Papers and Originals

Production of Lung Carcinomas in C57BL Mice Exposed to a Cigarette Smoke and Air Mixture

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[WITH SPECIAL PLATE BETWEEN PAGES 640 AND 641]

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There is no doubt that the tar produced by condensing cigarette smoke is carcinogenic for mice (see Day, 1967). However, lung epithelium differs from the skin and the condensate differs from fresh cigarette smoke in, for example, the absence of the gaseous components. It appeared worth while to us, therefore, to attempt to produce lung carcinomas in mice by allowing them to breathe a mixture of cigarette smoke and air for a prolonged period.

Stewart and Herrold (1962), in a "critique of experiments on attempts to induce cancer with tobacco derivatives," review negative mouse inhalation studies by Lorenz *et al.* (1943) and by Dunn (1943) using Strong A mice (a strain with a 90% spontaneous incidence of lung adenomas at 18 months of age). Essenberg (1952), however, had exposed the same strain of mice to cigarette smoke for 12 to 14 months in a ventilated chamber and *increased the incidence* of papillary adenocarcinomas in these mice.

Mühlbock (1955) also claimed to have *increased the incidence* of lung tumours in a hybrid 020 × DBA strain of mouse by puffing smoke from cigarettes into a sealed cage every two hours each day.

We believed, however, that we should use a strain such as C57BL, which apparently have a predominant gene for resistance to the induction of lung tumours (Bloom and Falconer, 1964), and in which Peacock and Peacock (1966) described only one deep-seated spontaneous papillary adenoma in an 817-days-old male.

We were thus aware of many negative results, but were not convinced that these experiments had been conducted under the optimum conditions. In most cases (except for Lorenz *et al.*) there was no evidence that the animals were actually taking the smoke droplets into their lungs.

Most observers agree that lung cancer in man is multifactorial, and that of these other factors chronic respiratory disease (bronchitis, bronchiolitis, and chronic pneumonitis) is probably the most important.

There are two ways in which lung disease might contribute to the occurrence of lung cancer. Firstly, the diseased lung might be less able to prevent retention of particulate carcinogens, such as smoke droplets; and, secondly, infectious agents such as respiratory viruses and mycoplasma might themselves produce areas of cellular proliferation which were susceptible to the effects of inhaled chemical (or viral) carcinogens.

As early as 1920 Winternitz *et al.* predicted that, since the changes produced by influenza viruses in the lung looked preneoplastic, the 1919 influenza pandemic should give rise

to lung cancers later. Straub (1937) observed similar lesions in the lungs of influenza-virus-infected mice, but single infections had no apparent effect in increasing the lung cancer incidence in A (lung adenoma-prone) or C57BL (lung adenoma-resistant) mice (Steiner and Loosli, 1950).

The studies of Kotin and Falk (1956) and Kotin *et al.* (1958), however, have now confirmed for mice the earlier hypothesis. They found, for example, that following infection with PR-8 and Lee strains of influenza the bronchial and bronchiolar epithelium of mice regenerated after about seven days, but that peribronchial hyperplastic foci remained for some time. Sendai (parainfluenza) virus produced extensive necrosis of the epithelium, followed by regeneration. Kotin *et al.* infected mice successively with these strains, hoping that the squamous metaplasias so produced would "progress" to squamous carcinoma.

The carcinogen employed by Kotin *et al.* was a "smog" of ozonized hydrocarbons. The animals given both influenza and smog showed areas of squamous metaplasia in their lungs, and where keratin was detected preneoplastic or neoplastic changes were also observed. The experiments ran for 31 months (Kotin, 1966), and whereas no squamous cell carcinomas (but only pulmonary adenomas) were found in control "smog"- or "flu"-treated groups the smog and influenza groups had 10% squamous cell carcinomas (33/328 mice). Kotin, however, gave no data concerning the transplantability of these tumours. Leuchtenberger and Leuchtenberger (1966) observed very similar histology for influenza-infected mouse lung. When mice were subjected to cigarette smoke in a large chamber "atypical proliferative lesions" were described by the same authors, and when these treatments were combined the dysplastic lesions of the bronchi and bronchioles were similar to those produced by influenza alone, but in addition there were histopathological features in the lung "resembling bronchogenic carcinoma."

We proposed, therefore, to study also the effect of previous infection with influenza viruses on the induction of lung tumours by cigarette smoke in the C57BL mice. There were, of course, a number of control groups of mice. Thus in summary:

Group 1: Exposure to infection by one or more serologically non-overlapping influenza virus aerosols.

