The Role of Bacteria in Pigment Gallstone Disease

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One hundred ten of nine hundred sixty consecutive patients who underwent surgery for gallstones (GS) had pigment stones (PS) (11.45%). Fifty brown PSs contained calcium bilirubinate, small amounts of cholesterol, and always calcium palmitate, were usually found in the common duct (96%), and were almost always associated with bile infection (98%) and diffuse erosion of the biliary mucosa. Fifty-one black PSs contained bilirubin polymers, calcium carbonate, and/or phosphate, seldom cholesterol, and never evident amounts of calcium palmitate, were mostly found in the gallbladder, and were associated with hemolysis or liver damage and with hyperplastic cholecystosis. Bile infection was found in 19.6% of cases, but bacteria were never found in the center of black PSs by scanning electron microscopy. Nine additional patients (8.2% of PSs, 0.9% of GSs) had concomitant black and brown PSs that were mostly found in the common duct and were always associated with bile infection. It is suggested that, even if PSs with concomitant black and brown material can be found, black and brown PSs greatly differ not only in pathogenesis but also in clinical behavior and treatment. In particular bacterial infection is important only in the pathogenesis of brown PSs while it plays no role in the initial formation of cholesterol, mixed or black GSs.

PIGMENT GALLSTONES ARE stones that contain large amounts of pigment material and little cholesterol.[1,2] They can be distinguished as black and brown stones simply on the basis of their color: brown stones are brownish yellow, soft, and show alternate dark and light layers in cross-section. They are said to occur principally in the Orient and usually are found in Western countries as recurrent common duct stones after cholecystectomy, even if they can be found in the common duct as well as in the gallbladder of old patients with no previous biliary surgery.[3,4] Brown stones consist mainly of calcium bilirubinate with little cholesterol and always contain calcium palmitate.[4] Infection has been documented at the time of stone removal in more than 90% of brown stones.[1-5]

Black pigment stones usually form in the gallbladder. They are generally small (less than 3 mm in diameter), irregular, with multiple spiculas. In cross-section they have a glasslike, featureless appearance. Black stones mainly contain bilirubin polymers, usually calcium carbonate or phosphate, seldom cholesterol, and never calcium palmitate.[3,4] They are rarely associated with bile infection. Patients in Western countries who have pigment gallstones usually have been considered to belong to this second category.[1,2] Previous studies suggested that bacteria play a key role in the pathogenesis of brown pigment stones.[1-10]

However the role that bacteria play in the formation of gallstones remains incompletely defined. Debate still continues as to whether other stones, in addition to brown stones, may be caused by bacteria.[8-13] In fact bacteria recently were identified in the majority of pigment stones and in the pigment portion of composite stones. Therefore bacterial infection has been considered a primary factor, not only in the pathogenesis of brown stones but also in the formation of black pigment gallstones.[8] Furthermore because bacteria were found by scanning electron microscopy on the surface of cholesterol gallstones, a possible role for bacteria in cholesterol stone formation also has been suggested.[11-13]

We documented for the first time that bile infection by *Escherichia coli* precedes rather than follows brown stone formation, and together with bile stasis is the main factor in their pathogenesis.[5]

In the present article, additional data on the relationship between bacteria and gallstones are reported that are derived from the study of 1000 consecutive patients who underwent surgery for biliary tract diseases.

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In particular, evidence is given that

(1) Brown and black pigment stones differ in composition and pathogenesis and should also require a different treatment.
(2) Sometimes, even if infrequently, pigment stones can be concomitantly black and brown. In these cases they behave as brown stones, *i.e.*, they are always associated with bile infection.
(3) Bacteria are crucial for the formation of brown stones, but not for that of black pigment or cholesterol stones.

Materials and Methods

The present data are part of a prospective study of 1000 consecutive patients who underwent surgery for bile tract diseases from 1980 to 1988. Gallstones were found in 960 cases. There were 895 patients with stones in the gallbladder. Two hundred one patients had stones in the common duct, which in 51 cases formed after cholecystectomy. In all of the patients, clinical, radiologic, and laboratory data were recorded. Histologic examination of the gallbladder was always performed. In some patients with biliary enteric anastomosis, specimens from the common duct also were examined. In addition to routine histology, scanning electron microscopy (ISI SX-25 SEM Pabisch, Milan, Italy) at 7 KV and transmission electron microscopy (Zeiss EM 109 TEM, Karl Zeiss, Oberkochen, FRG) at 80 KV of both gallbladder and common duct specimens also were performed in selected cases.[14,15] Bile specimens always were taken at operation. In patients with T tubes, bile specimens also were taken in the postoperative period. Bile pH analysis and bile culture always were performed. Stones obtained at operation were washed, dried, and photographed. After cross-section, they were classified according to previously reported morphologic criteria.[16,17] The analysis of stone composition was performed by x-ray diffractometry using a Siemens D 500 diffractometer (Siemens, Milan, Italy) and infrared spectroscopy, using a Perkin-Elmer 357 infrared spectrophotometer (Perkin-Elmer, South Pasadena, CA).[3-5] Identification of the various compounds was made by comparing the observed spectra and diffraction angles with those reported in the literature.[3,4,15-18] In a subset of patients, stone culture, stereomicroscopy, and scanning electron microscopy of stones (ISI SX-25 SEM Pabisch, Milan, Italy) were performed.[19]

Details of these methods have been reported elsewhere.[3-5,14,15,19]

In particular 15 patients with postcholecystectomy stones were included in the study both at cholecystectomy and at the second operation. Therefore they had stone and bile analysis in both cases.

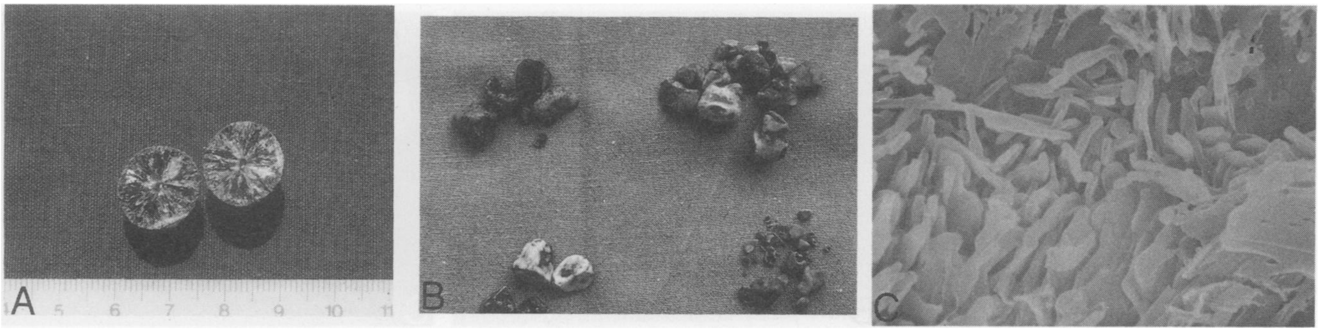
Results

Table 1 shows the data in the 960 patients with gallstones. There were 364 patients (37.9%) with ovoidal (or spheroidal cholesterol stones, 350 (36.5%) with faceted mixed stones (Fig. 1) and 110 with pigment stones (11.45%) (Figs. 1 to 4). Fifty had brown stones (5.2%) (Table 2, Fig. 2). Fifty-one had black pigment stones (5.3%) (Fig. 3). Nine had pigment stones that were concomitantly black and brown (0.93%) (Fig. 4). Seventy-

