THROMBOLYTIC AGENTS[1,2]

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Thrombolytic or fibrinolytic therapy is an area of great promise, for potentially it offers the physician a method by which the deleterious effects of acute thrombo-embolic vascular disease may be controlled or prevented by medical means. The practical urgency of developing such therapeutic measures is self-evident as, despite the introduction of anticoagulant therapy and the efforts of surgeons to extend the applicability of such techniques as thrombectomy, embolectomy, endarterectomy, vein ligation, and vascular grafting, acute thrombo-embolic vascular disease still remains the largest single cause of mortality and morbidity in the middle-aged and elderly populations of the Western World.

While the ability of the organism to both lay down and also remove fibrin was firmly established by classical pathological studies on wound healing, inflammation, etc., during the last century, it was only at its turn that the demonstrations by Sahli (1), that urine would dissolve crude fibrin clots, and by Goodpasture (2), that sterile whole blood clots underwent spontaneous lysis, suggested that humoral apart from cellular mechanisms might play a role in fibrin lysis. However, it was not until the thirties when Tillett & Garner's discovery (3) of a potent bacterial "fibrinolysin" (a substance now termed streptokinase, a plasminogen activator) provided the key reagent for biochemical and clinical studies, and Christensen & MacLeod's paper (4) on the enzymatic basis of fibrin lysis, in which the plasminogen-plasmin system terminology was introduced, that sustained research interest was aroused. Since that time, intensive basic, clinical, and therapeutic investigation of the plasminogen-plasmin system has occupied the attention of many investigators; the historical development of the field is adequately treated in a number of symposia and reviews (5–21).

Even though the past decade has seen important advances in understanding the biochemical interactions of the plasminogen-plasmin system, its individual components, its physiological function in the organism, its pathological malfunctions in disease states, and the principles that should govern its therapeutic applications, success at the applied therapeutic level has not matched other gains. Although this relative failure at the applied therapeutic level has been caused by many factors, i.e., inadequate knowledge of thrombolytic mechanisms, incomplete understanding of the interactions of the coagulation and plasminogen-plasmin systems, toxicity of the available thrombolytic agents, and other difficulties, it now appears that

[1] The survey of the literature pertaining to this review was concluded in June 1965.

[2] This work was supported by Grant H-3745 from the National Heart Institute, Bethesda, Maryland.

many of these former problems have either been solved or at least are on the verge of solution. Consequently, it will be the purpose of this review to evaluate current prospects and available thrombolytic agents, with only passing reference to discarded approaches and obsolescent drugs or drug combinations, to delineate current problems awaiting solution, and to attempt a projection of reasonable expectations and future developments. It will be apparent that in this field, as in so many other fields of pharmacological importance, the problems posed by accurate clinical evaluation of drug effect now rank equally with those problems incident to the acquiring of fundamental knowledge of drug action and in the development of new agents; therefore, we have attempted to emphasize both the scope and nature of the problem of clinical evaluation.

This review is designed to be selective, and since excellent reference coverage to the field has been provided by numerous reviews, monographs, and symposia (9–21) published over the last five years, we shall cite only such key references as appear pertinent to the subjects under discussion.

Introductory Considerations

In vivo fibrin lysis is controlled by an enzymatic process involving the conversion of an enzyme precursor, plasminogen, into a proteolytic enzyme, plasmin; a reaction mediated through specific plasminogen activators or kinases. In fact, useful biochemical and physiological analogies exist between the well-studied trypsinogen-trypsin system activated in the gut by enterokinase and the plasminogen-plasmin system activated intra- and extravascularly by specific activators. Both enzymes are formed as inactive enzyme precursors; both are activated by a similar mechanism of proteolytic cleavage by specific kinases (22, 23); both enzymes show only very limited substrate specificity and destroy many substrates of biological importance; both may under certain disease conditions, where enzymatic control mechanisms are disturbed, produce catastrophic consequences to the organism; and plasma contains powerful inhibitors against each.

The crucial differences between these enzymes occur with respect to their sites of action, for whereas trypsin activation occurs in the intestinal tract, a site which affords physical separation of the enzyme from other susceptible substrates of biological importance, the site of plasminogen activation is widespread throughout the extra- and intravascular spaces without physical separation of the proteolytic enzyme plasmin and its target substrate fibrin from other susceptible substrates of biological importance (24). Thus, the specific actions of the plasminogen-plasmin system depend upon certain special properties of the enzyme precursor plasminogen and its specific activators; these properties are considered later, for they are crucial to both the special problems inherent in thrombolytic therapy and also assure its feasibility and promise.

