These data suggest an explanation for the low plasma levels of epinephrine in patients suffering from phenylpyruvic oligophrenia(5) in that these aromatic acids which accumulate as a result of biochemical lesion may inhibit the synthesis of epinephrine in the adrenal gland.

The importance of epinephrine in normal nerve function is yet to be elaborated; however, its presence in brain tissue is well established(8). Weil-Malherbe has shown that epinephrine plasma levels were significantly lower in a group of mental defectives than in a group of mixed patients(5). As yet, information is not available which would allow us to speculate on the influence of a deficiency of epinephrine on the etiology of mental deficiency.

Summary. Phenylpyruvic acid, phenyllactic acid and phenylacetic acid inhibit beef adrenal medulla DOPA decarboxylase. The implications of these data in phenylpyruvic oligophrenia are discussed.

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Stability of the Activator of Bovine Plasminogen. (22774)

FRANK B. ABLONDI AND JAMES J. HAGAN (Introduced by Brian L. Hutchings)
(With Technical Assistance of Ralph E. Clarke)
Biochemical Research Section, American Cyanamid, Lederle Laboratories, Pearl River, N. Y.

Mullertz and Lassen(1) first reported that a mixture of streptokinase and human plasminogen acquired a characteristic which neither the streptokinase nor the plasminogen exhibited alone, namely, the ability to activate bovine plasminogen. They concluded that bovine blood lacks a factor (proactivator) which is present in human blood and suggested that this proactivator combines with streptokinase to form an activator of bovine plasminogen. The studies of Troll and Sherry(2) and Sherry(3) indicated that the activator of bovine plasminogen was also the activator of human plasminogen. It was further demonstrated(2) that the activator which appears after adding streptokinase to human plasminogen was lost by acidification and regained by the re-addition of streptokinase after neutralization of the fraction, indicating that streptokinase was required for activator activity.

This communication calls attention to the observation that a loss of activator can occur during the incubation of a mixture of streptokinase and human plasminogen.* The rate of disappearance of the activator has been found to be affected by the duration of incubation, the initial streptokinase concentration and the particular plasminogen preparation. These observations have significant bearing on the interpretation of the *in vitro* and *in vivo* activation of plasminogen by streptokinase.

Materials. Streptokinase (SK), Varidase (Lederle) was dialyzed before use. Bovine plasminogen was made according to Milstone (4) and then lyophylized. Lysine ethyl ester (LEE) was synthesized according to Werbin and Palm(5) and stored over phosphorous

* During preparation of this manuscript, Mullertz (*Biochem. J.*, v61, p424, Nov. 1955) reported on similar observations.

pentoxide. Several fractions of human plasminogen were prepared. The preparations were as follows: #1) A euglobulin precipitate, made from placental blood according to Milstone(4), #2) A lyophylized pooled sample of human plasma, #3) A pH 2.5 acid extract of placental Fr. III_2, prepared as described by Christensen(6) and further purified by an adsorption-elution technic,† #4) A Fr. III, obtained as described by Oncley *et al.*(7). These reagents were kept in a desiccator and weighed out immediately before use. A pH 6.4, 0.1 M phosphate buffer was prepared by mixing 300 ml of 0.1 M Na_2HPO_4 with 700 ml 0.1 M NaH_2PO_4, and a pH 7.15 0.1 M phosphate buffer, by mixing 700 ml 0.1 M Na_2HPO_4 and 300 ml of 0.1 M NaH_2PO_4.

Methods. Plasmin, the proteolytic enzyme resulting from the activation of plasminogen, exhibits lysine ethyl esterase activity. Thus, when L-lysine ethyl ester (LEE) is incubated with plasmin, lysine is formed(8). 'Activator' is measured by incubating mixtures of SK and human plasminogen with excess bovine plasminogen and LEE. The amount of lysine liberated under these conditions serves as a measure of the concentration of bovine plasmin, which, in turn, is related to the concentration of activator. The reagents are prepared as follows: the SK, human plasminogen and bovine plasminogen are prepared in pH 6.4 0.1 M phosphate buffer, the SK and human plasminogen at concentrations indicated in the text, the bovine plasminogen, at 4%. A 0.168 M solution of LEE hydrochloride is prepared just prior to use in pH 7.15, 0.1 M phosphate buffer. One ml of each of the 4 reagents is added to the reaction flask; the resulting pH of the reaction mixture is 6.4. Enzyme blanks are prepared by replacing the LEE solution with pH 6.4 buffer, and a substrate blank, by replacing the mixture of SK, human plasminogen and bovine plasminogen with pH 6.4 buffer. The order of addition of reagents is described under each experiment. In certain experiments one or more of the protein components were omitted from the reaction mixture and their blanks in order to establish adequate controls. These are described in each experiment. The tubes are incubated at 37° for 60 minutes. Aliquots are then removed and the lysine determined by a modification(9) of the Spies and Chambers method(10). The results are reported as % hydrolysis of LEE.

