Renal and Pulmonary Tumors in Rats Fed Dimethyl Nitrosamine

FREDERICK G. ZAK, J. HEINRICH HOLZNER,† EDWARD J. SINGER, AND HANS POPPER

(Department of Pathology, The Mount Sinai Hospital, New York, N.Y.)

SUMMARY

Renal and pulmonary neoplasms developed in male and female Sprague-Dawley rats fed a diet containing 125 ppm dimethyl nitrosamine (DMN) for longer than 80 days. The tumors found in the kidneys can be classified into two types: (a) benign solid and cystic adenomas occurring in rats fed DMN for less than 160 days; (b) anaplastic (possibly malignant) epithelial tumors in animals treated longer than 160 days. Characteristic nuclear alterations were observed in the cells of the proximal convoluted tubules and are interpreted as the earliest morphological manifestation in the kidney of DMN intoxication.

Stimulated by the observation of the occurrence of two cases of cirrhosis of the liver among workers handling dimethyl nitrosamine (DMN), Barnes and Magee (1) studied this compound in experimental animals, particularly in rats. These authors found that, when DMN was fed in a concentration of 50 ppm in a normal diet, primary liver neoplasms, seven of which showed metastases, developed between 26 and 42 weeks in nineteen of twenty animals (11). Recently, the same authors reported the occurrence of renal tumors in a relatively high incidence after feeding DMN to rats (12).

During a study of precancerous changes in the liver, with the use of this compound, among others, multicentric tumors arising in kidneys and in lungs were observed before the findings of Magee and Barnes (12) were known. These observations are reported in confirmation of those of the latter authors.

MATERIALS AND METHODS

Male and female Sprague-Dawley rats, 78 in number, with an average body weight of 100 gm., were placed on the standard low choline diet (0.03 per cent) used in this laboratory (6), supplemented by 125 ppm of DMN, while 33 animals were kept as controls on the basal diet alone. Subtotal hepatectomy was performed in twenty animals (thirteen experimental and seven controls), and sham laparotomy in 23 (seventeen experimental and six controls) before feeding of the toxic diet. Of the 111 rats employed, twenty died spontaneously and were excluded from the study.

The rats were sacrificed by cardiac exsanguination under ether anesthesia. Body weights at sacrifice are given in Table 1. After gross inspection tissues were fixed in formalin or Carnoy’s mixture, dehydrated, and embedded in paraffin. The following staining methods were used: hematoxylin-eosin, chromotrope-2 R aniline blue, Gomori silver impregnation, methyl green-pyronine, periodic acid-Schiff (PAS) reaction after diastase digestion, and toluidine blue. In some cases reactions for different enzyme activities (alkaline phosphatase at pH 9.2, adenosine triphosphatase) were performed.

RESULTS

Animals fed DMN for less than 80 days did not show any neoplastic process in the kidneys, although some atypism was seen in the cells of the cortical tubules.

1 The DMN was obtained from Delta Chemical Works, New York, the fraction distilling at 150°C. (uncorr.) being used.

† We are indebted to Dr. Kenneth Weinbren for his help with this phase of the study.
Small nodules, visible only microscopically, were found in the kidneys of six of eighteen rats treated for a period of 80–120 days, and in six of ten animals fed DMN for 120–160 days (Tables 2 and 3). Seven of eight animals, which were on the diet for more than 160 days, developed nodules varying from barely visible up to 9 mm. in diameter. The smaller nodules appeared as solid, well outlined structures of whitish-grey color, localized exclusively in the renal cortex. The larger neoplasms occupied extensive parts of the organs (Fig. 1) leaving only small areas of compressed renal tissue between them. These tumors displayed areas of hemorrhage and necrosis. They extended into the medulla and caused a bosselated appearance of the kidney without invading the capsule.

