animals were not in shock during hypothermia. Intraperitoneal injections of ox bile, and solutions of peptone produced a less intense hypothermia although ox bile caused severe shock.

Received August 8, 1949. P.S.E.B.M., 1949, **72.**

A Highly Active Material that Promotes Conversion of Prothrombokinase Complex.* (17419)

J. H. MILSTONE (Introduced by H. S. N. Greene)

From the Laboratory of Pathology, Yale University School of Medicine, New Haven, Conn.

A procedure has been described[1] for analyzing blood coagulation in three experimental steps: 1. activation of prothrombokinase; 2. activation of prothrombin; 3. coagulation of fibrinogen. Crude thrombokinase, besides activating prothrombin, also accelerated conversion of prothrombokinase. Both activities were lost upon adsorption with barium sulfate.

It is now reported that a material, prepared by elution from barium sulfate, promotes the activation of prothrombin, and also the conversion of crude prothrombokinase. Beginning with a solution of bovine plasma globulins, fraction "A" was adsorbed on Hyflo (Johns-Manville) and eluted with phosphate-potassium chloride solution. The unadsorbed globulins were exposed to adsorption by barium sulfate. From the latter, two successive fractions were eluted, with 0.2-0.3M phosphate, pH 6.6, and with 0.6M phosphate, pH 7.9. The pH 6.6 eluate had more prothrombin. From the pH 7.9 eluate, proteins soluble in 0.35, but precipitable by 0.45 saturated ammonium sulfate were selected as the "converter fraction." This involved 3 extractions and 20 precipitations. Proteins soluble in 0.45, but precipitable by 0.60 saturated ammonium sulfate, constituted the "thrombin fraction" (3 extractions, 7 precipitations). Fifty-five liters of plasma yielded 10 ml of each fraction. Both were dialyzed simultaneously in the same beaker.

Activations were studied with calcium present, giving results summarized in Table I. At high dilution, the converter hastened both the activation of prothrombin and the conversion of crude prothrombokinase. In comparing different preparations, converter activities estimated by the 2-stage and 3-stage procedures were roughly parallel. This is compatible with the view that the tests measured two different effects of the same substance.

In contrast, converter activity did not parallel thrombin activity. The ratio of converter to thrombin activity was 100 times as great in the converter fraction as in the thrombin fraction. Conceivably, varieties of thrombin can exist with different converter activities, or some factor modifies the behavior of thrombin in one of the fractions. If, as must be contemplated, the converter is distinct from thrombin, the results suggest how difficult would be its elimination from thrombin by salting-out. Moreover, the converter is manifest in sufficiently minute amount to cause confusion, even as a small contamination in a highly purified thrombin.

The relation of "A" and converter to thrombokinase activity resembles that reported for platelets plus "globulin".[2] Their effects are complementary, but not simply additive. Clear evidence has not been obtained that their mutual reinforcement is due chiefly to progressive activation of one by the other. An additional possibility must be considered, that one is thrombokinase and the other contains a co-factor or anti-inhibitor.

* Aided by a grant from the James Hudson Brown Memorial Fund of the Yale University School of Medicine.

1 Milstone, J. H., *J. Gen. Physiol.*, 1948, **31**, 301.

2 Milstone, J. H., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 225.

TABLE I.
Effective Amounts of Converter Fraction.

2-stage tests		3-stage tests
Activate half of prothrombin in 40 min.	Accelerate prothrombin activation in presence of aged fraction "A"	Accelerate conversion of crude prothrombokinase
2/11 dilution	1/1,000 dilution 1 μg protein-N/ml	1/45,000 dilution 0.02 μg protein-N/ml

Figures refer to final dilution or concentration in the respective activation mixtures. A concentration of 0.02 μg protein-N per ml prothrombokinase mixture becomes 0.002 μg per ml when the mixture is diluted tenfold in prothrombin. The tests were performed as previously described.[1,2]

Previous comparison[1] of prothrombokinase to Factor V was based on Owren's tentative *concept* of V as the precursor of VI.[3] If his V *preparation* did not so function, the comparison does not apply to it. Crude prothrombokinase "may contain an activator complex with more than one significant component."[1] More than one transformation may occur in the complex.

Although the converter fraction is highly active, it may be far from pure, chemically. It *may* represent a thrombokinase which activates not only prothrombin, but also prothrombokinase. Several years ago,[4] it was reported that concentrated prothrombin changed to thrombin in the absence of ionic calcium, without addition of activators; and the possibility was left open that a contaminant was responsible. The possibility must be entertained that the converter contaminates highly purified clotting preparations, and might be responsible for certain effects occasionally attributed to prothrombin or thrombin.

[3] Owren, P. A., *Acta Med. Scand.*, 1947, suppl. 194.

[4] Milstone, J. H., *J. Gen. Physiol.*, 1942, **25**, 679.

Received August 9, 1949. P.S.E.B.M., 1949, **72**.

A Modification of the Hemagglutination Test for Rheumatoid Arthritis.* (17420)

GEORGE HELLER, ABRAHAM S. JACOBSON, AND MAXWELL H. KOLODNY (Introduced by R. L. Cecil)

From the Veterans Administration Hospital, Bronx, N. Y.

A diagnostic test for rheumatoid arthritis based upon the agglutination of sheep erythrocytes has been recently reported by Rose, Ragan, Pearce and Lipman.[1] Serum from patients with rheumatoid arthritis agglutinates sheep erythrocytes sensitized with sheep cell hemolysin to a titer at least 16 fold higher than that obtained with normal sheep cells. These are expressed as a differential titer $\left(\frac{\text{titer with sensitized cells}}{\text{titer with normal cells}}\right)$. The test has been confirmed but may be of limited value for diagnostic purposes.[2,3] In the performance of the test as described by Rose, *et al.*,[1] we

* Published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the authors.

[1] Rose, H. M., Ragan, C., Pearce, E., and Lipman, M. O., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 1.

[2] Sulkin, S. E., Pike, R. M., and Coggeshall, H. C., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 475.

[3] Jawitz, E., and Hook, E. V., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 650.