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A SUSTAINED RELEASE BACTERIAL INOCULUM INFUSION MODEL OF INTRA-ABDOMINAL INFECTION IN CONSCIOUS RATS: BACTERIOLOGY, METABOLISM, AND HISTOPATHOLOGY

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ABSTRACT—This report describes the development of a rat peritonitis model that simulates a slow, sustained bacterial release from the gut. Septic animals (SEP) received an intraperitoneal infusion of a bacterial inoculum (6.5×10^8 colony forming units *Escherichia coli*) over 12 h, while control rats (CON) received a sterile inoculum. This model yielded a 52% mortality over 7 days in SEP, with deaths usually occurring 24–48 h after the onset of infusion. Septic rats showed greater febrile responses and body weight losses than those of CON, as well as mild hyperlactacidemia, hypoglycemia, and episodic bacteremia. Maximum bacterial counts in peritoneal fluid and several organs of SEP were observed at 36 h, with bacterial counts progressively decreasing by 7 days to levels similar to those observed at 12 h. Lung and spleen wet weights increased by 17% at 36 h and 35% at 7 days post-infection in SEP. Histological evaluation of random organ samples revealed mild to moderate morphological changes in SEP while CON showed no or minimal changes in the parameters measured during the study. This new model of chronic peritonitis in the rat reproduces many of the clinical features observed in human sepsis, and thus should prove to be a useful tool in further studies of the pathophysiology of peritonitis.

INTRODUCTION

Sepsis, a progressive systemic inflammatory response to infection (1), remains a leading cause of mortality and morbidity among hospitalized patients, despite careful monitoring and aggressive treatment including fluid replacement, wide-spectrum antibiotic therapy, surgical intervention, and nutritional support (1, 2). The increased use of immunosuppressive drugs over the last two decades and the fact that many critically ill patients are now surviving longer due to improvements in supportive medical care appear to contribute to enhance susceptibility to infection. However, it is difficult to perform controlled studies in septic patients, due to a wide diversity of underlying diseases, preexisting nutritional and health status of the individuals, type of infecting organisms, and severity and duration of septic illness. Therefore, the use of experimental models is considered useful in furthering the understanding of the complex pathophysiology of sepsis.

Several investigators have described animal studies that reproduce many of the clinical features (e.g., fever, tachycardia, low systemic vascular resistance, leukocytosis, thrombocytosis, and episodic bacteremia) observed in most septic patients. These models have included an intravenous bolus injection or short-term, slow intravenous infusion of either endotoxin or live bacteria in various animal species (3–6). These reproducible models of acute sepsis have been generally useful in clarifying the role of endotoxin in eliciting some key features of sepsis, studying blood bacterial clearance kinetics, and assessing the efficacy of various therapeutic agents. However, it is generally acknowledged that some models may not accurately reflect the pathophysiology of those septic cases where the patient may be inflicted with a sustained, slow release of bacteria from an infection site. This slow bacterial release mechanism is in sharp contrast to rapid bacterial release models where the host is exposed to a sudden and overwhelming bolus challenge, thus preventing the host from fully expressing its various defense mechanisms (7). The difference in animal modeling may partly explain the discrepancy between the results of some clinical trials and those expected from animal experimentation.

Because the most severe manifestations of human sepsis develop after penetrating trauma to the abdomen or following abdominal surgery, many attempts have been made to design animal models of chronic peritonitis. These models include intraperitoneal injection of pure bacterial cultures or viable bacteria trapped in a bovine fibrin clot, gelatin capsule, or mucus or fecal suspension (8–13); cecal laceration or perforation (14); and cecal ligation with or without puncture (7, 13–16). In most of these soilage models, however, a standardization of the septic challenge is difficult due to an unknown fecal bacterial load. Alexander et al. (17) described another model of peritonitis, where Alzet osmotic minipumps containing pre-determined quantities of viable *Escherichia coli* and *Staphylococcus aureus* were implanted in guinea pigs. Although this model also reproduced key features of chronic sepsis in humans, the use of this technique has not been widespread for unknown reasons.

The objective of this study was to establish a rat peritonitis model by the continuous intraperitoneal infusion of a bacterial inoculum over 12 h, simulating a trauma-induced, slow, sustained bacterial release from the gut. This report presents data

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characterizing this model with respect to bacteriology, histopathology, and metabolism.

MATERIALS AND METHODS

Animals

One hundred and fifty six male Sprague-Dawley rats with a mean body weight of 361 ± 4 g (mean ± SEM) were obtained from Charles River (St. Constant, Québec, Canada). The animals were housed individually, allowed to adapt to the environmental conditions (22°C, 12 h light/dark cycle), and handled daily before undergoing surgery 7 days later. All animals had free access to standard rodent chow and water at all times during the experimental period. All procedures described in this study were performed in adherence to the Canadian Council on Animal Care (CCAC) regulations for the use of experimental animals, and were reviewed and approved by the institutional animal ethics committee.

Intraperitoneal cannula

The intraperitoneal cannula was made of silastic tubing (.76 mm ID, 1.65 mm OD; Dow Corning Medical, Mississauga, Ontario, Canada) cut to 50 cm. Two 5 mm lengths of 18 gauge thin-wall needle were pushed into the lumen to lie 15 cm and 4 cm, respectively, from one of the extremities of the tubing; these metal supports prevented occlusion of the cannula at its points of ligation to the neck and abdominal muscles. Each intraperitoneal cannula was then steam autoclaved.

Surgical procedures

All surgical procedures were performed under aseptic conditions, with the animals under general anesthesia (2.0% halothane, 1:1 oxygen:nitrous oxide). The abdominal and interscapular regions were shaved and cleansed with povidone-iodine and alcohol. A sterile cannula was inserted 3 cm deep into the abdominal cavity through a 2 mm incision, located 5 mm below the xiphisternal cartilage. The distal end of the cannula was then tunneled subcutaneously to the interscapular region of the back where it emerged through the skin. The cannula was secured on the muscles of the neck and abdomen with 4-0 nylon sutures. All surgical wounds were closed in two layers with nonabsorbable interrupted 3-0 silk sutures.

