Nephroblastomas Induced in Ovariectomized Rats by Dimethylbenzanthracene

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SUMMARY

The effects of intragastrically administered 7,12-dimethylbenzanthracene were investigated in intact and ovariectomized 50-day-old Sprague-Dawley rats. Ovariectomy resulted in an 80% decrease in the incidence of mammary tumors after 7.5 months. On the other hand renal tumors developed between the 5th and the 8th month in 14% of the castrated animals, while not a single instance was recorded in intact rats. These tumors were histologically typical of nephroblastoma. The similarity to fetal kidney was confirmed by lack of reactivity of the newly formed tubules to both oxidative and hydrolytic enzymes normally present in differentiated epithelial cells of the nephron and in epithelial kidney tumors of adult type.

INTRODUCTION

Intragastric administration of 7,12-dimethylbenzanthracene to pubescent Sprague-Dawley rats results in a high incidence of mammary tumors after 2 or 3 months (6). The occurrence of these neoplastic growths is largely conditioned by the presence of ovarian hormones (5, 9, 10). Occasional renal tumors have been noted after oral or parenteral administration of large doses of DMBA; but their low incidence has precluded any thorough study (7, 8, 13). Investigations carried out in series of intact or castrated female rats have enabled us to observe a relatively high proportion of kidney neoplasms in the ovariectomized animals in contrast with their absence in the intact rats. The purpose of the present paper is to record this fact and to describe the histological appearance of the renal tumors thus induced. Data on the enzyme histochemistry of one of them are also included. All were typical nephroblastomas that developed with sufficient frequency to militate against mere coincidence. Moreover, the morphology of these DMBA tumors seems entirely different from that of the various renal growths induced by other chemical agents and raises a question as to the true nature of some kidney neoplasms currently labeled nephroblastoma.

MATERIALS AND METHODS

The animals used were 50-day-old female Sprague-Dawley rats weighing 140 to 160 g. They were fed Purina chow and water ad libitum. The observations outlined in Table 1 derive from 3 separate experiments using 102 animals divided into Group 1, 30 intact DMBA-treated rats, and Group 2, 72 ovariectomized, similarly treated rats. Ovariectomy was performed under ether anesthesia 48 hr prior to administration of the carcinogen in 1 experiment and 2 weeks after in the others. No differences in tumor induction were apparent in relation to the time of surgery. DMBA (Eastman Organic Chemicals, Rochester, N. Y.) solubilized in 0.5 ml sesame oil was administered through an intragastric tube in two separate doses of 10 mg each with a 4-day interval between them. The experiments lasted 7.5 months and the animals were regularly examined for early detection of tumors. Development, distribution, and size of mammary tumors were charted weekly. Tumor nodules exceeding 2 cm in diameter were excised surgically to ensure a longer life-span in animals. In each experiment a limited number of rats exhibited pulmonary rales especially after the 3rd month; such animals were given an aqueous solution of tetracycline HCl (Pfizer Co. Ltd., Montreal, Canada) p.o. at a daily dose of 20 mg during 3 consecutive days at variable intervals. At autopsy all kidneys were removed regardless of whether or not tumors were evident and the more representative mammary nodules as well as other incidental tumors were dissected and fixed in Susa fluid for subsequent histological examination. Tissues were embedded in paraffin in 5-μ sections were stained with hematoxylin-phloxine-saffron. Of the 8 renal neoplasms observed, 1 was investigated histochemically. The kidney was trimmed, mounted on a chock, and rapidly frozen with solidified carbon dioxide. Fresh 10-μ cryostat-frozen sections mounted on cover slips were incubated in appropriate substrates for the demonstration of succinic and hydroxybutyric dehydrogenases, cytochrome oxidase, acid phosphatase, 5'-nucleotidase, and nonspecific esterase, according to the methods described in previous reports (11, 12).

