TABLE II. Mouse Virulence of Colonial Variants of *Candida albicans*.

Culture	Conc. of packed organisms	No. of mice	Survival time (days) Avg	Range
#300 S*	1:10	16	5.1	<1 – >18
	1:50	10	2.8	<1 – 11
	1:100	12	4.4	<1 – >18
	1:500	11	7.8	2 – >18
	1:1000	6	8.2	2 – 12
#238 R	1:10	11	6.6	<1 – >18
	1:50	5	7.8	2 – >18
	1:100	11	16.5	2 – >18
	1:500	11	18.8	17 – >18
	1:1000	11	19.0	>18
#285 S	1:10	11	1.1	< 1
	1:50	10	4.6	<1 – >18
	1:100	8	2.1	<1 – 3
	1:500	8	6.5	2 – >18
	1:1000	8	16.2	4 – >18
#285 R	1:10	13	4.0	<1 – >18
	1:50	10	1.5	< 1
	1:100	8	9.6	<1 – >18
	1:500	8	9.1	2 – >18
	1:1000	8	12.6	2 – >18

* S = Smooth type; R = Rough type.

isms were resuspended at varying concentrations in Sabouraud's broth containing 4% gastric mucin. Male CFW mice were infected intraperitoneally with 0.5 ml amounts of the different concentrations of culture and observed for a total of 18 days. As seen from *Table II* the results of these experiments suggest that the different colonial types possess varying degrees of virulence for mice. This is indicated by a comparison of the rough and smooth types with regard to the average survival times for the different culture dose levels. In this connection, it is of interest to note that George and Plunkett(4) found no differences in fermentative properties among the dissociated strains.

3. *Chemotherapeutic studies.* All variants were used for chemotherapeutic assays. Elkosin did not influence the infections induced by any of our strains of *C. albicans*.

Summary. 1. Several variants isolated from 3 cultures of *C. albicans* exhibited differences with regard to colonial morphology, microscopic appearance as well as virulence. The smooth colonial type of strain No. 300 proved to be more virulent than the rough colonial form of Strain No. 238. 2. The results of the previous chemotherapeutic tests with Elkosin in *C. albicans* infections could not be reproduced in tests performed with the newly isolated variants.

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An Activator System in Blood Indispensable for Formation of Plasmin by Streptokinase.*† (20087)

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The fibrinolytic agent produced by certain streptococci (streptokinase) was originally thought to be a fibrinolytic enzyme(1). Milstone(2) showed that in addition to the streptococcal factor, a "lytic factor" in serum was necessary for the production of fibrinolysis. Later, Christensen(3) found that plasminogen, a precursor in blood, was transformed to the enzyme, plasmin, by streptokinase. Streptokinase is considered a direct activator of plasminogen. Discrepancies have been ob-

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served, when plasminogen preparations from different species were activated by streptokinase and by tissue activators(4-6). These observations and the demonstration of a plasminogen activator in spontaneously active human blood(7) prompted a reinvestigation of the action of streptokinase on the fibrinolytic enzyme system in blood.

Methods and materials. Fibrinolytic activity was estimated by: *1. The standard fibrin method*(8) (the substrate contains a large amount of plasminogen). *2. The heated fibrin plate method*(9) (plasminogen in the substrate is destroyed by heating). *3. Casein digestion*(10) (3% neutralized casein, "Hammarsten," in borate buffer. The transmission at 270 mμ of the perchloric acid precipitated solutions was determined by a Hilger spectrophotometer. Under the applied conditions the extinction was directly proportional to the plasmin concentration). *Human and bovine globulin* were precipitated isoelectrically from serum(2) and redissolved to 1/2 (human) and 1/1 (bovine) of the original volumes. *"Spontane activator."* Globulin containing a large amount of plasminogen activator was prepared from spontaneously active human plasma(7). *Streptokinase activated human globulin.* 10 ml normal human serum was precipitated isoelectrically and dissolved to 2 ml in borate buffer containing 5 mg streptokinase. *Streptokinase.* "Varidase" (Lederle). *Bovine plasminogen.* A lyophilized preparation was prepared from bovine fibrinogen(7). *Buffers.* Diethyl barbiturate buffer (Michaelis) M/10 (fibrin plate experiments). Borate buffer M/20 (casein experiments). Both: pH 7.75. Sodium chloride added to ionic strength 0.15.

