The etiologic role of a previous attack of influenza in human lung cancer has been under question since 1919. The pertinent evidence is histological, statistical, and clinical. The idea that this infectious disease might lead to cancer originated from the old concept that infections in general might, under certain conditions, cause cancers but more specifically from the proliferative changes that occur in the lung during recovery from influenza. Following a variable amount of necrosis and desquamation of bronchial and bronchiolar epithelium during the acute phase of the disease, a tremendous over-regeneration of these cells occurs during recovery. The hyperplasia results in intrabronchial papilloma formation, extension of epithelium into adjacent alveoli, and other features which resemble and suggest early cancer.

Study of this hyperplasia led Askanazy (1) and Winternitz et al. (9) to predict in 1919 and 1920, following the influenza pandemic, that an increase in the incidence of lung cancer might ensue. The latter authors state:

"In a number of cases, epithelial proliferation has been so extensive that it could not be differentiated histologically from an invasive, malignant neoplasm. There is no reason to believe that malignancy might not result from the continuous stimulation of the epithelium to proliferate, in the chronic inflammatory process of the lung in influenza, just as chronic infection in the lung of a mouse results in a much higher percentage of spontaneous neoplasms of the respiratory tract in this species than in those animals where chronic pulmonary inflammatory processes are uncommon. It will be interesting, indeed, to see whether, as a late manifestation, there is an increase in the number of more recently rare epithelial new growths in the respiratory tract of man."

Their forecasts seemed to be borne out when lung cancer became commonly recognized during the next two decades. Many observers, however, commented that many persons with tumors had not had influenza. Nevertheless, the idea of such a relationship has persisted (3, 6). Having at our disposal a suitable means, we have experimentally studied the problem of the carcinogenicity of human influenza-A virus in mice.

The laboratory mouse is an excellent animal to use for experimentally testing the relationship of influenza to lung cancer. It is susceptible to infection by human influenza virus. During recovery from influenza the bronchial and bronchiolar epithelium undergoes cell proliferation which resembles in an exaggerated degree the changes seen in man (4). Finally, inbred strains of mice are available in which the incidence of spontaneous lung tumors is accurately known. In some strains the incidence is low, so that an increase after an experimental procedure is easily detectable. Other strains, with high spontaneous incidence, are suitable to determine whether a procedure has caused tumors to appear at an earlier age and whether the number of tumors per mouse has been increased.

**EXPERIMENTAL METHODS**

Mice were exposed in a chamber to a mist containing human influenza virus, type A (PR-8 strain), according to a technic previously described (4, 5). Adequacy of the exposure was recognized because of deaths from influenza from the eighth to fourteenth days and because of characteristic lesions found by sacrificing mice at selected intervals. All other mice were permitted to live out their natural life span. After death, the condition of the lungs and the number of tumors were recorded. The lungs were then fixed by intratracheal injection of Zenker-formol or formalin solution. Microscopical sections, passing transversely through both lungs and the mediastinal structures, were cut from paraffin blocks and stained with hematoxylin-eosin-azure II. Final counts of tumor were made from these microscopical sections.

The mice, equally divided as to sex, were young adults, varying from 2.5 to 4.5 months of age at the time of infection. They were of the low spontaneous lung tumor strain, C57 black, and of the high lung tumor strain A. They were obtained from the Roscoe B. Jackson Memorial Labora-
tory; after mixing, part of each group was set aside as uninfected controls. In all, 639 mice were used, of which 351 were exposed in the chamber. One infection experiment was performed with the C57 black mice, and two separate experiments, at different levels of exposure, with the A strain. Thus, there were six experimental groups, of which three were infected and three kept as uninfected controls.