Half of a Velcro disk (Velcro Canada Ltd., Montréal, Québec, Canada) was then sutured in four locations to the skin of the back of the animals so that it laid out of reach of the animal, between its scapulae; care was taken not to puncture the lower surface of the skin to prevent infection and irritation. The distal end of the cannula was led through a 30 cm stainless steel tether (Ealing Scientific Ltd., St. Laurent, Québec, Canada), which was then secured to the disk with Velcro. In our experimental setting, this Velcro attachment of the tether was preferable to the wearing of a nylon harness by the rat, eliminating the problem of constriction of the intraperitoneal cannula. The operated animals were resuscitated with a subcutaneous injection of sterile saline (30 mL/kg body weight), topical antibiotic (Hibitane, Ayerst Lab., Montréal, Québec, Canada) was applied to the wounds, and analgesics were administered (buprenorphine i.m., .05 mg/kg body weight). The animals were housed individually, and kept under a heat lamp until recovery from the anesthesia (5–10 min). Each tether was then connected to the fecal inoculum infusion system via a swivel (Fig. 1); the latter allowed freedom of movement during the experiment. The animals were allowed to recover and familiarize themselves with the swivel and tether system for 8–10 h before induction of peritonitis.

Preparation of bacterial inoculum

Escherichia coli (ATCC 25922) was obtained from a commercial source (PML Microbiologics). This particular strain of *E. coli* was used in a previous study in our laboratory where we determined the tissue distribution of liposomal antibiotics in septic rats (18). These bacteria, harvested in the log phase of growth, were encapsulated, as assessed by Anthony's staining method (19). Preliminary experiments to determine the relative bactericidal action of various active human (n = 4) and rat (n = 4) sera *in vitro* revealed that this strain of *E. coli* is serum resistant; however, rat sera were bactericidal to a lesser degree than human sera, *E. coli* being killed at inoculum doses $<10^8$ colony forming units (CFU)/mL compared with $<10^9$ CFU/mL for human sera. Because most clinical isolates of *E. coli* are also encapsulated and serum resistant (20), the choice of this particular strain of *E. coli* for the study of sepsis seems appropriate.

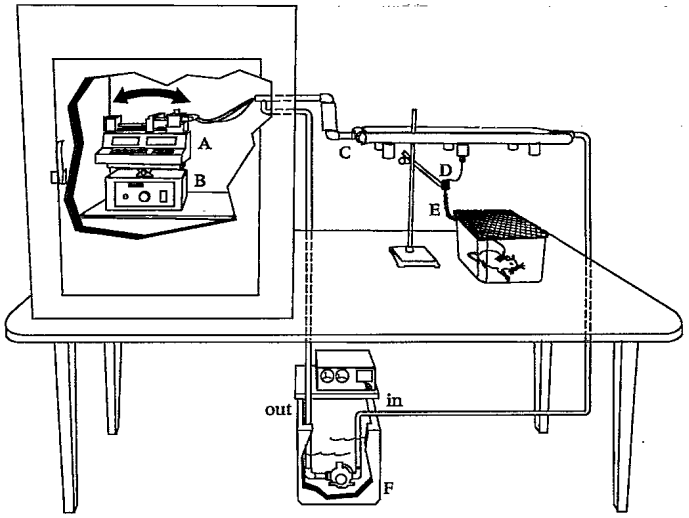

FIG. 1. **Schematic of the inoculum infusion system.** Up to 12 syringes containing either the bacterial or control inoculum were positioned on an automated pump (*A*) placed on a rocker (*B*) inside a refrigerated unit. The syringes were connected to different port lines enclosed in a common piping system (*C*), each line leading to a swivel (*D*) to which the intraperitoneal cannula, channeled in a stainless steel tether, was attached (*E*). The bacterial inoculum was kept at 3–5°C by recirculating cold water in the piping system (*F*).

E. coli were grown in brain heart infusion broth for 20 h at 37°C. The bacteria were then harvested after centrifugation, resuspended uniformly in sterile saline, and aliquoted as 10% glycerin stock. Before freezing at −70°C, a sample of each aliquot was cultured aerobically at 37°C for 24 h onto tryptic soy agar (TSA) to estimate the number of viable *E. coli*. On the experimental day, an aliquot was thawed and a volume containing an appropriate number of CFU *E. coli* was added to a mixture of 1.5 mL of sterile saline and .5 mL of a sterile rat fecal suspension. The sterile feces were prepared by removing and pooling the contents of 20–25 normal Sprague-Dawley rat ceca. The fecal slurry was autoclaved twice for sterilization, diluted 20:1 (saline:brain heart infusion broth), and filtered through sterile gauze pads to remove large particulate matter. Sterility of this fecal suspension was assessed by culturing aliquots onto TSA (aerobically), and *Brucella* anaerobic agar (anaerobically) plates (PML Microbiologics).

Induction of peritonitis

Eight to ten hours after implantation of the cannula, infusing syringes were filled with either the bacterial fecal inoculum or sterile inoculum, and positioned onto a programmable syringe pump (model 44, Harvard Apparatus, St. Laurent, Québec, Canada) placed on a rocker (Bellow Glass Inc., Vineland, NJ) inside a refrigerated unit set at 5°C (Fig. 1). The syringes were connected to different port lines enclosed in a common plastic piping system (each line leading to a swivel), and each inoculum was kept at 3–5°C by recirculating cold water in the piping system. We have determined in previous experiments that this infusion system ensured the following: 1) the fecal inoculum was homogeneously mixed by the rocker; 2) bacterial growth and bacterial cell death were minimized under the low temperature during the 12 h infusion period (± 10%); and 3) the *E. coli* dose infused to different animals was comparable in a given experiment (coefficient of variation ~10–15%).

