THROMBOSIS AND THROMBOLYSIS

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Collective Review

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OUR UNDERSTANDING of thrombosis and thrombolysis, like that of most other fields of medicine, has increased considerably during the past 2 decades. In addition to the normal interest in the clinical conditions of thrombophlebitis and phlebothrombosis, new impetus has been gained from the many improvements in the field of cardiovascular surgery. Stimuli have also been derived from evidence which suggested to investigators a possible relationship of thrombosis, arteriosclerosis (7, 83), and the collagen diseases (25, 46).

The twofold purpose of this communication is to review some of the literature pertaining to the mechanisms involved in thrombus formation and to discuss the means available for the prevention of thrombosis and the removal of thrombi. At present, it is difficult to evaluate the significance of the thrombolytic mechanisms, but it appears that the use of the enzyme system for the removal of fibrin may open new pathways in the clinical treatment of patients suffering from thromboembolic disorders.

Blood, as a tissue, operates within the confines of the vascular bed only as long as it exists in the liquid state; its characteristic functions depend upon a normal blood flow. Observation of the blood flow under a microscope shows that it occurs in a highly organized fashion. The middle of the blood stream is composed of a broad red current; at the side is a thinner zone consisting of plasma. The plasma zone is broadest in the veins and large capillaries. In these two regions the blood organization is particularly pronounced. In the narrowest veins and capillaries the lumen allows the passage of only single blood cells and no partition between the axial stream and the border zone is observed. At normal blood velocity, no details can be distinguished in the axial stream. White blood cells can be observed here and there in the plasma zone, appearing to roll, as it were, slowly along the vascular wall. Eberth and Schimmelbusch (31), in 1886, gave a mechanical interpretation to this picture of the blood stream when they attributed the distribution of the blood cells in the stream to variations in the specific gravity of the various cellular elements. The heavier cells, they claimed, remained in the axial stream and the lighter ones remained along the borders where the stream flowed more slowly. They observed that when the rate of blood flow was reduced, a considerable number of leukocytes would move from the axial stream toward the border zone, and that when the blood flow was retarded even more, the platelets would arrange themselves along the edges of the vessel. Total stagnation of the blood was accompanied by

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loss of the typical distribution of the formed blood elements.

These findings were subsequently confirmed by Fahraeus in 1929 (32). Fahraeus demonstrated that laminated flow is not the result of differences in the specific gravity of the different elements, as proposed by Schimmelbusch, but is the result of the variant size of the cellular elements. He noted that when the blood velocity is low, or when the suspension stability is upset, for example, in hyperglobulinemia, the erythrocytes tend to form large aggregates which, because of their size, force the leukocytes into the border zone.

In order to understand how the flow of the cellular elements is related to the problem of thrombosis, one must first define thrombosis.[1] Thrombosis is the localized conversion of blood from the liquid into the solid state. More than a century ago, it was realized that coagulation was somehow involved in the process of thrombus formation. Since 1854, the year in which Virchow (110) wrote his often quoted work on thrombosis, opinions as to the importance of blood coagulation in relation to the formation of thrombi have varied between two extremes. On one hand, thrombosis, as viewed by Virchow, was looked upon merely as coagulation of blood. On the other, thrombosis and coagulation were considered essentially different processes. The latter viewpoint predominated during the end of the nineteenth century and the first decades of the twentieth century, as a result of Aschoff's (4) investigation.

In the last 2 decades, however, a complete reversal has taken place as a result of the development of the various anticoagulatory agents which can prevent clotting as well as the further extension of a thrombus. The last development has led to our current understanding of thrombosis as a process which is closely akin to blood coagulation.

Through the recent work of Knisely (62), the significance of Aschoff's contribution to the problem has been re-evaluated. This investigator has demonstrated that, in addition to clotting, the blood flow rate and the suspension stability of the various cellular elements of the blood play a significant role in the causation of thrombosis.

