The Peritoneal Environment During Infection

The Effect of Monomicrobial and Polymicrobial Bacteria on pO_2 and pH

ROBERT G. SAWYER, M.D., MICHAEL D. SPENGLER, B.S., REID B. ADAMS, M.D., and TIMOTHY L. PRUETT, M.D.

Intraperitoneal (IP) abscesses frequently are composed of aerobes and anaerobes, and, in experimental models, a particulate adjuvant. The environmental changes effected by these components, either singularly or in combination, have not been well defined. The IP pO_2, pH, and recoverable bacteria from the peritoneum of rats were quantified over 6 hours during simple aerobic and anaerobic infections and during mixed peritonitis with and without a sterile feces–barium sulfate adjuvant (SFA). Fourteen groups were studied, receiving intraperitoneally, at time of oxygen probe placement, 1 mL normal saline (control), *Escherichia coli* (EC), *Bacteroides fragilis* (BF), SFA alone, or a mixture of EC and BF, EC and SFA, BF and SFA, or EC, BF, and SFA. Control animals exhibited a stable IP pO_2 and pH during 6 hours. In monomicrobial EC peritonitis, inocula well below the LD_{50} produced an increased IP pO_2 and reduced arterial–peritoneal gradient (APG), with a stable IP pH. By 6 hours lethal doses of EC produced a dramatic decline in IP pO_2, with no change in arterial pO_2 as well as acidic IP and arterial pHs. Simple BF peritonitis caused no or minor elevations in IP and arterial pO_2 with no change in pH. During mixed infections a significant decline in the IP pO_2 and pH at 6 hours in those groups infected with both SFA and EC of a moderate, normally sublethal inoculation was observed, while arterial pO_2 was unchanged and arterial pH was decreased only slightly. Concomitantly there was a significantly increased number of aerobic bacteria in those groups with SFA as adjuvant compared to similar inocula without SFA. This study demonstrates the complex interactions of bacteria, sterile particulate adjuvant (SFA), and the host peritoneum. It suggests that the combination of SFA and aerobic bacteria alter the peritoneal environment to one permitting anaerobic growth and promoting abscess formation.

From the Department of Surgery, Surgical Infectious Disease Laboratory, University of Virginia Medical Center, Charlottesville, Virginia

CLINICAL INTRAPERITONEAL (IP) INFECTIONS usually are polymicrobial, as they are comprised of both aerobic and anaerobic organisms.[1–4] Experimental models also have demonstrated a synergy between these two components,[5] and, in most cases, the necessity for anaerobes for consistent abscess formation.[6,7] Nonbacterial adjuvant have frequently been added as well to promote abscess formation while avoiding the lethality of higher bacterial inocula. Weinstein et al.[8] developed an early rat model of mixed peritonitis leading to IP abscess formation that relied on the presence of barium sulfate as a local irritant and two gelatin capsules to assure localization. Others have shown that other forms of inanimate material, including fibrin,[9] bran,[10] or agar,[11] can be used as well. The pathogenesis of this synergy between aerobes, anaerobes, and particulate matter is unclear. It has been proposed that the ability to effect abscesses by aerobes and anaerobes may be the consequence of alterations in the peritoneal environment resulting from the metabolism and by-products of the bacteria.[12] The nature of these environmental changes, however, has not been well characterized thus far.

In the only previous attempt at the direct measurement of IP pO_2 and pCO_2 during peritonitis, Renvall and Niinkoski[13] noted a steady decrease in pO_2 and increase in pCO_2 during 24 hours in animals during intra-abdominal infection. These experiments were hampered, however, in that the induction of peritonitis was by a poorly controlled transanal traumatic rupture of the colon. Close to 30% of their animals did not develop acute peritonitis and quantification of the bacterial inoculum was, of course, impossible. The effect of the different components of peritonitis on the peritoneal environment, therefore, remains unspecified.

Presented in part at the Surgical Infection Society meeting, Denver, Colorado, April 13–14, 1989, and the Virginia Surgical Society Meeting, Hot Springs, Virginia, May 4–6, 1990.

Supported by University of Virginia Research and Development Grant 5-507-RR05431-28.

Dr. Sawyer is an Infectious Diseases Society of America/Stuart Pharmaceutical Fellow in Surgical Infectious Diseases.

Address reprint requests to Robert G. Sawyer, M.D., Box 3154, Bldg. MR4, University of Virginia Medical Center, Charlottesville, VA 22908.

Accepted for publication April 24, 1990.

This study was designed to examine the early changes in peritoneal environment, specifically pO_2 and pH, and bacterial growth using a rat model of peritonitis. We first studied the effects of monomicrobial *Escherichia coli* and *Bacteroides fragilis* infections on sterile feces–barium sulfate suspension, which further allowed the assessment of these parameters in conditions known to be favorable for the formation of abscesses.

Materials and Methods

Animal Preparation

All experiments were performed in 350 to 400 g male Sprague-Dawley rats (Hilltop Laboratories, Scottdale, PA) housed in cages and fed Purina rat chow® (St. Louis, MO) and water *ad libitum*. Tonometers were constructed by inserting 22-gauge intravenous (IV) catheters, trimmed to 1 cm in length, into 5-cm segments of Silastic® tubing (internal diameter = 0.3 inches, external diameter = 0.65 inches; Dow-Corning, Midland, MI) selected for its documented gas permeability.[14] General anesthesia was induced in rats using a 5% halothane/air mixture and maintained with 1.25% halothane. The abdomen was prepped with 70% isopropyl alcohol, and, using sterile technique, was opened with a 2-cm midline incision. A puncture wound was created in the left flank using a large-gauge needle, and the tonometer was introduced through the abdominal wall into the peritoneal cavity with the catheter hub remaining outside. A second puncture wound was made in the left lower quadrant and the tonometer end without the IV catheter was brought out through this to project 0.5 cm from the skin. Both tonometer ends were secured to the rats skin using 4-0 silk (Fig. 1). Intraperitoneal pH was measured, 1 mL of normal saline and the appropriate infecting material, depending on the group (Table 1), was placed into all quadrants, and the abdominal wall and skin were closed using a single running 3-0 nylon suture. Tail arterial blood was obtained and rats were allowed to awaken.

