The Reaction between Streptokinase and Human Plasma

MOGENS LASSEN

Biological Institute of the Carlsberg Foundation, and University Institute of Physical Chemistry, Copenhagen, Denmark

1. The amount of activator formed from a given amount of streptokinase mixed with an excess of proactivator depends on the preparation of proactivator used. A probable explanation is that streptokinase is consumed by two competing reactions, the relative rates of which depend on the preparation of proactivator. The first reaction leads to the formation of the activator; the other is mentioned below (2).

2. Streptokinase is destroyed by small amounts of plasma. The reaction is probably catalyzed by the plasmin formed.

3. The reaction between streptokinase and proactivator initially leads to the formation of activator which is relatively stable at extreme pH values. The rapid transformation of the stable into the well known labile modification is probably catalyzed by the contaminating plasmin, the reaction resembling that mentioned above. Both reactions can be considered side reactions in so far as they are not involved in the formation of the activator.

The mixing of human plasma with the extracellular streptococcal product termed streptokinase results in the formation of a proteolytic enzyme termed plasmin. The following schemes summarize our present knowledge of the mechanism of this reaction:

References and detailed informations are given by Lassen ¹. Yet, it must be emphasized that there is some uncertainty on the nature of the proactivator and the plasminogen, since it is not known whether we are concerned with two different compounds, or with a single precursor which can participate in both reactions. From the results of this paper it is apparent that large systematical errors may interfere with the estimations of proactivator and of streptokinase. Therefore, the author does not feel absolutely sure that the results of the stability experiments previously reported (Lassen ²) substantiate the two component-theory; but to facilitate the discussion he wants to keep the double terminology.

The present paper deals with the reaction possibilities of the streptokinase after mixing with human plasma, including the first of the above reaction schemes, but excluding the last. The problem mentioned above is of minor importance for the conclusions drawn as the main assumptions are:

1. Activator and plasmin are chemically different. This assumption is based upon the fact that the activator can be destroyed without destroying the plasmin (Müllertz³).

2. When streptokinase and human plasma are mixed at neutral pH, activator as well as plasmin are always formed.

3. The activator is formed through a stoichiometric reaction between streptokinase and a plasma factor (termed proactivator). The most striking evidence for this mode of reaction was given by Troll and Sherry⁴, and in essentials their experiments were reproduced by Lassen (Ref.⁵, and present paper).

MATERIALS AND METHODS

Veronal-gelatin buffer. 0.05 M sodium diethyl barbiturate buffer pH 7.75 to which was added NaCl to give a final ionic strength of 0.15. Furthermore gelatin to a concentration of 0.2 % was added in order to prevent adsorption of the fibrinolytic factors on glass surfaces (Lassen⁶).

Streptokinase (SK). "Varidase" was kindly placed at my disposal by the Lederle Laboratories Division, American Cyanamid Company. The units are those stated on the packages as determined according to Christensen⁶.

Proactivator (PA). Human plasma or serum or acetone powders of these prepared as described by Lassen¹.

L-Lysine ethyl ester (LEe). The dihydrochloride of this compound was prepared according to the method of Werbin and Palm⁷.

Witte peptone (WP). A commercial product (made by pepsin hydrolysis of bovine fibrin). Before use the powder was dissolved in veronal buffer, heated at 100° for 15 min, and cleared by centrifuging.

Measuring methods. The activator was determined by the lysis time method as described by Lassen⁸. The reported methods for measuring the precursors were modified by adding an equal volume of a 10 % solution of WP to the incubation mixtures of SK and PA. The advantage of this addition will be obvious from the experimental results presented in section 1.

