HISTOGENESIS OF LUNG CARCINOMA IN MICE INDUCED BY 4-NITROQUINOLINE 1-OXIDE: CARCINOMA ARISING FROM AREAS OF ADENOMA\textsuperscript{*1}

(Plates XXVIII~XXIX)

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Synopsis

It has been reported that repeated subcutaneous injection of 4-nitroquinoline 1-oxide to mice produced lung cancers with metastases. Histogenetic study of induced cancers showed that they arose from adenomas. Cancer foci were often localized either on the margin or in the area of adenomas.

Recently, the adenomas and adenocarcinomas of the lung were induced in mice by the injection of 4-nitroquinoline 1-oxide. The tumors were almost invariably multiple, and lungs were extensively riddled with these growths. Grossly, there were little to differentiate the adenoma from the adenocarcinoma, as both growths were found intermixed on the surface of the lung. If anything, the adenomas appeared as soft, transparent and round nodules, while cancerous growths were often projecting prominently above the pleural surface and with irregular border. Usually, the most exact criterion for the diagnosis of cancer is the presence of neoplastic deposits in adjacent or distant tissues.

Microscopically, there are moderate variations in the appearance and density of the tumors. The tumor cells were columnar, cuboidal, round, or oval, arranged in acini, tubular and papillary, irregular in size and shape, showing papillary formation. The nuclei were single, round or oval in shape, and staining varied from light to deep. Growth of tumors was frequently expansive, and with a few mitoses.

The lesion diagnosed as adenoma could easily be mistaken for carcinoma, because of its size and hyperplastic appearance. Although the cellular elements of the lesion are hyperchromatic and appear to be adenomatous, they do not exhibit a wide range of variation in their patterns. It is noteworthy that the carcinoma is frequently found

\textsuperscript{*1} Presented in part at the 46th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 17, 1962.

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either adjacent to or within the lesion of adenoma (Photo 1). When serial sections of the lung were made and examined in detail, atypical growths were often found in the adenomatous pattern. All the examples described in the following pages have been found accidentally during the histological examination. Four examples of the origin of lung cancer from areas of adenomas were illustrated with photomicrographs.

In view of these findings, it was concluded that some of lung cancers in mice arise from the areas of adenoma. Histological evidence in support of this conclusion is presented in the following pages.

**Experimental**

As described in the previous paper, female mice of dd strain were injected subcutaneously with 0.1 ml of 0.25% solution of 4-nitroquinoline 1-oxide in a mixture of olive oil and cholesterol (100:5), i.e., 0.25 mg of the carcinogen. The injection was given once a week for 10 weeks, each time at a different site in the back. Ten out of twenty mice survived over 224 days, after which they died or were sacrificed. In all of these mice, varying number of tumor nodules were found adjacent to the pleura. Two out of ten mice had typical adenocarcinomas of the lung and these were accompanied by metastases into the diaphragm, the tracheal lymph nodes, and chest wall. No other type of the carcinomas was induced in the experiment.

Every lobe was removed from the lung of the remaining eight mice, and all were serially sectioned in 4 µ thickness. They were stained with Hematoxylin and Eosin, van Gieson, PAS, and Wilder’s silver, if necessary. The serial sections were used in the search for areas of transitional changes between hyperplasia, adenomatous, and cancerous lesions.

**Example 1:** In this specimen, an adenoma began to appear at the first section. It extended through 135 sections, and disappeared completely in the 136th section of the serial specimen, each of which was 4 µ thick. The adenoma measured 0.8×0.54×0.35 mm in size. In the last part of the sections, atypical growth was found forming a papillary appearance and staining rather basophilic. The nuclei were comparatively large and rather polygonal. In addition, other larger carcinomas were found formed by a mass of cells having the same characteristics as the above cells. It is suggested that these atypical growths are cancer foci. It began to appear in the 111th section and continued to the 135th section, and measured 0.20×0.15×0.09 mm in size. The relation between cancer and adenoma in this example is shown schematically in Fig. 1. In this graph, the dotted area represents adenoma and the black area cancerous focus. Nine of the sections are also shown in this diagram and one of them with cancer focus (No. 119) is shown in Photo 2. In view of the partial replacement of the adenoma by cancer, it is suggested that adenocarcinoma would arise from the border of an adenoma.
Adenoma (□) and adenocarcinoma (■) of lung in mouse

