UvA-DARE (Digital Academic Repository)

Analysis of occluded pancreatic stents and juices in patients with chronic pancreatitis

Smits, M.E.; Groen, A.K.; Mok, K.S.; van Marle, J.; Tytgat, G.N.J.; Huibregtse, K.

Publication date
1997

Published in
Gastrointestinal endoscopy

[Link to publication](#)

Citation for published version (APA):

Smits, M. E., Groen, A. K., Mok, K. S., van Marle, J., Tytgat, G. N. J., & Huibregtse, K. (1997). Analysis of occluded pancreatic stents and juices in patients with chronic pancreatitis. *Gastrointestinal endoscopy*, 45, 52-58.

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Analysis of occluded pancreatic stents and juices in patients with chronic pancreatitis

Marianne E. Smits, MD, PhD, Albert K. Groen, PhD, Kam S. Mok, Jan van Marle, PhD
Guido N.J. Tytgat, MD, PhD, Kees Huibregtse, MD, PhD

Amsterdam, The Netherlands

Background: Pancreatic stents may occlude with time, and there is little information available on the nature of the clogging process.

Methods: We analyzed the contents of occluded pancreatic polyethylene stents in nine patients with chronic pancreatitis. In the same patients, the protein patterns in the corresponding pancreatic juices were analyzed. The stents had been in place for a mean of 9 weeks (range 2 to 17).

Results: All stents were occluded at both ends, especially around side holes, with thick creamy-white precipitate. The average dry weight of occluding debris was 3 mg per 3.25 cm 10F stent. Total protein content was 50% (SD 16.3) and total calcium 0.8% of dry weight (SD 0.6). Light microscopy showed that proteinaceous material completely filled the stent lumen. Yeasts and plant material were seen in two stents. A variable number of bacteria of mixed species, sometimes in clumps, were patchily scattered in the protein matrix. Cultures of stent contents grew several species of Gram-positive and negative bacteria. Scanning and transmission electron microscopy showed an amorphous protein matrix in all stents, arranged as a network in some areas, but in layers in other areas. Sodium dodecylsulfate polyacrylamide gel electrophoresis showed that protein patterns of stent contents were remarkably different from the protein patterns of the juice samples of the same patient. A 66 kD band, identified as albumin, appeared in the protein patterns of stent content, whereas it was lacking in most juice samples.

Conclusions: Adherence of protein, especially albumin, plays an important role in the process of pancreatic stent clogging. Other factors, such as bacteria, refluxed duodenal contents, and calcium seem to be of less importance. (Gastrointest Endosc 1997;45:52-8.)

The pain in patients with chronic pancreatitis may be due to obstruction of the pancreatic duct with upstream dilatation because of strictures or stones.¹⁻³ Endoscopic pancreatic stent insertion may be used to relieve pancreatic duct obstruction. This results in symptom relief in 60% to 80% of the patients.¹⁻⁵ Stents

may also be used to predict symptom response in an attempt to select patients for surgery. In pancreas divisum, putative outflow obstruction from the larger dorsal part of the gland may be improved by stent placement across the minor papilla, which is particularly effective in a subgroup of patients with acute recurrent pancreatitis.⁶ Other indications for pancreatic stenting are ductal disruption and treatment of pancreatic ascites and pleural effusion, as well as draining of pseudocysts communicating with the pancreatic duct.^{5,7,8}

A major limitation of the use of pancreatic stents is stent occlusion, which causes recurrence of symptoms and necessitates repeated stent exchanges.^{1-3,5} The optimal time interval for elective stent exchange to prevent clogging is not known.

Received January 26, 1996. For revision February 8, 1996.
Accepted June 17, 1996.

From the Academic Medical Center, University of Amsterdam, Department of Gastroenterology and Hepatology, Amsterdam, The Netherlands.

Reprint requests: Marianne E. Smits, Gastroenterology and Hepatology, Academic Medical Centre, P.O. Box 22700, 1100DE Amsterdam, The Netherlands.

37/1/77269

A better understanding of the mechanism of pancreatic stent occlusion would allow the design of stents with a greater longevity. There is little information available on the nature of the clogging process. Provansal-Cheylan et al.⁹ analyzed the material blocking 10 pancreatic stents that had been in place for periods of 1 to 24 months. They found the sludge to be composed of CaCO₃ crystals, bacterial ghosts, and protein threads.⁹ The major component was pancreatic secretory protein. Schmalz et al.¹⁰ suggested that duodenal reflux was the cause of pancreatic stent occlusion. They found bacteria and necrotic cells in their pancreatic stents, mixed with calcium bilirubinate, calcium carbonate, and amorphous substance.

