

Effect of Cholecystoduodenostomy and Choledochostomy in Pancreatic Carcinogenesis¹

Parviz Pour² and Thorkatla Donnelly

Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska 68105

ABSTRACT

The pancreatic carcinogen *N*-nitrosobis(2-oxopropyl)-amine was administered to Syrian golden hamsters after cholecystoduodenostomy and choledochostomy to investigate a possible bile reflux mechanism as a factor in pancreatic carcinogenesis. The induced lesions were similar in morphology, multiplicity, and distribution to those of animals in other studies without surgery. Hence the findings contraindicate the importance of biliary reflux in pancreatic tumor induction. Other possible mechanisms are discussed.

INTRODUCTION

Among the etiological factors in pancreatic cancer, the bile reflux mechanism is presently the most popular hypothesis (9) and stems from the observation that most clinical pancreatic cancer develops in the head of the organ, in which bile-borne carcinogen could be effective. Although in hamsters the induced pancreatic neoplasms were randomly distributed among different pancreatic segments, much cancer developed in the pancreas head around the collecting main ducts (6, 7). This preferred cancer site was postulated to be due to the effect of either bile-borne or pancreatic juice-borne carcinogen. In the former case the bile reflux mechanism could be responsible, whereas in the latter it may be caused by a higher concentration of the carcinogen in the main collecting ducts in the head of the organ. In the present study the significance of bile reflux mechanism in pancreatic carcinogenesis was investigated in cholecystoduodenostomized and choledochostomized Syrian golden hamsters.

MATERIALS AND METHODS

Surgical Techniques. (For the anatomy and topography of the bile and pancreatic ducts in hamsters, see Ref. 8.) Under pentobarbital anesthesia (75 mg/g body weight) an approximately 2-mm-long segment of the common bile duct was dissected between 2 ligations (with silk suture) on the distal portion of the common bile duct just above the opening of the pancreatic duct (Chart 1a). A 2- to 3-mm-long incision was made in the gallbladder fundus near the

silk thread for bile flow (Chart 1a), and the gallbladder was then inserted into the duodenal lumen through a wound 5 to 10 mm distal to the opening of the common duct and secured by tightening the silk threads through a knot (Chart 1b). In 2 hamsters an operative cholangiogram through the duodenostomy prior to closure was performed to demonstrate the integrity of the anastomoses. By this method no contrast medium was visible in the common duct (and pancreatic ducts). Animals were treated during the next consecutive 3 days with Terramycin (Pfizer Inc., New York, N. Y.) in drinking water (0.1% solution). Only animals in normal condition 7 days after the operation were included in the experiments. Three hamsters were killed after 3 weeks for histological examination of surgical effects.

Treatment. Twenty female hamsters that had been operated on were used. Ten of these animals received weekly s.c. injections of BOP³ (5 mg/kg body weight) in 0.9% NaCl solution for 20 weeks, beginning 1 week after surgery (Group 1). Of the remaining 10 hamsters, which were designated as controls, 3 were treated erroneously once with BOP (5 mg/kg body weight), at the tenth week after the beginning of carcinogen treatment, and were considered as a single dose group (Group 2). The 7 remaining hamsters received weekly s.c. injections of the solvent for 20 weeks and served as untreated controls (Group 3). Animals were kept for life in plastic cages in groups of 5 (Group 2 consisted of 3 hamsters) and received Wayne pelleted diet (Allied Mills, Inc., Chicago, Ill.) and water *ad libitum*. After complete autopsies the organs were fixed in 10% buffered formalin and processed by conventional methods for histology (hematoxylin and eosin staining). A portion of the liver, an area of anastomosis (including the common duct stump), and the pancreas head were embedded *en bloc* and cut, as was the remaining portion of the pancreas, in step sections (6 sections/tissue). Pancreatic lesions were recorded by previously described methods (3, 6, 7). The durations stated are from the beginning of BOP injection.

