ON THE COLLAPSE OF BACTERIAL ENDOTOXIN RESISTANCE FOLLOWING HEMORRHAGE*

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During the past several years, considerable experimental evidence has been presented by Fine and his associates implicating bacteria and their products in the pathogenesis of "irreversible" hemorrhagic shock (1-8). On the basis of this evidence, these investigators have proposed that the irreversible phase of hemorrhagic shock is secondary to systemic absorption of gastrointestinal bacterial endotoxin coupled with collapse of the endotoxin defenses (8). The potential clinical implications of such a hypothesis for the treatment of advanced hemorrhagic shock are apparent—reduce gastrointestinal endotoxin absorption or attack the mechanisms leading to deterioration of the endotoxin defenses. The paucity of data concerning the latter mechanisms precludes a therapeutic approach at this level. The present investigation was designed to explore the nature of the collapse of bacterial endotoxin resistance consequent to acute hemorrhage. The findings indicate that in the immature albino rabbit, the several hundredfold increase in Escherichia coli endotoxin susceptibility observed following 2 hour exposure to reversible hemorrhagic shock cannot be attributed to blood loss per se, but reflects the combined influence of blood loss and the non-sterile femoral artery cut down and ligation employed for bleeding. At least two femoral wound factors appear to participate: clostridial wound infection and local plasma transudation subsequent to intense limb ischemia that follows femoral artery ligation in the presence of hemorrhagic shock.

Methods and Materials

Male albino rabbits 6 to 8 weeks old weighing 0.85 to 1.5 (average 1.1) kg. were utilized throughout. All animals, raised on antibiotic-free feed, were obtained from a uniform source of supply. Similar antibiotic-free feed was employed between arrival and the test procedures, an interval approximating 5 days. Studies were performed during fall and winter (1957-1959), with room temperatures at 24°C. ± 3°C. Hemorrhagic shock was induced by two methods:

1. Cardiac Puncture Technique.—The anterior chest wall and right femoral area of the unanesthetized animal were shaved and a 2-inch length of sterile polyethylene tubing (PE 90) inserted into a marginal ear vein. The chest wall was washed repeatedly with 70 per cent

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alcohol, and 15 to 20 ml. of blood withdrawn by cardiac puncture into a heparin-coated syringe through a sharp 20 gauge needle. The procedure required approximately 15 seconds. One hour later, the chest wall was retreated with alcohol and a second cardiac bleeding of 15 to 20 ml. performed. A third cardiac bleeding of 10 to 15 ml. was carried out 20 minutes thereafter. During any cardiac puncture, if blood was not immediately and easily aspirated, the rabbit was discarded. Approximately 4 of every 5 animals were discarded for this reason. The quantity of blood removed was determined such that replacement 2 hours after the last bleeding resulted in survival of the majority of animals whereas failure of replacement generally resulted in a lethal outcome within 5 hours. For this purpose, utilizing the method described, the mean femoral arterial blood pressure ranged between 46 and 60 mm. Hg. Femoral arterial blood pressures were determined by an indirect method employing a photoelectric cell over the shaved paw and a femoral pediatric blood pressure cuff, parallel to the technique described for the rat hind limb (9). Although initial pressures were regularly above 100 mm. Hg, only systolic levels could be measured. Moreover, following acute hemorrhage in the unanesthetized rabbits, peripheral vasoconstriction precluded subsequent indirect arterial blood pressure measurements. A preliminary study was therefore carried out to determine whether hypotensive levels of arterial blood pressure could be appraised reliably by clinical signs.

Femoral arterial blood pressures were measured directly with a mercury manometer and correlated with the clinical state. When hemorrhagic shock as produced by the three cardiac bleedings in the unanesthetized animal was sufficiently severe to (a) abolish the pain reaction induced by slowly pinching the tip of the tail in a Kelly forceps, and (b) inhibit the righting reflex during unmolested periods, mean arterial blood pressure was regularly below 60 mm. Hg. When shock was so severe as to (a) prevent the tendency to raise the head, (b) abolish nasal twitchings, and (c) induce marked pupillary dilatation, mean arterial blood pressure was consistently below 46 mm. Hg; convulsions and death occurred within 60 minutes unless a portion of blood was returned. With experience, it was found possible to estimate regularly mean arterial blood pressure reductions to the 46 to 60 mm. Hg range.¹

To maintain uniformity and objectivity in the subsequent studies, rabbits were paired and exposed to hemorrhagic shock by three cardiac bleedings estimated to reduce mean arterial blood pressure to between 46 and 60 mm. Hg; thereafter, if clinical signs indicated levels above 60 mm. Hg, a fourth cardiac bleeding of 5 to 10 ml. was performed. For levels below 46 mm. Hg, small quantities of blood were returned by the ear vein catheter; if more than 10 per cent of the maximum bleed-out volume was required, the animal was discarded. Sixty to 90 minutes after the estimated reduction of mean arterial blood pressure below 60 mm. Hg, one of each pair of animals was selected at random for a femoral artery cutdown and the blood pressure was measured by direct cannulation. Since all animals were paired, manifested initial systolic arterial pressures over 100 mm. Hg, were bled utilizing the same criteria, and when selected at random for femoral artery cutdowns consistently exhibited mean arterial blood pressures of 53 ± 7 mm. Hg, it seemed reasonable to assume that comparable shock states were produced in both rabbit groups; this was supported by the virtually identical mean maximum bleed-out volumes of 48 ± 1 ml./kg. per group.

Two hours from the time of last blood removal all blood (held at room temperature) was rein infused through the ear vein catheter, which was then removed; approximately 2 minutes was required for returning 45 ml. of blood. Four hours following the retransfusion, varying quantities of a purified E. coli endotoxin suspension in sterile pyrogen-free isotonic saline

¹ Transient increments of arterial blood pressure of 5 to 10 mm. Hg often occurred when the unanesthetized animal was moved or exposed to noise or pain. The blood pressure levels of 46 to 60 mm. Hg refer to unmolested states.
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(100 μg./ml.) were injected intravenously and the animals observed for the subsequent 96 hours; animals failing to survive 4 hours following retransfusion were not considered in the study. All syringes and needles employed for bleeding and retransfusion were washed and sterilized at 200°C. for a minimum of 5 hours prior to use; such treatment is more vigorous than that ordinarily required for pyrogen inactivation (10). The suspension of E. coli endotoxin was stored at 4°C. and its toxicity rechecked by intravenous injection into groups of 6 rabbits; no detectable loss of lethal potency occurred during the course of the experiments.