To shed some further light on this problem, we analyzed the material blocking pancreatic polyethylene stents in nine patients with chronic pancreatitis. In the same patients, protein patterns of pancreatic juice were analyzed.

MATERIALS AND METHODS

Materials

Ten pancreatic stents and pure pancreatic juice samples were obtained from nine patients with chronic pancreatitis who had pancreatic stents inserted between 1993 and 1994. Stents were 10 cm Amsterdam-type polyethylene stents, eight 10F and two 7F. The indication for stenting was chronic pain or recurrent pancreatitis and the presence of a pancreatic duct stricture with proximal dilatation. The cause of chronic pancreatitis was alcohol abuse in six patients and unknown in the other three. The stents had been in place for a mean of 9 weeks (range 2 to 17) and were removed endoscopically.

Simultaneously with stent removal a pancreatic juice sample was taken (2 to 5 ml) by placing the tip of a catheter in the pancreatic duct and aspirating the juice. Secretin (75 clinical units) was given intravenously to stimulate pancreatic juice secretion.

Methods

Pancreatic stents and juice samples were processed immediately or stored at -20° C. The stents were visually inspected and 2 to 3 mm segments were taken for scanning electron microscopy (SEM) and transmission electron microscopy (TEM). From the middle of the stent, a sample of contents was removed with a sterile scalpel for bacteriologic culture.

For further analysis, the stent contents were solubilized by repeatedly washing the inside of the stent, with 1 ml/cm sterile water, into a sterile container. Vortexing for 5 minutes completed the dissolution process. An aliquot of known volume was lyophilized and weighed to determine dry weight of stent contents. Total protein was determined with the Lowry method.

Protein patterns of stent contents and pancreatic juices were defined by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Specific protein bands were excised and sent for amino-terminal sequencing.

Microscopy

A sample of unfixed stent contents was placed on a clean slide, then examined with a polarizing light microscope (Zeiss, Oberkochen, Germany).

Fixed 2 to 3 mm stent sections were embedded in methacrylate resin, and sections 1 µm thick were obtained. Sections were stained with hematoxylin and eosin (H&E), Gram's stain for bacteria, periodic acid-Schiff (PAS) stain for mucin and polysaccharide, and van Kossa for calcium.

The resin-embedded processing of the stents ensured that CaCO₃ crystals would be detected if present. Calcium bilirubinate could be recognized as a golden pigment on H&E.

Scanning electron microscopy

Glutaraldehyde-fixed specimens were washed twice in phosphate buffer, dehydrated in ascending concentrations of ethanol, critical-point dried or treated with hexamethyl disilazane, sputter coated with approximately 15 nm of gold, and examined with an ISI SS40 scanning electron microscope at 10 kV acceleration voltage. Other samples were simply air dried, sputter coated and then examined.

X-ray microanalysis

To assess the calcium contents, selected areas of the scanning electron microscopy specimens were examined by electron beam microanalysis. By using this technique, local chemical composition of solid samples can be determined. A fine beam of monoenergetic electrons is focused within the area of interest on the specimen surface, using a Philips 505 scanning electron microscope (Philips, Eindhoven, The Netherlands) with a DX-4 EDS attachment at an acceleration voltage of 15 kV. As a result of interaction between impinging electrons and the local material, characteristic x-rays are emitted by the constituent chemical elements. Analysis of x-rays was accomplished by an energy dispersive spectrometer.

Transmission electron microscopy

The fixed material was washed three times in phosphate buffer, postfixed with 2% osmium tetroxide, washed twice in phosphate buffer, and dehydrated. The specimens were embedded in Epon embedding medium (Ladd Research Industries, Burlington, Vermont), sectioned, stained with uranyl acetate and lead citrate, and examined in a Philips 420 transmission electron microscope operating at 80kV.

SDS-PAGE electrophoresis

Sample buffer was added to the samples of pancreatic juice or stent contents. Phenylmethylsulfonyl fluoride, an inhibitor of proteolysis, was added (1 mM) first. These samples were heated at 100° C for 3 minutes and centrifugated. Electrophoretic separation of proteins was carried out in 12% gels according to the SDS-PAGE method.¹¹ Gels were stained with silver (Biorad) and with Coomassie blue. Molecular weights were determined by comparison with commercial standards (Biorad).

