Thrombosis and Urokinase

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PREFACE

This monograph represents an edited version of the proceedings of an international symposium on urokinase which was held in the Istituto Superiore de Sanita in Rome on October 30 – November 1, 1975. The symposium was organized by the editors and made possible by the Serono Institute.

The symposium was most timely because urokinase has recently become available in a number of countries for therapeutic purposes; consequently, its use as a thrombolytic agent in the management of a variety of acute thrombo-embolic episodes will now undergo a rapid expansion.

While there has been a number of individual reports and presentations on urokinase, this symposium was the first to bring together the accumulated knowledge on this agent. Accordingly this document provides an excellent source of information concerning its purification, biochemistry, actions, pharmacology, and application to clinical medicine; and this material is presented against the general background of fibrinolysis and thrombolytic therapy. Considering that interest in urokinase is accelerating at a rapid rate, this document should serve as an excellent resource for all investigators and students interested in this subject.

The Editors

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THERAPEUTIC THROMBOLYSIS: PAST, PRESENT AND FUTURE

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At the heart of this symposium is human urokinase, an enzyme made by and released from certain tissue cells and whose biological function is that of an activator of the naturally occurring mammalian fibrinolytic enzyme system. While the role of urokinase in human physiology is undoubtedly of great significance, the real interest in this substance, at this time, lies in its therapeutic potential for dissolving thrombi and emboli; these pathological events are of major importance in threatening the health and welfare of a large segment of the adult population of the Western World. Should urokinase fulfil our expectations, then it would provide for a significant therapeutic advance. Since one of the major purposes of this symposium is to review the ability and effectiveness of urokinase in producing thrombolysis *in vivo*, it is most appropriate that we begin with a perspective on thrombolytic therapy: its past, present and future; it is against this background that urokinase can best be viewed. Thrombolytic therapy is a vast subject and my remarks will present only a broad overview; however, many of the papers which follow will detail current developments on selected aspects. Furthermore since my involvement dates back to the very inception of therapeutic fibrinolysis, please excuse me for injecting a personal perspective and a few historical notes.

Looking back, one of the most fortunate experiences of my life occurred one day in the spring of 1946 when Dr. Tillett called me into his office in New York City's Bellevue Hospital where I was then completing residency training on his Medical Service, and asked me to work with him on a new project, namely the clinical investigation and possible therapeutic application of streptokinase as a fibrinolytic agent.

Tillett, who died in 1974, had originally discovered the existence of streptokinase in 1933, and his observation was a beautiful example of serendipity. During the course of studies on acute phase reactants, he noted that normal human

plasma was capable of agglutinating hemolytic streptococci while serum was not. Therefore he suspected that fibrinogen was, in some way, responsible for this agglutination. If this were correct, he reasoned that the addition of a fresh broth culture of hemolytic streptococci to a specimen of oxalated human plasma, would, after a short period of incubation, tie up all the fibrinogen; subsequent recalcification of the plasma should then no longer lead to clotting. When he ran the experiment, he was disappointed to find that clotting occurred just as rapidly as in the control. Later before discarding the tubes, he happened to look at them again. To his surprise, the tube to which he had added the culture was now liquid. He repeated the experiments several times and soon established that hemolytic streptococci elaborated a fibrinolytic principle which we now call streptokinase. Incidentally, the observation by Tillett (1934) of the agglutination of streptococci by fibrinogen subsequently was shown to occur with other bacteria and is the basis for the commonly used staphylococcal clumping test in assaying fibrinogen/fibrin degradation products in serum.

