

A Large Animal Model (Swine) to Study the Diagnosis and Treatment of Cholelithiasis

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Human gallstones were surgically placed into the gallbladders of 200 swine. Eight of these swine were used as a prospective series to verify that the placement and presence of human gallstones in their gallbladders caused no significant pathologic changes in the gallbladder and that the gallstones were not spontaneously dissolved. Although limitations exist, the advantages of this procedure demonstrate that the surgically prepared swine is an appropriate model for controlled in vivo experiments in radiologic imaging or interventional treatment. A 4 F pig-tailed catheter designed for Trocar insertion was developed as a companion to this model.

Key words: cholelithiasis; gallstone; model, animal; swine; catheter.

THE ATTENTION recently given to alternative methods of therapy for cholelithiasis requires a standard large animal model for this disease. An appropriate model is one

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that can be used in several types of research, such as radiologic imaging studies,¹⁻⁵ interventional techniques,⁵⁻⁷ lithotripsy⁸ and dissolution studies.^{5,9-12}

In establishing a multipurpose model, a surgical approach is fast, economical, and highly controllable. However, a search of the literature¹³⁻¹⁵ concerning surgical induction of cholelithiasis reveals no description of the methods of surgical implantation and its usefulness or associated problems.

We used young domestic swine as a model for cholelithiasis. A pigtailed catheter that can be placed percutaneously and transhepatically was developed for use in this animal model. A detailed description of the surgical methods of stone implantation is given, as well as the equipment and procedure of the percutaneous, transhepatic insertion of the catheter. The advantages, disadvantages, and sequelae of these procedures are discussed to demonstrate the utility of this model in studies involving the diagnosis and treatment of cholelithiasis.

Materials and Methods

Gallstones

Human gallstones were obtained after cholecystectomy from the pathology departments of three local hospitals. The stones were separated by gross observation into distinct groups with consideration given to color, size, shape, and surface texture. Based on laboratory and radiographic analyses, only gallstones that had cholesterol as the main constituent were used in this experimentation (ie, stones that were high in calcium salts or pigments were not chosen). The stones were cleaned of any debris and stored in a solution of 10% tincture of Zepharin Chloride (1:750, Winthrop Laboratories, Division of Sterling Drug, Inc., New York, NY) in normal Ringer's. This permitted complete hydration of stones and kept bacterial growth to a minimum.

Gallstone Implantation

Young domestic pigs (n = 200), weighing approximately 55-77 kg, were used. They were given water ad libitum and fed with standard pig chow once each morning. The pigs received no food 24-48 hours before the implantation surgery.

Initially, the pigs were sedated with an IM injection of Rompun (1-2 mg/kg, Haver of Miles Laboratories, Shawnee, KS) and Ketaset (10-15 mg/kg, Bristol Laboratories, Syracuse, NY). The pigs were maintained thereafter with 0.5% Halothane anesthesia through a large animal anesthesia mask.

The abdomen was prepped and draped according to sterile procedure. A longitudinal 8-10 cm midline abdominal incision was made through the skin, muscle, and peritoneum. Two 5-0 silk retention sutures were placed in the fundus of the gallbladder approximately 0.5 cm apart. Bile was aspirated with a 20-gauge needle. A small incision was made in the gallbladder between the retention sutures. After expansion of the opening with tissue forceps, preselected and preweighed human gallstone(s) were placed into the gallbladder. The cholecystostomy was closed with a running 5-0 chromic gut suture. The pig's abdomen was closed with 2-0 running sutures in the peritoneum and abdominal muscle layers and finally with a 2-0 running mattress closure of the skin. As a prophylactic, superficial antibacterial spray (Furazolidone 4% Aerosol Powder, Veterinary Products Industries, Phoenix, AZ) or cream (Furacin Veterinary Dressing, Eaton Laboratories, Norwich, NY) was applied to the incision area.

The entire procedure from induction of anesthesia to closure takes approximately 30 minutes, and recovery from anesthesia takes approximately 30 minutes, at which time the pig is ambulatory.