TABLE 1. *Classification of Gallstones According to Stone Morphology, Site, and Composition*

Stones	Type	n	Age	Sex M	Sex F	Site: Gall Bladder	Site: Common Duct	Positive Bile Culture	Composition %: Chol.	Bil.	Carb.	Palm.
Cholesterol stones	Pure	364 (37.9%)	54.2	136	228	360 (98.9%)	20 (5.5%)	49 (13.5%)	90.3 ± 5.5	0.8 ± 1.5	1.0 ± 0.5	—
	Mixed	350 (36.5%)	58	95	255	331 (94.6%)	93 (28.6%)	70 (20%)	85 ± 10	2.5 ± 1.2	1.3 ± 0.7	—
Pigment stones	Brown	50 (5.2%)	73.2	23	27	16 (32%)	48 (96%)	49 (96%)	10.4 ± 6	51 ± 17	traces	23 ± 11
	Black	51 (5.3%)	58.8	22	29	50 (98%)	11 (21.6%)	10 (19.6%)	7.5 ± 4.7	61.8 ± 11.6	6.5 ± 6.0	—
	Black and brown	9 (0.9%)	74.3	6	3	2 (22%)	9 (100%)	9 (100%)	7.8 ± 4.0	55.4 ± 14.5	1.0 ± 0.7	12.5 ± 6.3
Combination stones		72 (7.5%)	56.5	20	52	66 (92%)	9 (12.5%)	29 (40%)	Various			
Other stones		64 (6.7%)	58	25	39	60 (93.7%)	11 (17.2%)	13 (20.3%)	Various			
Total		960	56.7	327	633	833	201	229				

Composition according to infrared spectroscopy and/or x-ray diffractometry (percentage of stones dry weight) Chol, cholesterol; Bil., calcium bilirubinate; Carb, calcium carbonates (calcite, aragonite or vaterite); PALM, fatty acid calcium salts (mainly calcium palmitate).



FIGS. 1A–C. Ovoidal cholesterol (A) and faceted mixed stones (B). Scanning electron microscopy (C): no bacteria.

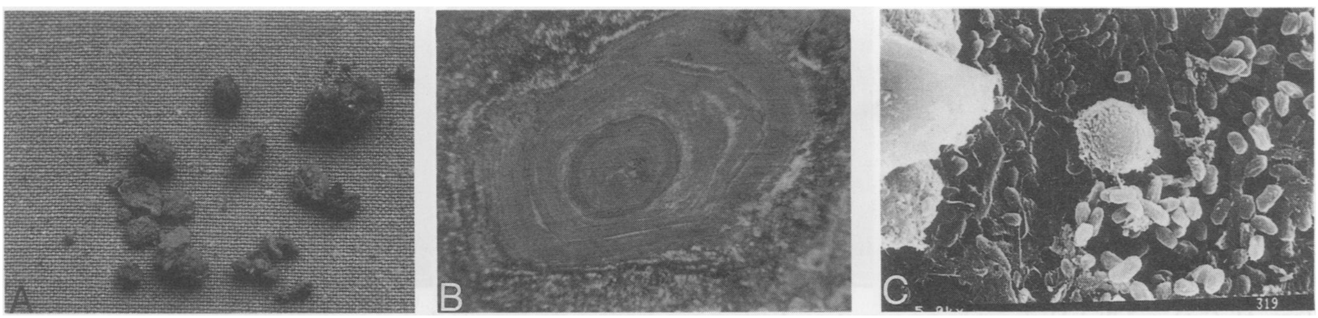
two patients had combination stones. Stones with a pigment coat but with a central nidus of a different composition from the periphery or stones entirely pigmented but found in the same site and concomitantly with cholesterol and mixed stones were not included in the pigment stone group. They were considered 'other stones' (n = 64), together with calcium salt stones and other rare types of stones (Table 1). A detailed analysis of patients with brown composite stones is given in Table 3.

Brown Stones

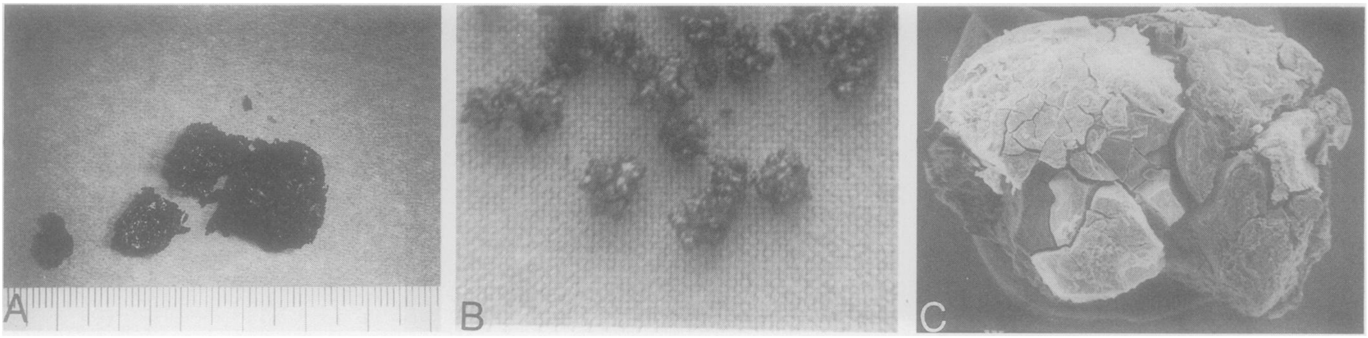
Brown stones were found as postcholecystectomy stones in 27 cases (Fig. 2A), in 21 cases in patients with no previous biliary surgery (Fig. 2B), and in 2 patients with spontaneous biliary enteric fistulas (Table 2). Twenty-two patients were men, 28 were women, with a mean age of 73.2 years (range, 50 to 87 years). Stones were found in 48 cases in the common duct, in 13 cases both in the duct and in the gallbladder, and in 2 cases only in the gallbladder. Positive bile culture was found in 49 cases. Analysis of stone composition demonstrated the presence of calcium bilirubinate (51% ± 17%) and cholesterol (10.4% ± 6%), which was found in all brown stones. However the most specific compound of brown stones was calcium palmitate (23% ± 11%), a crystalline calcium salt that was almost never found in other types of gallstones. In particular calcium palmitate was found in brown stones in a concentration greater than 10% in 48 cases, both in the stone center and periphery (Fig. 5A). All of these stones were found in the common duct and all had positive bile culture.