Each cannulated rat was infused with 2 mL of bacterial inoculum or sterile inoculum (i.e. saline with sterile fecal suspension) over a period of 12 h. This time period of infusion was selected based on the observation that in a large number of clinical cases, patients with slow-onset peritonitis are not treated for at least 8–12 h. At the end of the 12 h infusion period, the animals were anesthetized (1.5% halothane, 1:1 oxygen:nitrous oxide) and the tether was removed. The abdominal area was cleansed as previously described, and a 3 cm midline laparotomy incision was made through the skin and peritoneum. Five milliliters of sterile saline was then injected into the abdominal cavity and

thoroughly mixed with the peritoneal fluid, which was sampled and serially diluted. Aliquots were cultured on TSA plates (24 h at 37°C, aerobically) to determine bacterial content. The intraperitoneal cannula was exteriorized, sterilized (by flushing the inoculum out onto a gauze swab with 100% isopropyl alcohol), and cut down to the peritoneum. The distal end of the cannula was cut down to 1 cm above the skin of the neck and capped. The abdominal cavity was lavaged with 70 mL of warm (37°C) sterile saline, in 10–15 mL aliquots; the lavage fluid was aspirated with a sterile pipette before closure in two layers with nonabsorbable 3-0 silk sutures. Post-operative resuscitation procedures were performed as previously described before housing the animals individually.

Experimental protocol

The effect of altering the dose of the bacterial inoculum on survival rate was determined in preliminary experiments. Peritonitis was induced in four groups of 10 rats by infusing bacterial doses ranging from .5 to 8.0 × 10^8 CFU *E. coli*, while a group of six cannulated rats received the same volume of sterile inoculum. Laparotomy and peritoneal lavage were performed at the end of the 12 h infusion period, resuscitation procedures were implemented, and survival was monitored for 7 days, during which period analgesia (buprenorphine i.m., .05 mg/kg body weight) was provided twice daily to all cannulated animals. The guidelines of the Canadian Council on Animal Care for judging morbidity and moribund conditions, rather than death itself, was used as an end-point to calculate the mortality rate (21). Thus, animals were humanely euthanized before the end of the 7 day period if the nature of the signs of illness (e.g., distress behavior, excessive weight loss, abnormal breathing, or body temperature) and their rate of morbid onset and persistence strongly suggested an impending death.

The bacterial dose selected for further experiments was that yielding a mortality rate of 45–55% over 7 days (Fig. 2), similar to that observed in many clinical conditions. Thus, 80 cannulated rats received a bacterial inoculum containing approximately 6.5 × 10^8 CFU *E. coli* (SEP) while 24 animals received the same volume of sterile inoculum (CON). A group of six healthy, nonsurgical animals (HEA) was also included in the study to provide baseline values for the different parameters measured. Rectal temperature was measured twice a day in all animals (morning and late afternoon) until they were killed. Analgesia was provided twice daily to all cannulated animals during the 7 day study period. All animals were fed and weighed daily at the same time (8:00 a.m. to 9:00 a.m.) throughout the study. Peritonitis was induced in nine separate experiments, over a 14 week period during the winter; typically, each experimental group included eight to nine SEP, one to two CON, and one HEA.

The animals were randomly killed 12 h, 36 h, 60 h, or 7 days after the onset of infusion. Peritoneal fluid was collected in anesthetized rats and processed as previously described. After gross examination of the abdominal cavity, blood was withdrawn by cardiac puncture and a small aliquot was incubated for 24 h in brain heart infusion broth. An aliquot of the blood was immediately deproteinized in cold perchloric acid for subsequent determination of lactate concentration (22); another small aliquot was used for determination of hematocrit and hemoglobin using a Coulter Counter (model JT, Coulter Electronics, Burlington, Ontario, Canada). The remaining blood was centrifuged at 4°C within 30 min, and the plasma stored at −70°C until assayed for glucose levels (Boehringer Mannheim, Laval, Québec, Canada). The hepatic portal vein was catheterized and perfused with 100 mL of sterile saline. Lungs, liver, and spleen were dissected rapidly, surface sterilized by immersion for 15 s in 70% and 100% isopropanol, and weighed and homogenized. Aliquots of all body fluids and homogenates were then serially diluted and cultured on TSA to determine bacterial content. Random tissue samples of septic (n = 7) and control (n = 3) cannulated animals were taken from lungs, liver, spleen, kidneys, and mesentery (including lymph node), fixed in 10% buffered formalin, and processed for light microscopy evaluation. The histological evaluation of each tissue was performed blindly according to a pre-established five grade scoring system (Vita-Tech Canada Inc., Markham, Ontario, Canada).

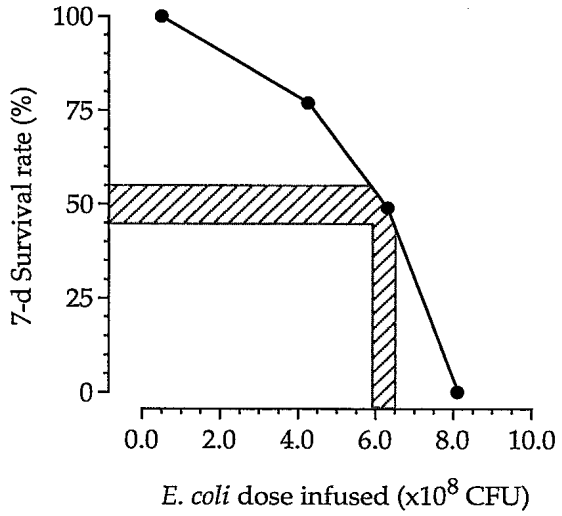

FIG. 2. **Survival in cannulated rats (n = 10 per group) during 7 days following the intraperitoneal infusion of different doses of *E. coli*.**

Statistics

Differences in bacterial content of body fluids and tissues were determined by a one way analysis of variance (ANOVA). Data were analyzed by a two factor ANOVA to determine the main effects of the time post-infusion and experimental group factors on metabolite levels, body weight, rectal temperature, and organ scores. When the p ratio proved significant ($p < .05$), the Duncan post-hoc test was used to locate significant differences between corresponding means. All data are expressed as mean ± SE.

