an upper limit around 40 percent. Above this presumed limit it seems that the TdR transport system is more active and more TdR is transported by the cell in response to the large increase in DNA synthesis. This suggestion is supported by the facts that the total uptake in the normal growing liver does not change during the day and the requirement of TdR due to the diurnal increase of DNA synthesis hardly goes over 40 percent of total uptake, and in the regenerating liver when the incorporation into DNA does not exceed 40 percent, the uptake is constant and about at the same level as normal liver, that is, about 40×10^4 disintegrations per minute per gram of fresh tissue (see data at 16 and 21 hours in Fig. 2A and at 16 hours in 2B and 2C).

Since our findings present clear evidence for a constant diurnal rhythm of DNA synthesis both in normal and in regenerating rat liver, we believe that this emphasizes the need for a precise knowledge and control of the feeding schedule on which the animals are maintained in order to obtain more useful information from the experiments.

The nature and the timing of the stimulus or stimuli responsible for the diurnal rhythm of DNA synthesis both in normal growing and regenerating rat liver are not known. Nevertheless, the present study makes it reasonable to believe that the stimuli may vary in relation to food intake and changes from the dark period to the light period of the day or vice versa. It appears that the first postoperative peak may represent a population of cells that has passed an environmentally controlled point in the cell cycle, while the following peak appears to reveal another population of cells that depend on environmental controls in order to move into the cell replication cycle (*19*).

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19. While our manuscript was in preparation H. Barbason (*20*) published the results of experiments on the effect of "circadian rhythm" on mitotic index after partial hepatectomy. He reports rhythmic periodicity of mitosis at 24-hour intervals. Nevertheless, it must be pointed out that his feeding schedule is a "natural" one as he did not use artificial light and reported 14 hours light and 8 hours darkness, with food always available. In this way the rats were in a rather reproducible environment, but, at the same time, their feeding schedule was unknown. We have also seen data from experiments by J. Echave-Llanos (*21*) demonstrating a correlation between labeling index, mitotic count, and diurnal variations in light, and voluntary food intake in rats.
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Cigarette Smoking: Objective Evidence for Lung Damage in Teen-Agers

Abstract. *High school students with 1 to 5 years' smoking experience have excessive cough, sputum production, and shortness of breath. When maximum expiratory flow is plotted against maximum expired volume, the curves of nonsmokers and smokers differ in shape. The smokers have lower flow rates at mid-vital capacity and at lower lung volumes. This probably reflects small airway obstruction in the smokers.*

We present objective evidence of functional changes in the lungs of teen-agers who smoke only a few years. At least part of this damage to the lungs might be reversible on cessation of smoking. However, permanent effects, including premature arrest of lung development, cannot be excluded at this time.

Although the effects of smoking as a cause of lung cancer and other diseases appear to be well known among young people, antismoking propaganda does little to reduce smoking (*1*), and it is even suspected that smoking among teen-agers may increase rather than decrease (*2*). College seniors and others in their twenties who smoke have reduced lung function (*3*), but at that age smoking habits may already be fixed (*4*). Since objective evidence of damage to their own lung function might be more convincing to teen-agers than the faraway danger of lung cancer, we therefore looked for such evidence in 365 students of four high schools in the New Haven area. There were 195 boys and 170 girls, aged 15 to 19 years; of these 50 percent of the boys and 37 percent of the girls were regular smokers. Most of the girls (23 percent) were light smokers, with only 1 percent smoking more than 20 cigarettes daily. Most of the boys (26 percent) were moderate smokers (11 to 20 cigarettes per day), with 8 percent smoking more than 20 cigarettes daily.

