Acute Effects of Selected Hepatocarcinogens on Polyribosomes and Protein Synthesis in the Livers of Rats Fed Purified Diets Containing Hepatocarcinogens

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ABSTRACT

This investigation was concerned with the acute effect of ethionine, thioacetamide, dimethylnitrosamine (DMN), or aflatoxin B₁ on hepatic polyribosomes and protein synthesis of rats fed purified diets either ad libitum for 3 to 29 weeks containing 0.025% N-2-fluorenylacetamide (2-FAA) or 0.06% 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) or by force-feeding for 3 days of diets containing 2-FAA, 3'-Me-DAB, DMN, 0.032% thioacetamide, or 0.25% ethionine. This study revealed that long-term feeding of 2-FAA or 3'-Me-DAB diminished the acute toxic effect of ethionine, disaggregation of polyribosomes, and inhibition of protein synthesis in liver. Likewise, short-term force-feeding (3 days) of hepatocarcinogens (2-FAA, 3'-Me-DAB, DMN, thioacetamide, or ethionine) diminished the acute toxic effect of ethionine on hepatic polyribosomes and protein synthesis. Rats force-fed diets containing 2-FAA, 3'-Me-DAB, DMN, thioacetamide, or ethionine for 3 days and then challenged acutely with thioacetamide, DMN, or aflatoxin B₁ revealed variable responses of hepatic polyribosomes and protein synthesis. Thus, long- and short-term feeding of several hepatocarcinogens leads to a resistance in hepatic polyribosomes and protein synthesis in response to the acute administration of ethionine while rats force-fed for 3 days the same carcinogens and then challenged acutely with carcinogens (thioacetamide, DMN, or aflatoxin B₁) other than ethionine develop variable effects.

INTRODUCTION

In an earlier study the response of hepatic polyribosomes and protein synthesis of rats that were fed a purified diet containing ethionine for days or months prior to the administration of ethionine 4 hr before being killed was investigated (21). The results revealed that rats fed a purified diet containing 0.25% ethionine ad libitum for up to 50 weeks failed to respond to the acute i.p. administration of ethionine with a change in hepatic polyribosomes (disaggregation) and protein synthesis (decrease) as did control rats fed the basal purified diet (21). As a follow-up to these earlier findings, we decided to study whether rats fed ad libitum a purified diet containing 0.025% 2-FAA or 0.06% 3'-

1 This investigation was supported by USPHS Research Grants CA-14156 and CA-22997 from the National Cancer Institute.
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: 2-FAA, N-2-fluorenylacetamide; 3'-Me-DAB, 3'-methyl-4-dimethylaminoazobenzene; DMN, dimethylnitrosamine; 4-FAA, N-fluoran-4-ylacetamide.

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sucrose. Rats were fed ad libitum or force-fed 3 times daily the basal diet alone or the basal diet containing 0.25% DL-ethionine, 0.025% 2-FAA, 0.06% 3'-Me-DAB, or 0.032% thioacetamide. In 2 experiments rats were force-fed for 3 days the basal diet containing 0.025% 4-FAA, a noncarcinogenic compound related to 2-FAA. When 3'-Me-DAB was added to the basal diet, the vitamin:sucrose mixture was changed so that it was low in riboflavin (33 μg/100 g diet) as was used earlier (24). Rats force-fed the DMN diet received DMN (0.8 mg/100 g of body weight) added to the third feeding each day but received the basal diet alone for the earlier 2 feedings each day. Animals were fed ad libitum the basal, 2-FAA, or 3'-Me-DAB diet for intervals of 3 to 29 weeks as indicated in Table 1. Before being killed, all animals were fasted overnight, and they received i.p. single injections of DL-ethionine (100 mg/100 g of body weight, 4 hr before being killed); DMN (10 mg/100 g of body weight, 14 hr before being killed); thioacetamide (5 mg/100 g of body weight, 4 hr before being killed); or aflatoxin B₁ (0.6 mg/100 g of body weight, 12 hr before being killed). In the force-feeding experiments, all rats were tube fed the basal diet for 1 day, and then groups were tube fed for 3 days the basal, 2-FAA, 3'-Me-DAB, thioacetamide, or basal diet with supplementation of DMN. In these experiments, the diets were made up with distilled water into a 67% suspension that was administered by stomach tube 3 times daily. Each animal of the different groups received daily on the average 0.86 g diet per 10 g of initial body weight. Rats were killed on the fourth morning after beginning the experimental diets, approximately 16 hr after the last evening tube-feeding. Before being killed, they received i.p. single injections of carcinogens as described previously. Rats had free access to water. They were housed in individual wire cages with raised bottoms.