RESULTS

Gross effect of surgical procedures and peritonitis

There were no apparent signs of stress or illness throughout the pre-infusion recovery period in any of the rats that underwent the intraperitoneal cannulation. In contrast, septic animals showed piloerection, diarrhea, nasal and/or ocular discharge, and hypoactivity at the end of the 12 h infusion period, the severity of these symptoms being relatively dependent on the bacterial dose infused; the control rats showed no or minimal symptoms. Postmortem examination confirmed ongoing peritonitis in the septic animals, as suggested by the presence of apparent Peyer's patches on the intestines, slightly edematous abdominal muscles with increased capillarization, and enlarged mesenteric lymph node. However, there was no sign of pus in the abdomen.

It is noteworthy that while the intraperitoneal cannulae in the control animals were relatively free of fibrinous adhesions at the end of the infusion period, those of septic rats were loosely wrapped in loops of omentum or epididymal fat infiltrated with inflammatory cells. However, this phenomenon was not severe enough to occlude the cannula, the tip of which remained visible or was free of tissue growth. The intraperitoneal cannula was completely blocked in only 4 of the 80 septic animals (5%) that received the selected bacterial challenge; data from these animals were excluded from analysis.

Lethality of sepsis model

Table 1 shows survival rates in nine experimental groups of rats infused intraperitoneally with 6.5 × 10^8 CFU *E. coli* over a 12 h period. There was a small intergroup variation in mortality in SEP, most deaths occurring within 24–48 h after the onset of infusion when a bacterial dose of 6.5 × 10^8 CFU was infused. All control animals survived throughout the 7 day study period.

TABLE 1. **Survival in different groups of rats infused intraperitoneally with 6.5 × 10^8 CFU *E. coli* over a 12 h period**

Group	No. of rats	Survival rate at a given time post-infusion (%) 12 h	36 h	60 h	7 d
1	8	100	50.0	50.0	50.0
2	8	100	50.0	37.5	37.5
3	9	100	55.6	55.6	55.6
4	8	100	62.5	50.0	50.0
5	9	100	55.6	44.4	44.4
6	9	100	44.4	44.4	44.4
7	8	100	50.0	50.0	50.0
8	8	100	62.5	50.0	50.0
9	9	100	44.4	44.4	44.4
mean ± SEM		100 ± 0	52.8 ± 2.3	47.4 ± 1.7	47.4 ± 1.7

Body weight and thermal response

Cumulative changes in body weight from baseline (i.e. before cannulation) for each experimental group are shown in Fig. 3*A*. Implantation of the intraperitoneal cannula and infusion of a sterile inoculum for 12 h had no effect on the body weight in CON compared with HEA. In contrast, there was a small but significant decrease in body weight (−3.3 ± .9%) in all septic animals killed at 12 h. Body weight loss was further exacerbated in both CON and SEP after the laparotomy and peritoneal lavage were performed, reaching a maximum value 60 h after the onset of infusion. However, body weight loss was greater in SEP than CON throughout the 7 day observation period (−13.3 ± 2.0% vs. −7.1 ± .8%).

Fig. 3*B* summarizes the thermal response of cannulated animals and healthy rats during the study period. Because the rectal temperature (T_{re}) of CON or SEP before the cannulation (37.1 ± .1°C) was slightly but significantly higher than that of HEA measured at the same time of the day (36.8 ± .1°C), data are expressed as the change of T_{re} from baseline value. T_{re} increased in both SEP and CON during the pre-infusion recovery period, this increase being significantly greater than that observed in HEA over the same period (1.1 ± .3°C vs. .4 ± .1°C). While T_{re} remained significantly elevated (1.2 ± .3°C) in SEP during the infusion period, there was a decrease in T_{re} in CON (.5 ± .1°C) comparable to that observed in HEA. The body temperature of control animals resumed a normal circadian cycle from the onset of the sterile inoculum infusion, but the change in T_{re} became comparable to that of time-matched healthy animals after 45 h (Fig. 3*B*). In contrast, the stress associated with the laparotomy and peritoneal lavage had differential effects in SEP (Fig. 3*B*). Although 52% of all septic rats became markedly pyrexic (39.3 ± .3°C) within 10 h after these surgical procedures, the remaining SEP became slightly hypothermic (36.3 ± .4°C); all these animals survived this critical period. Interestingly, the rectal temperature immediately before and 24 h after the laparotomy was comparable in these two subgroups of septic animals (Fig. 3*B*). T_{re} progressively returned toward baseline values but remained elevated for at least 84 h after the onset of infusion in SEP.

Organ wet weight

Changes in organ wet weights of the lungs and spleen throughout the experiment are shown in Fig. 4. Lung wet weights increased by 17% at 36 h in SEP compared with CON. Although lung weights appeared slightly reduced in CON compared with HEA for 60 h, this trend did not achieve statistical significance. There was also no change in spleen wet weight in CON compared with HEA throughout the study period (Fig. 4*B*). In contrast, septic animals showed a significant spleen hyperplasia compared with time-matched control animals (about 24% at 12 h), spleen wet weights being increased by about 43% after 7 days. Liver weights were reduced by 19% in CON (for 60 h) and SEP (for 7 days) compared with HEA (15.8 ± .4 g).

