Mitotic Index in the Regenerating Liver of Tumor-bearing Mice

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SUMMARY

The onset and the rate of cellular proliferation in the liver regenerating after partial hepatectomy was studied in C3H mice bearing a transplanted mammary carcinoma and in control animals.

The presence of the tumor elsewhere in the body resulted in an earlier onset, with an increased mitotic activity in the remaining liver during the period of 30 to 42 hours after partial hepatectomy. The control liver showed the rise in cellular proliferation at 48 hours. The rate of regeneration had the highest point at 48 hours in both the experimental and control animals. At that time, and throughout the rest of the study, the rate was similar in the two groups. The tumor showed a marked decrease in the number of mitoses at 18 hours, but it started rising thereafter and reached presurgery levels at 48 hours.

INTRODUCTION

The presence of a tumor elsewhere in the body alters markedly the metabolic patterns of the liver of the host, inducing measurable changes in the concentration, the turnover rates of various metabolic components, and the mitotic rate of the hepatic cells. The weight of the tumor-free livers of rats and mice with spontaneous or transplantable malignant tumors in the body is greater than that of the control animals without tumor (1, 20, 27, 28). The mitotic activity of the liver of the tumor-bearing animals is also higher (1, 19). This increased cellular proliferation in the liver has been documented recently by autoradiographic studies with tritiated thymidine (4, 23).

Little information is available with respect to any influence of malignant growth elsewhere in the body on liver regeneration following partial hepatectomy. About 24 to 30 hours after the removal of part of the liver in mice, a rise in the DNA synthesis appears, and it reaches its maximum at 36 to 42 hours and then declines gradually. A rise in the mitotic activity follows the DNA synthesis peak by a period of 6 to 12 hours (3). Although little is known regarding the cybernetics, the mechanism by which this process of regeneration is initiated, controlled, and terminated, extensive work has been done regarding the rate and the extent of this restoration (6).

The nucleic acid and phospholipid synthesis in the regenerating liver of tumor-bearing mice was investigated in a previous study (25). In the present work, the time of onset and the rate of mitosis of the tumor-free liver, regenerating after partial hepatectomy, were studied in tumor-carrying and control mice.

MATERIALS AND METHODS

C3H (Bittner Z) mice of both sexes, 6-10 weeks old, were used to receive the tumor transplant. Their weight varied from 18 to 24 gm, but mice of similar age and similar weight were used in a given experiment. The animals were fed Purina laboratory chow and water ad libitum. For at least one week prior, and also during the period of experiments, mice were kept under standardized environmental conditions (lights on at 6:00 A.M., off at 6:00 P.M., and temperature 78 ± 1°F).

For the periodic supply of a uniform epithelial solid tumor, a spontaneous mammary carcinoma that arose in a C3H female mouse was carried by successive subcutaneous transplantations of a small piece of tumor on the back of the recipient C3H mouse. After several passages, the tumor attained a relatively uniform rate of growth (2). This rate of growth was maintained throughout the study period and was such that at the time of the experiments, 5 weeks after transplantation, tumors of 1.5—2 cm in diameter were recovered. A piece of muscle from the thigh of the tumor donor was transplanted subcutaneously into the control mice. Ether anesthesia was used in these procedures. Each control and each experimental group consisted of at least 6 animals, with a range of between 6 to 12.

All partial hepatectomies were performed between 8 and 10 A.M., under Nembutal anesthesia, according to a modification (17) of the Higgins and Anderson technic (16). The animals were sacrificed by cervical dislocation at 6, 12, 18, 24, 30, 36, 42, 48, 54, and 60 hours after partial hepatectomy. The remaining lobes of the liver and the tumor in the tumor-bearing group were removed immediately. A piece from the right lobe was fixed in 10% formalin, imbedded in paraffin, then sectioned at 6 microns thickness. Sections were stained with hematoxylin and eosin. Mitoses were counted in random areas of the section. Fields of vision containing big blood vessels were not included in such counts. The counts included late prophase, metaphase, and telophase as cited elsewhere (29). Mitoses were calculated per 100 fields of high-power (X}

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RESULTS

The presence of the tumor elsewhere in the body affected the time of onset and the rate of regeneration in the remaining liver following partial hepatectomy. In the tumor-free animals, the rise in the number of mitoses occurred 48 hours after partial hepatectomy. In the tumor-bearing mice significant increase in the number of mitoses occurred at 30 hours, and the mitotic index kept rising for up to 42 hours. Both groups had the highest mitotic activity at 48 hours, and then the mitotic index started declining. There was no statistically significant difference in the mitotic index of the control and experimental groups from 48 to 60 hours posthepatectomy (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Hours posthepatectomy</th>
<th>Control</th>
<th>Experimental</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1.1 ± 1.1 a</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.6 ± 1.6</td>
<td>2.8 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>1.1 ± 1.1</td>
<td>1.2 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>1.4 ± 1.4</td>
<td>2.0 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.5 ± 0.3</td>
<td>7.8 ± 1.8</td>
<td>0.01 &lt; P &lt; 0.02</td>
</tr>
<tr>
<td>36</td>
<td>1.4 ± 1.4</td>
<td>17.5 ± 6.1</td>
<td>0.02 &lt; P &lt; 0.05</td>
</tr>
<tr>
<td>42</td>
<td>1.2 ± 1.2</td>
<td>40.0 ± 12.4</td>
<td>0.01 &lt; P &lt; 0.02</td>
</tr>
<tr>
<td>48</td>
<td>318.0 ± 111.6</td>
<td>207.1 ± 63.3</td>
<td>0.2 &lt; P &lt; 0.4</td>
</tr>
<tr>
<td>54</td>
<td>169.4 ± 41.0</td>
<td>138.8 ± 32.7</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>76.2 ± 22.7</td>
<td>103.7 ± 38.6</td>
<td></td>
</tr>
</tbody>
</table>

Mitoses per 100 high fields in the regenerating liver of control and tumor-bearing mice.

aMean ± S.E.

