Neoplasms in Rats with 2-Acetaminofluorene and Sex Hormones
II*

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In a previous communication (4) we reported that administration of estrogen, androgen or gonadotropins intensified and accelerated the development of the cystic and neoplastic hepatic lesions induced in Sherman rats by 2-acetaminofluorene. The purpose of the present report is to review the incidence and distribution of all neoplastic and other significant lesions in a larger series of animals treated over a longer period.

MATERIAL AND METHODS

Of an original group of 173 rats of the Sherman strain weighing about 75 gm., 80 served as controls and 93 received sex hormones. 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.

Groups 1 and 2 (Controls).—Sixty female and 20 male rats received no additional treatment; 18 females and 7 males died too early to permit their inclusion in the experimental data.

Group 3 (Exogenous estrogen).—Thirty-six female rats received 0.125 mgm. of estradiol dipropionate (in sesame oil) intramuscularly 3 times weekly; 15 survived sufficiently long to permit their inclusion in this report.

Group 4 (Endogenous estrogen).—Twenty female rats received 25 I.U. of pregnant mare serum (PMS) gonadotrophin (in aqueous solution) intramuscularly 3 times weekly; 17 survived sufficiently long to permit their inclusion in this report.

Group 5 (Exogenous androgen).—Twenty male rats received 0.5 mgm. of testosterone propionate (in sesame oil) intramuscularly 3 times weekly; 15 survived sufficiently long to permit their inclusion in this report.

Group 6 (Endogenous androgen).—Seventeen male rats received 25 I.U. of chorionic gonadotrophin (in aqueous solution) intramuscularly 3 times weekly; 9 survived sufficiently long to permit their inclusion in this report.

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 or scarlet red for fat and Best's carmine stain for glycogen (absolute alcohol fixation).

Vaginal smears were made repeatedly during the experimental period and in every instance at the time of sacrifice.

RESULTS

This communication is concerned with the gross and histologic findings in 111 animals examined after 61 to 375 days of treatment. No tumors have been observed in this strain of rats maintained on the basal diet without addition of a carcinogen.

Liver.—The hepatic lesions have been described in detail previously (4). Degenerative and regenerative changes occurred in some degree in all groups, being generally slight in the controls and most pronounced in those receiving testosterone.

Proliferation of young, cellular, connective tissue was observed in all groups; in no instance was it striking, the Masson stain failing to reveal a sharp increase in collagenous fibers.

There were focal abscesses and areas of chronic inflammation in 10 estradiol-treated animals that had pyometra.

Cystic changes were most striking and most constant. The cysts varied widely in size, shape and number, being most conspicuous in animals receiving estradiol. In some instances these were hemorrhagic and occasionally ruptured into the peritoneal cavity. Occasionally, lesions were observed that conformed to that designated as "cyst-adenoma" by Opie (8). This was encountered most frequently in animals receiving chorionic gonadotrophin.

The neoplastic lesions, similar to those induced
by p-dimethylaminoazobenzene (5, 8, 9), were class-
sified as (a) hepatomas (adenohepatoma and tra-
becular hepatoma) and (b) cholangiomas. Both oc-
curred in multiple foci throughout the liver. He-
patomas were found in 73 animals, in 9 of which chol-
angiomas also were present. Their occurrence in the
various experimental groups was as follows:

Control females (Tables I).—Earliest 265 days; pres-
ent in 23 of 25 animals examined after 265 to 375
days. Metastasis to lung was noted in 4 cases.

Control males (Table II).—Earliest 244 days; pres-
ent in all of 12 animals examined after 244 to 333
days. Metastasis to lung in 1 and mesenteric lymph
nodes in 1 case.

Estradiol (Table I).—Earliest 165 days; present in
10 of 14 animals examined after 165 to 263 days.

PMS Gonadotropin (Table I).—Earliest 199

Days of Treatment | Control | Estradiol | PMS gonadotropin |
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<td>150-200</td>
<td>10</td>
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<td>201-250</td>
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<td>301-350</td>
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<td>351-400</td>
<td>11</td>
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The liver weights, in grams per 100 gm. of body
weight, are indicated in Table III and the pertinent
comparative data for different periods of duration of
the experiment in Tables I and II.