Of the 27 patients with postcholecystectomy stones (Fig. 2A), 11 were men and 16 were women, with a mean age of 69.5 years (range, 50 to 76 years). Stones were single in 10 cases and multiple in 17 cases. Brown stones contained nonabsorbable suture material in five cases and phytobezoars in another five cases. Bile culture was always positive in a concentration greater than 10^5 CFU/mL. *E. coli* was found in all cases, alone in 11 cases, and in association with other bacteria (Proteus, Enterobacter, *Pseudomonas, Klebsiella*, Enterococcus) in the other cases. Bile pH was 6.9 ± 0.32 (range, 6.2 to 8.0). Mean lithogenic index (in 21 patients) was 1.44 ± 0.32 (range, 1.02 to 1.94).

Stone composition demonstrated, in all the patients, the presence of calcium bilirubinate, calcium palmitate, and cholesterol. In three patients with white material macroscopically evident both in the stone center and periphery, calcium carbonate also was found. X-ray diffractometry demonstrated in all three cases the presence of aragonite, which is the less frequent crystalline phase of



FIGS. 2A–C. (A) Recurrent common duct brown stones. They look like aggregates of brown mud. (B) Gallbladder brown stone of an 82-year-old woman. Note the alternate dark and tan layers. (C) Scanning electron microscopy of a fresh recurrent brown stone: bacteria are clearly evident in the stone center. Magnification, 5000×. Bile culture: *E. coli* 10^6 > CFU/mL.



FIGS. 3A–C. Black mud (A) and black microstones (B). Note the irregular surface with multiple spiculas. (C) Scanning electron microscopy of a black stone: typical amorphous aspect; no bacteria.

calcium carbonate in gallstones and which precipitates at lower pH.

All of these patients, in addition to cholecystectomy, had previous complementary surgical procedures on the bile tract. They were sphincterotomy in 19 cases, choledochoduodenostomy in 3 cases, hepaticojejunostomy in 1 case, and choledochotomy plus T-tube drainage in 4 cases.

The 21 noncholecystectomyzed patients with brown stones had stones in the common duct in 19 cases. Twelve of these patients had concomitant gallbladder brown stones (Fig. 2B). The last two patients had stones only in the gallbladder.

The 19 patients with common duct stones included 10 men and 9 women. Mean age was uniformly high: 80.36 years (range, 75 to 87 years). Age difference between patients with and without previous biliary surgery was highly significant ($p < 0.001$). Age difference also was significant between this group and the group with black pigment stones. Sex prevalence (10 men and 9 women) also was different from all other groups of patients with gallstones. Analysis of stone composition gave similar findings, as in cases of stones with previous cholecystectomy. Calcium palmitate always was present but usually it was found in smaller amounts (Fig. 5). Pigment material usually accounted for more than 50% of stone dry weight. Data from both x-ray diffractometry and infrared spectroscopy were extremely similar in both cases (Fig. 5). Bile culture was always positive and the same bacteria were found as in patients with postcholecystectomy brown stones. Mean lithogenic index (in four patients) was 1.56 ± 0.39. Mean pH of the common duct bile was 7.2 ± 0.50. Differences between lithogenic index and bile pH were not statistically different in the two groups ($p > 0.1$).

Brown stones were homogeneous in cross-section in both groups, with no evident difference between nucleus and periphery (Figs. 2A and B). In the same subject, gallbladder stones had the same composition as common duct stones. Mixed or composite stones, with a nucleus of different composition and origin from the periphery, were not included in this group. However it was noteworthy that (1) brown stones in the noncholecystectomyzed patients were usually larger in size and harder in density than postcholecystectomy stones, which often appeared as true aggregates of biliary mud (Figs. 2A and B); (2) a laminar structure, with eccentrically shaped rings in cross-section always was detectable in the former group (Fig. 2B), while it was almost never detectable in the latter with postcholecystectomy stones; and (3) the calcium palmitate content of brown gallbladder stones (16.0 ± 2.0; range,

FIGS. 4A–C. (A and B) A black and brown microstone, 4 mm in size, found in the commun duct of a patient with periampullary carcinoma. Stereomicroscopy clearly shows that it is not a combination stone, but black and brown material are tightly mixed both in the stone center and periphery. (C) SEM of a dry black and brown stone: note the typical bacterial casts, similar to those observed in brown stones. Magnification, 200×. Bile culture. *E. coli* 10^6 CFU/mL.

TABLE 2. *Brown Pigment Stones*

Stones		n	Age	Sex M	Sex F	Site GB	Site CD	Positive Bile Culture	CP
After cholecystectomy		27	69.5	11	16	—	27	27	27
Noncholecystectomyzed patients	CD	19	80.3	10	9	12	19	19	19
	GB	2	66	1	1	2	—	1	—
Spontaneous biliary enteric fistulas		2	68	1	1	1	2	2	2
Total brown stones		50	73.2	23	27	15	48	49	48
Black and brown gallstones		9	74.3	5	4	2	9	9	9
Total		59	73.4	28	31	17	57	58	57

GB, gallbladder; CD, common duct; CP, calcium palmitate.

5.5 to 35.0) was less than that of common duct stones in the former group and than that of postcholecystectomy stones ($p < 0.01$). In addition the calcium palmitate content of common duct stones was always greater than that of gallbladder stones in the same patient, even if the calcium palmitate content of the gallbladder stones of one patient could be greater than that of the common duct stone of another patient (Fig. 5A). Scanning electron microscopy of stones demonstrated the presence of calcified bacteria or bacterial casts in patients with recurrent common duct brown stones and in patients with brown stones without previous biliary surgery, while usually they were not found in cholesterol or black gallstones (Figs. 1C, 2C, 3C, 4C; Figs. 6A and B).

