of Geodesy and Geophysics, International Association of Seismology and Physics of the Earth's Interior, Programs of the Scientific Assembly (Lima, Peru, 1973), p. 85; J. H. Goncz and J. R. Cleary, *Geophys. J. R. Astron. Soc.*, in press.
31. I. S. Sacks, *Carnegie Inst. Washington Yearb.* **67**, 339 (1969); *Trans. Am. Geophys. Union* **53**, 452 (1972); ——— and H. Okada, *Phys. Earth Planet. Inter.* **9**, 211 (1974).
32. D. E. James, *Geol. Soc. Am. Bull.* **82**, 3325 (1971).
33. H. N. Pollack and D. S. Chapman, in preparation; D. S. Chapman and H. N. Pollack, *Earth Planet. Sci. Lett.* **28**, 23 (1975).
34. Pollack and Chapman take the temperature at the lithosphere-asthenosphere boundary to be 0.85 the temperature of dry melting of peridotite. This value is obtained empirically by considering the thickness of oceanic lithosphere as a function of varying oceanic geothermal gradients. The general form of continental geothermal gradients inferred from heat flow is corroborated by the results of geochemical studies in which experimentally determined phase relationships and mineral compositions of coexisting pyroxenes as functions of temperature and pressure are applied to nodules to obtain estimates of their equilibration temperatures and pressures [for example, see F. R. Boyd and P. H. Nixon, *Phys. Chem. Earth* **9**, 455 (1975)]. The persistence of subsolidus temperatures to great depth is further attested to by the absence of interstitial glass in ultramafic nodules [see (*35*)].
35. A. M. Kudo, D. G. Brookins, A. W. Laughlin, *Earth Planet. Sci. Lett.* **15**, 291 (1972).
37. S. P. Clark, Jr., and A. E. Ringwood, *Rev. Geophys.* **2**, 35 (1964).
36. J. G. Sclater and J. Francheteau, *Geophys. J. Astron. Soc.* **20**, 509 (1970).
38. See A. E. Ringwood [*Composition and Petrology of the Earth's Mantle* (McGraw-Hill, New York, 1975), pp. 100–114] for a general discussion and an extensive bibliography.
39. J. B. Dawson, *Philos. Trans. R. Soc. London Ser. A* **271**, 297 (1972).
40. F. R. Boyd and P. H. Nixon, *Carnegie Inst. Washington Yearb.* **68**, 324 (1970); A. E. Ringwood and J. F. Lovering, *Earth Planet. Sci. Lett.* **7**, 371 (1970).
41. P. A. Wagner, *S. Afr. J. Sci.* **25**, 127 (1928); P. G. Harris, R. Hutchinson, D. K. Paul, *Philos. Trans. R. Soc. London Ser. A* **271**, 313 (1972); P. G. Harris and E. A. K. Middlemost, *Lithos* **3**, 77 (1969); I. D. MacGregor, *Phys. Chem. Earth* **9**, 455 (1975); J. F. Lovering and M. Tatsumoto, *Earth Planet. Sci. Lett.* **4**, 350 (1968).
42. F. R. Boyd and P. H. Nixon, *Phys. Chem. Earth* **9**, 431 (1975).
43. I. D. MacGregor, *J. Geophys. Res.* **73**, 3737 (1968).
44. M. J. O'Hara, M. J. Saunders, E. L. P. Mercy, *Phys. Chem. Earth* **9**, 571 (1975).
45. J. M. Rhodes and J. B. Dawson, *ibid.*, p. 545.
46. D. R. Barrett, *ibid.*, p. 637.
47. W. I. Ridley and J. B. Dawson, *ibid.*, p. 559.
48. R. Hutchinson and J. B. Dawson, *Earth Planet. Sci. Lett.* **9**, 87 (1970).
49. Crustal heat production and steady-state heat flow are from Sclater and Francheteau (*36*) and are consistent with the relation between heat flow and surface heat production proposed by R. F. Roy, D. D. Blackwell, and F. Birch [*Earth Planet. Sci. Lett.* **5**, 1 (1968)]; K/U and U/Th ratios are from Clark and Ringwood (*37*).
50. H. L. Allsopp, L. O. Nicolaysen, P. Hahn-Weinheimer, *Earth Planet. Sci. Lett.* **5**, 231 (1969); R. H. Mitchell and J. H. Crocket, *Contrib. Mineral. Petrol.* **30**, 277 (1971).
51. P. G. Harris, R. Hutchinson, D. K. Paul, *Philos. Trans. R. Soc. London Ser. A* **271**, 313 (1972).
52. D. K. Paul, *Contrib. Mineral. Petrol.* **34**, 22 (1971).
53. W. Compston and J. F. Lovering, *Geochim. Cosmochim. Acta* **33**, 691 (1969).
54. A. M. Steuber and M. Ikramuddin, *ibid.*, p. 207.
55. A. D. M. Burwell, *Earth Planet. Sci. Lett.* **28**, 69 (1975).
56. E. J. Dasch and D. H. Green, *Am. J. Sci.* **275**, 461 (1975).
57. W. I. Manton and M. Tatsumoto, *Earth Planet. Sci. Lett.* **10**, 217 (1971); A. M. Steuber and M. Ikramuddin, *Geochim. Cosmochim. Acta* **38**, 207 (1974); J. A. Cooper and D. H. Green, *Earth Planet. Sci. Lett.* **6**, 69 (1969).
58. The mechanism of rise of diapirs from a buoyant mantle layer has been considered at length by H. Ramberg [*Phys. Earth Planet. Inter.* **5**, 45 1972)]. The formation of oceanic island volcanism by ascent of chemically distinct blobs or plumes forms a background to our model and has been discussed by J. G. Schilling and A. Noe-Nygaard [*Earth Planet. Sci. Lett.* **24**, 1 (1974)] and by J. G. Schilling [*ibid.* **25**, 103 (1975); *Nature (London)* **242**, 565 (1973); *Eos* **57**, 343 (1976)].
59. R. Kay, *Eos* **56**, 1077 (1975).
60. D. L. Anderson, *Geol. Soc. Am. Bull.* **86**, 1593 (1975).
61. The Usushwana Complex is particularly noteworthy since it exhibits an intrusion age of 2870 ± 42 million years while the mafic end members of the complex define a mantle isochron some 800 million years older (3685 million years) (*62*).
62. R. D. Davies *et al.*, *Geol. Soc. S. Afr. Spec. Publ. 1* (1970), p. 576.
63. R. F. Cormier, *Can. J. Earth Sci.* **6**, 393 (1969); H. W. Fairbairn, M. L. Bottino, W. H. Pinson, P. M. Hurley, *ibid.* **3**, 509 (1966).
64. S. E. Church and G. R. Tilton, *Geol. Soc. Am. Bull.* **84**, 431 (1973).
65. C. E. Hedge, R. A. Hildreth, W. T. Henderson, *Earth Planet. Sci. Lett.* **8**, 434 (1970).
66. K. S. Heier, W. Compston, I. McDougall, *Geochim. Cosmochim. Acta* **29**, 643 (1965).
67. D. E. James, C. Brooks, A. Cuyubamba, *Geol. Soc. Am. Bull.*, in press.
68. R. W. Kistler and Z. E. Peterman, *ibid.* **84**, 3489 (1973).
69. W. P. Leeman, *Geochim. Cosmochim. Acta* **34**, 857 (1970).
70. D. C. Noble, C. E. Hedge, E. H. McKee, M. K. Corringa, *Geol. Soc. Am. Bull.* **84**, 1393 (1973).
71. R. J. Pankhurst, *J. Petrol.* **10**, 115 (1969).
72. Z. E. Peterman, I. S. E. Carmichael, A. L. Smith, *Geol. Soc. Am. Bull.* **81**, 311 (1970).
73. Z. E. Peterman, B. R. Doe, H. H. Prostka, *Contrib. Mineral. Petrol.* **27**, 121 (1970).
74. J. L. Powell and K. Bell, *ibid.*, p. 1.
75. J. J. Stipp, thesis, Australian National University (1968).
76. We acknowledge A. Hofmann for many stimulating and productive discussions and M. Strobel for generously providing statistical programs and assistance in evaluating the Rb-Sr data. The drafting assistance of M. Demidoff is greatly acknowledged. This study has been supported in part by the National Science Foundation (grant GA 36094) and the National Research Council of Canada (grant A 5581 to C.B.). One of us (C.B.) gratefully acknowledges the Carnegie Institution of Washington for providing financial support and research facilities during his sabbatical year.

