All of the safety aspects of hydrogen utilization will have to be examined, especially the problems of safety in the domestic use and the long distance transport of hydrogen in pipelines at high pressures.

It is our opinion that the various energy planning agencies should now begin to outline the mode of implementing hydrogen energy delivery systems in the energy economy. The initial transition to hydrogen energy derived from available fossil fuels such as coal should be considered together with the long range view of all the hydrogen being derived eventually from nuclear energy. By the year 1985 when petroleum imports may be in excess of the domestic supply, these plans could set the stage for the transition period from fossil to a predominantly nuclear energy economy able to supply abundant synthetic fuels such as hydrogen. Synthetic fuels will obviously be more expensive than fuels now derived from petroleum; however, there may be no other viable choice. Thus, it is essential that the analysis and technological feasibility of a hydrogen energy system be considered now. It is of vital importance to the nation to develop some general-purpose fuel that can be produced from a variety of domestic energy sources and reduce our dependence on imported oil.

**References and Notes**


1. L. W. Jones, *Science* **174**, 367 (1971); G. De Beni and C. Marchetti, *Euro Spectra* **1970**, 46 (1970); D. P. Gregory, D. Y. C. Ng, G. M. Long, in *The Electrochemistry of Cleaner Environments*, J. O'M. Bockris, Ed. (Plenum, New York, 1971); D. P. Gregory, *Pub. Util. Fortn.* **89**, 21 (1972); L. O. Williams, *Astronaut. Aeronaut.* (Feb. 1972), p. 42.
2. B. W. Vincent, K. W. Webb, P. Don Vito, "A report on preliminary findings on innovative utility technologies of operation breakthrough" (Working paper 118-1, Urban Institute, Washington, D.C., 5 May 1971).
3. K. V. Kordesch, *J. Electrochem. Soc.* **118**, 812 (1971); G. Evans, "Hydrogen-air fuel cell for vehicle propulsion," presented at Symposium on Power Systems for Electrical Vehicles, April 1967.
4. R. J. Schoeppel, "Design criteria for hydrogen burning engines, final report" (Oklahoma State Univ. contract EHS 70-103 with the Environmental Protection Agency, October 1971); P. Underwood and P. Dieges, "Hydrogen and oxygen combustion for pollution free operation of existing standard engines," Proceedings of the Intersociety Energy Conversion Engineering Conference 1971 (Society of Automotive Engineers), p. 317; R. O. King, S. V. Hayes, A. B. Allen, R. W. P. Anderson, E. J. Waler, *Trans. Eng. Inst. Can.* **2**, 143 (1958).
5. National Urban Vehicle Design Competition, held in Detroit at the General Motors Proving Ground, August 1972.
6. K. C. Hoffman, W. E. Winsche, R. H. Wiswall, J. J. Reilly, T. V. Sheehan, C. H. Waide, "Metal hydrides as a source of fuel for vehicular propulsion," International Automotive Engineering Congress, Detroit, Mich., 13–17 January 1969, sponsored by the Society of Automotive Engineers.
7. Proceeding of a symposium on Abundant Nuclear Energy, held at Gatlinburg, Tenn., 1968 (U.S. Atomic Energy Commission, Division of Technical Information, Oak Ridge, Tenn., 1969).
8. R. L. Costa and P. G. Grimes, *Chem. Eng. Progr.* **63**, 56 (1967).
9. J. E. Mrochek, in *Ammonia, A Fertilizer*, A. V. Slack and G. R. James, Eds. (Decker, New York, in press).
10. *Encyclopedia of Chemical Technology* (Interscience, New York, ed. 2, 1966), vol. 10, p. 353.
11. W. E. Winsche, T. V. Sheehan, K. C. Hoffman, "Hydrogen—a clean fuel for urban areas," Proceedings of the Intersociety Energy Conversion Engineering Conference, August 1971, p. 38.
12. One British thermal unit is equivalent to 1055 joules; 1 mile is equivalent to 1.6 kilometers; a mill is a unit of monetary value equal to 1/1000 dollars; 1 lb. is equivalent to 0.45 kilogram.
13. D. P. Gregory, Institute of Gas Technology, private communication.
14. Committee on U.S. Energy Outlook, *U.S. Energy Outlook; An Initial Appraisal 1971–1985* (National Petroleum Council, Washington, D.C., 1971), vol. 2.
15. These demands are our estimates and represent the national average for a single household at the present time. It is assumed that in the future the pattern of domestic energy use will not change significantly.
16. U.S. Department of Interior and the Edison Electric Institute, *Underground Power Transmission* by Arthur D. Little, Inc. Electric Research Council of Edison Electric Institute, Publ. No. 1-72 (1966).
17. We have estimated that in the year 2000, off-peak power could be available to supply 50 percent of the energy required by private automobiles.
18. See Committee on U.S. Energy Outlook (*14*), pp. 53–54 for the cost of coal gasification to methane.
19. The Pratt and Whitney Aircraft Company is conducting limited tests of fuel cells produced for the "Target Project" which are intended for commercial residential applications.
20. Office of Coal Research, *Final Report Project Fuel Cell* (Research and Development Report No. 57, Office of Coal Research, U.S. Department of the Interior, Washington, D.C., 1970).
21. U.S. Federal Power Commission, *The 1970 National Power Survey* (Government Printing Office, Washington, D.C., 1971), parts 2 and 3.
22. U.S. Bureau of the Census, *Statistical Abstract of the United States; 1970* (U.S. Bureau of the Census, Washington, D.C., ed. 91, 1970), p. 515.


23. This work was performed under the auspices of the U.S. Atomic Energy Commission.


# Atherosclerosis and the Arterial Smooth Muscle Cell

## Proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis.


Russell Ross and John A. Glomset


Atherosclerosis is a disease of large- and medium-sized arteries, and is characterized by focal thickening of the inner portion of the artery wall in association with fatty deposits. Most commonly affected are the aorta and the iliac, femoral, coronary, and cerebral arteries. Because atherosclerosis can progressively or abruptly interfere with blood flow, particularly through the heart and brain, it often causes serious clinical consequences such as heart attack and stroke. Indeed, a recently published report (*1*) by a task force of the National Heart and Lung Institute (NHLI) indicates that atherosclerosis is the chief cause of death in the United States. Nevertheless, relatively little is known about the genesis of the disease. Studies have shown clearly that factors such as lipid concentrations of the plasma, blood pressure, and smoking habits strongly influence the development of clinical symptoms, but the sequence of pathological events at the cellular level remains to be clarified. Only recently have investigators begun to explore the disease process in terms of the biology of the major cell types involved. In this article we will focus on one of these, the arterial smooth muscle cell, with discussion of some fundamental questions regarding its biology and pathobiology, and description of some experimental approaches that we are using to investigate these questions.


