TABLE III. Reactions of Cerebrospinal Fluid.

Type of patient	No. in series	Spinal fluid mixed with human brain antigen — No. giving precipitin	Antibrain serum mixed with CSF — No. giving precipitin	Highest dilution of antiserum giving a precipitin
Non-neuropsychiatric	6	0	6	8 (2 cases) 16 (4 ")
Random neuropsychiatric	2	0	2	8, 16
Multiple sclerotic	2	0	2	8, 32

The sera from all severe schizophrenic patients were secured before ataractic drug or placebo treatment and each week for 6 consecutive weeks thereafter. Sera from 3 of these patients gave a positive precipitin reaction for all 7 specimens of each case. A wide range of antiserum dilutions was tried and led to clear precipitates from 80-fold to 5120-fold (Table II).

The sera of 13 of 41 paretic patients showed a strongly positive reaction at various antiserum dilutions; 6 sera tested a week later remained positive.

Of 10 spinal fluids tested, none reacted with precipitin formation when mixed with brain antigen, but all gave a definite precipitin when mixed with antibrain serum (Table III), regardless of disease state of patient.

Conclusions. Rabbit antiserum to a saline soluble component of human brain, when mixed with spinal fluids and with sera from patients with various illnesses, gave a precipitate with all spinal fluids and with 17 out of 244 human sera. These positively reacting sera were from 4 schizophrenic and 13 paretic patients. On the other hand, human brain antigen mixed with human serum gave a precipitate in 5 out of 244 cases. The 5 sera were from one arthritic, one paretic, one chronic schizophrenic and 2 alcoholic patients. The few sera reacting positively show that this is not a general flocculation phenomenon, but whether immune systems actually related to brain are being tested here, is the subject of further study. The relatively high number of paretic patients giving a positive reaction with antibrain serum may have a bearing upon this. These results may provide a lead for further investigation of brain disease.

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Reactions of Stuart Factor and Factor VII with Brain and Factor V. (24856)

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Several workers have shown that a potent prothrombin conversion factor forms on incubation of factor V (labile factor, Ac Globulin, proaccelerin) with serum and brain extract (1-4). This product can be sedimented by high speed centrifugation(1) and will, for convenience, be referred to as "extrinsic thromboplastin." The factor in serum which takes part in formation of this product was believed to be factor VII (stable factor, proconvertin, SPCA). The one-stage prothrombin time is prolonged in congenital deficiency of Stuart factor (SF) as well as congenital deficiency of factor VII so that it now appears

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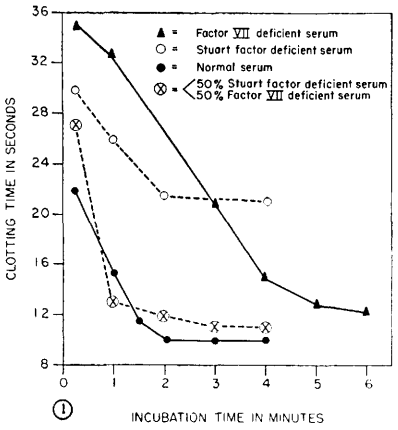
probable that SF is also involved. It is unlikely that PTC (factor IX, Christmas factor) plays a role since the one-stage prothrombin time is normal in congenital deficiency of this factor. There is, however, evidence that another factor named prephase-accelerator may be involved(5). The purpose of our work is to delineate the actions of PTC, factor VII and SF when incubated with brain and factor V. No attempt has been made to study the role of other factors such as "prephase-accelerator" in this reaction.