Hyperlipidemia and Atherosclerosis

Chronic hyperlipidemia initiates and maintains lesions by endothelial cell desquamation and lipid accumulation.

Russell Ross and Laurence Harker

The lesions of atherosclerosis are characterized by intimal proliferation of smooth muscle cells, accumulation of large amounts of connective tissue matrix including collagen, elastic fibers, and proteoglycans, and deposition of intra- and extracellular lipid. Many factors are involved in this process.

One of the original concepts regarding the pathogenesis of atherosclerosis was proposed by Virchow (*1*) who suggested that endothelial cell injury initiates atherogenesis. In a recent modification of this hypothesis (*2*), it was proposed 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 reduce the effectiveness of the barrier. Moreover, the lesions of atherosclerosis were considered to be potentially reversible, depending upon the nature, duration, and chronic nature of the injury.

Many investigators have subsequently confirmed the key role played by endothelial cell injury in atherogenesis. The various injurious factors that affect the endothelium somehow alter the threshold of endothelial stability so that local hemodynamic factors result in focal endothelial desquamation and exposure of subendothelial connective tissue. Recent evidence has also linked endothelial injury with platelet adhesion, aggregation, and release of platelet constituents at sites in the artery wall where endothelial cell desquamation has occurred.

Figure 1 illustrates the importance of endothelial injury and the resultant release and passage of platelet and plasma constituents into the artery wall in the genesis of the lesions of atherosclerosis. According to the injury hypothesis, everyone is potentially susceptible to many forms of endothelial damage as a result of mechanical (*3*), chemical (*4*), immunologic (*5*), or toxic sources of injury. Figure 1 shows that if the injury were a single event, the lesions would be revers-

Dr. Ross is professor of pathology and associate dean for scientific affairs, and Dr. Harker is professor of medicine, division of hematology, at the School of Medicine, University of Washington, Seattle 98195.

ible (see the outer regression cycle); if the injury were repeated or continuous, the lesions would progress and might become irreversible (see the inner progression cycle). Regenerating endothelium overlying lesions might be more susceptible to further injury because of the altered flow of blood necessitated by the intrusion of the lesion into the lumen of the artery.

Investigations in vivo with subhuman primates and studies of arterial smooth muscle cells in culture have demonstrated that platelets provide the principal mitogen present in blood serum responsible for the growth of the arterial smooth muscle cells in culture (*6*). When fibroblasts or smooth muscle cells are grown in a medium containing serum derived from platelet-free plasma, the cells remain quiescent for long periods. The addition of a factor purified from constituents released from platelets by thrombin or collagen restores all of the mitogenic capacity of serum.

We have demonstrated in baboons that chronic homocystinemia can produce lesions of atherosclerosis (*4*). During such lesion formation, there is a twofold increase in platelet utilization by the vascular desquamative lesions caused by the chronic homocystinemia (10 percent of the aortic endothelium was lost). Pharmacologic inhibition of platelet function interrupted platelet consumption and prevented intimal smooth muscle proliferation without altering the loss of endothelium.

The importance of platelet-derived factors for arterial smooth muscle proliferation has been confirmed by Friedman *et al.* (*7*). These workers prevented smooth muscle proliferation in rabbits following intra-arterial catheter-induced injury by making the animals thrombocytopenic with an antiserum to platelets. Additional evidence for the importance of platelets in atherosclerosis has been derived from studies with normal and diseased pigs. Pigs with von Willebrand's disease have a defect in platelet adhesion, and Bowie *et al.* (*8*) showed that such animals fail to develop the atherosclerotic lesions that occur in control animals that have been made hypercholesterolemic by being fed a fatty diet.

Thus, the fundamental response following some form of injury to the endothelium is platelet-mediated intimal smooth muscle proliferation. The studies presented in this article provide further insight into the role played by chronic hyperlipidemia in atherogenesis. The experiments we report indicate that chronic hyperlipidemia not only results in lipid accumulation in atheromatous lesions, but itself produces endothelial injury which is accompanied by platelet consumption and atherosclerosis.

Mechanical Injury and Hyperlipidemia

We used 32 pigtail monkeys (*Macaca nemestrina*) aged 1 to 2½ years, each weighing 1.5 to 3 kg. Eight animals served as controls for 18 hypercholesterolemic animals; six animals were used for platelet survival measurements.

The animals were made hypercholesterolemic by maintaining them on a liquid formula that provided additional cholesterol and triglyceride during the day (*9*). Purina monkey chow was given in the evening.

