INTRODUCTION

In 1948 it was reported from this laboratory (4) that the feeding of \(\alpha\)-acetylaminofluorene to weanling female rats on a semi-synthetic diet produced a 100 per cent incidence of mammary tumors—a much higher incidence than had been reported by other investigators. Following these observations, studies were initiated to determine whether or not modification of the diet would influence the tumor-inducing action of this chemical. It has already been reported that varying the riboflavin content of the diet had no influence on mammary tumor induction and that the inclusion in the diet of a synthetic detergent, or of teropterin, slightly enhanced the rate of tumor formation (5). More recently, results were obtained indicating that high levels of dietary protein were protective against the induction of mammary, ear-duct, and liver tumors by \(\alpha\)-acetylaminofluorene. The omission of fat from the diet markedly inhibited mammary tumor induction but promoted the induction of tumors of the eye (6,7,8). It is the purpose of this report to describe the eye tumor, the dietary conditions that promote its induction, and to present data that emphasize the importance of growth rate for the induction of mammary tumors by \(\alpha\)-acetylaminofluorene.

MATERIALS AND METHODS

Weanling female rats of the AES strain were used; they weighed from 35 to 65 gm. and varied from 19 to 23 days in age. The animals were kept in individual screen-bottomed cages; feed and water were supplied daily ad libitum, unless otherwise indicated. Records were kept of the amount of feed consumed during the first 16 weeks of the experiment. The diets were prepared at about 90-day intervals and were kept refrigerated.

The composition of the various diets is given in Table 1. Carotene, tocopherol, and calciferol were dissolved in normal hexane; the \(\alpha\)-acetylaminofluorene was dissolved in acetone; these solutions were mixed into the dry ingredients of the diet prior to the addition of fat and the solvents allowed to evaporate. The remainder of the vitamins, in a water solution, and the fats were then mixed into the diet with the aid of a mechanical mixer.

The animals were thoroughly examined for surface tumors once weekly and were usually continued on experiment until they died. At post mortem examination all tumors and other grossly abnormal tissues were preserved and carried through routine procedures for histologic study.

RESULTS

Eye tumors.—Eye tumors were induced with varying frequency in rats on the low fat diets containing 0.03 per cent \(\alpha\)-acetylaminofluorene. The data are summarized in Table 2. Out of a total of 31 rats receiving the various low fat diets \(\textit{ad libitum}\), 10 developed eye tumors—an incidence of 32 per cent. The highest incidence occurred among the rats receiving diet C-98. This diet, which produced eye tumors in six of eleven animals, was sufficiently low in fat to induce a moderate dermatitis indicative of essential fatty acid deficiency near the end of the experimental period of 34 weeks. Eye tumors did not appear in any of the 71 animals receiving the diets containing 15–30 per cent lard (Table 2).

During the course of these studies, it was observed that very poor body weight gains were made by the animals receiving the low fat diets containing 0.03 per cent \(\alpha\)-acetylaminofluorene.
Average body weights after the 16-week experimental period were only about 100 gm. In contrast, body weights of 175–200 gm. were usually attained in 16 weeks by the animals receiving similar diets containing from 15 to 30 per cent lard. To determine whether or not the development of eye tumors was favored by depressed growth rate, diet C-136 (low fat) and diet C-137 (high fat) were pair-fed, so that the intakes of calories, carcinogen, protein, minerals, and vitamins by the animals on these two diets were equal. Although two of the six animals receiving the low fat diet developed eye tumors, no eye tumors were observed in the six animals on the high fat diet. Despite the equi-caloric feeding, the animals on the high fat diet gained about 20 gm. more body weight than those on the low fat diet during the first 16 weeks of the experiment.

The average induction time for the eye tumors was 28 weeks. These tumors originated in the retrobulbar region of the orbital cavity. They were of a firm consistency and usually attained a size of 1–2.5 cm. in diameter in 2–3 weeks after they were first detected. Only one eye was involved in every case. The gross appearance of the eye tumor is shown in Figure 1. It was found to be closely associated with the harderian gland and probably arose from this gland. In some cases the growth of these tumors was so extensive as to occupy completely the orbital cavity with involvement of the eyelid. In such cases the eye was usually completely destroyed. In cases where the

### TABLE 1

**Percentage Composition of the Diets**

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>Diet Ingredient</th>
<th>C-5</th>
<th>C-96</th>
<th>C-94</th>
<th>C-98</th>
<th>C-127</th>
<th>C-131</th>
<th>C-136</th>
<th>C-137</th>
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<tbody>
<tr>
<td></td>
<td>Water-extracted casein*</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>12</td>
<td>16</td>
<td>16</td>
<td>16</td>
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<tr>
<td></td>
<td>Vitamin-test casein†</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Degerminated corn grits</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Alcohol-extracted peanut meal‡</td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Sucrose</td>
<td>60.67</td>
<td>66.67</td>
<td>66.67</td>
<td>59.87</td>
<td>53.17</td>
<td>51.57</td>
<td>53.67</td>
<td>51.56</td>
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<td></td>
<td>Salts §</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5.3</td>
<td>4</td>
<td>5.3</td>
<td>4</td>
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<tr>
<td></td>
<td>L-cystine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
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<tr>
<td></td>
<td>Lard</td>
<td>15</td>
<td>30</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
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<td>Cod liver oil</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>2-Acetylaminofluorene</td>
<td></td>
<td></td>
<td>.03</td>
<td>.03</td>
<td>.03</td>
<td>.03</td>
<td>.03</td>
<td>.03</td>
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</table>