RESULTS

1. Amount of activator formed in mixtures of its precursors.

The rate of formation. These experiments are preliminary investigations made in order to find an appropriate time of incubation usable in "equilibrium" studies. The rate of formation of the activator (A) was found to depend largely on the preparation of proactivator (PA) used. When different plasma samples were mixed with streptokinase (SK) in concentrated solutions the time necessary for obtaining maximum activity varied from 0 to 7 min at room temperature. With serum, the time was prolonged to 10 to 25 min. The rate of deterioration of A was found to depend mainly on the proportion of SK and PA, most commonly being at a maximum with equivalent amounts. Fig. 1 shows the results obtained by estimating A at different times in concentrated and diluted mixtures of SK with plasma or serum. The curves begin

Fig. 1. Formation and deterioration of activator at room temperature. *Diluted incubation mixtures*: 20 ml veronal-gelatin buffer + 500 units of SK + 100 µl plasma (Curve 1) or serum (Curve 2). A was estimated without further dilution. *Concentrated incubation mixtures*: 500 µl plasma (Curve 3) or serum (Curve 4) + 500 µl buffer containing 2 500 units of SK. 50 µl aliquots were diluted with 5 ml buffer before the estimation of A.

above zero concentration, indicating that the formation of A continues during the lysis time determinations. Accordingly, the experimental results do not represent the true variation of the concentration of A in the incubation mixtures.

However, an impression of the "time of formation" is obtained, and since concentrated mixtures of SK and PA generally form maximum amount of A within 20 min at room temperature, this incubation time was used in the experiments described below.

Relationship between the concentration of activator formed and the concentrations of streptokinase and proactivator used. Müllertz³ estimated the concentration of A formed in different mixtures of SK and human globulin and found it independent of the concentration of the precursor in excess and proportional to the concentration of the other precursor. The results obtained by investigating the relationship between the concentration of lysine esterase and of SK (Lassen¹), and between the concentration of A and of SK (Lassen⁵) are in accordance with the results of Müllertz. However, later experiments have shown that the results depend largely on the precursor preparations used. Thus, the results presented below differ considerably from those obtained by Müllertz, and show the reaction to be much more complex than assumed earlier. In Fig. 2 A the concentration of SK was constant and the concentration of PA (plasma, curve 1 and 2; serum, curve 3 and 4) was varied, and in Fig. 2 B PA

Fig. 2. Relationship between the concentration of activator and of PA (A) or SK (B) in solutions containing a constant concentration of the other precursor. All mixtures were incubated for 20 min at room temperature before estimating A. The mixtures contained: A: 625 units of SK per ml + the concentration of plasma acetone powder (Curves 1 and 2) or serum acetone powder (Curves 3 and 4) denoted on the abscissa. The mixtures used for the curves 2 and 4 contained additionally 25 mg of WP per ml. B: The concentration of SK denoted on the abscissa + 10 mg of plasma acetone powder (Curves 1 and 2) or 10 mg of serum acetone powder (Curves 3 and 4). The mixtures used for the curves 2 and 4 contained additionally 25 mg of WP per ml.

(plasma, curves 1 and 2; serum, curves 3 and 4) was constant and SK varied. For the curves designated 2 and 4, 2.5 % Witte peptone (WP) was present in all incubation mixtures. These curves resemble the curves obtained by Müllertz, but those without WP differ. The main differences in the shape of the curves are the obviously smaller equilibrium constants and the much more asymmetric behaviour of PA and SK in the experiments without addition of WP. The principal difficulties in understanding these curves become obvious by comparing the "saturation values" in the curves in Fig. 2 A. These values are the maximum amounts of A obtained in mixtures containing an identical amount of SK and excess of PA. If the large differences found are real (that is; really different amounts of A are present in the solutions) it means that the assumption that we are concerned with a simple equilibrium system must be wrong, since this assumption would require identical "saturation values" with different preparations of PA. That the difference between curves 1 and 2 is real is revealed by the experiment represented by Fig. 3. The figure shows

Fig. 3. At room temperature 75 mg of plasma acetone powder dissolved in 1 ml buffer were mixed with 1 000 units of SK dissolved in 1 ml buffer. At different intervals of time 100 μ l aliquots were withdrawn and added to either 10 ml buffer for immediate estimation of A (Curve 1) or to 50 μ l 10 % WP. After 20 min at room temperature the last samples were diluted with 10 ml buffer, and A was estimated (Curve 2).

that the effect of WP approaches zero, coincident with the completion of the reaction leading to the formation of A, and that WP is without effect on the activity of A already formed.

The stabilizing effect of WP. In addition to its activity-increasing effect WP was also found to have a stabilizing effect on A made from plasma but not on that made from serum. Table 1 shows that A in the case of serum with and without addition of WP, and in the case of plasma without addition of WP is very labile at 37° and nearly disappears within 24 h. By contrast A is nearly stable for 24 h at 37° in the case of plasma plus WP.

