

Bacteriologic Studies of Biliary Tract Infection

ROBERT J. FLEMMING,* M.D., LEWIS M. FLINT, M.D.,
SUYDAM OSTERHOUT, M.D., WILLIAM W. SHINGLETON, M.D.

*From the Departments of Surgery and Medicine, Duke University Medical Center,
Durham, North Carolina 27706*

ASPIRATION and culture of intrahepatic bile at the time of percutaneous transhepatic cholangiography have provided a unique opportunity to study intrahepatic bacterial flora in biliary tract disease. These culture data are particularly useful in correlating the pathophysiology and bacteriology of biliary tract infection since the type of obstruction is accurately shown with the percutaneous cholangiogram and the pathology confirmed by operation. The correlation of this objective data with the patient's symptomatology has led to a clearer understanding of the term "cholangitis." The major portion of this study is concerned with the data on intrahepatic bile culture at the time of percutaneous cholangiography. Additional bacteriologic data on a smaller group of patients undergoing cholecystectomy for chronic cholecystitis without common duct obstruction is presented.

Methods

The patients studied are divided into two major groups (Table 1). The largest consists of 75 patients undergoing percutaneous cholangiography. This procedure, previously described,⁴ is carried out observing careful aseptic technic. Aliquots of aspirated intrahepatic bile were placed in

sterile tubes and directly plated on appropriate media for aerobic and anaerobic culture. In addition a 1:100 pour plate dilution technic was used to quantitate the culture. Ninety per cent of "positive" cultures had colony counts greater than 100,000/ml. of bile.

The other group of 21 patients, had cultures obtained at the time of cholecystectomy for chronic cholelithiasis and/or cholelithiasis. Cultures of the gallbladder wall, gallbladder bile, common duct bile and the gall stones were performed. No patients with acute cholecystitis were included.

Blood cultures were performed in those patients in whom septicemia was suspected. The culture and identification of clostridia was carried out following operation on the biliary tract in two patients who developed signs and symptoms of clostridial gas gangrene.

Almost all patients in the major group undergoing percutaneous cholangiography had received broad spectrum antibiotics for 12 hours prior to the test.

Results

Patients studied at the time of percutaneous cholangiography fell into two ma-

Presented at the Annual Meeting of the American Surgical Association, May 11-13, 1967, Colorado Springs, Colorado.

* Present address: U. S. Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas 78234.

TABLE 1. Culture

Intrahepatic bile	75
Extrahepatic tract	21
Total	96

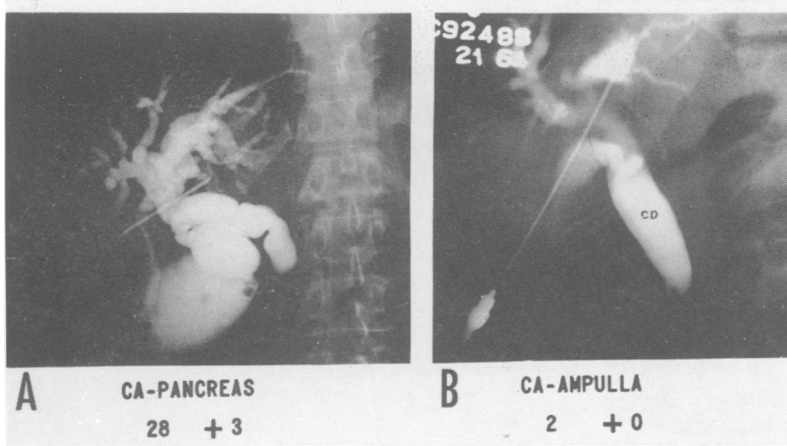


FIG. 1. A) The characteristic complete obstruction of carcinoma of pancreas. B) Characteristic ampullary carcinoma with complete obstruction and nipple-like projection upward into common duct (CD). Numbers indicate number of patients and incidence of positive cultures.

major groups: those with complete obstruction of the common duct as outlined by the cholangiogram and those with partial obstruction as evidenced by flow of some contrast material into the intestinal tract.

Intrahepatic Bile Culture. Seventy-five examinations were performed in 70 patients. Those examined more than once were studied at varying times after operation to evaluate the surgical result or recurrent symptoms.