RESULTS

The mortality rate was comparable in both groups (Table...
1) with approximately 20% of the animals dying during the first 3 months in a state of severe anemia. Autopsy revealed intestinal bleeding, liver steatosis, and adrenal hemorrhage; such manifestations are most probably related to the myelotoxic effects of DMBA (3, 8).

**Mammary tumors** developed earlier and with a higher frequency in intact animals, 1 to 3 nodules being already palpable by the end of the 2nd month in most cases; the larger tumors as previously mentioned were removed surgically. In ovariectomized rats tumors were detected much later; the first and often unique palpable nodule usually appeared after the 4th month. There was evidence of continuing growth in only 2 instances. The tumors were then excised and 1 was successfully transplanted, thus establishing its autonomy. On histological examination the mammary neoplasms exhibited the same features already reported by other investigators (4). Some appeared as nonsecretory intraductal papillary growths with one or several layers of epithelial cells supported by a more or less abundant stroma; in other milk-secretory activity was indicated by the presence of proteinaceous basophilic material within the distended acini. Smaller, slowly growing, or even regressive nodules were found in both intact and ovariectomized rats. They consisted predominantly of dense and partly hyalinized collagen fibers replacing the epithelial constituents.

**Kidney tumors** were found only in the ovariectomized DMBA-treated rats. They were unilateral and appeared as a single growth in the affected kidney. Two large tumors weighing 12 and 17 g, respectively, developed as early as 4 months after DMBA administration. These were soft, glistening, edematous, and hemorrhagic or necrotic in part. The others were small and could be detected on the uncut specimen only by a slight bulge on the surface of 1 pole of the kidney. After kidney sectioning they appeared as fairly well-circumscribed but not encapsulated gray-pearl nodules. There was some degree of compression of the surrounding renal tissues; but the growths did not seem purely expansive, as zones of fading, indicative of neoplastic infiltration, were often found at their periphery. This last finding was better seen on whole microscopic sections of the kidney (Fig. 1).

Histologically, all tumors were distinctly different from adenomas or carcinomas of adult type and reproduced the typical patterns of nephroblastoma. With a single exception, they consisted of a complex of large, well-delimited ducts (Figs. 2 and 3) and of masses of dark, poorly outlined cells (Figs. 2 to 4) on a background of looser mesenchyme-like tissue. The ductlike formations were seen in some instances to be continuous with kidney tubules of the surrounding renal parenchyma. In the intrapelvic projections of the tumors (Fig. 4) they were continuous with the superficial pelvic epithelium. The masses of undifferentiated cells tended to clump around the larger ducts. They were composed of spheroidal cells with scanty basophilic cytoplasm and round or oval hypochromic nuclei; mitoses were numerous. Very small, ill-delimited epithelial rosettes and large, curving primitive nephrons were seen to be formed in the clumps of undifferentiated cells (Figs. 3 and 7). The primitive nephrons were lined, in greater part, by prismatic cells but in places by flattened cells or by small tufts of cuboidal cells reminiscent of the fetal glomerular lining. However, typical embryonal glomeruli were not observed. The scanty, mesenchyme-like interstitial tissue was continuous with the nephrogenous cell masses (Figs. 2 and 6). It supported many blood vessels of simple structure. The component spindle or stellate cells showed lightly stained nuclei. They exhibited no tendency to differentiate into muscle, cartilage, bone, or to adult connective tissue. One single tumor was slightly different in that it lacked the large ducts and was chiefly composed of very small cuboidal cells arranged in innumerable, ill-delimited, scalloped tubules continuous with indistinct cords of identical cells in a stellate mesenchyme-like tissue (Fig. 5). However, large primitive nephrons with an undifferentiated prismatic lining were present in the peripheral invasive zone of this tumor.

The enzyme reactions within the nephroblastic cells were remarkably weak or absent. The contrast with normal nephronic tissue was easily discernible by comparison with the neighboring renal parenchyma. Newly formed tubules could be visualized only by a faint reactivity to succinic dehydrogenase (Fig. 8); the other oxidative enzymes investigated, i.e., hydroxybutyric dehydrogenase and cytochrome oxidase, were entirely negative. Except for the included larger vessels and engulfed normal collecting tubules the stromal tissue was also completely devoid of activity.