In in vitro incorporation experiments, postmitochondrial supernatants or microsomes of homogenates of pooled livers were used. The postmitochondrial supernatants were prepared in 0.25 M sucrose containing 0.05 M Tris-HCl, pH 7.5, 0.025 M KCl, and 0.005 M MgCl₂ and were used for protein synthesis in vitro or for size distribution analysis of polyribosomes after addition of deoxycholate (0.7%, final concentration) (25, 30). L-[U-¹⁴C]Leucine (10 μCi/mol), 0.5 μCi, was added to each incubation tube.

Table 1

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Duration (wk)</th>
<th>No. of experiments</th>
<th>(% inhibition)</th>
<th>Visual grading</th>
<th>Monomer-dimers/total ribosomes x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>3-4</td>
<td>2</td>
<td>78.9 ± 4.06</td>
<td>3.6+</td>
<td>73.0 ± 1.21</td>
</tr>
<tr>
<td>2-FAA</td>
<td>8</td>
<td>1</td>
<td>34.0 ± 14.00</td>
<td>1.0+</td>
<td>56.4 ± 14.11</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1</td>
<td>11</td>
<td>0</td>
<td>45.2 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>48.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>42.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>25-29</td>
<td>3</td>
<td>9.3 ± 5.49</td>
<td>0.7+</td>
<td>48.0 ± 0.95</td>
</tr>
<tr>
<td>2-FAA → basal</td>
<td>2</td>
<td></td>
<td>26.0 ± 9.00</td>
<td>1.5+</td>
<td>58.8 ± 4.58</td>
</tr>
<tr>
<td>Basal</td>
<td>4</td>
<td></td>
<td>77.3 ± 2.25</td>
<td>3.6+</td>
<td>73.3 ± 1.15</td>
</tr>
<tr>
<td>3'-Me-DAB</td>
<td>3-4</td>
<td>2</td>
<td>20.0 ± 38.00</td>
<td>1.0+</td>
<td>52.9 ± 9.95</td>
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<tr>
<td></td>
<td>6</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>52.9 ± 9.95</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Rats were fed ad libitum the basal diet, the 2-FAA diet (basal diet containing 0.025% 2-FAA), or the 3'-Me-DAB diet (basal diet containing 0.06% 3'-Me-DAB).
* In each experiment, livers from 3 to 6 rats of each group were pooled.
* All rats were fasted overnight and DL-ethionine (100 mg/100 g of body weight) was administered i.p. 4 hr before killing.
* In vitro protein synthesis was assayed with [¹⁴C]leucine and postmitochondrial supernatants of pooled livers of each group.
* Sucrose density gradients of deoxycholate-treated postmitochondrial supernatants. All gradients were compared with comparable controls not treated with ethionine. On (monomer-dimer)/total ribosome analyses, the mean value for control groups was 42.4 ± 1.15.
* In each experiment, ethionine-treated rats were compared with control (basal, 2-FAA, or 3'-Me-DAB diet without ethionine treatment) rats.
* Disaggregation was graded in each experiment, ranging from marked (4+) to none (0); each value is the mean.
* Mean ± S.E.
* p < 0.01, compared with basal untreated group or comparable control group.
* 0.05 > p > 0.01, compared with basal, ethionine-treated group.
* p < 0.01, compared with basal, ethionine-treated group.
* Rats were fed the 2-FAA diet for 21 to 24 weeks and then switched to the basal diet for 5 weeks.
the different experimental conditions was evaluated from the patterns obtained by sucrose density gradients in 2 ways: (a) by visual grading in which the shifts between heavier and lighter aggregates were scored; and (b) by calculating the relative distribution of monomer-dimers in relation to total ribosomes. Visual scoring was conducted in a manner similar to that described earlier (21, 27) and consisted of rating each gradient pattern from 0 (control) to 4+ according to degree of disaggregation (shift from heavier to lighter aggregates) or from 0 (control) to 4− according to degree of aggregation (shift from lighter to heavier aggregates). Relative distribution of monomer-dimers in relation to total ribosomes was determined on each gradient pattern by measuring the area under the monomer and dimer peaks and the area under the entire pattern (monomer-dimers plus the other polyribosome fractions).

