RESPONSIVENESS OF HYPERPLASTIC LESIONS AND HEPATOMAS TO PARTIAL HEPATECTOMY

Tomoyuki Kitagawa*
(Department of Pathology, Faculty of Medicine, University of Tokyo)

Synopsis
Responsiveness of hyperplastic lesions of the rat liver and hepatomas to partial hepatectomy was investigated by counting mitotic indices. Male Donryu rats fed 0.03% 2-fluorenylacacetamide (2-FAA) or 0.06% 3'-methyl-4-(dimethylamino)azobenzene (3'-Me-DAB) were used. Mitotic index 45 to 48 hr after a two-third partial hepatectomy was obtained and compared to that before partial hepatectomy. Areas of hyperplasia showed highly elevated mitotic indices, while the nodules of hyperplasia, nodules with atypia, and hepatomas with relatively low mitotic level responded with moderate elevation of the indices. In general the responsiveness of the nodules decreased gradually with the growing size, but it never became completely negative. Three hepatomas with high mitotic level and a cholangioma revealed slight or no change in the mitotic index after hepatectomy. In the liver of rats fed 0.03% 2-FAA, the original mature liver cells were quite inactive as long as the carcinogen was administered, but 6 to 24 weeks after discontinuation of the carcinogen their responsiveness returned to the same or even higher level than that of the areas and nodules of hyperplasia. The hypertrophic liver cells and areas of small cell hyperplasia, which were prominent in 3'-Me-DAB experiment, were apparently reactive to partial hepatectomy even during administration of carcinogen.

INTRODUCTION
Before development of apparent carcinoma, numerous hyperplastic lesions with different features appear in rat liver during and after the administration of carcinogenic diet. These hyperplastic lesions have been considered possible forerunners of carcinoma. Yet, despite obvious interest and numerous studies on these lesions, concrete and convincing evidence is still lacking as far as their exact nature is concerned, especially in their histogenetical relation to cancer.

Autonomy or indifference to homeostatic control mechanism has been regarded as one of the important characteristics of cancer. After partial hepatectomy there must be a powerful mechanism of homeostasis working in the body, as the normal liver regenerates and restores itself fairly quickly.3,12) Something essential for the clarification of the nature of the hyperplastic lesions may be contributed by the analysis of how such lesions and hepatomas are influenced by or respond to partial hepatectomy.

Nixon7) has reported decrease in incorporation of 14C-formate into nucleic acid of Novikoff hepatoma after partial hepatectomy of the host, and Maini et al.6) stressed unresponsiveness of hepatoma and "precancerous" liver during 3'-methyl-4-(dimethylamino)azobenzene (3'-Me-DAB) carcinogenesis to partial hepatectomy.

On the other hand, Paschkis8) reported a significant growth enhancement of Walker tumor and transplantable Morris 3924-C hepatoma in partially hepatectomized rats.

* Present address: Cancer Institute, Kami-Ikebukuro 1-37-1, Toshima-ku, Tokyo 170 (北川知行).
while no enhancement of Jensen sarcoma and Murphy lymphosarcoma was seen. Laws reported enhanced growth of small groups of hyperplastic cells and accelerated appearance of hepatomas in partially hepatectomized rats in 2-fluorenylacetamide (2-FAA) hepatocarcinogenesis. Guelstein reported slight elevation of mitotic index of transplanted hepatomas and high responsiveness of focal adenomatous nodules of the liver of 2-FAA-fed mice to partial hepatectomy, while the mitotic activity of the original liver cells was markedly inhibited. Wheeler reported a transient stimulation of synthesis of RNA and DNA in transplanted Morris 5123-C hepatoma without perceptive alteration in growth rate after partial hepatectomy.

Thus, there seems to be some inconsistency among the results, requiring further investigation. More analytical observation on the “precancerous” lesions or the hyperplastic lesions as well as hepatomas appears to be necessary.

The present author used rats fed 0.03% 2-FAA diet for analysis of areas and nodules of hyperplasia and hepatomas with low mitotic level, and rats fed 0.06% 3’-Me-DAB diet for that of hepatomas with relatively high mitotic level. Definitions of hyperplastic lesions were given in the preceding paper.

**MATERIALS AND METHODS**

**Animals**  More than 150 male Donryu rats (purchased from the Nippon Rat Co., Urawa), weighing 150~200 g, were used. The animals were housed two to a cage in screen-bottomed cages in an air-conditioned room.

