Specialty Conference

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Bacteriology of the Gut and Its Clinical Implications

The bacteriology of the gastrointestinal tract is rapidly changing in laboratory techniques and clinical correlations. The flora is found to be very complex, predominantly anaerobic, and importantly dependent on diet. An etiologic role for colon bacteria in colon cancer is suggested by correlations between epidemiologic data and prevalent dietary patterns and stool culture findings. Cultures from aspiration pneumonia, subphrenic abscess, and other intra-abdominal sepsis all yield anaerobes, and for best results antibiotic therapy should combat them as well as aerobes.

Surgical Considerations

DR. CLARKE:* Welcome to a tour of the darkest reaches of the gastrointestinal tract with swab and culture tube. We shall focus on selected topics of current interest to our faculty and we must here ignore other exciting fields of recent advance in which the bacteria in the bowel play a central role.

It seems valuable to recall that the lumen of the gastrointestinal tract is really outside of the body, part of the "milieu extérieure" as conceived by Claude Bernard. Topologically, it is the hole in the doughnut. Its contents do not achieve the same constancy of osmotic and chemical composition as do the blood plasma and extracellular and intracellular fluids. Moreover, its lower and

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uppermost reaches are the normal habitat of enormous numbers of bacteria, and in between the bacteria colonize with variable success. It is natural that these bacterial swarms affect in many ways the person on whose surface they live.

The magnitude of these interactions between intestinal bacteria and host becomes understandable when we note the surface area potentially involved. Davenport[1] states: ". . . the area of a normal man's small intestinal mucosa is of the order of 75,000 square centimeters and of his colon 2,500 square centimeters. Microvilli on the intestinal epithelial cells increase their surface area 600 times; therefore, the total absorptive area of the human small intestine is about 4,500 square meters." This is nearly the same surface area as that of our standard football field, which measures 300 feet between goal lines and 160 feet in width. Even ignoring the microvilli, the surface of the small intestine measures 7.5 square meters. Recalling that the external surface of a man 6 feet tall weighing 175 pounds is 2 square meters, it is clear that the intestinal surface is large enough to cause a lot of mischief as well as good.

In this conference our attention will be confined to the normal flora of the gut and the effects of these bacteria when in their usual location (Dr. Finegold), when displaced to other levels of the gastrointestinal tract (Dr. Clarke), and when displaced outside the gut lumen (Drs. Bartlett, Wilson, Gorbach).

There is now ample evidence that both aerobes and anaerobes usually increase in concentration in the small bowel after gastrectomy, vagotomy and drainage, ileostomy, and extensive small bowel resection.[2] The sharp increase in concentration of bacteria in the cecum over the terminal ileum suggests that the ileocecal valve is an important barrier to cephalad colonization of the colonic microflora.[3]

Greenlee and associates[4] performed vagotomy and antrectomy on 20 patients with duodenal ulcer requiring elective surgical operation. They found a consistent and pronounced increase in aerobic and anaerobic organisms of the jejunum, and could find no correlation between bacterial proliferation in the jejunum and gastric secretory output of acid. However, Broido and his colleagues[5] found in dogs that the intestinal bacterial content increased when acid secretion was reduced by gastrectomy or by truncal vagotomy and antrum resection, but not when the vagal fibers to the intestine were cut. From this they concluded that gastric acid is more important than vagal motor factors in regulating the microflora of the small bowel.

Small intestinal overgrowth of bacteria may be clinically benign or may cause one or more manifestations of the surgical malabsorption syndrome.[2] These manifestations include vitamin B_{12} deficiency due to bacterial binding of B_{12}, steatorrhea resulting from deconjugation or dehydroxylation of the bile salts required for micelle formation, diarrhea, protein malnutrition, and impaired absorption of sugars. These can be reversed by appropriate antibiotics taken orally.

The cause of diarrhea after vagotomy is unknown and may be related to the bacterial overgrowth in the jejunum found by Greenlee and associates.[4] Antibiotics have occasionally been found beneficial for continuous diarrhea,[6] but cases are rare and planned trials of antimicrobial agents lacking. Episodic diarrhea, however, occurs with sudden onset and brief duration of attacks, and it is hard to attribute this to vagal denervation of the small bowel, whose motor effect should be continuous. Perhaps newer knowledge of anaerobic bacterial overgrowth and appropriate antibiotics required to suppress it will lead to a solution of this problem.

Hepatic encephalopathy following portacaval shunt is another untoward surgical outcome due to excessive ammonia production by gut bacteria acting on a protein substrate. Peloso, Jacobson, and Silen[7] observed in patients with shunt that there was a worsening of the encephalopathy after a later acid-reducing operation for ulcer disease. Hypochlorhydria resulted in increased ammonia production, probably due to increased bacterial concentrations in the small intestine.

Finally, the value of oral non-absorbable antibiotics for bowel preparation before elective colon operations has been under study for 15 years. Although Gaylor et al[8] found no advantage for oral kanamycin or oral neomycin over a placebo with regard to postoperative infection and stool bacteriology, recent techniques permit quantitative study of anaerobes, which are now recognized to form the major constituent of the feces, and a combination of neomycin and erythromycin base has been shown to suppress both anaerobes and aerobes in the colon.[9] Preliminary studies show a pronounced reduction in incidence of wound infections in patients so prepared as compared with patients having only mechanical bowel cleans-

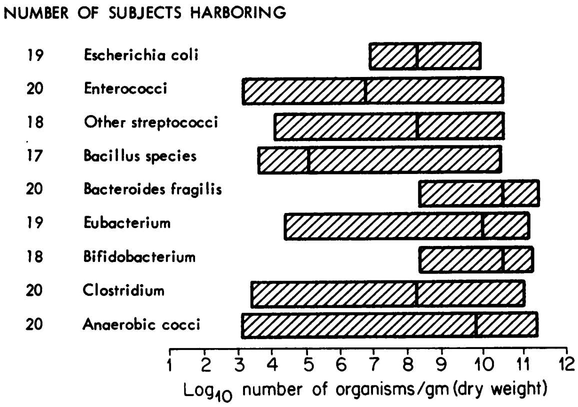

Chart 1.—Predominant human fecal flora: 20 specimens from 18 subjects. The length of the blocks represents the range; the vertical line within the block shows the median count in those subjects who harbored the organism.

ing.[10] Further randomized studies are needed to settle firmly this important question, since the incidence of wound infection in elective colon operations is reported as 10 to 20 percent.

