Note on the Electrophoresis of Thrombin.* (18316)

WALTER H. SEEGERS, ROBERT I. MCCLAUGHRY, AND EDNA B. ANDREWS.

From the Department of Physiology and Pharmacology, Wayne University College of Medicine, Detroit, Mich.

It has been possible to prepare prothrombin of sufficient purity to make it feasible to study its electrophoretic properties(1,2). Its isoelectric point is near pH 4.2 in buffer solution of 0.1 ionic strength, and the mobility is nearly the same as that of α-globulin. These electrophoretic properties of the protein change when it is activated to thrombin. This is most neatly demonstrated by dissolving the prothrombin in 25% sodium citrate solution. In this environment activation takes place by autocatalysis(2,3). There is no need to add such activators as calcium, thromboplastin, Ac-globulin, etc.; hence, upon activation there are no uncertainties introduced by these substances or by any impurities that might be associated with these activators. The products of prothrombin activation may thus be considered to be derived from prothrombin itself. Usually this has of necessity been considered only in terms of thrombin activity, but work with purified materials shows that more than one substance is derived from prothrombin as the result of its activation. In fact, at least three distinct entities are indicated by elecrophoretic analysis to be represented in thrombin preparations. These 3 substances need further investigation; but information gained from electrophoretic studies is reported in this paper. It was especially desired to determine the isoelectric point of the components in our thrombin preparations and to see whether it might be possible to make electrophoretic separations.

Experimental. The methods employed for the assay(4,5) and preparation of materials (1,2) have been described in detail in previous work. The prothrombin preparations did not have all impurities eliminated, because large quantities were needed in the macro-electrophoresis cells of the Tiselius apparatus and this made the cost of materials a factor to be considered. Consequently it seemed justifiable to compromise and work with prothrombin definitely known to contain impurity. The prothrombin was activated in 25% sodium citrate solution, precipitated with ammonium sulfate and freed of the latter by dialysis. All assays for activity were made as previously described.

Results. It was possible to follow the

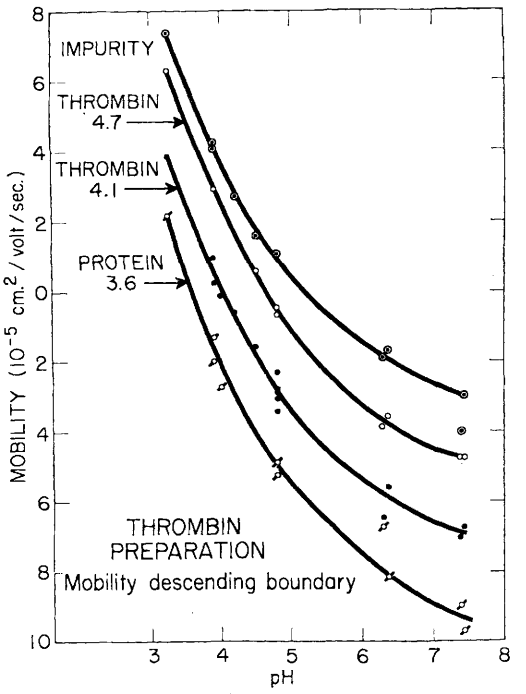

FIG. 1.

Electrophoretic mobility of purified bovine thrombin in barbiturate, phosphate, and acetate buffers of 0.1 ionic strength. The mobilities were calculated from observations of the descending boundary pattern.

* Aided by a grant from the National Institutes of Health, United States Public Health Service.

1. Seegers, W. H., Loomis, E. C., and Vandenbelt, J. M., *Arch. Biochem.*, 1945, v6, 85.

2. Seegers, W. H., McClaughry, R. I., and Fahey, J. L., *Blood*, 1950, v5, 421.

3. Seegers, W. H., *Circulation*, 1950, v1, 2.

4. Ware, A. G., and Seegers, W. H., *Am. J. Clin. Path.*, 1949, v19, 471.

5. Seegers, W. H., and Smith, H. P., *Am. J. Physiol.*, 1942, v137, 348.

mobility of 4 components (Fig. 1). One of these could be considered impurity because it was also found in the prothrombin preparations from which the thrombin was derived. The other 3 components have their isoelectric points at about pH 4.7, 4.1 and 3.6. One of these values is near that found for prothrombin which, under the same conditions, has an isoelectric point of about pH 4.2. The latter value is somewhat uncertain because prothrombin is not sufficiently soluble at its isoelectric point to permit accurate measurement. The value represents the result of extrapolating a curve rather than precise determinations. Such solubility difficulties are not encountered with thrombin preparations. The protein with an isoelectric point of pH 4.1 is quite soluble at that pH. It is thus different from the protein found in the prothrombin preparations. Of the other 2 proteins, one has an isoelectric point at pH 4.7 and the other at pH 3.6. Tentatively we are referring to these proteins by the terms 4.7-Thrombin; 4.1-Thrombin; and 3.6-Protein. This conveys the idea that two possess thrombic activity and that the third one does not.

Analytical solubility curves described by Seegers and McGinty(6) and repeated again later indicated the possibility that thrombin activity might be possessed by 2 different proteins. In electrophoretic separation experiments this idea received further confirmation. However, the work could not be done as nicely as we had hoped. Consequently it is only possible to say that it is quite likely that thrombic activity resides in 2 proteins, that the question is not satisfactorily answered, and that there are no indications to the contrary. Work on the 3.6-protein was somewhat more encouraging. This component is present in low concentration but has a high mobility and it was possible to separate material which most probably represented this protein. It was free of thrombic activity and it contained much less tyrosine than the thrombin preparation as a whole. It contained carbohydrate. The separations in several experiments were considered to be technically satisfactory and it seems justifiable to conclude that this protein is inert with respect to thrombic activity.

Summary. Results of electrophoresis experiments with purified thrombin preparations indicate that thrombic activity is probably associated with 2 proteins. Under the conditions of the experiments one has an isoelectric point of pH 4.1 and the other at pH 4.7. Another substance is derived from prothrombin and is always found in the thrombin preparations. It has a high electrophoretic mobility and its isoelectric point is 3.6. This is called 3.6-protein, and it is very likely devoid of thrombic activity.

6. Seegers, W. H., and McGinty, D. A., *J. Biol. Chem.*, 1942, v146, 511.

Received October 30, 1950. P.S.E.B.M., 1950, v75.

Cortisone-induced Transformation of Ovaries into Testes in Larval Frogs.* (18317)

EMIL WITSCHI, AND C. Y. CHANG.

From the Department of Zoology, State University of Iowa, Iowa City.

In continuation of earlier experiments with standard androgenic and estrogenic substances, a study was made of the effects of some alkyl substituted androstanes on the process of sex differentiation in larval frogs *(Rana sylvatica)*. The 4 investigated compounds all brought about inversions in genetic females. However, the required dosages are high in comparison with testosterone and its derivatives(1).

* Aided by grants from the National Research Council, Medical Division; Committee for Research in Problems of Sex. We are further indebted to Ciba Pharmaceutical Products, Inc. for the gift of Pregnenolone, Progesterone, and Desoxycorticosterone acetate.