Plasminogen is a serum globulin, which has been highly purified (25–

28), with a molecular weight of approximately 88,000 (29), which is distributed in both extra- and intravascular spaces and is found in body fluids and transudates. While its concentration in the plasma is relatively constant, its normally low concentration in such fluids as the aqueous humor of the eye or in the cerebrospinal fluid becomes greatly increased in the presence of inflammatory disease of the eye or the meninges. Similarly, inflammatory exudates in the pleural or joint spaces will show high plasminogen levels, while transudates show lesser concentrations.

Specific kinases (plasminogen activators) can be extracted from virtually all body tissues, except the full-term placenta and the liver, being particularly abundant in such tissues as the heart and lung (30, 31). Only very low levels of plasminogen activator are assayable in the plasma of resting man (32, 33), but five- to tenfold increases occur following the stress of exercise, and much higher levels after the administration of certain drugs, e.g., epinephrine, nicotinic acid, etc., and in disease states (pathological plasma proteolysis, "pathological fibrinolysis"). Plasminogen activator is also present in tears, breast milk, and, in particularly high concentration, in the urine; in all these fluids it appears to subserve the function of preventing fibrin blockage of the excretory ducts. The plasminogen activator present in urine, termed urokinase, has recently been crystallized in apparently homogeneous form (34), is assayable on synthetic substrates (35), and is presently being purified, on a large scale, for use as a thrombolytic agent. Similarly, recent substantial progress has been made in the purification of plasminogen activator of high specific activity from tissue (36), though to date no efforts have been made to utilize this material as a thrombolytic agent.

While it seems clear that the plasminogen activator, found in plasma, must represent that released from tissues, it is not established that urokinase, found in the urine, is wholly or even largely an excretion product of plasma, though several studies provide suggestive evidence that at least some part of the urinary plasminogen activator may represent excreted plasma activator.

In Vivo Thrombolysis[3]

The problem posed by the ability of the organism to utilize a proteolytic enzyme of relatively undifferentiated substrate specificity, plasmin, to lyse fibrin, without simultaneously destroying other substrates of biological significance, has excited considerable investigative speculation and interest. Moreover, since it was also known that over-activity of the plasminogen-plasmin system, in disease states, was productive of a severe coagulation defect and a sometimes catastrophic hemorrhagic diathesis (pathological

[3] The term thrombolysis will be used to describe the reaction by which thrombi (or clots) are lysed. The adjective thrombolytic will be used to designate biochemical moieties capable of inducing thrombolysis.

plasma proteolysis or pathological "fibrinolysis"), elucidation of the mechanisms by which *in vivo* thrombolysis occurred, under physiological conditions, became of crucial importance to the formation of a rational therapeutic approach.

Since several critical reviews (7, 11, 15) have traced the various controversies in this area, we shall confine our description of *in vivo* thrombolytic mechanisms to the current hypothesis and the therapeutic indications and contra-indications that have flowed from its development. This hypothesis (37, 38, 39), which appears to account adequately for the facts and is now almost universally accepted [for dissent see (40)], is founded on the concept that, *in vivo,* plasminogen exists as a "two phase" system; as a soluble phase form in the plasma and as a gel phase form in thrombi and fibrinous deposits. The actions of activators on plasminogen in the two phases and the consequence of plasminogen activation in these two sites are biochemically totally dissimilar. Activation of soluble or plasma plasminogen, because of the presence of plasma inhibitors, provided the activation is slow, will not result in detectable signs of plasma proteolysis, since the enzyme is effectively inhibited on its formation; however, rapid activation of plasma plasminogen does produce enzymatic effects (pathological plasma proteolysis). Fibrinogen, the most abundant available substrate, is the chief one degraded with the production of a severe coagulation defect. On the other hand, activation of gel phase or clot plasminogen produces thrombolysis, as the enzyme is activated in close spatial relationship with its substrate fibrin, and the reaction is, initially at least, independent of plasma inhibitors. Moreover, plasminogen activators differ in the ratio of their activities on soluble and gel phase plasminogens. Those tested all show a selective affinity for gel phase plasminogen activation but the bacterial activator streptokinase has a relatively lesser ratio of activity on gel phase plasminogen than do the naturally occurring tissue activators (36) urokinase (39) or plasma activator (41). The ratio of these respective activities may be quantitated by *in vitro* means and the accuracy of the predictions confirmed by *in vivo* methods (38, 41, 42).