Normal Intestinal Flora: Makeup and Possible Relation to Bowel Cancer

DR. FINEGOLD:* There are intriguing relationships between diet, intestinal microflora, and bowel cancer. We shall present some data on normal fecal flora, the influence of diet on this flora, and means by which intestinal bacteria may be involved in cancer of the colon.

Inadequacies of Bowel Flora Studies

Our knowledge of the normal colonic flora is far from complete; there are several deficiencies that account for inaccuracies in previously published studies:

- The moisture content of feces is variable and may significantly influence counts based on wet weight of stool. Correcting counts to dry weight avoids this problem.[11]
- Both the water content and bacteria are distributed irregularly through the fecal mass. Accordingly, one must homogenize the entire bowel movement (under anaerobic conditions) before taking an aliquot for culture.[12]
- There has been a failure to use adequate anaerobic technique; anaerobic jars are not adequate for normal flora studies. One must use either an anaerobic chamber (glove-box) or the "roll-tube" procedure, with reduced culture media.
- There has been a failure to use an adequate battery of selective and differential media. Organisms that are not numerically dominant may still be very important as to metabolic and other activities, but may be completely overlooked unless disclosed by selective or differential culture media.
- There has been a failure to provide proper cultural conditions for organisms with special growth requirements. Methane bacteria, for example, require an extremely low Eh (standard oxidation-reduction potential); it is desirable to incorporate hydrogen in the atmosphere.
- There has been a failure to characterize organisms thoroughly. Surprisingly, a number of studies have even failed to distinguish between facultative and obligate anaerobes. Even generic designations may be in error when shortcuts are taken, particularly with anaerobic bacteria.
- The present taxonomic schemes for anaerobes are not adequate and tests for characterizing these organisms are not all dependable. It is often difficult to demonstrate spores, and Gram-positive organisms not uncommonly de-stain and appear Gram-negative.
- There has been a failure to study mucosa-associated organisms.[13]
- There is no reliable information on the day-to-day variation in colonic flora related to minor variations in diet and other factors.

Study of Normal Fecal Flora

Utilizing techniques described in detail elsewhere,[12] we have determined the flora in 20 specimens from 18 adults without gastrointestinal disease. This study was part of a larger one, to be discussed below, to determine the effect of diet on fecal flora. The subjects were all of Japanese descent; they lived in Los Angeles and ate a predominantly Western diet (one Japanese meal daily or less). The data will be presented in greater detail elsewhere at a later date. The present report will summarize the results and give some specific examples of the complexity of the normal fecal flora.

Chart 1 is an overall summation of the predominant fecal microflora. Note that anaerobes are dominant numerically, outnumbering aerobic and facultative forms by almost 3 logs. Bacteroides fragilis and Bifidobacterium are present in the largest numbers, but Eubacterium (commonly

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TABLE 1.—*Fecal Flora: Facultative Streptococci (20 Specimens)*

Species	*No.**
S. faecalis var. faecalis	15
S. faecalis var. liquefaciens	3
S. faecalis var. zymogenes	3
S. faecium	10
S. durans	1
S. bovis	1
S. equinus ?	2
S. cremoris	4
S. equisimilis	2
S. lactis	2
S. mitis	1
S. salivarius	1
S. sanguis	1
S. species	13

*Number of subjects yielding organism.

TABLE 2.—*Fecal Flora: Eubacterium (20 Specimens)*

Species	*No.**
E. lentum	10
E. aerofaciens	6
E. rectale	4
E. ventriosum	2
E. cylindroides	1
E. limosum	1
E. moniliforme	1
E. tortuosum	1
E. species	19

*Number of subjects yielding organism.

overlooked in fecal flora studies) and anaerobic cocci are also found in high counts. The special techniques used in this study revealed Bacillus species to be present commonly, though in relatively small numbers.

Table 1 shows the large number of facultative streptococcal species present; despite this, 13 strains did not fit into established species. In the case of Eubacterium (Table 2), there were 19 distinct types not identifiable with any of the recognized species; in the case of Clostridium (Table 3), there were 22 unidentifiable groups despite the presence of 18 recognized species. The frequency with which C. ramosum may be found has not been generally appreciated. C. paraputrificum may be of special significance in relation to bowel cancer, inasmuch as this organism has been shown to carry out certain modifications of bile acids that might represent transformation of these compounds toward carcinogenic agents.[14,15] Table 4 shows that a variety of genera, as well as of species, of anaerobic cocci were recovered.

In order to determine to some extent the degree of variability that might be encountered in specimens taken at different times from the same person, duplicate specimens were run on seven subjects with an interval of two weeks to 19 weeks between specimens. The results are presented in Table 5. When organisms were analyzed primarily in broad groups, differences between the two specimens were relatively minor. Problems with the lactobacilli and with Eubacterium lentum were related to the lack of effective selective or differential media for these organisms, which are not numerically dominant. The results were also inconstant with the methane bacteria, perhaps relating to the fact that these organisms are extremely difficult to grow. When species were compared in duplicate specimens, there was quite

TABLE 3.—*Fecal Flora: Clostridium (20 Specimens)*

Species	*No.**
C. perfringens	12
C. ramosum	11
C. paraputrificum	7
C. glycolicum	6
C. aminovalericum	5
C. innocum	5
C. barkeri	4
C. bifermentans	3
C. oroticum	3
C. indolis	2
C. putrefaciens 'B'	2
C. sporosphaeroides	2
C. cochlearium	1
C. fallax	1
C. inulinum	1
C. irregularis	1
C. malenominatum	1
C. sartagoformum	1
C. species	22

*Number of subjects yielding organism.

