Effect of Hepatocarcinogens on Hepatocyte DNA Synthesis and Cortisone Induction of Tryptophan Oxygenase

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SUMMARY

The purpose of this study was to determine whether increases in adenosine monophosphate (AMP) deaminase activity in precancerous rat liver were correlated with proliferation among hepatic parenchymal cells. Rats were fed thioacetamide or the 3'-methyl- (3'-Me), 4'-methyl- (4'-Me), and 4'-fluoro- (4'-F) derivatives of 4-dimethylaminoazobenzene (DAB). Proliferation among hepatic parenchymal cells was assessed by 2 criteria: DNA synthesis determined autoradiographically or inhibition of cortisone induction of tryptophan oxygenase (E.C.1.3.1.12). Thioacetamide, 3'-Me-DAB, and 4'-F-DAB increased hepatocyte DNA synthesis, inhibited cortisone induction of tryptophan oxygenase, and stimulated hepatic AMP deaminase activity; 4'-Me-DAB did not alter any of these. 4'-F-DAB, when fed in a diet containing moderate riboflavin levels, was not hepatocarcinogenic and did not stimulate hepatocyte DNA synthesis or hepatic AMP deaminase activity. Injection of 3'-Me-DAB or thioacetamide also stimulated hepatocyte DNA synthesis, inhibited tryptophan oxygenase induction, and stimulated AMP deaminase activity. Simultaneous administration of ethionine with thioacetamide inhibited DNA synthesis.

AMP deaminase activity was not stimulated appreciably during liver regeneration, and the modest changes recorded could not be readily attributed to hepatocyte DNA synthesis or mitosis. The relationship between changes in cellularity in precancerous liver and stimulated AMP deaminase activity was discussed.

INTRODUCTION

Farber (11) believed that one of the characteristics of the carcinogenic process, especially in chemically induced liver cancer, was the need for cell proliferation at some time between exposure of the target tissue to a carcinogen and the recognition of neoplasia. Thioacetamide intoxication in rats is accompanied by increased synthesis of hepatic DNA (17, 40, 46) and by cellular proliferation (27, 32, 46). The administration of 3'-Me-DAB1 also results in increased synthesis of DNA and cellular proliferation (3, 10, 32, 38). Since these animals also have elevated levels of AMP deaminase activity (21–25), it was possible that increased enzyme activity was related to the onset of metabolic activities associated with DNA synthesis and proliferation.

This kind of relationship is not unknown in mammalian systems. For instance, increases in the activity of 3 hepatic enzymes; viz., deoxycytidylate deaminase, thymidylate synthetase, and thymidine kinase, have been observed in early stages of regeneration in rat liver (33, 34). Studies with mammalian cells in culture resulted in the demonstration of coincidence between DNA formation and increases in DNA polymerase and thymidine kinase activity (28). Later studies with cells in partial synchrony resulted in the demonstration of even greater parallelism between DNA synthesis and increased activity of thymidine kinase (4, 29, 47) or ribonucleotide reductase (48).

Recently, Seidman et al. (43) observed that 20–30 hr after partial hepatectomy, cortisone induction of tryptophan oxygenase in rat liver was inhibited, but substrate induction was not inhibited. Since this phenomenon occurred at a time when about 40% of the hepatocytes were synthesizing DNA (16), it appeared that this response was characteristic of rat liver in which hepatocyte DNA synthesis was stimulated by regeneration (1). Drews (9) afforded partial confirmation of this conclusion when he observed that although hypophysectomy delayed the mitotic wave about 24 hr, inhibition of cortisone induction of tryptophan oxygenase was not delayed.

In this study, we showed that feeding thioacetamide, 3'-Me-DAB, or 4'-F-DAB stimulated DNA synthesis in hepatocytes and inhibited cortisone induction of hepatic tryptophan oxygenase, but AMP deaminase activity was not appreciably altered during regeneration following partial hepatectomy.

