1) (F) H-2
2) (F) H-7

THE HUMAN MODEL*

H. C. McGill, Jr.

Of the two meanings of "model"--a pattern to be copied or a miniature representation--the latter is the concern of most of the rest of this program. Animal models are miniature representations of human disease in time, size, and cost. By contrast, during my discussion I will interpret "model" as the pattern that we are trying to copy, that is, the human model.

I don't think there is any question that our ultimate goal is to prevent human disease. Surrounded by rats, mice, and monkeys, we can easily forget that we are trying to generate knowledge applicable to humans. The final proof of validity rests with the application of this knowledge in humans--the drug given, the vaccine injected, the habit changed, or the exposure removed. Some knowledge gained from animals is applicable to humans and some is not. There are examples of both. Poliomyelitis vaccine was developed with chimpanzees as the model and it proved effective in humans. Thalidomide toxicity was tested in an animal model that followed its anticipated human use; its adverse effects on the human fetus were not anticipated.

There are several important differences between the human model and animal models. Epidemiologic studies deal with hundreds, thousands, and sometimes millions of people, many more than are possible with most animal models. The genetic heterogeneity of the human population contrasts with the relative homogeneity of special strains of laboratory animals, and produces idiosyncratic reactions to drugs and chemicals. Finally,

*Prepared with the support of grant HL-19362 from the National Heart, Lung and Blood Institute.

laboratory animals don't live as long as people and the exposure time to injurious agents cannot be the same as for humans.

To illustrate at least one disease that we are trying to simulate in animal models, I will describe briefly the process of human atherosclerosis and its sequelae. The common clinical syndromes of the atherosclerotic diseases begin to occur in middle age. Coronary heart disease may be manifested as myocardial infarction, sudden death, or angina pectoris. Other manifestations are stroke, peripheral vascular disease, and aneurysm of the aorta. The patient becomes aware of atherosclerosis only when one of these manifestations appears.

However, atherosclerosis begins many years before, at least in childhood and perhaps in infancy. The first step is deposition of abnormal fat in the inner lining of the artery. This lesion we call a fatty streak. Smooth muscle and connective tissue proliferate around the fat and encapsulate it. The resulting lesion projects into the lumen of the artery and is called a fibrous plaque.

In subsequent years, the fibrous plaque undergoes calcification, hemorrhage, necrosis, and finally ulceration and thrombosis on the surface. Thrombosis obstructs the blood supply. Depending on whether the heart, brain, or leg is affected, obstruction of the blood supply produces clinical symptoms related to the affected organ--heart attack, stroke, or peripheral vascular disease. This event may not take place until 30 to 50 years after the process begins.

Most current hypotheses about the pathogenesis of atherosclerosis center around the smooth muscle cell. The lipogenic hypothesis contends that the difficulty is with the lipid metabolism of the smooth muscle cell. The smooth muscle cell binds low-density lipoprotein to receptors in its plasma membrane, internalizes and metabolizes it, and esterifies the cholesterol that it carries. If excess low-density lipoprotein is absorbed by the smooth muscle cell, abnormal lipid accumulates in its cytoplasm and forms the fatty streak. Continued lipid accumulation forms the fibrous plaque.

Another leading idea is the myogenic hypothesis, which states that the basic lesion is proliferation of smooth muscle cells. Many endogenous and exogenous substances stimulate the growth of arterial smooth muscle cells, including environmental contaminants that are inhaled and absorbed, as from cigarette smoke and other industrial products.

A third idea about the origin of fibrous plaques is the mutagenic hypothesis. Smooth muscle cells in advanced lesions have characteristics that suggest they were derived from one cell, a finding that led to the proposal that a smooth muscle cell becomes transformed much as a tumor cell does. The transformed smooth muscle cell reproduces rapidly and forms a tumor-like mass in the wall of the vessel. One can imagine many substances with transforming activity, perhaps related to those with carcinogenic activity.

If any of these hypotheses are true, it is conceivable that the smooth muscle cell in tissue culture may become a model for testing potential atherogenic agents, such as components of cigarette smoke.

Fibrous plaques in the coronary arteries eventually reduce the lumen of the artery. As long as the endothelium remains intact, moderate constriction does not reduce blood flow enough to cause ischemia. However, when endothelium is damaged and thrombosis occurs over a plaque, the lumen is completely occluded; the victim develops pain in the chest and he may either die within a few minutes or develop myocardial infarction.

So many factors influence the final occlusive episode and so many different clinical syndromes result from atherosclerosis that it seems impossible, as a practical matter, to reproduce these endpoints in an animal model with sufficient reliability to use them for bioassay. Production of advanced lesions with necrosis, hemorrhage, ulceration, or thrombosis requires several years' administration of extreme atherogenic diets. Even then, there is a high degree of variability. To produce the uncomplicated fibrous plaque in conventional animal models also requires prolonged exposure to an atherogenic diet but fibrous plaques are associated closely enough with clinical diseases to provide a reasonable endpoint for testing atherogenic agents.

