portion moindre; pour 1.00 molécule de DNP-arginine, on trouve DNP-sérine 0.14, DNP-thréonine 0.13, DNP-aspartique 0.12; des coupures aberrantes s'effectuent donc en faible proportion au niveau des acides aminés hydroxyles; ceci expliquerait que LANDMAN *et al.*[4], utilisant uniquement la technique d'Edman, aient observé une libération de sérine à la cinquième scission. L'isolement d'un peptide *Val. Phe. Gly. Arg* après hydrolyse trypsique concorde avec la spécificité admise pour cet enzyme, et d'autre part est en accord avec l'enchaînement initial *Lys. Val. Phe. Gly. Arg* trouvé par les méthodes chimiques.

Le détail de ces investigations paraîtra ultérieurement dans ce journal.

BIBLIOGRAPHIE

1 J.C. LEWIS, N.S. SNELL, D.J. HIRSCHMANN ET H. FRAENKEL-CONRAT, *J. Biol. Chem.*, 186 (1950) 23.
2 R. ACHER, M. JUTISZ ET C. FROMAGEOT, *Biochim. Biophys. Acta*, 5 (1950) 493.
3 R. ACHER, J. CHAUVET, C. CROCKER, U. R. LAURILA, J. THAUREAUX ET C. FROMAGEOT, *Bull. soc. chim. biol.*, 36 (1954) 167.
4 F. SANGER ET H. TUPPY, *Biochem. J.*, 49 (1951) 463.
5 R. ACHER, J. THAUREAUX, C. CROCKER, M. JUTISZ ET C. FROMAGEOT, *Biochim. Biophys. Acta*, 9 (1952) 339.
6 R. CONSDEN, A. H. GORDON ET A. J. P. MARTIN, *Biochem. J.*, 41 (1947) 590.
7 F. SANGER ET E. O. P. THOMPSON, *Biochem. J.*, 53 (1953) 353.
8 W. A. SCHROEDER, *J. Am. Chem. Soc.*, 74 (1952) 5118.
9 S. MOORE ET W. STEIN, *J. Biol. Chem.*, 192 (1951) 663.
10 P. EDMAN, *Acta Chim. Scand.*, 4 (1950) 283.
11 P. EDMAN, *Acta Chem. Scand.*, 7 (1953) 700.
12 G. BIZERTE ET R. OSTEUX, *Bull. soc. chim. biol.*, 33 (1951) 50.
13 M. ROVERY ET C. FABRE, *Bull. soc. chim. biol.*, 35 (1953) 541.
14 W. A. LANDMANN, M. P. DRAKE ET J. DILLAHA, *J. Am. Chem. Soc.*, 75 (1953) 3638.

Reçu le 26 février 1954

THE ACTIVATION OF THROMBOPLASTIN BY CALCIUM*

by

GERARD F. LANCHANTIN AND ARNOLD G. WARE

Department of Biochemistry and Nutrition, University of Southern California School of Medicine, Los Angeles, California (U.S.A.)

The role of calcium in the physiological coagulation of blood has been a much disputed question for many years (for a review see WOHLISCH[1]). This preliminary report presents data which indicates that calcium is an activator of the thromboplastic enzyme. It has been a frequent observation in our Laboratory using one-stage clotting systems[2,3] that incubation of human brain thromboplastin with calcium greatly increases its clotting activity.

TABLE I

A RECONSTRUCTED COAGULATION SYSTEM COMPOSED OF PURIFIED CLOTTING FACTORS ISOLATED FROM HUMAN BLOOD AND TISSUES

Clotting factor	*Concentration*	*Activity*	*Method of preparation*
Prothrombin	0.09 mg protein/ml	142 units/ml*	(4)
Plasma Ac-Globulin	0.18 mg protein/ml	94%*	(4), (5)
Brain Thromboplastin	0.81 mg protein/ml	100%**	(3)
Fibrinogen	10 mg clottable protein/ml	—	(6)
Calcium chloride	0.008 *M*	—	—

* Activity comparable to normal human plasma.
** Activity giving 13.0 secs in reconstructed clotting system.

* Supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract # DA-49-007-MD-193 and by the USPHS RG 2684.

In order that this phenomenon could be studied in more detail, a purified coagulation system composed of the human blood clotting factors was devised. This reconstructed clotting system is presented in Table I. All the components listed in the table were added in 0.1 ml volumes to 75 × 10 mm test tubes at 37° C and the time of coagulation noted. Variation in the thromboplastin concentration from 100 % (arbitrary value) to 0.5 % produced clotting times the logarithm of which were directly proportional to the logarithm of the enzyme concentration. Such a pseudo-first order kinetic relationship served to standardize enzyme concentrations.

When the thromboplastin and calcium were incubated together at 37° C before adding them to the other components, the clotting time became progressively shorter. Such an experiment is presented in Fig. 1. The right and left ordinates of the Figure demonstrate the relation between thromboplastin concentration and clotting time. When thromboplastin of varying concentrations is activated maximally with calcium, the clotting times still exhibit a log-log relationship to concentration.

One of the necessary points which must be critically examined before one may postulate an activating effect of calcium on thromboplastin is that the enzyme preparation is not contaminated with some other clotting component. This does not appear to be the case. In the first place, purification of the enzyme does not appear to alter the calcium activating property. Secondly, the presence of prothrombin, thrombin, accelerator globulin or convertin (SPCA, Factor VII) in the thromboplastin could not be detected. Thirdly, experiments employing partial denaturation of the enzyme preparation with 1000 kc ultrasonic waves indicated that the degree of calcium activation of the enzyme was parallel in both partially denatured and untreated preparations of comparable initial activity. This would be very unlikely if the activation of thromboplastin with calcium were due to the presence of some other procoagulant.

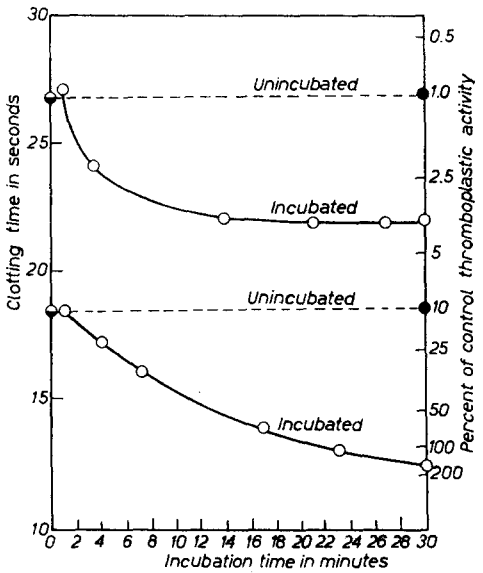

Fig. 1. The effect of incubation of human brain thromboplastin with calcium on the clotting time and thromboplastic activity of a reconstructed purified coagulation system.

In the two-stage determination of prothrombin[7] the activated enzyme appears to speed up the rate in the early stages of conversion of prothrombin to thrombin but does not increase the final thrombin yield. Strontium ion, which is capable of clotting decalcified blood in a physiological manner though less efficiently than calcium, is also an activator of human brain thromboplastin in a manner similar to that described above.

REFERENCES

1 E. WOHLISCH, *Ergebn. d. Physiol.*, 34 (1940) 174.
2 A. G. WARE AND R. STRAGNELL, *Am. J. Clin. Path.*, 22 (1952) 791.
3 G. F. LANCHANTIN AND A. G. WARE, *J. Clin. Invest.*, 32 (1953) 381.
4 M. L. LEWIS AND A. G. WARE, *Proc. Soc. Exptl. Biol. Med.*, 84 (1954) 636.
5 M. L. LEWIS AND A. G. WARE, *Proc. Soc. Exptl. Biol. Med.*, 84 (1954) 640.
6 A. G. WARE, M. GUEST AND W. H. SEEGERS, *Arch. Biochem.*, 13 (1947) 231.
7 A. G. WARE AND W. H. SEEGERS, *Am. J. Clin. Path.*, 19 (1949) 471.

Received March 5th, 1954