The sensitivity of the TBA test for linolenate was 30-80 times that for linoleate at the same peroxide values. The TBA test appears to be a reliable method of estimating the oxidation products of linolenic and linoleic acids in tissues and other biological material.

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Summary of the Collaborative Work on Total Nitrogen

Joint Committee of Association of Official Agricultural Chemists and American Oil Chemists' Society

Objective. The study was intended to provide the basis for elimination of numerous variations in the procedure for total nitrogen in fertilizers and feeds. Specifically, the work compared copper with mercury as a catalyst, compared boric acid with standard acid for absorption of ammonia, and gave some comparison of the heating devices used in digestion of the sample.

Collaborators. Of the 27 laboratories which participated in the work, 12 had expressed a preference for copper as the catalyst (Group 1), 11 others had expressed a preference for mercury (Group 2), and the remainder had indicated no preference. Four preferred boric acid over standard acid and used it in analyzing all samples. The participating laboratories were as follows:

Harry R. Allen, Agricultural Experiment Station, University of Kentucky, Lexington, Ky.
B. W. Beadle, Geo. W. Gooch Laboratories, Los Angeles, Calif.
A. T. Blackwell, Davison Chemical Corporation, Baltimore, Md.
W. A. Bridgers, Southern Testing Company, Wilson, N. C.
P. D. Cretien, Texas Testing Laboratories, Dallas, Tex.
H. A. Davis, Department of Agriculture and Biological Chemistry, University of New Hampshire, Durham, N. H.
E. A. Epps Jr., Department of Agriculture and Immigration, Baton Rouge, La.
C. W. Gehrke, Department of Agricultural Chemistry, Agricultural Experiment Station, Columbia, Mo.
C. W. Gourley, Beacon Milling Company, Cayuga, N. Y.
E. R. Hahn, Hahn Laboratories, Columbia, S. C.
G. C. Henry, Law and Company, Atlanta, Ga.
T. H. Hopper, Southern Regional Research Laboratory, New Orleans, La.
W. J. Ingram, Department of Agriculture, Salem, Ore.
L. A. Koehler, State Laboratories Department, Bismarck, N. D.
J. C. Konen, Archer-Daniels-Midland, Minneapolis, Minn.
H. R. Kraybill, American Meat Institute, Chicago, Ill.
R. F. Larsen, Feed and Fertilizer Laboratory, Department of Agriculture, Boise, Idaho.
C. A. Lathrap, Curtis and Thompkins Ltd., San Francisco, Calif.
C. L. Manning, Fort Worth Laboratories, Fort Worth, Tex.
C. V. Marshall, Department of Agriculture, Ottawa 2, Canada.
J. R. Mays Jr., Barrow-Agee Laboratories, Memphis, Tenn.
V. E. Munsey, Dept. Health, Education and Welfare, Food and Drug Administration, Washington, D. C.
B. O. Pattison, Pattison's Southwest Laboratories, Harlingen, Tex.
Willis Richerson, Department of Agriculture, Oklahoma City, Okla.
E. H. Tenent, Woodson-Tenent Laboratories, Memphis, Tenn.
W. S. Thompson, Department of Agriculture Laboratories, Reynoldsburg, O.
E. R. Toby, Agricultural Experiment Station, Orono, Me.

Samples. Ten samples were selected as follows: cottonseed meal, soybean meal, dried blood, digester tankage, meat scrap, fish meal, commercial fertilizer, commercial feed, ground hoofs, and S-benzylthiuronium chloride (m.p. 172°C.; N content, calculated 13.82%). This compound, recommended by Ogg and Willits [Ind. Eng. Chem., Anal. Ed., *18*, 334 (1946)], was prepared in the Purdue Laboratory by the method of Danbury [J. Am. Chem. Soc., *58*, 1004 (1936)] and was purified by recrystallizing three times from alcohol.

