PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 179–183 (1977)

Associated Leukocyte Responses in the Lethal Aspects of *E. coli* Shock[1] (39769)

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Dogs administered lethal injections of *E. coli* endotoxin or *E. coli* organisms develop systemic hypotension, hypoglycemia, and hepatosplanchnic dysfunction (1–4). Progressively decreasing blood glucose levels after endotoxin or *E. coli* administration are due in large part to depressed hepatic function, particularly gluconeogenesis (4–7). Accelerated glucose uptake has been reported following *in vitro* incubation of either endotoxin or live *E. coli* organisms in blood, and white blood cell (WBC) phagocytic activity has been implicated as the primary responsible factor (2). Increased phagocytic activity of the blood after endotoxin (8) has been traced to the buffy coat (9) and the leukocyte (10). Recent reports have shown circulating neutrophils to be of major importance in the clearance of bacterial organisms (11) or endotoxin (12) from the blood, while others have described beneficial effects of transfused WBCs in patients and animals in septic shock (13, 14).

The purpose of the present study was to explore the responses of canine blood to the separate effects of *E. coli* organisms and *E. coli* endotoxin, particularly emphasizing the role of the WBC in the uptake of glucose *in vitro* and its possible relationship to survival *in vivo*.

Materials and methods. In vivo experiments were carried out on 12 awake adult mongrel dogs during a 4-day period. On the fourth day, venous blood was drawn from each animal and additionally studied in the *in vitro* state. Animals, selected for robust health and absence of heart worms, were treated for intestinal parasites and conditioned in the animal facility for 3–6 weeks prior to use. Dogs with initial WBC counts between 7000 and 20,000/mm^3 and hematocrits exceeding 37% were utilized in the experiments.

In vivo studies. Unanesthetized, gently restrained animals were divided into paired control and experimental groups which were studied simultaneously. The experimental group received sublethal doses of endotoxin (Difco, Detroit, Mich.); 1/1000 LD_{100} on Days 1 and 2 (0.003 mg/kg body weight), 1 × LD_{100} on Day 3 (3 mg/kg), followed by 2 × LD_{100} live *E. coli* on Day 4 (2.5 × 10^{10} organisms/kg). The control group received equal volumes of saline on Days 1, 2, and 3, and on Day 4 received an identical dose of *E. coli* organisms as the experimental group. The LD_{100} of *E. coli* endotoxin and *E. coli* organisms was previously established in this laboratory. Animals living 6 days following injection of *E. coli* were considered permanent survivors.

In vitro studies. An *in vitro* system served as a test device to assay responses of the blood to *E. coli* endotoxin and *E. coli* organisms in the absence of the organs of gluconeogenesis and with the prevention of the cell migration which occurs *in vivo*. Accelerated uptake of glucose by the blood, ascribed to increased metabolic activity of white blood cells, was described in an earlier study (2). Blood for *in vitro* studies was drawn intravenously from the 12 awake dogs on the fourth day prior to their receiving *E. coli* injections and was incubated as previously reported (2). Three tubes of blood obtained from each control (saline-pretreated) and experimental (sublethal endotoxin-pretreated) animal were studied *in vitro* following separate additions of *E. coli* or endotoxin, at LD_{100} doses, or saline.

WBC counts were measured with an automatic particle counter (Coulter Z_F; Hialeah, Fla.) and the differential WBCs were measured by microscopic examination of blood

[1] This research was supported by the Veterans Administration Hospital and the U. S. Navy (Contract N00014-76-C-0229).

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ISSN 0037-9727

stained with Wright's stain. Blood glucose concentrations were determined with a Beckman glucose analyzer (Beckman Instruments; Fullerton, Calif.) possessing an accuracy of ±3 mg%. Venous blood samples for *in vivo* studies were placed in vacutainers containing ethylenediaminetetraacetic acid (EDTA; Becton-Dickinson). Blood samples for *in vitro* studies in 10-ml volumes were anticoagulated with heparin (0.1 ml; 20,000 units/ml) and incubated in a water bath at 37–38° for 3–6 hr. Results from all experiments were analyzed using the *t* test for paired or unpaired data.

