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Heparin and the Atherosclerotic Process*

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I. Introduction

HEPARIN is probably not a single substance but rather a generic designation for a heterogeneous mixture of highly sulfated glucosaminoglycans with a strong negative surface charge. Heparins are present throughout the animal kingdom (102). In vertebrate species the difference in tissue distribution between heparin and other sulfated glycosaminoglycans suggests a different role for the latter than for heparin (149). There is evidence that heparin-like material has been present throughout much of invertebrate and vertebrate evolution (214). It varies somewhat in different species. Heparins have been classified as acidic mucopolysaccharides, glycosaminoglycans, and most recently as linear polyelectrolytes (210). Heparins complex with specific proteins and dyes, primarily via electrostatic binding forces. Heparin-protein complexes are rather loose at physiologic pH and ionic strength (145), and the bond readily dissociates with changing conditions. When a protein complexes with heparin, conformational changes occur in the charged regions of the protein thus affecting its biologic activity (29). When the heparin-protein bond is broken the original activity of the protein is restored. The interaction between heparins and proteins represents a kinetic equilibrium which is readily altered if another protein or cation is added (208). These reactions with various proteins are the most significant biochemical action of heparins. Jaques (210) has discussed the complexity of heparin activity comprehensively.

The usual heparin preparation is a complex and heterogeneous mixture of polysaccharide chains which vary in molecular weight and degree of sulfation. It is composed of fractions which differ in their reaction with physiologically important proteins such as antithrombin (236, 192, 179, 336). As many as 21 different subfractions have been described in heparin (268). Only 25% to 35% of a given heparin preparation binds tightly to antithrombin and this high-affinity fraction is responsible for 85% to 95% of the anticoagulant activity (236). It is known that the degree of sulfation and the molecular weight affect the anticoagulant activity of heparin (58). There is also a relationship between the anticoagulant potency and the linear charged density of a series of heparin fractions (199). Fractions of the same molecular weight had increased anticoagulant potency associated with increasing anionic density. However, heterogeneity in molecular weight produced larger differences in anti-

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coagulant potency than heterogeneity in anionic density (200). It is also well documented that low molecular weight heparin is more effective in inhibiting the activation of factor X than in thrombin inhibition, while the converse is true for higher molecular weight heparin (9). Oligosaccharides derived from fractionation of heparin that essentially contain the antithrombin III binding region have very high anti-factor X_a activity but minimal antithrombin activity (75). It is obvious that the relationship between the structure of heparin and its effects on hemostasis and coagulation are complex. It has been suggested that the dynamic conformational properties of heparin are more significant than its static conformation (200). Also the carboxyl and sulfate groups of heparin have a somewhat different relative influence on the anticomplement activity of heparin than on its anticoagulant activity. Selective chemical modifications removed the anticoagulant activity while retaining its anticomplement activity (61).

It is probable that the different fractions in heparin account for many of its variable effects. Although there is much to learn about the specific actions and interactions of the fractions of heparin, the parent substance has a wide range of biologic activities. This review will consider how these known actions of heparin may influence the development of the atherosclerotic process. Since atherosclerosis and its complications are the leading cause of death in industrial societies it is unfortunate that the existing evidence about heparin and atherogenesis has been ignored (35).

Before discussing the actions of heparin it is necessary to summarize briefly what is known at present about the etiology of atherosclerosis. It is a multifactorial process involving the following (337):

1. Initial endothelial injury that impairs the endothelial barrier.
2. Platelet adherence to the subendothelium with release of platelet granule constituents including the platelet mitogenic factor.
3. Proliferation of smooth muscle cells that have migrated from the media into the intima.
4. Formation of connective tissue matrix material by the smooth muscle cells.
5. Extracellular and intracellular accumulation of lipid material from the plasma in the smooth muscle cell lesion and in macrophages.

After the initial endothelial injury there is usually overlapping of the subsequent steps in the reaction. Microthrombi may be involved in the atherosclerotic process even at an early stage. As the process advances, thrombus formation at atherosclerotic sites frequently contributes a major clinical role. Thus human atherogenesis involves the reaction of the endothelial and smooth muscle cells of the arterial wall, the actions of platelets and their products, factors contributing to abnormally elevated plasma lipid levels, and aberrations of coagulation that predispose to thrombus formation. Hemodynamic forces also play a role in the localization and acceleration of atherosclerotic lesions. It is interesting that, except for hemodynamic factors, injected heparin affects all of the physiologic systems involved in the atherosclerotic process.

The available evidence that will be presented suggests that the actions of exogenous heparin are related to the functions of endogenous heparin. It is my opinion that small amounts of exogenous heparin essentially act to reinforce the physiologic role of endogenous heparin. It should also be stated that, from the physiologic standpoint, the distinction between heparins, heparans, and heparin-like substances, while important, is only tangential. The key question is heparin activity.

Heparin acts in two areas relevant to atherosclerosis: at the endothelium and within the circulating bloodstream. Since the arterial wall is the disease site, let us first consider the actions of heparin at the endothelium. Injected heparin has an affinity for endothelium. Years ago uptake of heparin at endothelial cell junctions was shown by tissue staining techniques (348). Human and other animal arterial endothelial cell cultures showed metachromatic granules on the cell surface when heparin was added to the culture medium (239, 240). More recently the concentration of exogenous heparin on the endothelium of various species was demonstrated (182, 183). Studies with radiolabelled heparin showed a high binding affinity for endothelial cells even where large amounts of other glycosaminoglycans were present (142). This cell-surface-bound heparin retained its ability to bind antithrombin. However, after release from its binding sites, radioiodinated heparin no longer bound to antithrombin (72). Exogenous heparin taken up by liver sinusoidal and Kupfer cells was apparently not altered (181). It is not known how long injected heparin persists on the endothelium.

Older and more recent findings indicate that endogenous heparin activity is normally present on the endothelial luminal surface. Areas of metachromatic staining, characteristic of heparin and heparin-like substances, were demonstrated on the internal vascular surface of animals and man (412, 390). This metachromatic material had antithrombin cofactor and antithromboplastin-generating activities, properties of heparin (262). The glycosaminoglycans from the intima-media layer of human aorta had antithrombogenic activity (205). An extract from bovine aortic intima contained a heparin-like substance with heparin activity (284). Cultured endothelial cells synthesize and secrete sulfated mucopolysaccharides related to heparin, the bulk of which is apparently heparan (41, 42), which is also a surface component of other cell lines (230). Endothelial cell surface heparan from bovine aorta had several heparin-like features not possessed by standard heparan (132). The question of the presence of heparin itself as well as heparan at the

endothelial surface may be moot from the functional standpoint as X-ray diffraction methods showed that 20% of the heparan molecule is identical to heparin (17). This heparin moiety of heparan is probably the site of the heparin-like activity of heparan, and may well constitute more than 20% of the lower molecular weight and more highly sulfated heparans.