Group 2: Exposure to an aerosol of 3:4-benzpyrene.

Group 3: Exposure for life to a cigarette smoke and air mixture.

Group 4: A combination of group 1 followed by group 2.

Group 5: A combination of group 1 followed by group 3.

Group 6: Exposure to aerosols of the aqueous suspending media for the virus or benzpyrene.

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An interim account of the experiments is given by Harris and Negroni (1966).

Materials and Methods

Mice.—Young adult C57BL mice of both sexes, random bred in these laboratories, were used. Their past history showed that they had a long life-span, a low incidence of spontaneous tumours of all kinds (but *not* of leukaemia, see below), and a nil incidence of lung carcinomas, though *thymectomized* mice of a hybrid CBA × C57BL strain do develop these (D. M. V. Parrott, personal communication).

Aerosol Production.—The aerosol generator of “closed-circuit” design described by Henderson (1952) gave reproducible results not only for the influenza viruses but also for the benzo(a)pyrene.

Influenza Viruses.—Whereas Kotin and Falk (1956) had used PR-8, Lee, and Sendai, we employed W.S., Swine, Lee, and Singapore strains. These were grown from seed virus in RIF-free embryonated Brown Leghorn eggs from our own closed stock. These strains are antigenically unrelated, and the immune response of the mouse to one strain does not interfere with subsequent infection with another. Where mice were infected with more than one strain there was one month's interval between infection, so that interferon, which is believed to play a part in oncogenesis by other viruses, might not interfere with subsequent exposure to carcinogens. Small doses of the influenza viruses produce pneumonia in the mice but do not kill them, and so this optimum dose had to be determined by direct titration of each virus in mice and in the embryonated eggs. Intranasal instillation or aerosols were used, but the latter gave a more reproducible result and a more uniform distribution of lung lesions in the infected mice. Ten animals were exposed at a time, and the aerosol technique only was used for the combination treatment of mice with viruses and cigarette smoke.

Benzopyrene.—The aerosol was generated from a solution of 3:4 benzopyrene (32 mg.) in aqueous (0.5%) gelatin (250 ml.) containing 1:10,000 thiomersalate.

Cigarette Smoke Generator

In preliminary investigations groups of mice were exposed to a smoke and air mixture in a 400-litre plastic chamber originally designed by Amsco as a sterile isolator for housing germ-free mice. A standard smoking-engine (ITC) used for the production of tobacco smoke condensate was adapted by H. G. East and Co. (Sandy Lane West, Littlemore, Oxford) to produce a dilute mixture of fresh cigarette smoke in air. We chose a ratio of 1:39 (25 ml. smoke to 975 ml. air), which approximates to that of one puff of a cigarette in the tidal volume of the human lung.

Under equilibrium conditions (72 cigarettes smoked) the nicotine concentration in such a chamber reached 0.1 mg./l. (Harris and Negroni, 1966), which the mice tolerated for more than three hours without apparent distress. Moreover, analysis of their lungs after such a period failed to reveal the presence of nicotine. We concluded that, under conditions of high humidity—condensation was readily apparent on the walls of the chamber—the hygroscopic smoke droplets (containing the nicotine) had increased in size until they were too large to penetrate to the lower respiratory tract of the mice.

We then developed a new type of manifold (Fig. 1) based on the machine developed by Henderson (1952) for the exposure of animals to aerosols of bacteria, etc.

In such a machine a fresh cigarette smoke and air mixture is continuously passed across the noses of the mice, and the noses alone are in contact with it. The concentration of nicotine in the mixture was again about 0.1 mg./l. (and the CO

concentration—0.064 volume %—much less than that in the large chamber—0.16 volume %). Under these conditions we found that the mice were initially unable to tolerate exposure for more than 12 minutes, and that when they died under exposure the nicotine content of their lungs varied between 14 and 17 µg. (Harris and Negroni, 1966).

FIG. 1.—Plastic manifold for exposing mice to smoke and air mixture. The mice are held around the neck in metal boxes so that only their noses are in the smoke and air stream. The boxes are attached to the manifold magnetically. Reprinted by permission from *Lung Tumours in Animals*, edited by L. Severi, University of Perugia, 1966.)

We then adopted the procedure of exposing 10 groups of 20 male and female C57BL mice to the smoke/air from 12 cigarettes (taking 12 minutes) every other day for the duration of their lives.

