Relation of Hypertension, Lipids, and Lipoproteins to Atherosclerosis

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Introduction

For more than half a century, heart and blood-vessel disease has been the major cause of death in this country. It has been estimated that well over half of all deaths annually result from coronary heart disease (CHD), nearly three times the death rate from cancer, the second most prevalent disease. Cardiovascular disease causes two-thirds of all deaths among people over 65 years of age. About 30 million persons in the United States have disease of the heart and blood vessels; this country ranks second in the world in the incidence of CHD. Some 27 million of these victims of cardiovascular disorder suffer from hypertension (1).

Although intensive research efforts have begun to elucidate various facets of the etiology, development, and prognosis of the pathogenesis of atherosclerosis, we are still not sure how the initiating and accelerating factors associated with this degenerative disease process interact and manifest themselves. One major reason is that the disease process itself is complex, probably developing through a multitude of factors and processes, suggesting that atherosclerosis is a multifactorial disease. Another difficulty in understanding the disease process is that statistically valid data obtained on large populations and in prospective epidemiological studies do not necessarily apply directly to an individual. Risk factors acting intermittently or during the entire period of the developing degenerative disease are difficult, if not impossible, to evaluate. At any one time, primary risk factors (hypertension, smoking, elevated blood cholesterol, family history for coronary artery disease) as well as secondary risk factors (Figure 1) are probably simultaneously involved in the pathogenesis.

Still another difficulty is the definition of health-risk category. For example, the normal range may not in fact represent “health.” Many clinical laboratories and practicing physicians still use normal ranges in evaluating whether or not a sample can be considered normal or abnormal (a health risk). These nomograms are based on sampling of “apparently normal” individuals and arbitrarily define hyperlipidemia as being present when the plasma lipid concentrations are within the 95th percentile for the specified population to which the person belongs. Unfortunately, because of the way we have defined “normal” in the past, many abnormal test results do not predict disease states or health risk. Thus, a cholesterol value of 2.5 to 2.8 g/liter may be within the 95th percentile distribution of an apparently normal male population between the ages of 51 to 59 years in the U.S., but about 40–50% of the individuals later develop CHD. Epidemiological studies (2, 3) show that CHD risk is linearly related to serum cholesterol concentration down to 1.8 g/liter. Data from the Peoples Republic of China show that the mean serum cholesterol concentration in normal people is 1.36 g/liter, while in CHD patients it is 1.90 g/liter (4). Thus, even in a country with a low rate of CHD, there also appears to be a positive relationship between CHD and cholesterol concentrations. Perhaps the time has arrived when we must re-evaluate the term “risk factor.” In screening for the disease, we are most interested in the predictive value of positive results. Referent values (5) for serum lipids and lipoprotein concentrations should be established that are highly predictive of the disease or disease risk, irrespective of the normal distribution. It is now believed that atherosclerosis in man takes about three decades to develop and that treatment should be initiated when the serum cholesterol exceeds 2.20 g/liter in adults and 2.0 g/liter in children (6, 7). Until we obtain more reliable and predictive values for serum lipids, it will be difficult to understand the independent roles of the various other risk factors in CHD (Figure 1).

In spite of these overriding factors, there can be little doubt that the initiation, development, and progression—as well as the regression—of atherosclerosis are closely associated with hypertension. Although it has not been clearly established that antihypertensive treatment reduces the atherosclerotic complications of hypertension, the clinical and experimental evidence strongly suggests that the early detection and treatment of hypertension could result in substantial reduction in morbidity and mortality from CHD and atherothrombotic strokes. While there is little disagreement that a relationship exists between high blood pressure and increased incidence of cerebrovascular, coronary, and aortic atherosclerosis, little is actually known about the precise role that increased blood pressure plays in the development of the vascular disease process. It is not yet certain whether hypertension is an initiating factor or merely an accelerating factor in atherogenesis. Studies on the natural history and epidemiology of atherogenesis indicate that hypertension accelerates the progression of atherosclerosis in all populations (8). Autopsy findings show that atherosclerosis of the aorta, coronary arteries, cerebral arteries, and other major vessels is more extensive and more severe in hypertensive than in normotensive subjects. Although hypertension can aggravate atherosclerosis, it has not been shown from experimental, clinical, and epidemiological studies that hypertension itself, in the absence of other atherogenic risk factors, can cause atherosclerosis.

Hypertension has been shown to increase the severity of atherosclerosis in both man and experimental animals (9). This effect may be due to local damage to the vessel produced by the augmented pressure or turbulence of the blood in hypertension. This concept is supported by the more frequent occurrence and greater severity of atherosclerotic lesions at locations of high pressure or turbulence (10). There is also evidence to suggest that hypertension, per se, or hormones related to the etiology of hypertension (renin, angiotensin, epinephrine, and kinins) may be responsible for the increased vascular permeability to the serum macromolecules that may initiate the lesions (11). Hypertensive injury usually leads to...
fibromusculoelastic intimal thickening. The mature lesion is composed of smooth muscle (12–16), various connective tissue elements (17–20) and intracellular and intercellular lipids (21–28).

Hypertension, Lipids, and Atherosclerosis in the Rat

Many of the studies on the etiology and mechanism of elevated blood pressure were done on the laboratory rat, so it is not surprising that this animal model has been extensively used for studying the relationship of blood pressure to atherosclerosis. This section summarizes studies on rats as a model for experimental hypertension and its relation to blood lipids and lipoproteins and to atherogenesis. On occasion, other animal models will be mentioned when relevant data can be considered with those of the rat studies to emphasize certain key points. Since the variability in results may be attributed to the mode of induced hypertension, type of diet, age, sex and strain of the rat, and other factors, these variables will be described in greater detail to point out how these factors are all important considerations in an experimental study. This discussion will serve to emphasize certain concepts related to interaction of risk factors and to help explain variance in results and conclusions. Particular attention will be given to the various ways in which experimental hypertension was induced and the types of diets that were given.

Hypertension as an Accelerating Factor in Atherogenesis

Studies by Moss (29) in dogs, Bronte-Stewart and Hepinstall (30) in rabbits, and Daly and coworkers (31–33) in rats indicate that experimentally induced arterial hypertension intensifies atherosclerosis produced by dietary methods.

According to Freis (34), despite the present emphasis on the relation of disorders of lipid metabolism to atherosclerosis, the fact remains that neither hypercholesterolemia nor hypertriglyceridemia is essential for its development. In the presence of elevated blood pressure and “normal” concentrations of serum lipids, such lesions will develop over an appropriate length of time. This phenomenon appears to hold true even for rats, which normally are very resistant to spontaneous or diet-induced hypercholesterolemia and atherosclerosis (35). Only when the cholesterol (C) diet is supplemented with bile acid, cholic acid (C), and an antithyroidal (T) compound (TCC diets) will it produce marked hypercholesterolemia and atherosclerosis in a rapid and profound way (36). Even in rats with experimentally induced hypertension, atherosclerotic lesions generally only develop when they are given a high-fat diet supplemented with bile acid, a goitrogen, or the addition of NaCl and/or KCl to the drinking water. Possibly these factors, in addition to increased blood pressure, directly contributed to atherogenesis, because the method by which hypertension was induced (i.e., angiotensin infusion or deoxycorticosterone acetate (DOCA) implants) may not only have caused an increase in blood pressure but may also have altered arterial wall metabolism. Salts per se may have altered vascular permeability, directly or indirectly.

In 1958, Deming et al. (31) reported the relation of hypertension to the rate of development of atherosclerosis. Young Wistar rats were fed an atherogenic diet similar to that described by Fillios et al. (36). One group consisted of normotensive control rats, the other hypertensives. Hypertension was produced by subcutaneously implanting one 25-mg pellet of DOCA and adding NaCl and KCl to the drinking water. The rats were killed between the 17th and 20th weeks of this regimen. Both groups developed atherosclerosis, but the DOCA-treated hypertensive rats had a greater incidence of lesions, and their serum total cholesterol and total lipid concentrations were also higher. Unfortunately, no data were provided on DOCA-treated hypertensive rats and normotensive rats on a basal diet, to establish whether increased serum lipids were necessary for the generation of atherosclerosis. Studies were made to determine whether the differences shown between hypertensive and normotensive animals depended on the differences in blood pressure or whether they could be accounted for by the presence of DOCA or saline drinking water. The results indicated that the presence of DOCA in the absence of increased blood pressure increased neither the concentration of serum cholesterol nor the extent of intimal lesions. The study suggested, however, that the presence of hypertension, whether induced by DOCA + salt or by compression of one renal artery and removal of the contralateral kidney, resulted in a greater degree of hypercholesterolemia and hyperlipidemia than occurred in normotensive rats on the same “atherogenic” diet during the same period.

Daly et al. (32) later demonstrated that Carworth Farms’ Nelson-strain male rats, made hypertensive by DOCA implants and given saline drinking water, developed higher aortic cholesterol concentrations than normotensive controls, whether they were maintained on a regular chow diet or on an “atherogenic” diet (36). Serum cholesterol was higher in the hypertensive than in the normotensive rats when the basal diet was fed, but there was no difference in the serum cholesterol concentrations of the two groups when they were fed the “atherogenic” diet, in contrast to their previous study (31). Their report included an interesting study on parabiotic rats. Parabiotic pairs of rats were maintained in which the rat with a clip on one renal artery had a higher blood pressure than the other, unclipped control. The rats were on an “atherogenic” diet (36). In each parabiotic pair, the blood pressure of the two rats differed significantly, whereas serum cholesterol values were similar. However, the aortic cholesterol concentrations of the hypertensive rat of each pair was two- to three-fold higher than that of the normotensive rat. These findings suggested that the difference in aortic cholesterol concentrations in the parabiotic rats was independent of the serum cholesterol concentration, with the possible exception of relatively short turnover times in the blood. Turnover times, however, were not measured in the study. The authors suggested that the increase in aortic cholesterol concentration in rats with hypertension may be mediated through local factors operating in or on the vessel wall, rather than through increases in serum cholesterol concentrations. The role of the local factors may be an effect of the supranormal blood pressure on the movement of cholesterol between plasma and vessel wall, resulting in a greater net transfer of cholesterol into the vessel. If the high blood pressure increases the rate of transfer of cholesterol from plasma to vessel, it may have a more pronounced effect on aortic cholesterol accumulation at higher plasma cholesterol concentrations. The possibility that cho-
lesterol synthesis in the aortic wall may constitute another local factor affecting aortic cholesterol concentration was also considered in the study. Measurements of the incorporation of labeled precursors into cholesterol in vitro by aortas of rats on the regular diet indicated that aortas of hypertensive rats have a greater capacity to synthesize cholesterol than those of controls. The results suggested that this increased synthesis of cholesterol in the aorta may contribute to the increased aortic cholesterol concentration in hypertensive rats. It was not clear as to which of the two local factors played the predominate role in atherogenesis in the hypertensive animals.