One necessary precaution, discovered after loss of numerous animals, was that immediately following the last cardiac bleeding, each rabbit must be permitted to lie unrestrained on its side; rabbits immobilized on their backs following hemorrhage sufficient to lower mean arterial blood pressure below 60 mm. Hg do not survive for 2 hours.

2. Lamson Reservoir Technique.—Unanesthetized rabbits were immobilized on their backs and the right femoral groin shaved. Employing clean but not aseptic precautions, the femoral artery was exposed through a 1.5 inch incision, ligated, and cannulated with a polyethylene catheter (PE 90). A clean but non-sterile glass reservoir containing 2 mg. sodium heparin in 2 ml. isotonic saline was connected to the cannula. The animals were allowed to bleed spontaneously into the reservoir until mean femoral arterial blood pressure fell to 50 mm. Hg; maximum bleed-out volume was generally achieved by the end of the 1st hour. Blood clots within the system were flushed with 0.5 ml. aliquots of heparinized saline. Blood pressure was monitored continuously by means of a side arm tube attached to the polyethylene catheter and connected to a small bore U type mercury manometer. Whenever arterial pressure fell below 50 mm. Hg, blood was reinfused manually to maintain the 50 mm. Hg level. As observed by Schweinburg and Fine (11), animals requiring reinfusion during the 1st hour succumbed despite retransfusion and were discarded. Similarly, animals requiring reinfusion of more than 10 per cent of the maximum bleed-out volume during the 2nd hour succumbed consistently unless the remainder were reinfused rapidly. During the shock procedure, the animals were permitted to lie loosely restrained on their side. After 2 hours, all blood in the reservoir was reinfused under pressure, the femoral artery cannula removed, and the wound sutured. Four hours following retransfusion, most animals were ambulatory and made attempts at eating, similar to those observed following the cardiac bleeding procedure. Varying quantities of the standardized E. coli endotoxin suspension were now injected intravenously and the animals observed for the subsequent 96 hours.

Bacteriologic Techniques.—Bacteriologic studies on normal rabbit femoral skeletal muscle were performed by sacrificing 10 healthy rabbits with a head blow, immediately shaving the right groin, carbonizing the skin with a gas flame, and removing the skin and fascia with sterile instruments. With a second set of sterile instruments, 3 specimens of muscle (approximately 1 cm.) were removed from each animal, flamed briefly, placed into tubes containing 8 ml. thioglycollate (Brewer, Baltimore Biological Laboratory, Inc.), and incubated at 37°C. for 96 hours unless growth appeared earlier. Aerobic organisms were identified by routine bacteriologic methods. When smears of thioglycollate growth revealed large Gram-positive bacilli, aerobic subcultures were made to exclude bacilli of the subtilis group; in all cases the Gram-positive bacilli proved to be anaerobic. These anaerobes were further studied by Gram-stain morphology, capsule and hanging drop reactions, effect on litmus milk, and pathogenicity for the guinea pig. Nonmotile, anaerobic, Gram-positive encapsulated bacilli that produced rapid stormy fermentation of litmus milk were considered Clostridia perfringens provided they proved pathogenic when inoculated into guinea pig thighs prepared with mild aseptic hemostat crushing.

2 Supplied by Difco Laboratories, Detroit, as lipopolysaccharide E. coli 0127B8.

3 The shaved skin was not treated with antibacterial agents.
Bacteriologic studies of the femoral wound during hemorrhagic shock were made by swabbing the deepest portion of the wound, transferring to thioglycollate broth, and carrying out the above procedures. In addition, aerobic blood agar plates were streaked with the wound swabs. In 10 animals, specimens of femoral skeletal muscle were removed from the wound site several hours following retransfusion and endotoxin challenge, and the bacteriologic studies carried out as described above. Initial attempts to correlate clostridial wound counts with endotoxin susceptibility following hemorrhagic shock were abandoned when it became apparent that the trauma associated with femoral muscle biopsy caused the shocked animals to die much sooner. The feasibility of quantitating clostridia from wound swabs (12) was not appreciated at the time of this study.

Estimation of Extremity Plasma Transudation.—The effect of a femoral artery cutdown and ligation on plasma loss into the lower extremity following hemorrhagic shock was estimated by transecting the rabbit near the mid-abdominal level, resecting the tail, and bisecting the lower quarters. The weights of such hemic quarters were compared, and with allowance for the specific gravity of rabbit plasma, differences in plasma transudation were then estimated.

Unilateral Hind Limb Ischemia.—The effect of unilateral hind limb ischemia on endotoxin susceptibility was studied by wrapping one-quarter inch tubing tightly around the upper right thigh of normal rabbits for 2 hours followed by release. There was no observed mortality from this procedure per se. Four hours after tourniquet release, animals were challenged with single intravenous injections of E. coli endotoxin; plasma loss into the ischemic limb was estimated at death or after 48 hours as indicated above.

RESULTS

1. Effect of Cardiac Puncture on Cardiovascular Function.—Since hemorrhagic shock induced by cardiac puncture might be complicated by "cardiogenic" shock, the effects of multiple cardiac punctures on myocardial contractility were investigated. Direct mean femoral arterial blood pressure readings in 8 unanesthetized normal rabbits during four sham cardiac bleedings with 20 gauge needles, performed at 5 to 60 minute intervals, are indicated in Table I. The needles were left in situ for the same period required for blood withdrawal. Mean arterial blood pressure, monitored continuously during the cardiac punctures, showed virtually no tendency to fall, minimizing the possibility of compensatory reflex peripheral vasoconstriction masking a reduction in myocardial contractility.

Ten ml. aliquots of dextran solution were then injected intravenously into 10 normal unanesthetized control rabbits at 15 second intervals until acute pulmonary edema and death ensued; a mean of 106 ml./kg. of dextran was required. Six sham cardiac bleedings in each of another 16 animals failed to disclose impairment of myocardial contractility as indicated by the similar fluid loads required for acute pulmonary edema (Table II). The comparable initial arterial blood pressures in both animal groups minimize the possibility that a lowered peripheral resistance enhanced fluid load tolerance after cardiac puncture. The possibility is recognized, however, that large quantities of

4 Supplied by Wyeth Laboratories, Philadelphia, as plavolex, a 6 per cent solution of hydrolyzed fractionated dextran in isotonic saline.
dextran could have exerted a direct injurious effect on the pulmonary capillary
bed, thus leading to pulmonary edema prior to myocardial overloading. Isotonic
saline could not be substituted as it was found difficult to induce acute pulmo-
nary edema, the injected saline being rapidly transudated as ascitic fluid.

