Effect of Partial Hepatectomy on Tumor Incidence and Metabolism of Mice Fed Thioacetamide

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SUMMARY—Six-week-old male Swiss mice were given 0.03% thioacetamide (TAA) in the diet 24, 72, and 168 hours after partial hepatectomy. TAA-treated mice from all three groups were killed when they were 4, 9, and 13 months old. Intact and partially hepatectomized animals on normal diets served as controls. None of the controls evidenced neoplasms at any age. All three experimental groups developed liver tumors earlier than did intact mice treated with the TAA diet. Progressive metabolic studies on the livers or tumor tissues of treated mice showed that the levels of glucose-6-phosphatase, fructose-1,6-diphosphatase, and glycogen decreased significantly in the 4-month-old treated group when there was no significant alteration in liver histology. These parameters were lowest in the tumor tissues of treated mice.—J Natl Cancer Inst 56: 493-497, 1976.

Metabolic studies on thioacetamide (TAA) hepatocarcinogenesis have been done in this laboratory for several years. We observed that the continuous feeding of TAA induced 100% hepatomas in male Swiss mice approximately 17 months old (1). TAA treatments also decreased the level and biosynthesis of liver glycogen and the activities of gluconeogenic enzymes (2). Because of reports on the effect of liver injury or regeneration on hepatocarcinogenesis induced by diethylnitrosamine and urethan (3, 4), we wished to find out if TAA-induced malignant transformation of liver tissue could be accelerated by exposure of partially hepatectomized mice to TAA treatment.

Sequential metabolic changes in the livers or tumor tissues of TAA-treated partially hepatectomized mice were studied simultaneously with the biologic studies. Intact and partially hepatectomized mice on normal diets served as controls. This study reports the tumor incidence and sequential changes in carbohydrate metabolism in the livers and tumor tissues of partially hepatectomized mice fed TAA at different times after the operation.

MATERIALS AND METHODS

Male Swiss mice from the animal colony of the Cancer Research Institute, Bombay, were hepatectomized when 6 weeks old, according to the method of Higgins and Anderson (5) in which 75% of the total liver was removed under ether anesthesia and a tight knot was tied with a thread at the spot from which liver tissue was excised. Initially, histologic studies were done on regenerating liver tissue to follow the pattern of regeneration. Animals were autopsied at regular 6-hour intervals after partial hepatectomy up to 3 days, and thereafter every 24 hours up to 168 hours. Large numbers of newly formed liver cells were seen at 24 and 72 hours, but not at 48 hours. At 168 hours, the regenerating cells exhibited proliferation with fibrosis, mainly from the perportal area. We then introduced 0.03% TAA in the normal diet (6) 24, 72, and 168 hours after the operation, to see if the precise time of exposure to TAA was a critical factor.

Mice from these three experimental groups, and from intact untreated, and partially hepatectomized, untreated control groups, were given food ad libitum and killed when 4, 9, and 13 months old. At autopsy, all visceral organs, the liver in particular, were carefully examined for macroscopic pathology. Liver and liver tumors were fixed in 10% formalin for histopathologic studies. Paraffin sections 6 µ thick were routinely stained with hematoxylin and eosin (H & E).

Along with the biologic observations, metabolic studies on one representative experimental group of mice, i.e., the group given the TAA diet 72 hours after partial hepatectomy, were done and compared with the corresponding control groups. The following parameters of carbohydrate metabolism were assayed in the livers or tumor tissues of the 4-, 9-, and 13-month old mice given TAA 72 hours after the operation: 1) activities of glucose-6-phosphatase (G6Pase) and fructose-1,6-diphosphatase (FDPase); 2) content of glycogen and lactic acid. The blood glucose levels in the control and treated groups were also measured.

G6Pase activity was measured by the method of Cori and Cori (7) and that of FDPase by the technique of Poggel and McGilvery (8). Activities of both enzymes were expressed in terms of µg phosphorus liberated per mg tissue dry weight.

Glycogen content was measured as follows: Tissue was hydrolyzed with 30% KOH, and glycogen was precipitated and free glucose was determined by Nelson's method (9). Glycogen content was expressed in terms of µg glucose per mg tissue dry weight. Lactic acid content was measured by the procedure of Barker and Summers (10) and expressed in terms of µg lactic acid per mg dry weight. For the measurement of blood glucose, blood was withdrawn from the heart (before killing) with a hypodermic syringe in a heparinized tube. Proteins were precipitated and free glucose was determined by Nelson's method (9). Glucose content was expressed in µg glucose per ml of blood. Results were considered significant when P was less than 0.05.

RESULTS

Gross Observations

Liver tissues of 4-, 9-, and 13-month-old controls of both types, i.e., intact untreated mice and partially hepatectomized untreated mice, were normal. In all three TAA-treated groups, the liver tissues of 4-month-old mice...
were normal. At 9 months, livers from mice of the 24-hour group appeared normal; however, 3 of 4 mice from the 72-hour group and 3 of 5 from the 168-hour group had nodular livers. All animals from all experimental groups had liver tumors at 13 months.

