Induction in Hamsters of Biliary Carcinoma by Intra-cholecystic Methylcholanthrene Pellets*

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Because biliary steroids are related to certain chemical carcinogens, it has often been suggested that compounds of the phenanthrene type, normally present in bile, may be responsible for initiating neoplasia in the biliary tract. Certain foreign bodies implanted in the gall bladders of guinea pigs produce cholecystitis glandularis proliferans (3, 4), but the claim that they also result in the growth of malignant tumors (11) has been questioned (8). Fortner (7) succeeded in inducing carcinoma of the gall bladder in five of fourteen cats by placing single methylcholanthrene pellets in the gall bladder, followed by ligation of the cystic duct. Eight cats dying within 10 months after implantation of the carcinogen showed only non-neoplastic hypertrophic, hyperplastic, and metastatic changes in the gall bladder. Five of six cats surviving 23–32 months developed primary adenocarcinomas and adenosquamous carcinomas of the gall bladder. These were unquestionably malignant, invasive, metastasizing carcinomas, but the long induction period is inconvenient for laboratory studies.

An attempt was made in this laboratory to produce gall bladder tumors in guinea pigs by intracholecystic implantation of methylcholanthrene pellets without cystic duct ligation. Although 30 animals were observed for over 500 days, no tumors were seen. Only cholecystitis glandularis proliferans was observed.1 The experiment was therefore repeated with golden hamsters.

MATERIALS AND METHODS

Each of 50 male, golden hamsters, approximately 3 months of age, was anesthetized with sodium pentobarbital (30 mg/kg body weight), and the fundus of the gall bladder was incised. Into each gall bladder was placed a single, 6–8-mg. 3-methylcholanthrene pellet, and the incised fundus was ligated; the cystic duct was not ligated. The pellets were prepared as follows: 3-methylcholanthrene (Eastman Co.) was melted by heat and drawn into a glass tube 1 ml. in diameter. After being cooled, the hard methylcholanthrene cylinder was pushed out of the glass tube and cut into pieces 1.5–2.0 ml. long, which were then weighed. Twenty-five animals also received about one fourth of a 25-mg estradiol pellet (Progynon, Schering Co.), implanted subcutaneously in the back by trocar, immediately after the operation on the gall bladder and again 60 days later. Attempts to detect tumors by palpation were considered unreliable, and it was decided to sacrifice the animals in small groups at arbitrarily chosen times. When it was found that tumors appeared as early as 2–5 months, all remaining hamsters were sacrificed on the 240th day after implantation of the carcinogen. The weight of nonabsorbed pellets found at necropsy was not determined.

RESULTS

Thirty-nine hamsters survived longer than 1 month. Nineteen had received only methylcholanthrene pellets (Group I); twenty had received both methylcholanthrene pellets and estradiol (Group II). Times of sacrifice and tumor incidence are indicated in Table 1. There was no significant difference between the two groups. A relationship between duration of exposure to the carcinogen and tumor incidence was not elucidated by the data. As might be expected, mammary tumors occurred only in the estradiol-treated group.

Pathology.—In each of the two experimental groups there were twelve carcinomas of biliary origin. In most of the 24 hamsters so affected the gall bladder was difficult to identify grossly, the pellet being embedded in firm, grey tissue which infiltrated the gall bladder bed and adjacent hepatic substance. In a few instances the diaphragm and abdominal wall were adherent to the gall bladder region. In several animals firm tumor nodules were also present within the liver.

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1 Kowalewski and Shnitka, unpublished data.

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apart from the gall bladder region. The cut surfaces of such nodules were greyish-white and sometimes revealed small cysts. In one animal there were also metastatic tumor nodules in an adrenal gland and the cortex of a kidney.

In a few of the tumor-bearing animals the gall bladder could not be recognized upon histological examination, but in fifteen of the 24 there was microscopic support for a primary origin of the tumor in the gall bladder. Thus, in the majority a lumen could be identified, lined by hyperchromatic, atypical, columnar epithelium, and usually containing pus. In five animals papillary masses of neoplastic epithelium supported by delicate, branching, vascular stalks projected into the lumen (Fig. 1 and 2). In about two thirds of the carcinomas, a few of which contained varying numbers of dilated, pus-filled gland spaces. A few of the separate tumor nodules were small, papillary cystadenocarcinomas, lined by atypical epithelium and containing papillary masses of tumor.