Figure 1. End-on view of typical blocked pancreatic stent (10F).

Figure 3. SEM of protein as a network. Artefactual separation of matrix and stent wall (arrow) occurred during processing (Scale: 5µm.)

Figure 2. Protein matrix in an occluded stent (H&E). (Scale: 100 µm.)

Protein sequencing

Fractionated proteins were detected with SDS-PAGE under reducing conditions¹¹ using a small 11 gel system (Hoefer Scientific Instruments, San Francisco, Calif.). Gels were run and transferred to a polyvinylidene difluoride membrane using a 3-[cyclohexylamino]-1-propane sulfonic acid buffer as described by le Gendre et al.¹² The membranes were stained with 0.1% Coomassie blue R-250 in 50% methanol and destained with a 50% methanol/10% acetic acid solution. Protein bands were excised and subjected to amino-terminal sequencing.

RESULTS

Direct examination of the stents showed that all stents were completely blocked with a thick, creamy-white material (Fig. 1). The blocking material was gelatinous and closely attached to the stent wall. Minimal solid material was adherent on the outside of the stent. The stents were completely obstructed at both ends, and most were blocked along their entire length. Direct examination of pancreatic juice showed a cloudy juice containing particles. In some cases there was a tinge of blood.

The average dry weight of the stents was 3 mg per 3.25 cm 10F stent (SD 2.42). Total protein content was 50% of dry weight (SD 16.3). Total calcium content was 0.8% of dry weight (SD 0.6).

Light microscopy (LM) showed mesh-like eosinophilic proteinaceous material, completely filling the lumen (Fig. 2). PAS staining showed that no significant mucoproteins were present. There was minimal calcium bilirubinate. Calcium carbonate crystals were seen in the unfixed stent plug from only one patient and were few in number. Calcium, as shown by the van Kossa stain on LM, stained in patches colocalizing with areas staining for protein on H&E and was prominent in three stents.

At the duodenal end of the stent, characteristic budding yeast forms were seen in two stents and plant material in two. Birefringent globules with a "Maltese-cross" appearance were noted in the unfixed stent plug from two patients, indicating lipid globules. An occasional epithelial cell was found in one patient.

A proteinaceous matrix was present in all stents at SEM and TEM, confirming the LM findings. SEM showed that the protein was arranged as a network in some areas of the stent (Fig. 3), but in layers in other areas (Fig. 4). Both patterns were present in different areas of the same stent. TEM showed that the matrix had the ultrastructural features of protein (Fig. 5). The protein was arranged in lamellae in some areas; fibrils were seen less frequently. Protein coated the stent wall, and in no cases were bacteria seen in direct contact with the stent wall.

SEM revealed that some areas of the protein matrix were granular (Fig. 6). Energy-dispersive spectrometry (EDS) examination (Fig. 7A) revealed that these granular areas were approximately 100-fold enriched in calcium, as compared to surrounding areas that had the appearance of protein (Fig. 7B).

Figure 4. SEM of amorphous protein and protein in layers. (Scale: 5 μ m.)

Figure 6. SEM of coliform bacteria (*small arrows*) on and in the matrix. Granular projections (*large arrows*) are probably CaCO₃ microcrystals. (Scale: 5 μ m.)

Figure 5. TEM of amorphous protein matrix adherent to stent wall (*arrow*). (Scale: 1.0 μ m.)

Figure 7. X-ray microanalysis **A**, Areas appearing granular on SEM (Fig. 6) had a high calcium content on EDAX (CaKa elevation), suggesting they were modified CaCO₃ microcrystals (CaCO₃ protein complexes). **B**, Areas with a protein-like appearance (Fig. 6) had a low calcium signal on EDAX. C, Carbon; N, nitrogen; O, oxygen; Na, sodium; Au, gold; Ca, calcium; Ka, K shell α ; Kb, K shell β ; KeV, Kilo electron Volt. Gold peak is due to sputter coating of scanning electron microscopy specimens with 15 nm gold.

Figure 8. This stent had many bacteria, some in clumps. (Scale: 100 μ m).

At LM, a variable number of bacteria of different species, sometimes in clumps, were patchily scattered in the matrix (Fig. 8). The number of bacteria varied from patient to patient, and within the same stent. The predominant species cultured were Gram-negative bacteria (*Klebsiella* and *Escherichia coli*), Gram-positive (*Streptococcus faecalis*, *Staphylococcus epidermidis*, α -hemolytic streptococcus), and a mixture of gram-positive and negative anaerobes were also seen. Viability of the bacteria was 80% as assessed by plate counts.

