The Carcinogenic Activity of 2-Acetaminofluorene
Characteristics of the Lesions in Albino Rats*

Alvin J. Cox, Jr., M.D., Robert H. Wilson, Ph.D., and Floyd DeEds, Ph.D.

(From the Department of Pathology, Stanford University School of Medicine, San Francisco, California, and the Pharmacology Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Albany, California.)

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Attention has been called to the development of a variety of tumors in rats and mice after the administration of 2-acetaminofluorene by mouth (2, 11). Species differences in susceptibility and in distribution of proliferative lesions have been noted (1, 12), but animals of a single strain have shown fairly consistent development of characteristic lesions. We have used principally albino rats originally of the Stanford strain (11), so the discussion to follow will deal with tumors in these highly susceptible animals.

It is probable that the tumors are induced not by the acetaminofluorene itself, but by some derived product, possibly aminofluorene (13). The nature of the resultant tumors is similar whether acetaminofluorene or aminofluorene is administered to rats.

Focal proliferation of a number of different types of epithelium has appeared following continued feeding of acetaminofluorene in concentrations in the diet ranging from 0.5 per cent to 0.004 per cent. The other characteristics of the diet have been described previously (11). The nature of the resultant tumors is similar whether acetaminofluorene or aminofluorene is administered to rats.

Table I indicates the incidence and distribution of hyperplastic changes in the 84 rats that had more than one type of lesion and have given more information concerning the frequency of proliferative changes. Tissues from 108 rats which had lesions apparently due to acetaminofluorene have been studied histologically. The animals died or were killed at various intervals after administration of the substance in different quantities, so that the circumstances under which the tumors developed are not closely comparable. Since some of the animals, including all that were fed more than 0.125 per cent acetaminofluorene in the diet, were killed or died early in the course of an experiment before advanced lesions appeared, it was decided to include in this report only those rats which, because of the appearance of multiple lesions, had presumably received a thoroughly effective exposure to the compound. There were 84 such animals, 22 males and 62 females, and in most of them opportunity for the development of multiple lesions was great because they lived until their condition was poor or until large external lesions had appeared. The period of observation ranged from 104 to 695 days following the institution of the augmented diet, which contained from 0.008 per cent to 0.125 per cent acetaminofluorene for intervals not always as long as the observation periods.

Table I: Incidence of the Most Common Local Hyperplastic and Neoplastic Lesions in 84 Rats (22 Male and 62 Female) in Which Two or More Tissues Were Involved

<table>
<thead>
<tr>
<th>Organ</th>
<th>Number of animals with lesions</th>
<th>Total incidence, malignant,</th>
<th>Proportion %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>21</td>
<td>57</td>
<td>93</td>
</tr>
<tr>
<td>Bladder</td>
<td>16</td>
<td>51</td>
<td>80</td>
</tr>
<tr>
<td>Lung</td>
<td>8</td>
<td>24</td>
<td>38</td>
</tr>
<tr>
<td>Head</td>
<td>5</td>
<td>21</td>
<td>31</td>
</tr>
<tr>
<td>Breast</td>
<td>3</td>
<td>23</td>
<td>31</td>
</tr>
<tr>
<td>Kidney</td>
<td>3</td>
<td>17*</td>
<td>24</td>
</tr>
<tr>
<td>Uterus</td>
<td>13†</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>Thyroid</td>
<td>2</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>1</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

*Includes one subcutaneous fibroma.
†Includes one carcinoma.

Table I indicates the incidence and distribution of hyperplastic changes in the 84 rats that had more than one type of lesion. Since the acetaminofluorene was administered in different dosages in different animals, the indicated incidence of the various lesions is of value principally in showing the relative frequency of the different changes. After autopsy and fixation of the tissues in 4 per cent formaldehyde, histological sections stained with hematoxylin and eosin were prepared from all grossly abnormal tissues except for 2 mammary

*The experimental work on which this paper is based was completed in 1942, but because of the pressure of other work the authors were unable to prepare their results for publication.
tumors, which were lost. Many organs without gross lesions were also sectioned. In all there were sections from the liver of 83 animals, the urinary bladder of 79, the lung of 78, the kidney of 77, the pancreas from 76, the thyroid gland from 60, the ovary from 37, and the uterus from 32 animals. Numerous sections of heart, spleen, testis, stomach, intestine, thymus, hypophysis, salivary gland, skin and bone were examined histologically but they showed no proliferative lesions in addition to those identified by gross examination. Thirty-nine brains examined histologically have shown no lesions suggesting that described as a glioma by Lopez (7), and no other abnormalities have been found in them.

