Technical Papers

Cigarette Smoke and the Incidence of Primary Neoplasm of the Lung in the Albino Mouse

J. M. Essenberg

Department of Anatomy, The Chicago Medical School, Chicago, Illinois

Animal experimentation may be of service in resolving the controversy as to whether tobacco smoke is carcinogenic for the lungs. It is difficult—if not impossible—to set up adequate controls of human beings, whereas it can be done with ease in animals. With this in mind, preparations were made to test the effect of cigarette smoke on the lungs of more than one species of laboratory animals.

The literature in this field is conspicuous for its scarcity. The only experiment known to me was performed by Lorenz *et al.* (*1*) and reported at the 35th annual meeting of the American Association of Cancer Research. Lorenz and his associates exposed strain "A" mice to tobacco smoke for 28–250 days in an especially designed smoking machine. The maximum exposure was 693 hr. They reported that "no lung tumors are induced by the tobacco tar, for the average number of tumors are the same in the experimental as in the control animals."

It appeared to me that the exposure of mice to tobacco smoke for 250 days might be too short a period in the life span of the mouse to induce primary neoplasms of the lung. Longer exposure time, slightly higher dosage, and the use of cigarette smoke might make considerable difference in the results.

An automatic smoking machine was designed for this purpose (Fig. 1). It consisted of a chamber, 2

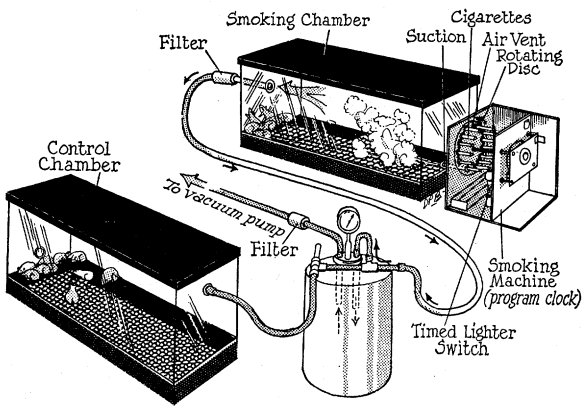

FIG. 1. The smoking machine, consisting of experimental and control units. Connection with the vacuum pump is indicated.

cu ft in capacity, in which the animals lived during the experiment, a rotary cigarette carriage holding 12 cigarettes, an automatic electrical cigarette lighter connected to a program clock[1] which lighted a cigarette every hour for 12 hr/day, and a vacuum pump creating suction just sufficient to burn the cigarette and circulate fresh air through the chambers containing the experimental and the control mice. The burning of the cigarette required 3–4 min. The chamber began to fill with smoke soon after the cigarette was lighted, and it remained filled for 6–7 min. It was almost clear at 9 min, and the mice began to feed 10 min after the cigarette was lighted.

TABLE 1

SUMMARY OF RESULTS

		Positive		Negative	
	Number	Number	Percentage	Number	Percentage
Experimental	23	21	91.3	2	8.7
Control	32	19	59.4	13	40.6

The strain "A" mice used had a hereditary tendency to lung tumors and were obtained from the Roscoe Jackson Memorial Laboratory. As there was a scarcity of these animals at the time, only 33 females and 3 males were obtained. It was decided to subject all 36 animals to cigarette smoke in a preliminary experiment.

At the end of 14 months, there were 25 mice well preserved by perfusion fixation. Those becoming ill or dying in the first 2 months of the experiment were not included. Both lungs were removed together, embedded in paraffin, sectioned serially, and stained by the hematoxylin and eosin method.

Of the 25 mice, 21 had definite primary neoplasms of the lungs. Two were negative and 2 were classified as uncertain. In the latter, epithelial proliferation was noted in one or several places, and the structure did not differ from those of definitely diagnosed tumors. The amount of cells proliferated was not considered sufficient for clear diagnosis, however.

A second experiment was started immediately after the completion of the first. Thirty-six mice were secured for experimental animals, and the same number for controls. The sexes were equally divided in both groups. Records of weight increase and reproduction capacity were kept of all animals. With the exception of the experimental period, which in the second experiment was one year, the procedure was identical in every respect. At the end of the experiment 23 experimental and 32 control animals were well preserved. The lungs were sectioned serially, with the results summarized in Table 1.

It will be noted that the preponderance of tumors in the smoked mice exceed those for the control mice

[1] The program clock was donated to me by the Zenith Electric Company, of Chicago, for which my gratitude is here expressed.

by 31.9%. When the figures are tested statistically by the χ^2 method, it is found that this is a significant difference, the probability of its occurring as a result of chance being less than 0.01.

