CIV. METHOD FOR THE RAPID AND QUANTITATIVE REMOVAL OF AMMONIA FROM SOLUTIONS, ESPECIALLY APPLICABLE TO THE MICROQUANTITATIVE ESTIMATION OF NITROGEN AND UREA IN PRODUCTS OF LIVING ORIGIN.

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Many analyses of biochemical importance end in the estimation of ammonia. The Kjeldahl process, in one or other of its countless modifications, is everywhere in use, and of recent years the estimation of urea by means of the Soya bean, or of the urease extracted from it, has become almost as much a matter of course.

All these processes leave the nitrogen in the form of an ammonium salt mixed with many other things. Several methods are in use to bring the ammonia into a pure solution in which it can be estimated. The mixture is generally made alkaline, and distilled, or alcohol is first added, or it may be distilled in steam. Another variation is the "aeration method" (drawing a vigorous current of air through it) as proposed by Folin and adopted by van Slyke.

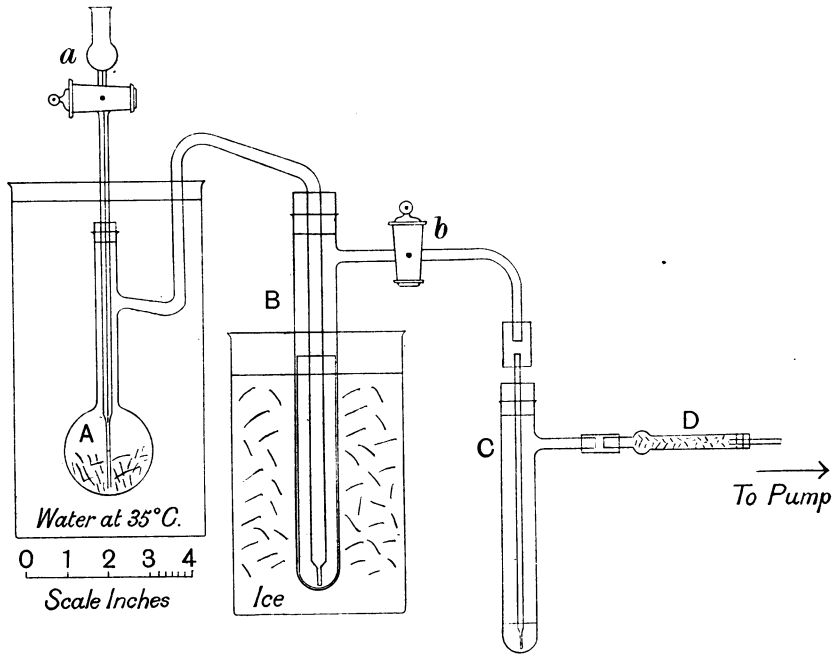
Folin has recently [Folin and Youngburg, 1918] sought a way out of the difficulty by estimating the ammonia by "direct nesslerisation." Unfortunately, owing to the presence of other colloids this nearly always gives turbid solutions (compare preceding paper) which are useless for colorimetric comparison.

The distillation and aeration methods are quite satisfactory as long as the quantity of ammonia to be measured is within volumetric limits, but in biochemical work the quantities to be measured are often so small that volumetric procedure would be absurd. In the case of the total nitrogen, non-protein nitrogen and urea nitrogen of cerebrospinal fluid or of blood, for example, microquantitative methods (in this instance colorimetric) are unavoidable, and it is therefore of importance to have some quick and quantitative means of removing the ammonia from the reaction mixture and getting it in the form of a pure solution which on nesslerisation will give a clear liquid for the colorimeter.

The aeration method is not applicable, for the slight losses which are bound to occur, and which are negligible in, say, a urine analysis, become comparable with the whole quantity of ammonia to be estimated—a quantity which may not exceed a few hundredths of a milligram.

Distillation may be employed, as in my method for the estimation of the total nitrogen of cerebrospinal fluid [Stanford, 1913], but would not be

suitable for urea estimations owing to the decomposition of the urease by the boiling alkali. The distillation method as there described has the advantages that it is easily carried out (except for the actual colorimetric comparison) by unskilled hands and it does not require any special apparatus. On the other hand it consumes a good deal of time, and this is inconvenient if a large number of estimations has to be made. For this reason we now do all our nitrogen estimations with the vacuum apparatus about to be described.



Vacuum Apparatus for the Distillation of Small Quantities of Ammonia.

The apparatus takes the form shown in the accompanying drawing. *A* is a small (100 cc.) distilling flask the side tube of which is lengthened and bent as shown, the end being drawn off to a fine jet. It is connected with the receiver *B* by means of a good rubber cork. In the receiver *B* is placed a thin, rimless test-tube of such diameter as to be an easy fit: this test-tube we term the container. *B* is connected through a tap *b* with the traps *C* and *D* and the pump. The distilling vessel *A* is provided with a capillary which carries a glass tap *a* and above that a small bulb.

