Certain Effects of Dietary Fats on the Production of Liver Tumors in Rats Fed p-Dimethylaminoazobenzene*

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Hydrogenated coconut oil and its component fatty acids appear to be the most striking examples of dietary lipid inhibitors reported for the carcinogen p-dimethylaminoazobenzene. When these substances were fed to rats as the sole sources of lipid in synthetic diets containing the azo dye very few liver tumors, if any, appeared by 6 months (3, 4). If, however, a fat such as corn oil was fed instead an incidence of 60 to 80 per cent at 6 months was obtained.

The effects of other fats on the carcinogenicity of p-dimethylaminoazobenzene has been the subject of two recent papers. György and his associates (1) have stated that Crisco and butter fat were co-carcinogenic as compared to lard when these fats were fed at high levels with diets low in protein, but no supporting data were offered. Opie (6) has compared diets containing various levels of Crisco, and has concluded that high levels of fat increase the rate of formation of liver tumors. The dye was fed continuously in these experiments and the animals that lived the longest (those on high fat) consumed more carcinogen than those that died early (those on low fat).

This report deals with the effects of the kind and the level of certain fats in the diet on the carcinogenicity of p-dimethylaminoazobenzene in rats fed a synthetic diet. Corn oil was compared with olive oil at a level of 5 per cent in the diet and corn oil, lard, and Crisco were tested at a level of 20 per cent. The series included also a diet free of added fat.

METHODS

Young albino Sprague-Dawley male rats, 130 to 170 gm. in weight, were fed 0.06 per cent of p-dimethylaminoazobenzene for 120 days. The animals were kept in groups of 7 and 8 in screen-bottomed cages; food and water were given ad libitum. After 120 days the livers were examined by laparotomy, and the animals (except Group 4) were continued on their previous diet without the carcinogen for another 60 days. At this time a final examination of the livers was made.

The diets consisted of purified casein (3) 120 gm.; salts mixture 40 gm.; one of the following quantities of fat: 50 gm. of either corn oil or olive oil, or 200 gm. of corn oil, rendered leaf lard, or Crisco (Primex); and cerelose (glucose monohydrate) to one kilogram. The diets were supplemented with the following levels of crystalline B vitamins per kilogram: 3.0 mgm. thiamin chloride, 2.0 mgm. riboflavin, 2.5 mgm. pyridoxine hydrochloride, 7.0 mgm. calcium pantothenate, and 30.0 mgm. choline chloride. Each rat received also 1 drop of halibut liver oil monthly. The carcinogen was dissolved with heat in the fat, or a solution in acetone was evaporated on the dry diet (Group 1). The rations were mixed in quantities sufficient for 1 to 2 weeks and stored at 0°C.

RESULTS

The data are presented in Table I. The general physical condition of the rats was good throughout the experiment until large tumors became evident. On the fat-free diet (Group 1) symptoms of fatty acid deficiency, consisting of a scaly dermatitis on the paws and tails, developed at about 3½ months; the dermatitis was absent in the other groups. The consumption of food, and hence of carcinogen, by this group was greater than for any other group, averaging 11 gm. per rat per day during the first 4 months. Only 2 rats in the low-fat group died before 4 months; 3 others developed hepatomas by 6 months, and 10 survived for this time without developing visible tumors. Gross cirrhosis at 4 months was absent or very mild. The final incidence of tumors found for this group, viz., 23 per cent, was comparable to a previous experience with this diet (2).
The inclusion of fat in the diet is shown by the other groups in the series. When 5 per cent of corn oil was added to the diet (Group 2) the incidence of tumors was 74 per cent, and increased to 94 per cent by 6 months. The figure is somewhat higher than our previous experience with this diet (3, 4, 5) would have led us to predict; in general an incidence of from 60 to 80 per cent at 6 months has been obtained in 8 previous trials. The substitution of olive oil for corn oil at a level of 5 per cent caused a substantial decrease in the incidence of hepatomas; to 13 per cent at 4 months and 53 per cent at 6 months (Group 3). Further, gross cirrhosis at 4 months was less severe on the olive oil diet, despite the fact that these 2 groups of rats consumed similar amounts of food; i.e., 9.7 gm. per rat per day.

When corn oil was fed at a level of 20 per cent a noticeable increase in toxicity and carcinogenicity occurred (Group 4). The higher level of this fat of the effect of the diet high in corn oil. The early palpation of tumors, the incidence of 100 per cent at 4 months, and the severe liver damage encountered in rats fed this diet leave no doubt as to the stimulatory action of corn oil under the conditions of these experiments. Even at a level of 5 per cent, corn oil was more stimulatory than the same level of olive oil; this comparison agrees with our previous data for these two fats (2).