Controls

Control groups of equal numbers of each sex were housed under identical conditions and fed the same diet and water *ad libitum*. Each control group (50 males and 50 females) had been exposed to an aerosol of an aqueous suspending medium used to produce aerosols of influenza viruses (the results of these experiments will be separately reported) or of benzo(a)pyrene. Another control group consisted of 200 C57BL mice exposed on four occasions at monthly intervals (beginning when they were 5 months old) for a period of 10 minutes to an aerosol of 3:4-benzopyrene (32 mg.) in aqueous (0.5%) gelatin (250 ml.) containing 1:10,000 thiomersalate as a preservative in the Henderson machine. We calculated that in such an exposure the mice would receive in their lungs about as much 3:4-benzopyrene as they would receive from two years' exposure to the smoke and air mixture.

When each animal died a careful search was made to determine the cause of death. Sick animals were usually killed so that it might be possible to attempt transplantation (and thus to confirm malignancy) of any lung tumours produced.

Results

We had originally believed that strain C57BL had a low incidence of lymphosarcoma/leukaemia, but it became apparent, as the groups of mice aged, that the studies of others had probably not been continued in sufficiently elderly mice. Table I shows the cumulative incidence of lymphosarcoma/leukaemia in all groups for each sex.

Table II shows the cumulative incidence for all deaths, except for malignancies, for each sex in each group.

Mice also died from other tumours, and these are listed in Table III. Table IV (p. 640) shows the incidence of lung carcinomas in the six groups.

TABLE I.—Cumulative Percentage Mortality from Lymphosarcoma/Leukaemia

Group	Sex	Month															
		16	18	20	22	24	26	28	30	32	34	36	38				
Control	M	1	2	5	7	9	16	25	31	39	43	48	53				
	F	2	3	4	8	13	19	29	35	41	55	57	60				
Influenza	M	—	2	4	6	13	19	29	37	41	43	44	45				
	F	—	1	3	6	10	14	23	30	34	36	37	38				
Benzpyrene	M	—	1	4	6	16	23	27	34	40	41	42	42				
	F	1	1	3	5	10	20	31	43	52	60	64	66				
Smoking	M	4	8	8	15	25	31	37	46	57	65	69	70				
	F	2	3	4	8	11	22	28	32	35	39	41	44				
Influenza + benzpyrene	M	—	—	3	8	14	20	26	33	47	48	50	51				
	F	—	—	4	8	15	29	34	45	49	61	62	63				
Influenza + smoke	M	—	1	1	1	6	10	10	14	17	21	21	26				
	F	2	4	5	8	14	19	23	30	40	46	49	—				

TABLE II.—Cumulative Percentage Mortality from "Non-malignant" Diseases

Group	Sex	Month															
		16	18	20	22	24	26	28	30	32	34	36	38	40			
Control	M	6	7	10	17	20	21	23	27	29	32	33	35	37			
	F	10	15	16	19	21	21	26	28	28	32	33	35	—			
Influenza	M	8	10	11	14	21	24	28	32	34	36	39	39	—			
	F	16	19	22	25	28	30	34	37	38	39	40	40	—			
Benz-pyrene	M	22	23	26	28	33	37	41	44	50	57	58	59	—			
	F	11	13	14	20	23	26	29	32	34	36	38	39	—			
Smoking	M	5	8	13	15	16	21	23	25	26	—	—	—	—			
	F	8	8	11	11	16	21	36	42	48	48	49	50	51			
Influenza + benz-pyrene	M	2	5	5	7	8	15	20	23	26	31	35	37	—			
	F	5	9	9	10	13	15	16	23	25	27	30	31	—			
Influenza + smoke	M	6	6	7	11	15	18	24	29	35	40	43	45	47			
	F	5	6	6	7	10	16	18	19	28	30	31	31	—			

TABLE III.—Incidence of Tumours (Other than Lymphosarcoma/Leukaemia or Lung Carcinomas) in Experimental and Control Groups

Group	No.		Hepatomas		Carcinomas		Sarcomas	
	M	F	M	F	M	F	M	F
Influenza ..	342	340	23 (29)	10 (25)	1 (18)	3 (28)	1 (27)	6 (26)
Benzpyrene ..	100	100	14 (29)	5 (33)	—	—	—	1 (34)
Smoking ..	100	100	3 (25)	6 (25)	1 (30)	—	1 (30)	1 (16)
Influenza + benzpyrene	100	100	3 (29)	6 (26)	—	—	—	—
Influenza + smoke ..	72	83	5 (30)	3 (25)	—	1 (20)	—	1 (26)
Control ..	100	100	5 (31)	6 (28)	1 (20)	2 (18; 33)	1 (30)	1 (31)

Figures in parentheses are mean ages at detection in months.