TABLE 4.—*Fecal Flora: Anaerobic Cocci (20 Specimens)*

Species	*No.**
Acidaminococcus fermentans	5
Veillonella alcalescens	5
Veillonella parvula	9
Ruminococcus albus	6
Ruminococcus bromii	2
Anaerobic Sarcina	1
Peptostreptococcus intermedius	6
P. productus	5
P. micros	4
Peptostreptococcus species	5
Peptococcus magnus	5
P. prevotii	4
P. asaccharolyticus	1
Peptococcus species	2

*Number of subjects yielding organism.

a bit of variation with some of the anaerobes; this very likely reflects problems in both the classification and characterization of these organisms.

Effect of Diet on Fecal Flora

The flora in 20 specimens from the above-discussed 18 persons eating a Western diet was compared with flora in 20 specimens from 15 subjects of Japanese descent also living in Los Angeles but eating a predominantly traditional diet (two Japanese meals per day). The data with regard to differences between the two groups are shown in Table 6. Aerobic counts were higher on the Japanese diet, whereas anaerobic counts and ratio of anaerobes to aerobes were somewhat higher on the Western diet. Despite the latter finding, certain fastidious anaerobes, such as extremely oxygen-sensitive (EOS) forms and spirochetes, were more prevalent on the Japanese diet. Certain Gram-positive nonsporeforming anaerobic bacilli (Eubacterium) were more prevalent on the traditional diet, whereas others (Bifidobacterium) were so on the American diet. Similar findings were also noted with different groups of anaerobic cocci. Clostridium paraputrificum was found somewhat more frequently in subjects on the Western diet. The differences noted have not yet been analyzed statistically.

TABLE 5.—*Fecal Flora: Comparison of Duplicate Specimens (7 Subjects)*

Group	A*	B†	C‡
Coliforms	6	..	1
Streptococci, Group D	6	1	..
Streptococci, other	4	2	1
Lactobacilli	4	3	..
Bacteroides fragilis	7	..	..
Fusobacterium	7	..	..
Eubacterium lentum	1	5	1
Bifidobacterium adolescentis	7	..	..
Bifidobacterium infantis	5	2	..
Peptostreptococcus	6	1	..
Veillonella	6	1	..
Methane bacteria (5 subjects)	3	2	..

*No difference between specimens.
†Present in one, absent in other.
‡Present in both, but 4 log or greater difference.

Relation of Bowel Flora to Colon Cancer

The incidence of colon cancer is generally much higher in developed countries. Japan is a notable exception—a highly developed country with a low incidence of bowel cancer. When Japanese migrate to the United States, however, their incidence of bowel cancer goes up considerably unless they maintain their traditional diet.[16-18] This, of course, indicates that diet plays an important role in bowel cancer. Studies such as that presented herein and others show that diet may influence the bowel flora significantly. The elements of the diet that are common to countries with a high incidence of colon cancer and that are not seen in areas with low bowel cancer rates are the high fat and animal protein content.

There are many reasons to consider that bile acids may be very important intermediaries in bowel cancer.[19] Certain bile acids and bile acid derivatives such as deoxycholic acid and apocholic acid are carcinogenic. A very potent carcinogen, methylcholanthrene, may be produced from bile acids by a series of reactions that can be carried out by bacteria. (However, it is not established

TABLE 6.—*Fecal Flora: Differences Related to Diet (20 Specimens for Each Diet Group)*

Species	Japanese Diet	Western Diet
Aerobic counts	Higher	...
Presence of EOS anaerobes	15/20	5/20
Spirochetes present	3/20	0/20
Lactobacillus	Greater prevalence and variety	...
Streptococcus bovis	Higher count	...
Bacteroides putredinis	0/20	2/20
Bacteroides pneumosintes	2/20	0/20
Unidentifiable bacteroides	More common	...
Eubacterium contortum	5/20	0/20
Eubacterium lentum	16/20	10/20
Bifidobacterium	...	More prevalent
Peptostreptococcus	Much more prevalent	...
Megasphaera	2/20	0/20
Other anaerobic cocci	...	More prevalent
Clostridium paraputrificum	5/20	7/20
Unidentifiable clostridia species	Much more common	...

EOS = extremely oxygen-sensitive.

TABLE 7.—*Predisposing Causes for Aspiration (70 Cases)*

Compromised consciousness	
Alcoholism	23
Cerebrovascular accident	13
Seizure disorder	9
General anesthesia	9
Drug ingestion	8
Miscellaneous	5
Dysphagia	
Neurologic disorder	7
Esophageal stricture	2

that these can be carried out by intestinal microorganisms.) It is known, nevertheless, that intestinal bacteria, particularly anaerobes, are capable of modifying bile acids in a variety of ways, including dehydroxylation, which is responsible for the production of the secondary bile acid deoxycholate.

It has also been shown that vegetarians have a lesser amount of bile acids in their feces than people on a mixed diet, and that fewer of the anaerobes from the former group are capable of dehydroxylating primary bile acids.[20]

In addition to direct production of a carcinogen by bacteria, other ways in which intestinal bacteria might lead to malignant change include modification of the host response to carcinogens or modification of the host response to cancer.[19]

The Bacteriology of Pulmonary Infections Following Aspiration

DR. BARTLETT:* Aspiration pneumonia encompasses a variety of pulmonary conditions with diverse manifestations. Often the clinical features suggest infection, but the pathogens in such cases have not been adequately studied because of the failure of previous investigators to obtain reliable specimens or to utilize optimal laboratory methods.

