in the titer of complement (Table I), which exceeded that in other groups and was significantly greater than in animals which received one dose of endotoxin (p < 0.05), but the decrease had no relation to the subsequent death or survival of the animal.

The highest sustained concentrations of histamine were observed in this group of animals (Table III). Within 60 seconds after endotoxin was injected there was a 13-fold increase. Increased concentrations were present 4 hours later in five animals.

The remarkable feature about these animals was the prompt fall in complement, the onset of hypotension, and a marked rise in plasma histamine, all of which suggested an antigen-antibody reaction of the immediate anaphylactic type. Nevertheless, eight of ten animals recovered.

7. Histamine concentrations in dogs pretreated with histamine and then given a lethal dose of endotoxin. Although dogs receiving the second dose of endotoxin exhibited the initial manifestations of severe anaphylactic shock and had sustained increases of histamine, only two of the ten animals died (Table II). This suggested that histamine might actually protect animals against the lethal effect of endotoxin, although one of its properties is the production of hypotension.

Ten adult dogs were each given an infusion of histamine phosphate, equivalent to 1 mg histamine base, in 100 ml 5% dextrose and water over a period of 15 to 20 minutes. Although some hypotension was induced with histamine, the average systolic blood pressure did not decrease below 90 mm Hg. After this infusion the animals were then given a lethal dose of endotoxin (0.55 mg per kg). The initial reaction to the endotoxin was mild, and eight of the ten animals survived. There was no rise in plasma histamine after endotoxin (Table II).

8. Control animals. a) Complement titers in dogs given Cr. terriclus venom. Six adult animals were given 50 mg of snake venom contained in 0.5 ml of saline. Although lethal shock was produced in all of the dogs the mean complement titer was 96% of the control titer 10 minutes after venom, and changed little thereafter. b) Complement titers in animals given 100 ml 5% dextrose in distilled water. Since EACA and cortisol were given intravenously in a solution of 5% dextrose and 100 ml of distilled water, this amount was infused into three anesthetized dogs. Complement titers fell approximately 19% during the infusion of dextrose, but thereafter complement changed little, and no change in blood pressure occurred. c) Plasma histamine concentrations in dogs given 2 ml of saline solution intravenously. After anesthesia, arterial catheterization, and iv injection of 2 ml
of saline, plasma histamine was measured serially in six dogs. No significant changes in concentration were obtained, with the mean (60 determinations) being 0.05 μg per ml.

Blood pressure always fell in animals given endotoxin, and the fall was associated with variable rises in histamine and decreases in complement titters. Nevertheless, calculation of correlation coefficients between the maximal fall of blood pressure and maximal change of histamine or complement in each of the six groups showed no statistically significant correlation.

Discussion

The foregoing experiments were primarily concerned with the initial hemodynamic alterations in the dog that are induced by endotoxin. The data support the concept that the initial phase of canine endotoxin shock involves an immune mechanism of the anaphylactic type in which endotoxin, acting as an antigen, reacts with antibody in the presence of complement, and histamine is liberated. This concept does not exclude the appearance of other vasoactive substances in the blood. Other studies also support such a concept. Gilbert and Braude (6) in immunologic studies on endotoxin shock in the rabbit concluded that endotoxin had an anaphylactic effect. Kostka and Sterzl (18) observed that the serum of piglets removed from their mothers at birth contained complement but no E. coli antibody. When endotoxin was added to piglet serum no inactivation of complement occurred. However, the addition of endotoxin to the sera of adult swine containing antibody did result in a significant decrease in complement. In contrast to the evidence suggesting that endotoxin acts as an antigen in the initial phase of shock, Franke (19) found that although a polysaccharide from Serratia marcescens was lethal for guinea pigs, the effects did not appear to be due to anaphylaxis. They measured only lung volume and specific gravity, however, and it has been shown in another species that many objective signs of toxicity during anaphylaxis may be absent despite the occurrence of chemical changes (20).

There is a considerable body of evidence indicating that the initial changes in vascular activity in endotoxin shock can be provoked by histamine. Unique in the dog is the immediate hepatovenous constriction with an increase in portal vein pressure that occurs after endotoxin and is associated with a pooling of blood along the intestinal tract. Essex and Thomas (21) observed in dogs that the intravenous injection of histamine resulted in constriction of the hepatic vein, and Weil and Spink (3) demonstrated a prompt rise in a histamine-like substance in the hepatic vein blood of dogs immediately after the injection of endotoxin. They were able to demonstrate histamine-like activity in hepatic vein blood but not in femoral vein blood, which is in accord with the variable increases in histamine found in this study in femoral artery samples. Histamine causes venoconstriction in the tissues and organs of other species (22, 23). Endotoxin produces a venous constriction of pulmonary veins in the cat and dog (24). Finally, the hypoxia and reduction of oxygen content in the dog’s blood that follows endotoxin has been produced by histamine (25).