† Unpublished data.

TABLE I. Effect of Streptokinase Concentration on Activation of Bovine Plasminogen.

Streptokinase in reaction mixture, units	% hydrolysis of LEE: Without bovine plasminogen	With bovine plasminogen
25,000	3.5	45.5
12,500	3.0	41.5
6,250	2.0	31.0
3,125	1.0	24.5

Results. 1) *Effect of streptokinase concentration on ability of a mixture of streptokinase and human plasminogen to activate bovine plasminogen.* Reaction mixtures with and without bovine plasminogen and containing 0.06 mg N of human plasminogen #1, LEE and varying amounts of SK were prepared by mixing the reagents in the above order. The results (Table I) show that with a constant amount of human plasminogen the ability to activate bovine plasminogen increases with increasing SK concentration.

2) *Effect of human plasminogen concentration on ability of a mixture of streptokinase and human plasminogen to activate bovine plasminogen.* This experiment was carried out with the same reagents and order of addition as used in Exp. 1. In this case, however, the SK concentration was kept constant at 6,250 units while the concentration of human plasminogen was varied. The results (Table II) demonstrate that the ability to activate bovine plasminogen is also dependent on the

TABLE II. Effect of Human Plasminogen Concentration on Activation of Bovine Plasminogen.

Human plasminogen reaction mixture, mg	% hydrolysis of LEE: Without bovine plasminogen	With bovine plasminogen
.10	2.0	47.0
.08	2.0	31.5
.06	2.0	31.0
.04	.0	22.5
.02	.0	6.0

TABLE III.* Effect of Preincubation Time on Ability of a Mixture of Streptokinase and Human Plasminogen to Activate Bovine Plasminogen.

Pre-incubation mixture	Time added, min.	Added to	% hydrolysis of LEE
(a) SK + human plasminogen	2	Bovine plasminogen and LEE	56
	30		39
	45		35
	60		31.5
	90		22

* Conditions described in text.

concentration of human plasminogen.

3) *Effect of preincubation time on ability of a mixture of streptokinase and human plasminogen to activate bovine plasminogen.* A mixture containing 1000 units of SK and 0.35 mg N of human plasminogen #3/ml was incubated at 37°. For assay, 1 ml aliquots were removed at intervals and added to freshly prepared solutions containing bovine plasminogen, LEE and buffer. The data are shown in Table III.

A gradual loss of ability of the mixture of SK and human plasminogen to activate bovine plasminogen is evident; SK and human plasminogen incubated separately were stable.

4) *Effect of addition of streptokinase or human plasminogen on a partially inactivated mixture of streptokinase and human plasminogen.* A mixture containing 200 units of SK and 0.6 mg N of human plasminogen #2 per ml was incubated at 37° and at the stated intervals samples were removed for assay. When partial inactivation had occurred (60 min.—see Table IV), 1 ml aliquots were removed from the solution. The first was used as a control; 0.6 mg N of human plasminogen #2 was added to the second; 200 and 1000 units of SK were added to the third and fourth, respectively. Each aliquot was then assayed for 'activator.' The results are tabulated in Table IV.

TABLE IV.* Effect of Streptokinase and Human Plasminogen on a Partially Inactivated Mixture.

Pre-incubation mixture	Time added, min.	Added to	% hydrolysis of LEE
SK + human plasminogen	2	Bovine plasminogen and LEE	61
	30		53.5
	45		49.5
	60		42
(1) Control	—	"	42
(2) Human plasminogen (.6 mg N)	2		47
(3) 200 SK	2		54
(4) 1000 SK	2		61

Component added to 60 min. preincubation mixture.

* Conditions described in text.