Very recent work more strongly suggests that heparin itself is normally present and functioning at the endothelial cell luminal surface. Cultured bovine aortic endothelial cells produce an inhibitor of smooth muscle cells which was apparently heparin and not heparan (47). Thrombin binds to high-affinity binding sites on endothelial cell surfaces which catalyze the inactivation of the bound thrombin by antithrombin (246). Antithrombin cofactor activity has been detected on endothelial cell surfaces in vitro (305). While heparan and dermatan also bind thrombin, antithrombin bound only to heparin (174). Heparan and dermatan sulfate from human aortas do show anticoagulant activity but it is less than 2% of the activity of heparin by the anti-factor X_a or activated partial thromboplast in time (APTT) tests (375). When polyethylene surfaces were coated with heparin, heparan, or dermatan sulfate, only the heparin-bound surface completely neutralized surface-bound thrombin upon exposure to antithrombin.

II. Endothelial Effects of Heparin

A. Restores Normal Electronegativity

Normal intima has a negative charge which becomes positive after injury (270). A metachromatic substance from mast cells (heparin) functions in the repair of injured endothelial surfaces (271). There is an inverse correlation between the endothelial surface negative charge and vascular injury in vitro (350). The normal negative charge on the surface of glomerular endothelial cells is a charge-based barrier to the passage of macromolecules (225). There is an increased adhesion of polymorphonuclear leucocytes when the negative charge of endothelial cells is decreased (193). The anionic sites on the luminal surface of mouse capillary endothelial cells are localized in differential microdomains and are apparently heparan and/or heparin (357). These anionic sites are distributed over the surface of vascular endothelial cells and are capable of lateral migration. They can be aggregated and depleted by cationized substances leaving most of the endothelial surface temporarily devoid of anionic sites (315, 360). The injection of heparin restores normal electronegativity (366) and normal glomerular filtration after the removal of the anionic sites by protamine (222). These observations indicate that anionic sites contribute to the normal negative charge of the endothelial surface, and that exogenous heparin corrects their depletion and restores normal function. Electrostatic and van der Waal's interactions between cell membranes and extrinsic molecules provide the basis for changes in packing and permeability. Effects at the outermost cell interface, not in the deeper regions of the membrane, affect permeability (356).

B. Prevents Endothelial Injury

Heparin binds and inactivates many of the substances that injure endothelium including histamine, serotonin, lysozymes, bradykinin, angiotensin, bacterial endotoxins, and many toxins (102, 109, 208, 209). Histamine injures endothelium (259, 260). Shear stress increases endothelial histamine synthesis which is accompanied by endothelial cell changes indicative of cellular contraction and increased intimal permeability (190, 334). Short-term cholesterol feeding increased aortic histamine synthesis in rabbit aorta (191). Histamine release may be involved in the increased vascular permeability resulting from immune complex deposition in vascular walls (178). Evidence suggests that de novo histamine formation is a mediator of the increased endothelial permeability which occurs early in the atherosclerotic process (306) and that histamine plays a role in lipid deposition in the plaque (189). Antihistamine drugs have anti-atherosclerotic effects in cholesterol-fed rabbits (173, 188) and antagonists of vasoactive amines suppressed the deposition of circulating immune complexes in vascular walls (60). Besides binding and inactivating histamine, heparin increases circulating histaminase activity (257) and suppresses the increased vascular permeability induced by histamine, bradykinin, and prostaglandin E_1 (46).

Aggregated leucocytes adhere to endothelial cells (206) and can damage them (346). In vivo white blood cell aggregation is associated with extravascular extravasation of plasma proteins suggesting endothelial damage (168). Heparin neutralizes lysosomal cationic proteins released by leucocytes (342) and so may decrease endothelial injury. The cytotoxic effect of eosinophilic major basic protein is decreased by large doses of heparin (126). Extremely small amounts of heparin prevent the endothelial desquamation that is caused by citrate injection (185, 186). Thrombin injures endothelial cells in culture (130, 249). Injected heparin on the endothelial surface would minimize thrombin formation and catalyze its rapid inactivation by antithrombin. This would also prevent the formation of fibrin which itself disorganizes endothelium (276). Fibrin is present in atherosclerotic plaques where it may enhance low-density lipoprotein deposition and smooth muscle cell proliferation (202, 361).

Low-density lipoproteins injure human endothelial cells in tissue culture (116, 176), and very low-density lipoproteins adversely affect bovine aortic endothelial cells (139). Lipoproteins increase platelet adherence to arterial walls and decrease the inhibitory effect of endothelial cells on platelets (13, 297). Hypercholesterolemia inhibited the recovery of prostacyclin production by previously injured rabbit aorta (90). Injected heparin rapidly

reduces the concentration of chylomicrons and very low-density lipoproteins in the bloodstream (153).

Viruses injure endothelium and, when associated with hypercholesterolemia, induce atherosclerosis in experimental animals that closely resembles the human disease (279). Mareks disease herpes virus leads to atherosclerosis in normocholesterolemic chickens (118). Several common viruses (herpes simplex type 1, adenovirus type 7, measles, parainfluenza type 3, mumps, polio type 1, and echovirus type 9) infect and replicate in human endothelial cell cultures causing varying degrees of injury (123). There is a marked increase in platelet adherence to virally injured human vascular endothelial cells (67, 151). Herpes virus infection increased the lipid content of chicken arterial smooth muscle cells (117). Receptors promoting the deposition of immune complexes were induced in human endothelial cells by herpes simplex type 1 virus infection (59). Humans are widely and persistently infected with up to five herpes viruses (118). Heparin inhibits the infectivity of many of the herpes viruses in cell cultures, but not herpes type 2 (383), possibly by interfering with viral absorption to the cell surface (56). Low levels of thrombin accelerate fusion of respiratory syncytial virus-bearing human cells in vitro (78). Bacterial adherence to the inner surface of urinary bladders of rabbits was markedly increased by protamine pretreatment (311). Small amounts of heparin restored the normal protective activity of the bladder mucin layer (170).

Immune vascular injury enhances atherogenesis in experimental animals (124, 278). IgG accumulates in injured endothelial cells and in the atherosclerotic lesions of rabbits (172). Immune complexes activate complement proteins leading to a chain of events ending in tissue injury including endothelial injury (380). However there is no proof, as yet, that immune processes contribute to human atherosclerotic disease although there is much circumstantial evidence to that effect. Circulating immune complexes are frequently found in patients with acute and chronic vascular disease (129, 131). Antibodies against homologous arterial tissue are present in the majority of patients with arteriosclerosis (137), although these findings could be a consequence of the disease. Even if so, such antibodies could then cause further damage. Soluble and tissue-bound immunoglobulins and the terminal component of activated complement have been found in atherosclerotic plaques of humans and rabbits (189, 310). It has been known for years that heparin ameliorates allergic reactions (76, 102, 208). It limits the activation of complement, it binds and inactivates histamine, and it increases circulating histaminase activity which would also decrease the noxious effects of histamine (257). In a study of mammalian species high amounts of heparin were found in lymphoid tissues which suggested that heparin is involved in defense mechanisms either directly or through the immune system (288). Several recent observations suggest that heparin may be helpful in other immune system abnormalities. Small doses of heparin corrected the deficiency of a T cell subset found in many patients with migraine (379). Heparin was mitogenic for human T cells and an activator of murine polyclonal B cells (371). These findings obviously suggest further studies.