The animals were fed the morning after the surgery. During a three-day recuperation period, prophylactic antibiotic therapy was given IM (Penstrap or Dicillin, 300,000 U/day). For studies requiring ultrasound imaging or interventional procedures, seven to ten days were allotted for reabsorption of free air. This postoperative period also allowed for healing of the incisions.

Percutaneous Gallbladder Access for Experimental Procedures

Access to the gallbladder lumen was achieved through a single-stick catheter. The custom 4F pigtail catheter of extruded nylon (Mallinckrodt, Inc., Angleton, TX) (Fig. 1) has both a distal opening and side ports positioned on the inside curve of the pigtail. The catheter is mounted on a 21-gauge thin-wall cannula (Cook, Inc., Bloomington, IN) with a diamond-tipped stylus. Upon removal of the solid stylus, a 0.018-inch Lunderquist guidewire (Cook, Inc.) can be inserted through the cannula and catheter.

Before catheter insertion, the swine were NPO for 24-36 hours, sedated, and anesthetized as described in the implantation phase of the procedure. Intubation is recommended for any experiment or procedure that lasts longer than approximately 30 minutes. To minimize gastric air, an Ewalt tube was inserted into the stomach and connected to a Gomco suction device (120 mmHg, intermittent). The skin in the upper right quadrant was shaved (or depilatorized) and scrubbed with Betadine.

To identify the most appropriate approach, we visualized the gallbladder ultrasonically with a Toshiba Diagnostic system (Model SSA-90A) using a 3.75 MHz sector scanner (Toshiba Model PSE-37H). Typically a subcostal, anterior, transhepatic approach was chosen for catheter insertion. Occasionally, an intercostal path was used when the subcostal approach was not optimal in terms of ultrasound imaging. With a 4 MHz linear

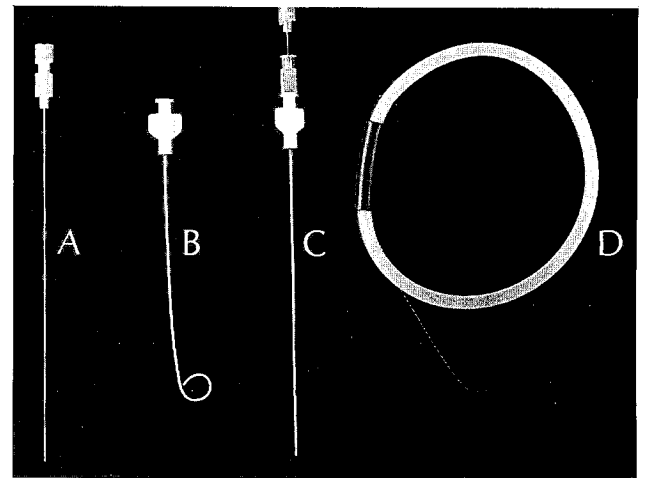


Fig. 1. 4F pig-tailed, extruded nylon catheter. The components of the 4F pig-tailed gallbladder catheter system are shown. The stylus/cannula assembly (A), the unmounted 13 cm catheter (B), and the J-guidewire (D) are shown. Also pictured is the catheter mounted (C) on the stylus/cannula assembly, as it would be inserted into the gallbladder.

array biopsy probe (Toshiba Model GCE-406M), a small incision (approximately 1 mm) was made in the skin with a No. 11 scalpel blade directly over the gallbladder.

Direct, real-time ultrasound visualization with a biopsy probe was used for the catheter placement. The catheter, mounted on the cannula/stylus assembly, was inserted into the gallbladder with one smooth motion. The stylus was removed and a small amount of bile was aspirated to ensure placement in the gallbladder. The 0.018-inch Lunderquist guidewire was inserted into the cannula and the floppy end allowed to curl into the gallbladder. The catheter was then advanced over the guidewire while the cannula was held fixed. The guidewire and cannula were retracted together leaving the catheter with the reformed pigtail in the gallbladder. The catheter can be left in place until just before the animal recovers from anesthesia.