RESULTS

Effects of Surgery. The weight lost by animals due to surgery was rapidly regained during the first postoperative week. Thereafter, no remarkable differences were clinically observed between these and control hamsters (of other parallel experiments). Three weeks after surgery the gallbladder in each of 3 sacrificed hamsters was found to have disappeared, and a free and intact communication between the cystic duct and duodenal lumen was observed (Chart 1c; Fig. 1). The epithelial lining of the intra- and extrahe-

¹ This work was supported by USPHS Contract NO1 CP33278 from the Division of Cancer Cause and Prevention, National Cancer Institute, NIH, and by Grant NO1 R26 CA 201 98-01 sponsored by the National Pancreatic Cancer Project, National Cancer Institute, Department of Health, Education, and Welfare.

² To whom requests for reprints should be addressed, at Eppley Institute for Research in Cancer, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, Nebr. 68105.

Received January 9, 1978; accepted April 14, 1978.

³ The abbreviation used is: BOP, *N*-nitrosobis(2-oxopropyl)amine.

patic and common ducts was intact; however, a slight distension of the common bile duct stump in all 3 hamsters and focal cholangitis and cholangiofibrosis in 1 hamster were observed. Among the hamsters that had been operated on, 1 untreated control (Group 3) was killed accidentally; hence no findings from this animal were considered. The remaining 6 untreated control hamsters that died between 19 and 39 weeks of severe generalized arterial calcinosis had unaltered anastomoses. Five had focal granulomatous tissue around the sutures; in addition 1 had focal abscess and necrosis of the duodenal wall, with perforation, and 1 had mild cholangiofibrosis. Among the 13 treated hamsters (Groups 1 and 2), common bile duct distension was found in 3, granulation tissue was found in 4, focal duodenal abscesses were found in 2, and small chronic liver abscesses were found in 2.

The Effect of BOP. Data relative to survival, body weight,

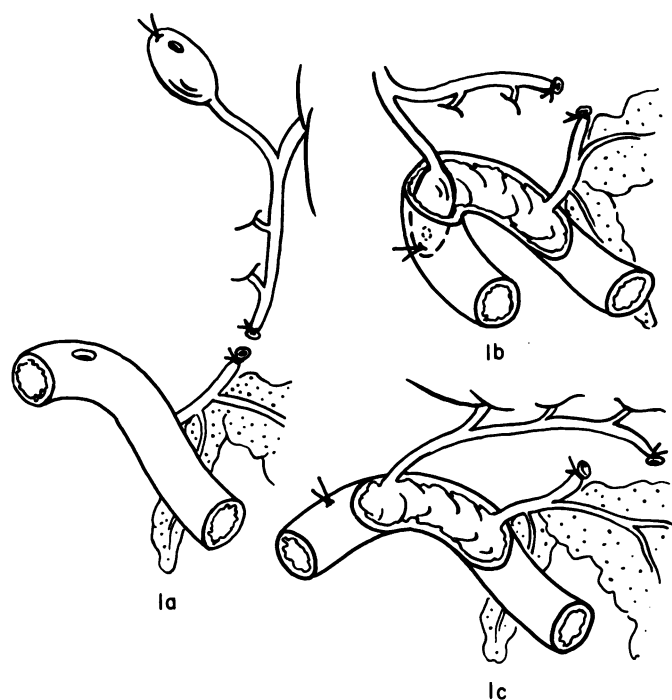


Chart 1. Schematized surgical procedure for cholecystoduodenostomy and choledochostomy. For detailed technique see the text. In *b* and *c*, part of the duodenal wall has been removed for better demonstration of the procedure.

