Some Effects of 2-Acetylaminofluorene on the Dog

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The compound, 2-acetylaminofluorene, was recognized as a carcinogen in 1941 by Wilson, DeEds, and Cox (15). This compound, incorporated in the diet in amounts varying from 0.03 to 0.15 per cent, will elicit the formation of tumors in various tissues of the rat, mouse, and cat (12, 13, 5). The hepatoma is one of the most common neoplasms produced by this drug in the rat. The purposes of the following experiments were to determine the carcinogenic activity of 2-acetylaminofluorene in the dog and to discover some of the changes in metabolism produced by the drug, particularly those associated with the induction of hepatomas. Changes in liver function and structure are revealed in the dog by an alteration in the urinary uric acid/allantoin ratio. If the liver cells are sufficiently destroyed or altered in character, the ratio increases reflecting a reduction in the activity of uricase (2, 6). This ratio was used, therefore, in the experiments described herein, to detect changes in liver function. Depletion in liver riboflavin and liver protein has been associated with the induction of hepatomas in the rat (7-11). A study of the effects of 2-acetylaminofluorene on the utilization of riboflavin and proteins by dogs was included, therefore, in the following experiments.

MATERIAL AND METHODS

The drug 2-acetylaminofluorene was incorporated (0.03 per cent) in the synthetic diet (Table 1) which was fed to twelve adult, male dogs (6 beagles and 6 mongrels, 2-6 years of age). Nine male control animals (3 mongrels and 6 beagles, 2-6 years of age) were fed the diet without the drug.

The technic of Najjar and Holt (14) was used to determine retention of riboflavin in dogs. For this determination, 0.02 mg. of riboflavin/kilogram of body weight was injected, and the quantity of the vitamin excreted in the urine (13) was measured over periods of 4 and 8 hours. The riboflavin content of the liver and feces were determined by the method of Arnold (4).

Received for publication, December 10, 1949.

RESULTS AND DISCUSSION

All the dogs fed the 2-acetylaminofluorene developed evidences of depletion in protein stores and in riboflavin. After 6 months of feeding, the urinary nitrogen excretion on a protein-free diet, the albumin/globulin ratio, hemoglobin content of the blood, and liver nitrogen were reduced to low levels reflecting a decrease in the protein stores. Data on six of the drug-fed animals and on six of the control animals are recorded in Table 2. The nitrogen balance index of the dietary casein also decreased significantly below control values during the feeding period of 6 months. The nitrogen balance index is a function of the retention of dietary nitrogen to build body protein stores, the index increasing as more nitrogen is utilized for this purpose. The decrease in index, therefore, indicates a reduction in the ability of the animals to retain dietary nitrogen in the body. With simple protein depletion there is an increase in the index rather than a decrease. The index for casein, for example, in a protein-depleted dog, will increase to values as high as 0.98 (1). Thus 2-acetylaminofluorene not only depletes the animal in body protein but also leads to reduced retention of dietary nitrogen.

The dogs fed the carcinogenic diet developed a seborrheic erythema with alopecia which is similar to skin conditions associated with riboflavin deficiency. A large excess of riboflavin in the diet of one of these dogs resulted in an improved condition of the lesions. Removal of the excess vitamin from the diet resulted in the reappearance of the lesions. Riboflavin retention tests were conducted on a number of the animals having lesions and on controls. The results of these tests are illustrated in Figure 1. The excretion of the vitamin was less in those animals with the lesions, demonstrating a greater retention of the riboflavin.

A reduction in the content of riboflavin in the liver was associated with the deficiency (see Table 2). The effect on this vitamin is similar to other carcinogens such as p-dimethylaminobenzene, and it is possible, as suggested by Miller (11), that riboflavin is involved in detoxication processes. It is interesting to note that the excretion of riboflavin in the feces is even reduced when the drug...
is added to the diet. This reduction in riboflavin is being correlated with a decrease in the bacterial count of the feces, a decrease which is being studied further in detail.

