INDUCTION OF PULMONARY TUMORS IN RATS BY SUBCUTANEOUS INJECTIONS OF 4-NITROQUINOLINE 1-OXIDE

(Plates XXXV~XXXVIII)

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Synopsis

Each of ten male rats, Buffalo and Long-Evans strains, received subcutaneous injections of 4-nitroquinoline 1-oxide. The final effective number of rats was 7 and 8 in the groups 1 and 2, respectively, after 164 days from the start. In most of rats, one or two subcutaneous sarcomas were induced, multiple pulmonary tumors were induced in 13 of 15 rats. Almost all the tumors were designated histologically as adenomas. Two cases of squamous cell metaplasia were found. Further, multicentric carcinomas, adenocarcinomas, and squamous cell carcinomas were induced in 3 rats among them, which survived more than 295 days. Sudden transitional connections between these adenomatous and cancerous growths or bronchi and frank carcinomas were found very frequently. From these evidences, it seemed that pulmonary carcinomas in rats will be derived from either the adenomatous growths or the bronchial terminals.

In the previous papers, it was reported that multiple pulmonary tumors including well-established adenocarcinomas with metastases in mice were exclusively obtained by subcutaneous injection of 4-nitroquinoline 1-oxide and it was pointed out that the lung in mice was a remote but target organ when the application of 4-nitroquinoline 1-oxide was made subcutaneously. From the above results, it seemed desirable to test the potential carcinogenic action of the compound against lung of rats when it was applied subcutaneously.

In the course of the present experiment, the induction of pulmonary tumors in rats was found to be similar to that in mice.

MATERIALS AND METHODS

Two groups of male rats, Buffalo and Long-Evans strains, were used in the experiment. They were 2 to 3 months of age, weighing over 100 g at the start of the experiment. All the rats were maintained on a mixture of rice and Oriental animal diet (1:1), with an unlimited supply of water. In addition, a small amount of green vegetables was given three times a week.

A 0.25% solution of 4-nitroquinoline 1-oxide in a mixture of olive oil and cholesterol (100:5) was prepared and was warmed to body temperature before use. Each rat,

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received 0.2~0.3 ml of the solution or 0.5~0.75 mg of the compound subcutaneously, each time in a different site on the back. The injection was repeated every week, 16 times in the first group (Buffalo) and 28 times in the second group (Long-Evans), for a total of 10.5 mg and 13.5 mg of 4-nitroquinoline 1-oxide, respectively. The injection was then discontinued and animals were kept under observation.

Generally speaking, Long-Evans group appeared to remain in a slightly better physical condition than the Buffalo group as is shown in Fig. 1. A few rats of both groups died or were killed early in the course of the experiment showing marked postmortem changes and these were excluded from the experiment.

In most of them, the development of subcutaneous sarcoma in the injected area began to be detected after six months. When sarcomas were found, they were surgically removed under anesthesia, before they had become larger than about 1 cm in diameter. This measure was taken to keep the animals live longer.

Complete autopsies were performed; lungs and any grossly abnormal organs were examined histologically. Tissues were fixed in neutral 10% formaldehyde solution. After fixation, the nodules in the lungs were counted and the number was recorded. Since the majority of pulmonary tumors in rats were found directly underneath the pluera, it was possible to count the tumors on the external surface of the lungs with fair accuracy when they attained a fraction of a millimeter in diameter, especially after fixation. The lung tissue was sectioned serially and stained with Hematoxylin and Eosine for histologic verification. Moreover, selected tissues were stained with van Gieson and silver.

RESULTS AND DISCUSSION

The result of the experiment is summarized in Tables I and II, where the body weight, and weight of liver, spleen, kidney, and lung, size of subcutaneous sarcoma, if any, and the nature of lung findings are tabulated for all the rats. The final effective number of rats was 7 and 8 in the groups 1 and 2, respectively.

One or two subcutaneous sarcomas were induced in 13 out of total 15 rats which survived for more than 164 days.