We have been conducting a prospective study of pulmonary infections following suspected aspiration during the past four years. The bacteriological findings in 70 patients are the subject of this report. A detailed analysis of 54 of these cases has been previously reported.[21]

The criteria for inclusion in this study were (1) observed aspiration or a predisposition to aspirate due to altered consciousness or dysphagia, (2) roentgenographic evidence of an inflammatory response in a dependent pulmonary segment, and (3) availability of a reliable specimen for culture before antimicrobial therapy. Culture sources utilized were percutaneous transtracheal aspirates (63 cases), empyema fluid (eight cases), or positive blood culture (four cases). (Two of these specimen sources were utilized in three cases.) Expectorated sputum cultures were not considered acceptable, as these specimens are not valid for anaerobic culture and are often unreliable with aerobic culture.[22-24]

Percutaneous transtracheal aspirates and pleural fluid specimens were processed immediately or were held in tubes containing oxygen-free gas. In all instances the duration of air exposure from the time of collection until incubation was limited to 20 minutes and seldom exceeded 10 minutes. Media routinely used were: eosin-methylene-blue agar for incubation in air; blood agar and peptic digest of blood (Fildes' extract) agar for incubation in 5 percent CO_2; Brucella base-menadione-blood agar and a selective agar (kanamycin-vancomycin laked-blood agar or neomycin-blood agar) for incubation in anaerobic GasPak jars; and pre-reduced, anaerobically sterilized, chopped-meat glucose broth inoculated under oxygen-free CO_2. Plates incubated in air and 5 percent CO_2 were read at 24 hours. GasPak jars were not opened until after 48 hours' incubation and were held a total of seven days. Chopped-meat glucose broth was subcultured at 72 hours and at three weeks. Aerobic and facultative isolates were identified by conventional schema. Anaerobic isolates were identified by criteria listed in the Anaerobic Laboratory Manual of the Virginia Polytechnic Institute and State University.[25]

Table 7 summarizes the associated conditions that predisposed these patients to aspiration. Most of the patients had altered consciousness as a result of alcoholism, cerebral vascular accident, seizure disorder, general anesthesia, or drug ingestion. Less common conditions causing compromised consciousness included cardiogenic shock, smoke inhalation, hepatic coma, encephalitis, and Korsakoff's syndrome. Nine patients had dysphagia as a result of a neurological disorder not associated with compromised consciousness or due to an esophageal stricture. (Six patients had two of the conditions listed.)

The types of pathologic processes according to chest x-ray appearance were pneumonitis (38 cases), solitary pulmonary abscess (20 cases) or

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multiple small pulmonary abscesses (12 cases). There was associated empyema in eight cases.

Localization of the pulmonary infection indicated the following sites were principally involved:

Site	*Number*
Right upper lobe, posterior segment	16
Right lower lobe, superior segment	9
Right lower lobe, basilar segments	13
Left upper lobe, posterior apical segment	4
Left lower lobe, superior segment	5
Left lower lobe, basilar segments	8
Bilateral lower lobes, basilar segments	15

Bacteriological results are summarized in Tables 8 and 9. In 52 cases (45 percent) anaerobic bacteria were the only pathogens isolated, nine patients (13 percent) had an exclusively aerobic flora, while 29 (41 percent) had a combination of both aerobes and anaerobes. In 16 cases a single organism was recovered in pure culture. The remaining 54 patients had multiple isolates with an average of 3.3 kinds of bacteria per case; this included 2.3 anaerobes and 1 aerobe. Thus, the flora in most of these patients was complex, a finding in contrast to our experience with other types of bacterial pneumonitis in which a single organism (usually the pneumococcus) is recovered in pure culture.

As to specific microorganisms (Table 9), the predominant anaerobic isolates were Bacteroides melaninogenicus, Fusobacterium nucleatum, and anaerobic Gram-positive cocci. Bacteroides fragilis was found in 10 instances; the recovery of this organism may have important therapeutic implications because B. fragilis is usually resistant to commonly used antibiotics such as penicillins, cephalosporins, and tetracycline.

The predominant aerobic and facultative bacteria were Diplococcus pneumoniae, Staphylococcus aureus, enteric Gram-negative bacilli, and pseudomonads. These organisms were seldom recovered in pure culture and in all but nine cases anaerobic pathogens were present concurrently.

Although bacteriological patterns were sought, consistent associations were generally not apparent. That is, the nature of the isolates was similar regardless of the roentgenographic appearance, predisposing condition, or eventual outcome. It was noted, however, that the 32 cases of aspiration pneumonia that occurred while the patient was in hospital were more likely to involve mixtures of aerobes and anaerobes (Table 8), in contrast to the 38 cases of community-acquired

TABLE 8.—*Types of Bacteria Recovered in Aspiration Pneumonia*

Origin	*Cases*	*Anaerobes only*	*Aerobes only*	*Anaerobes and Aerobes*
Hospital-acquired	32	7	6	19
Community-acquired	38	25	3	10
TOTALS	70	32	9	29

TABLE 9.—*Bacteriology of Aspiration Pneumonia (70 Cases)*

Anaerobic Gram-negative bacilli		
Bacteroides melaninogenicus	27	(1)*
B. fragilis	10	(1)
B. oralis	9	
B. corrodens	2	
B. pneumosintes	1	
Fusobacterium nucleatum	19	
F. necrophorum	1	
Unidentified	4	(1)
Anaerobic cocci		
Peptostreptococcus	23	(5)
Peptococcus	11	(1)
Microaerophilic streptococcus	9	(1)
Veillonella	4	
Anaerobic Gram-positive bacilli		
Eubacteria	6	
Propionibacteria	6	
Bifidobacteria	2	
Clostridia	2	
Aerobic Gram-positive cocci		
Diplococcus pneumoniae	11	(2)
Staphylococcus aureus	11	(2)
Enterococcus	3	
Group A β-hemolytic streptococci	2	
Aerobic Gram-negative bacilli		
Klebsiella sp.	8	(2)
Pseudomonas aeruginosa	7	
Escherichia coli	6	
Enterobacter cloacae	4	
Hemophilus influenzae	2	
Citrobacter freundi	1	
Pseudomonas maltophilia	1	

*In parentheses: number recovered in pure culture.

infections, which usually yielded only anaerobes. This finding was particularly apparent with the enteric Gram-negative bacilli and pseudomonads: 16 of the 22 patients with infections involving these organisms acquired their disease while in hospital.