Twelve of the 18 hypercholesterolemic monkeys were subjected to a single balloon-catheter deendothelialization of one iliac artery and the abdominal aorta while anesthetized with halothane as described (*2*). This procedure results in the removal of virtually all of the endothelium. The external femoral artery was then ligated and the wound closed. The animals remained hypercholesterolemic (Table 1) until they were killed at 1½ months, 3 months, 6 months, 9 months, and 1 year after the operation. The contralateral iliac artery served as a noninjured control of the vascular effects of chronic hypercholesterolemia (*10*).

Effect of Deendothelialization

At 6 weeks to 3 months after balloon injury to the iliac artery and abdominal aorta, the chronically hypercholesterolemic animals displayed a markedly thickened intima containing numerous smooth muscle cells with lipid inclusions (Fig. 2A).

The number of smooth muscle cells in the lesions were similar to those found in lesions induced with a balloon catheter in normolipidemic animals (*2, 3*), but the cells were changed in appearance because of the presence of numerous large amorphous lipid inclusions. The lipid-laden smooth muscle cells often con-

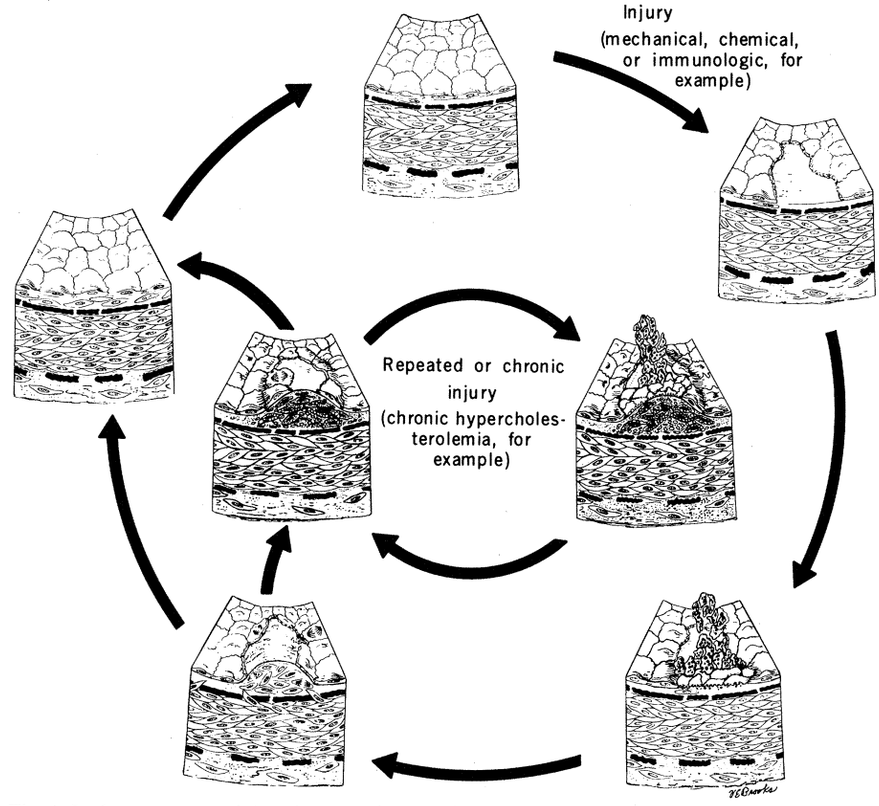


Fig. 1. In the response to injury hypothesis, two different cyclic events may occur. The outer, or regression cycle, may represent common single occurrences in all individuals in which endothelial injury leads to desquamation, platelet adherence, aggregation, and release, followed by intimal smooth muscle proliferation and connective tissue formation. If the injury is a single event, the lesions may go on to heal and regression occur. The inner or progression cycle demonstrates the possible consequences of repeated or chronic endothelial injury as may occur in chronic hyperlipidemia. In this instance, lipid deposition as well as continued smooth muscle proliferation may occur after recurrent sequences of proliferation and regression, and these may lead to complicated lesions that calcify. Such lesions could go on to produce clinical sequelae such as thrombosis and infarction.

Table 1. Platelet kinetics in normal and hyperlipidemic monkeys. Cell loss in the aortic endothelium is expressed as the percentage of surface lost.

Diet	No. of animals	Plasma lipids		Endothelial cell loss (% of surface)	Platelet		
		Cholesterol (mg/dl)	Triglyceride (mg/dl)		Count (No./ml)	Survival (days)	Turnover (platelets $(ml^{-1}\,day^{-1})$
Normal	8	88 ± 5.3*	30 ± 9.4	0	383,000 ± 62,000	8.0 ± 0.34	61,000 ± 11,000
Hyperlipidemic	6	223 ± 22	28 ± 14	5.0 ± 1.2	396,000 ± 70,000	5.8 ± 0.54	86,000 ± 13,000
P		< 0.01	> 0.75	> 0.001	> 0.75	< 0.01	< 0.05

*The variation is ± 1 S.D.

tained a well-developed rough endoplasmic reticulum and Golgi complex. The connective tissue surrounding the cells contained two types of lipid deposits. One of these was membranous in character (Fig. 3), the second was represented by round, globular, less dense deposits that were not clearly membrane bound.

Between 3 months and 1 year, the catheter-induced lesions of the chronically hyperlipidemic animals showed a persistence of the lipid-laden cells with no evidence of the regression that had been observed in the normolipidemic animals (*2*, *3*). Indeed, after 6 to 18 months, the lesions in the injured vessels were somewhat larger than those seen 3 months after injury. A distinguishing feature of the injury lesions in chronically hyperlipidemic animals was the progressive increase in the amount of extracellular membranous debris.

Most of the lipid-containing cells were identifiable as smooth muscle cells although they were altered in appearance. Many of the intracellular lipid inclusions were membrane bound and frequently contained concentric membranous whorls. These inclusions appeared like secondary lysosomes and contained numerous small membranous globules, indistinguishable in appearance from membranous deposits present in the space surrounding the cells (Fig. 4). Twelve months after injury, the membranes of many of the smooth muscle cells were disrupted, and these intracellular membranous deposits were in the process of being extruded. These observations suggested that many of the extracellular deposits were formed by the release of these bodies by exocytosis or from degenerating smooth muscle cells into the extracellular space. These were not considered to be artifacts since such necrotic cells were often surrounded by intact, healthy cells (Fig. 5).

Abundant quantities of newly formed collagen fibrils and elastic fibers, rich in elastic fiber microfibrils, were regularly seen at each interval after injury.

