Plasminogen and Proactivator Concentration in Human Plasma.*† (30732)

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The activation of plasminogen to plasmin by streptokinase (SK) occurs in at least two steps. A precursor in human plasma first reacts with SK stoichiometrically to form a plasminogen activator which then enzymatically transforms plasminogen to plasmin in virtually all species(1). Following the suggestive experiments of Norman(2) and Zylber, Blatt and Jensen(3), we have demonstrated that human plasmin can function as a precursor or proactivator, reacting with SK to form a plasminogen activator(4). These experiments, which have been confirmed(5), were performed with purified plasminogen, however, and the possibility was not excluded that plasma may contain another substance with potential proactivator activity. Such a claim has been advanced by Spritz and Cameron(6), who found a wide range of ratios of plasminogen (measured as plasmin) to proactivator (measured as activator) activities in human plasmas. If substantiated, this data would require the recognition that a substance other than plasminogen (or plasmin) can function as a proactivator.

The experiments of Spritz and Cameron are subject to two major possible errors: (a) the use of lysine methylester (LME) hydrolysis to measure proactivator and plasminogen concentrations in plasma and (b) the use of less than 2000 SK units per ml of plasma to convert the "proactivator" to an activator. This amount of SK corresponds to approximately 1000 SK units per Remmert and Cohen caseinolytic unit of plasminogen, a ratio of SK to precursor which is not only insufficient to produce complete activator formation, but also corresponds to the steeply ascending portion of the curve which describes activator formation as influenced by SK concentration so that reproducible measurements are virtually impossible under these conditions(4). Although we had found an excellent correlation between the proactivator values obtained by esterolytic and clot lysis methods in purified systems(4), justification for extension of this method to whole plasma has not been established. We, therefore, used the conversion of bovine plasminogen to plasmin, which defines the primary activity of the activator, in the clot lysis assay of Lassen(7). In this assay, as found by Lassen and confirmed by us, the high dilutions used render preformed plasmin activity negligible. Furthermore, and most importantly, the amount of SK required for complete activation of the plasma samples was carefully determined and used. Under these conditions, the ratios of plasminogen to proactivator activities agreed closely and the postulation of a proactivator distinct from human plasminogen or plasmin is not considered necessary.

Materials and methods. Blood was drawn from healthy human subjects into Vacutainers containing 0.05 ml of 30% EDTA (Becton, Dickinson & Co., Rutherford, N. J.). The blood was centrifuged without delay, and the plasma removed and placed in tubes in an ice bath for immediate proactivator analysis. Euglobulin was precipitated from a 20-fold dilution of plasma at pH 5.3. The precipitate was redissolved to the original volume in 0.15 M phosphate buffer, pH 7.5. SK (20,000 units per mg) was kindly supplied by Lederle Laboratories, Pearl River, N. Y.

Plasminogen was measured by caseinolytic and esterolytic assays and the results converted to Remmert and Cohen units from a standard plasmin curve. Proactivator concentration was estimated by the clot lysis method of Lassen(7). Proactivator units were arbitrarily defined from a standard plasma curve. Lysine methylester (LME) hydrolysis was estimated by methanol released as

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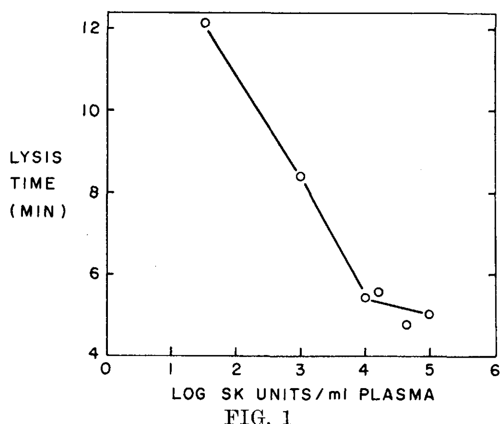

FIG. 1

measured by chromotropic acid reagent(8).

Results. In preliminary experiments, it was found that 10,000 SK units per ml of plasma produced full activator activity (Fig. 1). Three times this amount was not inhibitory and was used routinely to ensure completeness of activator formation in the plasmas tested. At this concentration, full activator activity developed immediately and was stable for at least 30 minutes. Since, in order to measure plasminogen, we separated it from antiplasmin by precipitation of the euglobulin fraction, it was essential to establish that the euglobulin precipitate contained all or nearly all of the plasminogen originally present in plasma. Plasma was acidified to destroy antiplasmin(9), and the plasminogen content after neutralization did not differ significantly from the values obtained with the euglobulin precipitate in caseinolytic as well as esterolytic assays (Table I). Caseinolytic assays for plasminogen in acidified plasma tended to be slightly higher than in euglobulin because of the high concentration of fibrinogen, an alternate substrate for plasmin, in the acidified plasma. Proteolytic activity

TABLE I. Recovery of Plasminogen in Euglobulin Precipitate.

