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SYNERGISTIC EFFECT OF POLONIUM-210 AND CIGARETTE SMOKE IN RATS

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by

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ABSTRACT

An experimental procedure was devised to test the possible synergistic effect of polonium-210 and cigarette smoke in rats. Appropriate techniques were developed to expose the rats to cigarette smoke through mouth-breathing and to add known amounts of polonium-210 to the cigarette smoke.

The findings from this experiment included: 1) lung deposition of polonium-210 was 31 ± 2%, 2) early retention of polonium was two-phased with half-times of 4 and 84 hours, and 3) bronchitis, emphysema and lung tumors were observed in the experimental animals. Though the spontaneous occurrence of two lung tumors in the number of animals at risk was highly improbable, any conclusion that this resulted from the exposure to cigarette smoke must be highly qualified.

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INTRODUCTION

Many hypotheses have been proposed to explain the relationship between cigarette smoking and lung cancer in humans. Because primary lung cancer was rarely produced in experimental animals by exposure to cigarette smoke alone, most postulates included one or more co-carcinogens. Of the multitude of co-carcinogens that may be involved in the production of primary lung cancer, ionizing radiation has been suggested as an important possibility. Such radiation has produced lung cancer in a variety of experimental animals as reported at a 1970 symposium.*(1)* The combination of smoking and radiation exposure has also been implicated in the elevated incidence of lung cancer among uranium miners.*(2)* In the non-mining population, lung tissue receives a substantial exposure from naturally occurring radioactive aerosols which are present in the atmosphere.*(3)* It has been suggested that volatilization of polonium-210, a naturally occurring alpha emitter which is present in tobacco, may add sufficient radiation exposure to lung tissue to potentiate the carcinogenic effect of cigarette smoking.*(4)*

To investigate the possibility that potentiation occurs, a two-part study was conducted. In the first part, the polonium-210 content of various tobaccos was measured. In addition, the amount of polonium in mainstream smoke under standard smoking conditions and the effect of filters on this amount were determined.*(5,6)* The amounts of lead-210 and radium-226 in mainstream smoke were negligible compared to the polonium. The second part of the study, investigating the effect of added polonium-210 on the carcinogenic activity of cigarette smoke, is reported herein.

PROCEDURES

Before starting the animal exposure, several problems had to be resolved. These were: 1) the animal species; 2) the treatment groups; 3) the method of adding polonium-210 to the cigarettes; 4) the exposure dose; and 5) the method of exposure.

The animal chosen was the albino rat. There were several reasons for this choice which outweighed the endemic murine pneumonia characteristics of this species. Firstly, a colony of the Wistar strain females had been maintained in our laboratory with little evidence of pneumonia. Secondly, the endpoint sought was frank lung cancer rather than precancerous changes which might be obscured by pneumonic changes.

Thirdly, the rat is of convenient size, and much experimental data on its response to various stresses has been accumulated. Lastly, the incidence of primary lung cancer in rats is very low and is well documented, namely, 0.03% for all lung tumors*(7)* (0.015% for squamous cell) and 0.013% for fibrous tumors in a 5-year study.*(8)*

The most satisfactory method of adding polonium-210 to cigarettes was by use of sewing thread. A solution of ^{210}Pb-Bi-Po in equilibrium was used which contained four microcuries per milliliter in 0.5*N* HCl. Mercerized cotton thread, button, extra strong, was cut to length, thoroughly soaked in the solution, and air dried. With a needle, one thread was placed lengthwise through the center of regular size non-filter cigarettes. Several cigarettes from each batch were then smoked on the smoking machine with the mainstream smoke being trapped on filters. Analysis of the filters yielded 12.5 ± 0.8 nanocuries of polonium-210 in the mainstream smoke of each of cigarettes to which polonium-210 had been added.

Fifteen of the Wistar rats were each exposed to the mainstream smoke of one cigarette containing the added polonium-210. The rats were sacrificed immediately after exposure and the respiratory tree analyzed for polonium-210. The results were 270 ± 30 picocuries (n=15) in the lungs, indicating an average deposition of 31 ± 2% of the amount inhaled. This was calculated by using the respiratory minute-volume of the rat,*(9)* the exposure time, and the picocuries per cubic centimeter of smoke; 100% deposition would have yielded 820-920 picocuries in the lungs per exposure. Another group of sixty rats was exposed to one polonium-210 labeled cigarette each and then serially sacrificed to determine retention. A least-squares analysis of lung content indicated a two-phase decrease for the polonium-210 representing biological half-times of 4 hours and 84 hours.