Prospective Protocol

Eight swine were used expressly for the purpose of verifying that the surgically prepared swine model was viable. These eight animals had preweighed gallstone(s) placed in their gallbladders according to the above protocol. Four of these pigs were allowed to survive for one week after surgery, and the remaining four survived for six weeks after surgery. The animals were killed, and the gallstones were extracted and weighed; both absolute and percentage weight losses were calculated.

The gallbladder and liver were analyzed histologically for any pathologic change that could be attributed to the surgery or the presence of the gallstone(s) in the gallbladder. Each gallbladder and liver was examined in multiple areas. Two to four cross-sections in different areas were mounted and stained with hematoxylin and eosin and viewed under the light microscope. These tissues were examined for edema, coagulative changes, inflammation, and fibrosis. Each parameter was assessed qualitatively with reference to normal swine tissue from a nonsurgical animal.

Normal bile from five swine was obtained before stone implantation. This bile was analyzed by gas-liquid chromatography to determine the composition of the bile acid pool and the lithogenic index.¹⁶

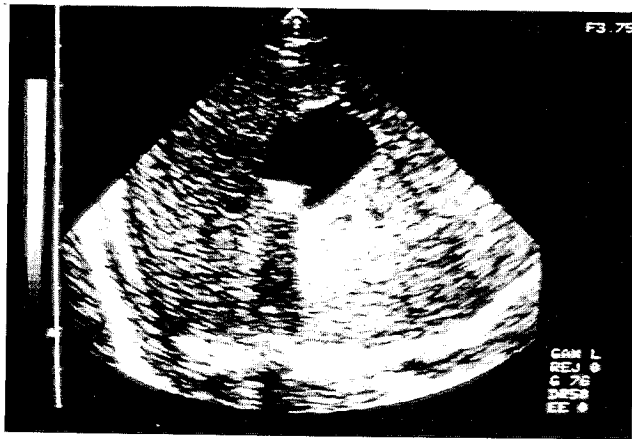


Fig. 2. Ultrasound image of cholelithiasis model in pig. This ultrasound image was obtained 4 weeks after implantation surgery. A 3.75 MHz transducer was used. In this animal, two gallstones were surgically placed in the gallbladder. The total mass of the gallstones implanted was 502 mg.

Results

Total Model Experience

Human cholesterol gallstones were implanted into the gallbladders of 200 young swine. No adverse effects directly attributable to the surgery were noted; the swine tolerated the procedure well. In every case, the gallstones were visible ultrasonographically at a minimum of seven days after surgery. Figure 2 is a typical ultrasonogram of an implanted human gallstone in a pig.

A total of 96 pigs were subjected to percutaneous, transhepatic insertion of the 4 F catheter. Successful catheter placement was achieved with 1-3 insertion attempts in 92.7% of the cases. For three of the pigs, it was necessary to repeat the catheterization attempt on two or three different days until successful placement was achieved. Generally, attempts were made as long as the gallbladder lumen was visible with ultrasound (up to 20 attempts). Two of the 96 pigs could not be catheterized even after repeated attempts (10-20 attempts on each day). In total, 94 of the 96 pigs were successfully catheterized for a success rate of 97.9%. Accounting for the pigs that were repeatedly attempted, 90 of 96 attempts were successful (93.9%). Figure 3 is an ultrasound image of a catheter placed into a pig gallbladder. Typically, catheter placement was achieved within 3 to 5 minutes under ultrasound guidance.

Of the 94 experiments that had successful catheter placement, 43 were acute experiments (ie, the pig was killed on the same day.) Two pigs were not killed until one week after catheterization; 15 animals were killed after two weeks; one was killed after three weeks; 15 were killed after four weeks, and 18 animals were killed at the end of six weeks. No adverse effects due to the catheter placement, such as intraperitoneal bleeding or bile leakage, were identified.

