Participation of Components of the Blood Coagulation System in the Inflammatory Response

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THE INFLAMMATORY RESPONSE and blood coagulation mechanism, as separate fields of study, have been analyzed continuously for many years. A great deal of information on both phenomena has been acquired through numerous investigations, and yet, no definitive explanation of the mechanisms of these basic biologic reactions has, as yet, been achieved.

In the recent past, several experiments provided evidence indicating that components of the blood coagulation mechanism play a role in certain types of inflammation—the Arthus reaction, arthritis produced by sodium urate crystals and the local Shwartzman reaction—each of which represents a special type of inflammation, although they obviously share mechanisms in common.

It is the purpose of this review to present known points of interdigitation between the blood coagulation mechanism and the inflammatory response and to question the extent to which they can be applied, as generalizations, to all inflammatory reactions. It is conceivable that from the backlog of information in each field, mechanisms now considered to apply exclusively to the clotting system may be found applicable to the inflammatory process and vice versa.

Fibrinogen—Fibrin

Fibrin deposition plays a major role in some inflammatory reactions, whether it is deposited within or outside of blood vessels supplying inflamed tissue. The deposition of fibrin in any location carries with it certain implications. Fibrin formation is the end stage of the blood coagulation mechanism. Thus far two pathways for initiating coagulation have been elucidated: a) the intrinsic system, initiated by surface contact with activation of Hageman Factor and Factor XI and b) the extrinsic system, initiated by tissue thromboplastin which requires Factor VII as an intermediary substance. These two systems converge

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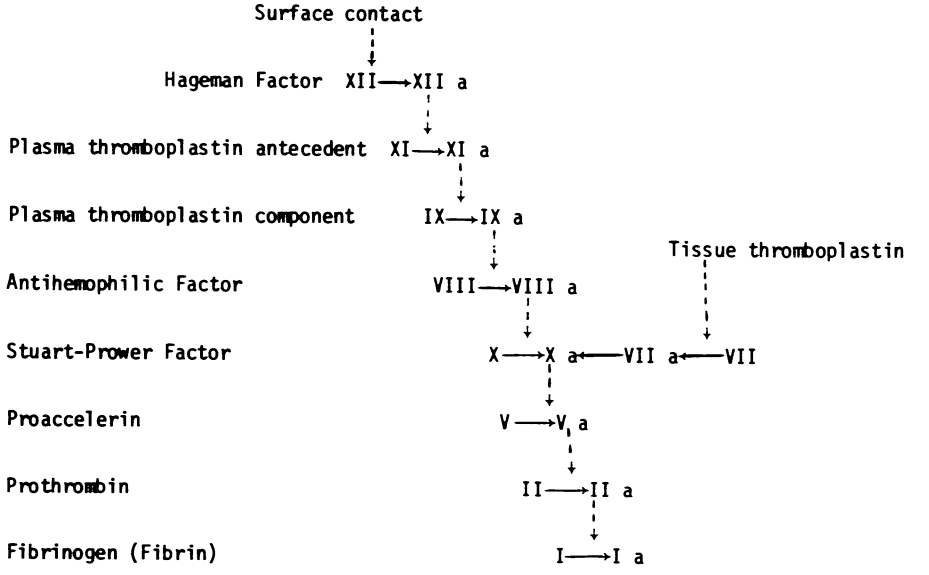
at the point in which Factor X is activated and from thereon the reaction is the same for both (Text-fig 1).

Fibrin deposition in any location implies that certain precursors were present. Factor X (Stuart-Prower), Factor V (proaccelerin), phospholipid (platelet factor 3), Factor II (prothrombin), thrombin and calcium are required in addition to an activator of the intrinsic or extrinsic system. The inactive precursor substances are all present in circulating blood and are readily available for intravascular fibrin deposition. Most often the presence of fibrin outside blood vessels probably is due to blood that has leaked from the vessel into the surrounding tissues.

Inflammation of Connective Tissue

Experimental (intravascular and extravascular fibrin). Two different types of experimentally induced inflammation illustrate the role of fibrin in the inflammatory response.

ACUTE INFLAMMATION PRODUCED BY BACTERIAL ENDOTOXIN: Small doses of lipopolysaccharide (bacterial endotoxin), injected subcutaneously, induce an inflammatory response characterized by: a) increased permeability of the microcirculation and b) leukocyte adherence to the endothelium and migration into connective tissue. As early as 4 hours



TEXT-FIG 1—The essentials of the coagulation cascade based on the concept of Davie and Ratnoff[1] and MacFarlane.[2]

after 0.1 mg of endotoxin (Difco Bacto-Lipopolysaccharide) is injected into rabbit skin, electron microscopy revealed small deposits of fibrin adherent to the capillary endothelium (Figure 2). A few capillaries contain small aggregates of platelets, and small amounts of fibrin can be seen in connective tissue.

Seventeen hours later, the intravascular deposits have become so large that focal thrombi occlude the lumens of capillaries (Figure 3) and the amount of extravascular fibrin is greatly increased. Exposure to bacterial endotoxin represents a nonspecific (nonimmune) inflammatory reaction clearly associated with fibrin deposition within the microcirculation (thrombosis) and outside the vessels (plasma leakage).

The manner in which the intrinsic and/or extrinsic activator systems are involved in this process has not been determined. Although bacterial endotoxin is known to activate Hageman Factor directly,[3] there is reason to suspect that *in vivo* it induces the appearance of an endogenous Hageman Factor activator. Collagen is capable of activating Hageman Factor *in vitro*,[4] but whether it acts on plasma that has leaked from the vessels *in vivo* remains to be determined. Extrinsic prothrombin activator may play a role, since potentially tissue thromboplastin is available from any cell damaged by the inflammatory agent.

THE DIRECT ACTIVE ARTHUS REACTION: A second type of inflammatory response, illustrating immunogenic inflammation, is the direct active Arthus reaction. This reaction takes place when precipitating antibody from the bloodstream and antigen from surrounding connective tissue unite, together with complement, in the vessel walls.[5,6] The early response is an intense polymorphonuclear leukocyte infiltrate and increased vascular permeability followed, at 5 to 6 hours, by grossly visible hemorrhagic necrosis of the region injected with antigen. The hemorrhagic necrosis is caused by thrombosis of the microcirculation. The fact that pretreatment with heparin prevents the thrombosis and hemorrhagic necrosis,[7] indicates that fibrin and the conversion of prothrombin to thrombin are essential to this phase of the reaction.

Although antigen-antibody complex is known to activate Hageman Factor, the same questions that were raised for endotoxin can be raised for antigen-antibody complex with respect to the precise manner in which extrinsic and/or intrinsic prothrombin activators are themselves activated.

For these two categories of inflammation, it is clear that: a) fibrin deposition is part of the inflammatory reaction, b) fibrin deposition occurs *late* in the reaction (4 to 8 hours), c) intravascular fibrin is responsible for ischemic tissue damage, d) extravascular fibrin deposi-

tion occurs in both types of inflammation and is indicative of leakage of blood from the vessels, e) since fibrin formation requires fibrinogen, thrombin, prothrombin, proaccelerin, Stuart-Prower Factor, calcium and either activation of the contact system or tissue thromboplastin, these components of the blood coagulation mechanism participate in the later phase of the inflammatory response.

Clinical–Ischemic Peripheral Corneal Disease. Knowledge that thrombosis or fibrin deposition is part of the inflammatory response is of more than theoretic significance; it has been put to practical use by Aronson *et al.*[8] Twenty-two patients with inflammatory reactions of the cornea, characterized by a progressive wasting away of the peripheral cornea, sometimes leading to perforation, were treated with heparin. The patients were classified as having: a) primary ischemic corneal disease (idiopathic) (including Terriens' senile marginal degeneration and Mooreus' ulcers) and; b) secondary ischemic corneal disease (including infections by *Pseudomonas aeruginosa,* paracolon bacillus and Herpes simplex; chemical inflammation produced by acid and alkali burns; postoperative necrosis and necrosis of corneal grafts). Patients with idiopathic necrosis were treated with heparin alone, while those with a known pathogenetic mechanism (secondary) were treated with adrenal corticoids and antibiotics as well as heparin. Ninety-six percent of the eyes involved improved and 71% improved dramatically; often, vision was restored completely.

