Cancer

A Journal of the American Cancer Society

Vol. 33

No. 1-6

JAN.—JUNE 1974

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Published Monthly for the American Cancer Society
by J. B. Lippincott Company
Philadelphia and Toronto

NATIONAL CANCER INSTITUTE MONOGRAPH 28

June 1968

Toward a Less Harmful Cigarette

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL CANCER INSTITUTE, BETHESDA, MARYLAND 20014

The Effect of Direct Cigarette Smoke Inhalation on the Respiratory Tree of Dogs [1]

OSCAR AUERBACH, M.D.,[2] E. CUYLER HAMMOND, *Sc.D.*,[3] DAVID KIRMAN, *B.S.*,[3] LAWRENCE GARFINKEL, *M.A.*,[3] *and* ARTHUR PURDY STOUT, *M.D.*,[4] *Veterans Administration Hospital, East Orange, New Jersey 07018; American Cancer Society, Inc., New York, New York 10019; and Department of Pathology, Columbia University, College of Physicians and Surgeons, New York, New York 10021*

WHILE dose-response studies on mouse skin and subcutaneous injection involve largely smoke aerosols, the total smoke can perhaps best be evaluated by direct inhalation studies. Such studies, for which a method shall be briefly outlined here, indicate that the longer the exposure to cigarette smoke, the greater the bronchial epithelial changes.

This method lends itself to a comparison of the effect of whole smoke from cigarettes that yield different amounts of particulate matter or that have different quantities of gaseous components.

METHODOLOGY

A tracheostomy was performed on 10 beagles, 9 males and 1 female, ages 9–30 months, and weighing 17–30 pounds. Ten additional dogs were used as controls. Two of these had tracheostomies kept open by hollow Teflon tubes.

Every morning and afternoon of the experiment 10 dogs smoked cigarettes through a specially designed tube with auxiliary apparatus for handling. The number of cigarettes smoked per day was gradually increased to a maximum of 12 per day per dog. Five of the ten dogs died during the experiment. Four of these had thrombi with pulmonary infarc-

[1] This is a condensation of article which appeared in "Cancer."
[2] Veterans Administration Hospital.
[3] American Cancer Society, Inc.
[4] Columbia University, College of Physicians and Surgeons (deceased, December 20, 1967).

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tion. The remaining 5 were killed after 421 days of smoking. Table 1 shows the total number of cigarettes smoked by each dog and the number of dogs from the start of smoking to death.

Immediately after death, the lungs were removed and filled with formalin instilled into the trachea. The tracheobronchial tree was then dissected out of the lungs and divided into portions by essentially the same procedure we have used with human material. We planned to divide each tracheobronchial tree into 133 portions (each from a specified location), embed each portion in paraffin, and then cut out one section from each block for microscopic examination.

FINDINGS IN GLANDS

Hyperplasia with distended goblet cells in the glands was not found in any of the sections from the 10 nonsmoking control dogs, but was observed in 96.7% of the sections from smoking dog 29 and in 98.9–100% of the sections from each of the other 9 smoking dogs. None of the glandular epithelium from the nonsmoking dogs showed any evidence of cells with atypical nuclei. The cells with atypical nuclei increased as the smoking habits of these dogs increased (fig. 1 and table 1).

FINDINGS IN BRONCHIAL EPITHELIUM

In 8 of the 10 nonsmoking dogs, no sections with areas of epithelium averaging more than 2 cell rows in thickness were found—that is, no more than 2 rows of basal cells, plus a surface row of ciliated columnar cells (fig. 2). The other 2 nonsmoking dogs showed minimal changes in this respect.

Every section from the 10 smoking dogs had epithelium consisting of 3 or 4 rows of basal cells plus a row of ciliated columnar cells, or 3 or more rows of cells but without ciliated columnar cells (fig. 3). Where ciliated columnar cells were lacking, the surface cells appeared typically squamous (fig. 4). Sections with areas having 6 or more rows of epithelial cells were not found in dog 29 that died on the 24th day and occurred more frequently in the 5 dogs killed after 421 days than in the dogs that died earlier (table 2).