In selecting a sensitive test for lung function we used the fact that expiratory flow rates are nearly independent of the subject's effort during the last portion of a maximally forced expiration after a full inspiration. During that maneuver, the large intrathoracic airways are compressed because of the high pressures generated in the chest. Under these conditions, more effort does not result in higher flow rates. Rather, the supply of air from the al-

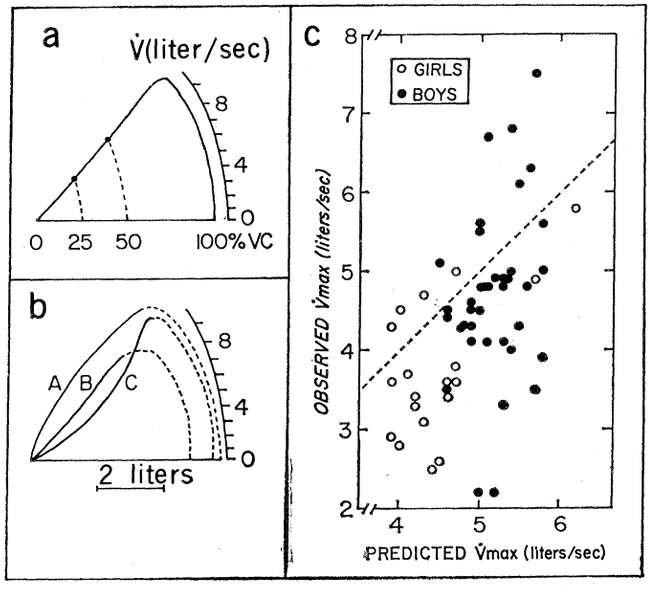

Fig. 1. Maximum expiratory flow-volume curves. (a) Schematic, to show readings of maximum flow rates at 25 and 50 percent of vital capacity (VC). Maximum inspiration is 100 percent VC; maximum expiration is 0 percent VC. Pen deflection is curvilinear; hence the curved ordinate. Curve starts at 100 percent VC. Initially, expired flow rates are high (to 9 liter/sec in this example); later, they decline to 0 at the end of the forced expiration. (b) MEFV curves in three 18-year-old boys. Subject A is a nonsmoker with no respiratory symptoms. Subject B smoked less than one cigarette per week for 2 years; he, too, had no respiratory symptoms. Subject C smoked 16 cigarettes per day since age 13, and has slight shortness of breath on exertion but no other respiratory symptoms. The differences in vital capacity are related to height (A, 190 cm; B, 171.5 cm; and C, 176.5 cm). (c) Observed compared to predicted values of $\dot{V}_{max}$ at 50 percent VC in boys smoking more than 15 cigarettes per day and in girls smoking more than 15 cigarettes per day. Line of equality (dashed) represents data of the average nonsmoker. The regression equations used for the predicted values (from data in nonsmokers) for $\dot{V}_{max}$ at 50 percent VC are (i) for boys $= -5.75057 + [0.28215 \times \text{age}] + [0.03755 \times \text{height}] - [0.00834 \times \text{weight}]$; and (ii) for girls $= -5.35737 + [0.20808 \times \text{age}] + [0.02828 \times \text{height}] + [0.03036 \times \text{weight}]$, where the age is in years, the height in centimeters, and weight in kilograms.

veoli sets the limits for maximum expiratory flow rates (*5*). Thus, these maximum flow rates are sensitive indices of slight narrowing of the small airways which is induced by drugs that cause contraction of the smooth muscle in the walls of the airway (*6*). In addition, measurements of maximum expiratory flow rates, at lung volumes from 10 percent to 75 percent of the vital capacity, have been used to distinguish between nonsmokers and smokers (*3*). This suggested to us that narrowing of small airways might be an early effect of smoking, and that maximum flow rates might detect these effects even when more conventional indices of lung functions are still normal.