C. Mitigates Harmful Effects of Thrombin

Thrombin formation is essential in the coagulation process and in normal hemostasis. However, the vascular damage present even in the early stages of atherosclerosis may initiate the generation of thrombin which, in these circumstances, has harmful effects. Injured endothelial cells have high thromboplastic activity (16) as does human atheroma material (252). Thrombin increases platelet adhesion to vessel walls (115, 297). Platelet adherence to both undamaged or damaged vessel walls was enhanced by prior exposure of the vascular wall to thrombin. Heparin prevented this effect of thrombin whereas drugs acting directly on platelets had much less effect on platelet adherence (307). The adhesion of platelets to endothelial surfaces is dissociable from the platelet release reaction (408). Irreversible platelet aggregation and the platelet release reaction usually are dependent on the generation of thrombin (2, 395). In vivo, platelet aggregates are unstable and disaggregate unless fibrin forms around them (309, 397). In the presence of thrombin even high concentrations of prostacyclin only partially prevented platelet adherence to cultured endothelial cells (127). There is evidence that the generation of prostacyclin by normal endothelium is not the key factor that prevents platelet adherence to the intact vessel wall (67). Platelet-derived growth factor is quickly released by small amounts of thrombin, as are platelet factor 4 and beta-thromboglobulin (403). There also may be a mechanism of thrombus development in which thrombin generation and fibrin formation precede platelet accumulation on the vessel wall (308). There is evidence in man that once an atheromatous plaque has formed it can grow by the accretion of thrombus material. Microthrombi have been identified on the endothelial surface and were incorporated into the thickened intima in areas of developing atheromatous plaque (391). Trace amounts of thrombin markedly suppress plasminogen activator production and release by endothelial cells (247). Thrombin is also a potent mitogenic agent (53). Thrombin is extremely potent in producing gaps in endothelial cell monolayers (237a) and this is prevented by heparin plus antithrombin.

The results of many investigations have shown that the anticoagulant activity of heparin results from its marked acceleration of the inactivation of coagulant serum proteases by antithrombin III (335). Heparin acts as a catalyst in this reaction facilitating the formation of the thrombin-antithrombin complex (157, 254, 323,

362). In the case of thrombin inactivation only a minute amount of heparin is required, 0.005 to 0.01 units per milliliter of blood (253, 363). Thus heparin acts to prevent the generation of thrombin or to facilitate its almost immediate inactivation. A recent comparative study in mammals, birds, reptiles, and amphibians indicated a high degree of evolutionary conservation for antithrombins and heparins and suggested an early origin for this regulatory mechanism of the clotting system (213). There are two binding sites for thrombin on platelets and heparin inhibits high-affinity binding (405). Thrombin-induced platelet aggregation and the subsequent release of platelet products is inhibited by heparin (86, 277, 381, 405). Heparin blocked thrombus formation in injured rat carotid arteries (318). Another heparin cofactor has been described (36, 156) which accelerates the inhibition of thrombin in the presence of heparin but does not inhibit the activation of factor X. Its physiological significance is unknown but it does not appear to function adequately enough to prevent thrombosis in patients with antithrombin III deficiencies (156).

D. Platelet Effects

As in the case of thrombin, the reaction of platelets, which are fundamental in normal hemostasis, contribute to the atherosclerotic process in the presence of repeated endothelial injury. Apart from inhibiting thrombin-mediated platelet aggregation, adhesion, and the release reaction, heparin protects against other harmful platelet effects. Human platelets contain an enzyme capable of degrading endothelial cell surface heparan. Heparin inhibits this degradation even though heparin itself is attacked and neutralized by the enzyme (303, 396). Heparin prevents the binding of released platelet factor 4 to endothelial cells (43). This factor enters endothelial cells after they are injured (147) and so might affect the nonthrombogenic role of the normal endothelium. At least six different proteins that bind to heparin have been identified in human platelets (146). It is probable that, in vivo, aggregated platelets adherent to the vascular wall are the ones that usually undergo the release reaction (403). Heparin minimized the adherence of platelets to endothelial cell junctions (270, 348), injured arterial walls, and artificial shunts (115, 155, 160, 238) although there is contradictory evidence (347). Some findings indicate that heparin has a generalized inhibiting action on platelet-collagen interaction (71). Postheparin plasma corrected postoperative increased platelet adhesiveness (166). In some patients with coronary heart disease, repeated small doses of heparin normalized the enhanced platelet adhesiveness to glass (267). The effect of heparin on platelet-derived growth factor will be discussed later in this paper.

In vitro observations have caused concern about the possible harmful effects of heparin in relation to platelets. Heparin, in a concentration as low as 0.06 units/ml, potentiates ADP- or epinephrine-induced platelet aggregation in citrated platelet-rich plasma (86, 277, 378). Unfractionated heparin, but not its low molecular weight fraction, increases the binding of fibrinogen to ADP-treated platelets (79). The effect of heparin on in vitro collagen-induced platelet aggregation is less clear (381). Both the rate and extent of platelet aggregation initiated by collagen or epinephrine in vitro were reduced in blood samples taken after the injection of heparin (24). Platelet aggregation by heparin is decreased in the presence of antithrombin (244, 347, 355). This would minimize platelet aggregation due to heparin if it occurs in vivo. Recently, no evidence was found of any major platelet aggregation following the intravenous injection of 10,000 units of heparin in man (28). Thus in vitro observations may be misleading about events in the body (54, 299). This is particularly true with citrated plasma in view of the observation that the inhibitory action of heparin on thrombin effects were not observed when citrate was present (2). Nevertheless, mild thrombocytopenia does occur with continued heparin therapy but this apparently is usually harmless, in contrast to the infrequent but severe thrombocytopenia that results from an immunological reaction (57). The bulk of the evidence thus far suggests that heparin-induced platelet aggregation is an in vitro phenomenon seen with citrated plasma, except for the rare immunological event which is poorly understood.

E. Heparin and Prostacyclin

Prostacyclin (PGI_2) is an endothelial prostaglandin which has potent vasodilator and antiplatelet-aggregation effects. Heparin, at the high concentration of 1 to 3 units per milliliter, reversed the inhibition of platelet aggregation by PGI_2 in vitro (23, 255), and by PGE_1 (343). Once again, however, the relevance of in vitro observations to actions in vivo is unclear. Even in vitro evidence was against a direct heparin-PGI_2 interaction (23). Heparin does not inhibit increased PGI_2 production by the vascular wall unless it is the result of thrombin generation (39, 333). Heparin did not block the in vitro coronary vasodilating effect of PGI_2 (89). In fact heparin itself, in minute amounts, relaxed coronary artery strips in vitro (89). In patients with coronary disease the injection of heparin increased the PGI_2 concentration in coronary sinus blood, a beneficial effect that was blocked by aspirin (394). Thus it appears that heparin does not neutralize the biologic activity of PGI_2 in vivo (89). In relation to the heparin inhibition of the PGE_1 antiaggregatory effect, this only occurred in vitro with broken but not with intact platelets, nor during the infusion of heparin in man (8).