One cannot discuss thrombosis or try to evaluate the cause of this condition without a discussion of Aschoff's work on the subject. Aschoff (3) stated that, as a rule, a thrombus is made up of three parts: the head or white thrombus; the neck or mixed thrombus; and the tail or red thrombus. When these different sections are examined histologically, one can differentiate in the head area a particular structure consisting of parallel beams which are so arranged that the whole has been compared to a colony of corals. The beams are made up of an aggregate of platelets with leukocytes along the free edges and in the interstices.

The predominance of platelets and leukocytes, and the absence of red cells, in the part of the thrombus which is formed first, made Aschoff adopt the concept, which he subsequently tried to prove through a number of ingenious in vitro experiments, that these cellular elements of blood somehow initiate the formation of a thrombus.

HEMODYNAMIC FACTORS

There is no question that the cellular elements as well as the clotting factors play a role in the genesis of thrombosis. As Bergquist (13) pointed out, the cellular elements of the blood play a role in thrombus formation, especially when the blood velocity decreases. Since thrombi will form more readily when the blood flow is slow than when it is rapid, stasis of blood promotes thrombosis. Zahn, in 1875, was the first to point out this fact, a point recently re-emphasized by Knisely who has developed further the concept of stasis.

Knisely and his co-workers (62) emphasized that "sludging" is associated with the settling out of cell masses from the blood stream when blood flow rates diminish. As they demonstrated, there exists in vessels a critical velocity or forward flow at which masses of a given size, such as blood cells, remain in suspension. How-

[1]Health, as conceived by the ancient Greeks, depended upon the normal mixture of the four fluids which form the contents of the vascular system. These were: serum or yellow bile—cholera, the blood cells of black bile—melancholia, fluid blood—sanguis, and fibrin—phlegma. An increase in the content of any one of these four components was thought to cause disease. Looked upon as the most important cause, however, was the accumulation of phlegma. This was based on the observation that the fibrinous layer which covers blood drawn from the sick is quantitatively increased when compared to that of normal persons. Furthermore, the Greeks seem to have known that coagulation, particularly after hemorrhages into the tissues, depended upon fibrin. It was thought, therefore, that disease was due to an increase in the intravascular quantity of fibrin, a view supported by the finding of extensive fibrin coagula at autopsy in the heart and vessels of people dying from diseases. These fibrin coagula were absent in some cases of violent death. The process of coagulum formation was referred to as θρόμβος, the Greek word from which the term "thrombosis" is derived.

ever, if the flow rate becomes less than the critical velocity, the masses begin to settle out. Factors, such as spasm, degree of angulation of the vessel, stickiness of the cells, and many others, determine the ease with which "sludging" will occur.

Changes associated with an extensive sludging of blood have been observed under various clinical conditions. The investigations of malaria, radiant heat, crush, and extensive burn injuries, spasm, and shock by Knisely and his co-workers (61); Ditzel's (24) work on diabetes; Gelin's (41) work with high molecular dextran, thrombin, and different types of experimental trauma; Marmont's (72) work on cryoglobulinemia; experimental evidence obtained by Long (68) on sludging during artificial perfusions or that of the sludging which results from injection of hypertonic solutions observed by Bernstein (14) —all of these have again demonstrated the importance of the rate of blood flow in the formation of thrombosis.

In spite of these considerations, we must point out that stasis of blood alone does not result in thrombosis as readily as could be imagined. This is clear when one considers a classical experiment which John Hunter (53) performed as early as 1784. Hunter found that blood remains in the liquid state for a long period of time in a doubly ligated vein. However, if the vascular wall is injured, rapid thrombosis takes place.

It was Frykholm (37) who explained why stasis and sludging will eventually result in thrombus formation. The intimal cells of veins and capillaries depend, in part, on the passing blood for their nutrition. Inadequate blood flow, as in cardiac disease, trauma, shock, anemia, or bed rest, results in intimal damage. This injury is thought to be the result of hypoxia and leads to thrombus formation.

