ENTEROTOXINS isolated from cultures of *Staphylococcus aureus* are protein exotoxins (28,000-29,000 daltons, molecular weight) which are capable of producing acute food poisoning in humans and other primates. Intravenous (iv) injection of enterotoxins in very small doses in rabbits and monkeys produces lethargy, fever, shock, and death. There is evidence that during staphylococcal wound infections and purulent skin lesions, enough enterotoxin is released into the circulation of an infected patient to stimulate synthesis of antibody specific to the toxins produced. Since circulating enterotoxins might contribute to the hypotension and shock often observed in these patients, we decided to study possible mechanisms by which enterotoxins might contribute to the hypotension and shock when injected (1 mg/kg, iv) into rhesus monkeys. Plasma levels of factors which have been implicated in the pathogenesis of other types of shock were measured. Endotoxin-like activity was measured by the Limulus lysate technique, fibrin degradation products (FDP) were quantified by electroimmunoassay, and activation of the complement system was assayed by measuring total hemolytic complement. Activation of the intrinsic coagulation cascade was assessed by measuring activated partial thromboplastin time (APTT). Activation of the kinin system was evaluated by measuring prekallikrein activity and kininogen. Myocardial depressant factor (MDF) was measured by paper chromatography. Endotoxin-like activity did not appear in plasma, and the complement system was not activated. The appearance of FDP and a significant trend for prolongation of APTT indicated activation of fibrinolysis and the intrinsic coagulation cascade, and suggested that disseminated intravascular coagulation was occurring. Activation of the kinin system was shown by a progressive and significant depletion of kallidin from 328 ± 37 to 226 ± 22 ng kallidin generated/ml, and a significant depletion of plasma prekallikrein activity from 169 ± 8 to 110 ± 15 tosyl arginine methyl ester (TAME) esterase U/ml. Analysis of covariance indicated that activation of the kinin system was related to changes in blood pressure. MDF did not increase until immediately before death (increase from 1.08 ± 0.15 to 1.92 ± 0.11 paper chromatographic U/µl, n = 6). We conclude that kinins, MDF, and disseminated intravascular coagulation, but not complement or endotoxin, may contribute to the pathogenesis of enterotoxic shock in rhesus monkeys.

**Methods**

Experimental animals were eight healthy, well-conditioned adult rhesus monkeys (*Macaca mulatta*) weighing 3–5 kg and seronegative for hemagglutinating antibody to SEB. Monkeys were anes-

**SUMMARY** Staphylococcal enterotoxin B, a protein exotoxin from *Staphylococcus aureus*, produced progressive hypotension and shock when injected (1 mg/kg, iv) into rhesus monkeys. Plasma levels of factors which have been implicated in the pathogenesis of other types of shock were measured. Endotoxin-like activity was measured by the Limulus lysate technique, fibrin degradation products (FDP) were quantified by electroimmunoassay, and activation of the complement system was assayed by measuring total hemolytic complement. Activation of the intrinsic coagulation cascade was assessed by measuring activated partial thromboplastin time (APTT). Activation of the kinin system was evaluated by measuring prekallikrein activity and kininogen. Myocardial depressant factor (MDF) was measured by paper chromatography. Endotoxin-like activity did not appear in plasma, and the complement system was not activated. The appearance of FDP and a significant trend for prolongation of APTT indicated activation of fibrinolysis and the intrinsic coagulation cascade, and suggested that disseminated intravascular coagulation was occurring. Activation of the kinin system was shown by a progressive and significant depletion of kallidin from 328 ± 37 to 226 ± 22 ng kallidin generated/ml, and a significant depletion of plasma prekallikrein activity from 169 ± 8 to 110 ± 15 tosyl arginine methyl ester (TAME) esterase U/ml. Analysis of covariance indicated that activation of the kinin system was related to changes in blood pressure. MDF did not increase until immediately before death (increase from 1.08 ± 0.15 to 1.92 ± 0.11 paper chromatographic U/µl, n = 6). We conclude that kinins, MDF, and disseminated intravascular coagulation, but not complement or endotoxin, may contribute to the pathogenesis of enterotoxic shock in rhesus monkeys.
thetized intramuscularly (im) with ketamine hydrochloride (5-10 mg/kg, calculated as the base), before implantation of femoral or carotid artery plus femoral or jugular vein catheters (polyethylene catheters). Monkeys were seated in Plexiglas chairs during the study. All catheters were coated with TDMAC-heparin complex (Polysciences) and infused with saline (1-2 ml/hour) to inhibit clot formation and maintain patency. In each experiment the first control blood sample was taken 1 hour before injecting SEB, and the second control sample was withdrawn at time 0, immediately before injection of the toxin. Each monkey was injected iv with highly purified SEB, 1 mg/kg, [lot 14-30, U.S. Army Medical Research Institute of Infectious Diseases, (USAMRIID), Fort Detrick, Md.]; this SEB was from the same lot used in other studies reported from this institute.9 Antigenicity and potency of the SEB were verified by Oudin immunodiffusion analysis.10 After injection of toxin, blood samples (8 ml per sample) were collected at various intervals from the arterial catheter; about 70% of erythrocytes removed were returned. In two control monkeys which were subjected to the same experimental procedure over an 18-hour period, but were not given SEB, this blood sampling procedure resulted in no appreciable change in hematocrit or plasma protein concentration.

