EFFECTS OF PENTOBARBITAL ANESTHESIA ON SURVIVAL, 
E. COLI CLEARANCE, GLUCOSE AND LEUKOCYTE CONCENTRATION 
IN DOGS SUBJECTED TO LD_{100} E. COLI 

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University of Oklahoma Health Sciences Center 
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EFFECTS OF PENTOBARBITAL ANESTHESIA ON SURVIVAL, 
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Leukocytosis and sustained gluconeogenic function have been suggested as important factors in survival of endotoxin shock (1). Recent studies in our laboratory have demonstrated that sublethal intravenous injections of E. coli endotoxin in the awake canine produces an initial leukopenia followed by a marked leukocytosis and a febrile response. These animals subsequently survive a 2x LD_{100} injection of either endotoxin or live E. coli organisms with an associated protection of liver function (1,2,3,4). In vitro portions of these recent studies reveal an increased glucose utilization in the endotoxin-pretreated leukocytotic blood compared with saline-pretreated normocytotic blood following incubation with live E. coli organisms. The neutrophil's phagocytic activity has been implicated as the primary factor accounting for this elevated glucose uptake (2,3). An increase in phagocytosis by the blood has been reported to occur after administration of endotoxin (5) with the leukocyte being responsible for this increased activity (6,7). The circulating polymorphonuclear leukocytes have been shown to be of major importance in the clearance of bacterial organisms (8).

The pathogenesis and mechanisms of septic and endotoxin shock in man have been obtained primarily from animal studies with the findings then being related back to man (8). General anesthesia in patients has been reported to decrease phagocytosis in vitro (9), while halothane anesthesia has been found to increase mortality in mice subjected to fecal peritonitis (10). Many animal studies have necessarily utilized anesthesia; however, the effects of the anesthetic on the host's defense mechanisms have not been fully assessed. The purpose of this study was to establish the effect of pentobarbital anesthesia on alterations in leukocyte and blood glucose concentration, clearance of E. coli from peripheral blood and survival in the dog subjected to LD_{100} injections of live E. coli organisms.
Methods. The experiments were carried out on 18 awake adult mongrel dogs during a 4-day period. The dogs were of random sex selected for freedom of clinical signs of disease and each was screened for microfilaria of heartworms, treated for intestinal parasites, and stabilized for a 3-6 week period. Animals were divided into three groups of six dogs each. Group A dogs, the control group, received saline equal to the volume of endotoxin administered the experimental groups. Group B and C dogs, the experimental groups, received a 0.003 mg/kg of \( E. \) \textit{coli} endotoxin (Difco; Detroit, Mich.) at 0 time on Days 1 and 2, and 2.25 mg/kg \( (LD_{100}) \) on Day 3. On Day 4, Group B dogs were anesthetized with sodium pentobarbital (28-30 mg/kg) 30 minutes prior to 0 time. All three groups received \( LD_{100} \) of live \( E. \) \textit{coli} (mean 1.3x10^{10} \) organisms/kg) at 0 time on Day 4 by a bolus injection. The \( LD_{100} \) of \( E. \) \textit{coli} organisms had been previously established in our laboratory (2). Dogs living seven days following injection of \( E. \) \textit{coli} organisms were considered permanent survivors. No supportive therapy, even to account for normal fluid loss, was given to any dog during this study.

Blood samples for leukocyte counts, hematocrits, and glucose concentrations were collected at control times on Days 1, 2 and 3 and at control 1, 2, 3, 4, 6, 8 and 24 hours on Day 4, while blood specimens were obtained for bacterial colony counts at control 5 and 15 minutes, 2 and 4 hours. The blood samples were obtained by venipunctures of either the cephalic or saphenous veins, then placed in vacutainers containing ethylenediamine-tetraacetic acid (EDTA; Becton-Dickinson) or sterile tubes containing saline (9 ml volumes) and immediately placed on ice. The injection of saline, endotoxin, or \( E. \) \textit{coli} was by the intravenous route utilizing either the cephalic or saphenous vein.