Directions to Collaborators. Laboratories were asked to number each burner to be used for digestion of samples, determine the time required to bring 250 ml. of water to a rolling boil and record the number of the burner used for each sample digested. With each catalyst, copper and mercury, samples were to be analyzed in duplicate and the analysis repeated at least one week later, for a total of four analyses per catalyst. Standard acid and standard alkali were to be prepared independently, then checked, one against the other. Boric acid was compared with standard acid on two samples (7 and 8). Collaborators were given detailed digestion procedures which were identical except for catalyst (copper *vs.* mercury). The mercury procedure was as follows:

Place weighed sample (0.7 to 2.2 g.) in digestion flask. Add 0.7 g. HgO (or 0.65 g. metallic Hg), 10 g. powd. K_2SO_4 (or anhyd. Na_2SO_4) and 25 ml. con. H_2SO_4. If sample larger than 2.2 g. is used, increase H_2SO_4 10 ml. for each gram of sample. Place flask in inclined position and heat gently until frothing ceases (if necessary, add small amount of paraffin to reduce frothing); boil briskly, until solution clears and then for at least 30 min. longer.

Cool, add *ca.* 200 ml. of water and 25 ml. of sulfide or thiosulfate soln. to ppt. Hg (thiosulfate or sulfide may be mixed with the NaOH before addition to flask). Mix thoroughly and cool to 25°C. or below. Add a few Zn granules to prevent bumping, tilt flask, and add layer of NaOH (25 g. solid reagent or sufficient soln. to make contents strongly alkaline) without agitation. Immediately connect flask to distilling bulb on condenser (end of condenser is immersed in standard acid or 50-75 ml. boric acid soln. in receiver) and rotate to mix contents thoroughly, then heat until all NH_3 has distilled (at least 150 of distillate). Titrate excess std. acid in distillate with std. alkali soln. (Me red indicator), or if boric acid is used in receiver, titrate direct with std. acid (bromocresol green—Me red or methylene blue—Me red indicator).

Summary of Results

It will not be attempted to reproduce the large amount of detailed data received from the participating laboratories. These data have been analyzed statistically[1] with the following conclusions:

1. Averaged over the 10 samples, mercury gave .05% more N than copper in the hands of Group 1 and .07% more in the hands of Group 2. The difference was highly significant for both groups.

2. The results with mercury were less variable than those with copper in the hands of both groups. Averaged over the 10 samples, the standard deviation of the 27 laboratories was 0.188% N with mercury and 0.238% N with copper. See table below:

STANDARD DEVIATION OF 27 LABORATORIES (% N)

Catalyst used	Catalyst preferred			Weighted av. all labs.
	Cu (Group 1)	Hg (Group 2)	Unknown	
Cu	0.267	0.198	0.243	0.238
Hg	0.210	0.178	0.133	0.188

3. Averaged over both catalysts and all samples, Group 2 obtained .02% more N than Group 1. This difference is significant.

4. There was no significant interaction between catalyst and sample. This means it is reasonable to believe that the difference between copper and mercury did not depend on the material which was being used.

5. The variation between analyses of the same sample as shown by standard deviations was only about half as great for parallel duplicates as for duplicates one week apart. This was true for both groups and all samples. There was no evidence that "difficult" materials such as tankage or hoof meal were more variable than others. Average results and standard deviations are shown in the following table:

Sample	Av. % N found[b]	Variation[a] between		Laboratories
		Parallel duplicates[b]	Duplicates a week apart[b]	
1. Cottonseed meal..........	6.74	0.038	0.064	0.115
2. Soybean meal..............	7.92	0.046	0.065	0.148
3. Dried blood.................	13.38	0.063	0.112	0.319
4. Digester tankage.........	8.60	0.047	0.125	0.174
5. Meat scrap..................	7.96	0.040	0.063	0.152
6. Fish meal.....................	9.66	0.051	0.092	0.219
7. Commercial fertilizer...	9.77	0.057	0.109	0.241
8. Commercial feed..........	3.97	0.047	0.049	0.117
9. Ground hoofs..............	15.51	0.044	0.106	0.338
10. S-BTC.......................	13.68	0.051	0.096	0.239

[a] Standard deviations per single analysis, by samples, averaged over both catalysts; in other words, the standard deviations measure the variation: (1) between 2 single analyses made in parallel, (2) between 2 single analyses made about a week apart by the same analyst, and (3) among laboratories if each laboratory made only 1 analysis. To convert the laboratory standard deviation to the basis on which it is usually reported, divide by the square root of the number of analyses which each laboratory made. This number varies for the various standard deviations we have calculated.

