The Effect of 2-Acetylaminofluorene on Growth and Composition of the Liver of the Rat*

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In the first study of the toxicity and carcinogenic activity of 2-acetylaminofluorene (AAF), Wilson, DeEds, and Cox noted that the compound caused a profound inhibition of growth, when it was fed in a 10 per cent casein diet to albino rats (21). Inhibition of the growth of the rat has also been obtained with a number of carcinogenic aromatic hydrocarbons and with p-dimethylaminoazobenzene (11, 19, 20), and the growth-retarding ability of carcinogenic compounds has been considered a specific property of carcinogens inasmuch as structurally related noncarcinogenic compounds do not appear to possess this property (10). It is conceivable, therefore, that carcinogenic amines in producing growth inhibition cause the same or a similar metabolic disturbance as carcinogenic aromatic hydrocarbons and p-dimethylaminoazobenzene.

Inasmuch as increasing the level of dietary protein has been shown to protect against growth inhibition by 1,2,5,6-dibenzanthracene (DBA) (7), there is some reason to believe that carcinogenic aromatic hydrocarbons cause a disturbance of protein metabolism. Whether the carcinogen interferes with protein metabolism in general, or with the metabolism of specific amino acids only, is an unsettled problem. In the case of DBA, addition of cystine or methionine to a 5 per cent casein diet did not prevent the growth inhibition, indicating that DBA did not interfere with the metabolism of the sulfur amino acids (5). In contrast, either cystine or methionine was effective in relieving the growth inhibition induced by p-dimethylaminoazobenzene in rats maintained on a 6 per cent casein diet (19). In the case of 2-aminofluorene, recent work indicates that the carcinogen decreased the in vitro incorporation of methionine into rat liver protein (18). This suggests that carcinogenic amines may cause a depletion of sulfur amino acids in rat liver protein.

The present study was undertaken to show whether the growth inhibition induced in the rat by a tumor-producing concentration of AAF (16) is influenced by the level of dietary protein. In addition, the livers of pair-fed rats were analyzed to determine any changes in the nitrogen and sulfur content following prolonged intake of the carcinogen.

MATERIALS AND METHODS

Animals and diets.—Female rats, which have been reported to be more resistant to the toxic action of acetylaminofluorene than male rats (21), were used in the experiments. The animals of the Sprague-Dawley strain1 had an average weight of 40 gm. and were 20–22 days old at the start of the experiment. The rats were caged individually in a room maintained at 26.5° C. and allowed water ad libitum. Food intake and body weight were recorded daily. The feeding jars were placed into tin cans (diameter, 8.4 cm.; height, 4.4 cm.) which were mounted in the center of 8-inch tin pie plates. This eliminated errors in the measurement of the food intake due to excessive spillage in the groups which consumed the carcinogen. Two basal diets, designated Diets A and B, were fed. The composition of Diet A was as follows: casein, 11; white corn dextrin, 66; sucrose, 16; salt mixture 12 (12), 4; corn oil, 2; cod liver oil, 0.05; inositol, 0.1; choline chloride, 0.2; liver concentrate (Wilson 1:20), 0.4. Diet B differed from Diet A only by containing 20 per cent casein and 57 per cent dextrin. Vitamins were added in mg/kg of diet as follows: thiamine hydrochloride, 5; riboflavin, 10; pyridoxine hydrochloride, 5; nicotinic acid, 5; calcium d-pantothenate, 25; p-aminobenzoic acid, 300; 2-methyl-1,4-naphthoquinone, 2; α-tocopherol, 210. Vitamin A was added to give 17,800 U.S.P. units/kg of food. In experiments 5 and 6 the rats were also given 2 drops of Navitol with Viosterol (Squibb) once weekly by mouth. 2-Acetylaminofluorene, m.p. 196°-198° C. (500 mg/kg of basal diet), was added in an acetone solution to the basal diets. The acetone was removed from the frequently stirred diets by a current of air. The control diets were treated with acetone alone in the same manner. The diets were stored in a cold room at 4° C.