Total leukocyte counts were measured with an automatic particle counter (Coulter \( Z_3 \); Hialeah, Fla.) and the differential WBC by microscopic examination of blood smears stained with Wrights stain (100 cells counted). Blood glucose
concentrations were determined with a Beckman glucose analyzer (Beckman Instruments; Fullerton, Calif.) with an accuracy of ±3 mg%, and rectal temperatures were obtained using a Tele-Thermometer probe (Yellow Springs Instruments; Yellow Springs, Ohio). Blood E. coli concentration was quantitated by serial tenfold dilutions of peripheral blood samples grown in tryptic soy agar pour plates incubated at 37°C for 18-24 hours.

The preparation of the E. coli organisms was as follows: E. coli Type B isolated from a stool specimen at Children's Memorial Hospital, Oklahoma City, Oklahoma, was maintained in a lyophilized state after growth on tryptic soy agar, (TSA). The E. coli was then initially grown in approximately 3-4 ml of tryptic soy broth at 37°C for 4-6 hours. Tryptic soy agar slants were inoculated from the broth suspension using sterile cotton swabs and incubated at 37°C for 18 hours. The E. coli organisms were washed from the slants with 2-3 ml of physiological sterile saline (PSS). The washing was then centrifuged, the supernatant was poured off, and the E. coli were resuspended in PSS. The E. coli suspension was then adjusted with PSS to a predetermined density using a spectrophotometer (Junior IIA. Coleman Instruments; Oak Brook, Ill.). The viability and quantitation of bacteria counts were done using serial tenfold dilutions on TSA pour plates. The results were analyzed using t tests for paired or unpaired data.

Results. Survival rates were markedly different in the control and the experimental groups (Table I). All dogs in Group A (saline controls) died within 10 hours post-injection of LD100 E. coli organisms. Four of six dogs in Group B (endotoxin pre-injected, anesthetized) were permanent survivors, with the two remaining animals surviving 30 and 54 hours after E. coli injection. All dogs in Group C (endotoxin pre-injected, awake) were permanent survivors.

Changes in blood bacterial concentrations can be seen in Table II. Both Groups B and C showed a significantly greater clearance of live E. coli from the
peripheral blood (p<0.005) at 2 and 4 hours post-injection of bacteria when compared with Group A (controls). Although at +15 minutes Group B had a significantly lower (p<0.05) E. coli concentration than Group C, there was no significant difference between the two groups at +2 and +4 hours.

Figure 1 illustrates the effects of LD100 live E. coli (mean 1.3x10^10 organisms/kg) on leukocyte concentration in saline pretreated dogs (Group A) or endotoxin pre-injected dogs (Groups B and C). Both Group B and C had significantly higher leukocyte concentrations (p<0.005) at control time of Days 2, 3, and 4 when compared with Group A, with the elevation of leukocyte count being accounted for primarily by increases in mature and immature neutrophils. After injections of the LD100 E. coli organisms on Day 4, all three groups became leukopenic (p<0.005) at +1 hour, while at +2 hours Group A (saline controls) had a significantly higher (p<0.005) peripheral leukocyte count than either Group B or C. The lymphocyte count did not change significantly at control times from Day 2 through Day 4 (p>0.05); however, on Day 4 the absolute lymphocyte numbers were significantly lower (p<0.02) in both Groups B and C when compared with Group A (control) at +1 and +3 hours after E. coli injection. There were no significant alterations (p>0.05) in absolute monocyte numbers either within or between the three groups at any of the sampling times.

Blood glucose concentrations were relatively constant at control times for Days 1 through 4 when comparing all three groups (Figure 2). In the control group (Group A) glucose concentrations progressively declined on Day 4 after E. coli administration and were significantly lower (p<0.05) at +6 and +8 hours when compared to the experimental groups (Groups B and C). There was no significant difference in blood glucose levels between the two experimental groups.