Table 2 demonstrates the correlation between the degree of obstruction and positive culture. All 30 patients with complete obstruction had carcinoma of the pancreas or ampulla. Figure 1A is a typical cholangiogram found with carcinoma of the head of the pancreas. Figure 1B is the configuration found in the two patients with complete obstruction secondary to ampullary carcinoma. Of these 30 patients only three (10%) had positive cultures. This is in marked contrast to the group with partial obstruction in whom positive

cultures were obtained in 29 of 45 examinations (64%).

The etiology of the partial obstruction was benign stricture in 23 instances, choledocholithiasis in 17 instances, and carcinoma of the bile ducts in five. Figure 2 shows the characteristic partial obstruction seen with stricture (A) and choledocholithiasis (B). The obvious difference in positive cultures obtained from patients with partial obstruction as compared to complete obstruction is also statistically significant with a $p < 0.001$.

Table 3 contains the entire data presented in a slightly different manner to

TABLE 2. *Intrahepatic Bile Culture*

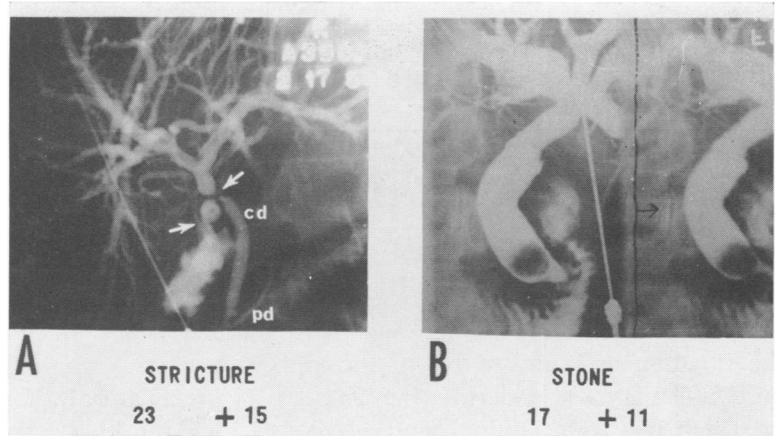
	Posi- tive	Nega- tive	% Positive
Complete obstruction	3	27	10
Partial obstruction	29	16	64

$p < 0.001$

TABLE 3.

Total No. Patients	96			
Obstruction	[Pos. 75]		Neg. 21	
Cultures	Pos. 32	Neg. 43	Pos. 11	Neg. 10
Cholangitis	20	6	0	0
No Cholangitis	12	37	11	10
% with Cholangitis	62.5	16.0	0	0
	$p < 0.001$		N.S.	

FIG. 2. A) Partial obstruction due to iatrogenic stricture (upper arrow). Note contrast entering intestinal tract via fistula in A and around calculus in B. Note the shift in position of calculus which quickly changes degree of obstruction. Numbers indicate the number of patients and incidence of positive cultures.



emphasize the relationship between obstruction and positive culture of intrahepatic bile. Also shown are data of 21 studies on extrahepatic bile of the patient with chronic cholecystitis and cholelithiasis.

The 96 patients are divided into 2 groups: 75 in whom obstruction or a history of obstruction was the indication for percutaneous cholangiography and culture of the intrahepatic bile; the other 21 studies were on the extrahepatic biliary tract at the time of elective cholecystectomy where obstruction was not a factor.

Of the 75 patients with obstruction, complete and partial, positive cultures were obtained in 32 and negative cultures in 43.

Of 32 patients with positive cultures 20 had a history of, or at the time of examination, had symptomatic cholangitis. There were 12 patients with positive bile cultures who had never had any symptoms of cholangitis. In the group with negative cultures only six of 43 patients had had symptoms of cholangitis. In 32 patients with positive cultures of intrahepatic bile, only 11 had chills or fever upon admission to the hos-

pital and prior to examination. Two-thirds of the patients with positive cultures in the presence of obstruction still were asymptomatic, i.e., no chills or fever at the time of bile culture (Table 4).