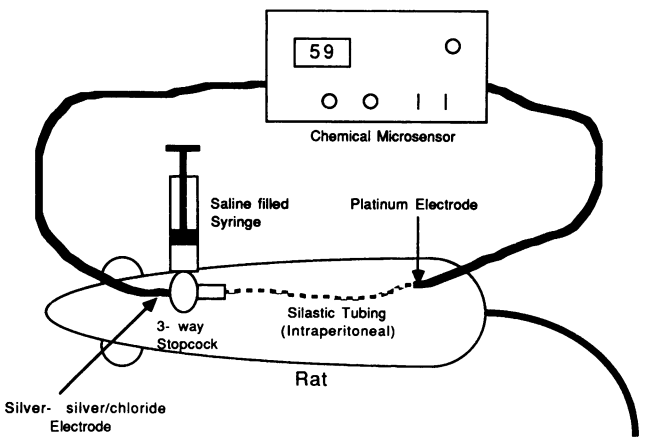

FIG. 1. Experimental design showing technique used for continuous intraperitoneal pO_2 monitoring.

TABLE 1. *Experimental Design as Number of Animals and Composition of Intraperitoneal Inocula Injected by Group*

Group	Animals Inoculated	Log CFU *E. coli* Inoculated	Log CFU *B. fragilis* Inoculated	MI Sterile Stool-Barium Sulfate Inoculated
1	7	0	0	0
2	6	5	0	0
3	5	8	0	0
4	6	9	0	0
5	5	10	0	0
6	4	0	6	0
7	4	0	8	0
8	6	0	9	0
9	5	0	10	0
10	5	8	9	0
11	4	0	0	1
12	5	8	0	1
13	5	0	9	1
14	6	8	9	1

PO_2 and pH Measurements

Intraperitoneal pH was measured at the time of tonometer placement and just before the animals were killed using a standard pH probe (American Scientific [McGaw Park, IL] H3725-103) and an Orion (Boston, MA) SA 720 pH meter. Rats were anesthetized hourly for 10 minutes with halothane for purposes of IP pO_2 measurement and blood drawing and were allowed to awaken between measurements until they were killed at 6 hours. As described by Gottrup et al.,[15] the Silastic® tubing was filled with normal saline at 37 C and a silver–silver chloride electrode placed through a three-way stopcock with a saline-filled syringe in the third port was attached to the IV catheter hub; a sheathed, open-ended platinum electrode was placed in the opposite end. Changes in electric potential and, therefore, IP pO_2 were assessed after stabilization (10 minutes) with a Transidyne General (now Diamond General) Chemical Microsensor 1201 with an electrode potential of −0.680 volts and calibrated with the pO_2 of normal saline at 37 C, as measured with a Dow-Corning 158 blood gas machine. Arterial blood was obtained percutaneously from the tail artery and pH, pO_2, and pCO_2 were determined with the blood gas machine.

Bacteria

Escherichia coli was grown from a single colony off a stock plate of a clinical isolate (obtained from G. T. Rodeheaver, University of Virginia) by incubating in brain-heart infusion (BHI) broth for 18 hours in a 37 C oscillating water bath. Cultures were centrifuged for 12 minutes at 2400 rpm at 4 C and pellets were further washed twice

in 0.9% normal saline. After the second wash, bacteria were quantified using serial dilutions and plate enumerations, and concentrations were adjusted as necessary. *Bacteroides fragilis* (ATCC #23745) was prepared by inoculating 1 mL of a stock chopped meat broth solution into supplemented BHI broth and incubated for 18 hours at 37 C. Cultures were centrifuged for 12 minutes at 2400 rpm and pellets were further washed twice in prereduced Hanks' balanced salt solution. After washing bacteria were diluted serially and quantified in a Coy anaerobe chamber (Coy Manufacturing, Ann Arbor, MI), and concentrations were adjusted as needed.

Sterile feces–barium sulfate suspension was prepared by mixing dried, weighed rat stool with an equal weight of BHI broth, straining through coarse mesh gauze, and mixing in a 1:1 volume:volume ratio with a 10% weight: volume barium sulfate suspension. The suspension was sterilized in a steam autoclave for 15 minutes at 121 C.

Quantitative peritoneal cultures were performed as previously described.[16] Briefly, 10 mL of anaerobic dilution solution was injected into the peritoneum after the animals were killed, animals were agitated for 60 seconds, and lavage fluid was removed after laparotomy, serially diluted, and plated quantitatively both aerobically and anaerobically. Colony-forming units were counted after 48 hours of incubation and total intraperitoneal bacterial counts were calculated.

Statistics

Statistical analysis was performed using unpaired, two-tailed Student's T test with the aid of a MacIntosh Plus® computer and StatWorks® software package (Cricket-Software, Philadelphia, PA).