2. Evaluation of Severity of Hemorrhagic Shock Induced by Cardiac Bleeding.—
Following the preliminary experiments described in the method section, a
group of 51 rabbits were bled by the cardiac puncture technique to a mean
arterial blood pressure between 46 and 60 mm. Hg as estimated from the

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Weight</th>
<th>Mean femoral arterial blood pressure*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td></td>
<td>kg.</td>
<td>mm. Hg</td>
</tr>
<tr>
<td>1</td>
<td>1.1</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>1.1</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>1.1</td>
<td>92</td>
</tr>
<tr>
<td>4</td>
<td>1.1</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>88</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>88</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>86</td>
</tr>
<tr>
<td>8</td>
<td>1.0</td>
<td>84</td>
</tr>
</tbody>
</table>

* Determined by direct femoral artery cannulation and a mercury manometer in unanes-
thetized animals immobilized on their backs.

† Refers to change from baseline determined during a 2 minute period prior to each cardiac
puncture. Such baselines remained within ±6 mm. Hg of the initial blood pressure values.
Cardiac punctures were performed with a 20 gauge needle, left in situ 15 seconds. Punctures
performed at 5 minute intervals in animals 1 to 4, and at 20 to 60 minute intervals in ani-
mals 5 to 8.

clinical state of the animal. Approximately 20 per cent of the animals required
bleedings of less than 43 ml./kg., these were excluded from the study. The
remaining animals required an average bleeding of 48 ml./kg. Subsequent
direct femoral arterial blood pressure readings, taken 60 to 90 minutes after
the last bleeding, consistently ranged between 46 and 60 mm. Hg. If retrans-
fusion was delayed 6 hours following the last bleeding, an 81 per cent mortality
ensued; 92 per cent of all deaths occurred 3 to 5 (mean 3.8) hours after the last
bleeding, prior to retransfusion. Retransfusion 2 hours after the last bleeding
resulted in a 15 per cent mortality within 4 hours and an additional 10 per cent
mortality during the subsequent 96 hours (Table III). Animals surviving the
initial 36 hours survived the 96 hour period. Gross postmortem examination of
those animals dying 4 or more hours after reinfusion revealed varying degrees of congestion of the liver, spleen, and intestinal wall, and scattered submucosal intestinal hemorrhages. All animals exhibited ecchymosis from 1 to 4 mm. in diameter on the epicardial surface at the cardiac puncture sites.

3. Alteration in E. coli Endotoxin Susceptibility Following Shock Induced by Cardiac Bleeding.—Susceptibility of untreated healthy rabbits to a single intravenous injection of E. coli endotoxin is shown in Table IV. Rabbits appearing ill or exhibiting diarrhea were excluded from the study since such animals were highly susceptible to the lethal action of endotoxin.

With the multiple cardiac puncture technique, a group of 19 rabbits paired

<p>| TABLE II |
| Effect of Multiple Cardiac Punctures on the Ability of the Rabbit to Tolerate Acute Intravenous Fluid Loading |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rabbits</th>
<th>Weight</th>
<th>Mean femoral arterial blood pressure prior to dextran*</th>
<th>Minimum dextran required for acute pulmonary edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>10</td>
<td>1.1 kg.</td>
<td>86 mm. Hg (82-96)</td>
<td>106 ml./kg. (90-130)</td>
</tr>
<tr>
<td>Subjected to six cardiac punctures‡</td>
<td>16</td>
<td>1.0 kg.</td>
<td>88 mm. Hg (76-98)</td>
<td>115 ml./kg. (100-140)</td>
</tr>
</tbody>
</table>

* Determined by direct femoral artery cannulation and a mercury manometer in unanesthetized rabbits immobilized on their backs. Dextran consisted of a 6 per cent solution in isotonic saline injected intravenously in 10 ml. volumes every 15 seconds.
‡ Cardiac punctures performed at 5 minute intervals in 6 animals and at 20 minute intervals in 10 animals employing a 20 gauge needle left in situ 15 seconds each trial.

with the animals to be described in the subsequent section, were exposed to hemorrhagic shock estimated to reduce mean femoral arterial blood pressure to 53 ± 7 mm. Hg. The mean maximum bleed-out volume was 48 ml./kg. Two hours after the last bleeding, the blood was reinfused and 4 hours thereafter, E. coli endotoxin was administered intravenously. It is emphasized that femoral trauma was entirely avoided. The survival results are given in Table V, group I. Employing the largest intravenous test dose of endotoxin uniformly nonlethal to healthy animals, 200 μg./kg., no increase in susceptibility could be demonstrated.

4. Alteration in E. coli Endotoxin Susceptibility Following Shock Induced by Cardiac Bleeding Complicated by Non-Sterile Femoral Artery Ligation.—A group of 19 rabbits, paired with the previous group and exposed to hemorrhagic shock by the identical procedure, were selected at random for a femoral artery cutdown and ligation. The cutdown and ligation were performed immediately
after the last cardiac bleeding using clean but not aseptic technique, and the
wound closed following blood replacement 2 hours later. Direct mean femoral
arterial blood pressure readings during shock were consistently between 46 and
60 mm. Hg. The mean maximum bleed-out volume was 48.5 ml./kg. Four

### TABLE III

*Appraisal of Severity of Shock Produced by Cardiac Bleeding Technique*

<table>
<thead>
<tr>
<th>Mean femoral arterial blood pressure*</th>
<th>Maximum bleed-out volume</th>
<th>Time blood reinfused (hrs. after last bleeding)</th>
<th>96 hr. survivals (Alive/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mm. Hg</strong></td>
<td><strong>ml./kg.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54 (46–60)</td>
<td>48</td>
<td>2</td>
<td>19/25‡</td>
</tr>
<tr>
<td>52 (46–58)</td>
<td>48</td>
<td>6</td>
<td>3/16§</td>
</tr>
</tbody>
</table>

* Determined 90 minutes after last cardiac bleeding by direct femoral artery cannulation.
Rabbit weight = 1.1 (range 0.9 to 1.5) kg.
† Four of the 6 animals succumbing died within 4 hours of retransfusion.
§ Twelve of the 13 animals succumbing died 3 to 5 (mean 3.8) hours after the last cardiac
bleeding, prior to retransfusion.

