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Experimental Intra-Abdominal Abscesses in Rats: Development of an Experimental Model

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An animal model has been developed to study the evolution of intra-abdominal abscesses. Gelatin capsules containing pooled colonic contents and barium sulfate were prepared in an anaerobic chamber and implanted into the pelvic region of Wistar rats. The natural course of the ensuing disease was studied in various groups according to the source of the inoculum and sex of the recipient. Colonic contents derived from rats fed a grain diet produced a highly lethal disease with an 80% mortality rate for males and 100% for females. Most deaths occurred within 3 days of implantation, and autopsies showed generalized peritonitis. The addition of blood to the inoculum caused a rapidly fatal peritonitis in all animals. With an inoculum derived from meat-fed rats implanted in male recipients, there was a biphasic disease. Initially, there was peritonitis associated with 43% mortality. All animals that survived this acute period developed discrete intra-abdominal abscesses by the seventh postoperative day. The latter stage was characterized by an indolent course and progressive enlargement of abscesses.

The pathophysiological events associated with intra-abdominal sepsis and subsequent abscess formation are poorly understood. In humans, this infection frequently causes prolonged morbidity and high mortality (2). The purulent material in the abscess cavity is usually found to be polymicrobic (15, 19, 27), but it is difficult to obtain specimens for sequential bacteriology. An animal model simulating large bowel perforation with subsequent abscess formation would be useful to study the development of abscesses in terms of bacteriology, immunology, and response to antimicrobial therapy. Previous attempts to produce such a model have been unsuccessful, either because of the rapid lethality of the disease produced (12, 25) or due to the lack of a defined inoculum (11). We report the development of a standardized peritonitis model which subsequently progresses to form mature, circumscribed abscess cavities. Since the disease follows a predictable course and the same inoculum is used in every animal, it should be possible to use this model for future experimental studies.

MATERIALS AND METHODS

Animals. Wistar rats (Simonsen Laboratories, Palo Alto, Calif.) weighing 160 to 180 g were used in this study. Initially, animals were caged by sex in groups of 10; after surgical procedures they were housed in individual cages. All animals were maintained on rat chow (Ralston-Purina) and water, except for those rats used as a source of the meat-fed inoculum (see below).

Inoculum. The preparation of the surgical implants was designed in a manner to insure a uniform inoculum of microorganisms for all animals. The specimens were obtained by pooling the ceca and large bowels of 15 rats. The abdomen of each rat was aseptically opened. The cecum and proximal large intestine were then clamped, excised, and immediately entered into an anaerobic glovebox (3). The contents of the cecum and bowel were carefully extruded into a sterile beaker, and the tissue was mascerated. An equal volume of prereduced peptone-yeast-glucose broth was added to this material and vigorously mixed. The resultant slurry was filtered through two layers of surgical gauze into a second sterile beaker to remove large particulate matter and tissue. Sterile barium sulfate (10%, wt/vol) was added, and the inoculum was then divided into small portions (approximately 5 ml) which were placed in glass vials fitted with rubber stoppers and screw caps. The closed vials were removed from the chamber, immediately immersed in liquid nitrogen for 4 min, and stored at −40 C until used.

An inoculum was prepared from two different groups of animals: one group was fed lean ground beef ("meat-fed") and the other was maintained on the regular chow diet ("grain-fed") for 2 weeks before being sacrificed. A sterile inoculum, used as a control,

was prepared by autoclaving a portion of the mixed cecal contents.

Bacteriology of the inoculum. Quantitative bacteriology was performed on the quick-frozen inocula obtained from meat- and grain-fed rats. All procedures were carried out within an anaerobic chamber (3). Samples of 0.1 ml were placed in 9.9 ml of prereduced Virginia Polytechnic Institute (VPI) dilution salts (17), and serial 100-fold dilutions were made. Samples of 0.1 ml of each dilution were spread on both prereduced and aerobic plating media to give final dilutions of 10^{-3}, 10^{-5}, 10^{-7}, and 10^{-9}/ml. Anaerobic media were: prereduced brucella agar base containing 0.5 mg of menidione per ml and 6% sheep blood (BMB), BMB containing 100 μg of neomycin sulfate per ml and laked blood agar containing 7.5 μg of vancomycin and 75 μg of kanamycin per ml. These three media were incubated at 37 C inside the anaerobic chamber and held for 3 to 5 days. The following media were employed for aerobic and facultative isolates: blood agar plates incubated with increased CO_2 and MacConkey agar and Pfizer Selective Enterococcus Agar. Incubation of these plates was at 37 C for 24 to 48 h. After incubation, colony types were enumerated, isolated, and identified. No attempt was made to isolate colony types on plates showing confluent growth, since enumeration was impossible. Anaerobic isolates were identified according to the procedures outlined by the VPI *Anaerobe Laboratory Manual* (17). *Enterobacteriaceae* were identified by the methods of Edwards and Ewing (13), and other isolates were identified by established procedures (4). Since several species were identified more than once, the population density for each isolate was recorded as the highest plate count observed. All counts were recorded as $\log_{10}$ colony-forming units per milliliter.

