The present series of experiments was carried out in order to see what role pre-existing localized fibrosis plays in carcinogenesis of the lung.

Hemorrhagic infarction was produced in the lung of 180 male Wistar rats by injecting 0.05 ml of hexachlorotetrafluorobutane into the tail vein. This resulted in localized fibrosis in the lung 3 months later. One hundred and fifteen rats were alive 3 months after administration of the chemical. Of these animals, 30 were given no further treatment (control). The remaining 85 rats were given intratracheal instillation of 0.2 µCi of polonium-210 once a week, a total of 15 times.

It was subsequently found that lung carcinoma was induced in close proximity to the localized pulmonary fibrosis in 3 of 26 rats (11.5%) during the period from completion of the 15 weekly administrations of polonium-210 until the end of this experiment (21 months after the 1st instillation of polonium-210). Polonium-210 was found to be deposited in the fibrous thickening of the alveolus around the subpleural fibrotic lesion, bronchial epithelium, and peribronchial lymph apparati at the initial period of administration of polonium-210, but during the period of pulmonary carcinogenesis, it was deposited in the localized fibrotic lesion in the lung and in a few cancer cells.

This suggests that polonium-210 deposited in the pulmonary fibrotic lesion remains there over a long period of time, indicating a reduced clearance ability at this site.

Key words: Lung cancer, experimental — Polonium-210 — Scar cancer of the lung — Histology of lung cancer

The incidence of carcinogenesis is different in each lobe of the lung.12 This may be related to the volume of each lobe, but also indicates that there are sites which may be susceptible to pulmonary carcinoma and suggests the presence of a local factor in carcinogenesis. It would be very difficult to identify local factors related to the induction of lung cancer by histopathological examination.

Since “scar cancer” was first reported by Friedrich5 in 1939, many cases with similar morphology have been described, both in Japan and other countries.4,7,11,19,22,24

We reported earlier that lung cancer was induced in close proximity to the localized fibrotic lesion produced by intravenous injection of hexachlorotetrafluorobutane followed by subcutaneous injections of 4-nitroquinoline 1-oxide as a carcinogen in male Wistar rats.23
In the present series of experiments, localized fibrotic lesion in the lung of animals was produced by the method used in the previous experiment, and then polonium-210 was given intratracheally as a carcinogen in order to determine by histopathological examination what role, if any, the pre-existing localized fibrotic lesion played in the induction of pulmonary cancer.

**MATERIALS AND METHODS**

One hundred and eighty male Wistar rats, weighing about 120 g each, were injected with 0.05 ml/rat of hexachlorotetrafluorobutane (HC-TFB) through the tail vein. Twenty male Wistar rats received intravenous injection of 0.05 ml/rat of tetrachlorodifluoroethane (F112). These two chemicals cause hemorrhagic infarction of the lung. Some of these 200 rats died of hemorrhagic infarction of the lung immediately after the injection of HC-TFB or F112, or died of bronchopneumonia later, but 115 animals were alive 3 months after the administration of the chemical. Of these animals, 30 rats were taken as a control group and were given no further treatment.

The remaining 85 rats were given intratracheal instillation of 0.2 ml of saline containing 3 mg of hematite and 0.2 μCi of polonium-210 (Radiochemical Centre, Amersham) once a week for a total of 15 times beginning 3 months after the injection of HC-TFB or F112. Four rats each time were killed and autopsied after the 1st, 7th, and 15th intratracheal instillations of polonium-210. Forty-one of the remaining 73 rats died 3–21 months after the 1st instillation of polonium-210.

Table I. Numbers of Animals with Localized Fibrosis, Epithelial Hyperplasia, and Lung Cancer

<table>
<thead>
<tr>
<th>Time after the 1st instillation of Po-210</th>
<th>Effective number of rats</th>
<th>Number of rats with localized fibrosis</th>
<th>Number of rats with epithelial hyperplasia</th>
<th>Number of rats bearing tumor (histology)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7 &quot;</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15 &quot;</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6 months</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>9 &quot;</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>15 &quot;</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>17 &quot;</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1 (Adenosquamous ca.)</td>
</tr>
<tr>
<td>19 &quot;</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1 (Adenocarcinoma)</td>
</tr>
<tr>
<td>20 &quot;</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1 (Adenosquamous ca.)</td>
</tr>
<tr>
<td>21 &quot;</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

All rats received an intravenous injection of HC-TFB, followed by 15 weekly intratracheal instillations of polonium-210 beginning 3 months after the injection of HC-TFB.

**RESULTS**

The injection of HC-TFB or F112 caused hemorrhagic infarction of the lung (Fig. 1). Twelve days after the injection of HC-TFB or F112, an increase of collagen fibers...
became apparent, and fibrotic thickening of the pleura and localized fibrosis beneath the pleura were observed. Details of these changes have been described previously.23,27)

The lungs of 30 control rats were examined histopathologically from 3 to 24 months after the injection of HCTFB.