In in vivo incorporation experiments, rats received i.p. L-[¹⁴C]leucine (10 μCi/mol). In these experiments, the rats received the acute administration of ethionine or 0.9% NaCl solution by stomach tube rather than i.p. Each animal received (2.5 µCi L-[¹⁴C]leucine per 100 g of body weight) 15 min before killing. The methods used for chemical analyses of protein and radioactivity have been described in detail in an earlier study (22). Radioactivity in protein was measured with a liquid scintillation spectrometer.

For hepatic ATP determination, animals were anesthetized with 2-bromo-2-chloro-1:1:1-trifluoroethane and then exposed to 100% O₂. A portion of liver was frozen between the faces of metal tongs precooled in liquid N₂ (2). The frozen piece of liver was then weighed and extracted with ice-cold 3.14% perchloric acid. The ATP in the neutralized perchloric acid extract was determined by the luciferin-luciferase reaction (31) with the use of desiccated firefly lanterns.

All rats in the chronic feeding experiments were necropsied. Tissues were fixed in Zenker-formol solution, and sections were stained with hematoxylin and eosin.

RESULTS

The Protective Effect of Hepatocarcinogens in the Diet against the Polyribosomal Disaggregation Caused by the Acute Injection of Ethionine

Ad libitum Feeding Studies. Female rats were fed the basal, the 2-FAA (basal plus 0.025% 2-FAA) or the 3′-Me-DAB (basal plus 0.06% 3′-Me-DAB) diet for 3 to 29 weeks. The animals were weighed at weekly intervals. The control rats fed the basal diet gained weight throughout (more during the first 10 weeks than later on) when compared with rats fed the 2-FAA or the 3′-Me-DAB diet without ethionine treatment (Table 1). In these experiments, a group of rats was fed the 2-FAA diet for 21 to 24 weeks and then was switched to the basal diet for 5 weeks. When rats of this group were then challenged by the acute administration of ethionine, the rats responded with some inhibition (26%) of hepatic protein synthesis and with some disaggregation of polyribosomes similar to that observed with rats fed the 2-FAA diet for 3 to 4 weeks and then challenged acutely with ethionine (Table 1).

In 2 experiments, in vivo L-[¹⁴C]leucine incorporation into hepatic proteins was determined in rats that had been fed ad libitum the basal or the 2-FAA diet for 12 or 22 weeks. Following an overnight fast and then the acute administration of ethionine, the rats that had been fed the basal diet for 12 weeks showed a 72% inhibition in incorporation into proteins (specific activity, cpm/mg of protein) while rats that had been fed the 2-FAA diet for 12 weeks showed no inhibition; the rats that had been fed the basal diet for 22 weeks showed an 83% inhibition, and those that had been fed the 2-FAA diet showed a 20% inhibition. In each case, the ethionine-treated rats were compared with rats fed the same (basal or 2-FAA) diet but without ethionine treatment.

In 1 experiment in which rats had been fed the 2-FAA diet for 27 weeks, primary hepatocellular carcinomas developed and were used for analyses. Tumor tissues from rats with or without ethionine treatment 4 hr before killing were analyzed for polyribosomes and in vitro protein synthesis. After ethionine treatment there was no disaggregation of polyribosomes and an 8% decrease in protein synthesis in tumor tissue in comparison with the findings in tumor tissue of untreated rats.

