The Effect of Added Dietary Tryptophane on the Occurrence of 2-Acetylaminofluorene-induced Liver and Bladder Cancer in Rats

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Recent reports by Bielschowsky (1), Harris (6), Engel and Copeland (5), Strombeck and Ekman (10), and Morris et al. (7), indicate that the genetic constitution of the rat plays a more dominant role than diet in the initiation of 2-acetylaminofluorene-induced neoplasms. While liver tumors occur in a majority of the rats and the frequency is altered somewhat by the protein content of the diet, the mechanism of tumor formation differs from that of dimethylaminoazobenzene carcinogenesis, in that liver extract and riboflavin exert no protective effect against the action of this drug. 2-Acetylaminofluorene-induced mammary gland and bladder neoplasms appear to be peculiarly strain limited.

In a preliminary survey of five strains of rats, Dunning, Curtis, and Madsen (2) showed that 2-acetylaminofluorene-induced bladder tumors were confined to the Copenhagen and AXC strains and that tumors of the mammary gland were relatively infrequent, with only two observed in rats of the Fischer strain and one each in rats of the August and AXC strains. Tumors of the liver occurred in rats of all five strains but were less frequent in rats of the Copenhagen strain. A later study (4) of the occurrence of diethylstilbestrol-induced tumors in rats of three of these strains showed that mammary tumors were most frequent in the AXC rats, occurred with low frequency in Fischer rats, and failed to occur in rats of the Copenhagen strain, while the induced bladder cancers were most frequent in the Copenhagen rats, occurred with low frequency in rats of the AXC strain, and failed to occur in Fischer rats.

Rats of the Fischer strain were chosen for the present study because of their relatively high incidence of liver tumors and a low incidence of mammary tumors which might serve as comparative material for the recently reported study (3) of diethylstilbestrol-induced mammary cancer in AXC rats on similar diets. This report showed that the addition of 1.4 per cent DL-tryptophane to a synthetic diet containing 25 per cent tryptophane-free casein hydrolysate increased the number and percentage of induced cancers, and significantly reduced the average latent period below that observed for the control group on 26 per cent casein. The substitution of 4 per cent dietary tryptophane for tryptophane-free casein hydrolysate, however, produced the reverse effect—a decreased number and percentage of induced cancers and a significantly prolonged latent period.

MATERIALS AND METHODS

Pedigreed female rats of Fischer Line 344 4-5 months of age were used for these experiments. Each rat was housed in an individual cage with free access to water. The daily portion of 7 gm. of food was weighed out and presented in a food cup, to which the daily supplement of crystalline vitamins was added. Attempts were made to recover, weigh, and record all food that was spilled.

The four diets differed only in quantity and composition of the protein, as shown in Table 1. The following crystalline vitamins1 per kilo of diet were fed as a supplement: thiamin, 4 mg.; riboflavin, 8 mg.; pyridoxine HCl, 4 mg.; niacin, 4 mg.; calcium pantothenate, 20 mg.; choline chloride, 2,000 mg.; and alpha-tocopherol, 150 mg. Each gram of diet was equivalent to 4.7 calories, and a daily portion of 33 calories was fed.

Each rat was weighed and inspected for tumors once a week. At death, post mortem examination included description of every visible tumor, gross sectioning of lungs and mammary glands, and the inspection and weight of the liver, kidneys, adrenals, pituitary, and sex glands. Representative

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1 Supplied through the courtesy of Dr. R. C. Pogge of Merck & Co., Rahway, N.J.
sections of each of these tissues and organs were preserved and examined microscopically.

RESULTS

The number of rats in each group, their average initial body weight, daily food consumption, and average daily and total dose of 2-acetylaminofluorene, is shown in Table 2. The twelve control rats received Friskie Dog Pellets ad libitum. The amount consumed was not recorded. Each group on the four synthetic diets averaged somewhat less than 7 gm. or 33 calories, which were fed to every rat daily.

The rats on diet No. 8, with the highest per cent of added tryptophane, most nearly met our experimental plan by the average consumption of 6.5 gm. of diet, or 51 calories containing 3.9 mg. of 2-acetylaminofluorene. The presence of as much as 0.06 per cent 2-acetylaminofluorene made the diet sufficiently unpalatable that the rats on all of these diets consumed less than the AXC rats (3) on similar diets without the drug. The rats on diet No. 9 with 26 per cent casein consumed an average of 5.9 gm., and those on diet No. 4 with 1.4 per cent tryptophane averaged only 5.8 gm. in daily consumption.