Fig. 1. Example 1

Adenoma (□) and adenocarcinoma (■) of lung in mouse

Fig. 2. Example 2
Example 2: Here is another example of a cancer focus in an adenoma. In this case, the carcinomatous focus was very similar in appearance, but larger than that of the above case. An atypical growth was also found in the serial sections of an adenoma. It can be assumed as a cancer focus for the same reason as in the above case (Photo 3). As seen in Photo 4, silver stain does not reveal any boundary between the two growths, adenoma and cancer. The adenoma was $0.90 \times 0.73 \times 0.55$ mm in size, extending through 183 sections, each of them being $4 \mu$ in thickness. The cancer focus was $0.35 \times 0.22 \times 0.20$ mm in size, extending through 49 sections. The relation between the two growths is shown schematically in Fig. 2, which shows that cancer focus is enclosed completely by but not localized in the center of the adenoma. The 155th and the 156th sections are shown in Photos 3 and 4.

Example 3: A study of the histological appearance at the point of the transition from adenoma to cancer reveals the following changes. The cellular elements of the lesion shown in the lower part of Photo 5 are hyperchromatic and do not exhibit a wide range of variation in the pattern. A well-differentiated adenocarcinoma is seen in the upper half of this photomicrograph, invading the surrounding tissue. In the center, the point of transition from adenoma into adenocarcinoma is seen clearly. The cells in the carcinoma have suddenly become large and irregular. Silver staining does not reveal any boundary between the adenoma and cancer (Photo 6). It is suggested that cancer is arising from an area of the adenoma.

Example 4: In this example, atypical growths are joined with the adenoma which was found adjacent to the subpleura (Photo 7). The cells lining this atypical growth become replicated and their nuclei are larger and more hyperchromatic than those of the adenoma. From the absence of boundary in the transition from adenoma, it is supposed that this cancerous growth also arose from the adenoma.

Among the eight mice which had previously been diagnosed as having adenomas, five were found to have cancer buds as described above. For the histogenesis of adenocarcinomas it may be supposed that adenomas was first induced by the injection of 4-nitroquinoline 1-oxide and these have developed into carcinomas in the course of time. Incidence of cancer in this experiment was 70%.

**DISCUSSION**

Histopathogenesis of lung cancer in mice has occasioned a great deal of controversy since Livingood first described spontaneous pulmonary tumors and advanced the opinion that they arose directly from the bronchus.\(^{3,4}\) However, few investigators proved constant relationship between lung cancer and adenoma.

Grady and Stewart\(^{1}\) demonstrated that all pulmonary tumors induced by subcutaneous injection of 20-methylcholanthrene and 1,2,5,6-dibenzanthracene in mice originated from alveolar cells. Lorenz and Stewart\(^{2}\) found atypical acini lined by a
single layer of cuboidal or columnar cells with basally situated hyperchromatic nuclei. However, they could not determine whether these lesions were neoplastic or non-neoplastic in nature. The evidence submitted in this paper is concerned only with histological transition from adenoma to carcinoma. There does not appear to be any investigation in the literature with which this evidence can be compared, in either human being or experimental animals.

In this experiment, we assume that the adenoma was first induced by 4-nitroquinoline 1-oxide and then developed into carcinoma in the course of time. This is similar to findings demonstrated in rats by one of the authors.6,7)

This work was supported partly by grant DRG-739 from the Damon Runyon Memorial Fund for Cancer Research.

REFERENCES

EXPLANATION OF PLATES XXVIII~XXIX

Photo 1. Section showing adenocarcinoma arising from an adenoma. The boundary of adenocarcinoma and adenoma is intermingled and is obscured. ×160.

Photo 2. Example 1. A cancer focus; section No. 119. Note the cancerous growth with several mitoses arising from adenoma abruptly. ×300.

Photo 3. Example 2. Section No. 155. In the center of the Photo, cancer bud is arising from the adenoma. ×200.

Photo 4. Example 2. Section No. 156. Silver stain. Compare with Photo 3. Note that the boundary of cancer and adenoma is not recognizable. ×200.

Photo 5. Example 3. Sudden transition from adenoma to adenocarcinoma is seen in the center of this Photo. ×170.


Photo 7. Example 4. A nodule of adenomatous growths is seen in the subpleural area. In the center of Photo, cancer focus with luminizing and irregular cells is seen. The adenomatous growth, in the right half, is not yet cancerized. ×160.

Photo 8. Silver stain of Photo 7. ×160.