SEM showed a few coliform bacteria scattered in foci (Fig. 6). TEM showed that most bacteria existed in colonies, sometimes consisting of different species. The number of bacteria differed from stent to stent, and within each stent. The bacteria were surrounded by a fibrillar glycocalyx that cemented them to each other to form colonies.

Stents that were processed immediately or after a period of storage at -20° C yielded comparable microscopic results. The results were also similar in patients whose chronic pancreatitis was due to alcohol or was idiopathic.

Figure 9 shows the SDS-PAGE patterns of samples derived from stent content and corresponding pancreatic juice samples of four different patients. The juice samples showed characteristic bands at around 57 kD and between 30 and 40 kD in all patients. In addition, most samples contained intense bands at around 14 kD, corresponding to the molecular weight of pancreatic stone protein (lithostatin). A 92 kD band, corresponding to GP2, was rarely seen. The protein patterns of the samples derived from stent content were remarkably different from the protein patterns of the juice sample. Notably, in the stent samples there was a general shift to higher molecular weight, little staining in 30 to 40 kD staining, and appearance of a

Figure 9. SDS-PAGE protein patterns of samples from pancreatic stents and corresponding pancreatic juices in four different patients. S, Stent; J, juice. In each lane about 10 μ g of protein was loaded. In the last lane a mixture of standard proteins (ST) was electrophoresed.

66 kD band, which was lacking in most juice samples. In most samples from stent content, the 66 kD and 57 kD bands were the predominant proteins present. Compared to the juice samples, the content of lithostatin (14 kD) was reduced.

Amino-terminal sequencing showed that the 66 kD protein band was human serum albumin and the 57 kD protein band was a degradation product of albumin.

DISCUSSION

Pancreatic stents were found to be blocked by a proteinaceous matrix. Bacteria, refluxed duodenal contents, and minimal calcium can be found in this matrix. The eosinophilic staining characteristics, the ultrastructural features, and the EDS composition all suggest that the matrix is predominantly proteinaceous in nature. There is no consistent organization or arrangement of the matrix, suggesting that particulate elements such as bacteria become more or less randomly trapped in the matrix as it forms. The matrix has different forms in different parts of the same stent: layered in some and network-like in other areas. It is possible that the different appearance of the network in different areas could be due to variation in local concentrations of the different proteins or trace elements, resulting in the formation of different structures with a variation in morphology.

Most likely, the initial step in stent blockage is the adherence of proteins to the inner surface of the stent (Fig. 3). The stent lumen is narrowed by the progressive deposition of protein, and possibly the adhesion of protein plugs, if present, in the pancreatic juice. Duodenal reflux may contribute some particulate matter to the matrix, particularly at the duodenal end of the stent, but does not seem to be mechanistically

important to stent blockage. The absence of calcium bilirubinate and of large quantities of plant material and mucous suggest that duodenal reflux was not important. Bacteria seem to colonize the matrix. However, bacterial biofilm, if present, does not prove to be a major mechanism contributing to stent occlusion. The amount of calcium in our stents was small. If the calcium was nonspecifically adsorbed from pancreatic juice, it would be expected to stain the matrix homogeneously. Compared with our results, Provansal-Cheylan et al.⁹ found a greater prevalence of CaCO_3 crystals in their study, which may be due to a longer stenting period. Alternatively, because their stents were left to dry at room temperature, calcium that was present in the stent plug may have precipitated in crystalline form.

Why do proteins precipitate in the duct lumen to form plugs? The pathogenesis of protein plugs is unclear, and it is unclear whether they are a cause or consequence of chronic pancreatitis, although the latter hypothesis is favored. Other authors noted that the duct epithelium in the immediate area of the protein plug is frequently damaged and they found that these protein plugs stained with antibodies against serum proteins, suggesting that serum proteins exuded through the damaged duct wall in chronic pancreatitis and contributed to the protein precipitate in the duct lumen.^{13, 14} In support, TEM of plugs showed fibrillar material resembling fibrin.¹³ An alternative theory suggests that premature pancreatic enzyme activation in the pancreatic duct can precipitate proteins, resulting in the formation of protein plugs, and is supported by the occasional finding of activated enzymes in pancreatic juice. Kern et al.¹⁵ found in a TEM study that pancreatic protein secretions in normal subjects had a tendency to aggregate even when they had been just secreted into the duct lumen. He postulated that the increased protein concentration seen in the pancreatic juice of patients with chronic pancreatitis may accelerate the process of aggregation, resulting in macroscopic plugs. Once formed, it is likely that these plugs obstruct some ducts, which can then accelerate the process of duct damage.

