# Note on Purification of Human Prothrombin.* (19289)

WALTER H. SEEGERS AND EDNA B. ANDREWS.

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

Work on methods for the purification of prothrombin has now advanced sufficiently so that products suitable for electrophoresis, ultracentrifugation, and other important studies can be obtained. Large quantities of bovine blood have been utilized for the many experiments performed in the development of these methods(1-3). It is not known whether these methods can be used successfully for the production of human prothrombin.

*Procedure.* Bovine blood is collected in a special sodium oxalate-oxalic acid anticoagulant(4). Use of the latter makes it possible to keep the ionic strength of the plasma low with the result that prothrombin can be precipitated quantitatively from diluted plasma. Human blood could, of course, also be drawn into such an anticoagulant but we were not in a position to do that for economic reasons. Discarded serologically positive plasma was available in citrate anticoagulant. This is old plasma; however, all of our studies of the past have indicated that prothrombin remains well preserved in such specimens(5-7). The ionic strength of the human plasma was reduced by overnight dialysis against cold distilled water. Pilot experiments showed that a specific resistance of 1,000 ohms was sufficient. Such plasma can be diluted with water and acidified to pH 5.1 and the precipitate will contain practically all of the prothrombin. All other procedures were as previously described (1-3). Arrangements were made to process six liters of plasma at one time, and to complete the work for each preparation in two days. When good results were constantly being obtained with bovine plasma a switch was made to human plasma, and then again back to bovine plasma. In this way, the best possible comparisons of human and bovine plasma could be made.

TABLE I. Yield and Specific Activity of Purified Human Prothrombin Compared with Bovine Prothrombin.

| Product No. | Liters plasma used | Total units yield | Specific activity | |
|---|---|---|---|---|
| | | | per mg | per mg tyrosine† |
| 510702 | 6.5 | 376000 | 1610 | 26100 |
| 510807 | 7.5 | 400000 | 1780 | 28000 |
| 510808 | 6.5 | 264480 | 1850 | 28000 |
| 510809 | 7 | 550000 | 1800 | 30300 |
| 510813 | 7 | 240300 | 1430 | 26000 |
| H510814* | 3 | 93500 | 2000 | 32400 |
| 510815 | 7 | 614400 | — | 30000 |
| 510816 | 6.5 | 366080 | — | 22130 |
| 510820 | 7 | 600340 | 2000 | 28500 |
| 510822 | 6.5 | 507840 | 1860 | 27130 |
| 510823 | 6.5 | 438370 | 1760 | 25230 |

* Human prothrombin.

† Method of Folin-Ciocalteau. This is not an accurate analysis for tyrosine but the figures can be compared with previous work from this laboratory. The prothrombin units are the same as previously employed(1-3).

*Results.* Table I shows the results obtained with 5 lots of bovine plasma and one lot of human plasma, followed again with 5 lots of bovine plasma. It is certainly possible to say that the human prothrombin product possessed at least as much potency as the bovine products, and we believe that the specific activity may be considered to be fully as high. Throughout the purification procedure, it was not possible to detect any gross differences in the way in which the various fractionation steps could be applied to either human or bovine plasma. The lower yield of human material was doubtlessly the result of narrowing the limits of the last fractionation steps. In this way a second fraction of less purity, but with many units of prothrombin, was obtained as a by-product.

*Summary.* Methods developed for the purification of bovine prothrombin can be applied to the purification of human prothrombin. By these procedures, human prothrombin has been obtained with a specific activity equal to

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the highest ever obtained with bovine material.

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## Stability of Purified Hyaluronidase. (19290)

MONROE E. FREEMAN AND MARION E. WEBSTER.

*From the Department of Biochemistry, Army Medical Service Graduate School, Washington, D.C.*

Highly purified bovine testicular hyaluronidase(1,2) has been found to be quite unstable compared to crude fractions containing less than 1000 turbidity reducing units per mg of nitrogen. McCullagh *et al.*(3) reported inactivation of dried enzyme and aqueous solutions at higher storage temperatures and some stabilization by gelatin and gelatin polypeptides. In this laboratory, however, hyaluronidase suspensions purified to 1,000,000 TRU per mg were rapidly inactivated above 0°C unless specific conditions were imposed. Assays(4) were difficult to evaluate and serious losses were encountered in dialysis, dilution, and lyophilization.

Ranges of pH, ionic strength, temperature, and enzyme concentration that seem to be critical for stability of the highly purified enzyme have been included in this report.

*Methods.* Hyaluronidase was prepared from fresh bovine testes(1,2) and assayed by the turbidity reducing method(4). Enzyme units were defined by this assay method and enzyme purity has been expressed as TRU per mg N. Enzyme solutions contained merthiolate, 1 to 10,000, and were kept in 0.022 M phosphate-citrate buffer at pH 5.2 containing 0.28% NaCl, ionic strength, 0.10, unless otherwise noted.

*Hydrogen Ion Concentration,* Table I, was not a critical factor for stability between pH 4.0 and 9.5. However, under less favorable conditions, optimum stability appeared to be between 5 and 7.

TABLE I. Effect of Hydrogen Ion Concentration on Stability of Buffered Hyaluronidase Solutions. Results in TRU/ml.

| | Purified hyaluronidase, 60000 TRU per mg N | | | | | |
|---|---|---|---|---|---|---|
| pH | 3.5 | 5.5 | 7 | 8.5 | 8.5 | 9.5 |
| 0 day | 800 | 750 | 950 | 800 | 750 | 920 |
| 7 | 290 | 855 | 1020 | 1030 | 880 | 1000 |
| 56 | 200 | 840 | 990 | 930 | 860 | 920 |

Enzyme sol. contained .1 mg protein per ml (of .022 M phosphate-citrate-NaCl buffer solution at −2°C).

*Total Protein Concentration,* Table II, was found to be the most critical factor, with rapid deterioration apparent at concentrations below 1 mg of total protein per ml. At the same total protein concentrations, purified enzyme was inactivated more rapidly than crude. The high dilutions necessary for assay (4), to about 3 TRU per ml, involved serious errors unless dilutions were made with 0.1% bovine serum albumin solution.

*Temperature.* Above 0°C the rate of inactivation of hyaluronidase, Table III, was increased with the noteworthy exception that purified enzymes were not more labile at higher temperature if total protein concentrations were high enough.

*Buffer Salts and Ionic Strength.* This factor played a significant role in the stability of hyaluronidase in aqueous solutions, Table III, and in ethanol suspensions at −2°C, Table IV. Phosphate-citrate buffers were most satisfactory in the presence of .9% NaCl.