

Passive Smoking and Heart Disease

Epidemiology, Physiology, and Biochemistry

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The first disease linked definitively to active smoking was lung cancer. It is, therefore, not surprising that the first disease identified as caused by passive smoking was also lung cancer.¹ Before the advent of mass-marketed cigarettes, lung cancer was a rare disease. Because smoking is the primary cause of lung cancer, identification of this link—for both active² and passive smoking³—was relatively straightforward. This situation contrasts with heart disease, which has many risk factors, and unsurprisingly, the scientific community was longer in concluding that active smoking caused heart disease.⁴ Once the link between smoking and heart disease was established, smoking was found to kill more people by causing or aggravating heart disease than lung cancer. In fact, smoking is the most important, preventable cause of coronary disease. Exposure to environmental tobacco smoke (ETS) has now been linked to heart disease in nonsmokers.^{5,6}

Much of the evidence for this link has appeared since 1986, when the US Surgeon General¹ and the National Academy of Sciences⁷ reviewed the evidence on the health effects of ETS. Based on the information available then, both reports concluded that the evidence linking ETS and heart disease was equivocal and that more research was necessary before any definitive statements could be made. These conclusions were reasonable in 1986. However, in the 4 years since publication of these reports, considerable information on both the epidemiology and biological mechanisms by which ETS causes heart disease has accumulated. Most of the results presented here were published after the 1986 Surgeon General and National Academy of Sciences reports.

There are now 10 epidemiological studies on the relation between exposure to environmental tobacco

smoke in the home and the risk of heart disease death in the nonsmoking spouse of a smoker and five epidemiological studies that examine nonfatal cardiac events. All but one of these studies yielded relative risks or odds ratios greater than 1.0. There are several lines of biological evidence that make this association plausible. There is evidence that exposure to ETS reduces exercise tolerance of healthy individuals and people with existing coronary artery disease. Such reduced exercise capability is one of the landmarks of acute compromises to the coronary circulation. There is good evidence, from both human and animal studies, that exposure to tobacco smoke, including passive smoking, increases aggregation of blood platelets. Such increases in platelet aggregation are an important step in the genesis of atherosclerosis. In addition, increasing platelet aggregation contributes to risk of coronary thrombosis, a cause of acute myocardial infarction. Last, carcinogenic agents in ETS, including benzo(a)pyrene, have been shown to injure the endothelial cells that line arteries. Such injuries are the first step in the development of atherosclerosis. Thus, exposure to ETS can contribute to short- and long-term insults to the coronary circulation and the heart. It is not surprising, therefore, that epidemiological studies have identified an increase in the risk of coronary artery disease in nonsmokers living with smokers.

Effects of Primary Smoking

Before reviewing the evidence linking ETS with coronary artery disease, summarizing the evidence that links active smoking with coronary artery disease is worthwhile. This evidence was summarized in the 1983 Surgeon General's Report,⁴ which was devoted entirely to cardiovascular disease; it concluded that cigarette smoking is one of the three major independent heart disease risk factors. It also concluded that the magnitude of the risk associated with cigarette smoking is similar to that associated with the other two major heart disease risk factors, hypertension and hypercholesterolemia; however, because cigarette smoking is present in a larger percentage of the US population than either hypertension or hypercholesterolemia, cigarette smoking ranks as the largest preventable cause of heart disease in the United States. Since 1983, an increasing body of evidence has shown that the polycyclic aromatic hydrocarbons

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TABLE 1. Epidemiological Studies of Environmental Tobacco Smoke and Coronary Heart Disease Death

Author	Type	Location	Deaths or cases (n)	Relative risk	95% Confidence interval	Dose* response?	Power† (%)	Controlling for
Males								
Gillis et al ⁸ (1984)	P	Scotland	32	1.3	0.7-2.6	-	5	Age
Lee et al ⁹ (1986)	C	United Kingdom	41	1.2	0.5-2.6	-	4	Age, marital status
Svensden et al ¹⁰ (1987)‡	P	United States	13	2.1	0.7-6.5	Yes	3	Age, blood pressure, serum cholesterol, weight, education, alcohol
Helsing et al ¹¹ (1988)	P	Maryland	370	1.3	1.1-1.6	No	40	Age, marital status, housing, education
Pooled§				1.3	1.1-1.6			
Females								
Hirayama ¹² (1984)	P	Japan	494	1.2	0.9-1.4	Yes	40	Age, diet
Gillis et al ⁸ (1984)	P	Scotland	21	3.6	0.9-13.8	-	2	Age
Garland et al ¹³ (1985)	P	California	19	2.7	0.9-13.6	-	2	Age, blood pressure, plasma cholesterol, weight, years of marriage
Lee et al ⁹ (1986)	C	United Kingdom	77	0.9	0.5-1.6	-	6	Age, marital status
Helsing et al ¹¹ (1988)	P	Maryland	988	1.2	1.1-1.4	Yes	2	Age, housing, marital status, education
He (1989) ¹⁴	C	China	34	1.5	1.3-1.8	Yes	3	Age, race, residence, occupation, hypertension, family history of hypertension or CHD, alcohol, exercise, hyperlipidemia
Humble et al ¹⁵ (1990)	P	Georgia	76	1.6	1.0-2.6	Yes	8	Age, serum cholesterol, blood pressure, weight
Butler ¹⁶ (1990)	P	California	64	1.4	0.5-3.8	-	4	Age
Pooled				1.3	1.2-1.4			
Both sexes combined								
Hole et al ¹⁷ (1989) ¶	P	Scotland	84	2.0	1.2-3.4	-	10	Age, sex, social class, blood pressure, cholesterol, weight
Pooled¶				1.3	1.2-1.4			

P, Prospective cohort; C, Case control; CHD, coronary heart disease.

*No entry in this column indicates no comment on the presence or absence of dose-response relation.

†Power to detect relative risk of 1.2 with 95% confidence.

