this animal in a substantial amount develops much more slowly if 10% of the fat in the diet is in the form of corn oil instead of Crisco. If vitamin B_6 is injected into corn oil fed rats the signs and symptoms of the egg white acrodynia almost entirely disappear.

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Studies on Identity of Prothrombin.

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Since Schmidt's[1] work in 1872, there have been many attempts to isolate and identify thrombin and its precursor, prothrombin. Chief among these have been those of Howell,[2] Eagle,[3] Mellanby,[4, 5] Seegers,[6] and Astrup and Darling.[7] Most of these workers have believed that prothrombin is a globulin. Mellanby, Seegers, and Astrup and Darling have probably isolated thrombin in its purest form, and Mellanby has investigated its chemical composition as well as its physiological properties. More recently, Astrup, *et al.*, have, on the basis of precipitation studies with ammonium sulphate, demonstrated that the precipitation pattern of thrombin was that of albumin.

In the course of plasma protein studies in multiple sclerosis,[8] using Butler's[9] method of phosphate buffer precipitation and the Tiselius electrophoresis technic, opportunity was presented to obtain additional data on the identity of prothrombin. Our aim was to show with what fraction of plasma proteins prothrombin precipitated, and how it migrated when subjected to electrophoresis by Tiselius's technic

Oxalated human blood plasma was mixed with Butler's phosphate

1 Schmidt, Alexander, *Pfluger's Arch.*, 1872, **6**, 445.

2 Howell, William H., *Am. J. Physiol.*, 1914, **35**, 474.

3 Eagle, Harry, *J. Gen. Physiol.*, 1935, **18**, 531.

4 Mellanby, J., *Proc. Roy. Soc.*, London, Ser. B, 1931, **107**, 271.

5 *Ibid.*, 1933, **113**, 93.

6 Seegers, W. H., Smith, H. P., Warner, E. D., Brinkhous, K. M., *J. Biol. Chem.*, 1938, **123**, 751.

7 Astrup, Tage, and Darling, Sven, *J. Biol. Chem.*, 1940, **133**, 761.

8 Putnam, Tracy J., to be published.

9 Butler, Allan M., and Montgomery, Hugh, *J. Biol. Chem.*, 1932, **99**, 173.

buffer in equal parts beginning at a concentration of 1.3 molar phosphate of resultant mixture, and increasing with an increment of 0.1 molar through 2.5 molar. The mixtures were allowed to stand several hours at room temperature, and filtered through Whatman No. 50 paper. Each precipitate and filtrate was saved. The precipitates were washed with buffer of molar strength equal to that used in the precipitation and washings discarded. The washed precipitates were dissolved in distilled water and all (filtrates and precipitates) were dialyzed for 12 hours in Viscose casings in running tap water to remove most of the phosphate. These resulting solutions were made up to equal volume,[5] and after activation with 3% rabbit brain extract and calcium they were tested for their thrombin content by the speed with which they clotted fibrinogen solution.