Calculation of the equivalent amounts of proactivator and streptokinase. This expression is defined on the basis of the following: From "saturation values" obtained with excess of one precursor in the presence of WP (curves 4 in Fig. 2) the maximum amounts of A which could be obtained from 1 ml of plasma (or from 1 mg of plasma acetone powder) and from 1 unit of SK were calculated. The proportion between these maximum amounts gives the equivalent amounts in units of SK per ml of plasma (or per mg of plasma acetone powder). Due to the uncertainty about the meaning of this value it has been used only for qualitative evaluations of the proportion between SK and PA in given mixtures. With six different plasma samples the equivalent amounts were found to be within the range of 3 500 to 6 000 units of SK per ml plasma.

Discussion. The results presented in this section indicate that the reactions which are initiated by the addition of SK to plasma or serum are much more complex than hitherto assumed. Especially, it must be emphasized that the results in Fig. 2 disagree with the simple introductory scheme. The assumption that A is formed through a stoichiometric reversible reaction between PA and SK or modifications of these compounds is probably still valid, since

Table 1. The stabilizing effect of Witte peptone.

The following solutions were incubated at 37°.

1. 100 μ l plasma + 100 μ l SK solution containing 10 000 units per ml + 200 μ l veronal-gelatin buffer.
2. 100 μ l plasma + 100 μ l SK solution as above + 200 μ l 10 % WP.
3. 100 μ l serum + 100 μ l SK solution as above + 200 μ l veronal-gelatin buffer.
4. 100 μ l serum + 100 μ l SK solution as above + 200 μ l 10 % WP.

Incubation mixture	Incubation time	Relative amount of activator
1	2 min	45
2	»	98
3	»	28
4	»	32
1	50 »	19
2	»	102
3	»	13
4	»	16
1	24 h	0.2
2	»	97
3	»	0.5
4	»	0.5

this assumption is strongly supported by experiments presented by Troll and Sherry⁴. However, it is evident that this reaction and the subsequent conversion of plasminogen into plasmin are only two of more reactions which are initiated by the mixing of SK and plasma. As an example the different "saturation values" of curves 1 and 2 in Fig. 2, A can most easily be explained by assuming that, while perhaps all SK is used for the formation of A when mixed with excess of plasma + WP, a considerable part of the SK must be

Fig. 4. Destruction of SK with small amounts of plasma. Curve 1: 1 ml of a solution containing 10 000 units of SK + 1 ml 20 % plasma were incubated at 25°. Curve 2: The same mixture as used for curve 1, but incubated at 37°. Curve 3: As for curve 2, but another plasma sample had been used.

consumed by another reaction when WP is omitted. More detailed investigations of this problem will be presented in the subsequent section.

2. Destruction of streptokinase.

This section deals with the possible side reaction mentioned in the above discussion. Below is shown that SK after mixing with plasma is consumed by two competing reactions; the first being the known reaction leading to the formation of A while the second leads to the destruction of SK. SK dissolved in veronal-gelatin buffer was found to be stable for hours at all temperatures below 50°. However, when small amounts of plasma or serum were added SK was found to be labile (Fig. 4). Comparing curves 1 and 2 of Fig. 4 it is apparent that for a rise of 10° the rate of reaction is increased tenfold.

In the next experiment (Fig. 5) solutions of SK were mixed with increasing small amounts of plasma acetone powder and incubated for 60 min at 37°, and the remaining concentrations of SK were then estimated. Although the reaction rates cannot be evaluated from these 60 min values it is evident however, that with small amounts of plasma acetone powder, the reaction rate is more than doubled when the concentration of plasma is doubled. With higher concentrations of plasma acetone powder this phenomenon is less significant, but a limited reaction rate was not obtained within the concentration range used.