Glomerulonephritis (Intravascular and Extravascular Fibrin)

In certain types of inflammation, extravascular fibrin deposition may play a demonstrably significant role in the inflammatory process. In some specific types of glomerulonephritis, both experimental and clincal, the deposition of fibrin appears to be the lethal factor.

Experimental (Nephrotoxic Nephritis)

The experimental model that best demonstrates the possible lethal effects of extravascular fibrin deposition is nephrotoxic nephritis. Antikidney antibody injected into the bloodstream of the animal species from which the kidney antigen was obtained results in glomerulonephritis characterized by: a) swelling of endothelial cells, b) fusion of foot processes of glomerular epithelium, c) glomerular capillary thrombosis, d) extravasation of plasma with fibrin deposition in capsular spaces (Figure 4) and e) eventual crescent formation in the capsular space (Figure 5). Fifty to ninety percent of the animals die of renal insufficiency.

Silfverskiold,[9] Kleinerman [10] and Halpern [11] demonstrated that heparin, administered systemically, reduced proteinuria, hematuria and the mortality rate from 50 to 10%. That this effect was due to the antithrombic (anticoagulant) activity of heparin and not to an "anticomplementary" effect was shown by Vassalli and McCluskey,[12] who successfully reduced the mortality rate by administering the anticoagulant warfarin.

Light and electron microscope studies revealed a significant diminution in glomerular thrombi and in the formation of crescents in these animals (Figure 6). The formation of thrombi, with rupture of capillary vessels, leakage of plasma with fibrin deposition in the capsular space and the subsequent formation of fibrous crescents appear to be the lethal factor in this disease process. The precise mechanism by which fibrin deposition results in crescent formation remains to be elucidated. One possibility is that leakage of plasma into the capsular space, with extravascular fibrin deposition, results in fibroblast proliferation so that fibrin is replaced by connective tissue, in essence, the chain of events in ordinary wound healing.

Clinical (chronic progressive glomerulonephritis)

The beneficial effects of anticoagulants on experimental nephritis led Kincaid-Smith [13] to use anticoagulant therapy in certain forms of human glomerulonephritis. In addition to heparin and phenindione, Kincaid-Smith used an anti platelet-aggregating agent, dipyridamole, in these patients. This agent produced beneficial effects on several severe and/or previously fatal renal disorders, but the disorder most germane to this discussion was chronic progressive glomerulonephritis. In 1 patient, clinical laboratory evidence of renal damage cleared completely after a year's treatment, and histologic examination revealed a return to essentially normal glomeruli. This effect was observed in a disease which, for all practical purposes, is irreversible with the usual modalities of therapy. Also, it demonstrates the important role of platelets and fibrin in this from of glomerular inflammation.

These experimental and clinical observations indicate that platelet damage and fibrin deposition are not only a part of the inflammatory process but, in certain specific diseases, they are the major factor, whether the end-point is local tissue necrosis or mortality.

Hageman Factor

Several recent studies of activated Hageman Factor indicate that it not only plays a role in fibrin deposition, but it also participates, as an integral part, in the early stages of the acute inflammatory response.

Inflammatory Response to Purified Activated Hageman Factor

Perhaps the keystone experiment in implicating Hageman Factor in the inflammatory response was that of Ratnoff and Miles [14] who, in earlier studies,[15] had obtained a highly purified Hageman Factor. Using this material, concentrated 5000 times and activiated by ellagic acid, Ratnoff and Miles injected guinea pigs intracutaneously and observed increased vascular permeability to circulating Pontamine Sky Blue at the site of injection. The reaction was inhibited by soybean trypsin inhibitor.

Subsequently, the influence of activated Hageman Factor on leukocyte sticking and migration was demonstrated by Graham *et al.*[16] Purified activated Hageman Factor (human), injected into the rabbit ear chamber, produced a delayed and prolonged inflammatory response characterized by prominent sticking and emigration of leukocytes. (In its inactive form Hageman Factor had insignificant inflammatory effects). These experiments indicate that activated, purified Hageman Factor is capable of inducing two major components of the local acute inflammatory response—increased vascular permeability and leukocyte migration.

Hageman Factor and Experimental Gouty Arthritis

The fact that activated purified Hageman Factor is capable of producing an inflammatory response experimentally does not necessarily mean that it participates in the inflammatory response caused by various agents. Therefore, Kellermeyer's studies provided an important extension of the theory, since he demonstrated that a known inflammatory agent (monosodium urate crystals) produced gouty arthritis by activating Hageman Factor.

Urate crystals play a primary role in the clinical syndrome of acute gouty arthritis.[17] Crystals of monosodium urate are found in the synovial space of affected joints and acute arthritis is produced by injecting urate crystals intra-articularly.[18]

Kellermeyer and Breckenridge [19] demonstrated that monosodium urate crystals activate Hageman Factor in normal blood plasma; amorphous urates failed to activate Hageman Factor, whereas other crystalline substances, including sodium orotate, hypoxanthine and calcium pyrophosphate, did. All of these substances migrated toward the anode in an electrophoretic field, indicating that the crystals carried a negative charge. The negative charge on the surface of glass is thought to be a major factor in the activation of Hageman Factor.[20]

Subsequently Kellermeyer and Breckenridge [21] showed that Hage-

man Factor (as well as plasma thromboplastin antecedent (Factor XI), predominantly in inactive form (99%), is present in normal synovial fluid.

In a later series of experiments, Kellermeyer [22] demonstrated that: a) urate crystals induce permeability enhancing activity in synovial fluid, b) an anitbody to purified Hageman Factor inhibited the permeability factor, c) ellagic acid could be substituted for urate crystals with the same results, and d) enhanced permeability could be produced in normal plasma but not in Hageman-deficient plasma.

He concluded that the high negative charge on the surface of urate crystals activated Hageman Factor which, in turn, activated the kallikrein system, causing local increased vascular permeability and, thus, gouty arthritis. What remained somewhat equivocal at this point was whether or not the kallikrein system was responsible for the cellular exudate. Kellermeyer suggested that leukocyte chemotaxis might possibly be due to the activation of complement.

Hageman Factor and the Kinin System

The kinin system has long been thought to play a role in the inflammatory response. As Lewis [23] pointed out, this hypothesis is based on the fact that even in small quantities, kinins increase blood flow as well as vascular permeability and induce pain and, possibly, leukocyte emigration. Concentrations of kinins are increased at sites of tissue damage and induce an inflammatory response when injected into the joint space. Kinins are released from kininogen by cellular elements of acute inflammatory reactions and their release is inhibited by anti-inflammatory compounds, such as cortisol and colchicine. Lymph draining a limb damaged by trauma, heat or ischemia contains increased concentrations of kinins.

Although there is little doubt that kinins and some of the precursor enzymes—*ie*, PF-dil, kallikrein—increase vascular permeability, the precise role of the kallikrein system in spontaneous acute inflammation is somewhat problematic. Also, there appears to be some question about the ability of kinin to cause leukocyte sticking and emigration.

Webster [24] pointed out that kinins are liberated into the plasma in one form of acute inflammation of circulating blood—anaphylaxis. Plasma kininogen levels fall [25] when circulating blood is exposed to bacterial endotoxin, another agent causing acute inflammation of circulating blood. Plasma kinin levels are elevated during the first hours after blood is exposed to bacterial endotoxin.[26] This finding constitutes

strong evidence for a role of the kallikrein system in spontaneous acute inflammation.