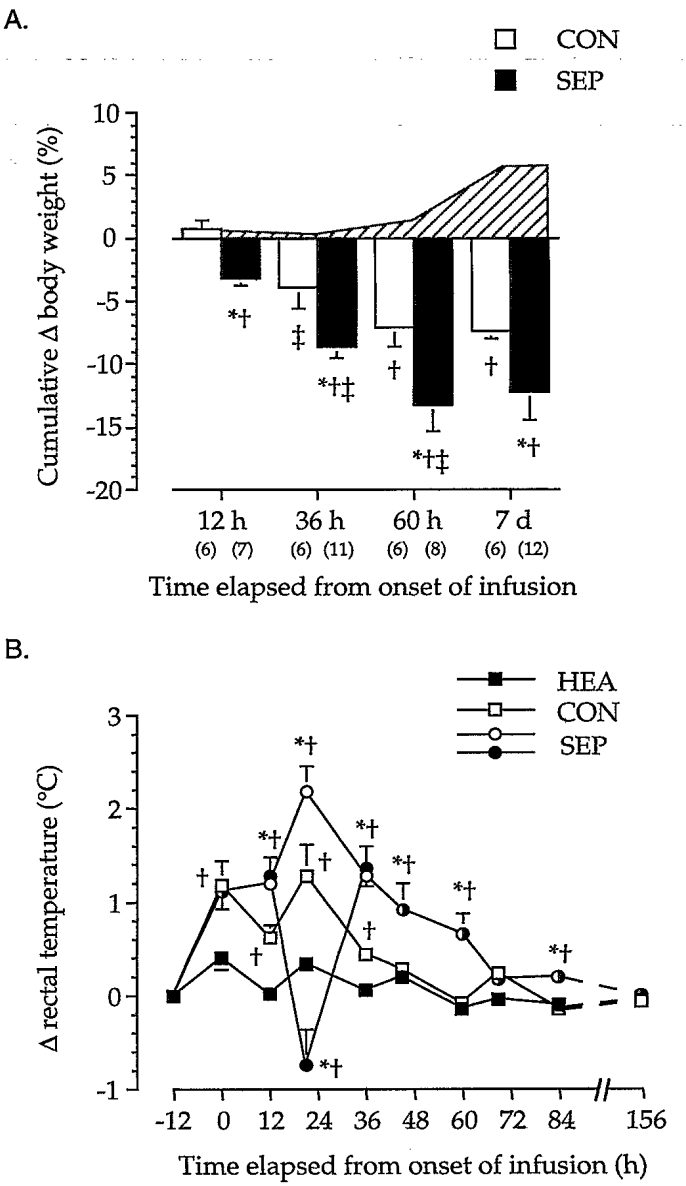

FIG. 3. **Cumulative changes in body weight and body temperature in septic (SEP) and control rats (CON) during the study period.** Dashed area represents baseline values obtained from six healthy, nonsurgical animals. Septic animals showed either a hypothermic (*closed circles*) or hyperthermic (*open circles*) response in the 24 h after the laparotomy and peritoneal lavage were completed. The number of experimental animals randomly killed at a given time period is indicated in parenthesis. *significantly different from CON ($p < .05$); †significantly different from HEA ($p < .05$); ‡significantly different from previous time period ($p < .05$).

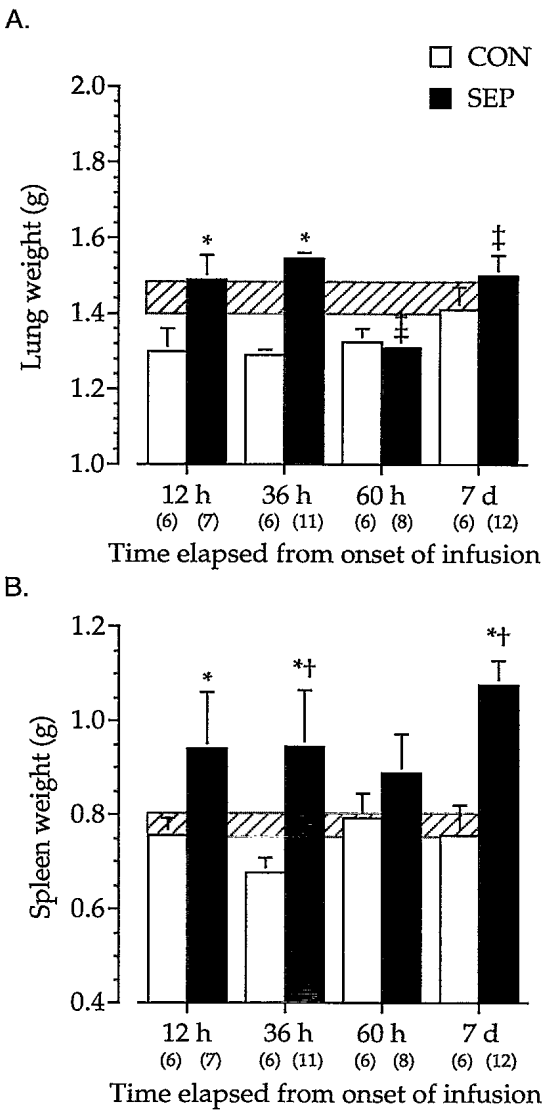

FIG. 4. **Spleen and lung weights in septic (SEP) and control rats (CON).** Dashed area represents baseline values obtained from six healthy, nonsurgical animals. Number of experimental animals randomly killed at a given time period is indicated in parenthesis. See legend of Fig. 3 for explanation of symbols.