Implantation of inoculum. The frozen inoculum was thawed in the anaerobic chamber. A 0.5-ml amount was placed in a sterile no. 1 gelatin capsule which was then inserted into a no. 0 capsule. (A double capsule was used, since a single capsule dissolved immediately after peritoneal implantation.) The double capsule was removed from the chamber for immediate placement into rats that had been anesthetized by intraperitoneal injection of 0.15 ml of Nembutal (50 mg/ml). The abdomen of each animal was shaved and cleaned twice with 1% iodine, and a 1.5-cm anterior midline incision was made through the abdominal wall and peritoneum. The capsule was inserted into the pelvic region of each rat. The incision was closed with three or four interrupted 3-0 silk sutures, and the animals were returned to separate cages and observed every 8 h for 2 weeks. There was a 5% acute mortality (within 4 h) regardless of the inoculum, secondary to either the anesthesia or the surgery. Those animals that died within 4 h of the procedure were eliminated from the study.

Control groups were implanted with gelatin capsules containing (i) sterile inoculum, (ii) barium sulfate, or (iii) sterile inoculum and barium sulfate (10%, wt/vol).

Experimental groups. Four groups of animals were studied. The inoculum prepared from rats on a meat-only diet was inserted into 106 male Wistar rats. To determine the role of diet on the morbidity produced by fecal material, 46 male rats received the inoculum prepared from grain-fed rats. This grain-fed inoculum was also implanted in 19 female rats to compare the effect of sex on the disease produced. In addition, 11 male rats received this inoculum with 0.5 ml of fresh rat blood. (The blood was obtained by transthoracic cardiac puncture of other rats.)

Gross pathological changes were noted immediately at the time of death. All surviving animals were sacrificed at 2 weeks, and an autopsy was performed.

RESULTS

Preservation of the inoculum. Two methods for preserving the inoculum after preparation were tested: lyophilization and quick freezing in liquid nitrogen. Quantitative assay of the inoculum before and after manipulation indicated that a decrease in total counts occurred with both methods. The total anaerobic count of the inoculum in the fresh state before manipulation was 10^9 colony-forming units/ml; after lyophilization counts were $10^{6.5}$ to 10^7 colony-forming units/ml and after quick freezing the total anaerobic population was $10^{7.5}$ to 10^8. No change in the relative proportions of the major isolates was seen with either method. Quick freezing of the inoculum in liquid nitrogen was selected as a means of preservation, since the decrease in total counts was less than that seen after lyophilization.

Bacteriology of inoculum. Bacteriological analysis of the quick-frozen inoculum from meat-fed animals yielded a total of 22 bacterial species, including 13 anaerobic and nine aerobic species (Table 1). In highest concentration were two species of *Eubacterium* which were present at levels of $10^{7.5}$/ml. These organisms outnumbered the most frequent aerobe in the inoculum by more than 2 logs. The next most frequent organisms were an anaerobic, pleomorphic, gram-negative bacillus and an anaerobic, nonsporulating, gram-positive bacillus. These organisms did not fit conventional classification schemes and could not be speciated. Several *Clostridia* species, *Bacteriodes fragilis*, peptococci, and *Fusobacterium varium*, were present in concentrations of 10^5 to $10^{6.1}$/ml. *Enterococcus* and *Escherichia coli* were the predominant aerobic species, occurring in concentrations of $10^{5.4}$ and $10^{5.2}$/ml, respectively. Several other aerobes were also present, including *Lactobacillus*, *Micrococcus*, *Corynebacterium*, *Proteus*, alpha-hemolytic streptococcus, and *Moraxella*.