In 3 of the preceding experiments, hepatic ATP levels were assayed. Rats fed diets for 3, 22, and 29 weeks were used. Rats fed the 2-FAA diet had essentially the same hepatic ATP levels (μmol/g of liver) as those fed the basal diet. The values for the control (basal diet) and experimental (2-FAA diet) rats at 3, 22, and 29 weeks, respectively, were: 2.62, 3.04; 2.32, 2.12; and 2.46, 2.13. Rats fed the 2-FAA diet for 3, 22, and 29 weeks and then treated with ethionine showed marked decreases in hepatic ATP levels similar to those found in rats fed the basal diet throughout and then treated with ethionine. Specifically, the results of hepatic ATP levels in the control and experimental rats at 3, 22, and 29 weeks and after ethionine treatment were, respectively: 0.53 (−88%), 0.45 (−85%); 0.51 (−78%); 1.03 (−51%); and 0.30 (−88%); 0.41 (−81%).

On histological examination, the livers appeared normal in rats fed the basal diet. The livers of rats fed the 2-FAA or the 3′-Me-DAB diet for varying periods appeared similar to those reported in detail in earlier studies (19, 20, 24, 28).
Force-Feeding Studies. For the avoidance of the decreased diet intake due to carcinogens added to the basal diet in the ad libitum feeding experiments and for the determination of how early feedings with diets containing 2-FAA or 3'-Me-DAB would influence the subsequent response to the acute administration of ethionine, force-feeding experiments modeled after an earlier study (21) were undertaken. Rats were force-fed the basal diet for 1 day, and then they were divided into groups. One group (control group) was force-fed the basal diet, and the other groups were force-fed different experimental diets for 3 days. All rats received ethionine i.p. on the following (fourth) morning 4 hr before being killed. The results of these experiments are summarized in Table 2. While the rats force-fed the basal diet responded to the administration of ethionine with a moderate degree of inhibition in in vitro protein synthesis (46.5%) and of polyribosomal disaggregation, the rats force-fed the ethionine diet showed only a minimal response to the ethionine administration (Table 2). These results were similar to those reported earlier (21).

Rats force-fed the 2-FAA, the 3'-Me-DAB, the thioacetamide, or the DMN diet and then treated acutely with ethionine showed only minimal inhibition of hepatic protein synthesis (11.4 to 23.5%) and minimal polyribosomal disaggregation in comparison to the rats fed the basal diet alone (Table 2). Thus the responses were very similar to those observed after feeding the ethionine diet in this (Table 2) and in an earlier study (21). Although the degree of hepatic polyribosomal disaggregation due to ethionine in the 2-FAA and 3'-Me-DAB groups was considered as minimal when compared to the basal untreated groups, the differences were somewhat more marked when each was compared to the comparable untreated 2-FAA or 3'-Me-DAB group, which had more aggregated patterns than did the untreated basal group (Table 2).

Since the administration of phenobarbital is known to lead to the induction of many liver microsomal enzyme systems (4) and since phenobarbital treatment has been demonstrated to influence the carcinogenicity of some chemical carcinogens (12, 14), several experiments were conducted in which phenobarbital was administered along with force-feeding of the basal or the ethionine diet. Sodium phenobarbital (3 mg/100 g body weight) was administered i.p. twice daily (10 a.m. and 3:30 p.m.) during the 3 days of

Table 2
Effect of ethionine administration on in vitro protein synthesis and polyribosome aggregation in livers of rats force-fed experimental diets for 3 days