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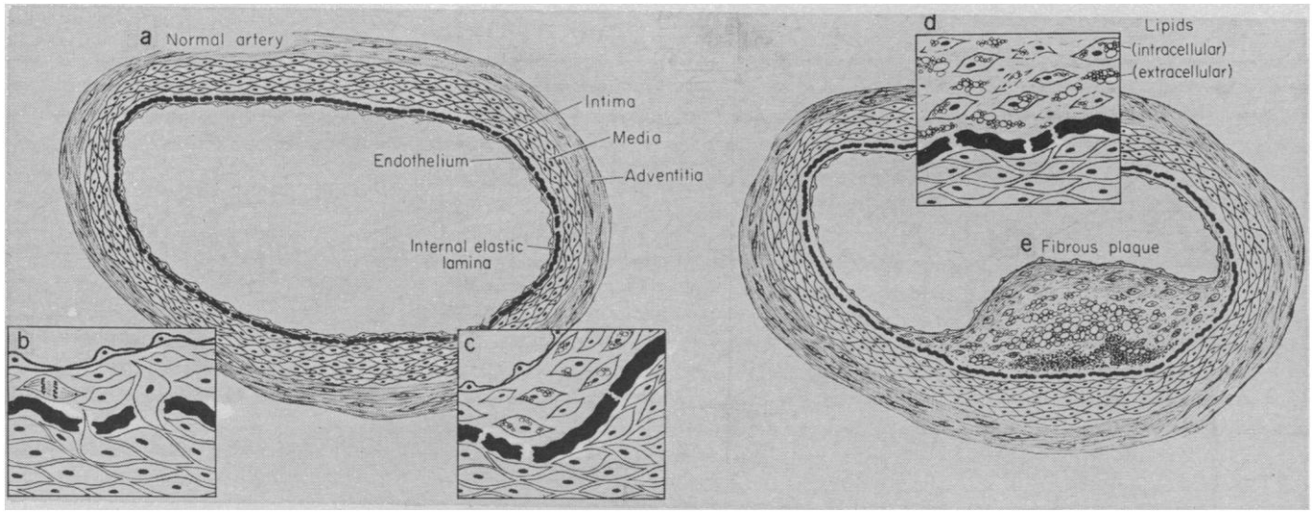
## The Normal Artery

To appreciate the role of the arterial smooth muscle cell in atherosclerosis, it is necessary to know something about the structure of normal arteries. Normal arteries are composed of three morphologically distinct layers (Fig. 1). The intima, or inner layer of the artery, consists in the newborn and in children of a narrow region bounded on one side by a single layer of endothelial cells that line the lumen of the vessel and on the other side by the internal elastic lamina, a perforated sheet of elastic tissue. Between these two boundaries are occasional smooth muscle cells surrounded by various extracellular components of connective tissue matrix. With increasing age, smooth muscle cells and extracellular matrix components accumulate slowly within the intima, increasing its thickness. The media, or middle layer of the artery, consists of large numbers of smooth muscle cells, each surrounded by small amounts of collagen and varying numbers of small elastic fibers and other connective tissue matrix components. There are no fibroblasts



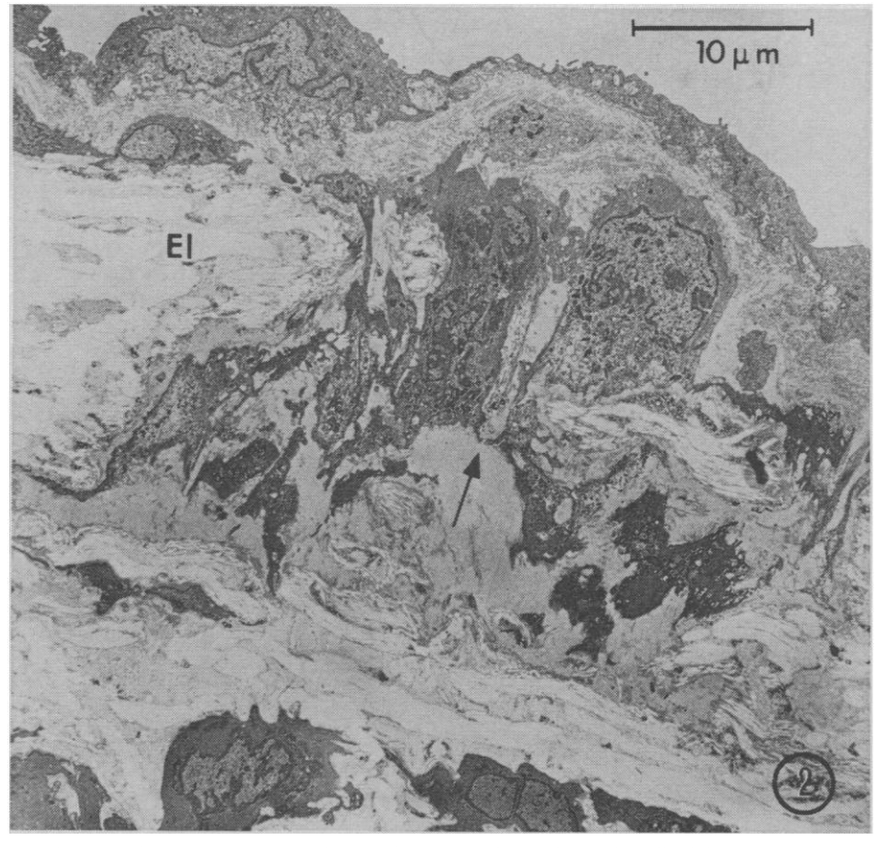




Fig. 1 (above). A series of possible stages in the development of the various lesions of atherosclerosis. (a) The appearance of a normal muscular artery and its component layers: the intima bounded by endothelium and internal elastic lamina, the media, and adventitia. In children and young adults the intima is thin and contains only an occasional smooth muscle cell; with age it slowly and uniformly increases in thickness and cell content. It is important to note that there are no fibroblasts present in either the intima or the media of mammalian arteries. Fibroblasts are found only in the adventitia. (b) The first phase of a developing lesion in atherosclerosis: a focal thickening of the intima consists of an increase in smooth muscle cells and extracellular matrix. Smooth muscle cells are shown proliferating within the intima; two are in the process of migrating through fenestrae of the internal elastic lamina, similar to those in Fig. 2. Subsequent to or possibly concomitant with intimal smooth muscle proliferation, accumulation of intercellular lipid deposits (c) or extracellular lipid (d), or both, occur resulting in a fatty streak. A fibrous plaque (e) may result from a continued accumulation of a connective tissue cap covering increased numbers of smooth muscle cells laden with lipids, extracellular lipid, and cell debris overlying a deeper extracellular pool of lipid. A complicated lesion may form as a result of continuing cell degeneration, ingress of blood constituents, and calcification superimposed upon the elements present in the fibrous plaque. Observations made at necropsy, and experiments such as those described in the text, suggest that this may represent the sequence of events that occurs in man. Fig. 2 (right). Electron micrograph of the right iliac artery from a primate (*M. nemestrina*) 10 days after the endothelium of the vessel was removed with an intravascular balloon catheter. The endothelium has regenerated and portions of several smooth muscle cells (arrow) can be seen within a fenestra of the internal elastic lamina (*El*) presumably in the process of migrating into the intima. The intact unoperated left iliac artery from this animal contained only an occasional smooth muscle cell within its intima.