The therapeutic implications of our findings indicate that, as with other types of infections, antibiotic selection is best determined by the results of proper processing of a reliable specimen. Expectorated sputum should not be used in these cases, as these specimens are not valid for anaerobic culture and are frequently unreliable with aerobic culture. When empirical therapy is necessary for infections following aspiration, agents that are active against anaerobes should be included, since these organisms were incriminated in 87 percent of the cases reviewed.

Changing Patterns in Diagnosis and Treatment of Subphrenic Abscess

DR. WILSON:* In the early 1900's, the likelihood of subphrenic abscess following appendectomy was approximately 0.9 to 1.5 percent.[26] Although the frequency of this postoperative complication has decreased to the present incidence of 0.3 to 0.4 percent, subphrenic abscess warrants reconsideration for several reasons. Almost half of all intra-abdominal abscesses are located in the subphrenic area. The alarming mortality of 20 to 40 percent appears relatively constant despite the general availability of skilled anesthesia, the use of antibiotics, and improved knowledge of surgical metabolic problems. This mortality is related in part to difficulties in diagnosis, with resultant delay of surgical treatment for weeks. The subphrenic abscess has not been a static syndrome, and perhaps its clinical vicissitudes account for the difficulty in prompt diagnosis and treatment.

Originally, perforated peptic ulcer and appendicitis accounted for the majority of subphrenic abscesses. Improved surgical treatment of these conditions has reduced their etiologic importance, and now most subphrenic abscesses occur as postoperative complications of elective duodenal ulcer and biliary tract procedures. Table 10 indicates the related procedures in a series of 88 subphrenic abscesses encountered between 1958 and 1971 at the UCLA Medical Center and the Veterans Administration Wadsworth Hospital Center. Gastric and biliary operations together accounted for almost 60 percent of the subphrenic abscesses. Surgical operation for disease of the large bowel, the third most common cause, was responsible for 15 percent of subphrenic infections.

The topography of the subphrenic area, long a matter of anatomical confusion, was clarified by Boyd's classical article in 1966.[27] He established that the coronary ligament has a dorsal attachment, with a large suprahepatic space on each side. Therefore, the area between the diaphragm and the transverse colon is divided into four subphrenic spaces: right and left subdiaphragmatic, subhepatic, and lesser sac. Previous anatomical descriptions were often based on pathological findings, so that the spaces described were actually defined by pyogenic membranes.

*Samuel E. Wilson, MD, Assistant Professor of Surgery, UCLA School of Medicine, and Chief, General Surgery Section, Surgical Service, Veterans Administration Wadsworth Hospital Center, Los Angeles.

TABLE 10.—*Subphrenic Abscess: Related Surgical Procedures UCLA-Wadsworth, 88 Patients*

Surgical Procedure	*No.*	*Percent*
Stomach	27	31
Biliary tract	25	28
Colon	15	17
Appendix	7	8
Other (Spleen, Pancreas, Trauma)	14	16

TABLE 11.—*Subphrenic Abscess: Location*

	1909		*1942*		*1973*	
Site	*%*	*No.*	*%*	*No.*	*%*	*No.*
Right subphrenic	56	469	50	50	30	26
Left subphrenic	37	296	10	10	45	40
Subhepatic	..	..	35	35	8	7
Combined	3.5	28	6	6	17	15

Reports: 1909, Piquand[28]; 1942, Hochberg[29]; 1973, UCLA-VA.

In the UCLA-Wadsworth group of 88 patients, the abscesses were mainly localized to the right and left subdiaphragmatic areas, with the subhepatic and lesser sac spaces involved less frequently (Table 11). Early reports[28,29] on subphrenic abscess indicate that the right subdiaphragmatic space was by far the commonest location. Appendicitis is now less frequently associated with abscess formation, and the trend is for the left side of the abdomen to be more frequently involved. In most series, infection of the right subphrenic space is still more common, probably due to localization of infection following cholecystectomy and appendectomy to this area. Of greater significance is the large percentage of multiple space involvement necessitating a transabdominal approach for drainage.

The bacteriology of the subphrenic abscess, although still quite similar to that of the preantibiotic era, has shown some changes. Multiple flora were encountered in 36 percent of the 67 patients who had adequate bacteriologic study, in contrast with the pure cultures usually found before general antibiotic use. The three predominant organisms are still E. coli, Streptococcus and Staphylococcus (Table 12), which was also the case in Ochsner and DeBakey's 1938 study.[30] The frequency of Streptococcus has declined somewhat, however, to be replaced by Gram-negative organisms such as Klebsiella-Aerobacter and Proteus. Early antibiotic administration has apparently reduced the number of positive blood cultures, and secondary complications such as

TABLE 12.—*Subphrenic Abscess: Bacteriology UCLA-VA Study, 65 Patients*

Organism	No.	Percent
Escherichia coli	39	60
Streptococcus	32	49
Staphylococcus	20	31
Klebsiella-Aerobacter	11	17
Proteus	7	11
Pseudomonas	5	8

TABLE 13.—*Subphrenic Abscess: Mortality*

Year	Study	Number Patients	Percent Mortality
1908	Barnard[32]	76	47
1938	Ochsner and DeBakey[30]	3,608	53
1955	Harley[34]	212	39
1962	Dineen and McSherry[33]	63	43
1973	UCLA-VA	88	33

diaphragmatic perforation, empyema, and free peritoneal rupture are now exceedingly rare.