Results. Activity of streptokinase activated human globulin. The fibrinolytic activity of streptokinase activated human globulin was estimated by means of the 2 fibrin plate methods. A large effect was always found on standard fibrin plates (containing plasminogen) while the effect on heated fibrin plates (no plasminogen) was very low (Fig. 1). A number of other proteases (trypsin, chymotrypsin, spontaneously active bovine plasmin, a *B. subtilis* protease and an aspergillus protease) showed only slightly different effects on the two fibrin substrates(9). These results

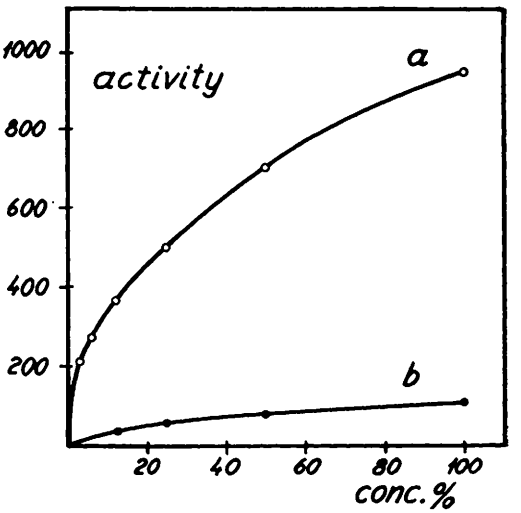


FIG. 1. Activity of serial dilutions of streptokinase activated human globulin measured on (a) standard fibrin plates (containing plasminogen) and (b) heated fibrin plates (no plasminogen). *Abscissa:* Concentrations in % of stock solutions. *Ordinate:* Activity as products of 2 diameters (mm^2) of the lyzed zones.

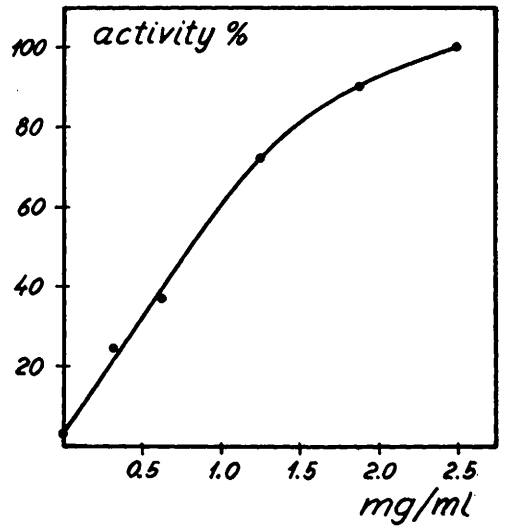


FIG. 2. Activity of mixtures of human globulin (dil. 1:60) and streptokinase (.006 mg/ml) with increasing concentrations of bovine plasminogen. *Substrate:* Heated fibrin plates. *Abscissa:* mg bovine plasminogen per ml. *Ordinate:* Activities as percentages of the maximal activity. (These values were obtained by interpolation of dilution curves prepared from each mixture(8). *Activity of controls:* Bovine plasminogen (2.5 mg/ml) + streptokinase (.006 mg/ml): 13%. Human globulin (dil. 1:60) + streptokinase (.006 mg/ml): 3%.

indicated that the large effect observed on standard fibrin plates was caused by an inter-

action of the mixture of human globulin and streptokinase with the bovine plasminogen contained in the substrate.

Effect of plasminogen. A mixture of streptokinase and human globulin was incubated with increasing concentrations of bovine plasminogen and the activity of the solutions was estimated on heated fibrin plates (Fig. 2). The activities were found to increase with the plasminogen concentration. Low activities only were produced, when solutions of human globulin and of bovine plasminogen were activated separately with streptokinase. These results suggested that the bovine plasminogen was activated by an agent formed by the interaction of streptokinase and human globulin.

Comparison of the activation of human and of bovine globulin. Bovine plasminogen preparations can be activated by tissue activators to much higher activities than those obtained by activation with streptokinase(4,5). Recently an excess of a plasminogen activator was demonstrated in spontaneously active human blood ("spontane activator")(7). These observations suggested the following explanation of our results. Streptokinase produces only a partial activation of the plasminogen in the bovine preparations. A complete activation of the bovine plasminogen is produced by an excess of activator formed by streptokinase in human globulin. This assumption was verified as follows. Solutions of human and of bovine globulin were activated by means of the "spontane activator." The resulting activities were compared with the maximal activities which could be produced by activation of the solutions by streptokinase. Heated fibrin plates were used (Table I). The "spontane activator" yielded the expected complete activation of all solutions. Twice as much plasmin could be produced by activation of bovine globulin with "spontane activator" as with streptokinase. Human globulin was activated to identical activities in both cases. The partial conversion of the bovine plasminogen by streptokinase was made complete when human globulin was added. Our bovine plasminogen yielded still smaller amounts of plasmin as compared with the high activities produced by addition of the "spontane activator."