These observations indicate that *in vivo* thrombolysis is mediated through a mechanism involving activation of intrinsically contained thrombus plasminogen and is not produced (except possibly in a very minimal and insignificant fashion) by the extrinsic actions of plasmin upon thrombi. They carry the therapeutic implication that the ideal thrombolytic agent will be a plasminogen activator of high affinity for gel phase plasminogen. The infusion of a proteolytic enzyme, such as plasmin, will prove ineffective in producing thrombolysis and hazardous through production of a state of pathological plasma proteolysis, if used in large doses.

Approaches to Thrombolytic Therapy

The clinical approach to thrombolytic therapy has involved the intravenous infusion of four main classes of agent. These comprise, firstly, the use

of a variety of proteolytic enzymes (capable of inducing *in vitro* lysis of fibrin), secondly, the naturally occurring enzyme plasmin,[4] thirdly, a variety of plasmin-plasminogen activator mixtures, and lastly, the use of plasminogen activators alone.

Proteolytic enzymes.—Intravenously administered trypsin, despite the unsatisfactory and contradictory evidence derived from animal experiment, received, some years ago, fairly extensive trial as a thrombolytic agent in man. Although initial clinical reports were encouraging, the lack of proven thrombolytic activity *in vivo,* the hazard of induced clotting *in vivo,* and the frequent production of local thrombophlebitis has resulted in its abandonment [for references see (11, 15)]. Although on the basis of animal experiment (45), chymotrypsin was shown to be an apparently superior thrombolytic agent, the advantages were marginal, and clinical trials have not been reported. While considerable investigative effort has been undertaken to purify aspergillin O (a proteolytic enzyme produced by *Aspergillus oryzae*) and define its properties (46, 47, 48), these are not of a nature to suggest therapeutic utility. Similarly, thrombin E (an acetylated thrombin derivative), although proposed (49) and tested as a thrombolytic agent in animals, has not, for various reasons (50), been tested in man.

Plasmin.—Activator-free glycerol-activated plasmin (22) has only had limited trial in man with discouraging results (51); neither do results with trypsin-activated porcine plasmin show greater promise (52, 53).

Plasmin-plasminogen activator mixtures.—Two plasmin-streptokinase mixtures have been commercially available, Actase (now withdrawn from the market) and Thrombolysin. Other plasmin-streptokinase mixtures and plasmin-urokinase mixtures have also undergone limited clinical evaluation (54, 55). The disadvantages attendant upon the use of such mixtures have been clearly defined (15, 43, 44) and, though initial clinical reports with all these agents were encouraging (8, 12), this approach is now largely discarded.

Plasminogen activators.—Only two plasminogen activators have undergone clinical study. The majority of work has been performed with streptokinase, a product of hemolytic streptococcal metabolism, but more recently, urokinase, extracted and purified, on a large scale from human urine, has become available.

While streptokinase has served as a model investigative drug to establish the scientific basis for (37) and the potential of thrombolytic therapy (38, 56), the variable clinical toxicity of streptokinase and streptokinase-plasmin mixtures, chiefly manifested by pyrogenic reactions, has proved to be a deterrent to its large-scale clinical trial. Since the pyrogenic effect has varied with different batches, has lessened as purification procedures have

[4] Several enzyme preparations, referred to in the literature as "plasmin," were in reality mixtures of plasmin and plasminogen activators and their *in vivo* activity later shown to be caused by this latter component alone (43, 44).

improved (57, 58), and in some instances nonpyrogenic batches of streptokinase have been produced (38, 59), it may be tentatively assumed that the pyrogenic batches contain some unidentified contaminant. Elimination of pyrogenicity from streptokinase preparations has proved to be an unusually difficult problem, since no reliable animal screening test exists for its detection, and the occurrence of pyrogenic reactions in individual patients, at a fixed dosage, is inconsistent and unpredictable. Moreover, streptokinase is highly antigenic to man and, because of wide variation in patient plasma antibody concentration (60) the correct dosage for the individual patient has to be determined by titration for plasma streptokinase antibody (60, 61). A full treatment course of streptokinase invariably immunizes the patient and may prevent patient re-treatment for a period of months until the plasma antibody level undergoes spontaneous decline (38). Despite these difficulties and the potential risk of inducing hypersensitivity reactions by drug infusion, streptokinase therapy has been successfully employed in a substantial number (several thousand) of patients.

Recently, in the search for a nonantigenic and nontoxic plasminogen activator suitable for clinical use, interest has focused on urokinase, the plasminogen activator excreted into urine. Although Ploug & Kjeldgaard (62), some years ago, described a urokinase purification method apparently suited to large-scale use, only extremely limited investigative studies with this preparation have been published (63, 64). Notable defects in these earlier preparations included those of low specific activity, contamination with urinary thromboplastic moieties, and the possibility of contamination with virus, derived from the bulk urine pools, used as starting material.

