Experimental Intra-Abdominal Abscesses in Rats: Quantitative Bacteriology of Infected Animals

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An animal model simulating intra-abdominal sepsis was produced by implanting large bowel contents into the pelvic region of rats. Bacteriological analysis of infected sites showed quantitative differences according to the stage of disease. During the initial, often lethal, peritonitis stage, Escherichia coli (mean concentration, 10^8/ml), enterococci (10^5) and Bacterioides fragilis (10^6) were always present. Blood cultures obtained during this phase were uniformly positive, with E. coli being the principal isolate. Animals that survived this early acute peritonitis stage developed indolent intra-abdominal abscesses. The major isolates in abscess contents were B. fragilis ($10^{8.7}$) and Fusobacterium ($10^{8.6}$); E. coli ($10^{7.8}$) and enterococci ($10^{5.7}$) were also present but in lesser concentrations. Rank order analysis of these four species in peritoneal exudates and abscess pus showed that the two aerobes outranked the two anaerobes during the early stage of the disease, whereas the reverse was true in abscesses. These experiments also illustrated that a major simplification of the original fecal inoculum occurred, even though the subsequent infection remained bacteriologically complex.

Rupture of the large bowel inevitably leads to contamination of the peritoneum with large numbers of bacteria (7). The diversity of microbes present after this traumatic event was initially observed by Veillon and Zuber (15) in 1898. These investigators noted that both anaerobes and aerobes could be recovered from infections which followed appendiceal rupture. In 1938 Altemeier (2) reported similar findings: cultures from 100 consecutive appendicitis specimens consistently yielded multiple bacteria, including both aerobic and anaerobic species. Recent studies have amply confirmed these early observations. In a study of 43 intra-abdominal abscesses, Gorbach et al. (8) noted an average of five bacterial species per case, including three anaerobes and two aerobes.

It is apparent that most intra-abdominal infections studied with optimal bacteriological techniques yield a diverse array of microorganisms. Since both aerobes and anaerobes are usually present, the question as to the importance of each component has generated considerable controversy. Several studies have utilized animal models in an effort to delineate the pathogenicity of anaerobes in abscesses (10, 11, 13). However, these models dealt with simple bacteriological systems that were not truly representative of the mixed flora associated with an intestinal perforation.

We have recently developed an animal model that utilized a uniform inoculum of cecal contents which was surgically implanted in the peritoneal cavity of rats. A predictable disease evolved that simulated the pathophysiological events of intestinal perforation (16). It is the purpose of the present study to define the evolution of bacterial populations from the initial peritonitis to final localization of intra-abdominal abscesses.

MATERIALS AND METHODS

Animal procedures. A uniform inoculum was prepared from the pooled cecal and large bowel contents of 15 rats maintained on a diet of lean ground meat and water for 2 weeks (16). This material was mixed with an equal volume of peptone-yeast-glucose broth and 10% (wt/vol) barium sulfate, frozen in liquid nitrogen, and stored at -40 C until used. The inoculum (placed in a double gelatin capsule) was inserted into the pelvis of 160- to 180-g male Wistar rats through a midline abdominal incision.

Five rats were sacrificed for bacteriological study at 1, 3, 7, and 14 days after surgical implantation of the inoculum. The animals were sacrificed with ether, and the abdomen was shaved and cleaned with iodine.

Each animal was opened aseptically; a sample of either peritoneal fluid or abscess pus was drawn into a sterile tuberculin syringe. The syringe was immediately placed into an anaerobic chamber (4) for bacteriological analysis.

The three control groups consisted of rats implanted with sterile gelatin capsules, gelatin capsules with barium sulfate, and gelatin capsules containing autoclaved intestinal contents. All control animals were sacrificed 2 weeks postoperatively, and material was obtained from the implantation site for bacteriological analysis.

Bacteriological procedures. Quantitative bacteriology was performed on peritoneal exudate and abscess contents that had been transferred immediately into the anaerobic chamber. Samples of 0.1 ml were placed in 9.9 ml of prereduced Virginia Polytechnic Institute (VPI) dilution salts (12), and serial 100-fold dilutions were made. Portions (0.1 ml) of each dilution were spread on both prereduced and aerobic plating media (16) to give final concentrations of $10^{-3}, 10^{-5}, 10^{-7},$ and 10^{-9}/ml. Colony types were enumerated, isolated, and identified. Anaerobic isolates were identified according to the procedures outlined in the VPI *Anaerobe Laboratory Manual* (12). *Enterobacteriaceae* and other aerobic isolates were identified by established procedures (5, 12).

Blood cultures. Samples of heart blood were obtained from infected rats by transthoracic, percutaneous cardiac puncture. Samples (2 ml) were drawn from 10 rats prior to sacrifice during the initial 1- to 3-day period and the later 7- to 14-day period. The blood was inoculated into 50 ml of prereduced brain heart infusion broth, supplemented with hemin and menadione (Scott Robbins, Fiskeville, R.I.), and incubated at 37 C. Blood cultures were routinely subcultured onto the aerobic and anaerobic media, described previously, at 1, 7, 14, and 21 days.

