THE STANDARDISATION OF THE DIGESTION PROCESS IN THE KJELDAHL DETERMINATION OF NITROGEN

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Although it is 70 years since Kjeldahl[1] first proposed his method for the determination of nitrogen in organic compounds, there is even now no general agreement regarding the details of the procedure. Various methods have been recommended for specific purposes, but the sphere of application of many is limited. A general method is needed for the digestion, suitable for all compounds for which the Kjeldahl method is applicable, and, in particular, capable of converting into ammonia the most resistant heterocyclic nitrogenous compounds. In order to devise such a general method, it is necessary to consider the limitations of existing procedures.

The method of determination of nitrogen under the Fertilisers and Feeding Substances Act is specified by Statutory Rules and Orders 1932, but these fail to give precisely either the quantity of sample or the amount of sulphuric acid to be taken; as regards the catalyst it is stated: "The operation may be accelerated by the addition of a small crystal of copper sulphate or a globule of mercury." The "Methods of Analysis of the Association of Official Agricultural Chemists" gives a choice of three methods[2], each of which includes alternatives which may or may not be used at will. In contrast, the monographs of the British Pharmacopœia, 1948, and its Addendum, 1951, are quite precise, assays, by the Kjeldahl process, being included for extract of malt, isoprenaline sulphate, mersalyl, riboflavine, promethazine hydrochloride and tryparsamide. Although the conditions for each assay are well defined, there are somewhat surprising differences; thus the quantity of sulphuric acid ranges from 3·5 ml. (for 0·1 g. of riboflavine) to 30 ml. (for 0·3 g. of tryparsamide); the ratio of sodium or potassium sulphate is equally flexible, and the catalyst may be copper, selenium, or a mixture of selenium and ferrous sulphate.

In the original proposal of Kjeldahl[1], sulphuric acid alone was used for the digestion, possibly with the addition of fuming sulphuric acid or of phosphorus pentoxide; oxidation was completed by the addition of potassium permanganate in powder. Wilfarth[3] discovered the accelerating action of mercuric oxide, while Gunning[4] recommended the addition of potassium sulphate. Arnold[5] considered copper and mercury in association to be more efficient than either metal singly and, in the so-called Gunning-Arnold process which combined the application of copper and mercury with that of potassium sulphate, Arnold and Wedemayer[6] recommended the use of a mixture of 40 g. of sulphuric acid,

20 g. of potassium sulphate, 1 g. of mercuric oxide and 1 g. of copper sulphate for 0·5 g. of the substance to be analysed.

In addition to these two catalysts, i.e., copper and mercury, many others have been proposed, about 50 being mentioned by Milbauer[7]. Of these the only one which has attained any practical significance is selenium, which has been used in the form of element, dioxide, oxychloride, selenates, etc. In micro-chemical work oxidising agents, generally hydrogen peroxide, are often added. Useful reviews of the method are given by Bradstreet[8] and Kirk[9].

Since the date of Kjeldahl's original publication, the number of communications dealing with the method has been so great that it might be supposed that nothing more remains to be said. Although every detail has been taken into consideration, the results have been confusing owing to the number of alternatives available. Many of these alternatives have been devised for a special purpose, e.g., rapidity of digestion, and are not suitable for a more general application. Moreover, a satisfactory procedure can only be obtained if the different elements of it are suitably adjusted to one another; in numerous publications one factor has been studied without reference to the general balance of the method.

With a large number of organic compounds the Kjeldahl digestion proceeds easily and there is no difficulty in obtaining correct results, so that attention has been directed mainly to reducing the time required for the digestion. With proteins, however, it is recognised[10,11,12,13,14] that low results may be obtained if the conditions are not carefully specified or controlled and also, for example, Dakin[15] has stated that the general method is inapplicable to tetramethylammonium salts.