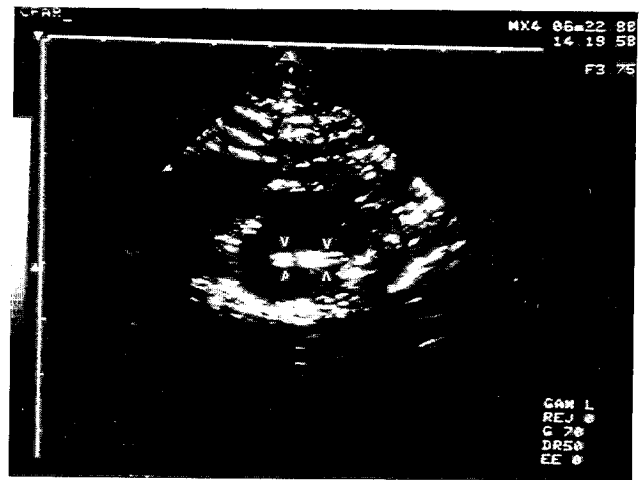


Fig. 3. Ultrasound image of 4 F catheter placed in the gallbladder. This ultrasound image shows the placement of the 4 F catheter in the pig gallbladder. The arrowheads indicate the catheter.

Prospective Study

A prospective series of eight pigs was conducted to specifically answer several questions concerning the viability of the model. Pigs were surgically prepared with human gallstones whose main constituent was cholesterol and allowed to survive either for one week (pigs 1-4) or six weeks (pigs 5-8). Table 1 summarizes these experiments. The range of spontaneous stone reabsorption was 0 mg to 120 mg (0 to 19.4%, by weight). The average stone reduction was $5.44\% \pm 6.84\%$.

Upon comparison of gallbladder tissue from these surgically prepared swine to gallbladders from nonsurgical animals, no differences were noted in edema, coagulative changes, or acute inflammation. Qualitatively, five of the surgically prepared swine had minor chronic inflammation. Fibrosis also was noted in three of these animals. The overlying liver tissue of these eight swine also was examined. Four swine exhibited mild fibrosis. Two of the swine demonstrated both chronic and acute inflammation.

Table 2 details the bile analysis of five pigs. The lithogenic index as described by Admirand and Small¹⁶ was less than 0.4. Table 3 shows the relative composition of pig bile in terms of the bile acid pool. The most prominent bile acid is hyodeoxycholic acid. Chenodeoxycholic acid is the second most prominent bile acid, followed by hyocholic acid, murocholic acid, and deoxycholic acid.

Discussion

A surgical approach was undertaken to produce cholelithiasis in swine. Several investigating groups have used surgery as the method of gallstone induction in experimental animals. Brendel et al¹⁷ reported using surgically prepared dogs for the study of shock wave therapy on

TABLE 1. Spontaneous Gallstone Dissolution in Surgically Prepared Swine (n = 8)

Pig Number	Number of Stones Implanted	Total Weight of Stones (mg)	Survival Time (wks)	Absolute Stone Reduction (mg)	Percentage Weight Loss
1	1	500	1	000	0.0%
2	4	530	1	000	0.0%
3	1	620	1	120	19.4%
4	4	570	1	000	0.0%
5	1	550	6	10	1.8%
6	4	470	6	40	8.5%
7	1	500	6	20	4.0%
8	5	510	6	50	9.8%

Average \pm 1.0 standard deviation for various parameters of interest: number of stones 2.6 ± 1.8 ; total weight of stones, 531 ± 48 mg; absolute stone reduction, 30 ± 41 mg; and percentage weight loss, $5.44\% \pm 6.84\%$.

gallbladder stones. McGahan et al¹⁸ also used a surgically prepared pig model. These reports do not detail the surgical methodology used, and they do not quantify any pathologic effects caused by the surgical intervention or evaluate gallstone dissolution in normal animal bile. Becker et al.¹⁹ proposed a surgically prepared swine as an animal model for interventional radiologic biliary procedures. Although this model provides a training tool for interventional procedures to study radiographic anatomy, no attempt was made to provide a model that could be used in the treatment of cholelithiasis. Although disadvantages and limitations exist, the advantages of our surgically prepared swine model make it appropriate for ultrasonography or interventional techniques in both diagnostic and therapeutic experimentation involving the gallbladder.