Spontaneous biliary enteric fistulas were found in 13 cases. Ten patients had a cholecystoduodenal and three had cholecystocolic fistulas. There were nine men and four women (sex prevalence completely different from all other groups of patients with gallstones). Mean age was 64.7 years (range, 46 to 80 years). Two patients with cholecystoduodenal fistula had no associated gallstones. Both had positive bile culture. Enterobacter was found in one case and Klebsiella in the other. Two patients (Table 2) had brown stones only. In one case, a 56-year-old man had a small brown stone in the gallbladder 3 months after a previous operation for gallstone ileus due to a large cholesterol stone. In the other case, an 80-year-old man had brown stones both in the gallbladder and in the common duct 1 year after a partial gastrectomy, complicated by duodenal leakage (and subsequent cholecystoduodenal fistula). In the other nine patients with spontaneous biliary enteric fistulas (mean age, 66.1 years) (Table 3), stones with a brown periphery or entirely pigmented were associated with cholesterol stones. In three cases stones were only in the gallbladder, in one case in the common duct, in the last five cases both in the gallbladder and in the common duct.

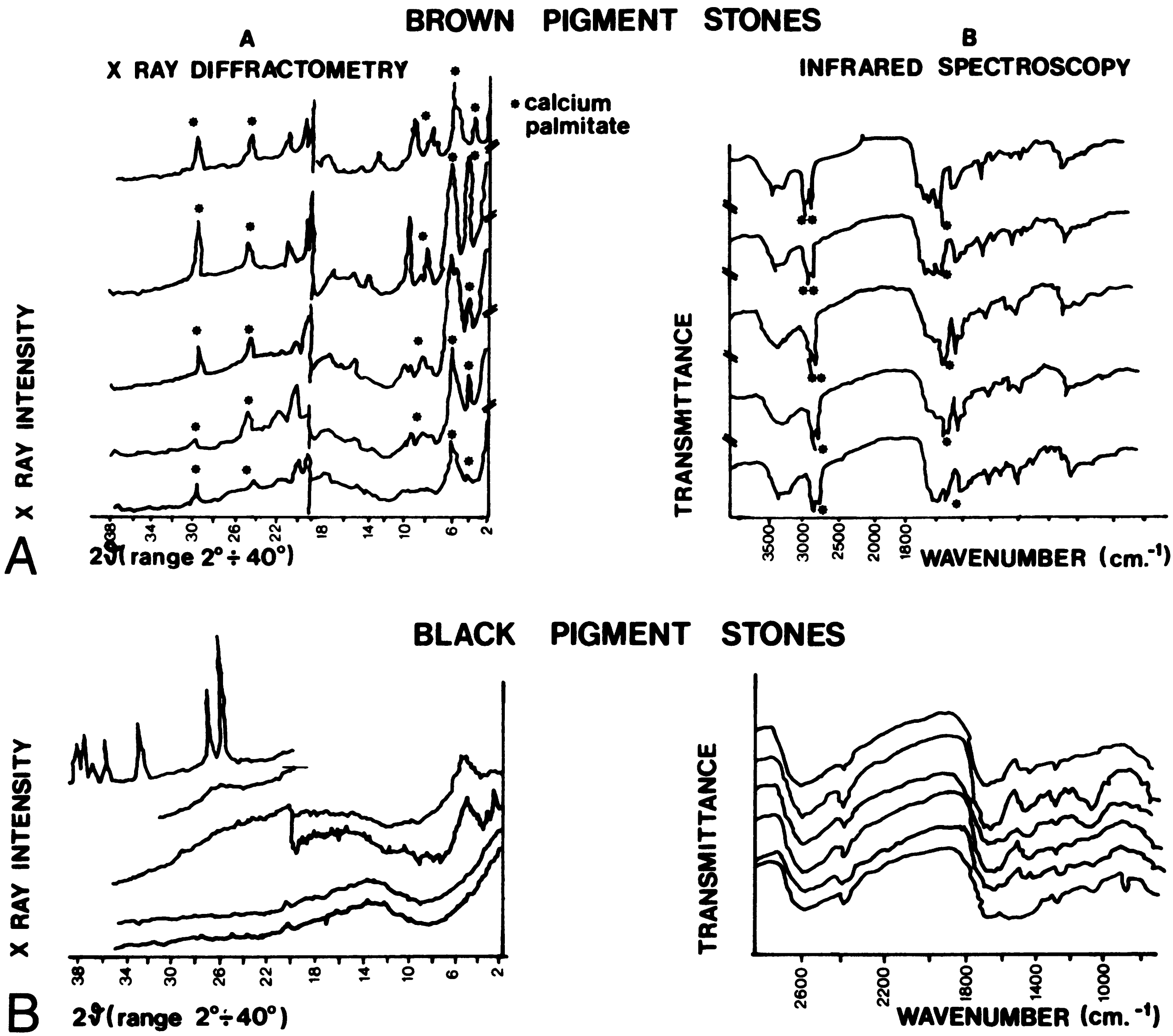
TABLE 3. *Composite Brown Stones*

Stones	n	Age	Sex M	Sex F	Site GB	Site CD	Positive Bile Culture	CP
Spontaneous biliary enteric fistulas	9	66.1	8	1	8	6	8	8
Postcholecystectomy retained stones	6	50.6	3	3	—	6	6	6
Cholesterol GB stones and composite CD stones	9	63	3	6	7	7	4	3
Cholesterol stones and brown mud in GB	4	51	2	2	4	—	1	—
Cholesterol stones with black coat in GB and brown coat in CD	1	63	1	—	—	1	1	1
Total	29	65	17	12	19	20	20	18

GB, gallbladder; CD, common duct; CP, calcium palmitate.

Stones with a cholesterol nucleus and a brown periphery also were found in the present series, in six patients after cholecystectomy. They were classified as other stones in Table 1. These stones were probably retained stones that formed a pigment coat in the common duct. Bile culture was always positive. Calcium palmitate was always present in the brown coat (Table 3).

The last two patients with gallbladder brown stones (a 50-year-old man and an 82-year-old woman) had typical single brown stones (measuring 0.7 cm and 4 cm, respectively). The former patient, an alcoholic, had a history of pancreatitis and cholangitis and had multiple hepatic abscesses at operation but no common duct stones detectable by intraoperative cholangiography. The latter underwent surgery for acute cholecystitis. Bile culture was positive for Klebsiella in the first case and negative in the other (*i.e.*, less than 10^5 CFU/mL). Bile pH was 7.2. and 7.4, respectively. Analysis of stone composition demonstrated the presence of calcium bilirubinate and cholesterol. Calcium palmitate was not detectable in a diffractometrically evident amount (*i.e.*, more than 5% of stone dry weight), even if small white beads, mainly consisting of calcium