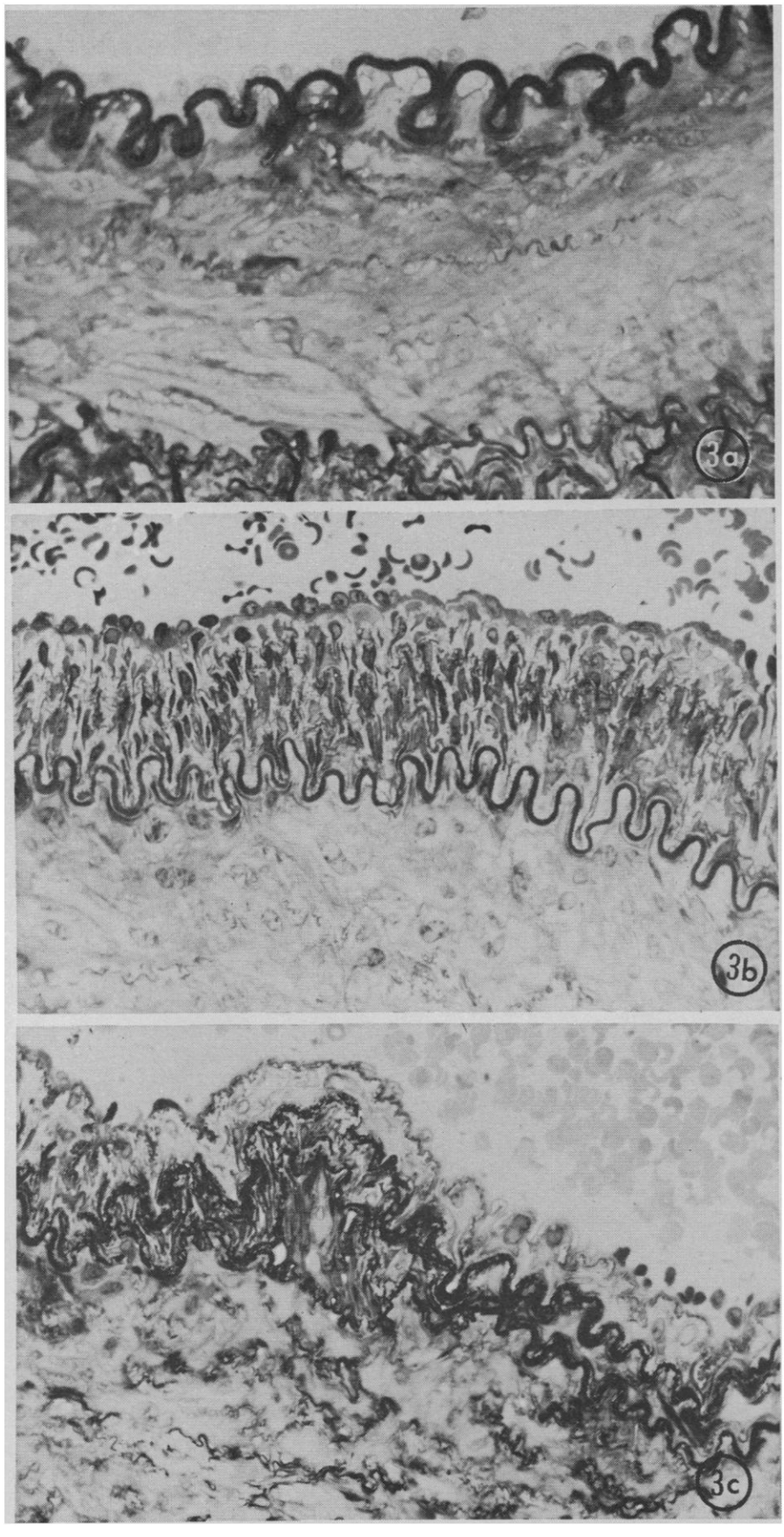



Fig. 3. Light micrographs demonstrating (a) a normal primate iliac artery (*M. nemestrina*) (× 550); (b) an iliac artery 3 months after deendothelialization, showing a marked increase in intimal thickness due to accumulation of smooth muscle cells and extracellular matrix (× 500); and (c) an iliac artery 6 months after deendothelialization, by which time the intimal thickness had returned to one to two layers (× 500). This sequence demonstrated the relative reversibility of an experimentally induced lesion in a monkey on a normal diet with normal concentrations of plasma lipid.

present in the media of mammalian arteries in contrast to the arteries of other species such as birds (*2*). Particularly large amounts of elastic tissue are found in the aorta as compared with the smaller, muscular arteries. The morphology of the media, in contrast to that of the intima, generally does not alter with age. The adventitia, or outer layer of the artery, consists mainly of fibroblasts and loosely arranged collagen and glycosaminoglycans (mucopolysaccharides); it is generally separated from the media by a more poorly defined sheet of elastic tissue, the external elastic lamina.

## Lesions of Atherosclerosis

Of the three layers mentioned, the intima is the one predominantly affected by the focal lesions of atherosclerosis. Three classic types of lesion are recognized: the fatty streak, the fibrous plaque, and the so-called "complicated lesion" (*1*). Fatty streaks are yellowish, relatively flat areas that are readily seen when the lumen of an affected artery is exposed by gross dissection. The yellow color is associated with the presence of lipid deposits, found mainly within intimal smooth muscle cells (foam cells) (Fig. 1c). These foamy smooth muscle cells may surround small irregularly dispersed extracellular deposits of lipids (Fig. 1d). Fibrous plaques are whitish in gross appearance and are elevated so that they protrude into the lumen. The elevation is associated with a focal accumulation of intimal lipid-containing smooth muscle cells. These cells, collagen, and elastic fibers form a cap that covers a large, deeper deposit of extracellular lipid and cell debris (Fig. 1e). Complicated lesions appear to be fibrous plaques that have been altered by hemorrhage, calcification, cell necrosis, and other changes. They are often accompanied by lumenal erosion of the arterial wall and mural thrombosis.

