MORPHOLOGY OF HEALING OF AN EXPERIMENTAL ANASTOMOSIS OF THE COMMON BILE DUCT FORMED BY MICROSURGERY

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The present state of development of the medical industry has resulted in the production of instruments for use in manipulations on small structures and, in turn, this has led to the development of a new branch of surgery, namely microsurgery. Microsurgical techniques are widely used in vascular surgery and traumatology for suturing vessels, nerves, and tendons. The need for its use in the surgical treatment of diseases of the gall bladder and biliary tract has grown progressively for a long time. The reason is that the use of ordinary suture material (silk, kapron, and so on) on such fine structures as the common bile duct and hepatic duct often leads to the development of inflammation, the formation of calculi on the sutures, sclerotic changes, and the development of strictures, requiring repeated surgical intervention. The performance of operations such as hemihepatectomy and manipulations on ducts of even smaller caliber require not only the development of techniques for the conduct of such operations, but also the study of their morphological basis.

The object of this investigation was to study healing of sutured wounds in the wall and an anastomosis of the common bile duct, performed end-to-end by a microsurgical technique in experiments on small laboratory animals.

EXPERIMENTAL METHOD

Experiments were carried out on 20 albino rats of both sexes weighing 130-180 g. The animals were anesthetized by intraperitoneal injection of 2.8-3.5 ml of 1% hexobarbital solution. The operations were performed under the Soviet MBS-2 bench microscope. The common bile duct was dissected under a magnification of 8 times. Sutures were applied to the wall of the duct under a magnification of 16 times. Microsurgical instruments were obtained from "Aesculap" and 10-0 suture material was used (dyed synthetic monofilament 30 μ in diameter).

At the beginning of the investivation the topographical anatomical features of the common bile duct of the albino rat were defined. This question has not been dealt with sufficiently accurately in the literature. The common bile duct in albino rats is formed in the porta hepatis by the union of five or six hepatic ducts carrying bile from the lobes of the liver. There is no gall bladder in rats. The main part of the common bile duct, up to 3 cm long, lies beneath the peritoneum in the superficial layers of the pancreas, and under the microscope it is whitish gray in appearance, elastic in consistency, with an outer diameter of 0.5-0.8 mm, and with walls from 150 to 200 μ in thickness. The pancreatic ducts empty into the common bile duct along its whole length; their diameter is 0.1 mm and their number varies from four to nine. To verify the duct it was punctured and 3 ml of dye injected in the retrograde direction. The staining of the liver and pancreas which quickly took place made it possible to determine the precise anatomical relations.

The experiment consisted of three series. In series I (eight animals) the possibility of using a microsurgical technique of suturing a wound in the wall of the common bile duct was studied. The bile duct was isolated through a longitudinal incision in the overlying peritoneum 8-12 mm long without dividing the pancreatic ducts emptying into it. Division of these ducts was undesirable because it would increase the operative

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Fig. 1. Semicircular suture (4 days). Scar is epithelized (arrow) and consists of proliferating fibroblasts. Inflammatory reaction in thickness of scar and around suture material absent. Here and in Figs. 2 and 3, stained by Van Gieson's method, 160 ×.

trauma and lead to the development of severe pancreatitis, followed by marked sclerotic changes in this region. The anterior wall of the duct was divided for half its circumference in the transverse direction. The lumen of the duct was not constricted: Bile entering during the operation was periodically removed with gauze swabs. The wound in the duct was sutured by means of three or four interrupted sutures. In some cases the sutured wound could be covered with a layer of peritoneum.

In the experiments of series II (eight animals) a circular suture was applied to the duct. In these operations the isolated part of the duct was divided completely in the transverse direction. Depending on the diameter of the duct, from six to eight sutures were needed altogether.

In the experiments of series III (four animals) a circular suture was inserted into the duct and reinforced by application of a thin layer of surgical glue to the outer surface of the anastomosis. To study the character of healing of the common bile duct in the early stages after the opeation animals were killed 4-9 days after the operation. The long-term results were studied after 2-3 months. Post mortem examination of the animals was carried out under an operating microscope. The results of the operations were studied *in situ* after dissection under magnifications of 8 and 16 times. A fragment of the duct 1 cm long in the center with clearly distinguishable suture line on account of the projecting ends of the suture material was taken for histological examination. The material was fixed in 15% formalin solution and embedded in eelloidin. The duct was oriented in the block both vertically and horizontally, so that circular and tangential sections could be cut. Considering the small size of the scar and the difficulty of finding it, serial sections were cut and stained by Van Gieson's method.