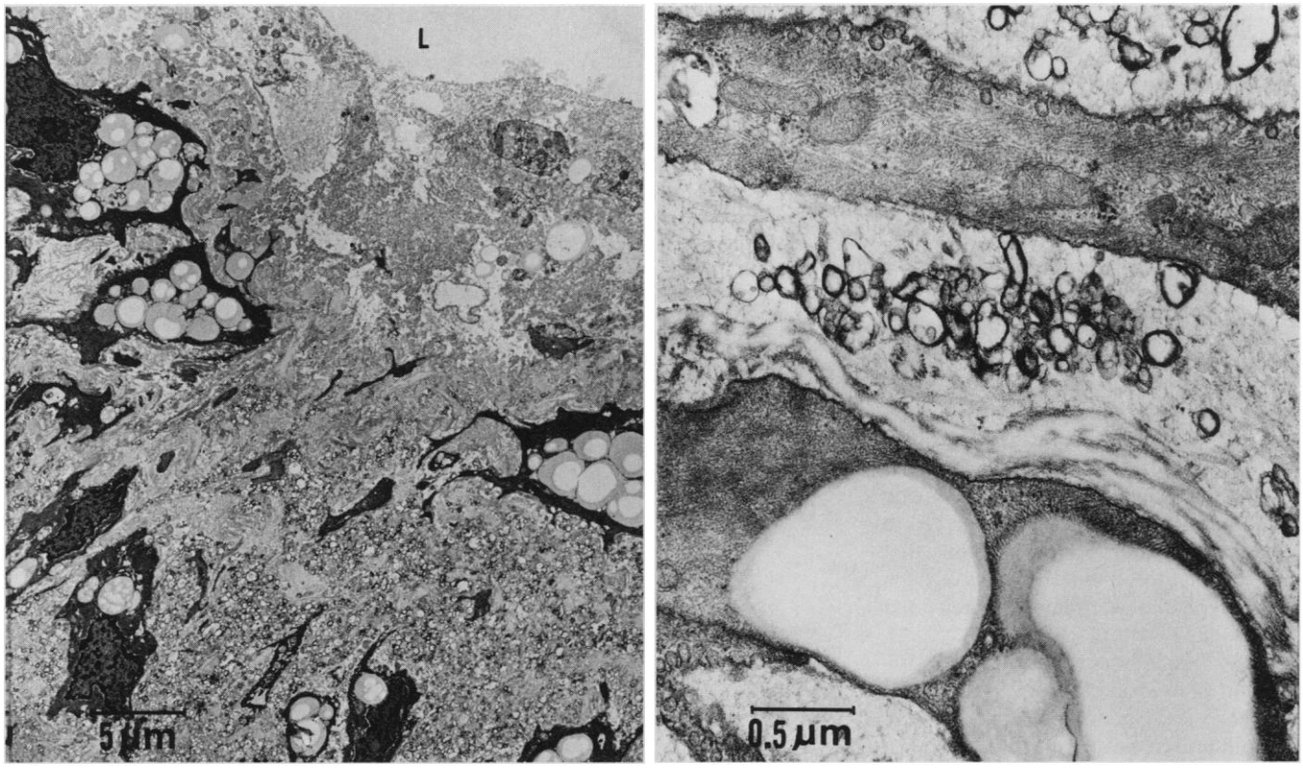


Fig. 2 (left). Electron micrograph of a portion of an intimal lesion in the iliac artery of a monkey on a hyperlipidemic diet 6 months after balloon injury. Most of the smooth muscle cells in the lesion contain large lipid deposits. The cells are surrounded by small globular membranous deposits in the connective tissue. An endothelial cover is lacking at the luminal surface (*L*) at the crest of the lesion. Fig. 3 (right). A small segment of a lesion induced by mechanical injury in a chronically hyperlipidemic monkey. A portion of one of the two smooth muscle cells in this micrograph contains three large lipid droplets. In the connective tissues surrounding the cells, there are numerous membranous deposits between the collagen and elastic fibers. These are located in sites usually occupied by proteoglycans.

The Nonballooned Iliac Artery

In the animals on the lipid-rich diet, there were very few alterations in the nonballooned vessels after 1½ months. An occasional lipid-laden cell was present in the still thin intima of the noninjured artery. Four months after commencing the diet, that is, 3 months after the contralateral artery was ballooned, the intima of the noninjured iliac was slightly increased in thickness and contained one to two layers of lipid-laden smooth muscle cells. Within 10 months after commencing the diet, there were no significant differences between the injured and noninjured iliac arteries. Both contained 10 to 15 layers of lipid-laden smooth muscle cells surrounded by extracellular lipid and large quantities of newly formed connective tissue matrix.

The remaining pigtail monkeys were maintained on the hypercholesterolemic diet (and showed similar elevations of serum cholesterol) for a period of 9 to 18 months; they were compared with eight control animals fed normal monkey chow. These animals were not mechanically injured in any way and were simply killed at 9 months, 1 year, and 1½ years. At the time they were killed, the animals were anesthetized and perfused with 0.3 percent silver nitrate solution under 100 cm of hydrostatic pressure in vivo. The entire endovascular surfaces of whole-aorta mounts were then examined by means of a grid micrometer to determine the proportion of the surface that was not covered by endothelial cells.

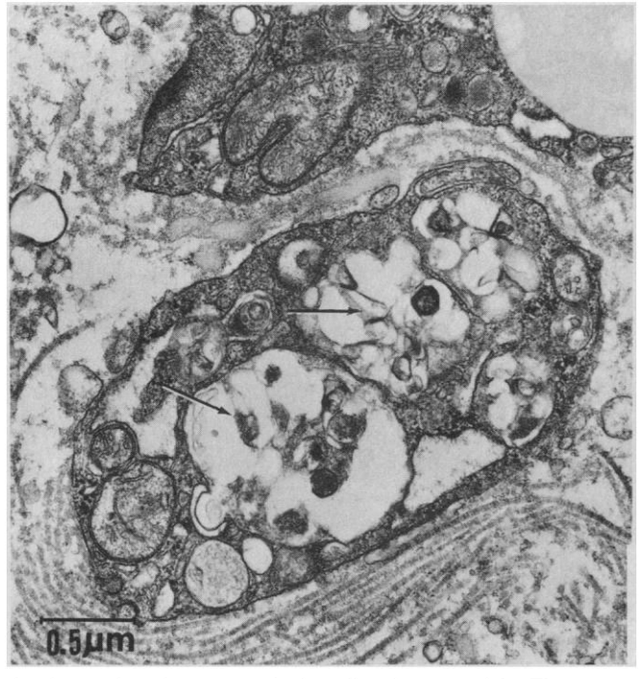
Endothelial Integrity

The series of chronically hyperlipidemic animals that received no mechanical injury permitted us to assess endothelial integrity by examining the entire aorta and iliac arteries after perfusion in vivo with silver nitrate and preparation of the arteries for viewing of the luminal surface. In all of the hyperlipidemic animals there was focal loss of endothelial cells amounting to a total loss of 5.0 percent (Fig. 6) (Table 1). In some regions in the chronically hyperlipidemic animals, the endothelial cells were altered in shape and appeared to have lost their characteristic longitudinal orientation. Many of them were polyhedral or round, suggesting abnormal endothelial regeneration (Fig. 6).