VASCULAR FACTORS

Physical factors have been recognized which, under normal conditions, aid in maintaining the liquid state of blood. Sawyer and his associates (95) have demonstrated that the normal intima of vessels is negatively charged and, therefore, repels the similarly charged formed elements. Plasma proteins are also negatively charged. Damage of the intima results in the reversal of this charge with the elimination of the normal repellent force which exists between plasma, blood cells, and intima. Both the platelets and the large size protein constituents of plasma, such as fibrinogen, are then attracted to the site of injury.

In addition to the difference in the electric potential, a second physical concept, namely, the wettability of the vessel wall, has been introduced into vascular physiology. As is generally known, platelets that have been collected in glass tubes will stick to the glass wall. By lowering the surface wettability, i.e., with silicone, Jaques and his associates (55) demonstrated a decrease in the adhesive tendency of the platelets. Moolten and his colleagues (77) demonstrated the changes in wettability of the vascular endothelium of rabbits after cessation of blood flow. Immediately after the rabbit was killed, the mesentery was exposed and a small air bubble was introduced into the vessels. As a result of changes in the wettability of the vascular wall, a gradual reshaping of the air bubble was observed when the plasma forced its way between the wall and the air bubble. They were able to demonstrate that as the wettability of the intima increased, the tendency of the platelets to adhere to that wettable surface also became greater.

Bizzozero (16) showed in 1882 that injury to the vascular wall, whatever its cause, will attract the platelets, a finding more extensively investigated by Fonio and Schwendener (36) with the aid of dark field microscopy. Fonio demonstrated that the platelets appear rounded in states of rest and show numerous offshoots in states of excitation. Moreover, he found that the platelets co-operate in the initiation of intravascular coagulation. In thromboplastic excitation, e.g., in vascular damage, the platelets send out protoplasmic offshoots which can adhere to the vessel wall and other cells. Aggregates of cells are formed in this way. Fonio photographed the sequence of thrombus formation and was able to show that the platelets stick to the injured site. More and more platelets agglutinate; some of them lyse. This process is associated with the concomitant appearance of fibrin. As the platelets continue to accumulate, the circulation slows down and final blocking of the vessel is associated with sludging. These platelets form the white thrombus, behind which form both the mixed and the red thrombus.

CELLULAR COMPONENTS

From this brief review of some of the etiologic factors in thrombus formation, it appears that the intricacies of thrombosis cannot be fully

understood simply by reference to coagulation; proper attention must also be paid to the role enacted by the cellular elements of the blood. Let us, therefore, summarize the role of the different cellular components in thrombus formation.

First, as mentioned, there are the platelets. Changes in the number of platelets as well as in their quality have been implicated as a cause of thrombosis. Statistically, an increased incidence of thrombosis has been observed under various conditions, such as splenectomy and polycythemia vera, which are associated with an increase in the number of platelets. A similar sequence is observed in thrombosis after stress, operation, and delivery, in patients with hyperadrenalism, or after cortisone or ACTH therapy (19, 108).

A great number of investigators have found that neither surgery nor delivery changes the platelet count remarkably unless extensive clotting or mechanical damage to the platelets has occurred. However, there is an increase in the number of platelets, with the maximum rise occurring on the fifth to seventh postoperative days. Helen Wright (113) noted an increase in platelet adhesiveness during this postoperative period. Heparin reverses this increased adhesiveness. It is interesting to note that some investigators, notably Kristenson (63), have attached clinical significance to this rise. He advised performing platelet counts routinely after operation. A decrease in the number of circulation platelets, or even no increase, is considered to be an indication of thrombus formation.

What is the relationship between the platelets and the increased tendency for thrombus formation in the immediate postoperative and postpartum period? Platelets lose suspension stability relatively easily, a point which was first emphasized by Osler (82) in 1882. Agglutination of platelets invariably results. In addition to the significance of the blood flow rate, other factors can be distinguished which may cause the noted effect. Thrombin, for instance, will activate the component in plasma which promotes the viscous metamorphosis of platelets, as demonstrated by Wright and Minot (114). An increased tendency of the platelets to agglutinate, a tendency associated with intravascular formation of platelet thrombi, has been described in peptone shock and after the injection of various colloids (10, 20). Similarly, masses of agglutinated platelets occlude small vessels of many organs in thrombocytopenic purpura, anaphylactic shock (84), and endotoxin shock (22). Under these conditions anticoagulants such as hirudin or heparin (22, 91) are unable to prevent the agglutination. At other times, however, under the influence of anticoagulants (113), the increased agglutination tendency is reversed.