The first evidence of a relationship between the kallikrein [27] and clotting systems came from Margolis' studies demonstrating the appearance of a factor that increases vascular permeability when human plasma is exposed to a glass surface. This reaction did not take place in Hageman Factor deficient "noncontact" or plasma heated to 60C for 20 minutes. Margolis stated: "although the permeability factor and plasma kinin are not the same, they seem to represent different products of the same chain of reactions."

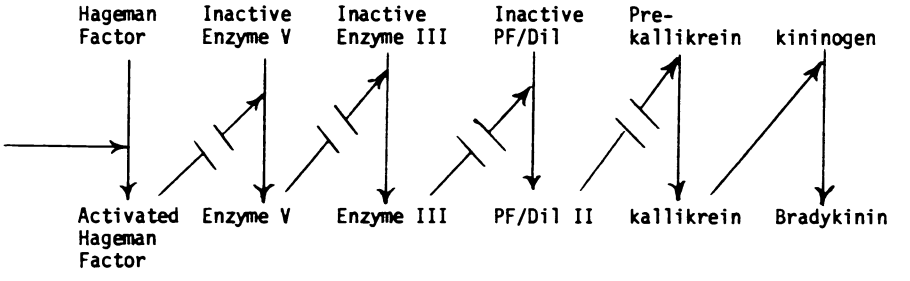
Webster [24] presented the current (1970) working hypothesis of the plasma kallikrein system (Text-fig 2).

Although she cautioned that this concept is subject to revision when further purification and characterization of these enzymes allow a more definitive sequence, there is little question that activated Hageman Factor is capable of activating the kallikrein system.

Activation of Complement by Hageman Factor

The complement system is composed of a chain of nine enzymes which act, in sequence, upon each other and are triggered by the activation of C1 esterase.[28,29] These enzymes can be derived from the α-, β- and γ- globulins of blood plasma and their molecular weights range from 79,000 to 400,000. When the complement system is activated, small molecular fragments are derived. These fragments have been shown to play an important role in certain types of inflammation.

Fragments C3a (MW 7,000) and C5a (MW 10,000 to 15,000) act as *anaphylotoxins* capable of releasing histamine from mast cells, causing smooth muscle contraction, changes in capillary permeability and hypotension. Another major action of these fragments is their ability to cause directed migration of leukocytes into areas of antigen-antibody



TEXT-FIG 2—Current Working Hypothesis of Plasma Kallikrein System.

reactions—*ie*, chemotaxis; C6 and C7 also appear to have chemotactic properties.

The role of complement as a permeability and chemotactic factor was first established for immune inflammatory reactions. Ward and Cochrane [6] prevented the reverse passive Arthus reaction by lowering complement levels in circulating blood. Similar results were observed in experimental glomerulonephritis and serum sickness.[30]

That complement may play a role in nonspecific (nonimmune) inflammation is indicated by the studies of Willoughby *et al.*[31] These investigators lowered complement levels in circulating blood to varying degrees by intravenously injecting heat-aggregated γ-globulin, antigen-antibody complex, antilymphocyte serum, carageenin, antipolymorphonuclear leukocyte serum and anticomplement serum. Using rats and guinea pigs, they induced a skin inflammatory response and quantitated inflammation by measuring the water content of skin 4 hours after injury. A direct relationship existed between the degree of blood complement depletion and the amount of increase in the water content of skin. They concluded that complement plays a significant role in nonallergic as well as allergic acute inflammation.

A connection between the complement system and Hageman Factor was first established by Donaldson.[32] She demonstrated that plasma from patients with hereditary angioneurotic edema lacked an inhibitor of C1 esterase. This lack seems to be the essential defect in these individuals. During clinical attacks of edema, evidence of spontaneous activation of C1 esterase is found in patient plasma.[33] In a later study, Donaldson used ALTEE (N-acetyl-L-tyrosine ethyl ester monohydrate) esterase activity, in the test system. This substance was identified with C1 esterase because it was associated with the development of a property which inactivated C4 and because its activity was reduced by a purified C1 esterase inhibitor.

In this study, purified activated Hageman Factor enhanced the generation of C1 esterase activity in plasma from patients with hereditary angioneurotic edema. Fractionation (purification) of plasma from patients with Hageman Factor-deficiency yielded a protein which failed to activate C1 esterase in hereditary angioneurotic edema plasma, and failed to activate the coagulation system.

The fact that Hageman Factor did not activate C1 esterase in normal plasma was thought to be due to the presence of normal inhibitor.

Two other substances, heparin and soybean trypsin inhibitor do not inhibit C1 esterase, but do have an inhibitory effect on the release of C1 esterase from the plasma of patients with hereditary angioneurotic

edema, when the plasma is exposed to activated Hageman Factor. These substances do not act directly to inhibit Hageman Factor itself. Ratnoff [34] demonstrated that heparin can block the interaction of PTA (Factor XI) and PTC (Factor IX), initiated by activated Hageman Factor. Breckenridge and Ratnoff [35] showed that soybean trypsin inhibitor interferes with activation of Factor V (proaccelerin) by Factor X (Stuart-Prower Factor); soybean trypsin inhibitor and heparin interfere with the release of kinin when plasma is exposed to glass. Thus, these two agents inhibited both the release of kinin and C1 activation under similar conditions. Donaldson concluded that soybean trypsin inhibitor and heparin inhibited some intermediate step in the chain of enzymes in the coagulation sequence.

Polymorphonuclear Leukocytes and Hageman Factor

The migration of polymorphonuclear leukocytes through vessel walls into connective tissue and their subsequent disintegration in the extravascular location is a long established feature of acute inflammation. That this process may be cyclic is suggested by the studies of Hurley [36] who showed that saline extracts of polymorphonuclear leukocytes were capable of inducing leukocyte migration *in vivo*. Concentrations of 50 million cells/ml or higher were required to induce leukocyte migration when the cells were incubated in saline, whereas concentrations as low as 6 million/ml were effective when the cells were incubated with serum. Similar results were obtained using the *in vitro* system—*ie*, leukocyte migration through the millipore filter of the Boyden chamber. Thus, leukocytes can generate leukocyte chemotaxis.

The relationship of polymorphonuclear leukocytes to the kinin system and Hageman Factor has been shown by Melmon and Cline,[37] who demonstrated that normal human granulocytes and eosinophils have both kinin generating and kininase activites *in vitro*. At physiologic pH, these activities were found predominantly in the soluble cell fraction. At pH 5.5, leukocyte granules exhibited a similar property. In these experiments, kinins were generated *in vitro* in normal plasma but not in plasma deficient in Hageman Factor. These findings may have an implication for the *in vivo* inflammatory response and not only suggest that plasma kallikrein is required for leukocyte-mediated release of kinin but that Hageman Factor is also necessary. Although a direct relationship between Hageman Factor and leukocyte migration has not been shown in a similar system, the demonstration by Graham *et al* [16] that activated Hageman Factor produces leukocyte migration *in vivo* is suggestive. It is possible that the perpetuating influence of extra-

vasated polymorphonuclear leukocytes on inflammation may be mediated by their action on Hageman Factor.

Plasmin

Whether or not the plasminogen-plasmin system plays a role in inflammation is problematic. Wherever fibrin is deposited, plasminogen is deposited. The active enzyme, plasmin, is capable of degrading fibrin and has been shown to clear unwanted fibrin from the vascular system. Plasminogen requires an activator, and endogenous activator has been demonstrated by Todd [38] in the vascular endothelium, predominantly in veins and venules. Thus, from the standpoint of anatomy, the proenzyme and activator are potentially available in blood and tissue for the inflammatory response.

Two studies suggest a link between plasmin and the inflammatory response. Ratnoff [39] demonstrated that plasmin was capable of producing increased capillary permeability in the guinea pig skin. Lepow, Ratnoff and Levy [40] showed the effects of plasmin on the early reacting components of the complement system (C1, C4, C2 and C3). Generation of the chemotactic activity was blocked by EACA.

