

## ESTIMATION OF NITROGEN BY FUMELESS DIGESTION. PART II.

Products of Oxidative Digestion of Organic Nitrogen and the Procedure for their Inclusion in the Estimate of Total Nitrogen.

BY C. R. HARIHARA IYER, M.A., R. RAJAGOPALAN, B.Sc. (HONS.),

AND

V. SUBRAHMANYAN, D.Sc., F.I.C.

(From the Department of Biochemistry, Indian Institute of Science, Bangalore.)

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It was shown in the previous communication (Narayanayya and Subrahmanyam, 1933<sup>3</sup>) that the digestion of organic nitrogen can be greatly accelerated by addition of oxidising agents. Attention was also drawn to the fact that the digestion proceeds to completion in presence of water, so that emission of fumes which is the most objectionable feature of the Kjeldahl method (or any of its modifications) is entirely eliminated. Based on these and other observations, a simple and rapid method for the estimation of nitrogen has been developed and shown to be applicable to soils and biological materials in general.

Although the above findings are of considerable practical interest, further information is, nevertheless, needed regarding the nature of the products formed during oxidative digestion and the manner in which they are included in the estimate of total nitrogen. Some of the conditions relating to the digestion also require elucidation and the details of procedure to be further simplified so as to facilitate easy adoption in routine practice. These and related problems have been studied and will be discussed in the present communication.

### *Experimental.*

It was suggested in some earlier communications (Narayanayya and Subrahmanyam, 1935<sup>1,2</sup>) that the material to be digested may be heated together with dichromate, water and sulphuric acid. When applied in practice, however, such a procedure was not found to be satisfactory. Thus, when mixtures of soil, dichromate and acid (2:1) were heated gently to boiling, the digests invariably yielded lower results than those obtained by the Kjeldahl "wet" method (Sreenivasan, 1932). The error was not constant but varied in the hands of different workers. The following results (Table I) obtained

with one specimen of soil will suffice for an illustration. The soil (a local specimen; 10 g.) was treated with potassium dichromate (5 g.), mercuric oxide (2 g.), water (20 c.c.) and sulphuric acid (40 c.c.) in succession and the mixture boiled for 30 minutes. The digest was treated with zinc (10 g.) and boiled for 15 minutes. It was then cooled and distilled with excess of alkali in the usual way.

TABLE I.

Observations by	Forms of zinc used for reduction	Alkali equivalent of nitrogen (as c.c. of 0.0352 N alkali)		Total Nitrogen in parts per million (Average estimate)
		Correction for zinc	Total nitrogen in digest	
A	Dust	6.3	16.1, 16.3	488
B	Do. (same sample)	6.2	16.3, 16.4	498
C	Do.	6.1	15.8, 16.0	483
D	Do.	5.5	15.6, 15.35	493
A	Turnings	0.6	10.1, 10.0	466
B	Do. (same sample)	1.0	10.7, 10.65	477
C	Do.	0.3	10.45, 10.35	498

A, B, C,.....were different workers. The value expected according to the Kjeldahl wet' method was 518 p.p.m.

The digestion was apparently complete in all the cases, as may be seen from the separation of white sand. The distillation was also quite smooth.

It was observed, however, that zinc was not digested to the same extent in all the cases. This was largely due to the presence of mercury, which formed a coat of hydride around unattacked zinc and thus retarded its reaction with acid. It is probable that this may have rendered the blank for zinc so variable. Even after allowing the minimum blank for zinc, the estimate of total nitrogen in the soil was still too low as compared with the expected value.

The discordant nature of the results, though coming within a narrow range, still suggested that certain conditions relating to the digestion were not adequately controlled. Further work was therefore carried out with

a view to throwing light on the nature of factors determining the efficiency of digestion.

*Effect of addition of mineral catalysts.*—Narayanayya and Subrahmanyam (*loc. cit.*) found that the acid digestion of cyanamide was catalysed by the presence of certain mineral salts. With a view to determining whether the estimate of nitrogen can be improved by such treatments, small quantities of these catalysts were added to mixtures of soil (10 g.) and acid (60 c.c., 2 : 1). In one set of trials, the mixtures were heated for 15 mins. under reflux and then cooled before addition of dichromate. In another set, the dichromate was added together with the catalyst and the mixtures raised to boil. After boiling for 30 mins., the digests were reduced with zinc (10 g.) and distilled with excess of alkali. The results (after deducting the average blank for zinc as expressed in parts per million) were as follows:—*Catalyst added before dichromate.* (a) *Potassium sulphide* (10 g.) added together with alkali.  $\text{MnSO}_4$  (2 g.), 735 p.p.m.;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (2 g.), 720;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (2 g.) +  $\text{HgSO}_4$  (3 g.), 695. (b) *Potassium sulphide not added with alkali.*  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 710;  $\text{MnSO}_4$ , 700;  $\text{SeO}_2$ , 715;  $\text{BaO}_2$ , 705. *Catalyst added together with dichromate.*  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (2 g.) +  $\text{HgSO}_4$  (3 g.), 666;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 656. Value expected (Kjeldahl 'wet' digestion), 764. It may be seen from the above that none of the treatments yielded the correct estimate for total nitrogen. The low estimates of total nitrogen should therefore be traced to other causes.

*Nitrogen content of zinc.*—It was found that even the purest specimens of zinc contained some nitrogen. The blanks were also variable. It was further noted that if the zinc was boiled with acid and the digest distilled with excess of alkali, the blank for nitrogen was slightly higher than when the metal was boiled directly with alkali. These observations would suggest that the blank for zinc, especially when used in such large quantities as 10 grams, was unreliable.

Attempts to remove the nitrogen associated with zinc were unsuccessful. A leading European manufacturing firm was consulted in the matter, but they were also unable to supply nitrogen-free zinc. It was considered necessary, therefore, to either eliminate the use of zinc altogether or, at any rate, apply it in such small quantities that the error due to the nitrogen present in the metal would be negligible.

*Reduced iron.*—It has already been shown (Narayanayya and Subrahmanyam, *loc. cit.*) that reduced iron can be used in place of zinc, though the formation of the heavy precipitate in alkaline medium renders the distillation rather difficult. In spite of this disadvantage, a few trials were carried out, using reduced iron either as such or after boiling with alkali (50 per cent.) to

remove as much of the associated nitrogen as possible. Two different specimens of soils (in 10 g. lots) were taken and, after being treated in the manner outlined previously, the digests were diluted with water, boiled with reduced iron (10 g.) and then distilled with alkali in the usual way. The results have been given in Table II.

TABLE II.

Material used for reduction	Alkali equivalent of nitrogen (As c. c. of 0.0352 N alkali)		Total nitrogen in soil (in parts per million)	
	Correction for N in iron	Total N in the digest	As found	As expected
Reduced iron (as such)	0.85	11.95	547	569
Do.	0.75	11.75	542	„
Do.	0.60	11.50	537	„
Do. (after boiling with alkali)	0.40	15.55	747	764
Do.	0.40	15.15	727	„
Do.	0.40	15.75	757	„

It may be seen from the above that reduced iron also contained nitrogen, though not in such large quantities as zinc dust. Boiling with alkali proved highly beneficial because, in addition to removing a part of the nitrogen and thus yielding a consistent blank, it also dissolved out the arsenic with which the iron was associated. If arsenic was not removed in this manner, the poisonous gas, arsine, used to be formed in considerable quantities and was given off freely both during boiling with acid and subsequent distillation with alkali.

As observed in the previous experiment, the final estimates of nitrogen were too low even after correction for the nitrogen content of the iron used for reduction. The deviations from the expected values depended largely on the mode of heating, though it was not possible to define as to how it was affected in each case.

*The nature and extent of reduction needed to release nitrogen from combination.*—The residue after oxidative digestion contains (a) unused chromic acid, (b) compounds containing nitrogen in association with chromium

mercury and other mineral constituents in the medium, and (c) nitric acid, part of which may have been derived from the nitrites and nitrates originally present in the medium and part formed through the oxidation of organic nitrogen. Of these three, the first requires no explanation though it may be pointed out that, on prolonged heating with acid, chromic acid will undergo steady decomposition, yielding oxygen, and thus getting reduced to the chromic ( $Cr^{+3}$ ) condition. This reaction takes, however, such a long time, especially in presence of water, that it may not be convenient, in practice, to reduce the unused chromic acid in that manner. Resistant compounds of mercury containing nitrogen are well known. The associated nitrogen can be released by either precipitation of mercury as the sulphide or by treating the digest with a reducing agent as suggested by Narayanayya and Subrahmanyan (*loc. cit.*). The latter procedure has also been applied for release of nitrogen occurring in association with chromium though it is not clear whether nascent hydrogen—as formed through treatment with zinc or reduced iron—is required for the purpose. Minute quantities of nitric acid are formed during oxidative digestion (Harilal Iyer, Rajagopalan and Subrahmanyan, 1935). The quantities actually formed—at any rate in the case of most soils—are so small that even if the digest is boiled in an open flask, the estimate of total nitrogen will not be appreciably affected. It is probable, however, that as recently reported by Acharya (1935), larger quantities of nitric acid may be formed from other biological materials.