and the incidence and sites of induced neoplasms are summarized in Table 1. All treated hamsters developed pancreatic neoplasms, which were classified as adenomas (50 and 33%), intraductal carcinomas (60 and 0%), and adenocarcinomas (100 and 67% in Groups 1 and 2, respectively). Adenomas (up to 50 in Group 1 and 15 in Group 2) were usually found in hamsters exhibiting fewer adenocarcinomas that had not destroyed the pancreatic tissue. However, in 50% of Group 1 animals, the entire pancreas was occupied by adenocarcinomas of various sizes (1 to 16 mm) and numbers (on the average, 11 tumors/animal with a range of 2 to 20 tumors/pancreas). In 4 hamsters the tumors had coalesced to about 4-cm-large nodules (Fig. 2). In 5 animals these lesions had invaded the peripancreatic lymph nodes, and in 1 animal they had invaded the spleen, liver, and entire peritoneum. Distant metastases were not found. Animals with larger numbers of tumors often exhibited serosanguinous ascites (5 to 50 ml), and nearly all had watery intestinal contents at death; this was not seen in controls. The localization of pancreatic neoplasms was in accordance with previous studies (3, 6, 7) and predominated in the tail of the splenic lobe and along the main ducts. Also, analogous to previous findings (3, 6, 7), > 90% of all tumors had ductular patterns, most of which arose from intra- and periinsular ductules. In animals subjected to a single BOP injection, peri- and intrasular ductular proliferations were the primary findings; 1 ductular carcinoma *in situ* in a hamster of this group had developed within the islet (Fig. 3). Hyperplasia and metaplasia of the pancreatic ductal epithelium and marked papillary proliferation of the common duct epithelium (Fig. 4) were found in all Group 1 animals and, to a much lesser extent, in Group 2 hamsters. Hyperplasia of the common bile duct stump and occasionally also of the hepatic duct occurred only in 3 hamsters of Group 1, 2 of which showed marked distension and papillary proliferation.

Proliferation of the small intrahepatic bile ducts, hyperplasia, and often goblet cell metaplasia of the larger hepatic ducts were found in all treated hamsters, but cholangiomas were found only in those of Group 1. Similarly, hyperplasia and dysplasia of bronchial epithelium and alveolar hyperplastic nodules (up to 5 in number) and 1 alveologenic adenoma were found only in Group 1 hamsters. Tumors of other sites included a forestomach papilloma (Group 1 at 25 weeks) and parathyroid adenoma (control at 24 weeks).

Table 1

Tumor patterns in cholecystoduodenostomized and choledochostomized female hamsters treated with BOP (5 mg/kg body weight)

| No. of hamsters | Group | No. of weekly treatments | Av. survival (wks) | Av. body wt (g) | % of tumor incidence | % of lung tumors | % of liver tumors | % of pancreatic tumors | | |
|-----------------|-------|--------------------------|---------------------|-----------------|----------------------|------------------|-------------------|------------------------|-----------------------|-----------------|
| | | | | | | | | Adenomas | Intraductal carcinoma | Adenocarcinoma |
| 10 | 1 | 20 | 32 ± 4 ^a | 103 ± 23 | 100 ^b | 10 | 60 | 50 | 60 | 100 |
| 3 | 2 | 1 | 34 ± 9 | 92 ± 20 | 100 | 0 | 0 | 33 ^c | 0 | 67 ^d |
| 6 | 3 | 0 | 33 ± 7 | 89 ± 28 | 16 ^e | 0 | 0 | 0 | 0 | 0 |

^a Mean ± S.D.

^b Including 1 forestomach papilloma.

^c At 35 weeks.

^d Both at 39 weeks; 1 was a ductular carcinoma *in situ*.

^e One parathyroid adenoma.

DISCUSSION

Compared to previous results (2, 3, 6, 7) the distribution and patterns of BOP-induced pancreatic neoplasms in this study were not altered by bypassing the bile through the common duct. Consequently, the effect of bile can be excluded as a mediating factor in pancreatic carcinogenesis. In the present study the possible regurgitation of bile-borne carcinogen from the duodenum into the pancreatic duct is most unlikely because in this species the pancreatic ducts enter the common duct well before it opens into the duodenum (8) and because no evidence for such a reflux could be demonstrated by cholangiograms. Nevertheless, in view of the similar location and morphology of these induced pancreatic lesions (especially with regard to the marked papillary proliferation of common duct epithelium), compared to previous findings, mechanisms other than bile reflux seem to operate in experimental pancreatic tumor induction.