Platelet Survival Measurements

In view of the endothelial cell loss in the hyperlipidemic animals, platelet survival (*11*, *12*) was measured in six hyperlipidemic monkeys (maintained on the hypercholesterolemic diet for longer than 3 months). These were compared with eight control animals. Platelet survival in the animals that were hyperlipidemic for more than 6 months averaged 5.8 days, which is significantly different from the mean platelet survival of the control animals, that is, 8 days (Table 2).

Since increased platelet reactivity in vitro has been reported in patients with familial hypercholesterolemia (*13*), it was necessary to determine whether this decreased survival was a direct effect of increased cholesterol (or lipoproteins) upon the platelets, or was a manifestation of increased platelet consumption by exposed subendothelial surfaces. Therefore, matched cross-over platelet survival experiments were carried out between six normolipidemic and six hyperlipidemic monkeys. Donor platelets were labeled with chromium-51 and their survival in the respective recipient was determined. Platelets from the hyperlipidemic monkeys survived normally after infusion into the normolipidemic animals. In contrast, platelets from normolipidemic animals infused into hyperlipidemic animals had a decreased survival time comparable with that of autologous platelets (Table 2). While these data do not rule out the possibility of a rapidly occurring lipid-mediated effect on the membranes of the transfused platelets, the reduction in platelet surviv-



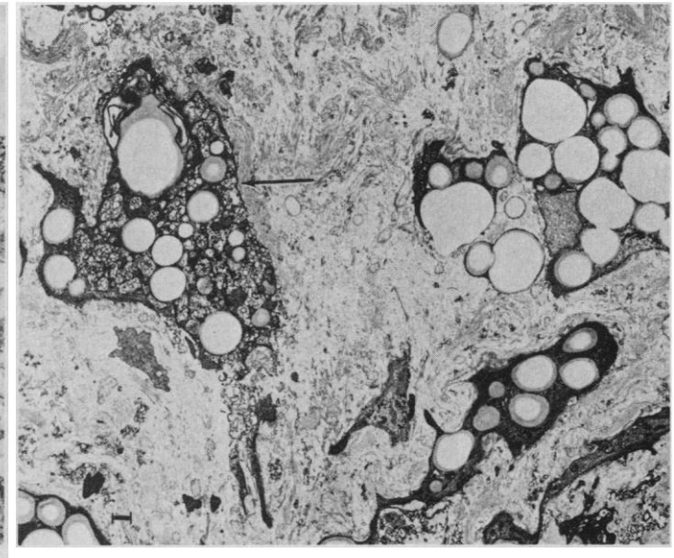


Fig. 4 (left). Electron micrograph showing portions of two smooth muscle cells from a 9-month lesion in a monkey with chronic hyperlipidemia. The cells are surrounded by numerous collagen fibrils. In one of the cells, secondary lysosomes (arrow) are visible which contain membranous globules or deposits that represent breakdown of the large amorphous lipid droplets such as the one seen in the cell to the upper right. The breakdown products are similar in appearance to the membranous deposits seen in the extracellular space surrounding these two cells. Fig. 5 (right). A lesion from a chronically hyperlipidemic monkey, in which the plasma membrane of the smooth muscle cell on the left is broken (arrow) demonstrating that the cell has undergone necrosis and is in the process of releasing its lipid inclusions into the extracellular compartment. Several other intact lipid-laden smooth muscle cells are also visible.

al is most consistent with increased platelet consumption on exposed subendothelium as observed in the desquamation associated with homocystinemia (*4*) and in prostheses such as artificial valves or vessels (*12*).

Experiments were performed to correlate the loss of endothelium with decreased platelet survival. A known amount of endothelium was removed from each of a series of monkeys with a balloon catheter, and platelet survival and the rate of return to normal were measured. A direct correlation was observed between the amount of endothelium removed and the extent of the decrease in platelet survival, and between the time required for reendothelialization and a return to normal levels of platelet survival.

The Response to Injury Hypothesis

These studies and those in which the endothelium is subjected to mechanical (*3*), immunologic (*5*), or chemical (*4*) injury support the hypothesis that the intimal smooth muscle proliferative lesions of atherosclerosis are the result of the response to injury (*2*). This response involves: (i) focal desquamation of endothelial cells; (ii) adhesion and aggregation of platelets to exposed subendothelial connective tissue; (iii) local release of platelet constituents, including a platelet mitogenic factor (*6*), and passage of plasma constituents into the underlying artery wall; (iv) migration of smooth muscle cells through gaps in the internal elastic lamina into the intima, and platelet-mediated intimal proliferation of smooth muscle cells; (v) formation of connective tissue matrix by the smooth muscle cells through synthesis and secretion of collagen, elastic fiber proteins, and glycosaminoglycans; and (vi) intracellular and extracellular lipid accumulation. After mechanical injury, maximal lesion formation occurs within 3 months. Such lesions appear to regress once the overlying endothelium has regenerated, and regression is virtually complete 3 months later in normolipidemic monkeys (*2, 3*). This formulation suggests that repeated or chronic endothelial cell loss may be the principal event leading to atherosclerosis (Fig. 1).

The importance of a platelet factor or factors in the focal proliferation of smooth muscle cells is supported by the identification of a factor from platelets that stimulates smooth muscle proliferation in cell culture (*6*). Furthermore, the intimal lesions that occur in homocystine-induced or mechanically induced arteriosclerosis can be prevented by pharmacologic agents (*4*) that interrupt platelet consumption, or by inducing a thrombocytopenia with an antiserum to platelets (*7*).

Table 2. Survival of donor platelets in matched cross-over studies between six normolipidemic and six hyperlipidemic monkeys. Platelet survival (days).