There was much variety in the degree of change in the affected organs of different animals, ranging from focal proliferative lesions which were visible only microscopically, to large grossly recognizable nodules which greatly distorted the shape of the involved organs. Multiple nodules in the liver commonly increased its size by more than 100 per cent. The lesions have not been classified according to size and no distinction between nodular hyperplasia and adenoma formation has been made because no basis has been found for anatomical separation of these states among the experimental lesions. However, an attempt has been made to classify lesions as malignant if there was infiltration of abnormal cells among pre-existing tissue elements. In cases where there was only questionable slight hyperplasia the tissue has been listed as unaffected, and no lesions are recorded as malignant unless distinct infiltration of adjacent tissues could be recognized histologically. It is likely that some instances of malignancy were not detected by this method. In general, the extent of the hyperplasia was great and cell differentiation was poor when infiltration of tumor cells was present, but there were several exceptions. Most of the tumors were composed of epithelial cells, although 5 animals had leukemia and 2 had sarcomas. One of the latter arose in the muscles of the leg and the other developed in the uterus.

The following paragraphs present the types of tissue change that have occurred with sufficient frequency to suggest that they are specific effects of the administration of acetaminofluorene.

Liver nodules.—These were the commonest and most prominent lesions, causing gross deformity of the liver in more than half of the animals. The lesions were always multiple and some reached a diameter of 3 or 4 cm. Most of the nodules were composed of cords of hepatic cells which were similar to, but distinctly different from the normal liver cells (Fig. 1). The cords were sometimes irregular and did not form a lobular pattern, although they bordered prominent sinusoidal spaces. No portal connective tissue spaces could be seen within the nodules. The cytoplasm of the abnormal cells had a varied appearance. In some parts it was more dense and more uniformly stained than normal, but in others it had a reticulated appearance or was distinctly vacuolated. Its volume per cell was distinctly greater than normal. The nuclei were large and quite varied in size and intensity of staining. Nucleoli were large and sometimes multiple. Mitotic figures were prominent in some of the larger nodules. Bordering the sinusoids there were occasional prominent endothelial cells resembling Kupffer cells. In a few places these contained a little light brown pigment that did not give the Prussian blue reaction for iron. There was no evidence that the abnormal nodules of hepatic cells were drained by bile ducts, yet most of them were not distinctly jaundiced. Several of the larger nodules, however, were grossly yellowish and they showed scattered small deposits of iron-free yellow pigment not only in the endothelial cells, but also in some of the hepatic cells.

In many of the livers there were additional nodules of quite different appearance. These were composed of small duct-like structures and cysts which had a lining of simple low columnar or flattened epithelium (Fig. 2). Separating these structures was a little dense fibrous tissue. Certain of the cysts were multicellular and among them in a few places were groups of hepatic cells which appeared to have been isolated from pre-existing

**DESCRIPTION OF FIGURES 1 TO 6**

**FIG. 1.**—Small nodule of hyperplastic hepatic cells adjoining normal liver tissue. At the edge of the nodule on the right is a central vein. Mag. X 120.

**FIG. 2.**—Nodule in liver composed entirely of cysts lined by low columnar epithelium. Mag. X 48.

**FIG. 3.**—Lung nodule formed by proliferated epithelial cells within alveoli. This is apparently benign. Mag. X 48.

**FIG. 4.**—Similar lung tumor. Mag. X 120. Clumps of cells in alveolar lumen can be distinguished from layer of alveolar lining cells.

**FIG. 5.**—Epithelial tumor arising from renal pelvis. Narrow rim of atrophic kidney tissue borders it on the left. Mag. X 48.

**FIG. 6.**—Border of a malignant tumor of squamous epithelium arising adjacent to the deep end of the external auditory canal. Mag. X 120. Projections of tumor cells have infiltrated skeletal muscle.
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Figs. 1-6
liver tissue. The cells forming these cysts were more uniform than those of many of the solid nodules.

