Studies on Specificity of Streptokinase.*† (24495)

FRANK B. ABLONDI AND JAMES J. HAGAN (Introduced by B. L. Hutchings)
(With technical assistance of Ralph Clarke and Emma Vergine)
Research Division, American Cyanamid Co., Pearl River, N. Y.

The specificity of streptokinase (SK) has been studied by a number of investigators(1-6) and possible explanations for its inability to activate plasminogen of certain animal species have been presented (7a, 7b, 7c, 8, 9). Geiger(10) suggested that SK required a complement-like factor present in human blood to activate guinea pig plasminogen which is not activatable by SK alone. Subsequent studies (11-14) have shown that activation of plasminogen by SK is preceded by reaction of SK with a proactivator, to form activator. It was also shown that the activator not only activated human plasminogen, but was also able to activate bovine plasminogen and the plasminogen of other species of animals(4) refractory to activation by SK alone. The purpose of this paper is to report the following: (a) When the plasminogen of a given species is activatable by SK, the SK-plasminogen mixture gains ability to activate plasminogen of a species refractory to SK. (b) The extent of activation observed in the activatable species seems to parallel ability of the SK-plasminogen mixture to activate a species of plasminogen refractory to SK.

Materials and methods. Plasmas were obtained from citrated blood of monkey, horse, sheep, goat and cow and from pooled citrated blood of cat, dog, rabbit, pig, guinea pig and man. *Euglobulin fractions* were prepared from aliquots of each plasma sample as described by Milstone(15) and resuspended at concentration 4 times that of original plasma sample. Guinea pig euglobulin was resuspended at 10 times its plasma concentration. *Human plasmin* was prepared by the method of Sherry and Alkjaersig (7c). *Lysine ethyl esterase activity* was measured as described previously (9) by incubating various dilutions of euglobulin with 30,000 units of SK‡ and 84 μMoles of lysine ethyl ester (LEE) for 60 minutes at 37°C. One unit of lysine ethyl esterase (LEEase) activity hydrolyzes 0.84 μMoles of LEE (1% hydrolysis) under the conditions of the assay. *Activator activity* was measured by adding 35 mg of a bovine plasminogen preparation(9,16) to lysine ethyl esterase test system. Any increase in esterase activity was considered to be due to activation of the bovine plasminogen by the SK-euglobulin mixture, since concentration of SK or euglobulin employed do not individually activate bovine plasminogen. *Plasma fibrinolytic activity* was determined by adding 0.9 cc of plasma diluted with equal volume of borate buffer to 0.1 cc SK, clotting the mixture with 0.1 cc rabbit clotting globulin and recording the lytic time during incubation at 37°C. Reagents used in this test are described by Christensen(17).

Results. 1. *SK activation of plasminogen of various species of animals.* (*a*) *Fibrinolytic activity of plasma after SK addition.* Results are tabulated in Table I. In the absence of SK all clots were stable for at least 72 hours.

(*b*) *LEEase activity of SK activated euglobulins.* Extent of activation of plasminogen by SK was also determined by measuring LEEase activity of the formed activator and plasmin(13,14,9,16). To reduce the inhibitor(s) content and to concentrate potentially available enzymes, the euglobulin fraction of each species was used. Results obtained are also shown in Table I.

The data indicate that ability of SK to induce clot lysis when added to a plasma, parallels its ability to induce LEEase activity when added to corresponding euglobulin fraction.

* This paper was presented in part at Am. Chem. Soc. Meetings, Chicago, Ill., Sept., 1958.

† The authors are grateful to Miss J. L. Prideau for assistance in certain experiments and to Dr. R. J. Price for supplying plasmas of the various species. We also thank Dr. E. C. De Renzo for helpful discussions and suggestions in preparation of this manuscript.

‡ SK added as Varidase®—Streptokinase-Streptodornase (Am. Cyanamid Co.).

TABLE I. SK Activation of Plasminogen of Various Species of Animals.

Species of plasminogen	Fibrinolytic activity*		LEEase activity‡	
	600 u SK	6000 u SK	No SK	30,000 u SK
Human	incoag.†		<1.5	130
Cat	" †		<6.0	130
Dog	ca. 1 min.		8.5	92
Monkey	"		<2.0	78
Rabbit	"		<6.0	35
Horse	no lysis	2 to 4 hr	<2.0	7
Pig	"	ca. 24 hr	<2.0	0
Sheep	"	no lysis	0	0
Cow	"	" §	<2.0	0
Goat	"	"	0	0
Guinea pig	"	"	9.0	17

* Values show clot dissolution times.

† Such large amounts of plasmin formed that defibrinogenation probably occurred.