F. Inhibition of Smooth Muscle Cell Proliferation

It is accepted that smooth muscle cells (SMC) from the arterial media are the key proliferating cells of the intimal atheromatous lesion. Unlike endothelial cells SMC growth appears to be dependent upon exogenous

growth factors (353). Platelet-derived growth factor (PDGF) plays an important role in SMC proliferation (337). A reduction in platelets markedly inhibits SMC proliferation in rabbits (125). It has been estimated that two-thirds of the growth-potentiating activity of normal human serum upon arterial SMC can be attributed to factors released from platelets (403). Heparin, in low concentration, inhibited the SMC growth-stimulating action of PDGF in vitro, although it did not bind to PDGF (194). Since heparin bound to the surface of SMC these investigators suggested that this binding was involved in the heparin inhibition of PDGF. In vivo studies showed that the antiproliferative effect of heparin amounted to about a 75% reduction in plaque volume (162). Another platelet mitogenic factor, a basic protein that stimulated the growth of mouse cells, was inhibited by small amounts of heparin which apparently complexed with this protein (313). There are no published studies of the effect of heparin on the recently described macrophage growth factor (141, 265).

Other investigations showed that bovine and rat aortic endothelial cells produce both positive and negative effectors of SMC growth (47, 218). The inhibitory activity had many properties characteristic of heparin. In vitro data afforded highly suggestive evidence that it was heparin. Exogenous heparin at 10 ng/ml inhibited SMC growth whereas other glycosaminoglycans including heparan had no inhibitory activity at 10 μg/ml. Other studies indicate that a platelet endoglycosidase may release heparin-like components from aortic endothelial cells (48). The authors suggested that alterations of the normal relationships between platelet endoglycosidases, endothelial cell heparin-like substances and SMC could, in part, be responsible for initiation of the atherosclerotic process. The above findings suggest that exogenous heparin reinforces the physiologic inhibiting influence of endothelial heparin on harmful SMC overproliferation.

G. Inhibits Lipoprotein Uptake by Endothelium

Minute amounts of heparin inhibited the imbibition of serum lipoproteins by human arterial endothelial cells in tissue culture (239, 240). Heparin inhibited the uptake of labelled lipid particles from human serum by 72% after 24 hours incubation. This apparently resulted from a charge effect at the cell membrane surface where metachromatic granules were observed when heparin was in the medium. This repellent action of heparin would apply to low-density lipoproteins which are anionic at physiological pH (351). Heparin was the mucopolysaccharide that most effectively interfered with the uptake of low-density lipoproteins by rabbit aortic tissue (74).

III. Effects of Heparin in the Blood

A. Corrects Hypercoagulability

For years reports have indicated an enhanced tendency to coagulation in many patients with atherosclerotic disease. Newer methods have afforded good evidence that hypercoagulability exists and plays a role in thrombogenesis and perhaps atherogenesis as well. Resistance to heparin was increased by abnormal plasma globulins (143, 187, 404), and by alpha-1-acid glycoprotein (11). The latter is elevated in acute inflammatory and some degenerative diseases, and may interfere with heparin activity by a steric hindrance of the heparin-thrombin interaction (12). Increased serum heparin-neutralizing activity, probably due to released platelet factor 4 which is the most potent inhibitor of heparin anticoagulant activity found in the blood (75), was found in patients with severe coronary heart disease (50, 69, 298).

High levels of heparin-neutralizing activity are present in and released from aggregated platelets of men with coronary disease (243). These factors markedly impair heparin anticoagulant efficacy (169). Markedly higher blood levels of fibrinopeptide A, a very sensitive index of thrombic activity in the blood, were found in patients with ischemic heart disease (289). Even minor respiratory illnesses may be associated with an accelerated heparin thrombin clotting time (300). Increased levels of factor VIII shorten clotting times (80) and raise the requirement for heparin to attain therapeutic anticoagulation. Increased levels of factor VIII were present in adults who had transient ischemic attacks or completed strokes before the age of 55 years (276). Mean levels of factor VII, factor VIIIc, and of fibrinogen were significantly higher in men who subsequently died of cardiovascular disease (275). Survivors of acute myocardial infarction who had recurrences during a 3-year follow-up period had higher fibrinogen levels and an increased maximum fibrin-growth rate as compared to control subjects who remained well (229). The children of men who had died of ischemic heart disease before the age of 45 years had lower antithrombin and higher fibringen levels, indicating an altered hemostatic balance (171). Lipoproteins interfere with the activation of antithrombin III by heparin (30) and hyperlipemia produces a hypercoagulable state in rats (133). Severe hypertriglyceridemia is associated with a hypercoagulable state which becomes normal when triglycerides are lowered (358). Estrogens decrease the activated factor X inhibitory activity of plasma without changing the level of antithrombin III, and so produce a hypercoagulable state that is completely reversed by trace amounts of heparin (400). Activation of the coagulation system increased the size of experimental thrombi in rats whereas platelet activators had no such effect (256). The 31% reduction in the mortality rate of seriously ill noncardiac patients in an intensive care unit who received low-dose heparin therapy probably resulted from the prevention of thromboembolic episodes related to hypercoagulability (164). Diabetic patients are particularly prone to develop atherosclerosis and many articles have reported increased

platelet aggregation in diabetics. There is also hypercoagulability. Insulin-dependent diabetics had evidence of increased activation of the coagulation system with thrombin generation (87). Increased factor VIII levels (266) are present in many diabetic patients. Decreased fibrinogen survival, probably resulting from an abnormal plasma environment, was found in patients with adult-onset diabetes (212). Heparin normalized the fibrinogen survival time whereas the antiplatelet drugs, aspirin plus dipyridamole, did not. Aspirin plus dipyridamole, or sulfinpyrazone, do not correct hypercoagulability (314). There is enhanced activation of the contact phase of the intrinsic coagulation system in diabetics (316).

There is a relation between viscosity and the extent of coronary disease determined by angiography (250). Increased blood viscosity, possibly secondary to a high hematocrit value, is associated with an increased risk of subsequent coronary disease (364). Heparin decreases blood viscosity (114, 340).

In addition to the above, heparin neutralizes many of the cationic proteins released by leucocytes and other sources. These proteins can influence blood coagulation and fibrin precipitation and so have potential significance in thrombosis and atherogenesis (287). Interestingly, the correction of hypercoagulability has often been accomplished with intermittent small doses of heparin, considerably less than those required for full anticoagulation.