Lung.—Metastases were found in the lung in 10
(13.7 per cent) of the 73 animals with hepatomas.
All occurred late, the earliest (PMS gonadotrophin)
at 278 days. The lesions consisted either of minute
nodular foci of pale polyhedral cells about or within
the lumen of blood vessels, or of grossly palpable
nodules replacing the lung tissue, with adjacent
areas of hemorrhage.

Metastasis to the lung from adenocarcinoma of
the breast occurred in 1 case. Inflammatory lesions
were present in the lungs in 3 animals and hemor-
rhage in 1.

Mammary glands.—Mammary adenocarcinoma
days; present in 9 of 16 animals examined after 199
to 321 days. Metastasis to lung was found in 2
cases.

Testosterone (Table II).—Earliest 206 days; pres-
ent in all of 11 animals examined after 248 to 329
days. Metastasis to lung was found in 2 cases.

Chorionic gonadotrophin (Table II).—Earliest
162 days; present in all of 8 animals examined after
162 to 295 days. Metastasis to lung was found in
2 cases and to mesenteric lymph nodes in 1 case.

**Table I: Primary Neoplasms in Female Animals**

<table>
<thead>
<tr>
<th>Days of Treatment</th>
<th>No. of rats</th>
<th>Primary neoplasm</th>
<th>No. of rats</th>
<th>Primary neoplasm</th>
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</thead>
<tbody>
<tr>
<td>150-200</td>
<td>10</td>
<td>Liver</td>
<td>7</td>
<td>Liver</td>
</tr>
<tr>
<td>201-250</td>
<td>6</td>
<td>Mammary gland</td>
<td>5</td>
<td>Mammary gland</td>
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<tr>
<td>251-300</td>
<td>5</td>
<td>Bladder</td>
<td>3</td>
<td>Bladder</td>
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<tr>
<td>301-350</td>
<td>10</td>
<td></td>
<td>3</td>
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<td>351-400</td>
<td>11</td>
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<td>3</td>
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**Table II: Primary Neoplasms in Male Animals**

<table>
<thead>
<tr>
<th>Days of Treatment</th>
<th>No. of rats</th>
<th>Primary neoplasm</th>
<th>No. of rats</th>
<th>Primary neoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>150-200</td>
<td>1</td>
<td>Liver</td>
<td>2</td>
<td>Liver</td>
</tr>
<tr>
<td>201-250</td>
<td>2</td>
<td>Mammary gland</td>
<td>5</td>
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<td>Bladder</td>
</tr>
<tr>
<td>301-350</td>
<td>8</td>
<td></td>
<td>6</td>
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**Table III: Liver Weight in Respective Groups in Grams per 100 Grams Body Weight**

<table>
<thead>
<tr>
<th>Days of Treatment</th>
<th>Control</th>
<th>Estradiol</th>
<th>PMS gonadotrophin</th>
</tr>
</thead>
<tbody>
<tr>
<td>150-200</td>
<td>5.6</td>
<td>4.3</td>
<td>6.5</td>
</tr>
<tr>
<td>201-250</td>
<td>6.0</td>
<td>5.1</td>
<td>8.5</td>
</tr>
<tr>
<td>251-300</td>
<td>8.5</td>
<td>8.3</td>
<td>15.3</td>
</tr>
<tr>
<td>301-350</td>
<td>3.5</td>
<td>17.4</td>
<td>13.5</td>
</tr>
<tr>
<td>351-400</td>
<td>3.6</td>
<td>10.0</td>
<td>12.0</td>
</tr>
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Since tabulation of these data, mammary carcinoma
has developed in 1 male rat receiving acetaminofluorone
and testosterone for 141 days, and in 2 male rats receiv-
ing estradiol dipropionate for 177 and 181 days.
treated animal (6.7 per cent). It should be pointed out that the highest incidence of mammary tumors in the control group occurred after 320 days and that only 2 of the PMS gonadotrophin-treated and none of the estrogen treated animals were maintained for this length of time. Metastasis from these lesions occurred in 2 instances, to the lung, (PMS gonadotrophin) and 1 to the liver (control) (Fig. 2).

The neoplastic lesions in the mamma consisted of single or multiple unilateral or bilateral nodules, varying in shape and size (diameter up to 2.5 cm.). They were irregularly lobulated, grey-brown in color and often cystic, containing hemorrhagic fluid or dark brown, chocolate-like material.