EXPERIMENTAL RESULTS

In the experiments of series I two rats died during the period when the technique was being perfected, on the 2nd-4th day after the operation, from biliary peritonitis, developing as a result of failure of the sutures in the common bile duct. The remaining animals were indistinguishable from normal on the day after the operation in their external appearance and behavior; three of these animals were killed 4-9 days after the operation and three were

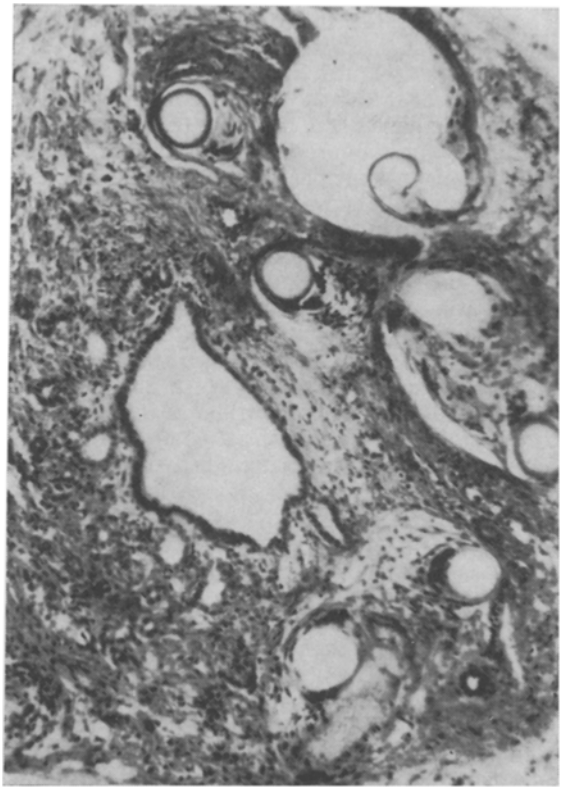

Fig. 2. Circular suture (3 months). Inflammatory reaction absent in thickness of scar and around suture material. Mild macrophagal giant-cell reaction around suture material.

killed after 2 months. The process of healing of the duct wall was largely complete 1 week after the operation. On examination under the microscope a recent scar could be seen in the suture zone. The duct was slightly distended proximally to the suture line and its external diameter was 20-30% greater than distally to the suture line. The sutures were clearly visible. Histologically, on the 4th day the scar consisted of proliferating fibroblasts and vessels and there was no inflammatory reaction. On the side of the lumen of the duct the scar was covered by flattened epithelial cells. Around the suture material no capsule had formed and there was no inflammatory reaction (Fig. 1). By the 6th day, single multinuclear foreign body cells could be found around the suture material. By the 8th-9th day collagenization of the scar had begun. After 2 months the morphological picture showed a slight change. The scars in the region of the sutures were coarser but the external appearance of the duct in the suture zone remained the same as in the early period after the operation. The absence of sclerotic condensation of the duct walls was noted: It remained just as thin-walled, transparent, and elastic as before the operation. The absence of any adhesions between the peritoneal organs must be emphasized.

In the experiments of series II two animals died from biliary peritonitis as a result of failure of the sutures of the anastomosis during the period when the technique of the operation was being perfected. Three animals were killed 7-9 days after the operation. Scaring in the zone of operation was varied in degree in these animals. In one case a fairly dense zone of infiltration measuring 3 × 4 mm was observed. In two other cases the zones of infiltration were smaller and located on the anterior and lateral walls of the anastomosis. Its posterior wall was firmly adherent to pancreatic tissue. In two cases the duct was 20-30% wider proximally to the anastomosis than the portion distally to the anastomosis. Sclerosis of the duct wall was slight. Histologically, on the 7th day epithelization of the scar was observed and it consisted of collagenized avascular connective tissue; no inflammatory reaction was present around the threads or throughout the thickness of the scar. Tangential sections showed that the layers of the duct wall were in accurate apposition. In the pancreatic parenchyma surrounding the duct a picture of acute inflammation was observed. The biliary capillaries of the liver were not dilated. Three animals were killed 2, 2.5 and 3 months after the operation. External examination in two animals showed condensation of the wall of the common bile duct, the proximal segment of which was 40% wider than the distal segment.