Frequently the same liver contained nodules of different types, and when one nodule showed malignant changes, others appeared benign. In the presence of large tumors, other parts of the liver regularly contained microscopic groups of enlarged cells forming discrete but non-encapsulated nodules. In some of the nodules of hepatic cells there were numerous small spaces often reaching a diameter of 1 mm. These were bordered by flattened cells and produced an appearance suggesting abnormal dilated bile ducts. These duct-like structures appeared to be integral parts of the hyperplastic nodules, although the manner of their development was not determined. These nodules containing hepatic cords and glandular structures correspond to the adenohepatomas described by Opie (8) after feeding p-dimethylaminoazobenzene. Two of the malignant liver tumors contained duct-like structures associated with other cells resembling hepatic cells.

Several of the large partially necrotic lesions were accompanied by infiltration of a variety of inflammatory cells but this was not observed in small lesions which were likewise unassociated with any fibrosis of the liver substance.

**Nodular proliferation of epithelium in the lungs.**—In lungs from 22 rats there was a characteristic sort of focal epithelial proliferation similar to that which has been observed in mice by many investigators. In 17 animals multiple lesions were found. A number were demonstrable only histologically, and since from most animals only one or two sections of lung tissue were prepared, it is certain that other undisclosed small lesions were present. Inflammation was prominent in some lungs and it is possible that instances of nodular epithelial proliferation were concealed by it. However, many of the nodules were not associated with inflammation. The lungs from control animals have not been examined by serial sectioning, but in numerous sections from old animals which did not receive acetaminofluorene, no lesions of this type have been seen.

Most of the pulmonary nodules were less than 4 mm. in diameter. They were localized though not encapsulated, and their appearance suggested a benign proliferative process. The principal constituents were abnormal epithelial cells within the alveolar spaces (Figs. 3 and 4). The pulmonary structure persisted within the nodules but the alveolar spaces were smaller than those elsewhere. Each alveolus in the altered region was lined by a layer of simple cuboidal or low columnar cells and in most of the spaces there were also small solid masses of polygonal, similarly stained cells. A small cleft usually separated these from the layer bordering the alveolar wall. The proliferated cells had nearly round nuclei with prominent small chromatin particles but no large nucleoli. Scattered nuclei were hyperchromatic and there were a few mitotic figures. The cells commonly appeared columnar on one side of an alveolus and cuboidal or even flattened on another. In a few places, clumps of 4 or 5 nuclei were very closely placed, suggesting formation of small syncytial masses. Most of the cells had scanty, slightly acidophilic cytoplasm. The appearance suggests that these may have been derivatives of normal alveolar lining cells. Small bronchi were present within some of the nodules but their epithelium was distinctly different from that of the intra-alveolar cells. The lesions in our rats were similar to those induced in mice with dibenzanthracene and methylcholanthrene by Grady and Stewart (5), who have presented satisfactory evidence based upon the study of serial sections, that the proliferating cells of their tumors originated in the alveoli.

The lungs from 5 additional acetaminofluorene-treated rats contained larger masses composed of less uniform cells which had destroyed lung tissue and had penetrated through the pleura or into large bronchi. The cells of these malignant tumors were small, they were arranged in small irregular clumps and strands, and their appearance suggested an epithelial origin. In places they showed a resemblance to the cells of the benign lesions. A lung from one additional animal showed a small papillary intrabronchial benign tumor in which the epithelium was similar to that of the bronchial wall.

It was suggested in our original report that the metaplastic squamous epithelium lining bronchi in certain regions of chronic inflammation in some lungs might be a specific effect of acetaminofluorene feeding. Similar regions of metaplasia have been found in additional animals, but they have also appeared in a number of old rats with chronic pulmonary inflammation which did not receive any carcinogenic substance and which showed no other lesions like those in the acetaminofluorene-treated animals. Therefore, this bronchial epithelial metaplasia cannot be regarded as a specific effect.

