Quantitative Relationship Between Cigarette Smoking and Death Rates

E. CUYLER HAMMOND, *Sc.D., Department of Epidemiology and Statistics, American Cancer Society, Inc., New York, New York 10019*

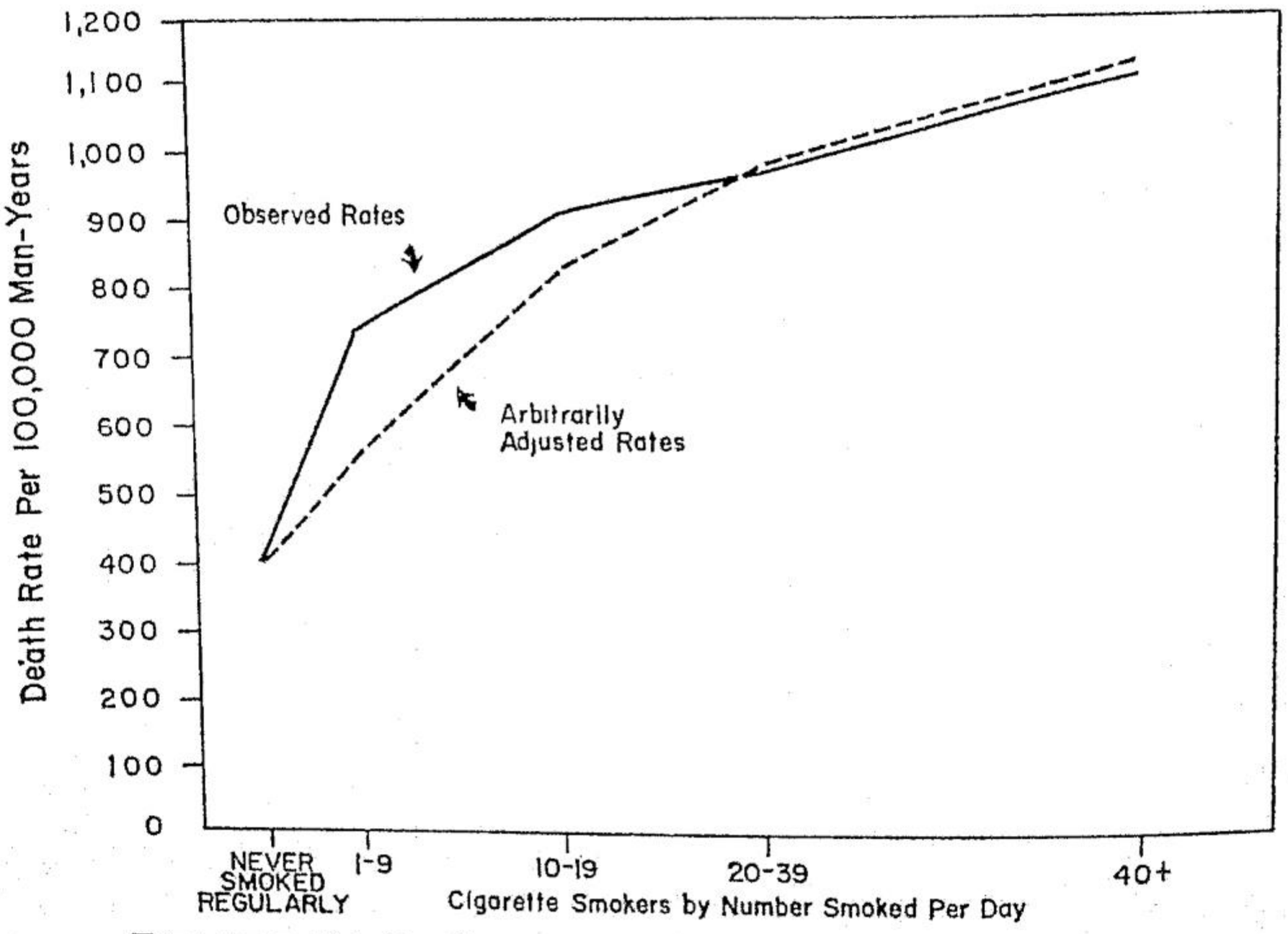
THE three most commonly used indexes of degree of exposure to cigarette smoke are: 1) number of cigarettes smoked per day, 2) depth of inhalation of the smoke, and 3) years of cigarette smoking (or age at start of cigarette smoking). No matter which of these is used, death rates of current cigarette smokers increase with degree of exposure (*1*). In this paper we will only consider the first of the three indexes. Our major concern is with the shape of the curve indicating the relationship between death rates and the number of cigarettes smoked per day.

The solid line in text-figure 1 shows the death rate of men who never smoked regularly and the death rates of men smoking various numbers of cigarettes per day as reported in one study (*1*) for men in age group 45–54. It is similar to the dose-response curve found in many other studies of this subject. The shape has aroused considerable curiosity. Smoking as few as 1–9 cigarettes a day appears to result in a surprisingly large increase in death rates. Considering this, smoking 40 or more cigarettes per day appears to produce less additional effect than might have been expected from usual experience with dose-response relationship.

I have long suspected that, in actual fact, light cigarette smoking produces considerably less effect upon death rates and heavy cigarette smoking produces somewhat more effect upon death rates than would seem to be the case if the observations are taken at face value. My reasons are as follows:

Many cigarette smokers smoke about the same number of cigarettes each day over a period of years (*2*). However, depending upon circumstances, an individual smoker may stop smoking, increase his daily consumption, or decrease his daily consumption. State of health has considerable influence on this (*3*). That is, cigarette smokers often stop smoking or reduce their daily consumption if they become ill or have a heart attack. Some-

289-019—68——2



TEXT-FIGURE 1.—Death rates by smoking habits for men 45–54 years old.

times the change is permanent, but often the individual returns to his former rate of consumption within a few weeks or a few months.

When classified on the basis of current number of cigarettes smoked per day, habitual heavy cigarette smokers who have temporarily reduced their smoking due to illness are counted as light smokers. This has the effect of artificially raising the apparent death rate of light smokers and lowering the apparent death rate of heavy smokers. Thus, the shape of the dose-response curve is distorted. Unfortunately, this problem has no easy solution.[1] However, using a hypothetical example, we can see the extent to which the shape of the curve could be altered by relatively slight temporary changes in daily cigarette consumption as outlined above.

In the study referred to earlier (*1*), among men in age group 45–54, death rates per 100,000 person-years were found to be: 402 for men who never smoked regularly, 741 for men currently smoking 1–9 cigarettes a day at the time of enrollment, 910 for men currently smoking 10–19 cigarettes a day, 970 for men currently smoking 20–39 cigarettes a day, and 1,109 for men currently smoking 40 or more cigarettes per day (*see solid line* in text-fig. 1).

The numbers of men in these 5 categories at the start of the study were: 36,863; 5,219; 12,172; 46,886; and 11,047, respectively. Altogether there were 36,863 nonsmokers and 75,324 current cigarette smokers.

Simply as an example, suppose that the 75,325 current cigarette smokers fell into the following 4 groups in relation to their *usual* smoking habits.

1) 11,047 men usually smoked 40 or more cigarettes a day and currently

[1] Some authors have classified their subjects by maximum number of cigarettes ever smoked per day but this leads to other complications.

smoked the same number. Their death rate was 1,109 per 100,000 person-years (*i.e.*, the observed rate). Another 750 men usually smoked 40 or more cigarettes a day, but due to illness had temporarily reduced their daily consumption. Because of ill health (and because they returned to heavy smoking), they had a death rate of 1,330 per year during the next several years. Of these 750 "temporary reducees," 275 currently smoked 1–9 cigarettes a day and 475 currently smoked 10–19 cigarettes a day.

2) 46,886 men usually smoked 20–39 cigarettes a day and currently smoked the same number. Their death rate was 970 per 100,000 person-years (*i.e.*, the observed rate). Another 3,200 men usually smoked 20–39 cigarettes a day, but due to illness temporarily reduced their daily consumption. Their death rate was 1,160 per 100,000 person-years; 1,150 of them currently smoked 1–9 cigarettes a day and 2,050 currently smoked 10–19 cigarettes a day.

3) 12,172 men currently smoked 10–19 cigarettes a day and their observed death rate was 910 per 100,000 person-years. As described above, 2,525 of these men usually smoked more than 10–19 cigarettes, but had temporarily reduced their consumption. The remaining 9,647 usually smoked 10–19 cigarettes a day.

4) 5,219 men currently smoked 1–9 cigarettes a day; 1,425 of them usually smoked more than this amount; and the remaining 3,794 usually smoked 1–9 cigarettes a day. (The observed death rate of the 5,219 men was 74.1 per 100,000 person-years.)

From these figures, it follows that death rates per 100,000 person-years were 571 for men who usually smoked 1–9 cigarettes a day, 836 for men who usually smoked 10–19 cigarettes a day, 982 for men who usually smoked 20–39 cigarettes a day, and 1,124 for men who usually smoked 40 or more cigarettes a day. These rates are shown by the dashed line in text-figure 1.

Obviously, changes in smoking habits, both temporary and permanent, are far more involved than those used in our example which was simplified for ease of arithmetic illustration. Nevertheless, state of health does influence smoking habits; this must tend to distort the shape of the dose-response curve if subjects are classified by current number of cigarettes smoked per day and then traced for several years. Perhaps an over-correction was made in our hypothetical example, but it serves to illustrate the point.

Why is it of interest to know the approximate shape of the dose-response curve?

Since the harmful effects of cigarette smoking increase with degree of exposure, then it is reasonable to suppose that nonselective reduction of all the components of cigarette smoke would result in a less harmful cigarette. If very low exposure produces only a slight effect upon death rates, then reducing the concentration of cigarette smoke would be highly beneficial. However, if even low exposure greatly increases death rate, then this approach to the "less hazardous cigarette" would appear to be less hopeful.

TOWARD A LESS HARMFUL CIGARETTE

Age Trends

The American (*1*, *4–6*) and Canadian (*7*) prospective studies of death rates in relation to smoking habits are in close agreement with each other. Tables 1–3 are from one of the American studies (*1*).

TABLE 1.—Age-standardized death rates, mortality differences, and mortality ratios for men with a history of only cigarette smoking who were currently smoking cigarettes at enrollment by current number of cigarettes smoked per day and age at start of study. Death rates for men who never smoked regularly are shown for comparison

Current No. of cigarettes per day	Age		
	45–54	55–64	65–74
	Death rates per 100,000 person-years		
Never smoked regularly	402	1, 187	3, 118
1–9	741	1, 815	4, 683
10–19	910	2, 280	5, 145
20–39	970	2, 437	5, 325
40+	1, 109	2, 680	5, 635
	Mortality differences		
Never smoked regularly	0	0	0
1–9	339	628	1, 565
10–19	508	1, 093	2, 027
20–39	568	1, 250	2, 207
40+	707	1, 493	2, 517
	Mortality ratios		
Never smoked regularly	1. 00	1. 00	1. 00
1–9	1. 84	1. 53	1. 50
10–19	2. 26	1. 92	1. 65
20–39	2. 41	2. 05	1. 71
40+	2. 76	2. 26	1. 81

Table 1 shows death rates (all causes of death combined) for men who never smoked regularly and for current regular cigarette smokers with a history of only cigarette smoking, classified by age and by current number of cigarettes smoked per day. Mortality differences and mortality ratios are also shown, the death rate of men who never smoked regularly being taken as a basis of comparison. By definition, men who never smoked regularly have a mortality difference of 0.0000 and a mortality ratio of 1.00. Table 2 shows death rates, mortality ratios, and mortality differences for coronary heart disease; table 3 shows the figures for lung cancer.

I strongly suspect that in each case the observed death rate of light smokers is artificially high and the observed death rate of heavy cigarette smokers is artificially low due to the factors discussed in the first part of this paper. We have found that a heart attack often leads a person to stop smoking or reduce his daily consumption of cigarettes. Undiagnosed lung cancer may or may not do so; but cigarette smokers who die of lung cancer also have some degree of emphysema, are typically short of breath, and typically have a chronic cough. There is reason to believe that these conditions often lead a cigarette smoker to stop smoking or reduce his daily consumption. Since the incidence of heart disease and ill health in general increases with advancing age, the effect of health on smoking habits almost

TABLE 2.—Coronary heart disease. Age-standardized death rates, mortality differences, and mortality ratios for men with history of only cigarette smoking who were currently smoking cigarettes at enrollment by current number of cigarettes smoked per day and age at start of study. Death rates for men who never smoked regularly are shown for comparison

Current No. of cigarettes per day	Age		
	45–54	55–64	65–74
	Coronary heart disease death rates per 100,000 person-years		
Never smoked regularly	150	542	1, 400
1–9	352	837	1, 758
10–19	463	1, 039	2, 254
20–39	467	1, 104	2, 188
40+	503	1, 152	*
	Mortality differences		
Never smoked regularly	0	0	0
1–9	202	295	358
10–19	313	497	854
20–39	317	562	788
40+	353	610	*
	Mortality ratios		
Never smoked regularly	1, 00	1. 00	1. 00
1–9	2. 35	1. 54	1. 26
10–19	3. 09	1. 92	1. 61
20–39	3. 11	2. 04	1. 56
40+	3. 35	2. 13	*

*Death rate is omitted when number of person-years times death rate for age group yields expected number of less than 10 deaths.

TABLE 3.—Lung Cancer. Age-standardized death rates, mortality differences, and mortality ratios for men with history of only cigarette smoking who were currently smoking cigarettes at enrollment by current number of cigarettes smoked per day and age at start of study. Death rates for men who never smoked regularly are shown for comparison

Current No. of cigarettes per day	Age		
	35–54	55–69	70–84
	Lung cancer death rates per 100,000 person-years		
Never smoked regularly	6	19	25
1–9	38	68	134
10–19	24	168	243
20–39	58	264	446
40+	47	334	754
	Mortality differences		
Never smoked regularly	0	0	0
1–9	32	49	109
10–19	18	149	218
20–39	52	245	421
40+	41	315	729
	Mortality ratios		
Never smoked regularly	1. 00	1. 00	1. 00
1–9	6. 17	3. 53	5. 32
10–19	3. 90	8. 77	9. 62
20–39	9. 37	13. 82	17. 62
40+	7. 67	17. 47	29. 84

certainly increases with age. These factors should be taken into consideration when viewing the figures shown in the tables.

In all age groups, total mortality as well as mortality from coronary heart disease and lung cancer increases with amount of cigarette smoking. In the case of total mortality and coronary heart disease, mortality differences increase with advancing age while mortality ratios decrease with advancing age. This means that with advancing age, the total impact of cigarette smoking increases, but the rate of this increase is less than the rate of increase in the impact of all other factors combined.

The picture is somewhat different for lung cancer. Both mortality differences and mortality ratios increase with advancing age. This probably results from the dose–time-response relationship in the production of cancer by exposure to carcinogenic chemicals.

REFERENCES

(1) Hammond, E. C.: Smoking in relation to death rates of one million men and women. Nat Cancer Inst Monogr 19: 127–204, 1966.

(2) Hammond, E. C., and Garfinkel, L.: Smoking habits of men and women. J Nat Cancer Inst 27: 419–442, 1961.

(3) ———: The influence of health on smoking habits. Nat Cancer Inst Monogr 19: 269–285, 1966.

(4) Hammond, E. C., and Horn, D.: Smoking and death rates—report on forty-four months of follow-up of 187,783 men. I. Total mortality. JAMA 166: 1159–1172, 1958.

(5) Kahn, H. A.: The Dorn study of smoking and mortality among U.S. veterans: Report on eight and one-half years of observation. Nat Cancer Inst Monogr 19: 1–125, 1966.

(6) Dunn, J. E., Jr., Linden, G., and Breslow, L.: Lung cancer mortality experience of men in certain occupations in California. Amer J Public Health 50: 1475–1487, 1960.

(7) Best, E. W. R., Walker, C. B., Baker, P. M., Delaquis, F. M., McGregor, J. T., and McKenzie, A. C.: Summary of a Canadian study of smoking and health. Canad Med Assoc J 96: 1104–1108, 1967.

Cigarette Smoking and Risk of Coronary Heart Disease. Epidemiologic Clues to Pathogenesis. The Framingham Study

WILLIAM B. KANNEL, *M.D.*, WILLIAM P. CASTELLI, *M.D.*, *and* PATRICIA M. MCNAMARA, *National Heart Institute*,[1] *Framingham, Massachusetts 01701*

MODERN technology has drastically altered man's environment, producing a considerable change in living habits. There is mounting evidence that this altered way of life has produced an environment that fosters the proliferation of coronary heart disease (CHD). One such habit, cigarette smoking, has proved distinctly hazardous.

Since the development and promotion of ready-made cigarettes, the inhalation of tobacco smoke has become a widespread practice. The substantial evidence concerning the hazard to health which this intense self-induced air pollution constitutes has evidently not gained the widespread acceptance necessary to promote the development of effective countermeasures.

As a result of substantial data from large retrospective studies and a variety of prospective ones, there is no longer any reason to doubt the existence of an association between the cigarette smoking habit and mortality from CHD (*1–5*). However, the reason for the association, the pathogenetic mechanism, involved has yet to be precisely defined.

The incidence of clinical manifestations of CHD developing in subgroups of the population in Framingham initially free of the disease, classified according to their tobacco smoking habits, was determined. In addition, by taking into account other factors also demonstrated to predispose to development of the disease, the independent contribution of the tobacco habit to the rate of occurrence of CHD may be assessed. In this way clues to pathogenesis may be discovered. Such information may provide the key to achieving a substantial reduction in morbidity and mortality from CHD. This is the purpose of this report.

---

[1] From the Heart Disease Epidemiology Study, Framingham, Mass., and the National Heart Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014.

METHODS

The methods employed, criteria for CHD, the sampling method, and prior findings at the Framingham Study have been described in detail in previous publications (*6–9*). The population sample under investigation in the Framingham Study consisted of 5,127 adults (2,283 men and 2,844 women, age 30–62 years)—respondents of a random sampling of the population of the town who were examined and found free of CHD. This analysis was confined to those in the 30–59 age group. The incidence of CHD over the subsequent 12 years was ascertained by biennial examination of this study group. Examination procedures included a detailed cardiovascular history and physical examination, a 13-lead electrocardiogram, chest roentgenograms, a vital capacity, and various biochemical laboratory tests. The results of these biennial examinations, as well as transcripts of hospital records from the local community hospital, information obtained from physicians and hospitals outside the community, and review of death certificates and medical examiners' reports provided a comprehensive assessment of the disease experience of the participants in the study.

The manifestations of coronary heart disease were subdivided into the following groups: angina pectoris, the coronary insufficiency syndrome, myocardial infarction, fatal coronary heart disease in general, and sudden death in particular.

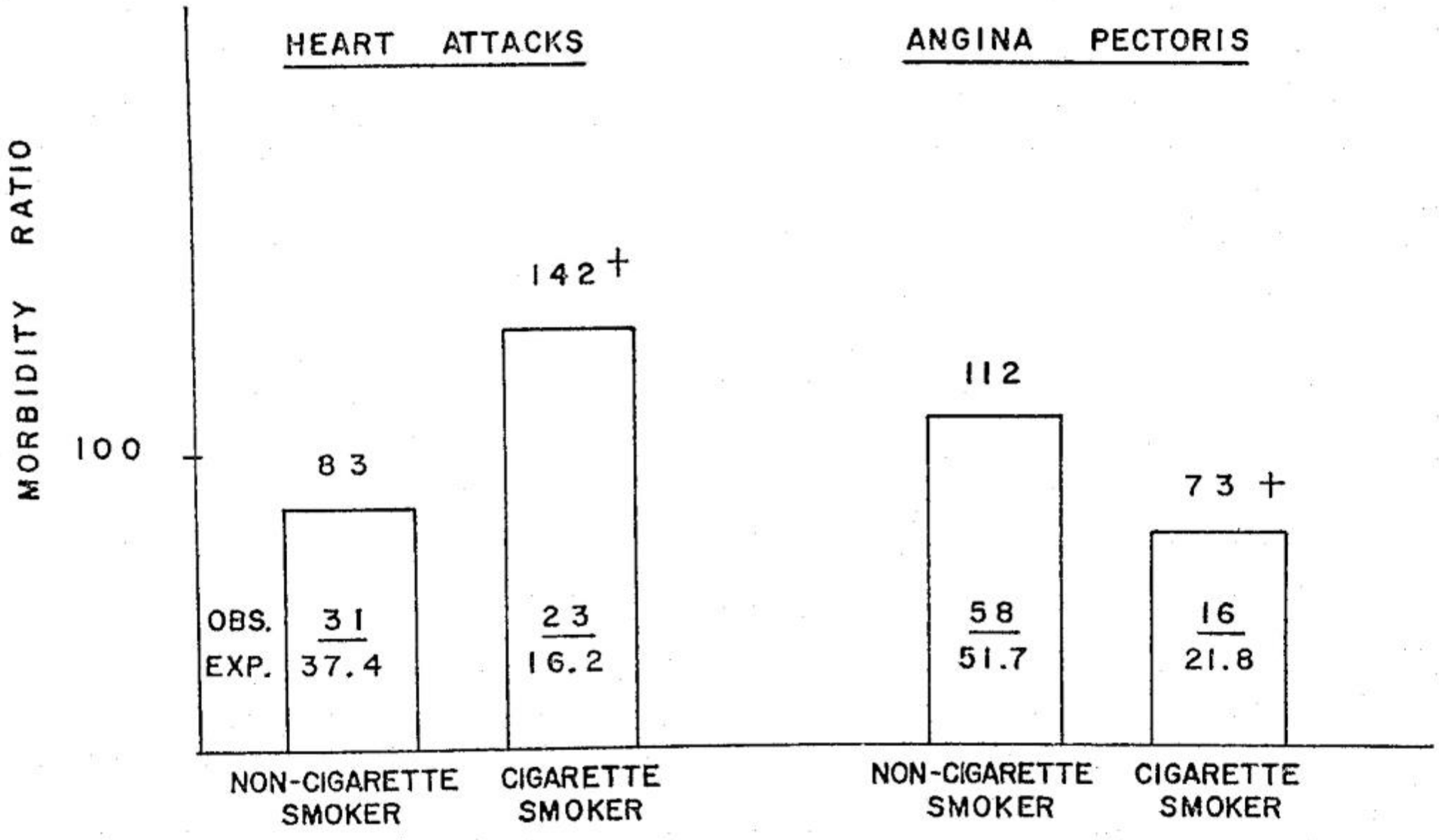
At the time of this report the population had been examined biennially seven times constituting a follow-up period of 12 years. The current health status of approximately 95% of the original 5,127 was known and less than 2% was considered completely lost to follow-up.

At the time of initial examination details of the tobacco habit of each individual were obtained as to type, duration, intensity, and any lapses in the use of tobacco. On the first examination a detailed smoking history was obtained for each participant. People were characterized as nonsmokers if they had never smoked, as exclusively pipe or cigar smokers, and as cigarette smokers according to the number of cigarettes smoked each day. Cigar and pipe smokers who also smoked cigarettes were classified according to the number of cigarettes they smoked. Blood chemistry determinations including lipids and blood sugar were also obtained. The methods employed have been described elsewhere (*6*).

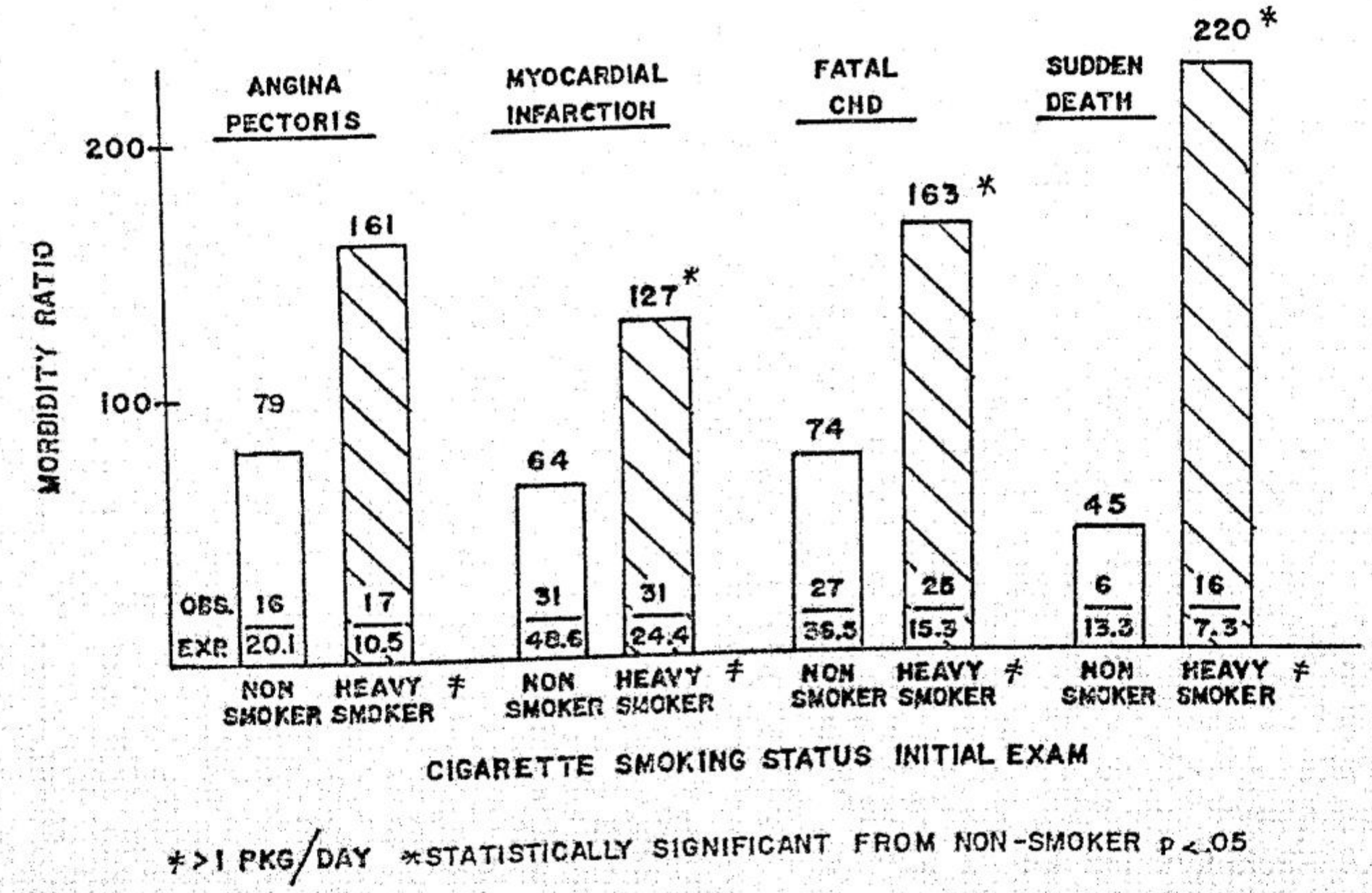
RESULTS

At the time of the initial examination close to 70% of the men and 40% of the women were cigarette smokers. A substantial increase in risk of developing every major manifestation of CHD over 12 years of follow-up was noted in the heavy cigarette smokers. This increased vulnerability was less evident (and not statistically significant) in women cigarette smokers (text-fig. 1) than in men (text-fig. 2). In both men and women the habit was particularly related to the occurrence of fatal attacks and, in men

especially, to sudden unexpected death. There were too few sudden deaths among the women to explore in them the effect of cigarette smoking on this catastrophic occurrence. Also it was not possible, because of limited numbers, to explore meaningfully the relation of the cigarette habit to the development of the coronary insufficiency syndrome in either sex.



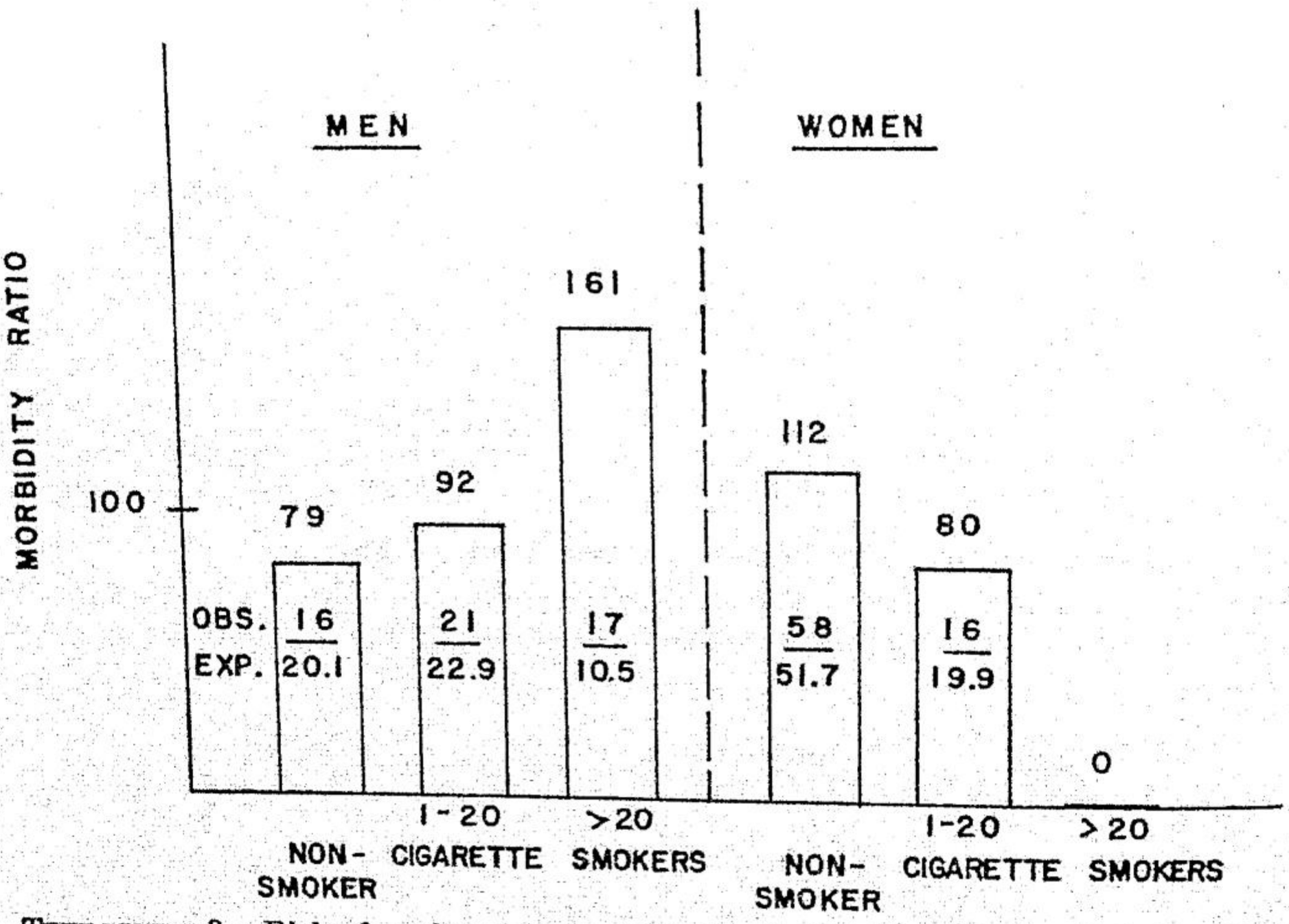
TEXT-FIGURE 1.—Risk of coronary heart disease (12 years) according to cigarette smoking habit (women 30–59 at entry). Framingham Heart Program.



TEXT-FIGURE 2.—Risk of manifestations of coronary heart disease (12 years) according to cigarette smoking status (men 30–59 at entry). Framingham Heart Study.

TOWARD A LESS HARMFUL CIGARETTE

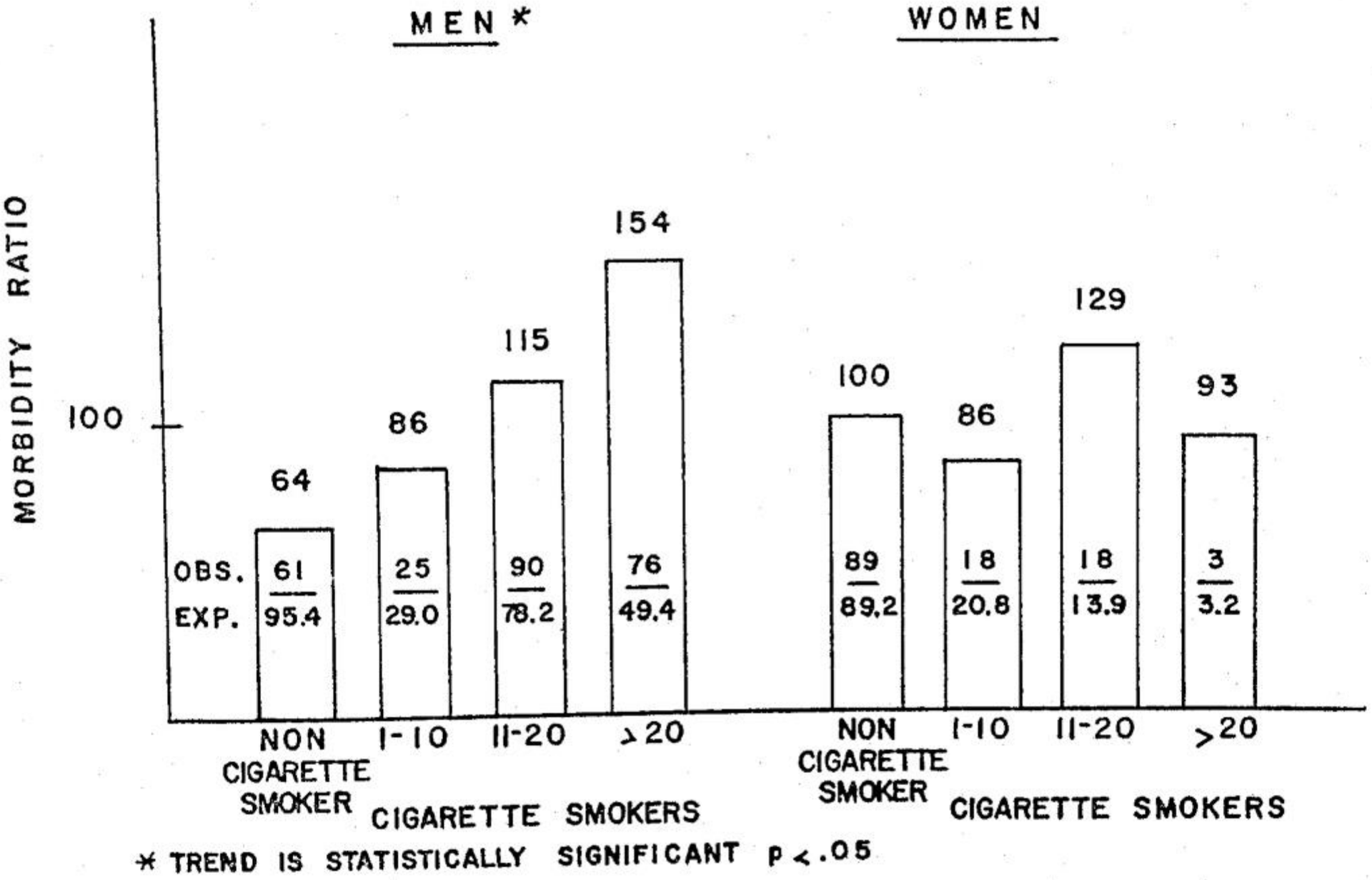
It now appears that in men but not women, the risk of uncomplicated angina pectoris may be increased in heavy cigarette smokers (text-fig. 3). In men, risk of CHD was not only greater in those who smoked cigarettes than in their nonsmoking cohorts, but was also proportional to the number of cigarettes smoked *each day* (text-fig. 4). On the other hand, the risk of developing CHD was unrelated to the *duration* of the habit, even in heavy cigarette smokers (text-fig. 5). Consequently, the *ex-smokers* were observed to have the same low risk as those who never smoked, suggesting that the adverse effect is reversible. The cigar and pipe smokers also had no increase in risk of attacks (text-fig. 6).



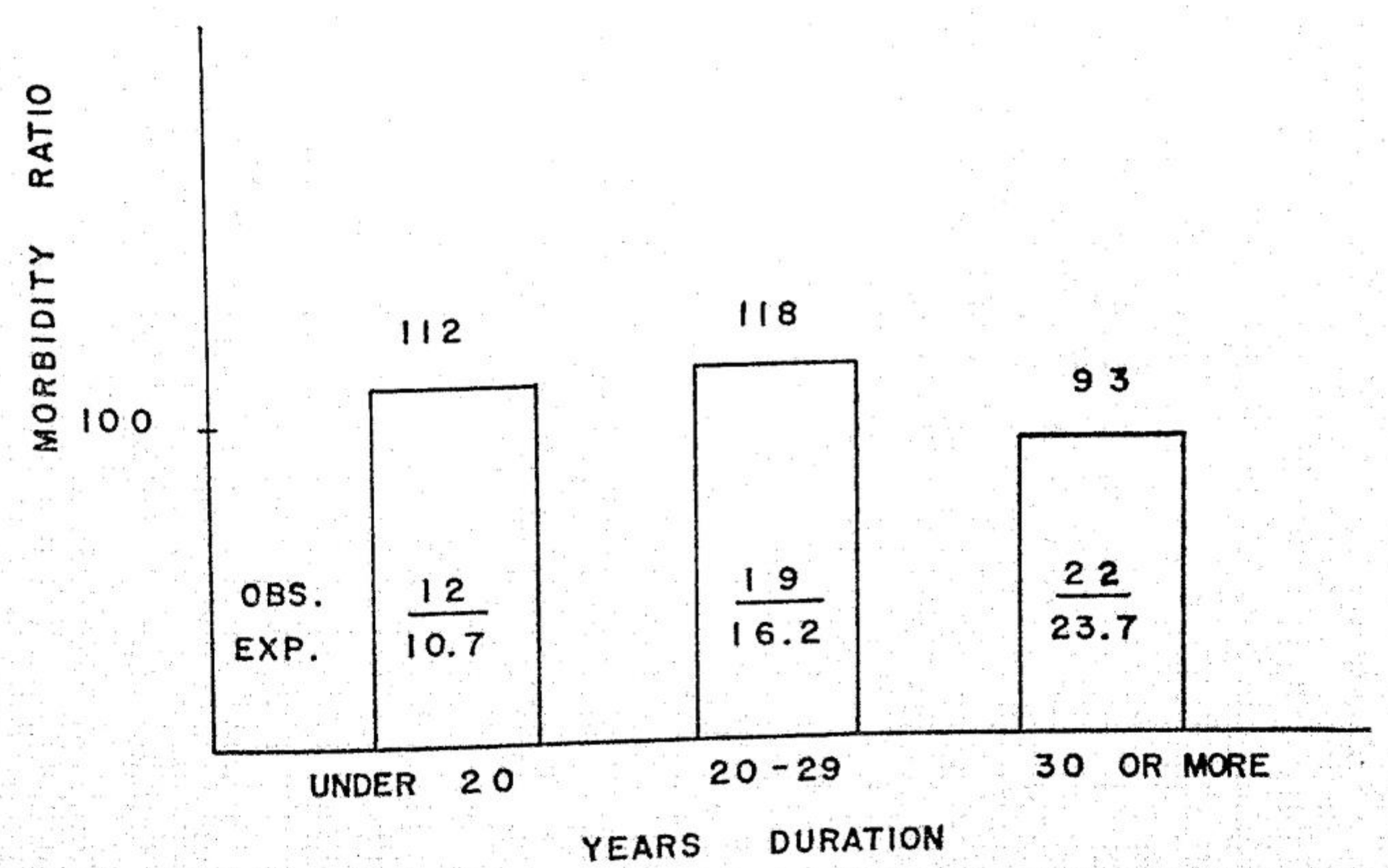
TEXT-FIGURE 3.—Risk of angina pectoris (12 years) according to intensity of cigarette smoking habit (men and women 30–59 at entry). Framingham Heart Study.

While the cigarette habit was especially related to the occurrence of a fatal attack, if the victim survived long enough to enter the hospital, the case fatality rate was little higher in cigarette smokers than in their nonsmoking cohorts.