Our subjects were studied with their own and with parental consent. This limited the study to those whose parents reacted positively to our explanatory letter. Most of the subjects (235 out of 365) were seen in two schools where the total response rate was 90.4 percent. That is, 235 out of a total of 260 students in specified classes participated. The remaining 130 subjects, in two other schools, were essentially volunteers. Since the results did not differ significantly between these groups, we pooled the data. The percentage of smokers (58 percent) among our subjects is similar to that found by Salber (*4*) among high school seniors. Although subtle sources of selection bias are always difficult to exclude in studies of this kind, we believe that our sample is representative of the general population of this age range. We avoided measuring acute effects of smoking (*7*) by asking students not to smoke on the day of the tests, which were done early in the morning in the schools. Two separate lung function tests which measured different aspects of maximally forced expirations were used. A direct-reading spirometer (*8*) was used to measure the air volume that can maximally be exhaled in 1 second (FEV_1) and the full volume range of the forced expiration, that is, the forced vital capacity (FVC). Each subject made five blows into the machine; the average of the two highest FEV_1 and FVC values was taken as the result. The same maneuver, a maximum inspiration followed by a forced and maximum expiration, was also used to record instantaneous expiratory flow rates versus lung volume with the flow-volume spirometer designed by Peters *et al.* (*9*). This recording is the MEFV curve. At least three curves were recorded, and the outer contour of these nearly superimposable curves was used. We read the highest values of the maximum expiratory flow rates ($\dot{V}_{max}$) at 50 percent and at 25 percent of the vital capacity (see Fig. 1a). The response characteristics of the flow-volume spirometer are insufficient for accurate recording of the very rapid events at the beginning of the forced expiration; these include the peak expiratory flow rate. After all function tests were completed, one of us filled out a questionnaire on respiratory symptoms for each student. The students themselves provided a detailed smoking history on a separate form that was later attached to the main questionnaire.

Cough, phlegm, and shortness of breath were much more common among smokers than among nonsmokers, with no significant difference between the sexes (Table 1). This striking preponderance of symptoms among the smokers is in agreement with previous studies of schoolchildren in Great Britain (*1*).

Since the daily amount smoked correlated highly with the number of years since the start of smoking, we cannot determine whether the daily amount smoked at present or the duration of smoking in years is more important in producing symptoms or in altering lung function. In comparison with the symptom prevalences, the differences in lung function between nonsmokers and smokers are more subtle and they require a detailed statistical analysis. However, the function data are much more objective as compared to the students' own statements about their symptoms.

For the analysis of the function data we used only results from students whose FVC's were closely the same on the two types of spirometers. This eliminated 22 boys and 12 girls whose flow-volume curve terminated abruptly well before residual volume was reached so that their FVC's were less on the flow-volume spirometer than on the other instrument. We have no explanation for these discrepancies other than insufficient practice since the MEFV curves were always recorded first. Thus, the analysis concerns 173 boys and 158 girls. A visual analysis of the shape of the curve (made without knowledge of smoking habits) placed each curve in one of three categories, as shown in Fig. 1b: (i) convex to the volume axis

(curve C), (ii) straight (curve B), or (iii) concave (curve A). This was judged from the part of the curve between approximately 10 and 60 percent of FVC. More nonsmokers had concave curves, and more smokers convex curves; the difference was highly significant ($\chi^2 = 18.7$; $P < .01$). Another observer, repeating this analysis independently, agreed with 90 percent of the classifications of the first observer; the disagreement was between adjacent categories in the remaining 10 percent.

For a more quantitative analysis we first computed linear regression equations on height for the different measurements; this is an accepted procedure for adults in a narrow age range. The mean adjusted values for FEV_1 and $\dot{V}_{max}$ at 50 percent of vital capacity (VC), calculated from these regressions for a standard height of 175 cm in boys and 162 cm in girls, were slightly lower in the smokers, but the difference was significant only at the 5 percent level in girls. However, the validity of this procedure in teen-agers is doubtful since it ignores such factors as changing muscle strength, shoulder width, and ratios of sitting height to standing height. These complexities of growth patterns during puberty and adolescence, particularly in boys, alter the relations between lung function, height, and sex (*10*). Maturity and body weight indirectly reflect these variables. Since both age and weight correlate positively with smoking and with flow rates, they cannot be ignored when seeking an effect of smoking on these measurements.