Taylor [41] and Ward [42] have shown that polymorphonuclear leukocyte chemotactic activity can be generated in rabbit serum by plasminogen streptokinase mixtures. In addition, they found that plasmin destroys the biologic activity of the trimolecular complex.

These observations, linking increased capillary permeability, complement activation and cell migration to plasmin, are of great interest, but the precise role of plasmin in inflammation is not clear. Studies of the effects of plasmin inhibitors on the inflammatory response *in vivo* have yielded conflicting results. Weimer [43] found that crystalline soybean trypsin inhibitor, applied to the cornea of rabbits up to 6 hours after injury, prevented not only exudation but most of the subsequent inflammatory response. This is difficult to reconcile with our own observation that soybean trypsin inhibitor not only failed to prevent the inflammatory response in the skin of rabbits injected locally with kaolin or bacterial endotoxin, but that the soybean trypsin inhibitor, by itself, produced an intense exudation of cells and fluid into connective tissue. It is also at odds with the observation by Allison *et al* [44] that soybean trypsin inhibitor, given intravenously, did not modify the reactions to thermal (burn) injury, as seen in the ear chamber of rabbits. They also reported that EACA, another plasmin inhibitor, failed to modify the thermal inflammatory response.

It may be that plasmin plays a role in the inflammatory response, but that it is obscured by more potent agents.

Platelets

Because of their exclusively intravascular existence and important role in blood coagulation, platelets are seldom considered participants in the inflammatory process. Yet, obviously, wherever fibrin is deposited within vessels in any inflammatory response where the intrinsic prothrombin activator system is the trigger mechanism, platelets participate through their required contribution of phospholipid (platelet factor 3).

In a much less obvious manner, platelets participate in at least one type of local inflammation—*ie*, the direct active Arthus reaction. Margaretten [45] has shown that the platelet is absolutely essential for the reaction. He sensitized rabbits with crystalline bovine serum albumin (BSA) and Freund's adjuvant and 6 weeks later challenged them with an intradermal injection of BSA. The animals were killed 8 hours after the challenging injection and the skin sites examined by light microscopy. An antibody to rabbit platelets, prepared in goats, was injected into the test rabbits 30 minutes before and 3½ hours after the challenging intradermal injection of BSA. A virtually complete thrombocytopenia, in the presence of an unchanged leukocyte count, was maintained for the 8-hour period of examination.

Removing the platelets from circulating blood not only prevented thrombosis and hemorrhagic necrosis characteristic of the late phase of the direct active Arthus reaction, but prevented endothelial sticking and the emigration of leukocytes into perivascular connective tissue. Since Ward and Cochrane [6] had shown that the reverse passive Arthus reaction was prevented by removing complement from the circulation and, since platelet antigen-antibody complex in circulating blood could be expected to activate (bind) complement, it was necessary to determine whether or not complement depletion prevented the direct active Arthus reaction. Measurements of C3 revealed that this component of the system was not depleted and, by using the Boyden chamber, it was shown that enough complement remained in rabbit serum to cause migration of leukocytes *in vitro*.

Subsequent studies by Margaretten,[46] using pharmacologic agents to prevent the aggregation and release of platelets confirmed the conclusion that the direct active Arthus reaction was prevented by removing circulating platelets. The intravenous infusion of prostaglandin E and dipyridamole, both of which prevent platelet aggregation, and neither of which is known to affect the complement system, prevented the reaction.

To understand the mechanism of action of platelets in the Arthus reaction, it must be realized that to produce local inflammation, antigen-antibody and complement must be present in perivascular connective tissue. Complement (C5 is probably the major component) is the proximate chemotactic agent for leukocytes, but in this reaction, antigen-antibody complex is required for activation. Since antigen had been injected into the connective tissue, in the thrombocytopenic animals either antibody or complement or both failed to cross the vascular barriers into the connective tissue. Fluorescein labeled γ-globulin was present in perivascular connective tissue in the Arthus reaction but was absent from the injection site in thrombocytopenic animals. This suggests that platelets are necessary for the exudation of γ-globulin from the blood vessel in this reaction. According to Humphrey,[47] thrombocytopenia does not prevent the reverse passive Arthus, indicating that platelets are not necessary for the diffusion of antigen from the blood vessel. The explanation may lie in differences in the molecular size of antigen (approximately 40,000 BSA) and antibody (γ-globulin 160,000) and/or the nature of the action of platelet permeability factor.

Since platelets seemed to be necessary for the diffusion of γ-globulin, they were first thought to act by releasing histamine and/or serotonin. Kniker and Cochrane[48] and Henson and Cochrane[49] showed that in serum sickness, platelets, histamine and serotonin influence the localization of antigen-antibody complexes. However, when histamine and/or serotonin were injected locally or systemically into thrombocytopenic animals they failed to restore the direct active Arthus reaction. However, the systemic infusion of platelets did restore the reaction and it seemed likely that platelets contained some other permeability factor. Mustard[50] demonstrated such a factor. This factor (or factors) which increases vessel permeability passes through a dialysis sac and is heat stable. Fractionation through Diaflo filters indicated permeability activity in substances of molecular weight 10,000 to 20,000, 1000 to 10,000 and in molecules smaller than 1000. Some of the activity in the low molecular-weight fraction may have been due to histamine. The exact nature of platelet permeability factor remains to be determined. It, or something like it, seems to be required for the exudation of γ-globulin in the direct active Arthus reaction.

It may be that platelets have this function only in the special case of the direct active Arthus reaction. The intradermal injection of heat-aggregated γ-globulin in thrombocytopenic animals resulted in a typical acute inflammatory response histologically,[45] indicating that platelets are not essential in this nonspecific inflammatory response. Nevertheless,

to what extent platelets participate in the inflammatory response in a general and nonimmune sense, remains to be determined.

Systemic Activation of the Coagulation Mechanism and Local Acute Inflammation

Depending on when it occurs, an episode of disseminated intravascular coagulation may produce more or less opposite effects on local inflammation. If it develops after local inflammation is established, the inflammatory site may be converted into an area of gangrenous necrosis. If it precedes the local injection of an inflammatory agent, it may completely prevent inflammation.

Hemorrhagic Necrosis—the Local Shwartzman Reaction

The injection of small amounts of bacterial endotoxin into the skin results in a typical nonspecific acute inflammatory response. This reaction will subside within a few days, leaving little or no evidence of its existence. However, if at some time between 6 and 72 hours after the skin injection a second injection is given intravenously, the area of local inflammation is converted into a hemorrhagic necrotic slough.[51] The hemorrhage and necrosis are due to the development of occlusive thrombi in the microcirculation (venules and capillaries) of the local area. This reaction was originally thought to be a "hypersensitivity" phenomenon. However, that it is predominantly a phenomenon related to the blood coagulation system and is not specific for the development or presence of specific antibodies, is indicated by the fact that the intravenous injection of endotoxin can be substituted for by such immunologically inert substances as kaolin, starch, glycogen, carageenin and India ink.[52] All of these substances act by virtue of their ability to agglutinate platelets, activate Hageman Factor and activate α-adrenergic receptor sites.

The conversion of a rather innocuous, transient, acute inflammatory process into a problem of gangrenous ischemic necrosis is a rather striking event. Clearly, it is mediated by a sudden increase in the local thrombotic phenomena initiated by the local injection of endotoxin, but the precise and proximate mechanism of this increase has not yet been elucidated.

The local Shwartzman reaction is only illustrative of this type of response which occurs in a variety of other clinical and experimental circumstances agents other than bacterial endotoxin.

Prevention of the Local Inflammatory Response Associated with Disseminated Intravascular Coagulation

The studies of Jancso[53] provide evidence that changes associated with the prior triggering of the blood coagulation mechanism sys-

temically, will prevent manifestations of a local inflammatory response.