MATERIALS AND METHODS

Animals. Male and female Holtzman rats weighing 140–180 gm were used in these experiments. They were housed in an animal room in which the lighting was controlled to provide 12-hr cycles of light and darkness. Later in the experimental period, a second animal room became available. Again, the lighting was controlled, but the light-dark cycles were exactly opposite to those in the first animal room.

Animals were fed Rockland Chow diet, a semisynthetic diet described by Farber (10), or a diet described by Medes et al. (35). The azo dyes, 3'-Me-DAB, 4'-F-DAB, and 4'-Me-DAB

1 The abbreviations used are: 3'-ME, 4'-ME, and 4'-F, 3'-methyl-, 4'-methyl-, and 4'-fluoro- derivatives of 4-dimethylaminoazobenzene; DAB, dimethylaminoazobenzene; AMP, adenosine monophosphate; ATP, adenosine triphosphate.

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were added to these diets to give concentrations of 0.06%; thioacetamide was added to give a concentration of 0.07%. When injected, the azo dyes were dissolved in corn oil and administered once intraabdominally at 250 mg/kg body weight; thioacetamide was dissolved in 0.9% saline and injected once daily at 50 mg/kg body weight.

Female rats were subjected to partial hepatectomy (18); controls for this group were laparotomized. All operative procedures were performed between 9 and 10 A.M. Half of the animals were taken from the animal room in the light cycle, while the other half were taken from the room in the dark cycle. Immediately after operation, the animals were returned to their respective rooms until killed for enzyme assays.

For determination of tumor incidence following the feeding of 4'-F-DAB, it was fed at 0.06% in Farber’s diet (10) to 2 groups of 12 female rats/group throughout 12 weeks. A third group of 12 animals were fed 4'-F-DAB at 0.06% in the semisynthetic diet described by Medes et al. (35) for 12 weeks. All 3 groups were returned to the Rockland Chow diet.

**Autoradiography.** Incorporation of thymidine-methyl-3H into nuclei of rat liver parenchymal cells was evaluated by the dip-coating method of autoradiography (2). Thioacetamide or the azo dyes were administered as dietary supplements or as injections. At intervals thereafter, tritiated thymidine was injected at 0.5 μg/gm body weight, and 30 min later the animals were killed. Livers were fixed in Carnoy’s fixative, embedded in paraffin, and sectioned at 4 μ. Slides were dip-coated with Kodak NTB emulsion. After exposure and development, they were stained with hematoxylin and eosin. The labeling index was determined by enumerating the number of hepatocyte nuclei containing grains among 3000–5000 nuclei/animal (44). In all experiments except one, animals were withdrawn from both light and dark cycles to minimize the influence of diurnal variation.

**Enzyme Assays.** AMP deaminase activity was assayed essentially as described previously (20), except that the substrate concentration was increased from 13.3 mM to 17.2 mM, and reaction mixtures contained 4 mM ATP and 50 mM KCl. Ammonia evolution was determined by the method of Chaney and Marbach (7). Tryptophan oxygenase was induced by intraabdominal injection of cortisone acetate at 10 mg/kg body weight into male rats (14). Tryptophan oxygenase activity was determined by the assay procedure of Feigelson and Greengard (12) modified by the addition of hematin (15).

**Source of Chemicals.** The azo dyes were synthesized using a procedure described by Giese et al. (13). Thioacetamide was purchased from J. T. Baker Company, Phillipsburg, New Jersey. Cortisone acetate was purchased from Merck and Company, Inc., Rahway, New Jersey. Thymidine-methyl-3H was purchased from New England Nuclear Corporation, Boston, Massachusetts.

### RESULTS

#### Labeling Index during Feeding of Hepatocarcinogens

The labeling index was determined. The data are shown in Table 1. Animals fed either 3'-Me-DAB or thioacetamide for 2 or more weeks had labeling indices that exceeded indices among animals fed the basal diets by 3-fold or more, but neither 4'-F-DAB nor 4'-Me-DAB feeding caused such increases. Marked histologic changes were observed among rats fed 3'-Me-DAB or thioacetamide, but these changes were notably absent among animals fed 4'-F-DAB. In separate experiments, we observed marked increases in hepatic AMP deaminase activity among animals fed 3'-Me-DAB or thioacetamide, but activity was not elevated appreciably among animals fed 4'-F-DAB or 4'-Me-DAB.