The method of exposure chosen for this study was oral inhalation because this simulates human smoking. The apparatus used and the reasoning used in its development are explained in a previous publication.*(10)* Initially, the rats were lightly anesthetized, placed in restrainers, their nostrils closed with collodion to force mouth breathing, and inserted in the smoking machine. With this procedure, several animals died each month. The procedure was then modified to eliminate the use of the anesthetic by gradually acclimating the rats to the smoking routine using a stepwise progression of the treatment. The death rate was substantially reduced, but was still higher than for non-smoking rats.

The amount of exposure for the rat was based on calculations using standard values. The standard cigarette smoking procedure used in our laboratory is 8 puffs per cigarette at the rate of 1 puff per minute with each 35-cubic centimeter puff lasting 2 seconds for a total volume in the mainstream smoke of 280 milliliters. For one rat, then, the exposure to 1 cigarette smoked under these conditions results in the

inhalation of approximately 20 milliliters of smoke. This is one-fourteenth of the amount available per cigarette. If a heavy smoker is assumed to smoke 40 cigarettes per day, then he will inhale 40 × 14 = 560 times as much smoke as the rat exposed to a single cigarette. Since the fresh weight of the adult human lung is 1169 grams*(11)* and that of the adult rat is approximately 2 grams, the ratio of lung weights is 580. Therefore, it was assumed that the amount of smoke per gram of lung for a rat exposed to one cigarette daily was the same as for a heavy smoker.

The polonium-210 exposure of the rat lung from inhaling the smoke of a regular-size cigarette was negligible. The average mainstream smoke concentration is 0.1 femtocuries per milliliter*(5)* for the standard smoking regimen so a 20-milliliter inhalation by the rat and an average deposition of 31% implies only 0.6 femtocuries deposited in the lung. Using the single exposure data from the 60 rats referred to above, a curve of lung content following multiple exposure was constructed. The area under the curve was integrated and the average lung content derived assuming 31% deposition. With this lung content and standard conversion factors, the lung exposure of rats smoking the cigarette having added polonium-210 would average 16 millirads per day, based on a 7-day week with 5 days of exposure. From the constructed multiple-exposure curve, the lung content 24 hours after the final of 25 exposures (5 exposures per week) could be estimated as 77 picocuries. The lung content of 3 rats sacrificed 24 hours after the last of 28 exposures averaged 84.5 picocuries, in reasonable agreement with the calculations.

The original plan was to conduct a modified factorial experiment using groups of 100 rats with daily treatment of the exposed rats. The groups would have included 1) an environmental control, 2) a collodion-only treatment (sham smoked), 3) a polonium-210 exposure, 4) a cigarette-smoke exposure, and 5) a cigarette-smoke plus polonium-210 exposure. Due to the exigencies of laboratory operation and limitation of resources, the groups were limited to 60 rats per group, exposures to 5 per week, and only treatments 1), 4), and 5) were used. The exposures continued over a two-year period with no periodic sacrifices scheduled. Those animals in the exposure groups that died were replaced by new rats up to the last three months of the experiment. Those that died were examined for gross pathology and lung sections were taken for histopathology. T Those remaining at the termination of exposure were maintained for three months, then sacrificed for pathological examination.

RESULTS

Because animals were entered in the experimental groups to replace those that died, a total of 381 rats was involved plus 38 used as room controls and another 150 used to determine exposure parameters. Retention of polonium in the rat lung following exposure to a single cigarette

containing added polonium-210 is shown in Figure 1. Analysis of the curve suggested in Figure 1 indicates a 2-phase retention function with 92% being retained with a biological half-time of 4 hours and 8% with a biological half-time of 84 hours.