TABLE 2. *Intraperitoneal pH at Time of Tonometer Implantation and Killing by Group*

Group and Inoculum	Intraperitoneal pH (±SEM) at Time of Tonometer Placement	Intraperitoneal pH (±SEM) at Time of Killing
1. Saline	7.40 ± 0.01	7.40 ± 0.02
2. 10^5 EC	7.39 ± 0.02	7.40 ± 0.01
3. 10^8 EC	7.40 ± 0.02	7.38 ± 0.02
4. 10^9 EC	7.41 ± 0.02	7.40 ± 0.07
5. 10^{10} EC	7.40 ± 0.03	6.60 ± 0.15*
6. 10^6 BF	7.38 ± 0.05	7.42 ± 0.01
7. 10^8 BF	7.39 ± 0.02	7.40 ± 0.02
8. 10^9 BF	7.40 ± 0.01	7.42 ± 0.03
9. 10^{10} BF	7.41 ± 0.01	7.47 ± 0.02*
10. 10^8 EC + 10^9 BF	7.39 ± 0.02	7.42 ± 0.01
11. 1 ml SFA	7.40 ± 0.02	7.41 ± 0.01
12. 10^8 EC + 1 mL SFA	7.41 ± 0.02	7.23 ± 0.02*
13. 10^9 BF + 1 mL SFA	7.42 ± 0.03	7.39 ± 0.01
14. 10^8 EC + 10^9 BF + 1 mL SFA	7.40 ± 0.01	7.15 ± 0.02*

All figures are ±SEM.
* $p < 0.01$ *vs.* control by Student's t test.

Results

Controls (Group 1)

Mean intraperitoneal pO_2 at catheter implantation in controls was 50 ± 0.92 mmHg, and remained stable between 53 and 58 mmHg throughout the study period (Fig. 2). Intraperitoneal pH was 7.40 ± 0.01 (SEM) in controls at time of laparotomy and remained neutral at 6 hours (Table 2). Initial arterial pO_2 was 74 ± 4.3 mmHg, and was noted to be stable through the course of the experiment with a slight but consistent decline at 6 hours, to 62 ± 1.2 mmHg (Fig. 3). At time of tonometer implantation,

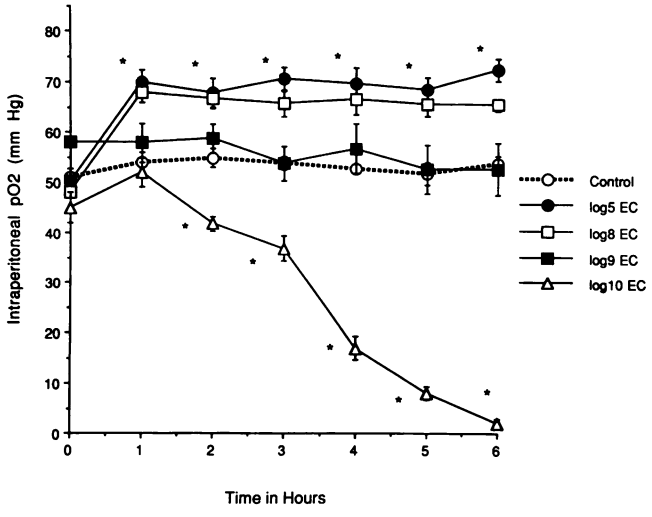

FIG. 2. Effect of different inocula of *Escherichia coli* (EC) on intraperitoneal pO_2. *Indicates $p \leq 0.05$ *versus* uninfected controls; error bars indicate standard error of the mean.

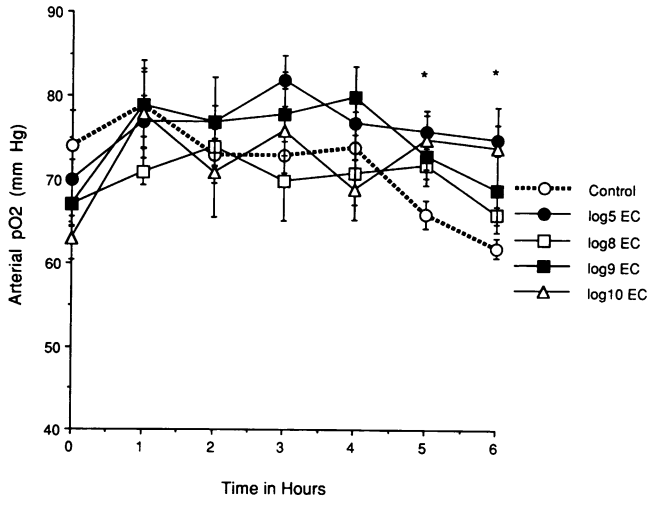

FIG. 3. Effect of different inocula of *Escherichia coli* (EC) on arterial pO_2. *Indicates $p \leq 0.05$ *versus* uninfected controls; error bars indicate standard error of the mean.

arterial pH was 7.38 ± 0.02 and remained neutral, with a final 6-hour value of 7.44 ± 0.02 (Fig. 4). Recovery of peritoneal bacteria from controls was limited to one case of of contamination with approximately 300 gram-positive cocci. Otherwise the peritoneum were noted to be sterile to the limits of detectability (Table 3).

E. coli *Peritonitis (Groups 2 to 5)*

In *E. coli* peritonitis, changes in the intraperitoneal pO_2 were highly inoculum dependent. At inocula well below the LD_{50}, 10^5 and 10^8 colony-forming units (cfu), intraperitoneal pO_2 increased significantly ($p < 0.01$ by unpaired Student's T test *versus* controls) by 1 hour and remained elevated through the ensuing 6 hours. With an inoculum of 10^9 cfu (approximate LD_{50}), the intraperitoneal pO_2 decreased back to that found in controls. With 10^{10} cfu, a lethal inoculum, intraperitoneal pO_2 was unchanged early, but by 3 hours had decreased significantly ($p < 0.01$) below controls, and by 6 hours had decreased to a mean of 6 ± 1 mmHg (Fig. 2). Frequently death ensued shortly thereafter. At time of tonometer implantation, intraperitoneal pH was neutral in all groups and was not different ($p > 0.1$) when the animals were killed in all but the animals receiving 10_{10} *E. coli*, where the intraperitoneal pH at 6 hours was 6.60 ± 0.15, significantly ($p < 0.01$) less than control (Table 2). The arterial pO_2 in the infected groups was similar to controls ($p > 0.1$) at time zero and sustained stable values through the time that the animals were killed; due to the decline in control arterial pO_2 at 5 and 6 hours, however, arterial pO_2 was slightly greater than controls in most groups (Fig. 3). From 0 to 2 hours, arterial pH was not significantly different from controls ($p > 0.1$). In the groups with the two lowest