One of the dogs, examined after approximately 6 months of receiving the carcinogen, had developed a tumor mass on the liver (see Fig. 4). It was believed that hepatomas such as this would increase the urinary uric acid/allantoin ratio. The ratio of excretion of uric acid to that of allantoin has been used in these laboratories for a number of years to detect liver damage in dogs, the ratio increasing as the severity of the damage increases. The data plotted in Figure 2 illustrate the effect of feeding the drug on the ratio. The two dotted lines drawn parallel to the X axis denote the upper and lower limits of the ratio in control dogs fed the diet (0.15 gm. casein nitrogen/kg body weight) without the carcinogen. A marked increase of the uric acid/allantoin ratio to 0.6 or more, illustrated by the large circles in Figure 2, was associated with abnormal proliferation of liver tissue and the development of neoplasms. Grossly, the livers of these animals showed abnormal growths, ranging

TABLE 1

**COMPOSITION OF THE SYNTHETIC DIET**

<table>
<thead>
<tr>
<th>Component</th>
<th>Calories</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (Labco)</td>
<td>3.75</td>
<td>1.10</td>
</tr>
<tr>
<td>Sucrose</td>
<td>12.25</td>
<td>3.08</td>
</tr>
<tr>
<td>Dextrin</td>
<td>13.04</td>
<td>3.26</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20.90</td>
<td>6.74</td>
</tr>
<tr>
<td>Lard</td>
<td>24.00</td>
<td>2.67</td>
</tr>
<tr>
<td>Salt*</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>

**TOTAL:** 80.00

* Wesson's Modified Salt Mixture.
† Mix 1.4 gm. of water with every gm.

**TABLE 2**

**THE EFFECTS OF 2-ACETYLAMINOFLUORENE ON NITROGEN AND RIBOFLAVIN RETENTION**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>AAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>1.2</td>
<td>0.53</td>
</tr>
<tr>
<td>Globulin</td>
<td>14.0</td>
<td>8.1</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>17.6 ± 0.03</td>
<td>14.1 ± 0.9†</td>
</tr>
<tr>
<td>Liver protein</td>
<td>gm/100 gm</td>
<td>2.12*</td>
</tr>
<tr>
<td>Urinary N</td>
<td>gm/day/sq. m.</td>
<td>0.78*</td>
</tr>
<tr>
<td>(Protein-Free Diet)</td>
<td>tissue</td>
<td></td>
</tr>
<tr>
<td>Nitrogen Balance Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>μg/gm tissue</td>
<td>33.8 ± 9</td>
</tr>
<tr>
<td>Feces</td>
<td>μg/day/kg</td>
<td>44.8 ± 4.9</td>
</tr>
</tbody>
</table>

* Values obtained on four animals.
† Standard error.
from pin-point nodules and cysts to well developed tumor masses. The small circles illustrate data obtained on dogs in which there were no marked gross lesions on the liver.

The gross and microscopic appearances of the livers of the dogs used to obtain the data in Figure 2 are summarized in Table 3 and illustrated in part in Figures 3 to 10.

Dog No. 1 (white circles in Fig. 2) developed multiple nodules and cystic areas in the liver (see Fig. 3) after 30 weeks on the carcinogenic diet. The microscopic appearance of this liver illustrated in Figures 6 and 7 can be compared with the normal liver in Figure 5. The abnormal growth (hepatoma) and surrounding tissues seen in Figures 6 and 7 can be described as follows: The cell size in the hepatoma was normal or reduced, with markedly granular cytoplasm which stained heavily with eosin. The nuclei varied in size—some were hyperchromatic, others pyknotic—but mitoses were not evident. Degenerate cells, signet ring in appearance, were present. The margin of the hepatoma was adjacent to normal liver cells at its entire circumference. Areas adjacent to central veins and the portal triad were more heavily stained than others, and the cells appeared normal. In between, however, fatty infiltration and fatty degeneration were marked. Slight fibrous infiltration was evident.

Dog No. 2 (white circles with crossed bars in Fig. 2) developed a tumor mass, as well as nodules and cysts, after 25 weeks on the carcinogenic diet (see Fig. 4). The microscopic appearance of this liver is illustrated in Figures 8 to 10. There was a complete loss of general liver topography, with a marked increase in fibrous connective tissues (cirrhosis), which had grown completely around liver areas. Fatty degeneration was almost absent. In the tumor, the liver cells were very compact and deeply stained with reduced cytoplasm. Mitotic figures were numerous. A definite cholangioma had developed. The tall columnar cells were in tubular or acinar arrangement, and numerous cross sections were observed.