The increased labeling indices among animals fed 3'-Me-DAB or thioacetamide agreed with previous reports by MacDonald (30) and Stoecker (45) respectively, while increased AMP deaminase activity among these animals agreed with data

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Number of labeled nuclei/1000 nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Basal</strong></td>
</tr>
<tr>
<td>0</td>
<td>5.0 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>6</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>8</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>10</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>12</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

The status of DNA synthesis in hepatocytes when azo dyes or thioacetamide (TA) were fed throughout 12 weeks. Female rats were fed the diet described by Farber (10), containing azo dyes or thioacetamide to give 0.06% or 0.07% respectively. They were housed in a room in which lighting was controlled to give 12-hr light-dark cycles. Thymidine-methyl-3H (0.5 μg/gm body weight) was injected into half of the animals on each point 6 hr after change to the light cycle, and in the second half 6 hr after change to the dark cycle. Animals were killed 30 min after injection of the isotope. Incorporation of tritiated thymidine was determined by the dip-coating method of autoradiography (2). Labeling indices were calculated by enumerating labeled hepatocytes among 3000–5000 nuclei/animal (44). 3'-ME, 4'-ME, and 4'-F, 3'-methyl, 4'-methyl, and 4'-fluoro derivatives of 4-dimethylaminoazobenzene (DAB).

aValues are averages ± S.E. based on 6 animals unless indicated by a number of parentheses.
bValues exceeding corresponding basal values by 3-fold or greater.
Hepatocarcinogenic Effects

published previously by us (24). On the other hand, the absence of histologic changes among rats fed 4'-F-DAB was not in accord with data published by Price et al. (38). Furthermore, we previously reported (24) that 4'-F-DAB feeding increased hepatic AMP deaminase activity. In that study (24), 4'-F-DAB was fed in a semisynthetic diet (35) which had a lower riboflavin content than the diet (10) used in the present experiments. Since Miller et al. (37) observed that carcinogenicity of 4'-F-DAB was altered by the level of dietary riboflavin intake, it was possible that discrepancies observed among animals fed 4'-F-DAB were associated with a failure to induce malignant transformations. Accordingly, 2 groups containing 12 female rats/group were fed 4'-F-DAB in the basal diet described by Farber (10), while a third group of 12 female rats/group was fed 4'-F-DAB in the lower riboflavin diet described by Medes et al. (35). After 12 weeks on these diets, the 3 groups were returned to the Rockland Chow diet. The 24 animals fed 4'-F-DAB in the diet described by Farber (10) were held in the colony for 12 months without fatalities, and they were then killed. There was no evidence of gross or microscopic pathology among these animals. Among the 12 animals fed 4'-F-DAB in the diet described by Medes et al. (35), none survived longer than 3 months after the dye-feeding period. Liver tumors were confirmed in 10 of 11 animals autopsied; one animal died but could not be autopsied.

MacDonald (30) fed 3'-Me-DAB in 2 diets and observed high labeling indices with one but not with the other; with either diet, tumor incidence was essentially identical. Therefore, it was appropriate to determine labeling indices when 4'-F-DAB was fed under conditions resulting in hepatocarcinogenesis. Animals were fed 0.06% 4'-F-DAB or 3'-Me-DAB in the diet described by Medes et al. (35). At 2-week intervals throughout 6 weeks, the uptake of tritiated thymidine into hepatocyte nuclei was determined. The data are shown in Table 2.

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Labeled nuclei/1000 nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td>0</td>
<td>5.3 ± 1.3 (6)</td>
</tr>
<tr>
<td>2</td>
<td>1.5 ± 0.7 (6)</td>
</tr>
<tr>
<td>4</td>
<td>2.3 ± 0.6 (5)</td>
</tr>
</tbody>
</table>

Stimulation of DNA synthesis in hepatocytes by hepatocarcinogenic azo dyes when fed in a low riboflavin diet. Female rats were fed 3'-Me-DAB or 4'-F-DAB (at 0.06%) in the diet described by Medes et al. (35). All were conditioned 7 days on the basal diet before beginning the experiment. During feeding, animals were housed in 2 rooms with 12-hr light-dark cycles in opposite phase; animals were withdrawn from both groups each time labeling indices were determined. Other experimental details were as described in Table 1. 3'-ME and 4'-F, 3'-methyl and 4'-fluoro derivatives of 4-dimethylaminoazobenzene (DAB).

