ENHANCEMENT OF AZO-DYE HEPATOCARCINOGENESIS WITH DIETARY PHENOBARBITAL IN RATS

Phenobarbital given together with the hepatocarcinogen, 4-(dimethylamino)azobenzene or with N-2-fluorenylacetamide (AAF), decreased the carcinogenic effect of the chemicals.1,4) On the other hand, phenobarbital given after the carcinogen treatment enhanced the hepatocarcinogenesis with AAF or with diethylnitrosamine (DEN).4,6,7) Phenobarbital also enhanced spontaneous hepatic tumorigenesis in mice.5)

We now examined the enhancing effect of phenobarbital in azo-dye carcinogenesis, because there is some uniqueness in morphological and/or biochemical aspects in the liver during carcinogenesis and in the carcinomas induced by azo dyes compared with those by AAF or DEN.3) The effect of phenobarbital was studied by checking the number and size of resulting carcinomas and also ATPase-deficient islands during carcinogenesis. The latter study seemed important since evidence so far suggests that phenobarbital most likely enhances the expression of preneoplastic lesions.6) ATPase-deficient islands (or hyperplastic areas and nodules) have been considered the most possible preneoplastic change.2)

Male Donryu rats at 21 days of age were fed a diet containing 0.06% 3'-methyl-4-(dimethylamino)azobenzene (3'-Me-DAB) for 3 weeks. After 2 weeks on basal diet, the rats were divided into 2 groups. The rats in group 1 were fed a diet containing 0.05% phenobarbital throughout the experimental course. The rats in group 2 were kept on the basal diet. Several rats were sacrificed at 6, 8, 12, and 24 experimental weeks. Frozen sections of the liver tissue were made in a cryostat and stained for ATPase by Wachstein and Meisel's method. The number and size of ATPase-deficient islands larger than 50 μm in diameter were scored from several sections, totally about 5 cm² in size, from a liver. All the remaining rats were killed at 36 weeks, and number and size of tumors larger than 5 mm in diameter were scored. Histological examination revealed that they were all well-differentiated carcinomas except one poorly differentiated in group 1.

As shown in Table I, the number of carcinomas per rat in group 1 was 14 times that in group 2. The size of carcinomas was also much larger in phenobarbital-fed rats. Thus the enhancing effect of phenobarbital in azo-dye hepatocarcinogenesis is very clear.

The number and size of ATPase-deficient islands were also significantly larger in the liver from group 1 rats (Figs. 1 and 2). In both groups the number and size of islands increased rapidly during 6 to 12 weeks. In group 1, there was further increase during 12 to 24 weeks, the datum at 36 weeks being not obtained because of the presence of multiple large tumors. In group 2, however, the number and size of islands remained relatively constant from 12 to 36 weeks. Apparent-

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**Table I. Number of Large Carcinoma at 36 Weeks**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of rats</th>
<th>No. of carcinoma relative to size</th>
<th>Total No. of carcinoma per group</th>
<th>No. of carcinoma per rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6~10 mm</td>
<td>11~20 mm</td>
<td>20 mm~</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>46</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>4</td>
<td>1</td>
<td>1</td>
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ly phenobarbital enhanced proliferation of azo dye-induced altered cells bringing them into detectable enzyme-deficient islands. In the great majority, however, their proliferative capacity was limited even under the influence of phenobarbital. Only a very small part of them (roughly one out of 1,000 islands in group 1) progressed to carcinoma larger than 5 mm by 36 weeks. Since the islands in group 2 did not increase in size and number from 12 to 36 weeks, it seemed that phenobarbital truly increased cancer production rather than merely accelerated cancer appearance of preneoplastic lesions that were destined to become cancers in any case.

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**Fig. 1.** Number of ATPase-deficient islands as a function of time

Numbers in parentheses indicate number of animals.

**Fig. 2.** Size distribution of ATPase-deficient islands at 12 and 24 weeks

**REFERENCES**