**Carcinogenic Diet and Grouping** Basal diet (CE-2, CLEA Japan Inc., Tokyo) containing 0.03% 2-FAA or 0.06% 3’-Me-DAB was used. Animals of Groups 2 to 12 were

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of effective rats</th>
<th>Weeks on carcinogenic diet</th>
<th>Weeks on basal diet</th>
<th>Expression on Figs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>3</td>
<td></td>
<td>A3W</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>6</td>
<td></td>
<td>A6W</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>12</td>
<td></td>
<td>A12W</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>18</td>
<td></td>
<td>A18W</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>24</td>
<td></td>
<td>A24W</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>30</td>
<td></td>
<td>A30W</td>
</tr>
<tr>
<td>Groups fed 0.3% 2-FAA diet and then basal diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>A6+3W</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>A6+6W</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>12</td>
<td>6</td>
<td>A12+6W</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>12</td>
<td>12</td>
<td>A12+12W</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>12</td>
<td>18</td>
<td>A12+18W</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>12</td>
<td>24</td>
<td>A12+24W</td>
</tr>
<tr>
<td>Groups continuously fed 0.06% 3’-Me-DAB diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>3</td>
<td></td>
<td>D3W</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>6</td>
<td></td>
<td>D6W</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>16</td>
<td></td>
<td>D16W</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>26</td>
<td></td>
<td>D26W</td>
</tr>
<tr>
<td>Controls, fed basal diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table I. Experimental Schedule
RESPONSE OF HYPERPLASIA AND HEPATOMA TO HEPATECTOMY

fed basal diet for 5 days at the 3rd week to prevent early death. Animals were grouped as shown in Table I.

Partial Hepatectomy The animals were anesthesized with ether and about two-thirds of the liver was extirpated according to Higgins and Anderson's method. When there were large tumors more than 1.0 cm in diameter in the remaining lobes, a wedge biopsy was performed. All hepatectomy was done between 9 and 12 am. The operated rats were fed basal diet and killed 45 to 48 hr later.

Staining and Counting Sectioned materials were fixed in Baker's cold formol-calcium for 24 hr and then impregnated with Holt's cold gum-sucrose. Frozen sections, 6 μ in thickness, were made in a cryostat and mounted on glass slides using egg albumin. According to Hayashi-Fishman's method, β-glucuronidase staining was performed by counterstaining with Hematoxylin. By using this preparation, precisely definable counting of enzymically mature and immature lesions became possible.

Semiserial sections were made for a few small lesions to cover a sufficient number of nuclei at counting. To assess the frequency of mitosis, 2500 to 5000 nuclei were counted for each lesion. Apparent anaphase, metaphase, and early telophase nuclei were counted as in mitosis. Though Maini et al. recommended counting of telophase nuclei only in order to avoid overestimation, such a precaution seemed not necessary in the present study. In spite of using the same procedure, the general mitotic level of the liver, 45 to 48 hr after partial hepatectomy, was found to vary with individuals. For analysis of areas and nodules of hyperplasia and hepatomas only the liver showing generally high mitotic level was used. The effect of the trauma of wedge biopsy on the remaining tumor was carefully checked. Usually the bleeding stopped in a moment and there were sharply demarcated necrotic zone at the cut end 45 to 48 hr after the operation. Mitosis was seen even at the very margin of demarcation mostly in the same frequency as in the deeper portion. In some, however, the mitotic index of the remaining tumor was much lower than that of biopsied specimen and such materials were discarded.

RESULTS

Mitotic indices of the liver cell of control rats, 48 hr after partial hepatectomy, are shown in Fig. 1. Mitotic level varied individually, but age-effect seemed negligible. Mitotic indices before partial hepatectomy were 0.01 to 0.02%.

![Fig. 1. Mitotic index in the liver cell of control rats, 48 hr after partial hepatectomy](image-url)
T. KITAGAWA

The results obtained from Groups 1 to 6 are presented in Fig. 2. High responsiveness of areas of hyperplasia is apparent while mitotic indices of mature liver cells are quite low, both before and after partial hepatectomy. All the rats of Group 1 died within 24 hr.

The results from Groups 7 to 12 are shown in Fig. 3. In these rats, the mitotic levels of areas and nodules of hyperplasia and hepatomas are quite low before partial hepatectomy and their responsiveness to hepatectomy is clearly noted; areas of hyperplasia show high responsiveness, nodules moderate to high, and nodules with atypia and hepatomas moderate. With two hepatomas indicated by asterisks in Fig. 3, their responsiveness was confirmed by biopsy. Histologically all the hepatomas are

![Fig. 2. Mitotic index in various hepatic lesions and original mature liver cells of individual rats in Groups 2 to 6](image)

- Original mature liver cells
- Area of hyperplasia
- Nodule of hyperplasia
- Nodule with atypia
- Hepatoma.

![Fig. 3. Mitotic index in various hepatic lesions and original mature liver cells of individual rats in Groups 7 to 12](image)

* * Biopsied cases
RESPONSE OF HYPERPLASIA AND HEPATOMA TO HEPATECTOMY

trabecular hepatomas. Recovery of original mature liver cells from toxic effect of carcinogen is already apparent at the 3rd week after the rats were returned to basal diet, and it is of note that the mitotic level of the mature liver cells in Groups 10 to 12 is equal to or even higher than the average value in hyperplastic lesions.