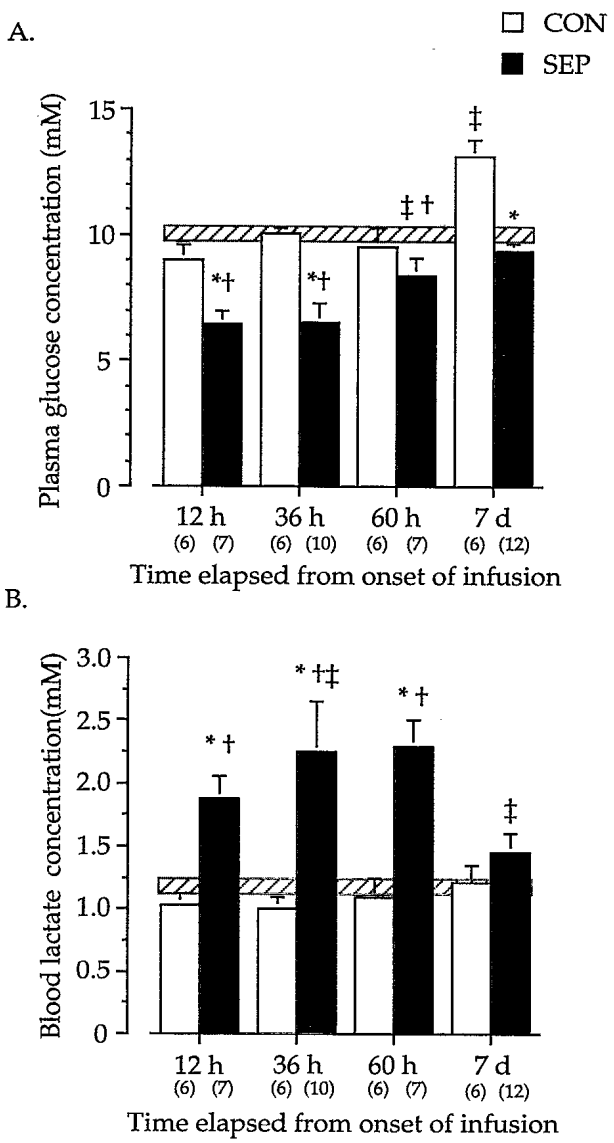

FIG. 5. **Plasma glucose and blood lactate levels in septic (SEP) and control rats (CON).** Dashed area represents baseline values obtained from six healthy, nonsurgical animals. The number of experimental animals randomly killed at a given time period is indicated in parenthesis. See legend of Fig. 3 for explanation of symbols.

Hematological parameters

Hematocrit levels remained unchanged in both SEP (38.7 ± .6%) and CON (39.6 ± .5%) compared with HEA (41.6 ± .7%) throughout most of the 7 day study period, except for a transient decrease at 36 h in both SEP (35.8 ± 2.1%) and CON (34.8 ± .5%), likely due to the small blood loss sustained during the laparotomy. This data would indicate that the septic animals did not suffer from dehydration or inadequate fluid replenishment during the 7 day study.

Circulating glucose and lactate

Figure 5 illustrates the differences in arterial blood glucose and lactate concentrations in control and septic rats throughout the study period. Arterial glucose levels in SEP were reduced by 28–35% for 36 h compared with those of time-matched CON; these levels progressively increased to reach values comparable to those of HEA after 7 days. Septic rats also exhibited a marked hyperlactacidemia (~2.3 mM) for at least 60 h after the onset of infusion (Fig. 5*B*). Both metabolite levels remained constant throughout most of the study period in CON. However, the control animals showed a significant hyperglycemia at 7 days compared with HEA (13.1 ± .7 vs. 10.2 ± .4 mM).

Bacteriology

Bacteremia was observed in five of seven septic animals at 12 h, and all SEP by 60 h; however, bacteria were detectable in the blood of only 16% of SEP after 7 days. Bacterial counts in the peritoneal fluid collected at the end of the infusion period were comparable in all groups of septic rats (Fig. 6*A*). Maximum bacterial counts in peritoneal fluid and the three organs of SEP were observed at 36 h, with bacterial counts progressively decreasing by 7 days to levels similar to those observed at 12