Through the first 2 weeks of feeding, neither 3'-Me-DAB nor 4'-F-DAB altered the labeling index, but after 4 weeks both hepatocarcinogens stimulated DNA synthesis among hepatocyte nuclei. Proliferation of bile duct cells and/or oval cells was observed in 4'-F-DAB-treated livers, but, in agreement with Price et al. (38), this proliferation was considerably less extensive when compared to the proliferation of these cells among rats fed 3'-Me-DAB. In agreement with our previous observations (24), we again observed elevated levels of AMP deaminase activity among animals fed 4'-F-DAB in the lower riboflavin diet (35).

**Tryptophan Oxygenase Induction during Carcinogen Feeding.** Our data presented a positive correlation between malignant transformation in rat liver, stimulation of DNA synthesis among hepatic parenchymal cells, and increases in hepatic AMP deaminase activity. Although DNA synthesis was stimulated (Tables 1, 2), the number of hepatocytes entering DNA synthesis was conservative compared to the situation following partial hepatectomy (16). Therefore, it was appropriate to determine whether a significant proportion of hepatocytes had entered DNA synthesis. Toward this end, we determined whether cortisone induction of tryptophan oxygenase was inhibited when DNA synthesis was stimulated by hepatocarcinogens.

Four groups of male rats were fed the low riboflavin diet (35) containing 3'-Me-DAB, 4'-F-DAB, 4'-Me-DAB, or thioacetamide through 6 weeks. At 2-week intervals, animals from each group were injected with cortisone, and the extent to which cortisone induced tryptophan oxygenase in these animals was compared to the induction observed among animals fed the basal diet alone. The data are shown in Table 3. Induction was inhibited in 2 weeks among animals fed 3'-Me-DAB or thioacetamide; by 4 weeks, inhibition was observed among animals fed 4'-F-DAB. Throughout the 6-week period, no inhibition was observed among animals fed 4'-Me-DAB. Thus, a positive correlation seemed to exist between stimulated DNA synthesis by hepatocarcinogens and interference with cortisone induction of tryptophan oxygenase.

It was possible that inhibitions seen in Table 3 were associated with delays in responses to cortisone among rats fed hepatocarcinogens rather than an inhibition of induction. Accordingly, male rats were fed basal, 3'-Me-DAB, or thioacetamide diets throughout 4 weeks. Then rats from each dietary group were sacrificed at various time-periods after a single intraabdominal injection of cortisone. The data are graphically depicted in Chart 1. When animals were killed immediately after injection of cortisone, there was no appreciable difference between levels of tryptophan oxygenase activity irrespective of dietary regimen. This agreed with the observation by Chan et al. (6), that 3'-Me-DAB hepatocarcinogenesis did not alter the noninduced level of tryptophan oxygenase activity. Throughout 11 hr after cortisone administration, however, induction of tryptophan oxygenase among animals fed 3'-Me-DAB or thioacetamide was appreciably less than that observed after 4–6 hr among animals fed a basal diet. Therefore, it appeared that differences seen in Table 3 were associated with inhibition of induction rather than delayed induction.

**Partial Hepatectomy Studies.** We reported that partial hepatectomy had no appreciable effect on hepatic AMP deaminase activity (24). But the experience of Seidman et al.,...
Cortisone induction of tryptophan oxygenase in the liver of rats fed azo dyes or thioacetamide. Male rats were fed azo dyes (0.06%) or thioacetamide (0.07%) in the diet described by Medes et al. (35). Three hr before killing, cortisone acetate was injected intraabdominally at 10 mg/kg body weight. Tryptophan oxygenase activity was assayed by procedures described by Fiegelson and Greengard (12) except that hematin (15) was added. 3'-ME, 4'-ME, and 4'-F, 3'-methyl, 4'-methyl, and 4'-fluoro derivatives of 4-dimethylaminoazobenzene (DAB).