In a more recent study (33), Daly could demonstrate that hypertension affects the concentration not only of cholesterol but also of other classes of aortic lipids, and that the concentrations of all lipids are not affected in the same manner. The rats with increased blood pressure and on a stock diet had increased concentrations of cholesterol (both free and esterified) and of phospholipid, but decreased concentrations of triglycerides in the aorta, while all classes of plasma lipids increased. Thus, the changes in aortic lipids were not directly related to the changes in plasma lipids. The author suggested that such alterations in certain lipid classes of the aorta may be induced primarily to medial hypertrophy, and an increase in size of the cellular compartment would be expected to require the presence of additional cellular membranes. Free cholesterol and phospholipid, which are the principal lipid components of these membranes, increased in the media of hypertensive rats on the stock diet, with essentially no change in the ratio of free cholesterol to phospholipid. It appears likely, therefore, that the increase in these lipids was related to hypertrophy of vascular smooth muscle. The decrease of aortic triglyceride was probably due to increased rate of triglyceride utilization by the aorta because of the increased rate of oxidative metabolism in hypertensive rat aortas (37). The higher concentration of cholesterol esters in the aortas of hypertensive rats and the general similarity in fatty acid composition of aortic and plasma cholesterol esters tend to suggest that most of the cholesteryl esters originated from the plasma.

Studies by Still (38) strengthen the concept that hypertension greatly accelerates the accumulation of lipids in the arterial intima and that this accumulation begins within a very short time after achievement of a mild hyperlipemia. Furthermore, the mode of formation of the intimal lesions emphasizes the role of blood mononuclear cells in the formation of arterial intimal thickenings. Male Holtzman rats were divided into three groups: (a) hypertensive on high-fat diet (40% egg yolk powder, 5% cholesterol, 20% casein, 20.5% sucrose, 4% salt, 6% non-nutritive fiber, 2% vitamins, 0.3% propylthiouracil, 2% sodium cholate); (b) hypertensive on laboratory chow; and (c) normotensive on high-fat diet. The hypertensive groups were uninephrectomized, had 75-mg DOCA pellets implanted subcutaneously, and then were placed on saline (10 g NaCl per liter) drinking water. The normotensives were uninephrectomized only. A sequential study was then performed by sacrificing two animals from each group on days 16, 18, 20, 24, and 28. The high-fat diet was begun two weeks after the operation (day 14). The serum cholesterol increased in Groups 1 and 3 owing to the diet, and the rate of increase in the two groups was similar, suggesting that hypertension was not a factor in the hypercholesterolemia. Numbers of mononuclear cells lined the endothelial surface of the aortas in Groups 1 and 2, and at times mononuclear cells appeared to be penetrating into the intima. Some of these penetrating cells seen in Group 1 contained lipids. Endothelial cells of the hypertensive rats showed lipid inclusions from day 18 onward. These changes were not observed in the normotensive group fed a high-fat diet. Lipid in the smooth-muscle cells was the exception rather than the rule in the aorta. In contrast to the aortas and other large arteries, the mesenteric artery showed more extensive deposition of lipid in the media than in the intima. For reasons that are obscure, the mesenteric arteries of the hypertensive rats are extremely susceptible to lesions and very frequently show a nodular polyarteritisic type of lesion. Hypertension accelerated influx of blood lipids into arterial intima. In addition, only a modest hyperlipemia of short duration was necessary to produce small fatty lesions in the aortic intima of the hypertensive rat. There is evidence that focal intimal thickenings composed of leukocytes and plasma-like material appeared in the larger arteries of rats in response to increased intramural pressure. Possibly hypertension accelerates the growth of atherosclerotic plaque in at least two ways: (a) in the early stages of the plaque formation by means of exaggerating the influx of plasma and cells into the intima and (b) at a later stage, when the plaques are larger and inclined to be brittle, by increasing the incidence of intimal hemorrhage and exaggerating the mechanical buckling stresses within the plaque and between the plaque and the more supple surrounding tissue. These observations lend support to the author's earlier works (39, 40).

More detailed, sequential study on structural changes in small arteries (mesenteric arteries) of rats with hypertension was described in a report by Guzelian and Matthews (41). From their observations they suggested that the sequence of changes at the onset of small-arterial injury in accelerated rat hypertension was as follows: (a) Within one week of inducing DOCA hypertension, alterations in the activity of certain vascular enzymes occurred, namely, increased alkaline phosphatase (EC 3.1.3.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity in adventitial cells, and glucose-6-phosphate dehydrogenase activity mainly in medial and endothelial cells. It appears that these changes in enzyme activity are a direct result of the agents used to induce hypertension and that in DOCA hypertension they are caused either by an action of DOCA upon the plasma and other membranes of vascular cells or indirectly by an influence on arterial electrolyte balance. At this stage in the experimental disease, no change in the vascular structure is seen by light microscopy. (b) The second stage involved the contractility of the peripheral blood vessels giving rise to the increased systemic blood pressure. Microscopy revealed no change in vascular structure at this stage, but increased vascular permeability was indicated by accumulation of particles focally beneath the arterial endothelium. These changes were initially reversible; arterial smooth-muscle cells have considerable powers of regeneration. When muscle cell necrosis is sufficiently severe or prolonged to lead to lyses of the elastic lamina, then the injury becomes irreversible.

Koletsky et al. (42) studied the relation of hypertension to the distribution and rate of development of atherosclerotic lesions. Young male rats were made hypertensive by constricting the aorta just above the ostium of the left renal artery. Two groups of hypertensive rats were fed Purina Chow for either one or two months before being transferred to a high-fat diet similar to that used by Still (38). Another group consisted of 35 normotensive controls, of which 18 were subjected to shan-cocartion of the aorta. Of these 18, 13 had simultaneous resection of the left kidney, to serve as a control for the ischemic left kidney that was generally nonfunctioning in the hypertensive animals. Of the remaining 17 control rats, seven had left nephrectomy only. All of the control rats remained on the Purina Chow diet for a month and were then transferred to the high-fat diet. The rats were killed at intervals of two weeks to three to four months. About one-third of the animals died during the course of the experiment and many of the remaining rats were in relatively poor condition when killed. Both normo- and hypertensive rats lost weight pro-
gressively after the high-fat regimen was instituted. The weight loss was accompanied by anorexia, poor bodily state, and apathy. At the time of dying or being killed, the rats were examined for the presence of atherosclerosis of the coronary arteries, aorta, and mesenteric arteries. The study showed striking differences between the hypertensive and normotensive rats in speed of development and severity of atherosclerosis. After three to four months of high-fat intake, the coronary arteries of normotensive rats showed only some lipid deposits, whereas at one to two months the coronaries of the hypertensive rats usually had well-developed atheromatous plaques with superimposed thrombosis. Also, at comparable time intervals, atherosclerosis of the intra-abdominal arteries was much more marked and widespread in hypertensive than in normotensive rats. The investigators regarded hypertension as an “aggravating” factor in the experimental production of atherosclerosis and the ingestion of large amounts of fat more important etiologically, because hypertensive rats that remained on a standard diet such as Purina Chow did not develop spontaneous atherosclerosis of any consequence. Normal rats will develop this pathologic process if large amounts of fat, particularly the TCC diet (36), are ingested for a sufficiently long time. There was no qualitative difference between the atherosclerosis in the hypertensive and in the normotensive rats. The earliest change appeared to be deposit of lipid particles—both extracellular and intracellular—in the intimal and medial layers. Subsequently, there was confluence of lipid material, formation of pools of fat and/or foam cells, and cellular proliferation. Hyperplasia of smooth muscle cells was seen in the hypertensive rats. It was suggested that these cells, which are exposed to the insult of high pressure forces, may have the potential for enhanced proliferation on exposure to excess lipids. In contrast to the lesion in hypertensive rats on standard diet, the polyarteritis in the rats on the high-fat diet was almost invariably associated with a fairly substantial fatty component in the vascular wall. Also, many vessels with lipid deposit or atherosclerosis showed varying degrees of superimposed proliferation, exudation, and fibrinoid necrosis—i.e., polyarteritis—which the authors postulated presents a secondary response (apparently peculiar to the rat) to lipid deposits. Association of atherosclerosis and polyarteritis in a similar way in many of the normotensive rats on the high-fat diet suggested that a previous interval of high blood pressure was not a requisite for the lesions. The authors attributed the rapid acceleration of atherosclerosis to the vascular damage caused by increased blood pressure mainly prior to initiating the high-fat diet. This damage resulted in a greater propensity of smooth muscle cells to accumulate lipid particles and in enhanced reactive hyperplasia of these cells on exposure to excess lipids. Another likely effect of hypertension was increased permeability of the arterial intima to the passage of lipids.

Reports of aortic lesions associated with hypertension have for many years been sparse. The mesenteric and renal arteries seem to have been studied in greater detail. In man, atherosclerosis appears to affect mainly large and medium-sized blood vessels, whereas hypertensive vascular disease is primarily a disease of small arteries and arterioles, although it also causes changes in large vessels. Thus, in the rat, it might be appropriate to study both large and small blood vessels when examining hypertensive vascular disease, since the distribution may differ between diet-induced atherosclerosis and hypertension-induced atherosclerosis. Salgado (43) studied the ultrastructure of the aorta in rats with metacorticotid hypertension. Under the conditions of the experiment, medial as well as intimal lesions were produced. Studies of aortic intimal lesions have been reported (29, 30, 36-41). Aortic medial lesions have been reported in rats with hypertension accompanying severe obstructive nephropathy (44), chronic renal failure (45), and anti-kidney serum nephritis (46). The lesions are basically similar in these three pathologic conditions and consist of extensive cellular necrosis and calcification with destruction of elastic lamellae. These types of lesions appear to be different from the type described by Salgado (43), in which necrosis occurred with increase of collagen and ground substance, but no evidence of calcification of the media, or invasion of the media by fibroblasts of inflammatory cells. Perhaps the differences observed were due to a difference in the phase of atherogenesis in the various experiments. Elastin was diminished or absent in areas of necrosis. Salgado postulated that normal structure and metabolism of smooth muscle cells play a decisive role in maintaining the structural integrity of elastin. It is possible that the resulting increased permeability to blood components causes smooth-muscle cells to be surrounded by blood constituents in excess of normal under conditions of hypertension. The cells then react to the changed milieu by changing from a contractile cell to a secretory cell, hence the accumulation of ground substance and collagen. Necrosis could be the result of exhaustion of overstimulated cells.

It has been reported that there is an increase in collagen but a decrease in elastin content in the thickened medial layer of the hypertensive arterial wall (37, 43). However, Wolinsky (47) has shown that the absolute amounts of both medial elastin and collagen were increased in male Carworth rats made hypertensive by subcutaneous DOCA-pellet implants and saline drinking water plus clipping of the left renal artery. The hypertensive vessel was unduly permeable to albumin, cholesterol, fibrinogen, leukocytes, erythrocytes, and other plasma components. Wolinsky suggested that the increased medial thickness associated with hypertension might interfere with “clearance” of increased amounts of intravascular substances entering the media, leading in turn to medial accumulation of these materials. If a vessel segment were normally avascular, i.e., totally dependent upon transmural filtration and diffusion for its nutrition, medial thickening consequent to hypertension could increase wall thickness beyond the critical depth of 0.5 mm, thereby rendering the cells of the deeper portions of the media relatively ischemic. This hypothesis is especially appealing since the rat has no vasa vasorum (48). In contrast, most species do have a vasa vasorum, and it is questionable whether the role of increased medial thickening does play a predominant role in the impaired process of transmural filtration or distribution of nutrients via the vasa vasorum.

