TABLE I.

Clinical diagnosis	No.	Electron microscopy: Spherical particles	Electron microscopy: Spherical particles in crystalline-like array	Electron microscopy: Brick-shaped bodies	Pathology: Intranuclear inclusion bodies	Pathology: Molluscum bodies
Intranuclear inclusion body papilloma	5	5	4	—	5	—
Molluscum contagiosum	2	—	—	2	—	2
Common warts	9	—	—	—	—	—
Normal skin	1	—	—	—	—	—

study of suspensions of 5 skin papillomas. Histological examination of the same tissues revealed intranuclear inclusion bodies and characteristic cytoplasmic masses in the cells of the hyperplastic epidermis. Thirty-one lesions with similar histological appearance were encountered along with 158 typical verrucae in a review of surgical pathological material.

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Studies on the Thromboplastic Agent in Plasma. (17329)

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Thromboplastic preparations can conveniently be divided into two categories, those containing proteins and those which are lipids free from protein. In a recent study on the mode of action of Dicumarol[1] evidence was presented indicating that the thromboplastic agent in plasma responsible for the clotting of recalcified plasma had the physiological characteristics of a lipid thromboplastic agent. Further evidence has been obtained consistent with this view and will be presented in this paper. This evidence depends on a demonstration of a direct proportionality between the coagulation time obtained with the thromboplastic lipid and the coagulation time of a control sample of the same plasma which was recalcified and to which no thromboplastic agent was added. Such a relationship can be explained most simply on the basis that the thromboplastic agent in plasma has the characteristics of the lipid thromboplastic agent. Such a relationship also implies, as will be discussed later, a mutual dependence on some limiting factor found in plasma.

Methods. The thromboplastic lipid was prepared from beef brain as outlined in the previous study.[2] It was stabilized by adding hydroquinone to make up 1% of its weight. This substance is an antioxidant that prevents the autoxidation of the preparation and its subsequent loss of activity.[3] In assaying the thromboplastic lipid a 0.2% emulsion in 0.9% NaCl was prepared from the dry material. The coagulation times of recalcified dog plasma were determined at room temperature with and without the thromboplastic lipid on 22 different occasions extending over a four month period. The plasma was obtained by withdrawing 9 parts of blood from the femoral artery of an unanaesthetized dog

1 Lein, J., and Lein, P. S., *Am. J. Physiol.*, 1948, **155**, 394.

2 Hays, H. W., and Lein, J., *Arch. Biochem.*, 1945, **7**, 69.

3 Lein, J., and Lein, P. S., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 446

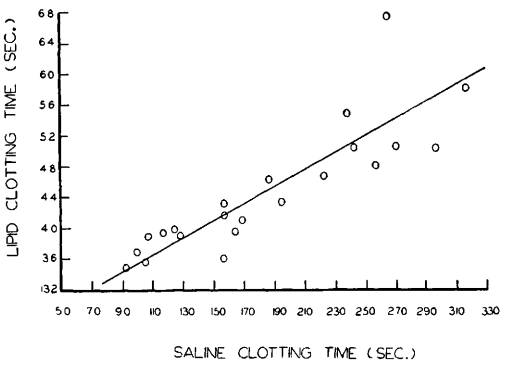

FIG. 1.
Linear relationship between clotting times obtained with and without the addition of the thromboplastic lipid to various samples of plasma.

directly into 1 part of 0.13 M sodium citrate. The blood was centrifuged for 30 minutes at 2,500 r.p.m. and the upper three-fourths of the plasma used. The clotting mixture consisted of 0.6 cc plasma, 0.2 cc of 1% $CaCl_2$, and 0.2 cc of either 0.9% NaCl or of 0.2% thromboplastic lipid emulsion in 0.9% NaCl. The $CaCl_2$ solution was always added last and the coagulation time determined with a stop watch from the time of mixing of the clotting mixture to the time the tube could be inverted with no flow of its contents. The tubes used were small test tubes 10 x 75 mm. Two determinations were done in each case and the average used providing the average deviation did not exceed 10% of the mean. In a few cases where the deviations were greater, additional determinations were carried out.

Results. There was a considerable variation in the coagulation times of different samples of plasma. This was true in those determinations in which the thromboplastic agent was added as well as those in which only saline was added. The variation, however, was not random. The results indicate a definite relationship between the clotting time obtained without the addition of the thromboplastic lipid and that obtained with its addition. The results are presented graphically in Fig. 1, each of the points representing determinations on a different sample of plasma. The points were fitted by a straight line using the method of least squares. The significance of the regression coefficient was tested statistically by the null hypothesis[4] and was found to be highly significant (df = 20, t = 12.3).