Results. Figure 1 presents *in vivo* WBC data obtained from animals receiving single sublethal injections of endotoxin on Days 1, 2, and 3, and superlethal administrations of *E. coli* organisms on Day 4. Daily values were obtained prior to injections of endotoxin in the experimental group or prior to saline injection in the control, and values on Days 2, 3 and 4 are seen to reflect the effects of the previous injections. Significant leukocytosis ($P \leq 0.05$) is observed in the experimental group on Days 2, 3, and 4, which is accounted for primarily by elevations in blood concentrations of mature and immature neutrophils, while insignificant changes occur in the lymphocyte and monocyte populations. Following injections of 2 × LD_{100} *E. coli* organisms on the fourth day, leukopenia and neutropenia were observed in experimental and control groups for 2 hr ($P < 0.01$), cell counts showing recovery to near preinjection values within 6 hr. Mean hourly WBC concentrations in the experimental group during Days 1, 2, and 3 are not shown but, by the first hour after sublethal endotoxin administration,

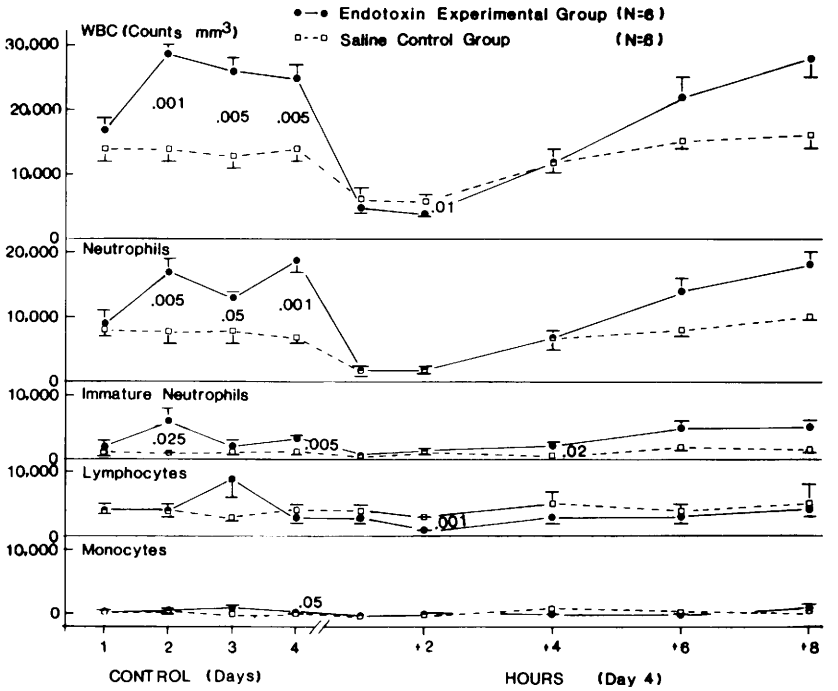

FIG. 1. White blood cell concentrations and differential white blood cell responses to superlethal dose of *E. coli* organisms in dogs following previous sublethal injections of *E. coli* endotoxin (mean ± SE; $N = 6$ in each group). The values at the "Control (Days)" time designations are initial measurements recorded prior to injection of endotoxin or saline on Days 1, 2, and 3, and *E. coli* organisms on Day 4; therefore, the Day 4 control value is actually the initial "control" measurement for the values in the "Hours (Day 4)" time designation. The values from 2–8 hr on Day 4 are recorded following intravenous administration of *E. coli* organisms; 2 × LD_{100} (2.5×10^{10} organisms/kg). The experimental (endotoxin) group received sublethal doses of *E. coli* endotoxin on Days 1 and 2 (1/1000 LD_{100}), on Day 3 (LD_{100}), and a challenge dose of *E. coli* organisms on Day 4 (2 × LD_{100}). The control (saline) group received equal volumes of saline on Days 1, 2, and 3, and on Day 4 received 2 × LD_{100} *E. coli* organisms. *P* values represent an unpaired comparison between control and experimental groups.

were lower than the control group ($P = 0.001$) and, within 6 hr, were elevated above it ($P < 0.05$). All dogs pretreated with sublethal endotoxin survived following superlethal *E. coli* administration, while every animal pretreated with saline died within 9 hr after *E. coli* injection, following massive intestinal bloody diarrhea and a protracted moribund condition.