With the reversal of experimental hypertension, there were significant decreases in water content of the arterial wall (49, 50) but Na+, K+, and Ca2+ concentrations remained increased in the treated animals (50). The decrease in vessel water content seen after the treatment of hypertension is not associated with a corresponding reduction of the increased wall thickness of these vessels (49). A study by Wolinsky (51) showed that male and female rats (Carworth Farms Nelson strain) with renal hypertension for 10 weeks showed increased wall thickness, medial area, collagen, elastin, and noncollagenous, alkali-soluble protein. When hypertension was reversed for 10 weeks, the vessels' diameter in both sexes and medial thickness in the male rats did not return to normal. The females responded to the reversal of hypertension by a decrease in the arterial medial thickness. This discrepancy between the sexes is not understood. Alterations in the thoracic aortic wall of renal hypertensive rats were evaluated, and the effect of treating the rats with estrogen or progesterone on these changes was determined (52). Estrogens had a distinct inhibitory effect on arteriosclerotic response of the rat vessel wall, and prostogestins slightly enhanced the effect of hypertension. Although a precise explanation for the etiology of the arteriosclerotic plaque is lacking, the hope of achieving specific inhibition of the factors responsible for its growth and
subsequent complications is stimulated by this study.

Eades (53) did a pertinent, "practical" study, in which he chose the factors that are recognized as having a high correlation with the presence or development of cardiovascular disease in man. These are aging, male sex, high blood pressure, a high-fat, high-protein diet, and high serum cholesterol concentration. He chose male Wistar rats, six to seven months of age (comparable to a young adult male human 25 to 30 years old). The rats were made hypertensive by two different methods: (a) unilateral nephrectomy and wrapping the remaining kidney with a figure-eight suture thread and (b) DOCA implantation. The animals were placed on either a chow diet or meat (ground beef) diet for a year or more after the operation. The renal hypertensive rats developed hypercholesterolemia, coronary artery lipid deposits, and atherosclerosis; the DOCA-implanted rats usually did not. When saline drinking water was substituted for regular drinking water, the incidence of hypertension was greater in the chow- and meat-fed normal control rats. The incidence of hypercholesterolemia was also increased in the DOCA chow-fed and meat-fed rats. Furthermore, drinking of saline increased the percentage of rats that developed coronary artery lipid deposits and coronary atherosclerosis in all groups—normal, DOCA-implanted, and unilaterally nephrectomized chow- or meat-fed animals. Even with added salt, the incidence and severity of lipid deposits in DOCA rats did not approach that caused by renal hypertension with or without added salt intake and regardless of whether or not the rats were on chow or meat diets. The author concluded that renal damage increases blood pressure and at the same time causes or permits the elaboration of some factor(s) that may cause irritation or injury to the coronary artery wall and possibly a derangement of the lipid metabolism in the body as well as in the arteries. The result of this series of events is the appearance of hypercholesterolemia and deposition of abnormal amounts of lipid in the coronary artery wall, leading to the development of severe atherosclerotic lesions. Since DOCA-treated animals developed neither hypercholesterolemia nor coronary artery lipid deposits (except in a small percentage of animals, in which the DOCA hypertension may have eventually caused some renal damage, leading to the observed lipid deposits), it appears that hypertension alone is not a major factor in the genesis of hypercholesterolemia and atherosclerosis. In contrast, increased blood pressure in rats caused by renal damage is associated with increased serum cholesterol, arterial lipid deposits, and augmented incidence of atherosclerosis. These events occur with low- or high-fat and low-cholesterol diets, but hypertension is more atherogenic when the rats receive high-fat, high-protein diets.

Hollander et al. (54) showed that experimentally produced hypertensive lesions in monkeys involved not only the larger arteries such as the coronary and cerebral vessels, but also the small branches of these arteries. The changes in the small and large arteries appeared to be similar and included thickening of both the intima and media, with luminal narrowing. The intimal lesions were usually focal, whereas the thickening of the musculoelastic media appeared to involve the entire vessel. The thickening of the intima and media in the hypertensive vessel appeared to be due to fibrocellular proliferation with deposition of acid mucopolysaccharides. These findings are consistent with other biochemical and metabolic studies, indicating that thickening of the arterial media in hypertension is due to hyperplasia and hypertrophy of the medial smooth-muscle cell in addition to increase in the content of collagen, elastin, acid mucopolysaccharides, and electrolytes and water in the arterial media. The actual mechanism by which hypertension accelerates and aggravates the atherosclerotic process has not been established. Some available evidence suggests that the changes in connective tissue metabolism and endothelial permeability associated with atherosclerosis are increased by hypertension. In both hypertension and atherosclerosis, vascular permeability is increased, followed by an augmented biosynthesis of collagen, elastin, and acid mucopolysaccharides.

Hypertension, Increased Permeability, and Atherosclerosis

Hatt and Doughticheff (55), in one of the earlier works in this field, suggested that the accumulation in medial cells of material derived from blood plasma might be a source for vasoconstrictor agents. Gardner and Matthews (41), however, suggested that it is probable that the observed permeability changes in arterial wall may be due to the abnormal activity of arterial cell-enzyme systems. A sequence of changes then begins in which a progressive and ultimately irreversible necrotic phase develops.

The renal factor(s) postulated by Eades (53) could involve the renin--angiotensin system, which may participate in the atherosclerotic process by increasing the arterial wall permeability. Koletsky et al. (56) infused synthetic angiotensin (valyl-5-angiotensin-II-amide) to produce acute and chronic hypertension in rats. After five consecutive infusions of angiotensin, the rats were killed. This group of animals showed vasoconstriction of the arterioles. The medial coat appeared to be hypertrophied; the internal elastic lamina was prominently scalloped, while the endothelial cells were enlarged and projected into the lumen. Such lesions are found in rats with acute renal hypertension (57). Some fibrinoid necrosis of the arterioles was found, mainly in the kidneys, adrenals, and gastrointestinal tract. Rats in the chronic hypertensive group were observed for four months after being infused with angiotensin. There was widespread thickening of the arterioles, especially the media. Vascular lesions involved mainly the larger arterioles, which showed hypertrophy and hyperplasia of the medial smooth muscles and in some instances an increase in PAS-positive material and collagen. The authors concluded that the lesions in the acute and chronic hypertensive rats may have been initiated by angiotensin itself or by the increased vascular permeability resulting from the injurious effects of vasoconstriction or increased intramural pressure or both. The altered permeability would permit the passage of blood constituents, especially plasma proteins, which may initiate the process of atherosclerosis. It would have been interesting to know whether acute or chronic hypertension induced similar changes in larger blood vessels such as the coronary arteries or the aorta. Arterioles respond to hypertension, morphologically, in a somewhat different manner from the major arteries and aorta. Although this study did not include the coronary arteries and aorta, a later report covered arteriolesclerosis of the mesenteric arteries of rats with renal hypertension (58). The authors reported that mesenteric arterial lesions in renal hypertensive rats occur in four stages: (a) arterial hypertrophy, (b) arterial hyperplasia, (c) arterial degeneration and fibrosis, and (d) fibrinoid necrosis. Arterial hypertrophy is characterized by the appearance of hypertrophic endothelial and smooth-muscle cells and arterial hyperplasia by medial thickening. Migration of smooth muscle cells was noted from the medial layer to the subendothelium through fenestrae or fragmented foci of the internal elastic lamina. Arterial degeneration was delineated by degeneration of the endothelial cells and smooth-muscle cells. Degenerative changes of smooth-muscle cells include increasing density of the cytoplasmic matrix, swollen mitochondria, vacuolization, disorientation of myofilaments, and appearance of osmiophilic bodies. Concomitantly, collagen fibers increased within the medial layer. Each smooth-muscle cell became irregular in shape and was finally obliterated by fibrous tissue. The ex-
tensive fibrosis of the artery appears to be the end stage of sclerosis produced by chronic hypertension and is characterized by deposition of electron-dense crystallloid materials in the vascular wall. Other nonpressor permeability factors of renal origin (58, 60) or from platelets (61) may also be involved. Whether increased diffusion could account for the relatively large amount of fluid and electrolytes (62, 63), plasma proteins, and lipoproteins passing through the vessel walls remains to be established.

Wiener et al. (64) demonstrated that vessels from rats with experimental renal hypertension are permeable to colloidal particles of carbon, fibrinogen, erythrocytes, and platelets. They suggested that the increase in vascular permeability may be due to alterations in the endothelial lining of these arteries and arterioles, where discontinuities have been found. Electron photomicrographs showed that these discontinuities varied in extent from separations of endothelial cell junctions to areas denuded of one to several endothelial cells. Furthermore, cytologic evidence of endothelial degeneration, such as swelling and increased electron density of the cytoplasm, were found in the vessels with increased permeability. It appears that the large endothelial gaps were caused by the degeneration of endothelial cells, rather than by retraction of separated endothelial cells. Similar findings were found in cerebral cortical arterial vessels (65). The concept that renin, or more strictly its active product, angiotensin II, may cause vascular lesions in hypertension is not novel. Studies designed to explore the role of the renin-angiotensin system in the genesis of hypertensive vascular disease have been difficult to interpret because it has been impossible to discriminate direct renin-angiotensin effects from those associated with the elevation in systemic pressure. In a more recent study, Wiener's group (66) demonstrated by electron microscopy that fragmentation of the medial layer and more extensive necrosis of certain blood vessels occurred in rats made acutely hypertensive by intravenous infusion of angiotensin for 4 h. This finding suggests that there is regional action of angiotensin on coronary circulation, which may lead to regional variation in permeability pathways across the arterial endothelium. They suggested that the increased permeability of epicardial arteries may be due to increased pressure, while altered permeability and vascular lesions of intramural arteries and arterioles are more readily attributable to the vasoconstriction by the direct action of angiotensin on the endothelium.