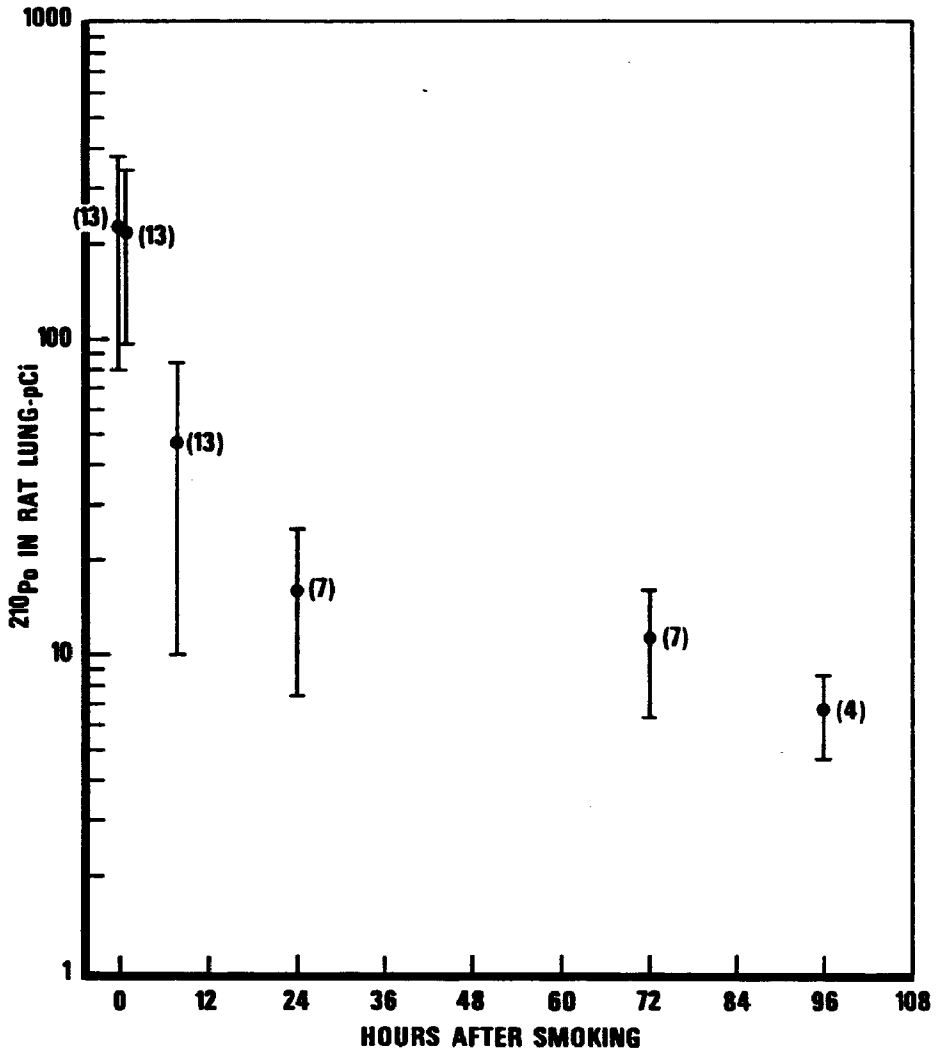

Figure 1. Retention of polonium-210 in rat lung after exposure to one cigarette with added polonium-210. Number of rats shown in parentheses.

Some of the data on the two experimental groups is shown in Figure 2 which is a long-probability plot of cumulative mortality. Also shown in this figure are the data for the rats exposed by the original technique. Cumulative mortality reached 50% in only 5½ weeks with the original technique compared to 28½ weeks with the modified technique. Also indicated on the figure are the two lung cancers detected in the experimental groups. One cancer was a bronchogenic carcinoma detected in an 8-months old rat which had been exposed for 15 weeks to cigarettes having added polonium-210, and the other a pulmonary adenoma detected in

an 18-months old rat which had been exposed for 49 weeks to normal cigarettes. The one other cancer discovered in lung tissue was metastasized from a mammary carcinoma.