Fatty streaks are produced easily by short periods of hyperlipidemia in many animal species, but they are so far removed from clinical disease that it seems unlikely they would be useful endpoints. Furthermore, there is considerable difference of opinion regarding whether the fatty streak becomes the fibrous plaque, or whether it is an innocuous and incidental lesion, and it would not be wise to accept fatty streaks as reliable endpoints for atherosclerosis.

Let us now look briefly at some aspects of the human model of inhalation of at least one toxic material--cigarette smoke--and its relationship to atherosclerosis. Atherosclerosis is present in all populations throughout the world (1). However, in the technically underdeveloped countries it is not severe enough to produce frequent clinical disease. Only in the technically developed countries does atherosclerosis affect the health of a significant number of persons. In these countries, the risk of incurring coronary heart disease or one of the other atherosclerotic diseases is related directly to the intensity and duration of cigarette smoking. A smoker does not inevitably get coronary heart disease nor is the nonsmoker guaranteed immunity; it is simply a matter of probability. The association with atherosclerotic disease is so strong and consistent that the causal relationship seems beyond question. The mechanism, however, is another matter about which we are not at all certain.

Cigarette smoking is associated with coronary artery fibrous plaques (2) as well as with clinical disease. It appears that cigarette smoking acts in part by aggravating atherogenesis, that is, by accelerating the progression of lesions, as well as by predisposing to the terminal occlusive episode.

The risk of coronary heart disease decreases when people stop smoking, fairly rapidly at first, and, after 10 years, returns nearly to that of nonsmokers (3). It will be important to determine whether cessation of cigarette smoking leads to regression of atherosclerotic lesions as well as to reduction in risk of clinical disease.

Many differences have been found between smokers and nonsmokers, and some of these may represent mechanisms by which cigarette smoking augments atherogenesis. Table 1 lists blood chemical variables that could be involved in atherogenesis and for which comparisons between cigarette smokers and nonsmokers have been reported. Two of these are risk factors for the atherosclerotic diseases--serum cholesterol concentration and triglyceride concentration. Both are slightly higher in smokers than in nonsmokers but the increase does not account for the increased risk of coronary heart disease associated with cigarette smoking.

Table 2 shows hematologic variables that have been compared. Increased hemoglobin, hematocrit, and red cell count probably are related to the increased carbon monoxide concentration in the blood of smokers. The increased white cell count may indicate tissue injury and an inflammatory reaction. It may be accompanied by increased endothelial permeability, which would be important in atherogenesis. No changes in coagulation factors have been observed in smokers but many studies have observed alterations in platelet functions that could be related to thrombosis.

Table 3 shows physiologic variables that have been studied. Smokers have lower weights and thinner skin folds, characteristics associated with lower rates of coronary heart disease. Remarkably, cigarette smoking has never been shown to be associated with increased blood pressure or hypertensive disease, and most studies have found lower blood pressures among smokers than nonsmokers.

Table 3 also shows endocrinologic variables that have been compared between smokers and nonsmokers. Since glucose intolerance is a risk factor for coronary heart disease, it will be important to seek confirmation of the report that glucose tolerance is impaired in smokers. Hyperinsulinemia is of interest because it accelerates atherogenesis in animal models, and it may be associated with more severe atherosclerosis in humans. There are no known relationships of the other two changes with atherogenesis.

In conclusion, we can see that the human model of atherosclerosis and the atherosclerotic diseases involves many biochemical and physiologic variables spanning several decades. It terminates in a variety of clinical syndromes in different target organs. The human model of cigarette smoking--that is, the human smoker--also involves alterations of many biochemical and physiologic variables, some of which may influence atherogenesis.

Atherosclerosis is only one of the diseases related to inhalation of cigarette smoke. Chronic obstructive pulmonary disease involves a different organ system with different anatomy, functions, and responses. Our knowledge of the natural history of chronic obstructive pulmonary disease is not as well developed as is our knowledge of atherosclerosis.

Finally, with regard to cancers associated with cigarette smoking, carcinogenesis also may be more complex than the effects of carcinogenic substances on cells. For example, we are learning about the role of viruses in causing cancer and also about the role of the immune system in preventing the spread of cancer cells in the body.

Table 1. Blood chemical variables potentially related to atherogenesis in cigarette smokers compared with nonsmokers.