Pathology

The following account of the pathological observations is reported in two sections: the first refers to the changes, other than lung lesions, observed in mice belonging to the various groups. The second is a detailed account of the macroscopic and microscopical changes in the lungs of mice which are summarized according to the treatment the animals received.

Lesions other than those Found in the Lungs

All the mice, no matter to which group they belonged, showed various lesions other than lung lesions. These, however, were common to all groups, and occurred quite independently of the treatment which the mice received. C57BL mice were chosen because, according to the current literature, they are supposedly free from tumours. This proved to be untrue, as lymphosarcoma/leukaemia occurred in a very high incidence in all groups of mice (Table I).

The incidence was the same in all groups, if one takes into account only animals which survived more than 20 months. A higher mortality rate before this age was observed in male mice treated with benzpyrene (Table II) and in females treated with influenza viruses (Table II). The incidence of leukaemia appeared to be lower in these groups than in the control group (Table II), but only because in these groups the mortality rate was higher up to 20 months of age.

Hepatitis of the type described by Tyzzer (1917), and more recently and carefully studied by Craigie (1966), in these laboratories was one of the major causes of death in one group. In other instances, enteritis, probably due to Tyzzer's micro-organism, was the cause of death. Salmonellosis does not occur in these laboratories.

Pneumonia, probably of viral origin, occurred sporadically. Urinary retention and cystic kidneys also occurred infrequently.

Tumours, other than leukaemia, were also observed—hepatomas, subcutaneous sarcomas, and carcinomas (Table III). Hepatomas in both male and female were the most frequent. Skin sarcomas and carcinomas, some of them originating from the skin glands, were also observed in very low incidence.

It is worth while stressing again that all these tumours occurred with equal frequency in mice which had received any of the treatments.

Lesions Found in the Lungs

This section refers to the lung pathology, and this is described separately according to the treatment each group of mice received.

Controls (Group 6)

The only lung lesions observed were those due to pneumonia, probably caused by viruses.

Mice Exposed to Cigarette Smoke (Group 3)

This group showed pneumonia occasionally, with an incidence not higher than that observed in the controls. Mice which had been exposed to cigarette smoke for a long time often showed emphysema. Marked peribronchial and perivascular infiltration with lymphocytes was seen, and this independently of the presence of tumours. Hyperplastic lesions similar to those described by Leuchtenberger and Leuchtenberger (1966) were not seen even in mice which survived smoking over a period of more than 30 months.

We did observe, however, a lesion which from its characteristics could be classified as hyperplastic. This had already been described by Kotin and Falk in mice which had breathed artificial smog (see Kotin, 1966). We have observed this in one mouse out of the 200 exposed to cigarette smoke. Macroscopically it was a small hard white nodule, 1 to 2 mm. in diameter, protruding from the surface of the lung. In this group it occurred alone, but may be found more frequently with the alveologenic carcinoma which is described below (for the influenza and smoke group). Microscopically they are little different from pearls of squamous cell carcinoma, having the pathological appearance of large cells and large empty nuclei, arranged in whorls with various layers of fibrous material which stains red in the H. and E. preparations. This fibrous material is also arranged in whorls. Despite the similarity of these lesions to highly malignant lung tumours in man we have been unable to transplant them serially from one mouse to another.

Adenocarcinomas occurred in eight mice exposed to cigarette smoke. Unlike the pearls described above some of these were

serially transplantable in mice, and to date three have survived respectively for 33, 51, and 67 transplant generations.

In the mice in which they originated they appeared as white round nodules, with a diameter of up to 10 mm., protruding from the lung tissue. Metastases were found in the chest wall or parietal pleura. Microscopically they are identical to those described by Andervont (1937) and classified as alveogenic carcinomas. The cells which compose these tumours are cuboid or columnar in shape and almost uniform in size and staining reaction. They contain round or oval nuclei with loose chromatin, and nucleoli are frequently seen. The cells are often present in mitosis. The tumour cells often form a continuous sheet, which distinguishes them from the adenomas which occur spontaneously, for example, in Strong A mice. In these adenomas the cells arrange themselves so as to give the appearance of a glandular structure. Infrequently we also observed these adenomas (Special Plate, Figs. 2 and 3).