At the time of his discovery, Tillett envisioned the possible application of streptokinase to clinical medicine; however more for its application to the lysis of thick fibrinous exudates as in empyemas and meningitis than for intravascular thrombo-emboli. Since techniques were not available for its purification, this goal could not be pursued until some thirteen years later when the first partially purified preparations of streptokinase were made available to us by Christensen; the latter worked in a nearby laboratory and recently had unequivocally demonstrated that streptokinase was an activator of human plasminogen (1945). Now, it was my task to initiate the clinical investigation of streptokinase in man. The preparations Christensen provided proved to be only 10% pure, and direct instillations into the pleural, cerebrospinal or other closed fixed body spaces were frequently accompanied by a severe febrile reaction; however, it was possible to document that the streptokinase extensively activated the fibrinolytic enzyme system locally and this resulted, on many occasions, in a dramatic lysis of a fibrinous exudate or clotted hematoma (Tillett and Sherry, 1949); this was readily demonstrable in the pleural space where large collections of clotted blood or loculated fibrinous exudates frequently followed trauma, pulmonary surgery or certain inflammatory diseases. These observations, made it possible to begin to envision using such a material, assuming it could be purified further, for the dissolution of thrombi. While awaiting further developments in purification, Johnson, who joined us in the late 1940's began to investigate the lysis of experimental thrombi in rabbit ear veins by streptokinase. The results of this study, published in 1952 (Johnson and Tillett, 1952), provided unequivocal evidence that streptokinase infused intravenously in appropriate dosage and at a distant site was capable of producing *in vivo* thrombolysis without significant toxicity to the animal.

Shortly thereafter a major development occurred when Kline (1953) reported on his technique for the purification of human plasminogen, the precursor of the fibrinolytic enzyme, plasmin. Now it became possible to consider two approaches to thrombolysis *in vivo*: one through the infusion of an activator, like streptokinase; the other through the infusion of plasmin, since the latter could be prepared by activating plasminogen with streptokinase *in vitro*.

In subsequent studies involving a number of animal species and several different groups of investigators, it was shown that the lysis of experimental venous and arterial thrombi could be achieved by either streptokinase alone or through

mixtures of streptokinase and human plasminogen. The question then arose as to whether an activator of plasminogen, or plasmin itself, would be the more desirable agent; this was a crucial question for its answer would determine the direction for future clinical studies. In a series of studies (Sherry *et al.*, 1959; Alkjaersig *et al.*, 1959; Fletcher *et al.*, 1962) my associates, particularly Fletcher and Alkjaersig, and I provided evidence that 1) the human body regulates endogenous fibrinolysis through control of the activator level and not that of free plasmin; 2) thrombolysis was most readily achieved by the activation of plasminogen within and in the immediate proximity of a thrombus; and 3) the maintenance of levels of free plasmin in the circulating plasma sufficient to lyse thrombi could only be achieved at the expense of extensive digestion of fibrinogen and other clotting components, notably Factors V and VIII, which are also native substrates for the action of plasmin. Out of this work emerged a fundamental consideration pertinent to the future development of this field, namely, that the lysis of thrombi could be achieved more rapidly by the regulation of the activator activity in plasmin, rather than through the level of free plasmin, and that the latter was responsible for the coagulation disturbances. Thus the key to effective thrombolytic therapy was in the maintenance of a high circulating activator level to effect rapid thrombolysis, and the control or prevention of significant hyperplasminemia so as to minimize the risk of serious hemorrhage. While this view never has been universally accepted despite the preponderance of evidence which remains in its favor, and studies with plasmin as a therapeutic agent continue, it proved to be the main determining factor in the therapeutic developments which followed.

By the late 1950's, preparations of streptokinase which were over 95% pure were made available by Lederle Laboratories for intravenous administration in man. Some batches were well tolerated, while others produced severe pyrogenic reactions; in the last analysis it was the inability of this firm to produce, regularly, an acceptable preparation which caused them to lose interest in trying to make this agent available at that time for therapeutic purposes in the United States. However with the preparations that were well tolerated, it was possible for Johnson and McCarty (1958) to demonstrate that the lysis of experimentally induced thrombi in the arm veins of human volunteers could be achieved through the intravenous administration of streptokinase, and for our group (Fletcher *et al.*, 1959) to show that this agent could successfully mediate the lysis of spontaneously occurring venous and arterial thrombi without undue hazard to the patient. In addition, we undertook the first trial in acute myocardial infarction; this limited study made no therapeutic claims but demonstrated the feasibility of using streptokinase in this disorder.