As regards heterocyclic compounds many references could be quoted[3,10,14,16,17,18,19] to illustrate the difficulties encountered in the application of the process, especially to those containing a pyridine ring. Some workers[11,13,16] have found it necessary to employ periods of heating of 8 to 16 hours or even longer; others consider the method to be fundamentally unsuitable for certain types of heterocyclic compounds. The United States Pharmacopœia[20] states that certain alkaloids and other nitrogen-containing compounds will not yield all of their nitrogen to digestion with sulphuric acid. The increasing employment, in medicine and other fields, of compounds containing the pyridine nucleus makes this problem especially topical at the present time, but even when dealing with proteins, possibly the most widely used application of the Kjeldahl process, the presence of tryptophane and of other heterocyclic compounds must be taken into account. In spite of all this, it does not appear that any extensive study of the applicability of the process to heterocyclic compounds has been made.

The general application of the Kjeldahl process has been extended by Friedrich[21], who employed a preliminary reduction with hydriodic acid; this process is specially valuable for those compounds which otherwise could not be assayed by the Kjeldahl method—where nitrogen is linked directly to nitrogen or to oxygen.

STANDARDISATION OF THE KJELDAHL DIGESTION

While it is often desirable to make use of special forms of the Kjeldahl digestion for particular purposes, and especially for serial determinations, there is a parallel need for a method applicable to the largest possible range of organic compounds. This is especially the case in pharmaceutical analysis where new compounds are continually being introduced, many of them being of a type not easily decomposed. Any proposed method must be devised with special reference to resistant substances, but should at the same time be applicable to as wide a range of compounds as possible. Before attempting to draw up such a method, it is necessary first to consider the different stages of the digestion process.

The Kjeldahl reaction generally takes the following course, a number of stages being observed:

1. Decarboxylation, with evolution of carbon dioxide (with derivatives of carboxylic acids, e.g., nicotinic acid, mersalyl).

2. Carbonisation, with resulting reduction of the sulphuric acid by the carbon and the evolution of sulphur dioxide and accompanying white fumes. This stage, with dense fumes, is not of great duration, but an appreciable time is generally required before the liquid becomes colourless. A few compounds, e.g., diphenan, form at this stage resistant coloured compounds which are only slowly oxidised. Others may give neither carbon nor coloured products, the mixture remaining colourless throughout and white fumes being absent.

3. Boiling of the now colourless mixture (the " after-boil "), without formation of dense fumes.

The loss of sulphuric acid which occurs during the course of these reactions may be ascribed almost entirely to the reduction in stage (2); loss during the boiling period is negligible, as is shown by the fact that the actual loss of acid agrees with that calculated (see below), and it is, therefore, possible to allow for this loss of acid in such a way that the proportion of sodium or potassium sulphate in the final mixture is always the same. This step is essential for the standardisation of the process; without it the loss of acid is so uncertain and variable that either on the one hand the boiling point of the final mixture is not high enough to give effective breakdown of resistant compounds, or on the other hand the boiling point is too high and there is danger of loss of ammonia (see below, page 835).

Formation of carbonaceous matter is normally observed in the early stages of the digestion, but may be almost or completely missing in the case of certain compounds, e.g., glycine, cyclic ureides, pyridine and some of its simpler derivatives, and tetramethylammonium salts. In such cases the conditions are different from those usually prevailing where carbon and sulphur dioxide are present in the mixture; it will be shown later that the addition of carbonaceous matter accelerates the breakdown in the case of resistant pyridine compounds.

Determination of the Acid Consumption During the Reaction

The amount of sulphuric acid used up in the oxidation process is greater than is generally realised. Self[22] found it to be as follows: 1 g. carbohydrate uses 7·3 g. (4·0 ml.); 1 g. of protein uses 9·0 g. (4·9 ml.); 1 g. of fat uses 17·8 g. (9·7 ml.).

These experimentally determined figures agree with those obtained by calculation on the assumption that the products of the reaction are ammonia, carbon dioxide, sulphur dioxide and water, i.e., 1 g. of sucrose requires 7·5 g.; 1 g. of triolein requires 18·0 g.

