A NOTE ON THE DETERMINATION OF NITROGEN BY THE KJELDAHL-FOLIN-FARMER METHOD.

By VICTOR JOHN HARDING AND FRANCIS H. S. WARNEFORD.

(*From the Biochemical Laboratory, McGill University, Montreal.*)

The use of the Kjeldahl-Folin-Farmer[1] method for the determination of the total nitrogen content of biological fluids has proved of such enormous value to chemists and physiologists that the authors were of the opinion that the method could be applied in pure organic chemistry to the determination of the percentage of nitrogen in organic compounds. Cases frequently arise in which the amount of an organic compound, produced in synthesis or by analysis, is too small for a determination of its percentage of nitrogen to be carried out by the Dumas method or the ordinary Kjeldahl-Gunning process. The identification of minute amounts of organic bases, the determination of the composition of rare alkaloids, where it is necessary to conserve the supply of material, are cases to which the determination of nitrogen by the two above mentioned methods could not be applied with success. Each of them requires at least 0.2 gram of substance for determinations in duplicate, whereas the small amount of material required in the micro Kjeldahl process of Folin and Farmer seemed to hold out a promise of success and open up fields of investigation which had previously been closed.[2]

The authors, however, were not aware at the time of the limits of accuracy of this method, and in order to ascertain this point, they made a series of determinations of the percentage of nitrogen in urea. Since this experimental work a paper by Bock and Benedict[3] has appeared in which the Folin-Farmer modification of the Kjeldahl process is adversely criticized. The number of experiments performed and the invariable discrepancy of the

---

[1] O. Folin and C. J. Farmer: this *Journal*, xi, p. 493, 1912.

[2] E. C. Grey: *Tr. Chem. Soc.*, cv, p. 2204, 1914.

[3] J. C. Bock and S. R. Benedict: this *Journal*, xx, p. 47, 1915.

results show quite clearly the limitations of the method. Our own results bring out the same point, but not to so great an extent; indeed, the errors of experiment are found to be compensated provided a sufficient number of determinations be performed. Such a number of experiments, however, from our point of view was not desirable; for the fact that a greater number than two has to be performed in order to obtain a result in harmony with theory destroys one's confidence in the method, and consequently we abandoned the process as a means of determining the percentage of nitrogen in minute amounts of organic compounds. Bock and Benedict's paper, however, opens the subject in a more general way and hence we think our experimental results will prove of interest to others besides ourselves.

In our experimental procedure we followed the details given by Folin and Farmer, except that our ammonia was aspirated in the $\frac{N}{10}$ HCl placed in a 100 cc. flask and Nesslerized direct without transference to another vessel.

0.02 gm. of urea was dissolved in water and made up to 10 cc., and the nitrogen was determined in 1.0 cc. The colorimeter readings given represent the mean of five consecutive closely agreeing readings.

COLORIMETER READING	N_2 PER LITER	N_2 IN UREA
cm.	gm.	per cent
2.17	0.922	46.08
2.15	0.930	46.51
2.12	0.943	47.12
2.16	0.926	46.30
Average 2.15	0.930	46.50
Theory..................	0.933	46.60

Thus it will be seen that the average of these four determinations gives a result in close agreement with theory. The errors of the individual determinations are compensated in this particular case, though they are large when reckoned in percentage of nitrogen. A difference of 0.02 cm. in the colorimeter readings means a difference of nearly half a per cent on the percentage of nitrogen, an error too great to be allowed in the calculation of the formula of an organic compound. That errors of this magnitude

are the normal errors of the method can be seen by comparing the results, when expressed as grams of nitrogen per liter, with those given in the original paper of Folin and Farmer. The following figures taken from that paper show the error of the method and the comparison with the Kjeldahl method. The results were expressed in grams of nitrogen per liter of urine.

FOLIN METHOD		KJELDAHL METHOD
gm.		*gm.*
7.9	8.1	8.0
3.7	4.1	3.7
10.5	10.0	10.2
9.4	9.3	9.2
9.3	9.1	9.2

The small amount of divergence of the colorimeter readings in our determinations is, we believe, a normal divergence, and is inherent not only in this particular colorimetric process but in all determinations depending on the use of the colorimeter. The results of one of us with Mr. MacLean[4] on a colorimetric determination of amino-acid α-nitrogen show this same divergence,— about 1 per cent. Small as this is, and confirming the value of the Kjeldahl-Folin-Farmer method for the uses for which it was designed, *viz.*, the determination of total nitrogen in minute amounts in physiological fluids, it is too large to allow of the extension of the method to those problems which we have indicated, and we are reluctantly compelled to abandon it.

[4] V. J. Harding and R. M. MacLean: *ibid.*, xx, p. 217, 1915.

A NOTE ON THE DETERMINATION OF NITROGEN BY THE KJELDAHL-FOLIN-FARMER METHOD

Victor John Harding and Francis H. S. Warneford

J. Biol. Chem. 1915, 21:69-71.