Of the three above-mentioned forms, the unused chromic acid which reacts with the major part of zinc or reduced iron can be reduced equally efficiently by oxalic acid, stannous salts, sulphites and such other reagents. It is difficult to state whether the nitrogen associated with mercury or chromium can also be released by such reagents. Nitrate nitrogen cannot be reduced to ammonia by such compounds. It thus appeared probable that one of the above-mentioned reagents—which may be expected to be reasonably free from nitrogen—can be used in conjunction with zinc or reduced iron in acid medium or Devarda's alloy or aluminium in alkaline medium. Some preliminary trials were accordingly carried out to determine the extent to which the different chemicals contained combined nitrogen as an impurity. In each case, a mixture containing dichromate (5 g.), mercuric oxide (2 g.), water (20 c.c.) and sulphuric acid (40 c.c.) was refluxed under water-cooled condenser for 30 mins. after which the unused chromic acid was reduced with excess of one of the reagents. The products were then cooled, treated with excess of alkali and distilled in the usual way. The average alkali (0.0352 N) equivalent of the ammonia distilling over in each case was as follows:—oxalic acid (pure), nil; sodium sulphite (A.R.), nil; potassium sulphide,

mercury and other mineral constituents in the medium, and (c) nitric acid, part of which may have been derived from the nitrites and nitrates originally present in the medium and part formed through the oxidation of organic nitrogen. Of these three, the first requires no explanation though it may be pointed out that, on prolonged heating with acid, chromic acid will undergo steady decomposition, yielding oxygen, and thus getting reduced to the chromic ( $Cr^{+3}$ ) condition. This reaction takes, however, such a long time, especially in presence of water, that it may not be convenient, in practice, to reduce the unused chromic acid in that manner. Resistant compounds of mercury containing nitrogen are well known. The associated nitrogen can be released by either precipitation of mercury as the sulphide or by treating the digest with a reducing agent as suggested by Narayanayya and Subrahmanyan (*loc. cit.*). The latter procedure has also been applied for release of nitrogen occurring in association with chromium though it is not clear whether nascent hydrogen—as formed through treatment with zinc or reduced iron—is required for the purpose. Minute quantities of nitric acid are formed during oxidative digestion (Harilal Iyer, Rajagopalan and Subrahmanyan, 1935). The quantities actually formed—at any rate in the case of most soils—are so small that even if the digest is boiled in an open flask, the estimate of total nitrogen will not be appreciably affected. It is probable, however, that as recently reported by Acharya (1935), larger quantities of nitric acid may be formed from other biological materials.

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nil; stannous chloride (1st sample), nil; stannous chloride (2nd sample, 15 g.), 1.90 c.c.; stannous sulphate (15 g.), 2.30 c.c.; ferrous sulphate (15 g.), 0.65 c.c.; and hydrogen peroxide (10 c.c., 30 per cent.), 1.35 c.c.

Of these, oxalic acid acted fairly rapidly on heating the diluted acid digest. Sodium sulphite acted readily in the cold and there was no smell of sulphur dioxide until all the dichromate was used up. This stage was further indicated by the development of a characteristic blue-green colour which was distinct from the bright emerald green of the earlier stages. All the stannous salts acted in the cold, but they contained varying quantities of nitrogen. Moreover, large quantities of these preparations had to be used because they had undergone partial oxidation to the stannic condition. Ferrous sulphate also acted in the cold, but considerable amounts of that chemical had to be used to ensure complete reduction of the dichromate. This resulted, naturally, in heavy precipitation of iron in the alkaline medium. There was violent bumping during distillation which could be prevented only by addition of small quantities of Devarda's alloy. Hydrogen peroxide was very effective in reducing the dichromate, but the analytical reagent used for that purpose contained some nitrogen.

*Comparative efficiencies of different reducing agents in releasing combined nitrogen.*—Samples (10 g.) of three different soils were digested in the manner previously outlined. The digests were treated with the different reducing agents, boiled to drive off excess of any acid vapour (such as sulphur dioxide) which may be present, cooled and finally distilled with excess of alkali. The results, after correction for the nitrogen contents of the reducing agents, have been presented in Table III.

There was general improvement in the estimate of total nitrogen when treatment with any of the above-mentioned reducing agents was followed by addition of zinc. The latter was added in the form of turnings (2 g.) and the digest then boiled for 15 mins. to complete the reduction (Table IV).

It may be noted, however, that in none of the cases was the result so high as that obtained by Kjeldahl 'wet' digestion.

The foregoing observations are highly significant. They show that mere reduction of dichromate is not sufficient to release all the nitrogen present in the digest. As already observed by Narayanayya and Subrahmanyam, even chromic salts can hold considerable quantities of nitrogen in combination, so that some special treatment will be needed to release all the nitrogen present in that form. The above results show that all reducing agents were not equally effective in that respect. The efficiencies of the different treatments also varied with the nature of the soil. These observa-

any, from combination and, at the same time, reduces any nitrate which may be present in the medium. The precise mode of action of the reducing agents requires further elucidation. It may, nevertheless, be concluded from the above that to ensure the inclusion of all the different forms in the estimate of total nitrogen, the digest should be treated with the most effective combination of reducing agents which will, at the same time, yield the smallest blank for nitrogen. From this point of view, the combination of sulphite with zinc was found to be the most satisfactory among those so far tried. Further results on this aspect of the problem will be considered in a later section.

*Order of addition of reagents.*—With a view to finding whether this was of any importance in determining the efficiency of digestion, some experiments were carried out with three different types of soils (in 10 g. lots) adding the reagents, potassium dichromate (10 g.), water (20 c.c.) and sulphuric acid (40 c.c.), in the order mentioned in Table IV. In all the cases, the mixtures were heated gently in the early stages followed by boiling under reflux for 30 mins. The digests were reduced with sulphite and zinc and then distilled with excess of alkali in the usual way.

TABLE V.

Soil from	Total Nitrogen in parts per million			
	Order of Addition			Value expected (Kjeldahl 'wet' digestion)
	(1) $K_2Cr_2O_7$ ; (2) $H_2O$ ; (3) $H_2SO_4$	(1) $H_2O$ ; (2) $K_2Cr_2O_7$ ; (3) $H_2SO_4$	(1) $H_2O$ ; (2) $H_2SO_4$ ; (3) $K_2Cr_2O_7$	
Tindivanam	116	112	108	137
Jacobabad	383	372	375	394
Nandyal	280	271	275	297

It may be noted that in all the cases the values were lower than those obtained by 'wet' digestion.

*Effect of conducting the digestion in two stages.*—It was first considered probable that the loss of nitrogen might have been due to the decomposition of some of the immediate products of oxidation consequent on the steady rise of temperature to the boiling point ( $170^\circ$ ). Some experiments were carried out, therefore, adding the reagents in the order, dichromate, water and sulphuric acid and maintaining the reaction mixture at  $100^\circ$  for 30 mins,



before raising it to boil. The digestion was then conducted in the usual way. (Table VI).

TABLE VI.

Soil from	Total Nitrogen in parts per million	
	Expected (Kjeldahl ('wet' digestion))	Found
Bangalore	764	729
Belgaum	466	436
Tindivanam	137	95

The values were all distinctly lower than those expected. Pre-heating for longer periods at 100° led to further fall in the estimate of total nitrogen. It may be concluded, therefore, that such a procedure is not only unnecessary but also undesirable.

*Effect of pre-heating with acid prior to addition of the oxidising agent.*—Some experiments were next carried out, heating the mixtures of soil (10 g.) and acid (2 : 1, 60 c.c.) to 80° and the boiling temperature (170°) respectively and then adding the dichromate (5 g.). The digestion was then carried out in the usual way followed by reduction with sulphite and zinc. The results have been presented in Table VII.

TABLE VII.

Soil from	Total Nitrogen in parts per million			
	No pre-heating (control)	Pre-heating to		Expected (Kjeldahl 'wet' digestion)
		80°	Boiling	
Tanjore	779	786	793	793
Nilgiris	1722	1767	1818	1816
Kandy	1700	1715	1766	1764

There was distinct improvement on adding the oxidising agent to the hot mixture (80°) of soil and acid. When the same treatment was repeated with the boiling mixture, correct estimates (within limits of experimental error) were obtained.