Microscopically the tumors present two different types. All the neoplasms arising in rats fed DMN for less than 160 days, i.e., all the nodules visible only microscopically, looked very similar. They consisted of nests and cords of large cells without lumen formation. The cytoplasm showed basophilic staining, and the nuclei were large, vesicular, and somewhat irregular in shape. Mitoses were common and sometimes atypical. The nuclei contained large nucleoli, often increased in number. Within these nodules smaller groups of cells were separated by a delicate collagenous stroma. The nuclei of the stromal cells could be easily differentiated from the tumor cells and correspond to typical fibroblasts and fibrocytes. The outline against the surrounding tissue was sharp, the tubules in the vicinity being slightly compressed. Nodules of larger size showed central necrosis. These solid nodules were combined in some cases with cysts of different size which resembled dilated tubules except for the quality of their epithelium (Fig. 2). The lining cells were similar to those forming the solid nodules. They were often arranged in two or more layers without regularity, sometimes forming papillary projections into the lumina. These cysts were also found without connection to solid structures. The arrangement of the stroma was the same as described above. Toluidine blue staining produced a

### TABLE 1

<table>
<thead>
<tr>
<th>ANIMALS</th>
<th>PERIOD (DAYS)</th>
<th>AVERAGE BODY WEIGHTS OF EXPERIMENTAL AND CONTROL RATS AT SACRIFICE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–79 (gm.)</td>
<td>80–120 (gm.)</td>
</tr>
<tr>
<td>Controls</td>
<td>214 ± 27 (14)*</td>
<td>233 ± 61 (7)</td>
</tr>
<tr>
<td>Experimentals</td>
<td>179 ± 24 (10)</td>
<td>206 ± 33 (10)</td>
</tr>
<tr>
<td>Partial hepatect., controls</td>
<td>243 ± 3 (2)</td>
<td>244 ± 30 (2)</td>
</tr>
<tr>
<td>Partial hepatect., experimental</td>
<td>147 ± 20 (5)</td>
<td>240 ± 10 (5)</td>
</tr>
</tbody>
</table>

* Means ± average deviation. Parentheses indicate number of animals sacrificed in each period.

### TABLE 2

<table>
<thead>
<tr>
<th>PERIOD (DAYS)</th>
<th>0–79</th>
<th>80–120</th>
<th>121–160</th>
<th>161–224</th>
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<tr>
<td><strong>Experiments</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No. surviving</td>
<td>57*</td>
<td>37</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td>No. dying</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>No. with kidney tumors</td>
<td>0/19</td>
<td>4/15</td>
<td>5/0</td>
<td>7/8</td>
</tr>
<tr>
<td>No. with lung tumors</td>
<td>0/19</td>
<td>0/15</td>
<td>2/0</td>
<td>5/8</td>
</tr>
<tr>
<td>No. with liver damage</td>
<td>5/19</td>
<td>11/15</td>
<td>6/0</td>
<td>8/8</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. surviving</td>
<td>26</td>
<td>12</td>
<td>5</td>
<td>3</td>
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<td>No. dying</td>
<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. with kidney tumors</td>
<td>0/14</td>
<td>0/7</td>
<td>0/2</td>
<td>0/3</td>
</tr>
<tr>
<td>No. with lung tumors</td>
<td>0/14</td>
<td>0/7</td>
<td>0/2</td>
<td>0/3</td>
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<tr>
<td>No. with liver damage</td>
<td>0/14</td>
<td>0/7</td>
<td>0/2</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* Denotes no. of animals surviving until time of sacrifice.
slight metachromasia of the cells of both the solid and the cystic tumors. In addition to the unquestionable neoplastic changes, solid nests of cells were found, not exceeding the size of a single tubule and not traversed by septa of connective tissue (Fig. 3). They consisted of the same large basophilic cells with large, pale, irregularly shaped nuclei. They could be found in animals treated for 80–120 days with DMN, even in the absence of tumors.

The second type of renal neoplasm developed in rats fed DMN for more than 160 days and consisted mostly of nests and groups of cells with a great variation in size and shape, separated quite irregularly by broad bands of connective tissue. The nuclei showed considerable polymorpha and polychromasia. Mitoses were numerous and often atypical. Large areas of these more anaplastic tumors were necrotic or were destroyed by extensive hemorrhages. In some cases, however, PAS staining after diastase treatment revealed an architecture similar to that of the renal cortex, namely, groups of tumor cells surrounded by thin PAS-positive membranes, very similar to those surrounding the renal tubules as basement membranes. In other cases the neoplasm consisted of an adenomatous papillary tissue with more advanced anaplasia, but somewhat reminiscent of the previously described cystic structures. These anaplastic types of tumors did not show any metachromasia.