### TABLE IV

*Lethality of E. coli Endotoxin for Normal Albino Rabbits*

<table>
<thead>
<tr>
<th>Endotoxin*</th>
<th>96 hr. survivals (Alive/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg./kg</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>30/30</td>
</tr>
<tr>
<td>500</td>
<td>19/20</td>
</tr>
<tr>
<td>1000</td>
<td>9/10</td>
</tr>
<tr>
<td>2000</td>
<td>5/6</td>
</tr>
<tr>
<td>4000</td>
<td>4/8</td>
</tr>
<tr>
<td>5000</td>
<td>4/6</td>
</tr>
<tr>
<td>7000</td>
<td>3/6</td>
</tr>
<tr>
<td>10,000</td>
<td>1/8</td>
</tr>
</tbody>
</table>

* Supplied by Difco Laboratories as lipopolysaccharide 0127B8. Administered as single
intravenous injections to rabbits weighing 1.1 (range 0.9 to 1.5) kg.

hours after retransfusion, the surviving animals appeared as alert and hungry
as in the previous experiments; *E. coli* endotoxin was now injected intra-
venously. The survival results are summarized in Table V, group II; suscepti-
bility to the lethal action of a single intravenous endotoxin injection as a result
of hemorrhage combined with a non-sterile femoral artery cutdown and ligation
had increased to the several hundredfold range. The importance of the hemor-
rhagic shock for this increased endotoxin susceptibility was indicated by the
resistance to endotoxin of animals subjected to sham cardiac bleedings combined with non-sterile femoral artery cutdowns and ligations (Table V, group III).

5. Alterations in E. coli Endotoxin Susceptibility Following Hemorrhagic Shock by the Lamson Reservoir Technique.—The effects of 2 hours of hemorrhagic shock induced by the elevated Lamson reservoir method on survival of 13 rabbits challenged with single intravenous injections of E. coli endotoxin are shown in Table VI. Mean maximum bleed-out volumes and susceptibility to endotoxin administered 4 hours after retransfusion were found to have increased into the same ranges as those following hemorrhagic shock induced by cardiac bleeding combined with a non-sterile femoral artery cutdown and ligation. The maximum bleed-out volumes of all animals fell within the 52 ± 10 ml./kg. range obtained by Fine et al. (13).

Unless otherwise specified, in the subsequent studies the quantity of E. coli endotoxin employed for testing the effect of a given procedure on the resistance of the rabbit to endotoxin was maintained constant at 200 μg./kg. It is emphasized that failure of the endotoxin to produce a lethal outcome within the 96 hour observation period indicates only that a given procedure did not increase the susceptibility of the rabbit to the 200 μg./kg. level and does not exclude increased susceptibility to higher endotoxin dosages.

### Table V

<table>
<thead>
<tr>
<th>Procedure*</th>
<th>Weight (kg)</th>
<th>Mean femoral artery blood pressure (mm. Hg)</th>
<th>Maximum bleed-out volume (ml./kg)</th>
<th>E. coli endotoxin (μg./kg)</th>
<th>96 hr. survivals (Alive/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Hemorrhagic shock</td>
<td>1.0 (0.9–1.1)</td>
<td>—</td>
<td>48 (43–58)</td>
<td>200</td>
<td>17/19</td>
</tr>
<tr>
<td>II. Hemorrhagic shock + femoral artery cutdown and ligation</td>
<td>1.0 (0.9–1.4)</td>
<td>52 (46–58)</td>
<td>49 (43–56)</td>
<td>50</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>1.1 (0.9–1.3)</td>
<td>54 (48–60)</td>
<td>48</td>
<td>10</td>
<td>8/9</td>
</tr>
<tr>
<td>III. Sham cardiac bleedings + femoral artery cutdown and ligation</td>
<td>1.1 (0.9–1.2)</td>
<td>—</td>
<td>200</td>
<td>9/10</td>
<td></td>
</tr>
</tbody>
</table>

* Animals of procedures I and II paired, exposed to hemorrhagic shock by the cardiac bleeding technique, and selected at random for femoral artery ligation. All animals retransfused 2 hours after last bleeding and challenged 4 hours thereafter with intravenous E. coli endotoxin.

† Determined 90 minutes after the last cardiac bleeding by direct femoral artery cannulation in unanesthetized rabbits lying loosely restrained on their side.

§ Only animals surviving 4 hours after retransfusion are considered.
6. Effect of Aseptic Precautions during Femoral Wounding.—Immediately after the last cardiac bleeding for production of hemorrhagic shock, 6 rabbits were subjected to a 1.5 inch superficial skin incision in the shaven right groin area. Aseptic precautions were not observed, but injury to the underlying covering fascial layers was carefully avoided. After 2 hours, the incision was closed with silk and the animals retransfused. Five of the 6 animals survived intravenous challenge with *E. coli* endotoxin administered 4 hours after retransfusion (Table VII, group IA). Since the expected 96 hour mortality from the hemorrhagic shock alone (considering only animals surviving at least 4 hours after retransfusion) is approximately 10 per cent, the superimposition of the superficial groin incision failed to increase *E. coli* endotoxin susceptibility significantly as tested at the 200 µg./kg. level. In contrast, of 8 rabbits (6 of which were paired with the previous group and selected at random) subjected to exposure of the right femoral artery without aseptic precautions following the last cardiac bleeding, 7 succumbed within 24 hours to *E. coli* endotoxin challenge (Table VII, group IB). Compared with the superficial groin incision mortality, this represents a significant increase in endotoxin susceptibility (*p* < 0.05). It is emphasized that the femoral artery was not ligated. The artery was approached through a 1.5 inch incision in the shaved groin, a three-quarter inch segment mobilized by blunt dissection, and each wound then covered with clean but non-sterile gauze until suturing and retransfusion 2 hours later.