*Effect of adding the oxidising agent after boiling with acid for varying periods of time.*—Samples (10 g.) of three different soils were treated with mercuric oxide (2 g.), water (20 c.c.) and acid (40 c.c.) and the mixtures raised to boil. One set of samples was treated with potassium dichromate (5 g.) immediately after commencement of boiling. The heating was then resumed and continued for a further period of 30 mins. Other sets were treated after 5, 10 and 20 minutes respectively after boiling and then digested for 30 mins. as before. The different digests were allowed to cool, diluted with water, reduced with sodium sulphite and zinc and finally distilled with excess of alkali (Table VIII).

TABLE VIII.

Soil from	Total Nitrogen in parts per million				Value expected (Kjeldahl 'wet' digestion)
	Oxidising agent added after boiling for				
	0 min. (Immediate)	5 mins.	10 mins.	20 mins.	
Chota Nagpur	209	209	207	212	209
Belgaum	466	468	468	466	466
Nandyal	293	293	295	295	295

Allowing for small experimental errors, it may be seen that the same values were obtained in all the cases. It may be concluded, therefore, that prolonged pre-boiling is unnecessary and that accurate results can be obtained by adding the oxidising agent even immediately after commencement of boiling.

*Comparative efficiencies of different reducing agents.*—The conditions for digestion having been standardised, attention was next directed to a comparison of the efficiencies of different methods of reduction in acid as well as alkaline media. Although the combination of sodium sulphite and zinc had proved highly useful, it was nevertheless realised that further improvement in the mode of reduction would be desirable. Thus, addition of sulphite had to be followed by boiling to drive off excess of sulphur dioxide, for, otherwise any acid vapour that may be present in the space above may pass over into the distillate and thus affect the titre value. Moreover, zinc had to be added only in the acid medium. Boiling was necessary to ensure complete reduction of nitrates and release of nitrogen combined with mercury or chromium. Although only small quantities of zinc (2 g.) were used, the action was

rendered rather slow by the presence of mercury salts which, as already explained, formed protective coats of hydride around unattacked zinc. These were not serious difficulties, but it was still considered desirable to avoid boiling in acid medium. Excess of chromic acid could be reduced in the cold and the reduction of nitrates and other complex forms, completed in alkaline medium, during distillation. A number of trials were accordingly carried out with a variety of soils employing different combinations of reducing agents. The results obtained with one soil have been presented in Table IX.

Samples (10 g.) of a specimen of Bangalore soil were digested according to the improved procedure and, after dilution, treated with different reducing agents followed by boiling. The digests were cooled and then distilled with excess of alkali in the usual way. The nitrogen content of the different reducing agents were determined separately. Alkali equivalent of total nitrogen as found by 'wet' Kjeldahl digestion was 17.25 c.c. (850 p.p.m.).

It may be mentioned that although two samples of zinc (dust and turnings) were first tried, only the turnings were used in the subsequent experiments where zinc was used in combination with other reducing agents. Potassium sulphide was generally added as a strongly alkaline solution, having been previously dissolved in the alkali to be used for distillation. Sodium sulphite, though highly reactive in acid medium, was quite ineffective in presence of excess of alkali. Reduction with ferrous sulphate was followed by heavy precipitation in alkaline medium, but this disadvantage can be overcome by addition of small quantities of Devarda's alloy. Distillation with more than about 1 g. of Devarda's alloy led to considerable frothing, the alkali being nearly always carried over with the spray. In consequence, the duplicates did not generally agree. The tendency to spray was greatly retarded in presence of tin or lead salts in the medium. This was due to the reduction of those salts to the metallic condition followed by the formation of the corresponding hydrides, which formed partially protective coats around unattacked Devarda's alloy. The vigour of the interaction with alkali was consequently retarded and the reduction proceeded smoothly, though somewhat more slowly, than might otherwise have been the case. This observation is of considerable practical significance and may be utilised with advantage when having to conduct reductions with Devarda's alloy or aluminium in alkaline media. Three or four brands of stannous salts were tried but they were generally impure and contained varying proportions of nitrogen. Moreover, with the exception of a few samples of analytical reagents in sealed bottles, all the others contained considerable amounts of the corresponding stannic salts having undergone spontaneous oxidation in presence of air. A similar observation was also made in regard to titanous chloride which was, in consequence, found to be unsuitable for the reduction.

TABLE IX.

Reducing agent employed	Alkali equivalent of nitrogen (as c.c. of 0.0352 N alkali)		Total nitrogen (in p.p.m.) after correction for blank
	Total nitrogen (without correction for blank)	Nitrogen content of reducing agent (blank)	
No reduction (control) ..	15.8, 15.95	..	783
Oxalic acid (10 g.) ..	16.4, 16.2	Nil.	803
Oxalic acid (10 g.) and zinc (2 g.)	16.5, 16.6	0.15	808
Oxalic acid (10 g.) zinc (2 g.) followed by potassium sul- phide (5 g.) in alkaline medium	16.65, 16.75	0.15	811
Oxalic acid (10 g.) followed by Devarda's alloy (2 g.) in alka- line medium ..	16.2, 16.1	Nil.	796
Zinc dust (10 g.) ..	23.25, 23.45	6.6 (?)	825 (?)
Zinc turnings (pure, 10 g.) ..	17.3, 17.4	0.6	821
Zinc dust (10 g.) followed by K <sub>2</sub> S (5 g.) in alkaline medium..	23.35, 23.40	6.6 (?)	827 (?)
Sodium sulphite (10 g.) ..	16.5, 16.7	Nil.	818
Na <sub>2</sub> SO <sub>3</sub> (10 g.) followed by K <sub>2</sub> S (5 g.) in alkaline medium ..	16.7, 16.9	Nil.	828
Na <sub>2</sub> SO <sub>3</sub> (10 g.) and Zn turnings (2 g.) in acid medium ..	17.4, 17.45	0.20	850
Na <sub>2</sub> SO <sub>3</sub> (10 g.) followed by Devarda's alloy (2 g.) in alka- line medium ..	16.8, 16.7	Nil.	826
Na <sub>2</sub> SO <sub>3</sub> (10 g.) and Devarda's alloy (2 g.) both in alkaline medium	Ineffective—Sodium sulphite does not reduce in alkaline medium.		
K <sub>2</sub> S (10 g.) followed by zinc (2 g.) in acid medium ..	16.8, 16.7	0.15	813

TABLE IX—(Contd.)

Reducing agent employed	Alkali equivalent of nitrogen (as c.c. of 0.0352 N alkali)		Total nitrogen (in p.p.m.) after correction for blank
	Total nitrogen (with- out correction for blank)	Nitrogen content of reducing agent (blank)	
K <sub>2</sub> S (10 g.) followed by Devarda's alloy (2 g.) in alkaline medium.	16.0, 15.95	Nil.	789
Reduced iron (10 g.) ..	18.05, 18.2	1.45	822
Reduced iron pre-boiled with alkali (10 g.) ..	17.65, 17.75	0.9	830
FeSO <sub>4</sub> (15 g.) in acid medium ..	16.95, 17.0	0.65	803
FeSO <sub>4</sub> (15 g.) and Zn (2 g.) in acid medium ..	17.25, 17.30	0.80	813
SnCl <sub>2</sub> (15 g.) in acid medium ..	16.4, 16.3	Nil.	805
SnSO <sub>4</sub> (15 g.) and zinc in acid medium ..	17.1, 17.2	0.15	838
SnCl <sub>2</sub> (15 g.) in acid medium and Devarda's alloy in alkaline medium ..	16.8, 16.8	Nil.	828

It may be seen from the results, that the most accurate results were obtained by using a mixture of sodium sulphite and zinc for the reduction. Some of the other combinations such as oxalic acid and zinc or stannous chloride and zinc, were, in some respects, easier to deal with but they generally yielded slightly lower estimates. In this connection, the results obtained with two of the soils previously examined would be of some interest (Table X).

The foregoing observations would suggest that although sodium sulphite was most potent in releasing combined nitrogen than either stannous chloride or oxalic acid, the difference could be largely made up by supplementing the reduction with zinc. It was not clear, however, whether addition of larger quantities of zinc would further improve the estimate of total nitrogen. With this in view, some experiments were carried out in which, after reduction with oxalic acid, parallel samples were treated with different quantities of zinc and boiled in the usual way. The results are presented in Table XI.

TABLE X.\*

Soil from	Total Nitrogen in parts per million after reduction with		
	Sodium sulphite (10 g.) and zinc (2 g.)	Oxalic acid (10 g.) and zinc (2 g.)	Stannous chloride (15 g.) and zinc (2 g.)
Kandy .. ..	1767	1653	1700
Nilgiris .. ..	1821	1757	1772
Bangalore .. ..	764	737	742

\* These results are distinct from those in Table IV. In the latter case, mixture of soil, dichromate and acid were heated together.

TABLE XI.

