Results.—Duplicate determinations made on the same milk agree with one another within the limits of error of the instrument (about 3%).

In the table below comparison is made of values obtained by the nephelometric method, the Babcock method and also in some cases, the Adams' method. In using the Babcock method, a calibrated pipette and specially prepared bottles were used. The cows' milk was mixed dairy milk collected from various sources. The human milk was from patients in the obstetric division of the Washington University Hospital, for which I am indebted to the kindness of Professor Henry Schwarz and Doctor Q. U. Newell. The samples were from cases in various early stages of lactation and were selected so as to obtain as wide range of fat values as possible.

TABLE I.—COMPARISON OF RESULTS OBTAINED BY THE NEPHELOMETRIC AND OTHER METHODS FOR FAT IN MILK.

Milk sample.	Babcock.	Nephelometric.	Adams (Soxhlet).
R. Kingston, Can	2.9	2.85	..
B. Kingston, Can	3.1	3.00	..
Hospital supply I., St. Louis, Mo	3.5	3.6	..
U. D. I., St. Louis, Mo	3.0	3.1	3.01
Hospital supply II, St. Louis, Mo	3.0	3.1	..
M. Kirkwood, Mo	3.6	3.8	3.80
J. A., St. Louis, Mo	3.5	3.6	3.77
Human milk.			
I. L. 22 days post partum	4.7	4.8	4.70
II. V. 10 days pp. (child still-born)	8.2	8.1	..
III. W. 11 days pp	3.9	3.83	..
IV. G. 4 days pp	3.75	3.8	..
V. N. 3 days pp. (colostrum)	3.3	3.4	..
VI. M. 16 days pp	2.0	2.2	2.15
VII. B. 8 days pp. (breast caked a little)	5.6	5.60	5.71

The preliminary work on this method was done in the Gordon Hall Laboratories of Chemistry of Queens' University and in the Kingston Dairy School at Kingston, Canada, and I wish in conclusion to express my appreciation of the kindness of Professor W. L. Goodwin, of the University, and of Mr. Zufeldt, Director of the Dairy School, in placing at my disposal the facilities of these institutions.

[CONTRIBUTION FROM THE HARRIMAN RESEARCH LABORATORY, ROOSEVELT HOSPITAL, NEW YORK.]

NEPHELOMETRY IN THE STUDY OF NUCLEASES.[1]

BY PHILIP ADOLPH KOBER AND SARA S. GRAVES.

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[1] Read at the Cincinnati Meeting, Am. Chem. Soc., April, 1914.

C. Interfering Substances; D. Technic; E. Applications to Physiological Material. III. Summary.

I. Introduction.

The study of nucleases has been hindered by the lack of suitable technic. Walter Jones[1] writes: "It's somewhat difficult to decide whether or not a ferment has brought about the decomposition of a nucleic acid, unless the decomposition is complete. The methods for the recovery of the undecomposed nucleic acids are so poor that one cannot always decide whether a partial disappearance of the substance is due to ferment action, or to analytical error."

It is the object of this paper to describe a new method for estimating undigested nucleic acids. Essentially, the method consists in adding a precipitant to a dilute solution of the nucleic acids and estimating the resulting suspensoids, nephelometrically. The results with yeast nucleic acid alone are given in this paper.

II. Method.

A. Precipitant for Nucleic Acids.—As strong hydrochloric acid in aqueous and alcoholic solutions has heretofore been used in the preparation of nucleic acids, attempts were made to use it as a nephelometric precipitant.[2] As the suspensoid gelatinized appreciably, it did not seem stable enough for quantitative work. Next, experiments were made, using dilute solutions of certain proteins, *i. e.*, casein and edestin[3] as precipitants.

Nucleic acids, as is known, combine in slightly acid solutions with proteins to form artificial "nucleins."[4] This reaction, preliminary qualitative experiments showed, was given by one part of yeast nucleic acid in a million parts of water. After considerable work, these two proteins were abandoned because uniform results were not obtained. The probable reason is that for the quantitative formation of these "nucleins" a certain concentration of hydrogen ions is necessary, which is too near the precipitation point of the proteins to be nephelometrically applicable. Therefore, a protein soluble at that hydrogen ion concentration was next sought. Egg white, which seemed to have this property, was tried and found satisfactory.

B. Experimental Work. (1) *Preparation of Egg Albumin.*—As commercial dried egg albumin was not completely soluble in water, albumin from eggs—in most cases not over 48 hours' old—was used. The egg white showed a slight turbidity after diluting to a 1% solution, but,

[1] *J. Biol. Chem.*, **9**, 130 (1911); also Levene and Medigreceanu, *Ibid.*, page 65.