At the invading tumor margins a narrow zone of liver parenchyma was usually infiltrated by lymphocytes in focal or bandlike distribution (Fig. 5). Gall bladder walls invaded by tumor showed a similar cellular reaction.

The livers of eleven animals showed varying degrees of central zonal necrosis. Nine were members of the estradiol-treated group. In some instances narrow zones of necrosis were also present around the portal tracts, and in a few instances

<table>
<thead>
<tr>
<th>Duration of Exposure to Carcinogen (days)</th>
<th>No. Hamsters</th>
<th>No. with Body Cancer</th>
<th>No. with Sarcoma</th>
<th>No. with Breast Cancer</th>
<th>No. without Tumor</th>
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</thead>
<tbody>
<tr>
<td>I (MCh)</td>
<td>140</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>3</td>
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<td>240</td>
<td>9</td>
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<td>3</td>
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<tr>
<td>Total</td>
<td>19</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>II (MCh and estrogen)</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>140</td>
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<td>4</td>
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<tr>
<td>Total</td>
<td>20</td>
<td>12</td>
<td>4</td>
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<td>5</td>
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<tr>
<td>Grand total</td>
<td>59</td>
<td>24</td>
<td>5</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>

MCh = Methylcholanthrene.
* Each had both biliary carcinomas and body wall sarcoma.
† One tumor metastasized to adrenal and to kidney.

24 hamsters atypical epithelial nests and glands could be seen invading the gall bladder wall and, in most instances, the adjacent liver substance (Fig. 3). In some animals the gall bladder had a small, irregular lumen lined by atypical epithelium and surrounded by a mass of solid tumor tissue of considerable depth. The latter had the pattern of adenocarcinoma composed of small glandular or ductlike structures supported by a moderate quantity of fibrous stroma. Some of the ductlike structures had well formed lumina lined by columnar or cuboidal epithelium (Fig. 4). Others were tubular structures with minute or absent lumina. The latter were composed of pale cells with abundant cytoplasm and a superficial resemblance to hepatic parenchymal cells (Fig. 5).

Tumor nodules in the liver at a distance from the gall bladder bed were usually solid adeno-
these necrotic zones contained proliferated small bile ducts. The necrotic areas were infiltrated by moderate numbers of lymphocytes, histiocytes, and macrophages filled with orange, finely granular pigment. In some instances a few polymorphs were also present. One animal of each experimental group showed focal necrosis of the liver, the necrotic foci being crowded with polymorphs.

The livers of 30 hamsters showed nonspecific inflammatory changes. Many showed focal infiltration of the lobules by small groups of lymphocytes, histiocytes, and, occasionally, a few eosinophils. Portal tracts were infiltrated by small to moderate numbers of lymphocytes and sometimes a few histiocytes and eosinophils. In some instances small groups of pigmented macrophages were seen, often near the central vein or at the margins of portal tracts, but sometimes scattered more diffusely.
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In a few hamsters an occasional small intrahepatic bile duct was moderately dilated and lined by hyperchromatic and somewhat atypical columnar epithelium. In an occasional animal perilobular cholangiocytes exhibited mild to moderate proliferation. In a few hamsters small groups of cysts lined by differentiated, flat to cuboidal epithelial cells were seen. They appeared to be distended and cystic ducts.

The degenerative, inflammatory, and hyperplastic changes described occurred in both experimental groups and in both tumor-bearing and tumor-free animals. Their relationship to the neoplastic process is not elucidated by the present study, but the presence of atypical biliary epithelium in the absence of, or at a distance from, a tumor of the gall bladder suggests the possibility that some of the multiple intrahepatic nodules could have been autochthonous rather than metastatic. There was, however, no evidence of nodular hyperplasia of hepatic parenchymal cells.