The widespread use of antibiotics accounts for the slow, insidious nature in which the subphrenic abscess may present today. In a typical case, two weeks after a surgical procedure the patient complains of malaise, mild abdominal pain, and low-grade fever; on examination there may be tenderness along the anterior costal margin, and leukocytosis is present in 90 percent of patients. Carter and Brewer in 1964[31] emphasized the thoraco-abdominal nature of the subphrenic abscess, pointing out that findings related to the chest predominated in 44 percent of their patients. Roentgenograms of the chest may disclose an area of pneumonitis, basal atelectasis, elevation of the diaphragm, or perhaps a small pleural effusion. Combined liver-lung scan is useful in delineating a "cold area" caused by depression of the superior surface of the liver when there is a right subdiaphragmatic abscess.

The mortality from subphrenic abscess has remained surprisingly high (Table 13) despite the promise of extraperitoneal drainage and newer broad-spectrum antibiotics. The prognosis is particularly severe in patients with multiple abscesses, 65 percent of whom may have a fatal outcome. Underlying disease processes (such as malignant disease) and secondary complications (including generalized peritonitis, sepsis, or intestinal fistulization) are associated with poor prognosis. Missed diagnosis and a prolonged interval before surgical drainage are related to increased mortality rates. The mean stay in hospital of 54 days for surviving patients at the UCLA Medical Center reflects the extensive morbidity of the disease.

An extraperitoneal approach has been advocated since 1939 as the optimal route, as avoiding contamination of the intraperitoneal cavity was presumed to lower mortality. In a critical analysis of this traditional view, Halasz[35] recently emphasized that multiple synchronous abscesses occur in as many as 25 percent of patients, and that this group has the highest mortality. The major cause of death was a missed second or even third abscess. In a prospective series of patients, a lower mortality was achieved utilizing a midline transperitoneal approach, careful abdominal exploration, and dependent drainage through the flanks with large-bore sump tubes.

Normal Bowel Flora and Intra-Abdominal Sepsis

DR. GORBACH:* The lower gastrointestinal tract harbors a high density of microorganisms. Anaerobic species—particularly bacteroides, clostridia, and peptostreptococci—are present in total concentration of 10^{11} per gram, outnumbering coliform species by approximately three logs.[36-42] It follows therefore that intra-abdominal sepsis, especially when associated with contamination by bowel contents, will have a mixed flora comprising both aerobic and anaerobic microorganisms. Although this formulation is eminently logical, recent studies[43-51] of abdominal infections do not consider anaerobic bacteria important, assigning this group of microorganisms to curiosities associated with rare diseases. With the advent of sophisticated techniques to culture anaerobes in clinical material,[52,53] this view may have to be revised.

We have studied 46 patients who were referred to the Infectious Disease Service for intra-abdominal sepsis. Included were 32 patients with intra-abdominal abscess, ten with generalized peritonitis, and four with miscellaneous infections such as retroperitoneal and prostatic abscesses and an infected aortic graft. All of these patients had had abdominal surgical operations and had received antibiotics postoperatively for signs of infection. The Infectious Disease consultation was requested because of persisting sepsis.

Predisposing conditions included trauma, generally due to gunshot or knife injuries (22 patients); carcinoma of the colon, pancreas, or kidney (17 patients); intestinal surgical therapy

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with postoperative complications (seven patients); perforated appendix (four patients); cirrhosis with spontaneous peritonitis (three patients); and peritoneal dialysis with intestinal perforation (three patients).

Bacteriologic cultures were obtained from 43 specimens of purulent drainage collected directly from surgical incisions. The other three patients had positive blood cultures, but no abdominal sites were available for culture. Anaerobic techniques utilized oxygen-free conditions and prereduced culture media; these methods have been shown to give a high yield of fastidious anaerobes from clinical material.[36,54] The flora recovered from 33 of the 43 specimens of purulent material contained a mixture of aerobic and anaerobic bacteria; an exclusively anaerobic flora was present in seven patients; three had only aerobic or facultative bacteria. Thus, anaerobic bacteria could be cultured from 40 of 43 wounds (93 percent) in patients with severe intra-abdominal sepsis.

The cultivable flora from intra-abdominal sites was characterized by several isolates of different bacterial species. There was an average of five microorganisms cultured from a single specimen, with a range of one to thirteen isolates; this included an average of two aerobes and three anaerobes. It can be appreciated that intra-abdominal sepsis represents a complex microbial flora with multiple types of bacteria.

The aerobic and facultative microorganisms (Table 14) were similar to those reported in other series. Of note is the low incidence of S. aureus, a reflection of the current era of hospital epidemiology. Gram-negative enteric organisms such as proteus, klebsiella, E. coli, pseudomonas and enterobacter are now isolated with greater frequency.

A large variety of anaerobic species was cultured from these intra-abdominal infections (Table 15). Bacteroides was the most frequent isolate, recovered on 36 occasions; the species Bacteroides fragilis represented 28 of these strains, and other species were present less commonly. Fusobacteria were isolated on six occasions. Clostridial species were isolated nearly as frequently as bacteroides (31 specimens); these included 13 known species and 10 strains that could not be further identified. It is interesting to note that Clostridium perfringens was isolated in only three patients, and was seen as frequently as Clostridium ramosum. None of the patients associated with histotoxic strains of clostridia had clinical signs of gas gangrene. Anaerobic cocci were present in 14 patients, with a preponderance of peptostreptococci. Peptococci were isolated in three patients, and veillonella in one. Gram-positive, nonsporing rods were recovered in 16 pa-

TABLE 14.—*Anaerobic and Facultative Microorganisms Isolated from 46 Intra-abdominal Infections*

Organism	*No.*
Escherichia coli	28
Staphylococcus epidermidis	15
Proteus	10
Klebsiella	9
Pseudomonas	8
Enterobacter	8
Candida	4
Streptococcus	3
Enterococcus	2
Staphylococcus aureus	1

