Pulmonary Neoplasms Produced by Methylcholanthrene in the White Pekin Duck*

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SUMMARY

Many neoplasms have occurred in the respiratory tract of white Pekin ducks following one intratracheal injection of methylcholanthrene suspended in a 1.0 per cent aqueous solution of polysorbate 80. No tumors occurred when only polysorbate was injected. The histologic type of the tumors varied. Some were neurofibromas, and others were ganglioneuromas, hemangiomas, squamous cell-like carcinomas, adenocarcinomas, whereas still others were unclassified types of neoplasms. Frequently a single tumor showed a variety of histologic patterns. No metastases occurred.

The first tumor was found 56 days after the injection of the carcinogen. Fluorescence studies showed the distribution of methylcholanthrene in the respiratory tract. Methylcholanthrene crystals persisted in the tract for long periods. This technic appears to be very satisfactory for the production of neoplasms in the respiratory tract of the duck.

Papillomas, fibromas, neurofibromas, ganglioneuromas, pacinian-corpuscle tumors, and hemangiomas have occurred in the skin of the white Pekin duck following local applications of methylcholanthrene in acetone (4-6, 13). Metaplasia in the trachea is a common finding in ducks following the intratracheal injection of methylcholanthrene suspended in liquid petrolatum (9). Recently, a brief reference was made to carcinomas and sarcomas observed in the respiratory tract of ducks following the intratracheal injection of methylcholanthrene suspended in polysorbate 80 (9). The present report is a more detailed discussion of neoplasms in the respiratory tract of white Pekin ducks given a single intratracheal injection of methylcholanthrene suspended in polysorbate.

MATERIALS AND METHODS

Ninety-one white Pekin ducks were used. They were usually 40-60 days of age; however, a few were 99 and 865 days old when the experiment was started. The ducks were kept in a large outside pen with water and food available at all times.

* Presented at a meeting of the Pan-American Medical Association, Mexico City, May 9-11, 1960.

This investigation was supported by grants from the National Cancer Institute, Public Health Service (USPH C-1469 (C7) and Tobacco Industry Research Committee.

Received for publication November 1, 1960.

Sixty-six ducks were given 3-methylcholanthrene suspended in a 1 per cent aqueous solution of polysorbate 80, U.S.P. (Tween 80), and 25 were given only the polysorbate. A small catheter was placed in the trachea through the external larynx, and one injection of 25 ml. of the solution was given within an interval of 2-3 minutes. Two hundred-fifty mg. of methylcholanthrene was given to 22 ducks, 125 mg. to 28, and 62.5 mg. to sixteen. Usually they showed no reaction to this injection. Some of the group were observed for as long as 400 days (Table 1). During this time some of the ducks died, and others were killed. The respiratory tract was removed in toto, and multiple sections for histologic study were taken from the lungs, the wall of the abdominal air sacs, and the different tumors. The specimens were fixed in a 4 per cent solution of formaldehyde. Paraffin sections were prepared and stained routinely with hematoxylin and eosin. The pulmonary tissues from several of the ducks were examined macroscopically for fluorescence with an ultraviolet lamp (Aloe, No. 52140 ultraviolet mineralight, high-intensity, long-wave 3,660 A).

RESULTS

No tumors occurred in the 25 ducks given a single intratracheal injection of 25 ml. of the 1.0 per cent solution of polysorbate. Eight of these
Ducks were observed for 365 days, six for 251 days, five for 175 days, four for 12-36 days, and two for only 30 minutes. Some of these ducks had small granulomatous lesions with masses of yellowish-brown pigment in the wall of the air sacs. Granules of similar pigment were present in groups of lymphocytes in the lungs of an occasional bird. Four of the ducks given only polysorbate had amyloid in the liver which was usually enlarged, yellowish-brown in color, and fluid was present in the abdominal cavity. Sections were removed from the striated muscle of fourteen ducks for histologic study. Eight of these had focal areas of necrosis.