Study pair	Normal animal baseline	Hyperlipidemic animal baseline	Normal platelets into hyperlipidemic animal	Hyperlipidemic platelets into normal animal
1	8.0	6.2	5.7	7.5
2	8.1	6.5	4.8	7.7
3	8.4	6.7	6.2	8.2
4	8.1	5.8	6.3	7.9
5	7.9	6.1	6.0	
6	7.5	6.0	5.9	8.0
Mean	8.0 ± 0.33	6.2 ± 0.3	5.8 ± 0.5	7.9 ± 0.3
P			< 0.02	< 0.01

The Role of Chronic Hyperlipidemia

Although the presence of lipids in the form of both free cholesterol and cholesterol esters has been recognized for many years in the lesions of athero-

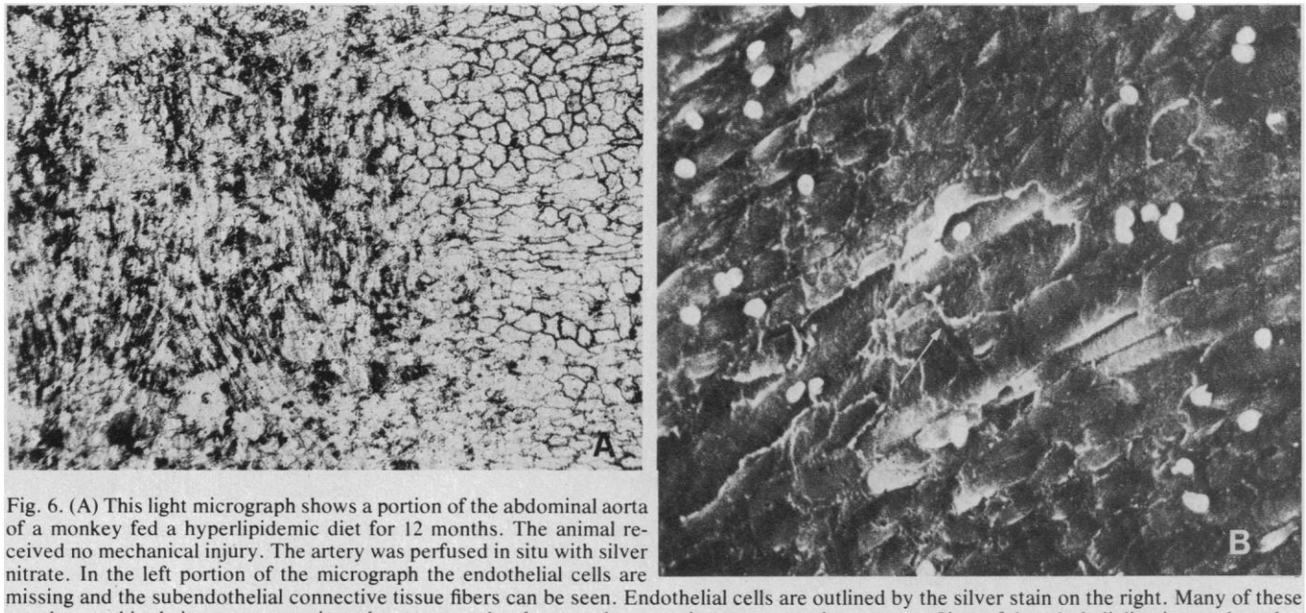


Fig. 6. (A) This light micrograph shows a portion of the abdominal aorta of a monkey fed a hyperlipidemic diet for 12 months. The animal received no mechanical injury. The artery was perfused in situ with silver nitrate. In the left portion of the micrograph the endothelial cells are missing and the subendothelial connective tissue fibers can be seen. Endothelial cells are outlined by the silver stain on the right. Many of these are abnormal in their appearance since they are rounder than usual, suggesting a regenerative process. Sites of deendothelialization such as that on the left are present over approximately 5 percent of the surface of the aorta and iliac artery of monkeys with chronic hyperlipidemia. (B) A scanning electron micrograph of a very small segment of the aorta of a monkey fed on a hyperlipidemic diet for 12 months. Several small areas of endothelial desquamation (arrow) can be seen in the center of the micrograph. Intact endothelial cells are outlined by the metal shadowing process used to prepare the tissues for scanning electron microscopy. [Magnification: (A) × 240; (B) × 580.]

sclerosis (*14*), the specific role of hyperlipidemia in lesion formation has not been well understood. Endothelial injury is now widely accepted as a possible antecedent to the formation of the lesions of atherosclerosis; it is important to understand the role of chronic hyperlipidemia in this regard.

While the nonmechanically injured iliac arteries of the lipid-fed monkeys were initially slower to develop lesions, the intimal lesions that eventually formed were comparable in size to the mechanically injured contralateral artery after the monkeys had been on the hyperlipidemic diet for 9 to 12 months. These lesions frequently appeared to lack an endothelial cover (Fig. 2A). To rule out the possibility of artifacts developing during tissue preparation, we conducted a second sequence of studies in which animals were maintained on the hyperlipidemic diet for 9 to 18 months without being subjected to balloon injury. At the time they were killed, the animals were placed under halothane anesthesia and were perfused with silver nitrate in order to measure the percentage of the surface covered by intact endothelium. These studies demonstrated focal sites of endothelial desquamation (Fig. 5), equal to approximately 5 percent of the endothelial surface area in the thoracic and abdominal aorta and iliac arteries. In contrast, the control, normolipidemic monkeys showed no endothelial cell loss.

Endothelial desquamation could result from a "lowered threshold" to the normal shearing stress of the flowing blood at given anatomic sites in the artery wall or from some form of direct "injury" to the endothelial cells. This phenomenon undoubtedly has some relation to blood flow characteristics since a correlation has been shown between sites of maximal shear forces, of endothelial injury, of increased permeability, and lesion formation (*15*).

There may be a number of ways in which chronic hyperlipidemia may "injure" the endothelium. It is not clear whether such "injury" results from the sustained increase in cholesterol (the concentration of triglyceride remained normal), or from complicated effects of hyperlipidemia or altered lipoproteins upon the endothelial cells. The development of methods to study endothelial cells in culture provides opportunities to answer these questions.

The endothelium normally regulates the entrance of low density lipoproteins (LDL), the principal cholesterol-carrying lipoprotein of the plasma, into the artery wall. The LDL also support smooth muscle proliferation in cell culture (*2*); however, although LDL may be mitogenic, they more probably provide nutrients necessary for cell proliferation in terms of new membrane formation. Human fibroblasts contain specific receptor sites for LDL (*16*), and arterial smooth muscle cells are thought to have similar receptors that play a role in controlling the intracellular synthesis of cholesterol (*17*).

A second difference observed in the present studies is the effect of chronic hyperlipidemia in lesion regression. After a single episode of mechanical injury to the endothelium, the smooth muscle proliferative response appears identical to the preatherosclerotic fibromusculoelastic lesion seen in man (*18*). In the primates, the mechanically induced lesion reaches its maximum size 3 months after injury and largely disappears within 6 months. In contrast, 6 months after mechanical deendothelialization in chronically hyperlipidemic primates, rather than regressing, the lesions remained unchanged or, in some instances, increased in size. Large amorphous intracellular lipid deposits were present, and accumulations of lipids in the form of membranous deposits and amorphous droplets were found in the surrounding connective tissue. These observations suggest that chronic hyperlipidemia prevents lesion regression and augments lipid deposition following a single episode of mechanically induced endothelial injury and, consequently, promotes lesion progression.