In this as well as in previous experiments (2, 6, 7), most induced pancreatic tumors were of ductular origin, and many seemed to develop within or around the islets (for definition see Refs. 2 and 7). The preference for the peri- and intransular ductules as sites of cancer induction could well be due to a greater exposure of the responsive ductular cells to the blood-borne carcinogen because of a marked vascularity of islets. This would explain why limited or single BOP doses in previous studies (2, 6, 7) and in this experiment primarily affected the intra- and periinsular ductules. Further support for a direct blood-mediated carcinogenic effect of BOP in the pancreas was found after p.o. BOP administration, which resulted in induction of only a few pancreatic tumors but a 100% incidence of liver neoplasms, apparently due to the primary effect of carcinogen in the liver (4). On the other hand, compared to BOP, lower equitoxic doses of *N*-nitrosobis(2-hydroxypropyl)amine and *N*-nitrosobis(2-acetoxypropyl)amine applied locally to the skin and vagina led to larger numbers of pancreatic tumors, mediated presumably by local absorption of carcinogen (5). Since the few pancreatic neoplasms induced after p.o. BOP application were primarily found around the main duct in the head region (4) and since there was a relationship

between the severity (and extent) of the alteration of these ducts and the BOP dose (2, 6, 7), it could be assumed that this pattern reflects an additional transluminal neoplastic effect of carcinogen and/or its metabolites excreted via pancreatic juice. Accordingly, the concentration of carcinogen would be higher in the large collecting ducts of the pancreas head, an assumption partially supported by common metabolites of pancreatic carcinogens identifiable in pancreatic secretions (1).

Although extrapolation of these experimental results to humans is still premature, there are circumstances that relate well to the human situation. For instance in the experimental model there was a direct correlation between the number of induced tumors and the size (and weight) of the pancreatic segments from which the tumors arose (7). The same situation could conceivably apply to humans since the pancreas head in humans constitutes a larger tissue mass. Consequently, the higher tumor incidence in this pancreas portion would be self-explanatory.