RESULTS

INFLUENZA

Following exposure, the mice appeared normal until shortly before the first deaths occurred on the eighth day—although it was known, from animals sacrificed earlier, that almost every mouse was developing characteristic lesions. The outbreak of fatal cases was short and sharp, as illustrated in Figure 1, which demonstrates the biological homogeneity of members of these two strains to this type of challenge. The mortality, occurring between the eighth and fourteenth days, was 6 per cent in the C57 blacks and 14 per cent and 53 per cent, respectively, in two experiments with the A strain. The mortality figures also indicate adequate exposure. Many of the surviving mice appeared externally normal, but others exhibited dyspnea, which was worse on exertion, for some weeks; and some of these mice later developed an asymmetrical thorax. This was found to be consequent to atelectasis of some lobes and compensatory emphysema of others. Almost every exposed mouse sacrificed during the experiment, as well as those that died, showed the evolving lesions characteristic of influenza in this species. None of the controls showed these lesions. The subsequent mortality during the course of the experiments in both the experimental and the control mice was excessive, and it was apparently due chiefly to intercurrent liver disease.

The characteristic lesions of influenza in the mouse have previously been described (4, 8), so that the details need not be repeated. Only those lesions which might be regarded as precancerous are stressed here. The disease may be divided into three stages, consisting of an acute, short, destructive and inflammatory period, a regenerative and proliferative stage, and a permanent phase showing a variety of reparative and retrogressive conditions.

During the first week, there was damage to, or necrosis of, bronchial epithelium in the areas most severely involved, followed by slight, progressive inflammatory exudation into bronchi, alveoli, and interstitial tissues by fluid and leukocytes. These changes may be designated as bronchitis and pneumonia, both of special types (Figs. 2, 3). During the second week these inflammatory changes continued, and regeneration of epithelium began. Epithelium rapidly proliferated into bronchi and out into adjacent alveoli. Thus, epithelial bodies, often circumscribed and tumor-like in appearance, were formed during the second week (Figs. 4, 5). These lesions were small or large, and focal, lobular, or even lobar in size and distribution. They were most conspicuous between 2–4 weeks. At that time they were visible to the naked eye at necropsy. They grossly resembled, to a certain degree, the common lung tumor of this species. They stood out sharply on the microscopic sections, because the acute pneumonitis elsewhere was subsiding.

After the first month reparative, regenerative, and degenerative changes were well advanced. In mildly affected parts the inflammatory process resolved, and the bronchi and lung were restored to normal. In more severely involved areas the hyperplastic proliferated epithelium underwent gradual but great atrophy, so that by 10 months only a layer of flat lining cells was found. The most severely affected parts became atelectatic with fibrosis, bronchiectasis, and cyst formation, the latter containing mucus. Such atelectatic areas were fo-
**FIG. 2.**—Photomicrograph (× 15) showing an acute lobular influenza 7 days after exposure. The epithelium has not yet proliferated.

**FIG. 3.**—Photomicrograph (× 200) showing the acute destructive bronchial lesions; 7 days after exposure. The bronchus above shows sloughing of the inner portion of the cytoplasm, leaving the nuclei; in the bronchus below the entire cells have undergone necrosis.

**FIG. 4.**—Photomicrograph (× 55) showing the nodular, tumor-like epithelial hyperplasia in the proliferative stage of recovery from acute influenza (14 days after exposure). Strain C57 black mouse.

**FIG. 5.**—High power (× 390) photomicrograph from the same mouse as shown in Figure 4 to demonstrate the papillary epithelial proliferation into a bronchus and the extension out into alveoli along alveolar walls. The cells are hypochromatic, but they appear neoplastic.
Carcinal, lobular, or lobar, and they were shrunken and depressed (Figs. 6, 7). Microscopically, such areas showed a slight diffuse increase in fibrous tissue. The larger bronchi and some alveoli became dilated, and there was some fusion of alveoli. These nonfunctional areas occupied as much as three entire pulmonary lobes.

The sequence of events was the same in both strains of mice. In the A strain, exposed at two levels of dosage, quantitative but not qualitative differences were seen in the two groups. The chronic changes persisted throughout the life span of the mice.