Easterly and Glagov (67) presented electron-microscopic evidence of increased arterial permeability and endothelial and medial smooth-muscle cell damage in hypertensive rats. The main renal artery of male Sprague-Dawley rats was selected for examination. The experiment was designed with four experimental groups: (a) control, (b) right nephrectomy, (c) right nephrectomy and saline drinking water, and (d) right nephrectomy and saline drinking water plus 75-mg DOCA pellet implantation. The rats were killed either four or eight weeks after initiation of the treatments. Only group d rats became hypertensive during the study and developed intimal and medial lesions in the main renal arteries sufficiently numerous to be found in a large proportion of sections examined. The findings included adhesion of blood cells to the luminal endothelial surface; the presence of macrophages, leukocytes, and erythrocyte fragments in enlarged subendothelial space; vacuolation; and degeneration of endothelial and medial smooth-muscle cells. Extracellular osmiophilic material had accumulated in both the intima and media. No types of blood cells or macrophages seen in the subendothelial space were found in the media. Cytoplasmic processes of leukocytes were observed in endothelial cell junctions. The authors suggested that the subendothelial blood cells must, therefore, have entered the intima from the lumen by passing through the endothelium. They also postulated that altered arterial permeability could be due to elevated pressure by (a) increased filtration, (b) direct mechanical effects of vessel distention on endothelial cells and cell junctions, (c) disruption of the media by excessive interstitial permeation by debris and blood constituents, and (d) direct mechanical damage to smooth muscle cells. The enlargement of subendothelial spaces in arteries of hypertensive animals in their study may suggest increased filtration. Endothelial cell changes could have resulted from mechanical disruption or increased pinocytosis. Though permeability to blood cells was increased, there was no direct evidence of structural damage to endothelial cell junctions. The presence of medial extracellular osmiophilic clumps and globules suggest that cell debris or altered blood constituents may indeed pass through gaps in the internal elastic lamina. These findings suggest that increased blood pressure or other hemodynamic changes may alter the permeability of the arterial wall, which may be one of the mechanisms by which hypertension enhances the development of atherosclerosis.

Giese (68) believes that the development of plasma leakage and plasma protein deposits in the arterial wall as a result of infusion of large amounts of renin indicates general increase in endothelial permeability. He studied the importance of pressor effects and possible alteration in permeability produced by renin and angiotensin. Acute pressure elevation was produced in rats by (a) application of narrow silver clips on both renal arteries, (b) infusion of renin-containing rat kidney extract into nephrectomized rats, and (c) continuous infusion of angiotensin into nephrectomized rats. All three groups of animals showed plasma leakage (i.e., edema of the mesenteric and pancreatic arteries) and vascular lesions with PAS-positive deposits. These changes were observed less than 24 h after the induction of hypertension. The effects of infusions of noradrenaline (3.4 µg/kg per minute) to nephrectomized rats produced an initial increase in pressure, but the increase was not present several hours later and none of the rats showed vascular lesions characteristic of renin- or angiotensin-induced vascular disease with plasma leakage. However, this was not conclusive, because some localized edema was seen in some rats. Infusion of methoxamine (115–140 µg/kg per minute) for 4 to 5 h to nephrectomized animals produced signs of edema of the mesenteric and pancreatic vessels. The arterial lesions caused by methoxamine differed somewhat from the lesions induced by renin or angiotensin in that no fibrin-like deposits were present. This study does not unequivocally answer the question of whether the renin-angiotensin system induces an exudation of plasma into the arterial wall by way of pressor effects or by way of increasing permeability of the vascular endothelium. The author postulated that the very acute occurrence of severe mesenteric or pancreatic edema in methoxamine-induced hypertension, under circumstances where any participation of the renal renin system has been excluded, would suggest the importance of high intravascular pressure in the genesis of this manifestation of plasma leakage. Likewise, the occasional finding of "fibrinoid" deposits in arterial walls after infusion of noradrenaline into nephrectomized rats tends to incriminate high blood pressure as the main factor. However, the possibility that the endothelial gaps were altered and caused a change in the permeability of the vessel wall could not be ruled out, because of lack of evaluation of the vessel wall by electron microscopy. In his following report (69) on vascular reaction patterns and permeability changes studied by means of vital microscopy and colloidal tracer techniques, Giese suggested that permeability changes do occur in the hypertensive rat. This study showed that the deposition of colloidal particles in the arterial wall takes place preferentially in dilated segments of the small arteries of hypertensive animals. Carbon deposits were never observed in severely constricted regions. The essential factor appears to
be a distension of the structures of the arterial wall due to physical forces, which in turn causes focal permeability changes. The latter may be the result of pressor agents working on focal segments of the blood vessel.

Robertson and Khairallah (70) introduced the concept of the "trap door" effect. In their previous observation (71) low "physiological" levels of angiotensin-II infusion resulted in rapid incorporation of labeled amino acids into the arterial wall. In their recent study (70) various vasoactive agents (angiotensin I, angiotensin II, norepinephrine, serotonin, and bradykinin) were studied, to determine the role of sudden changes of arterial permeability to circulating macromolecules. Young Sprague–Dawley female rats were infused with the various vasoactive agents. This was quickly followed by infusion of 2 ml of Ringer's solution (within 12 s), which was in turn followed by infusion of 10 ml of diluted fixative. Angiotensin II, but not angiotensin I, caused striking cytological changes of the vascular wall of the thoracic aorta. Endothelial contraction and widening of inter-endothelial spaces occurred at a dose of 0.1–10 ng. In contrast to control specimens, the average cross section of interendothelial junctions measured 75 nm or more in width, with occasional spaces 180 to 200 nm wide. Norepinephrine was considerably less effective than angiotensin II in inducing endothelial changes. Quantities 20 to 100 times greater were needed to induce overt contraction of endothelial and smooth muscle cells, and opening of intercellular junctions was less frequent. Serotonin, at the same concentration as norepinephrine, induced focal widening of cell junctions. These last two vasoactive agents seemed to be particularly effective in stimulating formation of pinocytic vesicles in both aortic and coronary endothelial cells. Bradykinin at concentrations 10-fold those needed for angiotensin II was found to have intermediate effects between the octapeptide and serotonin. It is interesting to note that endothelial responses to angiotensin II are short-lived, since in vitro fixation 8 to 10 min after injection of the peptide showed all inter-endothelial junctions closed in the thoracic aorta, main coronary vessels, and myocardial capillaries. In contrast, serotonin injection induced widening of inter-endothelial junctions for considerably longer periods (more than 10 min). Studies of the action of angiotensin II or arterial permeability to whole hyperlipemic sera showed lipid retention was highest in both intimal and medial layers when injected simultaneously with angiotensin II. This effect diminished rapidly after 60 s or longer. Both low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) fractions appeared to accumulate more rapidly in the medial than in the intimal layer of the coronary arteries; the opposite was true for the LDL fraction in the aortic intimal layer. The aortic wall also retained less VLDL lipids than those from LDL fractions. The retention of LDL lipids in the coronary artery wall after simultaneous injection of angiotensin II seemed to be localized primarily in the subendothelial space after endothelial cell contraction had subsided. This also was the case in the thoracic aorta, except that intracellular lipids were also found in medial smooth-muscle cells located immediately beneath the internal elastic membrane. This study showed evidence of coronary and aortic inter-endothelial junctions partly opened, with platelets very close to or actually attached to adjacent endothelial cells. Since platelets carry several vasoactive agents, the investigators proposed that local release of platelet vasoactive agents could maintain for periods longer than 6 min an open pathway for plasma macromolecules to enter the arterial wall—a series of events that predisposes to atherogenesis.

Hypertension and Immunologic Injury in the Vascular Wall

The focal accumulation of plasma proteins, lipoproteins, fibrinogen, and other plasma components (72–74) in the walls of arteries is an important characteristic of the vascular lesions of accelerated hypertension in the rat. The cause of this disturbed passage of plasma derivatives remains uncertain. At least three explanations appear possible. First, increased amounts of plasma proteins may gain access to the intima, media, and adventitia, owing either to increased blood pressure or to altered endothelial permeability; second, there may be obstruction to the continuous normal passage of proteins and other smaller molecules from the plasma to the perivascular lymphatics; or third, both mechanisms may operate simultaneously. Shinoff and Page (74) found an insoluble form of fibrinogen, differing from fibrin, in the intima of atherosclerotic aortas. It was suggested that clot formation may not be the principal pathway for deposition of the protein related to fibrinogen in the aortic intima. Possibly the deposits are formed by a process analogous to the formation and stabilization of cryofibrin in blood.

It has been proposed (40) that the mechanism that causes rat hypertension also directly or indirectly influences vascular tissue metabolism and the activities of vascular wall enzymes, and that these metabolic changes lead to altered vascular permeability. However, the consequences in accelerated hypertension of arterial protein aggregation and of the subsequent focal necrosis of groups of arterial smooth-muscle cells often include a perivascular lymphocytic infiltrate, and necrotizing arthritis may ensue. The possibility exists, therefore, that immunological mechanisms play a part in facilitating the accumulation of mural vascular protein—leading to the precipitation of a sequence of events characterized as vascular injury.

Gardner et al. (75) attempted to test this hypothesis by immunosuppression coincident with the onset of accelerated hypertension. Hypertension was induced in young male Wistar rats by unilateral nephrectomy and subcutaneous implantation of 50-mg pellets of DOCA and maintaining the rats on saline (10 g NaCl/liter) drinking water. The animals were simultaneously treated with one of three immunosuppressive procedures: cyclophosphamide, whole-body x-irradiation or rabbit anti-rat lymphocytic serum (ALS). Their findings suggested that ALS was entirely ineffective in suppressing hypertensive vascular disease, but cyclophosphamide and whole-body irradiation were effective. The authors concluded that the possibility remains that the latter two but not the former immunosuppressive agents partly suppressed hypertensive arterial injury by mechanisms in no way related to agents causing high blood pressure.