Discussion. The shortening of coagulation time by the addition of thromboplastic agents coupled with the fact that there is a great excess of prothrombin[5] and of fibrinogen[6] in normal plasma makes it evident that a limiting factor in coagulation when calcium ions are added in optimal quantities is the amount of thromboplastic agent present. The results have indicated that a linear relationship exists between the accelerated clotting time caused by the addition of the thromboplastic lipid and the clotting time of the plasma without this addition. The possibility that this dependence is due merely to the additive effect of 2 thromboplastic substances is excluded since the concentration of the lipid used was in excess of the amount necessary to produce the maximum shortening of coagulation time.[2] With this possibility ruled out, it becomes difficult to explain the results in terms of the classical ideas of thromboplastin action. If the lipid thromboplastic agent reacts with prothrombin in the presence of calcium ions to form thrombin, one would not expect there to be such a dependence as was demonstrated.

The results are believed to be explained most simply by the hypothesis that both the thromboplastic agent in the plasma and the thromboplastic lipid have the same mechanism of action and that the clotting of plasma by both of these agents depends on some limiting factor found in the plasma. The latter assumption is necessary since the results are obtained with optimal quantities of the thromboplastic lipid. The variation of clotting times of the different plasma samples is thought to be due to the variation in the plasma factor since prothrombin is present in more than adequate concentrations. According to this view the lipid thromboplastic agent prepared from tissues requires another factor found in plasma in order to become active. This is not true for protein thromboplastic agents since the work of Quick[7] and

[4] Snedecor, G. W., Statistical Methods, Iowa State College Press, Ames, 3rd ed., 118.

[5] Tanturi, C. A., and Banfi, R. F., *J. Lab. and Clin. Med.*, 1946, **31**, 703.

[6] Witts, L. J., *J. Path. and Bact.*, 1942, **54**, 516.

[7] Quick, A. J., *Science*, 1940, **92**, 113.

others showed that the plasma clotting times with these agents are absolute and depend only on the prothrombin concentration when it becomes limiting.

Summary. Statistical evidence is presented for a relationship between the coagulation time of recalcified plasma and the coagulation time accelerated by the addition of a thromboplastic lipid. This relationship is thought to imply that the thromboplastic substance in plasma responsible for the clotting of recalcified plasma has the same physiological characteristics as the lipid thromboplastic agent and that both require for activity some limiting factor present in plasma.

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Effect on Prothrombin of Acute Massive Plasmapheresis with Simultaneous Chloroform Intoxication. (17330)

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From the earliest work on blood clotting, plasma prothrombin has been recognized as an integral component in the mechanism of thrombin formation. The effects on prothrombin of diverse pathologic and physiologic states have been studied extensively in the clinic and laboratory. Because prothrombin is so intimately related to liver function, an appreciable understanding of the origin, mode of action, and utilization of prothrombin may be acquired from subjecting the liver to various abnormal chemical and physical conditions. Experimental evidence and clinical experience with certain forms of liver and biliary tract disease indicate that plasma prothrombin is produced through the vital activity of the liver.[1-7] Thus experimentally induced hepatic necrosis,[1,2] and partial[3] or complete hepatectomy[4,5] result in hypoprothrombinemia.

Many factors, in addition to the fundamental integrity of the liver, are known to operate in the maintenance of plasma prothrombin. An adequate intake of Vitamin K is essential. Certain drugs, notably dicumarol, cause hypoprothrombinemia without any morphologic evidence of damage to liver tissue. Yet very little is known about the mechanism by which prothrombin is produced and utilized in the body.

There is considerable evidence to suggest that plasma prothrombin will gradually disappear over a period of 1-3 days if the production of new prothrombin is impaired. Thus the minimum prothrombin concentration is reached in 36-48 hours following severe chloroform poisoning,[1,2] or partial hepatectomy.[3] If animals are rendered hypoprothrombinemic as a result of Vitamin K deficiency,[8] or dicumarol poisoning,[9] transfusions with normal blood result in a temporary elevation of prothrombin concentration. The effect of transfusions lasts only 1-2 days. Purified bovine prothrombin administered intravenously lasts 2-3 days in a dog made hypoprothrombinemic with dicumarol.[10] On the other hand, when prothrombin production is impaired, the tendency of plasma prothrombin concentration

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2 Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

3 Warner, E. D., *J. Exp. Med.*, 1938, **68**, 831.

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5 Andrus, W. D., Lord, J. W., and Moore, R. A., *Surgery*, 1939, **6**, 899.

6 Bollman, J. L., Butt, H. R., and Snell, A. M., *J.A.M.A.*, 1940, **115**, 1087.

7 Brinkhous, K. M., *Medicine*, 1940, **19**, 329.

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9 Quick, A. J., The Hemorrhagic Diseases, Charles C. Thomas, 1942, p. 283.

10 McGinty, D. A., Seegers, W. H., Pfeiffer, C. C., and Loew, E. R., *Science*, 1942, **96**, 540.