Purification and Physiological Properties of Factor VII from Plasma and Serum. Separation from Prothrombin. (20084)

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The existence of Factor VII was first recognized in serum*(1). A one-stage method for the quantitative determination of this factor has been proposed(1); it yields practically the same results with plasma or serum.† Separation of Factor VII from prothrombin offers considerable difficulties, although the physiological behavior of these two clotting factors are entirely different: Factor VII accelerates conversion of prothrombin to thrombin; prothrombin, on the other hand, determines quantity of thrombin formed. The two factors can be partly separated: a) by the clotting process itself, more than 95% of prothrombin being consumed, whereas Factor VII remains unchanged in the serum; b) by dicumarol and similar anticoagulants which cause a more rapid decrease of Factor VII-concentration at the beginning of therapy. The difficulty of the chemical separation of Factor VII and prothrombin is a consequence of the similarity of their physico-chemical properties. No separation can be obtained by fractional precipitation with different salts, organic solvents, or their combined use. Fractional adsorption on barium sulfate, tricalcium phosphate, etc., also is ineffective. The relative amounts of the two factors remain unchanged after such procedures. Only by means of *chromatography* is it possible to isolate prothrombin and Factor VII. Prothrombin and Factor VII are separated from fibrinogen and Factor V by adsorption on barium sulfate, the mixture prothrombin-Factor VII is then chromatographed. By this chromatographic procedure, first pure prothrombin and later pure Factor VII are obtained.

Method. Purification of Factor VII from plasma. The first two steps are carried out at 2°C, the third and fourth at room temperature. *Step 1. Adsorption.* 10 g of barium sulfate (Merck feinst gepulvert, Germany) are suspended in 200 cc of oxalated plasma. The suspension is stirred for 40 min., then centrifuged. The supernatant, containing fibrinogen and Factor V, is discarded. *Step 2. Washing.* The barium sulfate is washed twice with 30 cc of physiological saline. The solution is discarded. *Step 3. Preparation of column for chromatography.* A homogenous mixture of barium sulfate (10 g) and hyflosupercel (5 g) suspended in physiological saline is introduced into the column. The excess of saline is again discarded. *Step 4. Elution.* The prothrombin is eluted by means of 150 cc of .14 molar trisodium citrate-citric acid solution at pH 5.8. The elution is continued with a .14 molar trisodium citrate solution at pH 7.8. After addition of the latter, the pH of the eluate increases slowly until a final value of 7.8 is attained. First prothrombin is eluted, then a mixture of prothrombin and Factor VII, and finally Factor

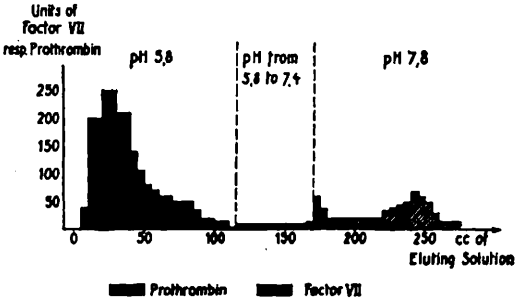

FIG. 1. Chromatographic separation of prothrombin and Factor VII. Eluting solutions: 1) Trisodiumcitrate-citric acid .14 m, pH 5.8. 2) Trisodiumcitrate .14 m, pH 7.8. Eluate fractions of 5 cc.

* Factor VII is almost certainly identical with SPCA of Alexander *et al.*(2), co-thromboplastin of Mann and Hurn(3), proconvertin of Owren(4), stable prothrombin conversion factor of Owen and Bollmann(5), and the accelerator factor of MacMillan(6).

† In undiluted plasma and serum, results obtained by the one-stage method are somewhat different; this is due to a difference in the prothrombin content of plasma and serum. After dilution (1:10) results of Factor VII assay are identical in plasma and serum.

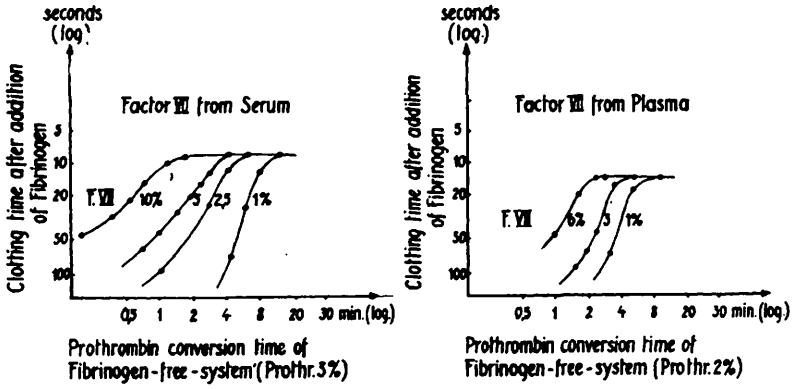

FIG. 2. Acceleration of thrombin formation by increasing amounts of Factor VII. Clotting system: .1 cc prothrombin-Factor V sol., .1 cc Factor VII sol. (variable), .1 cc thromboplastin, .1 cc $CaCl_2$ 1/40 m. Incubation of this mixture = prothrombin conversion time (abscissa). Ordinate, clotting time after addition of fibrinogen (.1 cc of 500 mg % sol.) is plotted in reverse order so that direction of the arrow signifies increasing amounts of thrombin.