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Three different types of growth experiments were performed which are summarized in Table 1. In experiments 1 and 2 the rats were allowed food ad libitum. In experiments 3 and 4 pair-fed litter-mates were used, and the control rats were allowed as much of the basal diet as was consumed by their litter-mates which received the basal ration plus AAF. In experiments 5 and 6 groups of four litter-mates were used, and the food intake of all litter-mates in each group was restricted to that of the rat which received the 11 per cent casein ration plus AAF. This feeding method is referred to in Table 1 as double-paired.

Preparation of tissues and analytical methods.—At the termination of the growth experiments the rats were killed by neck

stroke, the abdominal cavity and the chest were opened, and the animals were examined for the presence of grossly visible tumors. No tumors were found. The livers were removed, the abdominal cavity and the chest were opened, and the food intake of the rats which received the 11 per cent casein diet was determined by weighing the dry residue remaining after distillation of the ether. The dry tissues were ground to fine powders in a mortar, and the powders were extracted with diethyl ether in a Soxhlet apparatus for 24 hours. Total fat was determined on 5–6-mg aliquots of the fat-free liver powders by the micro-Kjeldahl procedure (15). Total sulfur was estimated gravimetrically as barium sulfate after oxidation of a suitable aliquot of the fat-free liver powders in a Parr bomb (2). The nitrogen and sulfur analyses were carried out in duplicate. In order to compare data obtained on rats of different weights, the analytical results, on a dry weight basis, were calculated and expressed as per cent of body weight. In all cases where the standard error was calculated, the statistical formulas applicable to small numbers of samples were used (1).

RESULTS

The effect of 0.05 per cent acetylaminofluorene incorporated into 11 and 20 per cent casein diets on the growth of the rat is shown in Table 1. When the rats were fed ad libitum, the addition of AAF to the 11 per cent casein diet produced a growth inhibition of 61 per cent. The growth inhibition was only 38 per cent when AAF was added to a 20 per cent casein ration. The extent of the growth retardation agrees with data of Wilson, DeEds, and Cox (21), who observed a 55 per cent growth inhibition when a 10 per cent casein diet containing 0.063 per cent AAF was fed ad libitum to female albino rats. Although in the above experi-

TABLE 1

THE EFFECT OF 2-ACETYLMAMINOFLUORENE ON THE GROWTH OF RATS ON 11 PER CENT AND 20 PER CENT CASEIN DIETS

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Diet</th>
<th>No.</th>
<th>Feeding method</th>
<th>Casein level</th>
<th>Av. food intake/initial wt.</th>
<th>Av. food intake/final wt.</th>
<th>Growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1C</td>
<td>Control</td>
<td>7</td>
<td>Ad libitum</td>
<td>11</td>
<td>12.7 ± 1.7</td>
<td>45.2 ± 1.7</td>
<td>105.4 ± 6.9</td>
</tr>
<tr>
<td>1E</td>
<td>AAF</td>
<td>7</td>
<td>Ad libitum</td>
<td>11</td>
<td>6.1 ± 1.4</td>
<td>42.7 ± 1.5</td>
<td>100.9 ± 4.6</td>
</tr>
<tr>
<td>2C</td>
<td>Control</td>
<td>7</td>
<td>Paired</td>
<td>20</td>
<td>18.0 ± 0.2</td>
<td>40.3 ± 0.9</td>
<td>37.6</td>
</tr>
<tr>
<td>2E</td>
<td>AAF</td>
<td>7</td>
<td>Paired</td>
<td>20</td>
<td>7.5 ± 0.9</td>
<td>40.3 ± 0.9</td>
<td>37.6</td>
</tr>
<tr>
<td>3C</td>
<td>Control</td>
<td>8</td>
<td>Paired</td>
<td>11</td>
<td>5.7 ± 0.9</td>
<td>40.5 ± 0.9</td>
<td>10.8 ± 0.6</td>
</tr>
<tr>
<td>3E</td>
<td>AAF</td>
<td>8</td>
<td>Paired</td>
<td>11</td>
<td>5.7 ± 0.9</td>
<td>42.7 ± 0.8</td>
<td>84.4 ± 2.4</td>
</tr>
<tr>
<td>4C</td>
<td>Control</td>
<td>8</td>
<td>Paired</td>
<td>20</td>
<td>7.5 ± 1.0</td>
<td>47.6 ± 1.0</td>
<td>38.5 ± 0.6</td>
</tr>
<tr>
<td>4E</td>
<td>AAF</td>
<td>8</td>
<td>Paired</td>
<td>20</td>
<td>7.5 ± 0.5</td>
<td>43.0 ± 0.5</td>
<td>35.2 ± 0.5</td>
</tr>
<tr>
<td>5C</td>
<td>Control</td>
<td>7</td>
<td>Double-paired</td>
<td>11</td>
<td>5.3 ± 0.8</td>
<td>35.1 ± 0.8</td>
<td>104.5 ± 3.8</td>
</tr>
<tr>
<td>5E</td>
<td>AAF</td>
<td>7</td>
<td>Double-paired</td>
<td>11</td>
<td>5.3 ± 0.7</td>
<td>35.2 ± 0.7</td>
<td>93.2 ± 5.6</td>
</tr>
<tr>
<td>6C</td>
<td>Control</td>
<td>5</td>
<td>Paired</td>
<td>20</td>
<td>5.5 ± 0.2</td>
<td>35.1 ± 1.1</td>
<td>121.9 ± 7.7</td>
</tr>
<tr>
<td>6E</td>
<td>AAF</td>
<td>5</td>
<td>Paired</td>
<td>20</td>
<td>5.5 ± 0.3</td>
<td>35.9 ± 1.3</td>
<td>122.6 ± 4.4</td>
</tr>
</tbody>
</table>