Thus by 1960, at a time when the clinical investigation of streptokinase ground to a halt in the United States because of the termination of Lederle's interest, its potential for therapeutic thrombolysis had been established. In addition, the nature and extent of the associated coagulation defect was reasonably well defined as was the elaboration of a number of pharmacological considerations, namely, the need for a loading dose to overcome the initial resistance, and a sustaining infusion to maintain a high level of circulating activator. However since patients varied considerably in resistance to streptokinase, presumably because of differences in their acquired antistreptokinase levels, it was our belief that proper use of this agent would require preliminary titration of the patient's blood as well as continuous monitoring of the fibrinolytic activity and coagulation defect as as to insure that

the desired effects were being achieved *in vivo*. The difficulties posed by these latter considerations, as well as the antigenicity of streptokinase, led us to turn our attention to urokinase, to which we will return later.

However, several of the difficulties which concerned us in these early days were overcome when the European firms, Kabi and Behringwerke, undertook the further development of streptokinase in the early 1960's. They improved on the quality of the preparations and minimized its pyrogenicity; this allowed them to make the agent available for clinical use. Equally important was that various European investigators helped solve other problems. For example, Verstraete and his associates (Verstraete *et al.*, 1966) introduced the concept of a fixed dose regimen. By using a large loading dose of streptokinase followed by a high sustaining infusion rate, the resistance of 90% or more of the patient population could be overcome, activator activity was maintained at a high level, and the period of brisk hyperplasminemia, which occurs during the phase of rapid activation of plasma plasminogen, was shortened. This allowed one to eliminate most of the complexities associated with the therapy, i.e., dose titration and careful laboratory monitoring both of the thrombolytic activity and the coagulation defect. These simplifications in the use of streptokinase made thrombolytic therapy with this agent practical for physician use. Furthermore it allowed for the extensive clinical studies which have taken place during the past decade, not only in Europe, but in other parts of the world as well; these continue to define and refine the indications for its use.

A vast literature has now accumulated on the use of streptokinase in a variety of clinical situations. Under certain circumstances, e.g., extensive deep vein thrombosis, life threatening pulmonary embolism or peripheral arterial thromboembolism, streptokinase has proven to be a useful adjunct in the initial management of many patients. More controversial is its usefulness in the management of acute myocardial infarction, and currently recommended regimens are not indicated for the management of an acute cerebro-vascular accident. Unfortunately, these are hazards associated with the use of streptokinase; these include an increased risk of bleeding complications, pyrogenic responses and occasionally, an anaphylactoid-like reaction.

It is these risks which have limited its more general use, and as will be discussed later, the challenge is to develop schedules which routinely are more effective therapeutically than current ones and/or minimize the bleeding hazard. Even should this be accomplished, streptokinase will remain a very potent antigen, and those problems which are related to its antigenicity are likely to persist; accordingly, the impetus for the development of urokinase arose from the desire for a non-antigenic activator of the fibrinolytic enzyme system. Its presence in human urine had been discovered independently by Williams (1951), Astrup and Sterndorff (1952) and Sobel *et al.* (1952) in the early 1950's, but it was not until 1957 when Ploug and Kjeldgaard published on the isolation and purification of this *human* activator that such a consideration became practical. Thereafter several pharmaceutical firms undertook the task of further purifying this material for clinical investigation, and Lesuk and associates (1965) reported the crystallization of urokinase. In 1963, the Committee on Thrombolytic Agents of our National Heart and Lung Institute, set up a standard for urokinase which subsequently served as the basis for the development of an International standard by Bangham and his group at Mill Hill; we shall hear more about this from him later.