**Epithelial proliferation in the urinary tract.**—With the exception of a benign tubular adenoma in one kidney, all of these lesions have been derived from transitional epithelium of the bladder, renal pelvis and ureter. The lesions were always focal.
and were usually multiple. There was wide variation in their size as well as their character. Small lesions sometimes consisted of no more than slight local thickening. The cells in the thickened zones were usually enlarged and arranged less regularly than in normal transitional epithelium. In many instances they resembled stratified squamous epithelium and some showed various degrees of keratinization. Most of the nodules were flat but elevated papillary lesions were common. Malignancy was recognized by deep penetration of irregular cords of tumor cells, which were usually less differentiated than the cells of the benign lesions. They frequently resembled stratified squamous epithelium rather than transitional epithelium. Some surrounded small foci of keratinization.

The hyperplastic changes were much more frequent and more prominent in the bladder than in the remainder of the urinary tract. Several instances previously reported as hyperplasia of the kidney pelvis are omitted from the present classification since they were minimal. Three carcinomas of the renal pelvis were present, although infiltration of adjacent tissue was not extensive. All these tumors were papillary in some parts; 1 was composed of keratinizing squamous epithelium, whereas in 2 the tumor cells resembled transitional epithelium (Fig. 5). A single tumor in the region of the upper end of one ureter was a squamous cell carcinoma. Since routine histological examination of the ureters was not made, no knowledge of the incidence of small ureteral lesions is available.

Subcutaneous tumors of the side of the head.—These tumors all appeared just anterior to the ear and were covered by intact movable skin when they were small. They were composed largely of squamous cells in abnormal arrangement. Usually the central part contained a cavity filled with desquamated keratinized material. The wall was formed by irregular masses of stratified squamous epithelial cells of varied size and staining reaction. These were supported by thin fibrous strands and frequently surrounded small masses of keratin. At the periphery in all but two instances there was infiltration of adjacent structures by tumor cells (Fig. 6), and the appearance was characteristic of squamous cell carcinoma. The two apparently benign lesions were papillary tumors nearly 1 cm. in diameter lying within cysts which occupied the same position as the infiltrating tumors. The cyst walls were lined by stratified squamous epithelium which had a smooth outer surface except for some blunt rounded projections of epithelium and a number of protruding lobules of sebaceous gland tissue in one place. The papillary portion of these tumors protruded into the cyst cavity and was composed of long thin folds covered by keratinizing stratified squamous epithelium. Parts of the cyst were packed with desquamated keratinized material.

The origin of all of the tumors of the head was apparently the same. They all developed in the same location and had a similar appearance. Although they sometimes perforated the auditory canal and were always closely attached to it, the lining of the canal was intact over several tumors except for a single small round hole in each case. The largest of these openings was 2 mm. in diameter; each had a smooth border and led into a cavity within the tumor. These openings suggested dilated duct orifices and together with the other features of these tumors, pointed to a probable origin from some adjacent accessory structure, rather than from the lining of the auditory canal. The most likely origin is the sebaceous glands which are normally prominent adjacent to the auditory canal in this region. The skin of the head was freely movable over the smaller lesions and can be eliminated as the point of origin even though the large tumors frequently produced ulceration of the skin. Parotid gland tissue was present adjacent to some of the tumors but there was a separating layer of fibrous tissue and the appearance did not suggest an origin from this tissue.

Tumors of the breast.—In our original report the mammary origin of several subcutaneous glandular tumors was not established with certainty, although it was suggested as the most likely possibility. In the animals studied subsequently, it has been possible to identify mammary tissue immediately adjacent to the tumors and there have been several instances of irregular hyperplasia of mammary tissue without distinct tumor formation (Fig. 7). The structural similarity of some of the subcutaneous tumors to portions of the hyperplastic mammary tissue provides further evidence that the tumors were of mammary origin.

In the hyperplastic glands there were usually irregular groups of mammary ducts, frequently somewhat dilated, and among some of them there was more than the usual amount of fibrous tissue, showing mild lymphocytic infiltration. Frequently there were prominent lobules of small acini. Thirty-two grossly visible tumors developed in 26 animals. Most of these were composed of irregular small glandular structures resembling mammary
ducts, lined by cuboidal or pseudostratified epithelium and scattered through different amounts of fibrous stroma. Some contained groups of structures resembling acini (Fig. 8). In some the connective tissue was prominent, as in fibroadenomas, but since there was no sharp dividing line among 19 of these benign tumors, they have all been classed as adenomas. One of the smaller lesions lay in a cyst-like space about 1 cm. in diameter lined by low columnar epithelium which was frequently piled up to a layer several cells thick. The intracytic nodule was 3 mm. broad and was attached on one side. It was composed of small irregular glands in a little cellular stroma.

