preparations of the red cells exposed to ozone confirm the formation of spherocytes. Erythrocyte fragmentation may be due to direct membrane damage, short of overt hemolysis, for which the normal reparative cell processes cannot compensate; or, to interference with the intracellular metabolic processes necessary for membrane integrity. The increase in TBA reacting substances suggests that the former mechanism is operative with peroxidation of unsaturated fatty acids contained in the cell membrane and the formation of free radicals perhaps causing damage to neighboring protein. However, interference with biochemical pathways necessary for the maintenance of the cell membrane cannot be excluded.

It is interesting to speculate that the hypothesized involvement of lipid peroxidation in the aging process(12) is related to the frequent observation of generalized aging in animals chronically exposed to ozone(4). A further speculation is that if emphysema be considered a normal aging change, the increase in chronic respiratory disease in areas of urban air pollution found in epidemiologic studies is due to the acceleration of aging in the lung by air pollutant-induced lipid peroxidation.

Summary. In vitro exposure of erythrocytes to ozone resulted in an increased osmotic fragility associated with the formation of TBA reactants. This suggests that lipid peroxidation may be involved in the mechanism of ozone toxicity.

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Caseinolytic and Fibrinolytic Activities of Human Plasma in Starch Block Electrophoresis. (32445)

MASAO NAKAHARA* AND YUZURA NAKANISH (Inroduced by D. R. Celander)
Orthopedic Department, Sapporo Medical College, Sapporo, Japan

Depending upon the circumstances of purification profibrinolysin (plasminogen) has been reported to appear in a number of different plasma fractions. For example, plasma fractionated on DEAE-cellulose columns yields the bulk of the profibrinolysin near the β-globulin fraction but traces appear also in the γ-globulin fraction(1). Profibrinolysin, purified by the method of Kline(2), has been found by several investigators to move toward the cathode in starch gel electrophoresis(3,4, 5).

Slotta and Gonzalez(6), using starch gel electrophoresis in the presence of epsilon-aminocaproic acid (E-ACA), separated purified profibrinolysin into 6 different bands of active protein. Cohly and Shulman have reported that human euglobulin migrates within the β- and γ-globulin region on cellulose acetate membrane in veronal buffer at pH 8.6(7). The heterogenous character has also been observed when profibrinolysin is subjected to chromatography on various cellulose preparations(8). It is apparent that the properties of purified profibrinolysin relate to the methods by which it was ob-

* Dept. of Anesthesiology Baylor Univ. College of Med., Houston, Texas.

tained. No single protein could possess all the properties reported for purified profibrinolysin. The object of this paper is to report the behavior of profibrinolysin as it occurs naturally in human plasma upon being subjected to starch gel electrophoresis.

Materials and methods. Streptokinase (SK) was purchased as Varidase at the hospital pharmacy. Urokinase (UK) was provided without cost by the Green Cross Laboratory, Osaka, Japan. Casein, Hammersten grade, made by Merck, was prepared by the method of Derechin(1). Bovine fibrinogen was purchased from Armour Pharmaceutical Co. Bovine thrombin was generously supplied by the Mochida Pharmaceuticals Co., Tokyo. Venous blood from 10 healthy subjects was drawn into 3.8% sodium citrate solution and the plasma separated by centrifugation at $2000 \times g$ for 10 minutes. E-ACA was supplied through the courtesy of the Daiichi Pharmaceutical Co., Tokyo. Dicarbobenzoxy-lysine (DCL) was kindly supplied by Professor Yamamura, Department of Medicine, Osaka University.

Starch block electrophoresis was performed according to a slightly modified method of Bloemendal(10), using veronal buffer of pH 8.6 and ionic strength 0.05. Potato starch was washed twice with a mixture of 3 parts alcohol and one part ether, four times with deionized water and twice with veronal buffer, pH 8.6. The blocks were 1.5 cm thick, 6.5 cm wide and 45 cm long. A trough 2½ mm wide was made in the starch and filled with starting material consisting of a starch paste containing 7 ml of plasma. Migration was carried out at 1°C for 42 hours at 220 volts, 20 ma. Each block was cut in 40 sections, 1 cm wide, and each section was eluted with 7 ml of veronal buffer. To a 0.5 ml aliquot of each eluate, deionized water (3 ml) was added and the protein content of the diluted mixture determined at 280 mμ. Caseinolytic activity was measured by a slight modification of the method of Derechin(9). An 0.8 ml aliquot of each eluate was mixed with 0.2 ml veronal buffer containing 1600 Christensen units(11) of SK or 10 Plough units(12) of UK and incubated for 10 minutes at room temperature. The solution was then added to 1.0 ml of 2.2% casein and the incubation continued for 2 hours at 37°C. After addition of 4 ml of 1.7 M perchloric acid the mixture was allowed to stand overnight at 5°C to permit complete precipitation. The suspension was filtered and absorption of the filtrate determined against a blank to which the perchloric acid had been added before the casein.

