

NOTE ON THE PURIFICATION AND PRECIPITATION OF CASEIN.

By JOHN H. NORTHROP.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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The numerous methods for the preparation of casein all make use of the fact that casein may be precipitated at its isoelectric point by the addition of acid to an alkaline solution of the protein. It is also known that in the presence of strong acid the protein is "denatured" and becomes permanently insoluble. Great care must be used therefore in the addition of acid. This point was recognized by Van Slyke and Baker,¹ who added the acid slowly with very rapid stirring. The method, however, required very careful handling, and even under the best conditions small amounts of denatured casein are formed, as can be seen from the cloudy solution obtained with alkali. Since it is the acid which causes the formation of this denatured casein, it would appear more logical to make the final precipitation with alkali, since small excess of alkali does not affect the protein. This procedure was tried and was found to yield a product free from denatured casein. The method adopted is briefly as follows.

Casein is precipitated from milk as described by Van Slyke and Baker, except that it is not necessary to take unusual precautions with the addition of acid. The precipitate is thoroughly washed to remove any soluble salts. About 10 gm. of the precipitate are then suspended in a liter of water and the suspension brought to a pH of 2.5 to 3.0 by the addition of HCl. A cloudy solution results. This is then filtered through hardened fluted filter paper, if necessary, several times. A perfectly clear filtrate is obtained which shows only a slight Tyndall cone, and which contains the casein. The filtration is very slow and may require several days, but no other method could

¹ Van Slyke, L. L., and Baker, J. C., *J. Biol. Chem.*, 1918, xxxv, 127.

be found to remove the very fine particles of denatured protein. This solution is then brought to pH 4.7 by the addition of NaOH. The precipitate of casein is washed several times with distilled water and either dried with acetone or kept as a fine suspension under toluene.

Casein prepared in this way gives an almost water-clear solution in either acid or alkali. If, however, the clear alkaline solution is precipitated by the addition of acid, a milky suspension results which cannot be centrifuged or filtered except in a very narrow range of pH near 4.7. It is possible, therefore, to have two entirely different solutions of casein in the pH range of 5.0 to 7.0 and from 4.5 to 3.0, depending on whether the acid or alkali was added to the precipitate of isoelectric casein or to the solution of casein. The suspension prepared from the precipitate by the addition of alkali consists of a perfectly clear liquid which may be easily filtered or centrifuged from a flaky white precipitate. If the clear alkaline solution is partly precipitated by acid, however, a milky liquid is obtained from which the solid casein cannot be separated by any of the ordinary procedure. This difference is due to the protective action of the casein in solution. As soon as a particle of isoelectric (or denatured) casein is precipitated in the solution, it is covered by a film of the soluble casein salt and therefore the particles do not coalesce. As the isoelectric point is approached, however, the concentration of the soluble casein becomes reduced, and near pH 4.7 becomes so small that it is no longer sufficient to protect the particles which therefore coalesce into large flakes. When, on the other hand, alkali is added to the isoelectric casein, some alkali salt is formed and dissolves. The presence of this salt cannot break up the large flakes of isoelectric casein in suspension, although, as was stated above, it can prevent their formation. The solution of isoelectric casein in alkali is a purely solubility effect, as stated by Loeb,² whereas the precipitation of casein from such solutions is complicated by the fact that the solid casein formed is kept in very fine suspension by the protective action of the soluble casein salt.

² Loeb, J., and Loeb, R. F., *J. Gen. Physiol.*, 1921-22, iv, 187. Cohn and Hendry (Cohn, E. J., and Hendry, J. L., *J. Gen. Physiol.*, 1922-23, v, 521) have shown conclusively that the solubility of casein in alkali is true solubility governed by the solubility product.