Pulmonary neoplasms occurred in each of the three groups of ducks given 250, 125, and 62.5 mg. of methylcholanthrene (Table 1). Fourteen (63 per cent) of the 22 ducks given 250 mg. of methylcholanthrene developed one or more pulmonary tumors; eleven (39 per cent) of the 28 receiving 125 mg. and only four (25 per cent) of the sixteen given 62.5 mg. developed tumors. The first tumor observed in each of these three groups was found on the 56th, 60th, and 147th day, respectively, following the intratracheal injection of the carcinogen.

**TABLE 1**

Ducks with Tumors in Respiratory Tract Following A Single 25-Ml. Intratracheal Injection of Methylcholanthrene (MC) in a 1.0 Per Cent Solution of Polysorbate in Water

<table>
<thead>
<tr>
<th>Interval in Days Between Injection and Death</th>
<th>250 mg. MC</th>
<th>125 mg. MC</th>
<th>62.5 mg. MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Ducks Treated</td>
<td>No. Tumors Treated</td>
<td>No. Tumors Treated</td>
<td>No. Tumors Treated</td>
</tr>
<tr>
<td>0-16</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>21-56</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>60-69</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>100-118</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>158-900</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>211-250</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>251-300</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>301-400</td>
<td>8</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

Totals: 22 14 28 11 16 4

mg. of methylcholanthrene (Table 1). Fourteen (63 per cent) of the 22 ducks given 250 mg. of methylcholanthrene developed one or more pulmonary tumors; eleven (39 per cent) of the 28 receiving 125 mg. and only four (25 per cent) of the sixteen given 62.5 mg. developed tumors. The first tumor observed in each of these three groups was found on the 56th, 60th, and 147th day, respectively, following the intratracheal injection of the carcinogen.

Figure 1 shows the normal respiratory tract of the duck after being filled with latex. The anatomic and histologic characteristics of the respiratory tract of the duck have been described previously (8, 10). Tumors occurred within the lung and on the wall of the cervical and abdominal air sacs in 29 of the 66 ducks given methylcholanthrene intratracheally (Table 1). The size of these tumors varied from a few millimeters to several centimeters. Necrosis was frequent in the larger tumors. Some tumors were circumscribed and well demarcated, whereas others infiltrated the adjacent stroma. Acute and chronic inflammation was conspicuous; sometimes the wall of the abdominal air sac was ½-1 cm. thick. Collections of lymphocytes and fibrosis were conspicuous within the lungs. There were no lesions that suggested metastases. The neoplasms were restricted to the respiratory tract, except for one duck in which a hemangioma (1.5 cm.) was present on the pericardium, a second hemangioma (0.8 cm.) was present on the capsule of the liver, and an embryonic-like tumor (11.0 X 8.0 X 7.0 cm.) was located in the retroperitoneal area. In the right and left lungs of this same duck (Fig. 2) were multiple firm nodules of neurogenic tissue. The tumor in the left lung showed innumerable ganglion cells and was considered to be a ganglioneuroma (Fig. 3). In the right cervical area of this duck was a firm, white mass, approximately 5.0 cm. in diameter, composed of spindle-shaped cells arranged in whorl-like formation consistent with a neurofibroma (Fig. 4). A fibrosarcoma was located adjacent to one of the major bronchi in duck 1596 (Fig. 5). A relatively firm, irregular, hemorrhagic area in the lower portion of the right lung of duck 1599 had the histologic features of a hemangiomatous tumor (Fig. 6). A tumor with a large amount of osteoid tissue was present in the lung of duck 1597 (Fig. 7).

Epithelial tumors were frequent in the lungs. In duck 1582 there was such a tumor with a small area of osteoid tissue adjacent to a major bronchus (Fig. 8). Frequently, the epithelial cells grew in sheets (Fig. 9), with a variation in the size and shape of the individual cells. Other of the epithelial tumors showed glandlike formations characteristic of an adenocarcinoma (Fig. 10). A variety of epithelial growths occurred in some of the tumor masses. Three different histologic patterns occurred in a tumor in the lateral wall of the abdominal air sac of duck 1571 (Fig. 11)—one squamous-like (Fig. 12), another adenocarcinomatous (Fig. 13), whereas the third was a solid carcinoma (Fig. 14).