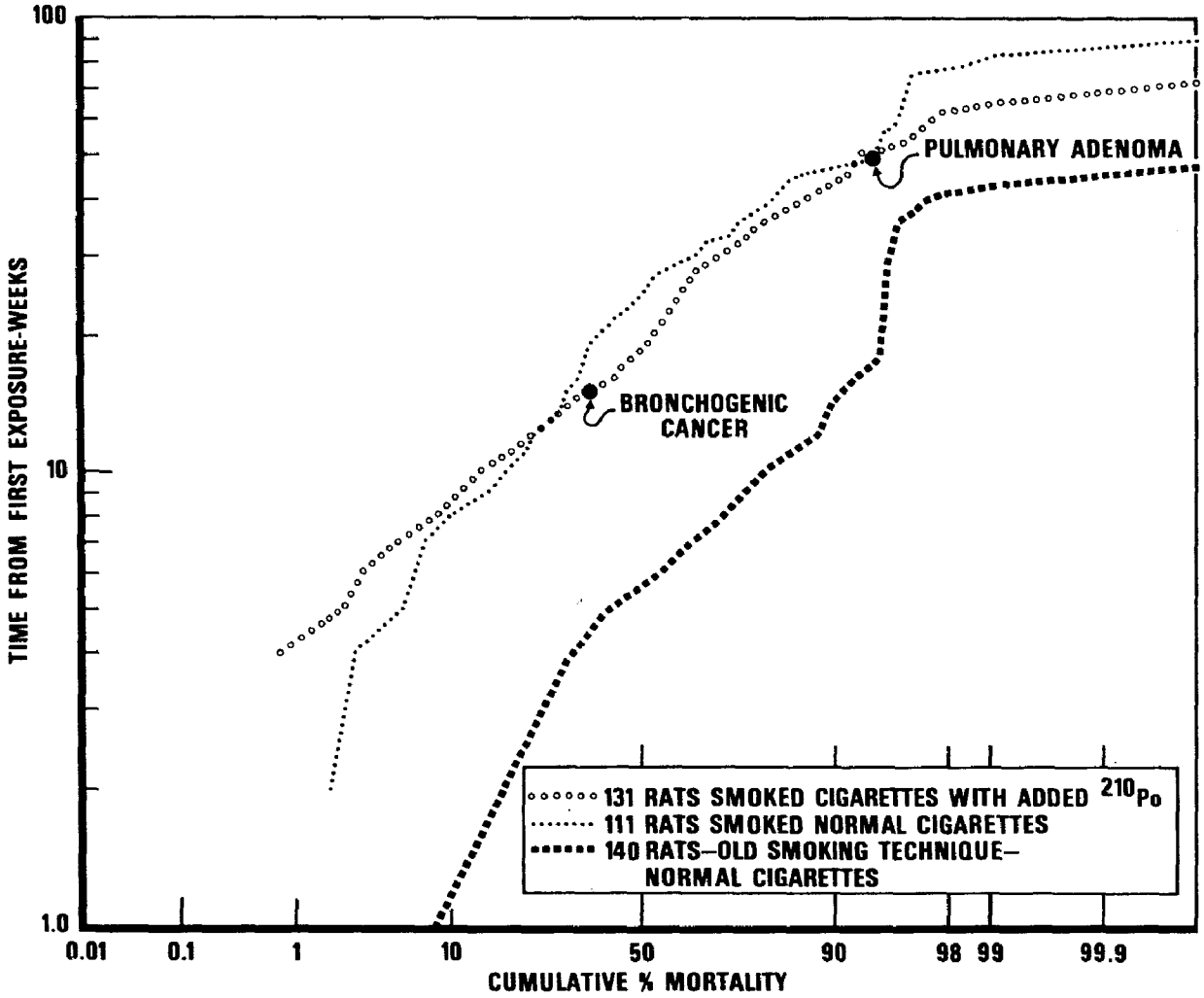

Figure 2. Cumulative mortality of rats in cigarette-smoking experiments.

The bronchogenic carcinoma is shown in Plate 1 and the adenoma in Plate 2. In three instances a morphology was noted that was suggestive of an early neoplastic change (Plate 3), but was considered more likely to be part of an inflammatory response.

Many of the lung sections exhibited other phenomena such as:

1) Lymphocytic cuffing

These are peribronchiolar accumulations of lymphoid cells, which are thought to occur normally, but proliferate in many disease conditions. It was possible that chronic bronchiolar