From the results (Chart 1) it is seen that prothrombin precipitates

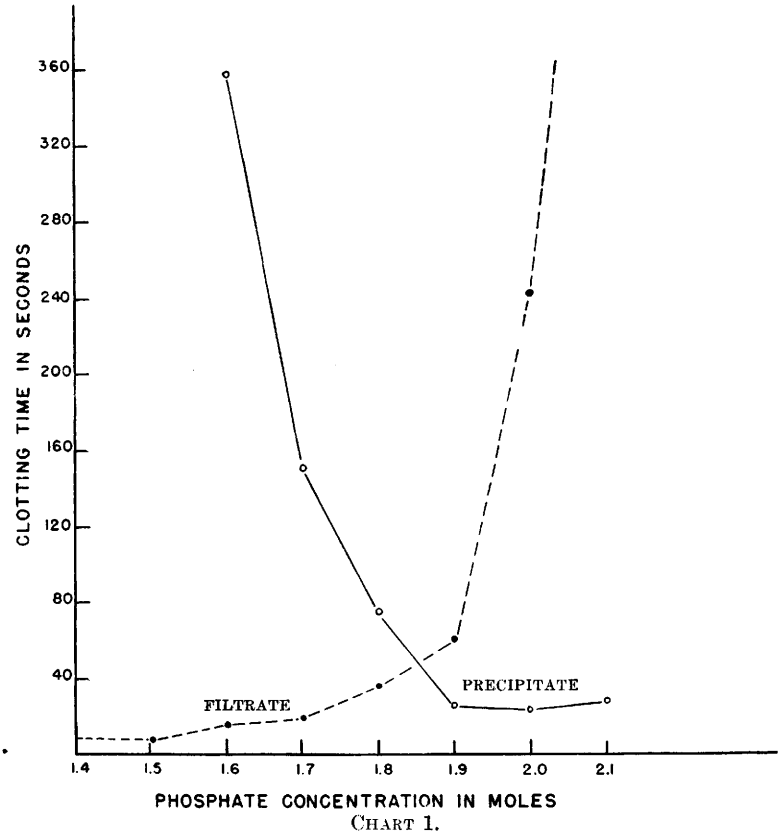

CHART 1.

Clotting time of fibrinogen by both precipitate and filtrate of plasma proteins plotted against molar strength of phosphate at which the division was made.

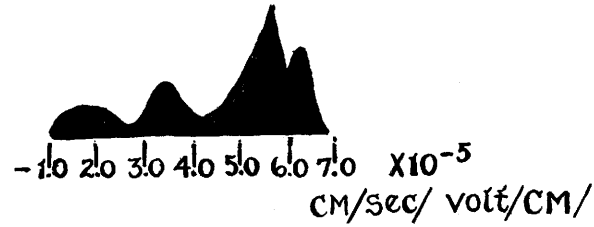

CHART 2.

Electrophoretic pattern of plasma proteins of high prothrombin content. Mobilities are given by position of peaks and relative concentration by areas under them.

between 1.7 and 2.1 molar phosphate which corresponds to the upper ranges of globulin solubility.

A similar preparation of plasma proteins precipitating between 1.7 and 2.0 molar phosphate was made, such that the final volume would be about 1/10 that of the original plasma, dialyzed 6 hours in running tap water and overnight in distilled water containing a small amount of potassium oxalate (about 0.01%) to precipitate any calcium which might be present. A considerable quantity of inert material precipitated during dialysis, and was spun out. One cc of the resulting solution, after activation, clotted 1.0 cc of fibrinogen solution in 3 seconds. Electrophoretic pattern (in 0.025 M barbiturate buffer at pH 7.76) of the above is found in Chart 2. The mobility of the chief peak in cm^2 $volt^{-1}$ sec^{-1} was 5.8 10^{-5} which most closely approximates that of alpha globulin.* It is to be observed, however, that the electrophoretic pattern appears to display all of the globulins and some albumin although the protein had been separated over a narrow range of salt concentration. This demonstrates again the inadequacy of salting methods for separating proteins.

Untreated oxalated plasmas from control healthy individuals and multiple sclerotic patients were subjected to electrophoresis in 0.025 M barbiturate buffer at pH 7.8, and the various components separated out in two parts. Each part was tested for prothrombin content (as before) with the following results:†

Prothrombin absent	Prothrombin present
1. gamma	phi alpha beta albumin
2. gamma phi beta	alpha; albumin
3. gamma phi beta alpha	albumin

* The mobilities of the peaks of the protein preparation differ markedly from that of whole plasma.

† It is interesting to note that the globulin fractions obtained by precipitation did not require with phosphate buffer the addition of thromboplastin to activate the prothrombin (though the reaction was accelerated by it). The Tiselius albumen fraction, however, could not be activated by calcium alone, and required the presence of thromboplastic substance.

Experiment 3 was repeated 3 times and on all occasions, the prothrombin was found to migrate solely with the albumin.

Summary. 1. With Butler's phosphate buffer as a salting solution, prothrombin precipitates between 1.7 and 2.0 molar strength, which places it in the upper globulin fraction. 2. The bulk of proteins of solubility similar to prothrombin migrates electrophoretically as alpha globulin. 3. On the electrophoresis of whole plasma, prothrombin migrates with the albumin fraction.