Soil from	Total Nitrogen in parts per million after reduction with				Total nitrogen expected (Kjeldahl 'wet' digestion)
	Oxalic acid (10 g.) alone	Oxalic acid (10 g.) and zinc (2 g.)*	Oxalic acid (10 g.) and zinc (3 g.)*	Oxalic acid (10 g.) and zinc (4 g.)*	
Nilgiris ..	1343	1757	1765	1770	1816
Kandy ..	1377	1653	1653	1681	1764

\* After correction for nitrogen in zinc.

It may be noted that although increasing quantities of zinc led to slight improvement in the estimate of total nitrogen, the values, thus obtained, were not so high as those by 'wet' digestion. When combined with sodium sulphite, however, even small quantities of zinc were quite effective, and as may be seen from the foregoing results, yielded correct estimates of total nitrogen in every case.

*Inclusion of nitrate in the estimate of total nitrogen.*—In the previous communication, a special procedure was suggested for the inclusion of nitrate in the estimate of total nitrogen. That would, no doubt, be needed if the quantity of nitrate is very large; but most soils do not contain more than traces of that form of nitrogen. Moreover, it is not probable that any nitrate will be lost when the digestion is carried out under reflux, so that if the subsequent procedure is adequately controlled, the entire quantity may be included in the estimate of total nitrogen. It may be reasonably expected that treatment with zinc in acid medium will help to reduce the moderate

quantities of nitrate which may be ordinarily present in the soil ; that in the case of soils containing useful amounts of nitrate, the method of oxidative digestion should give higher values than the Kjeldahl method. If the latter is modified, however, to include nitrates, then the two estimates should agree. Such was indeed the case as may be seen from the results obtained with some specimens of soils (Table XII).

TABLE XII.

Soil from	Total Nitrogen in parts per million by		
	Kjeldahl 'wet' digestion*	Wet digestion to include nitrate †	Oxidative digestion
Punjab .. ..	527	545	543
Sindh ( <i>Kalar</i> ) ..	513	522	524
Ahmedabad .. ..	372	394	397
Dacca .. ..	669	700	702

\* Soil (10 g.) digested with water (20 c.c.) and sulphuric acid (40 c.c.), K<sub>2</sub>SO<sub>4</sub> (10 g.) added at the fume stage.

† The same as above together with ferrous sulphate (2 g.) added to the digesting mixture. (Sreenivasan, *J. Ind. Inst. Sci.*, 1935, 18A, 25).

Although the specimens previously examined did not show any appreciable difference between the results of Kjeldahl 'wet' and oxidative digestions, the present set brought into relief a further aspect of the distinct superiority of the latter over the former. According to the usual Kjeldahl method any nitrate which may be present in the soil will be lost : special pre-treatment of the soil will be needed if the nitrate is to be included in the estimate of total nitrogen. On the other hand, the procedure adopted for oxidative digestion naturally includes nitrates and is, in consequence, more advantageous than the other methods.

*Effect of addition of nitrate to soil.*—Sreenivasan (*loc. cit.*) has already shown that zinc is only moderately effective in reducing nitrates in acid medium. It was considered desirable therefore to determine the extent to which the use of that reagent will help to include nitrates in the estimate of total nitrogen. To samples (10 g.) of two different soils, known quantities of nitrate were added. The mixtures were digested under reflux in the usual way after which they were reduced with the same quantities of sodium sulphite (10 g.) and zinc (2 g.) in each case. The results, which have been

given in Table XIII show that at least upto 50 parts per million of the nitrates (excluding any that may be already present in the soil) can be included in the estimate of total nitrogen. The average soil in any part of the world, rarely ever contains more than 50 p.p.m. of nitric nitrogen. The tropical soils generally contain very much less. It may not therefore be necessary to adopt any further modification to include nitrates.

TABLE XIII.

Soil from	Total Nitrogen in parts per million						
	Soil alone	Soil + nitrate (10 p.p.m.)	Soil + nitrate (20 p.p.m.)	Soil + nitrate (30 p.p.m.)	Soil + nitrate (40 p.p.m.)	Soil + nitrate (50 p.p.m.)	Soil + nitrate 100 p.p.m.
Nagpur-(Black cotton) ..	422	432	441	452	460	470	474
Mandalay ..	340	349	359	371	381	386	390

There are, no doubt, a few exceptional conditions in which special treatment may be needed. It is probable that in some cases fairly large quantities of soil (20–25 g.) may have to be taken for digestion. It may also occasionally happen that the soil is unusually rich in nitrates or has been recently fertilised with that form of nitrogen. In such cases more vigorous reduction than that provided by zinc alone may be needed. Reduced iron is known to be more efficient (Olsen, 1927), but generally large quantities of that reagent are required. Moreover, the nitrogen content of iron, as also the heavy precipitation during distillation, may militate against its use as a reducing agent. It would therefore appear to be preferable to use Devarda's alloy either before digestion, as outlined in the previous part, or together with alkali during distillation. Of the two, the former procedure is to be preferred because (a) it requires less attention and (b) there is no danger of any alkali being carried over into the distillate.

*Some of the factors determining the efficiency of oxidative digestion : Loss of nitrogen consequent on adding dichromate to cold mixtures of ammonium salts and acid.*—Attention has already been drawn to the fact that low estimates of nitrogen were invariably obtained when dichromate was added to a cold or even fairly hot mixture of soil and acid. It was not clear, however, as to whether the difference was due to loss of nitrogen in gaseous form or to its retention in the digest in some highly resistant form. With a view to throwing some light on this aspect of the problem, some experiments were carried out adding dichromate (5 g.) to dilute solutions of ammonium sulphate in sulphuric acid (2 : 1) and heating the mixtures to gentle boil. After boiling for 30 mins., the mixtures were cooled, reduced with sulphite and zinc and



distilled with excess of alkali in the usual way. In another experiment, a solution of ammonium dichromate was heated with excess of potassium dichromate while, in a third one, ammonium dichromate alone was boiled with 2 : 1 sulphuric acid. The results which have been presented in Table XIV show that there was very nearly complete recovery in the case of ammonium

TABLE XIV.

Treatment	Alkali equivalent of nitrogen (in terms of c.c. of 0.0352 N alkali)		Average recovery per cent.
	Expected	Found	
$(\text{NH}_4)_2\text{SO}_4 + \text{K}_2\text{Cr}_2\text{O}_7$ (5 g.) + $\text{H}_2\text{SO}_4$ (60 c.c., 2 : 1)	11.80	11.65, 11.60, 11.70, 11.45	98.3
$(\text{NH}_4)_2\text{Cr}_2\text{O}_7 + \text{K}_2\text{Cr}_2\text{O}_7$ (5 g.) + $\text{H}_2\text{SO}_4$ (60 c.c., 2 : 1)	12.85	12.10, 12.15	94.4
$(\text{NH}_4)_2\text{Cr}_2\text{O}_7 + \text{H}_2\text{SO}_4$ (60 c.c., 2 : 1)	233.5	231.8, 232.1, 232.9, 231.7	99.4

dichromate alone whereas, in the others, small amounts of nitrogen were lost. The latter observations are in agreement with the findings of Shewan (1935). The loss of nitrogen was completely avoided, however, by adding potassium dichromate to a boiling mixture of ammonium sulphate and acid (2 : 1). (The results actually obtained were 11.80, 11.85, 11.80 and 11.80 respectively against an expected value of 11.80.) The loss of nitrogen consequent on heating mixtures of ammonium sulphate and dichromate in acid medium may have been due to either or both of the following causes : (1) A part of the ammoniacal nitrogen may have been directly oxidised to the elementary condition. (2) Small quantities of ammonium dichromate, first formed in the cold, may have decomposed on heating, yielding elementary nitrogen. If the first explanation is correct, then there should have been some loss of nitrogen even on adding the dichromate to the boiling mixture of ammonium salt and acid. Moreover, other oxidising agents should also cause similar loss of nitrogen. The previous observations of Sreenivasan with hydrogen peroxide and of other workers with perchloric acid would show that there is no loss of nitrogen in those cases. The second explanation also, if taken as such, is comparatively weak. Pure ammonium dichromate itself—at any rate in such small quantities as are present in Kjeldahl digests—undergoes very little loss when heated with sulphuric acid. It is converted, more or less quantitatively, into ammonium sulphate. On

the other hand, the large excess of potassium dichromate (or the resulting chromic acid) in the digesting mixture would tend partly to reverse the reaction.



The quantity of chromic acid present in solution at any time may be small, but there is always excess of the precipitated trioxide, a part of which will pass into solution and make up for the saturation. In this manner, small quantities of ammonium dichromate may persist in the medium during heating and may decompose at some temperature below the boiling point of 2 : 1 sulphuric acid. If, on the other hand, the dichromate (or chromic acid) is added to the boiling, acid solution, then ammonium dichromate may not be formed at all, and loss of nitrogen may be entirely avoided. To verify this the following pieces of evidence are required : (a) that ammonium dichromate undergoes spontaneous decomposition at some temperature below the boiling point of 2 : 1 acid and (b) that nitrogen is lost from mixtures of ammonium sulphate, and chromic acid (in excess) at the same stage as the one at which ammonium dichromate decomposes.