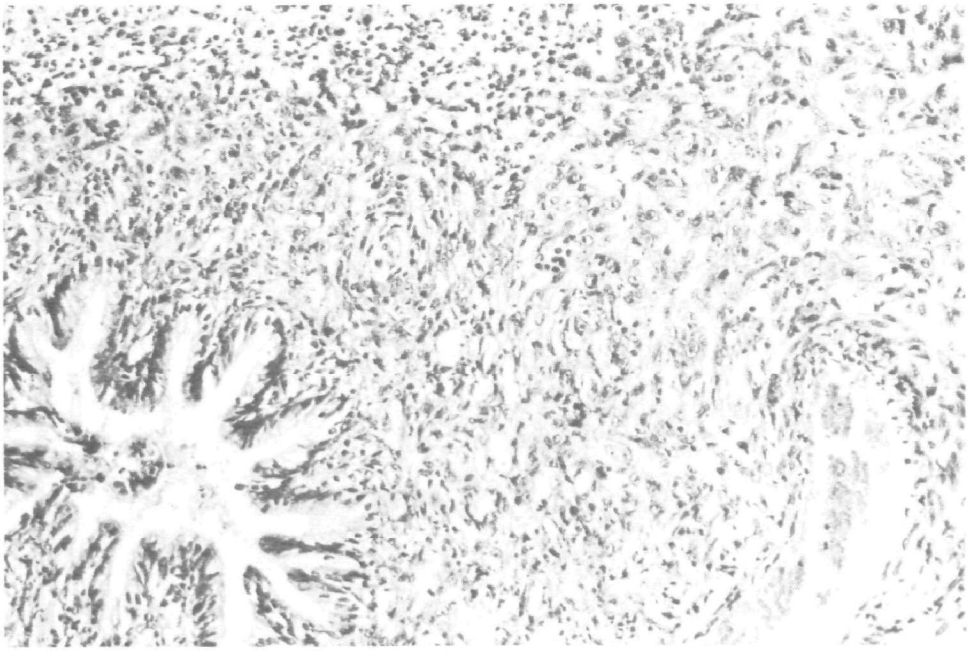

Plate 1. Primary pulmonary neoplasm (×140) composed of tumor cells that appear to be growing by expansion. Some of the cells are spindled while others are plump epithelial-like.

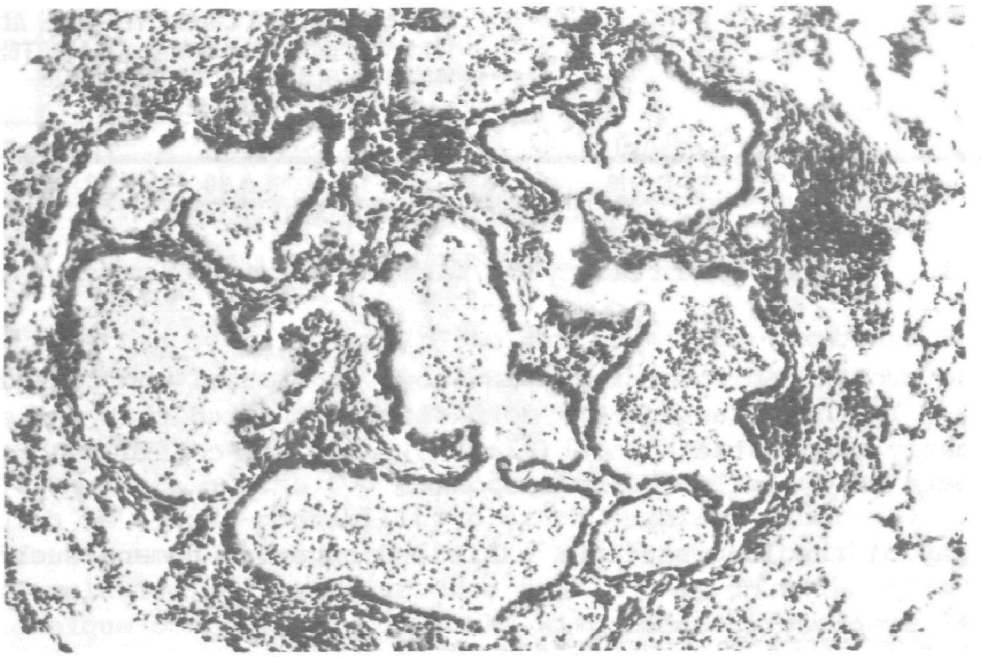

Plate 2. Small, well-circumscribed adenoma (×140) whose trabeculae are covered with cuboidal to columnar cells.