The inhalation of tobacco smoke increased the risk of CHD in the general population, but certain persons appeared to be particularly vulnerable to its adverse cardiovascular effects. Persons predisposed by factors known to accelerate atherogenesis (diabetes, abnormal blood lipids, hypertension) who also smoked, greatly increased their already heightened vulnerability to CHD. With each increment in susceptibility as a result of these factors, the cigarette smoker fared much worse than his nonsmoking cohort. An excess risk, although considerably attenuated, was also noted in cigarette smokers, even excluding those persons with any

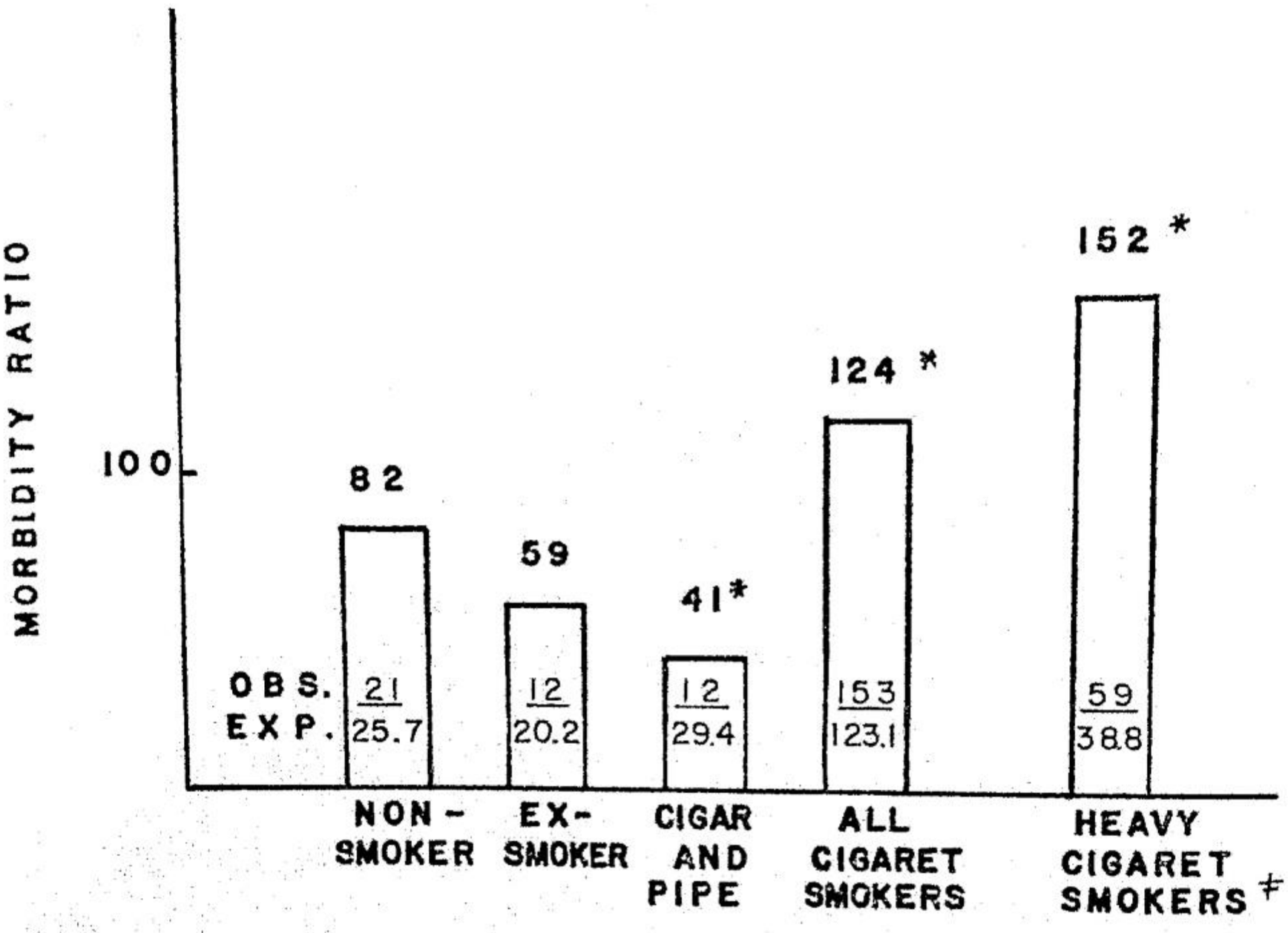


TEXT-FIGURE 4.—Risk of coronary heart disease (12 years) according to intensity of cigarette habits (men and women 30–59 at entry). Framingham Heart Study.



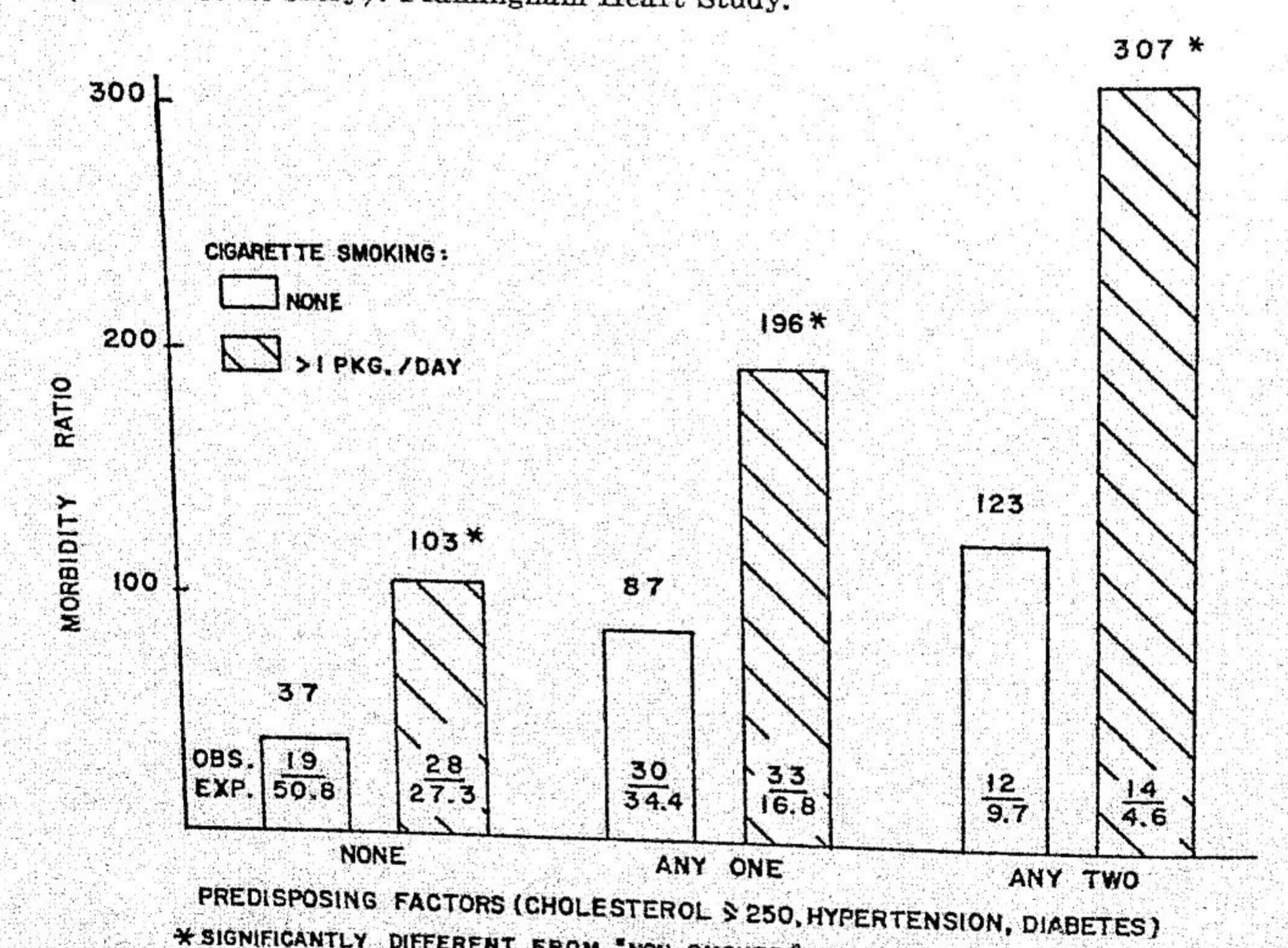
TEXT-FIGURE 5.—Risk of infarction or death from CHD in 12 years in heavy smokers (over pack/day of cigarettes) according to duration of smoking habit (men 30–59 at entry). Framingham Heart Study.

of these major factors contributing to CHD (text-fig. 7). Persons possibly afflicted with occult CHD as evidenced by the development of electrocardiographic abnormalities without other explanation, who also smoked heavily, had a pronounced increase in risk of developing overt CHD. However, this



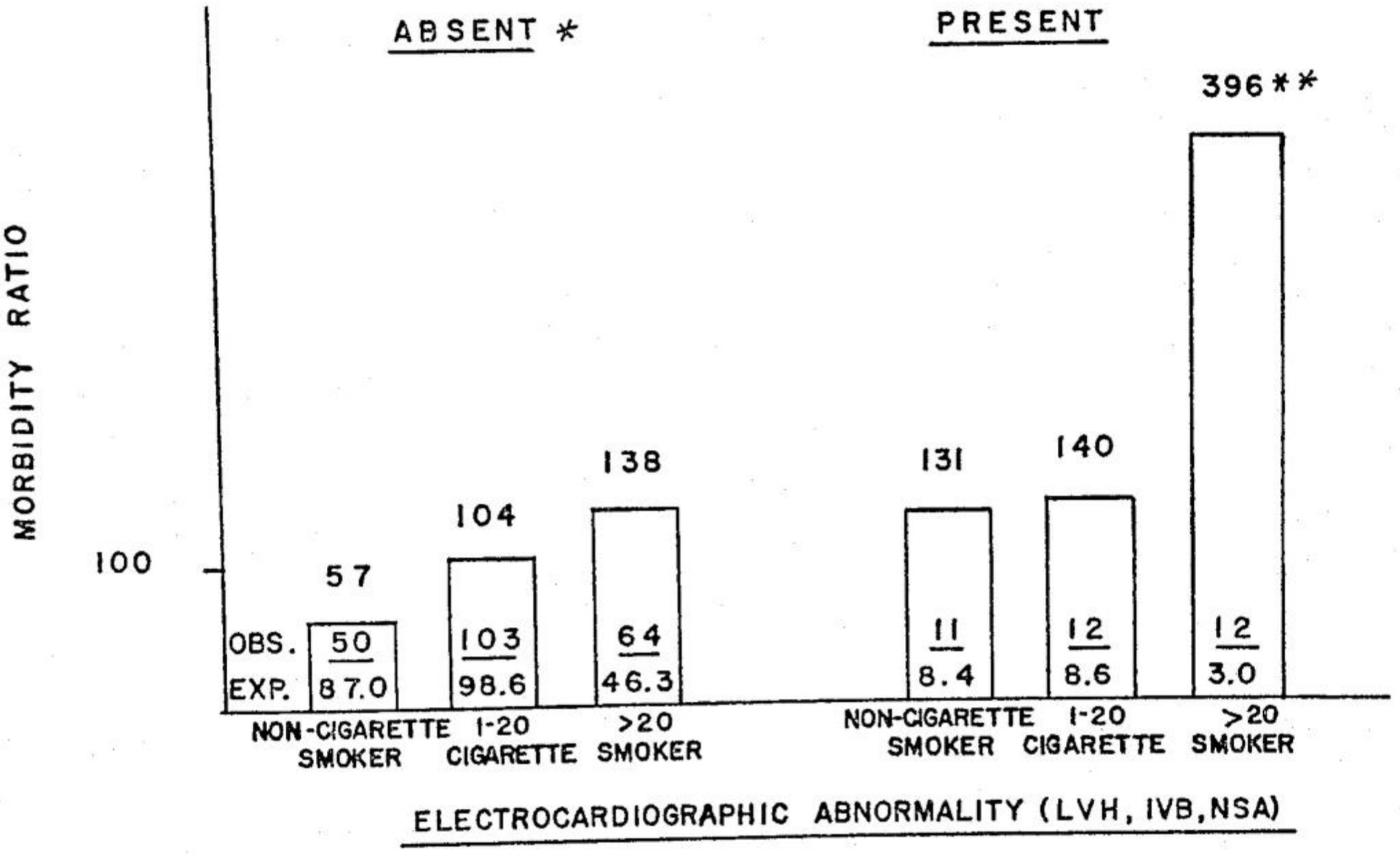
ǂ OVER 1 PACK/DAY * STATISTICALLY SIGNIFICANT FROM STANDARD $p < .05$

TEXT-FIGURE 6.—Risk of "heart attack" in 12 years according to smoking status (men 30–59 at entry). Framingham Heart Study.



* SIGNIFICANTLY DIFFERENT FROM "NON SMOKER" $P < .05$

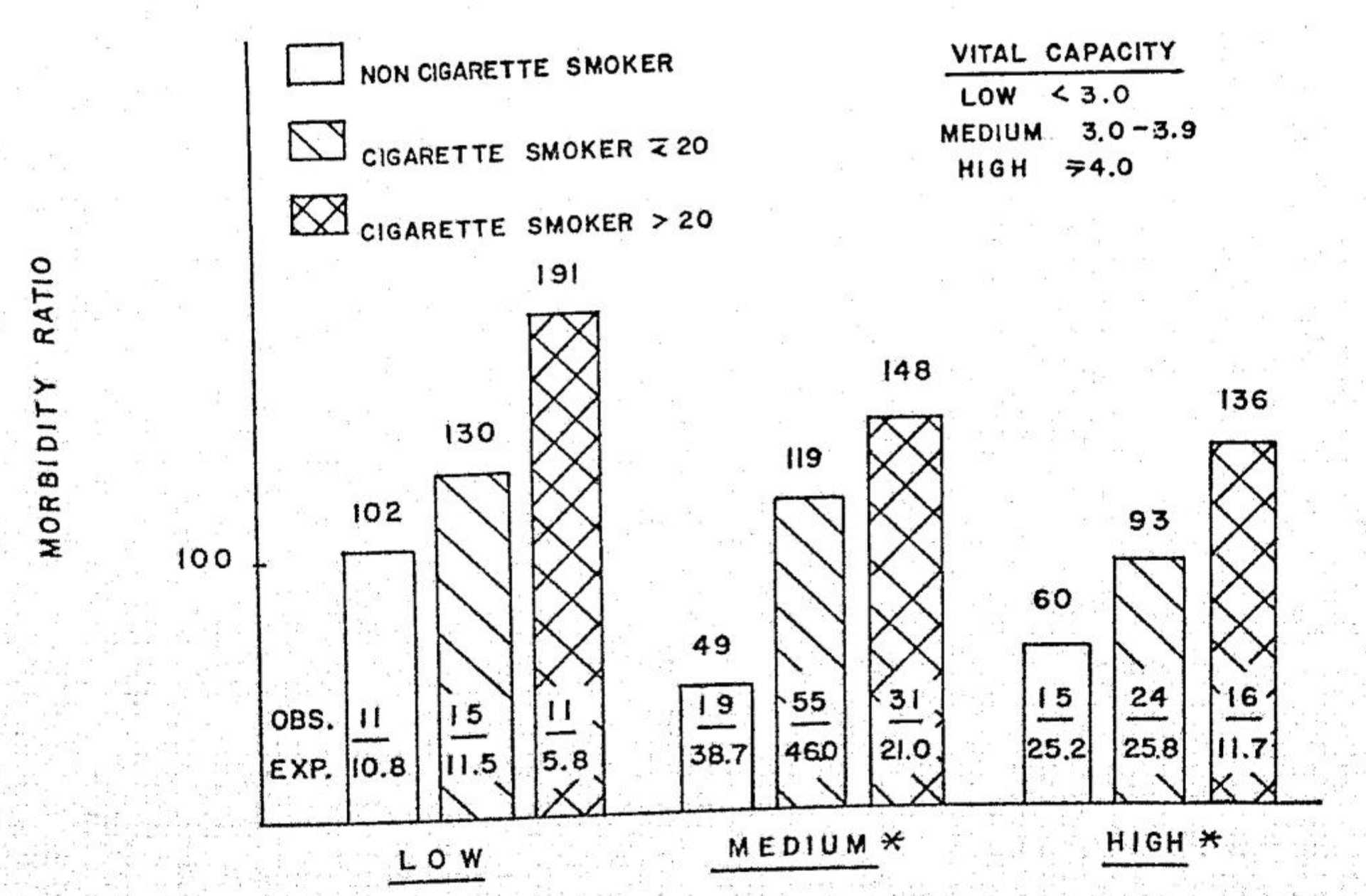
TEXT-FIGURE 7.—Risk of coronary heart disease (12 years) according to cigarette smoking habit and presence of "predisposing factors" (men 30–59 at entry). Framingham Heart Study.



*TREND IS STATISTICALLY SIGNIFICANT $p < .05$

**SIGNIFICANT FROM STANDARD $p < .05$

TEXT-FIGURE 8.—Risk of coronary heart disease (12 years) according to cigarette smoking habit and electrocardiographic abnormality (men 30–59 at entry). Framingham Heart Study.



*TREND STATISTICALLY SIGNIFICANT AT $p < .05$

TEXT-FIGURE 9.—Risk of heart attacks (12 years) according to cigarette smoking habit and vital capacity (men 30–59 at entry). Framingham Heart Study.

TOWARD A LESS HARMFUL CIGARETTE

was not an essential prerequisite since, even in the absence of electrocardiographic abnormalities, risk was related to the intensity of the habit (text-fig. 8). Persons with a low vital capacity were observed to develop an excess of heart attacks, but not uncomplicated angina pectoris. At any level of vital capacity, however, the risk of such "heart attacks" increased with the intensity of the cigarette habit (text-fig. 9).

Using discriminant function analysis to assess more precisely the relative contribution of each of a number of factors to the development of CHD again revealed in men an independent contribution of the cigarette habit to risk. This contribution was as weighty as that of any other factor, barring age and possibly lipids. The independent effect of cigarette smoking in women appeared more attenuated compared to that of men (table 1).

TABLE 1.—Risk factors in CHD linear discriminant function coefficients (standard units)

Men				
Risk factors	Combined ages	30–39	40–49	50–62
Age	.5934	.2394	.3334	.2370
Cholesterol	.4444	.9613	.3207	.3790
Systolic blood pressure	.3334	.3427	.1669	.3809
Relative weight	.1890	.1941	.3619	.1036
Hemoglobin	—.1050	.0313	—.0134	—.2206
Cigarettes smoked	.4192	.6823	.5084	.3004
ECG abnormality	.2626	.2685	.2556	.2197

Women	Combined ages	30–49	50–62
Age	.6259	.7325	.2600
Cholesterol	.2844	.7322	.1207
Systolic blood pressure	.5556	.1947	.4776
Relative weight	.0975	.0751	.1481
Hemoglobin	.0392	—.0304	.0734
Cigarettes smoked	.0625	—.0731	.1262
ECG abnormality	.3048	.2234	.2526

DISCUSSION

Confronted with the evidence presented, the conclusion that the cigarette smoking habit makes a potent contribution to the development of CHD proportional to the intensity of the habit seems inescapable. An examination of the details of the relationship demonstrated provides a number of clues to the pathogenesis of the association. It is more reasonable to regard the cigarette habit as playing a contributory rather than a primary role (as is likely the case in lung cancer and emphysema) in the causation of CHD. The fact that the risk of disease was proportional to the intensity of the habit but not to its duration strongly suggests a transient, noncumulative effect. Further support for this concept is afforded by the observation that the ex-smokers had the same low risk as those who never

smoked. Evidently there is much to be gained by giving up the habit, however long it was previously indulged in prior to its discontinuance. The fact that the risk of disease in those who gave up the habit was not intermediate between that of the nonsmoker and continuing smoker suggests a prompt loss of the increased vulnerability in quitting the habit. Examination of the case fatality rate in heart attack victims who survived long enough to be hospitalized lends additional support to the concept of a transient effect of the habit. Such persons, who are discouraged from continuing to smoke while in hospital, experienced very little increase in mortality compared to their nonsmoking cohorts. This too is consistent with an immediate, transient, noncumulative effect, since the habit predisposed especially to sudden death outside the hospital where no prohibition of smoking exists.

Lack of a cumulative effect is not a universal finding even in large prospective studies (*3*, *4*). It is possible that this stems from inability in some studies to place CHD events and change in cigarette habit in chronological sequence with certainty.

The fact that the smokers had a greater risk at any level of blood pressure or blood lipid concentration, whether or not diabetic, and at any level of vital capacity suggests an independent contribution of the habit to risk. In those already so predisposed to CHD the habit is particularly pernicious.

While the present studies at Framingham in general confirm previously reported findings of a combined study of the problem at Albany and Framingham (*1*), the present report further extends these findings by exploring the effect of the habit in women. Also, the results of this report are at variance with previous findings by suggesting that male cigarette smokers may develop an excess of angina pectoris as well as the more lethal manifestations of CHD. This finding is consistent with the experience of the H. I. P. of New York, which also reported an association of cigarette smoking and angina pectoris (*10*).

The fact that cigar and pipe smokers had no increased risk of developing CHD suggests that tobacco smoke must be inhaled to produce harmful cardiovascular effects.

The argument of some that persons who smoke are different to begin with and that this accounts for the apparently deleterious effect hardly seems credible. The reported findings that show an effect of cigarette smoking independent of most of the known constitutional factors contributing to risk of CHD, and the finding that giving up the habit results in a prompt disappearance of the adverse effect tend to make this argument unacceptable. Detailed analysis of the habits or traits of smokers failed to uncover any substantial differences in them from other persons that could explain their increased propensity to disease.

While the harmful consequences of the cigarette habit are certainly more pronounced in some persons than others as regards risk of CHD, it has not thus far been possible to identify persons who are completely immune

to its cardiovascular consequences. The less pronounced effect demonstrated in women at Framingham may well derive less from the fact that they are biologically female than the fact that so many of them who smoke cigarettes do not inhale.

This is at variance with reports of a number of studies of twins which have concluded that constitutional factors must be operative (*11*, *12*). As has been demonstrated, the cigarette habit is most strikingly related to the serious, lethal manifestations of CHD. These studies of survivors have of necessity focused on angina pectoris, the manifestation, in our experience, least strongly associated with the cigarette habit. These studies also suffered from small numbers, the inclusion of women, and the inability to compare sufficiently divergent smoking groups. Substantial differences in incidence of angina pectoris in the Framingham Study could be demonstrated only in men when nonsmokers were compared to *heavy smokers*, and not at all in women (text-fig. 3). None of the studies of twins were able to contrast such polar groups.

The reported findings demonstrating an effect of the cigarette habit independent of most of the known constitutional factors of importance and the suggestion that giving up the habit achieves a prompt loss of the adverse effect tend to implicate the cigarette, and not some associated factor, as the culprit.

In animals and in man it has been demonstrated that nicotine absorbed from inhaled tobacco smoke transiently stimulates chromaffin tissues to release catecholamines (*13*). In the irritable, ischemia-sensitized myocardium this may precipitate serious, life-threatening, arrhythmias, accelerate clotting in diseased vessels, and possibly affect coronary blood flow in the presence of a compromised coronary circulation (*14–16*). This hypothesized pathogenesis is consistent with a transient, reversible, noncumulative effect of the cigarette habit operating through mechanisms having little influence on the rate of intimal atherogenesis.

While the harmful effect of the cigarette habit on the respiratory tract is considerable and serious, its impact on cardiovascular disease rates should be emphasized. Large-scale prospective studies of death rates among male smokers have consistently revealed that CHD makes the major contribution to the excess mortality attributable to the smoking habit, with lung cancer in second place. In spite of a more potent effect of the cigarette habit in lung cancer, about half the excess deaths in smokers are from CHD and only one-sixth can be attributed to lung cancer. This is because coronary heart disease is such a frequent cause of death, exceeding that from lung cancer by sevenfold. The indication of a probable lack of cumulative effect plus the ranking of CHD as the principal cause of excess mortality attributable to the cigarette habit has very definite implications for prevention.

The inhalation of tobacco smoke makes a powerful independent contribution to the development of all manifestations of CHD and to its lethal consequences. While other associated factors including mental stress,

dietary excesses, and sedentary living could conceivably explain some of the association, the cigarette habit renders persons with coronary atherosclerosis more liable to the development of overt, often fatal CHD. Unlike the case for most of the factors demonstrated to be associated with an increased vulnerability to coronary attacks, evidence is already at hand to indicate the efficacy of discontinuing the habit. From estimates of excess mortality attributable to the cigarette habit and the anticipation of a prompt loss of the lethal effect (in CHD) on giving up the habit, it is conceivable that the appalling toll of deaths and illness from CHD might be reduced substantially. The potential for salvage of these needless victims seems too enormous to justify further temporizing.

Because of its apparent noncumulative effect on the cardiovascular system, there is much to be gained by giving up the habit entirely, or substituting noninhaled forms of tobacco. The development of a less harmful cigarette for the tobacco-dependent person may merit investigation.

The therapeutic implications are also clear. Cigarette smoking in persons with a compromised coronary circulation is bound to be a hazardous practice, proportional to the number of cigarettes smoked each day. In persons with overt symptomatic CHD, it would seem prudent that the habit be vigorously discouraged.

The psychosocial and economic considerations which promote and perpetuate the habit have proved powerful enough to frustrate efforts to curb this harmful practice. It would seem reasonable that, until such time as it is feasible to abolish the practice, ways to produce a less harmful cigarette should be explored.

SUMMARY AND CONCLUSIONS

The cigarette smoking habit of 5,127 men and women has been demonstrated to be distinctly related to subsequent morbidity and mortality from CHD. The risk of each manifestation of the disease in men and possibly in women was proportional to the intensity of the habit. While the risk was related to the *number* of cigarettes smoked *each day*, it was unrelated to the duration of the habit and ex-smokers had the same low risk as those who never smoked. This strongly suggests a noncumulative, transient, reversible effect, and that there is much to be gained by giving up the habit, or switching to pipe or cigar which was unassociated with any increase in risk.

While the cigarette habit was hazardous for the general population, it was particularly pernicious for those otherwise predisposed to CHD by factors such as hypertension, abnormal blood lipids, diabetes, and electrocardiographic abnormalities. Such persons who smoked had a pronounced excess risk of succumbing to CHD. At any level of these precursors of CHD the cigarette smokers developed more disease than their nonsmoking

cohorts, suggesting an effect of cigarettes independent of these atherogenic factors.

No difference between cigarette smokers and their nonsmoking cohorts could be demonstrated, which could account for the pronounced excess risk found to be associated with the cigarette habit. An independent effect of the cigarette habit could not be demonstrated in women.

The preventive and therapeutic as well as the pathogenetic implications of the data have been explored.

REFERENCES

(1) DOYLE, J. T., DAWBER, T. R., KANNEL, W. B., KINCH, S. H., and KAHN, H. A.: The relationship of cigarette smoking to coronary heart disease. JAMA 190: 886–890, 1964.

(2) DOLL, R., and HILL, A. B.: Lung cancer and other causes of death in relation to smoking: Second report on mortality of British doctors. Brit M J 2: 1071–1081, 1956.

(3) HAMMOND, E. C., and HORN, D.: Smoking and death rates—report on forty-four months of follow-up of 187,783 men: I. Total mortality. JAMA 166: 1159–1172, 1958: II. Death rates by cause. *Ibid* 166: 1294–1308, 1958.

(4) DORN, H. F.: Tobacco consumption and mortality from cancer and other diseases. Public Health Rep 74: 581–593, 1959.

(5) SIGLER, L. H.: Tobacco as a contributing cause of degenerative coronary disease. New York J Med 55: 3107–3113, 1955.

(6) DAWBER, T. R., MEADORS, G. F., and MOORE, F. E.: Epidemiological approaches to heart disease: The Framingham Study. Amer J Public Health 41: 279–286, 1951.

(7) KANNEL, W. B., DAWBER, T. R., GLENNON, W. E., and THORNE, M. C.: Preliminary report: The determinants and clinical significance of serum cholesterol. Mass J Med Tec 4: 11–29, 1962.

(8) FRIEDMAN, G. D., KANNEL, W. B., DAWBER, T. R., and MCNAMARA, P. M.: An evaluation of follow-up methods in the Framingham Heart Study. Amer J Public Health 57: 1015–1024, 1967.

(9) KANNEL, W. B.: Cigarette smoking and coronary heart disease. Ann Int Med 60: 1103–1106, 1964.

(10) SHAPIRO, S., WEINBLATT, E., FRANK, C. W., and SAGER, R. V.: The H.I.P. study of incidence and prognosis of coronary heart disease; preliminary findings on incidence of myocardial infarction and angina. J Chronic Dis 18: 527–558, 1965.

(11) CEDERLOF, R., FRIBERG, L., JONSSON, E., and KAIJ, L.: Respiratory symptoms and "angina pectoris" in twins with reference to smoking habits. Arch Environ Health 13: 726–737, 1966.

(12) LUNDMAN, T.: Smoking in relation to coronary heart disease and lung function in twins. Acta Med Scand, Suppl 455, 180: 1–75, 1966.

(13) KERSHBAUM, A., KHORSANDIAN, R., CAPLAN, R. F., BELLET, S., and FEINBERG, L. J.: Role of catecholamines in free-fatty acid response to cigarette smoking. Circulation 28: 52–57, 1963.

(14) BURN, J. H., and RAND, M. J.: Action of nicotine on the heart. Brit M J No 5063: 137–139, 1958.

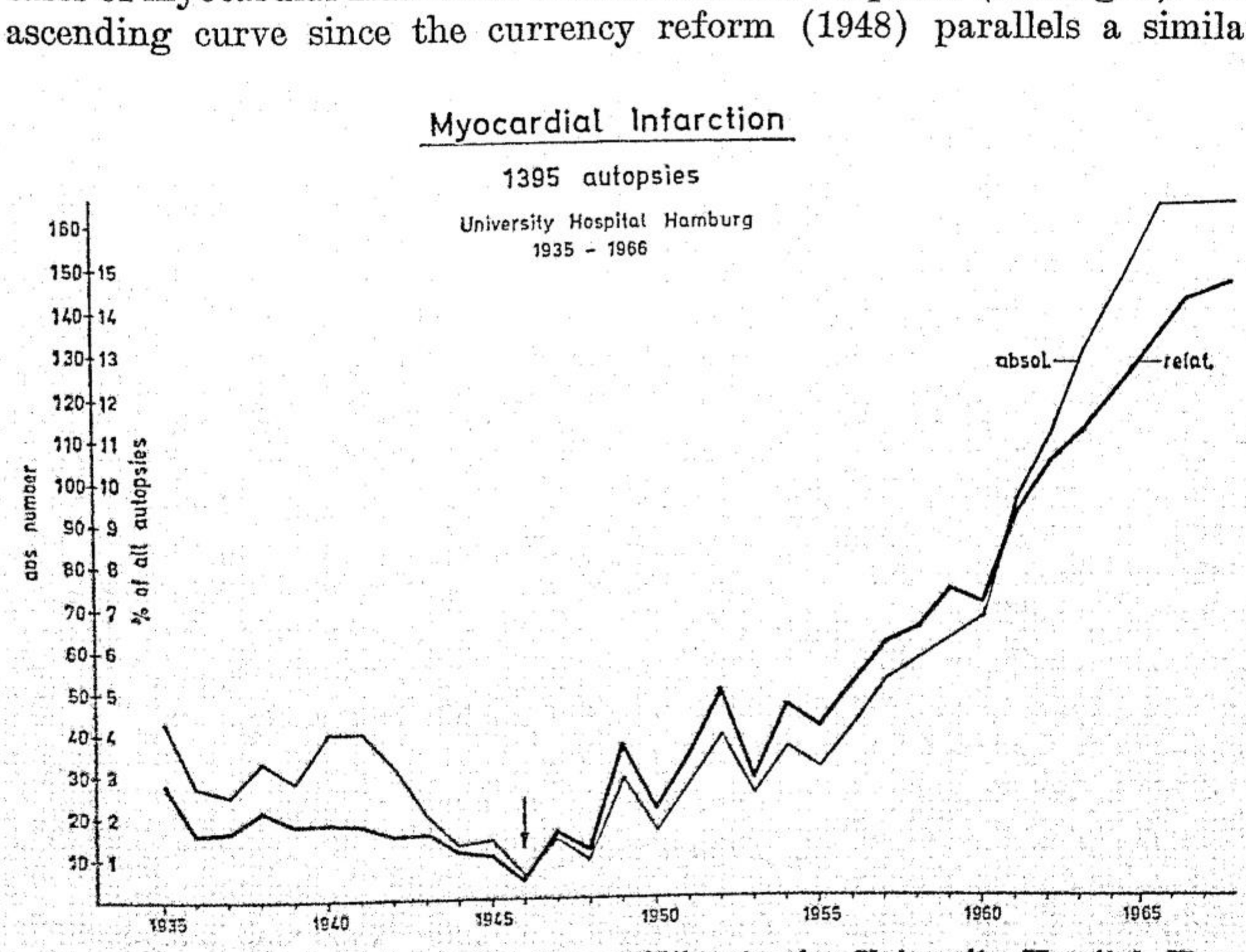
(15) GRASSI, B., and CALTABIANO, S.: Effeto della fatica, del fumo e della perfrigerazione su la coagulazione del sanque. Rass Fisiopat Clin Ter 28: 755–776, 1956.

(16) REGAN, T. J., HELLEMS, H. K., and BING, R. J.: Effect of cigarette smoking on coronary circulation and cardiac work in patients with arteriosclerotic coronary disease. Ann NY Acad Sci 90: 186–189, 1960.

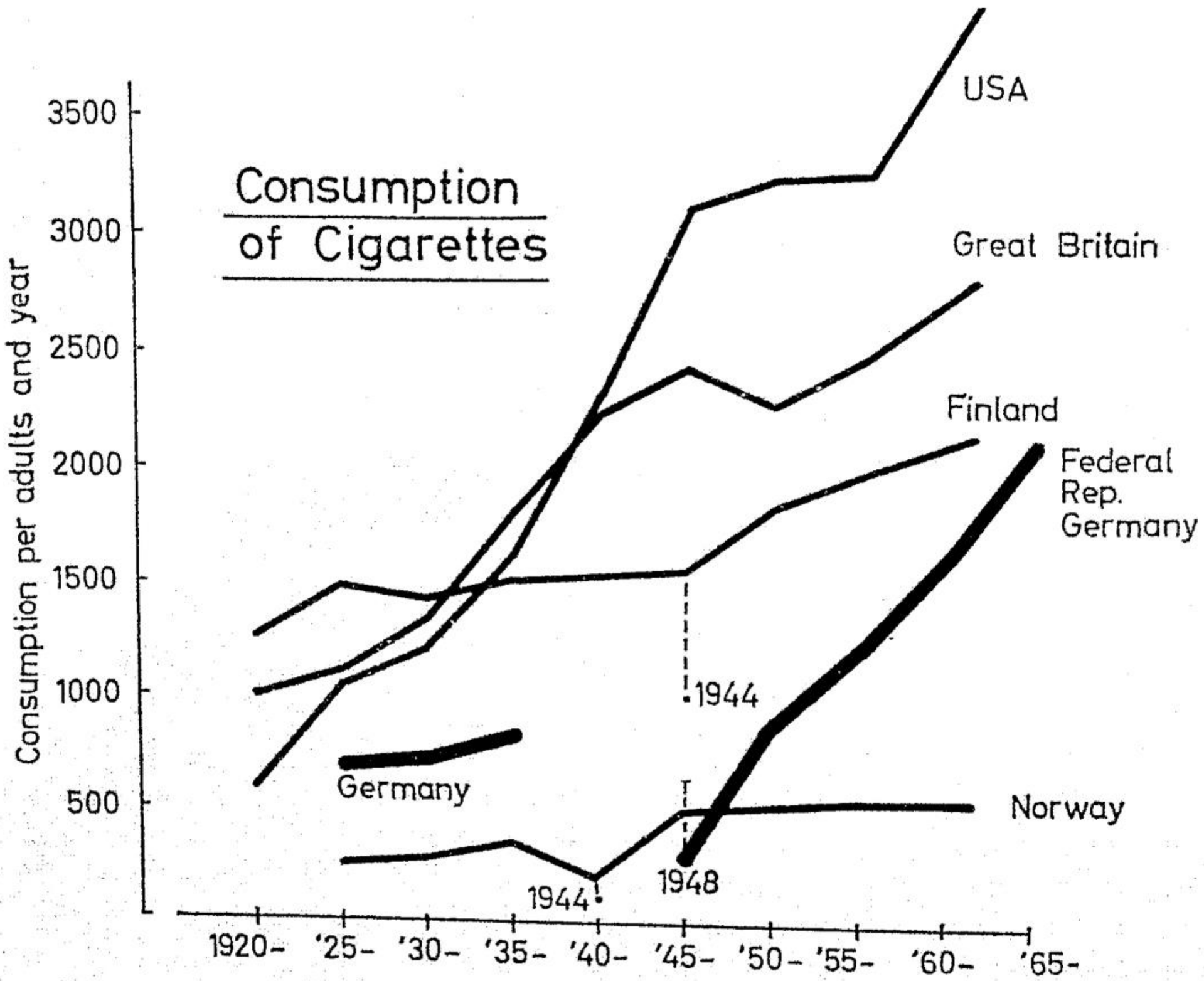
The Etiology of Myocardial Infarction—With Special Reference to Cigarette Smoking Among Young Coronary Patients and Those With Second Heart Attacks

HORST DOERKEN, *M.D.*, *First Medical Clinic, University Hospital, Hamburg, Germany*

STUDIES in Germany and other European countries have shown that the etiology of myocardial infarction is significantly affected by external factors, which is well documented by the changing pattern of frequency of this disease in the last decades (*1–4*). During the 4 years after the Second World War, only relatively few cases of myocardial infarction were seen in our hospitals (text-fig. 1). The ascending curve since the currency reform (1948) parallels a similar



TEXT-FIGURE 1.—Myocardial infarction—1,395 autopsies University Hospital, Hamburg—1935–1966.



TEXT-FIGURE 2.—Consumption of cigarettes.

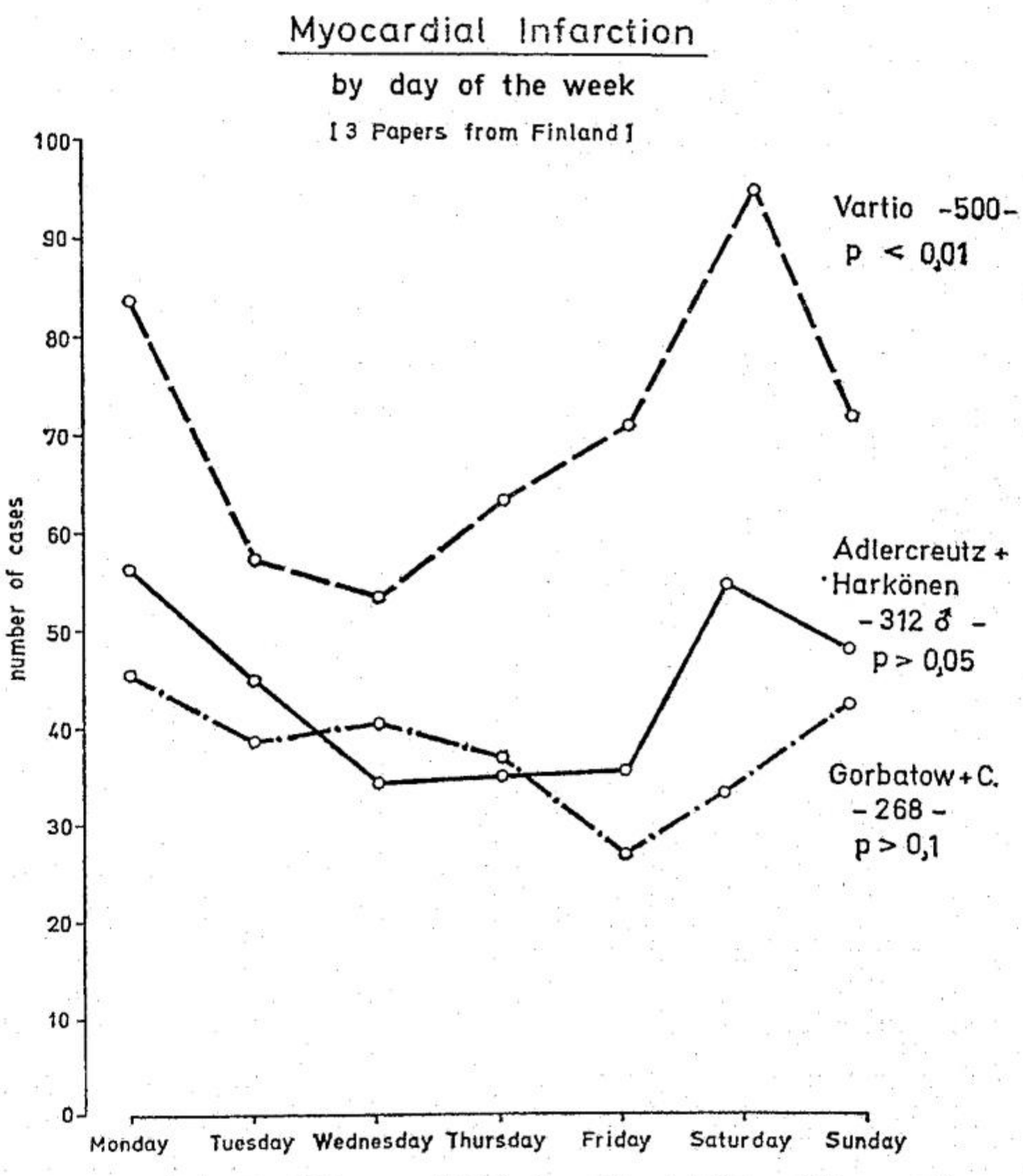
increase in cigarette consumption in Germany (*5*) (text-fig. 2). Along with Finland, Germany is one of the first countries where cigarettes were produced; manufacturing started in Dresden in 1862.