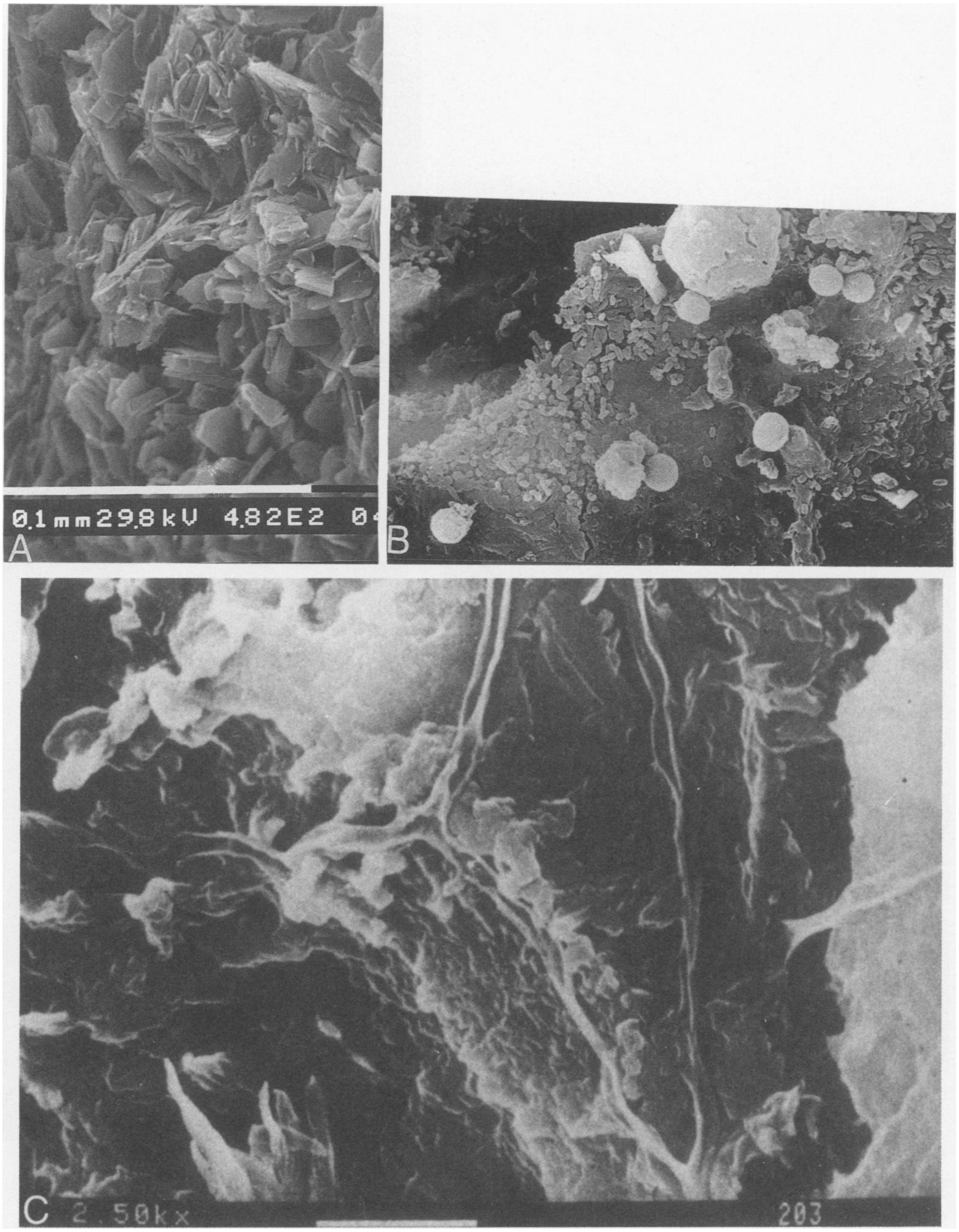


FIGS. 5A and B. (A) X-ray diffractometry of black stones and of brown pigment stones: five different patients with brown stones and six patients with black stones. Note the complete absence of spikes in amorphous black stones and the typical bands of calcium palmitate in brown stones. The first three diffractograms and IR spectra are of recurrent common duct stones. The last two are of brown stones in patients with no previous surgery. (B) Infrared spectroscopy: IR spectra of black stones are irregular, only slightly indented, with no evidence of spikes. Brown stones show more evident calcium bilirubinate bands and the typical bands of calcium palmitate.

palmitate, were macroscopically evident both in the center and in the periphery of the larger stone.

In 13 patients with no previous biliary surgery, brown stones were associated with cholesterol stones or contained a cholesterol nucleus in the common duct or in the gallbladder. In these patients bile culture was positive in only five cases while calcium palmitate was found in three cases (Table 3). Finally a patient who initially refused surgery underwent operation 4 months after the onset of jaundice and after suffering from multiple episodes of cholangitis. Gallstones were found in the gallbladder and in the common duct. They had a cholesterol nucleus in both sites. However gallbladder stones had a black periphery with multiple spiculas (containing bilirubinate and carbonate), while the three stones found in the common duct, which probably were responsible for jaundice, had a brown periphery containing calcium palmitate. Bile culture demonstrated the presence of *E. coli* and proteus sp. (Table 3).

Histologic examination of the gallbladder wall in patients with brown stones and gallbladder still *in situ* usually demonstrated the presence of chronic and acute inflammation, with diffuse erosion of the mucosa, loss of the epithelium, and microabscesses. The same findings also