‡High-risk population: members of Multiple Risk Factor Intervention Trial.

§Pooled relative risk computed as $R = \exp(\sum w_i \ln R_i / \sum w_i)$, where $w_i = (1/\ln R_i)^2$.

¶ This report is a later follow-up of the population reported in Gillis et al.⁸

¶ All studies combined without regard for sex, with Gillis et al⁸ excluded because Hole et al¹⁷ report later follow-up on the same people.

in cigarette smoke can injure the arterial endothelium and initiate the atherosclerotic process.

All the compounds from cigarette smoke that have been implicated as damaging to the cardiovascular system of active smokers have been identified in ETS.^{1,7}

Epidemiological Studies on ETS and Heart Disease

Since 1984, the epidemiological evidence linking exposure to ETS with heart disease has rapidly accumulated. The results of the 10 published studies⁸⁻¹⁷ that use death as an end point are summarized in Table 1 and Figure 1; four studies present data on men, eight on women, and one on both sexes combined. Despite minor differences in methodology or end points (some used death from ischemic heart

disease of any origin, and some were limited to death from myocardial infarction), the results of these studies are remarkably consistent. All the studies on men yielded relative risks of death from heart disease exceeding 1.0 when a nonsmoking man was married to a woman who smoked, with an overall risk of 1.3. All but one of the studies on women⁹ yielded relative risks exceeding 1, with an overall relative risk of 1.3. Five studies^{10,17-19,20} have also suggested an increase in the risk of nonfatal coronary symptoms, including angina and myocardial infarction. Consistency of an observation across different studies increases the confidence that a particular association is causal.

Several investigative teams also observed a dose-response relation between increasing amounts of

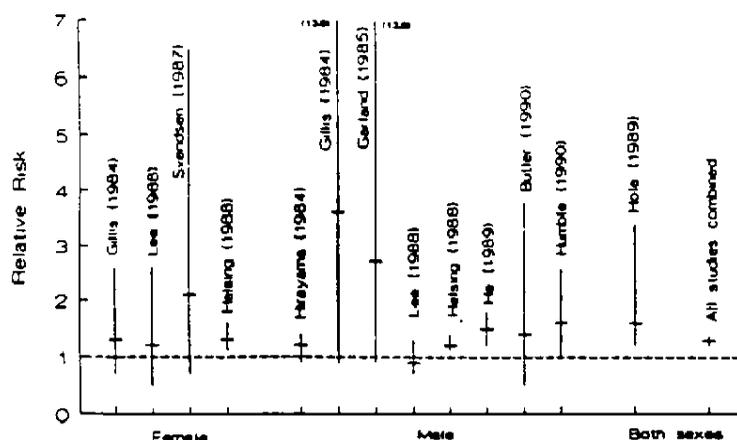


FIGURE 1. Graph of relative risk in epidemiological studies of the risk of death from coronary heart disease or myocardial infarction among nonsmokers living with smokers compared with nonsmokers living with nonsmokers. Lines indicate 95% confidence intervals. Note that two studies have upper bounds to the 95% confidence interval off the scale of the graph.

smoking by the spouse and the risk of heart disease in the nonsmoking spouse,^{11-13,17} which in most cases was statistically significant. The presence of such dose-response effects across multiple studies, conducted in different locations with different criteria, supports the hypothesis that ETS causes heart disease in nonsmokers.

While all but one of the studies in Table 1 and Figure 1 yielded relative risks greater than 1.0, the fact remains that three of the studies in men and five of the studies in women had 95% confidence intervals for the relative risk of passive smoking for heart disease that included 1.0, meaning that the risk was not statistically significantly elevated above 1.0 (with $p < 0.05$). Of note, the 95% confidence intervals do not lie symmetrically about 1.0 but are skewed toward higher risks. By examining the confidence intervals, the conclusion is reached that exposure to ETS elevates the risk of heart disease (Figure 1). Also, the results of these studies may be combined in a formal analysis to derive a global estimate of the relative risk and associated 95% confidence interval. By combining the studies, the sample size and, therefore, the power to detect an effect increases. Wells⁵ used then-available studies^{8,9,11-13,18} to compute a pooled relative risk of 1.3 (95% confidence interval, 1.1-1.6) for men and 1.2 (95% confidence interval, 1.2-1.4) for women. Our analysis on all the studies in Table 1 yields a combined relative risk of 1.3 (95% confidence interval, 1.2-1.4).

When interpreting the results of such epidemiological studies, it is always important to consider biological plausibility and potential confounding variables that can explain the results. Aside from noting that the hydrocarbons in mainstream smoke already implicated in heart disease are also in ETS, we will defer the discussion of biological plausibility until we discuss the effects of ETS on platelets and the atherogenic agents in ETS. For now, we will concentrate on potential confounding variables, which are particularly important in a disease like heart disease

because it is known to be caused by multiple risk factors.

All the studies controlled for the most important confounding variable, age, and several^{10,13-15,17} controlled for known risk factors for coronary artery disease, in particular levels of serum or plasma cholesterol, blood pressure, and body mass. Most of the studies also included one or more measures of socioeconomic status, such as housing or education. Indeed, studies that estimated the relative risk both with and without taking these confounding variables into account found an increase in risk associated with ETS after taking the confounding variables into account.^{10,15}

Lee²¹⁻²³ suggested that the elevated risk of heart (and other) disease with passive smoking may be due to misclassification of nonsmokers who are really smokers. In addition, Wald²⁴ noted that some people who say they live with nonsmokers have detectable levels of the nicotine metabolite cotinine in their blood, indicating that they are actually exposed to ETS, either at work or at home. The former type of misclassification tends to lead to overestimating the risks associated with ETS and the latter leads to underestimating the risk. Careful analysis of the question of misclassification, which applies generally to studies of ETS, has demonstrated that the observed risk cannot be explained by this problem.^{5,24-28}

The possibility always exists that some other confounding variable relates to cultural factors, such as the nature of housing or employment or the nature of time spent outside the home. Also, it is possible that there are other confounders, such as a correlation of spouses' poor health behaviors (e.g., diet), which are not controlled for in analysis. The fact that results are from all over the world in widely varying cultural settings—including several regions in the United States, the United Kingdom, Japan, and China—argues against this concern.