**Tumors**

Carcinogenicity of the influenza was evaluated by comparing the following five factors in the influenza-infected and nonexposed mice: (a) the per cent of mice with tumor, (b) the average number of tumors per tumor mouse, (c) the number of tumors arising in influenzal versus noninfluenzal areas in the exposed mice, and (d) the induction time for tumors. Finally, (e) evidence for direct origin of tumor from influenza was sought: the histological appearance of tumors was compared with the "precancerous" influenzal-induced hyperplasias, with special emphasis on transition or intermediate forms.

**C37 black strain.**—No lung tumors were seen in the low lung tumor C37 black strain in either the influenza-exposed or the controls (Experiments 1 and 2, respectively). Influenza virus, therefore, did not induce lung tumors. These mice were 4 months old at the time of exposure. The survivors in the experimental group numbered 65 at 12 months of age, 30 at 18 months, and 13 at 24 months (Tables 1 and 2). The corresponding figures for the controls were 65, 18, and 5 mice. The number of long-term survivors was less than ideal, but a fairly adequate test of carcinogenicity was obtained. Tremendous epithelial hyperplasia, often nodular and tumor-like (see Fig. 4), was seen in almost every mouse examined at an early age, but this process did not eventuate in tumor. Rather, it underwent gradual atrophy. It is concluded that influenza was not a carcinogen in this strain of mice.

**A strain.**—In the high spontaneous lung tumor strain A mice, tumors were found in the influenzal-infected and in the control groups. The per cent of mice with tumors in the two infected groups was 16.0 and 13.8 (Experiments 3 and 5 in Tables 1 and 2). The corresponding figures for the controls were 28.2 and 13.8 per cent. It is clear that the influenza did not increase the number of tumor mice, and by this criterion the virus was not a carcinogen. The number of mice with tumors was actually less in one infected group (Experiment 5) than in its control (Experiment 4). The difference is due to the greater number of tumors in the controls during the first months of the experiment. The difference is probably not statistically significant ($P = 0.2$).

The average number of tumors per tumor-bearing mouse was not greater in the infected than in the control mice. Influenza infection, although it often altered many areas in the lungs, did not increase the total number of areas from which tumors arose; and, judged by this criterion, it was not carcinogenic.

Lung tumors in mice, unlike those in man, are frequently multicentric and multiple. This affords an opportunity to study the relative incidence of tumors in influenzal and noninfluenzal areas within the same animal. While the lung tumors were studied and counted, efforts were made to determine whether they arose from normal or from influenza-altered areas. The results, given in Table 1, show that the great majority arose from normal-appearing lungs (Fig. 8). The sum of all normal areas, as seen on microscopical slides, greatly exceeded the diseased portions. An attempt was made to estimate the relative proportions of each. From a study of this data, not here presented because it was not quantitative, it was also clear that influenzal areas did not give rise to an undue proportion of tumors. One possibility, which cannot be eliminated, is that some tumors arose from small influenzal areas which became obliterated by subsequent growth of the tumor. This event is improbable, because all the small, early tumors seen were found to be originating from normal-appearing lungs (Fig. 9), and the incidence of tumors in uninfeeced controls was as great as in the exposed mice. By this criterion, also, influenzal infection did not induce tumors.

The age at which the first tumor appeared was less in both control strain A groups than in the infected mice (Tables 1 and 2). Judged by the induction time, influenza virus was not a carcinogen. Indeed, there is some question whether or not the virus is a tumor inhibitor or anticarcinogen. The cumulative yields and the induction times are shown graphically for the experiments with strain A mice in Fig. 10. It is clear that the virus was not carcinogenic, but uncertainty remains as to its anticarcinogenicity.

Finally, cytological study of the origins and critical comparison of the histological appearance of the epithelial hyperplasia of influenza and the lung tumors, in their early stages as well as in the fully developed lesions, showed that there were striking differences. The hyperplasia would hardly be expected to eventuate in the tumors, at least not directly as the result of failure to check the
FIG. 6.—Photomicrograph (× 7) showing the lesions of chronic influenza residues at 10½ months. Strain A mouse. Three lobes are shown of which three have lesions of chronic atelectasis and two show emphysema.