Extracellular Lipids

The membranous deposits found within the extracellular matrix appear to coincide with regions characteristically occupied by proteoglycans in atherosclerotic lesions. These lipid deposits may be formed by at least two mechanisms. These include the trapping of lipoproteins by glycosaminoglycans (*19*) or as a result of cell degeneration, and necrosis resulting in the release of intracellular lipid moieties into the extracellular environment.

Arterial smooth muscle cells form large quantities of dermatan sulfate and smaller amounts of chondroitin sulfates A and C and hyaluronic acid in vitro (*20*). Atherosclerotic lesions contain a meshwork of proteoglycans (*21*). This meshwork appears to interconnect collagen fibrils and elastic fibers with the cells. In chronic hyperlipidemia, the sites filled with proteoglycan particles and filaments are also rich in membranous deposits. Of the glycosaminoglycans formed by arterial smooth muscle cells, dermatan sulfate has the greatest affinity at physiologic *p*H and ionic strength for LDL (*19*) and has recently been found in high concentrations in lesions (*22*). The mitogenic factor released by the platelets could also have a tendency to bind with extracellular proteoglycans, perhaps competing with lipoproteins for these binding sites.

It is conceivable that the binding of lipoproteins with proteoglycans might inhibit the binding of proteoglycan with the platelet factor. This would make the platelet factor available for interactions with the smooth muscle cells, stimulate further smooth muscle proliferation, and result in a cycle that would promote lesion progression. Further examination of the interrelationship between proteoglycans and lipoproteins in vivo may clarify the mode by which lipids are trapped and held in the extracellular matrix of the lesions of atherosclerosis.

Conclusions

These studies provide new insight into the complex mechanisms whereby hyperlipidemia causes progressive atherosclerosis. It has been shown that physical injury to the endothelial lining of arteries sets off a process which probably is an attempt at healing the injury but which can lead to atherosclerosis. It has also been found that chemical agents such as homocystine can produce a similar series of events leading to atherosclerosis. These events include focal loss of endothelium, exposure of subendothelial connective tissue, and adherence of platelets followed by release of factors that stimulate intimal smooth muscle proliferation. The present studies indicate that the effects of chronic hyperlipidemia are complex in that the condition results not only in the deposition of lipids in the atheromatous lesions but that it may produce the primary endothelial injury that initiates the process of atherosclerosis as well.

References and Notes

1. R. Virchow, in *Gesammelte Abhandlungen zur Wissenschaftlichen Medicin* (Meidinger Sohn, Frankfurt-am-Main, 1856), p. 458.
2. R. Ross and J. A. Glomset, *Science* **180**, 1332 (1973); *N. Engl. J. Med.*, **295**, 369, 420 (1976).
3. M. B. Stemerman and R. Ross, *J. Exp. Med.* **136**, 769 (1972); S. Bjorkerud and G. Bondjers, *Atherosclerosis* **18**, 235 (1973); S. Moore, *Lab. Invest.* **29**, 478 (1973); J. A. Fishman, B. R. Graeme, M. J. Karnovsky, *ibid.* **32**, 339 (1975); H. R. Baumgartner, *Thromb. Diath. Haemorrh. Suppl.* **59**, 91 (1974); P. Helin, I. Lorenzen, C. Garbarsch, M. E. Matthiessen, *Circ. Res.* **29**, 542 (1971); J. F. Mustard and M. A. Pack-

ham, *Thromb. Diath. Haemorrh.* **33**, 444 (1975).
4. L. A. Harker, R. Ross, S. J. Slichter, C. R. Scott, *J. Clin. Invest.*, in press; L. A. Harker, S. J. Slichter, C. R. Scott, R. Ross, *N. Engl. J. Med.* **291**, 537 (1974).
5. C. R. Minick and G. E. Murphy, *Am. J. Pathol.* **73**, 265 (1973); N. J. Hardin, C. R. Minick, G. E. Murphy, *ibid.*, p. 301.
6. R. Ross, J. A. Glomset, B. Kariya, L. A. Harker, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1207 (1974); R. B. Rutherford and R. Ross, *J. Cell Biol.* **69**, 196 (1976); N. Kohler and A. Lipton, *Exp. Cell Res.* **87**, 297 (1974); B. Westermark and A. Wasteson, *ibid.* **98**, 170 (1976).
7. R. J. Friedman, M. B. Stemerman, T. H. Spaet, S. Moore, J. Gauldie, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 207 (1976); S. Moore, R. J. Friedman, D. P. Singal, J. Gauldie, M. Blajchman, R. S. Roberts, *Thromb. Haemostas (Stuttgart)* **35**, 70 (1976).
8. E. J. W. Bowie, V. Fuster, C. A. Owen, Jr., A. L. Brown, paper presented at the Fifth Congress of the International Society on Thrombosis and Haemostasis, Paris, 1975.
9. The supplement contained in each daily portion: (i) powdered egg yolk, 27.8 g; (ii) skim milk powder, 11.3 g; (iii) corn oil (Mazola) 15 g; applesauce, 87 g; apple juice, 31.3 g; and water to make 200 ml. The monkeys willingly drank the mixture from a standard water feeder bottle, and consumed 70 to 80 percent of the formula offered. The amount ingested was determined from measurement of the residual volume daily. The animals had unrestricted access to water. The amount of cholesterol ingested was calculated from the percentage of cholesterol in the formula that had been consumed. Estimation of total caloric intake, total fat intake, and percentage of calories as fat was made by a count of chow biscuits taken daily.
10. At the time they were killed, the animals were placed under halothane anesthesia and were perfused with buffered half-strength Karnovsky's fixative under 100 cm of hydrostatic pressure for 20 minutes in vivo. The arteries were subsequently fixed and prepared for light and electron microscopy.
11. Platelet counts were measured with an electronic particle counter on peripheral blood collected in EDTA (*12*). The platelet count of 20 normal animals was 372,000 ± 62,000 per microliter (± S.D.). Platelet survival was determined from the disappearance of radioactivity from blood sampled five to ten times after the injection of autologous ^{51}Cr-labeled platelets as described previously (*4*). Platelet consumption, measured as platelet turnover per microliter of blood per day, was calculated from the peripheral platelet count divided by the platelet survival time in days and corrected for recovery.