The post mortem weights for the rats which survived the various dietary regimens for at least 100 days are shown in Table 3. The control rats grew progressively, and the rats on diets Nos. 6 and 9 with 45 and 26 per cent, respectively, of casein, maintained their weight or gained slowly. Both groups, however, with the added tryptophane showed evidence of amino acid imbalance or caloric restriction. The weight loss was greater in the rats on the 4.3 per cent tryptophane diet.

The number of rats in each group, their average initial body weight, daily food consumption, and dose of 2-acetylaminofluorene

TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Body weight (gm.)</th>
<th>Daily ration (calories)</th>
<th>Average daily dose (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>130</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diet No. 6</td>
<td>13</td>
<td>150</td>
<td>5.9</td>
<td>27</td>
</tr>
<tr>
<td>Diet No. 8</td>
<td>12</td>
<td>130</td>
<td>5.8</td>
<td>27</td>
</tr>
<tr>
<td>Diet No. 9</td>
<td>13</td>
<td>150</td>
<td>6.5</td>
<td>31</td>
</tr>
<tr>
<td>Diet No. 4</td>
<td>12</td>
<td>144</td>
<td>6.1</td>
<td>29</td>
</tr>
</tbody>
</table>

The post mortem weights for the rats which survived the various dietary regimens for at least 100 days are shown in Table 3. The control rats grew progressively, and the rats on diets Nos. 6 and 9 with 45 and 26 per cent, respectively, of casein, maintained their weight or gained slowly. Both groups, however, with the added tryptophane showed evidence of amino acid imbalance or caloric restriction. The weight loss was greater in the rats on the 4.3 per cent tryptophane diet.

In all the 2-acetylaminofluorene-fed rats the livers, including the induced tumors, were relatively large and the uteri small and atrophic. Otherwise, there appeared to be no significant changes in the organs that were weighed.

The post mortem weights for the rats which survived the various dietary regimens for at least 100 days are shown in Table 3. The control rats grew progressively, and the rats on diets Nos. 6 and 9 with 45 and 26 per cent, respectively, of casein, maintained their weight or gained slowly. Both groups, however, with the added tryptophane showed evidence of amino acid imbalance or caloric restriction. The weight loss was greater in the rats on the 4.3 per cent tryptophane diet.

They averaged 150 gm. at the start of the experiment and progressively decreased to an average of only 86 gm., while those on the 1.4 per cent tryptophane diet averaged 130 gm. at the start of the experiment and 106 gm. at death.
tases were demonstrable in seven of these. One of these tumors has been successfully transplanted and is now in the seventeenth generation. Figures 5 and 6 are from the tenth transplanted generation. This tumor regularly metastasizes to the lungs from the subcutaneous growths and kills the host in an average of 60 days. Hepatoma No. 6, shown in Figure 7, has also been successfully transplanted subcutaneously. It is now in the 21st transplanted generation, and, although it shows more mitosis and less cellular differentiation, no metastases have been observed from the subcutaneous growths. In addition two rats on diet No. 4 developed squamous carcinoma of the external auditory meatus, three had what were considered benign hepatomas, and all eleven developed carcinoma of the bladder. Figure 10 shows a section of the first transplanted generation of one of these tumors. The majority of the tumors were papillary carcinomas, as shown in Figure 9, but areas of squamous carcinoma were not uncommon.

The rats on diet No. 8 with 4.3 per cent of

TABLE 3
THE NUMBER OF RATS WHICH SURVIVED FOR AT LEAST 100 DAYS, THEIR AVERAGE POST MORTEM BODY WEIGHTS IN GRAMS, AND THE PERCENTAGE WEIGHTS OF SOME OF THE ORGANS

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Average body wt.</th>
<th>Organ weights in per cent of body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>174</td>
<td>4.8</td>
</tr>
<tr>
<td>Diet No. 6</td>
<td>12</td>
<td>158</td>
<td>19.3</td>
</tr>
<tr>
<td>Diet No. 4</td>
<td>11</td>
<td>106</td>
<td>16.4</td>
</tr>
<tr>
<td>Diet No. 8</td>
<td>12</td>
<td>80</td>
<td>15.7</td>
</tr>
<tr>
<td>Diet No. 9</td>
<td>16</td>
<td>156</td>
<td>11.7</td>
</tr>
</tbody>
</table>

TABLE 4
THE AVERAGE SURVIVAL IN DAYS, THE NUMBER AND PERCENTAGE OF RATS IN EACH GROUP WITH LIVER, BLADDER, AND EAR CANCERS, AND THE NUMBER AND PERCENTAGE WITH LUNG METASTASES OF THE LIVER CANCERS

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Average survival</th>
<th>Liver ca. (No.) (per cent)</th>
<th>Lung met. (No.) (per cent)</th>
<th>Bladder ca. (No.) (per cent)</th>
<th>Ear ca. (No.) (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>406</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diet No. 6</td>
<td>12</td>
<td>385</td>
<td>11</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diet No. 4</td>
<td>11</td>
<td>400</td>
<td>8</td>
<td>78</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diet No. 8</td>
<td>12</td>
<td>320</td>
<td>9</td>
<td>75</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Diet No. 9</td>
<td>16</td>
<td>283</td>
<td>6</td>
<td>37</td>
<td>3</td>
<td>50</td>
</tr>
</tbody>
</table>
FIG. 3.—Hepatoma No. 9 from rat maintained 353 days on 45 per cent casein diet. X 280.