The resultant lesions in two experiments did not show any qualitative differences,
but varied in the number of adenomas. After 164 days, the lungs in the animals in both groups showed frequent changes. Of all 15 animals, 13 had multiple nodular tumors ranging in size from 1 to 5 mm in diameter, and the number of nodules varied from 6 to 55. The gross appearance of the pulmona\textted tumors was characteristic; they were greyish white, round areas, slightly raised on the lung tissue. Microscopically, the cells making up the tumors were fairly large, cuboidal or ovoid in shape, usually lying in a single layer on either side of a thin shred of stroma. Some tumor cells were hyperchromatic. The general pattern gave the impression that the tumor was composed of closely packed folds of an epithelial cord. Mitotic figures were not frequent. They were designated as papillary cystadenoma (Photo 1).

A few rats developed bronchitis and pneumonia, and in their lungs, metaplasia of squamous epithelia and proliferation of lymphoid tissue were sometime demon-

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<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Period of survival (days)</th>
<th>Weight (g)</th>
<th>Size of subcutaneous sarcoma (cm)</th>
<th>Lung findings</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Body</td>
<td>Liver</td>
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<td>335</td>
<td>13.0</td>
<td>1.4</td>
</tr>
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<td>10.4</td>
<td>1.6</td>
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<td>11.9</td>
<td>1.2</td>
</tr>
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<td>267</td>
<td>371</td>
<td>11.2</td>
<td>1.4</td>
</tr>
<tr>
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<td>223</td>
<td>9.6</td>
<td>1.1</td>
</tr>
<tr>
<td>6</td>
<td>343</td>
<td>310</td>
<td>9.2</td>
<td>1.8</td>
</tr>
<tr>
<td>7</td>
<td>400</td>
<td>280</td>
<td>8.0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table I. Lung Findings of Buffalo Rats receiving Subcutaneous Injections of 4-Nitroquinoline 1-Oxide

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Period of survival (days)</th>
<th>Weight (g)</th>
<th>Size of subcutaneous sarcoma (cm)</th>
<th>Lung findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Body</td>
<td>Liver</td>
<td>Spleen</td>
</tr>
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<tr>
<td>9</td>
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<td>15</td>
<td>344</td>
<td>342</td>
<td>11.3</td>
<td>0.7</td>
</tr>
</tbody>
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Table II. Lung Findings of Long-Evans Rats receiving Subcutaneous Injections of 4-Nitroquinoline 1-Oxide
strable. These metaplasia of squamous cells were present in two cases and a marked keratinization was observed in both (Photo 2).

One rat (No. 14) died on the 295th day, after the removal of a subcutaneous sarcoma. Two rats (Nos. 7 and 15) were sacrificed on the 344th and the 400th days, respectively. At autopsies, the pulmonary tumors were invariably multiple (Photos 3, 4, 5, and 6). Some nodules protruded from the pleural surface and the largest one measured 12 mm in diameter. Several nodules appeared to grow infiltratively with irregular border and they were opaque, grey in color except when they showed congestion. Microscopically, there was extensive variation in the appearance and in the density of the tumors. The cells were arranged partly in one layer and partly in many layers, often unaccompanied by stroma. The tumors apparently were columnar papillary carcinomas (Photos 7 and 8). There was another type; cuboidal tumor cells formed acini, irregular in size and shape, and with a homologous substance in the lumen. Some acini were jointed together, without showing limiting silver fibers between them (Photos 9, 10, and 11). These features suggested that the tumors were adenocarcinomas. Mitotic figures were numerous. The stroma was usually scanty. The growths of tumor, although not rapid, were expansive and infiltrative. Occasionally, tissues were necrotic in the center (Photo 12).