Substrate	Acidified plasma (units/ml)	Euglobulin (units/ml)
Casein	1.65	1.43
"	1.89	1.53
"	1.43	1.29
LME	1.60	1.64
"	2.40	2.46
"	2.14	2.21
Mean ± S.E.	1.85 ± .14	1.76 ± .18

was detectable in acidified plasma in the absence of casein. Complete recovery of plasminogen activity from plasma in the euglobulin precipitate has also been reported by others(10,11).

Having established that a comparison of plasminogen activity in whole plasma and in the euglobulin fraction of that plasma could be made with some confidence, the ratios of plasminogen to proactivator were determined in 12 normal human subjects (Table II). The average ratio found, 1.52, agreed well with the ratio obtained from an analysis of purified plasminogen, 1.65. In no instance was a ratio less than unity found. Considering the inherent uncertainties of the proactivator clot lysis assay and the fact that ratios are being compared, the scatter was small.

TABLE II. Proactivator and Plasminogen Content of Human Plasmas.

Subject	(A) Proactivator (units/ml)	(B) Plasminogen (units/ml)	(B/A) ratio
Hut	1.2	2.1	1.75
Coo	1.3	1.9	1.43
Get	1.7	2.3	1.34
Her	.65	1.8	2.76
Rob	1.2	2.1	1.75
Wei	1.2	2.0	1.65
Sem	1.5	2.2	1.47
Sto	1.5	2.3	1.53
Fro	1.7	2.0	1.18
Hum	2.0	2.0	1.00
D—	2.4	3.2	1.33
Bur	2.0	2.1	1.05
Average	1.53	2.20	1.52*
Plasminogen	20.0	33.0	1.65

* Correlation coefficient, 0.86, P <.001.

Discussion. The finding that human plasmin can react with SK to form a plasminogen activator(4), did not rule out the possibility that less purified materials such as plasma might contain another proactivator. If plasmin is the only proactivator in blood, the ratio between plasminogen and proactivator concentrations in various samples of plasma should be the same. Spritz and Cameron(6) found large differences with a correlation coefficient of 0.13 and a P value of >0.5. In their experiments, however, they used less than 2000 SK units per ml of plasma, an amount which we (Fig. 1) and other investigators(7,12) have found to be

insufficient for the complete formation of activator. Our data indicate that the ratios of proactivator to plasminogen are much more uniform than had been reported by Spritz and Cameron and agreed closely with the ratio obtained with a sample of purified plasminogen. The correlation coefficient of plasma plasminogen values compared with proactivator in our experiments was 0.86 with a P value of $<.001$, indicating a high degree of covariance. While these data undermine the experiments of Spritz and Cameron, which definitely suggested a proactivator other than plasminogen or plasmin, they do not, of course, rule out the possibility that other proactivators may exist.

Summary. When the concentration of SK was carefully controlled, a strong covariance relationship was found between the plasminogen and proactivator concentration in 12 normal human plasma samples. It is suggested that lack of such control had led earlier investigators to the erroneous conclusion that they had demonstrated the presence of a proactivator other than plasminogen or plasmin in human plasma. The euglobulin precipitate at pH 5.3 contained virtually all of the plasminogen present in the original plasma.

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Effect of Storage on Sheep Erythrocytes Used in Complement Studies. (30733)

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During investigations of the complement (C′) activity of humans with connective tissue diseases, it was observed that when erythrocytes from different sheep were employed, marked differences in the extent of immune lysis were obtained with a constant pool of human complement (HuC′). In addition, it was noted that progressive changes in the degree of immune hemolysis occurred during storage of an individual specimen of sheep red cells. Because sheep erythrocytes are an essential reagent in a number of serological and immunological test procedures, the present investigation was initiated to determine the effect of storage on the immune hemolysis, osmotic fragility and degree of spontaneous lysis of sheep red cells.

Materials and methods. Blood collection and storage. Nine healthy sheep (weight 225-325 lb) of mixed sex and breeding were bled aseptically by venipuncture. In each instance the blood was collected in an equal volume of modified Alsever's solution(1), pH 6.1, containing 20.5 g glucose, 8 g sodium citrate, 0.55 g citric acid, 4.2 g NaCl and sufficient distilled water to bring the volume to 1000 ml. The preserved blood specimens were stored in sterile 50 ml vaccine bottles at 3°C until tested. The osmotic fragility and susceptibility to immune lysis with a standard pool of C′ were determined at weekly intervals.

Immune hemolysis. Erythrocytes were washed and sensitized by the method de-