Hypertension, Lipids, and Atherosclerosis in Unique, Inbred Rat Models

Koletsky (76) developed a new mutant, obese, spontaneously hypertensive rat (SHR), by selective inbreeding of normotensive Sprague–Dawley and the original SHR, which initially were derived from a strain of Wistar rats by inbreeding (77). After several generations of selective inbreeding of hypertensive offspring from this cross, an abnormal phenotype resulted that is characterized by marked obesity, hypertension, hyperlipemia, endocrine and metabolic disturbances, and premature atherosclerosis. This phenotypic expression appears to be recessive, since its characteristic effect is not present when it is paired with its allele. Furthermore, it was represented as a homozygous trait that can be inherited only when both parents are heterozygous and each carries the same recessive allele. When heterozygous parents are mated, the probability is that about 50% of the offspring will be heterozygous carriers of the recessive allele similar to the parents and of normal phenotype, another 25% will be homozygous for the normal or dominant allele (and also show the normal
The remaining 25% will be homzygous for the recessive gene and thus represent the obese SHR. These rats gain weight rapidly and become rotund. This is apparently due to the fact that their food consumption is two to four times that of normal rats of similar age. Enormous deposits of fat occur throughout the body, especially subcutaneously, retroperitoneally, and in the mesentery. Koletsky suggested that hyperphagia and an abnormality in fat metabolism, perhaps resulting from excessive glucocorticoid activity (which was markedly increased), played major roles in the development of obesity. The hyperlipemia, of which triglycerides were the predominant fraction, developed as early as six weeks after birth and increased progressively. The serum electrophoretic pattern, which showed an increased concentration of pre-β-lipoprotein, resembles the pattern for type IV hyperlipoproteinemia of human beings described by Levy and Frederickson (78). The obese rats spontaneously developed increased blood pressure in a manner similar to their siblings of the original SHR. These unusual rats exhibited adrenal dysfunction and renal disease characterized by glomerular lesions and proteinuria. Atherosclerosis also developed in these rats. Koletsky reported that the obese and nonobese SHR developed vascular lesions of the pancreatic, mesenteric, hepatic, and, to a lesser extent, coronary arteries. No information was given on the susceptibility of the aorta to atherosclerosis in these two rat models. These obese SHR developed lesions even when fed the standard low-cholesterol, low-fat chow diet. The vascular lesions occurred at a much earlier age and were considerably more frequent among obese SHR than among the original SHR. Lesions were observed in 50% of the obese rats that died or were sacrificed between six and 12 months of age, whereas the corresponding incidence among nonobese animals in this age range was less than 10%. The hyperlipemia displayed by the obese SHR may have played a significant role in the increased incidence and accelerated development of vessel disease. The obese SHR described resembles the rat described by Zucker (79, 80) in respect to genetic obesity, endogenous hyperlipemia, and presence of endocrine disturbances. However, it is intriguing that the Zucker rat fat is not hypertensive and, although markedly hyperlipemic, apparently does not develop atherosclerosis. The group in Japan that established the original inbred strain of the SHR (81) reported the dietary effects of added neutral fats, cholesterol, NaCl, and their combination on cardiovascular lesions in the SHR (82). Their data showed that SHR in both the NaCl (i.e., 10 g/liter saline for drinking) and the fat–cholesterol–NaCl groups developed severe pathological changes in the kidney within two to three months, while the control Wistar rats maintained on the same dietary conditions showed no remarkable changes. The high-fat diet consisted of 20% tempura oil, 5% cholesterol, 2% bile powder, and 73% stock chow. Fibrinoid necrosis of intracardiac arteries and small arteries occurred in the experimental SHR on high-salt, high-fat, or a combination of the two diets between 30 and 100 days. These pathologic changes in the control SHR were apparent after 200 days, and the incidence of change was less than 10%. It appears that NaCl loading in addition to a high-fat diet accelerated development of ath- erosclerotic change. Little was mentioned about changes in the aortic wall. Okamoto et al. (83) reported the development of a high incidence of cerebrovascular disease, infarcts, and hemorrhages when these diets were used. It would have been interesting to see whether these changes could have occurred without the use of bile acids or tempura oil, which is generally considered a saturated fat. Hazama et al. (82) concluded that as a result of the abnormalities of the endothelium attributed to the hypertensive state and NaCl, the uptake or penetration of fatty substances as well as NaCl from the blood into the arterial wall is accelerated. The penetration of fatty substances seemed to depend on proliferation of smooth-muscle cells and sequential destruction of the normal arterial wall structure. In a more recent study (84), SHR (stroke-prone strain) were placed on a hypercholesterolemic diet (20% suet, 5% chole- terol, and 2% cholic acid) to examine the role of genetic disposition, hypertension, and salt in acute arterial (mesenteric arteries) fat deposition in various substrains of SHR and in various forms of experimental hypertension (DOCA and renal infarcted Kyoto/Wistar rats) treated with or without anti-hypertensive agents. Among the eight substrains of SHR studied, strain A₁-sb showed the greatest hypercholesterol- emic response (male = 5.07 ± 0.29 g/liter, female = 7.81 ± 0.43) compared with B substrains (male = 3.80 ± 0.43) and normotensive Wistar-Kyoto (2.39 ± 0.09). The A₁-sb group also showed the earliest signs of fat deposition compared to the rest of the groups. The fat deposition occurred in the intima and media. Salt loading in the drinking water accelerated the development of fat deposition in the various types of hyper- tension rats. In experimental DOCA and renal hypertensive rats, the fat deposition occurred, but at a later period than the SHR group. Whether the difference in rate of fat deposition is related to genetic differences, differences in mode of induction of hypertension, or differences in blood pressure is not discernible from their data. Because the fat deposition occurred in pre- and post-hypertensive rats (85), it appears that this strain of animal is more prone to either lesion for- mation or the marked hypercholesterolemia (or both) and that both are primary factors involved in the fat deposition. The investigators (86) believe that hypercholesterolemia is an essential factor for arterial fat deposit and that a genetic factor independent of hypertension is involved. Based on the intensity of sudanophilia of the arteries, they believe that increased blood pressure was the most important factor aff- ecting arterial fat deposition in these rats. Not to be neglected are the possible differences in hemodynamic parameters or hormonal states among the different hypertensive groups (89). Griffith and Hummel (88) found a close correlation between the degree of hypertension and the pathological changes produced in the coronary vessels. No fat was deposited in the arteries of hypertensive rats treated with antihypertensive agents. To determine the possible mechanism(s) of acute arterial fat deposition in hypertensive rats, Yamori et al. (85) studied the local factors, especially increased vascular permeability and its relation to vasospasm and vasodilation by vital and light- and electron-microscopic methods (85). Their results suggested that the fat deposition corresponded to the region of increased vascular wall permeability and that it was most likely related to vasoconstriction and dilatation. The degenerated vessel walls with fat deposition were easily dilated, with further increase in vascular permeability, which accel- erated the fat deposition and aggravated the vascular lesions. Since collagen content increases in hypertensive states, they also examined [3H]proline incorporation into collagenous protein of mesenteric arteries with fat-free mesenteric arteries in SHR (86). Newman and Langner (87) recently showed that [14C]proline incorporation into collagen proteins of the aortas from SHR and Wistar normotensive rats was increased only after 23 weeks of age in the SHR. Because the changes in SHR collagen synthetic activity occurred well after the increase in blood pressure, the data suggest that changes in collagen biosynthesis rate represent a secondary response to the me- chanical stress of the increased blood pressure. The spontaneously hypertensive rats may be a worthy animal model on which to study the effects of hypertension on vascular disease processes. The need to use DOCA, salt, or constriction of the renal artery to induce increased blood pressure can now be eliminated. Without these factors, the evaluation of hypertension and its effect on lipid and lipo-
protein metabolism, and on atherogenesis can be more directly investigated. The male SHR spontaneously develop increasing blood pressure from an early age, about 5 weeks. Pressure increases from about 110–120 mmHg to about 200–220 mmHg within five to seven weeks, at which time it remains unchanged for the remainder of the rat's life. We used this SHR model to study whether atherosclerosis will develop in the major blood vessels of the rat under the influence of a low-cholesterol, low-fat diet1 or of a high-cholesterol, low-fat diet.2 The sequential study (Table 1) was designed to examine the role of hypertension and diet in coronary and aortic atherosclerosis in the male normotensive (Wistar) rat and the SHR. Particular effort was made to determine which of the two factors, hypertension or cholesterol feeding, was of major importance in the etiology of atherosclerosis of the coronary and aortic blood vessels. In most of the previous studies on SHR the mesenteric arteries were examined, but these larger blood vessels, which are more frequently involved in CHD in man, were not. Since no serum lipoprotein studies were reported in previous works by the group in Japan, we included this variable in our present study. Unlike previous studies, no additional salt or bile acids were added to the diets, and no lard was included to complicate the evaluation of the two risk factors in question. The sequential study was used to detect the earliest signs of lesion formation under the influence of hypertension and cholesterol feeding. The mean systolic blood pressure and body weight was measured weekly by the tail-cuff method. Food and water were given ad libitum.

The study (Table 2) demonstrated that without the use of a drastic unphysiologic diet, such as the TCC diet (36) or the diet that Yamori et al. (84–86) used, serum triglyceride (triacylglycerol) concentrations of the normotensive rats do not change, even after a year on the low-fat, high-cholesterol diet. The serum phospholipid and cholesterol concentrations showed a slight increase in both the chow-fed and cholesterol-fed groups on the respective diets for one year (groups IV and VII). These serum lipid changes are not the result of diet, but a natural occurrence related to changes with age. The serum lipoprotein patterns determined by polyacrylamide-gel disc electrophoresis (90) of the normotensive chow-fed rats' sera showed that the predominant lipoprotein fraction is the α-lipoprotein (70–90% of the lipid-stainable fraction), while the β-lipoprotein (and to a slight extent, the pre-β-lipoprotein) constitutes 10–30% of the lipid-stainable fractions. During the course of the year on either diet, the percentage distribution of the lipoprotein fractions showed little change, which reflects the minimal changes observed in the serum lipid concentrations. The serum total protein concentrations of both dietary groups increased with age. An increase in serum protein concentration and change of distribution of the protein fractions with age has also been noted in the dog and other species (92). The liver lipids of the normotensive animals on the chow diet did not show any appreciable change with time (groups I to IV). There was a decrease in hepatic triglyceride concentration in the 40-week group (group IV) as compared to groups I to III. While the cholesterol feeding did not alter the serum lipid concentrations, the liver lipid concentrations increased significantly in the 10- and 20-week groups as compared to the respective age-matched groups fed no cholesterol. In the 40-week animals (group VII) liver lipid decreased as in the 40-week animals fed no cholesterol. The reason for this is not clear. The accumulation of liver lipid due to cholesterol feeding is not a new finding; it is generally thought to reflect hepatic cholesterol accumulation and fatty metamorphosis of the liver. The regression of lipid accumulation in the 40-week group was unexpected, perhaps demonstrating regeneration of the liver and an adaptive response to cholesterol feeding.

Histological studies of the coronary blood vessels revealed no evidence of atherosclerosis or thrombotic complications in either of the two normotensive dietary groups, even after 40 weeks. Their thoracic aortas also showed no evidence of atherosclerosis or abnormality in the arterial wall structure.