It is thus evident that the loss of acid may have a very considerable effect on the relative composition of the mixture, and it is apparently on account of this that unnecessarily large amounts of acid and sulphate are often used, i.e., in order that the proportion shall not be too greatly altered by the loss. This procedure is undesirable, not only because of the inconvenience associated with large quantities of acid, and subsequently of sodium hydroxide, but also because it is impossible to ensure optimum conditions for the digestion if this loss of acid is ignored.

The amount of acid required for any compound may readily be calculated from its molecular formula by deducting from the latter the elements of ammonia and carbon dioxide (or water) corresponding to the number of atoms of nitrogen and oxygen respectively. Any other elements present may be allowed for in a similar manner, e.g., sulphur, when combined with oxygen as in sulphonic acids, may be taken as converted to sulphur dioxide, while sulphur in a sulphide group is assumed to be eliminated unoxidised. In the cases of salts of alkali metals, allowance should be made both for additional acid used in forming the sulphate and for the extra alkali sulphate present. By the subtraction of these elements of ammonia, water, carbon dioxide, sulphur dioxide, hydrochloric acid, sulphur, sodium, etc., from the molecular formula there is left a residual formula containing C and H. The factor

Factors for Calculating Acid Consumption

For 1 g. of	CH_2	allow	11·4	ml. of	sulphuric	acid
,,	CH	,,	10·2	,,	,,	,,
,,	$C_{1·5}H$	,,	9·9	,,	,,	,,
,,	C_2H	,,	9·6	,,	,,	,,
,,	$C_{2·5}H$	,,	9·4	,,	,,	,,
,,	C_3H	,,	9·3	,,	,,	,,
,,	C_4H	,,	9·1	,,	,,	,,
,,	C	,,	8·9	,,	,,	,,
,,	carbohydrate	,,	4·0	,,	,,	,,
,,	fat	,,	9·7	,,	,,	,,
,,	protein	,,	4·9	,,	,,	,,
,,	salicylic acid	,,	5·5	,,	,,	,,
,,	Zn	,,	0·8	,,	,,	,,
,,	Na	,,	1·2	,,	,,	,,
,,	K	,,	0·7	,,	,,	,,

used in the calculation depends on the C/H ratio of this residual formula, and is taken from the table above, which also gives the general values for carbohydrates, fats, and proteins, and factors for salicylic acid and zinc for use when these substances are added in the determination of nitrogen present as nitrates. The acid consumption per g. is then equal to $\frac{\text{residual formula wt.}}{\text{molecular wt.}} \times$ factor, and the amount of acid, in ml., to be added for a particular assay is then equal to 6+ (acid consumption per g. × wt. of substance taken). Actually great accuracy is not generally required in the calculation of the factor, and the total volume of acid need not be measured with an accuracy greater than, say, ±0·25 ml.

EXAMPLES

Cinchophen $C_{16}H_{11}O_2N = 249{\cdot}3$
Subtract CO_2,NH_3, leaving $C_{15}H_8 = 188$
then acid consumption $\frac{188}{249} \times 9{\cdot}6 = 7{\cdot}25$ ml./g.

Glyoxaline dicarboxylic acid $C_5H_4O_4N_2 = 156{\cdot}2$
Subtract 2 CO_2, $2NH_3$, leaving $C_3—H_2 = C_{2\cdot5} = 30$ (since 2H = 0·5 C).
and acid consumption is $\frac{30}{156} \times 8{\cdot}9 = 1{\cdot}7$ ml./g.

Phenytoin sodium $C_{15}H_{11}O_2N_2Na = 274{\cdot}3$
Subtract CO_2, $2NH_3$, Na, leaving $C_{14}H_5 = 173$
and acid consumption is $\frac{173}{274} \times 9{\cdot}3 = 5{\cdot}9$ ml./g.
plus allowance for Na = $\frac{23}{274} \times 1{\cdot}2 = 0{\cdot}1$ ml./g.
Total = 6·0 ml./g.
Sodium sulphate allowance is $\frac{23}{274} \times 3{\cdot}2 = 0{\cdot}3$ g./g.