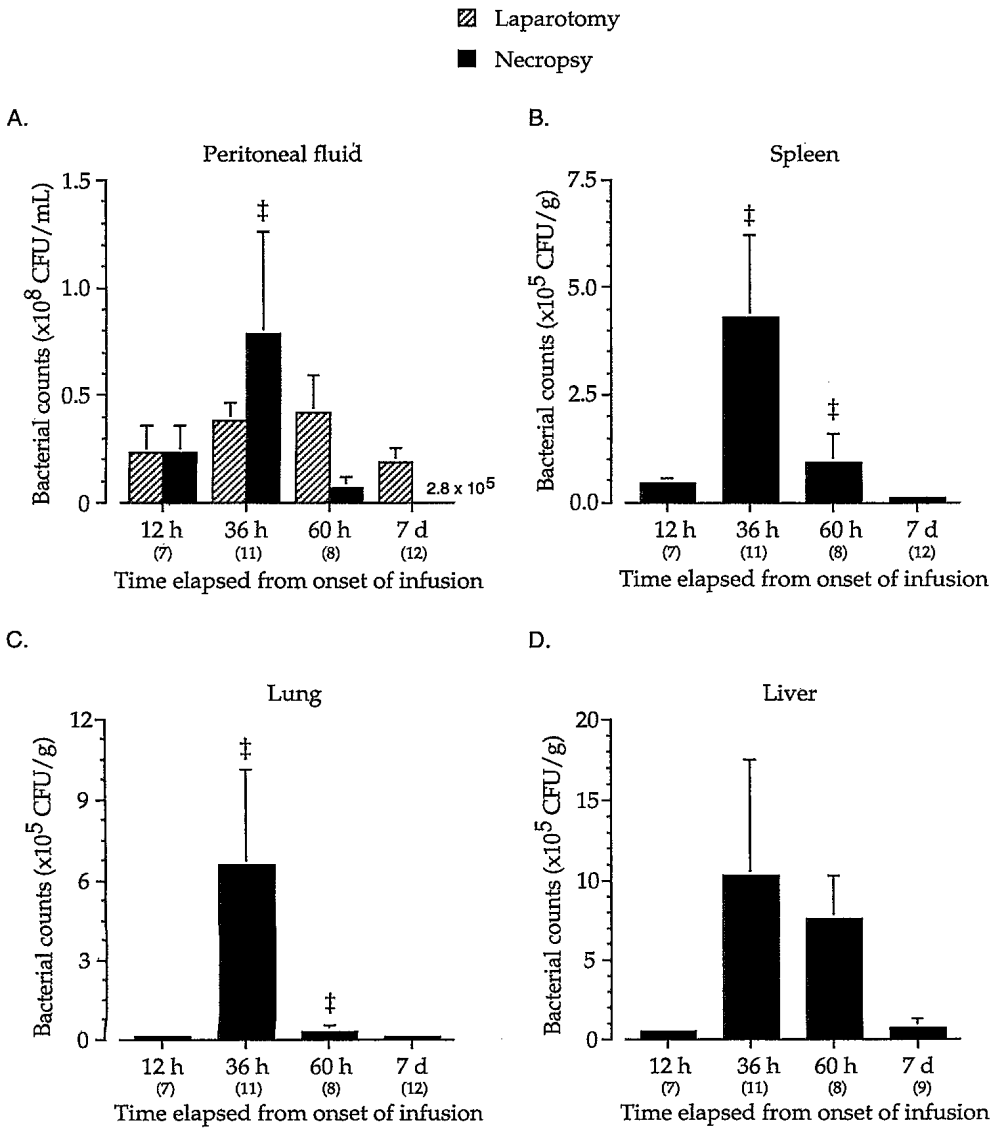

FIG. 6. **Bacterial counts in peritoneal fluid, spleen, lung, and liver in septic animals.** The number of experimental animals randomly killed at a given time period is indicated in parenthesis. See legend of Fig. 3 for explanation of symbols.

h (Fig. 6). Although bacterial sequestration was observed in organs of all septic animals after 7 days, bacteria were detectable in peritoneal fluid of only 50% of SEP. Bacterial sequestration in the liver ($10.3 \pm 7.2 \times 10^5$ CFU/g) was consistently greater than that in the lung ($6.6 \pm 3.6 \times 10^5$ CFU/g) and spleen ($4.3 \pm 1.9 \times 10^5$ CFU/g). There were no detectable bacteria in any body fluids or organs of the control animals.

Histopathology

Histological evaluation of the organs revealed mild to moderate morphological changes in the septic animals (Fig. 7). The spleen consistently showed vascular congestion and lymphoid hyperplasia, with increased presence of polymorphonuclear neutrophils (PMN) in the sinusoidal red pulp. Congestion was also observed in the lungs with moderate PMN aggregation in the septal capillaries and occasional macrophages present in the alveoli; however, this was not accompanied by edema (i.e., in the area of lung that was sampled). Foci of granulation tissue and microabcesses were observed only in the liver of two septic animals, while occasional PMN were present in the renal glomerular capillary tufts of two other septic rats killed at 7 days. The mesentery of all septic rats showed moderate to marked focal granulomatous serositis consisting of aggregates of macrophages, plasma cells, and lymphocytes, with acute diffuse lymphadenitis and mild apoptosis of lymphocytes in follicular centers and parafollicular areas of the lymph node. The control animals showed minimal or no (e.g., liver and kidney) morphological changes in the tissues that were sampled (Fig. 7).

DISCUSSION

We have described an animal model of Gram-negative peritonitis in conscious, unrestrained rats. Our model allows a continuous intraperitoneal infusion of a standardized bacterial inoculum over a period of time, simulating a trauma-induced, slow leakage of bacteria to the peritoneal cavity. The main characteristics of this experimental model are that the intraabdominal infection is induced by relatively simple surgical and bacteriological procedures; the septic challenge can be regulated to simulate a sustained and repetitive bacterial release; and, it is possible to control the severity and/or nature of the

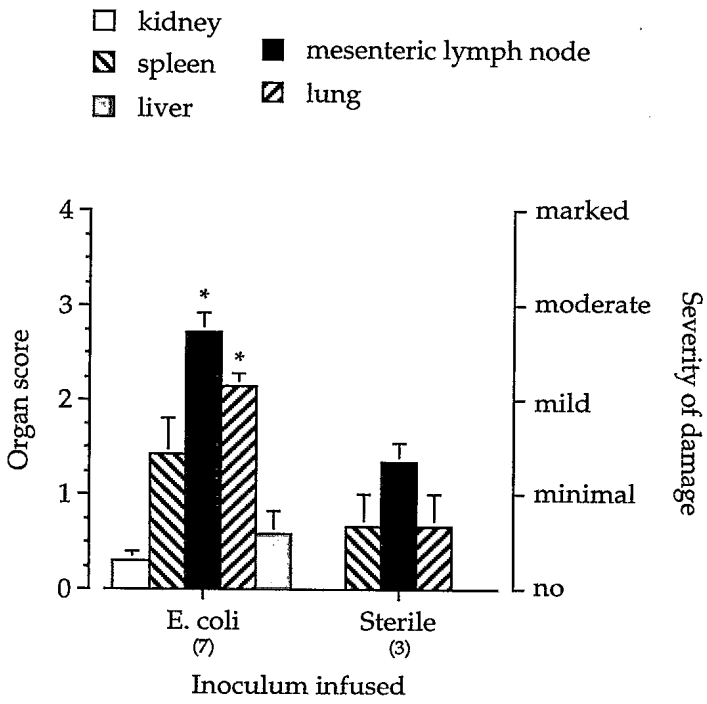

FIG. 7. **Histological evaluation of organ damage in seven septic (SEP) and three control rats (CON).**

septic insult by altering the number and strain of bacteria infused. It is noteworthy that the data presented reflect the "natural course" of peritonitis, but not the (clinically relevant) pattern resulting from the combined effects of sepsis plus routine supportive therapy. Nevertheless, because this experimental model exhibits many of the pathophysiological features (e.g., body weight loss, fever, lactacidemia, and episodic bacteremia) observed in the septic patient, it may be a valuable addition to the panoply of sepsis models available.

