The Influence of Sex Hormones upon the Hepatic Lesions Produced by 2-Acetaminofluorene*

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The carcinogenic action of 2-acetaminofluorene has been described by Wilson, De Eds and Cox (14) and by Bielschowsky (1). This compound is particularly interesting because of the fact that, when fed to stock albino rats (14) and to descendants of Wistar rats (1), it produced a greater variety of tumors than most known carcinogens. Bielschowsky (2) reported the development of benign and malignant tumors of the thyroid in rats following simultaneous administration of 2-acetaminofluorene and the antithyroid drug, allylthiourea, the carcinogen alone being without influence upon the normal gland. Adenomas of the thyroid, but not malignant tumors, have been observed in rats fed allylthiourea (3), brassica seed (5) and thiouracil (10) for long periods of time.

This observation of the susceptibility of the stimulated goitrous thyroid to the tumor-producing action of the carcinogen suggested that it might be interesting to investigate the influence in this connection of other agents that stimulate growth of specific tissues. Androgenic and estrogenic hormones were chosen for this purpose.

METHODS

Two hundred and three rats of the Sherman strain weighing about 75 gm. each were divided into 6 experimental groups. All received 0.03 per cent 2-acetaminofluorene in the following diet: corn meal, casein, alfalfa, linseed oil, bone ash, and NaCl, with supplements of brewers' yeast and cod liver oil.

**Group 1 (Control):** 80 rats received no additional treatment.

**Group 2 (Thiouracil):** 30 rats received 0.05 per cent thiouracil in the drinking water.

**Group 3 (Exogenous estrogen):** 36 female rats received 0.125 mgm. of estradiol dipropionate (in sesame oil) intramuscularly 3 times weekly.

**Group 4 (Endogenous estrogen):** 20 female rats received 25 I.U. of pregnant mare serum (PMS) gonadotrophin (in aqueous solution) intramuscularly 3 times weekly.

**Group 5 (Exogenous androgen):** 20 male rats received 0.5 mgm. of testosterone propionate (in sesame oil) intramuscularly 3 times weekly.

**Group 6 (Endogenous androgen):** 17 male rats received 25 I.U. chorionic gonadotrophin (in aqueous solution) intramuscularly 3 times weekly.

Treatment was continued in all instances until the time of death or sacrifice.

Tissues were fixed in Bouin's fluid and slides were stained with hematoxylin and eosin. Other slides were stained with Masson's trichrome stain for connective tissue, Marchi's fluid for fat, and Best's carmine for glycogen (absolute alcohol fixation).

RESULTS

It became obvious early in the course of the experiment that the hepatic lesions in the animals receiving sex hormones differed strikingly from those in the control (carcinogen only) and thiouracil-treated groups. This communication is concerned particularly with the 76 animals that have been examined after 44 to 316 days of treatment.

Mammary carcinoma occurred in 3 female controls. Of the thiouracil group, 7 animals developed tumors of the thyroid, 1 with pulmonary metastases, and another a carcinoma of the uterine horn. A squamous cell papilloma occurred in the bladder of 1 testosterone-treated rat.

All animals receiving thiouracil exhibited pronounced hyperplasia and hypertrophy of the thyroid. The majority of those receiving estradiol developed pyometra and one receiving PMS gonadotrophin had a pyosalpinx. The target organ response to testosterone was less marked than is usually observed in animals receiving this substance alone and the target organs in the PMS and chorionic gonadotrophin-treated animals showed no evidence of stimulation.

The hepatic lesions have been classified as (a) cystic and (b) neoplastic (hepatoma; cholangioma). The former were regarded as benign and the latter as malignant, solely on the basis of histologic characteristics, except in 3 instances in which there were pulmonary metastases. The incidence of these lesions in the various experimental groups is presented in Table I.

**Controls (carcinogen only): 21 animals; 18 females, 3 males.**—After 185 to 316 days of treatment, cystic lesions were observed in 15 (71 per cent) and neoplasms in 6 instances (29 per cent), the latter occurring in 2 of the 3 males and 4 of the 18 females of this group.

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group. No hepatic neoplasms were seen in animals treated for less than 180 days.

**Thiouracil** (15 animals; 9 females, 6 males).—After 44 to 316 days of treatment, cystic lesions were observed in 3 instances (20 per cent) at 130, 185 and 316 days, respectively. There were no hepatic neoplasms in this group and no sex difference was apparent.

**Estradiol** (13 animals).—After 61 to 256 days of treatment, cystic lesions were observed in 12 (92 per cent) and neoplasms in 9 (60 per cent) of instances; the earliest cysts occurred at 132 days and the earliest neoplasm at 165 days.