Values are averages ± S.E. Numbers in parentheses represent number of animals.

Values less than corresponding basal values at a probability level of 0.05 or greater.

Cortisone induction of tryptophan oxygenase in regenerating rat liver, together with our data on hepatocyte labeling (Table 2) and tryptophan oxygenase induction (Table 3), emphasized the propriety of a more detailed investigation on the status of AMP deaminase during liver regeneration. As shown in Table 4, enzyme activities among hepatectomized animals exceeded activity among laparotomized controls twice during a 7-day postoperative period; viz., at 24 hr and at 72–76 hr. At either point, the magnitude of the excess was not appreciable. Furthermore, neither point could be readily linked with metabolic events known to occur during regeneration. For instance, DNA synthesis was vigorous 20–30 hr postoperation (16), but during this period only one of 3 enzyme measurements exceeded controls. The period at 72–76 hr differs markedly from the 20–30 hr period in that both DNA synthesis and mitosis has subsided (5, 16), but enzyme measurements indicated a degree of metabolic homogeneity between these 2 periods. Therefore, it seemed unlikely that these increases were associated with metabolic shifts necessitated by DNA synthesis or by mitosis.

Labeling Index after Injection of Hepatocarcinogens. Injections of thioacetamide (21, 25) increased AMP deaminase activity. Simultaneous injections of ethionine inhibited these increases, but this inhibition was not associated with inhibition of protein synthesis (26). Since Schneider et al. (42) had reported that ethionine inhibited DNA synthesis in regenerating rat liver, we determined the labeling indices among rats injected once daily with thioacetamide, ethionine, or the combination. The data are shown in Table 5. DNA synthesis was stimulated 3- to 5-fold by thioacetamide injections as early as 24 hr after a single injection. Ethionine had no appreciable effect on the labeling index when injected alone, but when given in combination with thioacetamide, the incorporation of tritiated thymidine into hepatocyte nuclei was inhibited. This inhibition gradually eroded until, after 5 injections, the labeling index among animals receiving the combination was as...
Table 4

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>AMP deaminase activity* (µmoles NH₃/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
</tr>
<tr>
<td>3</td>
<td>2.78 ± 0.12b</td>
</tr>
<tr>
<td>6</td>
<td>2.93 ± 0.10</td>
</tr>
<tr>
<td>12</td>
<td>3.12 ± 0.11</td>
</tr>
<tr>
<td>20</td>
<td>3.26 ± 0.09 (9)</td>
</tr>
<tr>
<td>24</td>
<td>3.06 ± 0.07</td>
</tr>
<tr>
<td>30</td>
<td>3.14 ± 0.21</td>
</tr>
<tr>
<td>36</td>
<td>3.06 ± 0.13</td>
</tr>
<tr>
<td>48</td>
<td>3.37 ± 0.23</td>
</tr>
<tr>
<td>54</td>
<td>2.92 ± 0.11</td>
</tr>
<tr>
<td>60</td>
<td>2.64 ± 0.13</td>
</tr>
<tr>
<td>72</td>
<td>2.80 ± 0.11 (12)</td>
</tr>
<tr>
<td>76</td>
<td>2.68 ± 0.09</td>
</tr>
<tr>
<td>96</td>
<td>2.47 ± 0.14</td>
</tr>
<tr>
<td>120</td>
<td>2.89 ± 0.08 (12)</td>
</tr>
<tr>
<td>144</td>
<td>2.61 ± 0.14</td>
</tr>
<tr>
<td>168</td>
<td>2.79 ± 0.10</td>
</tr>
</tbody>
</table>

Adenosine monophosphate (AMP) deaminase activity in rat liver during regeneration following partial hepatectomy. Male rats were housed in 2 animal rooms as described in Table 2. During hepatectomy, approximately 65% of the liver was removed (18). Sham animals were subjected to laparotomy.