[2] For the definition of "Nephelometric Precipitant" see THIS JOURNAL, **35**, 1585 (1913).

[3] Reported at the Rochester meeting of the Am. Chem. Soc., September (1913).

[4] R. Altmann, *Archiv. Physiol.*, **1889**, 524; T. Milroy, *Z. physiol. Chem.*, **22**, 307 (1896–97).

upon shaking with 0.1 *N* acetic acid, a precipitate, probably a globulin, was thrown down and after filtration a clear solution was obtained. The amount of 0.1 *N* acetic acid necessary for the complete removal of this interfering precipitate was determined by the following experiment: 15 g. of egg white were shaken with 5, 7, 9, and 11 cc., respectively, of 0.1 *N* acetic acid; water was added to make 1% solutions which were then filtered, and the turbidity determined by comparing in the nephelometer with distilled water set at 15 mm. The following results were obtained:

0.1 *N* acid added. Cc.	Nephelom. reading. Mm.	Standard. Distilled water. Mm.
5	Heavy cloud	15.0
7	9.5	15.0
9	8.5	15.0
11	9.7	15.0

The variation of the readings being inappreciable, 7 cc. of acid were used—corresponding to 0.5 cc. of 0.1 *N* acetic acid per g. of egg white.

A clear 1% albumin solution was diluted and a 0.1% solution was found to give a suitable nephelometric curve with varying amounts of nucleic acids, but the reaction with some albumins took place slowly. To hasten the reaction and to insure an excess of precipitant a 0.2% solution was finally adopted.

It became evident, after obtaining several curves, that the alkalinity of the egg albumin varied considerably, and that additional acid was necessary to obtain a favorable hydrogen ion concentration. There being no rapid and accurate method for determining the alkalinity, varying amounts of acids were added and constants of the resulting curves determined by the means of the nephelometric formula, $Y = S/X - (1 - X)Sk/X^2$. For this purpose two points were found, usually the ratios of 1.0 and 0.70. To 250 cc. of 0.2% egg albumin were added 2, 2.5 and 3 cc., respectively, of 0.1 *N* acetic acid. The constants obtained with these amounts of acids for single eggs and for mixtures are given in the following tables:

Single eggs.	2 cc. 0.1 *N* acid. *k.*	2.5 cc. 0.1 *N* acid. *k.*	3 cc. 0.1 *N* acid. *k.*
I	0.23	0.24	0.25
II	0.13	0.16	0.13
III	0.33	0.36	—0.03
IV	0.28	0.12	0.17
V	0.30	0.17	0.25
VI	0.40	0.16	—0.04
Mixtures.			
I	0.38	0.18	—0.04
II	0.37	0.22	0.12
III	0.40	0.23	0.18
IV	0.31	0.30	0.25

(2) *The Use of Egg Albumin.*—The egg albumin solutions, although clear, have a tendency to form shreds on the surface of the liquid. These shreds increase considerably upon shaking. Therefore, like all solutions of colloids, these solutions must be used with caution.

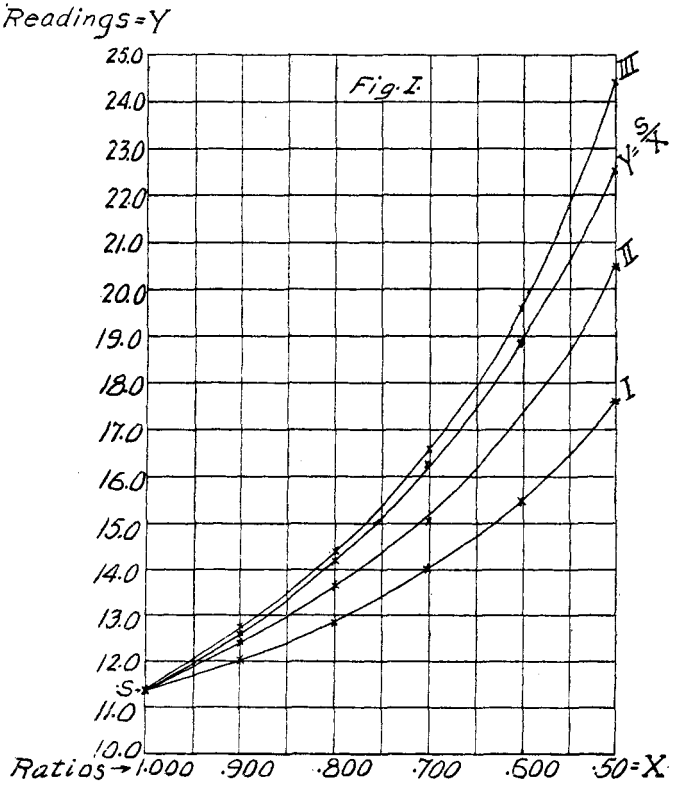
The formation of "nuclein" precipitates is strongly affected by hydrogen ions. This can be seen with a 0.2% egg albumin, which, without acetic acid, gives very little "nucleins," but on the addition of only 1 cc. of 0.1 *N* acetic acid to 50 cc. of albumin, the precipitation is complete.