Microscopic examination revealed proliferation of cuboidal or columnar epithelial cells, with basophilic cytoplasm and hyperchromatic nuclei, either forming cystic structures with papillary projections into the lumen, or tending to form acini or tubules. In some areas the stroma consisted of narrow, connective tissue septa, containing numerous fibrocytes, lymphocytes and polymorphonuclear leukocytes. In other areas the connective tissue was more prominent, with variable cellularity, separating groups of epithelial cells.

Female sex organs.—Examinations of the vaginal smears revealed cyclic changes in the control group and estrogenic stimulation in those receiving estradiol. No abnormality was observed in the vagina, uterus, tubes or ovaries in the control group.

Pyometra was present in 10 of the 15 estradiol-treated rats, the uterine horns containing pus and showing evidence of chronic supplicative metritis on microscopic study. The epithelium of the cervix uteri was commonly of the stratified squamous variety, with hyperkeratosis. Chronic salpingitis occurred occasionally and the ovaries were atrophic in 2 instances in this group.

The PMS gonadotrophin group showed no evidence (vaginal smears and uterine sections) of disturbance of the estrus cycle. The tubes were normal. The epithelium of the cervix uteri was frequently stratified squamous, with varying degrees of cornification. The ovaries contained cysts, varying in size and number, lined either by dark-staining cuboidal cells, as seen in maturing follicles or by pale oval or polyhedral cells with foamy cytoplasm.

Male sex organs.—The criteria proposed by Moore (7) were employed in evaluating cytological changes in the testis, epididymis, seminal vesicle and prostate. The presence of heads of spermatocytes in the tubular lumen or among the lining cells was regarded as evidence of mature type of spermatogenesis. The presence of spermatogonia only was considered indicative of an incomplete type of spermatogenesis and the absence of evidence of spermatogenesis was termed atrophy. The epididymis was examined for the presence of sperm and for evidence of hyperplasia. Seminal vesicles and prostate were studied particularly for the height of the epithelial cells, the location of the nuclei and changes in the cytoplasm, differentiating between the anterior (coagulating gland), middle and posterior lobes of the prostate.

The control animals presented no abnormality in the testes, epididymes, seminal vesicles or prostate.

In the testosterone-treated group, the changes in the sex organs were distinctly less pronounced than those usually observed in rats receiving this substance alone for comparable periods of time. Little testosterone effect was apparent in animals examined up to 300 days of treatment; there was comparatively little increase in size or weight of the prostate, seminal vesicles or preputial glands and little or no change in the testes. After longer periods, the testes revealed less active spermatogenesis, and the seminal vesicles and prostate contained numerous dilated acini, lined by flattened, low columnar or cuboidal epithelium, the lumens either being empty or containing pink granular or amorphous material.

The chorionic gonadotrophin group showed no disturbance of spermatogenesis and the Leydig cells were either normal or stimulated. The seminal vesicles and prostate rather consistently contained dilated acini lined by flattened epithelium.

Kidney.—No significant abnormality was observed in glomeruli or renal vessels. The tubular epithelium

**DESCRIPTION OF FIGURES 1 TO 6**

Fig. 1.—Adenocarcinoma of breast in control rat (carcinogen alone). Mag. X 125.
Fig. 2.—Liver of control rat (carcinogen alone), showing metastasis from mammary carcinoma. Mag. X 125.
Fig. 3.—Squamous-cell carcinoma of bladder in rat treated with chorionic gonadotrophin, Mag. X 60.
Fig. 4.—Papillary squamous-cell carcinoma of bladder in control rat (carcinogen alone). Mag. X 80.
Fig. 5.—Keratinizing squamous-cell carcinoma of external auditory canal in rat treated with PMS gonadotrophin, Mag. X 125.
Fig. 6.—Sarcoma of breast in rat treated with estradiol, Mag. X 160.
showed variable degrees of degenerative change, characterized by swelling and granulation or vacuolization of the cytoplasm, the nuclei being fairly well preserved. The severity of these changes in the control groups was not related to the duration of treatment. Severe tubular degeneration occurred most consistently in the chorionic gonadotrophin group.