The liver of dog No. 3 (circles with horizontal bar) developed a tumor mass at the periphery of one of the lobes after 34 weeks on the diet. There

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Weeks on diet</th>
<th>Weight, kg. start</th>
<th>Weight, kg. end</th>
<th>Appearance of liver</th>
<th>Gross</th>
<th>Microscopic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>10.1</td>
<td>9.0</td>
<td>multiple hard nodules and cysts</td>
<td>hepatoma, varied size cells, some cystic degeneration</td>
<td>cholangioma, glandular and cyst formation cirrhosis with general loss of liver topography</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>9.8</td>
<td>8.2</td>
<td>tumor mass, multiple nodules, and cysts</td>
<td>cholangioma, glandular and cyst formation cirrhosis with general loss of liver topography</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>10.2</td>
<td>9.8</td>
<td>tumor mass at periphery and cysts</td>
<td>fatty degeneration, necrosis, cyst formation (no sections of tumors)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>12.6</td>
<td>14.6</td>
<td>no gross pathology</td>
<td>cytoplasmic depleted cells, enlarged sinusoids</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>7.4</td>
<td>4.3</td>
<td>no gross pathology</td>
<td>normal</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>7.5</td>
<td>9.2</td>
<td>no gross pathology</td>
<td>normal</td>
<td></td>
</tr>
</tbody>
</table>
were also numerous small cysts. Unfortunately, sections of the tumor mass were missed, but a microscopic examination of the liver as a whole showed degenerative changes, necrosis, and cyst formation.

Thus, gross and microscopic examinations of the liver demonstrate that the feeding of 2-acetylaminofluorene in the diet can induce neoplasms in the dog. In all dogs examined, however, whether or not tumor masses developed, there was a depletion in protein and riboflavin stores of the body. It may be that the drug reduces the activity of some enzyme systems, possibly associated with riboflavin, creating an enzymatic environment more favorable for the growth of the cancer cell. Whether or not this growth takes place would be a function, in part, therefore, of the type of cellular environment produced. This environment is under investigation.

**SUMMARY**

1. The carcinogen, 2-acetylaminofluorene, fed in a synthetic diet over a period of 6 months to dogs, caused a reduction in the protein stores of

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**Fig. 3.**—Gross appearance of liver of dog No. 1 (see Table 3), the line equals 2 cm.

**Fig. 4.**—Gross appearance of liver of dog No. 2, the line equals 2 cm.

**Fig. 5.**—Liver of normal dog. ×560.

**Fig. 6.**—Liver of dog No. 1 fed 2-acetylaminofluorene for 30 weeks. Hepatoma bordering on normal liver tissue. ×110.
Fig. 7.—Liver of dog No. 1 fed 2-acetylaminofluorene for 30 weeks. Hepatoma. Cell cytoplasm is very granular. Cyst formation. ×330.

Fig. 8.—Liver of dog No. 2 fed 2-acetylaminofluorene for 25 weeks. General loss of liver topography. ×110.

Fig. 9.—Liver of dog No. 2. Fibrosis and liver cell degeneration. ×110.

Fig. 10.—Liver of dog No. 2. Gland formation and packing of cells. ×560.
the body. The plasma albumin/globulin ratio, hemoglobin, red blood cells, liver nitrogen, and protein-free urinary nitrogen all decreased over the feeding period—variables which also decrease when animals are fed a protein-free diet.

2. The retention of dietary nitrogen, as measured by the nitrogen balance index also decreased below control values, demonstrating that the depletion in protein stores was the result of a reduction of retention of both dietary and body nitrogen.

3. A riboflavin depletion was produced in dogs fed 2-acetylaminofluorene. This depletion resulted in the appearance of typical skin lesions, in the reduction of liver riboflavin, and in an increase in the ability of the animal to retain excess injected vitamin. The carcinogen also reduced the excretion of riboflavin in the feces.

4. Hepatic neoplasms developed in three of the six dogs fed the carcinogen from 6 to 9 months. The urinary uric acid/allantoin ratio increased markedly in these dogs, an increase which was associated with the alteration in liver function.

REFERENCES


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