The following results are typical of the readings and the curves obtained with albumins with varying constants:

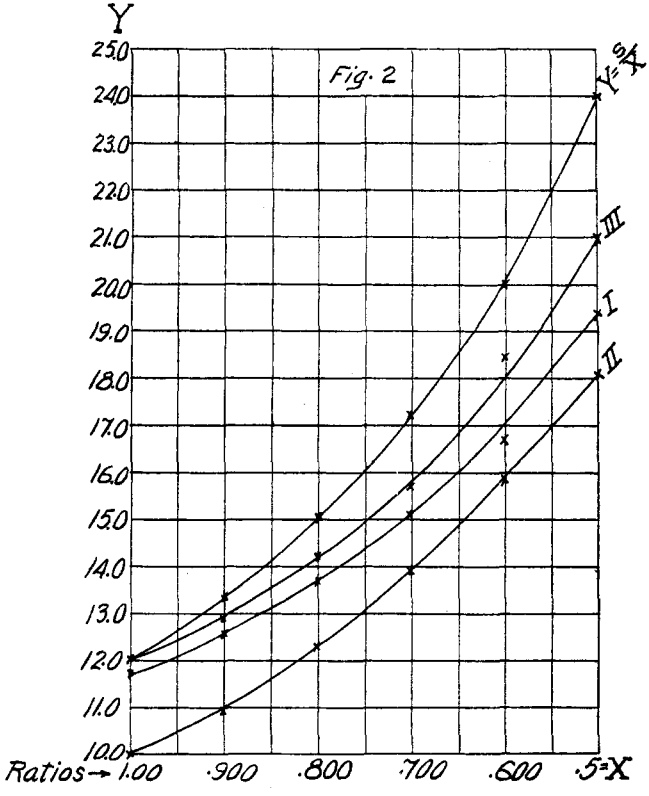
Curves.	Constant.	Ratio of sol.	1.0 mm.	0.9 mm.	0.8 mm.	0.7 mm.	0.6 mm.	0.5 mm.
I.....	0.34	..	11.4	12.0	12.8	14.0	15.5	17.6
II.....	0.24	..	11.2	12.2	13.5	14.9	..	20.3
III.....	—0.03	..	9.1	10.1	11.5	..	15.8	20.0

For the sake of comparison, Curves II and III were transposed to the same standard as I.

From Curve III it can be seen that the readings are higher than the hypothetical and therefore the conclusion can be drawn that, at least



in the more dilute solutions, the precipitation is not complete. From the curves it can also be seen that the greater the constant the greater the percentage error in determining the ratio of solutions, but it seems un-



likely that a large constant (greater than 0.30) can be a normal nephelometric phenomenon.

To show the sensitiveness of the reagent, the following experiments were made:

Solution I.—1 cc. 0.1% nucleic acid was diluted to a liter, making 1 part in 1,000,000.

Solution II.—5 cc. 0.1% nucleic acid was diluted to a liter, making 1 part in 2,000,000.

Compared to distilled water in the nephelometer, the readings were as follows:

Standard.		Unknown.	
Solution.	Reading. Mm.	Solution.	Reading. Mm.
H_2O	20.0	H_2O	20.0
H_2O	20.0	I	7.0
H_2O	20.0	II	11.0
H_2O	20.0	Reagent	13.0

C. Interfering Substances.—The formation of "nucleins" being easily affected by hydrogen ions, ought to be influenced by almost any substance, since most substances change the hydrogen ion concentration of a solution. However, when diluted as is necessary for nephelometric work, an equal weight of substance ought to have no appreciable effect. This was actually found to be the case.

One part by weight of the substances given below was dissolved with one part of nucleic acid and the latter estimated nephelometrically. Within experimental error, the controls gave the same readings as these mixtures:

Inorganic: NaCl, $NaNO_3$, Na_2SO_4, $(NH_4)_2SO_4$, NH_4Cl, $BaCl_2$, $Ba(NO_3)_2$, NaH_2PO_4, $CaCl_2$, $NaC_2H_3O_2$, $KC_2H_3O_2$, $Ca(NO_3)_2$.

Organic: Glycerophosphoric acid, Alanine, Tyrosine, Histidine, Asparagine, Phenylalanine, Urea, Peptone, Ereptone, Dextrose, Levulose, Urine (assumed 5% solids), Stomach contents (assumed 5% solids), Extract of pancreas (dried).

