Solubility of Bovine Fibrinogen in Ethanol-Water Mixtures.* (18171)

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The solubility of human fibrinogen in ethanol-water mixtures has been studied and on this basis there was developed a method for its purification from Fraction I, the crude concentrate formed by ethanol fractionation (1). The application of this procedure to the purification of bovine Fraction I has not been successful(2). This suggests that either the bovine fibrinogen or its impurities had different solubilities. It has also been observed that some of the properties of human and bovine fibrinogen differ slightly. For example, the bovine clotting time at pH 6.3 and ionic strength 0.45 is about twice as long and the opacity of the clot formed twice as great as the human. Further, a higher pH or ionic strength is required to cause the transition from production of coarse clots to production of fine clots(2-4). Rigidity measurements also reveal differences. At pH 7 and fibrin concentration 10 g/l, the rigidity of the human clot is about 6 times as great as that of the bovine; however, the rate of rigidity increase with increasing fibrinogen concentration is greater for the bovine material(6).

In view of these observations, it appeared worthwhile to study the solubility properties of bovine fibrinogen to establish differences and attempt to develop a satisfactory procedure for further purification.

Materials and methods. Armour bovine Fraction I was used as a source of fibrinogen. This material (lots C-107 and C-112) contained about 27% clottable protein. It was dialyzed at a fibrinogen concentration of about 20 g per liter against a buffer of 0.30 ionic strength (0.25 ionic strength sodium chloride plus 0.05 ionic strength sodium acetate or phosphate) for 20 hours with 2 changes of dialysis bath. Portions of this stock solution were then diluted with equal volume of (cold) diluent containing this same buffer composition and variable amounts of ethanol. After 3 hours of equilibration at 0°C the tubes were centrifuged and the supernatants decanted. The precipitates were redissolved at pH 6.3 and aliquots of these solutions and of the supernatants were clotted with thrombin in order to assay the fibrinogen(7). The supernatants and the redissolved precipitates were also analyzed for total protein, after an appropriate dilution, by measuring the light absorption at 2780 Å in the Beckman spectrophotometer. A linear relation between optical density (OD) and concentration (c) was obtained over the range, 0.05-1.25 g/l, such that OD = 1.476 c, as referred to Kjeldahl nitrogen determinations using a factor of 6.00(7).

Results and discussion. A representative series is shown in Fig. 1, where values for fibrinogen and total protein in the precipitate and supernatant phase as well as the sum of these 2 are plotted against ethanol concentration. Sum values remain satisfactorily constant for the various ethanol concentrations.

The results of the several series are summarized in Fig. 2, where the solubility of fibrinogen is shown in a 3-dimensional surface as a function of pH and ethanol concentration. Qualitatively this surface is similar to that found for human fibrinogen(1) in that it shows greater solubility at a high pH and low

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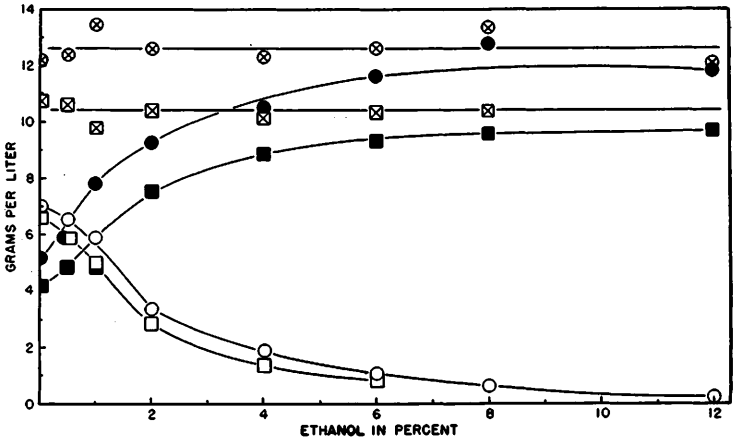

FIG. 1. The solubility of fibrinogen and total protein from Fraction I as a function of ethanol concentration. Temperature, 0°C; ionic strength, .30, phosphate (.05) and pH, 6.9. Squares, fibrinogen; circles, total protein; open symbols, precipitate; closed symbols, supernatant; half-closed symbols, sum of both phases.