The chow-fed SHR had slightly different hepatic and serum lipid profiles than the normotensive Wistar rats. At five weeks of age the liver lipid concentrations in the SHR (group VIII) were lower than in similar aged normotensive rats (group I). By the 40th week (group XI), all liver lipids in the SHR were lower than the initial level at five weeks, a finding that is consistent with the normotensive 40-week groups (groups IV and VII). However, feeding cholesterol to the SHR for 40 weeks caused a marked accumulation of liver cholesterol (about 700% increase). The normotensive rats fed cholesterol

1 Purina rat chow
2 Purina rat chow supplemented with cholesterol, 20 g/kg of diet.

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**Table 1. Experimental Design**

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>Blood pressure</th>
<th>Diet</th>
<th>Agea (weeks)</th>
<th>Systolic B.P. (mmHg)b</th>
<th>Body weight (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Wistar</td>
<td>Normotensive</td>
<td>Chow</td>
<td>5</td>
<td>115.2 ± 2.6</td>
<td>109.8 ± 2.5</td>
</tr>
<tr>
<td>II</td>
<td>Wistar</td>
<td>Normotensive</td>
<td>Chow</td>
<td>10</td>
<td>113.4 ± 2.0</td>
<td>179.6 ± 2.9</td>
</tr>
<tr>
<td>III</td>
<td>Wistar</td>
<td>Normotensive</td>
<td>Chow</td>
<td>20</td>
<td>111.0 ± 4.1</td>
<td>433.2 ± 33.2</td>
</tr>
<tr>
<td>IV</td>
<td>Wistar</td>
<td>Normotensive</td>
<td>Chow</td>
<td>40</td>
<td>104.6 ± 1.8</td>
<td>547.4 ± 28.3</td>
</tr>
<tr>
<td>V</td>
<td>Wistar</td>
<td>Normotensive</td>
<td>2% cholesterold</td>
<td>10</td>
<td>121.6 ± 2.8</td>
<td>307.4 ± 11.5</td>
</tr>
<tr>
<td>VI</td>
<td>Wistar</td>
<td>Normotensive</td>
<td>2% cholesterol</td>
<td>20</td>
<td>116.8 ± 7.5</td>
<td>447.2 ± 20.9</td>
</tr>
<tr>
<td>VII</td>
<td>Wistar</td>
<td>Normotensive</td>
<td>2% cholesterol</td>
<td>40</td>
<td>118.6 ± 7.2</td>
<td>475.0 ± 25.5</td>
</tr>
<tr>
<td>VIII</td>
<td>SHR</td>
<td>Hypertensive</td>
<td>Chow</td>
<td>5</td>
<td>125.1 ± 2.5</td>
<td>74.4 ± 3.2</td>
</tr>
<tr>
<td>IX</td>
<td>SHR</td>
<td>Hypertensive</td>
<td>Chow</td>
<td>10</td>
<td>182.6 ± 12.1</td>
<td>257.0 ± 11.3</td>
</tr>
<tr>
<td>X</td>
<td>SHR</td>
<td>Hypertensive</td>
<td>Chow</td>
<td>20</td>
<td>193.0 ± 5.4</td>
<td>276.4 ± 3.6</td>
</tr>
<tr>
<td>XI</td>
<td>SHR</td>
<td>Hypertensive</td>
<td>Chow</td>
<td>40</td>
<td>238.2 ± 2.3</td>
<td>330.8 ± 18.0</td>
</tr>
<tr>
<td>XII</td>
<td>SHR</td>
<td>Hypertensive</td>
<td>2% cholesterol</td>
<td>10</td>
<td>173.0 ± 7.1</td>
<td>178.2 ± 11.1</td>
</tr>
<tr>
<td>XIII</td>
<td>SHR</td>
<td>Hypertensive</td>
<td>2% cholesterol</td>
<td>20</td>
<td>190.6 ± 5.1</td>
<td>246.4 ± 12.7</td>
</tr>
<tr>
<td>XIV</td>
<td>SHR</td>
<td>Hypertensive</td>
<td>2% cholesterol</td>
<td>40</td>
<td>216.6 ± 4.1</td>
<td>357.0 ± 8.2</td>
</tr>
</tbody>
</table>

a Age of rats when sacrificed. All rats began either on chow diet or cholesterol supplemented diet at three weeks of age.
b Blood pressure represents pressure during the last week before sacrifice.
c Purina rat chow diet.
d Purina rat chow diet supplemented with 2% cholesterol by weight.
for 40 weeks did not demonstrate this propensity to accumulate liver cholesterol as did the SHR. Like the chow-fed SHR (groups VIII-XI), the liver triglyceride concentration of the cholesterol-fed SHR decreased with age. However, at the end of 40 weeks the concentration of the liver triglyceride of the cholesterol-fed SHR group (group XIV) was twice as high as in the chow-fed group (group XI). At five weeks of age, the SHR on the chow diet or on cholesterol-supplemented diet had slightly lower serum cholesterol and triglyceride concentration than the age-matched normotensive group. At the end of the 40-week period, only the serum phospholipid concentrations increased (in both SHR groups), but the cholesterol and triglyceride values did not. However, unlike the normotensive rats, cholesterol feeding caused an increase in serum total cholesterol in the SHR (group XIV). Despite a 60% rise (718 vs. 448 mg/liter) in serum cholesterol concentrations, they never surpassed the values of the group-fed (group IV) or cholesterol-fed (group VII) age-matched normotensive rats. Apparently the SHR has a less efficient system for maintaining constant liver and serum cholesterol concentrations with dietary feeding of cholesterol. Nominally, the rat has a very effective cholesterol negative-feedback system and an efficient system of increasing acidic and neutral steroid excretion with cholesterol feeding (91). This apparently may not be the case in the SHR when fed a cholesterol diet. The serum lipoprotein values of the SHR were much lower than those of the Wistar normotensive rats. On paper electrophoresis (93) the patterns were barely visible. Polycrylamide-gel disc electrophoresis showed that, as in the normotensive rats, the α-lipoprotein was the predominant fraction, consisting of 70–95% of the lipid-stainable fractions. Only after 40 weeks of cholesterol feeding did the relative percent distribution of the α-lipoprotein moiety go below 70% to 62% (group XIV). On paper electrophoresis, group XIV showed marked increase in intensity of the β-lipoprotein band as compared to the control groups (groups I or VIII). The increased serum cholesterol load due to long-term cholesterol feeding (group XIV) was probably carried in the β-lipoprotein, which increased in concentration. The serum total protein concentrations of both SHR dietary groups (groups VIII-XI and XII-XIV) followed a trend similar to that of the normotensive groups (I-IV, V-VII).

Regardless of diet, the protein concentrations in the SHR sera increased with age. It appears that this change is independent of hypertension, because it occurred in both the SHR and the Wistar normotensive rats. Hematocrit measurements showed no correlation with the changes in protein concentration with age. The hypertensive rats, whether chow-fed or cholesterol-fed, showed no evidence of coronary atherosclerosis even after 40 weeks. Early stages of atherogenesis were
seldom seen in the thoracic aortas in both dietary treatment groups. No lesions were seen in groups VIII and IX, but a few animals in groups X and XI showed slight intimal thickening, which may reflect age-related arterial wall changes (94). The endothelial cells showed no signs of lipid accumulation and there were no foam cells, fibroblasts, or smooth-muscle cells in the intima. The elastic lamellae in the media of groups X and XI showed some disorientation. Instead of the characteristic uniform laminar units, there was much fusion of adjacent elastic lamellae. It was uncommon to see any focal lipid accumulation in the media, while there was some smooth-muscle proliferation. The media of the SHR was much thicker than in age-matched normotensive rats. When the SHR were fed the cholesterol-supplemented diet, there was more necrosis and fibroblast accumulation in the media. Group XII showed evidence of focal necrosis of smooth-muscle cells and some medial lipid accumulation. Occasionally, fibroblasts were seen in the outer media in close proximity to the adventitia. Groups XIII and XIV showed not only necrosis but also proliferation of smooth-muscle cells. The occasional intimal thickening observed showed some lipid accumulation.

The results of the above study suggest that while the Wistar normotensive rat is resistant to atherogenesis, even with prolonged cholesterol feeding, the SHR is also resistant, but less so. SHR on Chow diet have lower serum lipid concentrations but as they grow older, they show signs of early thoracic intimal thickening (but not in the coronary arteries). These changes may be associated more with spontaneous changes related to aging (94). Our type of long-term dietary cholesterol feeding does not cause marked hypercholesterolemia, a requirement that is probably necessary for SHR to consistently develop atherosclerosis of the larger blood vessels, i.e., coronary and aortic. It is possible that not adding salt to the diet or the production of hypertension by a different method may explain the difference in results obtained by other investigators. The occasional lipid accumulation observed in our rats was restricted primarily to the intima, although there was some evidence that the media was undergoing some structural changes. Thus, it appears that chronic hypertension alone may initiate some pre-lesion atherosclerotic processes, but does not cause a dramatic change in the blood vessel in the SHR. With cholesterol feeding, the medial and intimal changes become slightly more pronounced. This was especially true at 40 weeks, when the serum lipid concentration had increased in the SHR on the cholesterol diet. Normotensive rats and normolipidemic rats are very resistant to spontaneous coronary and aortic atherosclerosis. Normotensive rats fed a low-fat, high-cholesterol diet did not develop a sufficient hyperlipidemic condition to cause lesion formation, despite the fact that they remained on a 2% cholesterol diet for about a year. Evidently, in a normolipidemic state, hypertension by itself is a risk factor for pre-lesion formation, but must persist for a long period of time to elicit any overt lesions in the arterial wall. The hyperlipidemic condition accelerates the disease process in the SHR. Of the two risk factors, it appears that hyperlipidemia has a more severe and rapid effect on lesion formation in rats. The coronary and aortic blood vessels of the rat are quite resistant to atherosclerosis, even with mild hypercholesterolemia and elevated blood pressure, unlike other animal species. The suggestion by other investigators (32, 38, 53, 76, 82) that overt hyperlipidemia initiates and accelerates hypertensive vascular disease is probably correct. With increased blood pressure, the sequence of events leading to atherosclerosis is further accelerated. The question still remains as to how either of these risk factors participate in the initiation, development, and progression of the atherosclerotic process. Orbetzova et al. (95) reported that SHR given commercial rat-chow pellets developed mild hyper-pre-β-lipoproteinemia and an increased cholesterol content in the aortic wall. Morphological and electron microscopic studies showed no evidence of atherosclerosis at the end of 34 weeks on the diets. Their studies emphasize that hypertension by itself is not a prerequisite for aortic lesion formation in rats. Experimental designs in which drastic measures are used to elevate serum lipid concentrations (i.e., thyroidectomy, addition of bile acid, lard, very high concentrations of cholesterol and salt in the diets) produce marked atherosclerosis in hypertensive rats. Whether this type of approach to the study of the role of hypertension and hyperlipidemia in early lesion formation in rats is the proper way of assessing the etiology of atherosclerosis in man is yet to be clarified. Figure 2 summarizes how hypertension may be participating in the etiology of atherosclerosis.

### Hypertension, Lipids, and Atherosclerosis in the Human Being

The relation of hypertension and hyperlipidemia to atherosclerosis in the human being has received extensive investigation for many years. During the past 25 years, knowledge concerning the nature of the lipoproteins and lipids, as well as the types, causes, and treatment of hypertensive disease, has expanded almost exponentially. Therefore, this discussion will summarize only the most relevant material, and we will not attempt a complete coverage of the field.

In a cooperative study on lipoproteins and atherosclerosis, a survey of serum lipid levels in normal persons was conducted (96). The survey studied 10,590 men and 3,404 women, 18 to 65 years of age. Data included serum lipoproteins of Sf 12–20, Sf 20–100 classes and data on cholesterol concentrations. Blood pressure correlated poorly with lipid values. Because the study did not include individuals with blood pressure higher than 170/100 mmHg, differences may have been less marked than would have been the case had those with higher pressures been covered. Lipid concentrations of individuals with lowest blood-pressure readings were compared with values of those with highest. The findings indicated that differences in blood pressure were associated with differences in lipid concentrations. This was especially true of the Sf 20–100 lipoproteins, which were significantly higher in the higher-blood-pressure group. It was emphasized in the report that while the differences were significant in the large population studied, they had little meaning for individual cases.