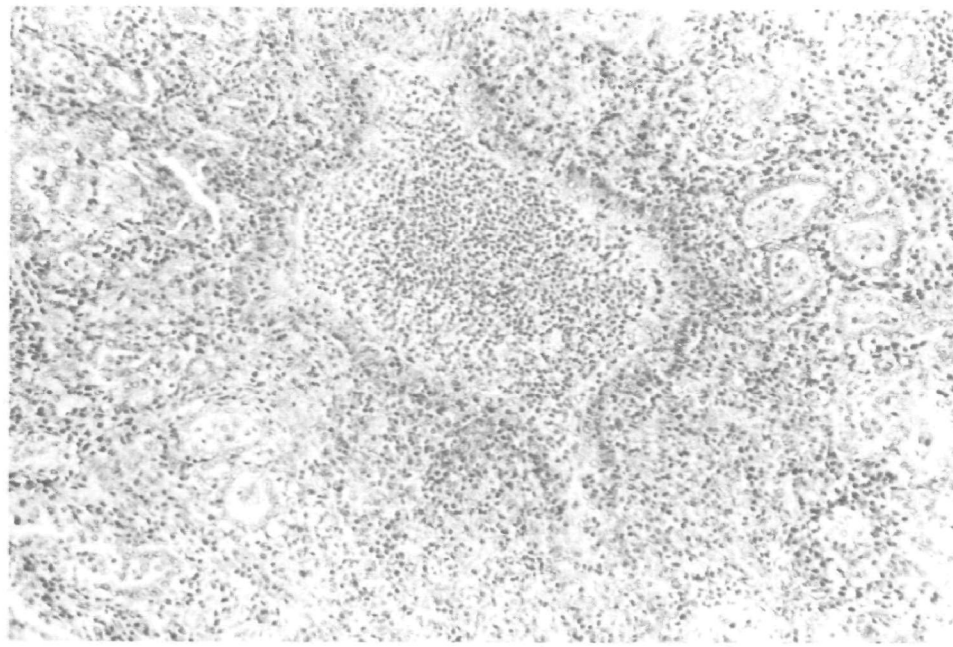

Plate 3. Adenomatous hyperplasia (×140) surrounding a small bronchus in an area of pneumonia. This may be an early neoplastic change but is more likely an inflammatory response.

irritation caused an increased amount of lymphocytic cuffing in these rats.

2) Focal macrophage response

These were focal accumulations of foamy macrophages, usually found near the pleura. These are frequently seen in normal rodent lungs and their significance is unknown.

3) A few of the lungs were pneumonic because debris and particles of hair were seen in bronchi; most were thought to be aspiration pneumonia. Bronchitis was seen in a few of the lungs examined.

4) The other changes seen in the lung were very non-specific and consisted of edema, congestion, emphysema and atelectasis.

Other changes detected in lung tissue of the rats exposed to cigarette smoke and in the 38 animals used as room controls are shown in Table 1.

Table 1. NON-NEOPLASTIC LUNG PATHOLOGY DETECTED IN EXPERIMENTAL RATS.

Group	Number of weeks exposure until appearance of: Pneumonia	Bronchitis	Emphysema	Early neoplastic** change	Atelectasis
Cigarettes with added polonium	13(12)*, 35(11), 36(13)	9(13), 65(21)	52(26)		
Cigarettes only	1(4), 1(4) 13(9)	5(5), 7(3)		6(9), 8(5)	38(13)
Control	(10), (10)			(30)	

* Number in ( ) is age in months.
** These are probably an inflammatory response to pneumonia (see text).

DISCUSSION

Before discussing the effects of cigarette smoking, it is useful to compare the retention data from this experiment with some of the published data. The initial lung deposition of 31 ± 2% and the half-times of 4 and 84 hours for retention as determined during this experiment were required to estimate the lung dose. Data from the literature are discussed below.

For a $PoCl_2$ solution injected into the trachea of rats, Thomas and Stannard*(12)* noted a 50% decrease in lung burden in the first few hours which changed after 10 days to an 18-day half-time. It was difficult to decide, on the basis of the published data, whether or not an intermediate half-time existed. Initial deposition was 100% because of the technique used. Casarett*(13)* used a "nose-only" exposure of rats to a $PoCl_2$-NaCl aerosol (0.046 μm CMD) and estimated that initial deposition was 33%. He noted half-times for retention of 2 hours and 3 to 4 days (72 to 96 hours). Both the initial deposition fraction and the retention half-times agree with our data. Yuile, *et al.(14)* also used chloride-type aerosols, although the CMD was 0.078 μm and the duration of exposure was 1.4 to 3.4 hours. Based on their earlier work, they assumed the initial deposition to be 46%. Since this was a "whole-body" exposure, the initial deposition could have been elevated by the rats licking their fur and paws. They observed retention half-times of "a few days" and 50 to 60 days with no estimate of half-time the first few hours after exposure.

Considering the differences in type of exposure and type of carrier aerosol, if any, the agreement with the data from our experiment is very good.

