Carcinoma of the Gallbladder Induced in Hamsters by Insertion of Cholesterol Pellets and Feeding Dimethylnitrosamine (35293)

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Diethylnitrosamine (DEN) and dimethylnitrosamine (DMN) given orally can induce hepatic tumors in various experimental animals. DEN had tumorogenic action on the liver in rats (1, 10), guinea pigs (2, 6), and in hamsters (7). DMN induced hepatic tumors in rats (1, 8), and in hamsters (7, 5). An interesting finding in DEN-treated guinea pigs was the presence of pre- and neoplastic lesions in bile duct epithelium (2). It was suggested that intrahepatic bile duct lesions might be the precursors of the carcinomas with adenomatous pattern (2). High incidence of cholangio-carcinomas in hamsters given DMN (11) seemed to indicate that both nitrosamines are carcinogenic for the biliary tract. However, the localization of neoplasm in the gallbladder is not frequently found in DEN-treated animals: Over 100 guinea pigs received DEN and most of them had lesions in intrahepatic bile ducts, but only in one animal gallbladder tumor was found (2). No gallbladder malignancy was found in 59 hamsters, treated with DMN (11).

We wondered, therefore, whether a chronic, nonspecific irritation of gallbladder mucosa, induced by an "experimental gallbladder stone" will predispose nitrosamine-treated animals to the appearance of gallbladder neoplastic lesions.

The association of gallbladder carcinoma with gallstones in man appears to be of high incidence (9) and it is possible that an etiological relationship between the two conditions does exist. Various intracholecystic pellets were used previously (3) in an attempt to produce gallbladder tumors in animals. We found that only pellets containing a carcinogen were able to induce biliary tumors in hamsters (3–5). However, we considered the possibility that, in the presence of prolonged exposure to an oral carcinogen, specifically affecting the biliary tract, an intracholecystic foreign body may enhance the neoplastic transformation of gallbladder mucosa.

Materials and Methods. Male hamsters (*Mesocricetus auratus*) having initial body weight 94 g (86–119) were used in this experiment. *N*-nitrosodiethylamine (DEN) and dimethylnitrosamine (DMN), purchased from Eastman Organic Chemicals (USA), were dissolved in tap water and given as sole drinking fluid, during the experimental period. Respective dosage, per 1000 ml of water, was 0.159 ml for DEN and 0.02 ml for DMN. Surgery was done under sodium pentobarbital anesthesia (30 mg/kg of body wt). After median epigastric incision 1.5 cm long, the gallbladder was exposed, the fundus was incised (3), and a cholesterol pellet was placed inside. The incision was closed with 6-0 silk. Muscles and skin were closed with 4-0 chromic gut. Cholesterol pellets (12) were 3 mm long and weighed from 13 to 15 mg. In sham-operated hamsters, a similar procedure, with exception of the implant of pellets, was followed. The exposure to carcinogen began 1 day after surgery. Hamsters were divided into the following groups: (i) normal controls, no surgery, no carcinogen; (ii) cholesterol pellets implanted, no carcinogen; (iii) receiving DEN and having cholesterol pellets implanted; (iv) receiving DEN and sham operated; (v) receiving DMN and having cholesterol pellets implanted; (vi) receiving DMN and sham operated.

Attempts to detect tumors by palpation were made but considered unreliable. It was decided to sacrifice the animals in small groups, when they were visibly ill. Some hamsters died spontaneously and were in-