The State of Hamburg with its 1.85 million inhabitants has 19 public hospitals, 17 of which belong to one ministry of health. In these hospitals about 8,000 autopsies are performed each year, which represented 40% of all deaths in 1966. It is therefore possible to collect suitable numbers of cases, even of rare diseases. One of the epidemiological results of these data is shown in text-figure 3 (*4, 6, 7*). Myocardial infarction has a changing frequency throughout the week, with a peak at weekends ("weekend pathology") and a low in the middle of the week. Suicide and some occupational diseases show a similar pattern.

Smoking habits have been investigated in patients with myocardial infarction from 3 different hospitals. Three groups of patients were analyzed: those up to 44 years of age (*8, 9*); survivors in contrast with men who had a second infarction; and nonsmokers with infarction.

METHODS

History of smoking habits and other data were obtained by personal contact with patients and/or relatives by a physician (*10*); some patients



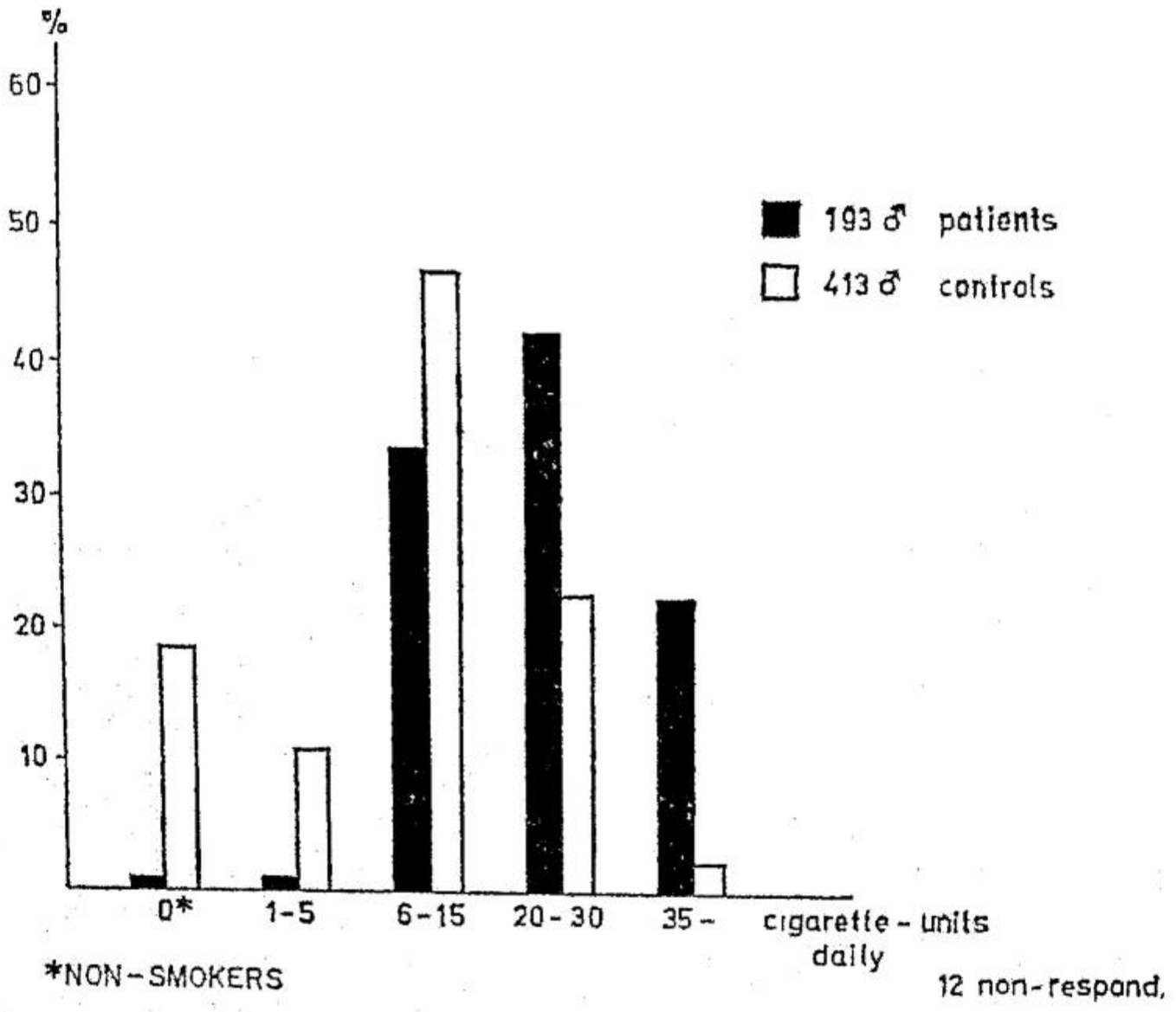
TEXT-FIGURE 3.—Myocardial infarction by day of the week.

were visited at home if necessary. A random series of 1,152 healthy men and 133 women served as controls.

RESULTS

The first data concern 205 men who suffered first myocardial infarctions and/or coronary death between the ages of 19 and 44. The high proportion of autopsies (131) resulted from a survey of all death certificates in Hamburg in 1956 and 1964 and from documents in the office of the coroner, who is obliged to clarify all sudden, unexpected deaths in Hamburg (*2*). There were only 2 nonsmokers in the group (1%). A heavy consumption of cigarettes is apparent in text-figure 4. The proportions of myocardial infarction tend to increase with heavier smoking, indicating a dose-response relationship. Among the smokers in the group, the average daily consumption was 25.9 cigarettes and, with 3 exceptions, all inhaled. Among the controls, 18.4% were nonsmokers and the remainder averaged 13.4 cigarettes per day.

A concurrent study of 33 women age 27–44 with infarction (text-fig. 5) showed only 2 nonsmokers (6.1%) while the rest of the group smoked an



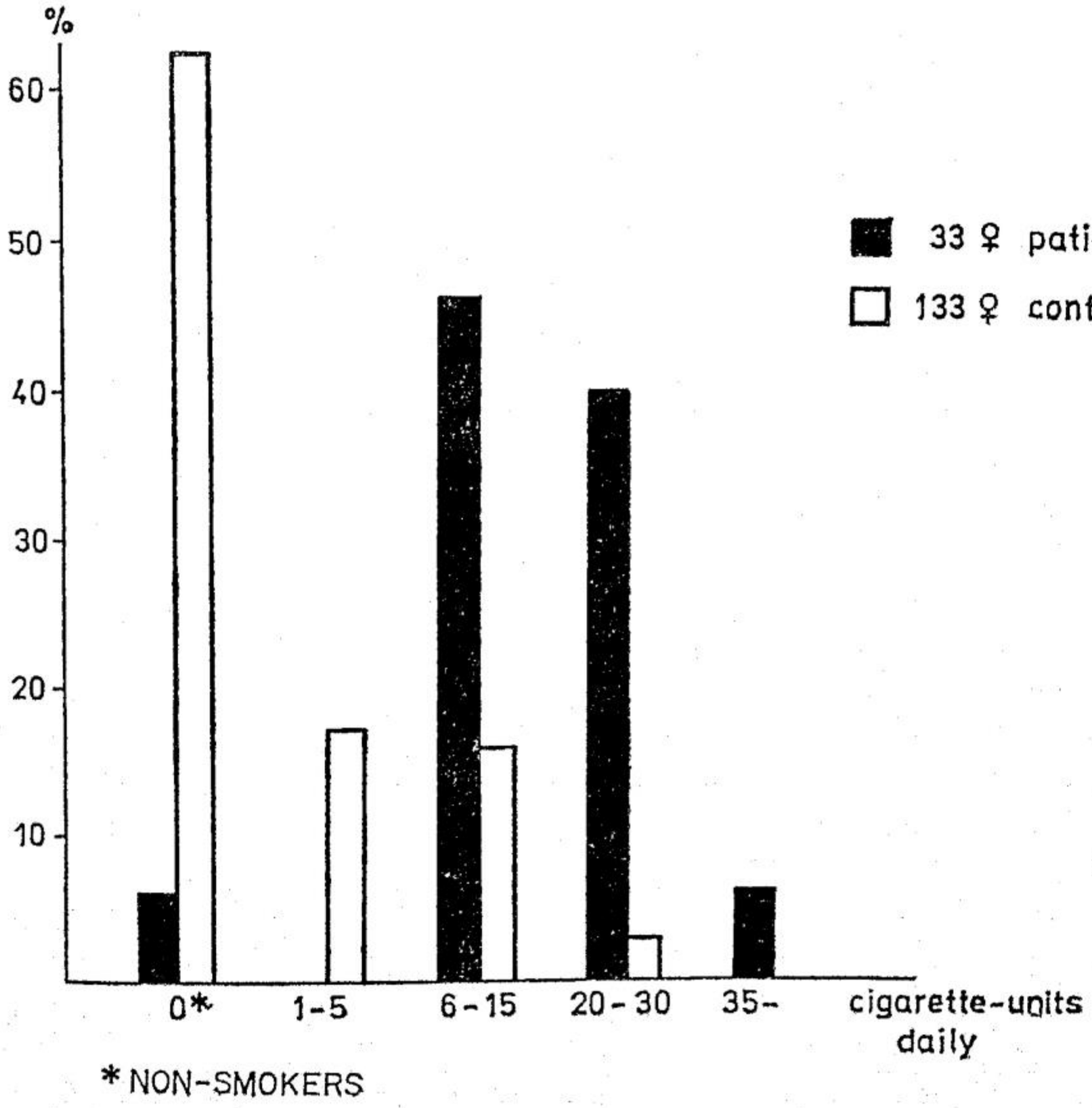
TEXT-FIGURE 4.—Smoking habits of younger men (19–44 years of age) with myocardial infarction and controls.

average of 18.4 cigarettes daily. The figures for the controls were 63.2% and 7.9 cigarettes per day. Because of the rarity of this disease in women, we had to go into the archives as far back as 1935.

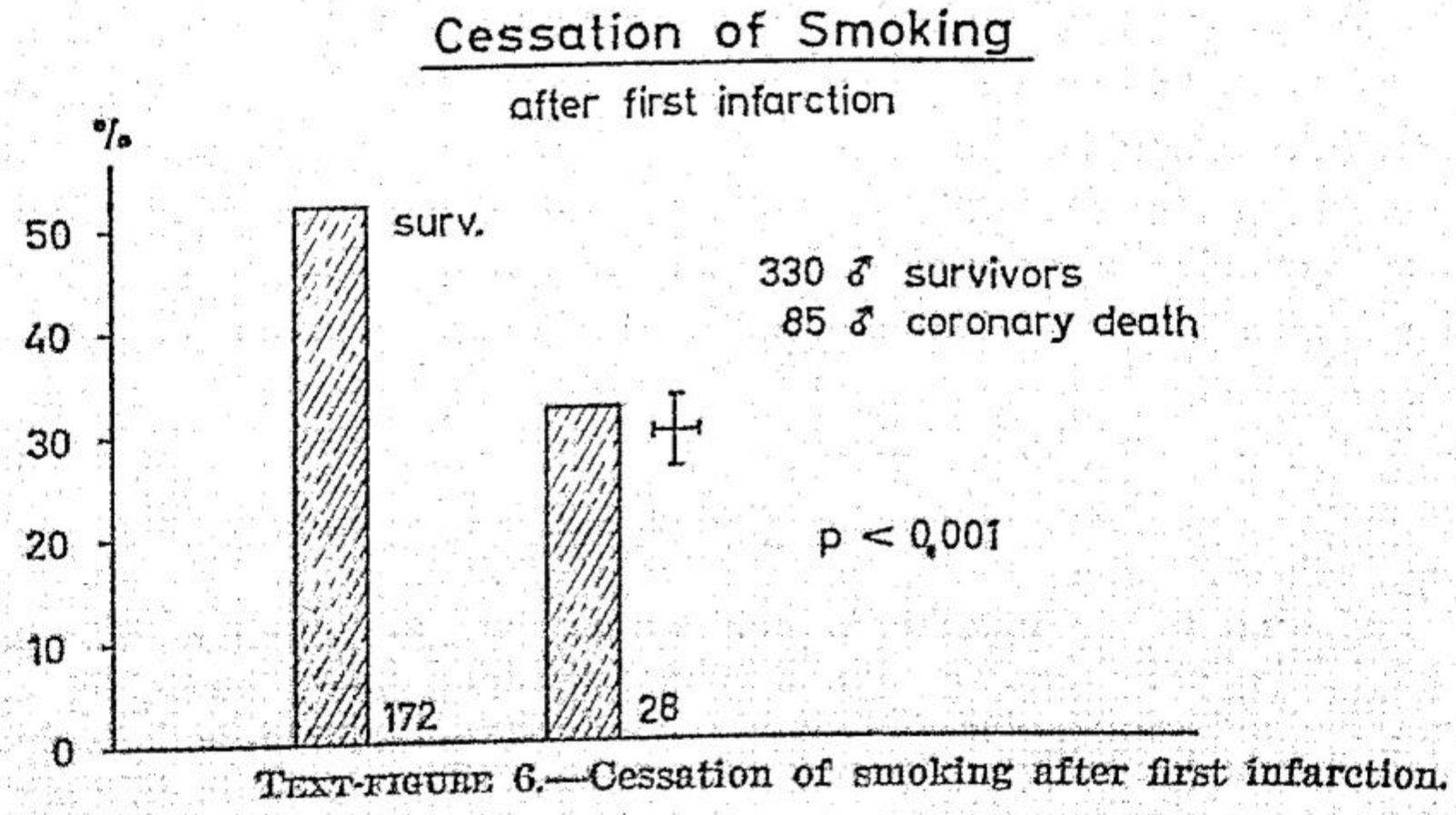
In a series of 330 men of all ages who survived a first myocardial infarction for at least 3 and up to 6 years, we found 14 nonsmokers (4.3%). One hundred and seventy-two (52.1%) had stopped smoking completely with the advent of the infarction. A great number of patients succeeded in stopping for a short time only—up to 1 or 2 years.

In contrast, among 85 subjects who had died from a second myocardial infarction or sudden coronary death after leaving the hospital, there were only 28 (32.9%) who had given up smoking completely (text-fig. 6). The differences between survivors and nonsurvivors in terms of giving up smoking are highly significant ($P<0.001$).

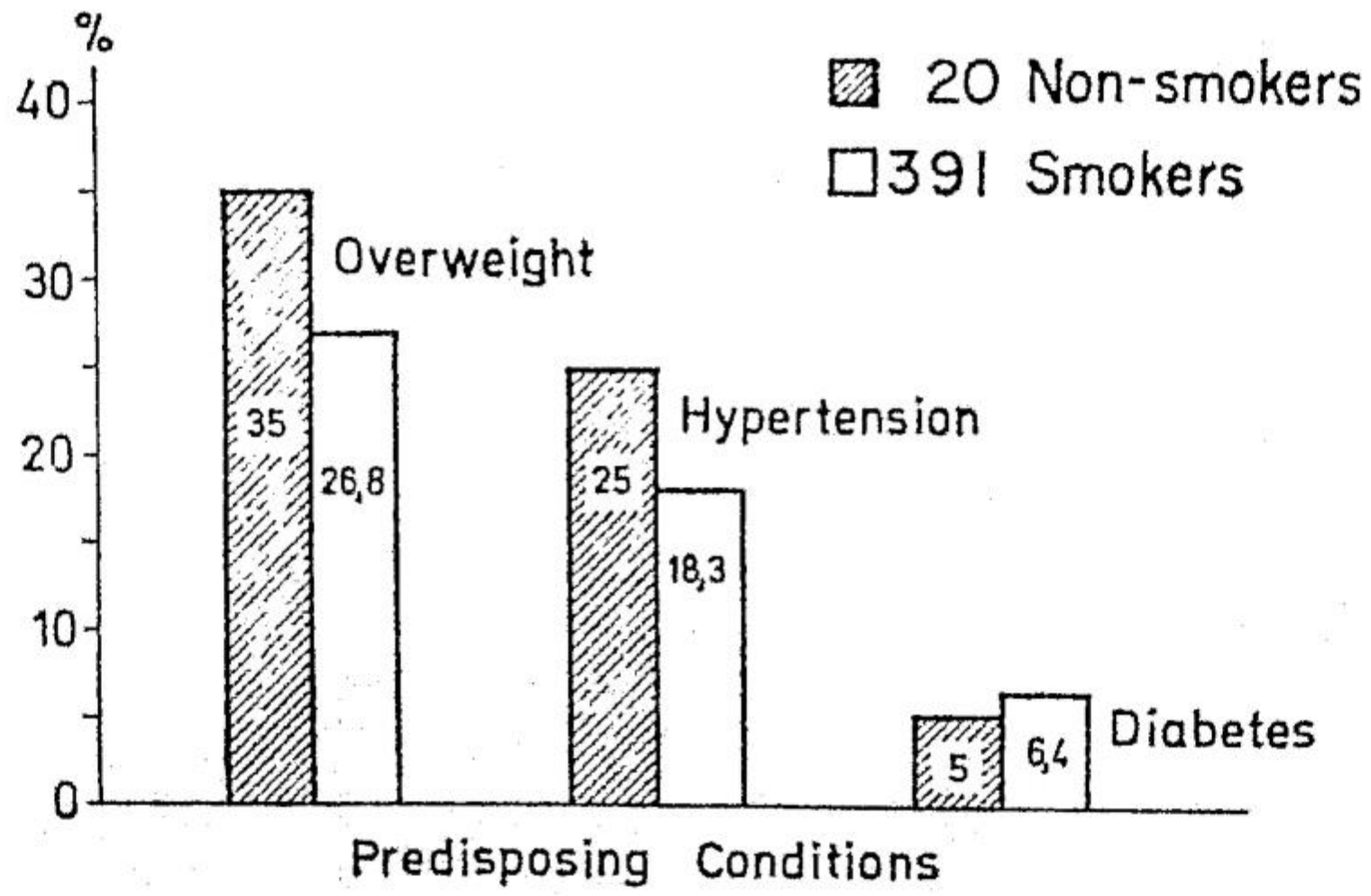
We next focused on the relatively few cases of men who had never smoked but nevertheless suffered a myocardial infarction. The group of 411 with myocardial infarction included 20 nonsmokers. Hypertension and overweight—other factors predisposing to myocardial infarction—were found more frequently among nonsmokers than among smokers (text-fig. 7). However, the differences are not statistically significant and more cases are necessary to confirm this finding.



TEXT-FIGURE 5.—Smoking habits of younger women (19–44 years of age) with myocardial infarction and controls.



TEXT-FIGURE 6.—Cessation of smoking after first infarction.



TEXT-FIGURE 7.—Myocardial infarction in men.

DISCUSSION

In addition to biochemical studies, the German literature includes epidemiological data on research in arteriosclerosis (*11*, *12*). The results of the well-known prospective studies carried out in the United States (*13–15*) and those of Gsell in smaller populations (*16*, *17*) were similar. Gsell also found a strong correlation between smoking and myocardial infarction in younger men and physicians.

A comparison of the smoking habits of survivors and nonsurvivors is of particular therapeutic interest. The variance between the 2 groups may be expected to increase over longer periods of time since we followed the patients for only 3–6 years. The impact of cessation is probably still greater in peripheral arterial disease, especially in thromboangiitis obliterans (*18*).

A study of nonsmokers with myocardial infarction should contribute to our understanding of other predisposing conditions, particularly among young people. While other factors, such as diets high in fat, especially of the saturated type, certainly contribute to the development of myocardial infarction, there can be no doubt that cigarette smoking plays a decisive role (particularly in the young coronary patient) and that cigarette smoking also affects the risk of a second heart attack. In both instances a definite dose-response relationship is established.

REFERENCES

(*1*) BERG, H. H., DOERKEN, H., and HARDERS, H.: Neue Befunde und Gedanken zum Koronarverschluss. Münch Med Wschr 99: 393–397, 1957.

(2) DOTZAUER, G., and NAEVE, W.: Statistische Erhebungen über den Panoramawandel des akuten Herztodes. Deutsch Zschr gerichtl Med 45: 30–42, 1956.

(3) DOERKEN, H.: Koronarverschluss. Klinische Beiträge zur Ätiologie und Pathogenese. Stuttgart, Thieme, 1961.
(4) GORBATOW, O.: Die Herzinfarktfrequenz in einem Material der Bevölkerung von Helsinki während der Jahre 1945–1952. Acta Med Scand 169 (Suppl 364): 2–94, 1961.
(5) TODD, G. F.: Tobacco comsumption in various countries. Tobacco Research Council, Research Paper No. 6, London, 1963.
(6) VARTIO, T.: Myocardial infarction. Ann Med Intern Fenn 49 (Suppl 32), 1960.
(7) ADLERCREUTZ, H., and HÄRKÖNEN, P.: Personal communication.
(8) DOERKEN, H.: Die Rauchgewohnheiten bei jüngeren Herz-infarkt-Patienten. Münch Med Wschr 109: 187–192, 1967.
(9) ———: Die Rauchgewohnheiten bei jüngeren Frauen mit Herzinfarkt. Münch Med Wschr 109: 2129–2134, 1967.
(10) FRIEDEMANN, M., STEIM, H., EMMRICH, J., and REINDELL, H.: Der Herzinfarkt in ätiologischer und katamnestischer Sicht. Deutsch Arch Klin Med 211: 261–296, 1965.
(11) SCHETTLER, G.: Arteriosklerose (Schettler, G., ed.). Stuttgart, Thieme, 1961.
(12) DOERING, H., and LODDENKÄMPER, R.: Statistische Untersuchungen über den Herzinfarkt. Z Kreislaufforsch 51: 401–442, 1962.
(13) HAMMOND, E. C.: Smoking in relation to mortality and morbidity. Findings in first thirty-four months of follow-up in a prospective study started in 1959. J Nat Cancer Inst 32: 1161–1188, 1964.
(14) KANNEL, W. B., WIDMER, L. K., and DAWBER, T. R.: Gefährdung durch coronare Herzkrankheit. Folgerungen für die Praxis aus 10 Jahren Framingham-Studie. Schweiz Med Wschr 95: 18–24, 1965.
(15) DOYLE, J. T., DAWBER, T. R., KANNEL, W. B., KINCH, S. H., and KAHN, H. A.: The relationship of cigarette smoking to coronary heart disease; the second report of the combined experience of the Albany, N.Y., and Framingham, Massachusetts, studies. JAMA 190: 886–890, 1964.
(16) GSELL, O.: Herzinfarkt und Tabakrauchen. Münch Med Wschr 108: 1210–1220, 1966.
(17) STROBEL, M., and GSELL, O.: Mortalität in Beziehungen zum Tabakrauchen—9 Jahre Beobachtungen bei Ärzten in der Schweiz. Helv Med Acta 32: 547–592, 1965.
(18) SCHATZ, I. J., FINE, G., and EYLER, W. R.: Thromboangiitis obliterans. Brit Heart J 28: 84–91, 1966.

TOWARD A LESS HARMFUL CIGARETTE

Relationship of Number of Cigarettes Smoked to "Tar" Rating

SELWYN WAINGROW, *B.S.*, *and* DANIEL HORN, *Ph.D.*, *National Clearinghouse for Smoking and Health, Public Health Service,*[1] *Arlington, Virginia 22203*

THE following information represents data gathered from a 1964–66 reinterview survey. In October and November 1964, data were obtained with a national sample involving some 4,700 interviews.

The sample plan called for a random selection of one adult to be interviewed in each sample household, and then all current and former cigarette smokers in the house, who were identified by the first respondent, were also to be interviewed. Those identified by the first respondent as having never smoked were (after confirmation) not interviewed.

It was necessary to weight the sample interviews so that the interviews with the first respondent and subsequent respondents could be added and also to assure proper distributions by age and sex.

After weighting, the total number involved became 5,794 with 2,337 classified as current cigarette smokers; that is, they reported they now smoke cigarettes and had smoked at least 100 cigarettes during their lifetime.

The average number of cigarettes smoked per day was ascertained by the simple question: "On the average, about how many cigarettes per day do you now smoke?" The data were grouped into six categories for the purposes of this paper, with 80.2% of the responses representing the midpoints of the categories.

The "tar" rating of the brands of cigarettes smoked most was based on ratings in mg that were published in the November 1966 and August 1963 editions of *The Reader's Digest* (*1*, *2*), as well as the 1960 issue of *Consumer Reports* (*3*). The latter source was used for the "unpopular" brands which were not listed in *The Reader's Digest* ratings.

The "tar" and nicotine rating for the mainstream smoke is not a fixed variable, as brands can and do change appreciably with time. However,

[1] U.S. Department of Health, Education, and Welfare.

it seems that little, if any, change has taken place in the ratings of most of the "popular" brands since 1960.

The Federal Trade Commission's plans to conduct periodic tests of the "tar" and nicotine content of cigarettes by brands should insure standardization of the ratings and thus provide a basis for the presentation of more meaningful data on changes.

The actual scale used in the present study of "tar" content is a 5-point scale obtained by the proportionate reduction of the original scale values ranging from 5 mg–45 mg. The values of 1 through 5 correspond roughly to the original rating values of 10, 20, 30, etc., mg of "tar" for the cigarette of a given brand. It should be noted that "tar" and nicotine content are closely related and, except in a few instances, a cigarette high in one is also high in the other.

The relationship between the "tar" rating score and the number of cigarettes smoked per day by sex is shown in table 1. Women are more likely than men to smoke fewer cigarettes per day and cigarettes with a lower "tar" rating score. There is little relationship, however, between the "tar" rating score and the number of cigarettes smoked.

In the spring of 1966, the attempt was made to reinterview all those who had been classified as current cigarette smokers in 1964. Of the number contacted and submitting to the reinterview, 1,466 could be used to analyze the change from 1964–66 in the number of cigarettes currently smoked per day and the "tar" rating score of the brand they smoked most. Those respondents for which such data were only available in 1966 from a short telephone interview schedule were not added to the above-mentioned group.

Change in the number of cigarettes smoked per day is expressed in table 2 by the shift from 1964–66 in terms of intervals where the range within each interval is 5 cigarettes per day.

More people (43.5%) claimed to have changed the number of cigarettes they smoked per day than were classified as having changed in terms of the "tar" rating score assigned the cigarette they smoked (21.5%). However, within each measure the percentage of smokers either increasing or decreasing was almost identical—26.6% increased and 26.4% decreased in terms of changes from one category of number of cigarettes smoked per day to another, while in the instance of changes in "tar" rating scores, 10.3% increased and 11.5% decreased. In addition, 46.5% were classified as not having changed at all in terms of either variable.

In examining the relationship between a change in the "tar" rating score and any change in the number of cigarettes smoked, no statistically significant relationship was found.

Of particular interest is the observation that of those who were categorized as having shown a reduction in their tar-rating score, exactly the same number (27.3%) were classified as smoking more cigarettes as were classified as smoking fewer cigarettes than previously, with 45.3% showing no change.

TABLE 1.—Number of cigarettes smoked per day by "tar" rating (by sex)*

Number of cigarettes per day	Sex	"Tar" rating of cigarette: Low 1 N	1 %†	2 N	2 %	3 N	3 %	4 N	4 %	High 5 N	5 %	No response N	No response %†	Total N	Total %
0-4	M	1	—	10	8.7	22	5.8	25	7.9	23	5.1	1	—	82	6.5
	F	1	—	27	12.8	45	11.6	29	11.5	20	9.8	—	—	122	11.4
5-14	M	—	—	21	18.3	51	13.5	67	21.2	86	19.2	—	—	225	17.8
	F	3	—	62	29.4	100	25.8	79	31.2	46	22.4	—	—	290	27.0
15-24	M	1	—	54	46.9	177	47.0	129	40.8	182	40.6	—	—	543	42.9
	F	5	—	86	40.8	180	46.4	93	36.7	101	49.3	—	—	465	43.4
25-34	M	1	—	12	10.4	57	15.1	47	14.9	73	16.3	—	—	190	15.0
	F	3	—	23	10.9	30	7.7	31	12.3	22	10.7	—	—	109	10.2
35-44	M	1	—	10	8.7	52	13.8	31	9.8	62	13.8	—	—	156	12.3
	F	2	—	10	4.7	24	6.2	17	6.7	12	5.8	—	—	65	6.1
45+	M	—	—	8	7.0	17	4.5	16	5.1	21	4.7	—	—	62	4.9
	F	—	—	1	0.5	6	1.5	3	1.2	4	2.0	—	—	14	1.3
No response	M	—	—	—	—	1	0.3	1	0.3	1	0.2	4	—	7	0.6
	F	—	—	2	0.9	3	0.8	1	0.4	—	—	1	—	7	0.6
Totals	M	4	—	115	100.0	377	100.0	316	100.0	448	99.9	5	—	1265	100.0
	F	14	—	211	100.0	388	100.0	253	100.0	205	100.0	1	—	1072	100.0

*National Survey on Smoking and Health, Public Health Service, October 1964.
†Percentages not computed for N of less than 25.

TABLE 2.—Changes in number of cigarettes smoked per day by changes in "tar" ratings of cigarettes smoked, 1964–66*

Change in No. of cigarettes smoked	Changes in "tar" rating											
	Increased 2 or more units (about 20 mg or more)		Increased 1 unit (about 10 mg)		Unchanged		Decreased 1 unit (about 10 mg)		Decreased 2 or more units (about 20 mg or more)		Total	
	N	%	N	%	N	%	N	%	N	%	N	%
Increase of 3 or more intervals (about 15 cigarettes or more)	—	—	3	2.5	37	3.2	7	7.0	3	4.9	50	3.4
Increase of 2 intervals (about 10 cigarettes)	4	12.5	9	7.4	131	11.4	10	10.0	5	8.2	159	10.8
Increase of 1 interval (about 5 cigarettes)	3	9.4	16	13.1	144	12.5	13	13.0	6	9.8	182	12.4
Unchanged	14	43.7	62	50.8	535	46.5	44	44.0	29	47.5	684	46.6
Decrease of 1 interval (about 5 cigarettes)	5	15.6	10	8.2	130	11.3	13	13.0	6	9.8	164	11.2
Decrease of 2 intervals (about 10 cigarettes)	3	9.4	15	12.3	112	9.7	6	6.0	7	11.5	143	9.8
Decrease of 3 intervals or more (about 15 cigarettes or more)	3	9.4	5	4.1	60	5.2	7	7.0	5	8.2	80	5.4
Not stated	—	—	2	1.6	2	0.2	—	—	—	—	4	0.3
Total	32	100.0 (2.2)	122	100.0 (8.3)	1151	100.0 (78.5)	100	100.0 (6.8)	61	99.9 (4.2)	1466	99.9 (100.0)

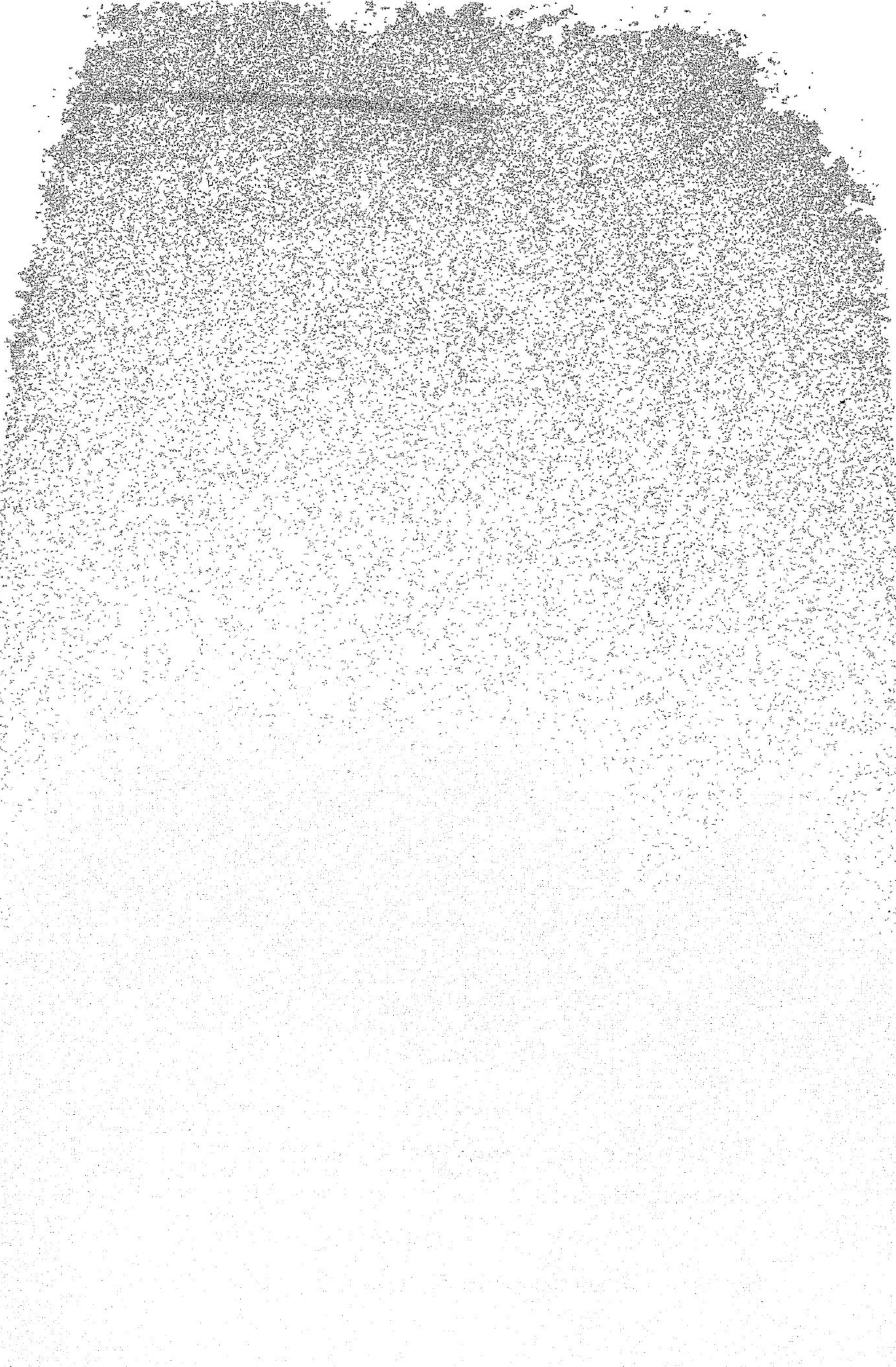
*1964-66 Reinterview Survey on Smoking and Health, Public Health Service.

REFERENCES

(*1*) MILLER, L. M., and MONAHAN, J.: To the cigarette makers: Just the facts, please. Reader's Digest 11: 61–67, November, 1966.

(*2*) ———: The cigarette controversy: A storm is brewing. Reader's Digest 8: 91–99, August, 1963.

(*3*) Cigarettes. Consumer Reports 25: 12–21, January, 1960.

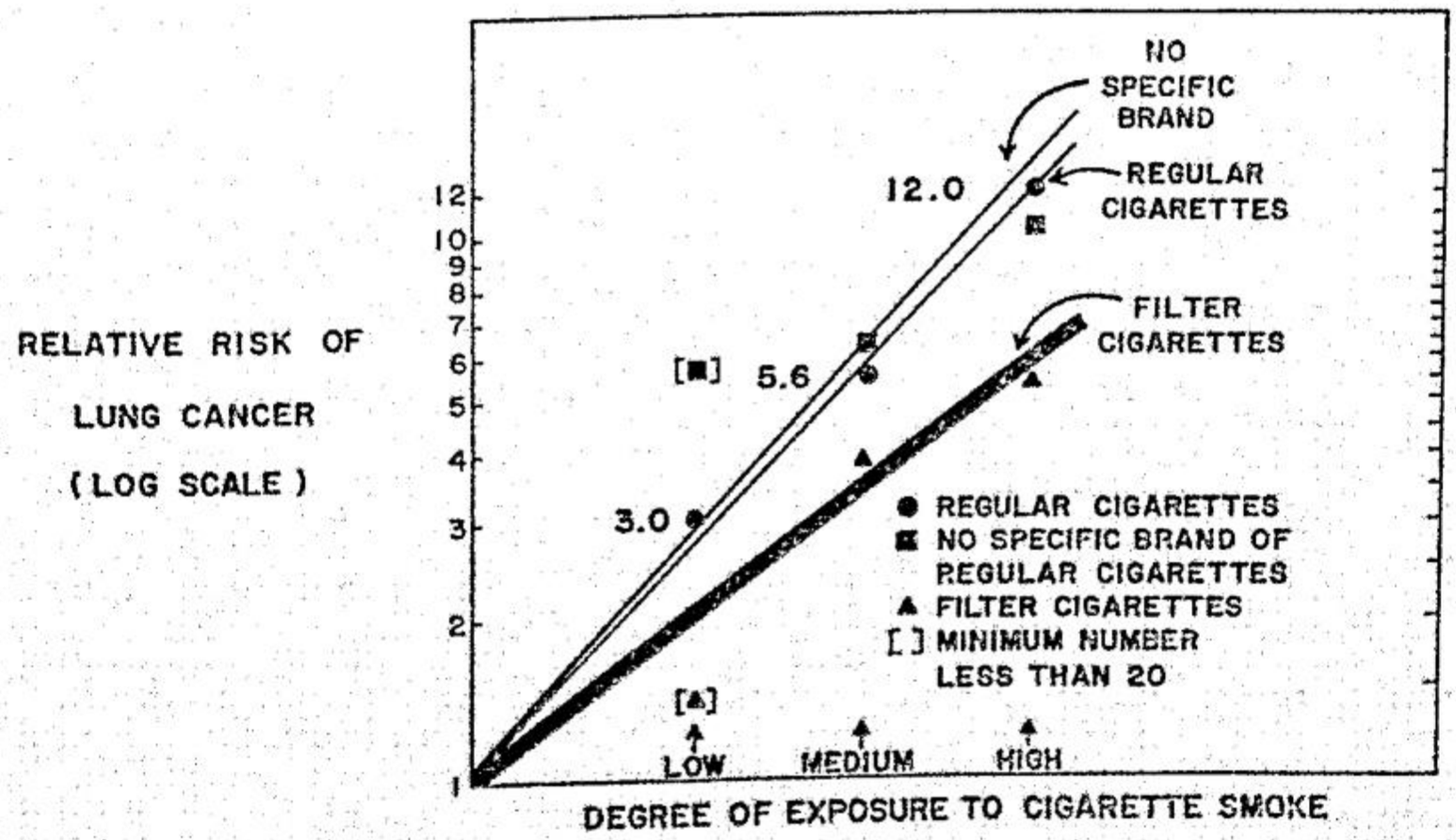


Effect of Filter Cigarettes on the Risk of Lung Cancer

IRWIN D. J. BROSS, *Ph.D.*, *Roswell Park Memorial Institute, Buffalo, New York 14203*

SOME epidemiological data are presented on the question: Does switching to filter cigarettes reduce the risk of lung cancer? On the basis of these data, the answer is: The risk seems to be reduced to about 60% of what it would have been if the smoker had not switched. Unfortunately, however, even with this reduction the risk is still 4 times that of a nonsmoker, and further steps toward a less harmful cigarette are needed.