Bacteriological analysis of the quick-frozen inoculum from grain-fed rats yielded a total of 16 bacterial species, including nine anaerobic and seven aerobic species. Among the anaer-

TABLE 1. *Bacteriology of the inoculum from meat-fed rats*

Bacteria	Log CFU/ml[a]
Anaerobes	
Eubacterium tenue	7.5
Eubacterium aerofaciens	7.5
Pleomorphic gram-negative rod	7.0
Nonsporeforming gram-positive rod	6.3
Clostridium perfringens	6.1
Clostridium paraputrificum	6.0
Clostridium sp.	6.0
Bacteroides fragilis	5.8
Peptococcus morbillorum	5.7
Peptococcus prevotii	5.8
Fusobacterium varium	5.2
Clostridium sartagoformum	5.2
Clostridium tyrobutyricum	5.0
Aerobes	
Enterococcus	5.4
Escherichia coli	5.2
Lactobacillus sp.	5.0
Micrococcus sp.	4.5
Corynebacterium sp.	4.4
Alpha-hemolytic streptococcus	4.0
Proteus mirabilis	4.0
Proteus morganii	3.9
Moraxella sp.	3.1

[a] CFU, Colony-forming units.

obes, two species of *Clostridia* and *Peptostreptococcus anaerobius* were isolated in highest numbers (10^7/ml). *Peptostreptococcus micros*, *Peptococcus constellatus*, two unidentifiable gram-positive anaerobic bacilli, *Bacteroides fragilis*, and *Fusobacteria* were present in lower concentrations (10^3 to 10^5/ml). Lactobacilli were the dominant aerobic species (10^8/ml), followed by *Staphylococcus epidermidis* (10^7/ml) and *Micrococcus* sp. (10^6/ml). *Proteus rettgeri*, enterococci, and *E. coli* were present in concentrations of 10^4 to 10^5/ml. Thus, in contrast to the inoculum from meat-fed animals, aerobes outnumbered anaerobes in this inoculum.

Effects of cecal inoculum from animals fed a meat diet. Using the inoculum from the meat-fed rats, 106 animals were studied (Fig. 1). Seven (6.6%) animals died between 8 and 16 h, and 21 (19.8%) were dead before 24 h. Autopsy of these animals revealed that the double gelatin capsule began to dissolve shortly after insertion, but even at 8 h the inoculum was usually still localized in the pelvis. Within 24 h a suppurative infection and ileus developed, and 0.2 to 0.5 ml of peritonitis fluid had accumulated. The fur appeared ruffled, and the animals were lethargic and cold. At 48 h, peritoneal adhesions began to appear anteriorly, and loosely attached collections of purulent material were noted. By 3 days, 38.7% (41/106) of the animals had died. Usually one or more abscess cavities were beginning to form either in the pelvis or along the peritoneal suture line. Marked abdominal distension could be easily palpated. Three additional animals died at 96 h, and by 104 h 43.4% (46/106) of the animals were dead. There was no further loss of animals after this time.

By 7 days a well-formed abscess was usually palpated inferiorly along the anterior abdominal wall (Table 2). The surgical incision was well healed, and peritoneal fluid was rarely present. The rats were now lively, began to gain weight, and did not appear ill. The abscess cavities continued to enlarge and by 2 weeks contained 0.1 to 0.3 ml of pus. Sterile barium granulomas could be seen on the surfaces of the liver and spleen, within the mesentary, and anteriorly in the peritoneal cavity.

In additional studies the course of the infection was observed for more extended periods. Some abscesses perforated at approximately 3 to 4 weeks, causing a generalized peritonitis. In many animals, however, they have persisted for many months. At autopsy, these animals had multiple large abscesses throughout the abdomen and in the subdiaphragmatic space. Liver, spleen, brain, and pulmonary abscesses have also occasionally been seen.

Effects of cecal inoculum from animals fed a grain diet (Fig. 2). The inoculum from grain-fed rats produced a disease of much greater lethality in the 46 male rats studied. There were no deaths before 16 h, but eight animals (17.4%) had died before 24 h. Most

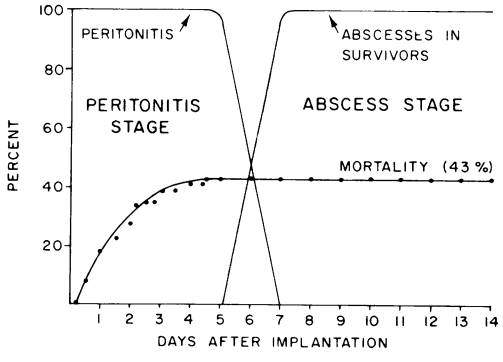

FIG. 1. *Mortality and abscess formation in 106 male Wistar rats receiving inoculum obtained from meat-fed animals. Mortality is expressed as cumulative percent.*

TABLE 2. *Factors influencing mortality and abscess formation*

Inoculum	Sex	No. of Animals	Mortality at 2 weeks (%)	Abscesses in survivors (%)
Meat fed	Male	106	43	100
Grain fed	Male	46	80	78
Grain fed	Female	19	100	
Grain fed + blood	Male	11	100	

remaining animals appeared ill, and only rarely was fecal material noted in the cases before 36 h. Fifty percent of the animals were dead before 48 h, and only 33% (15/46) survived 3 days. These animals all had distended abdomens with peritoneal fluid, but they were beginning to eat and drink fluid. Nine animals (20%) lived 2 weeks after the toxic insult and, at autopsy, seven had at least one large well-formed intra-abdominal abscess (8 to 15 mm in diameter). Multiple sterile granulomas were uniformly observed, but free peritoneal fluid was not seen. Occasionally as many as four distinct abscesses were found, usually located anteriorly or in the pelvis. A subdiaphragmatic abscess was noted in two animals, but none were seen within the liver, spleen, or thoracic cavity.