With a view to throwing some light on these and other aspects of the problem, the following experiments were carried out.

*Effect of heating ammonium sulphate with other oxidising agents in acid medium.*—To aliquots (10 c.c.) of an aqueous solution of ammonium sulphate, sodium bismuthate (5 g.), potassium persulphate (2 g.) and hydrogen peroxide (10 c.c. ; 30 per cent.) respectively were added and the mixtures treated with 10 c.c. each of concentrated sulphuric acid. The mixtures were then heated under reflux for 30 mins. after which they were distilled with excess of alkali in the usual way. Since bismuth was found to hold small quantities of nitrogen, the digests obtained after treatment with bismuthate were treated with excess of potassium sulphide together with alkali.

There was complete recovery in all the cases thereby showing that the presence of oxidising agents did not lead to any loss of nitrogen.

The above experiment was not repeated with permanganate because it is known that ammonium permanganate undergoes spontaneous decomposition on heating. Consequently, even if there was any loss of nitrogen, it would not have been possible to state whether it was due to direct oxidation or to intermediary formation of the ammonium salt.

*The stage at which ammonium dichromate undergoes spontaneous decomposition in acid medium.*—It is well known that the decomposition of ammonium dichromate does not start at any particular temperature. Our observations showed that even with the most careful heating, the decomposi-

tion of solid ammonium dichromate started sometimes in the vicinity of 130° and sometimes above 140°. When the dichromate was suspended in sulphuric acid, the decomposition did not generally commence until a temperature of 135° was reached. In some cases it began at a slightly higher temperature, but in no case did it exceed 145°. The decomposition was characterised by the entire medium turning blue green. There was also considerable evolution of gas. This was distinct from the rather slow decomposition of potassium dichromate with which control experiments were carried out.

*The stage at which nitrogen is lost when mixtures of ammonium sulphate and dichromate are heated.*—Aliquots (20 c.c.) of a standard solution of ammonium sulphate were treated with potassium dichromate (5 g.) and sulphuric acid (40 c.c.) and the mixtures heated to different temperatures. They were then treated with sodium sulphite (10 g.) and zinc (2 g.) and distilled with excess of alkali. The results (Table XV) show that the loss was significant at 130°, and quite pronounced at 150°. There was no further loss at 170°. These observations would show that the loss of nitrogen occurred between 130° and 150°. This range would correspond to the decomposition of ammonium dichromate.

TABLE XV.

Temperature	40°	60°	80°	100°	120°	130°	140°	150°	170°
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> present in terms of c.c. 0.0352 N alkali .. ..	23.6	23.6	23.55	23.5	23.45	23.25	23.05	22.9	22.9

Value expected = 23.6 c.c.

It would appear, therefore, that, with the steady rise in temperature, the ammonium dichromate formed in the medium underwent partial decomposition. The extent of decomposition was comparatively small because of the presence of acid which combined preferentially with ammonia. The loss of nitrogen was nevertheless quite significant.

*Effect of adding dichromate at different temperatures.*—The results of the previous experiment would suggest that if the dichromate is added in the cold or at any temperature below 150° there would be some loss of nitrogen. If, on the other hand, the digesting mixture is heated above this point, the intermediary ammonium salt will not be formed and the loss of nitrogen will be avoided. This conclusion is partly supported by the fact that there is no loss of nitrogen on adding the dichromate to the boiling mixture of ammonium salt and acid (temp., 170°). With a view to obtaining further

quantitative evidence, aliquots (20 c.c.) of a standard solution of ammonium salt were treated with concentrated sulphuric acid (40 c.c.) and raised to different temperatures. They were then treated with potassium dichromate (5 g.) and raised to boil. After boiling for 30 mins., the mixtures were reduced with sulphite and zinc and then distilled with excess of alkali. The results have been presented in Table XVI.

TABLE XVI.

Temperature	120°	130°	135°	140°	145°	150°	160°	170°
N recovered in terms of c.c. of 0.0352 N alkali	22.3	22.4	22.8	22.95	22.95	23.05	23.2	23.2

Value expected = 23.2 c.c.

It may be seen from the above that there was no loss of nitrogen when the dichromate was added at temperatures above 160°. These observations lend further support to the intermediary formation of ammonium dichromate at low temperatures. They also show that ammonium dichromate is not formed at higher temperatures so that the attendant loss of nitrogen is entirely avoided.

*Effect of re-heating chromic acid digest of soil.*—In view of the presence of ammonium sulphate and unused chromic acid in the final digest, it was considered probable that re-heating of such a mixture would cause some loss of nitrogen. Some experiments were accordingly carried out with a specimen of Bangalore soil which was first digested for 30 mins., by pre-heating to boiling followed by addition of dichromate. One set of digests was then reduced with sulphite and zinc in the usual way. The other was allowed to cool and then heated slowly to boiling. They were then reduced and distilled. The estimates of nitrogen thus obtained were as follows:—without re-heating (control), 603 p.p.m. (average value); with re-heating, 583.

In addition to supporting the previous findings, these observations are also of some practical interest. They show that after completion of digestion, the residue should not be re-heated. It is not also advisable to stop the heating before completion of digestion and then to resume it at a later stage.

Other evidence in support of the intermediary formation of ammonium dichromate will be considered elsewhere.

*Kinetics of digestion when the oxidising agent is added before or after boiling.*—With a view to determining the progress of digestion under the two sets of conditions, parallel samples (10 g.) of three different specimens of soils were treated with the same quantities of mercuric oxide (2 g.), water

(20 c.c.), sulphuric acid (40 c.c.) and dichromate and the digestion conducted in the usual way. In one set, the dichromate (5 g.) was added together with the other reagents, whereas, in the other, it was added only after the mixtures came to boil. The digestion of parallel samples was stopped at intervals of 5 mins. commencing from the moment of boiling. The digests were treated with sodium sulphite (10 g.) and zinc (2 g.) and then distilled with excess of alkali. The results have been presented in Tables XVII and XVIII.

TABLE XVII.

*Dichromate added in the cold.*

Soil from	Total nitrogen in parts per million as estimated after (time in mins.)						Value expected (control)
	0	5	10	15	25	30	
Cuttack .. ..	306	343	345	377	382	382	402
Gorakhpur .. ..	774	798	811	808	811	813	850
Gaya .. ..	325	387	432	438	438	440	451

TABLE XVIII.

*Dichromate added after commencement of boiling.*

Soil from	Total nitrogen in parts per million as estimated after (time in mins.)						Value expected (control)
	0	5	10	15	25	30	
Cuttack .. ..	392	394	397	399	402	402	402
Gorakhpur .. ..	806	821	831	850	850	850	850
Gaya .. ..	417	429	446	448	451	451	451
Nagpur .. ..	404	414	421	424	424	424	422

It may be noted that in both the sets of experiments, the digestion proceeded very rapidly, the estimates of total nitrogen reaching steady values in under 25 minutes. In the first series (Table XVII) the values were distinctly lower than those expected whereas in the latter (Table XVIII) they agreed closely. This may be traced to the difference in the starting values (0 min.) which were distinctly higher when the oxidising agent was added to the boiling mixture than when it was added in the cold. Since the progress of digestion was more or less the same after commencement of boiling, it may be inferred that in the first series (Table XVII) the loss had occurred

even before that stage. These observations thus lend support to the previous findings and show that the loss of nitrogen was due to the intermediary formation of ammonium dichromate.

The results presented in Table XVIII show that the moment the oxidising agent was added to the boiling mixture of soil and acid, over 90 per cent. of the nitrogen was digested. The remaining part was also rapidly attacked and the digestion was complete within 15 mins. after commencement. Although this observation would suggest further shortening of the period of digestion, it may, nevertheless, be desirable, in routine practice, to continue the boiling for at least 30 mins., so that even the most resistant forms may be included in the estimate of total nitrogen.

*Rate of digestion of urea.*—Attention has already been drawn to the difficulty in digesting urea by the oxidative method (Shewan, *loc. cit.*; Narayanayya and Subrahmanyam, *loc. cit.*). It was, nevertheless, considered probable that by adopting the improved procedure, the need for pre-boiling with acid may be avoided. Some experiments were accordingly carried out treating aliquots of a standard solution of urea with sulphuric acid (in the proportion 2:1) and adding dichromate after the mixtures came to boil. At intervals of 5 mins., representative specimens of digests were removed, and, after reduction with sulphite and zinc, distilled with excess of alkali. In another set of experiments, representative specimens (10 g. each) of soils were mixed with urea in solution and then digested in the same manner as in the previous experiment. The results have been given in Table XIX.

TABLE XIX.