In further developmental studies of urokinase as a therapeutic agent (18, 42, 50, 65), many difficult production and scientific problems have had to be surmounted.[5] These included not only the requirement that the preparations show negligible toxicity in animal studies, but also requirements for high preparation specific activity (35,000 C.T.A. urokinase units/mg protein), for preparation treatment at 60° C for ten hours (for virus inactivation), and for stringent precautions to eliminate the known urinary thromboplastic moieties from the final preparations. This latter requirement was a matter of particular difficulty and required the development of special assay procedures (66).

Though only limited clinical experience has been obtained with these

[5] Two pharmaceutical firms, the Abbott Laboratories, Chicago, and the Sterling-Winthrop Research Institute, Rensselaer, New York, have played the major role in this work.

Parenthetically it may be noted, especially in view of the present-day great costs and difficulties of drug development, that the evolution of urokinase as a therapeutic agent has only been possible through the development of the closest collaboration between pharmaceutical firms and university departments of medicine and with financial support from the National Heart Institute.

newer urokinase preparations (18, 42), they show considerable promise for inducing states of intense and predictable plasma thrombolytic activity immediately on their injection when administered on a dose-body-weight basis (42). Furthermore, they are apparently nonantigenic, nonpyrogenic, and show negligible signs of clinical toxicity, except insofar as their biochemical actions influence coagulation function (see later section).

Other Pharmacological Approaches

Though presently not of demonstrated practical value, two alternative pharmacological approaches to thrombolytic therapy require consideration. The pioneering observations of Biggs, Mcfarlane & Pilling (67) on epinephrine injection, and particularly the later studies of Weiner, Redisch & Steele (68) on injection of nicotinic acid have stimulated investigators to screen a large number of pharmacological substances for their effect on plasma fibrinolytic activity. The demonstration that enhanced plasma fibrinolytic activity following pharmacological stimulation resulted from release of plasminogen activator into the circulation (32, 33), the knowledge that organs and vessel walls contain high concentrations of this activator (30, 31), and the significant work of Kwaan, Lo & MacFadzean (69, 70) in which they attempted by a variety of ingenious studies to develop a general pharmacological theory to account for plasminogen activator release from the vessel wall, have maintained interest in this approach.

Nevertheless, the development of a practical pharmacological method of controlling plasma thrombolytic activity has been hindered by many difficulties. None of the drugs so far tested produces other than relatively mild and transitory enhancement of plasma thrombolytic activity, and most produce disturbing side effects which limit dosage; moreover, in certain cases (e.g., nicotinic acid) rapid refractoriness to drug action develops. While it has been reported that the long-term administration of certain drugs, e.g., sulfonylureas, testosterone, steroids, etc., will produce mild chronic elevation of plasma thrombolytic activity, these claims have been almost wholly based on the use of the euglobulin lysis time and similar tests, where assay values are partially dependent upon nonspecific changes. For instance, in one investigation (71) where it was claimed that steroid administration enhanced plasma thrombolytic activity, it seemed possible, at least from inspection of the other data furnished, that the apparent effect of steroid administration may have been wholly indirect and attributable to changes in patient plasma fibrinogen concentrations. While it is entirely possible that satisfactory methods for the pharmacological control of plasma fibrinolytic activity may be devised, an achievement that might have great clinical utility, it must be acknowledged that presently we lack effective pharmacological methods for this purpose. Fearnley (72) and von Kaulla (19) have recently reviewed this field.

A further potentially useful approach towards the goal of thrombolytic

therapy has been developed through the work of von Kaulla and his associates (19). These studies have involved the screening of a variety of chemical compounds in a simple *in vitro* plasma-plasma clot test system for evidence of "fibrinolytic" activity. Although a variety of active compounds have been discovered, the mechanism by which such effects occur is obscure and clinical studies have not yet been attempted.

In Vivo Thrombolysis in Man

Previous reviews (7, 11, 15) have cited the extensive and convincing evidence that the infusion of thrombolytic agents will lyse experimentally produced thrombi in a variety of animal species. Such evidence, though of important relevance to the general problem of thrombolytic therapy, does not offer certainty that similar results can be obtained in the human being where requirements for clinical safety are paramount and in whom thrombotic lesions will differ from those produced in the experimental animal. Indeed, several agents capable of producing thrombolysis in the experimental animal, have subsequently been shown to be unsuitable for human therapeutic use; the very success achieved in animal models has, in some cases, led to erroneous appreciation of the very different circumstances involved in clinical investigation. Consequently, the evidence, cited in this section, will be restricted to that derived from man.