Changes in the hematocrit can be seen in Figure 3. Although the hematocrit increased (p<0.05) in all groups when compared to initial control measurements on Day 4, the control group (Group A) developed a greater hemoconcentration
at +2, +4 and +8 hours after *E. coli* administration when compared to either experimental group. The anesthetized group (Group B) was significantly more hemocoagulated (*p*<0.02) at +8 and +24 hours after *E. coli* injection than the awake experimental group (Group C).

Figure 4 illustrates the changes in the rectal temperature of dogs in this study. There was no difference within each group or between groups for control values on Days 1, 2, and 3. Group B had lower (*p*<0.005) rectal temperatures at +1 and +2 hours on Day 4 than Group A (saline controls), while Group C exhibited an elevated (*p*<0.05) temperature from +1 through +3 hours compared to Group A. On Day 4 the anesthetized experimental group (Group B) had a significantly lower (*p*<0.05) rectal temperature than the awake experimentals (Group C) from control time through +4 hours.

**Discussion.** Recent studies from our laboratory have shown that the canine develops a rapid leukopenia followed by a leukocytosis in response to sublethal and lethal injections of endotoxin and subsequently survives a lethal challenge of either *E. coli* live organisms or endotoxin (1,2). The previous studies were conducted on dogs in the awake state since this setting appeared to be closer to the clinical condition of septic shock in man. Priano and associates established that sodium pentobarbital significantly depressed systolic blood pressure, stroke volume, pulse pressure, central venous pressure, *pO*₂, pH, and body temperature, and stated that serious consideration should be given to employing an unanesthetized model in physiologic and pharmacologic studies (11). Other studies have shown that barbituates as well as other anesthetics decrease hematocrit and peripheral leukocyte count in dogs (12,13). Since certain studies necessitate the use of anesthesia, this study was designed to investigate the effect of pentobarbital anesthesia on clearance of live *E. coli* from the peripheral blood, hematological, body temperature, and blood glucose concentrations, and survival in the leukocytotic dog pre-injected with sublethal doses of endotoxin.
Clearance of live *E. coli* from peripheral blood was significantly greater in both experimental groups compared to the control (normocytotic) group indicating that the leukocytosis (neutrophilia) might account for the increased survival by removing live *E. coli* from the peripheral blood. Since there was no difference between clearance of bacteria between awake and anesthetized experimental groups at +2 and +4 hours, it appears that pentobarbital did not influence host defense in eliminating the *E. coli*. These results are in agreement with a recent study that found the intact leukocyte to be the most important host defense mechanism for clearance of bacterial organisms from the peripheral blood (8). Data also suggest that the ability to clear *E. coli* organisms from the peripheral blood is not the only factor influencing survival in the endotoxin-pretreated dog.

The initial leukopenia and subsequent leukocytosis observed is in agreement with our earlier studies (1,2,3,4). The leukopenia is thought to occur when granulocytes adhere to the capillary endothelial cells and later leave the circulation moving into the tissue in response to endotoxin (14). A subsequent leukocytosis has been reported to occur when new leukocytes from the bone marrow enter the circulation (14). The protective role of leukocytes is emphasized in recent reports relating beneficial effects of transfused white blood cells in experimental bacteremia in the dog (15,16).

The progressive hypoglycemia which developed in Group A is similar to our laboratory's earlier studies (1,2,3,4) and has been suggested to occur as a result of impaired liver gluconeogenic capacity (17,18). Supportive of recent findings from this laboratory (4), this study appears to underscore a correlation between leukocytosis (neutrophilia), sustained gluconeogenesis, blood glucose concentration maintenance and canine survival. Since there was no significant difference between the awake and anesthetized experimental groups' blood glucose concentration, it seems that the pentobarbital had no adverse effect on liver gluconeogenesis.
The hematocrit increased in all groups and the degree of hemoconcentration appeared to be related to increased mortality as seen in the anesthetized leukocytotic dogs. Other therapy studies in canine endotoxin shock have associated hemoconcentration with mortality (19). The anesthetized group in this study was significantly hemoconcentrated although anesthesia alone normally produces a lowering of the hematocrit (11,12). This increase in hematocrit might be partially due to the animals inability to drink while anesthetized, since no fluids were given to account for normal body fluid loss.