Protein plugs may form the nidus for calcium deposition and are likely precursors of pancreatic stones. Pancreatic stones have a sponge-like organic matrix of protein and mucopolysaccharide. The protein matrix of pancreatic stones resembles the matrix of occluded pancreatic stents. However, the protein pattern of pancreatic stones and stent content is different. Stones have been reported to consist mainly of calcium complexes of pancreatic stone protein. The major component of the stent content was serum albumin. We can only speculate about the reason why this protein accumulates in the stent. Albumin is not a major protein in juice and probably ends up in pancreatic secretion

due to damage of the tissue wall. This leakage most probably increases after stent placement, since the stent inflicts damage to the pancreatic duct. It is well known that albumin contains hydrophobic binding sites, which could play a role in binding of the protein to the stent wall. But we have no explanation why the protein escapes degradation by the proteolytic enzymes present in the pancreatic juice. Perhaps the protein precipitates in a complex with calcium salts and the 57 kD breakdown product. Recently, it has been suggested that GP2, the pancreatic homolog of uromodulin, plays an important role in precipitation of protein plugs in chronic pancreatitis. We rarely observed a 92 kD band in the stent material, suggesting that precipitation of protein plugs occurs via a different mechanism.

Our observations suggest that bacteria are trapped singly or in colonies during matrix formation and, once trapped, they may continue to grow in situ. Bacterial survival in potentially unfavorable conditions such as in pancreatic juice and in pancreatic stents is aided by the presence of a glycocalyx coat.¹⁶ This glycocalyx is recognized on TEM as a fibrillar coat surrounding each bacterium, which cements bacteria to one another to form colonies, protecting and allowing the microorganisms to grow. Both TEM and LM showed that the bacteria were not adherent to the inner or outer wall of the stent, and in all stents a layer of protein separated any bacterium from the stent wall. Some areas of the matrix had no bacteria, suggesting that bacteria are not necessary for the formation of the protein matrix in pancreatic stents. Micro-organisms contribute a relatively small volume to the material blocking pancreatic stents. Bacteria most likely colonize the matrix once it has formed. The pancreatic duct of healthy individuals is normally sterile. Bacteria from the upper digestive tract may be introduced into the pancreas when the pancreatic duct is cannulated, and a pancreatic duct stent probably predisposes to reflux of duodenal contents, which can introduce more bacteria. A variety of gram-positive and gram-negative bacteria were found in our pancreatic stents, which has also been described by others.¹⁷

It would be useful to design pancreatic stents that remain patent for a longer period of time. Decreasing protein concentration in the abnormal pancreatic juice could decrease the rate of protein accretion onto the stent, but there are no methods available at present to accomplish this goal. Oral citrate and trimethadione therapy were useful in some patients to decrease pancreatic stone size, probably by an increase in the solubility of calcium carbonate, but the long-term use of these agents is not known.^{18, 19} Impregnation of pancreatic and biliary stents with the antibiotic cefoxitin did not alter the occlusion rate.²⁰ This finding is not surprising since it is not likely that

bacteria play a major role in stent occlusion. The use of metal stents in the pancreatic duct is not advocated at present, since these stents are nonremovable and may result in ductal obstruction due to mucosal hyperplasia.²¹ Prophylactic stent exchange has been advocated to prevent complications from blocked stents. However, repeated procedures are associated with an increased morbidity and higher costs, and it is not clear at which interval the stents should be exchanged.²² The most promising developments may lie in stent polymers to which proteins adhere less, perhaps by modulation of stent composition and its surface properties.