One can assess formally the confidence in reaching a negative conclusion by computing the power of the study to detect an effect of specified size.²⁹ Table 1

shows estimates of the power of each of the studies to detect a 20% increase in risk of heart disease (i.e., a relative risk of 1.2) with the available samples. The power was computed as described in Muhm and Olshan,³⁰ using a two-sided test for the relative risk with a type I risk of 5% (i.e., requiring the 95% confidence interval for the relative risk to exclude 1.0 before concluding a statistically significant elevation in risk in an individual study). Most of the studies have low power. This low power of the individual studies argues against drawing an overall negative conclusion concerning the link between ETS exposure and risk of death from heart disease, based on the individual studies taken one at a time.

Last, and of note, all these studies are based on the smoking habits of the nonsmoker's spouse and, therefore, the exposure to ETS at home. Household exposures to ETS at home are generally much smaller than exposures at work, where the density of smokers is generally higher.^{31,32} As a result, these studies generally underestimate the risk and attendant public health burden due to ETS-induced heart disease. Kawachi et al³³ adjusted Wells³³ relative risks to account for workplace exposures to ETS and found that the relative risks increase to 2.3 (95% CI, 1.4–3.4) for men and 1.9 (95% CI, 1.4–2.5) for women. Thus, any potential confounding of the results because of exposure to ETS outside the home will tend to produce underestimates rather than overestimates of the effect of ETS. Likewise, estimates of public health impact based on risks computed from household exposures³ will be lower than the true public health impact. In addition, Wells³ and Kawachi et al³³ indicate that the number of heart disease deaths due to passive smoking is an order of magnitude greater than the number of lung cancer deaths due to passive smoking. Even though the relative risks for heart disease and lung cancer caused by ETS are similar (about 1.3 for both diseases), the attributable deaths for heart disease is greater because heart disease is much more common than lung cancer. Of 53,000 annual deaths in the United States attributed to passive smoking,³ 37,000 are attributed to heart disease compared with 3,700 for lung cancer (Figure 2).

These epidemiological studies demonstrate a connection between ETS exposure and death from heart disease. We now turn our attention to possible physiological and biochemical mechanisms that explain these observations.

Short-term Effects of ETS Exposure

Long-term exposure to ETS exerts carcinogenic effects by increasing the cumulative risk that a carcinogenic molecule from ETS will damage a cell and then initiate or promote the carcinogenic process. The situation with heart disease is different. In heart disease, important long-term changes (i.e., the development of atherosclerotic lesions) and short-term changes occur. The latter include an increased myo-

Deaths from Passive Smoking Total Deaths: 53,000

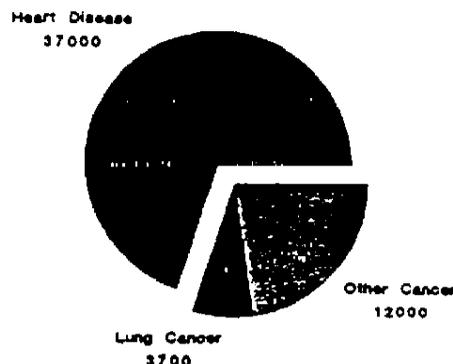


FIGURE 2. Pie chart of US deaths from environmental tobacco smoke. The majority of annual deaths are attributed to heart disease. Modified from Wells.³

cardial oxygen demand that may outstrip the oxygen supply and produce ischemia and an increased platelet aggregation that may lead to coronary thrombosis and acute myocardial infarction.

When the coronary circulation cannot provide enough oxygen to the myocardium to meet the demand, the result is ischemia, which can be a silent or an anginal episode. Earlier onset of angina or hypotension during exercise is a reflection of more severe heart disease. Oxygen supply can be reduced by atherosclerotic narrowing or vasoconstriction of the coronary arteries or by reducing the oxygen-carrying capacity of the blood because the carbon monoxide in the ETS forms carboxyhemoglobin, which, in turn, reduces the blood's oxygen-carrying capacity. Khalifen and Klochkov³⁴ confirmed earlier work by Aronow³⁵ demonstrating that exposure to ETS significantly reduced both the exercise ability in patients with coronary artery disease and the rate-pressure product (heart rate multiplied by systolic blood pressure). In both studies, patients were exposed to realistic levels of ETS by sitting in a waiting room while someone was smoking. These effects were present in smokers and nonsmokers³⁴ and regardless of whether the room was ventilated.^{34,35} Exposure to ETS also increased resting heart rate and systolic and diastolic blood pressure and resulted in a lower heart rate at the onset of angina.³⁵ Blood carboxyhemoglobin was increased by about 1% after exposure to ETS.³⁵ Thus, short-term exposure to ETS leads to an imbalance between myocardial oxygen supply and demand during exercise in patients with coronary artery disease. While this discussion has concentrated on the carbon monoxide in ETS as the active agent, some other component of the ETS may be causing or contributing to this effect.