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Ethionine administration</th>
<th>No. of experiments</th>
<th>Protein synthesis (%) inhibition</th>
<th>Disaggregation of polyribosomes</th>
<th>Monomer-dimers/total ribosomes x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>-</td>
<td>16</td>
<td>0</td>
<td>35.2 ± 1.77h,i</td>
<td>100</td>
</tr>
<tr>
<td>Ethionine</td>
<td>+</td>
<td>16</td>
<td>46.5 ± 4.11h,i</td>
<td>57.9 ± 1.85i</td>
<td>165</td>
</tr>
<tr>
<td>2-FAA</td>
<td>-</td>
<td>12</td>
<td>0.2±</td>
<td>35.3 ± 1.36</td>
<td>100</td>
</tr>
<tr>
<td>2-FAA</td>
<td>+</td>
<td>12</td>
<td>17.4 ± 6.57h,i</td>
<td>41.4 ± 1.57</td>
<td>118</td>
</tr>
<tr>
<td>3'-Me-DAB</td>
<td>-</td>
<td>7</td>
<td>21.7 ± 8.39h,i</td>
<td>41.5 ± 3.19</td>
<td>118</td>
</tr>
<tr>
<td>3'-Me-DAB</td>
<td>+</td>
<td>7</td>
<td>0±</td>
<td>26.6 ± 2.24</td>
<td>76</td>
</tr>
<tr>
<td>Thioacetamide</td>
<td>-</td>
<td>5</td>
<td>25.5 ± 7.09h,i</td>
<td>41.9 ± 4.72</td>
<td>119</td>
</tr>
<tr>
<td>DMN</td>
<td>+</td>
<td>5</td>
<td>0.5</td>
<td>29.8 ± 2.77</td>
<td>85</td>
</tr>
<tr>
<td>DMN</td>
<td>-</td>
<td>6</td>
<td>11.4 ± 3.85h,i</td>
<td>37.1 ± 3.03</td>
<td>105</td>
</tr>
<tr>
<td>DMN</td>
<td>+</td>
<td>8</td>
<td>0.3</td>
<td>36.7 ± 3.55</td>
<td>104</td>
</tr>
<tr>
<td>DMN</td>
<td>+</td>
<td>8</td>
<td>18.4 ± 3.44h,i</td>
<td>47.7 ± 1.55</td>
<td>136</td>
</tr>
</tbody>
</table>

- Rats were force fed the basal diet, the ethionine diet (basal diet containing 0.25% DL-ethionine), the 2-FAA diet (basal + 0.025% 2-FAA), the 3'-Me-DAB diet (basal + 0.06% 3'-Me-DAB), the thioacetamide diet (basal + 0.032% thioacetamide), or the DMN diet (basal + 0.8 mg DMN per 100 g of body weight added to the third feeding each day).
- Rats received DL-ethionine (100 mg/100 g of body weight i.p.) or 0.9% NaCl solution 4 hr before killing. All rats were killed 16 hr after the last evening feeding on the third day.
- In each experiment, livers from 2 to 6 rats of each group were pooled.
- In vitro protein synthesis was assayed with [14C]leucine and microsomes of pooled livers of each group. Supernatants of livers of rats fed the basal diet were used in all assays.
- Sucrose density gradients of deoxycholate-treated postmitochondrial supernatants were prepared.
- In each experiment, ethionine-treated rats were compared with control (basal, 2-FAA, or 3'-Me-DAB diet without ethionine treatment) rats.
- State of aggregation (+, lighter aggregates; −, heavier aggregates) was graded in each experiment ranging from marked (4) to control (basal) (0); values, mean.
- Mean ± S.E.
- p < 0.01, compared with basal (protein synthesis) or each control (polyribosomes), without ethionine treatment, group.
- 0.05 > p > 0.01, compared with basal, ethionine-treated group.
- 0.05 > p > 0.01, compared with basal, ethionine-treated group.
force-feeding of the diets. On the fourth morning the animals of each group were treated with ethionine 4 hr before being killed. The results of 3 experiments revealed that the phenobarbital treatment did not influence the responses to ethionine administration. Rats force-fed the basal diet with or without phenobarbital responded to the ethionine administration with a moderate degree of hepatic polyribosomal disaggregation and decreased in vitro protein synthesis while the rats force-fed the ethionine diet with or without phenobarbital responded to the ethionine administration with a minimal degree of hepatic polyribosomal disaggregation and decreased in vitro protein synthesis, similar to the results in Table 2.