There has been much speculation concerning the mechanism responsible for the changes in the suspension stability of the platelets. Intact platelets have been found to be negatively charged; their isoelectric point appears to be lower than that of globulin, but higher than that of albumin. Platelets, as demonstrated by Starlinger and Sametnik (101), have a tendency to agglutinate in blood with a high globulin or fibrinogen content. It has been reasoned that plasma changes, which include an increase in electronegative proteins, will partially cancel the negative charge on the platelet surface and will thus reduce the mutual repulsion which exists between them. Consequently, as electronegative charge decreases, the agglutination tendency of the platelets increases.

The possible role of the intact red cell in thrombus formation was evaluated by Fahraeus (32). This investigator found that erythrocytes, like platelets, are negatively charged. The suspension stability of the red cells is the result of the mutual repulsion of similarly charged cells. Loss of suspension stability of these cells is characterized by clumping and precipitation. This phenomenon was studied microscopically and physicochemically. He noted that aggregation of red cells occurs normally and that the increased aggregation of erythrocytes in disease seems to be only an exaggeration of a physiologic phenomenon. The increased aggregation, he found, depended not upon the cells, but upon the hydrophilic plasma proteins. Alterations in their concentrations or properties, as during pregnancy and disease, elicited an increase in aggregation tendency.

Loss of suspension stability could be experimentally produced with high concentrations of fibrinogen and globulin; a maximum suspension stability was observed with high concentrations of albumin. The loss of suspension stability resulted in the formation of large aggregates of cells, which were able to occlude capillaries, venules, and small arterioles. Stasis of blood in these vessels became readily apparent biomicro-

scopically, under conditions which altered the suspension stability of the erythrocytes.

Hemolysis of red cells can also induce thrombosis. Naunyn (80) in 1873 observed that intravascular, intracardiac, and intrapulmonary coagulation occurs after the injection of hemolytic substances. Recently, Leupold (65)—simultaneously with Hussey (54) and Georgatsos, Hussey, and Quick (44)—described a factor present in the stroma of erythrocytes which has been named "erythrocytin." It appears that this erythrocytin exerts a strong thromboplastin effect, an effect which may be brought about through its high phospholipid content. Hemolytic states resulting from a number of immunologic conditions, such as incompatible blood transfusion or cold agglutinins and hemolytic crisis in sickle cell anemia or other hemolytic anemias, are invariably associated with intravascular coagulation. Many of the acute symptoms during the hemolytic crisis can be attributed to thrombosis of blood vessels and the resulting infarction of parts of internal organs.

The leukocytes have also been implicated in thrombus formation. Changes in the number and in the adhesive properties of white cells have been studied extensively since Hankin's (51) work in 1892. This investigator noted that as soon as the blood leaves the vascular system, the leukocytes become highly viscous. Von Philipsborn (86) in 1930 developed a technique which enabled him and others to measure this adhesive tendency. Thoma (104) found that the adhesive tendency was a function of the amebic movements of the cell. Inhibition of the movements resulted in inhibition of the adhesive tendency as well. Vejlens (109), using this system, found that increase in fibrinogen concentration enhanced the adhesive tendency of the white cells.

A similar relationship was found between the adhesive property of the white cells and the sedimentation rate. These changes have been related to thrombus formation.

PLASMA FACTORS

From the preceding discussion, it should be clear that the blood cells play a significant role in the initiation and genesis of a thrombus. The fact remains that the formation or extension of thrombus can be effectively prevented by pretreatment with anticoagulants. Therefore, the general rule, "without coagulation no thrombus formation," still holds true.[2] The transformation of blood from the liquid into the solid state is the result of the conversion of fibrinogen into fibrin. With solidification of the plasma, the cells agglutinate and become incorporated in the thrombus.