This committee also defined the nature of a preparation which they would consider suitable for intravenous administration and human study. Such preparations were then made available about 10 years ago. The initial investigations were aimed at elaborating the pharmacological principles for its use in man, demonstrating its activity in instituting an appropriate thrombolytic state; and documenting its ability to dissolve thrombo-emboli. The initial studies reported by our group (Fletcher *et al.*, 1965) were very encouraging: there did not appear to be great variation in patient responsiveness when the agent was administered on a dose per body weight basis; an intense thrombolytic state could be achieved with a much milder coagulation defect than occurred with streptokinase; no pyrogenic or allergic reactions were noted; and no antibodies resulted from its administration. In effect, many of the problems initially encountered with streptokinase could be circumvented with urokinase, and therapy with this agent could be carried out simply on a dose-weight basis without the need for laboratory monitoring or control. As with streptokinase, Johnson and associates (1964) provided evidence that urokinase administered intravenously according to an appropriate fixed dosage schedule, dissolved experimental thrombi in the forearm of human volunteers, and we and others provided evidence that venous and arterial thrombo-emboli also could be lysed by urokinase (Fletcher *et al.*, 1965). Since acute pulmonary embolism lends itself to careful study and evaluation, this lesion was chosen for the first quantitative trial of the effects of this agent in man. As will be elaborated on later, by Bell, the data from phase I of the National Heart and Lung Institute's Urokinase-Pulmonary Embolism trial (1973) provided unequivocal evidence that 12 h of urokinase administration, in contrast to heparin, was associated with accelerated lysis of acute pulmonary emboli, improvement in pulmonary capillary perfusion and restoration of the hemodynamic abnormalities toward normal comparable in degree to those also described for streptokinase. In the second phase (Urokinase-Streptokinase Embolism Trial, 1974), the results of which also will be covered in Bell's presentation, a comparison was made between the effects of 12 and 24 h of urokinase and 24 h of streptokinase therapy. In brief, it was observed that all three regimens were effective; furthermore they produced equivalent benefits and only relatively minor differences existed among them. Thus we now have evidence of the comparability of these two agents, at present therapeutic regimens, for inducing effective thrombolysis *in vivo*.

Observations also exist on the use of urokinase in deep vein thrombosis, arterial thrombo-embolism, occlusive retinal vascular disease, acute myocardial infarction and cerebral vascular thrombosis. Some of these will be described in the various presentations dealing with urokinase therapy. Such studies have substantiated the ability of urokinase to induce quite regularly an active thrombolytic state *in vivo* and, in many instances, to achieve an extensive or complete lysis of an occluding thrombo-embolus; also confirmed has been the non-antigenicity of urokinase in man as well as its virtual freedom from inciting adverse reactions, except for bleeding.

At the present time it is difficult to document whether urokinase has virtues over streptokinase, other than for its non-antigenicity and freedom from those adverse reactions peculiar to streptokinase. Thus, at least, urokinase provides an acceptable alternative to streptokinase as a useful adjunct in the management of those thrombo-embolic disorders currently considered as indications for therapy with thrombolytic agents.

Unfortunately, the clinical investigation of urokinase has been hampered by the high cost of production of this activator and its relative lack of availability as compared to streptokinase. However, a number of pharmaceutical firms have developed techniques which should reduce its cost and improve its availability; included among these is the *in vitro* production of urokinase in tissue culture as will be presented by Barlow. Currently several laboratories, including our own, are involved in a pharmacological equivalence study comparing tissue culture urokinase with the urinary product. When these are completed, and assuming the materials are comparable in all aspects, the clinical investigation of urokinase in the United States should move forward at an accelerated pace.

While studies on the clinical evaluation of streptokinase and urokinase in various disease states will continue for many years to come, it has become increasingly apparent from the patient observations already made that many questions require further investigation if the full potential of therapy with these agents is to be properly exploited; such problem solving must concern itself with developments aimed at increasing the effectiveness of the lytic treatment and at diminishing the bleeding hazard. For example, if urokinase and streptokinase were almost always successful in completely or extensively dissolving an occluding thromboembolus and restoring the circulation to normal or near normal, rather than achieving a success rate of 50% or so, as usually reported in most studies, there would be much less hesitation to use these agents for initiating the treatment of all thromboembolic episodes even in the face of an increased bleeding risk. Alternatively the current success rate would be considered quite acceptable if the therapy could be carried out without hazard. Therefore the incidence of successful thrombolysis and the risk of bleeding are often overlapping considerations which currently restrict the widespread acceptance of thrombolytic therapy and limit the indications for its use even in those situations where its value has been demonstrated. While time will not permit an extensive discussion of the developmental requirements for improving thrombolytic therapy, several of the more important considerations deserve comment.