*Conditions for assay were: 17.2 mM AMP; 50 mM KCl; 4 mM ATP; 0.05 M Na citrate buffer, pH 6.0; 105,000 XQg supernatant protein to give 1.5-2.5 mg protein/ml; incubation at 37°C for 30 min. Ammonia was determined by the method of Chaney and Marbach (7).

bUnless indicated by a number in parentheses, values are averages ± S.E. based on 8 animals.

Values exceeding corresponding sham values by 3-fold or greater.

DISCUSSION

This study clearly demonstrated that hepatocarcinogens stimulated DNA synthesis in hepatocytes and increased hepatic AMP deaminase activity. Enhanced DNA synthesis was established directly by autoradiographic demonstration of the incorporation of tritiated thymidine. Indirect demonstration of enhanced DNA synthesis was accomplished when cortisone induction of tryptophan oxygenase was inhibited. Ancillary to this correlation was the demonstration that metabolism associated with DNA synthesis initiated in hepatocytes by partial hepatectomy must differ, at least quantitatively, from that initiated by hepatocarcinogens. Although regeneration was accompanied by DNA synthesis (16) and inhibited enzyme induction (43), appreciable increases in AMP deaminase activity were not seen. Evidence from other studies also indicated that these 2 proliferative responses differed. For high as those given thioacetamide alone. In the previous study (26), increases in AMP deaminase activity were initiated by a single injection and increased further with subsequent injections. These increases were inhibited by ethionine throughout 4, once daily injections of the combination but not after 5 injections (26).

Table 6 shows the labeling indices among hepatocytes 1, 3, 5, and 9 days after a single injection of oil, 3'-Me-DAB, or 4'-Me-DAB. Within 24 hr, the labeling indices among rats given 3'-Me-DAB exceeded controls and remained high throughout 9 days. In contradistinction with our experience when 4'-Me-DAB was fed, the labeling index among rats given a single injection of 4'-Me-DAB was elevated 3 days after injection but subsequently subsided to control levels. Histologic changes were readily seen after 3 days in the liver of rats injected with 3'-Me-DAB. The oval cell and/or ductal cell proliferation described by Price et al. (38) and Farber (10) was initiated by 3 days and was well established by 5 days. This response was not seen in animals injected with 4'-Me-DAB. Previously (23), marked increases in hepatic AMP deaminase activity were observed in rats injected with 3'-Me-DAB, and slight increases were observed 4 and 8 days after 4'-Me-DAB injections.

As shown in Table 7, injection of 3'-Me-DAB or thioacetamide inhibited cortisone induction of tryptophan oxygenase. Neither 4'-Me-DAB nor 4'-F-DAB injections were effective in this regard. Since these animals were maintained on Rockland Chow diet ad libitum, the ineffectiveness of 4'-F-DAB in this instance may be associated with dietary riboflavin levels. Repeated trials to stimulate hepatic AMP deaminase activity by injection of 4'-F-DAB also have been unsuccessful.
instance, regeneration following hepatectomy represents a more homogenous proliferation involving hepatocytes primarily (16), whereas proliferation stimulated by hepatocarcinogens is heterogenous usually involving major shifts in cellularity (10, 38). This shift was possibly associated with the observation that early stages of hepatocarcinogenesis with 3'-Me-DAB or ethionine were accompanied by stimulation of the functional capacity of the liver reticuloendothelial system (19). Recently, Machado (31) demonstrated diffuse proliferation of Kupffer cells after 2 weeks of DAB feeding. Although the greater stimulation of AMP deaminase activity in precancerous livers compared to regenerating livers may have been partially related to the greater stimulation of AMP deaminase activity in precan-
cerous livers compared to regenerating livers, we cannot exclude the possibility that both stimulated enzyme activity and the heterogenous proliferation were symptomatic of influences exerted on liver metabolism by hepatocarcinogens.