**Microscopic Observations**

Histopathologic studies revealed that, at 4 months, mice from the control groups and all the experimental groups had normal livers. No tumor was observed in any controls at 9 and 13 months. At 9 months, the 24-hour group showed vacuolation and fatty changes in the liver; in the 72-hour group, 1 had a hyperplastic liver and 3 had hepatocellular carcinomas (table I). In the 168-hour group, 3 of 5 mice had hepatocellular carcinomas. At 13 months, all treated animals of the 24-hour group had hepatocellular carcinomas. In the 72-hour group, 2 of the 4 hepatocellular carcinomas (figs. 1, 2) showed pseudoglandular formation with marked ductal proliferation (figs. 3, 4). In the 168-hour group, all 4 tumors were hepatocellular carcinomas with pseudoglandular formation and marked fibrosis with ductal proliferation. This pseudoglandular formation of malignant hepatocytes gave an appearance of mixed nature to these tumors.

**Biochemical Observations**

Tables 2 and 3 denote the activities of G6Pase and FDPase in the livers and tumor tissues of control and experimental groups. Compared to intact mice on normal diets, the partially hepatectomized control group showed a decrease in the functions of both enzymes at the age of 4 months. These increased at 9 and 13 months but were still lower than those of the corresponding normal intact groups. TAA-treated partially hepatectomized mice also showed a decrease in the activities of both the enzymes, but there was no recovery at subsequent age periods and the levels remained at the lowest in each age group.

Table 4 shows the glycogen content in livers and tumor tissues of control and experimental groups. Intact mice and those partially hepatectomized on normal diets showed a progressive decrease in glycogen content with increasing age. Partially hepatectomized mice given TAA had a lower glycogen content even at 4 and 9 months, followed by a further decrease in tumor tissue.

Table 5 shows the lactic acid content in livers and tumor tissues of control and experimental groups. Intact and partially hepatectomized mice 4 and 9 months old did not show significant variations in lactic acid content. Only the 13-month-old partially hepatectomized control mice...
The data on carbohydrate metabolism support earlier reports on minimal deviation hepatomas (14) and our earlier work (15, 16). However, the significant changes in activities of gluconecogenic enzymes and glycogen content, observed as early as 4 months of age, are not discernible in the 4-month-old intact mice on the TAA diet. In conclusion, partial hepatectomy accelerates TAA-induced hepatocarcinogenesis in Swiss mice.

REFERENCES

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(13) MARQUARDT H, STERNBERG SS, PHILLIPS PS: Dimethylbenzanthracene and hepatic neoplasia in regenerating rat liver. Chem Biol Interact 2:401-408, 1970
(15) BHIDE SV: Comparative study of metabolic profiles of primary hepatoma, regenerating liver and liver in newborn mice. J Natl Cancer Inst 47:797-800, 1971

### TABLE 6.—Blood glucose levels in partially hepatectomized and TAA-fed mice at different ages

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Normal untreated</th>
<th>Partially hepatectomized, untreated</th>
<th>Partially hepatectomized and TAA treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1,134.0 ± 30.5</td>
<td>1,160.0 ± 15.5</td>
<td>1,120.0 ± 20.0</td>
</tr>
<tr>
<td>9</td>
<td>1,181.0 ± 21.1</td>
<td>1,049.0 ± 21.1</td>
<td>973.0 ± 23.3</td>
</tr>
<tr>
<td>13</td>
<td>717.0 ± 33.6</td>
<td>902.5 ± 63.4</td>
<td>649.0 ± 30.5</td>
</tr>
</tbody>
</table>

* Mice were given TAA 72 hours after partial hepatectomy. Blood glucose is expressed in mg glucose per ml blood. Results are expressed as mean of 6 estimations.

** Denotes statistical significance when compared with partially hepatectomized untreated group. *P* <0.05.

DISCUSSION

As reported in (11-13), our data clearly show that liver injury or regeneration of the liver accelerates malignant transformation. In our earlier work (1) we observed that intact mice given TAA did not develop tumors at 9 months and only 33% had hepatocellular carcinomas at 13 months. In the present study we observed many tumors at 9 months and a 100% incidence of tumors at 13 months. The time of introduction of the TAA diet after partial hepatectomy is not critical for tumor induction, because all experimental groups showed a progressive decrease with increasing age. Partially hepatectomized mice given TAA also showed a progressive decrease with increasing age and had the lowest levels among each age group.

The 72-hour group had a decrease in lactic acid, when compared to the corresponding intact group. On the other hand, partially hepatectomized mice given TAA showed a decrease in lactic acid at the age of 4 months followed by a progressive increase in subsequent age groups.

Table 6 denotes the blood glucose levels in control and experimental groups at different ages. Intact and partially hepatectomized mice on normal diets showed a progressive decrease with increasing age. Partially hepatectomized mice given TAA also showed a progressive decrease with increasing age and had the lowest levels among each age group.
FIGURE 1.—Low magnification of hepatoma of 72-hour group of 13-month-old partially hepatectomized and TAA-treated mouse. H & E. × 140

FIGURE 2.—High magnification of hepatoma of 72-hour group of 13-month-old partially hepatectomized and TAA-treated mouse. H & E. × 280

FIGURE 3.—Pseudoglandular hepatocellular carcinoma of 72-hour group of 13-month-old partially hepatectomized and TAA-treated mouse. Note ductal proliferation. H & E. × 80

FIGURE 4.—High magnification of figure 3 showing ductal proliferation at left middle and malignant hepatocytes in center. H & E. × 220