RESULTS

Bacteriology of peritoneal exudates and abscesses. Previous studies of rats implanted with this inoculum indicated that a biphasic disease occurred. During the first 5 days, there was generalized peritonitis associated with exudation of inflammatory fluid and a mortality rate of 43%. Animals which survived this acute stage universally developed discrete intra-abdominal abscesses by the seventh postoperative day (16). Samples were obtained from infected rats at periods of 1, 3, 7, and 14 days after implantation. Analysis of these specimens indicated no differences in numbers of bacterial species between the 1- and 3-day samples nor between the 7- and 14-day samples. The results were therefore combined into two groups consisting of peritoneal exudates, which reflect the two early sample times, and abscesses, which refer to the 7- and 14-day sample times.

Peritoneal exudates consistently harbored three microorganisms, *Escherichia coli*, en-

terococcus, and *Bacteroides fragilis*. The concentrations of *E. coli* and *B. fragilis* were approximately equal (10^6/ml), whereas enterococcus was found at slightly lower levels (10^5/ml).

Abscess pus yielded greater total bacterial populations in which anaerobes outnumbered aerobes. Four microorganisms were always present in abscesses (Fig. 1): two aerobes, *E. coli* and enterococcus, were found at concentrations of $10^{7.8}$/ml and $10^{5.7}$/ml, respectively; two anaerobes, *B. fragilis* and a *Fusobacterium* species, were recovered at population levels of $10^{8.7}$/ml. The most common *Fusobacterium* was *F. varium*; however, *F. necrophorum* and *F. gonidiaformans* were also isolated from some animals.

These results indicate that the dominant components of the infected flora were *E. coli*, enterococci, *Bacteroides fragilis*, and *Fusobacterium*. An analysis of the differences in relative populations of these four microbes was made by comparing their mean rank order in peritoneal exudates and abscesses. The highest bacterial population in each rat was assigned a rank of 1, and the lowest was assigned a rank of 4. A mean rank value for the two aerobes and the two anaerobes was then obtained (Fig. 2). These results indicated that the two most common aerobes outnumber the two most common anaerobes during peritonitis. On the other hand, the anaerobes, *B. fragilis* and *Fusobacterium*, were consistently present in greater numbers in abscesses than were *E. coli* and enterococcus. A test for the significance of the differences was performed by a factorial analysis of variance (17); the difference between
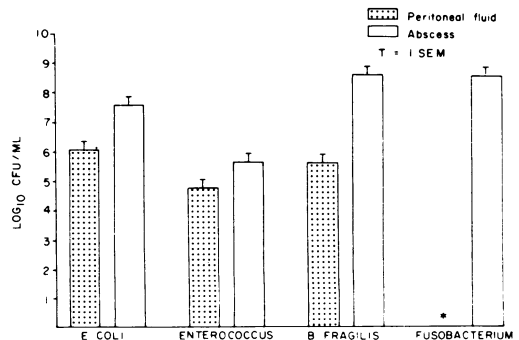

FIG. 1. *Comparison of population densities for the four major isolates recovered from rats after implantation of inoculum. The asterisk indicates that the numbers of Fusobacterium present in peritoneal exudates were found at 10^3/ml or greater in only three of 10 animals.*

aerobic and anaerobic populations in the two stages was significant at $P < 0.001$.

Several additional aerobic and anaerobic species were found in the infected sites, although this flora was not as complex as the original inoculum. A mean of 5.4 bacterial isolates was recovered from peritoneal exudates and included 3.2 aerobic species and 2.2 anaerobic species. Abscesses yielded a mean of 7.0 different isolates of which 4.0 were aerobes and 3.0 were anaerobes.

The bacteria recovered from infected rats were representative of the inoculum, with certain notable exceptions. Several microorganisms were present in large numbers in the inoculum, but were rarely found in rats after implantation, i.e., *Eubacterium*, *C. perfringens*, peptococci, *Lactobacillus*, *Micrococcus*, *Corynebacterium*, and alpha-hemolytic streptococci.

Bacteriology of control animals. Control groups consisted of rats implanted with autoclaved inoculum, gelatin capsules containing barium sulfate, and gelatin capsules alone. None of these animals developed abscesses, and cultures were uniformly sterile. Peritoneal tissues obtained at autopsy also failed to reveal bacteria by microscopic exam or culture.