B. Enhances Fibrinolysis

Fibrinolysin, or plasmin, activity is a physiologic process that is involved in thrombus dissolution and may relate to atherogenesis. Older studies showed contradictory results about the effect of heparin on this process. However, many observations indicated that polyelectrolytes in general, including heparin, are activators of fibrinolysis and thrombolysis (165). Heparin accelerated the neutralization of plasmin by antithrombin but the concentration of heparin required for this effect was 20 to 30 times that required for full anticoagulation (63). Only 3% to 11% of plasmin formed in vivo was neutralized (254). Thus, it is apparent that the inactivation of fibrinolysis is not a physiologically significant action of heparin. Therapeutic doses of heparin normalized prolonged postoperative clot lysis times (64). Endothelium is rich in plasminogen activator (382). Heparin-enhanced release of plasminogen activator occurred in pigs at heparin concentrations of 0.05 units/ml of perfusion fluid (263). Plasminogen activator release by heparin also has been observed in rabbits (384) and in man (385). Recent studies found that fibrinolysis was increased by heparin added to human blood in vitro and after its injection in vivo (121, 392). Heparin may also enhance fibrinolysis of non-cross-linked fibrin clots via a process that does not involve plasmin (261).

Evidence supporting the role of fibrinolysis in atherogenesis has been fully presented (233). This includes the demonstration of enhanced experimental atheroma formation when fibrinolytic inhibitors were administered, decreased circulating fibrinolytic activity in patients with atherosclerotic disease, and the inhibition of fibrinolytic activity by atherosclerotic risk factors such as increasing age, cigarette smoking, hypercholesterolemia, hypertriglyceridemia, and diabetes mellitus.

C. Inhibits Excessive Complement Activation

The complement system is an effector pathway of the inflammatory response that normally is present in the bloodstream in an inactive form. It is activated specifically by infections and immune complexes or it may be activated nonspecifically (339) by a wide variety of substances (320). Atheroma lipids may activate complement (167) as can high concentrations of membrane cholesterol (7). The consequences of complement activation include stimulation of neutrophiles, increased vascular permeability, and alteration in cell membranes that can lead to lysis and cell death (339). The neutrophile reaction involves aggregation and increased adherence to vascular endothelial cells (269, 354), liposomal enzyme release (148), and endothelial cell damage mediated by oxygen radicals (346). Complement activation also induces the platelet release reaction (20, 317, 359, 410). Complement proteins are rapidly metabolized, which may, in part, reflect their utilization by in vivo activation occurring under normal circumstances (338).

Activation of the complement system involves the sequential interaction of the plasma proteins comprising the system. Since complement activation as part of the normal defense system can potentially produce profound effects on cell membranes, the system has inhibitors. C1 esterase inhibitor is a serum protein which inhibits activation of the first component of complement. Heparin, at very low concentrations, greatly potentiates this inhibiting activity (330). It has been proposed that heparin simultaneously binds to C1 and C1-INH bringing the molecules into close proximity and thus stabilizing and kinetically favoring their interaction (49). The similarity to the catalytic action of heparin in the thrombin-antithrombin reaction is clear. The C1 macromolecule also may directly be inactivated by heparin (278). Purified C1q has two high-affinity binding sites for heparin, and heparin inhibited the ability of C1q to recombine with other C1 components to form hemolytically active C1 (3). Complement activation ultimately involves amplification of the third component of complement (C3) via the formation of an amplification convertase. Heparin inhibits the generation of this amplification convertase (398), and this is independent of antithrombin binding activity (220). C1 inhibitor activation by heparin may also be involved in the inhibition of C3 amplification convertase (49). Heparin also inhibits complement activity at a late stage, just prior to cell lysis (19). The classic pathway of complement activation is progressively inhibited by increasing concentrations of heparin whereas

its effect in the alternative pathway is more complex (245). Heparin augmented the action of control proteins in inhibiting one type of complement activation via the alternative pathway (219). Certain complement proteins bind strongly to heparin, and the authors suggested that this indicated a multifacetted regulatory role for heparin in the complement system (272, 273).

It has been proposed that activated complement is a key factor involved in endothelial injury (135). Rabbits with an inherited defect of the complement system (C6 deficiency) developed significantly less atherosclerosis on an atherogenic diet than controls (136). It is possible that the decreased production of complement proteins by the liver is at least partly responsible for the lack of atherosclerotic disease in chronic alcoholics (134).

There is substantial evidence that complement activation may contribute to myocardial damage (140). Human heart mitochondrial membranes are potent activators of human C1 (321). There is a significant depletion of C1, C4, and C3 within the first 24 hours after acute myocardial infarction in man (320). Inhibition of C3 by cobra venom decreased the extent of myocardial necrosis after coronary occlusion in experimental animals (264). C3 was found localized on swollen myocytes in areas of experimental myocardial infarction in baboons within 4 hours after coronary artery ligation (322).

D. Effect on the Reticuloendothelial System (Macrophages)

The reticuloendothelial system is a major host defense system (344). It monitors the vascular compartment and actively removes bacteria, immune complexes, injured platelets, effete red blood cells, denatured proteins, fibrin aggregates, circulating thromboplastin, tumor cells, and a variety of colloids. Thus macrophages remove some agents that injure endothelium or contribute to hypercoagulability. Plasma fibronectin, also known as opsonic alpha 2 SB glycoprotein or as cold insoluble globulin, plays a role in macrophage phagocytic activity (31, 281, 345). The ingestion of nonbacterial particulate matter and of some bacterial strains by Kupffer cells is augmented by preliminary coating by fibronectin. This action of fibronectin requires the presence of heparin in test systems using rat liver slices (6, 281) or isolated macrophages (32, 386). Human plasma fibronectin binds to heparin (341), as does cellular fibronectin from chick embryo fibroblasts (406). There is evidence that heparin enhances reticuloendothelial function in vivo (217, 235), and that fibronectin participates in this action (217). The authors suggested that endogenous heparin was a cofactor for fibronectin-mediated macrophage phagocytosis, that it might be depleted or inhibited in response to intravascular coagulation, and that this heparin depletion would explain the macrophage depression observed during intravascular coagulation. Heparin restored the antibody-forming capacity in cortisol-suppressed mice apparently by restoration of impaired macrophage function. The authors summarized considerable evidence that heparin beneficially influences the regulatory function of macrophages (211). Macrophages may be essential in the stimulation of T and B cell reactions by polyanions including heparin (5, 77, 81, 82, 293). The formation of pinocytic vessels in mouse macrophages was stimulated by microgram quantities of heparin (62). Heparin is retained by macrophages for a relatively long period apparently without degradation (302). Heparin increased the antileukemia cell activity of macrophages possibly via the involvement of interferon (352). A slight temporary impairment of reticuloendothelial function in rats after larger doses of heparin also has been reported (22).

E. Effect on High-Density Lipoproteins

In the past few years many epidemiologic studies have provided evidence that low levels of high-density lipoproteins are an independent and important risk factor for atherosclerosis, and that above average levels are a protective factor contributing to increased longevity (144). High-density lipoproteins inhibited the injury to human endothelial cells caused by low-density lipoproteins (177). When heparin is injected one of the lipid chages is an absolute increase in the alpha or high-density lipoproteins (33, 291). It was noted (291) that the redistribution of lipids elicited by heparin injection was towards the pattern found in normal subjects without known atherosclerotic disease. The relation of high-density lipoproteins to atherosclerosis, and the increase in the concentration of these particles in the blood after heparin- induced triglyceride lipolysis, fits well with the positive correlation that has been demonstrated between high-density lipoprotein cholesterol and the catabolism of triglyceride-rich lipoproteins (1, 349). Hypertriglyceridemia is frequently associated with abnormalities of the metabolism of the major high-density lipoproteins (325). Studies of human adipose tissue lipoprotein lipase (293) and of postheparin plasma lipases (374) also support the concept that a high rate of catabolism of triglyceride-rich chylomicrons and very low-density lipoproteins via lipoprotein lipase is an important factor contributing to elevated high-density lipoprotein levels.