Sulphathiazole $C_9H_9O_2N_3S_2 = 255{\cdot}3$
Subtract $3NH_3$, SO_2, S (one S atom is not linked to O)
leaving $C_9 = 108$,
then acid consumption is $\frac{108}{255} \times 8{\cdot}9 = 3{\cdot}8$ ml./g.

It is desirable that the amount of reagents should be as low as possible, especially where their quantity can become very great in proportion to the amount of substance taken for analysis; thus in the British Pharmacopœia 1948 process for the assay of tryparsamide, 30 ml. of sulphuric acid is taken for 0·3 g. of the compound: nearly 29 ml. should remain at the end of the reaction—at least three times as much as necessary. On the other hand, the 20 ml. of acid which is prescribed for the assay of extract of malt will be reduced to 5 ml. at the end of the reaction. Since there is 9·5 g. of potassium sulphate present, the composition of the final mixture corresponds to 14·9 g. of potassium bisulphate with 2·1 ml.

of sulphuric acid. In practice, of course, the analyst will add more sulphuric acid when the mixture appears to be getting too dry.

For organic compounds generally, it is convenient to take from 0·1 to 0·5 g. for the analysis according to the percentage of nitrogen anticipated. The amount of sulphuric acid should not be larger than necessary, and we have found 6 ml. to be a convenient figure, although this may, if desired, be reduced to one-half provided that greater care is used to avoid local overheating. The smaller the volume used the more important it is to apply correctly the correction for acid lost during the reaction. Thus the amount of acid actually added at the start of an assay will be 6 ml. plus a volume equal to that calculated to be lost in the reaction.

Temperature of the Reaction Mixture

The temperature of the digestion rises, after the conclusion of the first visible phase of the reaction, to the boiling-point of the mixture, and is therefore determined mainly by the ratio of acid to sulphate. It has been shown frequently that a high temperature (or a high proportion of sulphate) greatly speeds up the reaction[10,17,23]; on the other hand, if it is too high there is loss of ammonia[23]. It is difficult, and often impossible, to strike a satisfactory balance between these two factors unless allowance is made for the loss of the acid which is used up in oxidising the carbon and hydrogen.

A series of experiments was performed to determine the effect of different proportions of sodium or potassium sulphate on the temperature attained. As with all the work done in connection with the present paper, the neck of the Kjeldahl flask was not open to the air, but had a head connected to a fume removal system (this may have a slight effect on the temperatures attained). A short thermometer, with its bulb immersed in the reaction mixture, was used for measuring the temperature of the latter, which was prepared from a known weight of sulphate with a definite volume of sulphuric acid (d = 1·84). The actual weight of acid present was determined by titration after the completion of the observation. The mixture was heated by a small flame so that it was kept in steady ebullition. The results are shown in Figure 1.

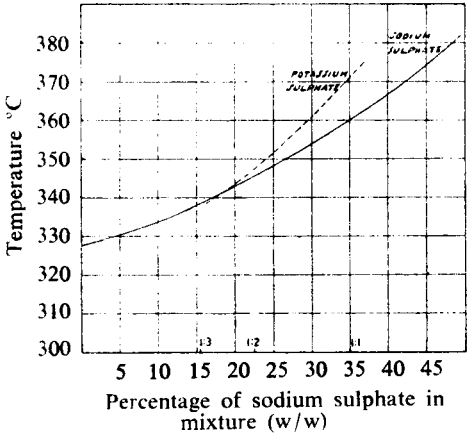

Fig. 1.

These results are similar to those obtained by Ogg and Willett[24], who

showed that the time of digestion could be decreased to one half, with a corresponding rise of temperature of 10°C., by increasing the proportion of potassium sulphate. Recently White and Long[25] have recommended carrying out the digestion on a micro scale, in a sealed tube at 470°C., with a great reduction in the time required. Dolique and Lacombe[26] also realised the significance of temperature, and used an electrical heating bath.

