only a small region of acidity and alkalinity near the neutral point, as shown in Fig. 2. We expect to apply the above accurate polariscopic method in a further study of the hydrolysis of salicin and other substances by emulsin, in order to learn the laws of the action of this enzyme.

[From the Laboratory of Physiological Chemistry, Department of Animal Husbandry, University of Illinois. Nutrition Investigations Publication No. 27.]

TOTAL NITROGEN DETERMINATION BY THE KOBER METHOD.

By F. W. Gill and H. S. Grindley.

Received September 6, 1909

In This Journal[1] Kober describes a new method for the quantitative estimation of ammonia as obtained in the total nitrogen determination of organic substances. Since this method of aeration has certain advantages in that it requires practically no attention when once started and uses no heat for distillation, we undertook a detailed study of the method in the hope of its adoption in this laboratory.

The very excellent results that we have obtained by the aeration process for urea in urine,[2] and for ammonia in urine, by the Folin[3] process also influenced us to investigate this method of Kober's for the total nitrogen in organic materials. The Kober method in substance is as follows: After the usual digestion of the organic material in the Kjeldahl flasks with 25 cc. of concentrated chemically pure sulphuric acid, mercury, and potassium sulphate, the liquids are cooled and from 90 to 95 cc. of ammonia-free distilled water is then added to each of the Kjeldahl flasks. The diluted acid solutions thus formed are cooled to room temperature. The Kjeldahl flasks are connected to 16-ounce bottles, each of which contains a measured excess of standard sulphuric acid plus 130 cc. of ammonia-free water for the ammonia absorption. The Kjeldahl flasks are also connected to vessels, from which is drawn through a widened tube, by the air current, the necessary amount of caustic-soda-sodium-sulphide solution.

After the alkaline solution has been drawn into the Kjeldahl flasks, wash bottles containing the dilute sulphuric acid for rendering the air for aeration ammonia-free, are attached in place of the vessels which contained the caustic alkali solution. The rapid, but not violent, aeration of the solutions in the Kjeldahl flasks is continued for at least one and one-half hours.

We have applied this modification of Kober's to the determination of

---

[1] **30,** 1131 (1908).

[2] This Journal, **31,** 1089 (1909).

[3] *Z. physiol. Chem.*, **37,** 161 (1902)

the total nitrogen in such substances as hay, corn, cornmeal, orts,[1] tankage, feces, urines, urea, acetanilide, and alpha-naphthylamine in comparison with the official distillation method and have obtained very concordant results between the two methods. We have found the same results with cottonseed meal as Davis,[2] namely, that the Kober method gives a low total nitrogen value on cottonseed meal as compared to the official Kjeldahl distillation method. It was also found that the Kober method gave practically the same results for the total nitrogen as did the official method when the solutions in the Kjeldahl flasks, by the Kober method, were kept warm for the one and one-half hours by means of small Bunsen flames placed beneath the flasks. This first exception of the adaptability of the Kober method without modification, for the determination of nitrogen in organic substances, led us to investigate the reason why cottonseed meal should not give up its ammonia, formed in the total nitrogen procedure, by this method unless it was modified as suggested by Davis and as stated above by us. Upon studying the quantitative mineral constituents of 21 vegetable substances used for the feeding of man and animals as given originally in Wolff's "Aschen Analysen"[3] and in quotations by Forbes,[4] it was noticed that the greatest variations in the mineral elements of these 21 vegetables substance existed in the magnesium and phosphorus content. The results expressed as parts per thousand of dry matter, group these materials as follows:

	Magnesium.	Phosphorus.
Cottonseed meal	6.86	15.01
Wheat bran	6.62	13.54
Rice bran	6.38	11.44
Linseed oil meal	5.58	8.06
The average of 17 other vegetable substances	1.61	2.74

Of these 17 materials of low magnesium and phosphorus content only three were much above the average in magnesium, namely red clover, white clover and alfalfa, which contain respectively 4.52, 4.18, and 2.19 parts per thousand on the dry matter basis. The phosphorus content of these three materials was, however, about the same as the average for the 17 vegetable substances. Since the liberation of ammonia from physiological solutions containing an excess of magnesium by means of sodium carbonate[5] and by means of magnesium hydroxide[6] have proven difficult or quantitatively impossible, it was thought that the presence of

---

[1] Orts is a term applied to the residues of feed left in animal feeding experiments.

[2] THIS JOURNAL, **31,** 56 (1909).

[3] Berlin, 1870, 1880.

[4] *Bull.* **201,** Ohio Exp. Station (1909).

[5] Steel and Gies, *J. Biol. Chem.*, **5,** 71 (1908).