ATYPICAL NUCLEI IN EPITHELIUM

Every section with epithelium was examined for the presence or absence of cells with atypical nuclei. The findings are shown in table 3. There were no atypical nuclei in any of the sections from the 10 nonsmoking dogs and

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TABLE 1.—Findings in glands in the walls of bronchial tubes in 10 smoking dogs and 10 nonsmoking control dogs. Sections are classified by the percent of gland epithelial cells with atypical nuclei

Dog No.	Days from start of smoking to death	Number of cigarettes smoked	Total No. of sections		Percent of glandular cells with atypical nuclei											
					No atypical nuclei		<30% atypical		30–39% atypical		40–49% atypical		50–59% atypical		60–69% atypical	
			Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Smoking dogs																
29*	24	73	123	100.0	122	99.2	1	0.8	—	—	—	—	—	—	—	—
30*	229	1,378	87	100.0	—	—	2	2.3	27	31.0	57	65.5	1	1.2	—	—
35*	278	2,298	127	100.0	—	—	1	0.8	14	11.0	97	76.4	13	10.2	2	1.6
32*	410	3,530	123	100.0	—	—	—	—	—	—	87	70.7	36	29.3	—	—
36*	415	3,598	119	100.0	—	—	—	—	—	—	54	45.4	64	53.8	1	0.8
31	421	4,104	131	100.0	—	—	—	—	—	—	7	5.3	94	71.8	30	22.9
15	422	4,114	129	100.0	—	—	—	—	—	—	40	31.0	81	62.8	8	6.2
34	422	4,116	129	100.0	—	—	—	—	—	—	12	9.3	93	72.1	24	18.6
37	423	3,702	131	100.0	—	—	—	—	—	—	4	3.1	98	74.8	29	22.1
28	423	4,084	127	100.0	—	—	—	—	—	—	66	52.0	56	44.1	5	3.9
Nonsmoking control dogs																
21	—	—	127	100.0	127	100.0	—	—	—	—	—	—	—	—	—	—
24	—	—	127	100.0	126	99.2	1	0.8	—	—	—	—	—	—	—	—
27†	—	—	125	100.0	125	100.0	—	—	—	—	—	—	—	—	—	—
38†	—	—	128	100.0	127	99.2	1	0.8	—	—	—	—	—	—	—	—
41	—	—	124	100.0	124	100.0	—	—	—	—	—	—	—	—	—	—
42	—	—	131	100.0	131	100.0	—	—	—	—	—	—	—	—	—	—
43	—	—	127	100.0	127	100.0	—	—	—	—	—	—	—	—	—	—
44	—	—	132	100.0	132	100.0	—	—	—	—	—	—	—	—	—	—
45	—	—	132	100.0	131	99.2	1	0.8	—	—	—	—	—	—	—	—
80	—	—	129	100.0	129	100.0	—	—	—	—	—	—	—	—	—	—

*Died during experiment. The other 18 were killed.
†Nonsmoking dogs 27 and 38 had tracheostomies.

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TABLE 2.—Findings in bronchial epithelium in 10 smoking dogs and 10 nonsmoking control dogs. Sections are classified by the number of cell rows exclusive of ciliated columnar cells when present

Dog No.	Days from start of smoking to death	Total sections with epithelium		Average number of cell rows in bronchial epithelium											
				1–2 rows		3 rows		4 rows		5 rows		6 rows		7+ rows	
		Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Smoking dogs															
29	24	123	100.0	—	—	28	22.8	81	65.8	14	11.4	—	—	—	—
30	229	86	100.0	—	—	7	8.1	47	54.6	30	34.9	1	1.2	1	1.2
35	278	127	100.0	—	—	4	3.1	40	31.5	69	54.4	12	9.4	2	1.6
32	410	14	100.0	—	—	3	21.4	6	42.9	4	28.6	1	7.1	—	—
30	415	29	100.0	—	—	8	27.6	13	44.8	8	27.6	—	—	—	—
31	421	131	100.0	—	—	—	—	30	22.9	73	55.7	22	16.8	6	4.6
15	422	129	100.0	—	—	10	7.8	14	10.9	61	47.2	29	22.5	15	11.6
34	422	129	100.0	—	—	7	5.4	25	19.4	53	41.1	31	24.0	13	10.1
37	423	131	100.0	—	—	3	2.3	11	8.4	71	54.2	30	22.9	16	12.2
26	423	127	100.0	—	—	9	7.1	33	26.0	62	49.6	18	14.2	4	3.1
Nonsmoking control dogs															
21	—	127	100.0	127	100.0	—	—	—	—	—	—	—	—	—	—
24	—	127	100.0	127	100.0	—	—	—	—	—	—	—	—	—	—
27	—	125	100.0	125	100.0	—	—	—	—	—	—	—	—	—	—
38	—	128	100.0	128	100.0	—	—	—	—	—	—	—	—	—	—
41	—	124	100.0	124	100.0	—	—	—	—	—	—	—	—	—	—
42	—	131	100.0	131	100.0	—	—	—	—	—	—	—	—	—	—
43	—	127	100.0	127	100.0	—	—	—	—	—	—	—	—	—	—
44	—	132	100.0	129	97.7	3	2.3	—	—	—	—	—	—	—	—
45	—	132	100.0	132	100.0	—	—	—	—	—	—	—	—	—	—
80	—	129	100.0	128	99.2	1	0.8	—	—	—	—	—	—	—	—