The inoculum from grain-fed animals produced a disease which progressed more rapidly than with the meat inoculum. There was no difference in mortality between animals at 1 or 2 days, but at 72 h they did show a significant difference ($P < 0.01$). At 4 days there was a marked difference ($P < 0.001$), with the increased mortality associated with the grain inoculum. Interestingly, only seven of the nine survivors (78%) developed an abscess at 2 weeks compared with 100% with the meat inoculum; however, this difference is not statistically significant (Table 2).

Effect of sex. Eight hours after surgery, the 19 female rats given the same grain inoculum all appeared groggy and lethargic. Four had died by 16 h, and the others were cold and dry (Fig. 2). Sixty-eight percent (13/19) were dead before 24 h, and there were no survivors at 48 h. At autopsy, all animals had large amounts of greenish-yellow peritoneal fluid, none of which was foul smelling. Often there was loosely adherent purulent material along the suture line. No pulmonary pathology was observed, and the gastrointestinal tract was intact. There was an increased mortality ($P < 0.001$) observed with the female rats compared with male rats of the same size receiving the same inoculum.

Effect of blood on virulence. The 11 male rats that received 0.5 ml of fresh rat blood with the grain-fed inoculum did poorly, with two deaths occurring before 12 h and six additional deaths before 48 h. All animals progressively deteriorated with abdominal distention and hypothermia (Table 2). The three remaining rats died before 72 h and had peritoneal fluid, adhesions, and purulent material seen at laparatomy. The addition of blood, even in small amounts, was sufficient to rapidly kill all the animals.

Controls. Animals that received the sterile inoculum in gelatin capsules showed no signs of toxicity. Upon awakening from the anesthetic agent, they were alert and active. After 6 to 8 h they drank water freely, and feces was noted in the cages at 16 h. At 14 days the animals were sacrificed, and no pathological changes could be found.

The barium sulfate alone or the combination of $BaSO_4$ and sterile inoculum produced a similar clinical picture. Initially, the animals looked like the group receiving the sterile inoculum. Their suture line began to heal, but at 3 to 4 days there was abdominal distention and sterile peritoneal fluid could be obtained by needle aspiration. They did not, however, appear ill, and they continued to eat and gain weight. The ascitic fluid gradually subsided over 3 to 4 days, and there were no deaths in either group. At autopsy the only findings in these animals were multiple granulomata, without pus, throughout the abdominal cavity.

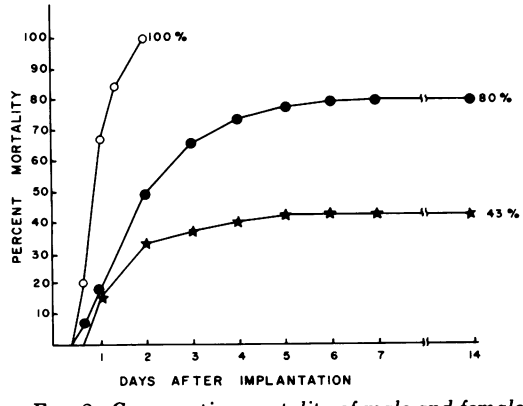

FIG. 2. *Comparative mortality of male and female rats receiving inoculum obtained from either grain- or meat-fed animals. Symbols:* (○), *female recipients with inoculum from grain-fed rats;* (●), *male recipients with inoculum from grain-fed rats;* (★), *male recipients with inoculum from meat-fed rats.*

These were principally located along the parietal peritoneum and in the pelvis, but they were also seen in the mesentary and on the surface of the spleen and liver. Culture and microscopic exam of peritoneal fluid and granuloma tissue in these animals failed to reveal bacteria. Although peritoneal inflammation and granuloma formation was related to the presence of $BaSO_4$, the absence of viable bacteria in the inoculum appeared to mitigate against abscess formation or death.