‡ Values expressed as units of LEEase activity/ml of concentrated euglobulin.

§ Addition of 200,000 units of SK to cow plasma resulted in clot lysis indicating activation of bovine plasminogen. However, activation is very weak since clot lysis results only after 4 to 12 hr.

2. *Activator activity of SK-euglobulin mixtures of various species.* The above findings suggested that those plasminogens activatable by SK might be endowed with a corresponding activator activity in presence of SK. Mixtures of various dilutions of euglobulins and 30,000 units of SK were assayed for LEEase activity in the presence or absence of bovine plasminogen.§ Those species activatable by SK displayed activator activity (Fig. 1). Those species incapable of activation by SK showed no activator activity (data not shown). Even though guinea pig euglobulin was slightly activatable by SK‖ no activator activity was observed.

3. *Activation of plasminogens not activatable by SK, by a mixture of SK and human plasminogen.* Fig. 1 shows that the SK-human euglobulin mixture appeared to be the most effective activator of bovine plasminogen. It was of interest, therefore, to determine ability of this mixture to activate those species of plasminogens not activated or only poorly activated by SK. The procedure was essentially that used in previous experiment except that the activating mixtures were composed of 30,000 units SK and 7.5 or 15 mg of dried human plasma (1 cc of plasma yielded 125 mg of dried powder on lyophilization). Bovine plasminogen used in experiments shown in Fig. 1 was replaced by 0.5 ml of the euglobulin fraction of the species to be activated. The results are summarized in Table II.

These results are in accord with those reported in the literature(4,5,12,14) and indicate that a mixture of SK and human plasma is capable of activating those species of plasminogen not activatable by SK alone.

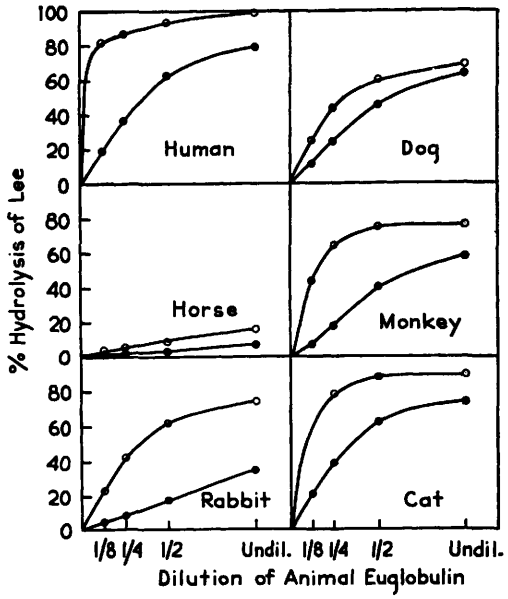

FIG. 1. LEEase (●) and activator (○) activity of a mixture of SK and animal euglobulins (see text for details of assays).

4. *Inhibition of human plasmin by various animal plasmas.* Ability of glycerol activated human plasmin (7c) to hydrolyze LEE in the

TABLE II. Activation of Animal Euglobulins by a Mixture of SK and Human Plasminogen.

Species of euglobulin	% hydrolysis of LEE*	
	15 mg human plasma	7.5 mg human plasma
Horse	48	45
Sheep	24	15.5
Pig	18	14.5
Goat	12.5	3.0
Guinea pig	74.5	43

* LEEase activity due to SK and human plasma alone was subtracted to obtain values shown.

§ pH optimum and effect of SK and/or euglobulin concentration for each species were not determined.

‖ It should be recalled that bovine and pig plasminogens are also weakly activatable by SK and similarly do not display activator activity.

TABLE III. Inhibition of Human Plasmin by Various Animal Plasmas.

Species of plasma added to human plasmin and LEE	Units of LEEase activity	% inhibition
None	336	
Human	216	36
Dog	168	50
Sheep	144	58
Cow	96	72
Horse	84	75

presence of plasma of various species was also investigated. The plasmin assay using LEE has been described(9). An equivalent amount of each plasma was added to various dilutions of plasmin and these mixtures were assayed for LEEase activity. The extent of inhibition of human plasmin by each plasma is tabulated in Table III. The plasma of all species studied showed a partial inhibition of plasmin activity. The quantity of inhibitor in a particular plasma must therefore determine in part the observed extent of activation of a given species of plasminogen.

Discussion. The data of our experiments show that the degree of fibrinolytic and LEEase activities induced by addition of SK to a given species of plasminogen parallel each other. That addition of small amounts of human plasminogen to a species of plasminogen refractory to SK activation leads to activation of the refractory plasminogen (Table II) is in accord with observations from many laboratories(4,5,10,11,12,13,14,19). Our experiments also show that any species of plasminogen activatable by SK forms, upon mixture with SK, an activator of a refractory plasminogen (Fig. 1).