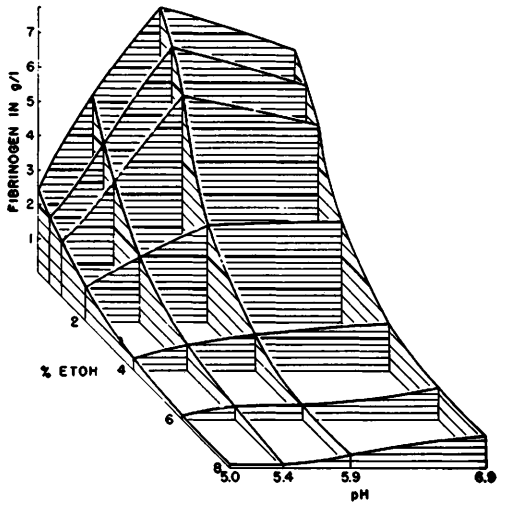

FIG. 2. The solubility of fibrinogen as a function of pH and ethanol concentration. Temperature, 0°; ionic strength, .30.

ethanol concentration. Quantitatively there are distinct differences in that the bovine fibrinogen shows a much smaller effect of pH with distinctly lower solubilities at pH 7 and higher solubilities at pH 5. The effectiveness of ethanol in reducing the solubility appears quite comparable in the two cases. This solubility behavior is in accord with the effect of pH on other properties of the clots formed from these fibrinogens. Thus in human fibrin clots there is a sharp change-over (at 0.15 ionic strength) from coarse to fine structured clots as the pH goes from pH 6 to pH 7. This correlates with a substantial shift in solubility in the human protein between these two pH values. On the other hand in the bovine substantially higher pH values must be reached before this transition takes place, and this appears in accord with the fact that little change in solubility was observed between pH 6 and pH 7 in the bovine fibrinogen.

Fractionation procedure. On the basis of the solubility results, a simple fractionation procedure was devised which gave a product with 90% clottability, as follows: Fraction I was dissolved in water to give about 3% protein. After cooling to 0°, an equal volume of cold 4% ethanol was added, containing 0.3 M sodium chloride and enough acetic acid to give a pH of 5.5 and a fibrinogen concentration of 1%. The precipitate was dissolved in 0.15 M disodium phosphate at 25° to give a pH of 7.6 and a protein concentration of about 2%. This was again cooled to 0° and an equal volume of cold 12% ethanol was added. The resulting precipitate was dissolved in the desired buffer solution at 25° to give the final solution. Yields of 30 to 35% were obtained. This procedure is similar to that developed for the human protein, but the middle step of removing a cold-insoluble fraction is omitted. Such a precipitate did not appear on simply cooling the solution of the first precipitate; this is in contrast to the be-

havior of the human Fraction I.

Summary. (1) The solubility of bovine fibrinogen in ethanol-water mixtures has been studied. The behavior was qualitatively similar to that of the human protein, but the influence of pH was much smaller. (2) A procedure for refractionating bovine Fraction I to give a 90% clottable product is described.

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Quantitative Studies on Prophylactic Effectiveness of Western Equine Encephalitis Antiserum in Mice.* (19172)

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The irregular, infrequent and unpredictable occurrence in the U. S. of sizeable epidemics of encephalitis due to the arthropod-borne viruses poses a difficult problem with regard to the various control measures which might be employed in the face of an epidemic. It has been demonstrated that the administration of suitable amounts of antiviral serum prior to infection, and under special conditions also at certain intervals after infection, can prevent development of the experimental disease (1-7). Previous studies were either of a qualitative nature in which large amounts of serum of unassayed potency were shown to have a protective effect, or, when sera of known potency were used in varying amounts, they were tested in animals infected by the intracerebral route. The purpose of the present communication is to present the results of certain pertinent quantitative studies which were carried out in 1942 and 1943 in relation to vaccination of human beings with western equine encephalitis chick embryo vaccine. The study was originally designed to indicate the significance of various levels of neutralizing antibody against western equine encephalitis virus as regards resistance to infection by a peripheral, extraneural route. The data, however, also throw light on the minimal amounts of passively introduced antibody which are required to protect against varying amounts of virus introduced by a peripheral, extraneural route, and thus provide a basis for estimating the amounts of antibody which may be required in humans.

Experimental procedure, methods and materials. The strain of WEE virus used was originally derived from California and since 1934 had been propagated in mice by Dr. P. K. Olitsky and his associates at the Rockefeller Institute and subsequently in this laboratory. The virus consisted of infected mouse brains, stored in dry ice as 10% centri-

TABLE I. Antibody Content of WEE Rabbit Antiserum Used in Prophylactic Tests.

Dilution of serum	Neutralization index* for .015 ml — Intra-abdominal test in 2-wk-old mice	Intracerebral test in 4-wk-old mice
Undiluted	5000000 +?	1600
1:10	2000000	200
1:100	1000000 +?	80
1:1000	6000	50
1:10000	13	6
1:100000	3	—
1:1000000	1	—

* Neutralization index = $\frac{\text{Titer of virus in mixtures with normal serum}}{\text{Titer of virus in mixtures with antiserum}}$

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