Most of the rats with tumors showed disseminated focal alterations of the epithelium of the cortical tubules. Large, sometimes monstrous nuclei exhibited an unusual variation in size, shape, and chromatin content (Fig. 4). These changes were most prominent in the proximal convoluted tubules. They could also be found in tumor-free animals treated with DMN for less than 80 days, but were absent from the controls.

Of the animals studied both with and without renal tumors, eight treated with DMN for more than 111 days developed multiple nodules in the lungs, ranging in size up to 1 mm. Histologically they consisted of epithelial tissue arranged in somewhat alveolar structure (Fig. 5). The cells were medium-sized, the nuclei polymorphic and varying in chromatin content. Bronchi were involved and were partly destroyed by the neoplastic tissue (Fig. 6). In one animal fed DMN for 209 days, a pulmonary neoplasm with a diameter of 1 cm. was observed. Microscopically, this was an anaplastic immature epithelial tumor arising from the bronchial lining, showing gradual transition to the normal epithelium. Histochemical demonstration of alkaline phosphatase activity showed a weak positive reaction in the cells of this tumor. No positive reaction was found in any of the renal tumors.

Other investigated organs, such as lymph nodes, lower urinary tract, and pancreas, did not show neoplastic changes. In the livers of animals treated for more than 15 weeks, however, hyperplastic and cystic nodules could be observed in a relatively high incidence.

Four rats were returned to stock diet (Rockland rat pellets) after a treatment of 202 days. One of them was found dead 9 weeks after the change of the diet and could not be used for further study. The three remaining animals were sacrificed after 22, 63, and 93 days, respectively. All of them had grossly visible kidney and lung tumors.

The effect of partial hepatectomy on the incidence of tumors in kidney and lung was negligible.

DISCUSSION

Spontaneous renal tumors in rats are rare. A few malignant embryonic nephromas, carcinomas,
Fig. 3.—Kidney of rat fed DMN for 86 days. Small solid cell nest not exceeding the size of a single tubule. H. & E. ×600.

Fig. 4.—Kidney of rat fed DMN for 106 days. Proximal convoluted tubule. Nuclear atypism. H. & E. ×950.

Fig. 5.—Lung of rat fed DMN for 167 days. Epithelial tumor with alveolar structure. H. & E. ×40.

Fig. 6.—Lung of rat fed DMN for 224 days. Tumor tissue invading a bronchus. H. & E. ×150.
and sarcomas have been reported (3, 4, 16-18). McCoy (15) found eleven kidney tumors, four of which were adenomas, in a series of 100,000 rats. More recently, the development of renal neoplasms following lead intoxication (19) and treatment with 4'-fluoro-4-aminodiphenyl (14) has been described. As mentioned above, Magee and Barnes (12) found a high incidence of tumors in the kidneys of rats after DMN feeding. These authors classified them into two types: benign adenomas, developing in older animals, and malignant anaplastic adenocarcinomas occurring in younger rats within a shorter period after the treatment. These results differ from our findings, as we observed the anaplastic types only in the older animals treated for a longer period. The difference in the dosage and the strain of rats used may have been responsible for the different results.

From a comparison of our results with the histologic description and the photographs published by others, it appears that most of the experimental kidney tumors present a similar appearance independent of the causative agent. Even spontaneous renal neoplasms observed by Eker (5) in an inbred strain of Wistar rats do not differ significantly.

The tumors found in the lungs were considered to be primary lesions, since the architecture was somewhat similar to the pulmonary parenchyma, and dissimilar to the renal tumors. Moreover, transition of lung tissue into tumor, infiltration, and loss of a sharp border were observed; lung tumors were found in rats in the absence of renal neoplasms; and histochemically a different enzyme pattern from that of the renal tumors was noted.

Biochemical studies have revealed (a) that DMN is rapidly metabolized by liver slices or homogenates and little or not at all by preparations of kidney or other organs (13), (b) that protein synthesis in the liver, but not in the kidney or spleen, is markedly decreased by DMN (10), and (c) that the microsomal fraction of the liver is primarily involved both in the degradation of DMN (13) and the metabolism of azo dye carcinogens (7, 9). Little is known about the metabolic products of DMN (8), however, and its mechanism of action has not been elucidated.

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REFERENCES

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