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TABLE 3.—Findings in bronchial epithelium in 10 smoking and 10 nonsmoking control dogs. The sections are classified by the percent of cells with atypical nuclei in the bronchial epithelium

Dog No.	Days from start of smoking to death	Total sections with epithelium		Percent of bronchial epithelial cells with atypical nuclei																	
				None		<20% of cells		20-39% of cells		40-49% of cells		50-59% of cells		60-69% of cells		70-89% of cells		100% of cells	
		Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Smoking dogs																			
29	24	123	100.0	122	100.0	1	1.3	1	24.4	83	72.1	3	2.5	1	2.1				
26	229	96	100.0			1	0.8	31	4.7	24	24.1	23	17.5	4	7.1				
35	273	127	100.0					6				10	71.4	4	28.6				
32	410	14	100.0																
36	415	39	100.0																
31	421	121	100.0																
33	422	128	100.0																
34	422	129	100.0																
27	423	131	100.0																
28	423	127	100.0																
Nonsmoking control dogs																			
21	—	127	100.0	127	100.0														
24	—	127	100.0	127	100.0														
37	—	125	100.0	125	100.0														
38	—	128	100.0	128	100.0														
41	—	124	100.0	124	100.0														
42	—	131	100.0	131	100.0														
43	—	127	100.0	127	100.0														
44	—	132	100.0	132	100.0														
45	—	122	100.0	122	100.0														
80	—	129	100.0	129	100.0														

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none in any of the sections from smoking dog 29. However, atypical nuclei were found in the epithelial cells of all the sections from the other 9 smoking dogs. Furthermore, the proportion of cells with atypical nuclei was considerably greater in sections from the 5 dogs killed after 421 days than in sections from dogs 30 and 35 that died on days 229 and 278, respectively (figs. 5 and 6). In the 7 smoking dogs that survived 410 days or longer, most sections had lesions in which 50–69% of the epithelial cells had atypical nuclei (fig. 8).

These findings parallel those in our study of human beings; namely, that as a result of the inhalation of smoke, there is an increase in the number of basal cells, and as the smoking habit continues, the nuclei become atypical, increasing and extending toward the surface. The studies of these dogs show dyskeratosis present in a number of sections from the heavy-smoking dogs.

As indicated, this method is presented to illustrate a procedure which can measure the effect of whole tobacco smoke and possibly its components. This method could be used not only to establish a dose response for whole smoke, but also to determine whether a selective reduction of different smoke components would be associated with a corresponding change in alterations of the tracheobronchial epithelium.

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FIGURE 1. Mucous glands of dog, showing basal cell hyperplasia and increased secretion after 229 days of smoking. Some nuclei vary in size and shape. × 425

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FIGURE 2.—Photomicrograph of bronchus of dog, showing normal ciliated columnar epithelium. × 170



FIGURE 3.—Basal cell hyperplasia in bronchial epithelium of dog after 21 days of smoking. Basal cells are uniform in size and shape. × 625



FIGURE 4.—Squamous metaplasia in a dog at site of tracheostomy after 21 days of smoking. Thick layer of epithelial cells resembles squamous epithelium in other sites. × 200



FIGURE 5.—Squamous metaplasia with atypism in a dog after 229 days of smoking. Some nuclei vary in size and shape. There is a disorderly arrangement of the cells. × 240



FIGURE 6.—Squamous metaplasia with atypism in a dog after 278 days of smoking. Some nuclei vary in size and shape. × 400



FIGURE 7.—*Greatly* thickened epithelium of a dog after 421 days of smoking. The nuclei vary in size and shape. Hyperchromatism is prominent. × 400