TABLE I. Activation of Bovine and Human Globulin with "Spontane Activator" and with Streptokinase.

Mixtures	Measured activities of dilutions: 100	50	25	12.5%	Activities as % of maximal activity
B* .50 ml H .50 S 1.00	148	115	84	61	100
B .50 ml H .50 St 1.00	148	104	81	64	100
B .50 ml S .50 Bu 1.00	120	94	64	(33)	60
B .50 ml St .50 Bu 1.00	91	68	50	36	30
H .50 ml S .50 Bu 1.00	100	76	54	42	37
H .50 ml St .50 Bu 1.00	97	75	59	44	37

* B = Bovine globulin; H = Human globulin; S = "Spontane activator"; St = Streptokinase; Bu = Buffer.

Substrate: Heated fibrin plates. *Measured activities:* Products of two diameters of the lyzed zones (avg of 3 determinations) in mm^2. *Activities calculated as % of the maximal activity* were obtained by interpolation on the curves drawn from the measured activities(8). *Controls:* "Spontane activator," streptokinase, bovine globulin: inactive. Human globulin: slightly active (25 mm^2). (The conc. of streptokinase and of "spontane activator" necessary to yield optimal activation of the mixtures were determined in a separate experiment).

The reaction between activator and plasminogen. In the following experiments bovine plasminogen was used as plasminogen and streptokinase activated human globulin as plasminogen activator. The *reaction time* (at 37°C) was estimated as follows. From a mixture of plasminogen and activator aliquots were withdrawn after various lengths of time and mixed with equal volumes of a casein solution. The digestion was followed by determination of the transmission at 270 $m\mu$ of the acid precipitated solutions (Fig. 3). The reaction was complete after an incubation period of 45 min., and a linear relationship between the extinction and the digestion time was found. The *nature of the reaction* was studied as follows. Plasminogen in varying concentrations was incubated with a constant amount of activator. Three series with 3 dif-

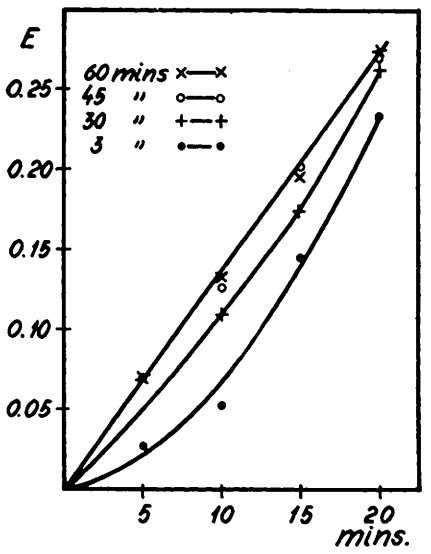


FIG. 3. Digestion of casein: Mixtures of bovine plasminogen (10 mg/ml) and streptokinase activated human globulin (dil. 1:100) were allowed to react for 3, 30, 45 and 60 min. Then equal volumes of the casein solution and of the mixtures were mixed and left at 37°C. After digesting for 5, 10, 15, 20 min., 2 ml aliquots were precipitated with 3 ml 10% perchloric acid. *Abscissa:* Digestion time. *Ordinate:* Extinction at 270 mμ measured with casein-buffer as reference and corrected for blank solutions.

ferent concentrations of activator were performed. The mixtures were allowed to react for 60 min. at 37°C. Casein was added and the transmissions of the acid precipitated solutions were estimated after digestion for 20 min. (Fig. 4). The curves show that the amount of plasmin produced depends: *1. In the case of an excess of activator,* on the plasminogen concentration; *2. In the case of an excess of plasminogen,* on the activator concentration.

This experiment indicates a stoicheiometrical mode of reaction between activator and plasminogen. It excludes the possibility of an autocatalytic reaction and of a catalytic action of human plasmin on bovine plasminogen. However, the instability of the activator in dilute solutions and the presence of an inhibitor in the plasminogen preparations complicate the quantitative evaluation of these preliminary experiments.

Discussion. The data presented here lead to the following conclusions: A. In preparations of bovine plasminogen streptokinase (in excess) produces low activities, which are smaller than those caused by other activators. B. In human globulin, streptokinase causes the formation of an activator in excess, which produces high activities in bovine plasminogen. C. Similar high activities are produced in bovine plasminogen by the activator found in spontaneously active human blood.