In addition to the 24 biliary neoplasms, seven other tumors occurred. Five were soft tissue sarcomas of the ventral abdominal wall having histological features similar to sarcomas produced by intramuscular implantation of methylcholanthrene pellets (1, 10). The other two were breast carcinomas arising in estradiol-treated hamsters. Both were of “carcinosarcoma” histologic type. Both breast tumors metastasized, the sites of secondary tumors being adrenal glands, renal cortices, and mesenteric lymph nodes. Multiple primary tumors occurred in three animals, each of which had a biliary carcinoma and a sarcoma of the ventral abdominal wall.

DISCUSSION

Comparison of these results with Fortner’s work in cats suggests that induction of biliary carcinoma in the hamster is more rapid. Regarding technique, it appears that ligation of the cystic duct is not necessary to prevent the overly rapid dissolution of the pellet. Significant intrahepatic hyperplastic and atypical epithelial changes apart from neoplasia proper were frequent in our material but were described by Fortner in only one animal. In this cat the cystic duct was left patent, and we wonder whether failure to ligate the cystic duct in our experiment permitted diffusion of carcinogen in the biliary tree, resulting either in back-diffusion into the intrahepatic bile channels or gastrointestinal “feeding” of the carcinogen, the latter reaching the liver in the portal venous blood. A second cat in which the cystic duct was not ligated showed central lobular necrosis such as was seen in our material. The intrahepatic non-neoplastic, perhaps pre-neoplastic, changes seen in our material bear considerable resemblance to changes described in the experimental induction of hepatomas (6). Also present in a few of our animals were small, multilocular intrahepatic cysts of ductal origin identical to those seen in rats fed carcinogens such as acetylaminofluorene (6).

Hepatic neoplasia induced by chemical carcinogen may be inhibited or potentiated by estrogen (5). The bile is an important medium for the excretion of this hormone. It might be expected, therefore, that administration of estrogen to animals having methylcholanthrene implanted in the biliary tract would influence the rate and/or incidence of tumorigenesis. Furthermore, since estrogen inhibits or stimulates various experimental tumors (9), administration of this hormone to animals with biliary cancer might be expected to influence the rate of growth and/or spread of these neoplasms. No such results were obtained in the present experiment. It can be concluded that induction and growth of the methylcholanthrene-induced biliary carcinoma are estrogen-independent under the described experimental conditions. Of probable significance in relation to the influence of estrogen on hepatic response is the fact that nine of the eleven hamsters showing central zonal necrosis were members of the estradiol-treated group.

The histological classification of the neoplasms was not difficult except for some of the “sarcomas of the body wall.” It is known that some hepatic tumors in mice may assume an anaplastic form very difficult to distinguish from fibrosarcoma (9). While the possibility that such might occur in a biliary carcinoma cannot be excluded, especially in the three animals in which biliary carcinomas were demonstrated, because of the relationship of the tumors to muscle of the body wall and their great similarity to methylcholanthrene-induced sarcomas, they were so classified.

SUMMARY

Methylcholanthrene pellets were placed in the gall bladders of 50 hamsters. In 25 animals estradiol was also implanted subcutaneously. Thirty-nine hamsters survived over 1 month. During 60–240 days 31 neoplasms developed in 28 of the hamsters. Twenty-four were biliary carcinomas, five were sarcomas of the body wall, and two were mammary carcinomas. Three hamsters had both biliary carcinoma and sarcoma of the body wall. Estradiol did not influence the development of the biliary neoplasms.
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4. ———. Gallstones and Cancer: A Problem of Etiology, with Special Reference to the Role of Irritation. Ibid., 27:166-69, 1939.


FIG. 4.—Adenocarcinoma of biliary origin, 240 days after methylcholanthrene implantation. This tumor invaded the gall bladder wall and adjacent hepatic substance. Hematoxylin and eosin. $\times 480$.

FIG. 5.—Carcinoma invading liver, 180 days after methylcholanthrene implantation. Tumor cells surround minute lumina (arrows). Groups of lymphocytes at tumor margin. Hematoxylin and eosin. $\times 480$. 

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