Platelets were determined as follows: 25 ml of whole blood was collected in 5 ml of ACD anticoagulant (acid, citrate, and dextrose) and centrifuged at 200*g* for 10 minutes at room temperature. The supernatant (platelet-poor) plasma was removed and adjusted to *p*H 6.5 with 0.15*M* citric acid, then centrifuged at 3000*g* for 15 minutes to form a platelet pellet. The platelets were then resuspended in 1 ml of supernatant plasma and incubated with 50 μc of radioactive chromium (New England Nuclear) for 20 minutes. Five milliliters of nonradioactive platelet-poor plasma were added and the pellet reformed by centrifuging at 3000*g* for 15 minutes. The resultant radioactive platelet-poor plasma was completely decanted, and the platelet pellet was washed by carefully layering over 2 ml of nonradioactive platelet-poor plasma, and then decanting. The platelet pellet was gently suspended in 3 ml of nonradioactive platelet-poor plasma. Contaminating red cells were largely removed by a final slow centrifugation of 100*g* for 5 minutes. A known amount of the supernatant ^{51}Cr-labeled platelet suspension was returned to the animal by intravenous injection after the preparation of a standard. Eight daily 2-ml samples of whole blood were collected in EDTA, lysed with sodium dodecyl sulfate, and counted for radioactivity in a gamma counter. The proportion of labeled platelets remaining within the systemic circulation after infusion (that is, recovery) was calculated from the platelet activity per milliliter at zero time, multiplied by the estimated blood volume, and divided by the platelet ^{51}Cr activity injected.
12. L. A. Harker and C. A. Finch, *J. Clin. Invest.* **48**, 963 (1969); L. A. Harker and S. J. Slichter, *N. Engl. J. Med.* **287**, 999 (1972).
13. A. C. A. Carvalho, R. W. Colman, R. S. Lees, *N. Engl. J. Med.* **290**, 434 (1974).
14. D. Vesselinovitch, G. S. Getz, R. H. Hughes, R. W. Wissler, *Atherosclerosis* **20**, 303 (1976); R. F. Scott, E. S. Morrison, J. Jarmolych, S. C. Nam, M. Droms, F. Coulston, *Exp. Mol. Pathol.* **7**, 11 (1967); H. C. Rowsell, H. G. Downie, J. F. Mustard, *Can. Med. Ass. J.* **79**, 647 (1958); R. W. Florentin and S. C. Nam, *Exp. Mol. Pathol.* **8**, 263 (1968); R. W. Wissler and D. Vesselinovitch, *Ann. N.Y. Acad. Sci.* **149**, 907 (1968).
15. D. L. Fry, *Circulation Suppl.* **4**, 38 (19[illegible] C. G. Caro, J. M. Fitzgerald, R. C. Schroter, *Proc. R. Soc. London Ser. B* **177**, 109 (1971).
16. J. L. Goldstein and M. S. Brown, *J. Biol. Chem.* **249**, 5053 (1974); M. S. Brown, J. R. Faust, J. L. Goldstein, *J. Clin. Invest.* **55**, 783 (1975); M. S. Brown, P. G. Brannon, H. A. Bohmfalk, J. C. Goldstein, *J. Cell. Physiol.* **85**, 425 (1975).
17. E. L. Bierman, O. Stein, Y. Stein, *Circ. Res.* **35**, 136 (1974); E. L. Bierman and J. J. Albers, *Biochim. Biophys. Acta* **388**, 198 (1975).
18. 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].
19. P. H. Iverius, *J. Biol. Chem.* **247**, 2607 (1972); *Ciba Found. Symp.* **185**, 185 (1973).
20. T. Wight and R. Ross, *J. Cell Biol.* **67**, 675 (1975).
21. ———, *ibid.*, p. 660.
22. B. Radhakrishnamurthy, D. A. Eggen, M. Kokatnur, S. Jirge, J. P. Strong, G. J. Berenson, *Lab. Invest.* **33**, 136 (1975).
23. Supported by grants HL-14823, HL-14860, and HL-11775 from NIH. We thank B. Kariya, T. Kirkman, and N. Joy for technical assistance, T. Wight for his help with the electron microscopy, and the Regional Primate Center of the University of Washington (supported by RR-00166). Statistical analysis was carried out through the PROPHET system developed by the Chemical Biological Information Handling Program of the biotechnology branch of the Division of Research Resource, NIH.

NEWS AND COMMENT

NIH Budget: Senate Committee Holds History's Quietest Inquiry

Some days Congress comes to grips with the world's harsh realities. Other times the nation's lawgivers cut corners and retreat into fantasy.

Take, for example, the scene that opened at 10 a.m. on 3 February this year in room 1223 of the Senate's Dirksen office building. Wielding the gavel is Senator Warren Magnuson (D–Wash.), chairman of the Senate's labor and health appropriations subcommittee. The business of the day is for the subcommittee to cross-examine the senior officials of the National Institutes of Health on the $2 billion they plan to spend for the betterment of the nation's health. And any citizen wishing to learn how diligently that business was performed may do so by consulting the 700-page hearing record which the Senate has published for his edification.*

What does so undramatic a proceeding have in common with the theater of the absurd? Only that it never took place. The appropriations hearings for the NIH and other health agencies were scheduled as usual this year but later canceled. Instead of rescheduling them, the Senate committee hit upon a quite novel way of conducting the public's business. Government health officials were asked to supply written testimony, together with the answers to written questions. Some playwright-manqué on the committee staff then wrote up the material as if the hearings had actually been held.

The script, it should be admitted, is not particularly inspired. The congressional roles, played by Senators Magnuson, Edward Brooke (R–Mass.), Richard Schweiker (R–Penn.) and William Proxmire (D–Wis.), are bit parts with lines that have scarcely a laugh between them.

But the scriptwriter has at least tried to insert a few dramatic touches of his own. For example, he has Magnuson say at one point, "Our next witness will be Dr. Donald Tower of the National Institute of Neurological and Communicative Disorders and Stroke. That's a mouthful."

After further imaginary mastication Magnuson inquires of Tower, "Now that you have communication as part of your name, what new initiatives are you planning in hearing disorders?"

The script to save the committee's hearings disorder places other witnesses in false positions. Donald S. Fredrickson, for example, tells Magnuson that "This is my first opportunity to appear before you as Director of the National Institutes of Health." Fredrickson is followed at the witness table by an insubstantial Frank J. Rauscher, director of the National Cancer Institute, and eight attendant wraiths. The next witness is Robert I. Levy, director of the National Heart and Lung Institute. "Mr. Chairman and members of the committee, it is a particular pleasure for me

*Departments of Labor and Health, Education, and Welfare and Related Agencies Appropriations for Fiscal Year 1977—Part 3. (Government Printing Office, Washington, D.C., 1976).