Of these rats, 26 showed localized fibrotic lesion in the lung. In these 26 rats, there was a partial fibrous adhesion of the pleura, and other parts of the pleura also showed fibrotic thickening. A localized fibrotic lesion was seen beneath this thickened pleura, with marked increase of collagen fibers and slight elastosis, periairteriolar fibrosis, and formation of a few cysts lined with a layer of squamous or cubic epithelium. A slight infiltration of inflammatory round cells was observed around these cysts, but such cell infiltration was virtually absent elsewhere in the localized fibrotic lesion. In some cases, the bronchiole was imbedded in part of the fibrotic lesion, and a lymph apparatus was seen around it. Lung tissue around the localized fibrous lesion showed scattered fibrous thickening of the alveolar wall, and emphysematous foci were observed (Fig. 2). There were large numbers of mast cells and sideroferous cells positive to Berlin blue staining in the localized fibrotic lesion in the lung. The lungs in these 30 control rats did not show any hyperplasia of epithelial cells. The localized fibrous lesions of the lungs in these 26 control rats were histologically identical.

In the experimental group, changes in the lungs of the 12 rats which were sacrificed at the 1st, 7th, and 15th intratracheal instillations of polonium-210 were approximately the same. In all the animals, there were partial fibrotic adhesions of the pleura and localized fibrotic lesions beneath the pleura. These pathological findings were almost the same as those in the control rats. There was no epithelial hyperplasia in the lungs of these 12 rats. In the autoradiograms of all these lungs, tracks of α-rays indicating deposition of polonium-210 were observed in the fibrotic thickening of the alveolar wall around the localized fibrotic lesion (Fig. 3), the margin of the localized fibrotic lesion beneath the pleura, the bronchial epithelium, and lymph apparatus around the bronchus. The amount of radioactivity deposited decreased in the above order. In each animal, the sites and amounts of polonium-210 deposited were as described above, but the amounts of the radionuclide deposited showed considerable differences among the animals in the same group given intratracheal instillations of polonium-210 the same number of times.

Thirty-two rats were examined histopathologically 6~21 months (the end of this experiment) after the 1st instillation of polonium-210. Among these animals, fibrotic adhesion and thickening of pleura, and fibrotic lesion in the lung were seen in 26 rats (81.2%). There was no infiltration of inflammatory cells nor neoformation of capillaries in the fibrotic lesion. A slight emphysema was seen around the lesion. In 19 (73%) of these 26 rats, hyperplasia of cubic epithelial cells or squamous metaplasia was seen on the alveolar wall of the marginal portion of the localized fibrotic lesion in the lung (Figs. 4 and 5). The nucleus of these epithelial cells showed a slight hyperchromasia, and these cells exhibited atypia and polymorphia. Foam cells were found in a mass in the alveoli near the localized fibrotic focus. In the autoradiogram, a large number of α-ray tracks were seen corresponding to the interstitium of the proliferating epithelial cells (Fig. 6) and foam cells in the alveoli of rats examined 6 months after the 1st instillation of polonium-210, but tracks were scattered in the marginal tissue around the localized fibrotic lesion 9~21 months after the 1st instillation of polonium-210.

Pulmonary carcinomas closely related to the localized fibrotic lesion were seen in 3 (11.5%) of these 26 animals. Two of these cancers were adenosquamous. These were found in two animals examined 17 months
Fig. 1. Pulmonary hemorrhagic infarction caused by intravenous injection of HCTFB. Hematoxylin-eosin stain. ×33.

Fig. 2. Localized fibrotic lesion 3 months after injection of HCTEB. Hematoxylin-eosin stain. ×13.2.

Fig. 3. Tracks of α-rays in the fibrotic thickening of the alveolar wall 2 days after completion of 7 weekly administrations of polonium-210. Autoradiogram. ×200.

Fig. 4. Hyperplasia of cubic epithelial cells and squamous metaplasia in the marginal portion of the localized fibrotic lesion 20 months after the 1st instillation of polonium-210. Hematoxylin-eosin stain. ×66.

Fig. 5. A higher-power view of the hyperplastic cells shown in the previous figure. Hematoxylin-eosin stain. ×132.

Fig. 6. Tracks of α-rays in the interstituium beneath the hyperplastic epithelium 6 months after the 1st instillation of polonium-210. Autoradiogram. ×400.
and 20 months after the 1st instillation of polonium-210 (Figs. 7 and 8). In these cases, squamous metaplasia was found in part of the marginal portion of the localized fibrotic lesion, together with adenomatous hyperplasia of epithelial cells. Continuous with these changes, epidermoid carcinonoma and adenocarcinoma were mixed irregularly. Morphological transition was observed between these two kinds of cancer cells. A part of the lesion showing the morphology of epidermoid carcinoma had destroyed the bronchial wall, and cancer tissue with marked keratinization was seen in the lumen of the bronchus. There was also infiltrative growth of cancer cells into the surrounding lung tissue, with destruction of elastic fibers in the alveolar wall. In the remaining animal, adenocarcinoma was found 19 months after the 1st instillation of polonium-210. The carcinoma was seen in the margin of the fibrotic focus in the lung. The cancer cells showed moderate atypia, with the structure of papillary adenocarcinoma (Fig. 9). Infiltra-
tive and destructive growth into the surrounding pulmonary tissue was apparent. In these 3 cases of lung cancer, α-ray tracks were seen in the autoradiogram corresponding to a part of the localized fibrotic lesion adjacent to cancer cells and in some of the cancer cells (Fig. 10).