**Vitamin supplements**

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>C-5</th>
<th>C-96</th>
<th>C-94</th>
<th>C-98</th>
<th>C-127</th>
<th>C-131</th>
<th>C-136</th>
<th>C-137</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-carotene (mg.)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Califerol (µg.)</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>16.7</td>
<td>10.5</td>
<td>16.7</td>
</tr>
<tr>
<td>Vitamin E (mg.)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6.7</td>
<td>5</td>
<td>6.7</td>
</tr>
<tr>
<td>B-vitamin solution** (ml.)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6.7</td>
<td>5</td>
<td>5</td>
<td>6.7</td>
</tr>
</tbody>
</table>

* Commercial casein percolated for 5 days with water acidified with 0.50 per cent acetic acid overnight and finally washed with 95 per cent ethanol and dried.
† General Biochemicals.
‡ Forty-five per cent protein meal percolated for 1 week with hot methanol.
† Obtained from Eastman Kodak Co.
** Equal parts of alpha-tocopherol and alpha-tocopheryl acetate.

### TABLE 2

**Tumor Induction by 2-Acetylaminofluorene in Weanling Rats Receiving Diets with and without Fat**

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>Food content (per cent)</th>
<th>C-5</th>
<th>C-96</th>
<th>C-94</th>
<th>C-98</th>
<th>C-127</th>
<th>C-131</th>
<th>C-136</th>
<th>C-137</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. body weight (gm.)</td>
<td>47</td>
<td>176</td>
<td>28</td>
<td>6.6</td>
<td>46</td>
<td>29</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Av. survival time (weeks)</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Av. daily intake per rat (gm.)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>No. of animals with Mammary Ear-duct Liver Eye</td>
<td>46</td>
<td>29</td>
<td>40</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Animals on diet C-136 each received 0.30 ml. of corn oil once weekly as a source of essential fatty acids.
† Animals on diet C-137 were restricted in food intake so that the consumption of total calories, vitamins, minerals, protein, and 2-acetylaminofluorene was equal to that of their litter mates receiving diet C-136.
tumors were smaller, the eye could be dissected free of tumor tissue.

Microscopic studies of slides prepared from the eye tumors revealed that they were carcinomas, although there was considerable variation in the cellular structure (Figs. 2 and 3). Glandlike arrangements suggestive of adenocarcinoma were found in some areas, and in other areas the arrangement was almost suggestive of sarcoma. The prominent cell type was a relatively large round cell with a hyperchromatic nucleus. There were variations to oval and odd-shaped nuclei. The cells were closely packed, with only a small amount of connective tissue stroma. There were numerous mitotic figures. Numerous lymphatic spaces with tumor cells packed closely around them were observed (Fig. 3). Local invasion of muscle tissue is illustrated in Figure 4. Tumor emboli were observed in blood vessels in the orbital region (Fig. 5), and metastases to the lung were observed in two of the ten eye-tumor cases (Fig. 6).

**Mammary tumors.**—The incidence of mammary tumors was extremely low among the animals receiving the low fat diets. Only 2 of the 31 animals developed this type of tumor (Table 2). In contrast, mammary tumors occurred in 55 of the 71 animals that received the diets containing 15—30 per cent lard. However, when a 26 per cent lard diet (diet C-137) was fed at a restricted level so that poor growth ensued, mammary tumors were completely inhibited. Thus, the poor body growth regularly observed in animals consuming 2-acetylaminofluorene in low fat diets could be responsible for failure of mammary tumors to develop. A relatively rapid growth rate is apparently essential for the uniform induction of mammary tumors by 2-acetylaminofluorene in weanling rats. This is further emphasized by the results obtained with diet C-5 (Table 3). All the 62 animals represented in these data received diet C-5 and served as controls in a series of studies designed to test the importance of various modifications of dietary vitamin levels on mammary tumor induction by 2-acetylaminofluorene. In Table 3, the body weights of these animals at 16 weeks have been grouped in ascending order. The body weights ranged from 144 to 235 gm. It is obvious from the data in Table 3 that the rats which made the more rapid weight gains also more consistently developed mammary tumors. Twenty-six of the 27 rats, weighing 176 gm. or more at 16 weeks, developed mammary tumors. In contrast, only 20 of the 35 rats, weighing 175 gm. or less at 16 weeks, developed mammary tumors.

The variation in weight gain among the rats receiving diet C-5 could not be attributed to differences in initial body weight. The 35 slowly growing rats had an average initial weight of 47 gm. as did the 27 rapidly growing rats. The differences in gain likewise could not be traced to any particular litters. The individual variations in gains exhibited by the data in Table 3 could be attributed directly to variations in total feed consumed.