## Role of Arterial Smooth Muscle

These descriptions of the classic types of lesion emphasize the accumulation of lipid, connective tissue matrix components, calcium, and cell debris. It is important to note, however, that focal accumulation of intimal smooth muscle cells is fundamental to the entire process. Indeed, it can be

argued that the accumulation of smooth muscle cells necessarily precedes or accompanies both the deposition of lipid and the accumulation of extracellular connective tissue matrix, because the lipid deposits occur either within smooth muscle cells or outside them in association with connective tissue matrix components which are secretory products of smooth muscle cells. Thus, one possible sequence of events in atherosclerosis is depicted in Fig. 1. This diagram suggests a focal increase in the number of intimal smooth muscle cells as the earliest phase of lesion development (see Fig. 1b), followed by the deposition of either intracellular or extracellular lipid (Fig. 1c). This sequence would also suggest that increased cell proliferation, lipid deposition, and synthesis of connective tissue matrix components ultimately lead to the formation of fibrous plaques (Fig. 1e), and that subsequent cell disintegration, calcification, and deposition of blood products eventually cause the formation of complicated lesions. It must be pointed out that this sequence represents only a possibility that remains to be established, since most of the details of the postulated relationships have not been clarified. Furthermore, a very real problem exists in establishing any type of sequence because, as the NHLI task force (*1*) pointed out, we are unable "to observe and study a single site in the arterial vasculature more than once." The fact remains, however, that arterial smooth muscle cells and their products are of central importance to all phases of atherosclerosis irrespective of the exact sequence of events. Without smooth muscle cell proliferation and the subsequent accumulation of connective tissue matrix components, the events that in many instances cause vascular occlusion and death could not occur.

### Experiments in vivo

What is known about the biology of arterial smooth muscle cells, and what are some of the approaches being used to investigate the role of these cells in atherosclerosis? It is not yet clear whether the increased numbers of smooth muscle cells found in atherosclerotic lesions are derived from a relatively small, preexistent population of intimal smooth muscle cells or from medial smooth muscle cells that have migrated into the intima and subsequently proliferated. However, there is evidence about the nature of the stimulus to their proliferation. Smooth muscle cells normally accumulate in the intima at arterial branch points, where endothelial permeability appears to be increased (*3, 4*). Similarly, accumulation of intimal smooth muscle cells can be caused experimentally by injuring the endothelium, thereby increasing its permeability. In our laboratory for example, arterial lesions identical in appearance to the "fibromusculoelastic" lesion seen in man were induced in nonhuman primates by removing the arterial endothelium with the aid of an intravascular balloon catheter (*5*). These catheters were inserted into the external femoral arteries of 1-year-old macaques (*Macaca nemestrina*), inflated, and passed through the iliac artery into the abdominal aorta, withdrawn back into the femoral artery, deflated, and removed. This procedure, similar to an earlier one used to induce microthrombi on denuded vessel walls (*6*), selectively removed the endothelium from the iliac artery leaving the remainder of the artery wall, including the internal elastic lamina, intact. From 10 minutes to 6 months after removal of the endothelium the arteries were examined by light and electron microscopy. During the first 24 hours, platelet microthrombi were seen adherent to the exposed internal elastic lamina; 3 to 5 days after injury the endothelium had begun to regenerate, and within 1 week after injury medial smooth muscle cells were observed extending through fenestrae of the internal elastic lamina into the intima (Fig. 2). By 14 days the endothelium had regenerated, as judged by its microscopic appearance, and the intima was thickened and contained five to ten layers of smooth muscle cells. This was in sharp contrast to the normal, uninjured intima which, like the intima of young human beings, contained only

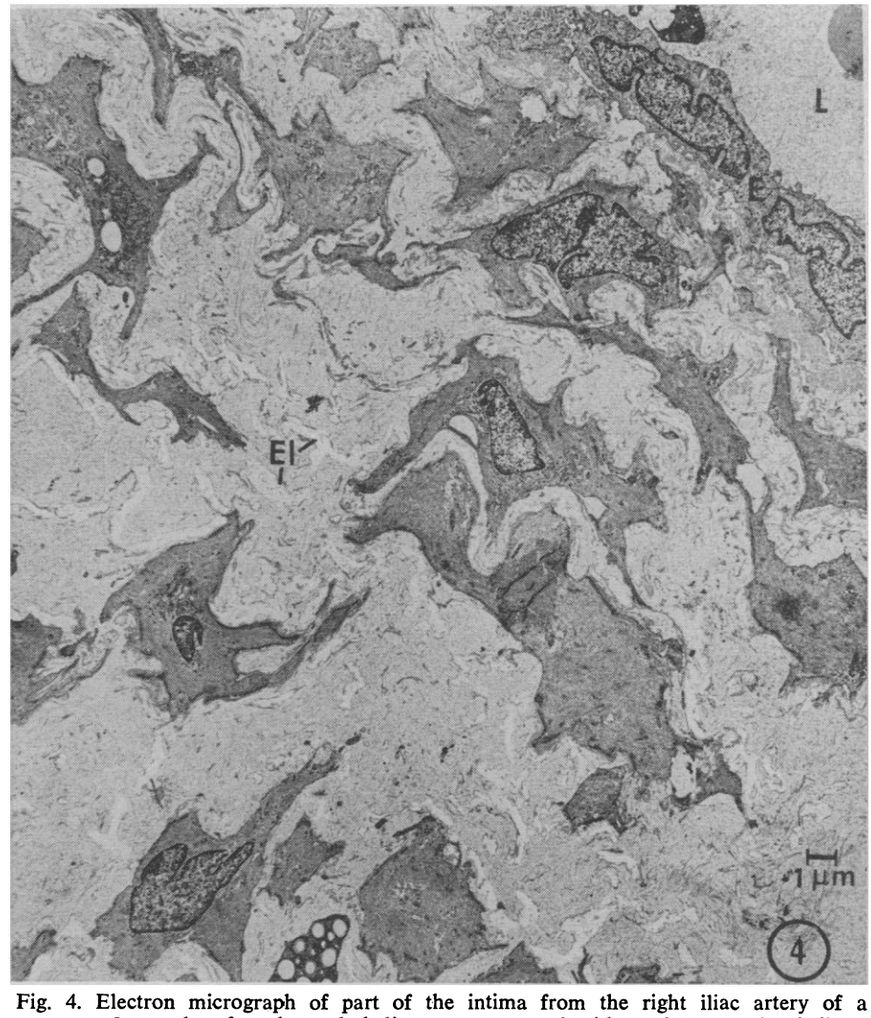



Fig. 4. Electron micrograph of part of the intima from the right iliac artery of a macaque 3 months after the endothelium was removed with an intravascular balloon catheter. The lumen (*L*) is to the upper right. Endothelial cells (*E*) cover the markedly thickened intima which contains large numbers of smooth muscle cells surrounded by a matrix of small elastic fibers (*El*), collagen, and proteoglycan.

an occasional smooth muscle cell. Three months after injury the lesion contained as many as 15 layers of smooth muscle cells surrounded by collagen and immature elastic fibers (Figs. 3b and 4). These experiments suggest that the intact arterial endothelium normally acts as a barrier to some substance or substances present in plasma which upon exposure to vascular smooth muscle promote cell proliferation.