Rectal temperatures of both awake groups exhibited initial increases in response to the E. coli injection which agrees with earlier data in endotoxin studies in the awake dogs (1) and is similar to clinical findings in man (20). Blunting of the pyrogenic response observed in the anesthetized experimental animals might be the result of temperature depression commonly found during anesthesia (11,13).

Results of this study reveal that anesthesia did not alter the clearance of live E. coli from the peripheral blood, blood glucose concentration, or leukocyte response; however, it did cause a hemoconcentration, depression of the pyrogenic response and decrease in survival. Findings suggest that the awake canine may be a better model for survival studies in septic shock unless a means of protecting the anesthetized dog from depression of body temperature and hemoconcentration is implemented.

Summary: This study was conducted to determine the effects of sodium pentobarbital anesthesia on survival of the dog, leukocyte response, E. coli clearance from the peripheral blood and blood glucose concentration in the leukocytotic endotoxin pre-injected canine subjected to a LD_{100} of live E. coli organisms. Our laboratory has shown that the awake leukocytotic endotoxin pre-injected canine survives lethal doses of E. coli live organisms or endotoxin. Sodium
pentobarbital anesthesia decreased survival in the leukocytotic canine which was associated with an increased hemoconcentration and hypothermia. Anesthesia did not alter either clearance of *E. coli* organisms from peripheral blood or blood glucose concentrations and there were only minor changes in leukocyte response. These data suggest that one should use the awake canine or provide means for preventing hemoconcentration and hypothermia in septic shock studies while using the anesthetized dog as the animal model.
REFERENCES

TABLE I. DOSAGES OF ESCHERICHIA COLI ORGANISMS AND SURVIVAL TIME OF DOGS

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Dose of E. coli Organisms Per Kgm.</th>
<th>Survival Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A^a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.3x10^10</td>
<td>5 hrs.</td>
</tr>
<tr>
<td>4</td>
<td>1.2x10^10</td>
<td>10 hrs.</td>
</tr>
<tr>
<td>7</td>
<td>1.4x10^10</td>
<td>7 hrs.</td>
</tr>
<tr>
<td>10</td>
<td>1.5x10^10</td>
<td>10 hrs.</td>
</tr>
<tr>
<td>13</td>
<td>1.1x10^10</td>
<td>5 hrs.</td>
</tr>
<tr>
<td>16</td>
<td>1.4x10^10</td>
<td>6 hrs.</td>
</tr>
<tr>
<td>Group B^b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.3x10^10</td>
<td>7 days*</td>
</tr>
<tr>
<td>5</td>
<td>1.2x10^10</td>
<td>54 hrs.</td>
</tr>
<tr>
<td>8</td>
<td>1.4x10^10</td>
<td>30 hrs.</td>
</tr>
<tr>
<td>11</td>
<td>1.3x10^10</td>
<td>7 days*</td>
</tr>
<tr>
<td>14</td>
<td>1.1x10^10</td>
<td>7 days*</td>
</tr>
<tr>
<td>17</td>
<td>1.4x10^10</td>
<td>7 days*</td>
</tr>
<tr>
<td>Group C^c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.3x10^10</td>
<td>7 days*</td>
</tr>
<tr>
<td>6</td>
<td>1.2x10^10</td>
<td>7 days*</td>
</tr>
<tr>
<td>9</td>
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<tr>
<td>12</td>
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</tr>
<tr>
<td>15</td>
<td>1.1x10^10</td>
<td>7 days*</td>
</tr>
<tr>
<td>18</td>
<td>1.4x10^10</td>
<td>7 days*</td>
</tr>
</tbody>
</table>

^a Group A (control group) received saline on Days 1, 2, and 3.