Although an enormous literature on the clinical effects of streptokinase infusion [for references see (6, 8, 12, 73)] has accumulated, most claims made for clinical efficacy and thus directly or indirectly for presumptive thrombolytic efficacy, have been based on purely clinical data. However, it is well recognized that the clinical outcome in patients suffering from many varieties of peripheral vascular disease is highly variable, and that clinical improvement, amounting in some cases to clinical "cure," may occur without resolution of the primary vascular thrombotic process. Thus, clinical evidence of improvement is insufficient to establish the reality of thrombolysis, and it is highly desirable that objective evidence of vascular function, such as is provided by pre- and post-treatment angiographic examination, be used to establish the precise cause of therapeutic benefit. Obviously, it is neither feasible nor desirable to employ such procedures in all patients or in all studies, but, in general, their use permits a degree of diagnostic and scientific precision obtainable in no other way.

In an important experimental study, Johnson & McCarty (59) induced localized thrombosis in the superficial arm veins of human volunteers, and 24 to 48 hours later infused streptokinase, using several dosage schedules. By means of pre- and post-treatment venography and with an "optimal" streptokinase dosage schedule, they demonstrated thrombolysis, without subsequent clot reformation, in 11 of 11 studies.

Treatment of the patient suffering from an acute thrombo-embolic vascular complication presents many problems (the uncertain age and extent

of the thrombo-embolus and often its occurrence in a severely atherosclerotic vessel) not encountered in the human volunteer with localized venous thrombosis. In such circumstances, it has been necessary to use considerably higher and more prolonged streptokinase dosage, and treatment failures have been frequent. Nevertheless, even under the adverse conditions of human thrombo-embolic vascular disease, clear evidence has been obtained that streptokinase therapy produces *in vivo* thrombolysis. Verstraete, Amery & Vermylen (74) treated 15 arteriographically proven arterial thrombi or emboli with high dosage streptokinase therapy (mean dose 3.22×10^6 units administered over a mean period of 65 hours). For a variety of reasons, including in some cases the patient's death, post-treatment arteriography could not always be performed, but in seven of nine such examinations, restoration of arterial patency was demonstrated; by clinical criteria, lysis had occurred in eight of 15 instances after 60 hours of therapy. Streptokinase was usually administered by intra-arterial catheter placed in close proximity to the thrombus or embolus, and thrombolysis was demonstrated from 34 to 108 hours after the commencement of therapy.

Salmon (20), who used very similar methods, reported on the effect of streptokinase infusion in 50 patients. Streptokinase therapy produced complete thrombolysis in 24 and partial lysis in a further nine. The majority of his patients were suffering from arterial embolism and his results in this group were superior to those obtained in patients with arterial thrombosis.

Since it has been suggested that the administration of streptokinase by intra-arterial catheter in close proximity to the obstruction might possess notable advantages over administration of this agent by the intravenous route, the studies of Winckelmann et al. (75) are of considerable importance. These investigators preferred the intravenous route for their treatment of 19 patients with acute recent arterial obstruction. In nine patients, all peripheral pulses returned and the oscillograms were restored to normal; in five patients there was partial return of these parameters to normal, while in five other patients there was no improvement. Arteriography in nine patients showed close agreement with the clinical findings and demonstrated complete lysis in four cases, partial lysis in three, and no change in two. Their streptokinase dosage varied from $2.2–13.4 \times 10^6$ units, and complete or partial thrombolysis was observed in from 15 to 144 hours. Other authors [including (56, 76, 77)] have published case reports with angiographic or other types of objective findings indicating that streptokinase produced thrombolysis in the patient with vascular disease.

While in such studies, there is sometimes doubt as to the etiological cause of the obstruction visualized by arteriography (thrombosis or embolism with or without atherosclerotic disease), it is believed that both thrombi and emboli have responded equally well to the therapy. Similarly, while authors have dated the time of arterial occlusion from the onset of symptomatology and have restricted treatment to relatively "recent" lesions, it is

probable that the occlusions visualized by arteriography were, in many instances, much older than would have been suspected by "clinical dating"; this could have accounted for the fact that some lesions were uninfluenced by therapy.

Studies with purified urokinase preparations are still very limited in number. However, Johnson, McCarty & Newman (65) have reported that following the infusion of low doses of urokinase and heparin for 25 hours, nine of 13 experimentally induced venous clots in human volunteers were successfully lysed, and Fletcher et al. (42) have demonstrated, by means of pre- and post-treatment venography, massive thrombolysis in two patients suffering from acute thrombophlebitis and treated with high urokinase dosage for 16 hours. Other investigator reports (78) suggest that purified urokinase acts as a powerful thrombolytic agent in man.