### Experiments in vitro

We are currently attempting to identify these growth promoting substances by growing arterial smooth muscle cells in vitro in the presence of various serum fractions. Medial smooth muscle cells derived from the thoracic aorta of macaques (*M. nemestrina*) are grown in a modified Dulbecco-Vogt modification of Eagle's medium containing serum from the same species (*7*). In the presence of 5 percent serum these cells maintain their differentiated appearance (Fig. 5), produce elastic fiber proteins and collagen, and grow logarithmically for 10 to 14 days before entering stationary growth. Their growth rate differs from that of fibroblasts, and their patterns of growth also differ (*7, 8*). In 1 percent serum the cells grow logarithmically for only 2 to 4 days before entering stationary phase. However, as in the case of the assay system used by Paul *et al.* (*9*), once the cells are stationary it is possible to stimulate them to grow logarithmically again by increasing the concentration of serum (Fig. 6). If dialyzed serum instead of whole serum is added, the cells also grow logarithmically, which suggests that the growth promoting factors are proteins. Since some serum lipoproteins have been strongly implicated in atherogenesis, we have been conducting experiments to examine the effect of adding serum proteins from which the lipoproteins have been removed by differential flotation (*10*). As shown in Fig. 6, the serum proteins of density greater than 1.25 grams per milliliter, that is, the fraction from which all of the low density lipoproteins (LDL) and most of the high density lipoproteins (HDL) had been removed, supported cell growth, but did so to a considerably lesser extent than did a mixture of these proteins with LDL, HDL, or both LDL and HDL. Presumably, the proteins of density greater than 1.25 grams per milliliter contain material similar to that which has been found to promote the growth of fibroblasts in culture (*9, 11*). The growth promoting effect of the lipoproteins is of special interest in view of the established relation between the concentration of plasma lipoprotein cholesterol and the extent of atherosclerosis. The basis for the growth promoting effect of lipoproteins remains to be studied. The role played by the protein components is unclear, but it seems likely that lipoproteins provide lipids that can be used in cell membrane formation. This has been suggested by experiments of fibroblasts in culture which have shown that cholesterol biosynthesis is effectively inhibited by the presence of lipoproteins in the medium (*11*). These experiments coupled with our own suggest that certain cells can use lipoprotein cholesterol to form membranes, and that the rate of biosynthesis of membrane cholesterol may limit cell proliferation in the absence of an external lipoprotein source.

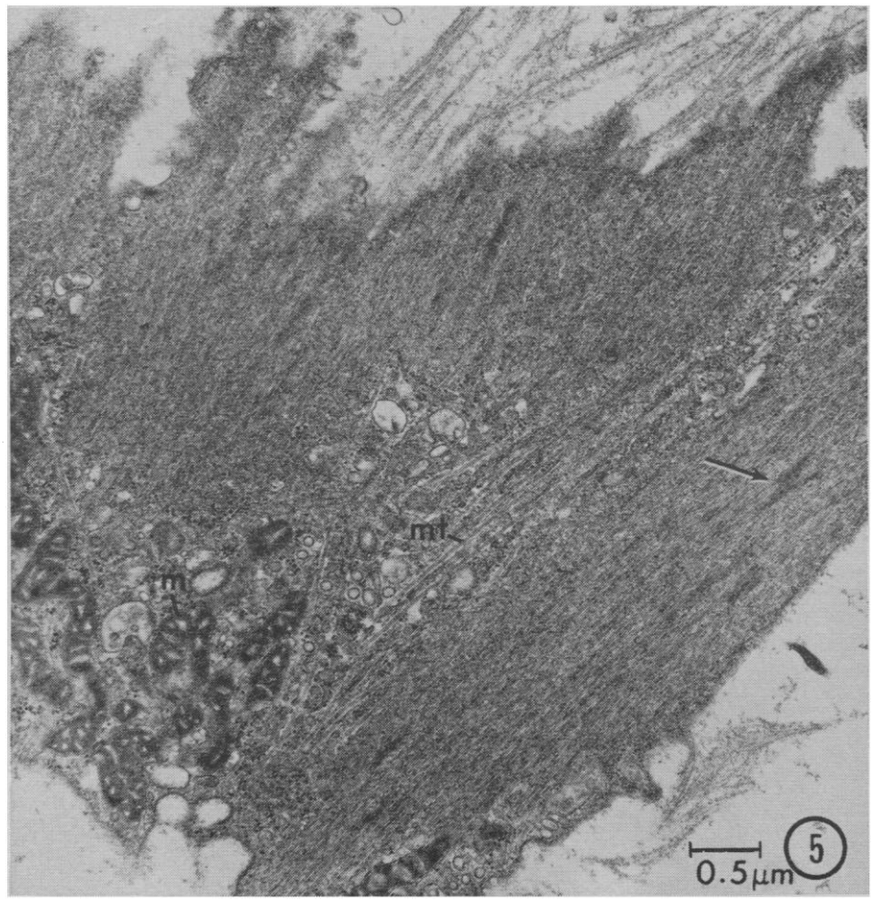



Fig. 5. Electron micrograph demonstrating the typical appearance of a macaque aortic medial smooth muscle cell after several generations of growth in culture. The cells were fixed in situ, embedded, and sectioned parallel to the plane of the surface of the culture dish so that an *en face* view of the cell is seen. The cytoplasm is abundant with myofilaments and dense bodies (arrow). Microtubles (*mt*) and mitochondria (*m*) also are visible.

Experiments such as that shown in Fig. 6 have demonstrated that LDL have a greater growth promoting effect than HDL, even when the concentrations of lipoprotein cholesterol are equal. The basis for the difference between lipoprotein fractions remains to be clarified, but a greater number of inclusion bodies are seen in cells grown in the presence of LDL than in those grown in the presence of HDL (*13*), and this may reflect differences in lipoprotein uptake. If the rate of uptake exceeds the rate of utilization of lipoprotein cholesterol, cholesterol might accumulate within cell inclusion bodies.

This type of phenomenon might also explain the extensive vacuolization seen in intimal smooth muscle cells in lesions resulting from removal of the arterial endothelium with a balloon catheter in macaques fed a high cholesterol diet (*14*). The diet increased the concentration of plasma cholesterol from a normal level of about 120 milligrams per 100 milliliters to approximately 400 milligrams per 100 milliliters, and this was associated with a severalfold rise in the concentration of LDL. Thus, it seems

likely that the vacuoles in the proliferating smooth muscle cells arose in connection with the increased uptake of LDL from the extracellular fluid in the intima. Smith and Slater (*12*) have already obtained evidence that the concentration of LDL even in the normal human intima with intact endothelium is directly proportional to the concentration of LDL in the plasma.