FIG. 7.—Higher power (× 35) of a small chronic influenza residue at 18 months. This is the late atrophic stage with distortion of architecture, bronchiectasis, and small alveolar cysts containing mucus.

FIG. 8.—Photomicrograph (× 7) showing two lung tumors, one originating from normal lung and the other from an influenza lobe although not from a influenza area.

FIG. 9.—Photomicrograph (× 200) of a very early lung tumor in an unexposed strain A mouse age 3½ months. Comparison with Figs. 4 and 5 show the differences between lung tumors and the influenza epithelial hyperplasia described in the text.
hyperplasia. The following differences, some of which may be seen by comparing Figures 5 and 9, were observed among others: (a) The influenzal areas are peri-bronchiolar, lobular or lobar in shape and distribution, while the tumors are round and chiefly peripheral; (b) The influenza areas apparently arise from bronchi and include alveoli, whereas the tumors begin from alveolar walls away from bronchi and only later enclose the latter; (c) The influenzal lesions incorporate the

**TABLE 1**

**EFFECT OF INFLUENZA ON INCIDENCE OF LUNG TUMORS IN MICE**

<table>
<thead>
<tr>
<th>Exp' no.</th>
<th>Strain of mice</th>
<th>Procedure</th>
<th>Mortality from influenza (per cent)</th>
<th>Age of mice when infected (months)</th>
<th>No. of mice</th>
<th>No. of mice at start</th>
<th>No. of mice in effectual total (months)</th>
<th>Age at first tumor (months)</th>
<th>No. mice with tumor per mouse</th>
<th>Av. no. of tumors per normal lung</th>
<th>No. tumors in areas of influenzal origin</th>
<th>No. tumors in normal lung areas</th>
<th>Per cent yield of tumors</th>
<th>Induction time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C57 Bl.</td>
<td>Infected</td>
<td>6</td>
<td>4.0</td>
<td>101</td>
<td>83</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>C57 Bl.</td>
<td>None</td>
<td>0</td>
<td>83</td>
<td>73</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>Infected</td>
<td>33</td>
<td>4.5</td>
<td>100</td>
<td>38</td>
<td>13</td>
<td>6</td>
<td>1.3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>None</td>
<td>0</td>
<td>108</td>
<td>92</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>Infected</td>
<td>14</td>
<td>3.5</td>
<td>150</td>
<td>121</td>
<td>10</td>
<td>14</td>
<td>1.2</td>
<td>16</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>None</td>
<td>0</td>
<td>102</td>
<td>87</td>
<td>4</td>
<td>12</td>
<td>1.1</td>
<td>13</td>
<td>0</td>
<td>13.8</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

**SUMMARY: SURVIVAL OF MICE AND OCCURRENCE OF LUNG TUMORS**

<table>
<thead>
<tr>
<th>Exp' no.</th>
<th>Strain of mice</th>
<th>Treatment</th>
<th>Survival time (in months after exposure) and mice with tumor*</th>
<th>Total no. of mice with tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C57 Bl.</td>
<td>Infected</td>
<td>0 11 3 6 9 14 18 31 54 81</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>C57 Bl.</td>
<td>None</td>
<td>83 78 70 69 58 28 16 11 2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>Infected</td>
<td>100 38 34 31 2/22 15 1/9 3/1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>None</td>
<td>103 3/6 2/64 2/37 5/4 4/37 9/25 7/0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>Infected</td>
<td>150 112 72 60 1/44 1/37 1/25 1/7</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>None</td>
<td>102 87 2/88 67 2/57 2/43 27 4/8</td>
<td>1/0</td>
</tr>
</tbody>
</table>

*The numerator is the number of mice with lung tumor in the previous months; the denominator is the number of surviving mice.
†The heavy losses were due to sacrifice and to influenza. For mortality from influenza see Table 1.