* Experiments 1 and 2 were carried out for 75 days, experiments 3 and 4 for 82 days, and experiments 5 and 6 for 69 days.
† Average weight ± standard error of the mean.
‡ Growth inhibition = Weight gain of control rats — weight gain of treated rats × 100.
§ See text.
|| Two rats died accidentally before termination of the experiment.
** This average is slightly higher than 0.5 gm/day, since the two rats which had died prior to the termination of the experiment belonged to groups of litter-mates which consumed only 8 gm/day.

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parable food and carcinogen intake on both diets was insured by the feeding method described above. Under these conditions, AAF retarded the growth of rats on the 11 per cent casein ration to the extent of 17 per cent. As in experiment 4, there was no growth inhibition on the 20 per cent casein ration. These experiments demonstrate conclusively, therefore, that the level of dietary protein determines whether AAF acts as a growth inhibitor for the rat. It will also be seen that the and heterocyclic carcinogenic compounds which were not aromatic amines (4, 6, 13). Enlargement of the liver despite subnormal body growth appears to be a common effect of a relatively large variety of carcinogens not closely related in structure.

The effect of AAF on liver fat, nitrogen, and sulfur is shown in Table 3. AAF had no effect on the percentage of fat, whereas the percentages of nitrogen and sulfur were decreased on both diets to approximately the same degree. However, the total quantities of fat, nitrogen, and sulfur, when expressed in per cent of body weight, were found to be significantly greater in the livers of rats which were fed AAF. The constant sulfur to nitrogen ratios in all groups indicate that there was no depletion in vivo of the sulfur amino acids due to AAF. The last column of Table 3 shows that part of the liver dry weight which was not accounted for by either protein or fat. It appears that this fraction, which undoubtedly consists of carbohydrate, was markedly increased (four to tenfold) in livers of rats consuming AAF. This suggests that AAF may stimulate deposition of liver glycogen. This point is being further investigated. Table 4 shows that the increase in liver size for a given AAF concentration in the diet is greater on a 20