The results from Groups 13 to 16 are shown in Fig. 4. In 3'-Me-DAB-fed liver, mature liver cells show moderate to high responsiveness, equal to areas of hyperplasia, even during administration of carcinogen. Areas of small-cell hyperplasia have relatively high mitotic level before partial hepatectomy and are responsive to it like areas of hyperplasia. Three hepatomas with relatively high mitotic level before partial hepatectomy show almost the same mitotic index after. These hepatomas belong to rather anaplastic

Fig. 4. Mitotic index in various hepatic lesions and original mature liver cells of individual rats in Groups 13 to 16

■ Mature liver cells ▼ Area of hyperplasia ▼ Area of small cell hyperplasia ○ Nodule of hyperplasia ○ Nodule with atypia ● Hepatoma ★ Cholangioma
*, **, *** Biopsied cases

Fig. 5. Mitotic index in various hepatic lesions of a rat in Group 11, relative to their size
hepatomas. One cholangioma showed the same mitotic index before and after partial hepatectomy.

The mitotic indices of various hepatic lesions of a rat of Group 11 and three rats of Group 12 are presented in Figs. 5 and 6 in relation to the size of lesions. Gradual decrease of responsiveness with the growing size of the lesions is noted but the responsiveness does not become negative.

DISCUSSION

A variety of hyperplastic changes can be observed during the course of hepatocarcinogenesis in rats fed 2-FAA or 3'-Me-DAB diet. They include areas of hyperplasia, nodules of hyperplasia with or without atypism, and small cell hyperplasia. When the responsiveness to partial hepatectomy of these hyperplastic lesions was tested,
RESPONSE OF HYPERPLASIA AND HEPATOMA TO HEPATECTOMY

none of them was found to have lost completely their ability to respond with cell division. They can be regarded as being still under the control of this kind of homeostatic regulation. However, there was a certain tendency for gradual decrease of responsiveness in the nodules according to their growing size. The results presented here are consistent with those of Lows and Guelstein, but contrary to that of Maini and Stich who reported unresponsiveness of the 'precancerous' liver after 3 to 11 week of 3'-Me-DAB feeding.

Concerning the hepatomas, elevation of mitotic index was apparent in two 2-FAA-induced hepatomas with low mitotic level, while it was slight or none in three 3'-Me-DAB-induced hepatomas with relatively high mitotic level. Though the rats of Groups 13 to 16 were continuously fed 3'-Me-DAB diet up to the time of partial hepatectomy, the toxic effect of the carcinogen on the hepatomas and hyperplastic lesions is reported to be negligible. The results with two 2-FAA-induced hepatomas coincide with the positive data of Paschkis, Guelstein, and Wheeler, while the results with three 3'-Me-DAB-induced hepatomas rather coincide with the negative data of Nixon and Maini. It may be that the effect of partial hepatectomy can be clearly observed only in hepatomas with relatively low mitotic level but may be overlooked when there is a considerably high mitotic level. Though Maini stressed unresponsiveness of hepatomas, his datum itself shows a certain elevation of mitotic rates in the majority of his hepatomas. Maini is the sole investigator who studied the responsiveness of hepatomas in vivo using the biopsy technique. However, he did not measure the general mitotic level of the liver. It may be meaningless to evaluate the effect of partial hepatectomy on a hepatoma when regenerative activity of the rest of the hepatic parenchyma is generally weak.

All the same, since hepatomas with relatively low mitotic level responded to hepatectomy beyond doubt, it appears that we should not characterize a hepatoma by unresponsiveness or indifference to homeostatic control mechanism. However, the gradual decrease of responsiveness of nodules according to their growing size is a fact and it may have some significance in the development or progress of a hepatoma.

Skoryna and Webster, and Guelstein demonstrated resistance of newly developed parenchymal cells to the toxic action of 2-FAA while Maini et al. and Guelstein did resistance of hepatomas and hyperplastic lesions to toxic action of azo dyes (o-azotoluene and 3'-Me-DAB). Vasiliev and Guelstein reported selective toxicity of azo dyes to normal liver cells and non-selective toxicity of 2-FAA both to normal and neoplastic liver cells in an in vitro system. The present results revealed that in 2-FAA carcinogenesis the mitotic activity of original liver cells was strongly inhibited as long as the carcinogen was administered and the original liver cells became fewer and fewer, while the areas and nodules of hyperplasia revealed resistance to toxic action of the carcinogen.

In contrast, in 3'-Me-DAB carcinogenesis, the original liver cells retained considerable responsiveness to partial hepatectomy instead of their rapid transformation to hypertrophic cells with prominent nuclear atypism and pleomorphism. Although histological observation tends to impress the stronger toxicity of 3'-Me-DAB than 2-FAA, the present results showed that the toxicity of 2-FAA on liver cells was quite intense, while deviation of cellular morphology remains unimpressive.
T. KITAGAWA

The author expresses his sincere gratitude to Prof. Kunio Oota, Department of Pathology, University of Tokyo, for his valuable suggestions and encouragement, and to Dr. Haruo Sugano, Department of Pathology, Cancer Institute, Tokyo, for his kind advices.

(Received February 22, 1971)

REFERENCES