pounds, some cases of this kind are not encountered. However, under the conditions of the determinations as described above (the very slow distillation over the very long and very hot carbon layer in a current of hydrogen) this objection does not apply, at least, to the compounds analyzed and these are fairly representative. Should this objection, however, in any case be observed, it could very probably be eliminated by the use of a small weighed amount of a pure oxidizing agent of definit oxygen content, as potassium permanganate, intimately mixed with the substance in the boat.

It is the intention to proceed in this laboratory with the investigations of this or other means of obviating this possible source of error, and also with the application of the method to compounds containing nitrogen and other elements.

UNIVERSITY OF TORONTO.

[HARRIMAN RESEARCH LABORATORY, ROOSEVELT HOSPITAL, NEW YORK CITY, N. Y.]

NEPHELOMETRY IN THE STUDY OF PROTEASES. II.

BY PHILIP ADOLPH KOBER.

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Introduction.—In a previous paper[1] a method was described for studying proteases and nucleases, based on the precipitation of the substrate as a suspensoid by a suitable precipitant, and the determination of this suspended substance with a nephelometer. Figures were given to show that by using a 12% solution of sodium chloride as a precipitant, quantitative results were obtained with 0.0005% to 0.005% edestin solutions.[2] The object of this paper is to show that by using the precipitant given below, equal accuracy can be obtained with casein, thus giving us extremely sensitive methods for peptic, tryptic and ereptic digestion.

General Considerations.—In choosing edestin and casein as substrates the following points were considered: (1) For the accurate standardization of these enzymes, it is essential not only to have sensitive instruments to measure the rate of digestion, but also to have a substance that is easily digested by these ferments, so that the measurement of the activity, like other speed estimations, will occupy as little time as possible. The object in view, of course, is to be able to determin the activity at any instant. (2) Not only are casein and edestin digested easily by most ferments but they may be useful in distinguishing trypsin from erepsin. Fischer and Abderhalden[3] showed that trypsin digested not only casein but proteins like edestin as well. Dox,[4] on the other hand, seems to have

[1] Kober, *J. Biol. Chem.*, 13, 485 (1913).

[2] This represents the range over which the best nephelometric work can be done.

[3] Fischer and Abderhalden, *Z. Physiol. Chem.*, 60, 81 (1903).

[4] Dox, *J. Biol. Chem.*, 6, 437 (1906).

been the first to point out that erepsin of certain moulds was not capable of hydrolyzing edestin, while it digested casein with ease. The clinical usefulness of distinguishing between trypsin and erepsin, when studying the functional activity of the pancreas or the presence of abnormal enzymes in carcinomatous stomachs, is sufficiently obvious. (3) Casein and edestin can be obtained fairly pure.

Precipitants for Casein.—The usual precipitant for casein, acetic acid, is unsatisfactory for the dilute solutions employed in nephelometric work. The amount of acid required to completely precipitate one concentration of casein is either too much or too little for another, so that it is almost impossible to gage the correct quantity of acid. Of the few precipitants that have been studied for this purpose, sulphosalicylic acid was found to be very satisfactory, small amounts precipitating casein completely while larger amounts did not cause resolution.

Roche and J. A. MacWilliam[1] used sulfosalicylic acid in testing for serum albumin in the urin and it is recommended for that purpose by Spaeth,[2] with the statement that it also throws down albumoses. Although this may be true of relatively strong solutions, yet under the conditions used in nephelometry, where the substrates are never stronger than 0.01%, solutions of Witte peptone, Merck's peptone (from egg albumen), Merck's meat peptone (from beef), peptone Roche (from silk), ereptone (mostly amino-acids), and diluted normal urin do not give any appreciable precipitation with 3% solutions of sulfosalicylic acid. Therefore, this reagent, under the conditions given in this paper seems, to be characteristic for albumins, globulins and native proteins,[3] *i. e.*, coagulable proteins, and does not precipitate amino acids, peptides, peptones, and urinary constituents.

To show the efficiency of this reagent with sodium caseinate, the following results, obtained as described in the first paper,[4] will suffice:

Number of solutions.	Standard "S". 3% sulphosalicylic acid. Cc.	Standard "S". 0.01% casein. Cc.	"Unknown" "Y". 3% sulphosalicylic acid. Cc.	"Unknown" "Y". 0.01% casein. Cc.	Mm. of "S".	Mm. of "Y".	Theoretical ratio X.	Found if $X=\frac{S+Sk+\sqrt{(S+Sk)^2-4Sky}}{2y}$
5	10	5	10	5	15.2	15.20	1.000	1.000
3	10	5	12	5	15.2	16.54	0.882	0.882
2	10	5	15	5	15.2	18.70	0.750	0.748
4	10	5	20	5	15.2	21.80	0.600	0.596
6	10	5	25	5	15.2	23.90	0.500	0.504

The figures in the first column show the number of determinations for

[1] *Brit. Med. J.*, 837 (1891).

[2] Edward Spaeth, Untersuchung des Harnes, p. 397.

[3] One exception was found thus far; yeast nucleic acid, even in 0.01% solutions, gives a precipitate with this reagent.

[4] *Loc. cit.*

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.

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

BY K. GEORGE FALK.

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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).