In a third group of 14 rabbits subjected to hemorrhagic shock by cardiac puncture, a femoral wound was produced with operating room aseptic precautions immediately after the last bleeding; tincture of merthiolate was em-
ployed for preparing the shaved skin. In 5 animals, the femoral artery was merely exposed as described above; in 9 animals the artery was also ligated. All wounds were closed immediately with sterile silk and sealed with a sterile collodion-like membrane (aeroplast). The sterile covering was replenished every 6 hours as necessary to maintain an unbroken surface. Two hours after the last bleeding the animals were retransfused and challenged intravenously with *E. coli* endotoxin 4 hours later. As seen in Table VII, group II, despite aseptic precautions, endotoxin susceptibility increased as tested at the 200

**TABLE VII**

<table>
<thead>
<tr>
<th>Wounding technique</th>
<th>Maximum bleed-out volume* (ml./kg.)</th>
<th>90 hr. survival† (Alive/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. No aseptic precautions (open wound sutured after 2 hrs.)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Superficial skin incision</td>
<td>47 (43-53)</td>
<td>5/6</td>
</tr>
<tr>
<td>B. Exposure of femoral artery</td>
<td>47 (43-51)</td>
<td>1/8</td>
</tr>
<tr>
<td><strong>II. Aseptic precautions (wound sutured immediately)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Exposure of femoral artery</td>
<td>46 (43-50)</td>
<td>1/5</td>
</tr>
<tr>
<td>B. Ligation of femoral artery</td>
<td>49 (47-52)</td>
<td>3/9</td>
</tr>
</tbody>
</table>

* Rabbit weight = 1.1 (range 0.9 to 1.4) kg.
† All animals received *E. coli* endotoxin, 200 µg./kg., 4 hours postretransfusion as single intravenous injections.

µg./kg. level. Nevertheless, almost 30 per cent (4 of 14) of these animals survived compared with the 10 per cent (1 of 10) survival of animals with non-sterile wounds challenged with 50 µg./kg. *E. coli* endotoxin. Although the significance of these differences cannot be evaluated (the expected difference between 50 and 200 µg./kg. endotoxin challenge being unknown), the findings suggest only slight protective effect of aseptic precautions against the subsequent *E. coli* endotoxin challenge.

7. **Bacteriologic Studies of Femoral Wounds.**—Of 30 specimens of femoral skeletal muscle removed from 10 normal rabbits after head blow sacrifice, employing rigid aseptic precautions including skin and muscle flaming, clostridia were cultured in 28 per cent; none were identified as *Cl. perfringens*. Schwein-
burg and Sylvester (14) reported a 23 per cent incidence of clostridia in 13 normal rabbit skeletal muscle specimens tested. Of interest, 50 per cent of 12 normal femoral skeletal muscle specimens from 4 rabbits sacrificed by cardiac exsanguination grew clostridia; *Cl. perfringens* was identified in each case. No bacteria other than clostridia were found in any normal rabbit muscle specimen.

In contrast to the findings with normal rabbit femoral muscle, all the swab cultures taken 15 to 30 minutes after exposure of the femoral artery without aseptic precautions during hemorrhagic shock grew clostridia, and 90 per cent of the specimens tested contained *Cl. perfringens*. The clostridia were invariably accompanied by staphylococci and/or streptococci (non-hemolytic). Although initial swab cultures from femoral wounds prepared with aseptic precautions during hemorrhagic shock failed to grow bacteria in 13 of 14 animals tested, 8 of 10 whole muscle samples removed with aseptic precautions from these same wounds 8 hours later (2 hours after intravenous *E. coli* endotoxin challenge) yielded *Cl. perfringens*.

In the absence of aseptic precautions, femoral wounds of animals surviving longer than 12 to 18 hours after hemorrhagic shock and endotoxin challenge exhibited overt signs of wound sepsis ranging from minimal local changes to extensive hemorrhagic myonecrosis, edema, and putrefaction. Such gross evidence of femoral wound infection was not usually encountered when aseptic precautions were employed, or in animals dying within 12 hours after hemorrhagic shock and endotoxin challenge.

8. **Effect of Topical Sulfanilamide during Hemorrhagic Shock.**—In 11 rabbits subjected to hemorrhagic shock by cardiac puncture, a femoral artery exposure without aseptic precautions was performed immediately after the last bleeding. The wound was covered with clean gauze and 2 hours later, 1 gm. of sterile sulfanilamide crystals (Hynson, Westcott & Dunning, Inc., Baltimore) sprinkled into the wound. The wound was then sutured and the animal retransfused. Intravenous challenge with *E. coli* endotoxin 4 hours thereafter yielded three survivors (Table VIII, group I). Sulfanilamide applied by this method thus did not significantly prevent increased susceptibility to endotoxin at the 200 μg./kg. level.

In contrast, when 11 rabbits, paired with the above group and selected at random, were subjected to a femoral artery exposure using aseptic precautions and the wound promptly sealed after instilling 1 gm. of the sulfanilamide crystals, 9 survivals resulted following *E. coli* endotoxin challenge (Table VIII, group II A). The combination of aseptic precautions and immediate application of sulfanilamide to the femoral wound during hemorrhagic shock inhibited the expected increase in endotoxin susceptibility. Compared with the non-sterile wound mortality, this effect appears significant (*p* < 0.05). However, when the femoral artery was both exposed and ligated, 3 of 4 animals succumbed to 200
COLLAPSE OF BACTERIAL ENDOTOXIN RESISTANCE

µg./kg. E. coli endotoxin challenge 4 hours after retransfusion despite the sulfanilamide (Table VIII, group II B).

9. Effect of Polyvalent Gas Gangrene Antitoxin.—Undiluted polyvalent gas gangrene antitoxin (Lederle Laboratories Division, Pearl River, New York), 2 ml./kg., containing approximately 2000 units Cl. perfringens, 2000 units Cl. septicum, 600 units Cl. histolyticum, 300 units Cl. novyi, and 300 units Cl. oedematoideus antitoxin, was administered intravenously over a 2.5 minute period to 19 rabbits 15 minutes prior to hemorrhage with the Lamson reservoir technique. All animals were exposed to 2 hours of shock at 50 mm. Hg using clean but not aseptic bleeding precautions. The antitoxin (a modified horse globulin preparation) impaired the ability of the rabbit to tolerate acute blood loss as indicated by an increased mortality after retransfusion and a reduction in maximum bleed-out volume. Thus only 9 animals (47 per cent) survived more than 4 hours post retransfusion; of these, maximum bleed-out volume averaged 42 ml./kg. (range 38 to 50 ml./kg.). This may be compared with a 92 per cent survival rate and a mean maximum bleed-out volume of 48 ml./kg. (range 42 to 60 ml./kg.) in 13 untreated animals. Moreover, reinfusion of 10 to 20 per cent of the total bleed-out volume was generally required towards the end of the 1st hour to maintain the mean arterial blood pressure of the antitoxin-treated animals at 50 mm. Hg. Similar results were obtained with 9 rabbits pretreated with 2 ml./kg. control modified horse globulin (Lederle) containing approximately 6000 units diphtheria antitoxin; of this group, only

TABLE VIII
Effect of Topical Sulfanilamide (1 Gm.) in Femoral Wound during Hemorrhagic Shock on the Susceptibility of the Rabbit to E. coli Endotoxin