Ratio of Sulphate to Acid

It has been shown by several workers that, with a high proportion of sulphate, ammonia is lost from a mixture of sulphuric acid and potassium or sodium sulphate on heating; that this loss may become considerable we have confirmed. Under these conditions, moreover, local overheating is another danger. It is thus necessary to adopt a ratio of sulphate to sulphuric acid such that there is no danger of loss from such causes, even after allowing for some incidental deviation in composition and possible local heating. A suitable proportion, which in extended experiments we have always found to be satisfactory, is that of 1 part of anhydrous sodium sulphate by weight to 2 parts of sulphuric acid (d = 1·84) by volume, and this ratio has been adopted as a standard in the present paper. It does not appear that potassium sulphate offers any advantage over sodium sulphate, and in view of the fact that sodium salts are more generally available at all times, it was considered to be better to standardise on the sodium salt.

The Catalyst

Contrary to the general opinion, digestion with sulphuric acid does not break up all organic compounds. It may be noticed, for example, that when animal matter is digested with nitric and sulphuric acids, even after repeated and prolonged treatment, considerable amounts of organic matter still remain in the liquid, while fats, if present, are lost mainly by volatilisation. Pyridine (and to some extent quinoline) heated with sulphuric acid and sodium sulphate, remains undecomposed even in the presence of a catalyst such as copper sulphate or selenium, and may be recovered in the distillate obtained after making alkaline; here it obscures the end-point, since it is not sufficiently basic to be titrated satisfactorily. On account of this Ploquin[27] has recently proposed the use of an indicator, changing colour at *p*H about 7·2, which gives a good end-point with ammonia, but does not respond to pyridine. This device may increase the sharpness of the end-point, but can hardly be expected to increase the accuracy of the determination.

Although many compounds can be broken down completely by a digestion mixture containing sodium sulphate and sulphuric acid alone, the process is often slow and it is usual to accelerate it by the addition of a catalyst, generally one containing copper, mercury or selenium. The efficiency in producing rapid clearing and decolorisation of the mixture increases in the order given. Many other substances have been sug-

gested as catalysts for the reaction, but none has found any general favour and it is not necessary to consider them here. Although speed of decolorisation (i.e., removal of carbon) is often taken as a measure of the efficiency of the catalyst, there is no reasonable basis for this assumption, and it will be shown below that it may in fact be definitely untrue.

Before considering the choice of a catalyst from the point of view of its efficiency in breaking down resistant organic compounds, it is advisable first to determine whether it can have any destructive action on ammonium salts under the conditions of the reaction. Results given in Table I show the recovery of added ammonium sulphate after prolonged heating in a digestion mixture composed of 3 g. of sodium sulphate with 6 ml. of sulphuric acid (see above). The mixtures were kept boiling steadily for 6 hours, and other tests were done with the addition of 0·1 g. of dextrose to give reducing conditions similar to those normally present.

TABLE I

Catalyst	Dextrose added	Titration ml. 0·1 N
Nil	Nil	15·60*
Nil	Nil	15·55
Nil	0·1 g.	15·60
Mercuric oxide, 0·5 g.	Nil	15·40
„ „ 0·5 g.	0·1 g.	15·50
Selenium dioxide, 0·5 g.	Nil	14·20
„ „ 0·5 g.	0·1 g.	14·45
Copper sulphate, 0·5 g.	0·1 g.	15·55

* Blank without heating.

These results confirm the loss of ammonia noticed by several workers when using selenium catalysts[18,25,28], and show that the use of selenium is not permissible when long periods of heating are needed. In view of its popularity, it is to be supposed that its use is justified in suitable circumstances, i.e., when heating is not continued after decolorisation, and when it is known that this is sufficient to give quantitative recovery of the ammonia. The figures given above show a slight loss of ammonia on heating, even in the absence of any catalyst, although this loss is negligible in the presence of carbonaceous matter. The loss with mercuric oxide or copper sulphate, under reducing conditions, is only equivalent to 0·1 ml. of 0·1 N acid after 6 hours' heating. The standardised method recommended below (page 839), requires only 2 hours' heating.

