each of the ratios given, and the results in the sixth and seventh columns, therefore, represent the average readings. The agreements between the theoretical and calculated ratios show that the nephelometric formula given in the first paper with edestin and silver chloride, also holds for casein, when 3% sulfosalicylic acid is used as a precipitant. In this case the nephelometric constant $k = 0.20$.

### Standard Solutions and Reagents.

*Casein solutions were made as follows:* (1) Stock solution, 0.1000 gram casein was dissolved in 1 cc. 0.1 *N* NaOH and after diluting and filtering, the solution was made up to 100 cc., thus giving a 0.1% solution. After adding chloroform and shaking, the solution was ready for use. (2) Standard solution, 10 cc. of stock solution were diluted to 100 cc., thus giving a 0.01% solution. One volume of standard solution with one or more volumes of reagent gave suitable suspensions for nephelometric work. These standards remained as suspensions twenty or more minutes.

*Precipitant for Casein:* 3% solution of sulfosalicylic acid. The commercial product was sufficiently pure for this purpose. Although dilute solutions of this reagent readily gave a precipitate with casein, yet the best results were obtained only with a 3% or stronger solution: 1.2% solution of sulfosalicylic acid precipitating about 97% of the casein in the standard solutions. The suspensions with this reagent have a greater tendency to agglutinate than edestin has with sodium chloride, and care should be taken that the solutions are not shaken too vigorously. When the solutions were shaken gently in a rotatory fashion, so that air-bubbles forming in the solution were avoided, the error due to agglutination was negligible. As sulfosalicylic acid slowly forms a red color with cellulose, the reagent should be filtered through paper rapidly or through a Gooch crucible containing asbestos.

### Summary.

The nephelometer can be used for studying the digestion of casein, when a 3% solution of sulfosalicylic acid is used as a precipitant.

This reagent does not precipitate amino acids, peptides, peptones and urinary constituents under the conditions given for nephelometry.

The nephelometric constant[1] ($k$) for casein with this precipitant was found to be 0.20.

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## STUDIES ON ENZYME ACTION. IV. NOTE ON THE OCCURRENCE OF A UREASE IN CASTOR BEANS.

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In the course of the study of the action of neutral substances upon the lipolytic activity of castor beans it was observed that a urea solution

[1] See first paper, *J. Biol. Chem.*, 13, 491 (1913).

containing the preparation became alkaline in reaction after standing for several days. That this was due to the formation of ammonia from the urea was proved in the experiments to be described.

The occurrence of a specific enzyme, urease, in many plants, capable of accelerating the reaction between urea and water whereby ammonia and carbon dioxide are formed, has been repeatedly demonstrated, especially in the last few years,[1] and the urease in some cases submitted to extensive study.

The castor bean material for this investigation was prepared from the cold pressed beans.[2] The shells were removed by hand, the kernels ground, extracted exhaustively with ether, and ground to an impalpable powder.

Four sets of solutions were prepared:

(*A*) Two solutions, each containing 0.5 gram castor bean preparation, 50 cc. 1.0 molar urea solution, 5 cc. toluene.

(*B*) Two solutions, each containing 0.5 gram castor bean preparation, 50 cc. water, 5 cc. toluene.

(*C*) Two solutions, each containing 50 cc. 1.0 molar urea solution, 5 cc. toluene.

(*D*) Two solutions, each containing 0.5 gram castor bean preparation which had been heated with 50 cc. water at 90–100° for $1^1/_2$ hours, enough water added to keep the volume of the solution 50 cc., and 3 grams urea (to make the solution 1.0 molar), and 5 cc. toluene.

The flasks containing these solutions were stoppered tightly and placed in a thermostat at 38–40° for 10 days. At the end of this time the solutions were tested for any possible formation of ammonia by the aeration method.[3] A rapid current of air was blown through the solutions after these had been transferred to the flasks used in this method, and the ammonia absorbed in the standard dilute sulfuric acid placed in the bottles following the solutions. No attempt was made to obtain a quantitative measure of the ammonia formed and therefore no alkali (sodium carbonate) was added to drive over all the ammonia, and the current of air was only passed for two hours. The sulfuric acid solutions were titrated with 0.1017 normal sodium hydroxide solution with alizarin sulfonic acid as indicator. Two solutions with the same amount of sulfuric acid used in the experiments, each required 23.35 cc. of the sodium hydroxide solution to attain neutrality. The results obtained in the experiments are as follows:

[1] Takeuchi, *J. Coll. Agric., Imp. Univ., Tokyo*, **1**, 1414 (1909); H. E. Armstrong and Horton, *Proc. Roy. Soc. London*, (*B*) **85**, 109 (1912); Zemplén, *Z. physiol. Chem.*, **79**, 229 (1912) and others.

[2] Supplied by the Baker Castor Oil Company, New York City.

[3] P. A. Kober, THIS JOURNAL, **30**, 1131 (1908); **32**, 689 (1910); Folin and Farmer, *J. Biol. Chem.*, **12**, 499 (1912).

Solutions (A) 19.04 cc. and 18.60 cc. 0.1017 *N* NaOH solution required
Solutions (B) 23.28 cc. and 23.31 cc. 0.1017 *N* NaOH solution required
Solutions (C) 23.05 cc. and 23.10 cc. 0.1017 *N* NaOH solution required
Solutions (D) 23.20 cc. and 23.17 cc. 0.1017 *N* NaOH solution required

These results show conclusively that in (*A*) where the castor bean preparation and urea were both present, ammonia was formed and carried over by the current of air; that in (*B*) where castor bean preparation but no urea was present, no ammonia was carried over; that in (*C*) where urea was present alone in solution, a minute quantity of ammonia was carried over; and that in (*D*) where the castor bean preparation had been heated in water solution before the addition of urea, a very minute quantity of ammonia (corresponding to less than 0.2 cc. of 0.1017 normal solution) had been carried over, less than in (*C*) where urea was present alone in solution.

Since it was evident that all the ammonia formed from the urea in experiments (*A*) had not been carried over by the air current into the acid solution, an attempt was made to obtain an approximate measure of the total quantity of ammonia by titrating the residues after the air current had been passed through the solutions. The end points were very poor, and the results show, after allowing for the blank experiments (*B*), (*C*) and (*D*), that about 10 cc. of 0.12 *N* hydrochloric acid were required to neutralize the ammonia remaining in flasks (*A*) after the air current had been stopped. This represents a minimum value if anything, as a certain amount of ammonia would be required to neutralize the acidity of the castor bean preparation. This amounts to about 0.4 cc. of 0.12 *N* hydrochloric acid for 0.5 gram substance when first added to water and increases on standing in aqueous solution or suspension. The above values found in titrating correspond to about 0.028 gram ammonia and represent an approximate minimum value for the amount of ammonia produced under the conditions of the experiments. Furthermore, the values found for titrating the residues of (*B*) and (*D*) gave results which, when the poor end point is considered, indicate the presence of practically no ammonia in (*D*).

Thanks are due Mr. K. Sugiura for his efficient aid in carrying out the experiments.

## Conclusion.

Castor beans contain a urease which is inactivated by heat in aqueous solution or suspension.

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