The effects of ETS on cardiac performance are, in fact, severe enough to affect exercise performance in

young healthy subjects with no evidence of heart disease. McMurray et al³⁶ exposed young healthy women to pure air and air contaminated with ETS while they exercised on a treadmill. The results were similar to those observed in patients with coronary artery disease. Resting heart rate was increased during exposure to ETS, which increased blood carboxyhemoglobin by about 1%. Exposure to ETS significantly reduced maximum oxygen uptake (by 0.25 l/min) and time to exhaustion (by 2.1 minutes). Exposure to ETS also increased the perceived level of exertion during exercise, maximum heart rate, and carbon dioxide output. It also significantly increased levels of lactate in venous blood (from a mean of 5.5 mM during the control period to 6.8 mM after exposure to ETS). This greater lactate at a lower oxygen consumption during the passive smoking trials indicates a greater reliance on anaerobic metabolism. The combined effects of the reduced oxygen-carrying capacity and increased lactate resulted in a reduction in maximal aerobic power and the duration of exercise. Thus, even in healthy subjects, exposure to ETS adversely affects exercise performance. Lamb³⁷ suggested that at maximal exertion levels, up to 90% of the oxygen-carrying capacity of the blood may be needed. Probably because of carbon monoxide, ETS reduces this capacity, so the muscle cannot maintain its high rate of aerobic metabolism unless cardiac output is further increased; people with heart disease and reduced ventricular reserve have difficulty meeting this demand. In sum, exposure to ETS increases the demands on the heart during exercise and reduces the capacity of the heart to respond. This imbalance increases the ischemic stress of exercise in patients with existing coronary artery disease and can quickly precipitate symptoms.

Moskowitz et al³⁸ found evidence that adolescent children of parents who smoked may suffer from chronic tissue hypoxia such as that observed in anemia, chronic pulmonary disease, cyanotic heart disease, or high altitude. These children had significantly elevated levels of 2,3-diphosphoglycerate (DPG), even after correcting for age, weight, height, and sex. DPG acts as a physiological modulator of hemoglobin oxygen affinity. It binds to specific amino acid sites and increases the P_{50} (lowers the oxygen affinity), thus making more oxygen available to peripheral tissues. This observation suggests that the body is attempting to compensate for hypoxia by increasing the DPG level in blood to meet tissue oxygen requirements. The changes were dose dependent; the greater the exposure to ETS (measured both in terms of parental smoking and serum thiocyanate levels in the children), the greater the increase in DPG.

There is also evidence that short-term exposure to ETS directly affects respiration of the myocardium at a cellular level. Gvozdjaková et al³⁹ exposed rabbits in a 50 l child's incubator to the smoke of three burning cigarettes smoked during a 30-minute period, and they measured several variables related to

the metabolism of cardiac mitochondria. They had three groups of rabbits: one group was exposed to a single dose of ETS, one group was exposed to 30 minutes of ETS twice daily for 2 weeks, and one group was exposed to 30 minutes of ETS twice daily for 8 weeks. They measured mitochondrial respiration as the consumption of oxygen after adding ADP to a vessel containing mitochondrial fragments. Using pyruvate as a substrate, mitochondrial respiration was reduced significantly compared with control (pure air) for all doses of ETS, by even a single exposure, to about half the control value. The oxidative phosphorylation rate was also reduced significantly at all exposures by about one third. There were no significant changes in the coefficient of oxidative phosphorylation with ETS exposure. Gvozdjaková et al³⁹ concluded that pyruvate as a substrate was a sensitive indicator of the toxic action of the ETS on the oxidative process.

Later, to further isolate where in the process of mitochondrial respiration the ETS acted, Gvozdjaková et al⁴⁰ and Gvozdjak et al⁴¹ reported data on succinate, NADH, and cytochrome oxidase activity in the mitochondria in the four groups of rabbits. Exposure to ETS affects the activity of NADH oxidase, succinate oxidase, and cytochrome oxidase of myocardial mitochondria. The activity of the first two oxidases exhibited no changes compared with the control group, neither after a single exposure to ETS or after exposures to 2 weeks. Cytochrome oxidase activity decreased both after a single exposure to ETS and over time, with greater decreases as the duration of exposure to ETS was extended. The observation that cytochrome oxidase and not NADH or succinate oxidase activity was affected by ETS suggests that the deleterious effects of ETS on myocardial mitochondrial respiration occur at the terminal segment of the mitochondrial respiration process. Prolonged exposure to carbon monoxide has been shown to induce ultrastructural changes in myocardium⁴²⁻⁴⁴ and may account for the adverse effects of ETS exposure on mitochondrial function.

Thus, short-term exposure to ETS not only increases the demand and compromises the supply of oxygen to the heart, but also reduces the myocardium's ability to use the oxygen to create ATP to provide energy to support the heart's pumping activity.

Effects on Platelets

The action of ETS to increase platelet aggregation is another way in which ETS can increase the risk of a coronary event. Platelets are important for the normal process of hemostasis, to prevent blood loss after an injury. When blood platelets aggregate inappropriately and form a thrombus in the coronary circulation, they can precipitate a myocardial infarction. Hemostasis depends on complex interactions among the dynamics of blood flow, components of the vessel wall, platelets, and plasma proteins. Definitive evidence has confirmed that platelets play a major role in thrombus formation and embolization.

TABLE 2. Effect of Passive and Active Smoking on Platelet Aggregation and Endothelial Cell Damage

	Platelet aggregate ratio			Endothelial cell count			n
	Before	After	Change	Before	After	Change	
Passive smoking (nonsmoker)	0.87	0.78	-0.09	2.8	3.7	0.9	10
Tobacco (nonsmoker)	0.80	0.65	-0.15	2.3	4.8	2.5	20
Nontobacco cigarette (nonsmoker)	0.81	0.78	-0.03	2.5	3.0	0.5	
Inhale cigarette (smoker)	0.81	0.68	-0.13	4.0	5.4	1.4	24
Not inhale cigarette (nonsmoker)	0.82	0.73	-0.09	3.3	4.7	1.4	22
Smoke (smoker)	0.85	0.70	-0.15	4.4	6.4	2.0	
Snuff (smoker)	0.82	0.76	-0.06	3.9	4.7	0.8	17

All studies are paired and reflect significant differences ($p < 0.005$). Platelet aggregate ratio is the ratio of platelet count of platelet-rich plasma, prepared immediately after venipuncture with a solution containing edetic acid and formaldehyde, to that of platelet-rich plasma prepared in the same manner, except for the absence of formaldehyde. A decrease in the platelet aggregate ratio reflects an increased formation of platelet aggregates. Endothelial cell count is mean number of anuclear cell carcasses in 0.9- μ L chambers. Modified from Davis et al.^{47,48,51,52}

especially in the arterial system. In addition, increasing evidence has shown that platelet deposition and thrombus formation can contribute to the growth and progression of atherosclerotic plaques.^{45,46} An arterial thrombus appears to develop in three phases: platelet adhesion, platelet aggregation, and activating of clotting mechanisms. Passive smoking increases platelet aggregation and, thus, increases the likelihood of thrombus formation and myocardial infarction.