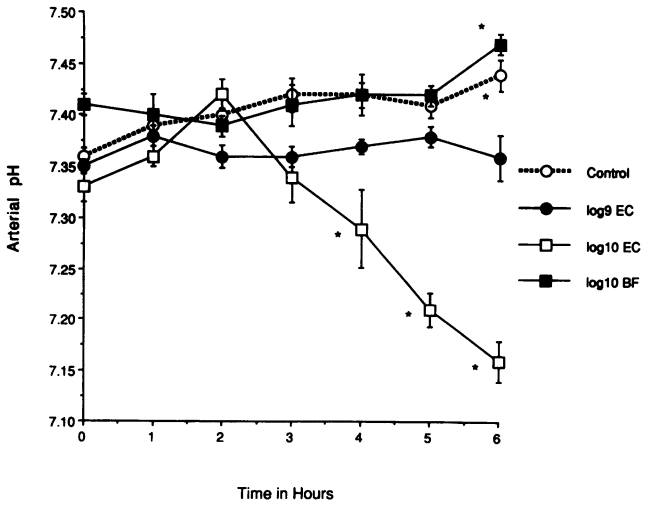

FIG. 4. Effect of selected monomicrobial *Escherichia coli* (EC) or *Bacteroides fragilis* (BF) inocula on arterial pH. *Indicates $p \leq 0.05$ *versus* uninfected controls; error bars indicate standard error of the mean.

TABLE 3. *Intraperitoneal Bacteria Recovered at Time of Killing in Groups with Monomicrobial Peritonitis*

Group and Inoculum	Log CFU *E. coli* Recovered	Log CFU *B. fragilis* Recovered
1. Saline	≤2	≤2
2. 10^5 *E. coli*	4.0 ± 0.3	≤2
3. 10^8 *E. coli*	7.3 ± 0.8	≤2
4. 10^9 *E. coli*	7.7 ± 0.2	≤2
5. 10^{10} *E. coli*	10 ± 1.1	≤2
6. 10^6 *B. fragilis*	≤2	4.9 ± 0.4
7. 10^8 *B. fragilis*	≤2	7.4 ± 0.8
8. 10^9 *B. fragilis*	≤2	8.3 ± 0.2
9. 10^{10} *B. fragilis*	≤2	9.3 ± 0.2

inocula, arterial pH remained neutral throughout the study period. With 10^9 cfu, however, arterial pH became mildly but significantly ($p < 0.05$) less than controls at 3 hours and remained that way through 6 hours, with the exception of the 5-hour time period; the final pH value was 7.36 ± 0.02. In animals receiving 10^{10} *E. coli*, the arterial pH became and remained significantly ($p < 0.01$) lower at 3 hours and declined thereafter, reaching a low of 7.16 ± 0.03 at 6 hours (Fig. 4). As would be expected, the number of *E. coli* recovered when the animals were killed was proportional to the inoculum. Roughly one log fewer bacteria were recovered than implanted; the notable exception was those animals receiving 10^{10} *E. coli*, where 10^{10} cfu were recovered as well (Table 3).

B. Fragilis *Peritonitis (Groups 6 to 9)*

With simple *B. fragilis* peritonitis, intraperitoneal pO_2 at time zero was similar to controls. Over time and through the time that the animals were killed, a very low inoculum, 10^6 cfu, produced no effect on intraperitoneal pO_2, while higher inocula of 10^8, 10^9, or 10^{10} organisms caused a significant ($p < 0.01$ *versus* controls) increase by 1 hour that was sustained through 6 hours (Fig. 5). Intraperitoneal pH was neutral at the time of laparotomy in all groups and was neutral when animals were killed in all except those animals receiving 10^{10} *B. fragilis*, where final intraperitoneal pH was 7.47 ± 0.02, significantly greater ($p < 0.01$) than controls (Table 2). Arterial pO_2 was similar to controls ($p > 0.1$) at time zero, and, with the exception of animals receiving 10^{10} cfu, remained stable through time of killing. Again, due to the decline in control arterial pO_2 at 5 and 6 hours, arterial pO_2 was usually slightly, but significantly, greater than controls at these times. In animals receiving 10^{10} organisms, arterial pO_2 peaked at 1 hour at 101 ± 6.6 mmHg, then declined to a stable 89 ± 5.1 mmHg, with a 6-hour value of 87 ± 5.0 mmHg. At all times this value was significantly greater than controls (Fig. 6). Through 5 hours arterial pH was not significantly different ($p > 0.1$) from controls in the experimental groups. In animals inoculated with 10^{10} *B. fragilis*,