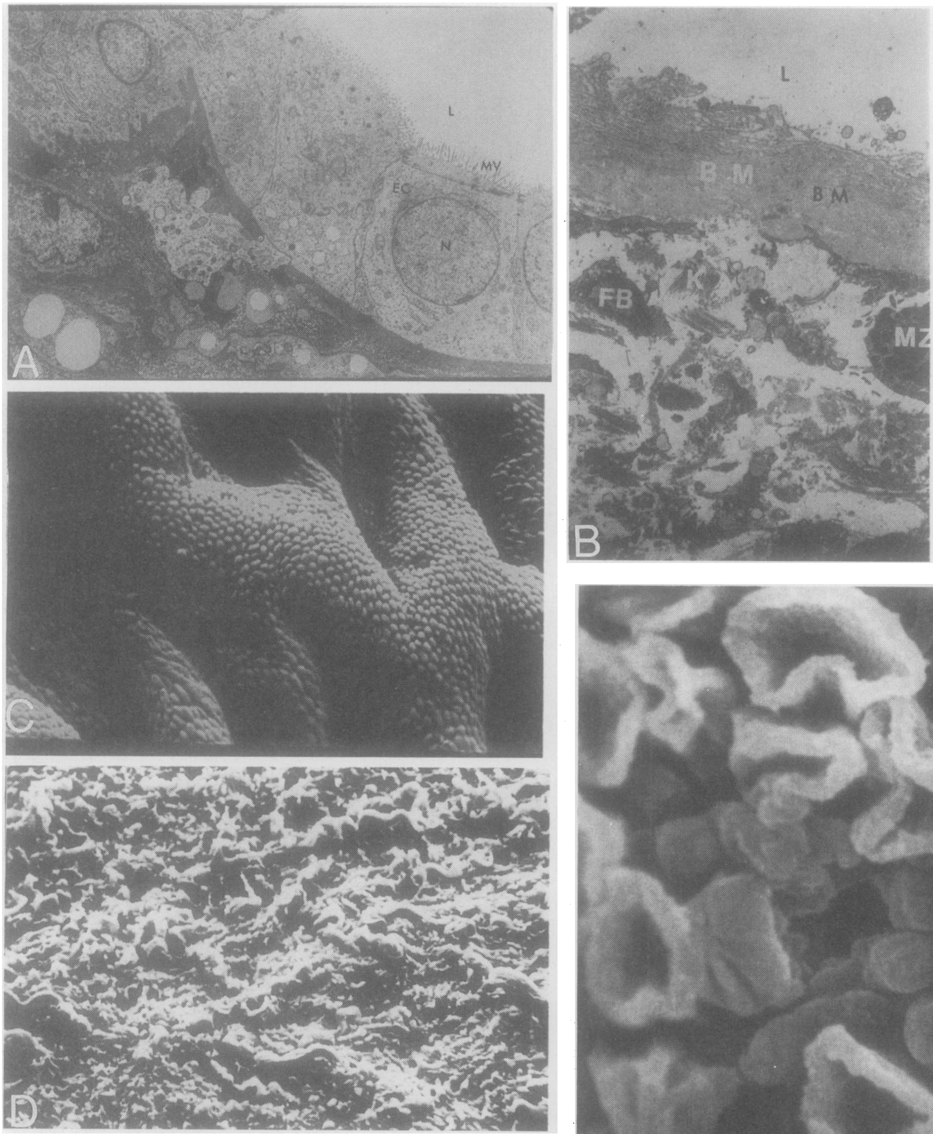
FIGS. 6A–C. (A) Cholesterol stone by SEM. Cholesterol crystals, no bacteria. Magnification, 480×. (B) Brown pigment stones by SEM. Note the typical presence of calcified bacteria both in the stone center and surface. (C) Mucus filaments both on the surface and in the center of a black pigment stone (SEM; magnification, 2500×).

were observed in common duct specimens obtained in patients who underwent biliary enteric anastomoses for postcholecystectomy brown stones. Both scanning and transmission electron microscopy showed diffuse alterations of the common duct mucosa (Figs. 7B and D).

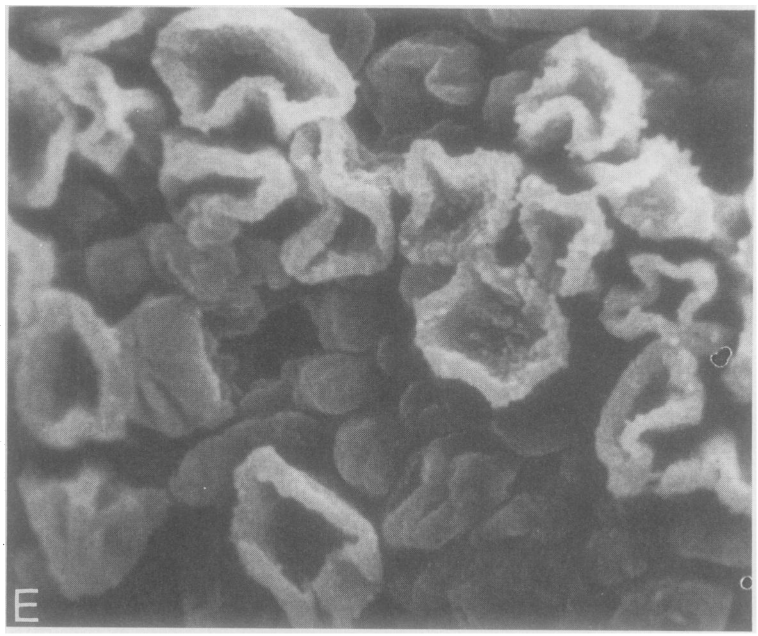
Black Stones

Black pigment stones were found in 22 men and 29 women. Mean age was 58.8 ± 12.5 years (range, 20 to 89 years). Stone size and site and bile culture are reported in Table 4 (Figs. 3A–C). Analysis of stone composition (Fig. 5B) demonstrated the presence of pigment material (61.8% ± 11.6%), calcium carbonate (6.5% ± 6.0%), calcium phosphate (3.0% ± 1.5%), and little cholesterol (7.5% ± 4.7%). In particular cholesterol was found in only 23 patients, while it was always found in brown pigment stones. Calcium palmitate was never found in diffractometrically evident amounts (*i.e.*, more than 5% of stone dry weight) in black pigment gallstones (Fig. 5B).

Associated clinical findings were cirrhosis in 11 cases, alcoholism in 5 cases, long-lasting use of drugs in 8 cases,



FIGS. 7A–E. Transmission electron microscopy of the gallbladder mucosa of patients with black (A) and brown stones (B). Scanning electron microscopy of the same patients (e = black; d = brown). Note the quite normal aspect of the epithelium, with well-preserved microvilli in the patient with black stones (A and C) and the complete loss of the lining epithelium, with the basal membrane (BM) directly facing the lumen (L) in patients with brown stones. (E) Glandular metaplasia with goblet cell hyperplasia in the gallbladder fundus of a patient with black microstones (SEM; magnification, 1500×).



and malignancies in 6 cases. It is noteworthy that patients with clinically evident hemolytic diseases were found in only 3 cases, aged 27, 47, and 49 years, respectively, despite the fact that in Italy thalassemia and sickle cell anemia have a high incidence in many areas.

Histologic examination of the gallbladder wall demonstrated in most cases the presence of hyperplastic cholecystosis with little or no signs of chronic inflammation (Figs. 7A and C). Acute inflammation was found in only 15 cases. Mucous cell metaplasia was found in 40% of black stones, which were analyzed by scanning electron microscopy (Fig. 7E). Black stones frequently were associated with mucus hyperproduction. This phenomenon could be detected both macroscopically (black microstones usually are embedded in a tight mucus network) and microscopically, either in gallbladder or in stone specimens. In particular scanning electron microscopy showed the presence of long mucus microfilaments, tightly adherent to black gallstones (Fig. 6C).