While these studies are consistent with the hypothesis that the early phase of canine endotoxin shock is related to an antigen-antibody reaction involving complement, resulting in histamine liberation, the precise role of histamine in the over-all genesis of irreversible shock is not clear. Schayer (26) has advanced the thesis that excessive amounts of histamine or epinephrine are deleterious to the microcirculation of the host. He has concluded that histamine appears in the blood after the onset of shock as a result of the liberation of preformed histamine and also because of continued synthesis of histamine from histidine through the action of the enzyme, histidine decarboxylase (27). The continued synthesis of histamine is more important in the genesis of shock than is the liberation of preformed histamine. Others (10) support this dual origin of histamine, although Watson (28) questions the continuing synthesis of histamine in cats, dogs, and man. Since the present experiments are concerned with acute and lethal endotoxin shock, no conclusive data on the continued elevation of plasma histamine over a period of several hours have been obtained except in those surviving animals given a second injection of endotoxin.

Several provocative points relating to the significance of histamine in endotoxin shock have been brought out in the present studies. First,
the highest sustained concentrations of plasma histamine were detected in those dogs that had been given a second lethal dose of endotoxin. This elevation of histamine was accompanied by the manifestations of severe anaphylactic shock. However, the majority of these animals survived. A state of tolerance to endotoxin was associated with acquired endotoxin hypersensitivity. The nature of this relationship is not clear. It is difficult to correlate the initial severe anaphylactic reaction with the mechanism or mechanisms by which recovery ensues, if indeed, a correlation exists. It is possible that the state of tolerance to the lethal effect of endotoxin is related to the reticuloendothelial system, as the studies of others would suggest (29, 30). Second, dogs given an infusion of histamine over a period of 15 minutes are rendered highly tolerant to the lethal effect of endotoxin, an observation that others have made in mice (31). An explanation for this phenomenon is not easily forthcoming. The injection of endotoxin was not followed by significant elevations of plasma histamine after histamine infusion. It is known that histamine stimulates the secretion of epinephrine from the adrenal medullae. Is it possible that the injection of appropriate amounts of histamine depletes or diminishes the potential secretion of epinephrine after the injection of endotoxin? This aspect is under investigation. Third, EACA or cortisol protects dogs against a lethal dose of endotoxin, although the mechanism by which each of these agents accomplishes this effect is probably different. EACA but not cortisol pretreatment significantly decreased the consumption of complement after endotoxin, when compared to the results in control animals (Table I). The rise in plasma histamine from endotoxin was no different in EACA pretreated animals, but was much less in cortisol-treated animals than in control animals given endotoxin (Table II). Liberation of histamine from cells is probably inhibited by cortisol at the cell surface, since the liberation of several cellular enzymes by endotoxin is considerably diminished both in vitro and in vivo in the presence of pharmacologic amounts of cortisol (32, 33).

It is apparent that endotoxin shock involves a chain of biochemical and functional alterations. Because of this complexity shock can be reversed at different stages and by a variety of agents (34). Further progress in the understanding of this type of shock is dependent upon a more precise chemical identification of endotoxin. Additional information is desirable on the quantitative relationship of histamine and catecholamines during the various phases of shock. Other vasoactive substances may also be involved. Data are especially needed in different species, including man.

Summary

Experimental data in canine endotoxin shock support the concept that the initial stage of hemodynamic alterations is due to an anaphylactic type of immune mechanism involving complement, with the liberation of histamine. However, the severity of the systemic reaction is not indicative of the final outcome of animals. Epsilon-aminocaproic acid and cortisol modify the severity of the initial reaction, and the majority of animals survive lethal doses of endotoxin. On the other hand, surviving animals given a second lethal dose of endotoxin have a severe initial reaction, a significant decline in complement, and a marked rise in plasma histamine. The majority of these animals also survive. Finally, dogs infused with histamine survive a lethal dose of endotoxin. The relationship of the initial anaphylactic activity to the ultimate outcome of an animal with endotoxin shock is not clear. Tolerance or intolerance to the lethal action of endotoxin is dependent upon other mechanisms and may be related to acquired humoral immunity and conditioning of the reticuloendothelial system.

References