Some of these tumors were composed of embryonic-like tissue in which a variety of cell types was present. Distributed through this growth were giant tumor cells and spaces lined with epithelial-like cells (Fig. 15). The wall of the air sacs normally is lined with a cuboidal type of epithelial cell; however, in the ducks given an intratracheal
injection of methylcholanthrene there were local areas of proliferated epithelial cells, at least suggestive of an adenocarcinoma (Fig. 16).

To ascertain the distribution of the methylcholanthrene following an intratracheal injection, duck 1572 was given 25 ml. of a 1 per cent solution of polysorbate containing 250 mg. of methylcholanthrene. Forty-eight hours later this duck appeared perfectly normal and was killed. Nothing abnormal was observed macroscopically in the respiratory tract. Microscopically there was an acute inflammatory reaction in the lungs. The respiratory tract when photographed under standard lights was normal, but when photographed with ultraviolet light the lungs and walls of the air sacs fluoresced. There were focal areas of granulomatous reaction in the air sacs of many of the ducks given methylcholanthrene and sacrificed several weeks later. In these areas were crystals considered to be methylcholanthrene that fluoresced. A proliferation of lymphocytes occurred in the lungs of some of the ducks, apparently as a reaction to this carcinogen. Some of the ducks given methylcholanthrene had amyloid in the liver and focal areas of necrosis in the striated muscle. The same liver and muscle lesions were present, however, in the ducks given only the polysorbate.

DISCUSSION

Neoplasms with the histologic characteristics of malignancy occurred in the respiratory tract of white Pekin ducks following the intratracheal injection of methylcholanthrene suspended in a 1 per cent aqueous solution of polysorbate 80. No tumors occurred in the 25 ducks given only polysorbate 80 intratracheally. This technic for the production of pulmonary neoplasms in the duck has proved to be most satisfactory. Carcinogens other than methylcholanthrene are now being given to a group of ducks.

The higher concentrations of methylcholanthrene, as used in these experiments, do not remain in a satisfactory suspension in a 1 per cent solution of polysorbate. This technic would not be satisfactory, therefore, for a quantitative study. The results, as observed in this experiment, with 250, 125, and 62.5 mg. of methylcholanthrene crystals may be considered only as a comparative study. It is of interest, however, to note that the first tumor occurred in a duck given the highest concentration of methylcholanthrene and also that the tumor yield was highest in this group of ducks. Sixty-three per cent of the ducks given 250 mg. of methylcholanthrene developed tumors, 59 per cent of those given 125 mg., and only 25 per cent of those given 62.5 mg. These percentages are only approximately correct, since some of the ducks in each group either died or were killed before enough time had elapsed for the development of a tumor. The age of ducks when tumors were observed is shown in Table 1.

The large number and the wide variety of neoplasms occurring in the respiratory tract of a single bird are of considerable interest, since the same phenomenon has been observed in the skin of ducks following the local application of methylcholanthrene in acetone. Neoplasms of the same histologic character as those observed in the skin of ducks occur in the respiratory tract. The presence of tumors in the respiratory tract 56 days after the injection of methylcholanthrene would suggest that an interval of 1 year after the intratracheal injection of this carcinogen would be a satisfactory period in which to observe ducks for the development of tumors. There is nothing in this experiment to suggest the maximum interval between the application of methylcholanthrene and the development of a tumor. Furthermore, there is no indication from this study to suggest that any of the tumors in the respiratory tract spontaneously regressed, as has been observed in some of the neoplasms that developed in the skin following local applications of this same carcinogen (4). It is of interest to note that the pulmonary neoplasms in this experiment occurred after a single injection of methylcholanthrene. However, crystals of this carcinogen have persisted in the respiratory tract of some of the ducks for a year.