Those receiving testosterone and PMS gonadotrophin for prolonged periods showed hyaline and occasional granular casts, in addition to degeneration and dilatation of the tubules.

Urinary bladder.—The transitional epithelium of the bladder was often thickened and stratified, with subjacent infiltration by chronic inflammatory cells. In some cases there were papillary projections into the lumen, lined by transitional or stratified squamous epithelium well-differentiated and with intact basement membrane. Evidence of malignancy was present in 6 instances, the epithelial cells, containing hyperchromatic nuclei, being irregularly arranged and extending beyond the fragmented basement membrane. In 4 of these the cells were squamous in type, with characteristic pearl formations and infiltration into the bladder wall (Figs. 3 and 4).

External ear.—A tumor was present in the external auditory canal in one animal (PMS gonadotrophin). It was a squamous cell carcinoma similar to that described by Bielschowsky (1) as a ductus acusticus tumor (Fig. 5).

DISCUSSION

Our findings differ from those reported by Wilson, DeEds and Cox (10) and by Bielschowsky (1, 3) in the comparatively limited distribution of primary malignant lesions, none occurring in the skin, subcutaneous tissue, muscle, ureter, renal pelvis, intestine, pancreas or lung. We have encountered no tumors of the submaxillary or parathyroid glands (6). Tumors of the thyroid and one of the uterus developed in rats receiving 2-acetaminofluorene and thioracil; these will be reported elsewhere as well findings referable to the hematopoietic system in the entire series.

In the females treated less than 250 days, mammary carcinoma occurred in 2 of 16 controls (12.2 per cent), in 1 of 12 receiving estradiol (8.3 per cent) and in none of 5 receiving PMS gonadotrophin. It was present in 8 of 26 female controls (30.8 per cent) treated for more than 250 days and in 3 of 12 PMS gonadotrophin-treated animals (25 per cent) during the same period. Although too few animals received estradiol to permit evaluation of its influence upon the incidence of mammary carcinoma after more than 250 days of treatment, the conclusion seems justifiable that exposure to increased amounts of estrogen had no significant effect in accelerating the development of this lesion. No mammary tumor was observed in male rats. Bielschowsky (1) found mammary cancer in 23 of 36 females (63.9 per cent) in 3 of 41 males (7.3 per cent), and in 1 of 11 female castrates (9.1 per cent). This difference is particularly interesting in view of the lack of effect of estradiol noted above.

Bladder tumor occurred in 2 females (2.7 per cent) and 4 males (10.7 per cent), hormone therapy being without apparent influence upon its development. No tumors of the bladder were observed by Bielschowsky (1) whereas it was the most common tumor (25.6 per cent) in the series reported by Wilson, DeEds and Cox (10). It should be noted that the latter encountered it most frequently in animals receiving relatively large doses of carcinogen (0.125 per cent of the diet) and that in our series it occurred after longer periods of treatment than were employed by Bielschowsky.

The comparatively low incidence of liver tumors (28.6 per cent) in control animals in our previous report (4) was due to the large proportion treated less than 250 days. This figure rose to 61.8 per cent (34 of 55 animals) in the present series. Bielschowsky (1) emphasized the higher incidence of hepatic malignancy in male rats receiving 2-acetaminofluorene (82.8 per cent as compared to 30.8 per cent in females). Although this observation is apparently corroborated by our data (54.8 per cent in females, 92.3 per cent in males), the difference may be apparent rather than real. Whereas 16 female controls were examined after less than 250 days of treatment, only 3 males were examined during this period, liver tumors being present in none of the former and in 2 of the latter. This apparent sex difference disappeared after more prolonged treatment, the incidence of this lesion being 88.5 per cent in females and 100 per cent in males treated longer than 250 days. Data must be obtained on a larger number of males treated for 170 to 250 days before a definite conclusion may be reached on this point.

The most striking effect of administration of estradiol and PMS gonadotrophin to females and of testosterone and chorionic gonadotrophin to males was intensification of the cystic and neoplastic hepatic lesions induced by 2-acetaminofluorene. Aggravation of the cystic lesions was most pronounced in the case of animals receiving estradiol, testosterone and chorionic gonadotrophin (4).