Table 6

<table>
<thead>
<tr>
<th>Time after injection (hr)</th>
<th>Labeled nuclei/1000 nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oil</td>
</tr>
<tr>
<td>0</td>
<td>2.23 ± 1.41</td>
</tr>
<tr>
<td>24</td>
<td>2.76 ± 0.22</td>
</tr>
<tr>
<td>72</td>
<td>0.39 ± 0.39</td>
</tr>
<tr>
<td>120</td>
<td>0.87 ± 0.33</td>
</tr>
<tr>
<td>216</td>
<td>1.32 ± 0.07 (2)</td>
</tr>
</tbody>
</table>

Effect of a single injection of 3'-methyl- or 4'-methyl-DAB upon DNA synthesis among hepatic parenchymal cells. Azo dyes were injected in oil at 250 mg/kg body weight into female rats. Tritiated thymidine was injected 3 hr after injection of oil or azo dyes. Technics of autoradiography were as described in Table 1. Unless indicated by a number in parentheses, values are averages ± S.E. for 3 animals. DAB, 4-dimethylaminoazobenzene.

Table 7

<table>
<thead>
<tr>
<th>Compounds injected</th>
<th>Tryptophan oxygenase activity (µmoles kynurenine/gm liver/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>4.68 ± 0.21 * (9)</td>
</tr>
<tr>
<td>3'-Me-DAB</td>
<td>2.27 ± 0.61 * (9)</td>
</tr>
<tr>
<td>4'-F-DAB</td>
<td>5.54 ± 0.63 * (9)</td>
</tr>
<tr>
<td>4'-Me-DAB</td>
<td>6.08 ± 0.14 * (9)</td>
</tr>
<tr>
<td>Saline, 3 injections</td>
<td>4.67 ± 0.15 * (6)</td>
</tr>
<tr>
<td>Saline, 7 injections</td>
<td>5.79 ± 0.34 * (10)</td>
</tr>
<tr>
<td>Thioacetamide, 3 injections</td>
<td>3.55 ± 0.19 * (6)</td>
</tr>
<tr>
<td>Thioacetamide, 7 injections</td>
<td>3.32 ± 0.11 * (10)</td>
</tr>
</tbody>
</table>

Cortisone induction of tryptophan oxygenase among male rats given single injections of azo dyes or multiple injections of thioacetamide. Thioacetamide or azo dyes were injected as described in Tables 5 and 6 respectively. Animals were injected with cortisone (intraabdominally at 10 mg/kg body weight) 3 hr before killing. Other conditions of assay were as described in Table 3. 3'-ME, 4'-ME, and 4'-F, 3'-methyl, 4'-methyl, and 4'-fluoro derivatives of 4-dimethylaminooazobenzene (DAB).

aValues exceeding corresponding oil values by 3-fold or greater.

MacDonald (30) concluded that increased proliferation during early stages of carcinogenesis with 3'-Me-DAB was not important in the development of hepatic tumors. But, in this study, when 4'-F-DAB induced liver tumors, DNA synthesis in precancerous livers was stimulated. Farber (10) believed that the oval cell hyperplasia which accompanied hepatocarcinogenesis with a number of agents might be necessary in “setting the stage” for neoplastic response. Later, Maini and Stich (32) concluded that both chromosomal injury and proliferation were necessary for azo dye hepatocarcinogenesis. The present work showed a positive correlation between hepatocarcinogenicity of the agents studied and their ability to stimulate DNA synthesis in hepatocytes. Even the slight stimulation of DNA synthesis following injection of 4'-Me-DAB fits this correlation. This dye was classified as a weak carcinogen (36) and caused mitotic irregularities in the study by Maini and Stich (32). Autoradiographic studies showed stimulated DNA synthesis during the administration of DAB (42), 3'-Me-DAB (3, 30), and thioacetamide (45), but not during the administration of aflatoxin (39). Although aflatoxin differed in this respect, it inhibited cortisone induction of tryptophan pyrrolase (8) as did the hepatocarcinogens in this study, (Table 3) or dimethylnitrosamine in a previous study (41). Thus, Farber’s contention (11) that liver carcinogenesis is mediated by cell proliferation or hyperplasia is supported.

ACKNOWLEDGMENTS

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Hepatocarcinogenic Effects


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