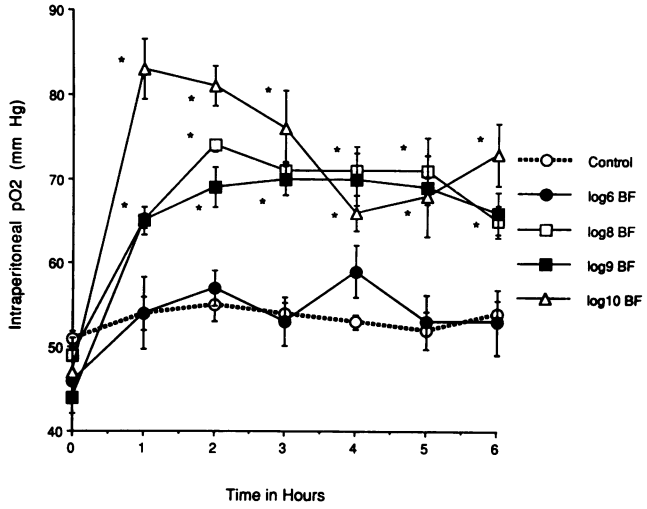

FIG. 5. Effect of different inocula of *Bacteroides fragilis* (BF) on intraperitoneal pO_2. *Indicates $p \leq 0.05$ *versus* uninfected controls; error bars indicate standard error of the mean.

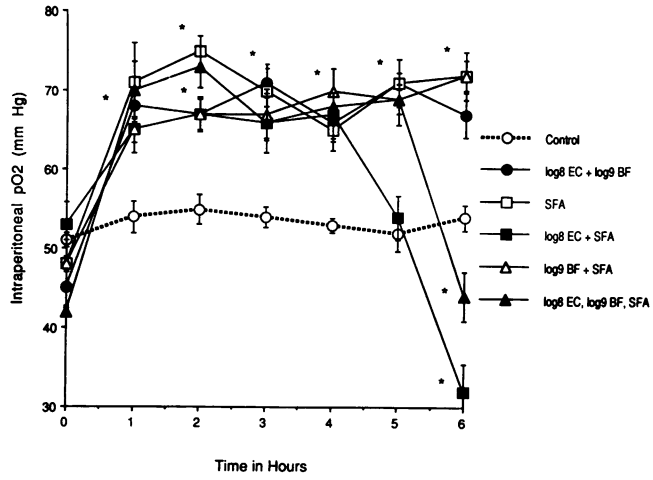

FIG. 7. Effect of mixed *Escherichia coli* (EC) and *Bacteroides fragilis* (BF) or sterile feces adjuvant (SFA) inocula on intraperitoneal pO_2. *Indicates $p \leq 0.05$ *versus* uninfected controls; error bars indicate standard error of the mean.

the arterial pH exhibited a significant ($p < 0.01$) increase to 7.47 ± 0.02 at 6 hours only (Fig. 4). In all groups the number of *B. fragilis* recovered at time of killing was about one log fewer than the inoculum (Table 3). There were no deaths in any *B. fragilis* group.

Mixed E. coli–B. fragilis *Peritonitis Without SFA (Group 10)*

At laparotomy there was no difference in intraperitoneal pO_2 between control and an inoculum of 10^8 *E. coli* plus 10^9 *B. fragilis*. By 1 hour intraperitoneal pO_2 was significantly greater than controls ($p < 0.01$) and remained so through 6 hours (Fig. 7). This increase was similar to that seen with either element of the inoculum alone. Intraperitoneal pH started at 7.40 ± 0.01 and remained neutral at the time animals were killed (Table 2). Arterial pO_2 was not different from controls until hours 5 and 6, when the experimental group maintained a steady value of 75 mmHg, but controls declined slightly to a significantly lower value (Fig. 8). Arterial pH was neutral throughout the study and at no times different from control (Fig. 9). When animals were killed, a mean of 2.1×10^7 *E. coli* and 3.1×10^8 *B. fragilis* were cultured from the peritoneum, with neither significantly different from when either component was inoculated separately (Fig. 10).

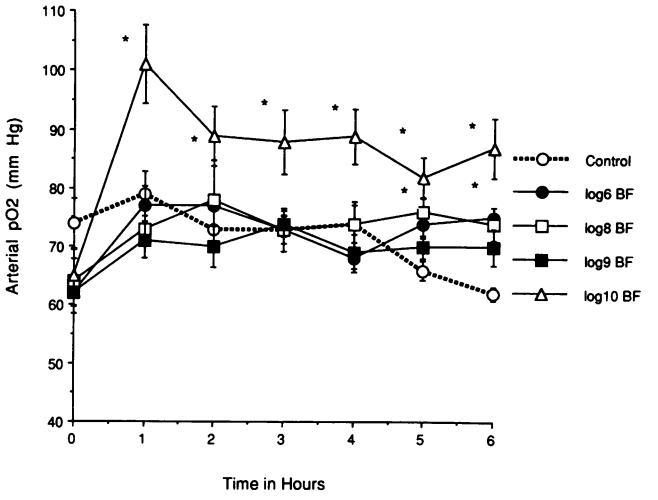

FIG. 6. Effect of different inocula of *Bacteroides fragilis* (BF) on arterial pO_2. *Indicates $p \leq 0.05$ *versus* uninfected controls; error bars indicate standard error of the mean.

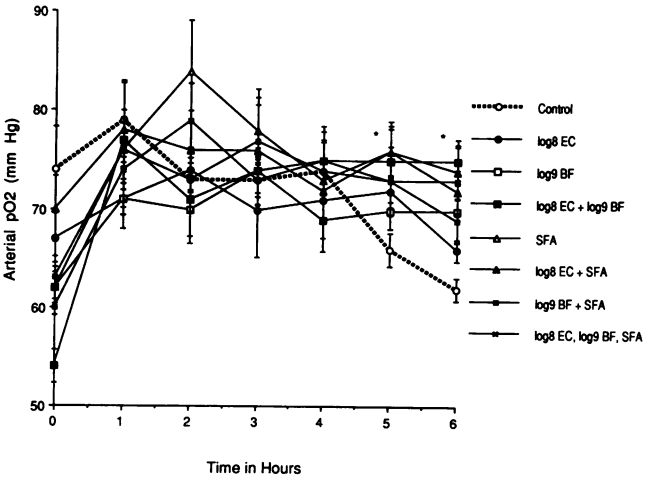

FIG. 8. Effect of mixed *Escherichia coli* (EC) and *Bacteroides fragilis* (BF) or sterile feces adjuvant (SFA) inocula on arterial pO_2. *Indicates $p \leq 0.05$ *versus* uninfected controls; error bars indicate standard error of the mean.