**PMS gonadotrophin** (endogenous estrogen; 12 animals).—After 185 to 300 days of treatment, cystic lesions were observed in 12 (100 per cent) and hepatic neoplasms in 4 (33 per cent) of the animals. One had pulmonary metastases. The earliest hepatoma occurred at 201 days.

**Testosterone** (10 animals).—After 206 to 302 days of treatment, cystic lesions were observed in 10 (100 per cent) and hepatic neoplasms in 8 (80 per cent) of instances. The earliest hepatoma occurred at 206 days.

**Chorionic gonadotrophin** (endogenous androgen; 5 animals).—After 160 to 250 days of treatment, cystic and neoplastic lesions were observed in all animals.

The significant histologic changes could be classified as (a) degenerative and regenerative, (b) cystic and (c) neoplastic.

**Degenerative and regenerative changes.**—There occurred in some degree in all groups of animals, being generally minimal in those receiving thiouracil, slight in the control group (carcinogen only) and most pronounced in those receiving testosterone. Even in the latter, these changes were seldom advanced.

The characteristic features of these lesions were as follows: The hepatic cells were swollen, with granular or vacuolated cytoplasm and well stained nuclei. The sinusoids were compressed by the swollen cells, but in early cases the normal lobular architecture was well preserved. In animals treated for longer periods the architecture became somewhat distorted, with variation in size and shape of the lobules, irregularity in position of blood vessels and variation in size and staining reaction of cells.

Proliferation of young, cellular, connective tissue was observed in all groups, paralleling the changes noted above; in no instance was it striking, the Masson stain failing to reveal a sharp increase in collagenous fibers. Regenerative phenomena, which paralleled the degenerative and proliferative changes, were represented by large hepatic cells with conspicuous nuclei and by increased mitotic activity, occurring particularly in the lobules exhibiting architectural distortion.

**Cystic changes.**—Both in the gross and microscopically, the most striking and most constant changes in the liver were cystic in nature. The cysts varied widely in size, shape and number and usually occurred in groups; the individual members of these groups were often partially separated by broken-down septa, suggesting that large cysts were formed by fusion of smaller ones. At autopsy, they contained clear fluid; in stained sections they were at times empty and occasionally contained a granular, pink-staining substance, or mononuclear cells. The smaller cysts were lined by cuboidal epithelium and the larger by flat epithelial cells. They were smallest and least numerous in the thiouracil-treated animals and most conspicuous in those receiving estradiol, in which they usually occurred as numerous collections of large cysts of a honey-combed appearance (Fig. 3).

Occasionally cysts were encountered, lined by tall columnar cells with a rather acidophilic cytoplasm, frequently forming papillary projections into the lumen (Fig. 4). This lesion conformed to that designated as “cystadenoma” by Opie (8), and occurred most frequently in the animals receiving chorionic gonadotrophin, rarely in the controls and never in the thiouracil group.

**Neoplastic changes.**—The neoplastic lesions were similar to those described by Orr (9), Edwards and White (4) and Opie (8) in rats treated with p-dimethylaminoazobenzene. They were classified as (a) hepatomas and (b) cholangiomas, on morphologic grounds. Both occurred in multiple foci throughout the liver.

The hepatomas were of two varieties conforming to those designated by Opie as “adenohepatoma” and “trabecular hepatoma,” with both types often repre-

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**Table 1: Incidence of Cystic and Neoplastic Lesions in Liver**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Cysts</th>
<th>Neoplasms</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Slight</td>
<td>Moderate</td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
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<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Estradiol</td>
<td>13</td>
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<td>4</td>
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<tr>
<td>PMS</td>
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<td></td>
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</tr>
<tr>
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<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Testosterone</td>
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<td>1</td>
<td>5</td>
</tr>
<tr>
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<td>12</td>
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<td>2</td>
</tr>
<tr>
<td>Gonadotrophin</td>
<td>5</td>
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</tbody>
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Fig. 1.—Liver of control rat (carcinogen alone) treated for 258 days.

Fig. 2.—Liver of rat treated with estradiol for 217 days. Note pronounced cystic changes.

Fig. 3.—Liver of rat treated with estradiol for 217 days, showing pronounced cystic lesions. Mag. × 30.