Table 2 summarizes the results of several studies by Davis et al.⁴⁷⁻⁵⁰ on the effects of cigarette smoke on platelet aggregation and damage to the arterial endothelium. Davis et al.⁵¹ also measured platelet aggregate ratios and endothelial cell counts in nonsmokers before and after exposure to 20 minutes of ETS while sitting in a hospital atrium. The platelet aggregate ratio in these studies is the ratio of the platelet count of platelet-rich plasma prepared from blood mixed immediately with EDTA and formaldehyde to the same mixture without formaldehyde. This method assumes that platelet aggregates circulating in blood are fixed in the EDTA-formaldehyde solution and that they break apart in the EDTA solution. Thus, a decrease in the platelet aggregate ratio reflects an increased formation of platelet aggregates. Mean values before and after passive smoking were 0.87 and 0.78 ($p = 0.002$) for platelet aggregate ratios and 2.8 and 3.7 ($p = 0.002$) for counts of anuclear endothelial cell carcasses in venous blood. These changes are intermediate between the effects observed after nonsmokers smoked two tobacco cigarettes and the effects observed after smoking two nontobacco cigarettes⁴⁷ and similar to the values observed in nonsmokers who smoked two cigarettes while trying not to inhale.⁴⁴ These effects were not correlated with the level of nicotine in the blood of the experimental subjects in any of these or other^{49,50} related studies on how drugs modify platelet aggregation and endothelial cell counts. In particular, the effects observed in nonsmokers who smoked without inhaling were similar to the effects on smokers who smoked two cigarettes even though the plasma nico-

tine levels in the nonsmokers were five times lower than those observed in the smokers.⁵⁰ Other work in the same laboratory comparing smoking with snuff use revealed similar changes in platelet function in response to these two forms of tobacco use.⁵² This result, combined with the finding that smoking nontobacco cigarettes⁴⁷ failed to produce changes in platelet function as large as observed with tobacco cigarettes, suggests that nicotine is an important active agent. Because nontobacco cigarettes also affected platelet aggregation somewhat, however, carbon monoxide or other combustion products may also influence the platelets.

Sinzinger and Kefalides⁵³ measured platelet sensitivity to antiaggregatory prostaglandins (E_1 , I_2 , and D_2) before, during, and after 15 minutes of exposure to ETS in healthy nonsmokers and smokers. Passive smoking reduced platelet sensitivity to the antiaggregatory prostaglandins I_2 and E_1 significantly ($p < 0.01$) by a factor of about 2 by the end of 15 minutes of exposure to ETS among nonsmokers. This effect persisted at 20 minutes after the end of exposure and ceased by 40 minutes. Platelet response to prostaglandin D_2 changed modestly in a similar pattern but was not significant. Among smokers, the control level of platelet aggregation was higher ($p < 0.01$), and the prostaglandins had no significant effects on platelet aggregation over time during or after exposure to ETS. Sinzinger and Virgolini⁵⁴ also showed that repeated exposure to ETS for 1 hr/day for 10 days produced lasting changes in platelet function in nonsmokers similar to those observed in smokers. Thus, nonsmokers' platelets seem much more sensitive to a single exposure to ETS than do smokers' platelets, and change in platelet sensitivity to disaggregating prostaglandins in nonsmokers exposed to ETS for short periods is similar to that observed in smokers.

Further evidence from the same laboratory that passive smoking increases platelet aggregation comes from work by Burghuber et al.⁵⁵ who studied smokers and nonsmokers who smoked two cigarettes and also exposed a different group of smokers and nonsmok-

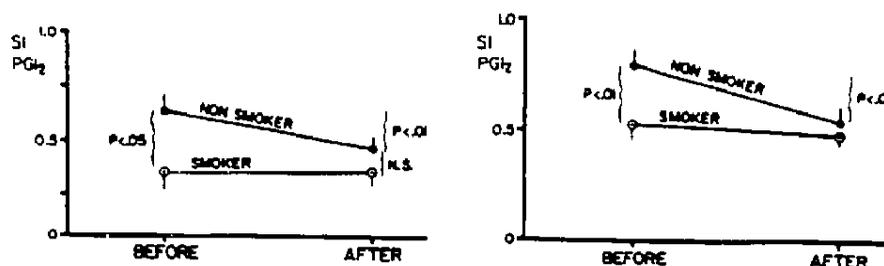


FIGURE 3. Plots of effect of active (left) and passive (right) smoking on platelet aggregation in smokers and nonsmokers. The sensitivity index, $SI\ PGI_2$, is defined as the inverse of the concentration of prostaglandin I_2 necessary to inhibit ADP-induced platelet aggregation by 50%. Lower values of $SI\ PGI_2$ indicate greater platelet aggregation. Adapted from Figures 3 and 4 of Burghuber et al.⁵³

ers to ETS in an 18 m³ room in which 30 cigarettes had been smoked just before exposing the nonsmokers. They measured the sensitivity of platelets to the disaggregating substance prostaglandin I_2 that is released by endothelium and inhibits platelet aggregation. Figure 3 shows the results of this experiment. In smokers, neither smoking nor passive smoking affected the sensitivity of the platelets to the disaggregating effect of prostaglandin I_2 . The sensitivity of platelets in smokers was also significantly lower than that of nonsmokers. In contrast, platelets were more sensitive to prostaglandin I_2 in nonsmokers, with both smoking and passive smoking producing a similar reduction in platelet sensitivity to prostaglandin I_2 . These results suggest that the platelets of smokers are already desensitized to the antiaggregatory substance prostaglandin I_2 , so that no further decrease in aggregation is seen. The significant decrease in platelet sensitivity to prostaglandin after short-term exposure to ETS suggests that after ETS exposure platelets are more likely to aggregate with adverse consequences.