The Effect of Acute Injections of DMN, Thioacetamide, or Aflatoxin B₁ on the Livers of Rats Force-Fed a Purified Basal Diet Containing Selected Carcinogens

On the basis of the preceding results, which indicated that the acute response of hepatic polyribosomes and protein synthesis to ethionine could be altered by each of a variety of hepatocarcinogens tested in long-term as well as in short-term experiments, it became of interest to determine whether rats force-fed a purified diet containing selected carcinogens (ethionine, 2-FAA, 3'-Me-DAB, thioacetamide, or DMN) for 3 days would respond in terms of hepatic polyribosomes and protein synthesis to the acute administration of carcinogens other than ethionine, such as DMN, thioacetamide, or aflatoxin B₁. Table 3 summarizes the results of such experiments. First, it is apparent that rats force-fed the carcinogen-containing diets for 3 days in comparison to rats force-fed the basal diet showed some differences in hepatic protein synthesis in vitro and in hepatic polyribosomal aggregation (Table 3). Hepatic protein synthesis in vitro was significantly increased in rats force-fed diets containing 2-FAA, thioacetamide, or DMN. Greater hepatic polyribosomal aggregation (less polyribosomal disaggregation) was present in rats force-fed diets containing 2-FAA, 3'-Me-DAB, thioacetamide, or 4-FAA.

Rats force-fed the carcinogen-containing diets all showed relatively similar decreases in hepatic protein synthesis in vitro and in hepatic polyribosomal disaggregation after the acute administration of DMN (Table 3). In general, hepatic protein synthesis in vitro was significantly diminished in the groups receiving acute injections of DMN in comparison to basal untreated controls or to carcinogen-containing diets but untreated controls (Table 3). Similarly, hepatic polyribosomal disaggregation was increased in experimental groups in comparison to control groups (Table 3).

The results following acute thioacetamide administration were different than after acute administration of ethionine, DMN, or aflatoxin B₁ (Tables 2 and 3). Thioacetamide administration caused increased hepatic protein synthesis in all groups except the ethionine-fed group.

The results following the acute administration of aflatoxin B₁ are summarized in Table 3. Rats force-fed the basal diet or the ethionine diet for 3 days responded to aflatoxin B₁ with marked decreases in protein synthesis and in polyribosomal aggregation (more polyribosomal disaggregation) more marked in the latter group than in the former group. Rats of the 2-FAA or the 3'-Me-DAB group showed little or
than to that of the group fed the 2-FAA diet.

**DISCUSSION**

The results of this and an earlier study (21) reveal that the chronic feeding of selected hepatocarcinogens (2-FAA, 3'-Me-DAB, or ethionine) in purified diets prevents the acute toxic effect of ethionine on hepatic polyribosomes (disaggregation) and on hepatic protein synthesis (inhibition). A similar resistance to the acute toxic effect of ethionine has been described in intrahepatically transplanted hepatomas (26), in primary hepatocellular carcinomas induced by feeding ethionine or 2-FAA (21), and, based upon a few preliminary observations in our laboratory, in hyperplastic nodules induced by chronic ethionine feeding (5) (where the resistance is greater than in nonhyperplastic nodular areas of the same livers). Others have reported that livers of rats fed hepatocarcinogens showed different properties in response to a variety of agents or conditions (1, 8, 15, 16, 18) and that hepatomas often fail to respond by changes in enzyme activity due to dietary inducing substances or to hormones as occurs in normal liver, a manifestation speculated to represent altered genomic expression in malignant cells (17).

The resistance to the acute toxic effect of ethionine by the livers of rats receiving chronic feedings of selected hepatocarcinogens occurs within 3 to 4 weeks, persists for months (as long as the hepatocarcinogen is in the diet), and is at least partly reversible in that after lengthy exposure and then return to basal diet the liver again becomes vulnerable to the acute toxic response to ethionine (Table 1; Ref. 21). The latter finding must be interpreted with caution since the total livers were studied and since even although the majority of hepatocytes may respond in this manner, the hyperplastic nodules, possible premalignant precursors (5, 6, 7, 33), could probably behave quite differently, as suggested by earlier mentioned preliminary observations. Studies by others (9, 29) also have reported that hyperplastic nodules induced by a variety of hepatocarcinogens become resistant to hepatotoxins or hepatocarcinogens.