The apparatus is used as follows. The "container" is removed from *B*, a wisp of glass wool is put in the bottom of it and the whole is then weighed to the nearest centigram. 4 cc. of very dilute (about $N/20$) sulphuric acid are run in, and the container is then replaced in *B*. The trap *C* is provided with about 3 cc. of dilute sulphuric acid and the tube *D* is filled with glass wool moistened with sulphuric acid. The receiver *B* is immersed in a beaker of ice and water containing enough water to enable the contents of *B* to be observed through it.

The liquid under examination is introduced into the distilling vessel *A*, together with a wisp of glass wool. If the solution is not faintly acid it must be brought to that condition, a scrap of litmus paper being thrown in to act as indicator. It is convenient so to arrange concentrations that the volume of solution taken shall be about 5 cc. The whole apparatus is now connected up as shown in the diagram, the mouth of *B* being previously smeared with glycerol, and, the tap *a* being closed, about 3 cc. of strong (5 *N*) sodium hydroxide solution are placed in the bulb above it. The distilling vessel is now surrounded with a beaker of warm water so that the cork at the top of the flask is completely in the water. The pump is turned on and the apparatus exhausted as completely as possible. With a good water pump this takes less than a minute. The most suitable temperature of the water in the bath will depend of course on the degree of vacuum obtained. A good pump should yield a vacuum of 12–13 mm., and in that case the warm water should be at 35°.

As soon as only an occasional bubble passes through *D*, the tap *b* is closed, and the tap *a* is opened to admit nearly all the sodium hydroxide solution. The apparatus is now left alone for five minutes, care being taken to maintain the temperature of the water-bath by placing a small flame underneath it. A continuous succession of bubbles will be seen being absorbed in *B*. The apparatus is functioning like the old cryophorus. During this period most of the ammonia passes over, and as the distillation is proceeding in a closed space there is no possibility of loss. At the end of five minutes connection with the pump is re-established by gradually opening *b*. Since the cork in *A* is sealed by immersion in water, and the cork in *B* by means of glycerol, no leaks can occur, but the vacuum is generally slightly impaired by reason of dissolved gases in the alkali solution which has been added. When connection with the pump has been completely re-established a fairly vigorous stream of bubbles will be seen passing through *B*, and an occasional bubble through the trap *C*. These are bubbles of water vapour which are only partially condensed in *B*, but they lose there any ammonia they may contain. The distillation is allowed to proceed in this manner for five minutes, counting from the time when the tap *b* first began to be opened. The tap *b* remaining open, the tap *a* is cautiously opened sufficiently to allow the slowest possible stream of air bubbles to pass through the distilling vessel, and this is allowed to continue for five minutes. The tap *b* is then closed, air is admitted through *a* and the apparatus is disconnected generally. The container is removed from *B* and weighed. In this way the total volume of the distillate is known, and it is then nesslerised with 1/10 of its volume of Nessler solution and compared with a standard in the dilution colorimeter.

It is obvious that the whole of the ammonia must be in this distillate unless any has remained in *A* or has passed over into the trap *C*. To check these possibilities, the liquid remaining in *A* is rinsed out into a Nessler cylinder, made up to 50 cc. and nesslerised. If the operation has been properly conducted not more than a barely perceptible coloration will be

observed. The contents of the trap *C* may then be poured in, and should cause no coloration either.

Summary of Procedure. The following summary of the procedure just described in detail should be intelligible on reference to the drawing.

(1) Exhaust the apparatus.

(2) Close *b* and admit sodium hydroxide solution through *a*.

(3) Both *a* and *b* remaining closed, allow the distillation to proceed for five minutes.

(4) Cautiously re-open *b* and let the distillation proceed a further five minutes.

(5) Admit the least possible stream of air through *a*, and continue for five minutes.

(6) Close *b*, admit air generally, re-weigh the container and nesslerise its contents.

(7) Nesslerise the liquid remaining in *A* and the acid in the trap *C* to check the completeness of the distillation.

Conclusion. Although, like most analytical schemes, rather lengthy in description the process is very quick and simple to carry out, for the distillation takes only 15 minutes, and during most of this time looks after itself.

The transference of the ammonia is almost strictly quantitative, for if any perceptible coloration is shown when the contents of *A* and *C* are nesslerised it does not exceed that due to one or two thousandths of a milligram of ammonia. It is true that even so small an amount makes a regrettable *percentage* error when only a few hundredths of a milligram of substance are there to be estimated, but percentage errors which could not be tolerated in ordinary analytical practice are inseparable from microchemical methods. Consider the experimental errors of the final colorimetric comparison, for instance.

It need hardly be pointed out that a blank experiment must be done to test the freedom from ammonia of any reagents that may have been used in arriving at the solution taken for distillation. Extensive use of the method has been made in this laboratory in connection with estimations of total nitrogen and urea in cerebrospinal fluid and in blood. In the case of the materials we have we find that blank experiments show the presence of less than half a hundredth of a milligram of nitrogen in the quantities of reagents used in any particular analysis, and as this is to a great extent off-set by the traces of ammonia remaining undistilled or passing through to the trap we do not take either error into calculation. Where reagents of a lesser degree of purity are met with the correction must of course be made.

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