The data here are on 974 white male patients with lung cancer who were seen at Roswell Park Memorial Institute between 1960 and 1966. These patients have been matched case-for-case on age and entry date with white male patients who had no diagnosis of neoplastic disease and who were seen at the same institution in the same time period. Table 1 gives the basic data. It also defines the degree of exposure categories used in text-figure 1. However the discussion will focus on text-figure 1 since this one



TEXT-FIGURE 1.—Relative risk of lung cancer by type of cigarette and degree of exposure: Risks relative to those of nonsmokers. Lines show filter risks are 60% of the regular risks.

289-019—68——4

TABLE 1.—Risks of lung cancer (relative to nonsmokers) for persons currently smoking regular and filter cigarettes by total years smoked cigarettes and daily amount smoked

Exposure			Regular cigarettes			Filter cigarettes		
			Number		Relative risk	Number		Relative risk
Years smoked	Daily quantity	Degree	Case	Control		Case	Control	
Under 30	1 pack or less	Low	15	20	3. 41	3	11	1. 23
	More than 1 pack	Low	6	12	2. 27	2	5	1. 82
30–39	1 pack or less	Medium	30	21	6. 50	11	16	3. 14
	More than 1 pack	High	28	10	12. 73	9	11	3. 73
40–49	1 pack or less	Medium	46	43	4. 86	15	15	4. 54
	More than 1 pack	High	24	9	12. 14	12	9	6. 04
50 and over	Under ½ pack	Medium	13	8	7. 27	2	2	4. 54
	½–1 pack	High	26	13	9. 09	9	6	6. 82
	More than 1 pack	High	12	2	27. 27	2	1	9. 09
	Total		200	138	6. 59	65	76	3. 90

graph tells a great deal about the human health hazards of our present cigarettes:

1) Persons who smoked specific brands of regular cigarettes for less than 30 years have been put in the category of "regular cigarettes: low degree of exposure to cigarette smoke." Their relative risk is 3—which means that their risk of lung cancer is 3 times greater than that of a nonsmoker.

2) Persons who have smoked specific brands of regular cigarettes for 30–50 years but who smoke a pack a day or less have (with one minor exception) been put in the category of "regular cigarettes: medium degree of exposure to cigarette smoke." Their relative risk is 5.6—almost double the risk in the low-exposure group. Persons who smoke more than a pack a day and have smoked for more than 30 years can be said to have a "high degree of exposure to cigarette smoke." They have roughly double the risk of the persons with "medium exposure." These smokers with "high exposure" have a 12 times greater risk of lung cancer than nonsmokers.

3) For various reasons some people did not give details on specific brands. They are shown in the text-figure under the heading "no specific brand." Except for the "low-exposure" risk, the relative risks in these patients are similar to those in the "regular cigarettes" series. The series might have been combined with the "regular cigarettes" series but has been kept separate here to show the reproducibility of the risk estimates in the most direct fashion. The reproducibility is quite good until the minimum number of patients in a category becomes less than 20.

4) Let us now consider the persons who switched to filter cigarettes. Text-figure 1 shows clearly that the risks for these smokers are lower than those for regular cigarette smokers who, on the basis of amount and duration of smoking, would have had a similar degree of exposure to cigarette smoke. In the high-exposure series, the relative risk is reduced from 12.0 to 5.4. In the medium-exposure series, the risk goes from 5.6 to 3.9. Even in the low-exposure series, there appears to be a reduction from 3.0 to 1.4, although these estimates are less reliable due to the smaller numbers.

5) The straight lines drawn on the text-figure are based on the overall risks for each type of cigarette. Most of the points lie fairly close to these lines. This indicates that the reduction in risk is similar for all degrees of exposure. Therefore, to obtain an overall estimate of the reduction in risk from a switch to filter cigarettes, we use the ratio of the slopes of the lines. This leads to the estimate that the person who switches to a filter cigarette has about 60% of the risk of a person in the same exposure category who continues to smoke regular cigarettes.

6) The reduction in risk for the filter smokers is of considerable scientific and practical importance. Therefore we would want some assurance that these results are not due to sampling variation alone. We can obtain this assurance in two ways. The first procedure is to set confidence intervals on the estimate of 60%. The confidence interval is fairly wide—from 38–91%—but it indicates that there has been some reduction in risk in the

filter series. A second procedure (which avoids the assumption that the same reduction occurs in all degrees of exposure) is called "Cochran's test." This test tells us that there is less than one chance in a hundred that this result could be due to sampling variation ($P=0.004$).

7) Text-figure 1 shows one further point that should not be overlooked. The filters seem to provide some protection, *but this protection is still inadequate*. Even with a switch to filters, a person with a high degree of exposure to cigarette smoke has over 5 times the risk of lung cancer for a nonsmoker. Filtration is a step in the right direction, but further steps are needed. Existing filter technology enables us to take such steps immediately.

8) There is another, more subtle, point of importance to public health action that this text-figure makes. With about 1,000 lung cancer patients it was possible to see clearly differentials in risk for the filter cigarettes. It took us 6 years to amass this series. But there are over 50,000 lung cancer deaths each year. If a nationwide, retrospective surveillance system were set up which was patterned after this study, it could easily get 5,000 cases in a single year. Such a system could monitor any steps toward safer cigarettes that might be taken. Definite answers to questions about reduction in human hazards should be obtainable in 3–5 years.

The above findings show that current filter cigarettes are *not* the answer to the problem of lung cancer, and to this extent they are discouraging. On the whole, however, they encourage the search for a less harmful cigarette. These findings provide the first human evidence that redesign of the product can reduce health hazards. They indicate that, if full advantage were taken of existing filter and other cigarette technology, a greater protection could be provided immediately. In the competitive situation in the cigarette market, however, government standards for filter cigarettes are probably a prerequisite to progress in this direction. Finally, the findings suggest the feasibility of monitoring progress toward a less harmful cigarette. A surveillance system would permit a direct test of the various speculative theories of carcinogenesis and of the different animal model systems and would speed development of less harmful cigarettes in other ways. The present findings should be viewed with some caution since they still require confirmation by other investigators, but they represent an encouraging result in an area where such results are infrequent.

APPENDIX

Due to the unexpected findings of this study, Dr. Bross answered specific questions about the validity and interpretation of his data.

* * *

Question: Isn't it true that some filters are not effective? Why didn't you separate the effective and noneffective filters?

Answer: Recent studies have shown a wide range of effectiveness for filters. We have looked into the individual filter brands. The problem is that this cross-tabulation

fractionates the data and the series become small and unreliable. We have found some suggestive differences. However, we felt that it might be misleading or unfair to try to separate the filter cigarettes on the basis of such fragmentary data. Furthermore, the brands which showed up poorly were not very common in this data and, therefore, including them with the other filters had little effect on the overall results.

Question: But doesn't it invalidate your results to lump together the effective and noneffective filters?

Answer: Not at all. Our focus here is on the differential between persons who smoke regular cigarettes and those who switch to filters. We have adopted a conservative analysis policy. Combining effective and noneffective filters would tend to reduce—not increase—any differential. This "bending over backwards" may lead us to understate the effect of filters, but in this first report an understatement seems preferable to an overstatement.

Question: Isn't it also true that a considerable portion of the patients in this study had smoked filter cigarettes for a relatively short time?

Answer: Yes, the arithmetic of the smoking habits insures this. The average age in this series is just over 60. Most people began smoking before they were 20. Since the interviews were in the 1960's, the patients began smoking around the 1920's. Filter cigarettes didn't become popular until the 1950's. By simple arithmetic, these people had smoked regular cigarettes much longer than they had smoked filter cigarettes.

Question: Why didn't you take the duration of filter cigarette smoking into account?

Answer: We were originally going to require that an individual who was smoking a filter cigarette at time of interview would only be classified as a smoker of this brand if he had smoked it for 3 years. Analysis on this basis gave results similar to those reported, but it markedly reduced the series size. It was also open to the objection that we were picking and choosing favorable groups among the persons who smoked filter cigarettes. Once again we adopted the conservative analytic policy of not subdividing the filter series in this presentation.

Question: Again, on the grounds that you prefer an understatement of the effect of filters to an overstatement—is that right?

Answer: Yes.

Question: Well I still find it hard to believe that filters could have even this much effect. You've just acknowledged that filter cigarettes were smoked for a relatively short time. What's more even the better filters are far from fully effective in reducing tar. Therefore I don't see how switching to filters could give much of a reduction in the *lifetime* exposure to cigarette tar. Do you agree?

Answer: Yes. The reduction in lifetime exposure is rather minor—less than 10% in most cases. The reductions in risks we are finding here are not proportional to the reduction in lifetime exposure.

Question: Since the reduction in lifetime exposure is so minor, why did you expect to find a reduced risk in filter cigarettes?

Answer: We didn't expect to find this reduction.

Question: Do you mean that you undertook this study although you didn't expect to find these results?

Answer: That's right. The data was pre-processed, we had a new automated data-utilization system, and it was relatively easy to take a look at the facts. Our analytic policy is to report the facts as we find them, irrespective of whether these facts fit in with preconceived notions or personal preferences.

Question: Weren't you suspicious? Weren't you afraid that your results might be due to an artifact of your retrospective analysis?

Answer: Yes, we were aware of this danger. We rather expected that, when we took into account the amount and duration of exposure to cigarette tars, the effects might disappear. When we ran off tabulations such as those in table 1, we found the dif-

ferential in risks for filters showing up consistently in the cross-categories. The effect didn't disappear.

Question: But couldn't it be somehow related to age?

Answer: We looked into this possibility. Although the lung cancer patients were originally age-matched, it was possible that partitioning by degree of exposure as in text-figure 1 had unbalanced the age distributions. We looked at the data and found this had not happened.

Question: What about the effect of switching, in and of itself?

Answer: We have looked at other switches—to regular menthol cigarettes, for example. The menthol series did not show a reduced risk of lung cancer.

Question: But you can't be absolutely sure that there isn't some artifact here, can you?

Answer: Of course not. In any actual scientific study—and particularly one that gets into a new area—there is always a risk of artifacts. Artifacts are tricky and there is a limit as to how much protection can be provided by internal checks. The best guard against artifacts is confirmation by independent investigators. Perhaps this cross-check will be available in the future.

Question: But doesn't there have to be an artifact here? Your data seem to show a reduction in the risk of lung cancer when filters are used which equals, if not exceeds, that of giving up smoking altogether. Isn't this illogical unless some artifact is operating?

Answer: The situation is puzzling and there does seem to be a strong artifact operating here. But it is the well-known selection effect in the ex-smokers: People who quit smoking tend to do so for health reasons. So it is not surprising to find that in the heavy-smoking lung cancer series there is quite a high proportion of ex-smokers. If there had been a similar selection effect in the filter switchers, we would not have found a reduction in risk. Apparently health problems are not an important factor in switching to filters. This in turn suggests that the key comparison—patients who continue to smoke regular cigarettes versus those who switch to filters—is valid.

Question: To sum up, where do you think we stand with respect to reducing risks of lung cancer by switching to less hazardous cigarettes?

Answer: We now have a substantial body of human data which suggest that it is possible to make progress toward control of lung cancer by the development and marketing of less hazardous cigarettes. This is one bright spot in an otherwise very gloomy picture. We should neither accept nor reject these findings merely because they are unexpectedly encouraging. Indeed, whether these findings should be wholeheartedly accepted or not is *not* really the crucial question.

Question: What is the crucial question then?

Answer: This study was originally undertaken as a pilot study to examine the feasibility of a retrospective surveillance system to guide the development of a safer cigarette. As a pilot study, it has been unusually successful—very few pilot studies lead to substantive results of the kind obtained here. The crucial question, then, is: What is the next step that can be justified on the basis of this evidence?

Question: And what would you say is the next step?

Answer: At present it does not seem feasible to proceed directly to the setting up of a nationwide surveillance system. On the other hand, these results suggest that further steps should be taken to exploit these new possibilities. With comparatively modest support from public health agencies, it would be possible to have a few large cancer hospitals form a voluntary surveillance system. This group could participate in a study to see if the findings reported here can be confirmed. At the same time the group could work out methodology, *e.g.*, standard interview procedures, for future studies. The evidence presented here is surely strong enough to justify taking this second step.

Chapter I

Part 2—LABORATORY EVIDENCE

Laboratory evidence also shows a dose response in terms of carcinogenic activity of cigarette-smoke condensate to mouse epidermis and of smoke aerosols and volatile components of cigarette smoke in respect to ciliatoxicity. A method was suggested whereby the effect of whole smoke and its various components could be tested by direct inhalation. Nicotine was considered to affect cardiovascular disease, possibly by metabolizing free fatty acids and by affecting blood coagulation. Significant differences were shown to exist in terms of "tar" and nicotine yields of American cigarettes.

Importance of Dose Response in Terms of Total Cigarette Smoke, "Tar," and Nicotine: Cardiovascular System

JOSEPH T. DOYLE, *M.D.*, *Albany Medical College, Albany, New York 12208*

PEARL observed that cigarette smokers die more rapidly of all causes than nonsmokers (*1*). Within the past decade the Framingham and Albany studies showed collaboratively that the consumption of 20 or more cigarettes daily is associated with two to three times the standard rate of myocardial infarction (*2*, *3*). Although our data include fewer cases than desirable, they do strongly suggest a dose response, which is in keeping with the graded response to the intensity of other factors considered to contribute to the likelihood of coronary heart disease. Men who smoke 60 cigarettes a day appear to be at substantially greater risk of sudden death or myocardial infarction than men who smoke 20 or perhaps only 10 cigarettes a day. Auerbach has shown most convincingly a strong correlation between the amount of cigarette smoking and the extent of atherosclerosis found at autopsy (*4*). Our data are not sufficiently refined to judge whether intensity or duration of smoking is more important. We are inclined, however, to believe that the smoking effect is acute and reversible, since the cessation of cigarette smoking appears to be attended by a prompt reduction in rate of myocardial infarction to that of nonsmokers.

There remains, however, one troublesome point. Although it is generally agreed that coronary atherosclerosis is the common morphologic denominator of myocardial infarction and of angina pectoris, our evidence fails to show any relationship whatsoever between cigarette smoking and angina pectoris. Cigar and pipe smoking appear to be relatively innocuous, since the smoke is too irritating to be inhaled.

The recognized circulatory responses to the inhalation of tobacco smoke appear to be due exclusively to nicotine, absorbed primarily from the respiratory mucosa (*5–7*). The urinary excretion of nicotine is almost three times greater in cigarette smokers than in cigar and pipe smokers, confirming the fact that despite their substantially higher nicotine content

less of these smokes are inhaled (*8*). Although the average nicotine content of cigarettes is well known, the actual amount absorbed must be extremely variable. The rate and vigor of puffing greatly influence the volume of mainstream and sidestream smoke and thereby the amount of nicotine absorbed compared with that burned or condensed on unburnt tobacco.

The depth of inhalation and the length of cigarette smoked are also important variables (*5–7*, *9*). Although the point is not settled, it seems more likely that the habituated smoker has learned how to regulate the rate of puffing and the depth of inhalation so as to afford maximum satisfaction and minimum unpleasant effects rather than that he has acquired pharmacological tolerance. Those personally familiar with cigarette smoking will surely agree that to depart from one's accustomed smoking pattern, particularly to smoke more rapidly and to inhale more deeply, is to invite the unpleasant symptoms of minor nicotine toxicity, such as giddiness, nausea, vomiting, sweating, acral paresthesias, intestinal overactivity, and rapid heart action.

Observations made in our laboratory by Kerrigan on the hemodynamic response to smoking in a large group of subjects indicate greater variability in individual response than is evident in several small published series (*10–12*). Smoking 2 standard filter cigarettes in 15 minutes was followed by small but significant increases in heart rate, cardiac output, and blood pressure, but no significant change in total peripheral vascular resistance both in regular smokers and in nonsmokers. These responses are similar to the effects of light exercise.

Cigarette smoking is associated with a substantial reduction in cutaneous blood flow, a matter of therapeutic importance in ischemic disease of the extremities (*13–15*). Although intravenous nicotine increases muscle blood flow, the inhalation of cigarette smoke has little effect (*16*). A recent elegant study shows that muscle capillary flow estimated by the clearance of an injected radioactive slug is augmented, while muscle flow measured by plethysmography is not much changed by smoking (*17*). It is difficult to state with assurance what effect cigarette smoking has on coronary blood flow in the human. Methods for measuring coronary blood flow are crude and, moreover, give no information on local blood flow. It is generally considered that coronary blood flow is increased in normal subjects by tobacco smoking and nicotine, but fails to increase in the patient with established coronary heart disease (*18*, *19*). The effects of cigarette smoking on platelet stickiness and on blood clotting are variable (*20*). Free fatty acids are briskly increased (*21*).

All of these responses are fully explained in terms of a powerful mobilization of catecholamines, almost exclusively epinephrine, by nicotine. Nicotine discharges the adrenal medulla as well as sympathetic ganglia in the myocardium and chromaffin tissue in the walls of blood vessels (*22*, *23*). The blood and urine of heavy cigarette smokers contain significantly more epinephrine than is found in nonsmokers (*24*). The catecholamine response to cigarette smoking, as judged by the mobilization

of free fatty acids, is not observed in completely sympathectomized subjects or after the administration of ganglioplegic agents, and is obtunded by pretreatment with *Rauwolfia* compounds (*21*, *25*).

The rapid degradation of nicotine in the body presumably accounts for the transience of its pharmacologic effects which do not long outlast the smoking period. The circulatory response is elicited just as briskly in the habituated smoker as in the novice (*10*). There are no recognized chronic or cumulative specific physiological or histological effects of smoking in human subjects or of nicotine administration in experimental animals. The apparent rapid reduction in risk of myocardial infarction when smoking is discontinued strongly supports the impression that the ill effects are acute.

It seems unlikely that the lethal effects of cigarette smoking, which are incontestable, can be ascribed to its acute and transient effects on the cardiovascular system. It seems more probable that immoderate cigarette smoking accentuates the deleterious effects of other risk factors, particularly in its lipid-mobilizing effects (*21*, *26*).

REFERENCES

(*1*) Pearl, R.: Tobacco smoking and longevity. Science 87: 216–217, 1938.
(*2*) Doyle, J. T., Dawber, T. R., Kannel, W. B., Heslin, A. S., and Kahn, H. A.: Cigarette smoking and coronary heart disease. Combined experience of the Albany and Framingham studies. New Eng J Med 26: 796–801, 1962.
(*3*) Doyle, J. T., Dawber, T. R., Kannel, W. B., Kinch, S. H., and Kahn, H. A.: The relationship of cigarette smoking to coronary heart disease; the second report of the combined experience of the Albany, N.Y., and Framingham, Mass., studies. JAMA 190: 886–890, 1964.
(*4*) Auerbach, O., Hammond, E. C., and Garfinkel, L.: Smoking in relation to atherosclerosis of the coronary arteries. New Eng J Med 273: 775–779, 1965.
(*5*) Pierce, I. H.: The absorption of nicotine from cigarette smoke, J Lab Clin Med 26: 1322–1325. 1941.
(*6*) Roth, G. M.: The effects of smoking and nicotine on normal persons. *In* Tobacco and the Cardiovascular System. Springfield, Charles C Thomas, 1951.
(*7*) Kensler, C. J.: The pharmacology of tobacco smoke: Effects of chronic exposure. *In* Tobacco and Health (James, G., and Rosenthal, T., eds.). Springfield, Charles C Thomas. 1962.
(*8*) Kershbaum, A., Bellet, S., *et al.*: Comparative effect of cigarette, cigar, and pipe smoking and the influence of inhaling on the nicotine content of the urine. Circulation 33–34 (suppl 3): 142, 1966.
(*9*) Lindsey, A. J.: Some observations on the chemistry of tobacco smoke. *In* Tobacco and Health (James, G., and Rosenthal, T., eds.). Springfield, Charles C Thomas, 1962.
(*10*) Kerrigan, R., Jain, A. C., and Doyle, J. T.: The circulatory response to cigarette smoking at rest and after exercise. Amer J Med Sci 255: 113–119, 1968.
(*11*) Thomas, C. B., Bateman, J. L., and Lindberg, E. F.: Observations on the individual effects of smoking on the blood pressure, heart rate, stroke volume, and cardiac output of healthy young adults; with statistical systems of Harold J. Bornhold. Ann Int Med 44: 874–892, 1956.

(12) Irving, D. W., and Yamamoto, T.: Cigarette smoking and cardiac output. Brit Heart J 25: 126–132, 1963.
(13) Freund, J., and Ward, C.: The acute effect of cigarette smoking on the digital circulation in health and disease. Ann NY Acad Sci 90: 85–101, 1960.
(14) Wood, J. E.: Effect of smoking on the peripheral circulation in relation to environmental temperature. Ann NY Acad Sci 90: 114–118, 1960.
(15) Juergens, J. L., Barker, N. W., and Hines, E. A., Jr.: Arteriosclerosis obliterans: Review of 520 cases with special reference to pathogenic and prognostic factors. Circulation 21: 188–195, 1960.
(16) Rottenstein, H., Peirce, G., Russ, E., Felder, D., and Montgomery, H.: Influence of nicotine on the blood flow of resting skeletal muscle and of the digits in normal subjects. Ann NY Acad Sci 90: 102–113, 1960.
(17) Coffman, J. D., and Javett, S. L.: Blood flow in the human calf during tobacco smoking. Circulation 28: 932–937, 1963.
(18) Regan, T. J., Hellems, H. K., and Bing, R. J.: Effect of cigarette smoking on coronary circulation and cardiac work in patients with arteriosclerotic heart disease. Ann NY Acad Sci 90: 186–189, 1960.
(19) Pentecost, B., and Shillingford, J.: The acute effects of smoking on myocardial performance in patients with coronary arterial disease. Brit Heart J 26: 422–429, 1964.
(20) Murphy, E. A., and Mustard, J. F.: Tobacco and thrombosis. Amer J Public Health 56: 1061–1073, 1966.
(21) Kershbaum, A., Khorsandian, R., Caplan, R. F., Bellet, S., and Feinberg, L. J.: The role of catecholamines in the free fatty acid response to cigarette smoking. Circulation 28: 52–57, 1963.
(22) Burn, J. H., and Rand, M. J.: Action of nicotine on the heart. Brit Med J 1: 137–139, 1958.
(23) Burn, J. H.: The action of nicotine on the peripheral circulation. Ann NY Acad Sci 90: 81–84, 1960.
(24) Westfall, T. C., and Watts, D. T.: Catecholamine excretion in smokers and nonsmokers. J Appl Physiol 19: 40–42, 1964.
(25) ———: Effect of reserpine on cardiovascular response to smoking. J Appl Physiol 17: 471–474, 1962.
(26) Gofman, J. W., Lindgren, F. T., Strisower, B., deLalla, O., Glazier, F., and Tamplin, A.: Cigarette smoking, serum lipoproteins, and coronary heart disease. Geriatrics 10: 349–354, 1955.

Smoking and Thrombosis

EDMOND A. MURPHY, *M.D.*, *Sc.D.*, *and* J. F. MUSTARD, *M.D.*, *Ph.D.*, *F.R.C.P.* (*C*), *Departments of Medicine and Preventive Medicine, University of Colorado, Denver, Colorado 80220, and the Department of Pathology, McMaster University, Hamilton, Ontario, Canada*

IN ANY attempt to establish a relationship between tobacco and thrombosis, one has to answer the following questions:

1) Does smoking lead to thrombus formation?
2) If so, which constituents in tobacco smoke produce the effect?
3) What are the mechanisms by which the compounds lead to thrombosis?

At the present time we know much more about the last question than either of the other two, though all our knowledge is very incomplete. It will be convenient to consider each question in turn.

DOES SMOKING LEAD TO THROMBUS FORMATION?

For various reasons, no satisfactory answer can be given to this question, despite the fact that it is in a sense the most important of all. We can use "thrombus" in two ways.

1) A thrombus is commonly viewed as a massive aggregation of the solid components of blood which arrests or gravely impairs the blood flow through a vessel. Though such occurrences are common enough in clinical practice, the incidence is not such that it would be easy to demonstrate an effect in any study of reasonable size. Furthermore, the means for diagnosis are poor: Only contrast angiography can establish with certainty that an occlusion has taken place, and even that technique cannot distinguish between thrombosis and other mechanisms of occlusion which have been conjectured (*1*, *2*). Also angiography is far too elaborate for routine use. A stream cannot rise higher than its source, and obviously, epidemiological studies are not going to overcome the inadequacies of diagnosis.

2) "Thrombus" may also be extended to include the microthrombus: the clumping of a small, perhaps microscopic, number of platelets asso-

ciated with a little fibrin which nonetheless constitute a thrombus in the sense of being exuberant—an aggregation more extensive than is required to meet the ordinary needs of hemostasis. There is a considerable body of evidence, which will not be reviewed here, that microthrombi may be important in the early as well as in the late stages of atherogenesis. The effect of microthrombi may be aggravated by certain serum lipids which may at once promote their formation and, by influencing their composition, interfere with the normal scavenger processes. This viewpoint is speculative, but provides a useful heuristic scheme which will accommodate the various factors known to be influenced by smoking. Such a process, if it is in any sense a usual one, is even more elusive than the first, though if one does not set unreasonable standards of proof it need not escape observation altogether.

As we have seen, epidemiological analyses will not help us to solve this problem. In the first place the occurrences are comparatively rare; in the second, the quality of the data is suspect; finally if an association were demonstrated, there would be no way of telling whether smoking caused thrombosis or whether it caused arterial disease and, only as a secondary consequence, thrombosis, or whether smoking and thrombosis reflect some common factor.

There has been considerable effort to develop an experimental approach. The ethical difficulties of such experimentation in man are insurmountable, but any alternative has its difficulties.

First, animals, lacking the superior intelligence of man, do not smoke and have been for the most part unteachable. Having animals inhale air charged with cigarette smoke simulates smoking, but this method is difficult and in most cases leads to a high mortality rate among the animals.

Second, assessing thrombosis is a problem. Some investigators, such as Ashwin (*3*) and Wessler *et al.* (*4*), have treated this as an all-or-none phenomenon, but this assay is not the most satisfactory method of dealing with the problem either from a statistical or a biological standpoint. The alternative is some approach that will allow thrombus formation under standard conditions to be more or less accurately measured. Of these, two have been extensively used—the Chandler loop which studies the time required for platelet aggregation and fibrin formation in shed blood kept perpetually in motion (*5*) and the extracorporeal circulation we have used in the pig and the rabbit in which flow is maintained by the circulatory system of the animal (*6*). The classical pathologist may object that both of these methods are in a sense studies of shed blood and therefore do not represent thrombogenesis but clotting. But there is reason to believe that the classical distinction should be related to movement of blood and not to whether it is strictly intravascular (*7*), and it is a fact that both forms of apparatus lead to a mass with the precise features of a coralline thrombus (*5,6,8,9*).

The actual experimental work is astonishingly scanty. The work of Shimamoto (*10*), which we shall discuss later, is really related to mecha-

nisms involved rather than to the demonstration that thrombosis is in fact produced by smoking. The only other evidence with which we are acquainted is that of Engelberg and Futterman (*8*, *9*).

In the first paper (*8*), Engelberg, using the Chandler loop, studied the time until the mass formed in subjects before and after smoking 2 cigarettes in 20 minutes: The 60 participants in this study were habitual smokers. The mean value decreased from 13.45–11.75 minutes. This response, though modest, was highly significant ($P<0.001$). It may be matched against an experimental error (based on 10 duplicate measurements) of 0.25 minutes. The times were read to the nearest half minute, and on this basis, in 37 of the 60 subjects the time was shortened, in 3 it was lengthened, and in the remainder it was read as the same on both occasions. These findings, though consistent, are not conclusive, since the effect of smoking is completely confounded with time: The observed change might have been spontaneous. To meet this criticism, Engelberg studied the same subjects "over several hours" when they were not smoking; the distribution of subjects in the "increased" and "decreased" groups was about equal. The difference from the distribution of the smoking subjects was highly significant ($\chi^2=12.32$ $P<0.005$). It is a pity, in the interests of scientific rigor, that the same interval (20 minutes) was not used in the nonsmoking as in the smoking studies.

In the second paper (*9*), Engelberg and Futterman studied 147 more subjects; all the individuals were fasting and all were evidently habitual smokers. Each subject smoked only 1 cigarette, but thrombus formation was studied not only immediately but also 20 minutes afterward, and in 17 subjects, it was studied 1 hour after smoking was completed. In 132 subjects, the time to the formation of the mass decreased by 1.5 minutes or more (*i.e.*, about 3 times the standard error of the test as determined in 47 duplicate determinations) and in only 2 subjects was it prolonged to a comparable extent. The overall average decrease was 3.5 minutes, though sufficient statistical details were not given to assess the precise significance of this change. The evidence suggests that this mean change was sustained for at least an hour and did not reach its minimum immediately after smoking—the 20-minute readings were a little lower. There were no control data, which the authors may have thought superfluous at that stage.

WHICH CONSTITUENTS IN TOBACCO SMOKE ARE PRODUCING THE EFFECT?

Though this question must be answered before there can be any hope of preventing thrombosis by the use of filters or other means, we have embarrassingly little information. Indeed we can make no formal conclusions at all; the evidence is purely circumstantial. However, we do know that nicotine may be responsible; we will discuss the pharmacological effects of nicotine later. Hass *et al.* carried out a pertinent study (*11*). They found that atherosclerosis could be simulated in rabbits by orally administering

cholesterol in corn oil and injecting moderate doses of vitamin D. Injections of nicotine in oil were given to some rabbits but not to others. For the first day, 5 mg nicotine was injected, and thereafter, the amount was increased by 1 mg/day. For a rabbit weighing 6.5–7.5 pounds, this dose would correspond weight-for-weight to about 50 mg in an adult human being, an amount equal to that absorbed from about 20–50 cigarettes (*12*, *13*). Thus the dosage is realistic. Some of those receiving nicotine developed thrombi in the arteries of the skeletal muscles, but none of those not receiving nicotine did. However, whenever thrombi formed, there was always some degree of inflammation in the arterial wall and it seems likely, as the authors pointed out, that the two are associated. Thus we would be unwise to conclude firmly that the nicotine influenced the thrombus directly and not indirectly through changes in the arterial wall.

WHAT ARE THE MECHANISMS BY WHICH THE COMPOUNDS LEAD TO THROMBOSIS?

In two recent reviews (*14*, *15*) the pharmacological actions of smoking on the several factors which contribute to thrombogenesis have been analyzed. That their contents should be so different indicates the activity in this field. Should proof become available that smoking causes thrombosis, there will be no lack of mechanisms to explain it. The evidence is scattered and, in the interests of brevity, the number of references will be kept small—further documentation can be found in the reviews referred to. For the most part, we shall have to be content to consider the pharmacological action of nicotine. There is good evidence that its action is mediated largely through the release of endogenous epinephrine.

In thrombogenesis, we have to consider the platelet, the arterial wall, and the coagulation system; and the stability of the thrombus is governed on the one hand by its composition and on the other by the activity of the fibrinolytic system.

The Platelet

In assessing the effects of smoking, it is desirable to distinguish between transient effects and long-term effects. A useful measure of platelet activity when undisturbed in the body is its half-life; since the measurement requires several days of observations, however, it is not available as an index of moment-to-moment changes in the physiology of the platelet. Consequently, much stress has been put on *in vitro* studies, the pertinence of which is hard to assess. In the past, before more refined techniques for handling the blood were available, the measurements obtained were artificial and probably rather irrelevant. But this defect was exaggerated, perhaps because of a confusion, still not completely exorcised, between coagulation on the one hand, and the hemostatic process, on the other—with its disorders, thrombosis, and liability to hemorrhage.

The most widely used test of the response of platelets to smoking is platelet adhesiveness. Various methods have been used and, perhaps as a consequence, the results have not been uniform. Many observations have been rendered suspect by the absence of suitable controls. A general conclusion is that there probably is some increase in adhesiveness after smoking, but there is evidence that this change may occur spontaneously. Whatever the effect, it is probably small, and Mustard and Murphy (*16*) were unable to show any long-term effect. By any technique so far devised, however, experimental error is large, and too much must not be made of negative results in small series.

Platelet survival studies in 7 subjects, kept throughout the experiment on a fixed diet and on a metabolic ward, showed that, when the subjects were habitually smoking, platelet survival was about 20% shorter than when they were not smoking at all (*16*). There was a corresponding increase in the turnover of platelets. Until such time as the fate of the platelet has been accurately worked out, the implications of this will not be quite clear, but the evidence is certainly compatible with the idea that microthrombi are more liable to occur.

The biochemistry of the platelet is now a large subject, and there have been several papers dealing with the effects of smoking on platelets. Glynn *et al.* (*17*) could show no change in the serotonin or nucleotide content of platelets after smoking. Murchison and Fyfe (*18*), however, found that the increase in adhesiveness of the platelet in subjects who were smoking was positively correlated with increase in glucose content of the serum, but negatively correlated with changes in serum nonesterified fatty acid.

Schievelbein and Werle (*19*) carried out more refined studies. They showed that serotonin (a constituent of platelets maintained at a much higher concentration than in plasma) is released from platelets when they are exposed to concentrations of nicotine of about 40–500 times those achieved in ordinary smoking. The release of serotonin from platelets has been used as a test of their function, and it thus seems that nicotine affects platelets, but perhaps indirectly, since the result is prevented by monoamine oxidase inhibitors.

The Arterial Wall

It is well known that gross injuries to the arterial wall may lead to thrombosis. There is a growing suggestion in the literature that minor changes not identifiable by light microscopy may have a similar effect. Of special interest are the claims of Shimamoto (*10*) on the transilluminated rabbit ear: If the animals inhale cigarette smoke, platelets tend to adhere to the endothelium and form clumps which, however, disintegrate when the stimulus is removed. This effect can be prevented by amino-oxidase inhibitors, and this suggests that it is mediated through the release of epinephrine. He attributes this to a change in the surface characteristics of the endothelial cells. Elsewhere (*20*), he reports that systemic administration of epinephrine leads to release of a thromboplastin-like substance

from the arterial wall. However, it is by no means clear that the arterial wall is the primary target and that it is not affected secondarily by changes in the platelet. The last-mentioned experiment would benefit from repetition with further studies in a platelet-free system.

Coagulation

We have already stressed that coagulation must by no means be identified with thrombus formation, and recently Solymoss, Selye, and Gabbiani (*21*) emphatically supported this viewpoint. There is a considerable literature (*14*, *15*) suggesting that coagulation is indeed affected by smoking, but much of it is uncontrolled and since platelet-free systems have not been used, the changes observed may be secondary. There is much conflict among the various writers about effects observed, but since the numbers of observations are for the most part small, there need be no major problem of reconciliation. The measurement, most widely used and most generally agreed upon, is the whole blood-clotting time which appears to be appreciably shortened in acute experiments. The largest study with the thromboelastogram, that of Kedra and Korolko (*22*), indicates that the speed with which the clot forms and the maximum tensile strength achieved are enhanced after smoking. Other smaller studies are in substantial agreement, though they have not produced statistically significant results.

Fibrinolysis

Platelet clumping, such as that described in the Shimamoto experiments (*10*), is now known to be reversible, at least in some instances. If fibrin forms in any quantity, however, reversibility depends mainly on the action of the fibrinolysins. Despite a large literature on this subject, little has been written about the effect of smoking. Gibelli *et al.* (*23*) and Kedra and Korolko (*22*) were unable to show any significant change in smokers.

Epinephrine and Thrombosis

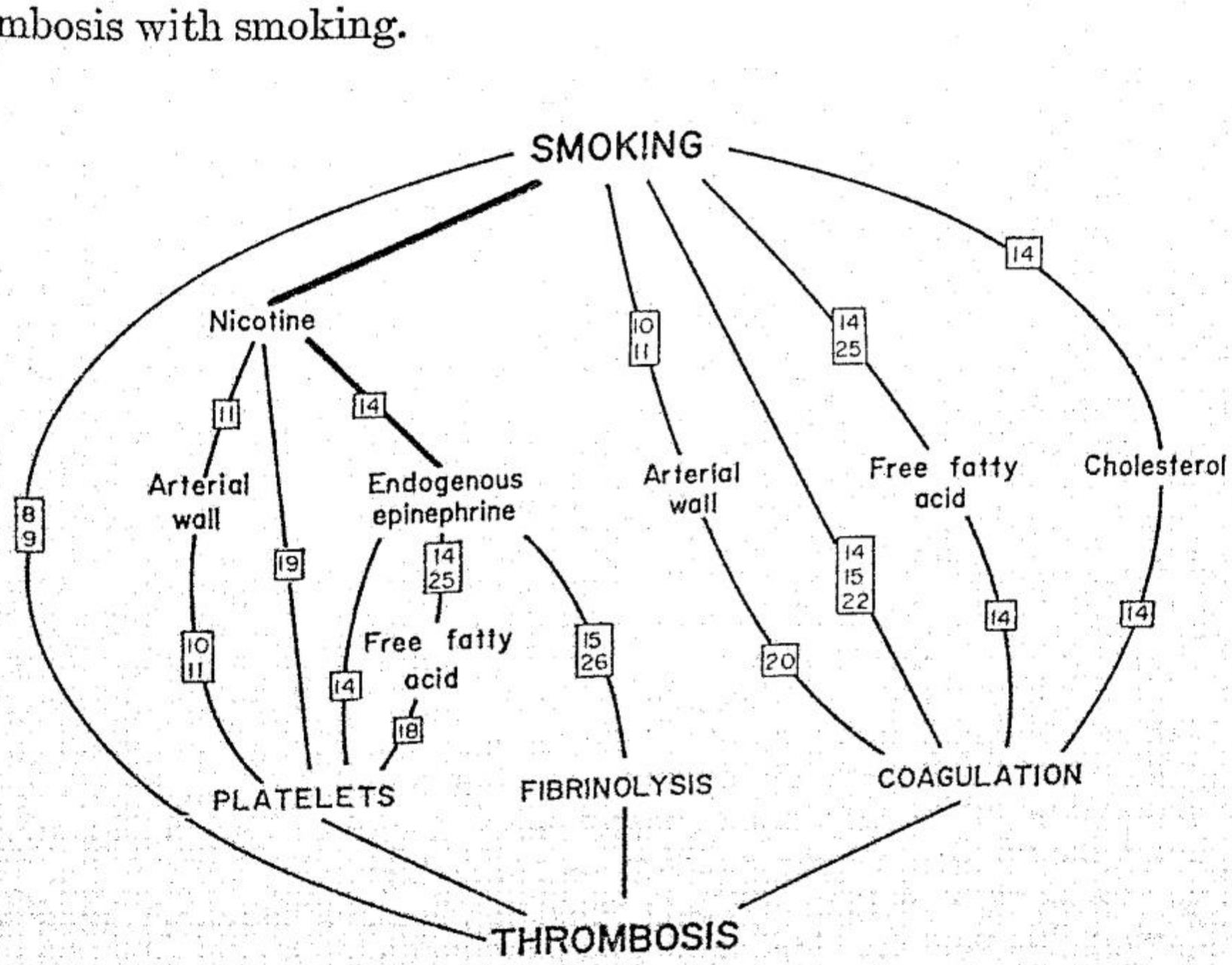
The relationship between epinephrine and thrombosis calls for special comment. It has long been known that in moderate doses epinephrine promotes coagulation, but in large doses it inhibits it. Recently, a similar biphasic effect has been shown in thrombus formation in the extracorporeal circulation, where, of course, the vital properties of the arterial endothelium cannot be invoked in explanation (*24*). Epinephrine, of course, has diverse effects, many of which may have an important bearing on thrombogenesis. Several writers have noted that serum lipids are higher in smokers than nonsmokers, and Kershbaum *et al.* (*25*) reported an increase in fatty acid in subjects after smoking, which may be prevented by

adequate ganglionic blockade. On the other hand, it is well known that epinephrine enhances fibrinolysis; however, to attain this effect it must be administered systemically and not merely added *in vitro* (*26*). Epinephrine aggregates platelets and enhances the effects of other stimuli which promote platelet aggregation (*27*).