Mice Infected with Influenza Viruses (Group 1)

These mice did not show the keratinizing lesions described above. Fifteen of them (Tables IV and V) have shown adenocarcinomas identical to those described in the group of mice exposed to cigarette smoke. Eight of these tumours were transplantable, and six of them have now undergone respectively 15, 18, 26, 71, 83, and 93 transplant generations (Special Plate, Figs. 4 and 5).

TABLE IV.—Incidence of Lung Carcinomas in C57BL Mice

Treatment	Number		Lung Carcinomas	
	M	F	M	F
Influenza	342	340	12 (26, 27, 28, 29, 30 (3), 31 (2), 32, 33 (2))	3 (29, 30, 34)
Benzpyrene	100	100	1 (25)	1 (34)
Smoking	100	100	4 (10, 27, 28, 29)	4 (11, 18, 21, 27)
Influenza + benzpyrene ..	100	100	3 (21, 28, 37)	
Influenza + smoke ..	72	83	1* (18)	2* (23, 27)
Controls	100	100	0	0

Figures in parentheses are ages of mice at detection of tumour (months).
* One other male and three other female mice had keratinizing preneoplastic lesions in the lungs.

TABLE V.—Lung Carcinomas in Relation to the Strain(s) of Influenza Virus Used

Sex	Age of Mouse (months)	Influenza Strain(s) Used
Females		
1	29	W.S.
2	30	Singapore, Lee, Swine, W.S.
3	34	Singapore
Males		
1	26	Singapore, Lee, Swine, W.S.
2	27	Swine
3	28	Singapore, Lee, Swine, W.S.
4	29	Singapore, Lee, Swine, W.S.
5	30	Singapore, Lee, Swine, W.S.
6	30	Lee
7	30	Swine
8	31	Singapore
9	31	Lee
10	32	Swine
11	33	Lee
12	33	Lee, Swine, W.S., Singapore

There is one lesion which is characteristic of mice infected with influenza virus and surviving the acute disease. These lesions have already been described by Kotin and Falk (1956) and by us (Harris and Negroni, 1966). They occur in almost every mouse infected with influenza viruses, and are of a hyperplastic type. Macroscopically they differ from the keratinizing lesion (or the adenocarcinomas), as they do not protrude from the lung surface, which, however, acquires irregularities. They may appear as well-defined nodules, but usually they have a very irregular edge. Microscopically they consist of cells characteristic of epithelial cells, which replace the alveolar tissue. They probably originate from the extension of the bronchial epithelium in the alveolar wall, and the arrangement of the

cells, disposed in one or two layers, seems to fill the old alveolar structure which they have replaced. When these lesions become infected pneumonia follows, with heavy infiltration of polynuclear cells. The extent of these lesions is directly proportional to the number of infections and to the dose of virus used for each infection, as may be demonstrated when the animals die or are killed, largely after recovery from the acute pneumonia caused by the influenza. However, if the animals die or are killed long after the virus infection there is no direct relation between the number and extent of the lesions and the virus dose or number of infections which the animal has received (Special Plate, Fig. 6).

Thus it appears that these lesions are progressive, though we have no evidence that they are malignant, since all attempts at transplantation have failed.

Mice Exposed to Benzpyrene (Group 2)

Only two mice have shown an adenocarcinoma as described above. The tumour arising in the female mouse was transplantable, and is now in its fifty-fifth generation.

Mice Treated with Benzpyrene and Infected with Influenza Viruses (Group 4)

These again show the typical metaplastic lesions which have already been described for mice infected with influenza viruses. These lesions occur with the same incidence. Three male mice had malignant adenocarcinomas of the lungs, all of which have been transplanted serially for 5, 10, and 20 generations respectively.

Mice Infected with Influenza Viruses and Cigarette Smoke (Group 5)

The metaplastic nodules described for mice infected with influenza virus only were also observed in animals of this group, with equal incidence (Special Plate, Fig. 7). Six have shown the squamous pearls described for mice exposed to cigarette smoke only (Special Plate, Fig. 8), and these had adenocarcinomas similar to those described in mice infected with influenza virus alone or exposed to smoke alone. One of these is in its eighty-third transplant generation.

In one instance in which this lesion occurred together with an adenocarcinoma pearls were detected in the tumour of the first transplant generation, but these were no longer found in subsequent transplantations.

Features of the Transplanted Tumours

Many of the adenocarcinomas that have been described as occurring in mice treated in these various ways grow readily when transplanted. The great majority maintain the histological features of the original tumour. Some of them, however, and mainly those originating in mice treated with benzpyrene or cigarette smoke, undergo changes. When this occurs the epithelial cells no longer form a continuous sheet; instead, they separate into clusters divided by well-developed connective tissue, which has pathological features. The tumour then acquires the appearance of a scirrhous carcinoma.