Sterile Feces–Barium Sulfate Adjuvant Enhanced Peritonitis (Groups 11 to 14)

Again there were no differences in intraperitoneal pO_2 between controls and any group at time zero. By 1 hour after tonometer implantation, all treated groups had significantly increased intraperitoneal pO_2 ($p < 0.01$ by Student's T test). In groups receiving the adjuvant alone or with 10^9 *B. fragilis*, this elevation persisted throughout the 6-hour study period. In rats infected with 10^8 *E. coli* and the sterile feces–barium sulfate combination, the intraperitoneal pO_2 elevation was seen through 4 hours, followed by a decline such that by 6 hours intraperitoneal pO_2 had decreased significantly below controls to 32 ± 3.4 mmHg ($p < 0.01$). Rats infected with *E. coli*, *B. fragilis*, and the adjuvant demonstrated an increase in intraperitoneal pO_2 through 5 hours ($p < 0.01$), followed by a rapid decline to 44 ± 3.1 mmHg, significantly less than control ($p < 0.01$) at 6 hours (Fig. 7). Intraperitoneal pH was again 7.40 ± 0.01 at the start in all groups. At 6 hours intraperitoneal pH was significantly different from controls ($p < 0.01$) in the *E. coli* and SFA adjuvant group, where intraperitoneal pH at 6 hours was 7.15 ± 0.02, and in the *E. coli*, *B. fragilis*, and adjuvant group where it was 7.23 ± 0.02 (Table 2). Arterial pO_2 was not different among any group and controls at time zero. In all infected groups, a mild increase was noted by 1 hour and was maintained throughout all 6 hours, with no difference between groups ($p > 0.1$). As previously noted the control group displayed a slight decrease in arterial pO_2 at 5 and 6 hours, yielding a slight but significant ($p < 0.05$) difference compared to the experimental groups (Fig. 8). Through 5 hours arterial pH was consistent and not different from controls ($p > 0.1$). At 6 hours only, the arterial pH was significantly lower ($p < 0.01$) in rats inoculated with *E. coli* and the adjuvant (7.35 ± 0.02) or *E. coli, B. fragilis*, and the adjuvant (7.37 ± 0.01) than in controls (7.42 ± 0.02) (Fig. 9). On culture fewer than 10^2 cfu of bacteria, all gram-positive cocci and diptheroids were recovered from rats inoculated with the sterile feces–barium sulfate combination alone. *E. coli* was recovered in significantly greater numbers ($p < 0.01$) when mixed with either the SFA adjuvant or the adjuvant and *B. fragilis* when compared to groups with *E. coli* alone or with only *B. fragilis*. The number of recoverable *B. fragilis* was not significantly different between groups (Fig. 10).

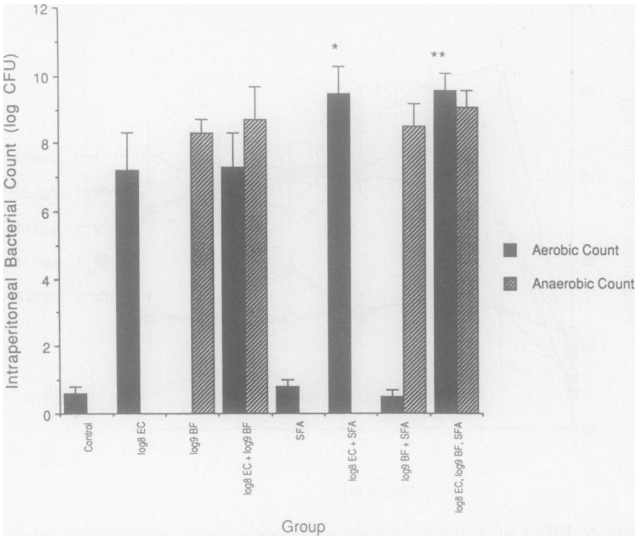

FIG. 10. Intraperitoneal *Escherichia coli* (EC) and *Bacteroides fragilis* (BF) recovered when animals were killed in selected monomicrobial, polymicrobial, and sterile feces adjuvant (SFA)-aided groups. *Indicates $p \leq 0.05$ *versus* log 8 EC without SFA, **Indicates $p \leq 0.05$ *versus* log 8 EC and log 9 BF without SFA.

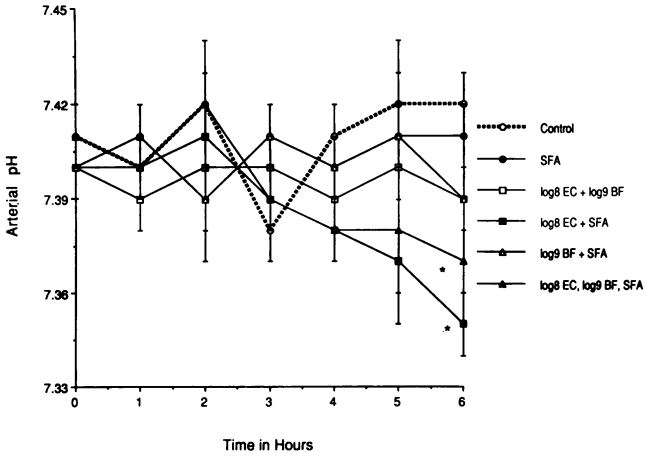

FIG. 9. Effect of mixed *Escherichia coli* (EC) and *Bacteroides fragilis* (BF) or sterile feces adjuvant (SFA) inocula on arterial pH. *Indicates $p \leq 0.05$ *versus* uninfected controls; error bars indicate standard error of the mean.