Although little can be said regarding the time of first appearance of neoplasms of the liver, their occurrence in less than 250 days in 8 of 12 estra-
diol-treated (66.7 per cent) and 2 of 5 PMS gonadotrophin-treated (40 per cent) females as compared with none of 16 female controls (Table I) suggests a significant influence of these agents in this connection. This is indicated also by a comparison of the liver weights of treated and control females during the first 300 days (Table III), which reflects the difference in extent of hepatic involvement in these groups.

The paucity of data on male rats treated for less than 250 days makes it impossible to evaluate the influence of testosterone or chorionic gonadotrophin in accelerating the development of hepatic neoplasms. However, the striking effect of these agents upon the extent of hepatic involvement is indicated by the enormously greater liver weights after 250 days in the hormone-treated as compared with the control males (Table III).

The hepatic lesions have been described and discussed in detail elsewhere (4). As in the smaller series reported previously the occurrence and extent of the cystic and neoplastic lesions was not paralleled by degenerative, regenerative or fibrotic processes in the liver.

The original purpose of this study was to determine whether hyperplasia of the uterus, ovaries, prostate, seminal vesicles, preputial glands and testes, hormonally induced during a period of exposure to 2-acetaminofluorene, would lead to the development of neoplasms in these organs, as in the hyperplastic thyroid produced by antithyroid drugs (2, 4). No tumors have as yet been found in the target organs of the hormones employed, nor was the development of mammary carcinoma in female rats accelerated by administration of estradiol. However, certain peculiarities have been observed in the action of some of these hormones. Whereas the target organ response to estradiol was comparable to that generally obtained in rats receiving this substance alone, that to testosterone was almost nonexistent in many instances and minimal in the remainder.

The absence of a significant testosterone effect upon the prostate, seminal vesicles, and testes is particularly striking in view of its pronounced effect upon the extent of the neoplastic change in the liver (Table III). The possibility is suggested that, under the existing experimental conditions, the metabolism of testosterone in the liver is so altered as to produce a substance which is carcinogenic or cocarcinogenic but only slightly if at all androgenic. A similar hypothesis may be invoked in explanation of the influence of estradiol upon the development of hepatic malignancy. The maintenance of an estrogenic effect may be attributed to a relatively high estrogenic potency of the hypothetical carcinogenic or cocarcinogenic metabolite or to the escape of a portion of the estradiol into normal metabolic pathways.

SUMMARY

Malignancy of the liver, breast, bladder and external auditory canal occurred in Sherman rats receiving 0.03 per cent 2-acetaminofluorene in the diet for 174 to 375 days.

Mammary carcinoma occurred only in females. Its development was apparently not accelerated by administration of estradiol or PMS gonadotrophin.

Malignant lesions of the liver occurred in 54.8 per cent of females and 92.3 per cent of males receiving the carcinogen alone. Administration of estradiol and PMS gonadotrophin to females and of testosterone and chorionic gonadotrophin to males intensified the cystic and neoplastic hepatic lesions induced by 2-acetaminofluorene. The latent period was considerably shortened in the females and the extent of neoplastic involvement of the liver was increased in both sexes.

No tumors occurred in the target organs of the hormones employed. The target organ response to estradiol was comparable to that generally obtained in rats receiving this substance alone, but that to testosterone was almost nonexistent in many instances and minimal in others. The possible significance of these findings is discussed.

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REFERENCES

U. S. Atomic Energy Commission Announces Distribution of “Heavy Water”

Heavy water and deuterium gas are now being made available by the United States Atomic Energy Commission for research purposes within the United States. The abundance of the heavy hydrogen isotope in the material is approximately 99.8 per cent. Quantities will be limited to normal research requirements.

The Stuart Oxygen Company of San Francisco, California will act as contracting agent for distribution. This company began small-scale production and distribution soon after the discovery of deuterium. In the post war period, because of the increased need for deuterium in research, the Stuart Company’s production has been far below the demand. The quantities being made available are from stock produced by other operators during the war for the Manhattan District.

The material will be distributed with charges based on the cost of handling and distribution. The cost of production of the material itself will not be included. Distribution in this manner is being effected under authority of the Atomic Energy Act of 1946 which provides for the fostering and assistance of research by the Commission.

Allocation will be handled in a manner similar to that for radioisotopes. For complete information write to U. S. Atomic Energy Commission, Oak Ridge, Tennessee, Attention: Isotopes Branch.
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