The first advantage is the lack of significant spontaneous dissolution of human gallstones in pig bile. The low lithogenic index of normal pig bile presents an environment that may enable the implanted cholesterol gallstone to dissolve spontaneously. This is a potential problem for studies examining the efficacy of experimental therapeutic techniques. The lack of gallstone dissolution in this model is supported by our data. We have not, however, studied spontaneous dissolution in low lithogenic swine bile beyond six weeks.

Second, histologic and gross changes in both the liver and the gallbladder are minimal at both one week and six weeks after implant. The surgical procedure produced no edema or coagulative changes in either tissue in any of the eight animals examined. No necrosis, ulceration, or thickening of the gallbladder were evident due to the introduction of the human gallstones into the swine's gallbladder. The fibrosis observed in the gallbladder generally was localized around the incision in the fundus. Because of the general lack of identifiable surgical complications, this model is appropriate for therapeutic studies that attempt to identify any adverse effects caused by the therapy in question. Surgically prepared swine offer several additional advantages.

The larger size of the domestic swine is conducive to ultrasonographic studies as well as interventional techniques, in contrast to the more prominent models in

rodents. The pig's gallbladder is anatomically accessible for imaging or percutaneous catheterization procedures; it can be approached either subcostally or intercostally.

Reproducibility and the ability to use a model soon after preparation or induction are two important characteristics in the evaluation of any proposed model. This model is easily reproducible, and there is a minimal time lag of no more than 10 days between the preparation of the animal and its use for experiments. These two factors are not consistent with other available models in which no guarantee of 100% induction can be given and an incubation period of several months may be required.¹⁴

This model allows control of parameters such as stone number, size, shape, and composition, which can be manipulated to define a controlled situation, an advantage over other models. The availability of stone analyses permits the preselection of a certain stone type to meet various experimental designs. In this study, stones that were high in calcium or pigments were avoided in favor of cholesterol stones.

Whereas these advantages establish our model's viability, some limitations exist that narrow its application. Surgical intervention is obviously not physiologic, so no useful information concerning bile physiochemistry or the pathogenesis of gallstones can be obtained. In addition, the bile acid pool of swine is dissimilar from that of humans. This may limit the model's usefulness in studies involving oral bile acid therapies such as chenodeoxycholic acid and ursodeoxycholic acid.

TABLE 2. Normal Pig Bile Analysis

Pig Number	Total Bile Acid (mM)	Cholesterol (mM)	Phospholipid (mM)	Lithogenic Index (Admirand/Small) ¹⁶
9	124.8	7.06	54.16	.3808
10	144.0	7.53	42.18	.3908
11	157.9	4.76	51.32	.3885
12	144.9	2.61	29.12	.1582
13	113.9	1.53	18.11	.2110

TABLE 3. Bile Acid Analysis of Normal Pig Bile (% Composition)

Pig Number	Hyodeoxycholic acid	Chenodeoxycholic acid	Hycholic acid	Murocholic acid	Deoxycholic acid
9	53.28	21.73	7.25	4.25	0.80
10	48.29	17.62	11.12	6.36	0.09
11	41.39	14.17	28.13	2.05	
12	35.05	16.41	22.40	4.47	0.295
13	44.87	18.20	8.57	3.91	0.90

Another disadvantage of this model is the rapid weight gain seen with these pigs. The initial weight of the pigs used was approximately 30 pounds. At the end of six weeks, the pigs had increased their weight to 40-45 pounds. Domestic pigs that are housed in animal quarters for up to three months reach a weight of 65-70 pounds. The use of this maturing domestic model poses a problem for any chronic study beyond three months because of its rapid increase in size and weight. This relative disadvantage could be avoided by using a different species such as miniature pigs.

Many studies that might use this model require a simple method of gaining direct access to the gallbladder lumen for bile sampling, infusion of chemical solvents, decompression, or opacification of the biliary tract. Therefore, a 4 F pig-tailed catheter was developed as a useful companion to this animal model. The percutaneous, transhepatic insertion of the catheter was successful in 97.9% of the animals attempted. In this animal model, the gallbladder's anatomic position makes it unlikely that anything but a transhepatic pathway results from the insertion. In the clinical situation, this may not necessarily be the case because of the inherent variability in human anatomy. The lack of observable complications is most likely attributable to the ease of the insertion (ie, single-stick trocar vs. Seldinger method) and the smaller size of the catheter (4 F) compared with currently available biliary catheters.