DISCUSSION

Experimental studies of intraperitoneal sepsis date to 1887, when it was noted that viable bacteria were necessary to produce death when intestinal contents were placed into the peritoneal cavity (23). Appendiceal ligation (9), ileal segment strangulation (24), and colotomy (18) have all been used to study peritonitis. However, the infection produced is generally fatal, the inoculum is not uniform with each animal, and there is no consistency of abscess formation in the surviving animals. In 1892, Massart produced abscesses by implanting open glass tubes containing bacterial cultures into the abdomen (20). Six years later, Metchnikoff inserted bacteria in collodion sacs into the abdominal cavities of guinea pigs (21). Others have used agar disks (29) or cellophane sacs (14) to localize purulent infection. Deysine et al. (11) produced intra-abdominal abscesses using fresh rat feces, but the inoculum was not standardized and the abscess contents drained spontaneously, making it impossible to do long-term studies. Others have studied abscess production in solid organs. Moore and Gross (22) produced liver granulomas in turkeys with intravenous injection of a gram-positive bacillus. More recently, Hill et al. (16) developed a progressive liver abscess in a mouse model using nonsporeforming anaerobic bacteria, suggesting that anaerobes may be important in that disease.

To prevent the normal body protective mechanisms from eradicating an intra-abdominal infection, either a continuous focus must be present or an area must be walled off. The use of a gelatin capsule permits ease in handling of the inoculum, and its slow dissolution and localization accounts for the development of abscesses without overwhelming systematic toxicity.

In dogs, intraperitoneal injection of barium sulfate alone produced a widespread peritonitis, with dense adhesions and a 70% mortality, within 2 weeks (1). The addition of $BaSO_4$ also increased the toxicity and tissue reaction of experimentally produced fecal peritonitis (30). It has been used to radiographically localize abscess cavities after perforation of a barium-containing viscus. In our model, barium was used to increase the local inflammatory response in order to restrict the dissemination of the fecal contents.

The animals receiving sterile cecal material and barium sulfate developed a reaction from the irritating effect of the inoculum. Despite this peritoneal inflammation, there was minimal morbidity and no mortality without an infectious component to their illness. Granulomas were noted whenever barium was inserted, but these did not appear to have clinical significance.

The natural history of the disease produced with the meat inoculum clearly showed two distinct stages (Fig. 1). The early peritonitis stage, lasting generally 4 to 5 days, was followed in all survivors by an abscess stage without obvious peritoneal fluid. The mortality was associated with the initial process. This progression simulates the mixed aerobic and anaerobic intra-abdominal infection after contamination by bowel or female genital tract pathogens (15, 27).

The grain inoculum, while producing a more lethal infection, did not always produce an abscess in the surviving animals. Since procedures were otherwise identical, the relative bacterial counts in the inocula were undoubtedly responsible for the changes in clinical disease. Although this may be a better inoculum for studying peritonitis, it is less desirable for investigations of long-term abscesses.

Other factors have been noted which alter the response of animals to infections. Childs et al. (7) showed that there is a much greater susceptibility and an earlier mortality with several diseases in the human male as compared with the female. During infancy and old age, men have a higher mortality from such infectious diseases as lobar pneumonia, pleurisy, respiratory and bone tuberculosis, osteomyelitis, diarrhea, appendicitis, and all forms of meningitis (8, 28). However, women do appear to have an increased incidence and toxicity from whooping cough and acute rheumatic fever. In experimental infection in rats, Campbell (6) revealed that sex hormones influence the degree of infection with *Cysticercus crassicollis*. The administration of testosterone to both sexes made the females as susceptible to the infection as the males. It is interesting that in our studies, the females developed greater toxicity from the experimental intra-abdominal infection. This

has not been observed in similar human infections.

Hemoglobin is capable of enhancing intraperitoneal growth of bacteria (5, 26). Studying experimental peritonitis in rats, Davis (10) found that neither whole blood nor 10^8 *E. coli* alone could produce any fatalities. However, by mixing the organisms with 1 g of hemoglobin per 100 ml, 17% of the animals died; the peritonitis was uniformly fatal when 4 g of hemoglobin per 100 ml was used. Rat blood used in our studies averaged 12.8 g of hemoglobin per 100 ml. The addition to the inoculum of only 0.5 ml of fresh blood was apparently sufficient to produce a lethal peritonitis in all animals. The importance of minimizing postoperative bleeding during intra-abdominal surgery should be obvious.

This method of producing intraperitoneal infection in rats does not eliminate all variations in intrinsic host resistance. However, all survivors do consistently develop abscess cavities with sufficient purulent material to do quantitative microbiology. In addition, the model should prove suitable for studying the pathogenesis of abscess formation, evaluating antibiotic efficacy, and investigating the immunological response to infection.

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