Mean arterial blood pressure (MABP) was measured with a Statham (P-23) pressure transducer coupled to an Electronics for Medicine (VR-6) recorder. Heart rate was obtained from the pressure tracings, and a water manometer was used to measure central venous pressure. The central venous pressure was measured at the phlebostatic level (right atrium) as described by Burch and Winsor.11 Plasma endotoxin levels were measured by the Limulus amebocyte lysate technique12 (E-Toxate, Sigma), which is reported to be capable of detecting very small quantities of endotoxin in plasma.13 Since evidence supporting the specificity of this test for endotoxin in plasma is not complete,13 positive test results are referred to as the ability of a plasma sample to cause gelation of Limulus amebocyte lysate. This gelation is scored (scale of 0 to +3) by the degree and rapidity of gel formation during incubation at room temperature. Under these conditions, addition of 1 μg of endotoxin (Escherichia coli, B:011, Difco) to 1 ml of freshly drawn plasma from normal monkeys resulted in the formation of a solid gel in less than 2 hours (+3); addition of 0.005 μg of endotoxin to 1 ml of plasma produced a solid gel within 18 hours (+1), and 0.001 μg of endotoxin in 1 ml of plasma gave equivocal results (i.e., a turbid solution) at 18 hours.

Myocardial depressant factor (MDF) was assayed by a modification (manuscript in preparation) of the paper chromatographic technique of Barenholz et al.14 Plasma samples were deproteinized, ether-extracted, and spotted (200 μl) on chromatographic paper (Whatman No. 3 MM) along with a serine standard. The paper was then placed in a tank and subjected to descending flow for 18 hours in a solvent-saturated atmosphere (n-butanol-glacial acetic acid-H2O, 25:25:6). The papers were sprayed with 0.3% ninhydrin in ethanol and developed at 90°C for 10 minutes. Spots corresponding to Barenholz' spot G (distance traveled relative to serine = 1.5-1.8), and the spots developed from serine standard were eluted from the paper with 3 ml of 1% NaHCO3, and the absorbance at 570 nm of each eluate was measured. Under these conditions we define 1 MDF paper chromatographic unit as equal to the A570 of 1.3 nmol of serine. With this technique we found excellent correlation (r = 0.92) between MDF measured by bioassay vs. MDF measured by paper chromatography.

Activation of the kallikrein-kinin system was assessed by measuring plasma prekallikrein and kininogen activities. Prekallikrein activity, measured according to the method of Colman et al.,15 is based on hydrolysis of tosyl arginine methyl ester (TAMe) after kaolin activation and is expressed as moles of TAMe hydrolyzed per milliliter per hour. Kininogen was measured by bioassay on guinea pig ileum (in vitro) according to the technique of Rocha e Silva16 as modified by Pierce and Guimarães.17 In this procedure an excess of human urinary kallikrein (supplied by Dr. J.V. Pierce, National Institutes of Health), followed by the plasma sample to be bioassayed, was added to the solution bathing the guinea pig ileum, and the magnitude of the isotonic contraction of the ileum loaded with 1 g was measured by a displacement transducer. Since human urinary kallikrein is a tissue kallikrein, the kinin generated from plasma kinogen is kallidin (lysyl-bradykinin) and results are expressed as micrograms per milliliter of kallidin generated per milliliter of sample. Each sample was assayed in duplicate and compared with samples of standard kallidin (Schwarz/Mann, lot 2039).

Complement levels in serum were measured using the hemolytic titration of whole complement described by Rapp and Borsos.18 Results are expressed as the reciprocal of the dilution of serum which causes 50% hemolysis (CH50).