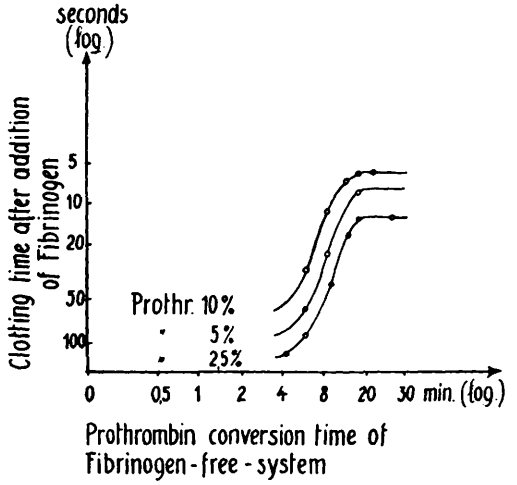

FIG. 3. Increase of quantity of thrombin formed by increasing amounts of prothrombin. Clotting system (see Fig. 2). In this system the Factor VII is constant, the prothrombin variable.

VII free from other clotting factors. The elution is performed under pressure in order to assure a sufficient speed of flow (one drop/12 sec.).

Purification of Factor VII from serum. Because of the minute amount of prothrombin in normal serum the purification of Factor VII offers fewer difficulties in serum than in plasma. All the operations are carried out between 2 and 5°C(7). *Step 1.* Factor VII is adsorbed on barium sulfate (Röntgen, Merck, Germany). 20 g of barium sulfate are suspended in 200 cc of diluted serum (9 parts of serum, 1 part of 0.1 M sodium oxalate). The suspension is stirred 45 min., then centrifuged. *Step 2.* The supernatant is discarded. The barium sulfate is washed twice with 30 cc of physiological saline and once with 30 cc of a .006 M sodium citrate solution. *Step 3.* Factor VII is eluted by .14 M solution of sodium citrate (pH 7.8). The barium sulfate is suspended in 30 cc of citrate, stirred 30 min., then centrifuged. The supernatant solution contains Factor VII (yield 90%) and can be used for the study of Factor VII.

Control of activity. The one-stage methods for the determination of prothrombin and Factor VII have previously been reported(1).

Results. The chromatographic separation of Factor VII and prothrombin from plasma results in clotting factors physiologically pure, *i.e.*, free of other coagulation factors (Fig. 1). The final prothrombin yield is comparatively high, 50% or more, whereas Factor VII recovery is very low and never exceeds 10%.

The effect of prothrombin and Factor VII from serum on the clotting process have previously been differentiated(1). Comparison of physiological properties of Factor VII obtained from plasma and from serum is now possible. Both preparations give the same acceleration effect on thrombin formation (Fig. 2). Prothrombin, on the other hand, prepared by the chromatographic method (Fig. 1) has no effect on velocity of thrombin formation; its concentration is proportional to the amount

of thrombin formed (Fig. 3). The curves of Fig. 2 and 3 were obtained in a fibrinogen-free system, all the clotting factors being kept constant except the one to be studied (Factor VII in Fig. 2, prothrombin in Fig. 3). The thrombin formed is determined by addition of fibrinogen. The identity in the mode of action of Factor VII from plasma and serum is obvious. Fig. 3 shows that the amount of thrombin formed depends on the quantity of prothrombin in the system. As already mentioned(1) the one-stage method for quantitative determination of Factor VII (using Seitz-filtered Factor VII-free oxalated ox-plasma) yields the same results with material obtained from plasma or serum.

Chemical and physical comparison of the two proteins is not yet possible, the quantity of the products being insufficient.

Summary. Methods of purification of Factor VII from plasma and serum are outlined. The separation of Factor VII and prothrombin from plasma is accomplished by means of chromatography. The mode of action of Factor VII from plasma and serum proved to be identical.

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Response of Young Dogs to West Nile Virus. (20085)

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The virus employed in this study was the B956 strain of West Nile virus furnished by the American Type Culture Collection in Washington, D. C. This virus was obtained by the above institution from Dr. K. C. Smithburn(1) of the Rockefeller Foundation Laboratories in New York. He had isolated this virus strain from a patient in Uganda, Africa and had passed it 25 times in Swiss albino mice by intracerebral inoculation. This virus has been cultivated in chick embryos(2) and in baby chicks(3). The virus, on receipt at this laboratory, was in the form of lyophilized mouse brain of the 25th passage. Three-weeks-old Swiss albino mice (Webster strain) were employed. This strain of mice was the one recommended by Webster(4) as being especially susceptible to neurotropic viruses. The dogs used were mongrels 5 to 6 weeks of age and averaged 4 to 5 lbs each.

The lyophilized mouse brain of the 25th passage was diluted to a 10% suspension with physiological saline. Each of 6 mice was inoculated intracerebrally with 0.03 cc of this 10% suspension. The mice showed involuntary motor reactions on the 3rd day post inoculation. When these symptoms of central nervous system involvement appeared, the mice were sacrificed, and the brains were removed aseptically. The pool of infected brains was ground with alundum and diluted to a 10% suspension with physiological saline. The suspension was then subjected to 5 minutes centrifugation in a horizontal centrifuge at 2,000 r.p.m. The supernatant was used as the inoculum for initiating the virus experiment in the dogs. The virus titrated 10^{-5} in Swiss albino mice inoculated intracerebrally. The supernatant material was injected intracerebrally into 4 unvaccinated mice and into 4 mice which had been immunized previously with the 25th mouse passage