Thrombolysis and the Blood Coagulation System

Fundamental to the development of thrombolytic therapy has been the relationship of plasma thrombolytic activity to the coagulation system and to hemostatic mechanisms. It is inferred that the action of physiological thrombolytic mechanisms is normally directed to the lysis of microthrombi rather than to the removal of large clinically evident thrombi. In this sense, the detection of clinical thrombosis may be regarded as indicative of either partial or complete failure of plasminogen-plasmin system activity to control intravascular fibrin deposition. Presumably, the failure of physiological thrombolytic mechanisms to prevent thrombus formation *in vivo* represents an accumulation of factors inimical to their actions (prior vessel disease, embolism, hemodynamic, coagulation, or platelet anomalies, etc.) rather than to primary failure of the plasminogen-plasmin system itself. Thus, mere therapeutic reinforcement of plasminogen-plasmin activity would be expected to constitute an ineffective approach (the available evidence would support such a concept) and, in the presence of established thrombosis, the physician should be prepared to use plasminogen-plasmin system components in a pharmacological, as apart from physiological, manner. Such pharmacological usage, involving the production of intense (several hundredfold normal) and prolonged states of plasma thrombolytic activity, though primarily directed at the target thrombus, also affects the integrity of the blood coagulation system and, in certain circumstances, hemostatic function.

The effects on the coagulation system are caused by activation of soluble phase plasminogen (because of infusion of plasminogen activator) with the production of a, usually mild, state of pathological plasma proteolysis. Fibrinogen, because of its abundance as a substrate, is the chief protein degraded, and these degradation products interfere with the polymerization of fibrin monomer, producing the coagulation defect, described by our group, as defective fibrin polymerization (79–82). However, even when

using streptokinase where virtually all soluble phase plasminogen must be activated prior to the development of substantial plasma thrombolytic activity (38), clinical studies indicate that, provided care is employed, the effects of the agent on coagulation function is not sufficiently severe as to preclude its use (38, 56). With urokinase, which possesses a very high gel/soluble phase plasminogen activity ratio, intense plasma thrombolytic activity may be induced and maintained with plasma plasminogen concentration at 20 to 30 per cent of normal, and a lesser degree of coagulation anomaly is produced than with streptokinase. However, clinical studies with urokinase have been limited and it is still not clear that the coagulation defect produced by urokinase infusion in man (18, 42) is precisely similar to that produced by strepokinase infusion under similar circumstances; in fact, it is possible that fibrinogen degradation products of a higher molecular range (83, 84) may be formed during urokinase infusion, and the coagulation anomaly, though of similar general characteristics, could be of a somewhat different type. Despite its propensity to affect the coagulation system, urokinase, on biochemical grounds, possesses two great advantages over streptokinase. Firstly, immediately on its infusion it induces an intense plasma thrombolytic state instead of after a variable time interval as with streptokinase; this permits immediate effective treatment in cases of clinical urgency. Secondly, states of intense plasma thrombolytic activity may be induced by its infusion in the presence of substantial concentrations of circulating plasminogen. This latter property partially obviates the difficulty previously observed with streptokinase (56, 59, 85), that plasminogen may be leached from the target thrombus during therapy (with the possibility that therapeutic action may cease), and also the danger that further thrombi laid down either during or shortly after the completion of therapy with streptokinase will be virtually devoid of plasminogen and thus insusceptible to lysis by thrombolytic agents.

Since thrombolytic agents are administered for the purpose of lysing fibrin clots, their use carries the hazard of inducing hemorrhage at local sites, where, owing to trauma or disease processes, fibrin, or fibrinous deposits contribute substantially to the hemostatic barrier. This phenomenon, in mild form, has been reported following both streptokinase (38, 79, 86, 87) and urokinase infusion (42). While normally this action of the drug, which is a reflection of its powerful therapeutic potential, is unimportant, it has sometimes constituted a severe practical limitation to drug evaluation, as many otherwise "suitable" cases are patients who have recently been subjected to an unsuccessful surgical procedure. The hazard of inducing hemorrhage in such patients at sites of recent surgical trauma is so high that thrombolytic therapy is definitely contra-indicated. Other relative or absolute contra-indications to thrombolytic therapy, at least as it is presently practiced, include any pre-existent disease where hemorrhage is a danger, i.e., recent peptic ulcer, bleeding from a chronic ulcer, other G.I.

bleeding, history of nonsurgical hematuria, etc. Moreover, prior anticoagulant therapy or other hemorrhagic diathesis may complicate the course of thrombolytic therapy, and careful laboratory study of the coagulation system is required before introducing anticoagulants into the therapeutic regimen of the patient who has been treated with thrombolytic agents.