Jancso's end points of observation of the inflammatory response were twofold: a) histologic observation of the deposition of intravenously administered colloidal silver on the endothelium of the microcirculation and in perivascular macrophages at the inflammatory site and; b) edema fluid was quantitated in the rat by measuring the increase in weight of the injected hindpaw. Unfortunately, the emigration of polymorphonuclear lukocytes was not studied. Jancso used a variety of inflammatory agents, including 5-hydroxytryptamine, compound 48/80, Wittes' peptone, dextran, kallikrein, human saliva, staphylococcus culture filtrate, bee venom and a variety of snake venoms.

Jancso believed that some kind of clotting process was involved in the mechanism of inflammation. Specifically, he evolved the theory that the fibrin which formed on the vascular endothelium at the site of inflammation, trapped colloidal silver and increased capillary permeability to fluid. He systemically administered "anticoagulants" and "defibrination" of blood to verify this theory. Although our own interpretation differs from the author's, the observations remain and seem to be of major interest in the light of newer knowledge of the action of the complement system and the blood coagulation system.

Jancso's first observation was that heparin, administered systemically, did not influence the inflammatory reaction. This finding has been confirmed by Allison and Lancaster [54] and constitutes strong evidence against the concept of fibrin deposition as the mechanism involved.

His next experiments involved the systemic administration of agents he classified as "anticoagulants." These agents included Liquoid (sodium polyanetholesulphonate) and the inorganic salts of the rare earths lanthanum, cerium, neodymium, praseodymium and samarium as well as the organic compounds Helodym 88 (didymius salt of β-acetyl propionic acid) and Thrombodym (neodymium salt of sulpho-isonicotinic acid). We now know that these substances, administered intravenously, are not "anticoagulant" but are procoagulant. In large concentrations they produce disseminated intravascular coagulation by activating Hageman Factor and platelet agglutination.

When high doses of these substances were given intravenously before the inflammatory agents were injected locally, evidence of inflammation did not appear. Our interpretation of these observations is that some change associated with disseminated intravascular coagulation prevented the inflammatory response to a wide variety of inflammatory agents.

Jancso's next experiment supports this concept. He induced disseminated intravascular coagulation to the point of "afibrinogenemia"

by infusing thrombin intravenously, which prevented the inflammatory response. With respect to animal experiments, the infusion of thrombin is the most direct and proximate means of producing disseminated intravascular coagulation.

In these experiments, a relationship between the systemic blood coagulation mechanism and the acute local inflammatory response is clearly established. What remains to be determined is the precise mechanism of this relationship and the points of interdigitation. The possibility that the points of overlap lie in activation of Hageman Factor and/or complement, raise a variety of intriguing questions.

As an addendum, Jancso produced local inflammation by injecting a variety of components of the coagulation mechanism, including thrombin, tissue thromboplastin and cephalin as well as Russell's viper venom. At first, this finding might seem to represent additional evidence of a relationship between the clotting mechanism and acute nonspecific inflammation. However, with the exception of thromboplastin, which was obtained from rat brain, all of these substances were heterospecific to the rate—*ie*, the source of thrombin was bovine; cephalin was obtained from pig brain; and the venom from the snake. The inflammation produced may have been an immune type related to heterospecific antibodies. Hence, the inflammation observed cannot be considered conclusive until the same result is obtained with species specific substances. Despite this, tissue thromboplastin can be accepted as a local acute inflammatory agent.

With a different purpose in mind and with a different ultimate interpretation, Willoughby[31] recently repeated the fundamental observations of Jancso. The two studies are mutually confirmative and, taken together, provide additional insight into each other.

With the knowledge that decomplementation of circulating blood prevents the immune inflammation of the indirect passive Arthus reaction, Willoughby raised the question of whether or not complement participated in nonspecific (nonimmune) acute inflammation.

His experimental procedure mainly involved heat as the inflammatory stimulus, but occasionally also turpentine. The end point in his study was the amount of edema at the inflammatory site based on wet weight/dry weight ratios. Complement levels in the circulating blood were reduced by a variety of means including antigen-antibody complexes (*ie*, antibodies to polymorphonuclear leukocytes, lymphocytes), heat-aggregated gamma globulin, antibody to complement and particulate matter (carrageenan).

There was a proportional relationship of the amount of edema and

the percent decrease in complement. His conclusion was that complement is as essential to the evolution of the nonspecific acute inflammatory response as to immune inflammation.

This was a major contribution to an understanding of the mechanism of inflammation. However, the materials and methods used contain other implicit interpretations which relate to Jancso's experiments. The main point is that the agents used to reduce (activate) complement in the circulating blood also produce disseminated intravascular coagulation by activating Hageman Factor and aggregating platelets. It is noteworthy that the greatest reduction in local edema was produced by the particulate matter carrageenan which also caused the greatest reduction in circulating complement. Carrageenan has long been known to activate the coagulation mechanism. Parenthetically, crystals of uric acid represent another particulate substance which has been demonstrated to activate both Hageman Factor and complement.

Complement depletion appears to be the proximate mechanism of preventing the local inflammatory response in Willoughby's and Jancso's experiments. But, since the coagulation mechanism was also triggered in both studies, the role of Hageman Factor or some other enzyme in the coagulation cascade is again questioned. It is possible that introducing these substances into the circulating blood activated complement directly and Hageman Factor simultaneously. It is equally possible that Hageman Factor was the primary target, triggering the complement system.

Basic Biologic Properties Common to Certain Inflammatory Agents

In addition to the specific experiments cited above, the inflammatory response and blood coagulation mechanism are brought close together by certain shared basic biologic properties of inflammatory agents.

Four inflammatory agents—bacterial endotoxin (lipopolysaccharide), antigen-antibody complex (protein + γ-globulin), heat-aggregated γ-globulin and particulate matter (carrageenan, sodium urate crystals)—commonly share the following biologic properties:

1. Produce local acute inflammation [5,19,55,56]
   a) Fluid exudation
   b) Sticking of leukocytes to endothelium and leukocyte emigration (chemotaxis)
2. Activate complement [6,31,57,58]
3. Activate Hageman Factor [3,19,59]
4. Aggregate platelets and release platelet constituents [60–64]

These lists are only partial and, undoubtedly, there will be additions in the future.

At least two ideas are implicit in the above experimental observations:

1. Agents capable of activating the blood coagulation system as well as the inflammatory response provide evidence that the two systems share mechanisms and components in common and;

2. Inflammatory agents, thus far studied, are of such widely divergent chemical composition that their shared biologic activities are not mediated by their chemical components but rather by a property which the surface of the molecules, aggregates and particulate substances have in common. Most likely this is a physical property of their surfaces and provides an interesting example of the interface between a physical force and a chain of biochemical reactions.

Melmon and Cline [26] pointed out other interesting analogies on the subject. They noted that "plasma kinin, complement and intrinsic blood coagulation systems are similar in several ways and share certain key intermediates. Each is a complex system of serum proteins whose activities are latent until activated. Activation of both the intrinsic clotting system and the kinin system is initiated by activation of Hageman Factor. . . . the clotting process proceeds through a complex series of steps in which each clotting factor is sequentially activated and, in turn, acts on the next factor. This series of activities has been called a *waterfall* or *cascade*. The process of complement activation probably occurs by a similar cascade mechanism. The release of kinin appears to follow a similar, if somewhat more restricted, pattern of sequential activation of components. In each of the three systems some of the components are inactivated or depleted after they have fulfilled their function. In each system, a naturally occurring serum inhibitor of activation exists—*ie*, C1 esterase inhibitor, kallikrein inhibitor and . . . [an inhibitor of Hageman Factor]."