In vitro experiments were carried out to determine the effects of *E. coli* endotoxin or *E. coli* organisms on glucose concentrations in blood drawn from animals pretreated with sublethal injections of endotoxin or saline as described above. Samples of blood were withdrawn from animals on Day 4 immediately prior to administering superlethal doses of *E. coli in vivo*. *Figure 2* illustrates the mean results from three paired experiments (total $N = 36$, i.e., three sets of 12 experiments each, including control groups). Endotoxin (LD_{100}), *E. coli* organisms (LD_{100}) or saline were added to separate tubes *in vitro* immediately after zero time and observed for 3–5 hr. Mean glucose concentrations are seen to fall significantly below control values in all experiments ($P < 0.05$). *In vitro* glucose concentrations in blood obtained from dogs pretreated with endotoxin *in vivo* were significantly lower than the *in vivo* saline-pretreated group within 60 min and were also lower than the group receiving only saline *in vitro* ($P = 0.05$). The endotoxin-pretreated blood groups receiving endotoxin and *E. coli* utilized significantly greater quantities of glucose within 2 hr than the saline groups ($P = 0.05$). The saline-pretreated blood receiving *E. coli in vitro* revealed similarly low glucose values by 3 hr. Both the *in vitro* saline control groups and the endotoxin group comprised of blood obtained from saline-pretreated animals demonstrated less marked declines in glucose concentrations during the 2- to 3-hr period. There were no significant differences between values of the endotoxin-pretreated blood administered endotoxin *in vitro* and the saline-pretreated blood to which *E. coli* was added ($P > 0.05$).

It was considered of interest to estimate the rate of glucose uptake per WBC in endotoxin- vs saline-pretreated blood to

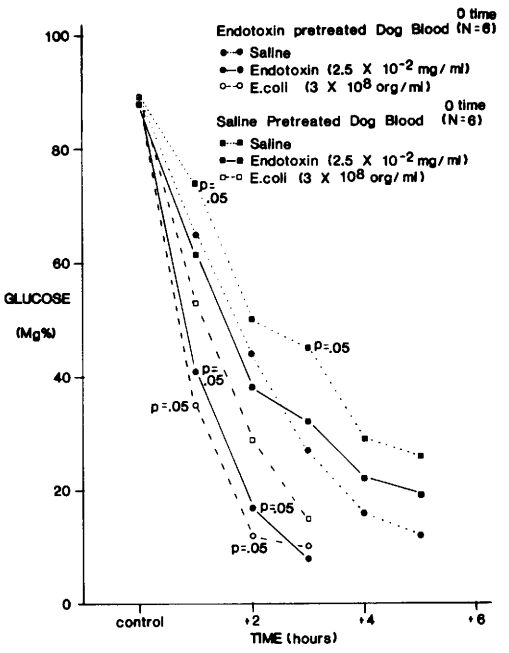

FIG. 2. Effects of *E. coli* organisms (LD_{100}) and *E. coli* endotoxin (LD_{100}) on blood glucose concentrations *in vitro* following previous sublethal injections of *E. coli* endotoxin *in vivo* ($N = 6$ in each group, total $N = 36$). Endotoxin, *E. coli*, or saline was administered immediately after zero time; LD_{100} endotoxin = 2.5×10^{-2} mg/ml of blood; LD_{100} *E. coli* organisms = 3×10^8 organisms/ml of blood. Mean glucose concentrations plotted; *P* values show statistical significances between groups of blood samples obtained from dogs pretreated with endotoxin and saline (see Fig. 1 for pretreatment data *in vivo*).