Discussion

Neither the peritoneal environment nor the response to various insults have been well characterized. Of particular interest in this study are the changes in local pO_2, especially as it affects the growth of aerobic and anaerobic bacteria and their interactions with adjuvant matter, and pH, which is known to be markedly decreased in the presence of ischemic or necrotic tissue.

Although it has been assumed, given the permeability characteristics of the peritoneum, that the peritoneal pO_2 under normal conditions approximates the capillary pO_2, this has not been easily demonstrated. In 1974 Klossner[17] described a technique for its intermittent measurement by the implantation of silastic tubing, which is freely gas permeable,[14] in the peritoneum, and the instillation of anoxic saline into this tubing. After an equilibration time, the saline was removed and assessed for pO_2 and pCO_2

using standard techniques. In 1983 Gottrup[15] developed a method for the continuous direct measurement of tissue oxygen tension using silastic tubing and oxygen polarography. We adapted a combination of these two means in our studies and obtained reproducible, consistent results, with a mean intraperitoneal pO_2 of 52 to 53 mmHg in untreated controls throughout the 6 hours of study. These values were consistently 10 to 11 mmHg higher than those cited by Klossner and probably were influenced by the specific kinds of silastic catheters used and different equilibration times: Klossner using only two minutes, a value derived from *in vitro* studies, and the method used in this study of continuous monitoring of intraperitoneal pO_2 until stabilization at each measurement, a procedure often taking 10 minutes or longer. This technique was used because of simplicity, durability, and the integration of values over a wider area of concern. The question of actual cellular oxygen tension, of course, remains unanswered. The animal model used here also appeared to be more reproducible than that used by Renvall[13] because peritonitis was achieved in all animals studied and was produced with a known inoculum. If one considers their model to be one of overwhelming infection analogous to the sterile stool, *E. coli*, and *B. fragilis* mixture used here, the results are somewhat similar in that they noted a steady decline in intraperitoneal pO_2.

It would be natural to assume that the intraperitoneal pH would approximate neutrality before catheter implantation and after 6 hours of study in the control group. This was observed in the control animals with a pH of 7.4 at initial laparotomy and 7.38 at 6 hours in uninfected animals. As with Renvall's earlier experiments, a decline in intraperitoneal pH was noted with severe peritonitis.

Although monomicrobial, polymicrobial nonadjuvant-aided peritonitis, or pure sterile fecal peritonitis are rare clinical entities, we studied each alone or as combinations as the components of polymicrobial, fecal peritonitis. In the sublethal inocula used here, infections induced with *E. coli* or *B. fragilis* alone, or in combination without sterile fecal adjuvant, effected an increased intraperitoneal pO_2 within 1 hour that persisted through the duration of study, yet no significant alteration in intraperitoneal pH was seen. The same effects were seen with the introduction of sterile feces alone. The lone exception was 10^6 *B. fragilis*, which produced no changes. These data suggest that the peritoneal inflammatory response is a stereotypic response to peritoneal irritation, with the arterial pO_2 unchanged or only slightly increased, indicating a decreased gradient between the vascular and peritoneal spaces. The cause of this phenomenon is not specifically addressed in this study, but it can be speculated that an enhancement of either mesothelial oxygen permeability or oxygen delivery occurs, most likely secondary to insult-induced hyperemia.

Conversely inocula of *E. coli* well above the LD_{50} induced a late and significant decrease in the intraperitoneal pO_2, despite a stable arterial pO_2. Both the intraperitoneal and arterial pH also decline, although the former to a much greater extent than the latter. These findings indicate an increased arterial–peritoneal gradient of oxygen and hydrogen ions. Again these studies do not elucidate the exact etiology of these findings, but there are three possible explanations for this induced local hypoxia and acidity. First the bacteria present may proliferate rapidly enough to exceed the delivery of substrates and the removal of waste products. Second the local host response *via* immune cells also could use local substrates and produce by-products at an increased rate. Third a generalized splanchnic vasoconstriction, secondary to local or systemic factors, could impede the delivery of oxygen and other nutrients to the site of inflammation. Whatever mechanism is present in these overwhelming infections, an environment is produced that encourages the proliferation of anaerobes and impedes the function of host-defense immune cells.

Fecal peritonitis, especially as a prelude to abscess formation, is a markedly more complex situation that is still not completely understood. Animal models have given some details of both host defense[16,18] and bacterial contributions[5,8,10] to this process. In early stages the host response includes the lymphatic absorption of bacteria, the influx of polymorphonuclear neutrophils and macrophages, and the release of opsonins, vasoactive substances, various enzymes, and fibrin-rich fluids. If the infectious insult is not quickly cleared or destroyed, fibrous adhesions form between serosal surfaces, producing loculations that eventually can lead to well-defined abscesses. The role of bacteria has similarly been examined clinically and experimentally.[1–4] Most abscesses are polymicrobial, and, in fact, it is close to impossible to induce abscesses experimentally with a pure culture of a single bacterium, except under very special, and usually artificial, conditions. Furthermore the bacterial synergy between aerobic and anaerobic bacteria in experimental models has been well described:[5] aerobes, usually *E. coli*, are associated with early death and positive blood cultures, while anaerobes, usually *B. fragilis*, are required for consistent intra-abdominal abscess formation.[6,7] The mechanisms underlying this interaction may include the production of growth factors, the release of substances interfering with the immune response, for example, succinate, and alteration of the local environment, *e.g.*, pH, pO_2, or redox potential. Various particulate adjuvants substances have also been shown to alter significantly the outcome of peritonitis, including sterile feces,[10] necrotic tissue,[19] barium sulfate,[8] and bran.[10] Consistent abscess formation, even with a mixed inoculum, also is quite difficult without the presence of adjuvants; whether this is due to decreased

bacterial clearance, augmented bacterial growth, or both is also unclear. This is the question we wished to examine, using a system devised to produce an environment conducive to the formation of abscesses.