Fibrin degradation products (FPD) in serum were measured by electroimmunoassay19 through agar containing antibody against monkey fibrinogen (Chapel Laboratories). Serum was obtained from blood collected in tubes containing sodium citrate (3.8 mg/ml of blood) and clotted according to the technique of Mersky et al.20

Activated partial thromboplastin time (APTT) in citrated plasma was measured by a one-stage assay using Platelin (General Diagnostics, Division of Warner-Lambert Co.) reagent.

Some of the data were subjected to linear regression (least squares) analysis,21 and the slopes and 99% confidence limits for the slopes of the lines were determined. Student's t-test was used for comparisons of data obtained immediately before injec-
tion of SEB and data obtained immediately before termination of the experiment. Analysis of covariance was used to compare changes in plasma kininogen and prekallikrein to changes in blood pressure.

Results

Six of eight monkeys died after SEB injection. The two survivors were included in the study because they became moribund, and their mean blood pressure dropped below 60 mm Hg. Mean time to death or lowest blood pressure (for survivors) was 18.6 hours (range, 6-35). Since the variability in time to death was so great, we calculated data as percent of time to final measurement for each monkey. For example, if a monkey died 6 hours post-SEB, his data for "50% of time to final measurement" would be the data taken at 3 hours post-SEB. For monkeys that died, the final measurements were the last ones before death; for survivors, the final measurements were those taken at the time of lowest blood pressure.

Cardiovascular Data

Intravenous SEB resulted in a progressive and significant decrease in mean arterial blood pressure (MABP) (Fig. 1). The slope of a regression line fit to the data was significantly negative ($P < 0.01$); a polynomial equation did not fit these data significantly better than a linear equation. Central venous pressure remained within normal limits and did not change significantly in any of the animals.

Heart rate data are also presented in Figure 1 (inset). Injection of SEB was followed by a rapid increase in heart rate which was maintained until death.

Kinin System

In our laboratory, mean kininogen concentration in normal control rhesus monkeys ($n = 15$) is $350 \pm 30$ ng of kallidin generated/ml of plasma. After SEB, plasma kininogen decreased significantly and progressively (Fig. 2). The slope of the regression line fit to these data was significantly ($P < 0.01$) negative.

Plasma prekallikrein activity (Fig. 3) also underwent a significant and progressive decrease. The slope of the regression line fit to these data was significantly negative ($P < 0.01$).

To investigate the possibility of a causal relationship between kinin system activation and blood pressure, we used analysis of covariance to compare blood pressure to changes in plasma kininogen and plasma prekallikrein. This test compared regression lines between prekallikrein vs. blood pressure and kininogen vs. blood pressure and indicated covariance between prekallikrein and blood pressure ($F$ statistic for slopes = 2.2) and even more marked covariance between kininogen and blood pressure ($F$ statistic for slopes = 0.643).

Complement

Total hemolytic complement (CH50) did not change. The slope of the regression line fit to the data was not significantly negative ($P > 0.05$) and the value for CH50 at $t = 100\%$ was $26 \pm 3$, which was not significantly ($P > 0.1$) different from the value of $29 \pm 3$ obtained at $t = 0\%$.

Myocardial Depressant Factor

Mean MDF was significantly increased in the preterminal sample (Fig. 4). The slope of the regres-

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Change in mean arterial blood pressure (MABP) in eight monkeys given staphylococcal enterotoxin type B (SEB) (●) and two controls (○) given saline. Mean time to final measurement was 18.6 hours (range, 6-35). Solid line is regression line for monkeys given SEB. Dashed line is regression line for control monkeys. Heart rate (HR) data are also included in the inset.
sion line fit to the data was significantly positive, but MDF varied widely before the preterminal sample.

Activated Partial Thromboplastin Time and Fibrin Degradation Products

APTT was measured in plasma from three monkeys given SEB and one given only saline (Fig. 5). By applying linear regression analysis to the data from enterotoxin monkeys, we found that there was a significant \( P < 0.01 \) trend for prolonged APTT as the shock progressed; this increase did not occur in the control monkey.

FDP (Table 1) were undetectable in all monkeys before injection of SEB \( (t = 0\%) \) and were not detected at any time in a control monkey given iv saline instead of SEB. Each monkey which received SEB developed FDP at some time during the course of enterotoxemia.

Plasma Endotoxin Levels

Endotoxin as measured by quantifying the gelation of Limulus amebocyte lysate (lower limit of assay = 5 ng endotoxin/ml of plasma) was not detected in plasma samples from any of the monkeys at any time.

Figure 2 Change in plasma kininogen in five monkeys given SEB (●) and two controls (○) given saline. Solid line is regression line for monkeys given SEB. Dashed line is regression line for control monkeys.