The results of this study reveal 2 additional observations that are interesting. First, the acute toxic response to ethionine administration is different in rats fed the basal diet ad libitum than in those force-fed the basal diet. The ad libitum-fed rats showed a more marked disaggregation of polyribosomes and a greater inhibition of in vitro protein synthesis than did the force-fed rats (Tables 1 and 2). This difference may be due to the longer fast (overnight) in the ad libitum-fed rats that normally eat at night than in the force-fed rats that were fed at 6 p.m. and killed the following morning. Indeed, prior nutritional state (fasted versus nonfasted) of rats prior to the administration of a hepatotoxin such as ethionine (21) or actinomycin D (27) has been reported to influence the degree of response of hepatic polyribosomes and protein synthesis. Second, hepatic protein synthesis appears to become enhanced in rats that have been force-fed for 3 days a hepatocarcinogen (ethionine, 2-FAA, 3'-Me-DAB, thioacetamide, or DMN)-containing diet (Table 3). Since these are hepatotoxic agents as well as hepatocarcinogenic agents, it is possible that the early response to low levels of the compounds in the diet is due to minimal cellular injury. Histologically, the livers did not show significant morphological alterations during the 3-day feedings, and there were no evident changes in cell populations. It is possible that the enhanced protein synthesis in the livers may reflect a reactive metabolic response similar to that that occurs with microsomal enzyme induction that acts to metabolize or detoxify low levels of carcinogens. Such effects have been demonstrated with compounds such as methylcholanthrene and others (4).

The acute feeding (3 days force-feeding) of selected hepatocarcinogens (ethionine, 2-FAA, 3'-Me-DAB, thioacetamide, or DMN) diminished the acute toxic effect of ethionine (Table 2; Ref. 21). This suggests that the use of a 3-day force-feeding protocol for acute toxicity studies may provide useful information. The results with this model, measuring the acute toxic effect of ethionine, suggest that a variety of hepatocarcinogens in the diet may cause or induce resistance to develop to acute ethionine toxicity. However, other studies with this model and measuring the acute toxic effects of different agents, DMN, thioacetamide, or aflatoxin B1, produced variable results (Table 3). First, we observed that the acute administration of some hepatocarcinogens, DMN and aflatoxin B1, to rats force-fed the basal diet produced hepatic polyribosomal disaggregation and inhibition of hepatic protein synthesis similar to that seen with ethionine while acute doses of one, thioacetamide, did not. Second, we found that force-feeding the basal diet containing some hepatocarcinogens protected against the hepatic polyribosomal disaggregation and the inhibition of hepatic protein synthesis seen with some acute toxic hepatocarcinogens, while force-feeding the basal diet containing other hepatocarcinogens did not. It is possible that the diversity of responses may be related to the overall metabolism of each carcinogen, which are known to be quite different (34), and to the means by which each agent produces acute toxic injury, which are again considered to be quite different (32). The nature of the resistance developed under certain conditions to the acute toxic action of selected hepatocarcinogens is not known, but it may be related to possibilities such as a reduced capacity for uptake of the toxic agent or from deficiencies in enzyme systems needed for metabolic activation of the toxin (3, 9, 11, 13). Further studies are necessary to elucidate the mechanisms responsible for the differences in responses.

Recently, a number of reports (9, 29) have dealt with the resistance of putative premalignant liver cell populations to acute cytotoxic effects. On the basis of these findings, Solt et al. (29) have postulated sequential steps in the development of liver cancer in experimental animals. Our own studies in which rats were force-fed purified diets containing hepatocarcinogens for 3 days reveal that in response to some carcinogens the liver becomes resistant to certain acute toxic responses relating to hepatic protein synthesis. Whether these responses are due to alterations in selected liver cells or in the total population is not known.
These changes occur very early, possibly before significant morphological alterations develop. Further studies are needed for the determination of whether the effects obtained by this acute experimental approach are in any way related to the subsequent course in carcinogenesis.

REFERENCES

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