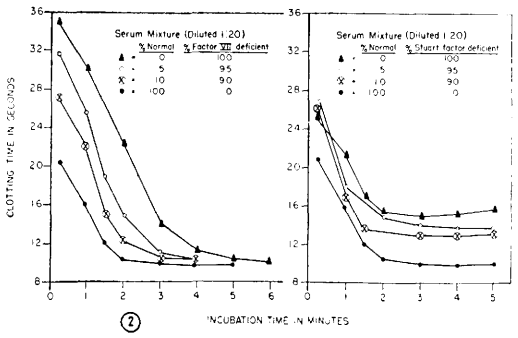
Materials and methods. Whole blood was collected in glass tubes and allowed to clot and to remain at 28°C for 24 hours before separating the serum. Plasma was obtained by adding 9 parts whole blood to 1 part 3.8% trisodium citrate and centrifuging at 3000 rpm. Plasma and serum were stored at –20°C in small aliquots and aged 2 to 3 months at time of experiments. SF-deficient plasma and serum were obtained from patient (R.S.), previously reported(6), with congenital deficiency of SF. Factor VII-deficient plasma and serum were obtained through courtesy of Drs. Harold A. Wurzel, University of Penn. and C. L. Johnston, Jr., University of N.C. from patient with congenital deficiency of factor VII; some studies on this patient have been previously made(7). *Factor V* was prepared from human blood by method of Biggs and Macfarlane(8). The *veronal buffer* (pH 7.2) used in reaction mixtures was prepared by method of Owren(9). Dilution of reagents was carried out with physiological saline. In experiments to be described 0.2 ml factor V, 0.2 ml rabbit brain (DIFCO) diluted 1:50, 0.2 ml buffer and 0.2 ml test serum diluted 1:20 were mixed in this order. Exactly 10 seconds after addition of serum, 0.2 ml of 0.025 M $CaCl_2$ was added and a stop watch started. At subsequent intervals 0.1 ml aliquots were removed from incubating mixture and added simultaneously with 0.1 ml 0.025 M $CaCl_2$ to 0.1 ml plasma substrate and clotting times recorded. All experiments were carried out in water bath at 37°C. In experiments in which mixtures of various sera were used, the individual sera were first diluted 1:20 with normal saline before mixing.

Results. Factor VII-deficient and SF-deficient sera were separately incubated with factor V, brain extract and calcium chloride and coagulant activity of each incubating mixture was then determined using normal plasma substrate (see Methods). The results (Fig. 1) show that in absence of SF, maximum activity or yield of "extrinsic thromboplastin" (reflected by shortest clotting time) was less than that of normal control. This experiment appears to show that factor VII deficiency affects rate of formation rather than yield of "extrinsic thromboplastin." There was a decrease of coagulant activity of normal incubation mixture (not shown in Figure) after minimum substrate clotting time (maximum yield) was attained; in such systems maximum yield of "extrinsic thromboplastin" in absence of factor VII was usually slightly less than that of normal control. In other test systems this apparently inhibitory phase was not seen during experiment and factor VII-deficient serum always produced normal yield. In all experiments, however, in which factor VII-deficient serum was used instead of normal serum, there was a delay in attaining minimum clotting time. A mixture of equal parts of SF and factor VII-deficient sera gave an essentially normal curve (Fig. 1) although the normal minimum was never quite reached. PTC-deficient serum gave normal result in respect of both rate and yield, and no further studies were carried out on reaction of this factor with brain.

Mixtures of SF-deficient or factor VII-deficient serum with normal serum in varying proportions were then prepared and tested as above. It was found (Fig. 2) that relatively small amounts of normal serum produced significant although not complete correction. It should be noted that in this particular experiment, yield of "extrinsic thromboplastin" in absence of factor VII was normal.

Effect of substitution of plasma deficient in factor VII or SF for normal plasma substrate. In preceding experiments, although normal substrate containing both factor VII and SF was used, an abnormal result was always obtained if one of these factors was excluded from incubation mixture. If normal serum was included in the reaction mixture, the results obtained using a substrate deficient in either SF or factor VII were identical to those obtained using normal plasma. When





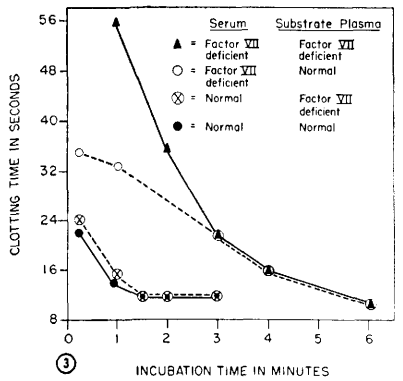


FIG. 1. Effect of substituting factor VII or SF-deficient sera for normal serum in incubation mixture containing factor V, brain and calcium using a normal plasma substrate.

FIG. 2. Effect of addition of small amounts of normal serum to factor VII or SF-deficient sera in formation of "extrinsic thromboplastin." Normal plasma was used as substrate.

FIG. 3. Effect of using factor VII-deficient plasma as substrate. Incubation mixture contained either normal or factor VII-deficient serum.