Fig. 4.—Liver of rat treated with chorionic gonadotrophin 250 days, showing epithelial proliferation into lumen of cyst, constituting a cystadenomatous lesion. Mag. × 60.
sent in the same nodule (Fig. 9). The constituent cells were large and palely acidophilic, resembling liver cells, with vesicular nuclei and distinct nucleoli. In the former the cells were arranged in acinar formation, the acini being surrounded by dilated sinusoids lined by endothelial cells (Fig. 5). These adenomatous areas gradually merged into relatively normal liver tissue. In the other variety the cells, which were often large and polyhedral, frequently with conspicuously irregular-shaped nuclei, exhibited no regularity in their arrangement. They were frequently fused together or formed giant cells, with multiple or lobulated, deeply-staining nuclei; many mitotic forms were present (Figs. 6 to 8); the cell outlines were often indistinct and irregular, the neoplastic cells frequently infiltrating between the cells of otherwise normal liver cords. Areas of hemorrhage and cystic change were commonly observed adjacent to the hepatomatous nodules. Both varieties of hepatoma occurred most frequently in animals treated with testosterone and chorionic gonadotrophin but were also common in those receiving estradiol. True hepatomas were differentiated from nodules of regenerating liver cells by the preservation in the latter of the normal, fundamental relationship between liver cells and sinusoids and by distortion of this relationship in the former, with marked irregularity in size and arrangement of the cells and in the staining reactions and appearance of their nuclei. Glycogen was present in the cells of the non-neoplastic areas of the hepatic parenchyma, although frequently in somewhat irregular distribution, but very few of the neoplastic cells contained glycogen granules. Multiple pulmonary metastases from these tumors were noted in 2 animals (Figs. 10 to 14).

The cholangiomas (Figs. 11 to 13) consisted of columnar cells, with a basophilic cytoplasm and oval nucleus, exhibiting a tendency to form acini and tubules. When arranged circularly, they resembled regenerating bile ducts. These gland-like structures were usually irregularly arranged, lined by one or several layers of tall, basophilic cells and separated by a cellular stroma containing lymphocytes, monocytes, and fibroblasts. There were many mitotic forms. This lesion closely resembled adenocarcinoma (Fig. 13).

**DISCUSSION**

These observations indicate that exposure to increased quantities of exogenous or endogenous estrogen or androgen hastens the development of and intensifies the hepatic lesions induced by 2-acetaminofluorene in the rat (Sherman). The lesions seem identical with those produced by p-dimethylaminoazobenzene. As noted by Opie in the case of the latter, both cystic and neoplastic lesions occurred frequently in the absence of significant cirrhosis or extensive hepatocellular damage. It is improbable, therefore, that this effect of the sex hormones is dependent fundamentally upon a hepatotoxic action, which has been attributed to estrogens by some (7, 13) and denied by others (11, 12). Moreover, no hepatotoxic effect has been reported for androgens or gonadotrophins.

Because of the absence at the time of sacrifice of evidence of significant stimulation of the ovaries and testes in the gonadotrophin-treated animals, their exposure to increased quantities of estrogen or androgen at some time during the experimental period must remain, for the present, an assumption. This seems justified, however, because of the dosage employed, and because of the similarity between the hepatic lesions in the animals receiving gonadotrophins and exogenous estrogen and androgen. One would otherwise have to assume a direct effect of gonadotrophin upon the liver identical to that of estradiol and testosterone, which is highly improbable. The absence of target organ response may be due to the development of antigonadotrophins.

The difference in response of the control, thiouracil-treated and sex hormone-treated animals does not appear to be due to variations in intake of food or carcinogen. In general, appetite was diminished in animals receiving thiouracil and especially in the estrogen-treated group, the intake of acetaminofluorene being correspondingly lowered. However, the fact that an adequate quantity of carcinogen was taken by both of these groups was evidenced by the consistent urinary excretion of a substance that stained the fur and pine shavings bright orange (14) and by the development of neoplasms, in the thyroid and uterus in the thiouracil series and in the liver in a large proportion of the estrogen series. Moreover, the food intake remained normal in the groups receiving testosterone and gonadotrophins during the greater part of the experimental period, as indicated by gain in weight. It has been noted previously, that the occurrence of the cystic and neoplastic lesions was not paralleled by degenerative or fibrotic processes in the liver, as might result from dietary causes such as increased cystine and decreased choline or methionine intake.

The accelerated development of these lesions under the influence of estrogen and androgen is particularly interesting because of the role of the liver cell in the intermediary metabolism and excretion of these hormones and related steroids. Any attempt to explain the phenomenon on this basis would be purely speculative, but the implications are obvious. The sex hormones or their metabolites may act as cocarcinogens, or carcinogens may be formed from them as a result of pereversion of their metabolism by the hepatic cells under the influence of aminofluorene. The effect of estrogen in males and androgen in females is being investigated.