REFERENCES

1. Cremer M, Deviere J, Delhay M, Baize M, Vandermeeren A. Stenting in severe chronic pancreatitis: results of medium-term follow-up in seventy-six patients. *Endoscopy* 1991;23:171-6.
2. McCarthy J, Geenen JE, Hogan WJ. Preliminary experience with endoscopic stent placement in benign pancreatic diseases. *Gastrointest Endosc* 1988;34:16-8.
3. Kozarek RA, Patterson DJ, Ball TJ. Endoscopic placement of pancreatic stents and drains in the management of pancreatitis. *Ann Surg* 1988;209:261-6.
4. Fuji T, Amano H, Ohmura R, Akiyama T, Aibe T, Takemoto T. Endoscopic pancreatic sphincterotomy—technique and evaluation. *Endoscopy* 1989;21:27-30.
5. Grimm H, Meyer WH, Nam VCh, Soehendra N. New modalities for treating chronic pancreatitis. *Endoscopy* 1989;21:70-4.
6. Lans JJ, Geenen JE, Johanson JF, Hogan WJ. Endoscopic therapy in patients with pancreas divisum and acute pancreatitis: a prospective, randomized, controlled clinical trial. *Gastrointest Endosc* 1992;38:430-4.
7. Dohmoto M, Rupp KD. Endoscopic drainage of pancreatic pseudocysts. *Surg Endosc* 1992;6:118-24.
8. Kozarek RA, Ball TJ, Patterson DJ, Freeny PC, Ryan JA, Traverso LW. Endoscopic transpapillary therapy for disrupted pancreatic duct and peripancreatic fluid collections. *Gastroenterology* 1991;100:1362-70.
9. Provansal-Cheylan M, Mariani A, Bernard JP, Sarles H, Dupuy P. Pancreatic stone protein: quantification in pancreatic juice by enzyme-linked immunosorbent assay and comparison with other methods. *Pancreas* 1989;4:680-9.
10. Schmalz M, Gavanis J, Mandel N. Analysis of occluded pancreas divisum stents: is duodenal reflux the cause? [abstract] *Gastrointest Endosc* 1988;34:A187.
11. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
12. le Gendre N, Mansfield M, Weiss A, Matsudaira PT. A practical guide to protein and peptide purification for microsequencing. New York: Academic Press, 1993:71-101.
13. Nakamura Y, Sarles H, Payan H. Three dimensional reconstruction of the pancreatic ducts in chronic pancreatitis. *Gastroenterology* 1972;62:942-9.
14. Payan H, Sarles H, Demirdjian M. Study of the histological features of chronic pancreatitis by correspondence analysis: identification of chronic calcifying pancreatitis as an entity. *Biomedicine* 1992;18:663-70.
15. Kern HF, Warshaw AL, Scheele GA. Fine structure of protein precipitations in acinar lumina of the normal human pancreas and in chronic pancreatitis. *Pancreatitis—concepts and classifications*. Amsterdam: Elsevier, 1984:101-5.
16. Speer AG, Cotton PB, Rode J. Biliary stent blockage with bacterial biofilm: a light and electronmicroscopy study. *Ann Intern Med* 1988;108:546-53.
17. Gregg JA, Chun CH, Thomas E. The bacteriology of pancreatic duct stents [abstract]. *Gastroenterology* 1993;106:A692.
18. Noda A, Shibata T, Hamano H, et al. Trimethadione (Troxi-done) dissolves pancreatic stones. *Lancet* 1984;11:351-3.
19. Noda A, Shibata T, Ogawa Y, et al. Dissolution of pancreatic stones by oral trimethadione in a dog experimental model. *Gastroenterology* 1987;93:1002-8.
20. Browne S, Schmalz M, Geenen J, Venu R, Johnson GK. A comparison of biliary and pancreatic stent occlusion in antibiotic-coated vs. conventional stents [abstract]. *Gastrointest Endosc* 1990;206:A87.
21. Davids PHP. Biliary endoprotheses: current indications, limitations and complications [thesis]. Meppel: Kripps Repro 1992.
22. Coene PP, Tytgat GNJ, Obertop H, Huibregtse K. Prophylactic versus symptomatic exchange of biliary stents [abstract]. *Gastrointest Endosc* 1995;41:394:394.

Availability of Journal back issues

As a service to our subscribers, copies of back issues of *Gastrointestinal Endoscopy* for the preceding 5 years are maintained and are available for purchase from Mosby until inventory is depleted at a cost of \$12.00 per issue. The following quantity discounts are available: 25% off on quantities of 12 to 23, and one third off on quantities of 24 or more. Please write to Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318, or call (800) 453-4351 or (314) 453-4351 for information on availability of particular issues. If unavailable from the publisher, photocopies of complete issues may be purchased from UMI, 300 N. Zeeb Rd., Ann Arbor, MI 48106 (313) 761-4700.