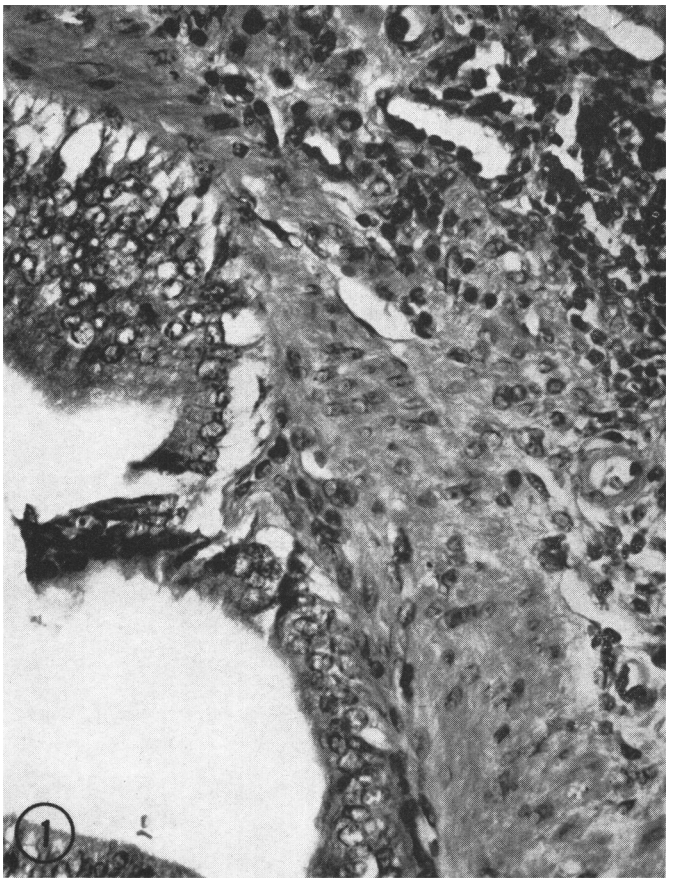

FIG. 1. Atypical epithelial cells in a gallbladder of hamster, exposed to oral DMN and having intracholecystic pellet; H & E, ×300.

cluded in these groups. Any remaining treated animals were sacrificed at the end of the 22nd week of treatment. Autopsies were concerned essentially with the livers and gallbladders, according to the purpose of this experiment. These organs were fixed in 10% neutral formalin and stained routinely with hematoxylin and eosin (H & E).

Results. Table I summarizes the data on pathological findings in six goups of hamsters. It shows that both nitrosamines used in this experiment produced hepatic tumors. Hepatocellular and cholangio-carcinomas found in our hamsters were identical to those described in detail by other investigators (1, 2, 7, 8, 10, 11). Previously reported bile duct proliferation in nitrosamine-treated animals (2, 11) were also present. We found, however, marked difference in the action of the two carcinogens on bile ducts. In DEN-treated animals proliferating bile ducts usually surrounded the regenerating hepatic nodules. Bile ducts were lined with cuboidal cells and areas of cystic formations were present. Bile duct proliferation in DEN-treated hamsters was merging into cholangio-carcinomas. These tumors showed budding of malignant cells from the ducts.

In DMN-treated animals the proliferating bile ducts were lined with atypical epithelial cells. These cells, present also in some gallbladders of DMN-treated hamsters (Fig. 1), showed abnormalities in nuclear polarity, crowding and variation in size and shape of nuclei, hyperchromatism and an increase in the number of mitotic figures. Only DMN-treated animals had carcinomas in their gallbladders. These were found in 68% of animals, having cholesterol pellets in gallbladder. In only one animal of the DMN series

TABLE I. Distribution of Pathological Lesions in Liver and Gallbladder of Hamsters, Drinking Water Containing Carcinogens Diethylnitrosamine (DEN) or Dimethylnitrosamine (DMN).

Numbers in parentheses give percentage of total number of animals studied in a group.

Group	No. of hamsters	Cholesterol gallbladder pellet	Carcinogen		Liver				Gallbladder		
							Bile duct proliferation[a]				
			Name	Exposure (weeks)	Hepatocellular carcinoma	Cholangiocarcinoma	Around regenerating nodules	With epithelial dysplasia	Cholecystitis glandularis proliferans	Epithelial dysplasia	Carcinoma
1	8	—	0	0	0	0	0	0	0	0	0
2	30	+	0	22–40[b]	0	0	0	0	8	0	0
3	21	+	DEN	10–22[c]	11 (52)	21 (100)	21	0	1	0	0
4	44	—	DEN	10–22[c]	21 (47)	44 (100)	44	0	1	0	0
5	19	+	DMN	8–22[c]	8 (42)	11 (58)	0	11	2	5	13[d](68)
6	16	—	DMN	8–22[c]	6 (38)	10 (62)	0	6	0	3	1[e](6)

[a] Bile duct proliferation in DEN-treated animals was localized around the regeneration nodules; in DMN-treated hamsters it was associated with dysplasia of lining cells.

[b] Exposure to pellet only.

[c] Liver tumors were already present in some hamsters treated for 8–10 weeks with carcinogen.

[d] Six gallbladder carcinomas were present already after 8–9 weeks of the exposure to DMN.

[e] Papillary adenocarcinoma after 15 weeks exposure to DMN.