Discussion

These data indicate the potential carcinogenicity of cigarette smoke and air mixture for the lungs of experimental mice. The animals are submitted to both the liquid and the gas

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FIG. 2.—Lung adenoma in a 22-months-old mouse exposed to smoke and air mixture. These lesions are benign. (H. and E. $\times 170$.)

FIG. 3.—Detail of Fig. 2. (H. and E. $\times 280$.)

FIG. 4.—Lung adenocarcinoma in a 33-months-old mouse, following influenza infection. (H. and E. $\times 85$.)

FIG. 5.—Detail of Fig. 4. (H. and E. $\times 280$.)

FIG. 6.—A metaplastic lung nodule originally induced by influenza in a 32-months-old mouse. Note the polynucleated white blood cells inside a bronchus and forming abscesses in the parenchyma. (H. and E. $\times 176$.)

FIG. 7.—Metaplastic nodule in the lung of a 31-months-old mouse, previously infected with influenza and then exposed to smoke and air mixture. Note the crystalline protein structure in spaces between the metaplastic cells. (H and E. $\times 165$.)

FIG. 8.—Keratinizing lesions in the lung of a 23-months-old mouse previously infected with influenza and then exposed to smoke and air mixture. Note the resemblance to the malignant keratinizing tumour of man. (H. and E. $\times 165$.)

phases of the smoke—an advantage over the technique of skin-painting with smoke condensate. The disadvantages from the point of view of an industry looking for a “safe” cigarette are manifold. It takes at least three years to make one test, and with the numbers of lung tumours so far produced it would be difficult to compare the carcinogenicity of different cigarette tobaccos. Moreover, as many other investigators have pointed out, the mouse lung cannot be equated histologically with the human lung, and so “degrees of carcinogenicity” derived in one system may have no validity for the other. Other tests may have to be used, though if the C57BL mice had been submitted more intensively to the smoke—for example, twice a day instead of once every two days—the lung tumour yield might well have been increased and the latent period decreased.

The smoke may not be the only cause of lung tumours, and endogenous factors such as latent viruses may be required.

It is difficult to explain the induction of lung tumours in C57BL mice by influenza viruses alone. It may be that these viruses are weakly oncogenic per se, or that there is a synergism between them and a natural but latent chemical or viral lung carcinogen in the mice, or that the influenza virus stocks were contaminated with a weakly oncogenic tumour virus for mouse lung. Polyoma would be excluded as a contaminant because the virus stocks were produced in RIF-free embryonated chicken eggs in which polyoma virus does not grow. If the third suggestion is correct, then all the virus stocks contain this virus, since lung tumours appeared in groups treated with some or all of the viruses (Table V). Moreover, since the incidence of other tumours in the influenza groups of mice was higher in the females than in the males (Table III), the hypothetical contaminant would be a specific weak lung carcinogen for males, but might produce other tumours in females. It is more probable that the second explanation is correct, and that the influenza infection “releases” a virus (such as polyoma) or a mycoplasma which is the immediate cause of the tumours.

We are currently investigating possible synergism between such chemical carcinogens as dimethylnitrosamine and cigarette smoke. If these lung tumours arose by some indirect mechanism—for example, the stimulation of a latent virus (such as polyoma)—then concomitantly one needs to postulate a general reduction in immunity which allows the “release” of virus-transformed tumour cells. It is necessary to have both steps because C57BL mice, even when newborn, are poorly responsive to large inocula of polyoma virus (Jahkola, 1965; Chang and Hildemann, 1964), though thymectomized animals (with reduced immunity) show a much higher yield of tumours. Moreover, the tumours induced in thymectomized mice by polyoma virus involve the salivary glands, kidney, bone, and hair follicles and *not* the lung.

Summary

C57BL mice subjected to breathing a cigarette smoke and air mixture for 12 minutes every other day for the greater part of their lives develop a low incidence (4%) of alveogenic carcinomas of the lung. Control animals subjected to non-specific lung damage did not develop tumours, but a group of 200 animals briefly exposed on four occasions to an aerosol of 3:4-benzopyrene developed two lung carcinomas. Attempts to influence lung tumour yield by infecting mice sequentially with influenza viruses, followed by exposure to benzopyrene or cigarette smoke and air mixture, showed that influenza viruses alone gave about 2% alveogenic carcinomas in C57BL mice—an incidence which was not exceeded in the other groups.

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