Fibrin formation represents the end stage of a chain of events, the details of which have puzzled investigators of coagulation for more than a century. Without entering into any controversies, it should be pointed out that a number of hypercoagulability states have been delineated, conditions which seem to predispose the patient to intravascular thrombosis. The thrombosis is the result of the presence of thromboplastic or thrombin-like substances in the bloodstream.

The direct intravascular administration of any of these substances in sufficient amounts over a short period of time will result in thrombosis. When the same substance is administered slowly, or in low concentration, no thrombosis will take place, although the fibrinogen concentration will be found to be markedly reduced (91).

In a number of conditions, substances which presumably contain thromboplastin, which induces intravascular coagulation and thrombus formation, have been found circulating in the blood. Obstetric conditions, such as eclampsia, dead fetus in utero, and the severe manifestations seen after abruptio placentae and amniotic fluid embolism, are all thought to be associated with intravascular clotting or pulmonary embolism by Schneider (97). Low fibrinogen levels are invariably found in these conditions. Other agents known to induce coagulation with subsequent thrombus formation are snake venom (30) and coagulase (52).

Thrombin and thrombin-like substances, such as trypsin and papaine, will result in intravascular thrombosis when introduced rapidly into the blood stream. The extensive thrombosis of the pancreatic veins in acute hemorrhagic pancreatitis reflects the possible result of trypsin activity.

It is interesting to note that in many of these conditions, intravascular clotting and thrombo-

[2]Fibrin formation is absent in lower animals, such as the horsecrab. The blood of these animals does clot, however. Upon agglutination, the amebocytes, which form the analogue of the platelets in these lower animals, disintegrate, a condition which is associated with gelation of the blood (98). Only when the blood pressure becomes sufficiently elevated to make this more primitive hemostatic mechanism inadequate, as noted by Quick, does the fibrin clot make its appearance phylogenetically. That clotting does occur in humans in the absence of fibrinogen is apparent from Pinniger and Prunty's report (87), in which they described the formation of white thrombi in a patient with congenital afibrinogenemia.

sis are concurrent with an increased bleeding tendency. It is well known that the first symptom of an incompatible blood transfusion is a markedly increased bleeding tendency, whereas the underlying intravascular clotting which is associated with consumption of platelets and various clotting factors remains obscure.

Dextran sulfate and several other acid polysaccharides are also known to induce intravascular coagulation (111). A material, identified as "fibrinoid," has been found to occlude the vessels after their injection (111). It is quite similar in appearance to the material identified by immunohistochemical methods as fibrin. This material has been described in the generalized Shwartzman phenomenon, thrombotic thrombocytopenic purpura, and the bilateral renal cortical necrosis accompanying premature separation of the placenta (25).

Three different mechanisms are known to operate in the removal of thrombin. Fibrin, as demonstrated by Quick, will rapidly absorb large quantities of thrombin. Antithrombin, described by Lenggenhager (64), consists of a plasma albumin fraction which slowly combines with and inactivates thrombin. Finally, there is heparin. Heparin, however, does not seem to play a significant role in human physiology. Heparin levels have not been known to rise in states of hypercoagulability or in conditions which, in animals, are associated with release of this substance (56).

In groups of postoperative and postpartum patients, approximately 10 to 20 per cent were found to have a marked decrease in the antithrombin activity of the plasma (13, 64). This suggests that a possible relationship between the deficiency of this factor and the tendency for postoperative thrombosis formation may, at times, be operative.

Some conditions predispose the patient to thrombosis. As Trousseau (106) pointed out more than a century ago, patients with malignant lesions—especially those with carcinoma of the pancreas—frequently have a migrating type of thrombophlebitis, which may persist in spite of adequate anticoagulant therapy. The exact nature of the condition is unknown.