A fundamental problem which undoubtedly influences therapy is the nature of the fibrin substrate which we are attempting to lyse. Fibrin during its formation first undergoes extensive polymerization both end to end and side to side; this occurs spontaneously once the fibrinopeptides are split from the fibrinogen molecules through the action of thrombin. This polymerized fibrin is readily digested and solubilized by plasmin, and clots made up of this type of fibrin can be lysed readily. However, as you know, thrombin also activates Factor XIII, the fibrin stabilizing factor, and this activated enzyme mediates crosslinking between the gamma and alpha chains of the fibrin molecules. Crosslinked fibrin is much more resistant to the action of plasmin, and clots containing this type of fibrin are much more difficult to lyse. While in the laboratory it is possible to prevent the crosslinking of fibrin through the use of simple competitive and non-competitive inhibitors, it has not been possible to unhinge crosslinking once it has occurred. Nevertheless this should be a goal for further investigation since a method for its accomplishment, if it could be applied *in vivo*, may profoundly influence the effectiveness of thrombolytic therapy.

Of more concern from a practical standpoint for improving the efficacy of *in vivo* thrombus dissolution is whether the *in vitro* measurements we have been making are appropriate to our needs. For example, in phase I of the Urokinase-

Pulmonary Embolism Trial (1973), no correlation existed between the various assessments of the resolution of a pulmonary embolus by urokinase and the observations made on circulating fibrinolytic activity, activation of plasma plasminogen and the fall in fibrinogen level. This lack of correlation is disturbing for it indicates that our present tests are not predictive of what will or will not occur in the patient, yet they served as the basis for the development of current therapeutic regimens. Thus, if we are to explore revisions in them, we would be aided immeasurably by tests which would correlate closely with resolution rates *in vivo*. Some of these may be close at hand. For example if confirmation can be obtained of the observation of Millar and Smith (1974), to be presented later, that technetium labelled urokinase rapidly localizes in preformed thrombi, not only could a test based on this observation prove useful for diagnostic purposes, but it might provide for a simple non-invasive procedure for the serial quantitation of resolution rates *in vivo*. Also the availability of a radioimmunoassay for d-dimer in plasma, which is under active development by Budzynski and Marder, could provide us with a measurement of the amount of crosslinked fibrin which is undergoing dissolution.

This matter of the need for simple but reliable techniques which would be indicative of *in vivo* events and allow for improvements in currently recommended schedules or the testing of new ones, other than through the laborious procedure of clinical trials or empirical observations, also was underscored by the findings in phase II of the Urokinase-Pulmonary Embolism Trial (1974). Here, twenty-four hours of urokinase therapy produced very little additional resolution of pulmonary emboli over that achieved with twelve hours of therapy. One can predict that most of the resolution which, on the average amounted to about 50% of the embolic material, was achieved in the first few hours, and thereafter relatively little additional thrombolysis occurred. Since the principles involved in developing a therapeutic regimen for urokinase were no different from those employed for streptokinase, it is likely that similar considerations pertain when the latter agent is used.

Thus, the time has come when we must actively investigate whether by altering or modifying dosage schedules or using different approaches, significant improvements in the efficacy of lysis can be achieved. While the availability of new tests would be most helpful for stimulating progress, it is encouraging to note that several leading scientists are already hard at work on this problem. For example, Cade and his associates (1974) have reported that low doses of streptokinase, i.e., one-tenth of the amount currently used, when combined with heparin therapy so as to prevent new fibrin formation, achieves as effective a lytic result as that accomplished with high dose streptokinase alone. To my knowledge this has not been tested for urokinase. Under any circumstance, the low dose streptokinase-heparin combination is unlikely to offer any major advantages; it does not provide for any improvement in effect, the bleeding hazard is not significantly reduced and the lysis is probably accomplished more slowly.

More exciting is the recent report of Kakkar and associates (1973) on a new approach to streptokinase therapy. In twelve patients with extensive deep vein thrombosis, the administration of a four to six hour infusion of plasminogen followed by 600,000 units of streptokinase given over a half-hour period only, and repeated on four successive days, resulted in complete lysis of all thrombotic material in eight patients and partial but extensive lysis in the other four. These

observations are better than previously reported with streptokinase alone, and currently a multicenter trial is underway to evaluate this new therapeutic scheme, particularly since hemorrhagic complications were not observed. Certainly if this regimen works more effectively for streptokinase, it deserves study with urokinase as well.