There was considerable variation in the size of the tumors. In some instances, the entire lobe of the lung was a mass of tumorous tissue (Fig. 2). In others, the

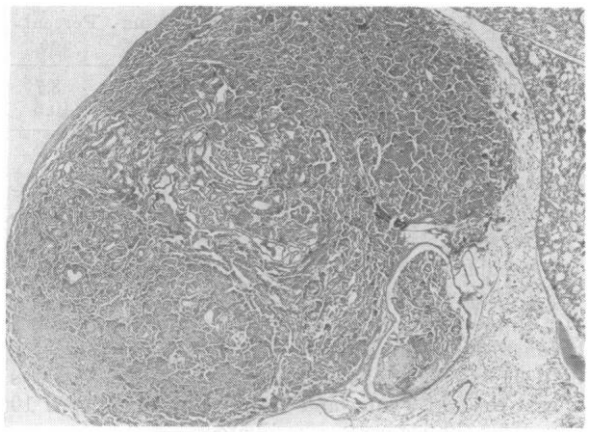

FIG. 2. Primary neoplasm of the lung of a mouse exposed to cigarette smoke for 1 year. It is of unifocal origin and involved practically the whole lobe of the lung. × 50.

tumor was of small size. In some of the lungs growth started from a single focus, part of a single bronchus. In others, it was multifocal (Fig. 3). Papillary adeno-

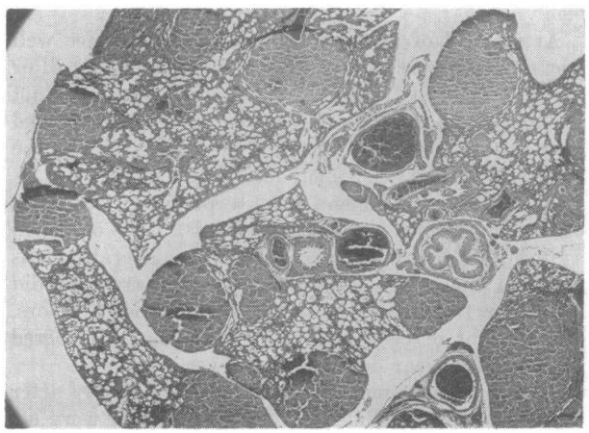

FIG. 3. Primary neoplasm of multifocal origin in the lung of a mouse exposed to cigarette smoke for 14 months. × 25.

carcinoma was the most common type of tumor, but adenomas and adenocarcinomas were also found. Lymphoid infiltration was a common occurrence; masses of lymph cells often surrounded branches of the bronchial tree and blood vessels. In some instances, lymphoid accumulation filled the greater part of a lobe of the lung. Pathology was noted in the organs of reproduction, endocrine glands, the kidney, and the liver.

The weight records showed that the smoked mice grew more slowly and failed by a large margin to attain the weight of the controls. Although sexes were equally divided in the second experiment, and there were some males in the first experiment, no young were obtained from the experimental mice. The controls, on the contrary, reproduced freely.

The smoked mice of the two experiments can be lumped together because they were exposed to cigarette smoke for at least one year and because the results of both experiments were similar, if not identical.

Since no other experimental differences existed between the smoked and the control mice except the smoke, it seems justifiable to conclude that the preponderance of tumors in the experimental mice was induced by the cigarette smoke. Cigarette smoke consists of many ingredients, some of which are considered carcinogenic. Of these, the tars and arsenic (*2*) are the foremost. There is some reason to believe that the alkaloids of nicotine have irritating properties that may act in the production of tumors. Which of these alone or in combination is responsible for the production of tumors in mice and possibly in other animals remains to be proved.

The weight and growth rates of the experimental animals were appreciably lower than those for the controls, but no explanation is available for this phenomenon at the present time. It could be due either to an inadequate intake of food or to the decreased utilization of food.

The lack of reproduction among the experimental animals is known to be caused by atrophic changes of the reproductive system (*3*). It is further known that the pathology caused by the injection of nicotine solution into mice or rats will parallel the pathology of the reproductive organs of mice exposed to cigarette smoke (*3*).

References

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Molecules of the Insulin Structure[1]

Dorothy Wrinch

Department of Physics, Smith College, Northampton, Massachusetts

Structures suggested for skeletons of protein molecules in 1948 (*1–3*) have become of greater interest in the light of results regarding insulin recently obtained.

The original C_n models (the skeletons of the cage-like, space-enclosing polycondensations of α-amino acid molecules proposed [*4*] as models for protein molecules in 1936) comprise residues interlocked at both terminals, with every atom of the $NC_\alpha C$ backbones (belonging to the constituent amino acid molecules $NH_2—C_\alpha HR—COOH$) on or near a point of

[1] This work is supported by the Office of Naval Research.