F. Displaces Lipoprotein Lipase Activity

It is generally believed that normally the bulk of triglyceride lipolysis occurs at the vascular endothelial surface. It has been proposed that the interaction of endothelial lipoprotein lipase with chylomicrons and triglyceride-rich lipoproteins leaves cholesterol-rich remnants (326) and beta lipoprotein in high concentration at the endothelium (409). Rat aortic medial smooth muscle cells showed an enhanced uptake of these remnant particles (26). Injections of heparin mobilize lipoprotein lipase and displace its activity into the circulating blood. A much greater portion of triglyceride lipolysis then takes place in the blood plasma leaving a lower concentration

of the cholesterol-rich remnant particles at the endothelial surface, available for uptake by endothelial cells. If this theory has validity, it would add another way in which injections of heparin might retard atherogenesis.

G. Lowers Serum Triglycerides

The mechanisms involved in the removal of ingested fat from the blood were not understood until it was accidentally observed that heparin abolished postalimentary lipemia in dogs (163). This finding was soon confirmed in other animals and in man (399). The presence of a lipemia-clearing factor in the blood after the injection of heparin was then demonstrated (10). Subsequent studies in rabbits and man showed that after the injection of heparin there was a rapid disappearance of triglyceride-rich lipoproteins from the plasma (153), a loss of neutral fat from the blood (38, 159, 387), an increase in alpha or high-density lipoproteins (33, 291), and a shift in cholesterol from the beta to the alpha lipoproteins (291). These significant changes in serum lipids were explained by the observation that postheparin lipemia clearing factor caused the lipolysis of serum triglycerides into their constituent fatty acids and glycerol (290) which then left the vascular compartment via the carrier protein, albumin (152, 332). The active lipolytic enzyme was extracted from rat heart and named lipoprotein lipase (226). It was also found in some human plasmas without the prior injection of heparin and its identity with postheparin lipoprotein lipase was shown (92). Although other lipolytic enzymes appear in the blood after heparin, many investigations have established that lipoprotein lipase enzymatic activity is the major physiologic pathway for the removal from the blood of both postalimentary and hepatically synthesized triglycerides, transported respectively as chylomicrons and very low-density lipoproteins. It is generally accepted that the enzyme normally functions at the endothelial surface where the bulk of triglyceride lipolysis takes place. However, a small amount of endogenous plasma lipolytic activity was present in over 75% of normal people when ideal methods for its demonstration were used (103, 105). In a few people this circulating lipoprotein lipase activity was substantial enough to account for the removal of the average daily fat intake from the bloodstream (96, 286).

Whether lipoprotein lipase can function without heparin in man remains an unsettled question. Highly purified enzyme obtained from postheparin rat plasma affects the hydrolysis of lipoprotein triglycerides (120). There was no evidence for a direct effect of heparin in the activity of bovine milk lipoprotein lipase with very low-density lipoprotein of rats as a substrate (21). These workers believe that heparin-binding is involved in the attachment of the enzyme to the capillary endothelium rather than in the lipolytic action, and that heparin increases the stability and solubility of lipoprotein lipase. Heparin had only a slight enhancing effect on the lipolytic activity of purified bovine milk lipoprotein lipase (204). The problem is that minute amounts of endogenous heparin may have been present. It has been pointed out (204) that even treatment with heparinase did not completely remove heparin from the purified enzyme. On the other hand, there is much evidence that endogenous heparin normally plays a role in the lipoprotein lipase mechanism. Heparin-neutralizing drugs increase the lipemia of rats and dogs (365), elevate serum lipoproteins in rabbits and rats (34), and increase chylomicronemia (161) and serum triglycerides (104) in man. Lipoprotein lipase is inhibited by heparin-binding agents (92, 103, 105, 226). Bacterial heparinase reduces the activity of chicken adipose tissue lipoprotein lipase (127). Tricalcium phosphate gel, which absorbs heparin, inactivates endogenous plasma lipemia clearing activity (lipoprotein lipase) (93). Circulating heparin-binding immunoglobulins are associated with markedly elevated serum triglyceride levels (143). Lipoprotein lipase is apparently inactivated as triglyceride lipolysis occurs and there is evidence that the inactivation is a result of breaking of the heparin-enzyme bond (97). This bond is probably ionic and reversible (83). Heparin stabilizes the activity of the enzyme and binds it to its substrate (228, 312), the negatively charged groups of heparin probably complexing with the positively charged amino groups of the lipoproteins (203). Exogenous heparin may also increase the net synthesis of lipoprotein lipase by displacing the enzyme into the surrounding medium from the cell surface (51). The action of heparin in facilitating the lipolysis of lipoprotein triglycerides via lipoprotein lipase resembles its role in catalyzing the thrombin-antithrombin reaction.

In general, after a 20,000-unit dose of subcutaneous heparin, it takes 48 to 72 hours for the decreased levels of the larger triglyceride-bearing lipoproteins to revert to their initial values if the diet has remained unaltered (95). Accompanying the reduction in serum triglycerides there is an increased excretion of cholesterol and bile acids in the feces (100). These changes in the circulating lipids have various beneficial effects.

To begin with the reduction in the average level of circulating atherogenic lipid particles results in a decrease of their insudation into the arterial wall. Although the magnitude of this response is hard to quantify, decreased infiltration of cholesterol-bearing lipoproteins would not only slow the atherosclerotic process but might allow mechanisms of regression or repair to operate more efficiently. Maintained lipemia continues to injure endothelium and also adversely affects regeneration of injured endothelial cells (328). Very low-density lipoproteins, which are markedly decreased after an injection of heparin, are the only plasma lipoproteins that accumulate in macrophages and cause them to become foam cells (258).

Lipemia also has harmful effects within the blood itself which would be decreased at lower lipid levels. Increased

agglutination and aggregation of erythrocytes with resultant capillary stasis has been observed after high fat meals (64, 401). The infusion of fat emulsions accelerated coagulation as shown by thrombin generation tests, an action completely prevented by low doses of heparin (37). Human plasma lipoproteins stimulated the activation of prothrombin by factor X_a and shortened the partial thromboplastin time (389). Fats increased the resistance of blood clots to lysis (241). Congenitally hyperlipidemic rats have shorter clotting times and faster formation of experimental aortic thrombi than normal control subjects (223). Platelets are more sensitive to thrombin-induced and ADP-induced aggregation when serum is more lipemic (296, 329). Both saturated and unsaturated fats increase platelet adhesiveness in man, with saturated fats doing so to a greater degree (282). This action of fats is more pronounced in patients with coronary disease (195). The correction of hyperlipidemia in coronary patients normalized their previously shortened platelet survival times (367).