For the first 12 hours after partial hepatectomy, the mitotic index of the tumor remained at the presurgery level. At 18 hours it dropped markedly and subsequently started rising. It reached the initial level at 48 hours (Chart 1).

DISCUSSION

The simultaneous growth of a normal, rapidly proliferating tissue and a malignant tumor in the same animal is an excellent experimental model for the study of tumor-host metabolic interrelationships. Liver regenerating after partial hepatectomy is an ideal normal, fast-growing tissue for such studies because the time of onset, the rate of proliferation, the metabolic turnover of various components, and the period of completion of growth are all well established.

The effect of tumor growth on liver restoration after partial hepatectomy has been studied by other investigators, but their findings remain controversial. Paschkis et al. (22) found that the final weight of the regenerating liver was greater in the presence of a growing tumor elsewhere in the body. Trotter (26) compared the mitotic activity of the regenerating liver two days after partial hepatectomy in hepatoma-bearing and tumor-free mice. The subcutaneous growth of the hepatoma had no effect on the mitotic index of the regenerating liver. This is in agreement with our finding of similar mitotic index at 48 hours posthepatectomy in tumor-bearing and tumor-free mice. Gershbein (12) demonstrated that the extent of liver regeneration in rats over a period of 10.5 days after removal of part of the liver was not influenced by the presence of Walker 256 and Flexner-Jobling carcinomas or by the Jensen sarcoma. The tumor was transplanted 5 days before or at the time of surgery. Rosene (24) compared hepatic cell mitotic rates after intrasplenic injection of Ehrlich ascites tumor or reticulum cell sarcoma in mice. One day later, partial hepatectomy was performed, and the hepatic mitotic index was determined daily for the subsequent 12 days. He observed a delay in the appearance of hepatic parenchymal cell mitosis. The rise of mitotic index did not occur on Day 3, but did occur on Day 6 after surgery. We observed exactly the opposite effect, that is, an earlier increase of hepatocellular proliferation in the tumor-bearing animal. Although our experimental conditions are completely different, these controversial findings remain intriguing.

Pregnancy causes an increase in the extent of liver restoration in partially hepatectomized rats (10, 21). The study of the simultaneous effect of pregnancy and transplanted tumor on liver regeneration showed no influence of the tumor on the effect of pregnancy (14). Thiouracil and other compounds known to stimulate or to inhibit liver regeneration were studied in tumor-bearing animals (13, 15). Thiouracil resulted in a more marked inhibition of liver regeneration in the tumor-bearing rats (15). Certain carcinogenic hydrocarbons caused definite increase in the extent of liver restoration (11), but most carcinogens had a suppressive effect (5, 9, 18).
It has been believed that the cancer growth is autonomous and independent of metabolic changes in the host. This belief has been strengthened by the fact that despite tissue wasting and cachexia in the host the tumor continues to grow. This autonomy is relative because it is well established that hormonal changes of the environment influence the growth rate of certain hormone-sensitive tumors. In this respect any influence of the regenerating liver, a normal, rapidly growing tissue, on the rate of tumor proliferation is of interest.

Paschkis et al. (22) were among the first to study tumor growth in partially hepatectomized rats for evidence on possible competition for growth between malignant tissue and normal, rapidly proliferating tissue. They failed to demonstrate any competition, but actually they observed that certain epithelial tumors, implanted subcutaneously, grew faster in the partially hepatectomized than in nonoperated control rats. Subsequent work by others gave conflicting results. Trotter (26), working with a subcutaneously transplanted hepatoma, observed earlier appearance of the tumor in the partially hepatectomized mice, but the growth rate and the mitotic index of the tumor were the same in the experimental and the control groups. Enchave Llanos and Saffe (8) observed that the tumor reached a greater size in the hepatectomized mice than in the control mice. De Peyster et al. (7) reported increased tumor growth in parabiotic rats where one parabiont had a partial hepatectomy and his partner had the transplanted tumor. After partial hepatectomy, Gershbein (12) injected Jensen, Walker 256, and Flexner-Jobling tumor suspensions into the remaining caudal or caudate lobes. The Jensen and Walker tumors grew larger in the experimental rats than in the control or sham-operated animals.

In the present study the effect of the regenerating liver on the rate of tumor cell proliferation was compared at different posthepatectomy times. There was an unexplained, marked drop of the mitotic index of the tumor at 18 hours. This cannot be attributed to the surgery because the mitotic rate remained normal at 6 and 12 postoperative hours. The mitotic rate kept rising thereafter, and it reached preoperative levels at 48 hours.

REFERENCES


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