Compounds offering maximum resistance to breakdown under the conditions of the digestion are those containing nitrogen in a ring, especially the pyridine ring. Nicotinic acid was, therefore, selected as a convenient test substance for comparing the efficiency of the catalysts. When

this compound, or pyridine itself, is heated with the digestion mixture, charring or discoloration is absent; parallel tests were, therefore, done with the addition of dextrose in order to give reducing conditions similar to those normally present in a Kjeldahl digestion. The digestion mixture for 0·25 g. of nicotinic acid was composed of 3 g. of sodium sulphate and 6 ml. of sulphuric acid, with an additional 1·1 ml. of sulphuric acid to compensate for that lost in the reaction with the nicotinic acid, and a further 0·4 ml. when necessary to allow for the dextrose. The periods of heating given are measured from the time that the mixture became clear and almost colourless.

TABLE II

COMPARISON OF CATALYSTS

Catalyst	Dextrose	Time of Heating	Titration ml. 0·1 N	Equivalent
		hours		per cent.
Selenium dioxide, 0·1 g.	nil	3	12·65	62·3
,, ,, 0·5 g.	nil	3	17·65	86·8
,, ,, 0·5 g.	0·1 g.	3	18·05	88·8
Mercuric oxide, 0·1 g.	nil	3	14·75	72·6
,, ,, 0·1 g.	0·1 g.	3	17·60	82·6
,, ,, 0·2 g.	0·1 g.	3	19·40	95·5
,, ,, 0·3 g.	nil	3	20·20	99·4
,, ,, 0·3 g.	0·1 g.	3	20·30	99·9
,, ,, 0·3 g.	0·1 g.	2	20·30	99·9
,, ,, 0·3 g.	0·1 g.	1	13·35	65·7
,, ,, 0·5 g.	nil	3	20·25	99·6
,, ,, 0·5 g.	nil	2	19·85	97·7
,, ,, 0·5 g.	0·1 g.	2	20·30	99·9
Copper sulphate, 0·5 g.	0·1 g.	3	14·40	70·9
Mixed				
Mercuric oxide, 0·1 g. Selenium dioxide, 0·2 g.	nil	3	20·15	99·2
Mercuric oxide, 0·1 g. Copper sulphate, 0·2 g.	0·1 g.	3	15·10	74·3

DISCUSSION OF RESULTS

The experimental results given show that, in order to devise a standardised method suitable for a very wide range of organic compounds, it is essential to use mercury as a catalyst; using 0·3 g. of mercuric oxide, decomposition is complete in 3 hours, provided that, in a few cases, a little organic matter is added. For many compounds a period of heating of half an hour after the mixture becomes colourless is sufficient.

The conditions of the reaction with pyridine, nicotinic acid, glyoxaline, and other simple ring compounds are somewhat exceptional since charring or separation of carbon does not occur, and there is little or no formation of sulphur dioxide. The importance of this factor is shown by figures in Table II, which show a distinct acceleration in the digestion under reducing conditions. The presence of substituents in the pyridine ring generally facilitates the breakdown. More complex condensed ring systems often require a comparatively prolonged period of heating before the mixture is fully decolorised.

There has been much discussion on the application of the Kjeldahl process to proteins, as some of the methods which have been used give somewhat low results, due no doubt mainly to the presence of heterocyclic compounds such as tryptophane, which are not easily broken down by a selenium catalyst. Lysine also, although not heterocyclic, must be put in the same category, as pyridine compounds are formed by condensation during the digestion[29,30].

It may be stated that, with compounds containing N not in a ring and not linked to O or to another N atom, there is generally no difficulty in carrying out the Kjeldahl process, and a very large range of conditions and catalysts may be suitable; this applies also to a large number of heterocyclic compounds such as, e.g., purines and pyrimidines. Thus simplified or rapid methods of digestion may often be used for special purposes, particularly when large numbers of similar samples have to be examined. On the other hand, there is a real need for the specification of a method which can be applied to all suitable compounds with the assurance that they will be completely broken down. Such a generally applicable method can only be based on the principle described above, of constant composition of the final mixture, and this carries the further practical advantage that the amount of acid, and therefore of alkali, used is reduced to a minimum and that the amount of alkali required for neutralisation is constant and predictable for all cases.