We therefore computed multiple regression equations of the type

$$y = a + [b \times \text{age (years)}] + [c \times \text{height (cm)}] + [d \times \text{weight (kg)}]$$

(where age is in years, height in centimeters, and weight in kilograms) for the 70 nonsmoking girls and also for the 73 nonsmoking boys. In these equations, y is the function measurement (FVC, FEV_1, $\dot{V}_{max}$ at 50 percent and at 25 percent VC), and a, b, c, and d are constants. We used these equations to predict which values the smokers, boys and girls, should have if they belonged to the same population as the nonsmokers from whose data the equations were calculated. Figure 1c compares the actual data in heavy smokers with those predicted by the regression equations for $\dot{V}_{max}$ at 50 percent VC. In most smokers, the values are less than those predicted. Chi-square tests on the differences between observed and predicted values showed that both $\dot{V}_{max}$ at 50 and at 25 percent VC were significantly below expected in boys who smoked more than 15 cigarettes per day, and in girls who smoked more than 10 cigarettes per day (χ^2 varying from 8.53 to 16.50, $P < .01$). In contrast, FVC's were close to expected values in all categories of smokers. In girls (smoking more than 10 cigarettes per day), values for FEV_1 were slightly below expected, but the difference was only probably significant ($\chi^2 = 6.13$; $P < .05$). In boys, smoking did not affect the FEV_1 significantly. Among the light smokers, the only significant effect was again in girls; those who smoked fewer than 10 cigarettes per day had a slightly lower than expected $\dot{V}_{max}$ at 50 percent VC ($\chi^2 = 6.03$; $P < .05$). Boys smoking up to 15 cigarettes per day had flow rates that were not significantly below expected.

Table 1. Respiratory symptoms in teen-agers (male and female). Symptoms are classified by positive answers to the following questions. (i) Cough: Do you usually cough during the day or at night? (ii) Phlegm: Do you usually bring up any phlegm from your chest during the day or at night? (iii) Dyspnea: Are you ever troubled by shortness of breath when hurrying on the level or walking up a slight hill? The excess of all three symptoms among smokers is statistically highly significant (χ^2 test; $P < .001$).

Cigarettes (No./day)	Subjects (No.)	Symptoms		
		Cough (%)	Phlegm (%)	Dyspnea (%)
0	152	2.0	3.3	5.3
<1	52	5.8	5.8	13.5
1–10	72	18.1	19.4	13.9
11–20	72	27.8	31.9	36.1
20+	17	64.7	58.8	58.8
Total	365	13.7	15.1	16.7

We conclude from these data that regular smoking for 1 to 5 years is sufficient to cause demonstrable decreases of lung function. In separate experiments we compared MEFV curves of 17 subjects (all regular smokers) before and after smoking of a single cigarette. There was a slight but significant decrease of $\dot{V}_{max}$ at 50 percent VC but no change of FEV_1 or FVC. However, such acute effects of smoking disappear in about 1 hour (*7*), and it is very unlikely that these affected our results. The lower maximum expiratory flow rates among smokers are probably due to partial obstruction of small airways, although loss of lung elastic recoil may be a contributory factor (*5*). Airways become obstructed when the smooth muscle in their walls contracts or when secretions accumulate in the lumen. The latter may, in turn, be due to hypersecretion or to failure of the mucociliary clearance mechanism, or both. Our results cannot distinguish between these various causes of airway obstruction. Emery (*11*) believes that damage to small airways early in life may cause premature arrest of lung development since new alveoli are in part formed by a process of "alveolarization" of terminal bronchi. Since Emery has also shown that new alveoli may continue to be formed at least until age 20, it is conceivable that damage by cigarette smoke might lead to developmental arrest, especially in students who have already begun to smoke before the age of 10. Follow-up studies of lung function in adolescents who stop smoking should clarify the question of whether arrest of lung development actually occurs. If the functional decrement in smokers can be reversed completely or nearly so after cessation of smoking, it is probably caused by a toxic effect of cigarette smoke on airways, rather than by interference with the final stages of lung development.

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