It may be that the activation of Hageman Factor is the center of both the blood coagulation system and the inflammatory reaction and that the activation of kinin, complement and coagulation systems are all secondary. The studies of Kellermeyer on sodium urate crystal arthritis provide the best experimental support for the theory, but whether or not this mechanism is operative for inflammation induced by other agents remains to be demonstrated.

Summary

1. Components of the blood clotting mechanism play a major role in certain types of inflammation. Fibrinogen (fibrin) and platelets in

vascular thrombi in the late stages of inflammation result in necrosis, which can be arrested experimentally and clinically by administering anticoagulants and antiplatelet aggregates.

2. Activated Hageman Factor, injected intradermally, produces an inflammatory response.

3. Monosodium urate crystals activate Hageman Factor in joint fluid and produce an acute arthritis which can be prevented by an antibody to Hageman Factor.

4. Hageman Factor activates the kallikrein system.

5. Hageman Factor is capable of activating complement in the plasma of patients with hereditary angioneurotic edema.

6. Platelets are required for the leakage of γ-globulin from the circulation and for the inflammatory response of the direct active Arthus reaction.

7. Plasmin is capable of eliciting increased vascular permeability and of activating a component of the complement system.

Disseminated intravascular coagulation may play a role in acute inflammation in two diametrically opposed ways: a) If it is induced after an acute local inflammation is established, it may increase the damage by increasing thrombotic vascular occlusion at the local site, leading to herorrhagic necrosis. b) If it is induced before the local inflammatory stimulus is applied, even the early phases of inflammation may be prevented. Nonspecific (nonimmune) decomplementation of the blood may be the responsible mechanism.

Such chemically diverse inflammatory agents as bacterial lipopolysaccharides, antigen-antibody complexes, heat-aggregated γ-globulin and a variety of particulate substances share four basic biologic properties—*ie*, production of acute inflammation, activation of complement, activation of Hageman Factor and aggregation of platelets with release of their constituents. These shared biologic properties imply a basic mechanism common for blood coagulation and acute inflammation.

These observations are fragmentary. They often deal with special cases or types of inflammation. Although overlapping is suggestive and proven in special cases, the extent to which they represent generalizations for other types, or for all inflammatory reactions, will require considerably more study.

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[*Illustrations follow*]

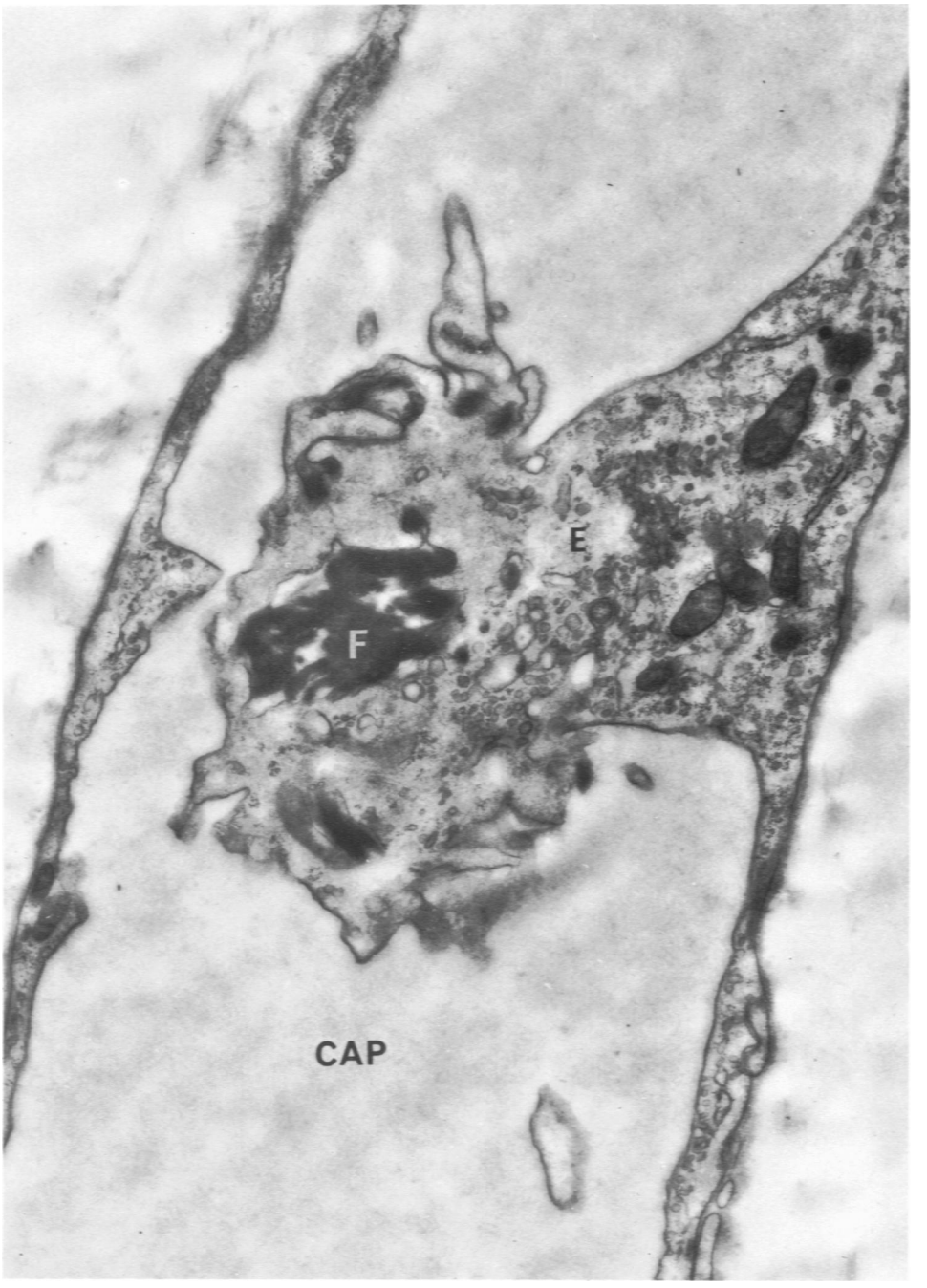

Fig 1—Local site of inflammation 4 hours after intradermal injection of bacterial endotoxin. The endothelial cell of the capillary has formed a pocket by extension of its cytoplasm and has trapped a small amount of fibrin (× 11,200). *CAP* = Capillary lumen; *E* = Endothelial cell; *F* = Fibrin.