[b] Averaged over all laboratories.

6. The average percentage nitrogen found in the crystalline S-benzylthiuronium chloride (Sample No. 10) with each of the catalysts was less than theoretical (theory, 13.82; found, Cu, 13.66, and Hg, 13.70). Nine of the 54 average values (Cu or Hg) fell between 13.82 and 13.87; 8 fell between 13.44 and 13.60. The difference between catalysts was highly significant, and that between theoretical and found values was also highly significant.

7. Standard acid gave 0.05% more N than boric acid when averaged over both samples (7 and 8) both catalysts and all laboratories. The difference was highly significant. For Group 1 the difference was .03%; for Group 2, .08%.

8. In the burner or heating test, gas was more variable than electricity, but wide variations were observed between laboratories and within laboratories. For gas burners the ranges within 15 laboratories were 3¼ to 8½ min. in the fastest laboratory and 13 to 25 min. in the slowest; laboratory averages ranged from 4½ to 16½ min.; over-all average for gas 8½ min. For electric heaters the ranges within 12 laboratories were 8 to 13½ min. in the fastest laboratory and 12½ to 16½ in the slowest; laboratory averages ranged from 9 to 13 minutes; over-all average for electricity, 11½ min.

Discussion

The results clearly indicate the superiority of mercury over copper as a catalyst for digestion of samples in the determination of total nitrogen. There would seem to be no important reason for retaining copper as an alternate reagent in official procedures.

Although boric acid has been gaining favor over standard acids for absorption of ammonia, the collaborative results fail to support its use. Accordingly, it is not recommended.

The wide range of results from the boil-test raises a question concerning optimal digestion time. The total digestion time reported varied from 55 min. to more than three hours, and while the statistical study relating digestion time, burner temperature, and total nitrogen found is not complete, no close relation is apparent except with the slowest heating equipment. Collaborator No. 19 reported a 13- to 25-min. boil-test range, used an 80-min. digestion time, and was lowest in percentage N with 8 of the 10 samples in Group 1. While in each group the laboratory with the most top values used a comparatively long digestion time (100 to 120 minutes), some laboratories got good results with shorter digestion times. Collaborator No. 6 digested 55 min. and was well above average in results. A new experiment should be designed to study these relationships specifically. Perhaps it will be found that a variable figure such as 10 or 15 times the boil-test value will provide an optimal digestion time for the various equipment. Until a further study is made, the digestion time should remain at the discretion of the analyst.

The committee wishes to thank the collaborators who so generously participated in the program. Special appreciation is expressed to Law and Company for preparation and distribution of the samples and to J. T. R. Andrews, H. A. Davis, E. A. Epps Jr., T. H. Hopper, J. R. Mays Jr., S. R. Miles, and C. H. Perrin for their contributions in planning the work.

Recommendations

It is recommended that:

1. The use of copper as a catalyst in official methods for total N be discontinued.

2. The directions for preparing standard acid and standard alkali (Sec. 2.20) be deleted; that new directions refer to Chapter 39 and require the use of a primary standard for each solution; the acid and alkali, so prepared, then to be finally checked one against the other.

3. The collaborative mercury procedure, changed to omit reference to boric acid, be adopted as the first action with a view toward its replacement of sections 2.20 through 2.24 and with a view toward its incorporation into sections 2.25 and 2.26.

4. Further collaborative work be done on the relation of the boil-test to the time necessary for digestion of the sample.

JOINT AOAC-AOCS COMMITTEE
R. W. BATES
M. P. ETHEREDGE
F. W. QUACKENBUSH, chairman

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[1] The statistical work was kindly performed by S. R. Miles and his staff at the Purdue University Agricultural Experiment Station.