**Table 1**

<table>
<thead>
<tr>
<th>Animals treated with DMBA</th>
<th>Survivors after 3 mo.</th>
<th>Mammary tumors</th>
<th>Renal tumors</th>
</tr>
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<tbody>
<tr>
<td>Intact</td>
<td>30</td>
<td>24 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Ovariectomized</td>
<td>72</td>
<td>11 (20%)</td>
<td>8 (14%)</td>
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<table>
<thead>
<tr>
<th>No. rats</th>
<th>Average time of appearance (mo.)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Other tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Carcinoma of pancreas (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thymus lymphoma (1)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Animals were killed after 7.5 months.<br>
<sup>b</sup>Mean ± S.E.
Nephroblastomas and Dimethylbenzanthracene

Acid phosphatase, 5'-nucleotidase, and nonspecific esterase were equally nondemonstrable. Even after prolonged incubation there was a sharp delineation between the neoplastic tissue and normal nephrons.

As listed in Table 1 a few other malignant growths were found in addition to the mammary and renal tumors. The ear dermal tumor detected in one of the ovariectomized DMBA-treated rats is a rare occurrence at the dose presently utilized, such tumors being more liable to develop in ovariectomized rats receiving a smaller amount of carcinogen, as will be reported in a separate paper, as yet unpublished.

DISCUSSION

To the best of our knowledge the occurrence of renal tumors as an incidental finding in DMBA carcinogenesis of rats has been reported in only 3 previous publications (7, 8, 13) each time as a single instance. They were encountered in long-term experiments on a limited number of survivors of the Sprague-Dawley or Wistar strain after the administration of relatively high doses of DMBA given as a single or fractionated treatment by the oral or intraperitoneal route. They were noted without comment and were referred to either as renal sarcoma or sarcoma embryonic type or as adenocarcinoma.

The present experiments were designed to increase the kidney tumor yield by extending the life-span of the animals, either through resection of mammary tumors in intact rats or through their prevention by castration. Although the relative number of survivors after 3 months was comparable in both groups, kidney neoplasms were found only in ovariectomized rats. No satisfactory explanation can now be offered for the increased susceptibility of renal tissue to carcinogenesis after castration.

On histopathological grounds it should be emphasized that neither sarcomatous nor epithelial tumors of adult type were observed. All the DMBA-induced kidney tumors were highly characteristic of nephroblastoma (2). Formation of epithelial rosettes or primitive nephrons in clumps of undifferentiated epithelial cells was observed in each instance. The continuity of the primitive epithelial clumps with the surrounding stroma make it likely that the cells involved in carcinogenesis are bipotential. The newly formed tubules did not exhibit any evidence of maturation to adult nephrons and lacked many enzymes found in normal nephrons (11), as in adenomas and carcinomas (12). These observations make it necessary to reappraise the nature of some experimental kidney tumors currently considered to be nephroblastomas although they are entirely different from the presently described growths. In our experience some of the dimethylnitrosamine-induced renal tumors in rats have morphological features suggestive of purely blastemal variants of Wilms' tumor. However, the constituent, primitive-looking cells differentiate into various mesenchymal cells, including rhabdomyoblasts, but consistently fail to form primitive nephrons (15). Whether this is due to failure of an inductive mechanism on competent, bipotential cells or to previous commitment of the cells to a stromal destiny is outside the scope of this paper. Suffice it to say that dimethylnitrosamine-induced tumors of this type should be labeled differently from the presently described DMBA tumors, which alone deserve the designation of nephroblastoma.