SF-deficient serum was substituted for normal serum in the incubation mixture, clotting times of SF-deficient plasma substrate were longer than those obtained using normal plasma substrate but in both instances low yields were obtained.

When factor VII-deficient serum was substituted for normal serum in the incubation mixture, the initial clotting times were longer with factor VII-deficient plasma substrate than with normal plasma substrate but the same minimum clotting times were eventually obtained (Fig. 3). SF-deficient plasma substrate gave results similar to normal plasma substrate when only factor VII was omitted from the incubation mixture.

Discussion. These results are understandable if factor VII and SF participate in a reaction with factor V and brain, producing an "extrinsic thromboplastin" which subsequently reacts with prothrombin to form thrombin. If this is what really occurs, SF and factor VII would no longer be required once "extrinsic thromboplastin" is fully formed. If, however, one of these factors is excluded from incubation mixture, presence of deficient factor in the substrate would compensate by promoting formation of "extrinsic thromboplastin" in substrate clotting tube; such substrate clotting times would be expected, of course, to be more prolonged than when all factors are present in incubation mixture.

In our experiments there is an unavoidable carrying over of factor VII and SF into substrate clotting tubes. However, when factor VII is omitted from incubation mixture but included in substrate, time required for maximum yield is same as when this factor is excluded from both substrate and incubation mixture. This finding suggests that factor VII is required only for formation of "extrinsic thromboplastin" and not for subsequent reaction of "extrinsic thromboplastin" and prothrombin. Although the yield of "extrinsic thromboplastin" is greater when SF is omitted from the incubation mixture but present in substrate than when it is absent from both substrate and incubation mixture, it should not be inferred that SF is required both for action of "extrinsic thromboplastin" on prothrombin and for formation of "extrinsic thromboplastin." It has already been men-

tioned that this greater yield would also be expected on the hypothesis that SF is required solely for "extrinsic thromboplastin." The work of Flynn and Coon(1) suggests that this hypothesis is correct. These workers sedimented "extrinsic thromboplastin" by centrifugation and then washed it by suspension in saline and recentrifugation; they showed that this washed material was active in converting purified prothrombin to thrombin in presence of calcium. The method of preparation of prothrombin was such that it is unlikely to have contained SF. However, this evidence is not entirely conclusive for some free SF not utilized in the initial formation of "extrinsic thromboplastin" might have been adsorbed onto this complex.

When factor VII is excluded from both incubation mixture and substrate clotting tubes in thromboplastin generation test, normal yield is obtained, and there is also normal rate of evolution. Therefore, the finding that absence of factor VII from incubation mixture and substrate tubes affects rate of "extrinsic thromboplastin" formation although having little or no effect on yield is interesting. SF is essential for "intrinsic thromboplastin" generation, primarily affecting yield, so that finding that this factor also primarily affects yield of "extrinsic thromboplastin" was not surprising.

Summary. Previous work showing that factor V, serum, brain and calcium react together to form an active prothrombin conversion factor ("extrinsic thromboplastin") is confirmed. The active components in serum in respect of this reaction include both factor VII and SF but not PTC. SF appears to influence yield while factor VII primarily determines rate.

ADDENDUM. Although SF appears to affect yield rather than rate of formation of both "extrinsic" and "intrinsic" thromboplastin formation, work of Fisch and Duckert (*Thromb. Diath. Haem.*, 1959, v3, 98) appearing since this paper was submitted indicates that SF acts as an enzyme in "intrinsic thromboplastin" formation.

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An Attempt to Recover WEE from Nasal Mites of Sparrows. (24857)

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Bird mites collected from various species of birds harbor the virus of Western Equine Encephalitis (WEE)(1,2,3), and some investigators have shown that transmission of St. Louis encephalitis virus occurs when infected mites were allowed to feed on susceptible chickens(4,5). Inasmuch as no experiments on nasal mites were reported in the literature an attempt was made to recover WEE virus from inoculated sparrows and nasal mites harvested from them.

Materials and methods. Adult English sparrows of both sexes were captured from roosting places at night when flight activity was at a minimum. These birds were banded and bled from the heart for pre-inoculation neutralization test (reference sera); inoculated subcutaneously with 0.1 ml of WEE virus containing 140 chick LD_{50}; and turned loose in screened flight cage approximately 8′ x 12′ x 6′. The WEE virus (3742-c2) was isolated from a pool of *Culex tarsalis* mosqui-