FIG. 4.—Lung metastasis of hepatoma No. 9. X 280.

FIG. 5.—Tenth transplanted generation of hepatoma No. 18 induced in rat maintained 460 days on 1.4 per cent tryptophane diet. X 280.

FIG. 6.—Lymph node metastasis from hepatoma No. 18. X 280.
FIG. 7.—Hepatoma No. 6 from rat maintained 326 days on 45 per cent casein diet. X 280.

FIG. 8.—Omental metastasis of hepatoma No. 6. X 280.

FIG. 9.—Papillary carcinoma No. 12 of bladder from rat maintained 376 days on 4.3 per cent tryptophane diet.

FIG. 10.—First transplanted generation of bladder carcinoma No. 19 induced in rat maintained 460 days on 1.4 per cent tryptophane diet. X 280.
added dietary tryptophane developed liver cancer in about the same proportion as rats of the former group. That is, nine of the twelve had malignant hepatomas, with demonstrable lung metastases in seven, and two had probably benign hepatomas. Eleven of these rats also developed bladder cancer and in a relatively shorter interval than those of the former group. The average survival period of these rats was 920 days, compared with 400 days for those on diet No. 4 with 1.4 per cent added tryptophane.

The increased proportion of dietary tryptophane, or the imbalance caused thereby, may be a real factor in the initiation of these bladder cancers. No spontaneous bladder cancers have been observed in rats of this inbred line, and no bladder cancers were initiated by the diethylstilbestrol technic that successfully initiated bladder cancer in rats of the Copenhagen strain. No bladder cancers have been observed in previous or subsequent series of rats of this line, on diets in which the protein source has been casein or whole wheat flour and whole milk powder (6). Furthermore, the low body weights of these rats should have exerted a protective effect, if the theory of Tannenbaum and Silverstone (8, 11) is tenable. Their caloric intake was approximately equal to that of the other groups of rats, but the absorption or utilization must have been disturbed. The experience of Strombeck (9) with azotoluene-induced hyperplasia of the bladder mucosa in rats in which a portion of bladder mucosa had been transplanted to the liver would suggest that the changes observed in the bladder result from direct contact with the chemical in the urine. In Strombeck’s experiment, sixteen rats with established grafts of bladder mucosa in the liver showed only normal mucosa in the transplant and papillomatous or hyperkeratotic changes in the mucosa of the bladder in situ. In the present experiment, combined elimination products of 2-acetylamino-fluorene and tryptophane may be a real factor in the initiation of these bladder cancers, and diet appears to exert a more predominant role than hereditary constitution.

SUMMARY
1. Sixty-five female rats of the Fischer Line 344 were placed on isocaloric synthetic diets containing 0.06 per cent 2-acetylamino-fluorene.
2. The rats were divided into four groups receiving, respectively, 45 per cent casein, 26 per cent casein, 25 per cent tryptophane-free casein hydrolysate plus 1.4 per cent DL-tryptophane, and 22 per cent tryptophane-free casein hydrolysate, plus 4.3 per cent DL-tryptophane.
3. Fifty-one rats survived for at least 100 days on the various dietary regimens and consumed a daily average of nearly 4 mg. of 2-acetylamino-fluorene.
4. The rats on the 45 and 26 per cent casein diets maintained their body weight, while those on the 1.4 per cent and 4.3 per cent tryptophane diets lost, respectively, 20 and 40 per cent.
5. Benign and malignant hepatomas were observed in the majority of rats in each group. The per cent of malignant hepatomas varied from 37 in the rats on the 26 per cent casein diet to 92 in the rats on the 45 per cent casein diet and was, respectively, 73 and 75 per cent in the rats on the 1.4 and 4.3 per cent tryptophane diets.
6. Squamous carcinoma of the external auditory meatus occurred in 20 per cent of the rats on the 26 per cent casein diet and 1.4 per cent tryptophane diet.
7. Bladder cancer occurred in 100 per cent of the rats on the 1.4 per cent tryptophane diet and in 92 per cent of the rats on the 4.3 per cent tryptophane diet. No bladder cancers were observed in the rats on the 45 per cent or 26 per cent casein diets.

REFERENCES
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