Oxidising Agents

An oxidising agent, as distinct from a catalyst, was used by Kjeldahl himself in the form of potassium permanganate, added cautiously in small portions to oxidise the carbon. In recent times, hydrogen peroxide, persulphates and similar additions have been used for the same purpose, mainly in micro analysis; the procedure is not generally favoured by the routine analyst. Kirk[9], who has published a useful report on the Kjeldahl process, points out that in the basic process it is necessary for oxidising conditions for the carbon and hydrogen to co-exist with reducing conditions for the nitrogen, and comments on unsatisfactory modifications in which strong oxidising agents are used or other conditions modified unfavourably. We agree with his conclusion that "judicious use of such agents in small quantity and with digests still rich in carbonaceous matter, is probably a safe procedure. The assumption of such judicious use may well be inapplicable to the routine analyist."

Effect of Halogens

It has been shown above that the rate of breakdown of resistant heterocyclic compounds depends on the amount of mercury catalyst used. When there are halogens present in the mixture, as when applying the method to hydrochlorides of organic bases, mercury is lost in the form of a mercury halide, which may be observed to condense on the neck of the flask. Experiments showed that in such a case 50 per cent. of the mercury added could easily be removed from the sphere of reaction. As

an alternative to the addition of more mercury to compensate for this loss (which will vary with the amount of halogen present and other conditions), the halogen may be eliminated by a preliminary heating before adding the mercury.

General Method

A quantity of substance, sufficient to give a final titration of 20 to 30 ml. of 0·1N acid, is taken for the assay together with 3 g. of anhydrous sodium sulphate, 0·3 g. of nitrogen-free mercuric oxide, and 6 ml. of nitrogen-free sulphuric acid *plus* a volume of acid equivalent to the amount calculated to be lost in the reaction (see pages 832-3 above). If halogen is present the substance is heated with the sulphuric acid for 15 minutes before the addition of the mercuric oxide and the sodium sulphate. The mixture is kept boiling gently for 2 hours after all carbonaceous matter has disappeared; i.e., when it becomes colourless. After adding water and cooling, 15 ml. of a solution containing 10 g. of sodium hydroxide and 2 g. of sodium thiosulphate (to decompose mercuric ammonium compounds) is run gently down the neck of the flask or is added in such a manner as to avoid mixing. A small fragment of zinc is added to ensure steady boiling, the flask is connected to a distillation apparatus, the contents are mixed, and the ammonia is distilled in the usual manner.

The list below, containing a selection from a very large variety of compounds to which the method has been applied, contains representatives of a varied selection of simple heterocyclic rings, condensed ring systems, and complex structures such as those of the alkaloids. In this list the figures in brackets represent the acid consumption, in ml./g. of substance.

Acridine (8·7)
Betaine hydrochloride (4·3)
Carbazole (8·6)
Caffeine (3·5)
Cinchophen (7·2)
Codeine (7·1)
Diphenan (7·3)
Haemin (7·3)
Histamine dihydrochloride (3·7)
Histidine hydrochloride (3·5)
Homatropine hydrobromide (5·6)
2-Hydroxyquinoline (7·0)
8-Hydroxyquinoline (7·0)
Indole (8·2)
*iso*Leucine (6·1)
Lysine monohydrochloride (4·4)
4-Naphthoquinoline (8·7)
5-Naphthoquinoline (8·7)
Narcotine (6·0)
Nicotine acid tartrate (3·8)
Phenytoin (6·4)
Physostigmine salicylate (6·2)
Pilocarpine hydrochloride (5·4)
Potassium oxyquinoline sulphate (3·8) (quantity of sodium sulphate reduced by 0·3 g./g.)
Proline (4·8)
Pyrrole (4·8)
Quinaldine (8·5)
Quinaldinic acid (6·2)
Quinine ($3H_2O$) (6·6)
Quinoline (8·3)
Isoquinoline (8·3)
Riboflavine (4·5)
Sulphadiazine (4·1)
Sulphathiazole (3·8)
Tetramethylammonium bromide (4·1)
Thiouracil (3·0)
Tryptophane (5·9)
Trigonelline (5·5)

A few simple pyridine derivatives, which do not char, require three hours' heating, the addition of 0·1 g. of dextrose and an extra 0·4 ml. of acid.