The following reactions have been proposed to represent activation of plasminogen by SK: 1. SK + proactivator (X) $\longrightarrow$ activator (SKX) 2. Activator (SKX) + plasminogen $\xrightarrow{\text{enzymatic}}$ plasmin. Astrup and Müllertz and their associates(8,18) and others(13,14) have suggested that inability of SK to activate plasminogen of certain species might be due to a deficiency of proactivator. This theory considers proactivator to be distinct from plasminogen. As a result of studies on the nature of proactivator and plasminogen in human plasma fractions, a simpler hypothesis was proposed in which plasminogen and proactivator were considered to be identical (7a, 9,19). Alternative suggestions, other than proactivator deficiency, were advanced to explain the inability of SK to activate refractory plasminogens.

Unfortunately, the data obtained in the present studies do not help preferentially to support or eliminate either of these concepts. Two other points of possible interest appear worthy of comment. First, the net plasmin activity observed for a given plasma sample must depend in part on the amount of plasmin inhibitor(s) present (Table III). Second, activation (Table I) of refractory plasminogens by large amounts of SK suggests that an inhibitor(s) of SK must be overcome before activation can occur. If the reaction depicted in equation (1) above is valid, small amounts of SK might be expected to activate, albeit weakly, a poorly activatable species of plasminogen. The presence or absence of SK and/or activator inhibitors and not the concentration of proactivator could be a primary factor governing the activation of a given species of plasma or plasma fractions by SK.

Summary. 1. Ability of SK to activate the fibrinolytic system in plasma or euglobulin fractions of various animal species has been studied. 2. Human, cat, dog and rabbit plasminogens were readily activated by SK. Pig, horse and cow plasminogens were much less readily activated. Sheep, guinea pig and goat plasminogens were not activatable by concentrations of SK employed. 3. When SK is able to induce formation of a fibrinolytic system on addition to a given species of plasma or euglobulin fraction, that particular mixture gains ability to activate plasminogen of an animal species not activatable by SK alone. 4. Mixtures of SK and a species of plasminogen in which fibrinolysis or LEEase activity does not occur are incapable of activating plasminogens refractory to SK activation. 5. Addition of plasma from all animal species studied partially inhibits the LEEase activity of a spontaneous plasmin preparation. 6. Possible implications of these findings are briefly discussed.

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Received October 16, 1958. P.S.E.B.M., 1958, v99.

Suppression of Acoustic Input by Thalamic Stimulation.* (24496)

J. E. DESMEDT AND K. MECHELSE (Introduced by P. Weiss)
Laboratoire de Pathologie générale, University of Brussels, Belgium

Studies by Hernandez-Peon and others(1,2) on conscious cats with chronically implanted electrodes have shown that voltage of the response evoked in the cochlear nucleus by a standard sound decreases reversibly when the animal's attention shifts from acoustic to other sense modalities. It so appears that irrelevant information can be prevented from entering the brain further than the first relay nucleus on the afferent path. The centrifugal pathways thrown into operation under such circumstances and which must convey the influence of higher levels of the nervous system to the gating process are unknown. The suggestion is currently put forward that the brainstem reticular formation, which is known to regulate the excitatory state of the cerebral cortex(3), might also exert a direct control over sensory input(*e.g.* 4). We feel however that a good case can be made for distinguishing the neurophysiological processes underlying attention and perceptual selectivity from the reticular arousal mechanism, the latter being non-specific in its intrinsic operation. Direct evidence in favor of such a dissociation has been provided by acute experiments on cats showing that cochlear nucleus response to sound could be suppressed by brainstem stimulation(5,6). The anatomical distribution of electrode locations from which such inhibitory effects were observed was quite restricted, and it was certainly not coextensive with the reticular formation. Indeed the positive points were situated laterally to the accepted limits of the reticular formation and in the close vicinity of the lateral lemniscus and inferior colliculus(6). The structures stimulated in these experiments apparently represent links on a very specific acoustic descending inhibitory pathway which, as shown in the present paper, can be traced further cephalad in the diencephalon.

Methods. The experiments were made on adult cats immobilized with decamethonium or flaxedil (gallamine) and artificially ventilated. The initial surgical preparation was performed under ether anesthesia. Skin incisions were infiltrated with procaine. Electrical stimulation of deep structures in the brain and recording from the cochlear nu-

* This work was supported by grants from Rockefeller and Lekime Foundations and from the U. S. Air Force.