REFERENCES

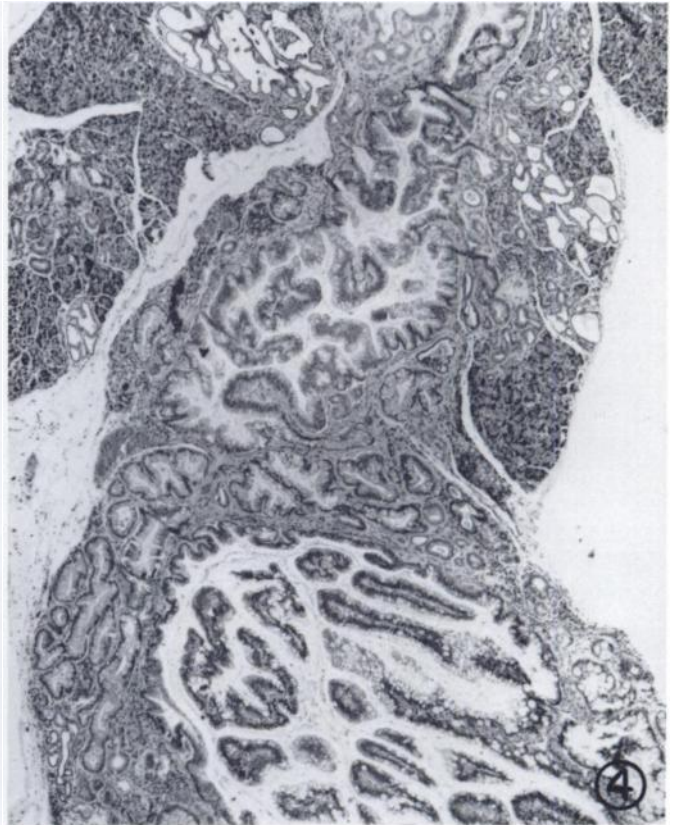
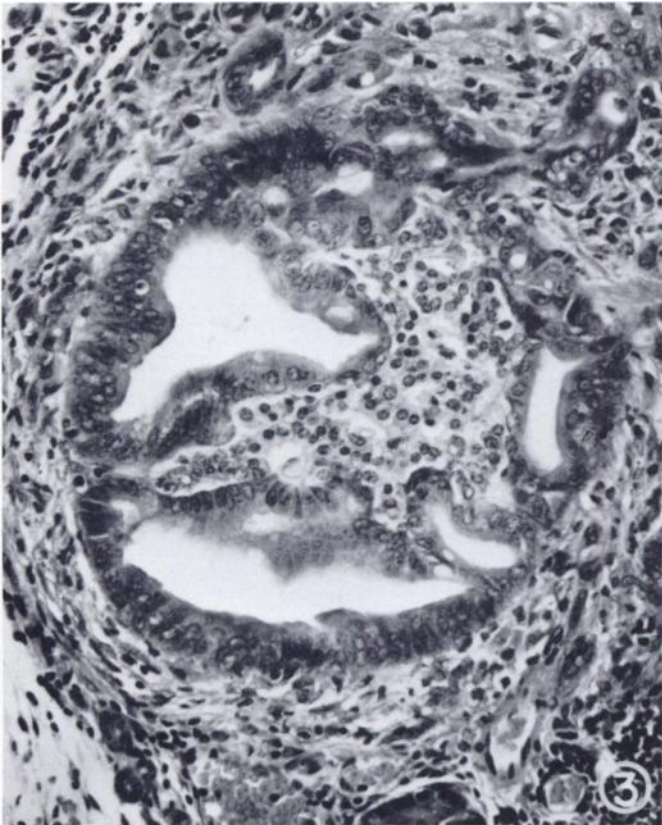
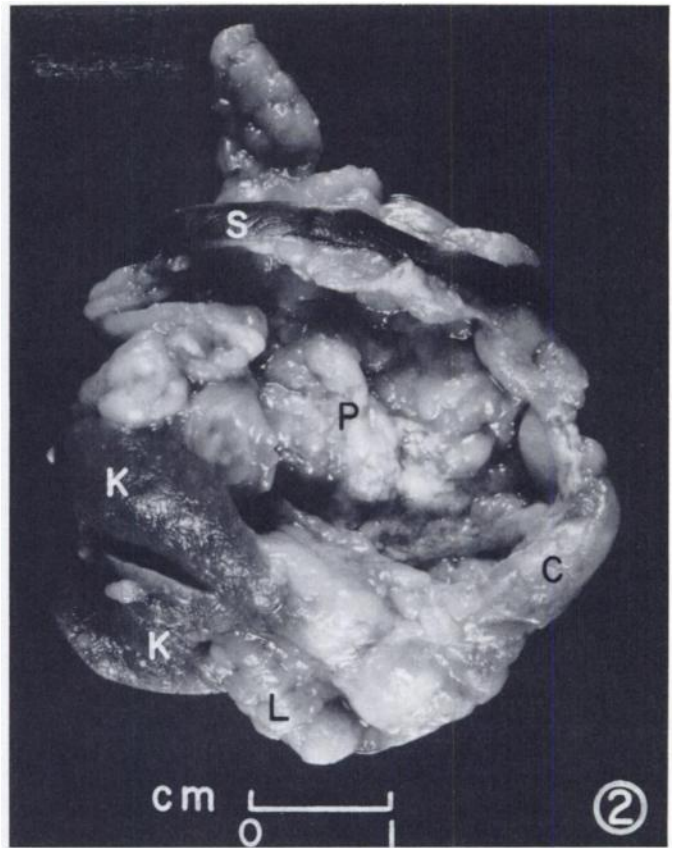
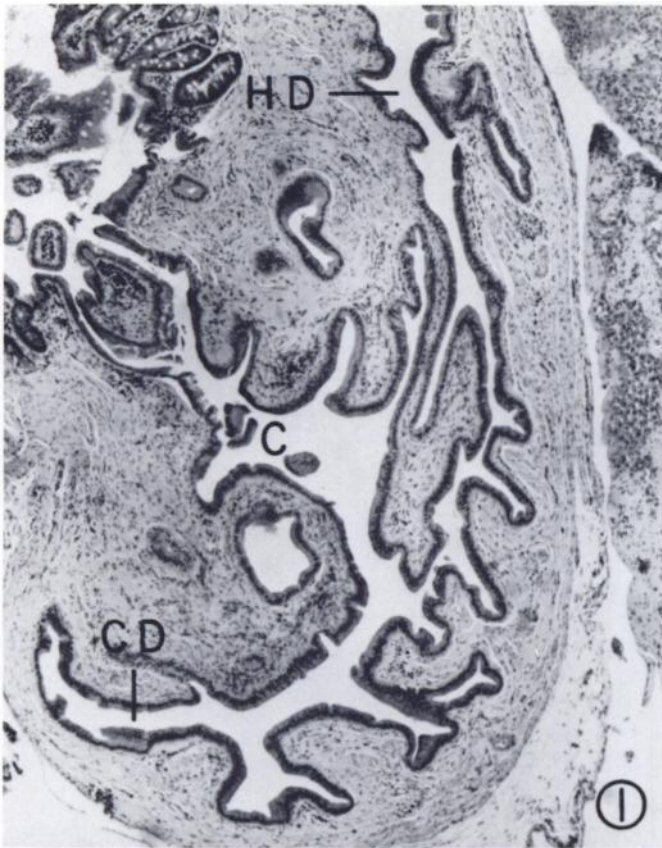
1. Gingell, R., Pour, P., and Langenbach, R. Metabolism of the Pancreatic Carcinogen *N*-Nitrosobis(2-oxopropyl)amine after Oral and i.p. Administration to Hamsters. *Proc. Am. Assoc. Cancer Res.*, **18**: 39, 1977.
2. Pour, P. Islet Cell as a Component of Pancreatic Ductal Neoplasms. I. Experimental Study: Ductular Cells, Including Islet Cell Precursors, as Primary Progenitor Cells of Tumor. *Am. J. Pathol.*, **90**: 295-316, 1978.
3. Pour, P., Althoff, J., Krüger, F. W., and Mohr, U. A Potent Pancreatic Carcinogen in Syrian Hamsters: *N*-Nitrosobis(2-oxopropyl)amine. *J. Natl. Cancer Inst.*, **58**: 1449-1453, 1977.
4. Pour, P., Althoff, J., Krüger, F. W., and Mohr, U. The Effect of *N*-Nitrosobis(2-oxopropyl)amine after Oral Administration to Hamsters. *Cancer Letters*, **2**: 323-326, 1977.
5. Pour, P., Althoff, J., and Nagel, D. Induction of Epithelial Neoplasms by Local Application of *N*-Nitrosobis(2-hydroxypropyl)amine and *N*-Nitrosobis(2-acetoxypropyl)amine. *Cancer Letters*, **3**: 109-113, 1977.
6. Pour, P., Althoff, J., and Takahashi, M. Early Lesions of Pancreatic Ductal Carcinoma in the Hamster Model. *Am. J. Pathol.*, **88**: 291-308, 1977.
7. Takahashi, M., Pour, P., and Althoff, J. Sequential Alteration of the Pancreas during Ductal Carcinogenesis. *Cancer Res.*, **37**: 4602-4607, 1977.
8. Takahashi, M., Pour, P., Althoff, J., and Donnelly, T. The Pancreas of the Syrian Hamster (*Mesocricetus auratus*). I. Anatomical Study. *Lab. Animal Sci.*, **27**: 336-342, 1977.
9. Wynder, E. L., Mabuchi, K., Maruchi, N., and Fortner, J. G. Epidemiology of Cancer of the Pancreas. *J. Natl. Cancer Inst.*, **50**: 645-667, 1973.