Material digested	Alkali equivalent of nitrogen after digestion for (as c.c. of 0.0352 N alkali) (time in mins.)						Value expected (control)
	0	5	10	15	20	30	
Urea (in solution, 10 c.c.) .. ..	5.25	5.45	5.55	5.55	5.55	5.55	5.55
Soil (from Cuttack)+ urea (in solution, 10 c.c.) .. ..	13.20	13.50	13.55	13.17	13.70	13.70	13.70
Soil (from Gorakhpur) +urea (in solution, 10 c.c.) .. ..	21.90	22.20	22.50	22.60	22.75	22.75	22.75

It may be noted that the digestion proceeded quantitatively in all the cases. The digestion of urea alone was complete in 10 mins., but when admixed with soil, a few more minutes were taken. As previously observed

in the case of soil, more than 90 per cent. of the digestion was complete immediately after addition of oxidising agent.

As already observed by Narayanayya and Subrahmanyan, urea can be easily digested by 2 : 1 acid alone. It is also rapidly decomposed by alkali yielding ammonia. These observations show that urea, by itself, does not require any oxidative digestion and will yield correct estimates even without treatment with dichromate. The loss of nitrogen consequent on heating mixtures of urea, dichromate and acid should therefore be traced to the intermediary formation of ammonium dichromate. As in the other cases, the formation of the intermediate compound and the attendant loss of nitrogen can be avoided by adding potassium dichromate to the boiling mixture of urea and acid.

*Trials with some representative specimens of soils.*—With a view to determining whether the method of oxidative digestion can be applied successfully to all types of soils, trials were carried out with representative specimens from India, Burma and Ceylon. In all the cases, air dry specimens (10 g.) passing the 100-mesh sieve were used. All the samples were treated with mercuric oxide (2 g.) to eliminate any possible error due to the presence of halides. Water (20 c.c.) and sulphuric acid (40 c.c.) were then added in succession and after the mixtures came to boil, they were treated with 5 g. each of dichromate. The digestions were continued for 30 mins. after which the digests were diluted with about 300 c.c. of water and then treated with sodium sulphite (10 g.) and zinc (2 g.). The mixtures were then boiled for about 15 mins. after which they were distilled with excess of alkali in the usual way. The results have been presented in Table XX.

It may be seen from Table XX that there was very close agreement between the two sets of values. In many cases, the same estimates were obtained, whereas, in others, the difference was less than what would be accounted by 0.1 c.c. of the alkali used for back titrations.

*Nitrogen contents of some biological materials.*—The estimations were next extended to biological materials some of which had offered considerable difficulty in the previous study. In all the cases, the oxidising agent was added to the boiling acid mixture after which the digestion was continued for only 30 mins. The digests were all reduced with sulphite and zinc and then distilled with excess of alkali. The results have been given in Table XXI.

There was close agreement in all the cases. Even cyanamide, which offered considerable difficulty in the previous study, was readily digested on adding the oxidising agent to the boiling mixture. Yeast is another biological material which is not easily digested. In the foregoing experiment,

TABLE XX.

Description of soil	Total nitrogen in parts per million	
	Expected*	Found
Sind (Willingdon Cattle Farm)— <i>kalar</i> —green manured ..	520	522
Tanjore (Madras)—deltaic area—alluvial—surface ..	793	793
Nandyal (Madras)—red clay—surface .. .. .	296	296
Cuttack (Orissa)—upland—surface .. .. .	399	402
Gaya (S. Bihar)—lowland, paddy—surface .. ..	451	451
Jacobabad—Govt. wheat farm—surface .. ..	397	394
Tindivanam (Madras)—dryland—surface .. ..	138	138
Gorakhpur—lowland—surface .. .. .	848	850
N. Bihar—paddyland—surface .. .. .	643	640
Devarshola (Nilgiris)—estate—surface .. ..	1819	1821
Kandy (Ceylon)—paddyland—sub-soil .. ..	1769	1767
Dharwar (Bombay)—old area—sub-soil .. ..	537	537
Sholapur—heavy black—surface .. .. .	244	242
Jaffna (Ceylon)—sub-soil .. .. .	397	399
Dacca—highland—cultivated—sub-soil .. ..	700	702
Chirakkal—sandy loam—surface .. .. .	315	315
Travancore—alluvial—surface .. .. .	1972	1973
Sind—uncultivated—surface .. .. .	352	350
Bangalore—sandy loam—surface .. .. .	850	850
Nagpur—black cotton—surface .. .. .	420	422
Mandalay (Burma)—paddy—sub-soil .. .. .	339	340



TABLE XX—(Contd.)

Description of soil	Total nitrogen in parts per million	
	Expected*	Found
Punjab-Barani area-sub-soil .. .. .	542	542
Ahmedabad-Kambha village-sub-soil .. .. .	394	397
Chota Nagpur-laterite-sub-soil .. .. .	209	209
Belgaum-cultivated-sub-soil .. .. .	466	466

\* Including Nitrates (Sreenivasan, *J. Ind. Inst. Sci.*, 1935, **18A**, 25).

TABLE XXI.

Material	Total Nitrogen per cent.	
	Expected	Found
Cyanamide .. .. .	16.51	16.50
Dried blood .. .. .	12.38	12.41
Groundnut cake .. .. .	7.72	7.68
Lantana leaf .. .. .	2.80	2.81
Paddy husk .. .. .	0.27	0.27
Ragi straw .. .. .	0.52	0.52
Yeast .. .. .	5.65	5.65

the Kjeldahl digestion was possible only after pre-treatment with alkali followed by prolonged digestion with concentrated acid (S. Rajagopal, private communication). On the other hand, oxidative digestion was comparatively simple and proceeded to completion, as rapidly as in any other case. Paddy husk is another material known to be highly resistant to Kjeldahl digestion: but even that was easily digested according to the new method.

*Is it always necessary to use a condenser during digestion?*—Attention has already been drawn to the fact that, at any rate in the case of soils, only traces of nitric acid are lost by volatilisation during oxidative digestion.

Since most soils are naturally poor in nitrates and since there is always a useful quantity of water in the digesting medium, it was considered probable that, even without any cooling arrangement, only traces of nitrates would be ordinarily lost by volatilisation. With a view to determining whether the condenser (air or water-cooled) can be dispensed with in routine practice, some experiments were carried out in which parallel samples of the same soils were digested with and without cooling. The details of digestion, reduction and distillation were the same as those already outlined. The results which have been presented in Table XXII show that although, in

TABLE XXII.

Soil from	Total Nitrogen in parts per million as estimated after digestion	
	With condenser	Without condenser
Nandyal ..	296	293
Tindivanam ..	138	133
Gorakhpur ..	850	835

two cases, very nearly the same estimates were obtained, the third one yielded distinctly lower values when the condenser was not used. This was traced to the presence of useful quantities of nitrate in the original soil itself. In view of this observation, it was considered desirable to use the condenser as an item of routine procedure. The air-condenser, if sufficiently long, is as efficient as the water-cooled one. It requires no attention, involves no extra expenditure and can be easily fitted to the digesting flask. It should, therefore, be preferred to the water-cooled condenser.

*Use of chromic acid in place of dichromate.*—The use of dichromate, though satisfactory in many respects, is attended by certain disadvantages. In ordinary practice it has to be weighed out (though only approximately) and has to be introduced into the hot boiling mixture after momentarily removing the condenser. This procedure, though fairly rapid, is rather inconvenient and should, if possible, be avoided. Some experiments were, therefore, carried out using aqueous solutions of chromic acid ( $\text{CrO}_3$ ) as the oxidising agent and comparing the values with those obtained with dichromate. The procedure adopted in the case of chromic acid was to add a saturated aqueous solution (5 c.c. for every 10 g. of soil) directly through the condenser as soon as the soil-acid mixture came to vigorous boil. The results which

have been presented in Table XXIII show that the same results were obtained in both the sets of experiments. Aqueous solution of chromic acid being more convenient to handle, should be preferred to solid dichromate.

TABLE XXIII.

Soil from	Total Nitrogen in parts per million after oxidative digestion with	
	Solid dichromate	Chromic acid (aqueous solution)
Bangalore ..	850	848
Kandy ..	1769	1767
Dharwar ..	537	537
Sholapur ..	244	244
Jaffna ..	399	396
Chirakkal ..	315	314
Travancore ..	1972	1969

When using the aqueous solution it would be useful to reduce the volume of water that is first added by that of the solution of chromic acid. This procedure is suggested so as to maintain the proportion of sulphuric acid to water in the neighbourhood of 2 : 1.

It has been found that commercial brands of chromic anhydride generally contain minute quantities of nitric acid. When using that chemical, it would be desirable, therefore, to perform control experiments and apply the necessary correction.

*Proportion of acid to water.*—It was found previously (Narayanayya and Subrahmanyam, *loc. cit.*) that a proportion of 2 : 1 between sulphuric acid and water was most suited to ensure rapid and complete digestion of organic nitrogen. In view of the later improvements in regard to procedure, it was considered desirable to determine whether the proportion of acid to water can be reduced with advantage.

