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The Diffusible Organic Blood-Clotting Factor.

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Larson and Greenberg[1] reported experimental evidence for the presence of a diffusible organic substance in serum which accelerates the clotting of blood. Briefly, it was found that prolonged dialysis of plasma against distilled water resulted in changes which prevented coagulation when calcium chloride was added, but the addition of a small amount of serum ultrafiltrate together with the calcium salt resulted in coagulation. This observation seemed to indicate that a diffusible substance necessary for coagulation was present in blood. The present report describes the results of experiments which were carried out in an effort to characterize the action of this factor.

Dialyzed plasma was found to vary considerably in its clotting characteristics, so purified clotting reagents were used to study the action of the filterable factor. The preparation of the reagents and the technic used in studying the activation of prothrombin have been described elsewhere.[2]

The results shown in Table I demonstrate that the ultrafiltrate factor accelerates coagulation due to a thromboplastic action, and that the active material is thermolabile.

TABLE I.

Test of Thromboplastic Activity.

0.7 ml of prothrombin solution, 0.2 ml of 0.025 M $CaCl_2$, and 0.1 ml of the solution being tested for thromboplastic activity were mixed and allowed to stand at 38°. After fixed periods of time (called "Activation Time"), the mixture was tested for thrombic activity by pipetting 0.1 ml into 1.0 ml of a fibrinogen solution or oxalated plasma diluted with 3 volumes of saline.[2] The coagulation time was noted and taken as an index of the amount of thrombin present.

A. Prothrombin + $CaCl_2$ + Saline (control): No activation in 10 min					
B. Prothrombin + $CaCl_2$ + Brain Thromboplastin:					
Activation Time (min)	1	2	3	4	9
Coagulation Time "	6	5.5	4.7	3.7	2.3
C. Prothrombin + $CaCl_2$ + Plasma Ultrafiltrate:					
Activation Time (min)	1.5	3	4	5.7	
Coagulation Time "	7	4	4	4	
D. Prothrombin + $CaCl_2$ + Plasma Ultrafiltrate (previously heated at 100° for one hour): No activation.					

[1] Larson, C. E., and Greenberg, D. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 305.

[2] Glazko, A. J., and Greenberg, D. M., *Am. J. Physiol.*, 1939, **125**, 108.

TABLE II.

Material added to 0.7 ml of dialyzed plasma and 0.2 ml of 0.025 M $CaCl_2$	Coagulation Time (min)
Saline (control)	no clot in 30 min
Saliva (heated)	5
Brain extract	4
Cephalin	6
Plasma ultrafiltrate	5

The apparent thromboplastic activity of the ultrafiltrate factor is supported by the enormous effect on the coagulation time produced by the addition of known thromboplastic materials, as shown in Table II.

Other experiments were carried out to study the effect of prolonged dialysis on the proteins involved in coagulation. The dialysis of plasma was accomplished by placing the plasma in a collodion bag together with a marble and dialyzing at 5° against running distilled water for 3 or 4 days with continuous rocking to keep the contents stirred. It was found that only a small quantity of prothrombin and fibrinogen could be isolated from the dialyzed plasma. The proteins undoubtedly were affected by the dialysis in spite of the low temperature employed. The results in Table III demonstrate the changed behavior of fibrinogen toward thrombin, due to prolonged dialysis.

TABLE III.

	Coagulation Time (sec)
Thrombin + Normal Fibrinogen	15
" + Dialyzed Fibrinogen	60
" + Dialyzed Plasma	45

Prothrombin which had been isolated from dialyzed plasma gave a very poor yield of thrombin on activation. Likewise, normal prothrombin which had been subjected to dialysis under the same conditions as the plasma was practically all destroyed, as is indicated in Table IV.

TABLE IV.

	Coagulation Time (min)
Normal Prothrombin + $CaCl_2$ + Cephalin + Fibrinogen	20
Dialyzed Prothrombin + $CaCl_2$ + " + "	150
" Plasma + $CaCl_2$	120
" " + $CaCl_2$ + Cephalin	10
" " + $CaCl_2$ + " + Normal Prothrombin	2

Summary. The diffusible substance present in blood acted as a thromboplastin in accelerating coagulation. The prolonged dialysis required to demonstrate this action produced destructive changes in the plasma factors involved in coagulation.