Fig. 1. Section from area of cholecystoduodenostomy. The gallbladder is not present. Direct communication of the cystic duct (C) with the duodenum (left upper corner) is shown. Sections of hepatic (HD) and common bile ducts (CD) are also seen. Liver (right) shows cholangiofibrosis. H & E, × 40.

Fig. 2. Numerous coalescing adenocarcinomas occupying almost the entire pancreas (dorsal view). C, transverse colon; K, kidney; L, paraaortal lymph node metastases; P, pancreas, S, spleen.

Fig. 3. Intransular ductular carcinoma *in situ* showing connection to atypical intralobular ductules (top). This lesion was found in the tail of the splenic lobe. H & E, × 200.

Fig. 4. Marked papillary proliferation of common duct epithelium (bottom) and merging common pancreatic duct (middle and top). Hyperplasia and metaplasia of the epithelium extends into the merging small ducts. Multifocal ductular proliferation and distension in the surrounding pancreatic tissue (upper one-half) are shown. H & E, × 40.



Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Effect of Cholecystoduodenostomy and Choledochostomy in Pancreatic Carcinogenesis

Parviz Pour and Thorkatla Donnelly

Cancer Res 1978;38:2048-2051.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/38/7/2048>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link <http://cancerres.aacrjournals.org/content/38/7/2048>. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.