The results indicate that although some increase in the concentration of activator may have occurred on addition of human plasminogen (see also Table II), a greater increase was observed when SK was added, suggesting that the loss of SK may be the cause of the decreased ability of the mixture to activate bovine plasminogen.

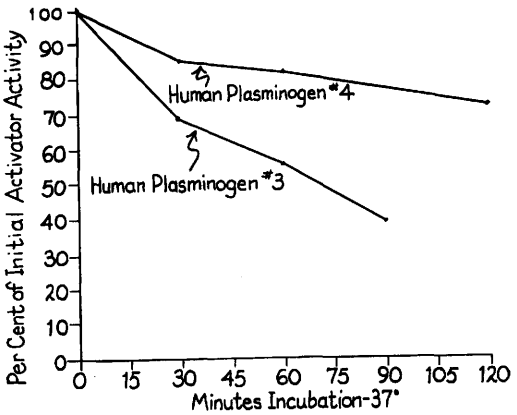

FIG. 1. Effect of plasminogen fraction on the stability of activator.

5) *Effect of human plasminogen preparation on the loss of the activator.* It was predetermined by activator assay that a 50-60% hydrolysis of LEE was obtained by a mixture of 1000 u SK and either 0.35 mg N of plasminogen #3/ml or 0.1 mg N plasminogen #4/ml. These mixtures were incubated at 37° and tested periodically for activator content. The rate of inactivation (Fig. 1) is shown to vary with the human plasminogen fraction employed.

6) *Effect of SK concentration on the loss of activator at pH 7.2.* Since the rate of inactivation could have physiological significance, it was decided to study the effect of SK concentration on the rate of disappearance of the 'activator' at physiological pH. The re-

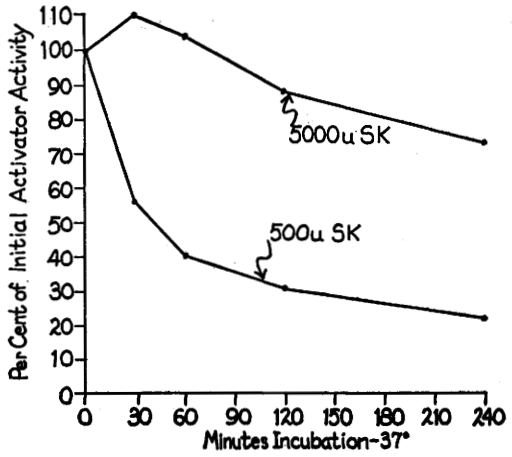

FIG. 2. Effect of SK concentration on stability of activator.

sults were essentially the same as those obtained at pH 6.4. The results (Fig. 2) show that with a constant amount of human plasminogen the rate of decrease of activator is greater at the lower SK concentration.

Discussion. The data presented are interpreted to indicate that SK may be consumed by a "deterioration route" as well as by the route in which it activates plasminogen. It can not be established from these experiments whether the deterioration of activator in a mixture of SK and human plasminogen occurs due to the loss of SK or to the loss of the formed activator, or both. Since variations in SK concentration as well as plasminogen concentration can affect not only the quantity of activator formed but also the rate at which deterioration of activator occurs, the relative rates at which these reactions occur present an obstacle to the study of the kinetics of the activation of plasminogen by SK.

Some speculation on the effects of *in vivo* administration of SK appears to be in order. If SK undergoes reactions *in vivo* as are described herein *in vitro,* its administration to humans(11) could result in variable activation of plasminogen depending on the mode and rate of administration of a given dose of SK. The *in vivo* activation of animal plasminogen by a mixture of SK and human plasma could depend on not only the total dose but also on such factors as the time of administration after mixing, the amount of SK and human plasminogen used, and the deterioration characteristics of the SK-plasminogen mixture.

It should be evident that the fibrinolytic activity appearing in an animal after administration of a mixture of SK and human plasminogen would reflect not only the human plasmin administered but also the activated plasminogen of the animal.

Summary. The ability of a mixture of SK and human plasminogen to activate bovine plasminogen was shown to be dependent upon the concentrations of both components. The 'activator' which was formed, may deteriorate on incubation even though the SK and human plasminogen when incubated separately were stable. The decrease was partially or completely restored by the addition of SK. The rate of disappearance of the 'activator' was affected by the plasminogen preparation and by the initial SK concentration. The inactivation occurred at physiological pH as well as at pH 6.4. The *in vitro* and *in vivo* significance of the data were discussed.

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