Table 5 lists the results of cultures of bile and blood in a group of five patients who had cholangiographic evidence of partial obstruction of the biliary tract. All gave a history of chills and fever. At the time of admission to the hospital and at the time of percutaneous cholangiograms, however, they were afebrile and completely asymptomatic. Within a few hours of percutaneous cholangiograms they experienced chills and subsequent significant fever. Blood cultures taken immediately after onset of symptoms revealed the same organism as had been obtained from the bile prior to the injection of the contrast material in all five patients. Each described

TABLE 4. Patient Status at Time of Positive Intrahepatic Bile Culture

Patients	32
Asymptomatic (No chills or fever)	21
Symptomatic (febrile or chills)	11

TABLE 5. Patients—Partial Obstruction—Asymptomatic—Cholangitis Induced by test

	Culture	
	Bile	Blood
M. H.	E. coli	E. coli
A. T.	E. coli	E. coli
M. P.	E. coli	E. coli
W. P.	E. coli	E. coli
W. C.	E. coli	E. coli
	Pseudomonas	

TABLE 6. *Biliary-Intestinal Anastomosis—15 Patients*

	Choledocho- jejunostomy Roux—Y	Choledocho- duodenostomy	Cholecysto- jejunostomy
Patients	6	5	4
Positive culture	5	4	3
Negative culture	1	1	1
Afebrile	5	4	3
History cholangitis	2	3	2
Mean serum bilirubin	5.1	15.3	7.2

the situation as "another one of those attacks." The fever subsided by the next day in all but one who required more vigorous treatment for gram-negative septicemia. All recovered and underwent successful operations for correction of the partial bile duct obstruction. The underlying pathologic entities in these five patients were benign stricture in two, choledocholithiasis in two, and carcinoma of the bile duct in one.

Fifteen of 75 patients who had percutaneous cholangiograms had had a previously constructed biliary-intestinal anastomoses. In 12 the procedure was done for nonmalignant duct obstruction, in 3 for carcinoma of the pancreas. All patients had partial obstruction on cholangiogram. Table 6 shows the results of culture related to the type of anastomoses previously performed. Twelve of the 15 patients had

positive cultures of intrahepatic bile. Approximately 80% of patients in each group had positive intrahepatic bile culture regardless of the type of anastomosis. All but one patient in each group was asymptomatic at the time of examination despite the presence of organisms and moderate to severe obstruction as estimated by serum bilirubin concentration. Only seven of the 15 patients had histories of cholangitis, emphasizing in this group as in the total group that a significant number of patients with biliary-intestinal anastomotic obstruction were asymptomatic even though positive cultures were obtained from their intrahepatic bile.

Table 7 lists the organisms found in the intrahepatic bile on positive culture. Flora is that which would be expected as all these organisms are found in the intestinal tract. The interesting point is that these organisms can exist in significant number within the bile ducts of the liver and yet cause little symptomatology.

Extrahepatic Bile Culture. A small group of patients (Table 8, 9) had cultures of various segments of the extrahepatic biliary tract at the time of elective cholecystectomy for chronic cholelithiasis and

TABLE 7. *Intrahepatic Bile*

Organisms Cultured	Total
E. coli	25
Pseudomonas	6
Aerobacter	5
Proteus	2
Enterococci	2
Lactobacilli	2
Microaerophilic Strep	2
Paracolon, Viridans Strep, S. aureus (one each)	3
Number of Organisms Isolated	
Pure culture	25
Two organism	5
Four organism	2

TABLE 8. *Culture at Time of Elective Cholecystectomy*

Patients	21
Positive culture (Total)	11
Febrile	0
Positive culture and history fever	5
Negative culture	10
Negative culture and history fever	2

TABLE 9. *Site of Positive Culture*

GB wall	GB bile	CD bile or stone
8	10	5

cholecystitis. To date studies have yielded results similar to previous reports indicating that approximately 25% of patients with chronic cholecystitis and cholelithiasis have organisms in the common duct bile. In this small series the incidence of positive cultures of gallbladder was approximately 50%, and we found no significant difference between the number of positive cultures of the gallbladder wall and gallbladder bile. All patients with positive cultures of the common duct stone or bile had positive cultures of the gallbladder wall or bile. Common duct bile was not infected without concomitant gallbladder involvement. Again, bacteria can exist in the gallbladder and extrahepatic bile ducts without symptoms referable to their presence. Only five of the 11 patients with positive cultures had histories of fever, which had occurred with previous episodes of acute cholecystitis. Three of these five had positive cultures of the common duct bile as well as the gallbladder.

Discussion

Percutaneous cholangiography provided a unique tool for the study of the bacteriology of the intrahepatic biliary tract. While many bacteriologic studies have been performed on the extrahepatic biliary tract, this represents the first detailed analysis of intrahepatic bile obtained during percutaneous cholangiography.