Some experiments were carried out, therefore, with parallel samples of two soils using the other reagents in the same proportions as in the previous experiments but only altering the proportion of sulphuric acid to water so as to correspond to 1 : 2, 1 : 1 and 2 : 1 respectively. The digestions were stopped at the end of 30 mins. in each case. The results, which have been

given in Table XXIV show that correct estimates were obtained after digestion for 30 mins. only when the proportion of acid to water was as 2 : 1. At the ratio 1 : 1, the values tended to approach the expected ones, while at 1 : 2 they were very much lower.

TABLE XXIV.

Soil from	Total Nitrogen in parts per million			Value expected
	Proportion of acid to water			
	1 : 2	1 : 1	2 : 1	
Sindh ..	219	318	352	352
Bangalore ..	633	836	850	850

With a view to determining whether the values obtained at the ratio 1 : 1 can be further improved, the period of digestion was increased to 60 minutes in the case of two soils. The results (Table XXV) showed, however, that the values did not show any appreciable improvement.

TABLE XXV.

Soil from	Total Nitrogen in parts per million				Value expected
	Period of digestion in minutes				
	30	40	50	60	
Bangalore ..	836	831	833	828	850
Nagpur (Black Cotton) ..	402	396	407	411	422

The above results were first inexplicable, but subsequent observations showed that the boiling temperature of soil suspension in 1 : 1 acid (by volume) was 140°, which comes within the range of decomposition of ammonium dichromate (*vide supra*). Since the boiling mixture was refluxed, the temperature remained more or less constant and consequently there was some loss of nitrogen. A similar argument would apply, even more strongly, to the suspension in 1 : 2 acid which boiled at 115°. On the other hand, the boiling temperature (170°) of the suspension in 2 : 1 acid was well above the decomposition point, so that when the dichromate or chromic acid (in small instalments) was added to the boiling mixture, there was no scope for the formation of ammonium dichromate as an intermediate product and, consequently, there was no loss of nitrogen.

*Effect of fineness of division on the efficiency of digestion.*—It has been shown by Subrahmanyam, Narayanayya and Bhagvat (1934) as also by later workers, that wet combustion of soil will yield accurate estimates of carbon only when the soil is ground to a very fine state of division (passing the 100-mesh sieve). With a view to determining whether a similar condition is necessary to obtain accurate estimates of total nitrogen as well, some experiments were carried out with specimens of two different soils, grinding them to varying degrees of fineness and then digesting them (10 g. each) with dichromate (5 g.) and sulphuric acid (60 c.c., 2 : 1) in the usual way. The results (Table XXVI) showed that the fineness of division did not make any appreciable difference to the accuracy of the estimate of total nitrogen.

TABLE XXVI.

Soil from	Total nitrogen in p.p.m. when the soil is ground to pass (in meshes to the inch)				Value expected as p.p.m.
	30-40	40-60	60-90	Above 90	
Bangalore ..	601	603	603	601	603
Tellichery (Red sandy loam) ..	418	414	416	414	416

*Efficiency of digestion at different proportions of soil to acid.*—If sufficient quantities of material are available, it would be generally desirable to digest as large a quantity as possible, using the minimum quantity of acid for the purpose. With this in view, some experiments were carried out maintaining the quantities of water (20 c.c.), acid (40 c.c.) and dichromate (10 g.) the same, but only increasing that of the soil. The results (Table XXVII) show that upto 15 g., the digestion proceeded quantitatively. With further increase in quantity, however, there was distinct fall in the efficiency of digestion.

TABLE XXVII.

Soil from	Total Nitrogen in p.p.m. on digesting (Wt. in grams)					Value expected (in p.p.m.)
	5	10	15	20	25	
Bangalore ..	601	603	603	593	581	603

In this connection, it may be mentioned that when all the components of the digesting mixture are increased in the same proportion, then accurate estimates of total nitrogen may be obtained irrespective of the quantity of

soil taken. Our observations have shown that using the distilling flask (cap. 1,500 c.c.) itself for the digestion, 25-30 g. of soil can be easily digested and distilled in the usual way. Even larger quantities can be efficiently digested, but considerable amounts of acid and alkali will be required. The distillation will also be rendered difficult. In ordinary practice, quite accurate results can be obtained by digesting in 10 g. lots and it will be rarely necessary to digest more than 15 g. at a time.

*Comparative efficiencies of digestion by different methods.*—The several methods now in vogue for the estimation of nitrogen by acid digestion may be classified, under the following main heads.—(1) Those involving 'dry' digestion with concentrated sulphuric acid. Such methods may or may not include additional procedure for inclusion of nitrates. This type of digestion is still most extensively adopted and forms the basis of 'official' methods recognised in different parts of the world. (2) 'Wet' digestion involving preliminary treatment with water or dilute sulphuric acid, but otherwise similar to the first group. The advantages of this type of digestion are now being steadily realised. (3) Methods involving pre-treatment with hydrogen peroxide, perchloric acid or other oxidising agent combined with dilute sulphuric acid. These require special modification to include nitrates. They are quite rapid but do not eliminate the emission of fumes. (4) Oxidative digestion involving vigorous oxidation in a strongly acid medium. As already explained, this type of digestion is fumeless and includes nitrates in the estimate of total nitrogen. With a view to comparing the relative merits of these four types, representative samples of two soils (both containing only traces of nitrates) were digested according to (1) the Kjeldhal method (*Chemists' Year Book*, 1935); (2) the 'wet' method as recommended by Bal (1925); (3) Sreenivasan and Subrahmanyam (1933) using hydrogen peroxide as the oxidising agent; and (4) the method of oxidative digestion as developed in the present paper. The digestion was stopped at definite intervals after commencement of boiling and the digests then distilled with excess of alkali. In the first three sets the samples were not taken for the first 30 mins. because the digestion was comparatively slow. The fourth one being very rapid, samples were taken at intervals of 5 mins. The results which have been presented in Figs. 1 and 2 show that (a) 'dry' digestion was incomplete even at the end of  $3\frac{1}{2}$  hours in one set and  $4\frac{1}{2}$  hours in the other; (b) 'Wet' digestion was speedier than (a) and was complete in under  $2\frac{1}{2}$  hours; (c) digestion in presence of hydrogen peroxide was still faster and was complete in  $1\frac{1}{2}$ —2 hours after commencement of boiling; and (d) oxidative digestion was the speediest and was complete in less than 30 mins. in both the sets. These results show conclusively that the last method was the most efficient.

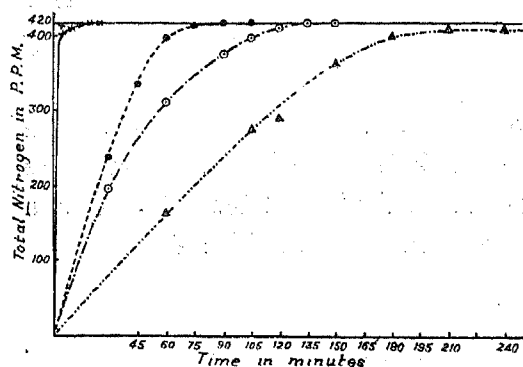


FIG. 1. Rate of Digestion of Black Cotton Soil from Nagpur.

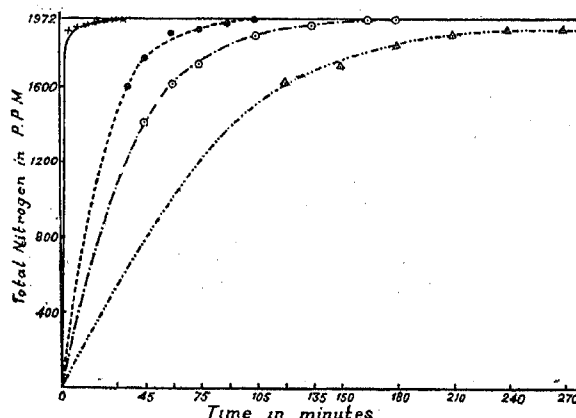


FIG. 2. Rate of Digestion of Alluvial Soil from Travancore.

FIGS. 1 and 2: Comparative Efficiencies of digestion by different methods:

- ×—× Values obtained by fumeless digestion.
- " " wet digestion with  $H_2O_2$ .
- " " " "  $H_2O$ .
- △—△ " " dry digestion official method.