^b Group B received E. coli endotoxin .003 mg/kgm on Days 1 and 2 and 2.25 mg/kgm on Day 3. On Day 4 these dogs were anesthetized with sodium pentobarbital 28-30 mg/kgm 30 minutes prior to injection of E. coli organisms.

^c Group C received E. coli endotoxin .003 mg/kgm on Days 1 and 2 and 2.25 mg/kgm on Day 3. They were not anesthetized on Day 4.

*Dogs surviving seven days were considered permanent survivors.
### TABLE II. E. coli CLEARANCE FROM THE PERIPHERAL BLOOD (MEAN ±SE)

<table>
<thead>
<tr>
<th>Time</th>
<th>+3 min</th>
<th>+15 min</th>
<th>+2 hrs</th>
<th>+4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>1.1x10⁷</td>
<td>1.0x10⁵</td>
<td>8.4x10⁴</td>
<td>6.2x10⁴</td>
</tr>
<tr>
<td></td>
<td>(.19x10⁷)</td>
<td>(.22x10⁵)</td>
<td>(1.8x10⁴)</td>
<td>(.9x10⁴)</td>
</tr>
<tr>
<td>Group B</td>
<td>8.2x10⁶</td>
<td>3.4x10⁴</td>
<td>1.8x10⁴</td>
<td>6.5x10³</td>
</tr>
<tr>
<td></td>
<td>(1.6x10⁶)</td>
<td>(.7x10⁴)</td>
<td>(1.8x10⁴)</td>
<td>(4.0x10³)</td>
</tr>
<tr>
<td>P#</td>
<td>NS</td>
<td>.01</td>
<td>.01</td>
<td>.001</td>
</tr>
<tr>
<td>Group C</td>
<td>4.1x10⁶</td>
<td>6.5x10⁴</td>
<td>1.5x10⁴</td>
<td>2.2x10³</td>
</tr>
<tr>
<td></td>
<td>(2.3x10⁶)</td>
<td>(1.0x10⁴)</td>
<td>(.5x10⁴)</td>
<td>(.7x10³)</td>
</tr>
<tr>
<td>P#</td>
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<td>NS</td>
<td>.005</td>
<td>.001</td>
</tr>
<tr>
<td>P*</td>
<td>NS</td>
<td>.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

a,b,c - See Table I for initial dose of live E. coli

P# - Unpaired between Groups A and B

PG - Unpaired between Groups A and C

P* - Unpaired between Groups B and C
Figure 1
Figure 2. Effects of intravenous LD$_{100}$ live E. coli organisms on blood glucose concentration in dogs following previous sublethal injections of E. coli endotoxin. (mean $\pm$SE; N=6 in each group). (See Figure 1 for details of experimental).
Figure 3. Changes of hematocrit after administration of LD$_{100}$ live E. coli organisms in dogs following previous sublethal injections of E. coli endotoxin (mean ±SE; N=6 in each group). (See Figure 1 for details of experiment).
Figure 4. Responses of rectal temperature in dogs administered LD$_{100}$ E. coli organisms after previous sublethal injections of E. coli endotoxin (mean ±SE; N=6 in each group). (See Figure 1 for details of experiment).
This study was conducted to determine the effects of sodium pentobarbital anesthesia on survival of the dog, leukocyte response, E. coli clearance from the peripheral blood and blood glucose concentration in the leukocytotic endotoxin pre-injected canine subjected to a LD50 of live E. coli organisms. Our laboratory has shown that the awake leukocytotic endotoxin pre-injected canine survives lethal doses of E. coli live organisms or...
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