<table>
<thead>
<tr>
<th>Wounding technique</th>
<th>Maximum bleed-out volume*</th>
<th>96 hr. survivals† (Alive/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. No aseptic precautions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure of femoral artery (sulfanilamide applied</td>
<td>46</td>
<td>3/11</td>
</tr>
<tr>
<td>after 2 hours and wound sutured)</td>
<td>(43-50)</td>
<td></td>
</tr>
<tr>
<td>II. Aseptic precautions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Exposure of femoral artery (sulfanilamide applied</td>
<td>45.5</td>
<td>9/11</td>
</tr>
<tr>
<td>immediately and wound sutured)</td>
<td>(43-50)</td>
<td></td>
</tr>
<tr>
<td>B. Ligation of femoral artery (sulfanilamide applied</td>
<td>45</td>
<td>1/4</td>
</tr>
<tr>
<td>immediately and wound sutured)</td>
<td>(43-48)</td>
<td></td>
</tr>
</tbody>
</table>

* Rabbit weight = 1.2 (range 1.0 to 1.4) kg.
† All animals received E. coli endotoxin, 200 µg./kg., 4 hours postretransfusion as single intravenous injections.
SHELDON E. GREISMAN

4 animals (44 per cent) survived longer than 4 hours post retransfusion and mean maximum bleed-out volume averaged 40 ml./kg. Employing the intramuscular route for antitoxin administration did not appreciably affect the results. The antitoxin thus altered the framework of the standardized Lamson reservoir shock preparation. Nevertheless, the prophylactic gas gangrene antitoxin exerted some protective effect against *E. coli* endotoxin challenge which could not be demonstrated with the control diphtheria antitoxin. Thus 7 of the 9 gas gangrene antitoxin-treated animals surviving 4 hours post retransfusion withstood challenge with 200 μg./kg. *E. coli* endotoxin, whereas only 1 of the 4 surviving control diphtheria antitoxin-treated animals withstood 50

### TABLE IX

<table>
<thead>
<tr>
<th>Antitoxin*</th>
<th>Initial mean femoral artery blood pressure</th>
<th>Maximum bleed-out volume</th>
<th><em>E. coli</em> endotoxin</th>
<th>% 96 hr. survivals (Alive/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gas gangrene</strong></td>
<td>mm. Hg</td>
<td>ml./kg.</td>
<td>μg./kg.</td>
<td></td>
</tr>
<tr>
<td>Gas gangrene</td>
<td>94 (88-102)</td>
<td>42 (38-50)</td>
<td>200</td>
<td>7/9</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>92 (90-104)</td>
<td>40 (36-45)</td>
<td>50</td>
<td>1/4</td>
</tr>
</tbody>
</table>

* 2 ml./kg, undiluted antitoxin (Lederle) injected intravenously over a 2.5 minute period 15 minutes prior to start of bleed-out.
† Rabbits weighing 1.1 (range 1.0 to 1.4) kg. were exposed to 2 hours of hemorrhagic shock at 50 mm. Hg with the Lamson reservoir technique employing clean but not aseptic precautions. Only animals surviving 4 hours after retransfusion, at which time intravenous challenge with endotoxin was instituted, are considered.

μg./kg. endotoxin (Table IX). More extensive studies of diphtheria antitoxin were precluded by lack of material.

10. Effect of Femoral Artery Ligation on Limb Weight and Skin Temperature.—The effect of a femoral artery cutdown and ligation on the weight and skin temperature of the leg will be considered in detail in a subsequent report. The data can be summarized as indicating that in the absence of hemorrhagic shock, femoral artery cutdown and ligation, even without aseptic precautions, do not increase limb weight significantly above the opposite control. Similarly, a simple exposure of the femoral artery without ligation during 2 hours of

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5 Tetanus antitoxin (Lederle) could not be employed for controls since the same donor horses may have been treated previously with gas gangrene toxoid (15). The one acceptable control, diphtheria antitoxin, was generously supplied by the Lederle Laboratories through the courtesy of Mr. Monroe. The material was the last batch available and was outdated by 2 years.
reversible hemorrhagic shock fails to increase limb weight appreciably. However, ligation during hemorrhagic shock causes a significant increase in limb weight within 24 hours following retransfusion, averaging 12 gm./kg. over the opposite control. This increase occurred even when aseptic precautions (including topical sulfanilamide) were employed, with either the cardiac or Lamson reservoir bleeding techniques, in the presence or absence of endotoxin challenge, and in animals pretreated with polyvalent gas gangrene or diphtheria antitoxin. The increment is equivalent to about 12 ml./kg. plasma transudation into the wounded limb and represents approximately 30 per cent of the original plasma volume (16). The presence of overt femoral wound infection was associated with augmented increments in limb weight; some animals with severe clostridial wound infection exhibited increases of 50 gm./kg.

Alteration in the skin temperature of the limb following femoral artery ligation also depended upon the presence or absence of hemorrhagic shock. Ligation in normal animals failed to lower the temperature of the distal third of the thigh. However, when hemorrhagic shock was superimposed, not only did the general skin temperature fall abruptly, but that with the ligated femoral artery fell 2–4°F. below the opposite control, reaching levels almost comparable to those seen at the same room temperature after application of a tourniquet.

11. Effect of Unilateral Hind Limb Ischemia.—In 10 normal rabbits exposed to 2 hours of unilateral hind limb ischemia, challenge with 200 μg./kg. E. coli endotoxin caused 50 per cent mortality within 48 hours. The plasma transudation into the ischemic limb at death or upon sacrifice after 48 hours was approximately 27 (range 8 to 36) ml./kg.

DISCUSSION

The series of provocative experiments described by Fine and his associates during the past several years (1–8) indicate the appearance of circulating bacterial endotoxins, or closely related substances during the course of hemorrhagic shock, accompanied by collapse of host resistance to these factors. The mechanisms for such deterioration of endotoxin resistance have not as yet been adequately defined. The inability of Sanford and Noyes to detect gastrointestinal absorption of Cr48-labeled E. coli endotoxin during hemorrhagic shock in the dog (17) renders less likely the possibility that endotoxin absorbed from the intestinal tract could account entirely for the injury to the endotoxin defense mechanisms. Among additional factors to be considered are the skeletal muscle trauma, major arterial occlusion, and bacterial wound contamination incurred when a femoral arterial cutdown and ligation are performed without aseptic precautions. The observation that failure to ensure asepsis during bleed-out favors the development of “irreversible” hemorrhagic shock in the rat (18), although contrary to that reported for the dog (2), suggests that a
non-sterile wound may exert some noxious effect upon host resistance to stress following hemorrhage. Moreover, employing aseptic bleeding precautions Sanford and Noyes (17) were unable to detect any remarkable increase in bacterial endotoxin susceptibility following hemorrhage in the dog; similar results were reported by Zweifach and Thomas (19) for the rat. It thus appeared essential to evaluate the contribution of inadequate aseptic technique, as well as of wound trauma, to those increments in bacterial endotoxin susceptibility detected following hemorrhagic shock.