which LD_{100} endotoxin was added *in vitro*. Previously reported work (2) and parallel studies carried out in this laboratory have implicated the white blood cell as the primary component of blood responsible for increased uptake of glucose following addition of endotoxin *in vitro*. Washed red blood cells, suspended in a glucose–saline solution, did not demonstrate an increased uptake of glucose following addition of endotoxin *in vitro* (2). On the basis of these earlier observations, calculations were carried out in the present study to estimate the increased rate of glucose uptake per WBC following addition of LD_{100} endotoxin *in vitro*. This excess quantity of glucose was obtained by subtracting the glucose uptake in blood to which saline alone was added from

that to which endotoxin was administered. The excess uptake occurring during the first hour was divided by the average WBC count during the same period, in order to estimate the quantity of excess glucose uptake per WBC. Calculations showed the quantity of excess glucose taken up per activated WBC from blood receiving prior sublethal injections of endotoxin was not different from the nonactivated WBC *in vitro* (11.7×10^{-9} vs 7.4×10^{-9} mg of glucose/WBC/60 min, respectively; $P > 0.05$).

Discussion. Progressively developing hypoglycemia in dogs administered endotoxin or *E. coli* organisms has been documented and found to be associated with systemic hypotension, hepatosplanchnic pathology, and death (1, 3). The cause of hypoglycemia has been the subject of much recent research in endotoxin or septic shock. Impaired glucose production as a result of depressed hepatic function has been suggested as a primary factor in the development of hypoglycemia because of adverse effects on gluconeogenesis (4–7). A recent publication from this laboratory suggests that endotoxin also eliminates the gluconeogenic ability of the kidney in the canine species (15).

Recent studies have documented increased uptake of glucose by the blood after endotoxin which partially accounts for the hypoglycemia of shock (2, 15). Results from the present study support these earlier observations and further suggest that the accelerated glucose uptake by the blood after endotoxin is primarily due to the increased activity of circulating white blood cells whose rate of glucose utilization varies directly with their total numbers.

Findings from the present investigation suggest a relationship between numbers of white blood cells, particularly neutrophils, and survivability to superlethal doses of *E. coli* organisms. Daily sublethal intravenous injections of endotoxin administered during a 3-day period resulted in a marked state of leukocytosis. The cause of the elevated numbers of white blood cells was not determined in the present study; however, it is known that endotoxin administration promotes the entry of new leukocytes from the bone marrow into the circulation (16). Animals receiving superlethal injections of *E. coli* organisms on the fourth day were completely protected against the pathophysiological and lethal effects of the organisms. It is possible that the significantly increased numbers of white blood cells, initially present on the fourth day and composed primarily of neutrophils, may have efficiently phagocytosed the injected organisms, thereby preserving hepatic function (7), including gluconeogenesis (6). Additionally, hepatosplanchnic pooling, extravasation, and bloody diarrhea may have been prevented by augmented white blood cell phagocytotic activity. The degree of protection seemed remarkable: Animals receiving prior sublethal injections of endotoxin were eating and drinking, and appeared normal in every respect within 12 hr, and all were healthy survivors at 6 days. On the other hand, all animals pretreated only with saline and challenged on the fourth day with superlethal doses of *E. coli* uniformly demonstrated the development of massive bloody diarrhea, vomiting, and a subsequent moribund state, dying within 9 hr postinjection.

The question of possible "activation" of the WBC, in which each cell becomes more phagocytically active, was not supported by the results of the present study. Enhanced phagocytic activity appeared to be due to the increased numbers of white blood cells, glucose uptake per cell being essentially equal in activated and nonactivated cells. The WBC types accounting for the total increase in numbers in the present study were shown to be the mature and immature neutrophils, cells which have been reported to be particularly active in phagocytosing endotoxin (12) or *E. coli* (11). Recent studies have documented beneficial effects of transfused white blood cells in animals and patients in septic shock (13, 14). Results from the present study suggest a relationship between leukocytosis and survivability in septic shock, lending support to the view that increased numbers of white blood cells by way of transfusion may augment the degree of protection.

Appreciation is expressed to R. T. Brantley and Ora Elmore for technical assistance and to Jeanette Glasgow for secretarial and editorial support.

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Received December 28, 1976. P.S.E.B.M. 1977, Vol. 155.