Figure 3 Change in plasma prekallikrein activity (TAME esterase activity) in seven monkeys given SEB (●) and in two controls (○) given saline. Solid line is regression line for monkeys given SEB. Dashed line is regression line for control monkeys.
**Discussion**

The dose of SEB which we used (1 mg/kg, iv), is lethal or near-lethal for nonimmune rhesus monkeys; it consistently produces severe hypotension. The progressive hypotension which we observed after SEB was similar to results obtained by others,[3,9] but appears to be at variance with observations reported by Liu et al.[2,22] Liu's group found that MABP in monkeys was relatively constant for at least 6 hours after iv SEB and then decreased, but never to values less than 90 mm Hg until immediately before death. These different results probably are related to the fact that monkeys used by Liu's group had higher initial blood pressures (MABP = 135 ± 10 mm Hg) than did our monkeys (MABP = 112 ± 5 mm Hg) or those used by Elsberry et al.[9] (MABP = 118 ± 4 mm Hg); others[23,24] have reported that the MABP of a normal unstressed monkey is about 110 mm Hg.

The immediate and sustained increase in heart rate which we observed after SEB has been re-

---

**Figure 4** Change in plasma myocardial depressant factor (MDF) in seven monkeys given SEB (●) and one control (○) given saline. Solid line is regression line for monkeys given SEB. Dashed line is regression line for control monkeys.

**Figure 5** Change in activated partial thromboplastin time in (APTT) three monkeys given SEB (●) and one control monkey (○) given saline. Solid line is regression line for monkeys given SEB. Dashed line is regression line for control monkey.
progressive and significant depletion of kininogen and prekallikrein indicated that iv SEB causes activation of the kinin system in monkeys. The sequence by which the kinin system is activated could involve damage to capillary endothelium which resulted in exposure of collagen to the vascular lumen and, thereby, caused activation of the kinin system through activation of Hageman factor (factor XII of intrinsic coagulation cascade). However, it was found (personal communication, J.L. Middlebrook) that a very high concentration (1 mg/ml) of SEB applied to vascular endothelium in tissue culture does not cause damage evidenced by morphological changes (light microscopy); nor does it cause inhibition of growth. Alternatively, SEB may cause activation of the kinin system by interaction of toxin with leukocytes. Both in vivo and in vitro evidence indicates that this is the mechanism by which endotoxin causes kinin system activation in subhuman primates and in man. In this regard, it has been demonstrated that, like endotoxin, SEB binds to leukocytes, which are subsequently bound to capillary endothelium in the lung. Pulmonary edema is the most severe and consistent pathological lesion in monkeys given SEB, and areas of endothelial cell necrosis in the lung are associated with sequestered leukocytes. Furthermore, it has been shown that iv SEB causes increased lymph flow in the thoracic duct and altered radiiodinated serum albumin kinetics, indicative of increased vascular permeability. Thus, the SEB-leukocyte complex may be involved in the increased permeability and damage to capillary endothelium observed in enterotoxic monkeys. Activation of the kinin system and subsequent generation of kinin in the area around these leukocytes is an attractive explanation for the increased capillary permeability observed in enterotoxic monkeys.

Kinins also cause peripheral vasodilation. The covariance found between prekallikrein and blood pressure and the very marked covariance found between kininogen and blood pressure support but do not prove the hypothesis that there is a cause-effect relationship between kinin system activation and the hypotension observed in enterotoxic shock. It is not surprising that covariance between kininogen and blood pressure is especially marked, because depletion of kininogen is a direct reflection of generation of kinin.

Complement

Activation of the complement system may be involved in the early hypotension which occurs after iv endotoxin. The monkeys used in our experiments had no hemagglutinating antibody to SEB, so we would not expect SEB to activate the complement cascade via the classical pathway. Experiments in vitro performed by Craig et al. indicated that SEB does not activate the complement cascade via the alternate pathway (properdin). However, we considered it possible that complement could be generated in vivo as a result of damaged capillary endothelium, and subsequent activation of Hageman factor (factor XII) resulting in production of plasmin which, in turn, activates complement. Therefore, we measured total hemolytic complement (CH50) during the course of these experiments. Our results indicate that complement activation did not occur. However, it must be emphasized that our monkeys did not have hemagglutinating antibody to SEB in their serum. Antibody (IgG) to SEB is common in rhesus monkeys and is often very high in humans. Thus, SEB could activate the complement system via the classical pathway, in individuals with antibody, by forming antigen-antibody complexes. Indirect evidence for occurrence of such activation is contained in work by Denniston et al. who noted a sudden decrease in blood pressure after giving SEB iv to immune monkeys. When the immune monkeys of Denniston et al. were skin-tested with SEB, an Arthus reac-
tion (intermediate hypersensitivity) was elicited. Since antigen-antibody complexes and resultant activation of complement are involved in the Arthus reaction, its occurrence in monkeys immune to SEB indicates that complement is probably activated in immune monkeys challenged with SEB.