HEREDITARY FACTORS

There remain those cases of recurrent thrombophlebitis or phlebothrombosis in which there is an apparent hereditary predisposition to the disease. These are the patients whose parents or whose paternal or maternal relatives have suffered or died from recurrent thrombosis and embolism. In these families, Banti's disease, Chiari's syndrome, coronary occlusion, and migrating thrombophlebitis occur regularly. There are very striking examples recorded in the literature of families whose members appear to have had an hereditary tendency to thrombosis (58, 113).

After an enumeration of the various factors involved in thrombosis, brief consideration of the mechanisms available to the organism for the prevention of thrombus formation and the removal of thrombi seems indicated. Recently, emphasis has been placed on the fibrinolytic enzyme system as a means of protecting the individual against thrombosis. More and more data have become available which indicate that intravascular clotting occurs continually. This fact was postulated years ago in an attempt to explain the relationship between bleeding and clotting. Coagulation defects result, as a rule, in an increased bleeding tendency. Deficiency of a clotting factor, it was reasoned, resulted in poor fibrin formation. Since fibrin was thought to "plug up" injured sites, bleeding appeared to be a logical consequence of coagulation defects.

That fibrin formation does, indeed, occur quite extensively intravascularly was first described by von Rokitansky (94) and has been reemphasized by Duguid (26). Under normal conditions, this fibrin is removed at a rate which approximately equals the rate of fibrin formation. Fibrin, as it appears, is continually broken down in the organism not only in vessels, but also in inflammatory reactions and in wounds. Typical examples of the removal of fibrin are, for instance, the revascularization of thrombosed veins and the resorption of enormous quantities of fibrin during the resolution stage of a lobar pneumonia. Consequently, deficient breakdown of fibrin results in a prolonged retention of this substance with excess connective tissue formation. Deficient breakdown of fibrin has been implicated in many conditions, including: keloid and adhesion formations, consolidation of the lung after lobar pneumonia, thrombophlebitis, the Leriche syndrome, and arteriosclerosis.

Although there are ample instances of inadequate removal of fibrin, there are also conditions which can be ascribed to an opposite effect. This effect, which consists of an excessive break-

down of fibrin, was termed "fibrinolysis" by Dastre (21) in 1893 when he first observed the autolysis of blood clots. He proposed the term "fibrinolysin" for the active agent responsible for the lysis. Since other proteolytic enzymes, notably trypsin and papaine, show fibrinolytic activity, the enzyme in plasma which has a specific thrombolytic property has been called "plasmin." Its active precursor is called "plasminogen." Delezenne and Prozerski (23) found that chloroform accelerates clot lysis, and Tillett and Garner (105) observed a similar effect after the injection of streptokinase.

The first person to single out the basic phenomenon was undoubtedly John Hunter (53) when he wrote in 1794, "In many modes of destroying life, the blood is deprived of its power of coagulation, as happens in sudden death produced by many kinds of fits, by anger, electricity or lightning; or by a blow on the stomach, etc. In these cases we find the blood, after death, not only in the fluid state as in the living vessels, but it does not even coagulate when taken out of them." Morawitz (78) showed in 1906 that the blood to which Hunter had referred remained fluid because it was free from fibrinogen. Yudine (116) turned this phenomenon to practical use in the preparation of cadaver blood for human blood transfusion, a procedure still used today in Russia (85). Mole (76) linked incoagulable cadaver blood to conditions with increased plasmin activity, the appearance of which was considered to be part of the body's general reaction to injury. "This," he writes, "accounts for the presence of fibrinolysin in the blood after death from a wide variety of causes, and for the frequency with which fluid and incoagulable blood is found at autopsy." Subsequently, Mullertz (79) related the plasminogen activation to certain acute and violent forms of death, especially those associated with asphyxia, such as drowning, hanging, carbon monoxide intoxication, electrocution, and sudden cardiovascular deaths.

PLASMINOGEN ACTIVATION

From the plasma of mammals, a protein called "plasminogen" can be isolated in the euglobulin fraction. This protein is the inactive precursor of the proteolytic enzyme, plasmin. Besides fibrin, plasmin will break down fibrinogen, AC globulin, complement, and factors V and VII (100).