Earlier work by Saba and Mason⁵⁴ also indicated that nicotine increased a variety of measures of platelet aggregation in nonsmokers and smokers. Although the *in vitro* effects of nicotine on platelets from smokers was greater than that in nonsmokers, the effect generally did not vary with dose (between 2×10^{-9} and 2×10^{-8} M), suggesting that the effects of nicotine on platelets occur at low doses and that the system saturates quickly. This observation may explain why passive and active smoking have such similar effects on platelets.^{51,52,55}

The probable link between nicotine and adverse physiological effects is nicotine-induced release of catecholamines. Catecholamines are then responsible for increased platelet aggregation. This reasoning suggests that β -adrenergic receptor blockers may provide some protection in smokers. This premise is borne out by a trial comparing the effects of the β -blocker metoprolol to a thiazide diuretic in the control of moderate hypertension.⁵⁷ For the same reduction in blood pressure, the metoprolol-treated group had a significantly lower mortality rate than did the thiazide-treated group. Practically all of this

reduction in mortality, however, was seen in smokers and not nonsmokers. This study provides evidence that blocking the effects of catecholamines (released by nicotine) was the cause of the reduced mortality in smokers who were receiving metoprolol.

In sum, passive smoking increases platelet aggregation, with a magnitude similar to that observed in active smoking. Moreover, the response of nonsmokers to both active and passive smoking appears to be different from smokers, with nonsmokers being more sensitive to lower exposures to cigarette smoke than are smokers. This observation indicates that the pharmacology of ETS in nonsmokers may be different than in smokers, with nonsmokers being more sensitive to low doses of ETS. In particular, it invalidates attempts to estimate "cigarette equivalent" doses of ETS in nonsmokers or extrapolating from risks of smoking in smokers to effects of ETS on nonsmokers.⁵⁸ The resulting increase in platelet aggregation can contribute to acute thrombus formation and myocardial infarction.

In addition to the role of platelets in acute thrombus formation, platelets are also important in the development of atherosclerosis.⁴⁶ Once there is damage to the arterial endothelium, either through mechanical or chemical factors, platelets interact with or adhere to subendothelial connective tissue and initiate a sequence that leads to atherosclerotic plaque. When platelets interact with or adhere to subendothelial connective tissue, they are stimulated to release their granule contents. Endothelial cells normally prevent platelet adherence because of the nonthrombogenic character of their surface and their capacity to form antithrombotic substances such as prostacyclin. Once the endothelial cells have been damaged, the platelets can stick to them. Once the platelets are bound to the endothelium, they release mitogens such as platelet-derived growth factor, which encourage migration and proliferation of smooth muscle cells in the region of the endothelial injury.⁵⁹ If platelet aggregation is increased because of exposure to ETS, the chances of platelets building up at an endothelial injury will be increased. Thus, in addition to contributing to short-term effects through increasing the likelihood of thrombus formation, the

effects of ETS on platelets also increase the chances that endothelial injury will lead to arterial plaque.

ETS also plays a role in causing damage to the endothelium and initiating the atherosclerotic process. As discussed above, Davis et al⁵¹ found that short-term exposure to ETS, like active smoking⁴⁷⁻⁵⁰ and use of chewing tobacco,⁵² leads to a significant increase ($p < 0.002$) in the appearance of anuclear endothelial cell carcasses in the blood of people exposed to ETS (or tobacco product) constituents. The appearance of these cell carcasses indicates damage to the endothelium, which is the initiating step in the atherosclerotic process. As noted above, the appearance of endothelial cells after passive smoking is almost as great as after primary smoking (Table 2). Exposure to ETS has been shown to produce injuries similar to those observed with exposure to primary smoke and also affects platelets in a way that increases the chances that they will bind to the injured area and promote growth of smooth muscle cells.⁴⁶

Role of the Polycyclic Aromatic Hydrocarbons in ETS

Many atherosclerotic plaques in humans are either monoclonal or possess a predominantly monoclonal component,⁶⁰ which indicates that the smooth muscle cells of each plaque have a predominant cell type. Several animal studies have also shown that injections of polycyclic aromatic hydrocarbons (PAHs), in particular 7,12-dimethylbenz(a,h)anthracene (DMBA) and benzo(a)pyrene,⁶¹⁻⁶³ accelerate the development of atherosclerosis. Benzo(a)pyrene is an important element in ETS.¹ The effects of PAHs or other carcinogenic or mutagenic elements in ETS⁶⁴ relate directly to the response to injury theory of atherogenesis discussed above.⁴⁶ Changes in the underlying smooth muscle stimulated by these agents can then initiate the "injury" that leads to platelet aggregation and plaque formation. Thus, long-term exposure to ETS can affect plaque formation through mechanisms similar to those by which long-term exposures produce cancer in other organs.

Albert et al⁶¹ gave chickens weekly intramuscular injections of DMBA and benzo(a)pyrene for up to 22 weeks, then killed the chickens at various times beginning after 13 weeks and measured the plaque volume in the chickens' aortas. They found that both DMBA and benzo(a)pyrene significantly increased the volume of plaque compared with control chickens who had just received injections of the solvent used to carry these agents. This study provided the first evidence that known carcinogenic chemicals can be atherogenic as well.