Production of atraumatic and aseptic hemorrhagic shock poses serious difficulties. The problem was approached in the rabbit by employing graded hemorrhage utilizing cardiac rather than femoral artery bleedings. Although some tissue injury and potential microbial contamination were unavoidable, these could be minimized if performed properly. Since such a method may represent hemorrhagic plus cardiogenic shock, the effects of sham cardiac bleedings on cardiovascular function were studied initially. Control cardiac punctures without hemorrhage, when performed carefully, failed to indicate impairment of myocardial contractility as evidence by a virtually unwavering mean arterial blood pressure and by a maintained ability to tolerate acute circulatory fluid overloading. However, to minimize the possibility that superimposition of blood loss and heparinization might unmask a myocardial defect, animals with maximum bleed-out volumes less than 43 ml./kg. were excluded from the study.

The problem of obtaining reproducible degrees of hemorrhagic shock by cardiac bleedings of unanesthetized animals, avoiding arterial cutdowns and apparatus that could not be sterilized at 200°C., was never entirely solved. Nevertheless, one approach appeared promising. After preliminary studies had indicated good correlation between hypotensive arterial blood pressure levels and certain clinical signs related to cerebral function, it became possible to predict blood pressures in the hypotensive range by inspection of the animal. Paired rabbits were then bled by cardiac punctures to induce clinical signs compatible with severe shock. The arterial blood pressure in one of each pair of animals, chosen at random, was then confirmed by direct femoral artery cannulation. With experience, mean femoral arterial blood pressure levels between 46 and 60 mm. Hg were obtained consistently. Of the rabbits acceptable for the present study, the resultant mean maximum bleed-out volume was 48 ml./kg. Based upon 96 hours of observation, such a procedure produced a 76 per cent survival if blood was retransfused 2 hours after the last bleeding, and an 81 per cent mortality if retransfusion was delayed 6 hours.

With this technique, rabbits exposed to 2 hours of hemorrhagic shock in the absence of femoral trauma exhibited no detectable increase in susceptibility to E. coli endotoxin administered 4 hours after retransfusion. These results are based upon testing with single intravenous injections of 200 μg./kg. E. coli
endotoxin, the largest dosage uniformly non-lethal to healthy animals. It is emphasized that the findings do not exclude enhanced susceptibility to higher endotoxin dosages. In contrast, the paired animals selected at random and exposed to similar hemorrhagic shock but also subjected to a femoral artery cutdown and ligation without aseptic precautions, exhibited several hundred-fold increases in susceptibility to the same *E. coli* endotoxin. This increase in susceptibility is significant (*p* < 0.005) since mean age, weight, initial systolic arterial blood pressure, and maximum bleed-out volumes in both rabbit groups were comparable. Femoral arterial cutdown and ligation in the absence of shock did not appreciably increase susceptibility to endotoxin; the superimposition of hemorrhagic shock constituted an essential factor, as indicated by the resistance to endotoxin of animals subjected to femoral artery ligations after sham cardiac bleedings.

To consider more critically the status of hemorrhagic shock induced with the multiple cardiac bleeding technique, the results of the preceding studies were reevaluated in animals of the same stock subjected to 2 hours of hemorrhagic shock at 50 mm Hg by femoral arterial bleeding into an elevated Lamson reservoir without aseptic precautions. It was reasoned that if the cardiac bleeding data were reliable, the Lamson technique results would be at least roughly comparable, since it is the duration and intensity of arterial hypotension, not the rapidity or frequency of bleeding that is of primary importance in the standardization of hemorrhagic shock (20). And indeed, both maximum bleed-out volume as well as susceptibility to single intravenous *E. coli* endotoxin injections 4 hours after retransfusion fell into the same ranges as those when cardiac bleedings were combined with femoral artery cutdowns and ligations in the absence of aseptic technique. Such findings strongly suggest that the cardiac puncture technique was a valid method for studying the effect of hemorrhagic shock on bacterial endotoxin defense mechanisms.

The present results with the Lamson reservoir technique can be compared with previous comparable studies indicating a 100,000-fold increase in susceptibility of rabbits to *E. coli* endotoxin (11). The greater increment in susceptibility in the previous study might be attributable to variations in endotoxin, animal strain, and details of the shock procedure; however, one factor that may account for much of the observed variation is the age differences in the rabbits employed: immature animals averaging 1.1 kg. were used in the present study, whereas older rabbits weighing approximately 2.3 kg. were employed previously. Such older animals are not only significantly more susceptible to the lethal action of single intravenous injections of bacterial (meningococcal) endotoxin (21) but also exhibit lower tolerance to acute stress (22). Nevertheless, despite the quantitative differences, there is definite agreement that in the presence of a non-sterile femoral artery cutdown and ligation, remarkable increases in the susceptibility of rabbits to bacterial
endotoxin follow exposure to 2 hours of severe but reversible hemorrhagic shock.

The possible mechanisms of action of the femoral arterial cutdown and ligation on endotoxin resistance were considered. By employing aseptic cardiac bleeding precautions, it was found that a superficial femoral skin incision during hemorrhagic shock failed to increase the susceptibility of the rabbit to *E. coli* endotoxin when tested at the 200 μg./kg. level. However, if the underlying fascia and muscle were traumatized by mobilizing the femoral artery without aseptic precautions, significant increases (p < 0.05) in susceptibility developed. Since such findings strongly suggested the participation of wound infection, subsequent studies were directed at determining the effect of wounding under aseptic conditions.