Another concern relates to the bleeding problem, a risk which currently limits the application of lytic therapy in the post-operative state and other instances where this hazard may outweigh the clinical benefits which can be achieved. In the earlier work with streptokinase, the bleeding hazard was attributed to the brisk hyperplasminemia often seen in the early treatment phase; fibrinogenolysis was extensive and large quantities of fibrinogen degradation products accumulated in the circulation and were claimed to be responsible for impaired platelet function and the coagulation defect. Urokinase, in the dosages currently being utilized, activates plasma plasminogen at a significantly slower rate and to a lesser degree extent than observed during streptokinase therapy; consequently fibrinogenolysis is not as extensive, fibrinogen breakdown product concentrations are lower and the coagulation abnormalities milder. Yet the bleeding risk with urokinase therapy has been as great as that encountered with streptokinase. Accordingly other factors, possibly more important than those previously described, are likely to be involved in the bleeding diathesis associated with thrombolytic therapy. These should be identified and, if they can be prevented or controlled without sacrificing the ability to lyse thrombi, then the acceptance and usefulness of lytic therapy would be considerably extended. In this regard, Niewiarowski's recent observation (1973) that the platelet release reaction can be induced by plasmin, and that the loss of this important hemostatic function in animals receiving streptokinase can be prevented by the prior administration of dipyradimole is worth investigating in man.

Finally, a brief comment on the problem of clinical evaluation. The establishment of clinical benefit is an increasingly arduous task. Nowhere is this more evident than in the various trials aimed at evaluating whether lytic therapy has a role to play in the management of acute myocardial infarction. For a number of reasons, mortality studies will not easily provide us with an answer. If the aim of thrombolytic therapy is to reduce the size of a cardiac infarct, then we must first devise an acceptable method for measuring infarct size in the living patient; then, it may become possible to demonstrate in a logistically feasible, reasonably sized, well-controlled trial whether lytic therapy is or is not a useful adjunct in the management of this disorder. A similar consideration most likely will pertain to its eventual application to the stroke problem.

In concluding, I would remind you that, viewed in perspective, thrombolytic therapy has come a long way in a relatively short period of time. It is only thirty years since the first foundations were laid for the investigation and appreciation of fibrinolysis as a biological event of importance both for human physiology and pathology. And, as for thrombolytic therapy, this has become a subject of considerable interest only in the past decade or so. Compare that with our first knowledge of blood clotting, and of the many years which followed before anticoagulants were successfully introduced into clinical medicine.

Today, thrombolytic therapy with streptokinase or urokinase, already has emerged as a practical and useful method for the management of certain forms of thrombo-embolic disease. This by itself is a considerable accomplishment.

And, as for the future, this is bright indeed. We know what the problems are, and though we have not solved or circumvented them all as yet, we certainly can be optimistic that, in time, they will be coped with very well indeed. While anticoagulants or other antithrombotic agents always will be useful for preventing thrombus growth or embolization, optimal treatment of such pathological events should include their initial lysis and rapid restoration of the circulation. The ultimate benefits to be derived from this approach to management must await a more perfect form of therapy; and, I am confident that we will achieve this in the not too distant future.

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THE PHYSIOLOGY OF FIBRINOLYSIS

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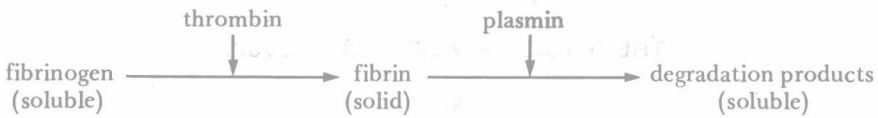
Fibrin formation is an early phase of tissue repair. When tissues are injured an exudative process ensues, by which the fibrinogen-containing exudates undergo coagulation and form fibrinous deposits. The solid deposit provides a barrier against hemorrhage or continued exudation. In this manner the formation of fibrin deposits functions as a mechanism by which the organism tries to restrict the damage to the living tissue and to prevent injurious effects on adjacent areas, including the loss of fluids from the blood to the surface or into the interstitial space. Secondly, the fibrin deposit forms the matrix for the subsequent tissue repair process. Fibroblasts migrate into the fibrin layer accompanied by capillaries and resulting in the formation of reparative connective tissue. Later the vessels undergo involution and scar tissue is formed.