Other changes in the mucosa were also observed; in some there was a proliferation of the basal cells and in others there were localised proliferation of mucosal cells forming a polypoid, sometimes papillomatous, projections growing into the lumen. Two rats (Nos. 7 and 15) with squamous cell carcinomas showed advanced bronchiectasis with marked squamous metaplasia. The tumors were well-differentiated keratinizing squamous cell carcinomas (Photo 8). No definite metastases from these carcinomas were found.

No spontaneous pulmonary tumors were found in control rats of both strains in this laboratory for the past 15 years.

In view of the current interest in the problem of lung cancer, because of its increasing incidence, numerous attempts have been made to induce lung cancer experimentally. Perhaps the most consistent results have been obtained using radioactive substances, carcinogenic hydrocarbons, and derivatives of aminofluorene. Various routes of administration were used for getting the carcinogen into contact with the epithelia of the lung in rodents. Few of these methods induced pulmonary tumors which simulate the bronchogenic carcinoma of man in type and behavior.

In the present experiment, 4-nitroquinoline 1-oxide was found to be effective in inducing adenomas, and subsequent adenocarcinomas and squamous cell carcinomas in the lungs of rats when administered subcutaneously. This method has the advantage of simplicity when compared with that used by previous investigators.
Besides, squamous cell metaplasia was the rule rather than the exception and it showed high degree of keratinization.

To find out any relationship, if any, between the two definite histologic changes diagnosed as adenoma and adenocarcinoma, serial sections were made in most of the adenomatous nodules in the lungs and they were examined in detail. Very frequently, atypical growths were observed among the structure of adenomas as shown in Photo 13. The characteristics of these atypical growths are replaced upon an epithelium in adenomatous type. They seem cancerous rather than adenomatous in their pattern. Silver staining does not reveal any boundary between the two growths, adenomatous and cancerous. It can therefore be assumed that these atypical growths are cancer buds, in a broad sense. Thus, it may be presumed that an adenomas are first induced by 4-nitroquinoline 1-oxide and some of them would develop into carcinomas in the course of time, similar to the case of mice.9)

On the other hand, another finding in the lungs of rats is noteworthy in indicating that the pulmonary carcinomas were derived directly from hyperplastic bronchial epithelium, because sudden transition from bronchus to carcinoma was frequently observed and no boundary was demonstrated between them by silver staining (Photos 14, 15, and 16).

There does not appear to be any investigation in the past literature with which this evidence can be compared. However, it is interesting to contrast the histological appearance of these rats with those in mice which received 4-nitroquinoline 1-oxide subcutaneously.

For the present, it is suggested that the lung carcinomas in rats seem to originate from either the site of adenomatous growths or the terminal bronchi. Extensive experiments along this line are now underway in this laboratory and the results will be presented in the near future.

REFERENCES

**EXPLANATION OF PLATES XXXV~XXXVIII**

Photo 1. Adenoma encapsulated (Rat No. 8).
Photo 2. Squamous metaplasia. The lumens are filled with keratin (Rat No. 5).
Photo 4. Dorsal view of the lung of Rat No. 7. Multiple cancerous nodules are seen diffusely.
Photo 5. Ventral view of the lung of Rat No. 15. A large cancer nodule (1.2cm in diameter) is seen in the hilar portion.
Photo 6. Gross longitudinal sections of the above photo.
Photo 7. An example of the usual type of adenocarcinomas induced in Rat No. 15.
Photo 8. A type of squamous cell carcinomas induced in Rat No. 15.
Photo 9. Other type of adenocarcinomas (Rat No. 14).
Photo 10. High-power of view of the above photo.
Photo 11. Silver staining of the same.
Photo 12. An adenocarcinoma with necrotic areas in the center (Rat No. 14).
Photo 13. The characteristics of the carcinoma are superimposed upon adenomatous growths (Rat No. 14).
Photo 14. Sudden transition from bronchus to carcinoma (Rat No. 14).
Photo 15. Silver stainig of Photo 14.
Photo 16. Bronchial epithelia are changing into malignant growths in the center (Rat No. 14).