This test done preoperatively allows correlation of pathologic anatomy, physiology, and bacteriology with clinical history and course. In reviewing this data and the literature on biliary tract infection it has become apparent that the term "cholangitis" is used loosely and is not accurately defined.

Cholangitis is defined as inflammation of the bile ducts, but general clinical usage includes intermittent jaundice, chills and fever. The definition and the clinical situation are not necessarily mutually inclusive nor do they necessarily exist together. The data in Table 3 reveal that clinical cholangitis is associated with obstruction and bacteria in the bile. However, these two factors often coexist and do not produce symptomatic cholangitis. In this study, at the time of demonstrated partial obstruction and positive culture, 66% of the patients had no chills or fever or other criteria of clinical cholangitis (Table 4).

Studies of patients with biliary intestinal anastomosis revealed bacteria within the intrahepatic bile ducts in 75 to 80%.

This study suggests the need for reappraisal of the factors causing symptomatic cholangitis. Since there was no difference in the type or number of bacteria in these different situations a correlation of the presence or absence of symptomatic cholangitis to the type obstruction is of interest. The difference between partial and complete obstruction and associated presence or absence of cholangitis reveals that cholangitis is more commonly associated with partial duct obstruction, and the difference is statistically significant.

In considering cholangitis, an analogy to pyelonephritis is helpful. Culturable bacteria in bile would represent a carrier state of "asymptomatic bactibilia" which could be likened to the well-known asymptomatic bacilluria. The organisms could exist causing the definition of cholangitis and inflammation of the bile ducts, without causing clinical cholangitis. It seems likely that the classic triad is caused by an acute change in partial obstruction leading to a sudden increase in intraductal pressures rupturing the biliary mucosa and allowing the organisms to enter the blood and the resultant septicemia creating the clinical entity of cholangitis with chills, fever and jaundice. Surgical trauma and the injection of saline

or contrast under pressure into the biliary tract at the time of T-tube, operative or percutaneous cholangiography can precipitate sudden overdilatation and rupture of the biliary mucosa. In support of this hypothesis is the data in five patients in whom percutaneous cholangiography precipitated an attack of clinical cholangitis in the presence of partial obstruction, bacteria in the bile and a sudden increase in pressure by the injection of contrast. Blood cultures taken at the onset of chills revealed septicemia with the same organisms in the blood as had been isolated from the bile prior to the injection of the contrast. Ong,⁸ in reviewing a large group of patients with recurrent cholangitis collected a small group with histories of sudden fever, chills, jaundice and hypotension. He was able to get positive blood cultures in most cases and believed that septicemia caused the clinical manifestations of cholangitis.

Further support comes from the well-documented appearance of clinical cholangitis with chills and fever after T-tube cholangiography as described by Mixer⁷ in 1947. Experimental work by Hultborn⁵ on cholangiovenous reflux during T-tube cholangiography revealed the ease with which bacteria injected into the bile ducts could be immediately recovered and cultured from the blood stream if there was just an insignificant increase in intrabiliary pressure over the resting secretion pressure.⁶ Chills and fever after T-tube cholangiography prompted their experimental work. All our data support this analogy between cholangitis and pyelonephritis. Recognizing this and having the data to predict which patients are most likely to have the factors associated with clinical cholangitis, i.e., partial obstruction and positive culture, it behooves the surgeon to prevent conversion of asymptomatic bacteremia to florid gram-negative septicemia associated with chills, fever, and jaundice of cholangitis,

The bacteriology of the extrahepatic biliary tract including the gallbladder wall and bile, common duct bile and stones has been reported many times. One observation generally agreed upon is that the gallbladder wall is a better source of positive cultures than is the gallbladder bile. The range of incidence of positive cultures in several reported studies^{1, 3} is 20 to 40% in gallbladder bile, and 25 to 50% in the gallbladder wall, in patients with chronic cholecystitis. Our results in a limited number of patients lean toward the higher end of the spectrum. The normal gallbladder rarely yields a positive culture.³ Edlund *et al.*³ obtained a 44% positive culture yield from the common duct of patients with chronic cholecystitis without stones in the common duct and a 93% positive culture in patients with chronic cholecystitis plus choledocholithiasis. Cultures of open liver biopsies were almost all negative despite positive common duct culture. Anderson and Priestly¹ studying common duct bile at operation found positive cultures in 75% of patients with choledocholithiasis.