Determination of SK destroying factor. The SK destroying factor present in plasma can be measured by determining the destroying effect in defined manner, provided that the relationship between concentration and effect is known. The following method was found convenient: The experimental sample was diluted to give a concentration of PA equivalent to less than 500 units of SK per ml. By reason of accuracy the concentration of destroying factor

Fig. 5. Destruction of SK at 37° with small amounts of plasma. Mixtures containing 5 000 units of SK per ml and the concentrations of plasma acetone powder denoted on the abscissa were incubated for 60 min at 37°, and the residual concentrations of SK were then estimated.

must be within the range of 2 to 10 % of that found in plasma. 50 μ l of the diluted sample was incubated for 60 min at 37° with 50 μ l of a solution containing 5 000 units of SK per ml, which is a large excess of SK. The SK not destroyed was then converted into activator by adding 100 μ l plasma, which now provides an excess of proactivator, and incubating for 20 min at room temperature. Finally, 10 ml veronal-gelatin buffer was added, and lysis time determined as described. Lysis times were converted into concentrations of destroying factor by interpolation on a standard curve obtained by testing serial dilutions of plasma as described for the experimental sample. Good results could be obtained within the range of 2 to 10 % of undiluted plasma (before mixing with SK).

Attempts to identify the streptokinase destroying component. 1 a. Plasmin (Müllertz ⁹) and A (Müllertz ⁹, Lassen ⁵) are adsorbed on fibrin. To find out whether the SK destroying factor is also adsorbed the following experiment was performed: 1 ml of plasma was mixed with 1 ml of a solution containing 5 000 units of SK (nearly equivalent amounts) plus 50 μ l thrombin solution. After incubation for 20 min the fibrin clot was removed. Estimations revealed that plasmin, A, PA, and SK destroying component were completely absent from the serum (plasmin was estimated by the manometric method described by Lassen ¹).

1 b. In a control experiment in which no SK was added only slight differences between plasma and serum were found. The activities in serum expressed as percentages of the corresponding activities in plasma were: PA 55 %, plasminogen 90 %, and SK destroying component 70 %. Plasminogen was measured by adding an excess of SK and estimating the plasmin activities as above, ignoring the effect of plasmin inhibitors. These two experiments show that the SK destroying component resembles plasminogen and PA by being present in plasma as a precursor which is not adsorbed to fibrin, and which is transformed into another component by addition of SK; this component is strongly adsorbed to fibrin.

2. SK was found to be rapidly destroyed by preparations of human as well as bovine plasmin. The human plasmin was prepared according to Müllertz ³, but since the preparation contained considerable amounts of PA the observations obtained with this did not give further information as to the nature of the destroying component. The bovine plasmin was prepared according to Lassen ¹ with the modification that a smaller amount of activator was employed (100 μ l human plasma + 1 000 units of SK (excess) to 10 ml of 1.3 % of bovine fibrinogen). The plasmin activity of the final preparation was compared to that of SK activated human plasma. Assuming no inactivation of the original amount of A present in the preparation the proportion A/plasmin was about 1/50 of that in SK activated human plasma. Solutions of bovine plasmin containing the same plasmin activity as did SK activated human plasma were found to destroy SK somewhat faster than did human plasma.

3. Inhibition studies: The destruction of SK by human plasma was found to be inhibited by L-lysine ethyl ester (Fig. 6) by WP (Fig. 7), by benzoyl-L-arginine ethyl ester and, to a smaller extent, by casein. The synthetic esters are known to inhibit plasmin and A (Troll and Sherry ⁴, Lassen ¹), and in the

Fig. 6. The effect of LEE on the destruction of SK with small amounts of plasma at 37°. The following mixtures were employed. Curve 1: 1 000 units of SK dissolved in 100 μ l buffer + 100 μ l 10 % plasma + 100 μ l 0.3 M NaCl. Curve 2: As for curve 1, except that 100 μ l 0.1 M LEE were substituted for the NaCl solution.

lysis time method WP and casein were found to inhibit the fibrinolytic effect of bovine as well as human plasmin. Contrary to the inhibition of plasma neither WP nor casein seem to inhibit A as judged from preliminary experiments on the activation rate of bovine plasminogen in the presence or absence of these compounds. The formation of plasmin was followed by the semi-quantitative method described by Lassen¹.