These nephroblastomas were observed in 14% of the castrated rats. In spite of this relatively small incidence, their presence cannot be ascribed to mere coincidence, as the spontaneous occurrence of this entity in the rat is not seen in more than 1 out of 5000 animals (2, 14). Lack of data has made it difficult to comment on the discrepancy with the few previously recorded DMBA kidney growths (7, 8, 13). The 2 tumors labeled "sarcoma" may have represented instances of predominantly mesenchymal differentiation in nephroblastoma (2) and the adenocarcinoma could have been a purely tubular variant of Wilms' tumor (1); on the other hand, they may have been different neoplastic entities arising in animals of heterogenous genetic constitution. In this connection it would seem to be most pertinent to carry out studies in the same inbred strain of animals for comparative purposes as well as for reproducibility. A third possibility should also be considered, namely, that the carcinogenetic process taking place in intact rats differs from that of castrated animals with resulting changes in tumor morphology and histogenesis. Further elucidation of this point would require larger series of animals of the same strain, taking into account their sex, age, and hormonal status at the time of induction.

Up to now, we have not detected any localized kidney changes that could be interpreted as incipient stages of the carcinogenetic process induced by DMBA. Histogenetic studies on the presently described nephroblastomas could help in the solution of many controversial questions concerning Wilms' tumor. In our opinion, these studies would be particularly rewarding if, at the same time, work was conducted in parallel with similar research on the several categories of mature or immature renal tumors which have been produced in recent years by chemical means. As a prerequisite to research at the level of molecular pathology, such investigations could help to elucidate whether the observed differences are due to variations in cellular origin or merely to different modes of action of the carcinogens on the same cell line.

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REFERENCES


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Fig. 1. Whole section of a small nephroblastoma developed at 1 pole of the kidney, demonstrating the expansive and invasive character of this growth. The apparently well-delimited "pushing" nodule is capped in the upper part of the figure by an ill-delimited dark fringe that corresponds to a zone of interstitial infiltration of the tumor. Hematoxylin-phloxine-saffron, X 10.

Fig. 2. Central part of a nephroblastoma showing a large branching collecting duct, intraluminal projections of nephrogenous tissue, and numerous incompletely formed epithelial rosettes. Another collecting duct is seen in the left upper corner. Hematoxylin-phloxine-saffron, X 150.

Fig. 3. Large ducts partly filled with proteinaceous material with adjoining clumps of primitive epithelial cells and small epithelial rosettes. In the left is a group of primitive nephrons. The clear mesenchymal interstitial tissue is continuous with the clumps. The constituent cells are stellate or spindle-shaped. Their nuclei are distinctly smaller and more lightly stained than the nuclei of epithelial cells. Hematoxylin-phloxine-saffron, X 100.

Fig. 4. Intrapelvic projection of a nephroblastoma, showing the continuity of the urothelium of the fornix with a tubule-like recess (arrow) and the tendency of the nephrogenous tissue to differentiate in the vicinity of the urothelial lining. Hematoxylin-phloxine-saffron, X 50.

Fig. 5. A variant of nephroblastoma consisting of undifferentiated epithelial and stromal cells, and of ill-shaped tubules suggestive of abortive formation of glomeruli. No large duct was seen in the tumor. In the zone of invasion a higher degree of differentiation was observed as illustrated in Fig. 7. Hematoxylin-phloxine-saffron, X 250.

Fig. 6. Invasion of the kidney parenchyma by clumps of undifferentiated cells to show the microscopic appearance of the darker and somewhat large undifferentiated epithelial cells, which are continuous with lighter stained stromal cells. Hematoxylin-phloxine-saffron, X 250.

Fig. 7. Zone of invasion of the tumor appearing in Fig. 5. The picture shows intertubular infiltration of undifferentiated epithelial cells in situ formation of a curved pronephron, large and small nephric tubules, and a pseudoglomerulii (lower right corner). Hematoxilin-phloxine-saffron, X 250.

Fig. 8. Fresh frozen section of a portion of nephroblastic renal tissue stained for the demonstration of succinic dehydrogenase activity. There is almost no enzyme activity in cells of newly formed tubules by comparison with those of adjacent normal nephrons. Tetranitro blue tetrazolium, X 60.
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