Since that early study, numerous investigations involving smaller population groups have been made, sometimes in much greater detail. During the early 1950s, more effective means of controlling blood pressure were becoming available. Such advances led many clinicians to raise the question of
whether the reduction of blood pressure might also reduce the atherosclerotic complications of high blood pressure. In a study reported by Corcoran et al. (97) in 1956, it was shown that atherosclerotic complications were more common in patients who did not respond well to antihypertensive treat-
ment. Serum lipoprotein and lipid values showed little relation to responsiveness to antihypertensive treatment. Serum li-
poprotein concentrations of the hypertensive patients, with the exception of the patient with malignant hypertension, varied little from those of normals.

A survey was made of “diet,” serum cholesterol, protein, blood hemoglobin, and glycemia in a West Indian community (St. Kitts, W.I.), with observations on ischemic heart disease (98). The serum cholesterol and lipoprotein patterns, determined ultracentrifugally on normo- and hypertensive St. Kittian women, were similar. Serum low-density lipoprotein concentrations of St. Kittian women, unlike those of women of the Cleveland area, did not increase significantly with age, the levels in 20–34 and 35–59 age groups being nearly the same. If there was any tendency for familial hyperlipidemia among the St. Kitts population, it was not manifest when they were on their low-fat, low-calorie diet and doing hard work. Ischemic heart disease had a minimum prevalence of 1.2 per 100 in men age 40–49 and 0.75 in women age 45–49 years. Thus, while hypertension, a contributory factor in atherogene-

The occurrence of hypertension in family members with hyperlipidemia and in members with normal lipid values was the same. These results indicate the great importance of type II hyperlipidemia as an accelerating factor in atherogenesis and lend additional evidence for the need for early cholesterol control. The presence of hyperlipidemia per se did not appear to be related to the occurrence of hypertension of these kind.

It has been suggested that one explanation for the develop-
ment of atherosclerosis may be the consequence of a time-
dose-product of “acquired risk” factors. There is also the possi-

Possible part of the problem in correctly assessing the sig-
ificance of some of the risk factors is in establishing the cor-
rect cut-off level at which a value should be considered normal. This was well demonstrated for serum cholesterol, very-low-density ($S_f 20–100$), and $S_f 12–20$ lipoproteins, in which relatively wide ranges of values were found (96). Simi-
larly, systolic blood pressures in men 30 to 62 years of age on entry to the Framingham study showed a relatively wide spread (104). The distribution curves of systolic blood pres-
sure of those who developed coronary artery disease during the ensuing 16 years and those who did not were very similar.

Risk of atherosclerotic disease was, however, proportional to the pressure in the systemic circulation. The importance of blood pressure as a risk factor was greatly augmented by ele-

The Framingham study showed that all of the variables were continuous, and in the multivariate analysis, two or three small abnormalities carry as much risk as one large one, since the increased risk with increased factors is exponential. Kannel (106) interpreted the results of the Framingham study as lending considerable support to the filtration or perfusion hypothesis of atherosclerosis suggested by Page (1).

A significantly decreased plasma volume (10 to 15%) has been observed in essential hypertensive patients (107, 108). When their blood pressure is reduced to the normal range by treat-
ment with antihypertensive agents, the plasma volumes increase. Study of serum lipid and lipoprotein concentrations in the hypertensive patient has shown, as had previously been reported (97), a relatively slight increase or alteration in dis-
tribution. (This has not been found to be true in the malignant
hypertensive individual.) No correlation between serum lipoprotein or lipid levels and altered plasma volume, however, has been noted. In animal studies on experimental atherosclerosis produced by hypercholesterolemic diets or hypertension, studies of blood volume changes are lacking.

Hypertension is often associated with an accelerated development of atherosclerosis; however, in certain selected groups such a relationship may not be observed. Twenty-four subjects with human somatotrophin deficiency but otherwise with normal pituitary function were studied for five years (109). It was found that 12.5% of the dwarfs, 36% of age-matched diabetics, and 18.5% of a control group consistently had hypertension (>150/90 mmHg). The diastolic pressure of none of the dwarfs exceeded 100 mmHg, whereas 11 of the diabetics had values between 100 and 120 mmHg. Twelve of the 24 dwarfs showed hypercholesterolemia and 10 showed hypertriglyceridemia; their lipoprotein patterns were typical of a type IIa or IIb abnormality. α-Lipoprotein concentrations were normal. Twenty-eight percent of the diabetics had increased serum cholesterol concentrations, and 30% had increased triglyceride concentrations. The incidence of atherosclerosis in the dwarfs was low: only two subjects, 68 and 77 years of age, showed its presence. In contrast, atherosclerosis was found in 40% of the 20 diabetics, some of whom were in the younger age groups, two in the 30–40 year group, two in the 40–50 year group, and two in the 50–60 year group. Deficiency of somatotropin seemed to confer upon the dwarf population studied a certain degree of protection from atherosclerosis. This factor was effective despite a high incidence of other atherogenic factors: hypertension, hyperlipidemia, and diabetes. Further understanding of this means of protection should be profitable.

Hypertension, hypercholesterolemia, and cigarette smoking are recognized as important etiological factors in coronary heart disease. Their association has been demonstrated by careful epidemiological investigations (110). They are major risk factors for premature atherosclerosis. As presented in 10-year morbidity–mortality tables, presence of one of the factors increased the probability of a fatal event, including total morbidity, by twofold, while presence of two of the factors increased the risk fourfold. For the person with all three factors, the risk was eight times that of the non-smoking person with normal serum cholesterol and normal blood pressure. Only 17% of the male subjects up to 59 years of age were in this fortunate group. In these studies, a serum cholesterol concentration of 2.50 g/liter or greater was considered high. Diastolic blood pressures were divided into four groups: less than 85, 85–94, 95–104, and 105 mmHg or greater. Even mildly increased diastolic blood pressure was associated with large increases in risk of a major coronary event.

An unusual situation was observed in the Framingham study (111), which included an evaluation of brain infarctions. The investigators observed a very significant increase in risk of “atherothrombotic” brain infarction in normotensive people with increased serum cholesterol or $S_f$ 20–400 (i.e., pre-β-) lipoproteins. There was an even greater increase in risk with increased blood pressure, but this was apparently independent of the serum lipid values, as the risk in hypertensives with normal and with high serum lipid concentrations was similar.

Two questions have frequently been asked over many years: Are small vessel changes a cause of hypertension? Are such vascular changes always associated with hypertension? Examination of a large series of renal biopsies obtained at the time of sympathectomy for the relief of hypertension showed that, in many cases, hypertension could be present with only minor vascular change (112). The authors concluded that these findings indicated that vascular change was not essential for hypertension.

The fact that the pulmonary artery, normally exposed to low blood pressure, may develop atheromatous plaques in the patient with pulmonary hypertension was described more than 50 years ago. It has more recently been noted that plaque development does not depend on the presence of abnormal serum cholesterol concentrations. Thus, in the pulmonary vasculature, increased pressure seems to be the dominant factor in the etiology of arteriosclerotic plaques (113). Other parts of the venous vasculature with relatively high blood pressure are the proximal portion of the right iliac vein and the distal portion of the inferior vena cava. These areas may show typical atherosclerotic plaques (114). It was also found in a study of 52 children with nephrotic syndrome, all of whom had increased serum cholesterol concentrations, that 36 also had elevated blood pressure (115). At autopsy, none of the children with normal blood pressure showed coronary arteriosclerosis, but 11 of the 36 with elevated pressure did so. Unfortunately, this particular experiment designed by nature did not include hypertensive children with normal blood lipids. Fortunately, in the children, as has been observed in epidemiologic studies, hypertension and hyperlipidemia had a definite atherogenic effect.

Diabetes is a recognized increased risk factor for the development of coronary heart disease. When increased blood pressure and high concentrations of very-low-density and low-density lipoproteins are present in the diabetic patient, as occurs in the Kimmel–Stiel–Wilson syndrome patient, atherosclerotic complications are greatly accelerated (116). The atherosclerotic involvement in these patients is greater than in unselected diabetic patients. Arterial hypertension is more frequent in different stages of diabetic nephropathy than in an unselected group of diabetics matched for age. While the arterial wall of persons showing no clinical evidence of atherosclerosis showed presence of some lipoproteins of all flotation classes, the arterial wall showing atherosclerosis contained greatly increased amounts of $S_f$ 20–100 lipoproteins, which comprised the major fraction found. The concentration of lipoproteins of $S_f$ 20–100 class showed similar increase in the serum (116).

In a long-term study, Page and Dustan (117) found that many hypertensive patients required less drug to maintain their blood pressure at desirable levels after a number of years than they had needed during the early treatment periods. It was not known what produced this change. It would be important to know whether “vascular and myocardial restructuring” had occurred. It has been clearly demonstrated that atherosclerotic plaques in experimentally induced disease may regress after withdrawal of the atherogenic stimuli, and that size of atherosclerotic plaques in the human being may decrease if blood lipid levels are reduced by as much as 40% (118).

Recent studies of the turnover of cholesterol in human atherosclerotic arteries have been reported (119). The equilibration of cholesterol between plasma and atherosclerotic areas of arteries was followed for periods ranging from two to 96 days in 13 patients who were in a metabolically steady state. The arterial samples were obtained from 12 patients during surgery and in one at autopsy. After two to four days, the specific activity of cholesterol ranged from 0.3 to 4.5% and had increased to 6 to 30% in different arteries after 17 to 27 days. This represented a very low uptake in comparison with that of skeletal muscle, which had a relative specific activity of 96% by 22 days. After periods of 60–96 days, cholesterol in atheroma of abdominal aortas, common iliac, and femoral arteries had equilibrated to 55, 30, and 29%, respectively. From the one patient in whom coronary abdominal and thoracic aorta atheroma were available, it appeared that the degree of equilibration in different vessels was very similar, being 66, 66, and 57%, respectively, after 96 days. No information was
given concerning the blood pressure; thus, its part (if any) in rate of equilibration cannot be estimated. It is significant that equilibration of free cholesterol was greater in the media than in the intima. The approximate turnover time of cholesterol in atheroma was estimated to be between 442 days in coronary and abdominal aorta to 580 days for common iliac, 821 days for the femoral, and 904 days for the carotid arteries. Although these rates are slow and vary from artery to artery, and undoubtedly are affected by the stage of development and characteristics of the atheroma, the results nevertheless suggest the possibility of potential regression of the atherosclerotic lesion in the human being.

Such possible regression of atheroma was suggested in a study reported in 1958 (120), in which the changes observed in renal lesions in malignant nephrosclerosis of the treated patient were compared with those found in the untreated patient (120). The data showed that in patients with malignant nephrosclerosis, clinical remission effected by intensive treatment with antihypertensive drugs also resulted in remission of the histologic lesions of renal vascular disease. Of particular interest in a consideration of the relation of lipids, hypertension, and atherosclerosis was the observation that the small focal accumulations of lipid-laden histocytes evident within the intisters of the thickened walls of small arteries and arterioles of 40% of the untreated cases were not present in those of the treated cases. Because it was felt that the lipid-laden histocytes represented an early phase of atheroma formation, their absence in the treated groups indicated that the process was reversible.