**Myocardial Depressant Factor**

MDF is a low molecular weight polypeptide that decreases myocardial contractility and has been shown to be present in the plasma of humans and animals in a variety of types of shock. 49 Our data demonstrate that preterminal levels of MDF are significantly increased in monkeys during enterotoxic shock. In other work (unpublished observations) we found that an increase in MDF (measured by paper chromatography) equal to the mean levels we found in our monkeys is indicative of activation of the fibrinolytic and intrinsic coagulation systems, respectively; activation of fibrinolysis has come to be regarded as secondary to activation of intravascular coagulation plus the thrombocytopenia observed by others 50 in enterotoxic animals. As in other types of shock, this increased level of MDF could be partially responsible for the demise of the animal.

**Fibrin Degradation Products and Activated Partial Thromboplastin Time**

Appearance of FDP and prolongation of APTT in our monkeys are indicative of activation of the fibrinolytic and intrinsic coagulation systems, respectively; activation of fibrinolysis has come to be regarded as secondary to activation of intravascular coagulation in shock. 42-44 Activation of fibrinolysis and coagulation plus the thrombocytopenia observed by others 50 in enterotoxic animals are all laboratory criteria consistent with the diagnosis of disseminated intravascular coagulation. 49 Although these laboratory data indicate the presence of disseminated intravascular coagulation, we could not find consistent gross or histopathological evidence for this diagnosis in the monkeys that died; Hawley et al. 50 obtained similar results in rhesus monkeys infected with *Salmonella typhimurium* in which they found laboratory evidence of disseminated intravascular coagulation with no concomitant pathological evidence.

**Endotoxin**

In studies on rabbits, 47 we found that iv injection of SEB resulted in appearance of appreciable levels (up to 1 μg/ml) of endotoxin in plasma, presumably absorbed from the gut; death was associated with endotoxemia, and survival was associated with lack of endotoxemia. In the present study we obtained contrasting results in that endotoxin never was detectable in any of the monkeys despite measurement at repeated time intervals during endotoxemia. Lack of enterotoxin-induced endotoxemia dispels the notion that SEB may be exerting its toxic action in monkeys via secondary release of endotoxin from the gut; such a hypothesis is attractive because there are many similarities between endotoxic and enterotoxic shock in monkeys. 48

As in other types of circulatory shock, a variety of factors appear to contribute to hypotension and death in enterotoxic shock. In addition to pulmonary edema and alterations in circulatory physiology, metabolic derangements might also be involved in enterotoxemia. Canonico and Van Zweiten 46 documented mitochondrial swelling and subsequent loss of mitochondrial function in rabbits given SEB. Crawley et al. 25 described mild hypoglycemia followed by progressively developing modest hypoglycemia in enterotoxemic monkeys; these changes were similar to those seen after endotoxin, in which case glucose and insulin initially rose, then subsequently fell, and the animal eventually reached a state of hypoglycemia associated with glycogen depletion and hyperinsulinemia. 50 The present study addresses the cardiovascular derangements present in enterotoxemia; our data indicate that activation of the kinin, fibrinolytic, and coagulation systems plus increased plasma levels of MDF may contribute to enterotoxic shock.

**Acknowledgments**

We thank Steven Tobery, Ken Esclito, Cathy Dickerson, and Don Smith for technical assistance. The secretarial assistance of Regina Staley and typing assistance of Jenny Kennard and Linda Stup are greatly appreciated. We are indebted to Phebe Sumners for her editorial work, to Drs. Duane Hilmas, William Beisel, Virginia McGann, Michael Elwell, and Michael Kastello for their helpful suggestions, to Dr. Clayton Hadick for performing the necessary surgery, to Glenn Higbee for his statistical analyses, and to Dr. J.V. Pierce, National Institutes of Health, for generously providing the kallikrein used in this study.

**References**

9. Elsherry DD, Rhodes DA, Beisel WR: Hemodynamics of


42. Minna JD, Robboy SJ, Colman RW: Disseminated Intravascular Coagulation in Man. Springfield, Ill., Charles C Thomas, 1974


Enterotoxic shock in rhesus monkeys. The role of selected bloodborne factors.
G W Pettit, T Yamada, D A Wing and P B Jahrling

Circ Res. 1978;43:398-405
doi: 10.1161/01.RES.43.3.398
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1978 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/43/3/398.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/