D. Technic.—The albumin was obtained from egg white calculating it as 10% by weight. A 1% solution of egg albumin was made by roughly weighing the whites of from 4 to 6 strictly fresh eggs, shaking well with 0.1 *N* acetic acid (0.5 cc. per gram of egg white) diluting with 10 volumes of water (allowance being made for the original acid) and filtering twice. 50 cc. of the clear 1% solution were put into a flask, acetic acid added (usually 3 cc.) and made up to 250 cc. with distilled water. This 0.2% solution was used as a precipitant.

The nucleic acid solution was made by dissolving 0.1 g. of nucleic acid in 100 cc. of distilled water. This 0.1% stock solution was further diluted before using.

One volume (10 cc.) of nucleic acid (not stronger than 0.01%) was precipitated by running in from a pipet[1] two volumes (20 cc.) of 0.2% albumin solution. After shaking gently in a rotary fashion, the solution was ready for reading.[2] In most cases 0.007% nucleic acid was used as a standard.

E. Application to Physiological Material.—To show that this method is applicable in physiological work, normal specimens of urine, stomach

[1] It is well to dip the point of the pipet below the liquid to avoid the entrance of air bubbles which sometimes cause agglutinations.

[2] The nephelometric tubes and plungers must be kept clean. In the nephelometers heretofore described the plungers and the receptacles for the nephelometric tubes were painted with asphaltum paint. As this paint is somewhat affected by alkaline and other solutions, it became necessary to avoid the use of paint on surfaces which came in contact with the solvents. By making the plungers of hollow glass and painting the interior sides and by making the receptacles of glass and painting them on the outside, this difficulty has been overcome. These new plungers also seem better adapted for the albumin solutions, as there is less tendency for the fibers and air bubbles to stick upon the plungers.

contents and pancreatin were added to nucleic acid solutions and precipitates formed in the usual way. The following figures were obtained:

Mixtures.	k.	Ratio of sol.	1.0 mm.	0.9 mm.	0.8 mm.	0.7 mm.	0.6 mm.	0.5 mm.
I Urine and nucleic acid	0.23	...	11.7	12.6	13.7	15.1	16.7	19.4
II Pancreatin and nucleic acid	0.11	...	11.2	12.1	13.5	15.2	17.1	19.3
III Stomach contents and nucleic acid	0.19	...	12.0	12.9	14.2	15.7	18.5	21.0

These figures are the average of 5 or 6 readings. In addition to 0.05 g. of nucleic acid, solution I contained 2 cc. of urine; solution II 0.02 g. of pancreatin, and solution III 2 cc. of filtered stomach contents. As may be seen, the curves with the mixtures run practically the same as nucleic acid alone.

To determine the presence of nucleases and their relative activity, solutions of nucleic acid were incubated with pancreatin, and the digestion determined by comparing at intervals with known amounts of nucleic acids and precipitating in both cases with 0.2% solution of egg albumin.

Solution I.—Contained 0.05 g. of nucleic acid, 2.5 cc. of 2% pancreatin solution (Mercks) 2.5 cc. of 2% tricresol, made up to 50 cc. with distilled water. *Solution II* was made in the same manner, except that it contained 5 cc. of 2% pancreatin solution (Eimer & Amend).

NUCLEIC ACID DIGESTED.

Pancreatin.	0 time. Per cent.	1 hr. Per cent.	2 hrs. Per cent.	3 hrs. Per cent.	4 hrs. Per cent.	5 hrs. Per cent.
I Merck	0.0	38.0	65.0	75.3	76.8	78.3
II Eimer & Amend	0.0	21.5	28.5	33.0	(33.0)	41.5

III. Summary.

The nephelometer can be used for the study of digestion of yeast nucleic acid, when a 0.2% solution acid egg albumin is used as a precipitant. This reagent is not appreciably affected in dilute solutions by most substances met with in physiological work, and will easily detect one part yeast nucleic acid in 1,000,000 parts of water.

NEW YORK, N. Y.

A PROPOSED MODIFICATION OF THE KOBER METHOD FOR QUANTITATIVE AMMONIA DISTILLATION BY AERATION.

BY F. L. DILLINGHAM.

Received March 25, 1914.

The method outlined by P. A. Kober and S. S. Graves under the title, "Quantitative Ammonia Distillation by Aeration" in THIS JOURNAL[1] seemed of such great value that it was given a series of trials by the writer.

For this purpose ammonium sulfate, 99.80% $(NH_4)_2SO_4$, was used. The details of the method employed are as follows: A weighed quantity of ammonium sulfate was placed in a Kjeldahl flask of 800 cc. capacity

[1] THIS JOURNAL, 35, 1594 (1913).