Blood cultures. All rats sampled between 1 and 3 days after implantation had positive blood cultures (Table 1). The most frequent isolates were *E. coli* (9/10) and *P. morganii* (5/10). The blood culture isolates were also present in the peritoneal exudates obtained from these animals. Multiple bacterial isolates were found in several cases and included *B. fragilis* (2/10) and enterococcus (1/10). Blood

TABLE 1. *Results of blood culture obtained from rats after implantation of inoculum*

Time after implant (days)	% Positive	Microorganisms	No. positive/total No.
1–3	100	Escherichia coli	9/10
		Proteus morganii	5/10
		Bacteroides fragilis	2/10
		Enterococci	1/10
7	60	Escherichia coli	5/10
		Fusobacterium varium	1/10
		Proteus morganii	1/10
14	20	Alpha-hemolytic streptococci	1/10
		Clostridium perfringens	1/10
Control (not implanted)	20	Diphtheroid	1/10
		Alpha-hemolytic streptococci	1/10

samples obtained at 7 days gave positive cultures in 60% of animals. *E. coli* was found in five of 10 animals, and one rat yielded *F. varium*. Cultures were positive in only two of 10 animals tested at 14 days. One culture was positive for *C. perfringens*, and the other contained alpha-hemolytic streptococci; these figures are consistent with the rate of spontaneous bacteremia and contamination found in 10 control animals from which blood cultures were obtained.

DISCUSSION

The bacteriological results in this study indicate that the microflora of experimentally induced intra-abdominal sepsis is a dynamic system which changes during the course of disease. The initial inoculum consisted of intestinal contents harboring at least 22 bacterial species. The average number of bacterial species recovered from peritonitis exudate and abscess contents was 5.4 and 7, respectively. Thus, the flora associated with infection, although complex, represented a major simplification of the original inoculum.

It is interesting to note that certain organisms were seldom isolated from intra-abdominal sepsis. For example, *Eubacterium*, the dominant organism in the fecal inoculum, was conspicuously absent in cultures from the infected sites. On the other hand, four organisms emerged as the dominant populations in infected material. These were *E. coli*, enterococci, *B. fragilis*, and *Fusobacterium*. The relative proportions of these four microbes showed significant differences according to the stage of disease.
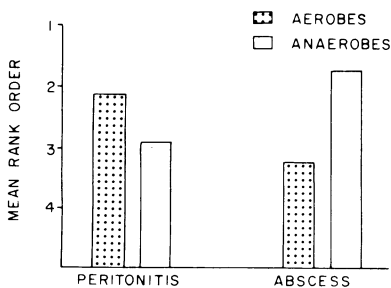
As previously noted, this animal model was

FIG. 2. *Comparison of the mean rank order for the two major aerobic isolates (E. coli and enterococcus) and the two major anaerobic isolates (B. fragilis and Fusobacterium) in peritoneal exudates and abscesses. In peritonitis exudates, the rank order of the two aerobes was greater than that of the two anaerobes; in abscesses, anaerobes outranked aerobes. Factorial analysis of variance showed that this difference in rank order was significant ($P < 0.001$).*

characterized by a biphasic disease. Initially, there was an acute, often lethal, generalized peritonitis; animals that survived this acute stage universally developed intra-abdominal abscesses by the seventh postoperative day. During the peritonitis stage, *E. coli* and enterococci were the major isolates. Blood cultures obtained within 3 days of implantation yielded *E. coli* in nine of 10 samplings. By contrast, the abscess stage of infection proved clinically indolent, and bacteremia was seldom detected. Two anaerobes, *B. fragilis* and *Fusobacterium*, consistently outnumbered the facultative bacteria in quantitative cultures of abscess contents. These results suggest that aerobic bacteria play an important role in producing acute peritonitis with its associated bacteremia and high mortality. Anaerobes, however, emerged as the dominant isolates concurrent with abscess formation.

Factors responsible for sequential changes in the infecting flora and pathological events are unknown. Previous experimental studies by Altemeier (1–3) may be relevant. This investigator inoculated bacteria recovered from appendicitis specimens intraperitoneally into guinea pigs. Enterococci, *E. coli*, *Bacteroides fragilis*, and *Bacteroides thetoides* proved nonvirulent in pure culture. Combinations of these organisms, however, produced severe peritonitis. Hite et al. (11) also showed that recombined cultures of intestinal microbes, including both aerobes and anaerobes, were required to produce necrotizing lesions of the abdominal wall in mice. These studies suggest that certain intestinal organisms exist in a state of symbiosis, and that synergistic mechanisms are responsible for their pathogenic effects.

The bacteriological verisimilitude of this model to intra-abdominal sepsis in humans is worthy of comment. The inoculum contained a polymicrobial flora in which anaerobes outnumber aerobes by a factor of 100:1. Intestinal flora studies in humans have shown bacterial populations similar to those obtained from rats fed a meat diet (9, 14). It was also noted that the number and types of bacteria recovered from infected sites in rats were comparable with reports of intra-abdominal sepsis in humans. It appears that this experimental model is useful in understanding the clinical and bacteriological events pursuant to colonic perforation in humans.

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