The data fail to show any properties of Crisco, as compared to lard, that favor carcinogenesis, or any inhibitory effects of lard when compared to Crisco. The level of 20 per cent at which these fats were fed is close to the level of 23 per cent employed by György and his associates (1), although these workers fed the fats in a diet containing only 6 per cent of casein. Under our conditions the high levels of lard and Crisco gave results similar to those usually obtained with 5 per cent of corn oil.

### Table I: The Effects of the Kind and the Level of Certain Fats on the Carcinogenicity of p-Dimethylaminoazobenzene

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Average starting weight, gm.</th>
<th>Average weight at 4 mos., gm.</th>
<th>Average food consumption, gm./rat/day</th>
<th>Survival * at 4 mos.</th>
<th>Hepatomas † at 4 mos.</th>
<th>Negative survivors at 6 mos.</th>
<th>Gross cirrhosis at 4 mos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low-fat</td>
<td>162</td>
<td>221</td>
<td>11.0</td>
<td>13/15</td>
<td>1/13</td>
<td>3/13</td>
<td>none</td>
</tr>
<tr>
<td>2</td>
<td>5% Corn oil</td>
<td>161</td>
<td>213</td>
<td>9.7</td>
<td>15/15</td>
<td>11/15</td>
<td>14/15</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>5% Olive oil</td>
<td>153</td>
<td>236</td>
<td>9.7</td>
<td>15/15</td>
<td>2/15</td>
<td>8/15</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>20% Corn oil</td>
<td>162</td>
<td>176</td>
<td>7.3</td>
<td>15/15</td>
<td>15/15</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>20% Crisco</td>
<td>151</td>
<td>200</td>
<td>8.4</td>
<td>15/15</td>
<td>7/15</td>
<td>13/15</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>20% Lard</td>
<td>156</td>
<td>212</td>
<td>7.8</td>
<td>15/15</td>
<td>9/15</td>
<td>13/15</td>
<td>2</td>
</tr>
</tbody>
</table>

* Survival = Number living over number at start.
† Hepatomas = Number with hepatomas over number surviving at 4 months.

Opie (6) has studied the carcinogenicity of p-dimethylaminoazobenzene in diets containing either no fat of 23.8, 36.5, or 68.2 per cent of Crisco. While the amount of casein in these diets differed from one another the addition of Crisco greatly increased the ultimate carcinogenic response to the azo dye. Although the rats fed the high-fat diets lived longer and consumed more carcinogen than those given the low-fat diet, the effect of added fat is nevertheless evident in the data presented by Opie. In the present experiments the dye was fed to all groups for 4 months, and the only variation in this respect was in the different amounts of food consumed by each group. The group fed the diet low in fat received the greatest quantity of dye, yet this diet was the least toxic. The addition of only 5 per cent of corn oil caused a nearly tenfold increase in tumors at the end of the period of dye feeding, while the ingestion of 20 per cent of this fat resulted in a tumor incidence of 100 per cent at this time. Furthermore, the latter group consumed the least amount of dye.

In this paper and in previous communications we...
have presented data on the effects of corn oil, olive oil, coconut oil, hydrogenated coconut oil, Crisco, and lard on the carcinogenicity of \( p \)-dimethylaminoazobenzene in a synthetic diet. In all these experiments rats of the same strain, sex, and age were used, and the same synthetic diet was fed throughout. It is evident that the kind as well as the level of fat in the diet is important in determining the response obtained with this agent.

**SUMMARY**

Six groups of 15 rats each were fed 0.06 per cent \( p \)-dimethylaminoazobenzene for 4 months in synthetic diets containing: no fat, 5 per cent of corn oil or olive oil, or 20 per cent of corn oil, Crisco, or lard. The dye-free diets were then fed for 2 more months. A final incidence of liver tumors of 23 per cent was produced by the low-fat diet, as compared to 94 and 53 per cent respectively when the diet contained 5 per cent of corn oil or olive oil. When 20 per cent of corn oil was fed large tumors were palpated before 4 months, and the incidence of tumors at this time was 100 per cent. When 20 per cent of Crisco or lard was given, the incidence of tumors at 6 months was 87 per cent. These 2 groups were most comparable to the group fed 5 per cent of corn oil.

The data demonstrate that in general \( p \)-dimethylaminoazobenzene is more carcinogenic when the diet contains fat, and further show that the incidence of hepatomas is higher in rats given a diet containing 20 per cent of corn oil instead of 5 per cent. It is therefore apparent that the level of fat in the diet, as well as the type of fat, is important for the formation of hepatic tumors by \( p \)-dimethylaminoazobenzene.

**REFERENCES**

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