Variable	Comparison	Reference
Albumin	Decreased	Dales, Friedman, Siegelaub, and Seltzer, 1974 (4)
Calcium	Equal	Dales, Friedman, Siegelaub, and Seltzer, 1974 (4)
Carcinoembryonic antigen	Increased	Alexander, Silverman, and Chretien, 1976 (5)
Cholesterol	Increased	Karvonen, Orma, Keys, Fidanza, and Brozek, 1959 (6) Blackburn, Brozek, Taylor, and Keys, 1960 (7) Pincherle, 1971 (8) Dales, Friedman, Siegelaub, and Seltzer, 1974 (4) Billimoria, Pozner, Metselaar, Best, and James, 1975 (9)
	Equal	Acheson and Jessop, 1961 (10) Konttinen, 1962 (11) Higgins and Kjelsberg, 1967 (12) Andrus, Miller, Stallones, Ehrlich, and Jones, 1968 (13) Howell, 1970 (14)
Creatinine	Decreased	Dales, Friedmann, Siegelaub, and Seltzer, 1974 (4)
	Equal	Andrus, Miller, Stallones, Ehrlich, and Jones, 1968 (13)
Globulin	Decreased	Dales, Friedman, Siegelaub, and Seltzer, 1974 (4)
Glucose	Increased	Blackburn, Brozek, Taylor, and Keys, 1960 (7) Szanto, 1967 (15) Higgins and Kjelsberg, 1967 (12)
Glutamic oxalic transaminase	Equal	Dales, Friedmann, Siegelaub, and Seltzer, 1974 (4)
Triglyceride	Increased	Billimoria, Pozner, Metselaar, Best, and James, 1975 (9)
	Equal	Konttinen and Rajasalmi, 1963 (15)
Uric acid	Decreased	Higgins and Kjelsberg, 1967 (12) Dales, Friedmann, Siegelaub, and Seltzer, 1974 (4) Howell, 1970 (14)
	Equal	Andrus, Miller, Stallones, Ehrlich, and Jones, 1968 (13)

Table 2. Hematologic variables potentially related to atherogenesis in cigarette smokers compared with nonsmokers.

Variable	Comparison	Reference
Hemoglobin	Increased	Eisen and Hammond, 1956 (17) Isager and Hagerup, 1971 (18) Billimoria, Pozner, Metselaar, Best, and James, 1975 (9)
	Equal	Blackburn, Brozek, Taylor, and Keys, 1960 (7) Howell, 1970 (14)
Hematocrit	Increased	Eisen and Hammond, 1956 (17) Andrus, Miller, Stallones, Ehrlich, and Jones, 1968 (13) Isager and Hagerup, 1971 (18) Billimoria, Pozner, Metselaar, Best, and James, 1975 (9) Helman and Rubenstein, 1975 (19)
Erythrocyte count	Increased	Eisen and Hammond, 1956 (17)
White blood cell count	Increased	Howell, 1970 (14) Corre, Lellouch, and Schwartz, 1971 (20) Billimoria, Pozner, Metselaar, Best, and James, 1975 (9) Fisch and Freedman, 1975 (21) Helman and Rubenstein, 1975 (19)
Platelet count	Equal	Eisen and Hammond, 1956 (17)
Platelet adhesiveness	Increased	Ashby, Dalby, and Millar, 1965 (22)
Platelet aggregation	Increased	Glynn, Mustard, Buchanan, and Murphy, 1966 (23) Hawkins, 1972 (24) Levine, 1973 (25)
Platelet life span	Decreased	Mustard and Murphy, 1963 (26)
	Equal	Steele, Weily, Davies, and Genton, 1973 (27)
Fibrinolytic activity	Increased	Janzon and Nilsson, 1975 (28)
Fibrinogen	Increased	Ogston, Bennett, and Ogston, 1970 (29)

Table 3. Physiologic and endocrinologic variables potentially related to atherogenesis in cigarette smokers compared with nonsmokers.

Variable	Comparison	Reference
Body weight	Decreased	Karvonen, Orma, Keys, Fidanza, and Brozek, 1959 (6) Blackburn, Brozek, Taylor, and Keys, 1960 (7) Higgins and Kjelsberg, 1967 (12)
Skin fold	Decreased	Karvonen, Orma, Keys, Fidanza, and Brozek, 1959 (6) Blackburn, Brozek, Taylor, and Keys, 1960 (7) Higgins and Kjelsberg, 1967 (12) Andrus, Miller, Stallones, Ehrlich, and Jones, 1968 (13)
Blood pressure	Decreased	Karvonen, Orma, Keys, Fidanza, and Brozek, 1959 (6) Blackburn, Brozek, Taylor, and Keys, 1960 (7) Higgins and Kjelsberg, 1967 (12) Andrus, Miller, Stallones, Ehrlich, and Jones, 1968 (13)
Heart rate	Increased	Blackburn, Brozek, Taylor, and Keys, 1960 (7) Higgins and Kjelsberg, 1967 (12)
Serum insulin	Increased	Szanto, 1967 (15)
Glucose tolerance	Reduced	Dales, Freidman, Siegelaub, and Seltzer, 1974 (4)
Salivary antipyrine disappearance rate	Increased	Hart, Farrell, Cooksley, and Powell, 1976 (30)

DRAFT

40

NATIONAL CANCER INSTITUTE
SMOKING AND HEALTH PROGRAM

76 X Go-8v

PROCEEDINGS OF THE TOBACCO SMOKE INHALATION WORKSHOP on BIOASSAY MODELS AND INHALATION TOXICOLOGY

Held in
Tampa, Florida on
October 20-22, 1976
Gio B. Gori, Editor

A-3-F

F-2,3

A-2-F

A-7-F

Department of Health, .
Education and Welfare
Public Health Service
National Institutes of Health
DHEW Publication No.

1981 DRAFT

13

TABLE OF CONTENTS