Although a number of authors have advocated the concurrent use of thrombolytic and anticoagulant agents, it is our view that if a sufficient dosage of thrombolytic agent is infused to raise plasma thrombolytic activity to any significant degree, this combination with anticoagulants will prove hazardous and if, in the interest of patient safety, a lower dosage of thrombolytic agent is administered, the benefits to be expected from thrombolytic therapy are unlikely to be achieved.

Considerable space has been devoted to the relationship between thrombolysis and the coagulation system, as this relationship has been of fundamental importance to the development of the field. It should be emphasized that in the past this problem has proven to be controllable at the bedside and, as knowledge increases and improved agents are developed, its importance may be expected to diminish.

The Future

The investigative advances of the past decade have included (*a*) the development of a rational approach towards the problems of thrombolytic therapy; (*b*) an understanding of the difficulties that may complicate this approach; (*c*) the devising of practical laboratory means of monitoring thrombolytic therapy (37, 88, 89, 90); (*d*) the development of two therapeutic agents, streptokinase (which has less than ideal properties as a thrombolytic agent) and urokinase, which on the basis of laboratory and limited clinical investigative studies (18, 42) more closely approximates the ideal thrombolytic agent (except insofar as its production is severely limited and it is presently far too costly to be explored in any except an investigative setting); and lastly (*e*) the crucial demonstration that, at least under certain circumstances, such agents will induce thrombolysis in man (20, 42, 74, 75, 76).

These remarkable achievements have faced the investigator with further problems of which the chief are (*a*) in what way can thrombolytic agents be best employed as therapeutic tools; (*b*) how are appropriate dosage schedules for clinical trial best established, especially in view of the great cost and scarcity of urokinase; and (*c*) how is the clinical utility of the agent to be tested in the most economical fashion? Though at first sight these appear to represent somewhat elementary questions, in actuality each is complicated by formidable difficulty at the clinical research level. Indeed, it is common knowledge that the trial of many other promising pharmaceutical agents is presently hindered by similar evaluation difficulties at the clinical research level.

Previous studies of thrombolytic agents have usually involved study of *in vivo* biochemical effects with limited pharmacological, toxicity, and clinical studies, the better of the latter having been designed to obtain objective evidence of drug action. Thus, selection of patients for study has primarily involved the selection of those in whom objective evidence of improvement could be obtained, rather than those in whom the treatment might be thought to be, on theoretical grounds, of greatest benefit. While numerous purely clinical studies (mostly uncontrolled) have been published, these have not been of a nature to contribute, in any substantial fashion, to the solution of the clinical evaluation problem. Indeed, investigators have tended, for reasons of convenience in obtaining sufficient patient material and to limit the hazards of investigation, to utilize patients with either peripheral arterial or venous disease. While studies on such patients have served many useful purposes, it has unfortunately become clear, as a result of the increasing use of angiography in clinical practice, that clinical concepts concerning the age and extent of certain apparently "acute" thrombotic lesions are in need of revision. Not all patients with the diagnosis of acute thrombophlebitis or acute arterial occlusion of a limb vessel suffer from thrombotic vascular disease or, if such is the case, the main lesion is not necessarily acute. Further, such studies suggest that clinical "cure" of these vascular lesions is not necessarily associated with lysis of the causative thrombus. These angiographic studies have introduced a degree of complication into therapeutic studies of such peripheral vascular diseases as to necessitate either intensive study of a small selected group of patients or the collection of very large groups, studied by clinical means, for assessment of therapeutic effect.

Similarly, while such apparently straightforward and uncomplicated lesions as thrombosis of the central artery of the retina may appear as ideal for assessing the effects of thrombolytic agents (since visual and photographic assessment of progress is easy), increasing pathological and clinical knowledge of this lesion suggests that in many cases both the etiology and prognosis are so uncertain that extensive studies involving a large number of patients would be required to establish the true value of thrombolytic agents in this condition.

There remain three extremely important disease classifications in which thrombolytic agents might be of great utility and in which valid assessment of their therapeutic utility might prove feasible; these are pulmonary embolism, myocardial infarction, and acute cerebral thrombosis. It has been previously suggested that cerebral thrombosis was the area of greatest difficulty (11, 15) and in some ways that of least promise, because of the difficulties inherent in the differential diagnosis of hemorrhage from thrombosis, the peculiar nature of infarction in the brain as compared to that of other organs and the difficulty of evaluation in clinical response, etc. However, recently Meyer et al. (91, 92) have published the first large-