SUMMARY

The evidence, so far, suggests the tentative conclusion that smoking is associated with a transient increase in tendency to form thrombi and this result could be largely explained by the release of endogenous epinephrine by absorbed nicotine. There are no grounds for excluding the possibility that other, as yet unidentified, compounds are involved. *In vitro* tests of coagulation also show transient changes, while there seems to be an effect on platelets sufficient to change their external economy. No biochemical changes have been demonstrated in the platelet, but nicotine, in concentrations far in excess of those produced by smoking, has profound pharmacological effects on the platelet. Fibrinolysis is known to be enhanced by systemic administration of epinephrine, but attempts to show a change after smoking have so far failed.

Text-figure 1 summarizes the known or probable pathways connecting thrombosis with smoking.



TEXT-FIGURE 1.—Known or probable pathways connecting thrombosis with smoking. *Numbers* indicate the references (in accordance with the numbering of the present bibliography) where pertinent evidence or pertinent sources can be found. The *thickness of the lines* is more or less related to the uncertainty of the evidence.

REFERENCES

(1) SPAIN, D. M., and BRADESS, V. A.: The relationship of coronary thrombosis to coronary atherosclerosis and ischemic heart disease. (A necropsy study covering a period of 25 years.) Amer J Med Sci 240: 701–710, 1960.

(2) CONSTANTINIDES, P.: Plaque fissures in human coronary thrombosis. J Atheroscler Res 6: 1–17, 1966.

(3) ASHWIN, J. G.: The effect of drugs on experimental thrombogenesis. *In* Anticoagulants and Fibrinolysins (MacMillan, R. L., and Mustard, J. F., eds.). Toronto, The MacMillan Co., 1961, p 117.

(4) WESSLER, S., REIMER, S. M., FREIMAN, D. G., and THOMAS, D. P.: Factors involved in the initiation of thrombosis. *In* Anticoagulants and Fibrinolysins (MacMillan, R. L., and Mustard, J. F., eds.). Toronto, The Macmillan Co., 1961, p 108.

(5) CHANDLER, A. B.: In vitro thrombotic coagulation of the blood. Lab Invest 7: 110–115, 1958.

(6) DOWNIE, H. G., MURPHY, E. A., ROWSELL, H. C., and MUSTARD, J. F.: Extracorporeal circulation: A device for the quantitative study of thrombus formation in flowing blood. Circ Res 12: 441–448, 1962.

(7) MUSTARD, J. F., ROWSELL, H. C., and MURPHY, E. A.: Thrombosis. Amer J Med Sci 248: 469–496, 1964.

(8) ENGLEBERG, H.: Cigarette smoking and the in vitro thrombosis of human blood. JAMA 193: 1033–1035, 1965.

(9) ENGELBERG, H., and FUTTERMAN, M.: Cigarette smoking and thrombotic coagulation of human blood. Arch Environ Health 14: 266–270, 1967.

(10) SHIMAMOTO, T.: Damages to "silicone-like property" of vascular endothelial cells and prevention by monamine oxidase inhibitor, Nialamide. Asian Med J 3: 479–492, 1960.

(11) HASS, G. M., LANDERHOLM, W., and HEMMENS, A.: Production of calcific atheroarteriosclerosis and thromboarteritis with nicotine, vitamin D and dietary cholesterol. Amer J Path 49: 739–771, 1966.

(12) JOHNSTON, L. M.: Tobacco smoking and nicotine. Lancet 2: 742, 1942.

(13) LARSON, P. S.: Absorption of nicotine under various conditions of tobacco use. Ann NY Acad Sci 90: 31–35, 1960.

(14) MURPHY, E. A., and MUSTARD, J. F.: Tobacco and thrombosis. Amer J Public Health 56: 1061–1073, 1966.

(15) MURPHY, E. A.: Platelets, coagulation and thrombosis. *In* Nikotin—Pharmakologie und Toxikologie des Rauchens in Experiment und Klinik (Schievelbein, H., ed.). Stuttgart, Georg Thieme. In press.

(16) MUSTARD, J. F.: and MURPHY, E. A.: Effect of smoking on blood coagulation and platelet survival in man. Brit Med J 1: 846–849, 1963.

(17) GLYNN, M. F., MUSTARD, J. F., BUCHANAN, M. R., and MURPHY, E. A.: Cigarette smoking and platelet aggregation. Canad Med Assoc J 95: 549–553, 1966.

(18) MURCHISON, L. E.: and FYFE, T.: Effects of cigarette smoking on serum-lipids, blood-glucose, and platelet adhesiveness. Lancet 2: 182–184, 1966.

(19) SCHIEVELBEIN, H., and WERLE, E.: Freisetzung von 5-Hydroxytryptamin durch Nikotin. Psychopharmacologia 3: 35–43, 1962.

(20) SHIMAMOTO, T., and ISHIOKA, T.: Release of a thromboplastic substance from arterial walls by epinephrine. Circ Res 12: 138–144, 1963.

(21) SOLYMOSS, B., and SELYE, H., and GABBIANI, G.: Predisposition to thrombosis not reflected by the blood coagulogram. J Clin Path 19: 331–333, 1966.

(22) KEDRA, M., and KOROLKO, A.: Tobacco smoking and blood clotting. Bull Pol Med Sci Hist 8: 145–148, 1965.

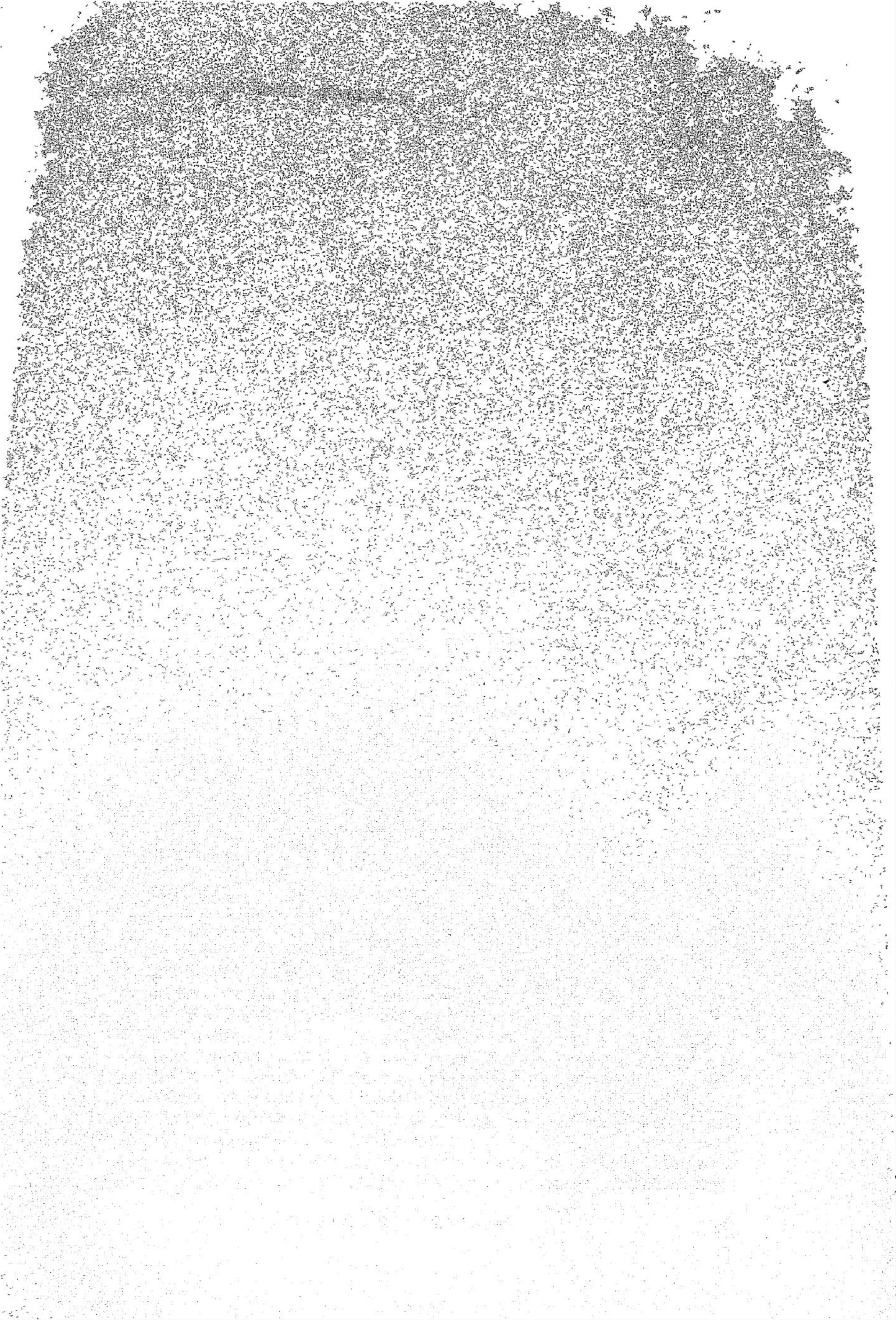
(23) GIBELLI, A., BOLANDRINO, E., DEL FAVERO, A., and PASOTTI, C.: Modificazioni emocoagulative e della fibrinolisi in seguito a carico lipidico orale associato a fumo da sigaretta. G Geront 12: 31–41, 1964.

(24) ROSWELL, H. C., HEGARDT, B., DOWNIE, H. G., MUSTARD, J. F., and MURPHY, E. A.: Adrenaline and experimental thrombosis. Brit J Haemat 12: 66–73, 1966.

(25) KERSHBAUM, A., BELLET, S., CAPLAN, R. F., and FEINBERG, L. J.: Effect of cigarette smoking on free fatty acids in patients with healed myocardial infarction. Amer J Cardiol 10: 204–208, 1962.

(26) MATHUR, K. S., GUPTA, S. C., GUPTA, D. K., and WAHAL, P. K.: Spontaneous fibrinolysis and the changes produced by adrenaline, fatty meal storage and diurnal rhythm on fibrinolytic activity. Ind J Med Res 52: 38–48, 1964.

(27) ARDLIE, N. G., GLEW, G., and SCHWARTZ, C. J.: Influence of catecholamines on nucleotide induced platelet aggregation. Nature (London) 212: 415–417, 1966.



Dose Response: Experimental Carcinogenesis[1]

FRED G. BOCK, *Ph.D.*, *Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14203*

TO a large extent, dose-response effects in experimental carcinogenesis by tobacco "tar" resemble those that have been discovered with known carcinogens. Polynuclear aromatic hydrocarbons serve as a model for neutral fat-soluble contact carcinogens of cigarette smoke; croton oil may serve as a model for tumor-promoting agents. A comparison of cigarette-smoke condensate with these pure compounds will be of value in evaluating the available experimental data.

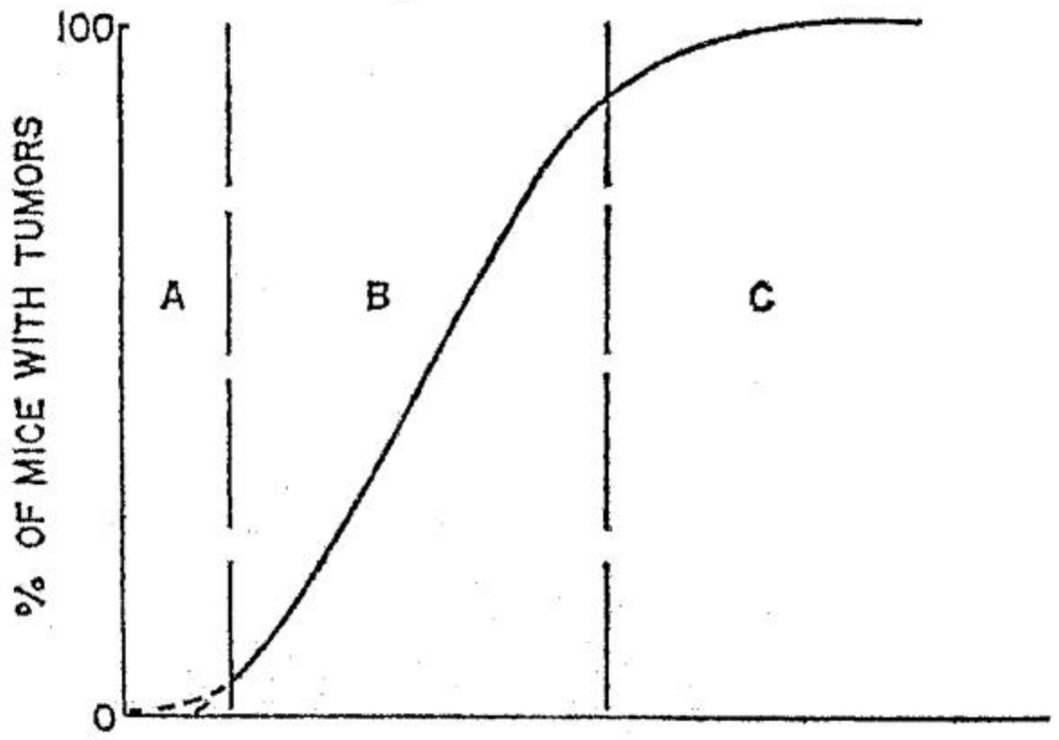
COMPLETE CARCINOGENESIS

Pure polynuclear aromatic hydrocarbons give a dose-response curve that consists of either two or three separate segments. At the higher dose levels (text-fig. 1-C) the dose-response curve is nearly horizontal at the 100% level. Obviously, no dose-response effect can be seen in this part of the curve. The central part of the curve is nearly linear (text-fig. 1-B) and dose-response effects are dominant. Although data are insufficient for generalization, there may be a region at very low dose levels where no response is observed, *i.e.*, there may be a threshold (text-fig. 1-A). No convincing evidence of a true threshold for carcinogenic activity by polynuclear aromatic hydrocarbons has been produced, but in any experiment with limited numbers of animals, an apparent threshold is possible.

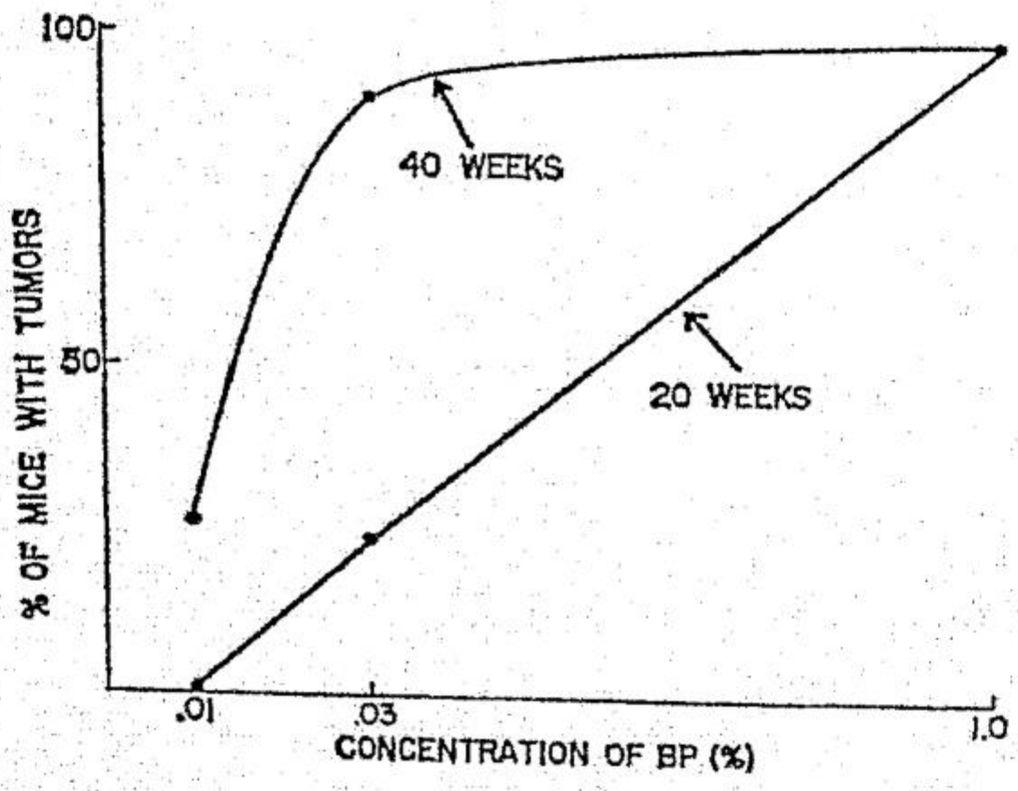
These effects can give somewhat different results from one experiment to the other unless conditions are rigidly standardized. For example, the dose-response curve may be either linear or curvilinear after different periods (text-fig. 2). Similar curves are also obtained with a fixed time schedule and carcinogens of different potencies.

Tobacco "tar" behaves in much the same way. Wynder *et al.* showed that mice exposed to decreasing quantities of tobacco "tar" suffered de-

[1] This work was supported in part by Grant E 358 from the American Cancer Society, Inc.

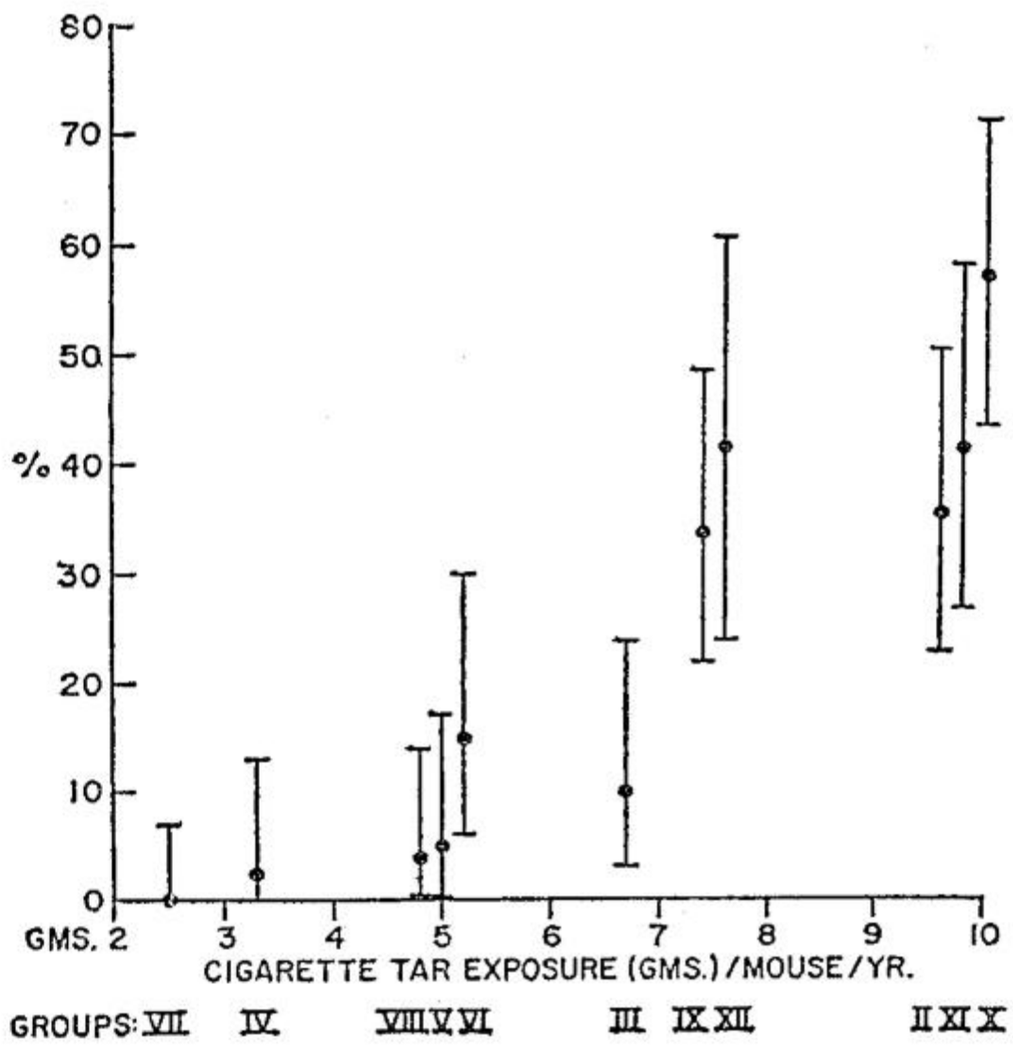


TEXT-FIGURE 1. — General dose-response curve for mouse skin carcinogenesis by polynuclear aromatic hydrocarbons.



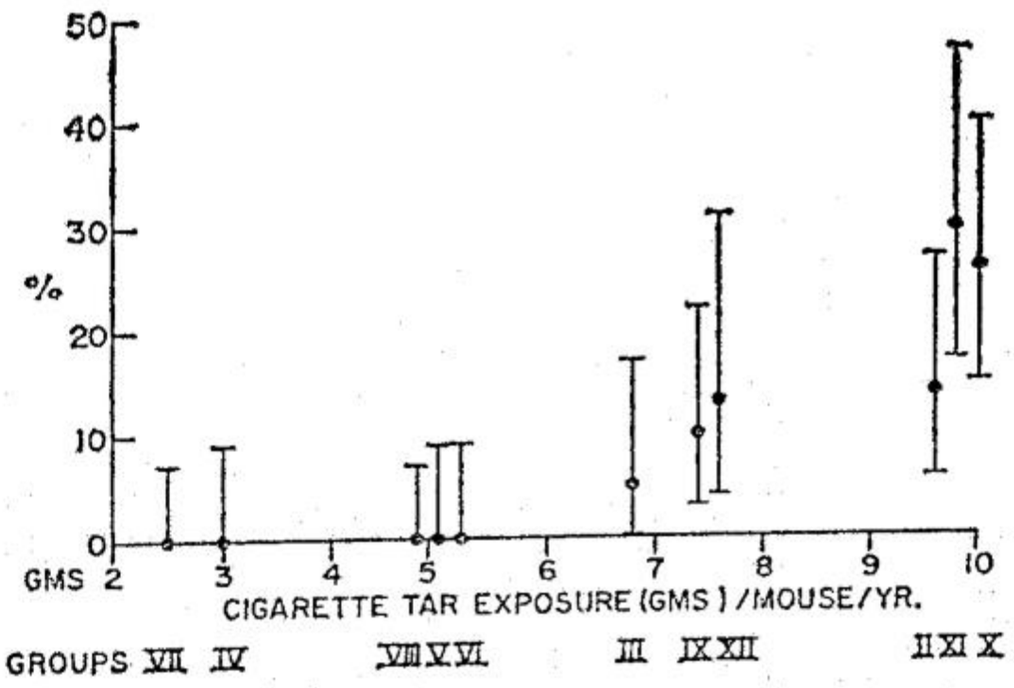
TEXT-FIGURE 2.—Dose-response effects in mice painted 4 times weekly with 0.25 ml of benzo[*a*]pyrene in acetone.

creasing numbers of tumors (text-figs. 3 and 4) (*1*). A linear response with a possible threshold was observed. Recently, Day (*2*) reported a nearly linear response without a threshold in studies with large numbers of animals. Nearly linear dependence of response on dose seems to be the rule whenever moderate levels of dosage are used (*3–7*). This is true, not only for mouse skin tumors and cigarette "tar" but also for skin tumors produced by "tars" from other tobacco products and for subcutaneous tumors induced by cigarette "tar" injection (*8*, *9*). In contrast, a dose-response effect may not be seen when very high levels of "tar" carcinogens are used. This can be seen most easily in an experiment with partially refined cigarette "tar" (text-fig. 5) (*3*). Muñoz *et al.* (*7*) likewise did not see a dose-response effect with "tar" from Colombian cigarettes which appears to be more carcinogenic than "tar" from American cigarettes.



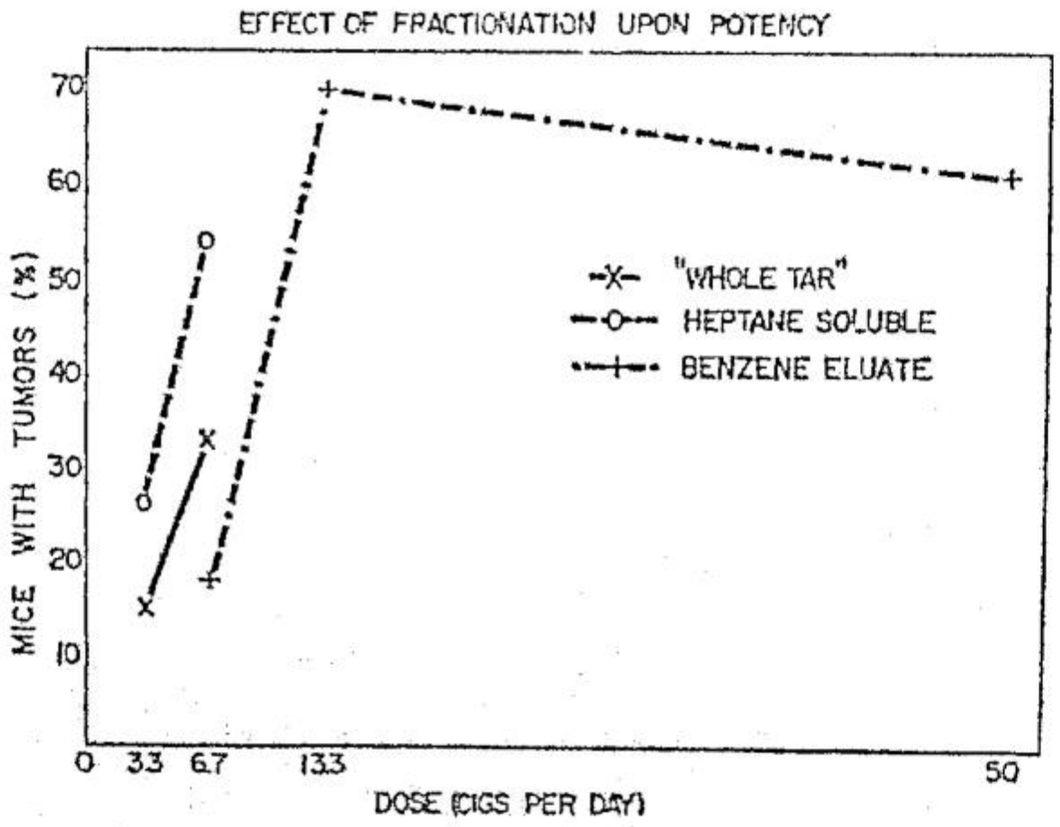
TEXT-FIGURE 3.—Dependence of total tumor incidence upon dose of cigarette-smoke condensate. [from Wynder *et al.* (*1*)].

Text-figures 3 and 4 are reproduced through the courtesy of the editor of "Cancer."



TEXT-FIGURE 4.—Dependence of carcinoma formation in mice upon dose of cigarette-smoke condensate [from Wynder *et al.* (*1*)].

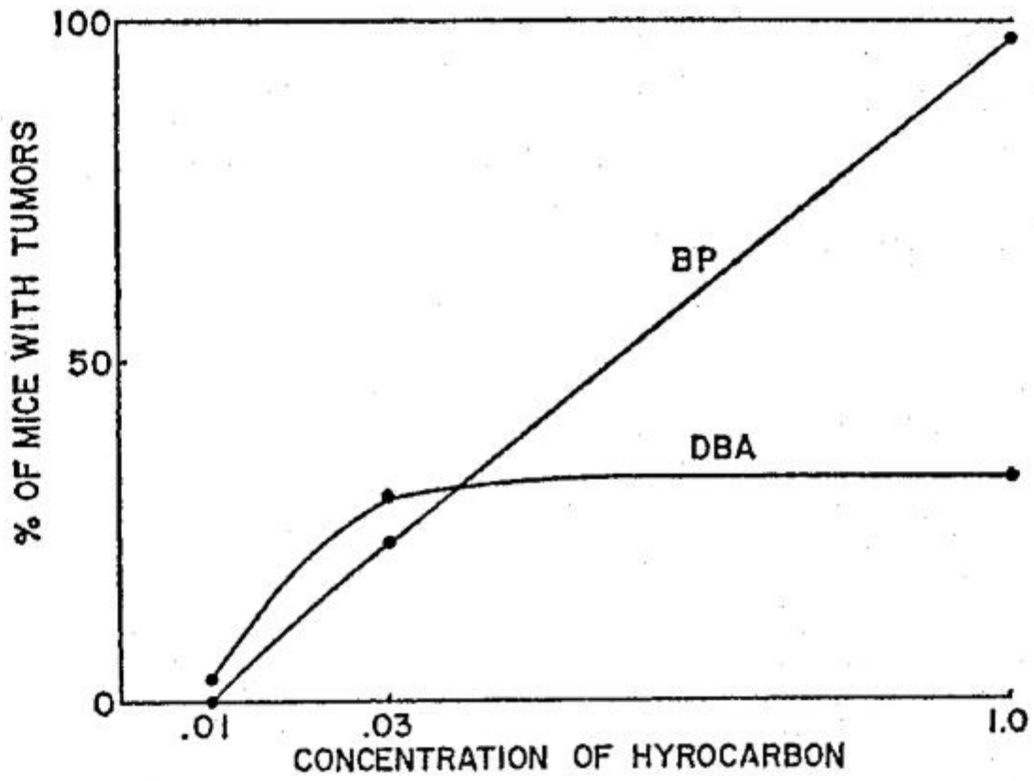
Often, the toxicity of carcinogens also places an upper limit on the numbers of tumors produced. This effect is not seen with moderate levels of polynuclear aromatic hydrocarbons. However, the toxicity of cigarette "tar" is such that when increasing doses are used, the mortality increases to such an extent that the total tumor incidence may be reduced. The highest incidence of tumors in mice painted with crude cigarette-smoke condensate appears to be in the range of 55–70% (*10*). This is true even when partially refined fractions of cigarette-smoke condensate have been tested.



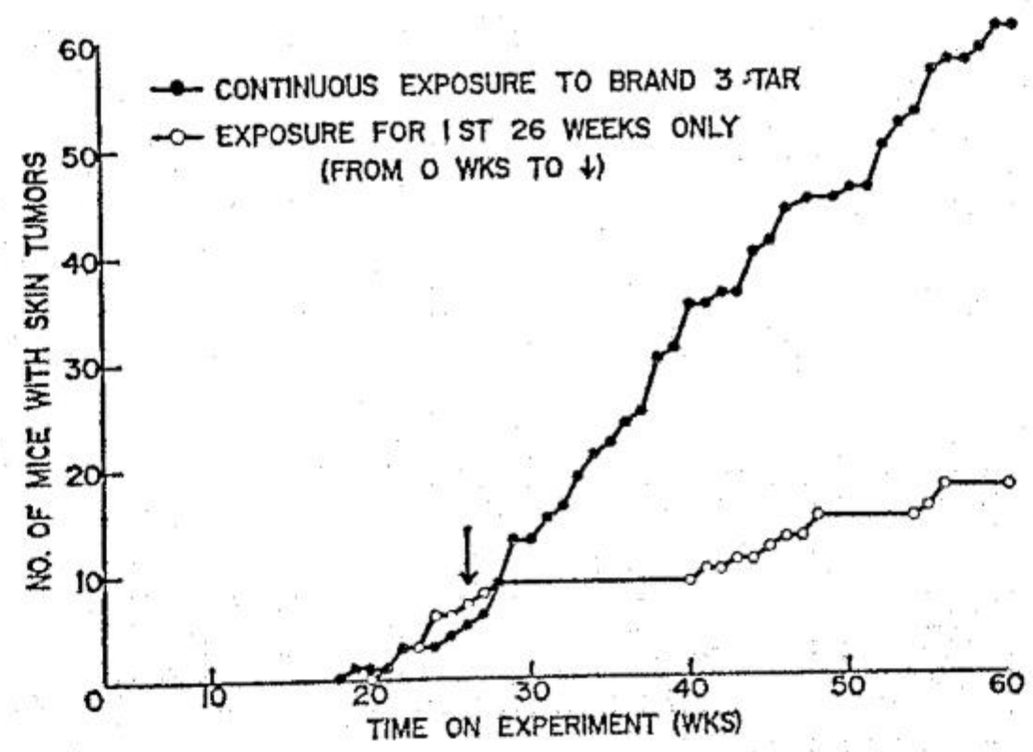
TEXT-FIGURE 5.—Dependence of total tumor incidence in mice painted with crude cigarette-smoke condensate and two fractions of cigarette-smoke condensate at various doses [from James and Rosenthal (*3*)].

Another characteristic of dose-response curves, the dependence on tissue penetration is exemplified by dibenz[*a*,*h*]anthracene (DBA). When concentrated solutions of DBA are applied to the surface of the skin, penetration into the skin is inefficient, thus imposing a limit to the carcinogenic effect. Compared with benzo[*a*]pyrene (BP), DBA is equally carcinogenic at low concentrations but much less carcinogenic at high concentrations (text-fig. 6). This apparent loss of effect at high concentrations is related to the limited solubility of the compound in the surface skin lipids which aid in the movement of carcinogen into the deeper skin structures. Skin penetration may be important in studies with refined fractions of smoke condensate.

Most experiments with cigarette-smoke condensate involve the continuous administration of the material throughout the experimental period. However, dependence of the response upon continued application is pronounced. Wynder *et al.* showed that when "tar" was applied for limited periods, the numbers of tumors appearing were drastically reduced (*1*). This observation was confirmed in our laboratory where we painted mice with identical "tar," one group for 60 weeks and the other group for only the first 26 weeks. The results showed that after 26 weeks, without further treatment of tobacco "tar," development of additional tumors was greatly retarded (text-fig. 7). The tumors that appeared in the early part of the experiment did not regress in the absence of continued treatment, but new tumors did not appear.



TEXT-FIGURE 6.—Dose-response effects with benzo[*a*]pyrene and dibenz[*a*,*h*]anthracene. The mice were painted 4 times weekly with 0.25 ml of hydrocarbon in acetone for 20 weeks.



TEXT-FIGURE 7.—Effects of cessation of treatment on the incidence of skin tumors in mice painted with heptane-soluble fraction of cigarette-smoke condensate [from Bock *et al.* (*5*)].

TUMOR PROMOTION

Dose-response effects with croton oil as a model tumor promoter have been summarized by Boutwell (*11*). He has shown that the dose-response relationships are substantially different from those of complete carcinogenesis by polynuclear hydrocarbons. Tumor promotion involves a distinct threshold for individual treatments, below which no effect is elicited. Furthermore, tumor promotion is reversible. However, within these limitations, the response is dose dependent. Wynder and Hoffmann have reported the effect of two doses of the phenolic fractions of cigarette-smoke

condensate (*12*). A clear dose-response effect was seen. A similar dose-response effect was also seen when crude smoke condensate was applied to mice after the application of 7,12-dimethylbenz[*a*]anthracene.

It can be concluded that a nearly linear dose-response effect with tobacco "tar" is the rule in carcinogenesis experiments. In most cases, the reduction of tumor yield is roughly parallel to the reduction in dosage. If very high levels of "tar" are used, a maximum tumor yield of 70% may be found, with no further dependence of results on dose. The most extensive data do not disclose a threshold to the carcinogenic effect of tobacco "tar"; with small numbers of animals, threshold-like effects are seen. But inasmuch as most laboratories use intermediate doses of tobacco "tar," a linear dose-response effect is usually observed.

SUMMARY

Dose-response effects of cigarette-smoke condensate in experimental carcinogenesis are quite comparable to those of pure carcinogenic compounds. At very high concentrations, increasing doses do not increase the response, which appears to reach a maximum when about 70% of the animals develop tumors. In the most extensive experiment, no evidence of a threshold was seen; but in experiments involving a few animals threshold-like data have been obtained. Most experiments show a nearly linear dose-response effect.

REFERENCES

(*1*) WYNDER, E. L., KOPF, P., and ZIEGLER, H.: A study of tobacco carcinogenesis. II. Dose-response studies. Cancer 10: 1193–1200, 1957.

(*2*) DAY, T. D.: Carcinogenic action of cigarette smoke condensate on mouse skin. Brit J Cancer 21: 56–81, 1967.

(*3*) BOCK, F. G., and MOORE, G. E.: The significance of mouse skin tests of cigarette smoke condensate. *In* Tobacco and Health (James, G., and Rosenthal, J., eds.). Springfield, Charles C Thomas, 1962, pp 72–86.

(*4*) BOCK, F. G., MOORE, G. E., DOWD, J. E., and CLARK, P. C.: Carcinogenic activity of cigarette smoke condensate. JAMA 181: 668–673, 1962.

(*5*) BOCK, F. G., MOORE, G. E., and CLARK, P. C.: Carcinogenic activity of cigarette smoke condensate. III. Biological activity of refined tar from several types of cigarettes. J Nat Cancer Inst 34: 481–493, 1965.

(*6*) WYNDER, E. L., and HOFFMANN, D.: A study of air pollution carcinogenesis. III. Carcinogenic activity of gasoline engine exhaust condensate. Cancer 15: 103–108, 1962.

(*7*) MUÑOZ, N., CORREA, P., and BOCK, F. G.: Comparative carcinogenic effect of two types of tobacco. Cancer 21: 376–389, 1968.

(*8*) CRONINGER, A. B., GRAHAM, E. A., and WYNDER, E. L.: Experimental production of carcinoma with tobacco products. V. Carcinoma induction in mice with cigar, pipe, all-tobacco cigarette tar. Cancer Res 18: 1265–1271, 1958.

(*9*) DRUCKREY, H., and SCHILDBACH, A.: Quantitative Untersuchungen zur Bedeutung des Benzpyrens für die carcinogene Wirkung von Tabakrauch. Z Krebsforsch 65: 465–470, 1963.

(*10*) WYNDER, E. L., and HOFFMANN, D.: Experimental tobacco carcinogenesis. Advances Cancer Res 8: 249–453, 1964.

(*11*) BOUTWELL, R. K.: Some biological aspects of skin carcinogenesis. Progr Exp Tumor Res 4: 207–250, 1964.

(*12*) WYNDER, E. L., and HOFFMANN, D.: A study of tobacco carcinogenesis. VIII. The role of the acidic fractions as promoters. Cancer 14: 1306–1315, 1961.

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.*,[2] 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).

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

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	3,539	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
26	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 15 were killed.
†Nonsmoking dogs 27 and 38 had tracheostomies.

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	—	—
39	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	63	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	—	—	—	—	—	—	—	—

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		< 30% of cells		30–39% of cells		40–49% of cells		50–59% of cells		60–69% of cells		70–99% of cells		100% of cells	
		Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Smoking dogs																			
29	24	123	100.0	123	100.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
30	329	86	100.0	—	—	1	1.2	21	24.4	62	72.1	2	2.3	—	—	—	—	—	—
35	278	127	100.0	—	—	1	0.8	6	4.7	94	74.1	22	17.3	4	3.1	—	—	—	—
32	410	14	100.0	—	—	—	—	—	—	—	—	10	71.4	4	28.6	—	—	—	—
36	415	29	100.0	—	—	—	—	—	—	—	—	—	65.5	10	34.5	—	—	—	—
31	421	131	100.0	—	—	—	—	—	—	2	1.5	32	24.4	97	74.1	—	—	—	—
15	422	129	100.0	—	—	—	—	—	—	15	11.6	75	58.1	37	28.7	—	—	2	1.6
34	422	129	100.0	—	—	—	—	—	—	4	3.1	38	29.5	74	57.3	8	6.2	5	3.9
37	423	131	100.0	—	—	—	—	—	—	1	0.8	35	26.7	91	69.4	3	2.3	1	0.8
26	423	127	100.0	—	—	—	—	—	—	39	30.7	50	39.4	37	29.1	1	0.8	—	—
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	132	100.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
45	—	132	100.0	132	100.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
80	—	129	100.0	129	100.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—

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.