2-Aminopyridine (5·3)	Nicotinic acid (4·7)
αα′-Dipyridyl (6·8)	Piperidine (8·4)
γγ′-Dipyridyl (6·8)	Pyridine (7·5)
2-Hydroxypyridine (5·3)	

SUMMARY

1. Nitrogen can be quantitatively recovered in the form of ammonia from a very wide range of heterocyclic compounds, and also from tetramethylammonium compounds, by a standardised method using a mercury catalyst.

2. The method is based on the principle that the final ratio of sulphuric acid to sodium sulphate is kept constant, compensating for the acid consumed in the reaction, by the addition of an extra amount of acid at the commencement of the digestion which will equal that consumed in the destruction of the compound under test. The amount of additional acid required is obtained from the formula of the substance by a simple calculation.

3. The addition of a little dextrose accelerates the breakdown in the case of simple pyridine derivatives which do not char with sulphuric acid.

4. A constant amount of alkali is used before the distillation.

5. It is anticipated that the standardised process will be found capable of breaking down any type of heterocyclic or other compound; it will not, however, deal with those substances containing linkages for which the direct Kjeldahl process is inapplicable.

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DISCUSSION

The paper was presented by DR. R. E. STUCKEY.

DR. D. C. GARRATT (Nottingham) asked whether the authors had considered systematically the time factor after the clearing of the digestion mixture. Was there any significance in the use of zinc as distinct from porous pot during the boiling, in particular did the hydrogen evolved assist in the reduction? Was the method suitable for all types of organic compounds, for example those containing nitro groups and also certain complex dystuffs?

DR. K. BULLOCK (Manchester) asked whether the method had been tried with procaine hydrochloride or similar substances, as even with various modifications of the process he had never been able to obtain satisfactory results with such compounds.

PROFESSOR H. BRINDLE (Manchester) said that he had used zinc on numerous occasions and had always assumed that it was the steady evolution of hydrogen which was responsible for the reduced bumping. Reduced iron had been found to be equally useful for the purpose.

DR. D. C. GARRATT added that he never used porous pot but always employed steam distillation.

DR. G. FOSTER (Dartford) said that he had used a selenium catalyst without experiencing any difficulty. Could the author suggest the reason for the loss of ammonia on heating with selenium? He also asked whether there was any theoretical reason why mercury was the most suitable catalyst.

DR. STUCKEY, in reply, said that the question of examining systematically the time factor had been considered. According to the literature, confirmed by their own work, it would appear that there was always a possibility of loss of ammonia on prolonged heating even in the absence of a catalyst. Therefore it was important to use the most efficient catalyst compatible with the minimum of ammonia. There was no particular significance in the use of zinc. As to the suitability of the process generally, work was proceeding on its applicability to organic compounds which formerly were not assayed by that method. The method was

directly applicable to quaternary ammonium compounds. Preliminary reduction with hydriodic acid enabled groupings such as NO_2,NHOH, and N-N to be determined. The process had not been specially tested with procaine hydrochloride, but there appeared to be no reason why it should not be suitable. He did not dispute that selenium was satisfactory provided the process was in the hands of experienced workers and long boiling was avoided. It had been suggested that loss of ammonia was due to thermal decomposition of ammonium bisulphate in the reaction mixture although in his opinion that was unlikely as the neck of the flask was usually wet with sulphuric acid. He pointed out that there were two processes, the reduction of nitrogen to ammonia and the oxidation of carbon, and when the latter was complete it was possible that some oxidation of ammonia might also occur. The authors could offer no theoretical reason for the preferred use of mercury as a catalyst.