Penn et al⁶³ extended this result in a similar experiment by showing that the effects of DMBA on the extent of plaque buildup in chickens was dose dependent. The median cross-sectional area of plaques on individual aortic segments and the plaque volume index (an approximate measure of the total volume of plaque per aorta) increased in a nearly linear fashion with DMBA dose. In contrast to the marked increase in plaque area in the DMBA-

treated animals, the percentage of aortic sections with plaques in carcinogen-treated animals was only slightly higher than in controls. Plaques with a small cross-sectional area were present in all animals. Lesions of widely differing cross-sectional areas appeared to be similar histologically under the light microscope.

Together, these data suggest strongly that a major effect of long-term DMBA exposure is to increase the size of spontaneous aortic lesions. Rather than inducing a cancerlike change in an individual cell that begins the process that ultimately leads to plaque formation, Penn et al⁶³ suggested that long-term DMBA exposure causes preferential division of individual cells or patches of cells within the preexisting spontaneous lesions. From this perspective, DMBA and other exogenous compounds would be acting as a mitogen, similar to that released by activated platelets, to stimulate division of aortic smooth muscle.

Revis et al⁶² found similar results in White Carneau pigeons injected with DMBA and benzo(a)pyrene weekly for 6 months, beginning when the pigeons were 3 months old. Compared with the work described above, they found that benzo(a)pyrene had a greater effect on atherogenesis than did DMBA, and they also failed to observe a dose-response relation between the dose given and the amount of aortic plaque. These differences from the work just described may be related to species differences, differences in the carrier used to inject the PAHs (dimethyl sulfoxide in the previous studies compared with corn oil in this one), or differences in the age of the pigeons or dosing schedule. They also found an increase in aortic plaques in pigeons treated with the PAH 3-methylcholanthrene but not the carcinogen 2,4,6-trichlorophenol or the PAH benzo(e)pyrene, which is not considered a carcinogen. This result suggests that carcinogenic PAHs, rather than carcinogens or PAHs in general, are implicated in the atherosclerotic process.

Revis et al⁶² also studied the distribution of these compounds after they had been radiolabeled. Forty-eight hours after the injection of PAHs, radioactivity in the liver, aorta, and lung accounted for 75% of the injected dose, whereas in animals injected with 2,4,6-trichlorophenol, radioactivity in the liver and kidney accounted for 80% of the dose. In addition, 80% of the radioactivity observed in the plasma immediately after injection of radiolabeled PAHs was associated with the low density and high density lipoprotein cholesterol fractions compared with only 24% of the 2,3,6-trichlorophenol, suggesting that plasma lipoproteins are an important vehicle for transporting PAHs to their sites of activation in the arteries.

There is also evidence that ETS directly affects plasma lipoproteins. Moskowitz et al⁵⁸ showed that adolescent children whose parents smoked had elevated levels of cholesterol and depressed levels of high density lipoproteins, even after correcting for age, weight, height, and sex. These effects were dose dependent; the greater the exposure to ETS, the

greater were the changes in these variables. Pomerehn et al⁶⁷ observed similar effects of ETS on high density lipoprotein in children whose parents smoked and in children who smoked or chewed tobacco themselves. High levels of total cholesterol and low levels of high density lipoprotein are important for the development of plaque. Data on total cholesterol and high density lipoprotein from non-smokers married to smokers are inconclusive.^{10,14}

To further elucidate the possible mechanisms by which PAHs induce atherosclerotic changes, Majesky et al⁶⁵ administered a single injection of benzo(a)pyrene to White Carneau and Show Racer pigeons, then looked for metabolites of the benzo(a)pyrene in aortic and hepatic tissues 48 hours later. White Carneau pigeons typically develop severe atherosclerosis by 3 years of age, whereas Show Racer pigeons are relatively resistant to aortic atherosclerosis. Aortic preparations of the White Carneau strain exhibited a much greater inducibility of the microsomal monooxygenase system than did those of the Show Racer strain, particularly in young pigeons. Aortic tissues from White Carneau pigeons aged 6–12 months exhibited a threefold to 12-fold inducibility, whereas aortic tissues from the same strain at 2–5 years of age exhibited only minor (maximum, 3.3-fold) and, for the most part, statistically insignificant increases. No age differences in inducibility could be detected in the Show Racer strain. Interestingly, the differences in inducibility manifest in aortic tissues were greater in aortic tissues than in hepatic tissues from the same birds. Thus, the PAHs seem to accelerate any preexisting tendency to develop atherosclerosis.

Regardless of the ultimate mechanism by which PAHs exhibit atherogenic effects, it seems logical to suppose that the reactive intermediary metabolites of these chemicals are the proximate atherogenic or coatherogenic agents because the parent compounds are relatively inert both chemically and biologically. Thus bioactivation and inactivation (and regulatory control of these processes) may be presumed to play extremely important roles in their atherogenic properties. Bioactivated chemicals vary in their stability and reactivity according to four general categories: 1) those that are extremely unstable and persist only at the immediate site (enzyme) of bioactivation, 2) those that persist only within cells in which bioactivation occurs, 3) those that persist primarily only within tissues in which bioactivation occurs, and 4) those capable of being transferred in the circulation from one organ to another. For the first three of these four categories, biotransformation in the aorta *per se* (target tissue activation) would be of prime interest and importance. Thus, it appears that PAHs could be playing either a mutagenic or mitogenic role in beginning the atherosclerotic process in susceptible cells or individuals, depending on how the PAHs in ETS are metabolized in the aorta.