Many experimental models of peritonitis such as cecal ligation and puncture (CLP), cecal puncture, or cecal laceration also mimic a focal release of bacteria leading to systemic infection (7, 9, 13–16). However, it is generally acknowledged that the bacterial challenge in these models is difficult to control because of the wide variety of strains and numbers of bacteria in the ceca of the experimental animals at the time of surgery. These models are further complicated by an unpredictable necrosis that may occur in the affected tissues, leading to a highly variable intestinal leakage. These drawbacks are likely responsible for the significant variation in mortality rates often observed in fecal contamination models within and/or between laboratories (16, 17, 23) and may create serious problems for evaluating potential therapies. For example, Bohnen et al. (16) have reported that if a random sample of their rat CLP groups was considered, it would have appeared that saline was superior to cefoxitin in treating the intra-abdominal infection, with day-to-day mortality rates ranging from 14 to 100% in the saline-treated and 0 to 57% in the cefoxitin-treated groups. Alexander et al. (23) have reported that CLP typically resulted in mortality rates ranging from 50% to 80% in their laboratory. There was only a small intergroup variability in the lethality of the present model, likely due to the use of a prospectively quantitated bacterial dose administered under controlled experimental conditions. This model, when used simultaneously with vascular catheterization for repeated blood sampling or drug administration, might circumvent the use of large numbers of experimental animals when testing different therapies for the treatment of sepsis.

Human clinical intra-abdominal infections following perforation of the gut due to various diseases or penetrating trauma are usually characterized by a polymicrobial contamination with an average of three species of aerobic bacteria and up to nine anaerobes (24). In the present series of experiments, we selected *E. coli* as the sole infecting pathogen, because it is the most frequent Gram-negative organism isolated from septic patients, and it is generally held responsible for the high mortality and morbidity observed both clinically and experimentally (2, 11). The intraperitoneal bacterial infusion resulted in a systemic response and mortality, but did not lead to abscess formation, which is an important component of the host defense. However, several investigators have shown that monoclonal *E. coli* abscesses are formed only if the contents of the inoculum injected are not scattered into the abdominal cavity (12, 25).

Kazarian et al. (26) have reported that the physiological responses of minipigs challenged intraperitoneally with a bolus dose of a pure *E. coli* culture are consistent with those of a rapid intravenous bolus injection of a high dose of LPS and lead to circulatory collapse. However, our use of *E. coli* mixed in a sterile fecal carrier, albeit diluted, may have impeded the rapid systemic absorption of the bacteria and thus, promoted the development of a sustained intra-abdominal infection as opposed to inducing fulminant peritonitis. Furthermore, our findings of a marked and persistent fever, systemic bacteremia, as well as mild hypoglycemia and hyperlactacidemia in the septic animals, during the observation period, are consistent with experimental data obtained using fecal soilage models of polymicrobial chronic sepsis (6, 7, 11, 15). Nevertheless, the composition of the bacterial inoculum in the present model could easily be altered to include other species commonly associated with Gram-negative sepsis, such as *Bacteroides*, *Proteus*, and *Enteroccocus* (2).

The mortality rate associated with intraperitoneal infusion of the bacterial inoculum was dose dependent, which is consistent with other models involving intraperitoneal injection of pure cultures of *E. coli* in rats (13, 27). However, the bacterial dose in our study was approximately an order of magnitude lower than those reported in peritonitis models yielding similar mortality rates. This discrepancy may be due partly to differences in the capacity of various strains of *E. coli* to induce peritonitis or alterations in virulence of the infused *E. coli* caused by the laboratory culturing procedures (11). One can also speculate that the nature of the intraperitoneal bacterial administration (i.e. continuous infusion versus bolus injection) might also be important in determining the lethality of an experimental model by eliciting different responses of the host defense mechanisms. An interesting topic for future research would be to compare some of the immune responses elicited by these different methods of induction of peritonitis.

Although the treatment of intra-abdominal sepsis usually includes early cleaning of peritoneal contamination and removal of free particulates and foreign bodies, these surgical procedures are very seldomly reproduced in experimental models of sepsis (16, 28), despite their obvious relevance to the clinical setting. Although excision of the cecum and peritoneal lavage 16 h after CLP markedly improved survival outcome (28), the lavage procedures may also reduce the number of peritoneal macrophages and/or alter their ability to mount an antimicrobial attack (29). Furthermore, Edmiston et al. (30) have shown that the microbial recovery of the mesothelial surface of the peritoneum, measured 24 h after CLP rats underwent saline lavage, was equal to or exceeded pre-lavage values. These effects, taken together with the lack of concomitant antimicrobial therapy, likely contributed to the secondary bacterial growth observed 24 h after completion of the surgical procedures in the present series of experiments.

Although the septic rats received comparable bacterial challenges, the numbers of viable bacteria present in the peritoneal fluid and sequestrated in the different organs were quite variable; this likely reflected inter-individual differences in defense against infection. It is also noteworthy that the average bacterial count in the peritoneal fluid immediately at the end of the 12 h infusion period was approximately half of the bacterial dose infused hourly in these animals. In contrast, these bacterial counts have been shown to be markedly increased (up to two logs) after peritoneal injection of either live *E. coli* or exogenous feces (9, 31). Although some bacterial death due to the intraperitoneal acidic conditions cannot be ruled out (32), our finding would suggest that the septic insult was not overwhelming to the host, and allowed any *in vivo* bacterial multiplication to be adequately offset by bacterial uptake by the resident peritoneal macrophages as well as by polymorphonuclear leukocytes recruited to the peritoneum. However, in agreement with Zanetti et al. (31), we observed a trend toward more deaths among the rats with the greatest bacterial counts in the peritoneal fluid.

In summary, we have developed a model of chronic peritonitis in the rat that mimics the slow, continuous release of bacteria from the gut. This experimental model reproduces many metabolic, bacteriological, and histological features of human sepsis, and may thus prove useful for studies of the pathogenic mechanisms of sepsis. Compared with the most commonly used animal models where sepsis is induced by an intravenous administration of bacteria or endotoxin, this intraperitoneal bacterial infusion model may more closely simulate some clinical situations observed post-traumatically before treatment. Studies are now underway to determine the efficacy of antimicrobial therapy in this model.

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