Conclusion. SK is destroyed by small amounts of plasma. Studies on the adsorption on fibrin indicate that the destroying factor is identical with plasmin and/or activator. The isolated effects of human plasmin and activator on SK could not be studied as the necessary preparations were not available. However, the fact that the SK destroying activity of bovine plasmin is comparable to that of human plasma gives some evidence for the assumption that the destruction of SK caused by human plasma, is due to the proteolytic effect of the plasmin formed. This assumption was further supported by inhibition studies. Accordingly, the reaction may be described by:

As emphasized in the discussion of the preceding section the existence of a reaction like this is a necessary condition for explaining the fact that the maximum amount of A which can be formed from a given amount of SK depends on the source of PA (Fig. 2 A). The amount of A formed in a given mixture of PA and SK depends on the original concentrations of the precursors

Fig. 7. The effect of WP on the destruction of SK with small amounts of plasma at 37°. The following mixtures were employed. *Curve 1*: 200 units of SK dissolved in 200 μ l buffer + 200 μ l 10 % plasma + 200 μ l veronal buffer. *Curve 2*: As for curve 1, except that 20 mg of WP were dissolved in the buffer solution.

as well as on the relative rates of the two competing reactions; namely the stoichiometric reaction leading to the formation of A and the above mentioned destruction of SK which is probably caused by the plasmin formed. The formation of A proceeds at a slower rate in serum than in plasma and supposing nearly identical rates of destruction the different "saturation values" of curve 1 and 2 in Fig. 2 A becomes understandable. The effect of WP on the "saturation values" can at least partly be explained by its inhibitory effect on the destruction of SK.

3. The transformation of stable into labile activator.

Troll and Sherry ⁴ found that a transformation of A into PA could be achieved by heating at 100° for 15 min at pH 2. Since SK is labile under these conditions they assumed the reaction to be caused by the isolated destruction of SK, leaving the more stable PA unchanged in the solution. Lassen ⁵ found the same transformation to proceed at milder conditions (37° and pH 3.5). The inactivation did not run to completion as a certain part of A was found to be stable under the experimental conditions used. It was later found that the same reaction can proceed at alkaline pH. Fig. 8, curve 1, shows the inactiva-

Fig. 8. Inactivation of activator in acid and alkaline solutions. Ordinate: Concentration of A (full lines) or PA plus A (dotted line) in per cent of the untreated solutions of A. The activator solution was prepared by adding 1 ml plasma to 1 ml of a solution containing 5 000 units of SK and incubating for 20 min at room temperature. The inactivation at acid reaction (Curve 1, (O)) was performed by adding 1 ml A solution to 1 ml 0.1 M HCl in water bath at 37°, yielding a pH of about 3. The inactivation at alkaline reaction (Curve 2, (×)) was performed by adding 1 ml A to 1 ml 0.1 M NaOH in an ice bath, yielding a pH of about 13. A was estimated in 100 μ l aliquots diluted with 10 ml veronal gelatin buffer. For the estimations of PA + A 100 μ l samples were diluted with 10 ml-veronal-gelatin buffer containing 500 units of SK. These solutions were incubated for 60 min at room temperature before the lysis time determinations.

tion of A at 37° and pH about 3.0, while curve 2 shows the inactivation of the same preparation of A at 0° and at a pH between 12 and 13. The amount of stable A is seen to be independent of the mode of inactivation. This probably excludes the interpretation that the stable residue represents an equilibrium concentration in a reversible denaturation, and supports the assumption that labile and stable A are chemically different components. SK itself was found to be stable for at least 1 h under the conditions used for the inactivation of A. (At the acid pH this is true only when proactivator or denaturated activator is present in the solution, at the alkaline pH also uncontaminated SK was found to be stable). This makes the situation a little more complicated than assumed by Troll and Sherry⁴. The assumption that the denaturation of the activator at extreme pH values is caused by the isolated destruction of the SK part of the activator molecule may still be valid; but this SK part

Fig. 9. The formation (and deterioration) of A (Curves 2 and 4), and the degree of inactivation at alkaline reaction (Curves 1 and 3) followed with time in mixtures of SK and PA without addition of WP (dotted lines), and with addition of WP (full lines). The experiments were performed at room temperature (22°). For the dotted curves a mixture of 1 ml plasma with 0.5 ml of a solution containing 4 000 units of SK was used. The mixture used for the full drawn curves contained in addition a final concentration of WP equal to 2.5 %. The activator was estimated in 100 μ l aliquots diluted to 10 ml with veronal-gelatin buffer. The inactivations were performed by adding 100 μ l samples to 50 μ l 0.2 M NaOH at 0° . After standing for 10 min at 0° the residual amount of A plus SK was estimated by adding 50 μ l 0.2 M HCl + 200 μ l buffer containing 25 % plasma (v/v) and 10 % WP (w/v), incubating for about 20 min at room temperature, diluting with 10 ml veronal-gelatin buffer, and finally determining the lysis times as described. All results were converted into concentrations of A by interpolation on the same standard curve.