In this pattern of normal tissue repair thromboplastic agents from injured cells or formed by components of the humoral system (intrinsic activation) induce the formation of fibrin. The subsequent dissolution of the fibrin deposits is chiefly caused by the fibrinolytic system. Fibrinolysis is induced by activators released from injured cells or produced in the humoral system. These activators convert the humoral zymogen: plasminogen, to the active blood protease: plasmin. Fibrinolysis acquires its physiological significance by its role in the regulation of fibrin deposits in the body. In this manner fibrinolysis is a mechanism of major importance in the regulation of connective tissue formation during tissue repair. Fibrinolysis also influences the permeability of certain membranes by its dissolving action on a fibrinous layer. This chapter intends to give a brief description of the physiological significance of fibrinolysis. Several selected examples will illustrate the role of fibrinolysis in the organism. Specific problems related to urokinase and to fibrinolysis in the urinary tract will be discussed in more detail.

* Current address.

1. The Hemostatic Balance

The balance between the fibrin formation and the dissolution of the resulting deposits of fibrin was termed the hemostatic balance (Astrup, 1958, 1959, 1965a). This term was selected because of the obvious role of fibrin formation and fibrin dissolution in the process of hemostasis. However, as indicated in the introduction, this balance has a much broader physiological role than solely in the regulation of the hemostatic process. It is evident, and was pointed out by Pierre Nolf already in 1905, that fibrin formation cannot be the true physiological endpoint of coagulation because the fibrin must be removed again in order to restore normal conditions. It is this balance which lends to fibrinolysis the role of a funadmental process in physiology. Schematically the balance can be depicited as follows:



It is believed that the major role of this balance consists in the regulation of tissue repair as described in the introduction. An indirect support of this concept was provided when Beck and his associates observed that wound healing and the growth of fibroblasts are impaired when the fibrin-stabilizing factor (Factor XIII) is lacking (Beck *et al.*, 1961, 1962). The insufficiency of a non-crosslinked fibrin as a matrix for normal cell growth and wound healing is a good indication of the importance of a fully-stabilized fibrin in the early phase of tissue repair. The findings support the role of the hemostatic balance in the regulation of fibrin deposition during normal tissue repair. It should perhaps be pointed out, that we are talking about tissue repair following tissue damage with little or no inflammatory reaction so that the influence of leukocytic immigration is insignificant. This is the case in the healing of the minute tissue injuries without contamination by bacteria or other foreign matter, which each of us sustains during our normal life. The same process occurs in the involution of organs during embryogenesis or following changes induced by hormones. We call this process "physiological tissue repair" to distinguish it from the process of healing in which a defence against foreign, toxic matter (such as bacteria) is involved, leading to an activation of the immune system and other defence mechanisms, including the participation of white cells – reactions which in themselves characterize this injury and healing process as belonging to the realm of pathology. As a matter of fact, already in 1907 Opie noticed that leukocytes disappeared from a fibrinous exudate before the fibrin had been dissolved (Opie, 1907). We observed the same phenomenon when a homologous plasma clot was implanted in the rat (Kwaan and Astrup, 1964). A mild tissue reaction followed, with little leukocytic attraction. Later, fibroblasts and highly fibrinolytic capillary endothelial cells migrated into the clot followed by resolution of the fibrin and formation of connective tissue. Resolution was delayed when inhibitors of fibinrolysis were present, and an increased amount of connective tissue was formed (Kwaan and Astrup, 1969). In contrast, healing of a mild injury to rat skin is very rapid because of the rich vascularization and the high fibrinolytic activity of the vessels in the skin of the rat (Tympanidis and Astrup, 1972). Additional examples of the influence of the fibrinolytic activity of tissues on repair processes will be mentioned in the following sections.