Brown and black pigment stones were found in nine cases: five men and four women (mean age, 74.3 years) (Table 5). This finding also was reported by others.[8-10] Most of them (seven of nine) were found in patients with periampullary carcinoma. They usually appeared as small conglomerates that were crushed easily with forceps or fingers and had alternate irregular black and brown concentric layers in cross-section (Figs. 4A–C). Analysis of stone composition showed the presence of calcium pal-

TABLE 4. *Black Pigment Gallstones (n = 51)*

	Sex		Stones			Bile				
			Site							
Age	M	F	GB	CD	Size	pH	L.I. (n = 18)	Positive Culture	Clinical Findings	Associate Pathologic Findings
58.8 ± 12.5 + – 12, 52	22	29	50	11	6 single (5–10 mm) 2 double (10 m) 5 black mud 5 multiple (3-6 mm) 33 multiple (3 mm)	7.27 + – 0.47	1 + – 0.28	10	11 Cirrhosis 5 Alcoholism 8 Drugs 6 Cancer 5 Gastric surgery 2 Colon surgery 3 Hepatitis HBS + 3 Recent Weight Loss 5 Nonspecific	15 Pancreatitis 5 Hyperplastic cholecystosis with adenomyomatosis 2 Empyema 2 Hemorragic cholecystitis 1 Septicemia 1 Liver abscesses

mitate, which is the typical calcium crystalline compound of brown stones.

Bile Infection

Bile infection (*i.e.*, a bacterial concentration in the bile greater than 10^5 CFU/mL) was found in 229 of the 960 patients (23.8%). Bile was infected in 49 of 364 patients with ovoidal cholesterol stones (13.5%), 70 of 350 patients with faceted mixed cholesterol stones (20%), 49 of 50 patients with brown pigment stones (98%), 10 of 51 patients with black pigment stones (19, 6%), 9 patients with black and brown pigment stones (100%), 29 patients with combination stones (40%), and 13 patients with 'other stones' (20.3%) (Table 1). *E. coli*, alone or in association with other bacteria, was found in 153 of the 229 positive bile cultures (66.8%). Seven hundred thirty-one patients had negative cultures. Fifty-eight patients with homogeneous brown stones (49 brown and 9 black and brown) had both bile infection and calcium palmitate in gallstones. Eighteen additional patients with composite stones, including brown material, also had both bile infection and calcium palmitate (Table 3). On the contrary there were (1) two patients with bile infection and calcium palmitate in nonbrown stones; and (2) two patients with brown stones and calcium palmitate, not associated with evident bile infection, and six patients with brown stones not containing calcium palmitate and not associated with evident bile infection (*i.e.*, less than 10^5CFU/mL). All of these six patients had brown stones in the gallbladder, while common duct brown stones always contained calcium palmitate and always were associated with bile infection. In particular *E. coli* always was present.

Among the 153 patients with bile infection by *E. coli*, brown stones were associated in 81 cases (52.9%) and calcium palmitate was found in 79 (51.6%). In one of the two patients with brown pigment gallbladder stones not containing calcium palmitate and in the two patients with spontaneous biliary enteric fistulas with no associated stones, bile culture was positive but *E. coli* was absent. Klebsiella was cultured in two cases and Enterobacter in the last case. Sixty-three patients had stone culture. Fifty-five patients had scanning electron microscopy (SEM) of stones. In all of the patients with positive stone culture or with bacteria demonstrable in the center of stones by SEM, bile culture also was positive. In the present series, no patient was found with bacteria demonstrable in the stone center by SEM, both directly and indirectly (bacterial casts), who had negative bile culture (Figs. 1C, 2C, 3C, 4C; Figs. 6A and B).

In black stones bile culture was associated in 19.6% of cases, mostly in stones that were found concomitantly in the gallbladder and common duct or in patients with previous pancreatitis. In 10 patients with black stones, who were analyzed by SEM, bacteria were never found (Figs. 3 and 6C), while they were always found in the four (of nine) patients with black and brown stones in whom SEM was performed (Fig. 4C).

Discussion

In a surgical series of consecutive patients from a Western country such as Italy, pigment stones were found as unique stones in 11.45% of cases. Pigment stones were brown in 45.4% and black in 46.3% of cases. In nine additional patients (8.2% of pigment stones, 0.9% of all gallstones), concomitant black and brown stones were found. In addition pigment material was found in 53 other patients (29 with brown pigment and 24 with black pigment), with combination stones or stones classified as 'other stones' (5.52 of all gallstones). In these cases pigment material usually formed the peripheral coat of stones with a different nucleus (usually cholesterol).

Brown stones were found mostly in the common duct (48 of 50 cases), almost always (48 of 50) contained calcium palmitate as typical compound,[20] and were associated with bile infection (*i.e.*, a bacterial concentration greater than 10^5CFU/mL) in 49 of 50 patients. In particular common duct brown stones always were associated with bile infection, while brown stones from the gallbladder sometimes were not associated with bile infection (1 of 19) and did not contain calcium palmitate (2 of 19). These observations correlate with previous findings reported by Tabata and Nakayama.[7] In addition present findings suggest that not only the mere positivity of bile culture but also the site of stones (gallbladder or common duct: see also data in patients with composite stones or with biliary digestive fistulas) and the type of bacteria (*E. coli*) are possible determinants of the brown pigment and calcium palmitate concentrations in the stones. On the other hand, black stones usually were found in the gallbladder, never contained evident amounts of calcium palmitate, and were associated with bile infection in only 19.6% of cases.

Other associated findings also were very different. Brown stones usually were found as postcholecystectomy stones (n = 27), in older patients with bile stasis (n = 19), or in patients with spontaneous biliary enteric fistulas (n = 13) (Table 2). In most of these patients bile stasis was evident, due to a stricture of a previous sphincterotomy or of a biliary enteric anastomosis or to age-dependent degenerative alterations of the sphincter of Oddi or of the lower portion of the common duct.[4] Black stones, on the other hand, were associated with hemolysis or with a previous or current liver damage (cirrhosis, hepatitis, long-term use of psycodrugs, and so on) (Table 4). Mean age of patients was also different, particularly in patients with no previous biliary surgery (80.36 in brown stones, 58.8 in black stones) ($p < 0.001$). In particular black stones were found in patients as young as 27 years, even in the absence of evident hemolysis, while brown stones were never found as unique stones in patients younger than 50 years.

Histologic examination of the biliary mucosa also gave different results in the two groups. Diffuse erosion of the gallbladder and common duct mucosa, loss of the lining epithelium, and parietal microabscesses were found in patients with brown stones (Figs. 7B and D). On the other hand, hyperplastic cholecystosis, associated in 40% of cases with mucous cell metaplasia, was found in patients with black stones. A quite normal mucosa, with no or poor signs of chronic inflammation, was another common finding in the black stone group (Figs. 7A and C).