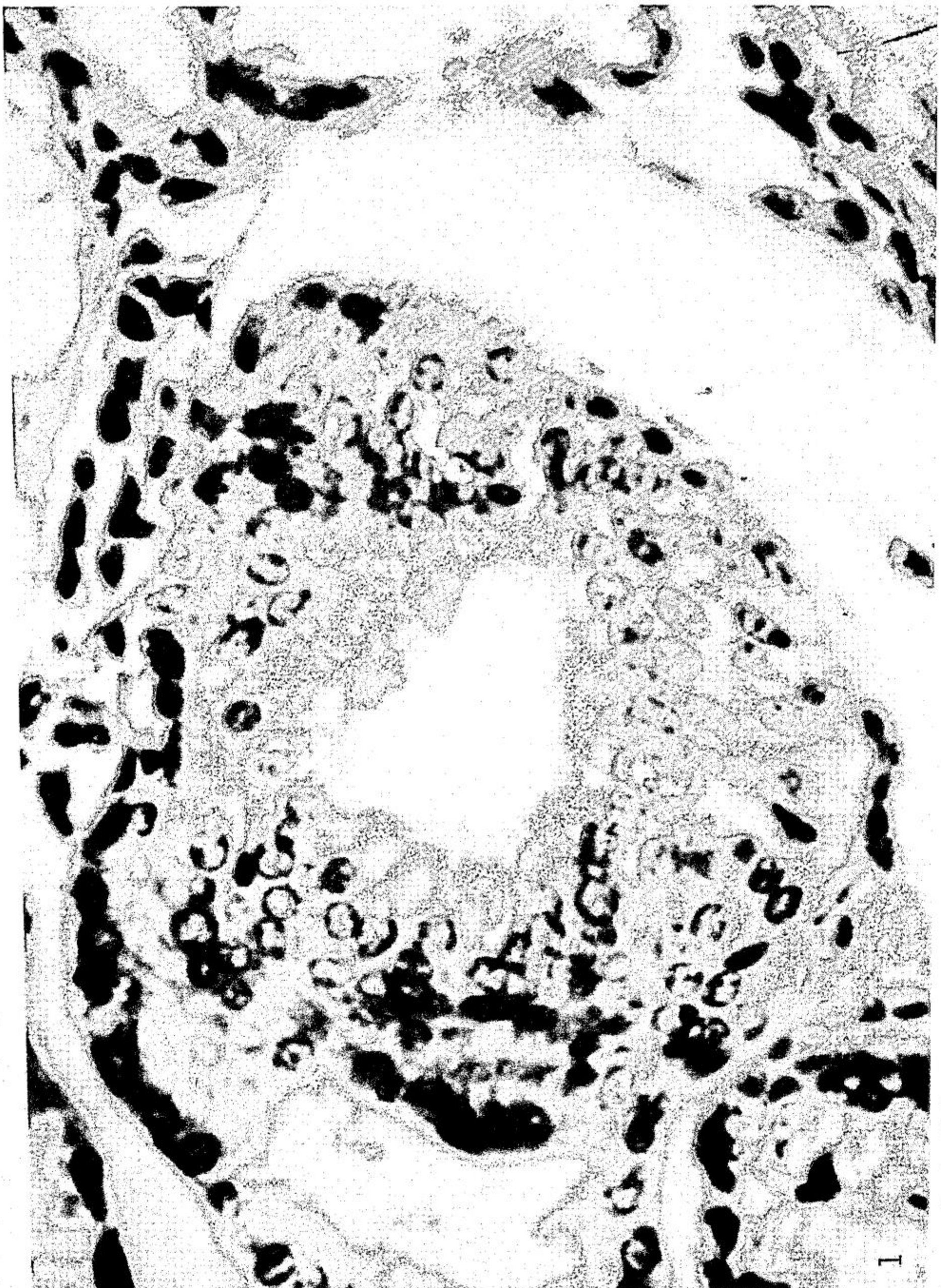


FIGURE 1.—Mucous glands of dog, showing basal cell hyperplasia and increased secretion after 229 days of smoking. Some nuclei vary in size and shape. × 425

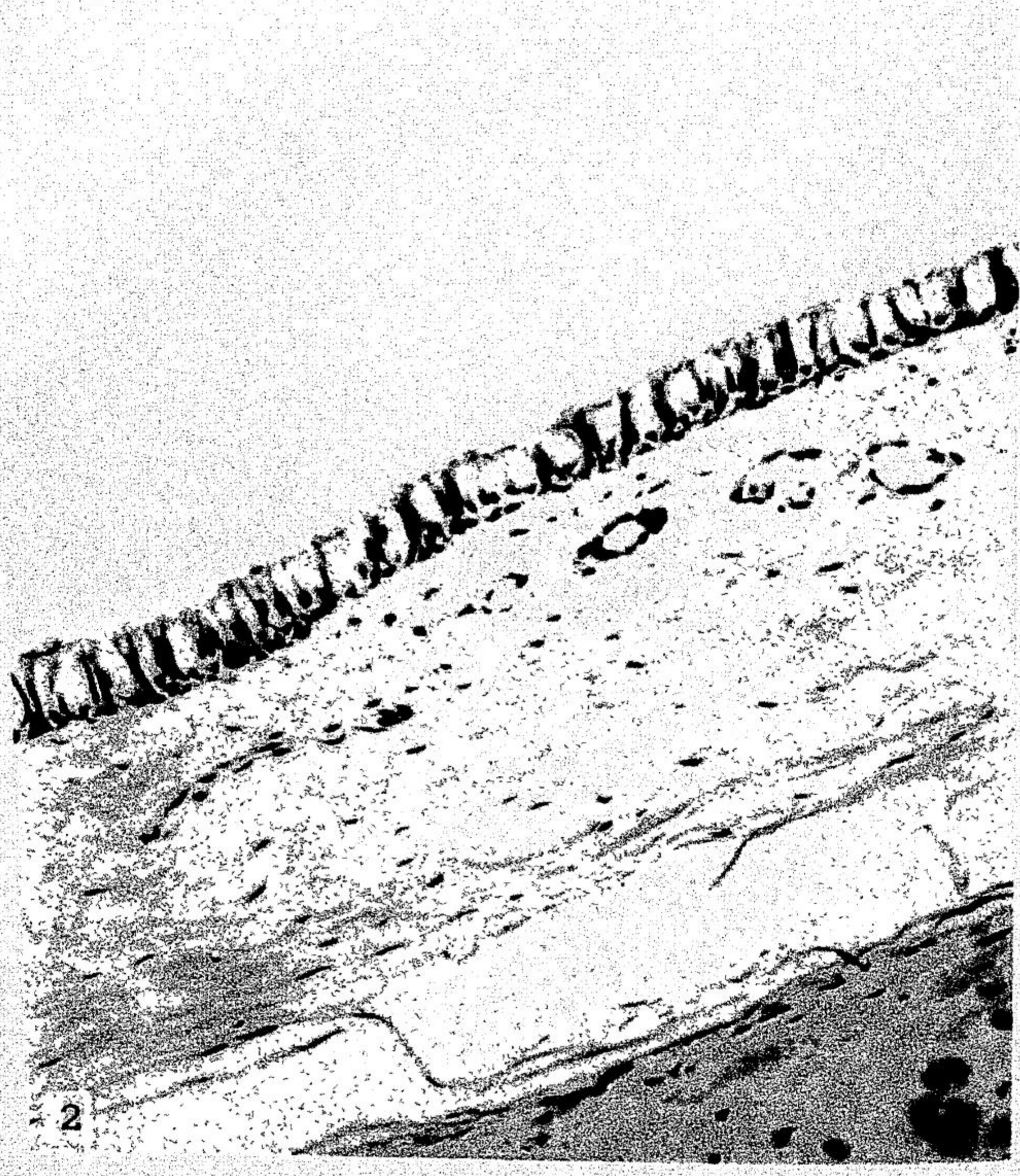


FIGURE 2.—Photomicrograph of bronchus of dog, showing normal ciliated columnar epithelium. × 170

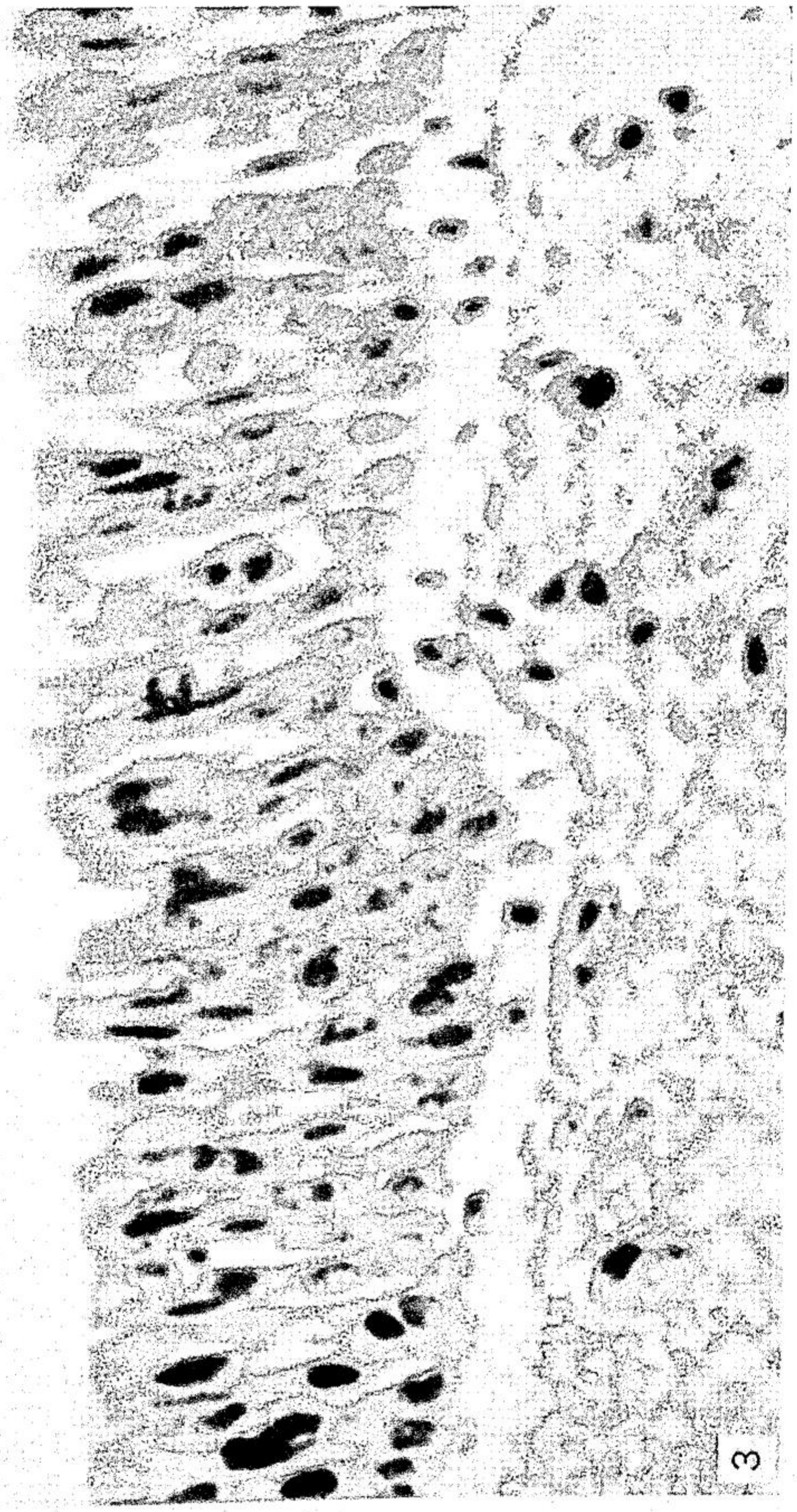


FIGURE 3.—Basal cell hyperplasia in bronchial epithelium of dog after 24 days of smoking. Basal cells are uniform in size and shape. × 425

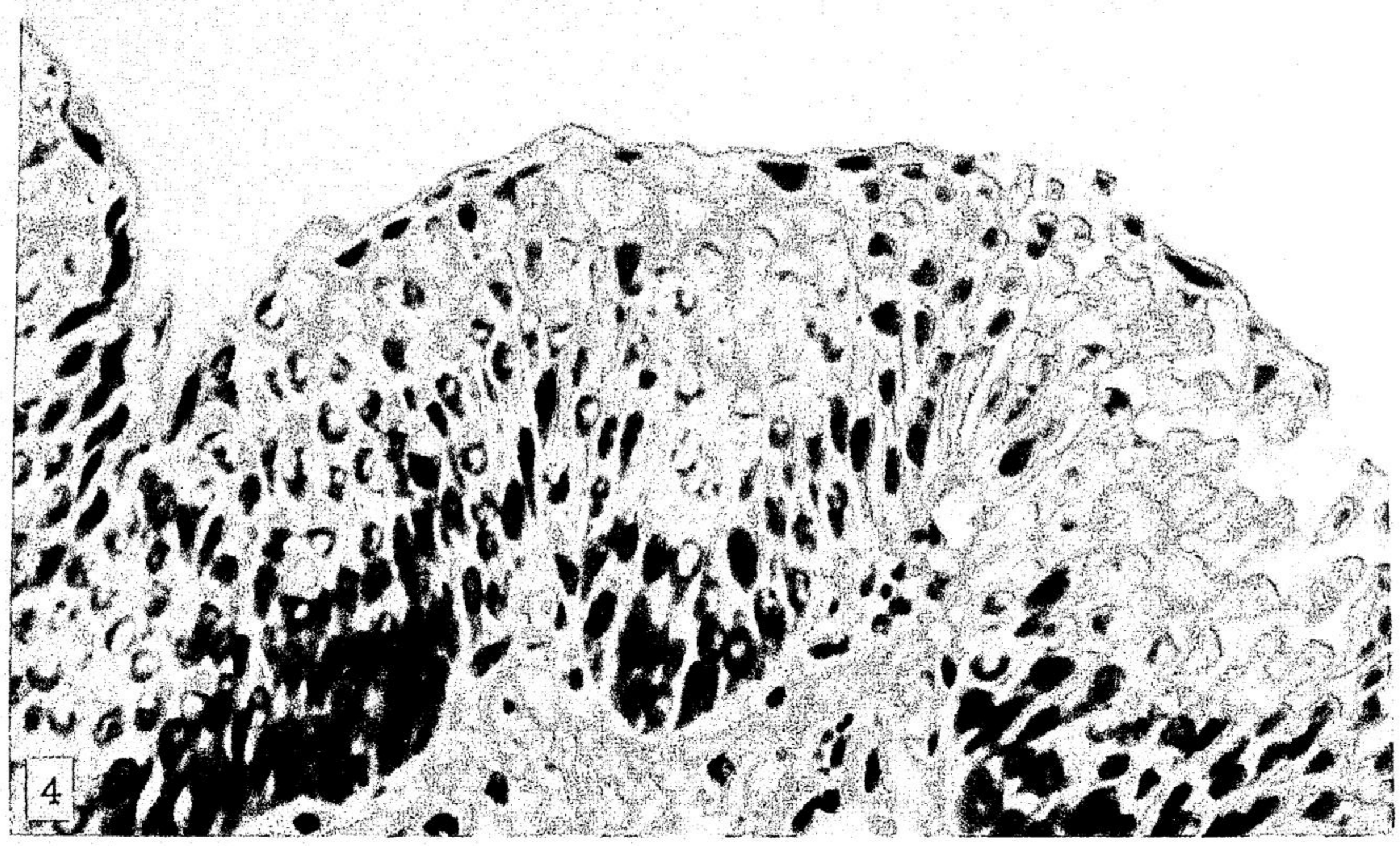


FIGURE 4.—Squamous metaplasia in a dog at site of tracheostomy after 24 days of smoking. Thick layer of epithelial cells resembles squamous epithelium in other sites. × 300

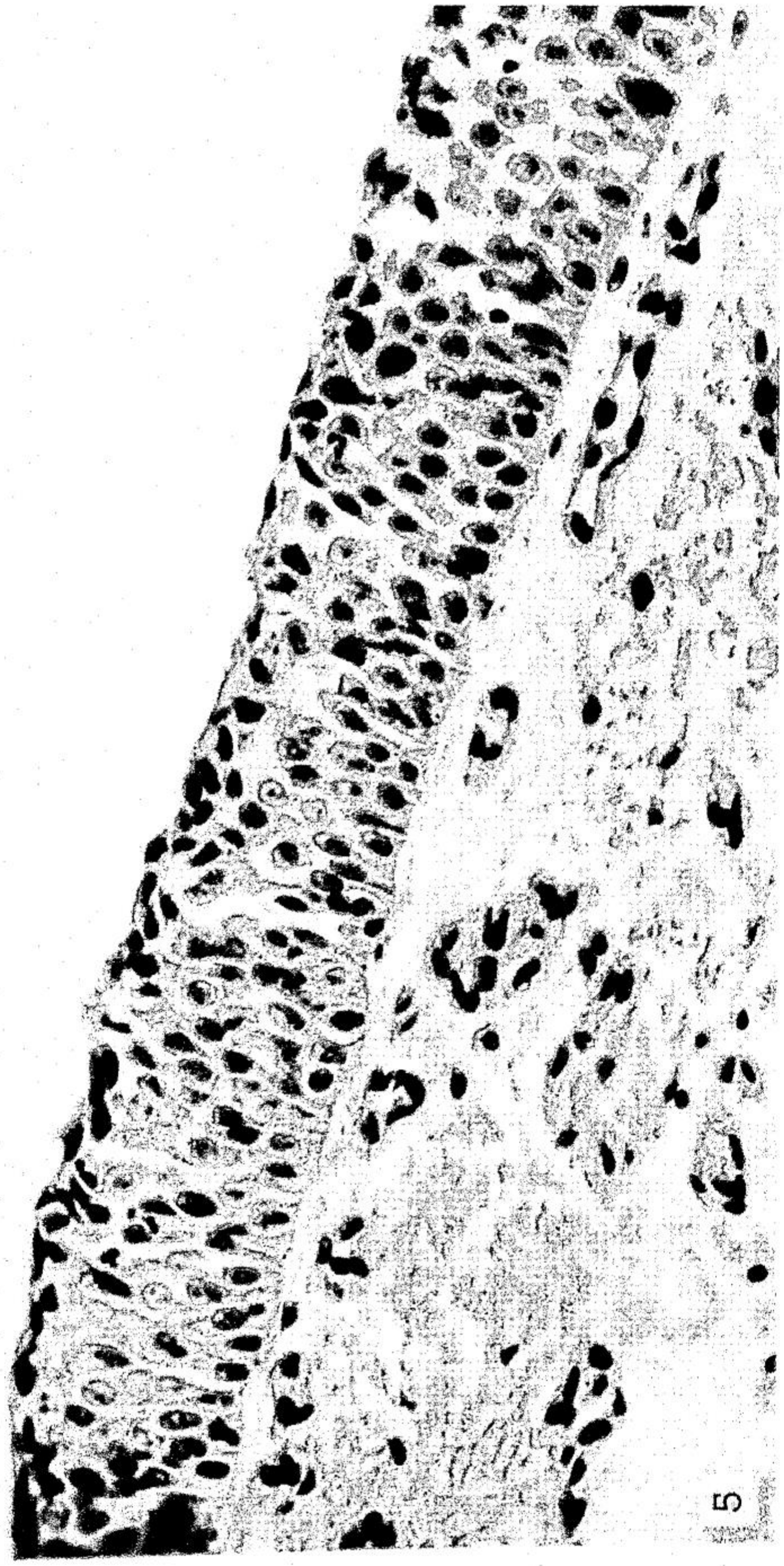


FIGURE 5.—Squamous metaplasia with atypism in a dog after 229 days of smoking. Some nuclei vary in size and shape. There is a disorderly arrangement of the cells. × 240

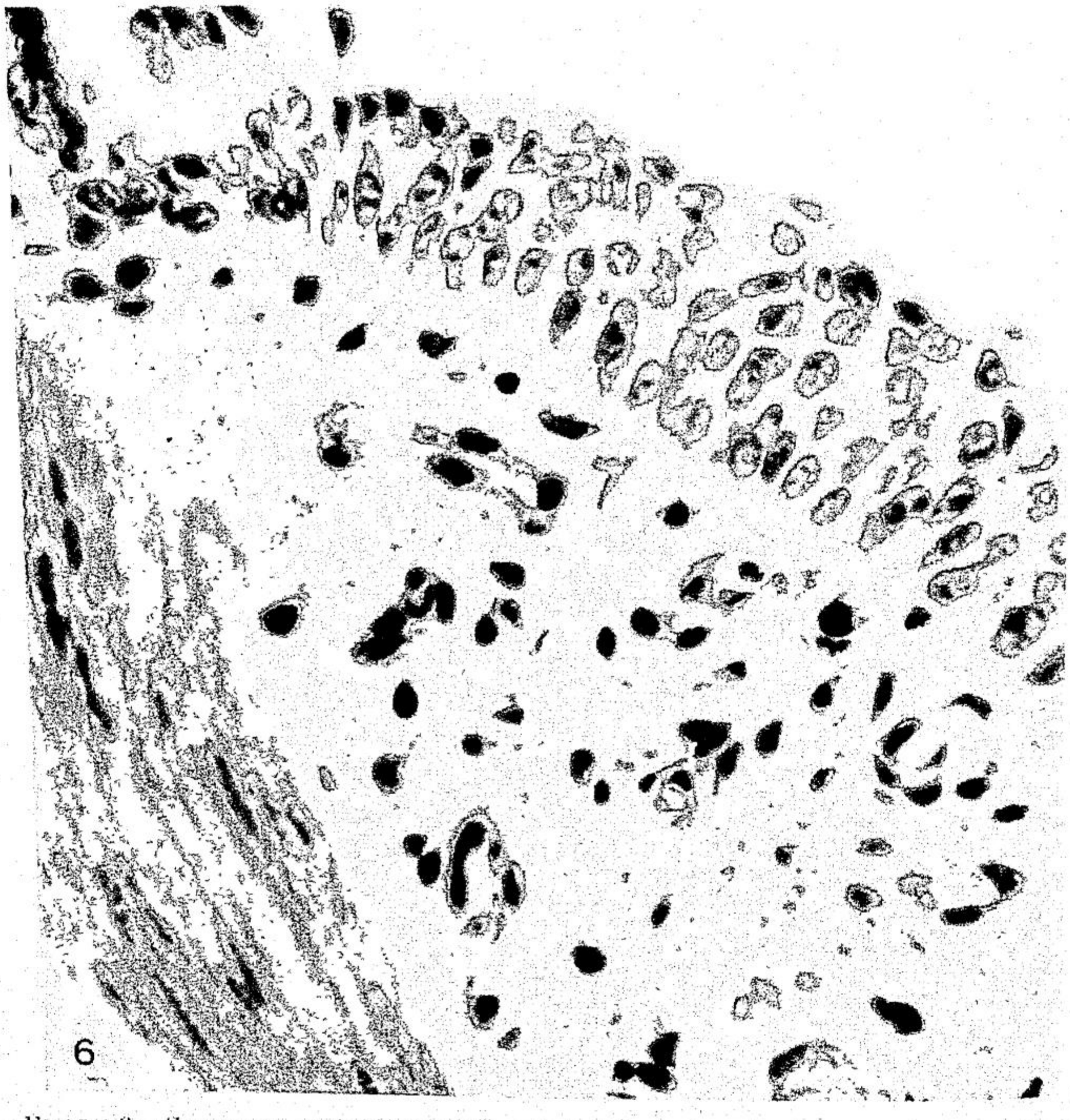


FIGURE 6.—Squamous metaplasia with atypism in a dog after 278 days of smoking. Some nuclei vary in size and shape. × 400

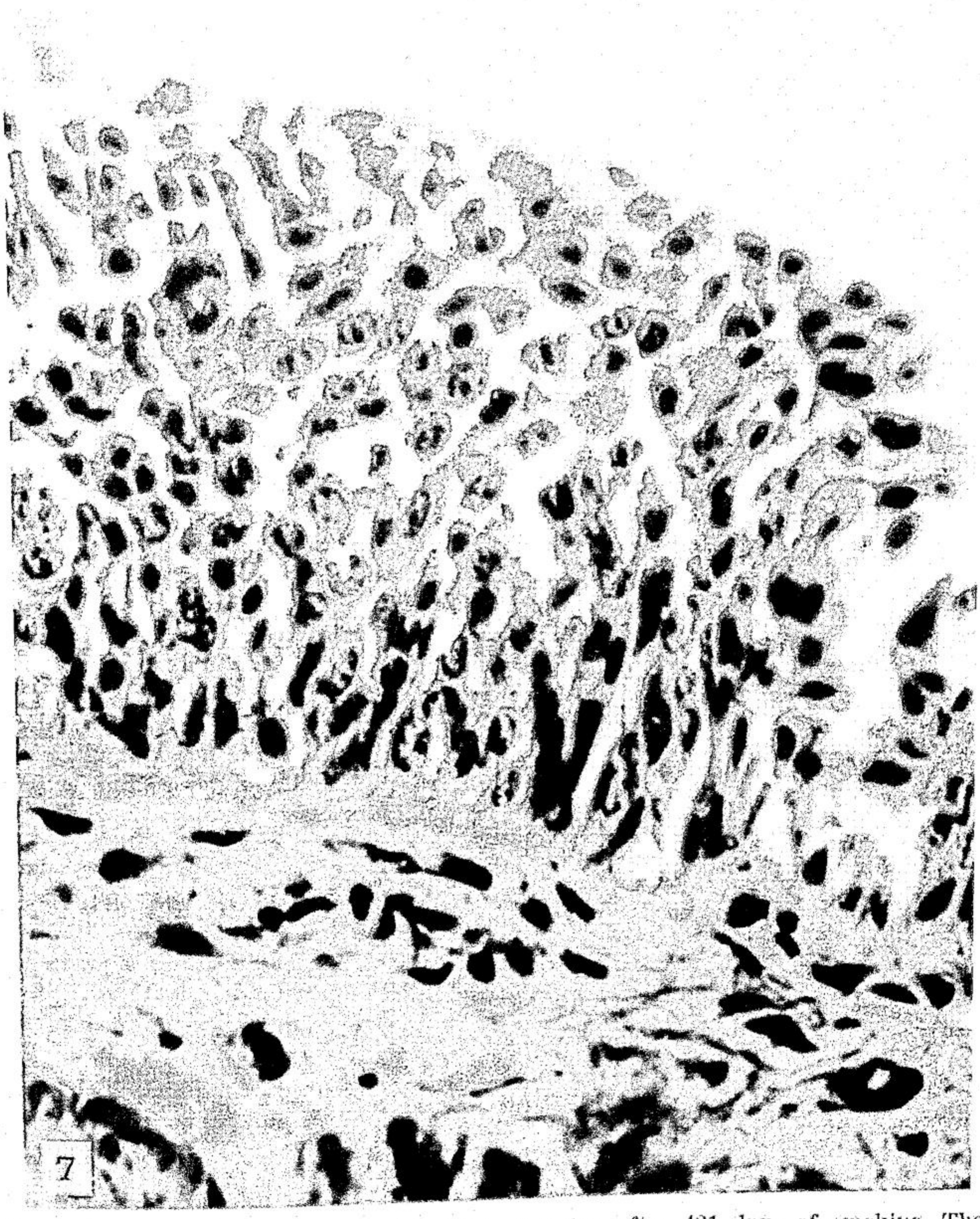


FIGURE 7.—Greatly thickened epithelium of a dog after 421 days of smoking. The nuclei vary in size and shape. Hyperchromatism is prominent. × 400

Effect of Different Doses of Tobacco Smoke on Ciliary Activity in Cat. Variations in Amount of Tobacco Smoke, Interval Between Cigarettes, Content of "Tar," Nicotine, and Phenol

TORE DALHAMN, *M.D.*, *Institute of Hygiene, Karolinska Institutet, Stockholm, Sweden*

INTEREST in the multitudinous effects of tobacco smoke on the biological organism increases. This attention chiefly concerns the reactions in the airways of mammals—particularly, mucosal changes (*1*, *2*), changes in pulmonary clearance (*3*), and ciliotoxic and mucotoxic effects.

The experiments described in this paper summarize our results from earlier work and unpublished studies concerning the ciliotoxic effects of cigarette smoke as outlined below. The latter will soon be published in greater detail (*4*).

Experiments on the effect of cigarette smoke on ciliary activity

Various factors

A. Components in smoke:
   1. Total particulate phase
      Total gas phase
   2. Particle phase ("tar" 6–19 mg/cigarette)
   3. Nicotine 0.7 and 1.8 mg/cigarette
   4. Phenol 2.7 and 18.8 mg/100 cigarettes

B. Interval between cigarettes: 0, 2.5, 5, 10, and 15 minutes
   Filterless
   Cellulose acetate filter

C. Amount of smoke 0.5, 1, 5, 10, and 20 ml
   Filterless
   Cambridge filter

METHODS

Two techniques were mainly used in the study of exposure to smoke. The first was fully mechanized, the cat being exposed every third breath to 1 ml of fresh tobacco smoke taken from a 35 ml puff. After 25 puffs, the

TABLE 1.—Ciliostatic effect of tobacco smoke of varying composition (identical tobacco—filters varied)*

Cigarette	Number of puffs for ciliostasis (mean of 5 animals)	Tar —	Nicotine 247.3	Phenol 195.5	Toluene 111	Acetonitrile 82	Methyl ethyl Ketone 79.6	Acetone 56.5	Isopyrene 34	Acetaldehyde 21
1	91	20.3	1.33	6.2	25	31	37	57	56	94
2	170	19.4	1.27	6.4	1	1	1	8	9	27
3	194	12.8	0.87	1.6	20	20	28	49	52	88
4	512	14.6	0.99	2.6	1	1	1	8	8	31
5	600	0.5	0.02	0.4	6	16	25	50	40	89
Correlation coefficient		−0.74	−0.73	−0.69	−0.56	−0.36	−0.27	−0.16	−0.28	−0.11

*Tar and nicotine expressed as mg/cigarette, other compounds as tenths of μg/cigarette. Numerical values under compounds indicate boiling point in centigrade degrees.

Effect of Different Doses of Tobacco Smoke on Ciliary Activity in Cat. Variations in Amount of Tobacco Smoke, Interval Between Cigarettes, Content of "Tar," Nicotine, and Phenol

TORE DALHAMN, *M.D.*, *Institute of Hygiene, Karolinska Institutet, Stockholm, Sweden*

INTEREST in the multitudinous effects of tobacco smoke on the biological organism increases. This attention chiefly concerns the reactions in the airways of mammals—particularly, mucosal changes (*1*, *2*), changes in pulmonary clearance (*3*), and ciliotoxic and mucotoxic effects.

The experiments described in this paper summarize our results from earlier work and unpublished studies concerning the ciliotoxic effects of cigarette smoke as outlined below. The latter will soon be published in greater detail (*4*).

Experiments on the effect of cigarette smoke on ciliary activity

Various factors

A. Components in smoke:
   1. Total particulate phase
      Total gas phase
   2. Particle phase ("tar" 6–19 mg/cigarette)
   3. Nicotine 0.7 and 1.8 mg/cigarette
   4. Phenol 2.7 and 18.8 mg/100 cigarettes

B. Interval between cigarettes: 0, 2.5, 5, 10, and 15 minutes
   Filterless
   Cellulose acetate filter

C. Amount of smoke 0.5, 1, 5, 10, and 20 ml
   Filterless
   Cambridge filter

METHODS

Two techniques were mainly used in the study of exposure to smoke. The first was fully mechanized, the cat being exposed every third breath to 1 ml of fresh tobacco smoke taken from a 35 ml puff. After 25 puffs, the

animal was allowed to rest for 5 minutes before the exposure was repeated (*5*).

Because it proved impossible to avoid a certain amount of leakage with this technique (so that 1 ml fresh smoke corresponded to only about half this amount, *i.e.*, 0.5 ml), subsequent experiments were performed manually. One ml was taken from 35 ml fresh smoke at 1 puff a minute and the cigarette was smoked until a 28 mm stub remained. There was no leakage. These two series are thus not entirely comparable, but comparisons can be made between the experiments in each series.

The methods for observing ciliary activity have been described in detail elsewhere. Ciliary activity in the trachea was observed after tracheotomy with an Ultropak microscope (*6*).

RESULTS

Components in Smoke

1. *Total particulate phase, total gas phase:* These experiments (*7*) concern the effect of variations in the gaseous and/or the particulate phases (table 1), by use of the method described elsewhere (*5*).

The results showed that an average of 91 puffs of unfiltered smoke was required to elicit ciliostasis. When the gaseous phase was reduced to only a small fraction of its original value and the particulate phase was left largely intact, the ciliostatic effect diminished from 91–170 puffs. Reduction of the particulate phase and maintenance of the gaseous phase gave a figure of 194 puffs. When both components were reduced at the same time, the ciliostatic effect of the smoke was greatly diminished to 512 puffs.

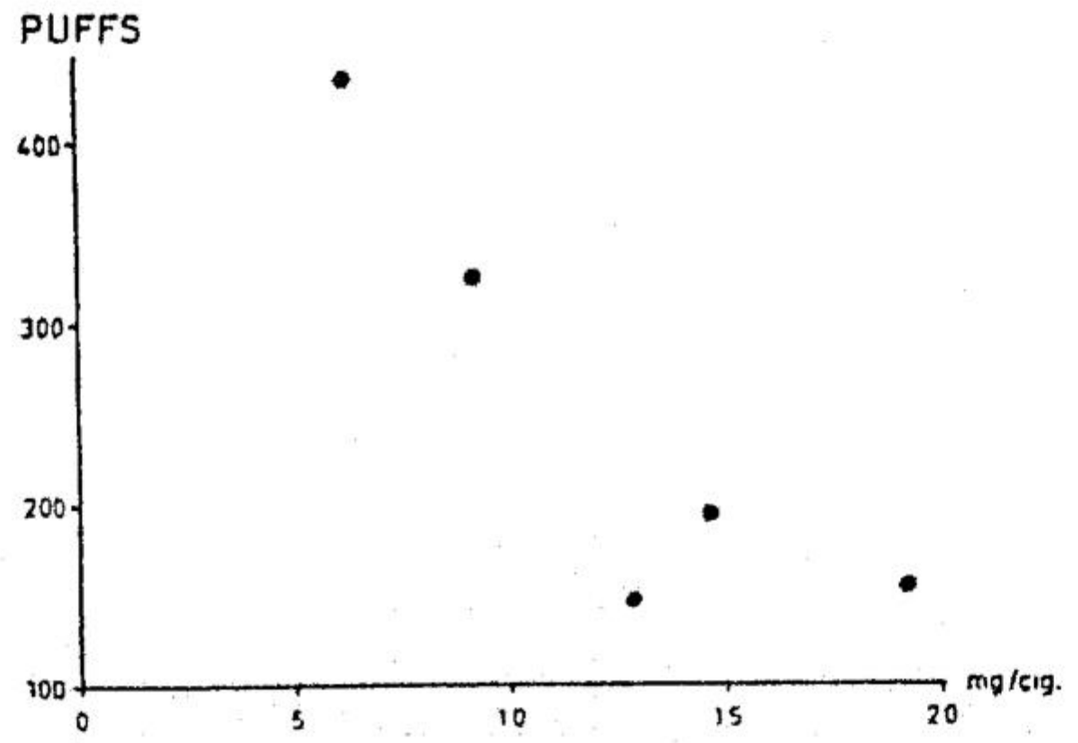
However, the ciliostatic effect of the smoke could also be substantially diminished by complete elimination of only the particulate phase. More than 600 puffs were then required to elicit ciliostasis. The correlation coefficients for the relationship between the particulate phase ("tar" including nicotine and phenol) and ciliotoxicity were high throughout (0.74–0.69). High correlations were also found for certain components in the gaseous phase, for instance for toluene (0.56). In view of the relevance of the particulate phase in the present context, subsequent experiments were confined to varying the measured components of this phase, that is, the "tar," nicotine, or phenol, and keeping the other two components more or less constant.

2. *Particulate phase*: Experiments (*8*) were conducted by the same techniques as described in Section 1. Five series of 5 animals each were studied, the "tar" content of the smoke varying from 6.2–19.2 mg/cigarette. The number of puffs before ciliostasis, recorded as in previous experiments, is given in text-figure 1. These experiments again showed a strong correlation between tar content and ciliotoxicity. Control experiments with expo-

TABLE 1.—Ciliostatic effect of tobacco smoke of varying composition (identical tobacco—filters varied)*

Cigarette	Number of puffs for ciliostasis (mean of 5 animals)	Tar —	Nicotine 247.3	Phenol 195.5	Toluene 111	Acetonitrile 82	Methyl ethyl Ketone 79.6	Acetone 56.5	Isopyrene 34	Acetaldehyde 21
1	91	20.3	1.33	6.2	25	31	37	57	56	94
2	170	19.4	1.27	6.4	1	1	1	8	9	27
3	194	12.8	0.87	1.6	20	20	28	49	52	88
4	512	14.6	0.99	2.6	1	1	1	8	8	31
5	600	0.5	0.02	0.4	6	16	25	50	40	89
Correlation coefficient		−0.74	−0.73	−0.69	−0.56	−0.36	−0.27	−0.16	−0.28	−0.11

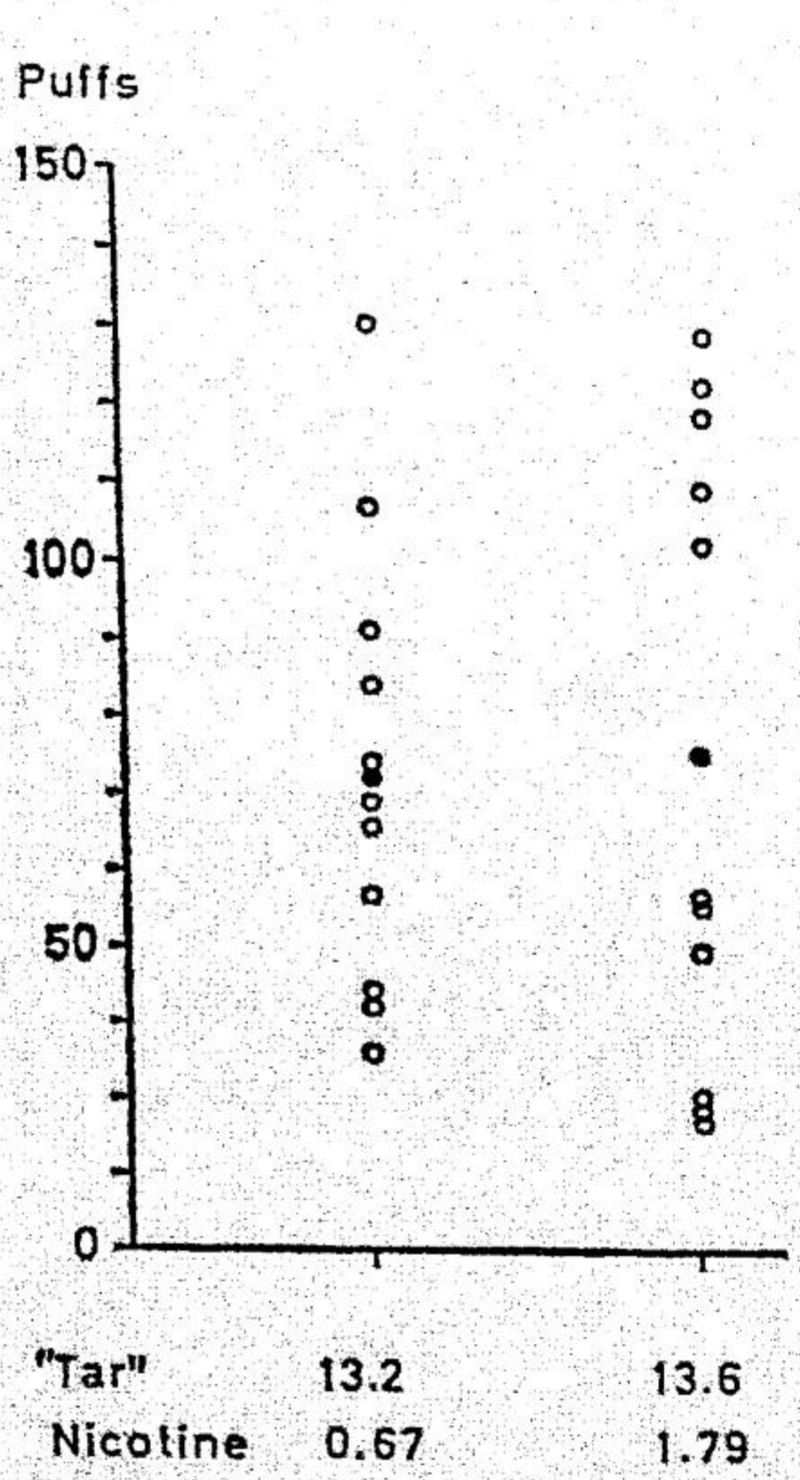
*Tar and nicotine expressed as mg/cigarette, other compounds as tenths of μg/cigarette. Numerical values under compounds indicate boiling point in centigrade degrees.



TEXT-FIGURE 1.—Effect of different tar content of cigarette smoke on ciliary activity.

sure to air alone were conducted in this, as well as in all the other studies, without displaying any effect on the ciliary activity.