There were 6 cases without any localized fibrotic focus in the lung during the 6–21 month period (end of this experiment) after the 1st instillation of polonium-210. In these 6 animals, there was no pulmonary carcinoma nor epithelial hyperplasia in the lung. A few α-ray tracks indicating deposition of polonium-210 were dispersed in the alveolar wall in these cases.

**DISCUSSION AND CONCLUSIONS**

Various kinds of environmental pollution have been cited as external factors in lung carcinogenesis,1–10 but the internal factor inherent in an individual is also considered to play an important role in carcinogenesis. It is generally difficult to establish morphologically the internal factor responsible for pulmonary carcinogenesis. However, a group of cancers called “scar cancer of the lung” is presumed to arise from a localized fibrotic focus present in the lung, i.e., the scar.2–4,19 Further, the fact that diffuse interstitial fibrosis5 and honeycomb lung21 are considered to be involved in lung carcinogenesis suggests a close relationship between lung scars and carcinogenesis. However, there has been a report in Japan casting doubt on the existence of true scar cancer of the lung.9 Uncertainty remains for many reasons, including the lack of a method for determining accurately which of the two, the scar or cancer, appears first, the small number of reported cases in which growth of cancer cells was seen only in a region surrounding the scar in the early period of scar cancer, and the difficulty in producing scar cancer in experimental animals similar to that observed in the human lung, at least until recently.

Stanton and Blackwell26 obtained lung cancers in rats given an intravenous injection of 3-methylcholanthrene with HCTFB. Blenkinsopp3 reported a study on pulmonary carcinogenesis. In his experiments, rats were given HCTFB followed by intravenous injection of 3-methylcholanthrene in arachis oil, and no cancer was found. They did not determine the intrapulmonary distribution of the carcinogen, however.

Polonium-210 is a naturally occurring, alpha-emitting radio-nuclide of the uranium decay series and is contained in plants and foods. Some reports have appeared on the relationship between polonium-210 and lung cancer.15,16,17,18,20 Kennedy, Worcester, and Little13 gave polonium-210 intratracheally to untreated hamsters.

In our experiments, rats were pre-treated with HCTFB to produce a localized fibrotic lesion after hemorrhagic infarction, and then polonium-210 was given intratracheally to see whether or not a carcinogenic substance given by intratracheal instillation would be deposited in the localized pulmonary fibrotic focus. It was found that polonium-210 deposited in epithelial cells of the bronchus and in the lymphatic apparatus around the bronchus disappeared relatively quickly from these sites, but that polonium-210 deposited in the localized fibrotic lesion in the lung remained there for a long period. These findings indicate that the radionuclide disappeared rapidly into the blood and trachea from the parts without any lesion.13 In contrast, the clearance ability was greatly reduced in the localized fibrotic focus of the lung. With regard to clearance ability, a localized fibrotic lesion located in the peripheral part of the lung is often accompanied by pleural adhesion, interfering with normal movement of the lung, and thus affecting aeration and blood or lymph flow.8 The finding that the localized fibrotic lesion is poorly supplied with blood (or lymphatic)
vessels may be significant. These factors would lead to reduced clearance ability, which means that the carcinogen might remain there over a long period of time. Straus and others\textsuperscript{28} stated that anoxia, disturbance of circulation, and accumulation of carcinogen at the scar are responsible for carcinogenesis.

In our present series of experiments, there was a proliferation of cubic epithelial cells continuous with the bronchiole along the alveolar wall around the localized fibrotic lesion and this is considered to correspond to what Lisco and others\textsuperscript{14} called "alveolar epithelization." It seems to indicate that this epithelial hyperplasia is related to carcinogenesis, since the hyperplasia was observed in close proximity to the localized fibrotic focus, and lung cancer was found there at the same time.

In the present experiments, lung cancer was induced in 3 of 26 rats (11.5%). These cancers were in close proximity to the pre-existing fibrotic lesion in the lung. This incidence is similar to the results obtained by Little and others,\textsuperscript{18} Yuile and others,\textsuperscript{29} and Sanders\textsuperscript{25} using polonium-210 or plutonium-238 in hamsters or rats. We were not able to find any epithelial hyperplasia or carcinoma in the intact lung tissue apart from the localized fibrotic lesion in the rats which had received the injection of HCTFB and instillations of polonium-210. We should have given intratracheal instillations of polonium-210 to untreated rats, but in our present experiments, there were 6 rats without any fibrotic lesion in the lung, even though they had received the injection of HCTFB followed by 15 weekly intratracheal instillations of polonium-210. In these rats, there was no epithelial hyperplasia or cancer in the lung. We could not find any published report of a localized fibrotic lesion in close proximity to carcinoma induced by intratracheal administration of polonium-210 to untreated rats. These findings indicate that pulmonary cancers may arise in close proximity to localized fibrotic lesions, and we consider that localized pulmonary fibrosis is a contributing factor in lung carcinogenesis.

(Received October 20, 1979)

REFERENCES