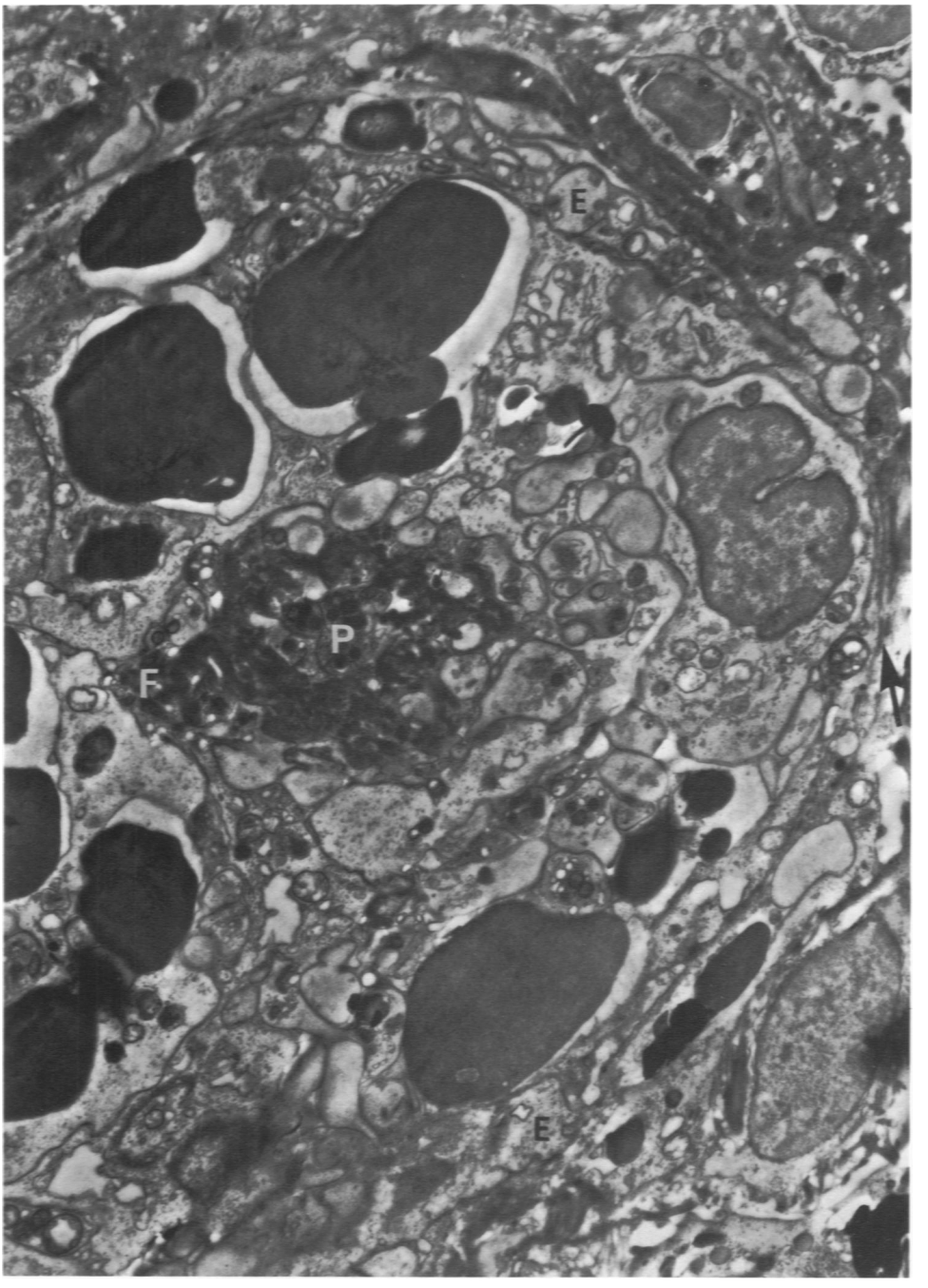

Fig 2—Local site of inflammation 21 hours after intradermal injection of bacterial endotoxin. This capillary is occluded by a thrombus composed of a central nidus of fibrin surrounded by platelets in various stages of degeneration. Red cells are trapped in the thrombus and the endothelium shows evidence of degeneration (× 6000). *E* = Endothelial cells; *F* = Fibrin; *P* = Platelets.

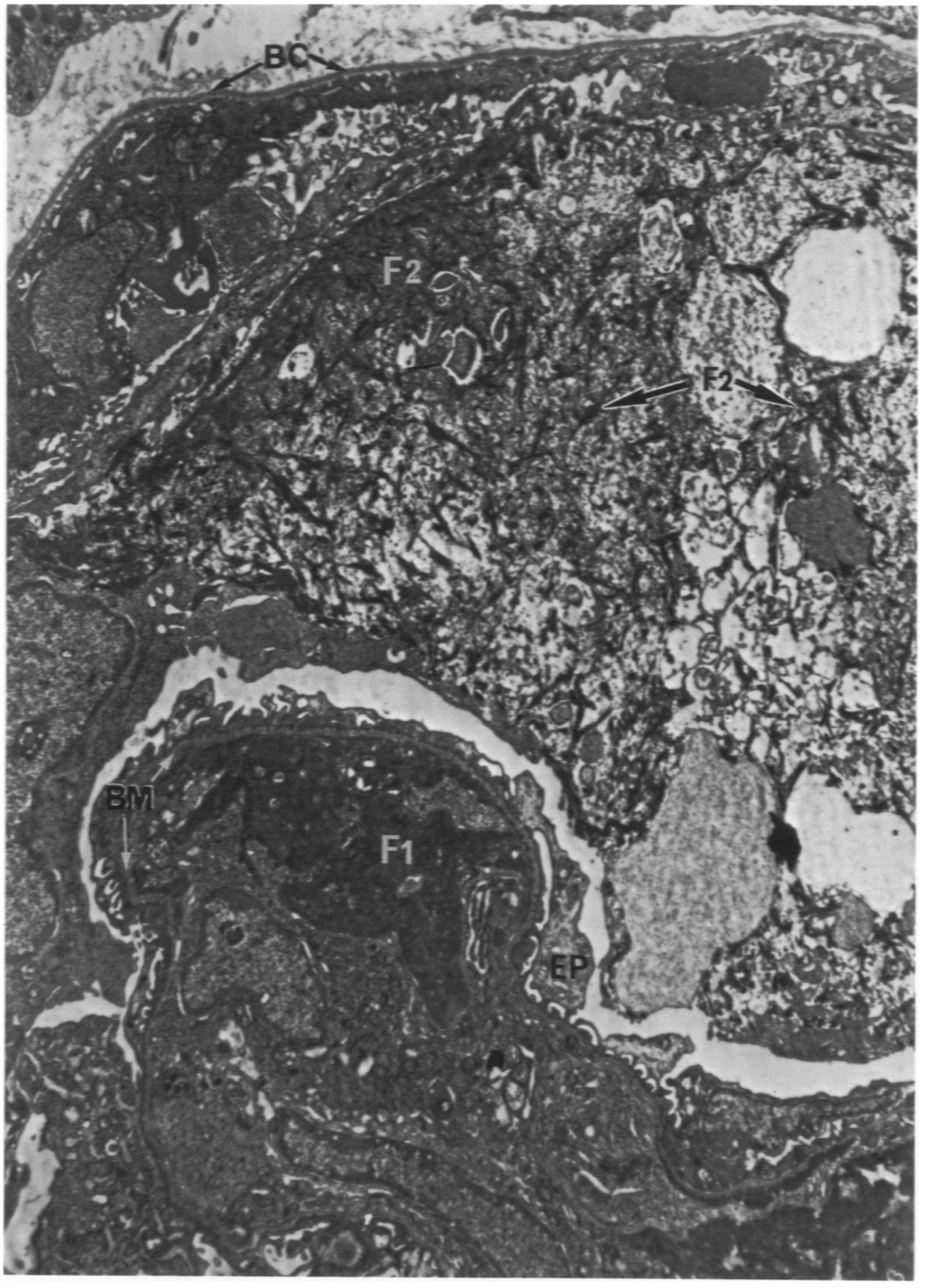

Fig 3—Nephrotoxic nephritis. Glomerulus 8 days after injecting anti-kidney antibody. The capsular space is filled with a loose meshwork of generally fine strands of fibrin (× 4000). F_1 = Fibrin in capillary lumen; F_2 = Fibrin in capsular space; *BC* = Basement membrane of Bowman's capsule; *EP* = Epithelium (visceral); *BM* = Basement membrane (capillary).