The average induction time for mammary tumors was 19 weeks. The average induction time for the rats weighing less than 176 gm. at 16 weeks was 20 weeks (range, 12—30 weeks), as compared to an average of 18 weeks (range, 15—27 weeks) for those weighing more than 175 gm. at 16 weeks. However, with the wide variations apparent in both groups, the significance of this difference is questionable.

**Other tumors.**—Tumors of the ear duct developed in an average of 24 weeks. This type of tumor appeared to develop less frequently under conditions of rapid growth associated with high feed consumption and a high incidence of mammary tumors (Table 3). For example, only 4 of the 18 most rapidly gaining rats on diet C-5 had ear-duct tumors. In contrast, 25 of the 44 more slowly growing rats on this diet developed ear-duct tumors. The development of liver tumors did not appear to be influenced by changes in dietary fat level. The rate of growth of the animal likewise did not appear to influence the induction of liver tumors. No attempts were made to establish the induction time for internal tumors.

Three unusual types of tumors were observed among the animals receiving diet C-5. One rat had a tumor on the lateral aspect of the buccal cavity, another rat had a kidney tumor, and a third rat had an intestinal tumor.

**DISCUSSION**

The appearance of eye tumors in rats fed low fat diets and the absence of this type tumor in rats
Fig. 1.—Photograph showing the gross appearance of a tumor of the orbital cavity of a rat fed a low fat diet containing 0.03 per cent 2-acetylaminofluorene.

Fig. 2.—Photomicrograph illustrating the general histology of a tumor of the orbital cavity (harderian gland). Note the "glandlike" arrangement of the cells and the numerous mitotic figures. Hematoxylin and eosin. ×375.

Fig. 3.—Photomicrograph of a section from another area of the tumor showing closely packed tumor cells around a lymph vessel. Hematoxylin and eosin. ×350.

Fig. 4.—Photomicrograph of a section of the same tumor to illustrate the local invasion of muscle tissue by the tumor cells. Hematoxylin and eosin. ×375.

Fig. 5.—Photomicrograph of a section through a blood vessel which contains a tumor embolus in the orbital region of the rat shown in Figure 1. Hematoxylin and eosin. ×325.

Fig. 6.—Photomicrograph of a section of lung from the rat shown in Figure 1 illustrating metastasis of the orbital tumor (harderian gland tumor) to the lung. Hematoxylin and eosin. ×325.
receiving similar diets with fat indicate that the site of action of 2-acetylaminofluorene in the animal body can be influenced by diet. Eye tumors induced by feeding 2-acetylaminofluorene apparently have been observed only rarely by other investigators using this carcinogen. Bielschowsky (1) reported a cancer of the eyelid in one animal in a series of studies which included several hundred animals. A detailed description of this tumor was not given. Morris and associates (6) reported a transplantable tumor involving the harderian gland, which was originally induced by feeding 2-acetylaminofluorene and thiouracil. The type of diet fed was not mentioned in either of these reports. That tumors can be produced in the eye of rats by 2-acetylaminofluorene should perhaps be expected in view of the results of Weisburger and associates (8). These workers observed that the harderian gland accumulated appreciable quantities of this carcinogen after the C14-labeled compound was administered to rats.

One of the requirements for mammary tumor induction by 2-acetylaminofluorene in rats appears to be that the animal must grow at a normal or nearly normal rate. Underfeeding and caloric restriction are known to inhibit the induction of various types of spontaneous and induced tumors (7). A lowered caloric intake, probably due to the presence of the carcinogetic in the diet, explains the lowered incidence of mammary tumors in slowly growing rats in the present study. Furthermore, a diet known to produce a high incidence of mammary tumors when fed ad libitum produced no mammary tumors when fed at a restricted level. The ear-duct tumor incidence appeared to decrease when diets were used that promoted rapid growth (high caloric intake) and produced a high incidence of mammary tumors. A high caloric intake did not appear to be essential for the induction of either ear-duct or liver tumors.

The present results thus indicate that the type of diet fed and the quantity consumed can have a very definite influence upon the site of action of 2-acetylaminofluorene.

SUMMARY

Eye tumors, presumably originating in the harderian gland, were induced in 10 of 31 weanling rats fed 0.03 per cent 2-acetylaminofluorene on low fat diets for 7—8 months. Rats fed such diets made very poor weight gains. Attempts to induce eye tumors by feeding fat-containing diets ad libitum or at a restricted level of caloric intake were unsuccessful. Whether or not fat was present in the diet, mammary tumor induction was greatly decreased when the animals made poor weight gains. Conditions that favored mammary tumor induction, rapid growth, and high caloric intake appeared to decrease the induction of ear-duct tumors. The type or quantity of diet consumed had little or no influence upon the induction of liver tumors. The results emphasize that the site of carcinogenic action of 2-acetylaminofluorene can be markedly influenced by the type and quantity of diet fed.

REFERENCES

Influence of Diet on the Relative Incidence of Eye, Mammary, Ear-Duct, and Liver Tumors in Rats Fed 2-Acetylaminofluorene

R. W. Engel and D. H. Copeland


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