[6] Kober, THIS JOURNAL, **30,** 1279 (1908).

a large amount of these elements, magnesium and phosphorus, may be the reason for the non-removal of all the ammonia by the Kober procedure for the total nitrogen. We therefore obtained samples of these high magnesium and phosphorus content vegetable substances and determined the total nitrogen therein by the Kober and official Kjeldahl processes, the results of which are here given:

PER CENT. NITROGEN. AVERAGE DETERMINATION.

Materials.	Official method.	Kober method.	Per cent. Kober is of official.
Cottonseed meal	7.25	7.06	97.38
Wheat bran	1.98	1.96	98.99
Rice feed	1.06	0.99	93.40
Linseed oil meal	5.61	5.49	97.86
Magnesium ammonium phosphate sol.[1]	5.66	5.48	96.82

It is evident from the above table that the Kober procedure with all of the materials which contain comparatively large amounts of magnesium and phosphorus, does not obtain all of the nitrogen unless modified so that the solutions are kept warm during the period of aeration, under which condition all the nitrogen is obtained. The reason for this non-removal of the nitrogen probably is that magnesium ammonium phosphate is formed during the digestion of the organic materials, and the nitrogen of this, as shown by the data in the above table, is not completely removed by the Kober aeration method.

The following table gives the results of a set of materials, the total nitrogen of which was determined by the Kober and official methods:

PER CENT. NITROGEN. AVERAGE DETERMINATION.

Material.	Kober method.	Official method.
Hay	1.63	1.58
Tankage	7.00	7.01
"	5.70	5.63
Cornmeal	1.24	1.24
Orts	2.10	2.11
"	3.09	3.08
"	2.29	2.31
"	2.12	2.06
Urine	2.42	2.40
Feces	0.57	0.56
"	0.62	0.61
"	0.66	0.65
"	0.55	0.55
Alpha-naphthylamine, C. P.	9.50	9.52
Acetanilide, C. P.	10.10	10.07
Urea, C. P.	46.15	45.90

---

[1] Aeration of the phosphate solution with sodium carbonate as in the ammonia determination gave as a result of six tests 3.68 per cent. nitrogen, which is 65.02 per cent. of that obtained by the official distillation procedure.

NOTE.—Since the writing of this paper the work of Sebelien, Brynildsen and Haavardsholm (*Chem.-Ztg.*, **33**, 795 (1909)) has come to our notice. These investigators report the very satisfactory results obtained by the aeration procedure applied to total nitrogen determination, but they do not give data upon the nitrogen in organic materials.

Conclusions.

1. The Kober aeration process for the total nitrogen determination in organic materials can be successfully used, except in cases where the elements magnesium and phosphorus are present together in relatively large amounts.

2. Materials which contain relatively large amounts of both magnesium and phosphorus can be correctly analyzed for the total nitrogen by the Kober method when it is so modified as to keep the aerating solutions in the Kjeldahl flasks warm for the entire period of aeration.

3. The Kober aeration procedure has many advantages over the official distillation procedure and the results are as accurate except as mentioned above.

URBANA, ILLINOIS.

[FROM THE LABORATORY OF AGRICULTURAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN.]

THE DISAPPEARANCE OF PENTOSANS FROM THE DIGESTIVE TRACT OF THE COW.

BY E. V. McCOLLUM AND W. A. BRANNON.

Received September 18, 1909.

Within recent years a great deal of energy has been devoted to the study of the physiology of the pentosans and their derivatives, the pentose sugars.[1]

The pentosans make up a large part of the "nitrogen-free extract" of the hays and coarse fodders used in animal feeding, and the degree in which they contribute to the maintenance of the animal is a matter of the utmost importance. The present paper is a report of a preliminary series of experiments which is being supplemented by further work on special phases of this subject.

It has been repeatedly confirmed that the pentosans of the food disappear from the digestive tract in rabbits and sheep to the extent of 40–80 per cent.[2] It seems also demonstrated that the pentose sugars, when absorbed by the animal, are utilized for energy production, and may lead to glycogen formation.[3]

[1] C. Neuberg, *Ergebnisse der Physiologie*, Bd. 3, 1, p. 373 (1904), gives an excellent résumé of the work in this field and makes reference to individual papers unnecessary.

[2] Slotzoff, *Z. physiol. Chem.*, **34**, 181 (1901–2). Stone, *Ber.*, **25**, 563 (1902). Weiske, *Z. physiol. Chem.*, 20, 489 (1895). Stone and Jones, *Agric. Science*, **7**, 6–20 (1893). Lindsay, *Mass. Exp. Sta. Report* (1894), p. 175, 12th annual Rept.

[3] Ebstein, *Virchow's Archiv.*, **129**, 401 (1892); **134**, 361 (1893). Voit, *Deut*