### Connective Tissue Metabolism

The mechanisms that promote cell proliferation are not the only ones relevant to the pathobiology of arterial smooth muscle. Arterial smooth muscle cells synthesize extracellular connective tissue matrix components including collagen, elastic fiber proteins and glycosaminoglycans (mucopolysaccharides) and so can be regarded as analogs of fibroblasts of tendon, the chondroblasts of cartilage, and the osteoblasts of bone (*15, 16*). This is important because at least one of these components, collagen, is a major constituent of fibrous and complicated atherosclerotic plaques (*3, 4, 17, 18*). Evidence that arterial smooth muscle cells synthesize collagen and elastic fibers in vivo (*15*) and in vitro (*7*) has been obtained by autoradiography, and by electron microscopic and chemical analysis. Recent experiments in our laboratories (*19*) also have shown that arterial smooth muscle cells in culture incorporate tritiated lysine into a protein of approximately 70,000 daltons. This protein serves as a substrate for the enzyme lysyl oxidase, and is presumably soluble elastin. Other recent experiments in our laboratory (*20*), have shown that the cells incorporate isotopically labeled sulfate into glycosaminoglycans under similar conditions, demonstrating their capacity to make this connective tissue matrix component. The factors that promote the synthesis of these connective tissue matrix constituents in atherosclerosis are not understood nor has the "program" of matrix component biosynthesis in the experimentally injured artery been extensively studied. Furthermore, essentially nothing is known about the mechanisms that remove extracellular matrix components during the "healing" of injured arteries. However, evidence does exist that at least partial "healing" occurs. In the balloon catheterization experiments already described, the intimal lesion which contained as many as 15 layers of smooth muscle cells with associated connective tissue matrix 3 months after injury, was much thinner and contained only one to two layers after 6 months in animals that were normocholesteremic (*5*) (Fig. 3c).

If "healing" occurs after experimental injury to the intima of nonhuman primates, why do atherosclerotic lesions progress relentlessly in susceptible humans? One possibility is that the injury in man is repetitive or continuous. Another is that humans who are at increased risk of developing atherosclerotic heart disease tend to have increased concentrations of LDL in the plasma (*21*) and probably also in the extracellular fluid of the intima (*12*), so that the intimal LDL might increase smooth muscle cell proliferation or in some unknown way interfere with the metabolism of the cells. Alternatively, the LDL might become associated with extracellular glycosaminoglycans (*22*) or with elastin (*23*) and interfere with matrix turnover. Still another possibility is that lipoproteins become entrapped within the matrix, in time spontaneously denature, and yield insoluble deposits of lipid, no longer capable of diffusing away upon removal of the matrix.

### Other Factors in Atherosclerosis

Although we and others (*3, 4, 17, 18, 24, 25*) have focused on the role of the arterial smooth muscle cell in atherosclerosis, there are other factors which are also important. Implicit in this discussion are the assumptions that the arterial endothelium normally provides a barrier to circulating plasma factors that influence the metabolism of

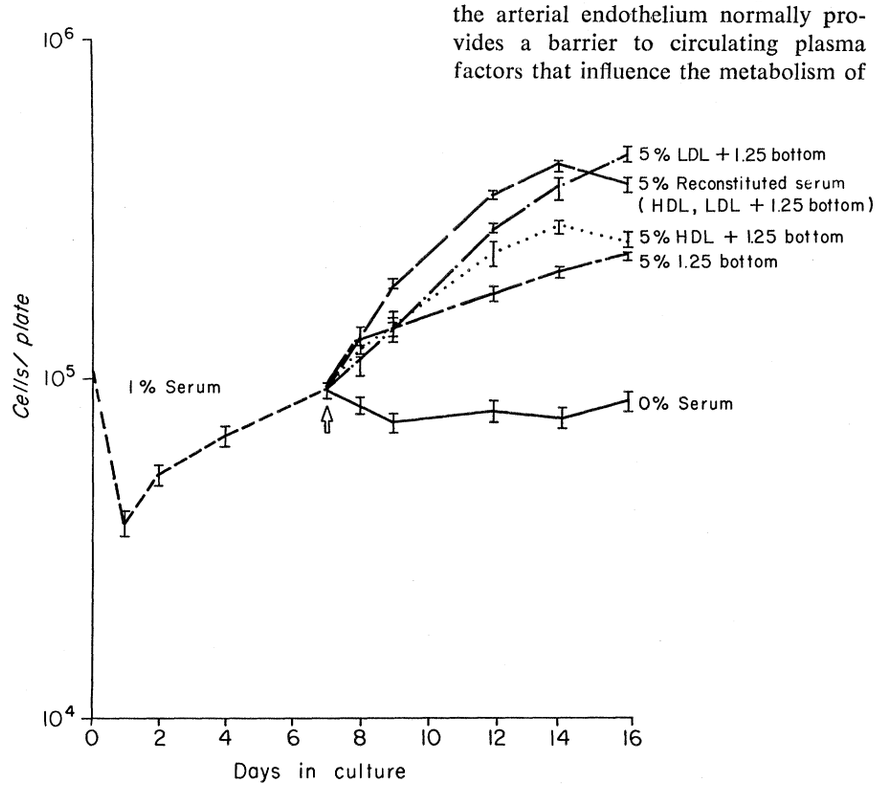



Fig. 6. Response of arterial smooth muscle to serum fractions. Equal numbers ($10^5$) of smooth muscle cells were added to a large series of petri dishes and incubated in a modified Dulbecco-Vogt modification of Eagle's medium (*7*) containing 1 percent serum pooled from several *Macaca nemestrina*. After 7 days (arrow), the dishes were separated into five groups to be further incubated. One group was incubated in serum-free medium. The remaining groups were incubated in media containing: dialyzed protein of density greater than 1.25 g/ml from the equivalent of 5 percent serum; this protein fraction contained very little high density lipoprotein (HDL) or low density lipoprotein (LDL); proteins of density greater than 1.25 g/ml plus HDL (154 nmole of cholesterol per milliliter of medium); proteins of density greater than 1.25 g/ml plus LDL (154 nmole per milliliter of medium); and reconstituted serum containing proteins of density greater than 1.25 g/ml plus HDL (77 nmole of HDL cholesterol per milliliter of medium) plus LDL (77 nmole of LDL cholesterol per milliliter of medium). The pooled primate serum used as a source of lipoprotein in these experiments contained 154 nmole of lipoprotein in 5 percent whole serum. This experiment demonstrates that both serum lipoproteins and proteins of density greater than 1.25 g/ml stimulate smooth muscle cell proliferation in vitro and that LDL is at least as effective as a combination of LDL and HDL. Thus, these observations support the concept that endothelial injury in vivo could promote smooth muscle cell proliferation by increasing the concentration of plasma proteins in the extracellular fluid of the vessel wall and points to the potential importance of plasma LDL in this response. Vertical bars represent standard error of the mean.

smooth muscle cells, and that atherosclerosis is associated with the diminution of this barrier because of endothelial injury. The concept that endothelial injury promotes atherosclerosis dates back to Virchow (*26*), who in 1856 noted degenerative changes associated with developing atheromata and suggested that irritation of arterial intima by mechanical forces causes degenerative and inflammatory consequences that stimulate a proliferative response of the cells. Virchow's hypothesis has subsequently been modified by many investigators including Duncan (*24*), who suggested that "injury" to the endothelium causes insudation of inflammatory fluid from the plasma into the intima followed by degeneration and proliferation. French (*3*) and others (*25*) have also pointed out that endothelial injury promotes the formation of microthrombi that participate in the developing atherosclerotic lesions. In view of the probable importance of the arterial endothelium in atherosclerosis it is clearly desirable that the normally functioning role of the endothelium and the response of the endothelium to injury be understood. In this connection it must be emphasized that, although capillary endothelium has been extensively studied, we know little about the functional capabilities of the arterial endothelium, its permeability characteristics, turnover, and ability to produce extracellular components.

