Enterotoxic Shock in Rhesus Monkeys

The Role of Selected Bloodborne Factors

GEORGE W. PETTIT, TADATAKA YAMADA, DAVID A. WING, AND PETER B. JAHRLING

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. Enterotoxin-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 kininogen from 338 ± 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.

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 enterotoxin causes circulatory shock.

When Josefczyn measured anti-enterotoxin antibodies in normal patients and patients with staphylococcal infections, antibody to staphylococcal enterotoxin type B (SEB) was the most common type in each group. SEB has been isolated in purity greater than 99%, and its amino acid sequence has been determined. To help to elucidate the mechanisms by which enterotoxins produce shock, we injected SEB (1 mg/kg, iv) into rhesus monkeys and measured plasma levels of various factors (complement, endotoxin, fibrin degradation products, myocardial depressant factor, and kinin system components) which have been implicated in the pathogenesis of other types of shock.

Besides the possibility that enterotoxins are released into the circulation from staphylococcal infections, enterotoxic shock possesses unique aspects for study as a shock model; animals have natural antibody to endotoxin, but it is possible to screen monkeys for presence or absence of antibody to enterotoxins and, thereby, study the influence of specific antibody on the progression of shock.

Rhesus monkeys were chosen for the present studies because most of the previous work on enterotoxin has been done in this species. Also, since the cardiovascular responses of endotoxemic rhesus monkeys have been shown to be similar to the responses of humans to Gram-negative sepsis, we hypothesized that the response of the rhesus monkeys to SEB might be similar to that of man.

**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-
terized intramuscularly (im) with ketamine hydro-
chloride (5–10 mg/kg, calculated as the base), be-
fore 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 in-
fused with saline (1–2 ml/hour) to inhibit clot for-
formation and maintain patency. In each experiment
the first control blood sample was taken 1 hour
before injecting SEB, and the second control sam-
ple 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 Dis-

eases, (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 anal-
ysis.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 mea-

ured with a Statham (P-23) pressure transducer
coupled to an Electronics for Medicine (VR-6) re-
corder. Heart rate was obtained from the pressure
tracings, and a water manometer was used to mea-
sure 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 con-
ditions, 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 as-
sayed by a modification (manuscript in preparation)
of the paper chromatographic technique of Bare-
holz et al.14 Plasma samples were deproteinated,
ether-extracted, and spotted (200 μl) on chromato-
graphic 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-gla-
cial acetic acid-H2O, 25:25:6). The papers were
sprayed with 0.3% ninhydrin in ethanol and devel-
oped 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 mea-
sured by paper chromatography.

Activation of the kallikrein-kinin system was as-
sessed by measuring plasma prekallikrein and kin-
inogen 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 bioas-
sayed, was added to the solution bathing the guinea
pig ileum, and the magnitude of the isotonic con-
traction 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 milli-
liter of kallidin generated per milliliter of sample.
Each sample was assayed in duplicate and com-
pared with samples of standard kallidin (Schwarz/Mann, lot 2039).

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

Fibrin degradation products (FDP) 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 regres-
sion (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 com-
parisons 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 ± 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 \( (F \text{ statistic for slopes} = 2.2) \) and even more marked covariance between kininogen and blood pressure \( (F \text{ statistic for slopes} = 0.643) \).

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 ± 3, which was not significantly \( (P > 0.1) \) different from the value of 29 ± 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.
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, but appears to be at variance with observations reported by Liu et al.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); others23, 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-
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 and in 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 reaction was observed by others and probably is due to the increase in plasma epinephrine level which occurs soon after administration of the toxin. In later stages, hypotension would result in tachycardia through the baroreceptor mechanism.

The absence of change in central venous pressure corroborated similar results obtained by Elsberry et al., who noted only a very slight fall (decrease of 1.5 cm H2O).
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. 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) is equal to the mean change which we observed in our enterotoxemic monkeys would result in a 30% increase in bioassayable MDF activity. This means that the plasma from our monkeys in enterotoxemic shock, when compared to preshock plasma, would cause a 30% depression in the contractility of an isolated papillary muscle. 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 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 in shock. Activation of fibrinolysis and coagulation plus the thrombocytopenia observed by others in enterotoxemic animals are laboratory criteria consistent with the diagnosis of disseminated intravascular coagulation. 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. 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, 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 enterotoxemia. 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.

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 documented mitochondrial swelling and subsequent loss of mitochondrial function in rabbits given SEB. Crawley et al. 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 hypoinsulinemia. 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 Escoto, Cathy Dickerson, and Don Smith for technical assistance. The secretarial assistance of Regina Staley and typing assistance of Jenny Kennard and 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. Elsbbery DD, Rhoda 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/