is not identical with the original SK-molecule, as some transformation into a more labile modification must have occurred during the previous incubation at neutral reactions. The nature of the stable residue cannot be evaluated on the basis of the above experiments. We may be concerned either with a stable activator molecule or with a residue of unreacted SK which during the assay reacts with PA to form A.

Since total A and labile A can be measured independently it might be possible to obtain further information of the whole problem by comparing the relative rates of the formation of these quantities, especially if it could be possible to find conditions under which these formation rates differ significantly. Nearly equivalent amounts of plasma and SK were mixed at room temperature and at a neutral pH. At different intervals of time samples of this mixture

were treated at alkaline reaction, and the residual amounts of A plus SK were estimated by neutralizing, adding an excess of proactivator and measuring the total amount of activator formed (Fig. 9, curve 1). On the condition that SK and the activator formed is stable in the neutral mixture of plasma and SK the difference between the ordinate at zero time and that at the time t represents the amount of labile A present in the neutral solution at the time t . Since this condition is not fulfilled the curve obtained does not permit an exact calculation of the amounts of labile A formed, but a rough picture of the rate of formation can be obtained. Simultaneously with the performance of the estimations used for curve 1 the formation and deterioration of A in the original mixture were followed with time (Curve 2). Unfortunately, there is a high initial lability of A, and therefore the curve does give any information of the time of formation. Accordingly, no conclusions can be drawn from the results of the whole experiment.

More valuable information was obtained by adding Witte peptone (WP) to the neutral incubation mixture of SK and plasma. The rate of formation of labile A appears from curve 3 of Fig. 9 which correspond to curve 1 in the above experiment. Obviously the formation of labile A was strongly inhibited by WP, the rate of formation being reduced to about 1/10 of before. The formation of A in the mixture of plasma SK and WP is represented by curve 4. The activator formed is seen to be stable throughout the whole experiment and evidently the formation ceased 2 to 3 min after mixing. At this time nearly all activator was present as a stable modification (stable at alkaline pH), but during the next few hours this modification was transformed into the known labile modification. These results strongly suggest that PA and SK initially react to form an activator which is stable at extreme pH values. In mixtures of SK and plasma, this primarily formed activator is rapidly transformed into a labile modification having the same activating properties. (The transformation of the stable into the labile modification does not alter the activities as judged from curve 4). The rate of the formation of stable A is not significantly influenced by WP, while the secondary transformation is inhibited by this agent.

The effect of L-lysine-ethylester (LEe) was found to be comparable to that of WP. The experiment was performed as described in the legend to Fig. 9, except that 0.05 M LEe was substituted for WP. After incubation for 30 min only 5 % of the total amount of A plus SK were present as labile A. However, after 1 h the rate of reaction increased, and after 3 h only 10 % remained in the form of stable A. This increased rate was accomplished by a drift downwards in pH due to hydrolysis of the ester, and therefore quantitative details other than those given above are probably without significance.

Conclusion. The activator formed by the reaction between SK and PA is extremely labile at acid and alkaline reactions. The experimental results presented above strongly indicate that the reaction sequence leading to the formation of this labile A involves at least two consecutive steps, the first being the stoichiometric reaction between the precursors, during which a more stable modification of A is formed, while the last step is a transformation of the stable into the well known labile modification. The temporary existence of the stable A was demonstrable in mixtures of plasma and SK to which

Witte peptone or L-lysine-ethylester was added. This was possible since these agents proved to reduce strongly the rate of the secondary transformation without significantly affecting the rate of the primary formation of the stable A.