Significant amounts of a specific streptokinase inhibitor could not be demonstrated in our preparations. Plasmin inhibitors cannot be the cause of the low activity observed in case *A*. Such inhibitors must be as effective in case *B* and *C* as in *A*. Hence the low activity in *A* must depend on an incomplete conversion of the bovine plasminogen to plasmin. This result excludes a direct reaction between streptokinase and plasminogen and presupposes the involvement of a plasminogen proactivator. The formation of a large excess of plasminogen activator in human globulin by streptokinase shows that a proactivator (as well as plasminogen) is present in human

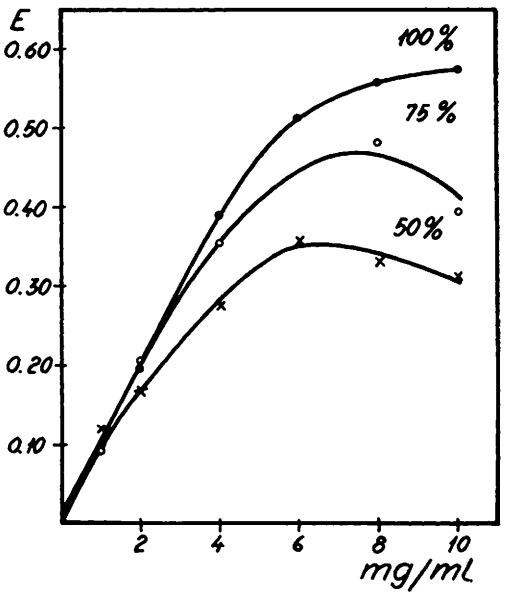
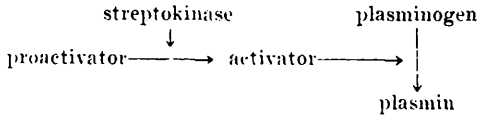


FIG. 4. Digestion of casein by increasing concentrations of plasminogen activated for 60 min. (37°C) by 3 different concentrations of streptokinase activated human globulin (dil. 1:200, streptokinase concentration: .012 mg/ml). Digestion time: 20 min. *Abscissa:* mg bovine plasminogen per ml. *Ordinate:* see Fig. 3. *Activity of controls.* Bovine plasminogen (10 mg/ml) + streptokinase (.012 mg/ml): .020. Streptokinase activated human globulin + buffer: .012.

blood. The formation of plasmin by streptokinase evidently follows this scheme:



Christensen(3) found the formation of plasmin in human globulin by the action of streptokinase to be a catalytic process. Therefore, at least one of the reactions mentioned here must be catalytic. The fact that preparations of bovine plasminogen are incompletely activated by streptokinase strongly indicates a stoicheiometrical reaction between the activator formed by streptokinase and plasminogen. Further evidence in support of this view was presented here (Fig. 4). Similarly, Astrup (11) found that plasminogen was activated stoicheiometrically by fibrinokinase.

Our experimental results are now easily understood. A complete activation of plasminogen by streptokinase can be reached only when sufficient amounts of plasminogen proactivator are present. Insufficient amounts are present in bovine preparations, especially in our bovine plasminogen preparation. Somewhat larger but still insufficient amounts are present in the fresh bovine globulin. Large amounts of proactivator are present in human globulin. Therefore, a large excess of activator is formed in human globulin by addition of streptokinase, and all plasminogen present is activated.

Bovine fibrin normally contains large amounts of plasminogen. In this substrate, therefore, activators contained in the test samples activate plasminogen of the substrate and produce activities, which are much larger than those corresponding to the plasmin content of the samples. Human fibrinogen contains a large excess of proactivator in addition to plasminogen. Therefore, the results of estimations of plasminogen or plasmin depend on the substrates applied and on the mode of activation of the samples. Discrepancies in previous streptokinase studies are caused by these facts. With bovine fibrin as substrate, preparations of human plasminogen activated by streptokinase produced activities much larger than those obtained with preparations from a number of other animals(4). With casein as substrate smaller differences only were found(12). With bovine fibrin as substrate, fibrinokinase produced activities of the same magnitude in human and bovine plasminogen(4). With the same substrate, human plasminogen was activated by streptokinase to activities several hundred times larger than those obtained with fibrinokinase(6). The observations presented here necessitate a reconsideration of all previous studies on the action of streptokinase.

The plasminogen activator recently demonstrated in spontaneously active blood obtained from the human organism was assumed to be formed from a precursor in blood(7). This assumption is also strongly supported by the present findings.

Summary. 1. Streptokinase transforms a proactivator in blood to an activator, which converts plasminogen to plasmin. Streptokinase does not react directly with plasminogen. 2. A low content of plasminogen proactivator in bovine blood is the reason for the incomplete activation of preparations of bovine plasminogen by streptokinase. In human globulin a large excess of plasminogen activator is formed by streptokinase. 3. The large activities usually observed by the activation of human globulin with streptokinase is caused by the action of this activator on the plasminogen contained in the fibrin substrate.

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