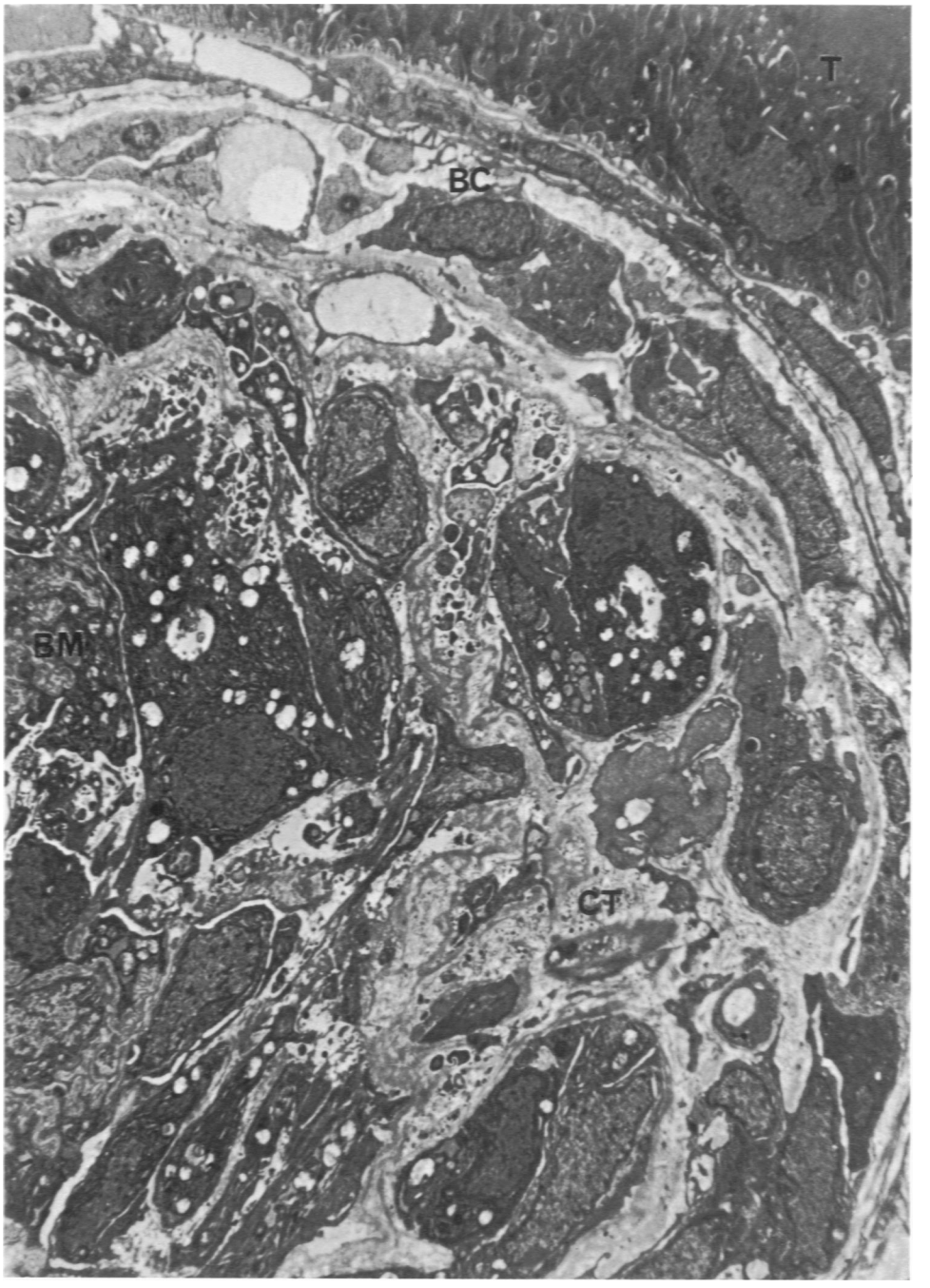

Fig 4—Nephrotoxic nephritis. Glomerulus 18 days after injecting anti-kidney antibody. The capsular space is filled with inflammatory cells, fibroblasts and epithelial cells with strands of collagen and fibrin interspersed (A crescent, × 2800). *T* = Tubular cell; *BC* = Bowman's capsule; *CT* = Connective Tissue; *BM* = Wrinkled collapsed capillary basement membrane.

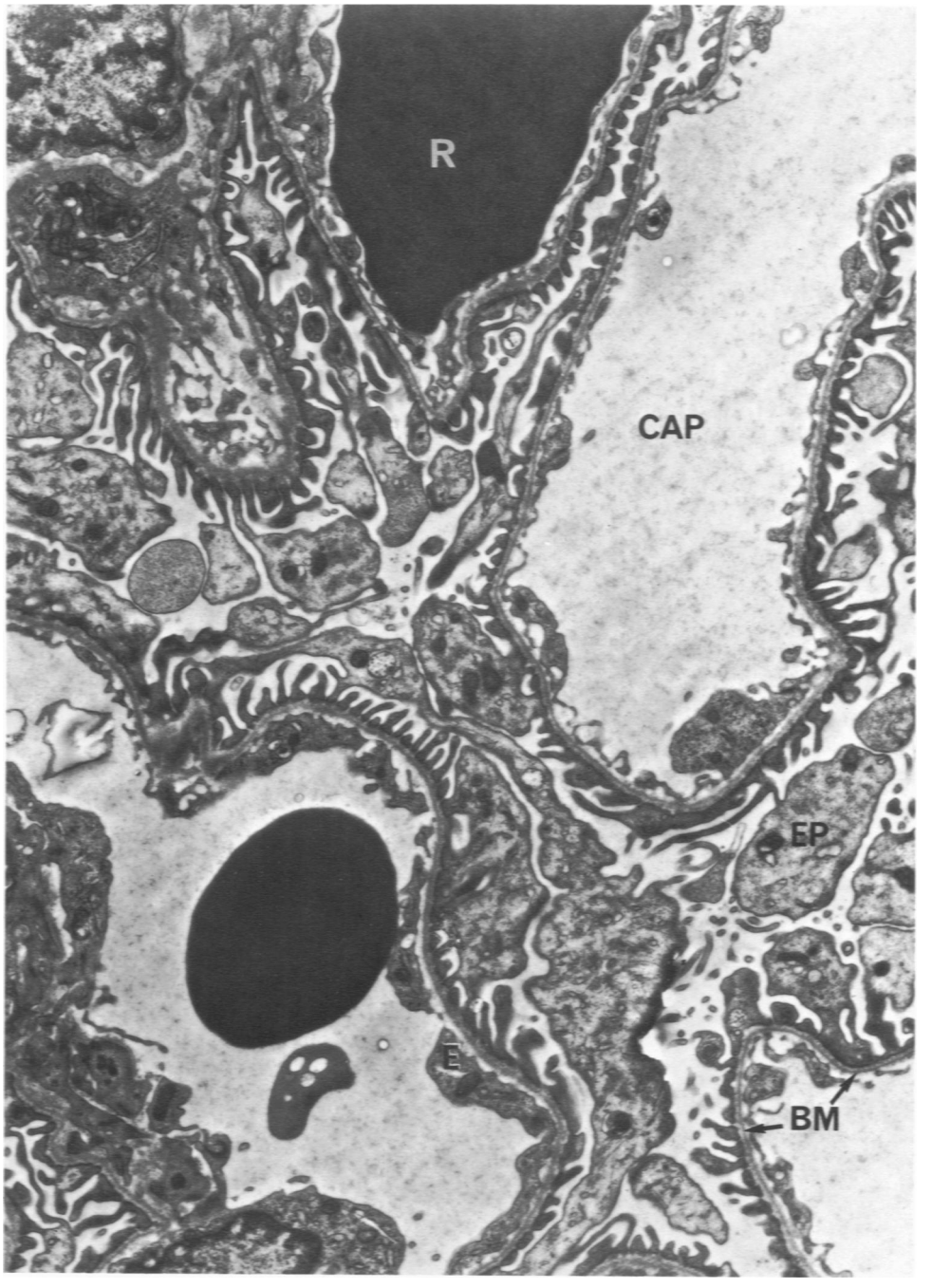

Fig 5—Nephrotoxic nephritis treated with heparin. Glomerulus 8 days after injection of anti-kidney antibody. This is essentially a normal capillary tuft (× 8000). *CAP* = Capillary lumen; *R* = Red blood cell; *EP* = Epithelial cell; *BM* = Capillary basement membrane.

[*End of Article*]