Nine additional tumors were classed as malignant. These showed less uniform glandular structures than did the benign tumors, and there were commonly thin cords of atypical epithelial cells without demonstrable lumen. Two of the tumors contained cystic spaces lined by columnar cells and enclosing protruding masses of glandular tumor tissue, suggesting pre-existing papillary adenomas.

Two benign subcutaneous tumors were composed only of fibrous tissue without any glands. The origin of these is not certain, although their location was similar to that of the glandular tumors, and bordering the one which appeared in a male rat was a layer of mammary tissue. Two other subcutaneous tumors apparently of mammary origin were not examined histologically because the tissue was lost. They have been classified as benign lesions.

Nearly all the subcutaneous tumors developed in female rats, but 3 appeared in males. None of the tumors in the males has been regarded as malignant.

These observations indicate that the mammary gland in acetaminofluorene-treated rats undergoes changes comparable to those of other organs showing nodular hyperplasia in association with, and presumably preceding, tumor formation. The frequency of this hyperplasia is not known, since the mammary glands were not subjected to routine histological study.

**Nodular proliferation of endometrial glands.**—Eight uteri from the experimental animals showed local enlargements up to a diameter of 5 mm., and of 32 uteri which were examined histologically, 13 showed irregularities of the endometrium. These lesions all appeared in animals surviving for more than 250 days following the onset of treatment; most of the animals were more than 400 days old. It is possible that ageing played a more important part in the development of these changes than in most of the others observed in this group of animals, although comparable alterations have not been seen in other old rats.

Some of the glands in the altered zones resembled normal endometrial glands. They were small, fairly uniform, and lined by a single layer of cuboidal or low columnar epithelium. There was an accompanying increase in endometrial stroma, which sometimes contained a few scattered lymphocytes and brown pigment-filled macrophages, suggesting that bleeding had occurred. Not infrequently some glands were dilated to a diameter of more than 1 mm. These cyst-like spaces contained a small amount of eosinophilic coagulum and their lining epithelium was usually flattened.

The proliferation was eccentric with respect to the lumen and the myometrium was expanded over the surface of the proliferated nodules (Fig. 9). There was no evidence of malignancy. The lower portion of one uterus was destroyed by a large sarcoma composed chiefly of spindle-shaped cells. It apparently arose in this organ.

**Thyroid gland nodules.**—In our first animals no proliferative nodules were noted in the thyroid gland. Bielschowsky (3) has stated that acetaminofluorene alone does not evoke thyroid hyperplasia. However, in 11 of the animals more recently studied and included in the present series there was distinct nodular irregularity of the histological structure of this gland. A possible factor of importance is that these animals were older at death than most of the animals studied previously. In four glands the nodular appearance was due principally to variation in size of follicles and in amount of colloid contained. There were several distinct but not encapsulated nodules up to 1 mm. in diameter (Fig. 10). Among abnormally large colloid-filled follicles in the nodules there were usually small empty follicles. The proliferative nature of the process is evidenced by epithelial cells of normal or even increased height forming the large follicles. In five adenoma-like nodules much of the epithelium was composed of tall

**DESCRIPTION OF FIGURES 7 TO 10**

**Fig. 7.**—Irregular hyperplasia of mammary tissue without formation of distinct tumor. Mag. × 48.

**Fig. 8.**—Benign fibroadenoma of mammary glands, remnant of which appears above the tumor. Mag. × 120.

**Fig. 9.**—Nodule of hyperplastic endometrium. This presents no evidence of malignant growth. Mag. × 48.

**Fig. 10.**—Nodule in thyroid gland formed by large follicles with abnormally tall lining epithelial cells. Mag. × 120.
columnar cells. It formed projecting folds into the lumen of moderately large collapsed follicles which contained very little colloid. The structure in these nodules had little resemblance to the normal thyroid, but their origin in this organ seems certain. Several lay deep in the substance of the thyroid gland and none were situated upon the surface as would be expected if they were derived from the parathyroid glands. These in some sections were demonstrable as separate structures.

Tumors of parathyroid glands, which have been reported after acetaminofluorene administration by Heiman and Meisel (6), have not been recognized in our animals.