No experience was gained in chronic installations of this percutaneous catheter (ie, the catheter left in place after recovery from anesthesia while the pig is ambulatory). The lack of associated complications from single insertions suggests that repeated percutaneous catheter insertions over a period of time are achievable and indeed have been accomplished in several animals.

Many experiments investigating cholelithiasis require a readily available and reliable model. This simple catheter and swine model is a combination that is easy to use, produces minor adverse effects, and allows much flexibility in the design and implementation of experiments in the diagnosis and treatment of cholelithiasis. The use of these

research tools will enable further progress toward a more effective therapy for gallstone disease.

References

1. Harbin WP, Mueller PR, Ferrucci JT. Transhepatic cholangiography: complications and use patterns of the fine-needle technique. *Radiology* 1980;135:15-22.
2. McGahan JP, Phillips HE, Nyland T, et al. Sonographically guided percutaneous cholecystostomy performed in dogs and pigs. *Radiology* 1983;149:841-843.
3. Phillips G, Banks S, Kumari-Subaiya S, Kurtz LM. Percutaneous ultrasound-guided puncture of the gallbladder (PUPG). *Radiology* 1982;145:769-772.
4. Teplick SK, Haskin PH. Percutaneous transcholecystic cholangiography: experimental study. *AJR* 1985;144:1059-1063.
5. vanSonnenberg E, Wittich GR, Gerhard R, et al. Diagnostic and therapeutic percutaneous gallbladder procedures. *Radiology* 1986;160:23-26.
6. McGahan JP, Walter JP. Diagnostic percutaneous aspiration of the gallbladder. *Radiology* 1985;155:619-622.
7. Kerlan RK, LaBerge JM, Ring EJ. Percutaneous cholecystostomy: preliminary experience. *Radiology* 1985;157:653-656.
8. Sackmann M, Delius M, Sauerbruch T, et al. Shock-wave lithotripsy of gallbladder stones: the first 175 patients. *N Engl J Med* 1988;318(7):393-397.
9. Allen MJ, Borody TJ, Bugliosi TF, et al. Rapid dissolution of gallstones by methyl tert-butyl ether. *N Engl J Med* 1985;312(4):217-220.
10. vanSonnenberg E, Hofman AF, Neoptolemus J, et al. Gallstone dissolution with methyl tert-butyl ether via percutaneous cholecystostomy: success and caveats. *AJR* 1986;146:865-867.
11. Mack E, Crummy AB, Babayan VF. Percutaneous transhepatic dissolution of common bile duct stones. *Surgery* 1981;90:584-587.
12. Teplick SK, Haskin PH. Mono-octanoic perfusion for in vivo dissolution of biliary stones. *Radiology* 1984;153:379-383.
13. Freston JW, Bouchier JAD. Experimental cholelithiasis. *Gut* 1968;9:2-4.
14. Gurl N, DenBesten L. Animal models of human cholesterol gallstone disease: a review. *Lab Anim Sci* 1978;28(4):428-432.
15. Holzbach RT. Animal models of cholesterol gallstone disease. *Hepatology* 1984;4(Suppl 5):191s-198s.
16. Admirand WH, Small DM. The physiochemical basis of cholesterol gallstones in man. *J Clin Invest* 1968;47:1043-1052.
17. Brendel W, Enders G. Shock waves for gallstones: animal studies. *Lancet* 1983;1:1054.
18. McGahan JP, Lee LL, Tesluk H, Nyland TG, Ruebner B, Schmidt B. Dissolution of gallstones using cholecystostomy tube in the pig. *Invest Radiol* 1987;22:201-205.
19. Becker CD, Burhenne HJ. An animal model for radiologic biliary interventional procedures. *Invest Radiol* 1988;23:30-32.