Another important factor in atherosclerosis, stressed by investigators since Anitschkow (*27*), is lipid. Anitschkow and Chalatov (*28*) showed in 1913 that if cholesterol is fed to rabbits there is a great increase in the concentration of cholesterol in the plasma and cholesterol-containing lesions develop in the aortas; and Parker and Odland (*18*) showed in 1966 that much lipid accumulated within altered intimal smooth muscle cells. Other investigators (*29*) have subsequently reported similar effects in primates. In addition, the fact that cholesterol is a major component of human atherosclerotic lesions (*30*) and the well-established correlation between the concentration of lipoprotein cholesterol in plasma and the incidence or prevalence of atherosclerotic heart disease (*31*) support the concept that cholesterol, like the arterial smooth muscle cell, may be essential to the development of atherosclerosis. Unfortunately, we understand neither the factors that control the concentrations of cholesterol in the plasma nor the factors that influence the distribution of lipoprotein cholesterol between the blood and tissues. It is clear that cholesterol is insoluble in aqueous solutions except when present as a lipoprotein complex, that most of the cholesterol in plasma is a component of lipoproteins synthesized in the liver or the intestine, and that most cells cannot degrade cholesterol; but it is by no means clear how cholesterol is deposited in arteries, how it influences the tissue response of arteries, or how it could ever be transported away from arteries once deposited. Thus, as in the case of the endothelium, many more studies need to be done to clarify the physiologic and pathologic roles played by cholesterol.

## Conclusions: A Modified Hypothesis

Arterial smooth muscle cells clearly play a fundamental role in atherosclerosis. They are the principal cells that accumulate, they undoubtedly form the extracellular matrix components of atherosclerotic lesions, they accumulate intracellular lipid in the presence of increased concentrations of extracellular lipoprotein, and they may also promote the deposition of lipid in the extracellular matrix. Although the basis for these changes remains to be established, there is much to recommend the hypothesis (*24*) that endothelial injury is involved. Our studies support a modified hypothesis that the endothelium normally influences the behavior of arterial smooth muscle cells by providing a barrier to the passage of plasma proteins and that the major effect of hemodynamic or other factors that injure the endothelium is to decrease this barrier.

According to this hypothesis, local injury to the endothelium increases the concentration of plasma proteins in the vicinity of medial smooth muscle cells, and in response to some of these proteins the cells migrate into the intima and proliferate. When these changes in tissue architecture are accompanied by restoration of the endothelial permeability barrier, the lesion becomes self-limited and may regress. However, with continued injury to the endothelium, the smooth muscle cells that have already migrated into the intima are stimulated to proliferate further, and a critical balance between cell proliferation and cell destruction may determine whether the lesion enlarges or remains of relatively constant size. It may be by affecting this balance that factors such as hypertension, hormonal imbalances, and plasma constituents exert their greatest effects. Indeed, plasma lipoproteins such as LDL may convert what would ordinarily be a limited tissue response to injury into what is classically recognized as atherosclerosis by introducing an additional problem of balance: that of removing cholesterol from arteries once that cholesterol has been deposited and is no longer a component of a soluble lipoprotein complex. When cholesterol deposition exceeds cholesterol removal the balance is tipped toward irreversibility of the lesion and the development of clinical disease. Thus, according to this hypothesis, all individuals could have continually forming and regressing lesions, while clinical sequelae develop only in those in whom imbalance has occurred.

Clearly, this hypothesis and others related to atherosclerosis need to be carefully tested. Fortunately, techniques are now available for studying the responses of smooth muscle cells at the molecular level, so that new opportunities exist for investigators interested in studying the fundamental biology of cells having immediate relevance to a catastrophic human disease.

### References and Notes


1. National Heart and Lung Institute Task Force on Arteriosclerosis, *Arteriosclerosis* (National Institutes of Health, Bethesda, Md., Department of Health, Education, and Welfare Publ. No. (NIH) 72-219, June 1971), vol. 2.
2. P. H. Cooke and S. C. Smith, *Exp. Mol. Pathol.* **8**, 171 (1968).
3. J. E. French, *Int. Rev. Exp. Pathol.* **5**, 253 (1966).
4. M. D. Haust and R. H. More, in *Evolution of the Atherosclerotic Plaque*, R. J. Jones, Ed. (Univ. of Chicago Press, Chicago, 1963), p. 51; R. Jones, A. S. Daoud, O. Zumbo, F. Coulston, W. A. Thomas, *Exp. Mol. Pathol.* **7**, 34 (1967); H. C. McGill, Jr., and J. C. Geer, in *Evolution of the Atherosclerotic Plaque*, R. J. Jones, Ed. (Univ. of Chicago Press, Chicago, 1963), p. 65.
5. M. B. Stemerman and R. Ross, *J. Exp. Med.* **136**, 769 (1972).
6. H. R. Baumgartner and T. H. Spaet, *Fed. Proc.* **29**, 710 (1970); H. R. Baumgartner, M. B. Stemerman, T. H. Spaet, *Experientia* **27**, 282 (1971); M. B. Stemerman, H. R. Baumgartner, T. H. Spaet, *Lab. Invest.* **24**, 179 (1971); S. Bjorkerud, *Virchows Arch. Abt. A, Pathol. Anat.* **347**, 197 (1969); P. Helin, I. Lorenzen, C. Garbarsch, M. E. Matthiessen, *Atherosclerosis* **13**, 319 (1971); P. Helin, I. Lorenzen, C. Garbarsch, M. E. Matthiessen, *Circ. Res.* **29**, 542 (1971).
7. R. Ross, *J. Cell Biol.* **50**, 172 (1971); Proceedings of the Sigrid Juselius Foundation Symposium, Turku, Finland, August, 1972, in press.
8. R. W. Holley and J. A. Kiernan, *Proc. Nat. Acad. Sci. U.S.A.* **60**, 300, 968 (1968); L. N. Castor, *Exp. Cell Res.* **68**, 17 (1971); C. J. Bates and C. I. Levene, *J. Cell Sci.* **7**, 683 (1970).
9. D. Paul, A. Lipton, I. Klinger, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 645 (1971).
10. R. J. Havel, H. A. Eder, J. H. Bragdon, *J. Clin. Invest.* **34**, 1345 (1955).
11. R. W. Holley and J. A. Kiernan, *Growth Control in Cell Cultures*, G. E. W. Wolstenhome and J. Knight, Eds. (Churchill, London, 1971), p. 3; G. D. Clarke and M. G. P. Stoker, *ibid.*, p. 17; G. Y. Todaro, Y.