The use of aseptic wounding precautions during hemorrhagic shock conferred only minimal protection against *E. coli* endotoxin challenge at the 200 μg./kg. level. The aseptic technique appeared to be satisfactory as judged by initial sterile femoral wound swab cultures; however, specimens of whole femoral wound muscle cultured several hours after retransfusion and endotoxin challenge yielded *Cl. perfringens* in 8 of 10 animals tested. Whether the clostridia were introduced at operation, arrived as metastatic deposits, or existed *in situ* prior to wounding was not determined. All sources appear capable of contributing these organisms. The present findings conform with the conclusion of Schweinburg and Sylvester (14) that "Most of the tissues of healthy dogs, and rabbits to a lesser degree, harbor clostridia..." Obviously, experiments with aseptic precautions in these species cannot be accepted as excluding a possible role of femoral wound infection in the collapse of bacterial endotoxin resistance following hemorrhagic shock.

Since it was not possible to eliminate clostridial wound contamination by aseptic precautions, sulfanilamide was applied topically to suppress microbial growth. When 1 gm. of sulfanilamide was placed into a femoral wound prepared with aseptic precautions and the wound promptly sealed, *E. coli* endotoxin susceptibility 4 hours after retransfusion showed no increase at the 200 μg./kg. level. These findings appear significant (p < 0.05) and support the concept that microbial activity in the femoral wound may be an important factor in enhancing the susceptibility of the rabbit to endotoxin following hemorrhagic shock. A definite conclusion from this observation alone cannot be drawn, however, since sulfonamides (and certain antibiotics) possess "anti-endotoxic" activity (23–28), which according to Schweinburg *et al.* (29) is induced largely by suppression of endogenous gastrointestinal bacterial endotoxin production. The possibility that the topically applied sulfanilamide might act after systemic adsorption to suppress such a release of endotoxin cannot be excluded in the present study. It appears unlikely, however, that the protection was due to systemic action. Oral sulfathalidine, which should be equally effective in
suppressing release of endotoxin from the intestines, fails to inhibit the irreversible phase of hemorrhagic shock (30). Sulfanilamide did not exert a direct anti-endotoxic effect; when applied to non-sterile femoral wounds after a 2 hour delay, or to sterile wounds after the femoral artery was ligated, it was ineffective.

Since aseptic precautions eliminated all bacteria except clostridia from the femoral wound, it follows that if topical sulfanilamide acted by local bacteriostasis, clostridia in some way play a significant role in lowering endotoxin resistance of the rabbit following hemorrhagic shock. Subsequent studies with polyvalent gas gangrene antitoxin prophylaxis support this hypothesis. Protection against *E. coli* endotoxin was obtained with gas gangrene antitoxin which could not be demonstrated when diphtheria antitoxin was employed as a control. It must be emphasized, however, that both the gas gangrene and diphtheria antitoxin reduced the maximum bleed-out volume, increased the rate of blood uptake, and accelerated the onset of irreversibility. In addition, only 4 diphtheria antitoxin-treated animals were suitable for study, and the diphtheria preparation was outdated. The antitoxin studies must therefore be interpreted as suggestive, rather than as providing definitive evidence for the role of clostridia in altering resistance to endotoxin.

The manner by which clostridial wound infection may lower host resistance to bacterial endotoxin during hemorrhagic shock is unknown, although recent studies implicate certain contributory mechanisms. Thus clostridial wound infection intensifies low blood volume shock (31–33) and presumably accentuates the underlying mechanisms by which shock impairs endotoxin resistance. In addition, potentiation of specific toxic effects of endotoxin is possible since filtrates of *Cl. perfringens* duplicate endotoxin activity by inhibiting leukocyte phagocytic activity and sensitizing the vascular system to epinephrine (34–37). The ability of endotoxin to lower host resistance to infection (38–43) may augment these effects by enhancing the clostridial infection.

Although clostridial wound infection appears to be an important factor, this does not imply that the local alterations following femoral wounding are the only mechanisms that increase susceptibility of the rabbit to bacterial endotoxin 4 hours after reversible hemorrhagic shock. Other studies have shown that hemorrhagic shock impairs reticulo-endothelial function (44), an important component of the endotoxin defense system (45–47), and the present studies show that following retransfusion after 2 hours of reversible shock approximately 30 per cent of the original plasma volume is transudated in the wounded lower extremity. This plasma transudation is a consequence of the intense hind limb ischemia that follows femoral artery ligation during hemorrhagic shock. On the basis of the present and previous (48, 49) studies with unilateral hind limb tourniquet shock, it is likely that such ischemia not only potentiates clostridial infection, but that the transudation of plasma further enhances
endotoxin susceptibility by augmenting the shock state and intensifying the injury to the reticulo-endothelial system.

SUMMARY

Unanesthetized immature albino rabbits exposed to 2 hours of severe but reversible hemorrhagic shock induced with aseptic precautions by multiple cardiac bleedings exhibited no increase in susceptibility to single intravenous injections of 200 µg./kg. E. coli endotoxin administered 4 hours post retransfusion, a quantity of endotoxin that was found to be the largest dose uniformly non-lethal to normal rabbits. Paired and randomly selected rabbits treated identically except for the additional procedure of a femoral arterial cutdown and ligation (without aseptic precautions) exhibited increases in susceptibility to the same endotoxin of several hundredfold. This effect could not be attributed to the femoral cutdown and arterial ligation alone since such trauma when coupled with sham cardiac bleedings failed to increase susceptibility to 200 µg./kg. of endotoxin. These data appear valid since sham cardiac bleedings produced no detectable impairment of myocardial contractility, while 2 hours of hemorrhagic shock at 50 mm. Hg with the Lamson reservoir technique caused an increase in endotoxin susceptibility comparable to that seen when cardiac bleedings were combined with a non-sterile femoral arterial cutdown and ligation.

The mechanisms increasing the susceptibility to E. coli endotoxin were investigated. It was found that (a) rabbit femoral skeletal muscle is normally contaminated with clostridia, (b) the use of aseptic femoral wounding precautions exerted some suggestive protective influence, (c) the use of aseptic wounding precautions combined with immediate topical sulfanilamide and wound closure exerted a significant protective influence, and (d) prophylactic polyvalent gas gangrene antitoxin protected in a manner not demonstrable when diphtheria antitoxin was employed as a control. These observations suggest that clostridial wound infection is one mechanism whereby a femoral arterial cutdown lowers endotoxin resistance of the rabbit following hemorrhagic shock.

It is, however, not the only mechanism since the ligation of a femoral artery during hemorrhagic shock led to edema following retransfusion equivalent to a mean of approximately 30 per cent of the original circulating plasma volume. The intensification of shock caused by this transudation presumably intensified reticulo-endothelial injury, and thus further lowered the resistance of the rabbit to an intravenous injection of endotoxin 4 hours following retransfusion.

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