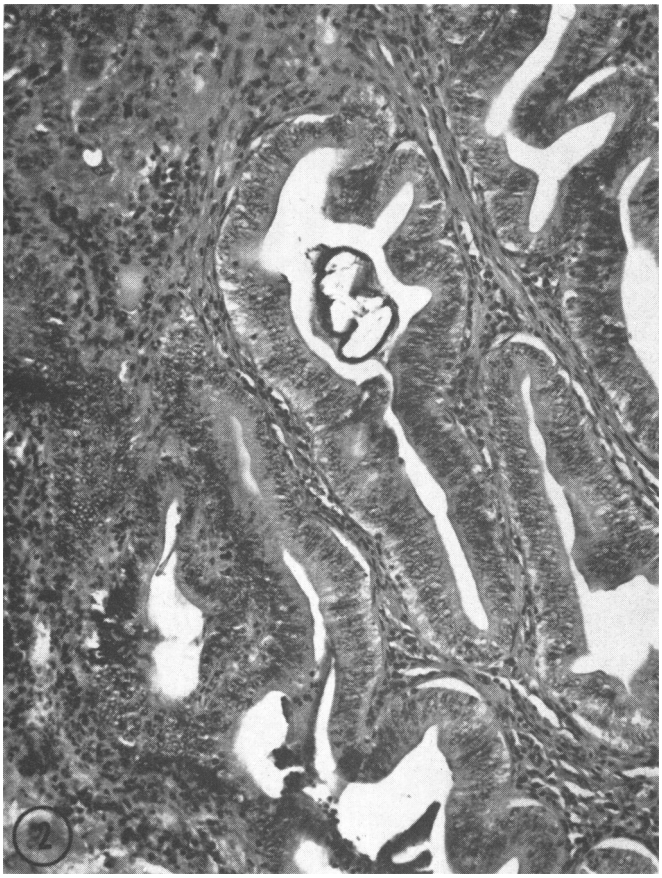

FIG. 2. Papillary adenocarcinoma of gallbladder in a hamster, exposed for 9 weeks to oral DMN and having intracholecystic pellet; H & E, ×300.

having no cholesterol pellet, gallbladder carcinoma was diagnosed. From 13 gallbladder tumors, 5 were of the papillary variety (Fig. 2), and 8 were diagnosed as poorly differentiated tumors. A detailed description of gallbladder tumors in hamsters was given previously (5), and will not be repeated in the present preliminary report. A separate study on histopathology of DMN induced changes in biliary tract and gallbladder carcinomas is now in preparation.

Discussion. It appears from this preliminary study that the presence of an intracholecystic foreign body, combined with exposure to an oral carcinogen specifically affecting the biliary tract, did indeed enhance the neoplastic transformation of hamster's gallbladder. Both carcinogens studied induced hepatic cellular and cholangiocarcinomas. Both had a very marked effect on the proliferation of bile ducts. These results agree with previously reported studies on postnitrosamines hepatic tumors (11). However, a very marked difference was observed in their action on bile ducts. In DEN-treated hamsters, essential component of hepatic pathology were regenerating nodules surrounded by proliferating bile ducts. No gallbladder carcinomas were found in DEN-treated hamsters.

In DMN-treated animals, marked dysplastic changes were observed in the intrahepatic bile ducts and were associated with bile duct proliferation.

It is probable the DMN-induced epithelial dysplasia of bile ducts and gallbladder represents an important premalignant transformation. It is unlikely that this is an accidental finding. Gallbladder pellet alone had no carcinogen effect even after 40 weeks fol-

lowing pellet implantation. Such a pellet did not enhance gallbladder malignancy in DEN-treated hamsters, in which no epithelial dysplasia occurred in bile ducts. One can conclude that the presence of experimental gallbladder stones did enhance malignant transformation of gallbladders in hamsters exposed to the carcinogen DMN, which is able to induce epithelial dysplasia of bile duct and gallbladder epithelium.

Summary. Hamsters having or not surgically implanted intracholecystic cholesterol pellets were given carcinogen diethylnitrosamine (DEN) or dimethylnitrosamine (DMN), dissolved in drinking water. Exposure to these carcinogens lasted from 8 to 22 weeks. No gallbladder tumors were found in DEN-treated hamsters, having or not intracholecystic "experimental stones." In 68% of hamsters exposed to DMN and having gallbladder pellets, adenocarcinomas were found. Only one gallbladder tumor was detected in DMN-treated animals who had no gallbladder pellet. It was concluded that "experimental gallbladder stone" enhanced the malignant transformation of gallbladder mucosa in DMN-treated hamsters. It was considered probable that dysplasia of bile duct and gallbladder epithelium observed only in DMN-treated animals represents a premalignant lesion. Presence of this premalignant lesion associated with nonspecific irritation of gallbladder mucosa by a "stone" may be considered an important etiological factor contributing to gallbladder malignancy in hamsters.

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