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Diet†</th>
<th>Total fat (per cent)</th>
<th>Nitrogen (per cent)</th>
<th>Sulfur (per cent)</th>
<th>Total fat/100 gm BWT (Mg.)</th>
<th>Total nitrogen/100 gm BWT (Mg.)</th>
<th>Total sulfur/100 gm BWT (Mg.)</th>
<th>Residue§/100 gm BWT (Mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3C</td>
<td>Control</td>
<td>12.6±0.37</td>
<td>14.8±0.05</td>
<td>0.94±0.08</td>
<td>104±4</td>
<td>109±5</td>
<td>6.9±0.5</td>
<td>0.06±3</td>
</tr>
<tr>
<td>3E</td>
<td>AAF</td>
<td>13.4±0.64</td>
<td>12.2±0.05</td>
<td>0.75±0.01</td>
<td>151±6</td>
<td>120±2</td>
<td>7.4±0.8</td>
<td>0.06±2</td>
</tr>
<tr>
<td>4C</td>
<td>Control</td>
<td>15.1±0.78</td>
<td>15.5±0.08</td>
<td>1.10±0.08</td>
<td>95.2±4</td>
<td>103±3</td>
<td>7.5±0.8</td>
<td>0.07±3</td>
</tr>
<tr>
<td>4E</td>
<td>AAF</td>
<td>12.5±0.44</td>
<td>12.6±0.06</td>
<td>0.83±0.01</td>
<td>188±9</td>
<td>142±6</td>
<td>9.8±0.8</td>
<td>0.069±3</td>
</tr>
</tbody>
</table>

* All data are arithmetic means ± standard error of the mean.
† AAF was added to the basal diets to give a concentration of 0.05 per cent.
§ Mg. residue = Mg. dry weight — (mg. total nitrogen x 6.25 + mg. total fat).
per cent casein ration than on an 11 per cent casein diet. In addition, it will be seen that on the high casein ration protein contributed to the liver enlargement to a greater extent than fat or carbohydrate. As had been noted in the growth experiments, utilization of dietary protein in the presence of AAF was, therefore, better on a 20 per cent than on an 11 per cent casein ration.

DISCUSSION

The present experiments allow the conclusion that the action of AAF as a growth inhibitor for the rat depends on the level of dietary protein. However, even on a low protein diet AAF is not so powerful in retarding growth as might appear from earlier work (21). The present study shows that control of food intake is essential for the evaluation of a carcinogen as a growth inhibitor.

TABLE 4

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Diet</th>
<th>( \Delta ) dry weight/100 gm body weight</th>
<th>( \Delta ) total fat/100 gm body weight</th>
<th>( \Delta ) total protein/100 gm body weight</th>
<th>( \Delta ) residue/100 gm body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>11 per cent casein</td>
<td>280 ± 25</td>
<td>47 ± 7</td>
<td>70 ± 22</td>
<td>107 ± 22</td>
</tr>
<tr>
<td>4</td>
<td>20 per cent casein</td>
<td>600 ± 51</td>
<td>95 ± 10</td>
<td>244 ± 39</td>
<td>228 ± 26</td>
</tr>
</tbody>
</table>

* All values are differences of arithmetic means ± standard error of the mean.

The data of Elson and Warren obtained with DBA (7) and the present experiments with AAF demonstrate that both carcinogens, when administered by routes and in quantities which will produce tumors, exert a growth retardation which is manifest only on diets which are relatively low in protein. It appears likely from these observations that carcinogenic polycyclic hydrocarbons and carcinogenic amines cause disturbances of growth by similar mechanisms, and the available evidence indicates that both types of carcinogens interfere in some way with protein metabolism.

It might be inferred from the work of Wase, Allison, and Migliarese (18) that AAF has a specific effect on the metabolism of methionine and thus retards growth. However, if AAF interfered only with the metabolism of methionine, one would hardly expect that a 20 per cent casein diet, which supplies just enough methionine for growth (3, 17), would be effective in protecting against the growth-inhibiting action of AAF. Further experiments are necessary to determine whether the action of AAF on growth involves specifically the metabolism of methionine.

Although AAF has no effect on growth on a 20 per cent casein ration, the incidence of mammary, per cent but not on the 20 per cent casein ration. 2. AAF caused enlargement of the liver on both diets. Determinations of the fat, nitrogen, and sulfur content of the liver indicated that the composition of the livers of rats which had consumed AAF differed significantly from the composition of the livers of normal rats. There was no evidence for a sulfur depletion of the livers of rats consuming AAF.

The significance of the results is discussed and compared to similar data obtained with DBA.

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The Effect of 2-Acetylaminofluorene on Growth and Composition of the Liver of the Rat

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