In discussing bacteriology of the biliary system some mention of clostridial organisms should be made. The percentage of patients who harbor clostridia in the intrahepatic or extrahepatic biliary tract is unknown. None of our intrahepatic anaerobic bile cultures revealed clostridia in the human liver. This agrees with Sborov's¹⁰ culture studies of a large series of liver biopsies wherein only three of 86 liver biopsies were positive and none contained clostridia. Stormont¹¹ in a similar study of 20 patients with hepatocellular disease found only one positive culture of liver biopsy and this patient turned out to have choledocholithiasis. In light of our studies and those in the literature it is highly unlikely that clostridia are a normal inhabitant of the intrahepatic biliary system. This may not be true for gallbladder for studies^{9, 12} of the gallbladder wall yield clostridia in 2 to 14% of patients with chole-

cystitis. However, in a thorough bacteriologic study Edlund⁸ found no clostridia in all normal biliary samples or in association with the usual afflictions of the extrahepatic biliary system. He did, however, encounter anaerobic streptococci, lactobacilli and undifferentiated gram-positive rods which were not clostridia.

During the period of our study two patients were seen with clinical symptoms of gas bacillus infection following biliary tract surgery. Clostridial organisms were cultured from the necrotic abdominal musculature in one and from the drain site of the other. Both were successfully treated with hyperbaric oxygen therapy in addition to massive antibiotics and surgery. Both patients showed excellent initial clinical and bacteriologic response to treatment. One made a complete recovery, the other developed cardiovascular complications and died 17 days postoperatively. Further bacteriologic studies are needed to define the incidence of clostridial organisms in the biliary tract and, when such infections develop, the role of hyperbaric oxygen therapy needs further evaluation.

On the basis of this data we advocate broad spectrum antibiotic administration during the diagnostic and operative procedures carried out on patients with partial common bile duct obstruction. Cultures should be taken in all instances and should infection become manifest the specific antibiotics to which the organisms are sensitive should be employed.

The use of antibiotics during routine cholecystectomy for chronic cholelithiasis is debatable. No data is yet available to decide this issue. Further evidence is needed which will correlate culture data with past operative infectious complications.

Summary

Results of cultures of intrahepatic bile at the time of percutaneous cholangiography reveal that 64% of patients with partial

common duct obstruction due to stricture, choledocholithiasis and bile duct carcinoma have positive bile cultures. Only 10% of patients with complete obstruction secondary to carcinoma of the pancreas or ampulla have positive cultures.

Correlating the objective culture and x-ray with the patient's symptomatology indicates that bacteria in concentrations of 10^8 /ml. or more may exist in the presence of obstruction with no symptoms, i.e., chills and fever, referable to their presence.

The clinical manifestations of cholangitis may be precipitated by percutaneous T-tube or operative cholangiography.

The analogy between cholangitis and pyelonephritis is suggested by the evidence that chills and fever were accompanied by septicemia in 5 patients undergoing percutaneous cholangiography. The same organisms were grown from both blood and the bile aspirate.

It is postulated that "asymptomatic bacteribilia" is converted to clinically manifest cholangitis by trauma. Disruption of the biliary tract mucosa allows bacteria to enter the blood stream and septicemia instigates the chain of events leading to chills and fever.

Cultures of the extrahepatic biliary tract in a small group of patients confirm previous reports indicating that approximately 50% of gallbladders will yield positive cultures in the presence of chronic cholelithiasis and cholecystitis.

An unknown percentage of patients with chronic gallbladder disease have clostridia in the gallbladder wall. Rare instances of clostridial gas gangrene follow operations of the gallbladder. Two such cases have been presented and briefly discussed. However, no clostridia were found in 75 cultures of intrahepatic bile.

The implications of these culture data provide a clearer understanding of cholangitis. The knowledge of which asymptomatic patients are likely to have bacteria

in the biliary tract provides the foreknowledge which will enable the surgeon to exercise the precautions to prevent conversion of "asymptomatic bactibilia" into cholangitis. The role of trauma, care in diagnostic procedures, and antibiotics in this respect can be further elucidated by continuing study.