Our findings differ in certain respects from those
Fig. 5.—Liver of rat treated with PMS gonadotrophin for 195 days, showing a hepatoma, with adenomatous arrangement of proliferating cells, with dilated sinusoids and cysts. Mag. X 110.

Fig. 6.—Liver of rat treated with PMS gonadotrophin for 162 days. Hepatoma, with marked variation in size and shape of cells and area of hemorrhage at corner. Mag. X 86.

Fig. 7.—Higher magnification of Fig. 6, hepatoma, showing the syncytial, giant neoplastic cells with bizarre, hyperchromatic nuclei. Mag. X 312.

Fig. 8.—Liver of rat treated with estradiol for 165 days, showing margin of hepatoma infiltrating adjacent liver tissue. Mag. X 86.

Fig. 9.—Liver of rat treated with testosterone for 252 days. Hepatoma, exhibiting both adenomatous (a) and trabecular (b) arrangements in the same nodule. Mag. X 86.

Fig. 10.—Metastatic lesion in lung from a hepatoma in a rat treated with testosterone for 297 days. Mag. X 55.
Fig. 11.—Liver of rat treated with estradiol for 176 days, showing cholangioma with irregular gland and cyst formation. Mag. X 110.

Fig. 12.—Liver of rat treated with estradiol for 289 days. Tall columnar cells of cholangioma in tubular or acinar arrangement. Mag. X 400.

Fig. 13.—Liver of rat treated with chorionic gonadotrophin for 250 days, illustrating close resemblance of cholangioma to adenocarcinoma. Mag. X 65.

Fig. 14.—Lung of rat treated with PMS gonadotrophin for 278 days, showing metastatic hepatoma cells in bronchial blood vessel (V). Mag. X 150.
reported previously. Wilson, DeEds and Cox (14) found malignant tumors in 19 of 39 (48.7 per cent) and Bielschowsky (1) in 93 of 104 rats (89.4 per cent), as compared with only 7 in our control group of 21 animals (33.3 per cent). Hepatic neoplasms were observed by Bielschowsky in 59 per cent of cases (83 per cent males; 30 per cent females; 100 per cent castrate males; 45 per cent castrate females) and in only 29 per cent of our controls. This discrepancy may be due in part to the difference in strain. It may also be due to the fact that the basic diet in the present experiment, which was the same as that employed by Wilson, DeEds and Cox (14), was supplemented by brewers’ yeast. This may have made the liver more resistant to the effect of the carcinogen, but it is difficult to understand how it could have affected other tissues in this manner.

The extremely low incidence of degenerative, fibrotic and cystic changes and the absence of neoplasms in the livers of the thiouracil-treated animals was most striking. As indicated above, it does not seem to be due to dietary factors or to inadequate intake of the carcinogen. No such protective action was apparent in Wistar rats receiving allylithiouracil (2), 50 per cent of which developed hepatomas. It is of interest in this connection that György and Goldblatt (6) found that thiouracil exerts a preventive effect on the production of dietary cirrhosis in rats. They suggested that this phenomenon may be due to decrease in the requirement for methionine incident to the lowered metabolic rate. Further observations will be reported elsewhere.

The original purpose of this study was to determine whether hyperplasia of the uterus, ovaries, prostate, seminal vesicles, and testes, induced during a period of exposure to acetaminophenurea, would lead to the development of neoplasms in these organs, as in the case of the hyperplastic thyroid. We have observed thyroid adenomas following prolonged treatment (360 days) with thiouracil alone. Simultaneous administration of acetaminophenurea may merely accelerate this process. It is of interest, however, and possibly of significance, that the hyperplasia of target organs of the sex hormones is a functioning hyperplasia, whereas that induced in the thyroid by thiouracil is functionless, may possibly be a determining factor in the relationship between simple and neoplastic growth of tissue.

SUMMARY

Excessive quantities of endogenous or exogenous estrogen and androgen accelerated and intensified the development of cystic and neoplastic hepatic lesions induced in Sherman rats by 2-acetaminophenurea. This phenomenon may be related to the role of the liver in the intermediary metabolism and excretion of the sex steroids.

Thiouracil appeared to exert a strikingly protective action upon the liver as regards the development of both cystic and neoplastic lesions.

No tumors occurred in the hyperplastic target organs of the sex hormones, in sharp contrast to the high incidence of tumors of the thyroid in rats receiving thiouracil simultaneously with the carcinogen. The fact that the hyperplasia induced by the sex hormones is a functioning hyperplasia, whereas that induced in the thyroid by thiouracil is functionless, may possibly be a determining factor in the relationship between simple and neoplastic growth of tissue.

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