Statistically the appearance of lung cancers in each of the two experimental groups represents a significant incidence since the incidence of spontaneous lung tumors in this strain of rats would suggest <0.05 tumors for either of the groups of 131 or 111 rats involved. The fact that one of the tumors was a bronchogenic carcinoma is particularly noteworthy.

However, any significance which may be attached to the preceding observations must be highly qualified. The appearance of the bronchogenic cancer in an 8-months old rat after only 15 weeks exposure leads one to suspect that the cause of the cancer was that exposure. Admittedly these animals were subject to considerable stress, such as forced mouth breathing, and multiple inhalation insults; *i.e.*, either from the collodion, tobacco smoke, or alpha radiation from the polonium-210 added to the cigarettes; but the latent period appears too short. The radiation exposure, for example, represents a cumulative alpha dose of only 1.7 rads or 17 rems if the RBE is assumed to be 10. Other experimenters have produced cancers in rodents with relatively small amounts of radiation dose. For example, Yuile, *et al.(14)* observed a 3% incidence of lung cancer in rats (1 squamous-cell carcinoma of the trachea and 3 adenomas) after a cumulative dose of 71 rads from polonium-210 inhalation and Grossman, *et al.,(15)* observed a 43% incidence of lung cancer in hamsters with a cumulative dose of 225 rads from polonium-210 administered by intratracheal instillation. Of particular interest, Yuile, *et al.*, observed adenomas only in the low- and medium-dose groups; not in the control animals or high-dose group.

The adenoma detected in the rat exposed to normal cigarette smoke, however, cannot be dismissed so readily. This animal had lived nearly half its normal lifespan and had been exposed to cigarette smoke for nearly one-third its normal lifespan. The uncertainty arises because of the lack of an adequate control group (sham smoking), which was not included for the reasons mentioned in the Procedures section, and the <u>possibility</u> that this tumor was spontaneous even though the <u>probability</u> was extremely small.

The induction of lung cancers in rodents by exposure to tobacco smoke has generally been unsuccessful though inhalation of a constituent of tobacco smoke (3,4-benzopyrene) attached to hematite aerosols*(16)* has produced numerous cancers. The observation of lung cancers in dogs following cigarette smoking*(17)* may be qualified somewhat since the dogs smoked cigarettes through a tracheostomy. This procedure delivers the smoke more directly to the lung than does inhalation through the mouth, and therefore may increase the exposure to carcinogens in the smoke.

The other effects on the rat lung which could possibly result from cigarette smoking, bronchitis and emphysema, also cannot be attributed

solely to the smoke as the contribution of the collodion vapors cannot be assessed with the data from this experiment.

Relative to the cumulative mortality curves, apparently the added polonium exposure did not change the mortality rate. The non-parametric Kolmogorov-Smirnov test of the hypothesis that the two groups of rats were representative of the same population failed to reject that hypothesis.

SUMMARY AND CONCLUSIONS

A method for exposing rodents to cigarette smoke in a manner which closely simulates human smoking was developed and tested. Two technicians trained in the procedure could expose 120 rats each day to one cigarette-smoking experience. The cumulative mortality from the stress of the smoking procedure reached 50% in 29 weeks, but continued exposures would eventually result in a cadre of animals which could survive a year or more.

Also tested was a method for adding polonium-210 to the cigarettes which, when smoked by Wistar-breed rats, added an alpha-radiation exposure of 116 mrad/day to the lung, based on exposure to one cigarette/day for five days/week. About 31% of the polonium-210 was deposited in the rat lung of which 92% was eliminated with a half-time of 4 hours and the remainder with a half-time of 84 hours.

Lung pathology which may have been produced by exposure to cigarette smoke included bronchitis, emphysema, a bronchogenic carcinoma, and an adenoma. The small number of rats exposed suggests that spontaneous occurrence of the neoplasms was highly improbable. The uncertainty in this conclusion arises because adequate control groups could not be established with the limited resources available for this experiment.

Note added in proof:

A recent publication (Little, J. B., A. R. Kennedy and R. B. McGandy, *Science* 188:737-38, May 16, 1975) tentatively supports the carcinogenic effect of low dose of alpha radiation on the rodent lung reported herein. Little, *et al.*, report that polonium-210 delivered in 15 weekly intratracheal instillations of 250 pCi, delivering a cumulative lung dose of 15 rads, resulted in 9 malignant tumors for the 83 hamsters so exposed.

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16. ABSTRACT

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