The activation of the plasminogen involves a loss of protein moiety and there is evidence to suggest that the plasmin obtained by different modes of activation may vary in composition. The different activators may be divided into those which occur naturally, such as urokinase, trypsin, streptokinase, and staphylokinase, and those which induce activity after being injected, such as protamine, nicotinic acid, pyrogens, epinephrine, and acetylcholine. Some of these drugs have been used rather indiscriminately in clinical medicine. Serious bleeding problems have sometimes resulted and these substances are therefore no longer used.

Naturally occurring activators have been found to circulate in the blood under various conditions, for example, after stress, hypoxia, surgery—especially extensive pulmonary (73), hepatic (118), and cardiovascular surgery (39, 59, 81)—, electroshock (33), cancer of the prostate (103), cirrhosis of the liver (50, 93), and leukemia (45). Poor wound healing and the breakdown of barriers against infection with subsequent dissemination is, in part, the result of excessive plasmin activity. Some tissues are very rich in activator content. The lung, for instance, is one such organ. Extensive manipulation of the lung during operation may, for that reason, result in severe bleeding. The high activator concentration of the lung might explain why pneumonia usually resolves. Moreover, the lung as a filter for the peripheral bed is furnished with the means to break down considerable amounts of fibrin.

The kidneys, too, are richly supplied with plasminogen activator. Urine contains large quantities; this is responsible, in part, for the persistent blood loss after many of the transurethral resections, according to McNicol and his associates (74). On the other hand, there are organs whose plasminogen activator activity is much more limited, such as the liver (9). This deficiency is probably one of the reasons why the liver heals with extensive scar formation, as it does in cirrhosis.

Recently, pulmonary changes in several diseases, notably congenital conditions such as hyaline membrane disease and pancreatic fibrosis, have been explained on the basis of deficiency in the tissue plasminogen activator by Lieberman (66, 67).

Finally, it should be pointed out that a marked difference exists in the amount of plasminogen activator activity released under the influence of one and the same stimulus in ani-

mals of different species. This may explain some hitherto poorly understood differences which have been observed in experimental animals.[3]

INHIBITORY AGENTS

We have come to distinguish natural—alpha 1-antiplasmin and alpha 2-antiplasmin—and synthetic—soybean trypsin inhibitor, amines (methylamine, laurylamine, quaternary amines), basic amino acids (epsilon amino caproic acid, lysine and arginine esters), and urea—inhibitors as counterparts of the activators. At least one of those which occur naturally is probably lipoid in nature. Some of the fat dissolving agents, such as chloroform, are thought to activate the enzyme system by eliminating this inhibitor (6). The clinical significance of the inhibitor is seen in its protective action. If all the plasminogen of blood were to be activated, there would not be enough plasmin generated to overcome the inhibitory level. This is why, in normal individuals, the activation of all plasminogen is seldom associated with free plasmin activity because of the excess of inhibitor present in the blood. Only when the plasminogen concentration is markedly increased or the inhibitor level is markedly decreased can a situation occur which is characterized by active fibrinolysis. The clinical manifestations of active fibrinolysis are those of a hemorrhagic diathesis resulting from digestion of AC globulin and fibrinogen.[4] A similar hemostatic breakdown is created artificially when free plasmin is administered to patients as treatment for thrombophlebitis. Only free plasmin exerts this action. In the presence of sufficient inhibitors, natural or synthetic, plasmin is rapidly inactivated.

The first principle in treatment with fibrinolytic enzymes is, therefore, to abstain from the use of the active enzyme plasmin. The hemorrhagic diathesis resulting from such treatment is quite severe and does not respond to the administration of fibrinogen alone. In some clinical cases of this free plasmin activity, severe thrombocytopenia has been observed as well (18). Other defects usually seen are: a hemophilia-like bleeding tendency as a result of breakdown of AC globulin and a decrease in complement concentration. At this point, one wonders how this enzyme system can possibly be utilized in the future treatment of thromboembolic diseases.