Because we have analyzed constantly stones in relationship to bile and parietal wall (gallbladder and bile duct mucosa), we can state that not only composition of stones but also parietal and environmental characteristics are very different in patients with black and brown stones. Therefore present findings confirm our previous data published in 1983 to 1986[3–5] and lend support to the suggestions by Malet et al.[20] and Kaufman et al.[9] that differences in structure between black and brown stones reflect fundamentally different mechanisms of stone formation.[21] On the other hand, our data do not correlate, at least in part, with the conclusions of Stewart et al.[8] and Smith et al.,[12] who do not distinguish between brown and black stones because of the frequent finding of concomitant black and brown stones. In addition they classify together as 'infectious stones' (1) brown pigment stones that contain bacteria (and calcium palmitate) both in the center and in the periphery; (2) combination or composite stones that have a brown periphery, usually associated with infection, but not always containing calcium palmitate (and that had at least two different mechanisms in their pathogenesis, the former responsible for the cholesterol nucleus and the latter for the pigment periphery); (3) all gallstones with bacteria found on the stone surface.

In our series, in addition to 50 patients with homogeneous brown stones and 51 with black stones, we also found 9 patients with concomitant black and brown stones. These stones were never combination or composite stones with a black nucleus and a brown coat or vice

TABLE 5. *Black and Brown Stones*

n	Age (years)	Sex	Site: GB	Site: CD	Bile Culture	CP	Associated Findings
1	67	M		+	+	+	Periampullary carcinoma
2	81	F	+	+	+	+	Multiple liver abscesses
3	69	M		+	+	+	Cirrhosis, ascitis, carcinoma of the colon
4	89	F		+	+	+	Pancreatic carcinoma
5	77	M	+	+	+	+	Periampullary carcinoma
6	81	M		+	+	+	Periampullary carcinoma
7	66	M		+	+	+	Periampullary carcinoma
8	65	F		+	+	+	Periampullary carcinoma
9	74	M		+	+	+	Periampullary caricinoma
9 (Total)	74.3	6M − 3F	2 GB	9 CD	9+	9	6 periampullary carcinoma 1 pancreatic, 1 colon carcinoma 1 multiple liver abscesses

GB, gallbladder; CD, common duct; CP, calcium palmitate.

versa, but they always had typical brown and black material both in the center and in the periphery (Figs. 4A to C). They were found mostly in the common duct, were always associated with bile infection, and always contained calcium palmitate. Therefore they can be considered to share the same pathogenesis as brown stones. Excluding this small group of nine patients, all other pigment stones can be distinguished as brown and black, which greatly differ not only in composition, structure, and pathogenesis but also in clinical behavior and thus they require a different treatment.

In fact infectious brown stones are more likely to cause symptoms and require surgery than other types of stones.[12] In our study patients with brown stones presented with acute cholangitis in 60% of cases, while patients with noninfectious stones had associate cholangitis in only 23% of common duct stones. These figures correlate with those previously reported by Smith et al.[12] (52% in infectious stones, 18% in noninfectious stones). However it must be stressed that not all brown stones produce symptoms because typical cholangitis with fever and chills only occurs when sudden hypertension in the bile ducts is also associated, thus determining the passage of bacteria from the biliary tract into the blood stream. In a long-term follow-up study of patients with sphincterotomy or sphincteroplasty, we found that two patients with recurrent stones (including one patient with a 4-cm large stone, resembling in shape a true cast of the confluence between the main hepatic ducts and the common duct) were asymptomatic 5 years after sphincterotomy.

On the other hand, we recently documented that black pigment stones frequently are associated, because of their size (microlithiasis) (Table 5), density (high content in calcium carbonate and phosphate), and structure (presence of multiple spiculas on stone surface) (Fig. 3B) with pancreatitis, probably due to the easier obstruction of the ampulla of Vater by such stones.[22] However, in almost 50% of these cases (7 of 15), black stones were found only in the gallbladder but not in the common duct at the time of cholecystectomy. Therefore, because black stones never reform after cholecystectomy,[2] simple cholecystectomy could have been curative not only in the 35 patients without pancreatitis but also in one half of the patients with associated pancreatitis.

In addition we suggest that both cholesterol and black pigment stones form with a mechanism that is completely different from the stasis–infection mechanism. We also can provide evidence that the two mechanisms (metabolic and stasis–infection) are mutually exclusive of each other and that, particularly in the common duct, no stone other than brown pigment can reform after the initial formation of infectious brown stones. In our prospective study we documented that brown stones can form after cholecystectomy in patients with previous cholesterol or black stones,[5] but the opposite was never observed. In particular, in a 67-year-old woman who had cholecystectomy and sphincterotomy for a single ovoidal cholesterol stone (saved by the patient) in 1968, brown stones were found at the first reoperation (repeated sphincterotomy in 1971) and always were found at the second operation (choledochoduodenostomy in 1974), third reoperation (sphincteroplasty in 1984), and fourth reoperation (hepaticojejunostomy in 1987).

Therefore, even if common duct parietal alterations due to bile stasis (Figs. 7B and D) were shown to be reversible after removal of the stenosis (Lygidakis, oral personal communication, 1988), in our opinion, when the vicious circle of infection–stasis–infection, which is crucial for brown stone formation, has begun and brown stones have formed, they will recur almost invariably, regardless of the treatment performed. Drainage procedures (large biliary enteric anastomoses, not sphincterotomy, should be used liberally in patients with brown pigment stones. They cannot prevent permanently brown stone reformation, but if they can assure good biliary clearing for a long period, they could minimize symptoms, even if aggregates of brown mud (*i.e.*, brown stones) continue to form in the ducts. On the other hand, drainage procedures[12] (sphincterotomy, cholechoduodenostomy) are not only unnecessary but also harmful in patient with 'pure' black pigment gallstones, which never reform after cholecystectomy.[2]

We documented that recurrent common duct brown stones form mostly in patients with a previous sphincterotomy or sphincteroplasty for cholesterol, mixed, or black pigment stones. It can be hypothesized that sphincteric damage and bypass allow bile and duodenal juice to mix in the common duct. If duodenal content is sterile, bile is not infected and brown stones do not form. When duodenal content becomes infected with age and associated intestinal motor disorders or gastric pathology, bile is also contaminated and, if bile stasis is concomitant, due to a stricture of the sphincterotomy, brown stones can form.

In conclusion (1) bacteria actually constitute a substantial portion of brown stones and are primarily responsible for the formation of brown stones, which account for the majority of pigment stones also in Western countries as well as in the Orient[3–6] (53.5% of pigment stones, 6.1% of all gallstones in the present series). On the other hand, bacteria are not involved in the formation of cholesterol stones[10–12] and in that of the great majority of black stones,[12] which have a pathogenesis different from stasis–infection; (2) a sharp distinction between black and brown stones is not only useful for a better knowledge of the pathogenesis of the two main types of pigment gallstones but is also crucial for clinical and therapeutic purposes, including the proper selection of the therapeutic options on the basis of stone type and etiology.

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