TABLE 15.—*Anaerobic Microorganisms Isolated from 46 Intra-abdominal Wounds*

Organism		*No.*
Gram-negative nonsporing rods		
Bacteroides		36
B. fragilis (28)		
B. melaninogenicus (4)		
B. clostridiiformis (2)		
B. biacutus (1)		
B. species (1)		
Fusobacterium		6
F. varium (1)		
F. necrophorum (1)		
F. species (4)		
Cocci		
Peptostreptococci		11
Peptococcus		3
Pc. magnus (2)		
Pc. prevotii (1)		
Veillonella		1
Clostridia		31
C. ramosum (3)	C. fallax (1)	
C. perfringens (3)	C. beijerinckii (1)	
C. butyricum (2)	C. barati (1)	
C. bifermentans (2)	C. difficile (1)	
C. sporogenes (2)	C. ghoni (1)	
C. innocuum (2)	C. sphenoides (1)	
C. sordellii (1)	C. species (10)	
Gram-positive nonsporing rods		
Eubacterium		11
E. lentum (6)		
E. filamentosum (2)		
E. multiforme (1)		
E. limosum (1)		
E. species (1)		
Propionibacterium		2
Lactobacillus catenaforme		2
Bifidobacterium		1

TABLE 16. *Microorganisms in 13 Cases of Septicemia Associated with Intra-abdominal Infections**

Organism	*No.*
Bacteroides fragilis	8
Bacteroides melaninogenicus	1
Clostridium perfringens	2
Clostridium tertium	1
Clostridium difficile	1
Fusobacterium fusoforme	1
Klebsiella	2

*Two patients had B. fragilis and Klebsiella; one patient had two strains of Clostridia.

tients. We believe at present that Eubacterium species are nonpathogens; all of these isolates were present in mixed cultures, usually associated with bacteroides.

To summarize: of the types of anaerobes isolated from cases of intra-abdominal sepsis, three groups of organisms were most frequently involved: Bacteroides fragilis, clostridia, and anaerobic streptococci, especially the peptostreptococci. This pathogenic triumvirate has been cultured repeatedly from patients with intra-abdominal infections.

Positive blood cultures were present in 13 of the 46 patients (28 percent). Eleven patients had exclusively anaerobic growth and two had mixed aerobic and anaerobic growth, but there were none with exclusively aerobic organisms. As in the cultures from pus, Bacteroides fragilis was the predominant organism isolated in the bloodstream, found in eight of the thirteen positive cases (Table 16). Two of these patients had a mixed culture of Bacteroides fragilis and klebsiella; clostridia septicemia was noted in three patients; one patient had two strains of clostridia—C. perfringens and C. tertium—in his blood on repeated occasions; Bacteroides melaninogenicus and fusobacteria were present in one patient each.

Previous studies[43-51] of the bacteriologic features of intra-abdominal sepsis have suggested that the offending bacteria were usually aerobic, enteric bacilli. Anaerobes were considered a curiosity that caused only special types of infections such as gas gangrene, burrowing ulcers, or necrotizing fasciitis.

The application of rigorous techniques for culturing anaerobes in clinical material has produced a new understanding of the microflora of intra-abdominal infections. It appears that the flora is often a complex ecosystem. There are on the average five bacteria in each infection, and we have seen patients with as many as 13 distinct microbial species in a single infected site.

The recognition of anaerobes in abdominal wounds has important implications for antimicrobial therapy. On the basis of *in vitro* testing, it would appear that certain drugs are acceptable for treatment of mixed aerobic and anaerobic infections, whereas other antibiotics are unacceptable.[55-57] For example, when considering Bacteroides fragilis, the most frequent isolate in intra-abdominal sepsis, the acceptable group of antibiotics includes chloramphenicol and clindamycin as the most satsfactory agents.[58-63] On the other hand, there is good evidence that penicillins, cephalosporins, and aminoglycoside antibiotics have relatively poor activity against B. fragilis. New informaton on antibiotic susceptibilities, combined with the data on anaerobic bacteriology in intra-abdominal infections, should cause a reassessment of the use of antibiotics in such conditions.[64-67]

Discussion

DR. HEWITT:* Dr. Gorbach, actually you are showing the isolation of many clostridia in these patients. I wonder what you think the role of these organisms may be in patients who really don't show gas in tissue and other signs that are ordinarily associated with clostridia infections.

DR. GORBACH: Gas gangrene is a rare event in civilian hospital practice, even when many histotoxic strains of clostridia can be cultured from open wounds. In other studies of penetrating intra-abdominal trauma we showed that the flora of healing wounds, including clostridia, was essentially the same as that of infected wounds. Thus, the decision concerning the presence of infection was clinical, and not based on the bacteriologic findings. This is especially pertinent to the study of clostridia. The most common setting of positive cultures for this organism is simple contamination. We could speculate on the conditions that transform contamination into pathogenic circumstances, such as necrotic tissue, low oxidation-reduction potential, low pH, and calcium. The important lesson for the clinician is not to panic in the face of a positive clostridial culture. More often than not, the organism is either a contaminant or part of the complex flora of an abscess,

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rather than producing the classic picture of gas gangrene and myonecrosis.

DR. CLARKE: Dr. Gorbach, would you comment on the role of sensitivities when you have 20 different bacteria and you are trying to pick an antibiotic?

DR. GORBACH: I will have to take the Fifth Amendment on that question. In fact, it becomes an impossible task for the laboratory to do antibiotic sensitivity determinations on each isolate in the complex flora of an abscess, and an intolerable task for the clinician at the bedside to interpret the data. In my view, our current priorities are misplaced. Rather than the relentless pursuit of individual sensitivity tests, we should be asking basic biologic questions. It is far more pertinent to understand which organism(s) is the pathogen, which is the symbiont, and which is the harmless commensal. It is quite obvious that treating commensals will neither help the patient nor cure the disease. Until these basic questions are answered, we have taken the empirical approach of using two broad-spectrum drugs for mixed infections associated with intestinal perforation. One agent is an aminoglycoside such as gentamicin or kanamycin to control the facultative Gram-negative bacilli; the other agent is selected to control anaerobic bacteria, the current choice being either clindamycin or chloramphenicol. We have now treated more than 50 patients with this empiric regimen, and the results have thus far been satisfying.