THERAPEUTIC CONSIDERATIONS

There appear to be several ways in which patients with thromboembolic disease are benefited by enzyme therapy. As mentioned, Astrup and co-workers have demonstrated that plasminogen is present in considerable quantities in thrombi. It seems to be absorbed into the fibrin mesh. In treating patients with thromboembolic phenomena, one must confirm that the amount of natural inhibitor present in the patient's blood is sufficiently large to neutralize the plasmin activity which results from the activation of plasminogen. Then, activator is administered. Theoretically, in these instances, no free plasmin is formed and, consequently, no breakdown of the hemostatic mechanism can occur. In practice, however, administration of activator is at times associated with serious difficulty.

On a theoretic basis the idea of furnishing activators such as urokinase or purified streptokinase is sound. Several investigators have demonstrated that rather than giving plasmin, which will break down valuable coagulation factors, activator is preferred (34, 35, 57, 75). When given in liberal amounts, part of the activator will diffuse into the clot. The plasminogen[5] attached to the fibrin is activated in the process. The clot is subsequently broken down by the plasmin so formed. Of course, at the same time, the circulating plasminogen is converted into plasmin. This plasmin, however, is readily inactivated by the circulating inhibitors.

Back and his associates (11) have recently demonstrated that activators are capable of lysing clots in this fashion even days after the thrombus is formed. Since organization of the fibrin substrate of thrombi and emboli does not

[3]With regard to the activation of the plasminogen-plasmin system, a definite species difference can be distinguished. The plasminogen-plasmin system of dogs was found to be readily activated during shock as a result of injection of adrenalin (69, 70) and of endotoxin (38), whereas no activation occurs in the rabbit after injection of endotoxin (39) or adrenalin (112). However, in the rabbit marked activation of the enzyme system could be demonstrated during anaphylaxis (40) and hemorrhagic shock (106). Since intravascular clot formation takes place in most of these shocklike states, the presence or absence of thrombi in the different species appears, at least in part, to be related to a difference in reaction of the plasminogen-plasmin system. This difference in reaction could possibly be due to a different effect of these agents in releasing plasminogen-activator from the various shock organs. Data obtained so far implicate the plasminogen-plasmin system as a protection of dogs against thrombosis, whereas under similar conditions this system fails to protect the rabbit from thrombosis after endotoxin injection (39).

[4]Epsilon amino caproic acid and other synthetic inhibitors of the plasminogen activation or plasmin inhibitors can be used successfully in combating the conditions of active fibrinolysis (1, 2, 40).

[5]It is obvious that if the patient has low plasminogen levels to start with, therapy with activator is a priori doomed to failure.

seem to start for several days after their formation, it would seem that enzyme therapy definitely has something to offer to these patients.

One fact has to be considered in this type of therapy. This is that the activator will convert all circulating plasminogen, thereby exhausting the patient's natural reservoir of plasminogen. Consequently, any new thrombus formed immediately after termination of therapy will be devoid of plasminogen. To prevent new thrombus formation, activator administration must be combined with anticoagulant therapy.

Several series of patients have been treated according to the principles described. When purified streptokinase was given, the antistreptokinase titer had to be determined prior to the infusion. It is still too early to discuss the results of these clinical trials. Several investigators, however, claim to have obtained good results, including clinical cures of thrombophlebitis after one or two 30 hour perfusions with activator (57, 98). However, in some patients, in spite of all precautions, a bleeding tendency developed.

With regard to this type of therapy, it is the general consensus of those working actively in this field that when activator therapy is instituted for thrombosis, close supervision of such treatment with frequent determinations of activator levels and of the clotting parameters, as well as clinical evaluation of the patient, will be necessary. The feeling prevails that, since these medications are not free from serious hazards, the time for their routine use is not yet here.

Let us fully realize that several problems have been presented in simplified fashion, and that a great deal has been omitted for the sake of clarity. This presentation will have served its purpose, however, if it has helped toward an understanding of these complex problems.

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