The presence of multiple tumors, the large size of some, and the accompanying inflammatory reaction make it most difficult to form an opinion as to the cell and site of origin of any of these tumors. Additional studies are now in progress to ascertain the point of origin of these neoplasms. Birds are being given small amounts of methylcholanthrene and sacrificed at frequent intervals to obtain sections of lung for histologic study. The influence of polysorbate on tumor occurrence is also being investigated. Some investigators (13, 14) consider the Tween compounds to be "tumor promoters." It is not possible to say from this experiment whether or not polysorbate 80 acted as a "tumor promoter." Technics now being used may contribute to this phase of the problem. Obviously in this study very large amounts of methylcholanthrene were given only to demonstrate that pulmonary neoplasms would occur in the duck following a single intratracheal injection.

Andervont (1) in 1937 apparently was the first
to produce pulmonary tumors experimentally in the mouse. This was done by passing threads impregnated with 1,2,5,6-dibenzanthracene through the intact chest wall. Kuschner and associates (3) in 1956 produced tumors in the lungs of rats by the implantation of methylcholanthrene pellets into the bronchi, but were unable to produce tumors by inhalation and intratracheal injection of the carcinogen. Blacklock (2) in 1957 introduced 3,4-benzpyrene, methylcholanthrene, and the condensate from cigarette smoke into the lungs of rats and obtained very few tumors. He states that "when known and potent carcinogens are introduced directly into the lung in large amounts, a long interval must elapse before neoplastic change occurs in the lung." The occurrence of pulmonary neoplasms in the duck 56 days following the intratracheal injection of methylcholanthrene would indicate that tumors may appear much earlier in the duck than in the rat.

Amyloid has been observed frequently in ducks given methylcholanthrene (7, 11). It was thought at one time that this amyloid was associated in some way with the methylcholanthrene; however, some of the ducks in this experiment given only polysorbate have amyloid in their tissues. This problem of amyloidosis in the duck is being studied now in our laboratory. Focal areas of necrosis in the striated muscle of ducks given both methylcholanthrene and polysorbate would indicate that this lesion is not specifically related to methylcholanthrene. Similar areas of necrosis occur in the striated muscle of untreated ducks.

**REFERENCES**

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Fig. 7.—Duck 1597 was given 250 mg. of methylcholanthrene and killed 188 days later. A small firm nodule was present in periphery of left lung. Histologically it is composed of fibroblasts and osteoid tissue. H. & E. stain, X140.

Fig. 8.—Duck 1582 was given 250 mg. of methylcholanthrene and killed 254 days later. This is an adenoma-like tumor occurring in right lung adjacent to a major bronchus. Observe small area of bone formation. H. & E. stain, X30.

Fig. 9.—Duck 1575. An epithelial tumor in the wall of a small bronchus 224 days after an intratracheal injection of 250 mg. of methylcholanthrene. H. & E. stain, X400.

Fig. 10.—Duck 1575. An adenocarcinoma in lung 224 days after an intratracheal injection of 250 mg. of methylcholanthrene. H. & E. stain, X400.
Fig. 11.—Duck 1571 was given 250 mg. of methylcholanthrene and killed 388 days later. Lesser abdominal air sac is filled with hemorrhagic necrotic material. In lateral wall of air sac (A) is a firm, white tumor. Histologic characteristics of this tumor are shown in Figures 12, 13, and 14. A second tumor with similar histologic characteristics is present in area of cervical air sac indicated by arrow.

Fig. 12.—Duck 1571. A portion of the tumor shown in Fig. 11 is a squamous-like-cell carcinoma. H. & E. stain, X150.

Fig. 13.—Duck 1571. Same tumor shown in Figs. 11 and 12. This is a typical adenocarcinoma. H. & E. stain, X160.

Fig. 14.—Duck 1571. Same tumor shown in Figs. 11, 12, and 13. This is a carcinoma that is quite different histologically from type of growth shown in preceding two illustrations. H. & E. stain, X160.

Fig. 15.—Duck 1595 was given 250 mg. of methylcholanthrene and was found dead 309 days later. Tumor mass 4X 3X 1.5 cm. was present on lateral side of right lung. It contains both epithelial and mesothelial elements. H. & E. stain, X120.

Fig. 16.—Duck 1602 was given 250 mg. of methylcholanthrene and killed 313 days later. There is an adenocarcinoma-like tumor on wall of an air sac. Observe normal epithelial lining of air sac at margin. H. & E. stain, X150.
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