*General Procedure for the Estimation of Nitrogen by Oxidative Digestion.*—

Based on the foregoing and other observations, the following improved method may be recommended for adoption in routine practice. The material to be digested (10-15 g. in the case of soil; others in proportion) is weighed out into the distilling flask (Cap. about 1,500 c.c.) and then treated with mercuric oxide (2 g.), water (15 c.c.) and sulphuric acid (40 c.c.). The flask is then fitted with water- or air-cooled condenser and the mixture heated to boiling. After the mixture has started boiling, saturated aqueous solution of chromic anhydride (about 5 c.c.) is introduced through the condenser in small instalments at a time. Boiling is stopped after 30 mins. and the digest, after being cooled for about 5 minutes, diluted with water (about 300 c.c.). It is then treated with sodium sulphite (about 10 g.) and again raised to boil. (The sulphite need not be weighed out but may be added in small instalments at a time until there is a pronounced smell. When the reduction of unused chromic acid is complete, there is also a characteristic change in colour from dark green to blue.) To the boiling mixture, pure zinc (2 g.) is added and the heating continued for a further period of about 5 minutes. The contents of the flask are then cooled and distilled with excess of alkali in the usual way.

Attention has already been drawn to the presence of minute quantities of nitric acid in commercial brands of chromic anhydride and the consequent need for applying some correction. This can be avoided, however, by using pure solid potassium dichromate which can be introduced after momentarily removing the condenser. A very small correction will also be needed for the

nitrogen present in the zinc used for reduction. Commercial samples of dichromate and sulphite may contain some chloride, but the error due to this is entirely eliminated by the presence of the mercury salt in the digesting mixture.

In the hands of the experienced worker, each estimation (commencing from the weighing to the completion of the back titration) will occupy less than 2 hours. If a number of distilling sets are available, at least two dozen determinations can be easily carried out in the course of a working day.

#### *Discussion.*

The present enquiry has led to a number of findings of considerable scientific interest. It has thrown fresh light on the mechanism of oxidative digestion; on the nature of the products formed and the manner in which they are retained in the digest. It has also led to the development of an improved method of fumeless digestion which is quite rapid and can be easily applied in routine practice.

The main product of oxidative digestion is ammonia, but a useful proportion is also present in combination with the chromium in the digest. It is difficult to state how exactly the nitrogen is retained by chromium, but there is no doubt that the resulting complex requires treatment with a reducing agent to convert into ammonia. Further work, analytical as well as synthetic, is needed to define the precise nature of the chromium complex and to determine whether one or more of such compounds are present in the digest.

Nitrogen is retained not only by chromium but also by other metals present in the digest. Thus, mercury, copper and bismuth hold a part of the nitrogen and have to be generally treated with a chemical precipitant (usually a sulphide) before the nitrogen can be distilled with alkali in the usual way. In the case of mercury, the nitrogen can also be released by treatment with a reducing agent. The available evidence is not sufficient to show whether nitrogen is retained in the same manner in all the cases.

There are no quantitative data relating to the formation of nitric acid during oxidative digestion. Judging however from the facts that (a) only minute quantities of that acid were lost on heating the digest in open flasks and (b) the zinc used for reduction cannot deal with more than about 50 p.p.m. of nitric acid, it has to be inferred that the quantities actually formed—at any rate, in the case of soil—are quite small. The mechanism of formation of nitric acid is still obscure.

The most noteworthy feature of oxidative digestion is that no nitrogen is lost through *direct* oxidation. The loss observed in some of the earlier experiments was due to the intermediate formation of ammonium dichromate



which, in turn, was due to the use of potassium dichromate (or chromic acid) as the oxidising agent. Although this difficulty has been overcome by adding the dichromate to the boiling acid mixture, it would nevertheless be desirable to use another oxidising agent, equally efficient in digestion but free from the above defect. Some work in this direction is already in progress.

The advantages of the new method over the others have already been enumerated. Its rapidity and freedom from fumes should commend itself to all workers in the field. The procedure for digestion and reduction are, no doubt, capable of further improvement. The use of the condensers should, if possible, be dispensed with and some simple device introduced for trapping the small quantities of nitric acid that may escape during digestion. The procedure for reduction is rather elaborate and occupies nearly as much time as the digestion itself. It should, if possible, be combined with the distillation. Although the results so far obtained in this direction have not been encouraging, it should still be possible to devise some treatment that can facilitate distillation almost immediately after digestion.

Using dichromate or chromic acid as the oxidising agent, it would be impossible to reduce the proportion of sulphuric acid to water to less than 2 : 1 or, at any rate, below 3 : 2. The reason for this has already been discussed in the text. Although the proposed procedure does not necessitate the use of larger quantities of acid than in the usual Kjeldahl method, it should still be possible to economise further by using other oxidising agents that would function in presence of still more dilute acid. In this manner, the cost of digestion can be reduced and the undue wastage of chemicals (chiefly acid and alkali) avoided.

Among the future lines of interest, mention may be made of the extension of the method to the digestion of organic compounds, particularly those to which the Kjeldahl method is not applicable. The procedure should also be capable of being adapted for micro analysis. Some useful progress has already been made in this direction.

The large amount of information now available in regard to the conditions of digestion should render it possible to include the wet combustion of carbon with the estimation of nitrogen. Some preliminary work in this direction has already shown that using an apparatus of the type described by Subrahmanyam, Narayanayya and Bhagvat (1934), the following conditions should be observed.—(1) for the estimation of carbon, the oxidising agent (chromic acid) should be added to the boiling mixture of the material to be digested with 2 : 1 acid ; (2) the distillation of the vapours (containing  $\text{CO}_2$ ) should be continued for at least 20 minutes ; and (3) after titrating the unused alkali, the distillate (containing all the nitric acid which might have passed over)

should be added to the digest and reduced, in the usual way, with sulphite and zinc prior to distillation with alkali. In this manner, accurate estimates of both carbon and nitrogen in the same samples have been obtained. Attempts are now being made to further simplify the procedure and it is hoped that, before long, it will be possible to develop a comprehensive method that can be easily adopted in routine practice.

*Summary.*

1. When an organic substance containing nitrogen is digested with a mixture of dichromate (or chromic acid), mercuric oxide, and sulphuric acid (2 : 1), the digest contains the major part of the nitrogen as ammonium sulphate. A small part remains in association with chromium or mercury. Minute quantities of nitric acid are also formed. The estimates of total nitrogen obtained after reducing the digest are lower and less concordant than those obtained by Kjeldahl 'wet' digestion. This is traced by two causes.— (1) loss of nitrogen in the elementary form when the digestion is conducted according to the above-mentioned procedure and (2) variable nitrogen blank yielded by the large quantities of reducing agent (zinc or reduced iron) used.

2. Comparative study of a number of reducing agents showed that sodium sulphite was the most efficient. When that reagent was combined with small amounts of zinc (for which the nitrogen blank is almost negligible), the highest and the most concordant estimates of total nitrogen were obtained.

Attempts to combine reduction in alkaline medium with distillation of ammonia have not, so far, yielded satisfactory results: nor has combination of other reducing agents (such as oxalic acid or stannous chloride) with zinc or Devarda's alloy proved so efficient as that of sulphite and zinc.

3. The efficiency of digestion was not improved by addition of mineral catalysts. Change in the order of addition of reagents did not lead to any appreciable improvement in the estimate of total nitrogen: nor was any benefit derived by conducting the digestion in two stages.

4. Addition of dichromate (or chromic acid) to the boiling mixture of the material to be digested (soil or other biological material) with sulphuric acid (2 : 1) followed by reduction with sulphite and zinc yielded correct estimates of total nitrogen. There was no need for any extended pre-boiling before addition of the oxidising agent.

5. The small quantities of nitric acid formed during digestion, as also any that may be already present in the original material, are retained in the digest by the use of a condenser. They are reduced by zinc upto a maximum of 50 p.p.m. and included in the estimate of total nitrogen.

## REFERENCES.

- Acharya .. .. *J. S. C. I.*, 1935, 54, 596 (Abstract).  
 Bal .. .. *J. Agric. Sci.*, 1925, 15, 454.  
 Harihara Iyer, Rajagopalan  
 and Subrahmanyam .. *Curr. Sci.*, 1935, 4, 98.  
 Narayanayya and Subrah-  
 manyam .. .. *Curr. Sci.*, 1935, 3, 423; *J. S. C. I.*, 1935, 54, 106T.  
 Do. .. .. *Proc. Ind. Acad. Sci.*, 1935, 2, 213.  
 Olsen .. .. *Compt. Rend. Trav. Lab. Carlsberg*, 1927, 17, No. 3.  
 Shewan .. .. *J. S. C. I.*, 1935, 54, 172T.  
 Sreenivasan .. .. *Ind. J. Agric. Sci.*, 1932, 2, 525; *Ibid.*, 1934, 4, 320, 546.  
 Do. .. .. *J. Ind. Inst. Sci.*, 1935, 18A, 25.  
 Sreenivasan and  
 Subrahmanyam .. .. *Ind. J. Agric. Sci.*, 1933, 3, 646.  
 Subrahmanyam, Narayanayya  
 and Bhagvat .. .. *J. Ind. Inst. Sci.*, 1934, 17A, 197.