DR. CLARKE: What are the chances that anaerobes will develop resistance to the two drugs that you have now? Do you have other drugs waiting in the wings?

DR. GORBACH: It is not a certainty that resistance will develop in microorganisms to all antimicrobials. For example, we have not seen significant resistance to penicillin in Streptococcus pyogenes or Diplococcus pneumoniae, even though this antibiotic has been used in treating disease caused by these organisms for over 30 years. Similarly, we have not noted significant resistance of Bacteroides fragilis to chloramphenicol despite the availability of this agent since 1948. With regard to newer drugs such as clindamycin, we will have to adopt a "watchful waiting" approach. It may require five years of observation with standardized techniques in order to determine whether organisms are progressively developing resistance. We should be aware that it is not inevitable that organisms develop resistance to antibiotics the way we are accustomed to seeing in staphylococcus or pseudomonas.

Question: Dr. Gorbach, do you think the 33 percent mortality that Dr. Wilson mentioned in subphrenic abscess is going to be considerably reduced by the advent of clindamycin? Why is that mortality occurring? Is it because the surgical operation is inadequate or is it because the infection is not well controlled?

DR. GORBACH: This is a difficult question, as there are many factors that relate to surgical mortality. The patients described in our series were often healthy young men who incurred gunshot or knife wounds to the abdomen. On the other hand, Dr. Wilson's patients were generally older persons who had rather low resistance. Thus, the two groups are not readily comparable. I would emphasize that expertise in anaerobic bacteriology and the use of appropriate drugs in these settings may improve the outcome for such patients. This is not to deny the primary importance of adequate surgical drainage of closed-space infections, but we must recognize that proper use of effective antibiotics is a significant adjunct. Some patients will continue to have suppurative infections, bacteremia, and even insidious extension of the process if they are treated with ineffective drugs. Hence, all three elements are important in the cure of chronic intra-abdominal abscesses—good host defenses, judicious surgical drainage, and effective antibiotics, with attention paid to both the aerobic and anaerobic components.

DR. MELLINKOFF:* Dr. Finegold, I thought there was a high incidence of gastric cancer in Japan, and I wonder whether the apparent rise in colonic cancer that you noted when the patients came here from Japan and changed to a Western diet is an effect of the diet that increases the incidence of colonic cancer or whether it simply reflects a reduction in gastric cancer. In other words, if somebody doesn't die of gastric cancer, does he then die of colonic cancer?

DR. FINEGOLD: Well, there certainly is a reduction in gastric cancer as well. Whether this is the only explanation or not I don't know, but I think this is probably not the major explanation. It is

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kind of discouraging, though, when you realize that the Japanese diet is very palatable and that it might be good for reducing the incidence of colon cancer, to think that it might increase the likelihood of gastric cancer, which is certainly a worse disease. Just what the mechanism might be in leading to stomach cancer is entirely unknown. It may be a function of the type of processing of food. The basic food itself may be fine for minimizing risk of colonic cancer, but perhaps something in the processing results in aflatoxins or other carcinogens that might account for the gastric cancer.

Question: Dr. Finegold, suppose a stool culture is submitted from a patient who has diarrhea and who hasn't been receiving antibiotics. The laboratory reports Staphylococcus aureus. What are the conditions that allow that to happen, aside from possibly some error in the procedure?

DR. FINEGOLD: Was this patient in hospital? Our experience is that it is uncommon to get S. aureus in the stool unless the patient has recently been receiving antimicrobial therapy, which suppresses some of his indigenous flora, allowing the staphylococcus to colonize. It is true that staphylococci and other potential pathogens—klebsiella and pseudomonas, for example—may colonize the upper respiratory tract in the absence of antimicrobial therapy in sick patients who are in hospital, and perhaps this happens in the gut too. Small numbers of staphylococci may be seen in the absence of disease, and the use of a highly selective culture medium might reveal them.

Question: Dr. Bartlett, your implications about flora in aspiration pneumonia are very interesting. Men in practice often treat these patients with penicillin. Since you isolated B. fragilis in ten cases, do you advocate another agent?

DR. BARTLETT: This is a difficult question—one that cannot be answered with currently available information. When we recovered B. fragilis it was always part of a complex flora which included other anaerobes as well. It is conceivable that antibiotic therapy directed against other components of the infection would be adequate. In our patients, having isolated B. fragilis, we were unwilling to test this hypothesis.

Question: Was there anything unique about the bacteriology of the various types of infections? Did any particular anaerobe show a propensity to cause a particular type of x-ray picture?

DR. BARTLETT: We have subsequently done that type of analysis by correlating the bacteriological results with the type of pathogenic process. To summarize the findings among 140 cases of anaerobic infection: There was no particular pattern for pneumonitis, empyema, or cavitating pulmonary infections. In other words, given the various forms of anaerobic infections, the bacteriology was similar both in total and specific isolates.

DR. CLARKE: Dr. Bartlett, I'd like to ask why there are so many anaerobes in the lung infections. Are you implying that there is overgrowth in the upper gastrointestinal tract in those patients; are those anaerobes in the food that those patients have eaten recently, or what?

DR. BARTLETT: Most of these cases of aspiration pneumonia presumably result from aspiration of oropharyngeal contents. The mouth harbors a very rich flora that includes both aerobes and anaerobes. In our laboratory, analysis of saliva in people with good dental hygiene yields five to ten anaerobes for every aerobe. The relative proportion of anaerobes would be even higher in the presence of dental infections. I assume that saliva is the inoculum in most patients with aspiration pneumonia.

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