References

1. Anderson, R. E. and Priestly, J. T.: Observations on the Bacteriology of Choledochal Bile. *Ann. Surg.*, 133:486, 1951.
2. Andrews, E. and Dell, H. L.: Bacteriology of Normal and Diseased Gallbladders. *Arch. Int. Med.*, 56:1171, 1935.
3. Edlund, Y. A., Mollstedt, B. O. and Ouchterlony, O.: Bacteriological Investigation of the Biliary System and Liver in Biliary Tract Disease Correlated to Clinical Data and Microstructure of the Gallbladder and Liver. *Acta Chir. Scandinav.*, 116:461, 1958.
4. Flemma, R. J., Schauble, J. F., Gardner, C. E., Anlyan, W. G. and Capp, M. P.: Percutaneous Transhepatic Cholangiography in the

- Differential Diagnosis of Jaundice. *Surg. Gynec. Obstet.* 116:559, 1963.
5. Hultborn, A., Jacobsson, B. and Rosengren, B.: Cholangiovenous Reflux During Cholangiography. An Experimental and Clinical Study. *Acta Chir. Scandinav.*, 123:111, 1962.
6. Jacobsson, B., Kjellander, J. and Rosengren, B.: Cholangiovenous Reflux. An Experimental Study. *Acta Chir. Scandinav.*, 123:316, 1962.
7. Mixer, H. W., Rigler, L. G. and Oddone, M. G.: Experimental Studies on Biliary Regurgitation During Cholangiography. *Gastroenterology*, 9:64, 1947.
8. Ong, G. B.: A Study of Recurrent Pyogenic Cholangitis. *Arch. Surg.*, 84:199, 1962.
9. Pyrttek, L. J. and Bartus, S. H.: *Clostridium welchii* Infection Complicating Biliary-Tract Surgery. *New Eng. J. Med.*, 266:689, 1962.
10. Sborov, V. M., Morse, W. C., Giges, B. and Jahnke, E.: Bacteriology of the Human Liver. *J. Clin. Invest.*, 31:986, 1952.
11. Stormont, J. M., Mackie, J. E., Kass, E. H. and Davidson, C. S.: Bacteriologic Culture of the Diseased Human Liver. *Ann. Int. Med.*, 51:17, 1959.
12. Thornsness, E.: Bacteriology of Cholecystitis, The Virulence and Spore Formation of *Clostridium welchii*. *Surg. Gynec. Obstet.*, 59:752, 1934.

DISCUSSION

DR. FRANK GLENN (New York): Mr. Chairman and Gentlemen: Yesterday we had an excellent presentation on the problem of infection, and today, whereas that was general, we are focusing on the biliary tract. The facts that have been brought out here this morning are of great practical importance to us, because of the prevalence of this disease.

The essayists, Dr. Flemma, Dr. Flint, Dr. Osterhout, Dr. Shingleton emphasized the significance of the organisms in the biliary tract and the predominance of the coliform when there is a "bactibilia."

My associates and I have long been interested in the source of bacteria in the bile and the factors that contribute to transform "asymptomatic" bactibilia into a cholangitis.

Infection, bacterial growth within the biliary ductal system is indeed a pertinent subject for discussion among surgeons. The essayists have stressed in their presentation that the trauma of percutaneous transhepatic puncture of the distended bile duct and the distention of the communicating ramifications of that system may be followed by a flagrant cholangitis. The bile ducts in a patient with calculous disease may contain within their walls many and various bacteria. Colon organisms are the most common. Dineen¹ in our laboratories, has demonstrated that guinea pigs that have had

colon bacilli introduced into the portal circulation via the spleen give a high concentration of these bacteria in the bile, much greater in fact than when bacteria are placed in the circulation through a vein of the ear or the lymphatics of the duodenum. (Slide) The viscera, the intestines can thus be the source of organisms that cause a cholangitis. Organisms seemingly quiescent and well tolerated in the mucosa and submucosa of the bile ducts become the cause of symptoms when the ductal system is distended. They multiply and further invade the duct wall and the blood vessels as is also the case with their products.

Sudden distention of the ductal system by an occluding calculus or introduction of fluid under pressure may well occur in doing a postoperative cholangiogram or by percutaneous transhepatic cholangiogram (slide).

The examples cited in this paper also confirm the well appreciated observation that the slowly developing ductal obstruction and associated jaundice in patients with neoplasm so located as to impinge upon the lumen of the common duct is not ordinarily associated with cholangitis, pain and fever. Such information is to be kept in mind in our management of patients with biliary tract disease. In acute suppurative obstructive cholangitis now being encountered by us in patients over 65 years of age with long-standing calculous biliary tract disease, ductal decompression accomplished by the minimum of operative burden and