**Tumors of the stomach and intestine.**—One small adenocarcinoma of the stomach was situated beneath a shallow ulcer 2 mm. broad in the pyloric zone where a mass of atypical small glands extended into the submucosa (Fig. 11). There was also heavy infiltration of small mononuclear cells. There was not much variation of the tumor cells but the structures formed did not closely resemble normal mucosal glands. They reached the muscularis but did not penetrate it. The tumor cells contained a number of mitotic figures. Another glandular tumor of the submucosa of the stomach has been classed as an adenoma because the tubular structures forming it were more uniform than those of the tumor described above and it formed a well circumscribed, though not encapsulated, nodule.

A somewhat similar tumor was situated in the ileum of another animal. It lay principally in the submucosa and elevated the mucosa, which was not sharply distinguishable from the tumor in some places. Most of the tumor cells contained vacuoles and at the deep margin there were several glands containing Paneth cells. Although this tumor reached the muscularis, it had a sharp border and has been classified as benign.

The colons in 2 animals contained extensively infiltrating adenocarcinomas composed of moderately irregular, coarse, glandular structures with a lining of columnar epithelium. These tumor cells penetrated the entire thickness of the intestinal wall (Fig. 12). In one of these animals there were multiple metastatic growths.

**Changes in the pancreas.**—In our original report, microscopic nodules of prominent pancreatic acini were described in more than half of the animals in which the pancreas was examined histologically. There was also one carcinoma apparently of pancreatic origin. These lesions have been seen much less frequently in subsequent animals and no further instances of tumor of this organ have been found. There appears to be little doubt of the proliferative character of this change when the lesions are marked, but review of the sections from the original series throws doubt upon the significance of those changes reported as slight. Further observations are necessary to determine the importance of the pancreatic changes. In an accessory lacrymal gland of one rat a tiny sharply defined group of slightly enlarged acini resembled the pancreatic lesions. In several others clusters of tiny cystic spaces were seen in the pancreas, but these were not numerous and it is not possible at present to relate them to the acetaminofluorene feeding.

**Leukemia.**—Five of the animals had leukemia, with infiltration of multiple organs. In each case the liver and spleen were involved. Leukemic cells were present in all parts of the liver lobules, and many cells lay within the sinusoids. The appearance of these abnormal cells suggested that they were myelogenous, although studies of the cells in smears have not been made. The incidence of leukemia in the acetaminofluorene-treated animals is considerably higher than that in control animals of our colony, but leukemia has been observed frequently under other experimental conditions (13), and therefore the relation of leukemia to the acetaminofluorene feeding is less distinct than in the case of most of the other lesions described above.

**Miscellaneous proliferative lesions.**—A sarcoma situated among the leg muscles was described in our original report. The other sarcoma that we observed is discussed in conjunction with the uterine changes. The development of only 2 sarcomas in contrast to 76 malignant epithelial lesions emphasizes the relative rarity of tumors in the connective tissue of animals fed this agent. A malignant tumor in the mediastinum of one animal infiltrated the pericardium extensively to form a firm white layer 2 to 3 mm. thick completely en-
casing the heart. The tumor cells were polygonal in places, but elsewhere they were elongated and spindle-shaped. Around a number of small necrotic foci the cells were larger and had a palisade arrangement. Similar palisading of the cells occurred at the surface of the epicardium. The cells were not uniform, many contained mitotic figures, and groups of them frequently lay within small vessels bordering the myocardium. It is suggested that this is a tumor of the thymus, although proof of its origin has not been obtained.

**Metastasis from malignant tumors.**—Metastatic lesions were found in 13 of the 52 animals bearing carcinomas. These secondary tumors were usually multiple but were of only 2 types. No animal showed metastasis from more than 1 tumor. In 11 instances metastasis occurred from liver tumors. The secondary nodules, which were structurally like the hepatic cell tumors in the liver, were found in the lungs (Fig. 13) of 8 rats, in the peritoneum and adjacent tissues of 4, and in the lymph nodes of 1 animal. No metastatic nodule contained any cyst-like structures. The other type of metastasizing tumor was adenocarcinoma (Fig. 14). Secondary growths of this type occurred in only 2 animals and were limited to tissues covered by peritoneum. In one the primary tumor arose in the colon and in the other the primary tumor was not found.