Another harmful effect of increased lipemia is upon tissue oxygen supply. This is an important area which is relevant both to atherogenesis and to myocardial function, but it has been ignored. Before discussing it further it is necessary briefly to consider how the oxygen demand of the arterial wall is met. Oxygen is supplied to the arterial wall from the vascular lumen and from the vasa vasorum. The latter nourish the outer two-thirds of the vessel wall. The intima and inner media are entirely dependent on the diffusion of oxygen from the lumen. In larger animals and in man the avascular wall thickness of large arteries is approximately 1 mm, close to the limiting distance over which oxygen diffusion will adequately supply tissue needs (138). Oxygen tension is lowest in the media with a sharp rise in the tissue just below the normal endothelium (40, 295). Thus the media and its smooth muscle cells are very sensitive to even slight decreases in tissue oxygen tension. In man, due to intimal thickening, the distance over which oxygen must diffuse to reach the media increases with age. In addition, early fatty lesions of atherosclerosis markedly interfere with oxygen diffusion through the intima (180). These anatomic facts implicate the oxygen economy of the arterial wall in the pathogenesis of atherosclerosis.

Does lipemia affect tissue oxygenation? In 1952 it was found that abnormal ballistocardiograms improved after an injection of heparin, and it was suggested that the decrease in serum triglycerides resulted in an increased supply of oxygen to the myocardium (91). This led to studies of total oxygen consumption in patients with coronary heart disease which showed an increase after heparin in those patients (one-half of the total group) whose control levels were below normal (94). The increase in oxygen consumption coincided with maximal lipid clearing. From the opposite standpoint, the induction of angina at peak serum lipemia after high fat meals in ischemic heart disease patients was observed (231). This postlipemic angina was quickly relieved by intravenous heparin (232). Studies of subcutaneous oxygen tension in man (215) showed a decrease coincident with postalimentary lipemia, with a slow rise to normal levels as the serum lipemia spontaneously cleared. After a small dose of intravenous heparin, the lowered skin oxygen tension rapidly rose. In the nonlipemia state heparin had no such effect. Lipemia prevented the usual enhancement of mycardial oxygen extraction after exercise (327). Oxygen availability in the brain tissues of hamsters was decreased after high-fat meals (372). Ear oximeter measurements showed an increase in the rate of oxygen transfer from the blood to the tissues after the injection of heparin in atherosclerotic patients (106). High fat meals caused hypoxic changes in the electrocardiograms of patients with angina, and a decrease in the arteriovenous oxygen difference; both improved after heparin (128). Heparin also corrected the decreased diffusion through pulmonary membranes found in some normal subjects after intravenous lipid emulsions (154). These various studies show that lipemia impairs oxygen diffusion and that this impediment is corrected by heparin.

There is considerable evidence that suggests how serum lipemia interferes with tissue oxygenation but only a brief discussion of the mechanisms involved will be presented. One such mechanism is by the formation of surface films. Such films of fat have been observed on red blood cells after fat ingestion (128). Due to their property of combining with oxygen, fats affect the diffusion constant of oxygen (70). The diffusion of oxygen through plasma was decreased by increasing concentrations of plasma proteins and lipoproteins even over normal physiologic ranges (55). It is also probable that the increased adhesiveness, aggregation, and roulleaux formation of erythrocytes observed after fat meals (66) limits the amount of cell surface available for oxygen diffusion. The increased cholesterol content in the red blood cells of hypercholesterolemic animals occurs primarily in the cell membrane where it may serve as a barrier to oxygen transfer (368).

Many years ago the early evidence supporting anoxemia as a major factor in the genesis of atherosclerosis was reviewed (198). There is more recent evidence that hypoxia accelerates the atherosclerotic process (15, 175). Hypoxia injures endothelium (285), stimulates fibroblast production of mucopolysaccharides and collagen (407), and impairs lipid degradation by smooth muscle cells (4) and so decreases lipid removal from the arterial wall. It increases lipoprotein insudation into the vascular wall which in turn increases the metabolic requirements for oxygen thus aggravating the hypoxia (138). Studies of human endothelial cells in tissue culture indicated that local hypoxia at the cellular level may accelerate atherogenesis by initiating a series of self-sustaining metabolic abnormalities (331). Thus, decreasing the lipemic imped-

iment to oxygen diffusion would improve the oxygen supply to the arterial wall and minimize the many harmful effects of hypoxia. It is probable that this action of heparin is one of its important effects relative to the prophylaxis of atherosclerosis (110).

H. Corrects Deficiency of Plasma Endogenous Heparin Activity

Many early studies with fat tolerance tests showed that there is a delay in the removal of ingested fats from the blood in atherosclerotic patients. Since it is highly probable that heparin plays a normal role in the function of lipoprotein lipase, one of the factors that could contribute to the delay might be an inadequate amount of endogenous heparin activity in the bloodstream or at the endothelial surface. Inhibitors of or interference with heparin could also impair the clearance of fat from the blood.

The normal presence of chemically defined heparin in the human bloodstream has not been proven as yet. It has been shown in rat blood (196). However, extracts have been obtained from normal human plasma which showed the biologic activity of heparin. In 1947, such material was isolated from a large volume of human and horse plasma (14). In 1954 an anticoagulant heparin-like substance was extracted from small quantities of human plasma in two independent laboratories (122, 294). The next year, increased yields were obtained by a new extraction method involving tryptic digestion of the plasma proteins thus freeing protein-bound substances (112). The extracted material showed anticoagulant activity on recalcified sheep plasma; it was metachromatic, moved identically with commercial heparin on paper electrophoresis, and was neutralized by protamine. A later study showed that the extract inhibited the generation of intrinsic plasma thromboplastin (101). It is possible that the plasma extract contained other mucopolysaccharides besides heparin. However, it was quantified by anticoagulant activity and, of the possible contaminants, the one closest to heparin is heparan sulfate which has only a small fraction of the anticoagulant activity of heparin. A small amount of heparan sulfate has been identified in human plasma (45). As noted earlier, x-ray diffraction studies have shown that 20% of the heparan molecule is identifical to heparin (17), and it is probable that the heparin-like activity of heparan resides in its heparin moiety. Also, from the standpoint of physiological function, it matters very little whether the material present in human plasma is heparin, heparan, or both. What is important is that normal human plasma contains biologic heparin activity.

However, some leading investigators doubt that this has been proven. Oddly, even in more recent published studies where circulating heparin activity was not demonstrated, the essential step of freeing heparin from its protein bonds was omitted (207). When one considers that heparin binds to many plasma proteins (272, 376) it is apparent that small quantities of heparin normally would not circulate in the blood as free or unbound heparin. When trypsin was used, endogenous heparin was found in normal dog blood (84). It was also demonstrated in ox blood after tryptic digestion (52). There is evidence that the anticoagulant activity of heparinoid drugs results from their displacement of endogenous circulating heparin from protein to which it is bound (324). Recently, after pronase digestion, a substance was demonstrated in human plasma that competed with commercial heparin for attachment to a protamine-sepharose column (73). Chondroitin sulfates were also present but were eliminated by digestion with chondroitinase ABC. The release of heparin from human platelets adherent to a solid surface, but not in suspension, was also recently shown (388).