scale study in this field. They studied the response of a streptokinase-anticoagulant-treated group of patients suffering from advancing stroke as compared to the response of a similar anticoagulant-treated group. Despite the finding, by means of serial angiography, that the apparently causative thrombus was lysed more frequently in the streptokinase-anticoagulant-treated group rather than in the control anticoagulant-treated group, the response of the former as assessed by mortality and clinical response was inferior to that of the control group. While this was a pioneering study and certain details such as the decision to use combined streptokinase-anticoagulant therapy, uncertainties as to the precise basis of drug dosage, and the significance of certain coagulation findings should be classified as arguable, the study does indicate the need for caution in this difficult therapeutic area. On the other hand, it is not unreasonable to believe that further research into the problem of acute cerebral thrombosis may delineate a class of patients in whom a trial of thrombolytic therapy might be deemed desirable.

Pulmonary embolism would appear to offer a highly suitable condition for a trial of thrombolytic therapy. Embolism occurs into a presumably normal vascular tree and initially, at least, the embolus is unorganized and nonadherent to the vessel wall. Moreover, the condition is frequent, carrying both high mortality and morbidity, and surgical treatment, when indicated, is often hazardous.

Studies of thrombolytic therapy, in this condition, have hitherto been sporadic and isolated, largely because of difficulties in making and confirming the diagnosis and in assessment of treatment. However, recent improvements in the technique of pulmonary angiography and, in particular, in the use of pulmonary isotopic scanning methods, after the injection of I^{131} labeled macroaggregate albumin (93, 94), have largely overcome former difficulties. It is to be expected that this lesion will attract considerable sophisticated investigative attention.

The main impetus to the development of thrombolytic agents has been the hope that these agents would prove useful in the treatment of acute coronary thrombosis. There have been several studies of streptokinase-plasmin (95-101) (Thrombolysin) and one of a urokinase-plasmin mixture (55) in the therapy of acute myocardial infarction. None of these studies provided evidence that the agent under investigation was of value but, since in no case did the scanty laboratory data substantiate the author's claim of effective thrombolytic therapy, these studies are without direct relevance to the problem.

The hypothesis that effective thrombolytic agents may prove beneficial to patients suffering from acute myocardial infarction rests on several grounds [review and references in (102)]. First is the belief that in a substantial proportion of patients, probably a majority, the proximate etiological factor precipitating myocardial infarction is acute thrombosis of

the artery supplying the involved region and that by the use of thrombolytic agents, arterial continuity may be restored. Second is the concept that the blood supply of the infarcted area may be improved by the action of the agent in preventing microthrombus formation or by the lysis of microthrombi. These two effects might be expected to reduce the final area of myocardial death and in this way, reduce the degree of electrical instability of the heart during the early critical stages of infarction at a time when death from arrhythmia is a considerable danger, and finally, prevent or lyse mural thrombi or extracardiac thrombi.

On the other hand, the treatment could exert deleterious effects and the hypothetical dangers previously raised in the case of anticoagulant therapy in acute myocardial infarction (now disproved) could apply with greater force to thrombolytic therapy. Clearly, studies of thrombolytic therapy in acute myocardial infarction should be designed first to test the feasibility of the approach and only later to assess therapeutic value. Data on the feasibility of using thrombolytic therapy is already available. Fletcher et al. (56, 103) used streptokinase to induce and maintain intense states of enhanced plasma thrombolytic activity over a 30 hour period in 19 patients suffering from early acute myocardial infarction; all survived the acute phase of the illness, one patient dying three weeks later. Although the treated group fared better than the controls selected by a double-blind method, and better than other patients treated in the hospital during the same period, the results were not statistically significant. However, these results, in conjunction with the follow-up studies, demonstrate that the infarcted myocardium is tolerant of the induced plasma thrombolytic state and constitute an important demonstration of feasibility. Only limited data are available (104) concerning a rather similar, larger, but still unpublished, European study of streptokinase treatment in myocardial infarction (Verstraete et al.). Stage 1 of the study has been completed, ten of 75 patients having died in the streptokinase-treated group and five of 57 in the heparin-treated control group. Although benefit was not demonstrated to result from streptokinase treatment, it is reasonable to regard these results as offering a further demonstration that the infarcted myocardium is tolerant of the induced plasma thrombolytic state.

The problems involved in the running of adequately controlled therapeutic studies in acute myocardial infarction are of unusual difficulty, a fact emphasized by the controversy that has surrounded the many studies of anticoagulant therapy in this condition. Nevertheless, provided that feasibility studies of urokinase therapy in acute myocardial infarction yield satisfactory results, the potential utility of this therapeutic approach and the serious nature of the myocardial infarction problem will require that consideration be given to the development of full-scale clinical trials with this agent.

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