Matsuya, S. Bloom, A. Robbins, H. Green, *Wistar Inst. Symp. Monogr.* No. 7, 87 (1967).
12. E. B. Smith and R. S. Slater, *Lancet* **1972-I**, 463 (1972).
13. R. Ross and J. A. Glomset, unpublished data.
14. R. Ross, T. Wight, J. Glomset, M. Riddle, unpublished data.
15. R. Ross and S. J. Klebanoff, *J. Cell Biol.* **50**, 159 (1971); J. Kunz, D. Kranz, O. Keim, *Virchows Arch. Abt. A, Pathol. Anat.* **342**, 345 (1967); R. C. Buck, in *Atherosclerosis and Its Origin*, M. Sandler and G. H. Bourne, Eds. (Academic Press, New York, 1963); R. W. Wissler, *Circulation* **36**, 1 (1967).
16. R. Ross, in *Treatise on Collagen*, B. S. Gould, Ed. (Academic Press, New York, 1968), vol. 2, pp. 1–82.
17. E. R. Dalferes, Jr., and H. Ruiz, *Atherosclerosis* **13**, 121 (1971); S. A. Bencosme and J. U. Nalis, *Exp. Mol. Pathol.* **6**, 300 (1967).
18. F. Parker and G. F. Odland, *Amer. J. Pathol.* **48**, 451 (1966).
19. D. Layman and S. Narayanan, unpublished data.
20. T. Wight, unpublished data.
21. W. B. Kannel, M. J. Garcia, P. M. McNamara, G. Pearson, *Human Pathol.* **2**, 129 (1971).
22. S. R. Srinivasan, S. A. Lopez, B. Radakrishnamurthy, G. S. Berenson, *Atherosclerosis* **12**, 321 (1970); P.-H. Iverius, *J. Biol. Chem.* **247**, 2607 (1972).
23. D. M. Kramsch, G. Franzblau, W. Hollander, *J. Clin. Invest.* **50**, 1666, 1677 (1971).
24. L. E. Duncan, in *Evolution of the Atherosclerotic Plaque*, R. J. Jones, Ed. (Univ. of Chicago Press, Chicago, 1963).
25. M. D. Haust, in *Atherosclerosis: Proceedings of the Second International Symposium*, R. J. Jones, Ed. (Springer-Verlag, New York, 1970), pp. 12–20.
26. R. Virchow, in *Gesammelte Abhandhungen zur Wissenschaftlichen Medicin* (Meidinger Sohn, Frankfurt-am-Main, 1856), p. 458.
27. N. N. Anitschkow, *Beitr. Path. Anat. Allg. Pathol.* **56**, 379 (1913).
28. ——— and S. Chalatov, *Zentralbl. Allg. Pathol.* **24**, 1 (1913).
29. O. W. Portman and M. Alexander, *J. Lipid Res.* **11**, 23 (1970); R. L. Robinson, K. C. Hayes, H. L. McComb, T. P. Faherty, *Exp. Mol. Pathol.* **15**, 281 (1971); P. J. Manning and T. B. Clarkson, *ibid.* **17**, 38 (1972).
30. E. B. Smith, *J. Atheroscl. Res.* **5**, 224 (1965).
31. S. Dayton and S. Hashimoto, *Exp. Mol. Pathol.* **13**, 253 (1970); P. J. Scott and P. J. Hurley, *Atherosclerosis* **11**, 77 (1970).
32. This study was supported by grants HL 14823, RR 00166, and HD 04872 from the National Institutes of Health. We thank Dr. Frank Parker for his help and Dr. Michael Stemerman with whom the initial studies in vivo were performed. We are indebted to Beverly Kariya, Lynne Phillips, Mary Stewart, and Eunice Wong for their technical assistance.

# Health Care Delivery and Advanced Technology

## A more significant role is proposed for those who develop technology.

Charles D. Scott

The current drive to upgrade the health care delivery system lacks a vital factor—significant involvement of those who develop new technology. A whole spectrum of development engineers and applied scientists is necessary to provide support for any massive venture requiring the development and application of advanced technology, and this is exactly the kind of effort that will be necessary to help solve our "health care crisis." Further, the effective implementation of such an involvement will require the integration of many elements in biomedical science and engineering into well-defined, multidisciplinary teams organized to solve specific problems.

The problems associated with the delivery of health care have sometimes been compared with the problem that the National Aeronautics and Space Administration (NASA) faced in sending men to the moon, or with the concentrated, cooperative effort necessary for developing a viable nuclear energy industry. These are examples of Herculean endeavors that required the development of advanced technology to successfully achieve national goals. In each of these examples, the objective was realized when engineers and applied scientists became intimately involved with the problems, not only on an operational basis, but also in the formative and conceptual phases.

Development personnel are not effectively employed when the problems are narrowly defined and when the allowable solutions lie only within the realm that is well known to the more basic scientist or scientific administrator. In the biomedical area, all too often a contract approach has been used, where the engineer is contracted to add the details to a preconceived technological concept. As a result, the innovative development engineer has not been attracted to this area, and much of the resulting technology has not been founded on the best engineering principles.

It is true that we have spent, and are still spending, vast sums of money in the general area of health care. Large amounts are allotted by the National Institutes of Health (NIH) for studying the causes of disease; billions are disbursed every year for medical services; and the number and sophistication of our current health care facilities are being increased by the Health Services and Mental Health Administration (HSMHA). However, the amount spent on research and development for health care delivery by the federal government through the Department of Health, Education, and Welfare (HEW), recently estimated to be $18 to $59 million per year (*1*), is very small in comparison. The higher figure also includes funds used for the development of manpower, system analyses of alternative plans, and so forth. This by no means represents a national commitment or dedication to solving the health care problem, at least in the same sense that we committed ourselves to traveling to the moon or developing nuclear energy.

An additional point should be stressed. We will not achieve an "ultimate" health care delivery system, although we must maintain a continuing effort in this direction. For as new insights into the prevention and treatment of diseases are attained through biomedical research, they must also be applied to the health care of the general population.

### Why "Advanced" Technology?

The patient frequently points to two major problems in his involvement with the health care delivery system: the unresponsiveness of the system and the high cost. The first problem manifests itself in the difficulty of entering the health care system,

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