3. *Nicotine*: The next step was to vary only the content of nicotine. These experiments were conducted by the manual technique described; the cigarette was smoked down to a 28 mm stub at a rate of 1 puff a minute and



TEXT-FIGURE 2.—Effect of nicotine on ciliary activity.
o=individual values; •=mean value.

1 ml from a 35 ml smoke puff was manually injected into the trachea. The study was made with 2 series of 6 animals each. The nicotine contents were 0.67 and 1.79 mg/cigarette, respectively, the "tar" content being kept practically constant (13.2 and 13.6 mg/cigarette). The results are shown in text-figure 2. Ciliostasis was elicited by the cigarettes with a low nicotine content after 75 puffs, while 67 puffs were required with the cigarettes with a high nicotine content. The difference between these values is not significant.

4. *Phenol*: Finally, the phenol content was varied while the contents of nicotine and "tar" were kept largely constant. Two series of 5 animals each were studied by the manual technique used for the investigation of nicotine effect. The concentration of phenol was low and very high (2.7 and 18.8 mg/100 cigarettes) compared to the concentration in normal cigarettes (ca. 7 mg/100 cigarettes). The results are given in table 2. Ciliostasis was induced by a mean of 135 puffs in the first series and by 72 puffs in the second, indicating a clear correlation between ciliostasis and the amount of phenol in the concentrations studied.

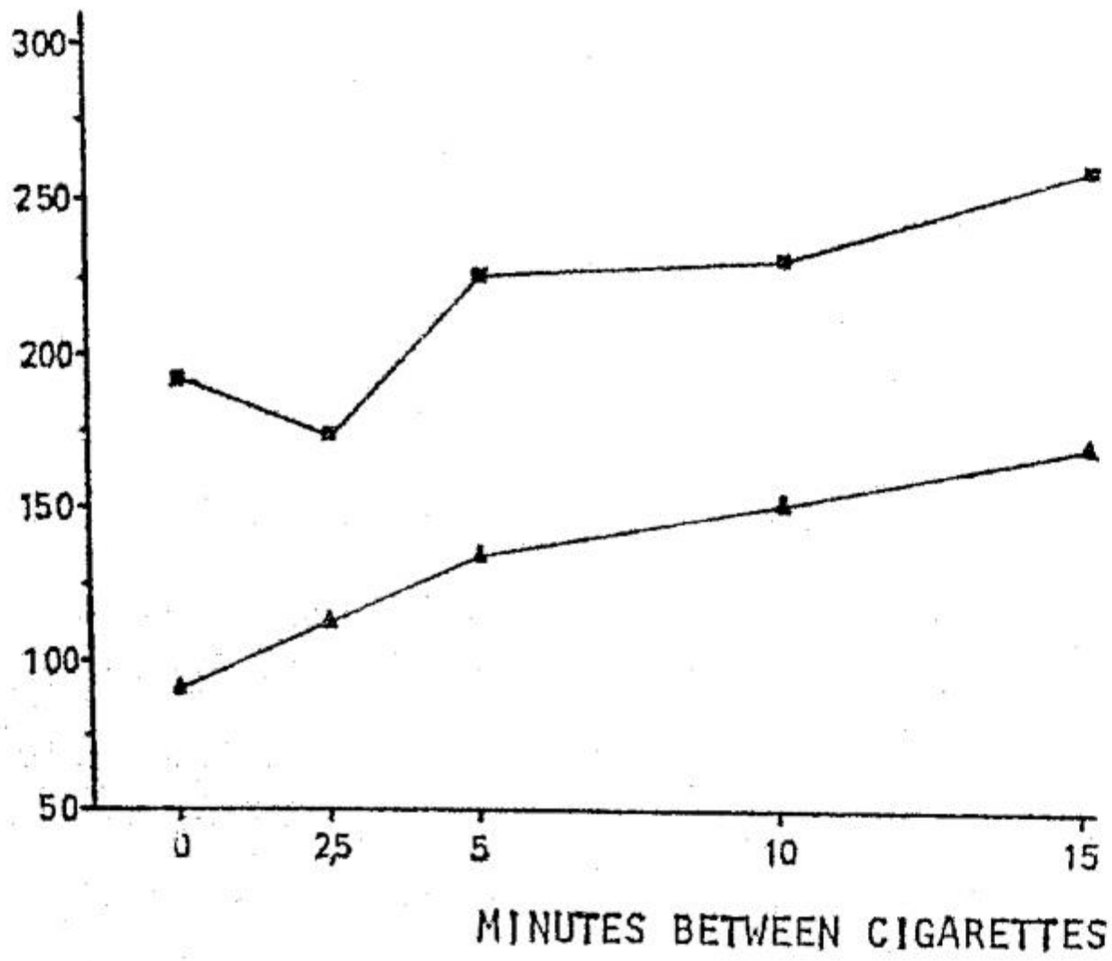
TABLE 2.—Effect of different phenol content on ciliary activity

Phenol concentration (mg/100 cigarettes)	Puffs to ciliostasis	"Tar" and nicotine (mg/cigarette)
2. 7	135	19
18. 8	72	1. 2

Interval Between Cigarettes

To study the relationship between the ciliostatic effect of cigarette smoke and the length of the interval between cigarettes, this interval was varied from 0 minutes to 2.5, 5, 10, and 15 minutes. In addition, two types of cigarettes were used, one without a filter and one with a filter of cellulose acetate. A series of 5 animals was studied for each type of cigarette and each interval (*9*). The cigarettes were commercial brands.

Text-figure 3 shows the results of these experiments, which were conducted by the fully mechanical technique. For both the filterless cigarettes and the filter-tipped, there is a clear correlation between the toxicity of the smoke and the length of the interval between cigarettes: the longer the interval. the greater the number of puffs before the onset of ciliostasis—in other words, a clear dose-response relationship. There is also a significant difference between the two commercial cigarettes, the one with a filter always requiring a greater number of puffs to elicit ciliostasis than the filterless.



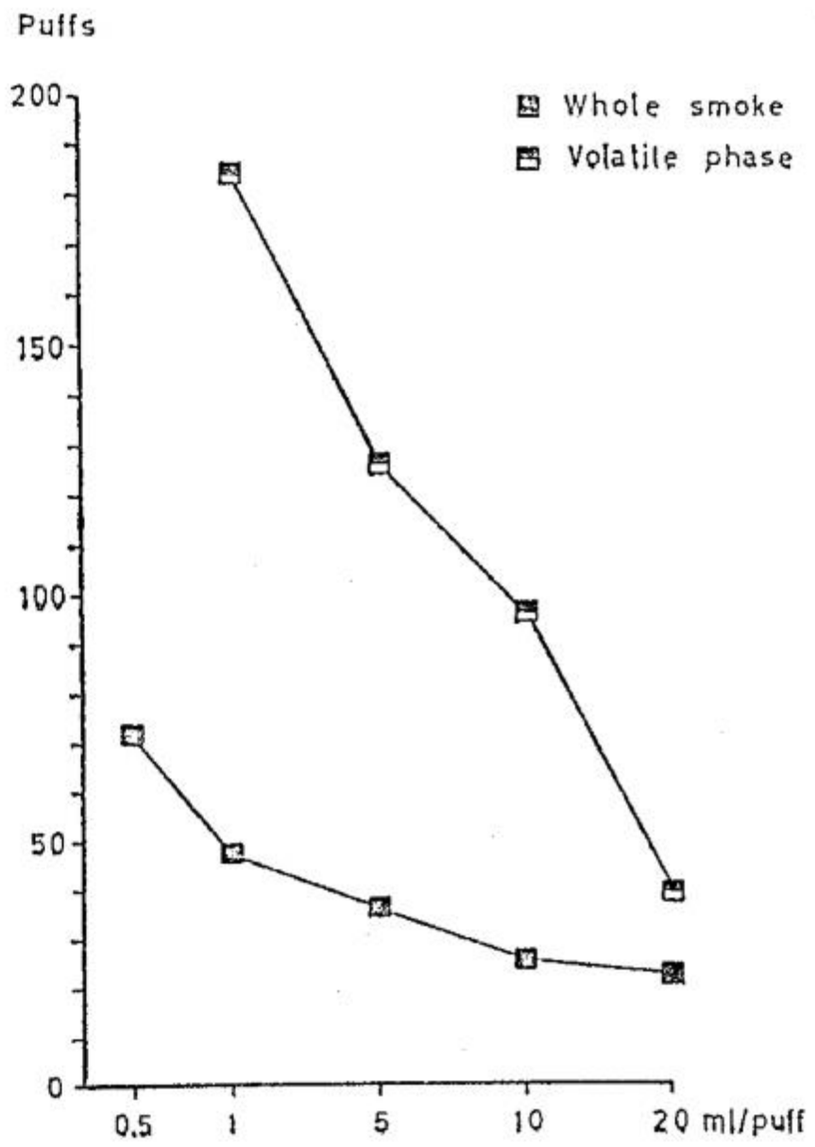
TEXT-FIGURE 3.—Ciliostatic effect of smoke from filter-tipped (*squares*) and filterless (*triangles*) cigarettes, with varying time intervals between cigarettes. The means from groups of five animals are plotted.

Amount of Smoke

Several authors have discussed the effect of cigarette smoke on the airways in terms of its ciliotoxic influence as well as its effect on the transport of mucus and pulmonary clearance (*10–13*). The experimental methods used for exposure to cigarette smoke have varied considerably, ranging from the use of mussels and similar animals through *in vitro* studies on frogs and mammals to *in vivo* investigations of mammals. Various techniques have also been used for producing the smoke. The experiments described below concern the effect of different amounts of smoke (*4*).

The experiments were made in two series, one with unfiltered smoke and the other with smoke filtered through a Cambridge filter. The amounts of smoke administered once a minute until the time that a 28 mm stub remained were 0.5, 1.0, 5, 10, and 20 ml. Each group comprised 5–10 animals. The results are shown in text-figure 4.

A clear dose-response relationship was found for both types of cigarette smoke. Moreover, at all dose levels studied, the unfiltered smoke proved more toxic than the same amount of filtered smoke, though it is interesting that this difference appears to be less for the larger amounts of smoke administered. When 20 ml puffs were used, the difference between the number of puffs of filtered and unfiltered smoke required to elicit ciliostasis was about 20, whereas for 1 ml puffs the difference was no less than 150.



TEXT-FIGURE 4.—Ciliotoxicity of whole smoke (*lower curve*) and Cambridge filtered smoke (*upper curve*), at varying dose levels.

CONCLUSIONS

The experiments presented concern the effect on ciliary activity in the trachea of the cat of short-term exposure to tobacco smoke. It should be emphasized that from such experiments one naturally cannot draw any conclusions about the genesis of various respiratory diseases in man in connection with cigarette smoking. No detailed investigations have been made of the correlation between ciliotoxic and mucotoxic agents of the above type and respiratory diseases, though the probability of such a relationship exists. To judge from results of the experiments, both the particulate and the gaseous phases possess ciliotoxic properties. This raises the question of which individual component or components are responsible for this effect. Several different substances are probably biologically active in this respect and there are no doubt synergistic effects as well. The particulate phase is generally regarded as consisting of "tar," nicotine, and phenol, though some phenol is also found in the gaseous phase.

The present investigations started in an attempt to establish the ciliotoxicity of the various components of the particulate phase. The content of "tar" (which is itself a mixture of more than 500 compounds) thus displayed a strong correlation to the ciliary activity, whereas nicotine, in the concentrations studied, had no detectable effect. The phenol experiment admittedly showed a clear correlation, but it must be borne in mind that the higher concentration (18.8 mg/100 cigarettes) is much more than one

finds in a normal cigarette. Smaller variations in the content of phenol have produced contradictory results.

Individual compounds in the gaseous phase were not investigated, but Kensler and Battista (*13*) report that, with their technique, both hydrogen cyanide and acrolein are essential in the ciliotoxic effect of the gaseous phase.

Concerning the length of the interval between cigarette-smoke exposures, a clear dose-response relationship was found to ciliotoxicity.

Finally, it is quite clear that the smaller the dose of smoke, the smaller the acute toxic effect. This applies to the unfiltered smoke as well as to the smoke passed through a Cambridge filter. The difference between these two types of smoke is not constant, however; it diminished with increasing doses. This relationship should be borne in mind when deciding on the technique of exposure; otherwise it is easy to overestimate or underestimate the toxicity of the total smoke or of its individual components.

SUGGESTIONS

To study the ciliotoxic and mucotoxic effect of cigarette smoke in a meaningful manner, it seems necessary to design more experiments with conditions that agree as closely as possible with the conditions obtaining when a person smokes.

It has been pointed out that the technique used for exposure to smoke is important in the interpretation of the results obtained. The technique and the biological system involved in the observation of the ciliary activity appear equally important. These problems thus warrant re-examination, without which it will be difficult to make further progress in the study of ciliotoxic effects of cigarette smoke.

REFERENCES

(*1*) Gheen, G. M., and Carolin, D.: The depressant effect of cigarette smoke in the in vitro antibacterial activity of alveolar macrophages. New Eng J Med 276: 421–427, 1967.

(*2*) Leuchtenberger, C., Leuchtenberger, R., Ruch, F., Tanaka, K., and Tanaka, T.: Cytological and cytochemical alterations in the respiratory tract of mice after exposure to cigarette smoke, influenza virus, and both. Cancer Res 23: 555–565, 1963.

(*3*) Rylander, R.: Pulmonary defense mechanisms to airborne bacteria. Acta Physiol Scand Suppl 306: 1–89, 1968.

(*4*) Dalhamn, T., and Rylander, R.: Ciliatoxicity of cigarette smoke and its volatile components. Submitted for publication in Amer Rev Resp Dis.

(*5*) Dalhamn, T., von Essen, E., Kajland, A., and Rylander, R.: A machine for introducing a regulated amount of tobacco smoke into an animal. Air Water Pollut 7: 511–515, 1963.

(*6*) DALHAMN, T.: The determination in vivo of the rate of ciliary beat in the trachea. Acta Physiol Scand 49: 242–250, 1960.

(*7*) DALHAMN, T., and RYLANDER, R.: Cigarette smoke and ciliastasis. Effect of varying composition of smoke. Arch Environ Health (Chicago) 13: 47–50, 1966.

(*8*) ———: Tar content and ciliatoxicity of cigarette smoke. Acta Pharmacol Toxicol 25: 369–372, 1967.

(*9*) ———: Ciliastatic action of cigarette smoke: Varying exposure times. Arch Otolaryng (Chicago) 81: 379–382, 1965.

(*10*) CARSON, S., GOLDHAMER, R., and CARPENTER, R.: Responses of ciliated epithelium to irritants. Mucus transport in the respiratory tract. Amer Rev Resp Dis 93: 86–92, 1966.

(*11*) FALK, H. L., TREMER, H. M., and KOTIN, P.: Effect of cigarette smoke and its constituents on ciliated mucus-secreting epithelium. J Nat Cancer Inst 23: 999–1012, 1959.

(*12*) GUILLERM, R., BADRÉ, R., and VIGNON, B.: Effets inhibiteurs de la fumée de tabac sur l'activité ciliaire de l'épithélium respiratoire et nature des composants responsables. Bull Acad Nat Med (Paris) 145: 416–423, 1961.

(*13*) KENSLER, C. J., and BATTISTA, S. P.: Components of cigarette smoke with ciliary-depressant activity. New Eng J Med 269: 1161–1166, 1963.

"Tar" and Nicotine Levels of American Cigarettes[1]

GEORGE E. MOORE, *M.D., Ph.D., and* FRED G. BOCK, *Ph.D., Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14203*

THE simplest proved procedure available today for reducing the hazard of cigarettes is the design of a product that delivers less "tar" and nicotine. The formation of cancer depends on "tar" dose, both in the laboratory and in the human population (*1–5*). Probably the incidence of various smoking-connected cardiovascular fatalities is dependent on the consumption of nicotine (*6*). Ultimately, it may be possible to design cigarettes which provide satisfaction without harmful "tar" and nicotine. Until the efficacy of such cigarettes has been demonstrated in the consumer population, it is desirable that efforts be made to reduce the nicotine and general "tar" level of existing cigarettes as much as possible.

The levels of "tar" and nicotine delivery by various types of cigarettes have been published. The methodology and terminology varied, but comparisons among different types of cigarettes within the same series are valid and much can be learned from them. Reports of "tar" delivery by specific brands of cigarettes have been published in the "Reader's Digest" (*7*) and in "Consumer Reports" (*8*). Wynder and Hoffmann tested the "tar" delivery of various brands of cigarettes in 1959 (*9*). In 1967, the "tar" and nicotine levels of American cigarettes were reported by Moore *et al.* (*10*). This presentation concerns additional comments on this study and recent assays conducted at our laboratory. The methods by which the data were obtained were described earlier (*10*). They differ from those adopted by the Federal Trade Commission in 1967 in that the "tar" delivery is reported as total wet "tar." Subtraction of water and nicotine levels gives smaller absolute values for the "tar" delivery but does not affect the relative values among presently available brands.

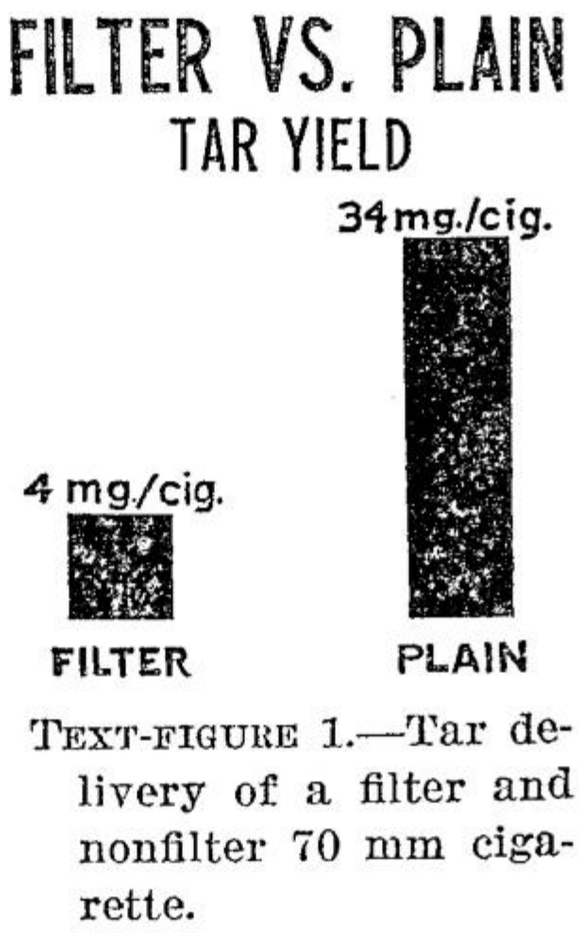
[1] This work was supported by Public Health Service research grant 1053.

TAR LEVELS

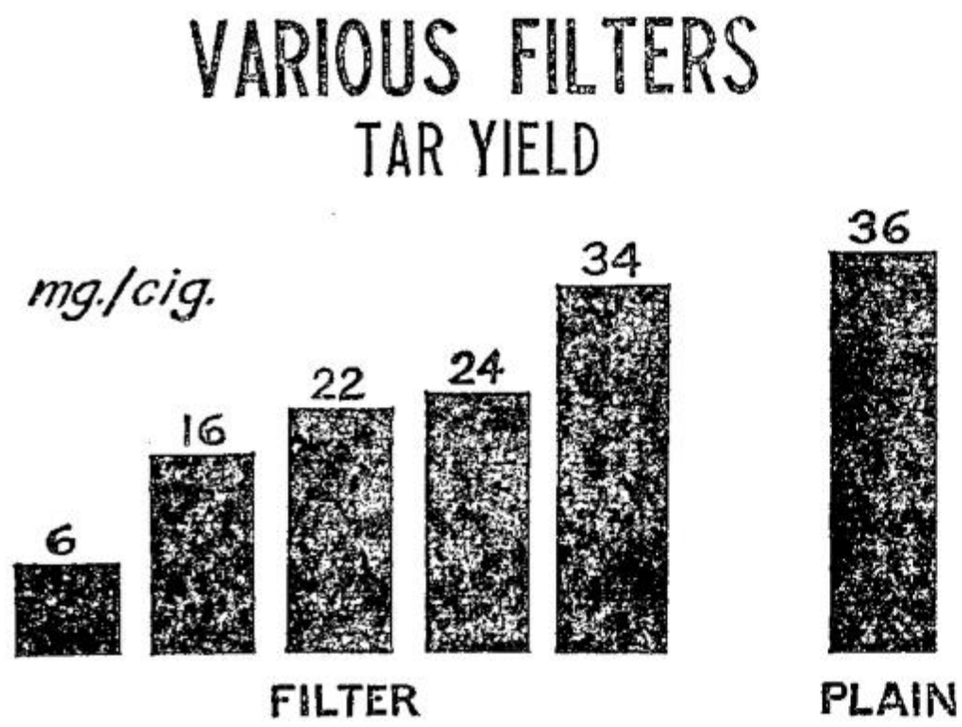
The diversity of cigarettes presently on the market shows the technical capability of the industry in manufacturing numerous kinds of cigarettes with rather exact characteristics. Presently available 70 mm cigarettes range in "tar" delivery from 4–34 mg/cigarette (text-fig. 1). If all sizes of cigarettes are considered, the range extends from 4–48 mg/cigarette, a twelvefold range. This difference is truly enormous and the maximum is unparalleled by any approved level of noxious major material allowed in other kinds of ingested products. The range of "tar" delivery by nonfilter cigarettes is relatively narrow, from 27–36 mg/70 mm cigarette. Most of the diversity in commercial cigarettes appears among the filter-type brands. This is understandable, since many of the brands have been marketed recently, and the most acceptable (salable) characteristics are not known. Filters range in effectiveness from very good to nearly useless. In addition, other techniques such as air dilution are used to provide a "milder" product. For example, among the kingsize cigarettes a representative nonfilter cigarette delivered 36 mg "tar"/cigarette, whereas various filter-tipped cigarettes delivered from 6–34 mg/cigarette (text-fig. 2). The fact that a cigarette has a filter is not an indication that it is necessarily yielding a low quanity of "tar." Indeed, our earlier analyses of commercial cigarettes showed that for some brands, the filter-tipped representative delivered as much "tar" as, or more than, its regular namesake (*10*). In these instances, use of a filter was apparently more of a merchandising technique than one aimed toward reducing the hazard of the smoke.

The data obtained from various types of filter-tipped cigarettes must be considered relevant to consumer acceptance. Interestingly, of the 5 most popular brands, 3 are below the average in "tar" delivery and 2 are in the lower third. Obviously, the cigarette industry can produce filters that have either very little effect or a profound effect on the delivery of "tar" by the cigarettes. Unfortunately, a major segment of the cigarette manufacturing companies subscribes to an advertising code which prohibits them from exploiting the few truly effective filters they produce. Accordingly, the consumer has been obliged to pick and choose new brands without information concerning the delivery of "tar" and nicotine. However, most of our associates do recognize that low "tar" delivery cigarettes also deliver less flavor. Although many smokers would choose a low flavor cigarette if they knew it delivered substantially less "tar," it is obvious that the high "tar" delivery cigarette will be more popular in the absence of full disclosure of "tar" yields. The fact that cigarettes low in the delivery of "tar" exist on the market at all demonstrates clearly that many consumers desire the truly effective filters.

A second way in which "tar" delivery of commercial cigarettes is reduced is by the use of a long filter or heavy overwrap which insures a long butt. Text-figure 3 illustrates the average amount of "tar" recovered

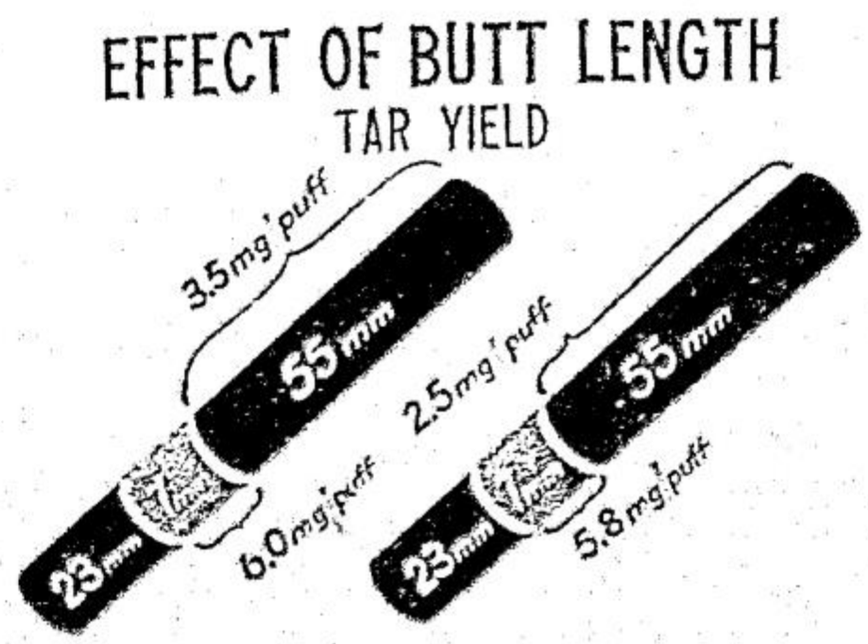


TEXT-FIGURE 1.—Tar delivery of a filter and nonfilter 70 mm cigarette.

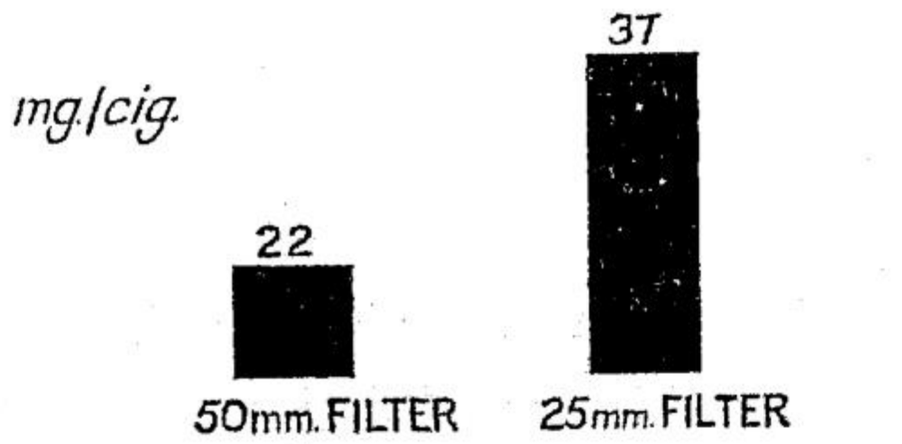


TEXT-FIGURE 2.—Tar delivery of various 85 mm cigarettes.

per puff when a commercial cigarette is smoked to a moderate butt (30 mm) versus a short butt (23 mm). The last puffs that reduce a moderate butt to a short butt contain substantially more "tar." It is significant that in the United States, 28% of smokers of nonfilter cigarettes leave a butt shorter than 25 mm (*11*). Any means that would insure a long butt on cigarettes would reduce "tar" delivery. Several existing commercial cigarettes now produce a long filter. In fact, one brand has a 47 mm filter with the overwrap extending another 3 mm. That this is an effective approach can be seen in text-figure 4 where the cigarette described is compared with another of the same length but with a short filter and overwrap. The use of the heavy paper overwrap is probably sufficient to insure a minimum butt length. However, an even more effective method would be to use an overwrap of some nonflammable material such as aluminum. An aluminum foil overwrap in our experience will effectively extinguish the coal of the cigarette, insuring a long butt. Cigarettes containing such an overwrap may also be much less of a fire hazard.



TEXT-FIGURE 3.—Average "tar" delivery per puff from different lengths of cigarette. Upper cigarette, nonfilter; lower cigarette, filter tip.



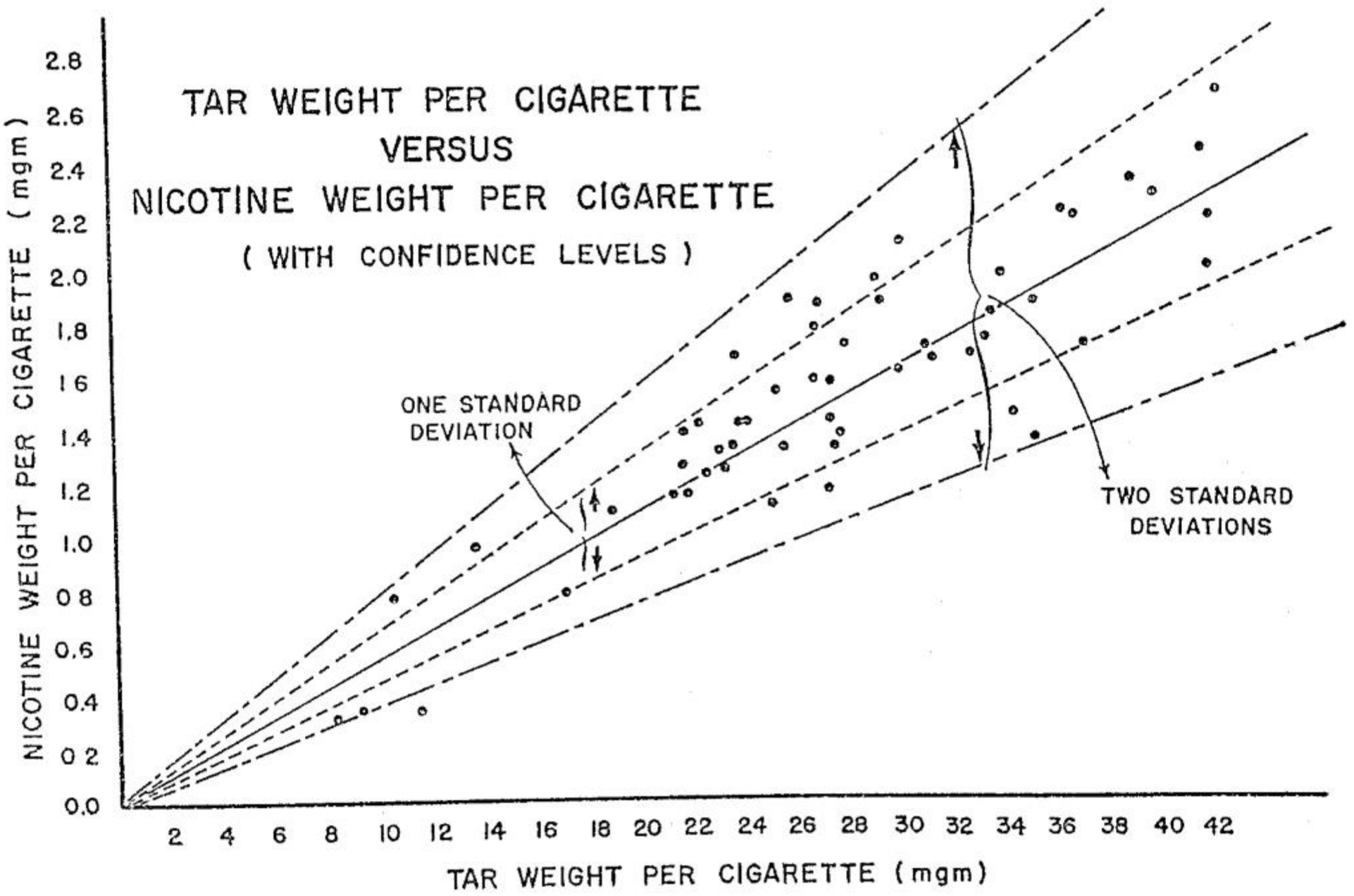
TEXT-FIGURE 4.—"Tar" delivery by 100 mm cigarettes with long and short filters.

Dilution of the mainstream smoke by air entering through porous paper or perforations near the butt also reduces "tar" yields. In the low delivery cigarettes shown in text-figures 1 and 2 this technique was employed. Combinations of effective filters and dilutions of smoke by air are very effective. They provide effective filtration with reasonable resistance to draw and, at the same time, reduce the proportion of air that is drawn through the burning portion of the cigarette.

Length of the cigarette is a very important factor in "tar" delivery. The recent development in cigarette merchandising of "super king" lengths (100 mm) is particularly disturbing. Overall, the 100 mm cigarettes deliver far more "tar" to the consumer than shorter cigarettes. In our tests, the median delivery of "tar" of 12 brands of 100 mm cigarettes was 37 mg/cigarette in comparison to 25 mg/cigarette of "tar" per king size cigarette. Only 1 of the 100 mm brands delivered less than 25 mg of "tar," that being the brand with a 50 mm overwrap. The other 11 all delivered at least 30 mg and 8 delivered more than 35 mg/cigarette. Therefore, they are very high delivery cigarettes and are the most dangerous cigarettes introduced on the market for some time.

NICOTINE LEVELS

This report does not dwell greatly on the levels of nicotine in commercial cigarettes. Generally, any method that reduces the delivery of "tar" will also reduce the level of nicotine delivery. Indeed, for most commercial cigarettes the levels of "tar" and nicotine in the smoke are closely correlated, as seen in text-figure 5. For most of the brands, the nicotine level falls within one standard deviation of a regression line. However, it is possible for the industry to reduce the nicotine level independently of "tar" levels or to increase it. A few brands are noteworthy in that they have proportionately less nicotine than would be expected on the basis of "tar" delivery. Methods by which nicotine can be lowered include selection of



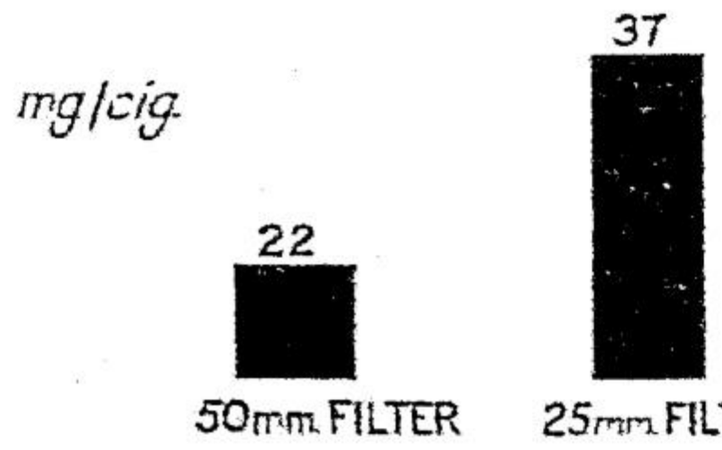
TEXT-FIGURE 5.—Dependence of nicotine delivery on "tar" delivery of commercial cigarettes [from Moore *et al.* (*10*)].

Text-figure 5 is reproduced through the courtesy of the editor of *Cancer*.

varieties of tobacco, cultivation practices on the part of the farmers, the use of reconstituted tobacco sheet, and selective removal of nicotine from the tobacco during cigarette manufacture. Presently, delivery of nicotine among commercial cigarettes ranges from 0.2 mg/cigarette–2.6 mg/cigarette. Thus, there is evidence that low nicotine cigarettes are also acceptable to a segment of the population.

SUMMARY

The range of "tar" delivered by commercial American cigarettes is from 4–48 mg/cigarette. The range of nicotine delivery is from 0.2–2.6 mm/cigarette. Generally, reduction of "tar" delivery is accompanied by a corresponding reduction in nicotine delivery. However, a few brands with proportionately low nicotine levels do appear on the market. "Tar" levels can be reduced with an effective filter, a long overwrap, perforations near the butt, and by restriction of the length of the cigarette. The existence of low "tar" and nicotine cigarettes on the market, despite the lack of extensive promotion by the industry, indicates consumer acceptability of such products.



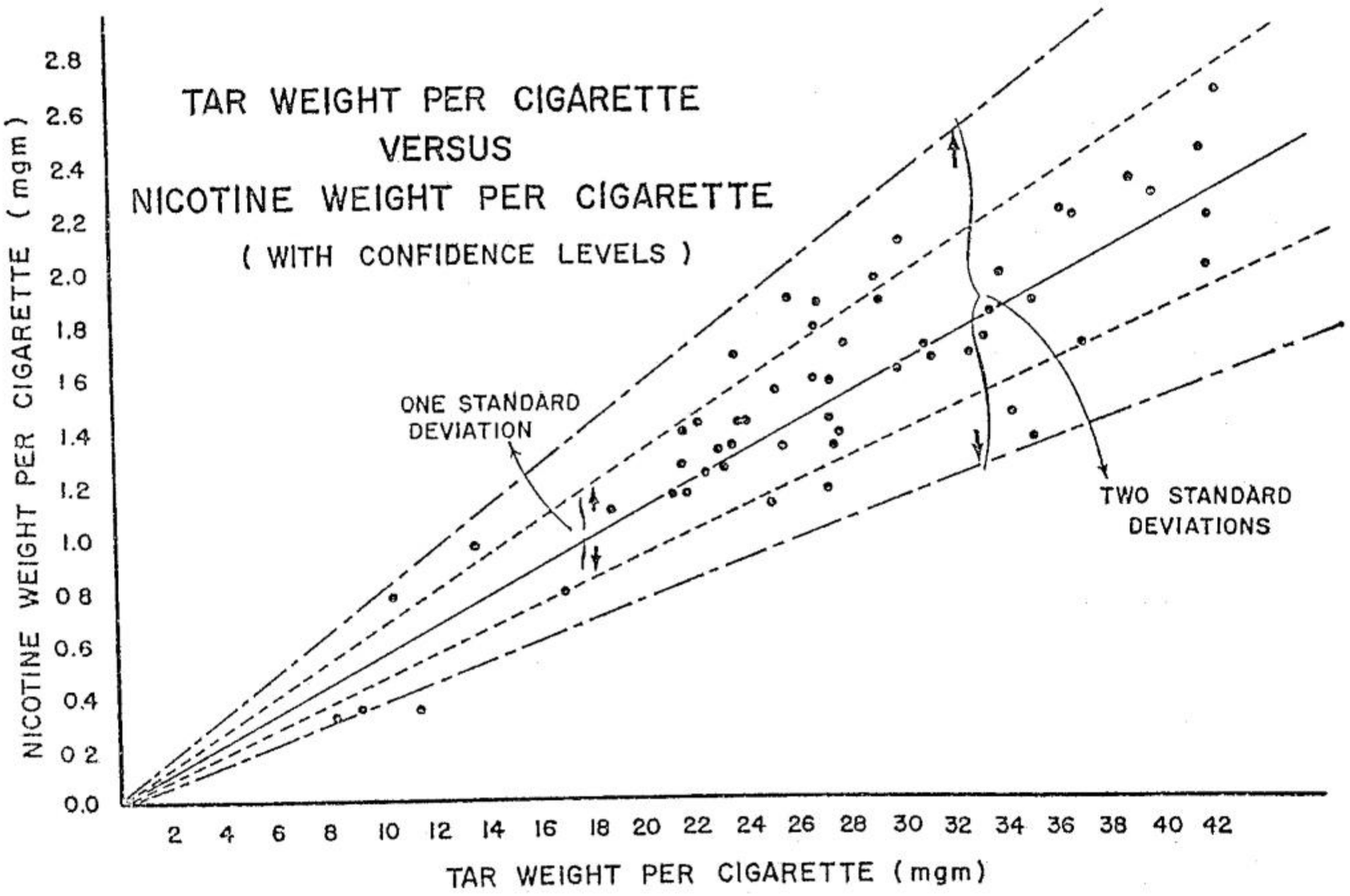
TEXT-FIGURE 4.— "Tar" delivery by 100 mm cigarettes with long and short filters.

Dilution of the mainstream smoke by air entering through porous paper or perforations near the butt also reduces "tar" yields. In the low delivery cigarettes shown in text-figures 1 and 2 this technique was employed. Combinations of effective filters and dilutions of smoke by air are very effective. They provide effective filtration with reasonable resistance to draw and, at the same time, reduce the proportion of air that is drawn through the burning portion of the cigarette.

Length of the cigarette is a very important factor in "tar" delivery. The recent development in cigarette merchandising of "super king" lengths (100 mm) is particularly disturbing. Overall, the 100 mm cigarettes deliver far more "tar" to the consumer than shorter cigarettes. In our tests, the median delivery of "tar" of 12 brands of 100 mm cigarettes was 37 mg/cigarette in comparison to 25 mg/cigarette of "tar" per king size cigarette. Only 1 of the 100 mm brands delivered less than 25 mg of "tar," that being the brand with a 50 mm overwrap. The other 11 all delivered at least 30 mg and 8 delivered more than 35 mg/cigarette. Therefore, they are very high delivery cigarettes and are the most dangerous cigarettes introduced on the market for some time.