Significantly decreased intraperitoneal pO_2 and pH values by 6 hours in the presence of *E. coli*, *B. fragilis*, and SFA were noted in this study. Although this is similar to the environment previously found in mature abscesses, the rapidity with which these changes occur was somewhat unexpected and could not be attributed to a well-localized infection, but, rather, to a generalized condition. The pathogenesis of these changes is clarified somewhat by the observation that the same alterations occur in the presence of *E. coli* and SFA alone, but not with *B. fragilis* and SFA; it would appear that it is the combination of an aerobe and particulate matter that accounts for the hypoxic/acidic milieu. The implication regarding bacterial synergy is obvious.

Regardless of the presence of anaerobes, *E. coli* was noted to be recovered in significantly greater numbers when SFA was added, probably through decreased clearance leading to more viable, reproducing bacteria. The increased oxygen consumption and production of metabolites of these aerobes yields an environment able to promote anaerobic growth. Although *E. coli* proliferation may enhance *B. fragilis* growth and abscess formation seemingly by producing anaerobic conditions, it is still possible that *E. coli* could produce other substances that directly promote anaerobic growth or blunt the immune response. Nevertheless these rapid changes in local environment must be taken into account in the further study of intraperitoneal infection.

The effects of bacteria and particulate matter and their interactions on early and late intra-abdominal infections are complex. These experiments studied the local environment during early peritonitis only but have demonstrated that under certain conditions known to favor the formation of abscesses, such as the presence of *E. coli* and sterile feces, the local environment is altered rapidly. A marked decline in both intraperitoneal pO_2 and pH appears to enhance the ability of anaerobes to multiply and produce chronic mixed infections that are seen commonly in the clinical arena.

References

1. Altemeier WA. The bacterial flora of acute perforated appendicitis with peritonitis: a bacteriologic study based upon one hundred cases. Ann Surg 1938; 107:517–528.
2. Altemeier WA, Culbertson WR, Fuller WD, et al. Intra-abdominal abscesses. Am J Surg 1973; 125:70–79.
3. Lorber B, Swenson RM. The bacteriology of intra-abdominal infections. Surg Clin North Am 1975; 55:1349–1354.
4. Stone HH, Kolb LD, Geheber CF. Incidence and significance of intraperitoneal anaerobic bacteria. Ann Surg 1976; 181:705–715.
5. Onderdonk AB, Bartlett JG, Louie T, et al. Microbial synergy in experimental intraabdominal abscess. Infect Immun 1976; 13: 22–28.
6. Onderdonk AB, Kasper DL, Cisneros RL, et al. The capsular polysaccharide of *Bacteroides fragilis* as a virulence factor: comparison of the pathogenic potential of encapsulated and unencapsulated strains. J Infect Dis 1977; 136:82–89.
7. Weinstein WM, Onderdonk AB, Bartlett JG, et al. Antimicrobial therapy of experimental intraabdominal sepsis. J Infect Dis 1975; 132:282–286.
8. Weinstein WM, Onderdonk AB, Bartlett JG, et al. Experimental intra-abdominal abscesses in rats: development of an experimental model. Infect Immun 1974; 10:1250–1255.
9. Dunn DL, Rotstein OD, Simmons RL. Fibrin in peritonitis: IV. Synergistic intraperitoneal infection due to *Escherichia coli* and *Bacteroides fragilis* within fibrin clots. Arch Surg 1984; 119:149–144.
10. Nulsen MF, Finlay-Jones JJ, Skinner JM, et al. Intra-abdominal abscess formationin mice: quantitative studies on bacteria and abscess potentiating agents. Br J Exp Path 1983; 64:345–353.
11. Walker CB, Williams TD. Use of semisolid agar for initiation of pure *Bacteroides fragilis* infection in mice. Infect Immun 1976; 14:721–725.
12. Mackowiak PA. Microbial synergy in human infections. N Engl J Med 1978; 298:83–86.
13. Renvall S, Niinkoski J. Intraperitoneal oxygen and carbon dioxide tensions in experimental adhesion disease and peritonitis. Am J Surg 1975; 130:286–292.
14. Niinkoski J, Hunt TK. Measurement of wound oxygen with implanted silastic tube. Surgery 1972; 71:22–26.
15. Gottrup F, Firman R, Chang N, et al. Continuous direct tissue oxygen tension measurement by a new method using an implanted silastic tonometer and oxygen polarography. Am J Surg 1983; 146:399–403.
16. Dunn DL, Barke RA, Knight NB, et al. Role of resident macrophages, peripheral neutrophils, and translymphatic absorption in bacterial clearance from the peritoneal cavity. Infect Immun 1985; 49: 257–264.
17. Klossner J, Kivisaari J, Niinkoski J. Oxygen and carbon dioxide tensions in the abdominal cavity and colonic wall of the rabbit. Am J Surg 1974; 127:711–715.
18. Dunn DL, Barke RA, Ewald DC, et al. Macrophages and translymphatic absorption represent the first line of host defense of the peritoneal cavity. Arch Surg 1987; 122:105–110.
19. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock—a review of laboratory models and a proposal. J Surg Res 1980; 29:189–201.