None of the tumors of the breast, urinary tract, lung or head, and neither of the 2 sarcomas, had spread to distant sites, although in several instances small satellite tumor nodules were seen adjacent to a primary tumor.

**DISCUSSION**

The present discussion of tumors resulting from the feeding of acetaminofluorene has been limited to their appearance in rats, but we have observed many of the same types in several strains of mice subjected to similar treatment. The frequency of involvement of particular organs differed with species and with different mouse strains, but when they appeared in mice the lesions were like those of the rat, similarly located (12).

It has been mentioned above that acetaminofluorene may not itself be the active carcinogenic principle. Bielschowsky (2) has reported the appearance of tumors when aminofluorene is applied to the skin. Histological study of tissues from 6 rats and 7 mice developing lesions after aminofluorene feeding has revealed lesions that are essentially the same as those produced by acetaminofluorene (5).

Most of the distinct tumors observed have appeared in organs which were also the site of frequent irregular hyperplasia of cells of the same type as those producing the tumors. In each such organ it has been impossible to draw a sharp line of distinction between non-neoplastic hyperplasia and tumor formation. The hyperplasia never involved all parts of an organ or tissue uniformly, but appeared only in foci, which suggests that there was gradual development of some of these foci until large, well defined nodules were formed, or until sufficient penetration of adjacent tissues had taken place to classify the process as malignant growth. In portions of lesions which showed such "invasion," the cellular structure was sometimes indistinguishable from that of other lesions which were localized. No evidence was seen to suggest a sudden transformation of hyperplastic to neoplastic cells, or of benign to malignant cells. Many authors have expressed similar conclusions with respect to experimental skin tumors and Willis (9, 10) has collected evidence suggesting that human skin tumors do not arise by a single sudden transformation of epithelial cells.

The great frequency and invariable multiplicity of nodules in the liver may be the result of greater susceptibility of the liver cells to the effect of the carcinogenic agent or to more intimate contact of the agent with the liver cells than with those of other organs. Our observations do not clarify this problem. The findings of Bielschowsky (3) who reported that combined administration of allylthiourea and acetaminofluorene will produce benign and malignant tumors of the thyroid in rats, suggest that some factor in addition to the acetaminofluorene is important in the localization of the carcinogenic effect, and that this element may be provided or enhanced by certain types of stimulation of tissue growth. While it is possible that such factors may play a part in the development of all tumors following acetaminofluorene administration, it is impossible at present to identify them. The livers in our animals did not show frequent cirrhosis such as that found after feeding p-dimethylaminoazobenzene to rats, and we have seen no other morphological changes that might suggest a predisposing condition. Similarly, in other organs which were the site of proliferative changes, there was no reason to suspect any specific predisposing factor. Inflammation was associated with some of the pulmonary lesions, but a number were entirely without evidence of inflammation. Also, a number of inflammatory lesions in the lungs and elsewhere were not accompanied by any unusual proliferative changes.

The rarity of the development of tumors in any but epithelial cells following the administration of
acetaminofluorene, and the repeated localization of lesions in the same organs, suggest a specificity of the carcinogenic effect, possibly related to the metabolism or excretion of the substance. On the other hand, the appearance of nodules of two types of proliferating cells in frequent association in the same liver emphasizes the lack of complete specificity of the carcinogenic agent. The possibility that the agent acts merely by acceleration of a latent tumor-forming capacity of the treated animals, as has been visualized by Engelbreth-Holm (4) and others, cannot be excluded. Such a mechanism might account for the differences in frequency of the various tumor types in different strains of animals after administration of acetaminofluorene.

SUMMARY AND CONCLUSIONS

1. Oral administration to rats of small quantities of acetaminofluorene has been followed by the development of a wide variety of tumors in different tissues. Most of the tumors are derived from epithelial cells.

2. Most tissues that give rise to tumors are also the sites of nodular epithelial hyperplasia which is not distinctly neoplastic. No sharp distinction can be made between these hyperplastic nodules and the tumors formed by similar cells.

3. Malignancy can be recognized in some of the tumors by the occurrence of tumor cell infiltration and metastasis.

4. The factors determining the localization of the experimental tumors are not known.
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