Current work in our laboratory has confirmed the presence of endogenous heparin activity in normal human plasma. The material must first be freed from its binding proteins by proteolytic digestion, and then residual interfering peptide fragments removed by adsorption chromatography. The plasma extract prolongs the partial thromboplastin time, binds to human antithrombin, and is absorbed by heparin-binding gel (111).

The anticoagulant activity previously measured in normal human plasma ranged from 0.1 to 0.24 units of heparin per milliliter of plasma (98), a value very close to that obtained earlier (14). This low level of activity is physiologically significant, however, when it is considered that only 0.005 to 0.01 units of heparin per milliliter of blood facilitates thrombin inactivation (253, 363). More relevant to this discussion, however, was the finding of an inverse relationship in man between endogenous plasma heparin and the triglyceride-bearing very low-density (Sf 12-400) lipoproteins (99). The results were statistically significant (P 0.01) but the relatively low correlation coefficient (−0.3) indicated that other factors besides plasma heparin activity affected the lipoprotein levels. It is unfortunate that these findings have not been investigated by others by using reliable methods for the determination of circulating endogenous heparin activity for they are both physiologically and clinically significant. The results indicate that hypertriglyceridemia, with its secondary hypercholesterolemia and accelerated atherogenesis, results, in part, from a deficiency of endogenous intravascular heparin activity, analogous to the insulin deficiency in some patients with diabetes mellitus. A decrease in protamine-binding capacity of the blood in patients with atherosclerotic disease, which was attributed to heparinoid substances, was noted years ago (292). However, protamine neutralization may not be specific for heparin activity. Studies of mast cells, which synthesize and store heparin, in experimental animals have also led to the suggestion that a high susceptibility to atherosclerosis might be related to a deficiency of endogenous heparin supply (65, 370).

Apart from a low supply of endogenous heparin, there might be a relative deficiency in the face of an excessive functional demand or in the presence of inhibitors. Different substances which contribute to hypercoagulability or to hyperlipemia by blocking heparin activity were described earlier in this paper. Thrombin (319), extracts of white blood cells and platelets (119), and tissue extracts (224) inhibit heparin-activated triglyceride lipolysis to some extent. Circulating clotting factors and lipoproteins compete for heparin (184). Heparin in vivo is not irreversibly bound to antithrombin III, and may be mostly bound to other proteins (373). The addition of lipoproteins to a mixture of heparin and serum prevented the activation of antithrombin by heparin (30). Antithrombin functional activity is depressed for several hours after fatty meals without any decrease of the antithrombin level itself (150, 411). There is a negative correlation between plasma antithrombin activity and serum triglycerides which suggest that the latter have an inhibiting effect on the antithrombin III–thrombin reaction, reducing the amount of antithrombin consumed during clotting (402). Low-density lipoproteins, which specifically bind to high molecular weight heparin and form a soluble complex (309), inhibit the action of heparin in catalyzing the inactivation of activated factor X by antithrombin (237), but do not inhibit the heparin enhancing action on the rate of thrombin inactivation by antithrombin (221). This differential effect probably indicates that the binding of heparin to thrombin is strong and so the heparin–low-density lipoprotein complex is dissociable by thrombin, whereas the binding of heparin to activated factor X is weaker and so the latter will not break the heparin–low-density lipoprotein complex. Human histidine-rich glycoprotein, a plasma protein of unknown function, efficiently competes with antithrombin for binding to heparin and so impairs the heparin anticoagulant effect (242). Fibronectin-mediated macrophage phagocytosis, which requires heparin, is depressed during thrombin-induced intravascular coagulation and it is corrected by low doses of heparin (217). The binding of low-density lipoproteins, coagulant proteins, released platelet factors, complement proteins and other normal plasma proteins to heparin (272) clearly suggests that they may compete for available heparin and so impair its functions to some extent unless endogenous heparin supplies are abundant. Injection of exogenous heparin would correct any inadequacy of endogenous heparin production, or supplement its normal functions (107) in the face of excessive demand. This reinforcement of normal function usually is the modus operandi when biologic substances such as insulin or thyroid are used therapeutically.

IV. Conclusions

There is at present much interest in the therapeutic possibilities of fractions of the parent heparin molecules. It may therefore be of some value to assess which of the different effects of heparin are more important in relation to the atherogenic process. Such an assessment is necessarily speculative in the present state of our knowledge. Certainly the reinforcement of normal endothelial function by exogenous heparin, and the protection against endothelial damage by various noxious agents, has substantial value. In view of the role of platelets in atherogenesis and thrombogenesis, the decrease in platelet adhesiveness by heparin and the substantial inhibition of the effect of platelet growth factor on medial smooth muscle cell proliferation should be quite beneficial. The effect of heparin on circulating lipids, the decreased insudation of atherogenic lipoproteins, and the decrease of the hypoxia stemming from hyperlipidemia must be important. The recent prospective studies showing the role of hypercoagulability in thrombotic events indicates that its efficient correction by heparin would be helpful. Finally, if further investigation substantiates that endogenous circulating heparin deficiency is etiologically involved in hyperlipidemia and its harmful consequences, the administration of exogenous heparin should be valuable. The role of heparin in relation to immune vascular damage, and the tantalizing suggestions about its relation to the immune process and macrophage function, require much more investigation before their importance can be evaluated.

It is not the intent of this paper to present a thorough discussion of the results of heparin therapy in the prevention of atherosclerosis. This has been done elsewhere (109, 110). Briefly, however, heparin decreased the extent of atherosclerosis in experimental animals on an atherogenic diet. In patients who have recovered from an acute myocardial infarction, heparin has been used subcutaneously in long-term studies in total doses of 30,000 to 40,000 units per week. Injection schedules varied from 5,000 units daily to 20,000 units twice a week. Except for the first postinfarction year, when the majority of deaths result from severe pump failure or unstable electrical mechanisms, the results have been excellent. There was a 75% reduction in mortality from coronary disease in the heparin-treated patients as compared to control groups thus corroborating the findings of the initial study (113). Side effects have been minimal, and fractures or fatalities due to heparin have not occurred when heparin is given in small intermittent doses even over a 10- to 25-year period. In preinfarction or unstable angina, the use of heparin has also markedly reduced mortality (340, 377). The benefits of heparin therapy were maintained for several months after heparin was stopped (377). In view of these good results with heparin in controlled studies in experimental animals and in man, and the extensive rationale presented in this manuscript, the use of heparin for the prophylaxis and therapy of atherosclerotic disease merits a wider application.

V. Addendum

Heparin increased the rate of development of coronary collateral vessels about 50% in dogs subjected to inter-

mittent occlusion of the circumflex artery, a stimulus known to promote collateralization (121a).

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