DISCUSSION

The present work is an experimental investigation on the reaction possibilities of streptokinase (SK) after mixing with the fibrinolytic precursor(s) contained in human plasma. It is considered an experimental fact that SK reacts stoichiometrically with a precursor in human plasma termed proactivator (PA) giving rise to the formation of an activator of plasminogen (A) (Müllertz and Lassen ¹⁰, Troll and Sherry ⁴ and Müllertz ³). It seems probable that this reaction can be described by one of the following two schemes:

This reaction is followed by the catalyzed transformation of plasminogen into plasmin:

In all probability the last reaction involves the splitting of a peptide bond (Troll and Sherry ⁴).

The "equilibrium" studies presented in the first section of this paper indicate that these schemes do not represent all reaction possibilities of the precursors concerned, and therefore suggested a search for competing reactions. Evidence for the existence of such competing reactions were obtained in the case of SK as well as in the case of PA (not published) but owing to the irritating situation that the nature of the PA is still unknown a discussion of the last case is omitted. In the case of SK the competing reaction probably consists in a proteolytic splitting of one or more peptide bonds in the SK molecule caused by the plasmin formed, which is not surprising since SK is a protein and plasmin is a proteolytic enzyme. The quantitative importance of this reaction appears from Fig. 2 A. With excess of PA supplied as serum at least 70 % of the SK is consumed by the side reaction and only 30 % by the main reaction leading to the formation of A. Substitution of plasma for serum results in a higher rate of the main reaction and accordingly a smaller fraction of the SK is destroyed by the side reaction.

The last section deals with a reaction which seems to have a certain resemblance to this side reaction. The results show that the reaction between SK and plasma results in the formation of two chemically different modifications of A. One of these is relatively stable at extreme pH values, and may be considered the primary product of the reaction between SK and PA. This modification does have a temporary existence only, since it is rapidly transformed into the labile A ordinarily dealt with. If the above mentioned reaction (1) is valid the formation of labile A may be described by:

During the inactivation of labile A at acid and alkaline pH the sum of A and PA is constant, which means that the reaction consists in a transformation of labile A into PA plus some sort of inactivated SK.

Since stable A as well as the original SK is stable under the conditions used for the inactivation of labile A the difference between stable and labile A must be due to a difference in the SK part of the activator molecule. Still supposing that the reaction (1) is valid the whole series of reactions may be described by:

If reaction (2) is valid the SK molecule must be present — partly or as a whole — in either the A molecule or in the inactive component. In this case more detailed conclusions must be omitted, but the general conclusion that it is this "modified SK molecule" on the right side of the equilibrium sign which is changed during the formation of labile A is still probable.

Since we have already seen that plasmin may attack SK there is some probability that the formation of labile A is due to a similar effect of plasmin:

This probability is further supported by the fact that both reactions are inhibited by L-lysine-ethylester and by Witte peptone.

Acknowledgements. The author is indebted to Dr. Tage Astrup of the Biological Institute and to Dr. Daniel L. Kline, Yale University, U.S.A. for valuable discussions and helpful advice. This investigation was made possible by a grant from *Statens almindelige Videnskabsfond*, Denmark. It forms part of the investigations on fibrinolysis for which Dr. Astrup receives support from the *Josiah Macy, Jr. Foundation*, New York, and the *National Danish Association against Rheumatic Diseases*.

REFERENCES

1. Lassen, M. *Biochem. J.* **69** (1958) 360.
2. Lassen, M. *Acta Chem. Scand.* **13** (1959) 1064.
3. Müllertz, S. *Biochem. J.* **61** (1955) 424.
4. Troll, W. and Sherry, S. *J. Biol. Chem.* **213** (1955) 881.
5. Lassen, M. *Acta Chem. Scand.* **12** (1958) 1825.
6. Christensen, L. R. *J. Clin. Invest.* **28** (1949) 163.
7. Werbin, H. and Palm, A. *J. Am. Chem. Soc.* **73** (1951) 1382.
8. Lassen, M. *Scand. J. Clin. Lab. Invest.* **10** (1958) 384.
9. Müllertz, S. *Mechanism of activation and effect of plasmin in blood* (Diss); *Acta Physiol. Scand.* **38** (1956) Suppl. No. 130.
10. Müllertz, S. and Lassen, M. *Proc. Soc. Exptl. Biol. Med.* **82** (1953) 64.

Received April 29, 1959.

Acta Chem. Scand. **13** (1959) No. 7