The severity of atherosclerosis as measured by the amount of lipid extracted from segments of the coronary, cerebral, and femoral arteries and the abdominal aorta was determined as part of a long-term study of factors affecting atherogenesis in each of 184 fatalities in a large series of patients permanently confined to a hospital (121, 122). The severity of atherosclerosis thus determined was compared with the presence or absence of hypertension during the patient's hospital stay. A significant relationship was consistently found between the severity of atherosclerosis in the coronary and cerebral arteries and hypertension. This positive correlation of presence of hypertension and severity of atherosclerosis contrasted with the lack of correlation found by the same group in a study of serum cholesterol, determined at least once a year for eight years, and atherosclerosis. Most of the 800 patients having blood studies were in the 50- to 90-year age group, and 191 of them succumbed during the study. The severity of atherosclerosis was estimated by gross and histologic examination of the arteries and by chemical analysis for total lipid and calcium in the intima carefully stripped from the artery. Only when the serum cholesterol exceeded 3.00 g/liter was there any significant correlation with the severity of atherosclerosis, and then it was weak. The study was limited because it covered an older age group, but the investigators had the advantage of knowing the diet and treatment of the patients for a longer time.

A study of the prognosis of women and men after myocardial infarction (MI) with elevated blood pressure or elevated serum cholesterol showed that the relative influence of these parameters differed in the two sexes (123). During the first 3½ years after MI, women with or without increased blood pressure had the same probability of death, while those with a serum cholesterol concentration greater than 2.70 g/liter had approximately three times the cumulated probability of death at 3½ years as those with levels 2.70 g/liter or less. The male subjects with increased blood pressure showed about twice the probability of death after 6½ years as those with normal blood pressure, while those with high or low serum cholesterol concentrations had about the same probability.

In Jerusalem, Tel Aviv, and Haifa, Medahe et al. (124) found that systolic and diastolic blood pressure readings showed a direct significant association with the five-year incidence of myocardial infarction in permanently employed government and municipal workers 40 years of age and older at the beginning of the study. There was also a direct association of serum cholesterol and of $\beta$-lipoprotein concentration with incidence of myocardial infarction, but an inverse association between the serum $\alpha$-lipoprotein cholesterol concentration and incidence was observed.

In an epidemiological-genetic study at Tecumseh, Michigan, it was clearly demonstrated that serum cholesterol concentrations of children were related to those of their parents (125). This relation was not the type dictated by a single gene inheritance; rather, when the parents' values differed greatly, the child's value tended to be an average of the two. A similar type of inheritance seemed to hold for blood pressure, height, relative weight, and glucose tolerance. The inheritance mechanism of triglyceride concentration may also be similar (126). The permanence of the serum lipid and lipoprotein pattern, once established, was demonstrated in a study of 107 company executives, carried out annually at the Cleveland Clinic for seven years (127). Serum cholesterol concentrations and all classes of lipoproteins, when studied ultracentrifugally, and blood pressures were very constant if the men continued with their usual physical activities and diet. Hatch (128) believes that inheritance is the primary factor in determining the magnitude of the risk factors, while the influence of dietary factors is simply superimposed on the genetic. He also feels that energy balance is the environmental factor that most influences most of the major risk factors. Furthermore, heredity plays such an important role that even if environmental factors were advantageously altered so that the risk value was halved, there still would be the same proportion of men in the high-, medium-, and low-risk categories.

Friedman and Roseman have divided patients into two groups according to their behavior pattern (129). Individuals in Group A were found to be coronary-prone; those in Group B showed a lower incidence of CHD. The group A coronary-prone subject showed traits indicating the presence of an "excessive struggle against the exigencies of time, socioeconomic pressures, or the competitive efforts of other persons." The subjects of this personality type also showed increased serum cholesterol and lipid values, and type-IV serum lipoprotein patterns were frequently observed. When even a mild degree of hypertension is present in such patients, Dr. Friedman believes that it should be treated vigorously. This group also recognized the enhanced proneness of the hypertensive subject to CHD if an increased serum cholesterol concentration is present.

The effect that modification in coronary heart disease risk factors may have on incidence of the disease can only be fully appreciated by long-term epidemiologic factors. However, a recent report from Australia indicates that improved control of blood pressure may have played an important part in the substantial reduction in mortality due to CHD during the late 1960s and early 1970s (130). No information was available about serum cholesterol or lipoprotein concentrations, but no major changes in diet or other factors were known that would have altered these values. Smoking was known to have increased rather than decreased, so it appeared likely that the improved control of hypertension was the causative factor in this very desirable shift in incidence of CHD deaths.

High blood pressure and hyperlipidemia are two of the major risk factors in atherogenesis. Further understanding of the metabolic processes occurring in the cells of the blood vessel and set by "genetic templates," which in many aspects are not currently understood, may ultimately permit more adequate control of these factors. Some of the pathways are now known, but methods of altering or correcting the errors,
if present, are usually lacking.

Predisposing factors of hyperlipoproteinemia and hypertension to the development of atherosclerosis have already been considered. Mention will now be made of the level of these risk factors in populations relatively free of atherosclerosis. Nomadic populations such as the Masai of East Africa have a serum cholesterol concentration similar to that of young Americans or young Europeans, despite the fact that their diet, which consists of milk, blood, and meat, is high in animal fat and cholesterol (131). Their blood pressure is, however, generally low in comparison with that of Americans, and unlike that of the American, does not increase with age (131, 132). With this balance in severities of atherogenic factors, as might have been predicted, the incidence and rate of development of atherosclerosis as judged from the nomadic group studied was very low.

A study of a group of 590 men and women (60 to 90 years of age) living in a home for the aged in Toronto, Canada, also showed a low incidence of atherosclerosis associated with serum cholesterol values less than 1.70 g/liter and a significantly greater incidence in those people with values above 2.50 g/liter. No correlation was found between serum cholesterol and blood pressure. It was pointed out that the serum cholesterol concentration had only limited diagnostic or prognostic value for the individual patient (133).

In a study of the correlation of cine coronary arteriographic and clinical findings in 1000 patients, serum cholesterol concentrations were correlated with arteriographic findings in 147 of the men younger than 40 years. In general, if the serum cholesterol value was less than 2.00 g/liter, the incidence of normal findings in the coronary arteries was high. If the value exceeded 3.00 g/liter, the frequency of abnormal findings was high. As in many other studies and the preceding citation, data on the cholesterol concentration were of limited diagnostic value in the individual patient (134).

A study (135) of the effect of plasma of patients with essential hypertension on the response of an isolated artery preparation showed that the hypertensive plasma augmented the response of the artery to the drug more than did plasma from normotensive controls. Because plasma cholesterol in these patients was increased, the possibility that hyperlipidemia was a contributing factor was investigated. Preparations of β-lipoprotein from the plasmas were found to potentiate the arterial preparation response. Thus, hyperlipidemic plasma may influence atherogenesis not only by providing excess lipids for deposition, but by modifying the response of the vessel to physiologic agents.

Until the last decade, studies of serum cholesterol values in children were fragmentary. As a result, the question of what, if any, effect early hyperlipidemia may have on subsequent development of hypertension has not been adequately documented. Blumenthal, in a review entitled "Pediatric aspects of atherosclerosis," mentioned that some abnormal plasma lipoprotein patterns seen in late childhood (specifically Type-IV) are associated with some increased risk of coronary heart disease and hypertension (136).

A study in a Dusseldorf hospital of 51 patients with intermittent claudication (137) showed that those patients with type-IIa hyperlipidemia were younger than those with other types of lipoprotein abnormality, and the atherosclerotic lesions in this group were primarily in the pelvic area while those of the other types were more distal. Patients with type-IV patterns generally had at least three risk factors; 46% had all five (i.e., hypertension, smoking, obesity, abnormal glucose tolerance, abnormal lipoprotein pattern). The study emphasized the multifaceted etiology of peripheral vascular disease.

Risk factors in the development of atherosclerosis were evaluated in rural and urban populations in Puerto Rico. While cholesterol values and blood pressures were found to be significant risk factors in both groups, cholesterol was not considered of major importance as the values in both groups were lower than in similar populations on the mainland U.S. The incidence of aortic arteriosclerosis was similar in the two populations studied, but that of coronary atherosclerosis was greater in the urban group (138).

In 1949, the death rate from coronary heart disease in Japan was 9.9 per 100 000, one tenth the U.S. rate (139). By 1970, it had increased in Japan to 25.9. Studies devoted to exploring the cause for such differences have included an epidemiologic investigation of risk factors including smoking, blood pressure, blood lipids, body weight, glucose tolerance, and postmortem examination of the hearts (139). In this study, serum cholesterol concentrations of less than 2.00 g/liter did not indicate abnormality of lipids as a major risk factor, but among other factors hypertension was most strongly associated with development of stroke and ischemic heart disease.

Among the many important observations in the Framingham study, one of the most enlightening was the evaluation of serum lipids and hypertension in relation to atherothrombotic brain infarction (ABI). In normotensive patients, increased serum lipids seemed to be of importance in predisposing to ABI, whereas in the hypertensives, the incidence of ABI was similar in those with normal and hyperlipidemic serum (140).

The clinical course of 500 patients, some with normal and others with slightly or moderately abnormal arteriograms, was followed for a minimum of five years at the Cleveland Clinic (141). During the follow-up period, 26 patients died. In 15 of these, coronary disease was not a cause of death. Of the remaining 11 cases, five had a history of hypertension. Eight of the 11 died suddenly, and there was some question as to whether coronary disease was responsible in two of these. In case 5, severe generalized atherosclerosis was found. The patient had had "extremely severe" hypertension and hypercholesterolemia. Thus, in the patients whose deaths were probably due to coronary artery disease, hypertension was a common finding.

Summary and Conclusions

The evidence of work by many investigators, summarized above, lends further support to the concept that atherosclerosis is a multifaceted disease that may be accelerated by the presence of hypertension and hyperlipidemia. The results of experiments on rats suggest that hypertension alone or hyperlipidemia under certain selected conditions can induce atherogenesis. Genetic factors play an important role both in the laboratory rat and in the human being in establishing the serum lipoprotein pattern and lipid concentration. Genetic factors also may be important in determining blood pressure. Studies on the spontaneously hypertensive rat showing the importance of the genetic factor have been described in detail. Lipid and lipoprotein deposition in the arterial wall is accelerated by increased blood pressure, and if the plasma lipid and lipoprotein concentrations are also high, the process is further accelerated. Vascular permeability may be altered by the action of numerous hormones including renin, angiotensin, epinephrine, and norepinephrine, and thus may accelerate the passage of lipids into the arterial wall. Serum lipoprotein concentrations also may be greatly affected by hormonal actions, including that of growth hormone, insulin, various steroids, angiotensin, epinephrine, and norepinephrine, and by physical activity. Quality and quantity of diet, including minerals consumed (especially NaCl), also may play an important role. Neurological factors may affect not only lipid and lipoprotein levels, but also blood pressure.

Evidence that is slowly accumulating suggests that ather-
omatosus lesions may regress if the precipitating factors, including hypertension and hyperlipidemia, are controlled.

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