Fibrinolytic activity was determined by measurement of the time required for lysis of a standard clot formed in the presence of the enzyme to be tested. To a 1 ml aliquot of each eluate mixed with 0.2 ml SK solution (1600 units) and incubated for 10 minutes at room temperature, were added in order 0.4 ml veronal buffer containing 40 units of thrombin and 2 ml 0.3% fibrinogen. Lysis time was determined by tipping the tube to the horizontal. Clot lysis was considered to be complete when the solution flowed freely. In each series of experiments, no control clot dissolved in less than 48 hours.

Caseinolytic and fibrinolytic activities of each eluate were also determined when activation by the addition of SK was performed in the presence of E-ACA (2×10^{-3} M) or DCL (2×10^{-2} M).

Results. The experimental values represent the average of individual analyses of plasma samples obtained from 3 to 10 healthy subjects.

Caseinolytic activity in SK activated plasma protein fractions. Caseinolytic activity, although somewhat variable, could be induced by SK in the α_1-globulin fraction (Fig. 1) of all individuals tested. Six out of ten exhibited high caseinolytic activity with optical densities (O.D.) higher than 0.20. The remaining 4 had moderate (O.D. $= 0.1 - 0.2$) to low (O.D. < 0.1) caseinolytic activity.

Inhibition of caseinolytic activity in SK activated plasma protein fractions by E-ACA or DCL. Plasma samples from the 3 individuals possessing the highest SK elicitable caseinolytic activity in the α_1-globulin fraction were selected for this experiment. The caseinolytic activity of the α_1-globulin fraction was not inhibited by 2×10^{-3} M E-ACA but it was inhibited by 2×10^{-2} M DCL (Fig. 2).

Caseinolytic activity in UK activated plasma

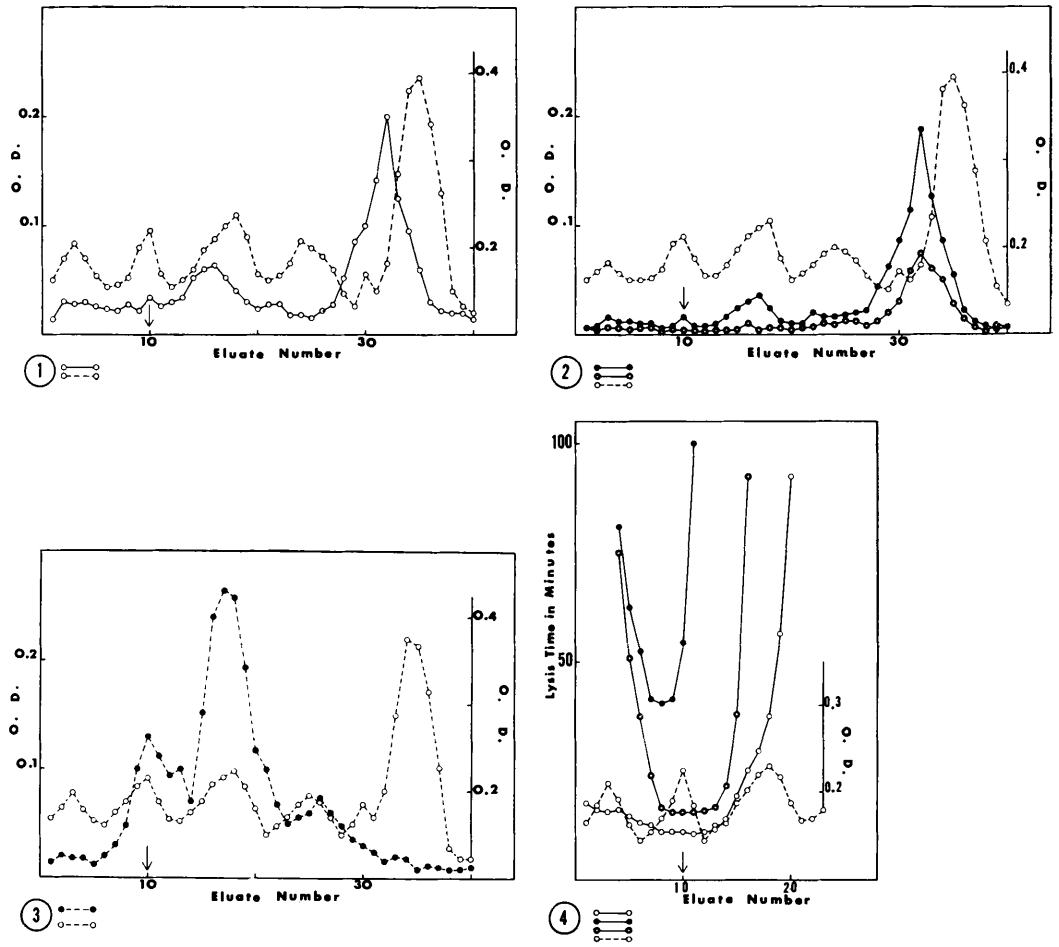

FIG. 1. Caseinolytic activity in SK activated plasma protein. O———O, SK activated plasmin; O - - - O, plasma protein. O. D. on left ordinate is a measure of protein content; O. D. on right ordinate, a measure of the perchloric acid-soluble products in filtrate from SK-activated plasmin-casein digest. Numbers on the abscissa refer to the segment of the starch block cut after electrophoresis and eluted with veronal buffer. Segment 10 (↓) is point at which serum sample was applied.