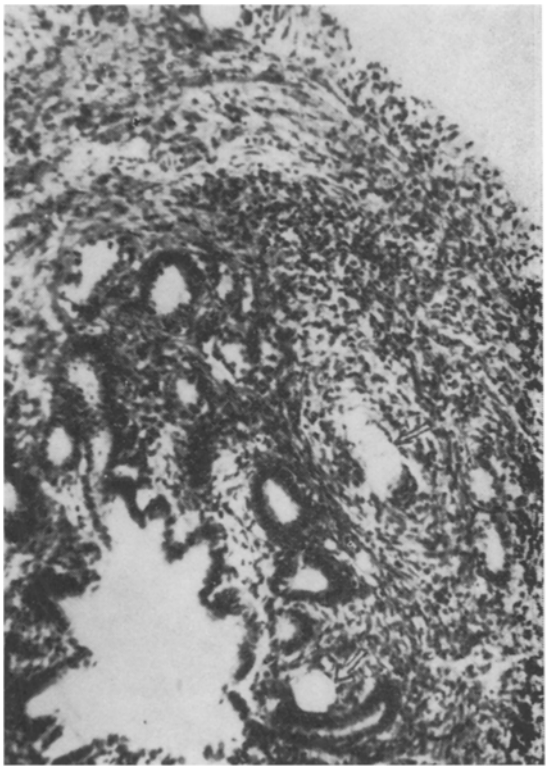

Fig. 3. Circular suture reinforced with glue (2 months). Inflammatory infiltration in substance of duct wall, migration of suture material (arrows) from outer layers toward lumen of duct.

Inflammatory infiltration in the zone of the anastomosis was slight. In one case the duct had the normal appearance. Sutures in the region of the anastomosis lay satisfactorily and no tissue sclerosis could be seen in the zone of the anastomosis. Histologically there was no inflammatory reaction around the suture material or in the substance of the scar. The threads were immured in fibrous tissue without the formation of a capsule around them, and near each of them there was a single giant multinuclear foreign-body cell (Fig. 2). Marked sclerosis was present in the adjacent areas of pancreatic parenchyma. The liver tissue was unchanged.

In the experiments of series III one animal died from spreading biliary peritonitis. Three animals were killed 2 months after the operation. In two cases dilatation of the proximal segments of the anastomosis by 2 and 5 times respectively compared with the distal segments was observed. In one case the anastomosis line was located in the zone of infiltration. In all cases a well-marked picture of aseptic inflammation was found in the tissues surrounding the duct, with the development of sclerosed granulation tissue. Inflammatory infiltration spread throughout the thickness of the duct wall along the course of the channel of the suture material. Around the threads fibrous capsules with a picture of inflammatory infiltration were formed. In serial sections it could be clearly seen how suture material migrated into the lumen of the ducts on account of the inflammatory reaction (Fig. 3). As the threads came out the inflammatory reaction underwent resolution. A picture of marked inflammation and sclerosis was seen in the pancreatic tissue.

Analysis of these observations (series I and II) shows that the use of microsurgical techniques in operations on bile ducts of small caliber in combination with unabsorbable suture material leads to the rapid formation and epithelization of the scar as a result of exact apposition of the layers of the duct wall, minimal trauma to the tissues, and absence of an inflammatory reaction to the suture material. Covering the anastomosis line with surgical glue to improve airtightness in these small structures gives worse results because of the development of an inflammatory reaction, both around the duct and in the thickness of its walls, and this leads to migration of the sutures. Ectasia of the ducts observed above the zone of the anastomoses was due primarily to defects in the technique of anastomosis formation. With the gaining of experience this defect was found less often. Ectasia was due,

second, to the marked sclerotic changes taking place around the duct as a result of the inflammatory reaction in the pancreatic parenchyma, due to its trauma during the operation.

ERRATA

To the article by B. A. Khaw, V. P. Torchilin, V. R. Berdichevskii, A. A. Barsukov, A. L. Klibanov, V. N. Smirnov, and E Haber, "Enhancing Specificity and Stability of Targeted Liposomes by Coincorporation of Sialoglycoprotein and Antibody on Liposomes," Bulletin of Experimental Biology and Medicine, 95, No. 6, pp. 776-781 (1983).

The fourth sentence in the abstract on page 776 should read: In this study, the binding of the sialoglycoprotein, fetuin, to the liposome surface by hydrophobic interaction significantly diminished hepatic concentration of substituted liposomes in mice.

The last sentence on page 778 should read: A liposome suspension containing 200,000 cpm of cholesteryl-[1-^{14}C]oleate was injected intravenously into groups of five 20- to 25-g female CBWA mice.