HÆMATOLOGY

Fibrinogen Free of Plasminogen as Substrate for Fibrinolytic Assays

THE most physiological substrate for fibrinolytic assays is non-heated fibrin. However, the clot formed from ordinary fraction-I, or even from highly purified fibrinogen, is generally heavily contaminated by plasminogen; this interferes with the protease content of the test solution.

Lassen therefore proposed the use of fibrin plates heated for 1 hr. at 80° to destroy the pro-enzyme activity[1]. On the other hand, several authors have published techniques for the purification of fibrinogen involving repeated precipitation in order to overcome the intimate association of plasminogen with fibrinogen[2]. The difficulty of preparing fibrinogen free from plasminogen and the complexity of these rather time-consuming techniques led other workers to use artificial substrates such as casein, *TAME*, *LME*[3]. However, the lysis of a normal but plasminogen-free clot formed under well-defined conditions seems to be the ideal substrate for the evaluation of plasmin activity. Such a preparation can be obtained by treatment of fraction-I with tricalcium phosphate.

We first noticed that certain preparations of fraction-I obtained from blood collected on ion-exchange resin showed less lytic activity than ordinary fibrin clots[4]. The fraction-I under consideration, which we call 'fraction-I*A*', is prepared by alcoholic precipitation as usual, but the starting material is plasma which was previously adsorbed by 0·5 per cent tricalcium phosphate. (All tricalcium phosphates have not the same adsorbing capacities; we used the light triphosphate from Baker Manuf., which has outstanding adsorbing properties.) We then re-adsorbed this fraction I*A* with increasing amounts of tricalcium phosphate (10 min. at room temperature) to remove the residual plasminogen contaminant (Table 1). Fibrinogen, free of plasminogen, or contaminated with trace amounts only, is obtained when a 1·5 per cent solution of clottable protein is adsorbed by 15 per cent of tricalcium phosphate. The solution remaining after centrifugation of the adsorbing powder contains about 15 per cent of the original clottable protein, the rest having been adsorbed together with the pro-enzyme. The solution can be stored in the frozen state or lyophilized. The stock solution is diluted with veronal buffer, *p*H 7·35 (Owren-Koller), and the lysis test performed in the following way: 0·1 ml. of the test solution, or buffer + 0·1 ml. of streptokinase (66 u./ml. dornokinase); incubation for 3 min. at 37° C.; + 0·1 ml. thrombin (thrombin time 15 sec. with oxalated plasma) + 0·1 ml. of fibrinogen (1 gm./litre).

Only small amounts of fibrinogen are needed for each test, and we noticed that tiny clots gave reliable results and are more sensitive to small concentrations of protease. Ordinary fibrinogen will give a lysis time of about 3–5 min. under these experimental conditions.

Plasminogen is not removed from ordinary fraction-I by the adsorption technique described. This material is obtained from blood collected in citrate. We therefore studied the adsorption, on tricalcium phosphate, of plasminogen in the presence of citrate, and we found that this anion is a powerful inhibitor of the removal of plasminogen from fibrinogen[4]. This action of citrate is not a simple effect of salt concentration (Table 2). Standard fraction-I can be used as starting material for the preparation of fibrinogen for fibrinolytic assay if the citrate concentration is first lowered by washing the crude paste, followed by re-precipitation or resin treatment. Further details will be given elsewhere.

Table 1. QUANTITY OF TRICALCIUM PHOSPHATE (BAKER) NEEDED FOR COMPLETE ADSORPTION OF PLASMINOGEN.
Fraction 1 obtained from plasma treated with ion-exchange resin and containing trace amounts of citrate

		Thrombin time*	Lysis time
Percentage of tricalcium phosphate. (Adsorption, 10 min.: centrifugation, 10 min.; 3,600 r.p.m.; 1·5 per cent clottable proteins)†	0	5 sec.	9 min. 30 sec.
	5	6 sec.	12 min. 30 sec.
	10	12 sec.	17 min. 30 sec.
	15	15 sec.	> 1 hr. 30 min.
	20	42 sec.	> 1 hr. 30 min.
	25	No clot	No clot
Fraction 1—Standard (0·5–1 gm./litre clottable proteins)		5 sec.	5 min.

* Purified thrombin, free of plasminogen, giving a thrombin time of 7 sec. with normal oxalated plasma and 5 sec. with standard fibrinogen.
† A considerable amount of fibrinogen is adsorbed together with plasminogen; to avoid the necessity of re-concentration, we use concentrated solution of fraction I (1·5 per cent clottable proteins) as starting material.

A. VIGNAL
CH. BLATRIX
M. STEINBUCH

Centre National de Transfusion Sanguine,
6 rue Alexandre-Cabanel, Paris, XV^e.

[1] Lassen, M., *Biochem. J.*, **69**, 360 (1958). Astrup, T., and Mullertz, S., *Arch. Biochem. Biophys.*, **40**, 346 (1952).
[2] Kekwick, R. A., Mackay, M. E., Nance, M. H., and Record, B. R., *Biochem. J.*, **60**, 671 (1955). Laki, K., *Arch. Biochem. Biophys.*, **32**, 317 (1951). Ware, A. G., Guest, M. M., and Seegers, W. H., *Arch. Biochem.*, **13**, 231 (1947).
[3] Remmert, L. F., and Cohen, P., *J. Biol. Chem.*, **431**, 181 (1949). Schultz, R. L., Moorman, J. A., Matorish, L. O., and Lincoln, A. F., *Proc. Soc. Exp. Biol. et Méd.*, **94**, 198 (1957).
[4] Dormont, J., Steinbuch, M., Blatrix, Ch., and Weilland, C., *Path. et Biol.*, **7**, 2439 (1959).

ANATOMY

Shape and Structure of the Kidney

WORK on the action of the heart and the flow of blood, reported by me[1], has now reached the stage at which it may be used to identify the shape and internal structure of the kidney.

It is necessary to assume that the structure is organized and that the proteins thereof, although crystalline in nature, exist in skeleton form. They conform, however, to the systems, classes and space-groups of crystalline substances in general, producing a composite crystal similar in complexity, for example, to that found in rock crystals where isomorphous crystalline compounds occur in juxtaposition, associated with replacement and interpenetration. Cell fluid is present in the interstices of the skeleton lattice work and fibrous forms constitute hollow tubes wherein flows blood or other fluid such as glomerular filtrate.

Table 2. ROLE OF CITRATE AND OF IONIC STRENGTH
Precipitate of Fraction 1*A* dissolved in citrated buffers of increasing concentration

Trisodium citrate (*M*)	0·00045	0·0009	0·0018	0·0036	0·009	0·018
Sodium chloride (per cent)	0·45	0·45	0·45	0·45	0·45	0·45
Clottable proteins before adsorption, 11·5 gm./litre. Adsorption, 10 min. with tricalcium phosphate (Baker, 15 per cent).						
Clottable proteins (gm./litre)	1·0	1·5	1·8	3·0	9·0	11·0
Lysis time*	> 2 hr.	> 2 hr.	> 2 hr.	3 min.	2 min. 45 sec.	2 min. 30 sec.

* After dilution to approximately 1 gm./litre clottable protein.

© 1962 Nature Publishing Group