FIG. 2. Inhibition of caseinolytic activity in SK activated plasma protein by E-ACA and DCL. ●———●, SK activated plasmin + 2 × 10^{-3} M Σ-amino caproic acid; O———O, SK activated plasmin + 2 × 10^{-2} M dicarbobenzoxy-lysine; O - - - O. plasma protein. See legend of Fig. 1 for details.

FIG. 3. Caseinolytic activity in UK activated plasma protein. ● - - - ●, UK activated plasmin; O———O, plasma protein. See legend of Fig. 1 for details

FIG. 4. Fibrinolytic activity in SK activated plasma protein and its inhibition by E-ACA and DCL. O———O, SK activated plasmin; ● - - - ●, SK activated plasmin + 2 × 10^{-3} M Σ-amino acid; O———O, SK activated plasmin + 2 × 10^{-2} M dicarbobenzoxy lysine; O - - - O, plasma protein.

protein fractions. UK-activatable caseinolytic activity was found in both β- and γ-globulin fractions, but the activity of the β-globulin fraction was much higher (Fig. 3).

Fibrinolytic activity in SK activated plasma protein fractions and its inhibition by E-ACA or DCL. SK-elicitable fibrinolytic activity was highest in the γ-globulin fractions but could also be demonstrated in the β-globulin fraction and in the fraction moving to the cathode. The SK-induced fibrinolytic activity or the activation process itself was markedly inhibited in all fractions by 2 × 10^{-3} M E-ACA but was only slightly in-

hibited by 2×10^{-2} M DCL (Fig. 4).

From the foregoing it is clear that in addition to caseinolytic activity which can be elicited in the β- and γ-globulin fractions of human plasma by both streptokinase and urokinase, there is also a precursor of caseinolytic activity which appears in the α_1-globulin fraction. In addition to a different electrophoretic mobility, the caseinolytic activity of the α_1-globulin fraction could not be elicited by urokinase nor was it inhibited by E-ACA. It was inhibited by DCL which was only slightly effective as an inhibition of the caseinolytic activity of the β- and γ-globulin fractions. The α_1-globulin fraction differed in still another respect when compared on the basis of fibrinolytic activity in the presence of streptokinase. Whereas an abundance of such activity existed in the β- and γ-globulin fractions, almost none was found in the α_1-globulin fraction. Fibrinolytic activity of urokinase-treated plasma fractions was not tested because a profibrinolysin-free fibrinogen and thrombin were not available. Failure of urokinase to elicit caseinolytic activity in the α_1 fraction might be explained by the presence in this fraction of the slow-acting inhibitor reported by Norman and Hill(13). It is likely that a far larger amount of activator developed in the presence of streptokinase than was brought to bear by the relatively small amounts of urokinase added. However, since this inhibitor could be expected to affect fibrinolysis less than caseinolysis, there is no ready explanation for the lack in the α_1-globulin fraction of streptokinase-elicitable fibrinolytic activity in the presence of streptokinase-elicitable caseinolytic activity.

That plasminogen can change its properties depending upon how it is purified is well established(1-8). However, the findings here were obtained with native plasma, a medium in which little opportunity for modification of plasminogen exists. That error of technique was not responsible is shown by the fact that the same electrophoretic distribution of proenzyme was observed for every plasma tested.

Summary. Human plasma was subjected to starch electrophoresis with veronal buffer at pH 8.6, $\mu = 0.05$. The veronal buffer eluates of each of the 40 sections into which the block was cut were used for the study of caseinolytic and fibrinolytic activity after activation with streptokinase or urokinase. A high fibrinolytic activity was induced by streptokinase in the γ-globulin fraction. A high caseinolytic activity was induced by urokinase in the β-globulin fraction and by streptokinase in the α_1-globulin fraction. Caseinolytic activity in the α_1-globulin fraction was not inhibited by a concentration of epsilon-aminocaproic acid (2×10^{-3} M) which gave a marked inhibition of the fibrinolytic activity elicitable in the β- and γ-globulin fractions. Inhibition of the caseinolytic activity in the α_1-globulin fraction was effected by dicarbobenzoxy-lysine (2×10^{-3} M) which had only a slight inhibitory effect on the fibrinolytic activity of the β- and γ-globulin fractions.

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