The finding that enzymes that metabolize DMBA and benzo(a)pyrene are in the artery wall led Penn et al⁶⁴ to search for specific molecular events in plaque

cells that would lead to DNA changes similar to those previously found in tumors. Identification of such processes would be supportive of the monoclonal hypothesis of atherogenesis. They obtained human DNA samples from coronary artery plaques as well as DNA from normal sections of the coronary arteries at surgery to remove the plaque. These DNA samples were tested with the NIH 3T3 cell transfection assay. Foci arose in cells transfected with each of the DNA samples obtained from the human coronary plaque, with an efficiency (number of foci/ μ g of DNA) ranging from 0.016 to 0.060 (mean, 0.036). The transfection efficiencies for DNA from normal coronary artery, liver, spleen, lung, kidney, and trachea were all less than 0.008. The transformed cells were also injected into the scalps of nude mice, where they developed tumors. These results provide direct evidence for similarities on the molecular level in the development of plaques and tumors. Human coronary artery plaque DNA contains sequences capable of transforming NIH 3T3 cells, and these transformed cells can cause tumors after injection into nude mice. Control experiments verified that the transforming cells did indeed contain human DNA and that the tumorigenic (or transforming) activity was not due to the *ras* oncogene family. Although these results clearly demonstrate that human plaque DNA has transforming ability, the temporal expression of this activity *in vivo* is not known. The plaques were taken from adult patients in late stages of vascular disease. Thus, we cannot determine from these samples whether the manifestation of transformation is a relatively late event in plaque development or an early but stable event. Oncogene activation and expression is an important early event in transformation and tumor genesis. These results identify specific molecular events that may underlie the proliferation of smooth muscle cells that is a hallmark of atherosclerotic plaque development and demonstrates that plaque cells exhibit molecular alterations that had previously only been thought to be present in cancer-cell transformation and tumorigenesis. These results provide direct support for the monoclonal hypothesis.

Randerath et al⁶⁶ also demonstrated that constituents of cigarette "tar," including benzo(a)pyrene, are preferentially attracted to the heart and damage DNA there. They studied molecular mechanisms of smoking-related carcinogenesis by examining the induction and distribution of covalent DNA damage in internal organs of the mouse after topical application of cigarette smoke condensate daily for 1, 3, or 6 days then killed 24 hours later. DNA samples were obtained from skin, lung, heart, kidney, liver, and spleen. Adducts containing benzo(a)pyrene-derived moieties were identified, together with others. At all three times, the number of adducts in heart and lung DNA was about five times higher than that in liver and slightly higher than that in skin. Covalent DNA damage was estimated to be 6.2, 5.7, 3.9, and 1.9 times higher, respectively, in lung, heart, skin, and

kidney than in liver, ranging from approximately 1 adduct/ 5.4×10^6 DNA nucleotides in lung to 1 adduct/ 3.3×10^7 DNA nucleotides in liver. Spleen DNA was practically adduct free. Although the DNA adduct profiles resembled each other qualitatively among the different tissues, there were major quantitative differences between the different tissues, with the highest DNA binding occurring in the lung and heart. The reasons for the high incidence of DNA adducts in the heart are not known but may be related to the role of plasma lipids in transporting PAHs such as benzo(a)pyrene and binding of these lipids to coronary arteries.

In sum, there is a growing body of evidence at a molecular level supporting the monoclonal hypothesis of atherogenesis, with compounds in tobacco smoke and ETS strongly implicated as agents that stimulate the development of coronary lesions. Regardless of whether the monoclonal hypothesis proves to be true (or, more likely, one of several initiators of the atherosclerotic process), there is clear evidence that components of ETS, in particular PAHs such as benzo(a)pyrene, initiate or accelerate the development of plaque. These biochemical findings are consistent with the epidemiological finding that chimney sweeps, who are exposed to high levels of PAHs in soot, have an increased risk of heart disease (as well as cancer) and tend to develop these diseases earlier than do members of other, comparable, occupations that are not exposed to PAHs.⁶⁹ The PAHs in ETS are clearly implicated at epidemiological, physiological, and biochemical levels in the genesis of heart disease.

Summary

The evidence that ETS increases risk of death from heart disease is similar to that which existed in 1986 when the US Surgeon General concluded that ETS caused lung cancer in healthy nonsmokers.¹ There are 10 epidemiological studies, conducted in a variety of locations, that reflect about a 30% increase in risk of death from ischemic heart disease or myocardial infarction among nonsmokers living with smokers. The larger studies also demonstrate a significant dose-response effect, with greater exposure to ETS associated with greater risk of death from heart disease.

These epidemiological studies are complemented by a variety of physiological and biochemical data that show that ETS adversely affects platelet function and damages arterial endothelium in a way that increases the risk of heart disease. Moreover, ETS, in realistic exposures, also exerts significant adverse effects on exercise capability of both healthy people and those with heart disease by reducing the body's ability to deliver and utilize oxygen. In animal experiments, ETS also depresses cellular respiration at the level of mitochondria. The polycyclic aromatic hydrocarbons in ETS also accelerate, and may initiate, the development of atherosclerotic plaque.

Of note, the cardiovascular effects of ETS appear to be different in nonsmokers and smokers. Non-

smokers appear to be more sensitive to ETS than do smokers, perhaps because some of the affected physiological systems are sensitive to low doses of the compounds in ETS, then saturate, and also perhaps because of physiological adaptations smokers undergo as a result of long-term exposure to the toxins in cigarette smoke. In any event, these findings indicate that, for cardiovascular disease, it is incorrect to compute "cigarette equivalents" for passive exposure to ETS and then to extrapolate the effects of this exposure on nonsmokers from the effects of direct smoking on smokers.

These results suggest that heart disease is an important consequence of exposure to ETS. The combination of epidemiological studies with demonstration of physiological changes with exposure to ETS, together with biochemical evidence that elements of ETS have significant adverse effects on the cardiovascular system, leads to the conclusion that ETS causes heart disease. This increase in risk translates into about 10 times as many deaths from ETS-induced heart disease as lung cancer; these deaths contribute greatly to the estimated 53,000 deaths annually from passive smoking.¹ This toll makes passive smoking the third leading preventable cause of death in the United States today, behind active smoking⁷⁰ and alcohol.⁷¹

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