The typical gross changes produced by influenza and the relation of subsequent lung tumors to these lesions is further illustrated by Figures 11–16. Chronic, permanent influenzal residues may exist without developing related tumors (Figs. 13 and 14). Tumors were found in normal-appearing areas of uninfected mice (Fig. 12), in the normal-appearing portions of influenza-exposed lungs (Fig. 15), or in the atelectasis of influenza residue.
(Fig. 16). The relative numbers of tumors under these different conditions are given in the tables and in Figure 10.

COMMENTS

In searching for agents which might induce human lung cancer, it is desirable to keep in mind not only the well known physical and chemical carcinogens which, under special conditions, cause cancers in man, but also the possible carcinogenicity of microorganisms, including viruses. It is sufficient to recall, in this connection, the role played in their respective tumors by the Shope papilloma virus, the Rous sarcoma virus, Bittner’s mammary tumor agent, and the Lucké renal carcinoma virus. Studies on possible pulmonary chemical carcinogens have been reported in a previous paper (7).

In mice, the human influenza virus was not carcinogenic according to the five criteria used for analysis. The virus was a powerful epithelial growth stimulant, however, inducing nodular hyperplasias which were suspected in mice, as they had earlier been in man, of being precancerous. Subsequent observations showed that they did not go on to tumors, and hence they were not truly precancerous. The virus was a powerful growth stimulant (i.e. an epitheliagen) but not a carcinogen. This again illustrates that cancer is not merely a simple problem in growth.

The experimental results should not, of course, be transferred to the problem in man. From all the evidence, however, there is little reason to suspect any longer that influenza does play a role in causing human lung cancer. The epithelial hyperplasias induced in the mice were relatively much greater than those described in man. One strain of mice used has a strong predisposition to spontaneous and induced lung tumors. Despite these facts, the lesions did not progress to neoplasms.

The possible inhibitory or anticarcinogenic effect of influenza virus to lung tumors deserves further study. Campbell (9) obtained evidence for the same phenomenon by a different method. He vaccinated mice by intraperitoneal injection of virus vaccine, and followed this by instillation of live virus intranasally. The mice were then exposed daily to a dust containing coal tar. He observed two tumors in mice so treated. This figure was greater than that for his controls but less than that for the dusted mice without influenza virus. The statistical significance of his results, however, is questionable.

SUMMARY

The carcinogenicity of human influenza virus (type A, PR-8 strain) for lung tumors was studied in mice. The infection produced great, tumor-like, epithelial hyperplasia during the recovery stage. Some influenza-induced lung lesions persisted throughout the life of the animals. These changes did not eventuate in lung tumor in mice of the low

lung tumor strain C57 black. Neither was the infection carcinogenic nor the epithelial hyperplasia precancerous in the high spontaneous lung tumor strain A as judged by (a) no increase in the number of mice with tumor, (b) no increase in the average number of tumors per tumor mouse, (c) no
greater number of tumors arising from influenzal lesions than from noninfluenzal areas, (d) no reduction in the tumor induction time, and (e) no visible microscopic transformation of influenzainduced hyperplasia to tumor.

There was a suggestion that the influenza infection might have been anticarcinogenic as indicated by a lower per cent yield of tumors (in one experiment), and a lengthening of the tumor induction time (in two experiments) in the virus-infected as compared with the unexposed control mice.

CONCLUSIONS

Human influenza virus (type A, PR-8 strain) was a powerful growth stimulant to mouse regenerating bronchial epithelial cells, but it did not induce tumors. It was a growth stimulant—an epitheliagen—but not a carcinogen. The experimental work with mice did not support the theory of a causal relationship between influenza infection and lung cancer.

REFERENCES

The Effect of Human Influenza Virus (Type A) on the Incidence of Lung Tumors in Mice

Paul E. Steiner and Clayton G. Loosli