NICOTINE LEVELS

This report does not dwell greatly on the levels of nicotine in commercial cigarettes. Generally, any method that reduces the delivery of "tar" will also reduce the level of nicotine delivery. Indeed, for most commercial cigarettes the levels of "tar" and nicotine in the smoke are closely correlated, as seen in text-figure 5. For most of the brands, the nicotine level falls within one standard deviation of a regression line. However, it is possible for the industry to reduce the nicotine level independently of "tar" levels or to increase it. A few brands are noteworthy in that they have proportionately less nicotine than would be expected on the basis of "tar" delivery. Methods by which nicotine can be lowered include selection of



TEXT-FIGURE 5.—Dependence of nicotine delivery on "tar" delivery of commercial cigarettes [from Moore *et al.* (*10*)].

Text-figure 5 is reproduced through the courtesy of the editor of *Cancer*.

varieties of tobacco, cultivation practices on the part of the farmers, the use of reconstituted tobacco sheet, and selective removal of nicotine from the tobacco during cigarette manufacture. Presently, delivery of nicotine among commercial cigarettes ranges from 0.2 mg/cigarette–2.6 mg/cigarette. Thus, there is evidence that low nicotine cigarettes are also acceptable to a segment of the population.

SUMMARY

The range of "tar" delivered by commercial American cigarettes is from 4–48 mg/cigarette. The range of nicotine delivery is from 0.2–2.6 mm/cigarette. Generally, reduction of "tar" delivery is accompanied by a corresponding reduction in nicotine delivery. However, a few brands with proportionately low nicotine levels do appear on the market. "Tar" levels can be reduced with an effective filter, a long overwrap, perforations near the butt, and by restriction of the length of the cigarette. The existence of low "tar" and nicotine cigarettes on the market, despite the lack of extensive promotion by the industry, indicates consumer acceptability of such products.

REFERENCES

(1) WYNDER, E. L., KOPF, P., and ZIEGLER, H.: A study of tobacco carcinogenesis. II. Dose-response studies. Cancer 10: 1193–1200, 1957.

(2) BOCK, F. G., MOORE, G. E., and CLARK, P. C.: Carcinogenic activity of cigarette smoke condensate. III. Biological activity of refined tar from several types of cigarettes. J Nat Cancer Inst 34: 481–493, 1965.

(3) HAMMOND, E. C., and HORN, D.: Smoking and death rates. Report on forty-four months of follow-up of 187,783 men. II. Death rates by cause. JAMA 166: 1294–1308, 1958.

(4) DORN, H. F.: Tobacco consumption and mortality from cancer and other diseases. Acta Un Int Cancr 16: 1653–1665, 1960.

(5) BROSS, I. D. J.: Effect of filter cigarettes on the risk of lung cancer. Nat Cancer Inst Monogr 28: 35–40, 1968.

(6) U.S. PUBLIC HEALTH SERVICE. The health consequences of smoking. A Public Health Service Review: 1967. Pub Health Service Publ No. 1696.

(7) MILLER, L. M., and MONAHAN, J.: To the cigarette makers: Just the facts, please. The Reader's Digest 89 (No. 535): 61–67 (Nov) 1966.

(8) Cigarettes: Consumer Reports 29: 114–115 (Mar) 1964.

(9) WYNDER, E. L., and HOFFMANN, D.: Some practical aspects of the smoking-cancer problem. New Eng J Med 262: 540–545, 1960.

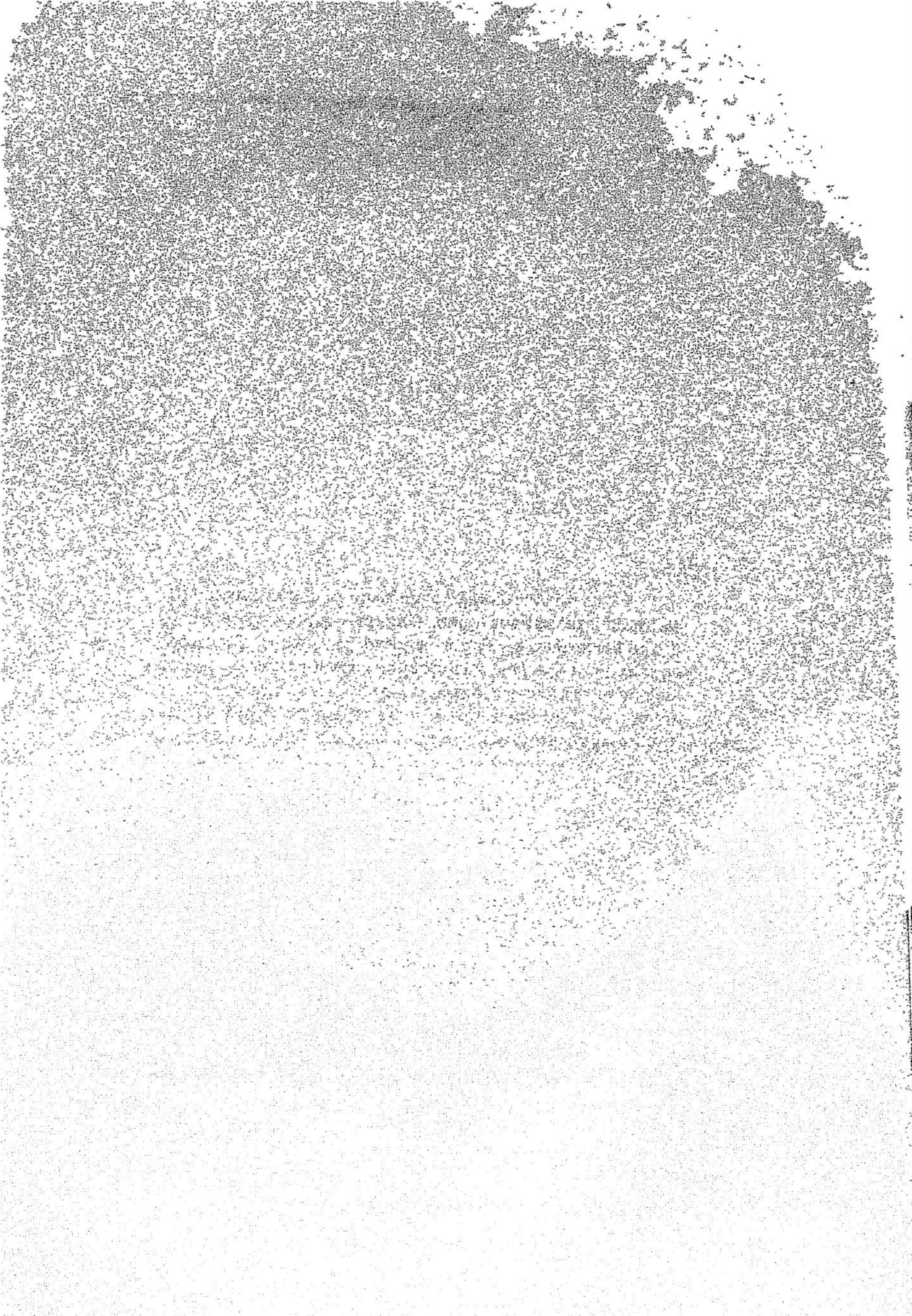
(10) MOORE, G. E., BROSS, I., SHAMBERGER, R., and BOCK, F. G.: Tar and nicotine retrieval from fifty-six brands of cigarettes. Cancer 20: 323–332, 1967.

(11) HAMMOND, E. C.: Lung cancer death rates in England and Wales compared with those in the U.S.A. Brit Med J 2: 649–654, 1958.

Chapter II

MEASURES FOR REDUCING "TAR" AND NICOTINE LEVELS IN CIGARETTE SMOKE

The "tar" and nicotine yield of cigarettes can be significantly affected through farm production practices, the use of reconstituted tobacco leaves, and mechanical filtration. In addition, means are available of selectively reducing the nicotine in tobacco through a variety of treatments of the tobacco. More work was suggested in the area of increasing the amount of bound nicotine in tobacco smoke.



Effect of Farm Production Practices on Nicotine and Total Particulate Matter in Cigarette Smoke

T. C. Tso, *Ph.D.*, *U.S. Department of Agriculture, Agricultural Research Service, Crops Research Division, Beltsville, Maryland 20705*

THE physical and chemical properties of leaf tobacco are determined by genetic makeup, weather conditions, cultural practices, soil differences, and curing methods. Characteristics of tobacco smoke are largely functions of chemical and physical properties of the leaf. A change in the production practice can change tobacco-leaf composition and therefore may affect the properties of tobacco smoke.

For many years plant scientists have "synthesized" new tobacco varieties for disease resistance. Such varieties may differ in botanical characteristics as well as in chemical constituents. This principle may well be applied to health-related tobacco research, if special chemical compounds are shown to be important in this regard. For example, in an examination of some randomly selected tobacco samples from various genetic backgrounds and produced under various cultural and curing practices, wide differences in major chemical components were found in the cured leaf. There are 3-fold differences in sterol content (0.1–0.3%), 10-fold differences in nitrate content (0.14–1.48%), 30-fold differences in alkaloid content (0.17–4.93%), and 5-fold differences in phenolic content (0.52–2.61%) (*1*). Still greater variations probably exist among samples not yet studied. By incorporating such variations through breeding and tobacco production, modification of smoke characteristics appears feasible.

MATERIAL AND METHODS

To examine possible differences in nicotine and total particulate matter (TPM) content in smoke from cigarettes made of tobacco obtained by variation in farm production practices, a special study was conducted with three groups of samples: samples of different genetic makeup, or varietal differences; samples of various cultural practices, including rate

of nitrogen fertilization and methods of sucker control; and samples of various postharvest handling, including tobacco harvested at different degrees of ripeness and at different speeds of curing. These samples were produced through the cooperation of many U.S. State Experiment Stations and involved flue-cured and Burley tobacco. Detailed treatments are clarified under "Results." Manufacture of cigarettes and determination of nicotine and TPM in cigarette smoke were through the courtesy of the tobacco industry.[1]

Results of cigarette and smoke characteristics on variety, nitrogen fertilization, and curing method are based on 20 ports smoking tests, while results on sucker control material and ripeness-curing material are based on 10 ports smoking tests. Cigarettes were of normal firmness and 70 mm long, and they were smoked under conditions described by Ogg (*2*).

RESULTS

In examining these data, one should bear in mind that they are based on only a limited sampling of 1 year's crop. Also, in the absence of any information relating to the specific composition of the TPM from each sample, the relative amount of TPM reported here is only an indication of variation among different treatments.

Samples of Different Genetic Makeup

We observed 6 flue-cured varieties, including Coker 139, PD 33, N.C. 402, N.C. 95, Hicks, and S.C. 58. Coker 139 is a discount variety with a low nicotine content and "flat flavor"; PD 33 is a low-nicotine breeding line; N.C. 402 is an older broadleaf, flue-cured type; N.C. 95 is a multiple disease-resistant tobacco; Hicks is a popular flue-cured variety, and S.C. 58 is a black shank-resistant Hicks type.

As shown in table 1, nicotine content in cigarette smoke, on either a per cigarette or a per puff basis, is in proportion to nicotine content in tobacco leaf. Because current knowledge of the inheritance of alkaloid in the tobacco plant is well advanced, it is possible to breed varieties of tobacco with a wide range of nicotine content (*3*).

Table 2 shows smoke properties and physical properties of cigarettes made from these 6 varieties. These varieties appeared to vary in weight per cigarette, which contributed to differences in pressure drop, rate of burn, and number of puffs per cigarette. These differences contributed to considerable differences in TPM yield per cigarette as well as per puff.

The above-mentioned results show that Coker 139 delivers less nicotine and TPM to the smoke than some other varieties. However, this variety

[1] We thank The American Tobacco Co., Liggett and Myers Tobacco Co., Philip Morris, Inc., and R. J. Reynolds Tobacco Co. for their cooperation.

TABLE 1.—Variety differences (flue-cured tobacco) and nicotine content

	Nicotine in tobacco (%)	Nicotine in smoke* (mg/cig.)	Nicotine in smoke* (mg/puff)
Coker 139	1. 5	1. 3	0. 16
PD 33, low alkaloid	1. 6	1. 4	. 17
Hicks	2. 7	2. 5	. 31
N.C. 402	2. 9	2. 3	. 29
N.C. 95	2. 9	2. 5	. 32
S.C. 58	3. 4	3. 0	. 37

*70 mm cigarettes were smoked as described by Ogg (*2*).

TABLE 2.—Variety differences (flue-cured tobacco) and smoke properties

	Smoke properties				Physical properties	
	Number of puffs	Burn* rate (mm/min)	TPM† (mg/cig)	TPM/puff (mg/puff)	Weight (g)	Pressure drop (cm H_2O)
Coker 139	8. 0	3. 77	27	3. 3	0. 97	11. 3
PD 33, low alkaloid	8. 3	3. 51	28	3. 4	. 99	8. 6
Hicks	8. 0	3. 63	33	4. 1	. 99	7. 0
N.C. 402	7. 9	3. 73	29	3. 7	. 99	10. 5
N.C. 95	7. 7	3. 66	30	4. 0	. 97	8. 9
S.C. 58	8. 0	3. 27	34	4. 3	. 96	6. 2

*mm of cigarette burn per minute.
†Includes water.

lacked the flavor, showed no improvement in quality through aging, and has not met with public favor. The phenolic and sterol content in Coker 139 is much higher than in Hicks. The total phenolic content in Coker 139 is 1.4% versus 0.5% in Hicks, and the total sterol content in Coker 139 is 1.97 mg/g versus 1.38 mg/g in Hicks (*1*).

During recent years, many old varieties were discarded and many new ones were developed either for disease-resistance purposes or for high yield. An experiment is under way to compare 2 flue-cured varieties, Virginia Bright Leaf representing an old type and Coker 319 representing a modern disease-resistant variety. Smoke condensates from cigarettes made of these 2 varieties will be bioassayed to compare their biological activities.

Samples of Various Cultural Practices

Rate of nitrogen fertilization.—Air-cured Burley 37 tobacco and flue-cured Hicks tobaccos were produced with different rates of nitrogen fertilization and were used for cigarette smoking tests. Tables 3 and 4

TABLE 3.—Rate of nitrogen fertilization and nicotine content

	Nicotine in leaf (%)	Nicotine in smoke*	
		mg/cig.	mg/puff
Burley 37:			
0	2. 8	1. 7	0. 28
333 kg/ha (300 lb/acre)	5. 4	4. 1	. 57
Hicks:			
55.5 kg/ha (50 lb/acre)	1. 5	1. 4	. 17
122.1 kg/ha (110 lb/acre)	2. 1	1. 8	. 24

*70 mm cigarettes were smoked as described by Ogg (2).

TABLE 4.—Rate of nitrogen fertilization and smoke properties

	Smoke properties*				Physical properties	
	Number of puffs	Burn rate (mm/min)	TPM		Weight (g)	Pressure drop (cm H_2O)
			mg/cig.	mg/puff		
Burley 37						
0	6. 0	—	22	3. 7	0. 82	—
333 kg/ha (300 lb/acre)	7. 2	—	26	3. 6	. 97	—
Hicks						
55.5 kg/ha (50 lb/acre)	8. 2	3. 66	29	3. 5	1. 10	7. 9
122.1 kg/ha (110 lb/acre)	7. 5	4. 07	28	3. 7	. 94	7. 8

*70 mm cigarettes were smoked as described by Ogg (2).

show the nicotine content and smoke properties, respectively. The nicotine content is high whenever a high rate of nitrogen fertilization is used.

Regarding smoke properties, the two types of tobacco seemed to respond differently to nitrogen fertilization. A higher rate of this fertilization in Burley production resulted in more weight of tobacco per cigarette, higher number of puffs, and correspondingly more TPM. In flue-cured tobacco, a higher rate of nitrogen fertilization resulted in lesser weight of tobacco per cigarette, fewer puffs, and a higher burn rate and, therefore, less TPM.

Nitrogen fertilization of tobacco plants plays an important role in the smoke characteristics of cigarettes. It is well known that a higher nitrogen rate in tobacco fields results in a higher nitrogenous fraction in leaf tobacco. Recent reports have indicated that the addition of KNO_3 and $NaNO_3$ to tobacco reduces the concentration of benzo[*a*]pyrene and other polynuclear aromatic hydrocarbons (PAH) in cigarette smoke (*4, 5*). It is also reported that catechol content in cigarette smoke is inversely proportional to the amount of nitrate in the tobacco (*6*). This evidence suggests that a higher nitrogen fertilizer rate is desired in tobacco production. However, an increase of nitrogen in tobacco also increases the level of volatile bases,

including some secondary amines and also oxides of nitrogen. It has been speculated that this may create a favorable environment for possible formation of nitrosamines and other derivatives (*7*). Furthermore, one recent finding (*8*) showed that the amount of phenolic compounds, including chlorogenic acid, rutin, scopolin, and scopoletin, appears to be positively correlated with the rate of nitrogen fertilization. Similar increases of aromatic amino acids, phenylalanine, and tyrosine and their selective incorporation into phenolics are also observed (*9*).

This information illustrates the complexity of the metabolism within a tobacco plant and also the interrelationship among leaf composition and smoke characteristics. To study these problems and to see if they have any effect on observable biological activity, two experiments are in progress. Burley tobaccos were produced at Greeneville, Tennessee, with high levels of nitrogen (300 lb/A) and without nitrogen fertilization. These tobaccos were made into cigarettes and the smoke condensates are in the process of bioassay. Another experiment in progress is the production of tobacco under conditions believed favorable for the formation of precursors of nitrosamines. This tobacco is now harvested and will be made into cigarettes to study the level, if any, of nitroso compounds.

Aside from nitrogen, factors such as soil parent material and source of phosphate fertilizer may also affect smoke characteristics, especially radioelements (*10–12*).

Sucker Control Materials

In tobacco production, decapitation of the plant is necessary to obtain leaves with desired physical properties and chemical composition. The consequent growth of axillary buds, or "suckers," and the problem of inhibiting such growth are of great economic importance. A number of compounds, synthetic or natural, are known to effectively inhibit the growth of tobacco suckers. Some of them may induce further metabolic changes which affect the leaf and smoke characteristics.

Burley and flue-cured tobacco were used for this study. Cigarettes used were from 1966 Advanced Regional Sucker Control Tests and obtained from G. L. Steffens (*13*), who also provided information on leaf characteristics. Burley cigarettes were a blend of tobaccos produced at Waynesville, North Carolina; Greeneville, Tennessee; and Lexington and Princeton, Kentucky. Cigarettes made of flue-cured tobacco were separated into two different groups: One was from tobacco produced in North Carolina; the other was a mixture from Florida, Georgia, and South Carolina.

Tables 5 and 6 show levels of nicotine content in leaf tobacco and in smoke of Burley and flue-cured tobaccos, respectively. In Burley tobacco smoke, nicotine content was lowest in hand-suckered tobacco, while closely hand-suckered had the highest nicotine content. Among the others, methyl-pelargonate-treated tobacco delivered the lowest nicotine per cigarette.

TABLE 5.—Nicotine content in Burley sucker control samples

	Nicotine in leaf (percent)	Nicotine in smoke*	
		mg/cig.	mg/puff
Methyl pelargonate, 6%	3. 6	3. 0	0. 48
Methyl caprate, 6%	3. 2	3. 3	. 45
Octanol and decanol (1:1), 5%	4. 0	3. 4	. 46
Maleic hydrazide	3. 7	3. 6	. 46
Control			
Hand-suckered	4. 1	2. 9	. 49
Closely hand-suckered	4. 8	3. 8	. 60

*70 mm cigarettes were smoked as described by Ogg (2).

Methyl caprate (95% C_{10}, 3% C_8, and 2% C_{12} saturated) was second, and fatty alcohol (octanol and decanol 1:1) was third. Maleic-hydrazide-treated tobacco gave highest nicotine delivery among the four chemicals tested and was only next to closely hand-suckered tobacco.

Results given in table 6 on flue-cured cigarettes show that 2 groups are almost identical, in that tobacco treated with fatty alcohol, octanol, and decanol (1:1) delivered the lowest nicotine per cigarette among 8 treatments, including 6 chemicals and also hand-suckered and closely hand-suckered controls. However, when the nicotine content is calculated on per puff basis, some of the results are not so consistent.

The results of TPM contents of these samples are shown in tables 7 and 8. In Burley tobacco, hand-suckered controls delivered the lowest TPM per cigarette, closely hand-suckered tobacco the highest, and four chemically treated tobaccos were intermediate. Comparatively, methyl caprate

TABLE 6.—Nicotine content in flue-cured sucker-control samples

	A*			B†		
	Nicotine in leaf (percent)	Nicotine‡ in smoke		Nicotine in leaf (percent)	Nicotine‡ in smoke	
		mg/cig.	mg/puff		mg/cig.	mg/puff
Octanol and decanol (1:1), 5%	2. 5	1. 6	0. 25	3. 3	2. 6	0. 41
Methyl caprate, 6%	2. 4	1. 9	. 28	3. 0	3. 0	. 38
Octanol, 5%	2. 4	2. 0	. 29	3. 3	3. 1	. 41
Methyl pelargonate, 6%	2. 3	1. 9	. 27	3. 1	3. 0	. 39
MH analog§	2. 8	2. 2	. 30	3. 2	3. 4	. 41
Maleic hydrazide	2. 6	2. 3	. 28	3. 2	3. 3	. 41
Control						
Hand-suckered	2. 8	2. 0	. 29	3. 4	3. 3	. 42
Closely hand-suckered	3. 1	2. 5	. 32	3. 8	3. 9	. 46

*1960 samples from N.C.
†1960 samples from S.C., Ga., and Fla.
‡70 mm cigarettes were smoked as described by Ogg (2).
§Potassium salt of 6-hydroxy-2-methylol-3(2H)pyridazinone.

TABLE 7.—Characteristics of Burley tobacco, cigarettes, and smoke from sucker-control samples

	Methyl caprate, 6%	Methyl pelargonate, 6%	Octanol and decanol (1:1), 5%	Maleic hydrazide	Control	
					Hand-suckered	Closely hand-suckered
Leaf tobacco, filling value (60% RH) (ml/g)	4.19	4.47	4.17	4.05	4.34	4.30
Smoke*						
Number of puffs	7.3	6.2	7.4	7.8	5.9	6.3
TPM						
mg/cig.	23.0	24.0	24.0	24.0	22.0	25.0
mg/puff	3.1	3.8	3.2	3.1	3.7	4.0

*70 mm cigarettes were smoked as described by Ogg (3).

appeared to be the lowest among those treatments. In flue-cured samples, tobaccos treated with the fatty alcohol, octanol, and decanol mixture delivered the lowest TPM per cigarette in both areas. However, the data for TPM per puff do not show such consistent differences. Since chemical and physical properties of leaf tobacco, such as sugar content and filling value, may affect the weight per cigarette and its pressure drop, they may also affect the burn rate, and thus the number of puffs per cigarette. These and many other variables contribute to the differences in the amount of TPM per cigarette as well as per puff. The present information may well illustrate the importance of farm production practices on the composition of cigarette smoke.

Samples Resulting From Various Methods of Postharvest Handling

Tobacco harvested at various degrees of ripeness and cured at different yellowing rates.—Coker 139, produced in 1965 by J. M. Carr at Oxford, North Carolina, was used. Leaves were harvested at three different stages of ripeness—underripe, ripe, and overripe—and were cured at four different speeds—fast, normal, slow, and very slow. Cigarettes were supplied by J. A. Weybrew of North Carolina State University, who also provided the information on nicotine value of these tobaccos (*14*). The data for nicotine content in leaf tobacco and cigarette smoke are shown in table 9. These results, as expected, indicate that, when underripe tobacco was harvested, it had a lower nicotine content. The nicotine content increased gradually as the degree of leaf ripeness increased. The rate of leaf curing, however, appeared to have no effect on nicotine content in cigarette smoke.

The TPM content in cigarette smoke (table 10) appeared to follow the same pattern as nicotine. The TPM increased with ripeness and was not affected by curing rate.

Method of curing.—Curing is an important process in tobacco production and falls into the category of starvation phenomena of excised plant parts. It produces dried leaf of suitable physical properties and chemical composition. The 2 principal curing methods are air-curing and flue-curing. Air-curing is a slow and mild drying procedure in which tobacco leaves are usually not removed from the stalk. In flue-curing, leaves are primed from the stalks as they ripen and are cured in 4 or 5 days at elevated temperature, with ventilation and humidity controlled. In addition to dehydration, 2 most conspicuous chemical conversions take place during curing. The first phase of conversion is dominated by activities of hydrolytic enzymes and occurs in either flue-curing or stalk-curing; for example, disaccharides and polysaccharides hydrolyze to simple sugars, and protein hydrolyzes to amino acids which undergo oxidative deamination. The second phase of conversion is dominated by oxidative reaction and takes place only in stalk-cured tobacco; *e.g.*, simple sugars oxidize to acids, CO_2

TABLE 11.—Method of curing and nicotine content

	Nicotine in leaf (%)	Nicotine in smoke*	
		mg/cig.	mg/puff
Burley 21			
Air-cured	3. 2	2. 1	0. 31
Flue-cured	3. 1	2. 4	. 34
Hicks			
Air-cured†	3. 1	2. 5	. 35
Flue-cured‡	2. 7	2. 5	. 31

*70 mm cigarettes were smoked as described by Ogg (*2*).
†Sample from Oxford, N.C.
‡Mixed tobacco from Florence, S.C., and Oxford, N.C.

TABLE 12.—Method of curing and smoke properties

	Smoke properties*				Physical properties	
	Number of puffs	Burn rate (mm/min)	TPM (mg/cig.)	TPM (mg/puff)	Weight (g)	Pressure drop (cm H_2O)
Burley 21						
Air-cured	6. 9	4. 0	22	3. 2	0. 90	13. 3
Flue-cured	6. 9	3. 9	26	3. 8	. 92	10. 1
Hicks						
Air-cured†	7. 2	4. 0	37	5. 2	. 91	7. 1
Flue-cured‡	8. 0	3. 6	33	4. 1	. 99	7. 0

*70 mm cigarettes were smoked as described by Ogg (*2*).
†Sample from Oxford, N.C.
‡Mixed tobacco from Florence, S.C., and Oxford, N.C.

In recent years frequent changes have been made in curing methods, due to economics or fuel supply. In flue-curing, the old method was to use wood-curing furnaces and sheet metal flues with wood as a fuel; one current method is the use of open-flame, wick-type oil burners with kerosene. To study the influence of these different processes, we subjected tobaccos from two flue-cured varieties to these two curing methods. Smoke condensate from cigarettes made of these tobaccos will be assayed for their biological activities.

DISCUSSION

Plant growth and development are complicated biological phenomena dependent on genetic and environmental variables. In tobacco, these factors are further complicated by postharvest handling, such as curing, aging, and fermentation, which drastically change the leaf characteristics.

Leaf composition differs from crop to crop. Even within the same plant, leaves from various positions on the stalk vary considerably in their physical properties and chemical composition (*15–18*), as shown in table 13.

TABLE 9.—Nicotine content in smoke and leaf tobacco of varied ripeness and curing rate

		Curing rate				
		Fast	Normal	Slow	Very slow	Average
Underripe:	Leaf (%)	2. 8	3. 0	3. 1	3. 2	3. 0
	Smoke*					
	mg/cig.	2. 3	2. 3	2. 2	2. 4	2. 3
	mg/puff	0. 25	0. 25	0. 26	0. 26	0. 25
Ripe:	Leaf (%)	3. 0	3. 0	3. 3	3. 3	3. 1
	Smoke*					
	mg/cig.	2. 5	2. 4	2. 6	2. 5	2. 5
	mg/puff	0. 28	0. 27	0. 29	0. 28	0. 28
Overripe:	Leaf (%)	3. 5	3. 5	3. 6	3. 7	3. 6
	Smoke*					
	mg/cig.	2. 6	2. 7	2. 6	2. 7	2. 6
	mg/puff	0. 30	0. 31	0. 31	0. 30	0. 31
Average:	Leaf (%)	3. 1	3. 2	3. 4	3. 5	
	Smoke*					
	mg/cig.	2. 5	2. 5	2. 5	2. 5	
	mg/puff	0. 28	0. 28	0. 28	0. 28	

*70 mm cigarettes were smoked as described by Ogg (2).

TABLE 10.—TPM content (mg) in cigarette smoke* from flue-cured tobacco varying in degree of ripeness and curing rate

		Curing rate				
		Fast	Normal	Slow	Very slow	Average
Underripe:	Per cig.	24	23	23	24	24
	Per puff	2. 5	2. 6	2. 6	2. 6	2. 6
Ripe:	Per cig.	25	25	25	24	25
	Per puff	2. 7	2. 8	2. 7	2. 7	2. 7
Overripe:	Per cig.	26	25	25	26	26
	Per puff	3. 1	2. 9	3. 1	2. 9	3. 0
Average:	Per cig.	25	25	24	25	
	Per puff	2. 8	2. 8	2. 8	2. 7	

*70 mm cigarettes were smoked as described by Ogg (2).

and H_2O, amino acids to ammonia and amides, and oxidation and polymerization of phenols to brown products.

Different types of tobacco, such as Burley and flue-cured, are from different varieties and are grown under different field culture practices and cured according to type. The purpose of the present study was to subject the green tobacco produced under one condition to two different curing methods. Burley 21, an air-cured type, was air-cured and also flue-cured. Hicks, a flue-cured type, was flue-cured and also air-cured. Table 11 shows the effect of the curing method on nicotine content, and table 12 shows the effect on smoke properties. Air-curing resulted in a higher nicotine content in the leaf in both cases. Flue-curing appeared to decrease the filling power, and hence the flue-cured cigarettes are heavier.

TABLE 11.—Method of curing and nicotine content

	Nicotine in leaf (%)	Nicotine in smoke*	
		mg/cig.	mg/puff
Burley 21			
Air-cured	3. 2	2. 1	0. 31
Flue-cured	3. 1	2. 4	. 34
Hicks			
Air-cured†	3. 1	2. 5	. 35
Flue-cured‡	2. 7	2. 5	. 31

*70 mm cigarettes were smoked as described by Ogg (*2*).
†Sample from Oxford, N.C.
‡Mixed tobacco from Florence, S.C., and Oxford, N.C.

TABLE 12.—Method of curing and smoke properties

	Smoke properties*				Physical properties	
	Number of puffs	Burn rate (mm/min)	TPM (mg/cig.)	TPM (mg/puff)	Weight (g)	Pressure drop (cm H_2O)
Burley 21						
Air-cured	6. 9	4. 0	22	3. 2	0. 90	13. 3
Flue-cured	6. 9	3. 9	26	3. 8	. 92	10. 1
Hicks						
Air-cured†	7. 2	4. 0	37	5. 2	. 91	7. 1
Flue-cured‡	8. 0	3. 6	33	4. 1	. 99	7. 0

*70 mm cigarettes were smoked as described by Ogg (*2*).
†Sample from Oxford, N.C.
‡Mixed tobacco from Florence, S.C., and Oxford, N.C.

In recent years frequent changes have been made in curing methods, due to economics or fuel supply. In flue-curing, the old method was to use wood-curing furnaces and sheet metal flues with wood as a fuel; one current method is the use of open-flame, wick-type oil burners with kerosene. To study the influence of these different processes, we subjected tobaccos from two flue-cured varieties to these two curing methods. Smoke condensate from cigarettes made of these tobaccos will be assayed for their biological activities.

DISCUSSION

Plant growth and development are complicated biological phenomena dependent on genetic and environmental variables. In tobacco, these factors are further complicated by postharvest handling, such as curing, aging, and fermentation, which drastically change the leaf characteristics.

Leaf composition differs from crop to crop. Even within the same plant, leaves from various positions on the stalk vary considerably in their physical properties and chemical composition (*15–18*), as shown in table 13.

Leaves from the lower positions deliver less TPM in smoke than those from middle and upper positions. However, leaves from upper and middle positions are more aromatic than those from lower positions (table 14). Analysis of linear correlations between the chemical components and certain physical quality factors was attempted on flue-cured and Burley tobacco (*19*). A few of these correlations are listed in table 15. Leaves of greater porosity generally have a higher rate of burn.

TABLE 13.—Stalk positions and leaf characteristics

	Lower leaves	Middle leaves	Upper leaves*
Flue-cured tobacco			
Cell membrane substances (*15*)	Comparatively higher	Comparatively lower	Comparatively lower
Total sugar (*16*)	Lower	Higher	Lower
Total acid (*16*)	Higher	Lower	Medium
α-amino-N (*16*)	Higher	Lower	Higher
Nicotine (*16*)	Lower	Medium	Higher
Water-soluble N, total N (*16*)	Medium	Lower	Higher
Soluble ash (*16*)	Higher	Lower	Medium
Tannins, resins (*16*)	Lower	Higher	Higher
*p*H (*16*)	Higher	Lower	Lower
Air-cured Burley (*17, 18*)			
Color	Lighter	Darker	Darker
Porosity	More	Less	Less
Density	Lighter	Heavier	Heavier
Ammonium-N, Amino-N, Amido-N	Lower	Medium	Higher
Nicotine-N	Lower	Medium	Higher

*Not including uppermost tips.

TABLE 14.—Stalk positions and smoking properties (*15*)

	Lower leaves	Upper and middle leaves
Strength (N compounds)	Relatively light	Relatively strong
Aromaticity (tannins, resins)	Aromatic	Highly aromatic
Mildness and aromaticity (sugars, starch, oxalic acid)	Mild	Sharp
Sharpness (cell membrane substances, ash constituents, organic acid-citric)	Mild	Sharp

Any change in farming practices will affect the chemical components of tobacco and hence its smoke composition. The problem is not merely how to reduce TPM or nicotine content in smoke, but also how to maintain desirable leaf quality at the same time. Basic knowledge is needed on the metabolism of tobacco plants and its regulation in a favorable direction. For example, it will be most desirable to permit leaves of all stalk levels

TABLE 15.—Linear correlation between chemical components and certain physical quality factors of 1952 crop (*19*)

	Flue-cured		Burley	
	Rate of burn	Porosity	Rate of burn	Porosity
Alkaloids	−0.19	−0.44	+0.16	−0.70
Ash	+.37	+.78	+.44	+.83
Cellulose	+.44	+.74	+.23	+.75
Crude fiber	+.44	+.70	+.27	+.65
Total N	+.07	−.32	−.44	−.93
Petroleum ether extract	+.27	+.75	+.27	+.32
Total reducing sugar	−.36	−.26	−.11	−.52
Total volatile acids	+.36	+.46	+.47	+.24
Total volatile bases	+.03	−.33	−.31	−.92
Coefficient of variation	.08	.24	.18	.18

to mature uniformly and to harvest the leaves at the same time, to narrow the chemical and physical differences among lower, middle, and upper leaves. This can be achieved only by the joint efforts of research workers in the genetic, physiological, and phytochemical fields who should take part in the search for breeding lines, studies of growth characteristics, and the use of growth regulators.

Studies are in progress on the genetics, cultural effects, biosynthetic pathways, and metabolism of several groups of organic components, including phenolics, flavonoids, sterols, fatty acids, and nitrogenous compounds, especially secondary amines. Preliminary findings indicate that farm production practices account for wide variation in the levels of these compounds (*1*, *20*). Experience in the development of disease-resistant lines in tobacco, as well as the selection of quantity and quality of various tobacco alkaloids (*3*), may well serve as reference in our approach to research problems related to health.

SUMMARY

Information presented in this report demonstrates that genetic background, cultural practices, and curing methods affect the chemical and physical properties of leaf tobacco and, therefore, the nicotine and total particulate matter (TPM) content in cigarette smoke. The nicotine content in tobacco smoke seems largely dependent on nicotine content of leaf tobacco, but TPM of the smoke seems to be more related to the amount of tobacco required to make the cigarette, as well as the density and total number of puffs. Additional studies are under way to compare the biological activity of smoke condensates from tobaccos of old and new varieties, high and low nitrogen fertilization, open-flame and closed flue-curing systems, and also to examine for the presence of a number of chemical substances in leaf tobacco and in cigarette smoke from tobacco

produced under specially designed conditions. Studies are also in progress on the genetics, cultural effects, biosynthesis, metabolism, and means of regulation of several groups of organic components suspected of being related to health problems. From these findings, adjustment can be made in breeding and in farm production practices to modify nicotine content of tobacco smoke in a desirable direction. To a lesser degree, the TPM content can also be modified. Perhaps more importantly, the opportunity exists through genetics and cultural practices to modify the composition of the TPM.

REFERENCES

(*1*) Kentucky University, Lexington, Kentucky, and CRD, ARS, U.S. Department of Agriculture contract and in-house research: Unpublished data, 1967.

(*2*) Ogg, C. L.: Determination of particulate matter and alkaloids in cigarette smoke. J Assoc Offic Agr Chem 47: 356–362, 1964.

(*3*) Moore, E. L., Gwynn, G. R., and Powell, N. T.: Some alkaloid relationships among seedlings and mature plants of flue-cured tobacco. Tobacco Sci 7: 170–175, 1963.

(*4*) Bentley, H. R., and Burgan, J. G.: Polynuclear hydrocarbons in tobacco and tobacco smoke. III. The inhibition of the formation of 3,4-benzopyrene in cigarette smoke. Analyst 85: 727–730, 1960.

(*5*) Hoffmann, D., and Wynder, E. L.: Selective reduction of the tumorigenicity of tobacco smoke. Experimental approaches. Nat Cancer Inst Monogr 28: 151–172, 1968.

(*6*) Kallianos, A. G., Means, R. E., and Mold, J. D.: Effect of nitrate in tobacco on the catechol yield in cigarette smoke. In 20th Tobacco Chemists' Res. Conf., Winston-Salem, North Carolina 1966, pp 21–23.

(*7*) Neurath, G., Pirmann, B., Lüttich, W., and Wichern, H.: Zur Frage der N-Nitroso-Verbindungen im Tabakrauch, II. Beitr Tabakforsch 3: 251–262, 1965.

(*8*) Tso, T.C., Sorokin, T. P., Engelhaupt, M. E., Andersen, R. A., Bortner, C. E., Chaplin, J. F., Miles, J. D., Nichols, B. C., Shaw, L., and Street, O. E.: Nitrogenous and phenolic compounds of *Nicotiana* plants. I. Field and greenhouse grown plants. Tobacco Sci 11: 133–136, 1967.

(*9*) Tso, T. C., Sorokin, T. P., Chu, H., Schaeffer, G. W., and Burk, L. G.: Nitrogenous and phenolic compounds of *Nicotiana* plants. II. Selective incorporation of aromatic amino acids in phenolic compounds of tumorous *Nicotiana* hybrids. Botan Bull Acad Sinica. In press.

(*10*) Tso, T. C., Hallden, N. A., and Alexander, L. T.: Radium-226 and polonium-210 in leaf tobacco and tobacco soil. Science 146: 1043–1045, 1964.

(*11*) Casarett, L. J.: Role of radioactive substances in effects of smoking. Nat Cancer Inst Monogr 28: 199–209, 1968.

(*12*) Tso, T. C.: Po^{218} and Pb^{210} in tobacco. Nat Cancer Inst Monogr 28: 211, 1968.

(*13*) Steffens, G. L., and Spaulding, D. W.: Unpublished information from 1966 Regional flue-cured and Burley sucker control tests.

(*14*) Weybrew, J. A.: Personal communication.

(*15*) Brückner, H.: Die Biochemie des Tabaks und der Tabakverarbeitung. Verlagsbuchhandlung Paul Parey, Berlin, Germany, 1936, pp 280–330.

(*16*) Darkis, F. R., Dixon, L. F., Wolf, F. A., and Gross, P. M.: Flue-cured tobacco —correlation between chemical composition and stalk position of tobaccos produced under varying weather conditions. Ind Eng Chem 28: 1214–1223, 1936.

(*17*) HARLAN, W. R., and MOSELEY, J. M.: Tobacco. *In* Encyclopedia of Chemical Technology, vol 14. New York, Interscience Encyclopedia, 1955, pp 242–261.

(*18*) MOSELEY, J. M., HARLAN, W. R., and HANMER, H. R.: Burley tobacco—relation of the nitrogenous fractions to smoking quality. Ind Eng Chem 43: 2343–2347, 1951.

(*19*) U.S. Dept. Agr., AMS Tobacco Div. Techn. Res. Paper Series 1–4, June 1958.

(*20*) CHENG, A. L. S., CHAPLIN, J. F., and TSO, T. C.: Sterol variation in flue-cured tobacco varieties. Tobacco Sci 12: 33–36, 1968.