

LXI. THE ESTIMATION OF NON-PROTEIN NITROGEN IN BLOOD BY A MICRO-KJELDAHL METHOD.

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THE following description shows the application of a micro-Kjeldahl method to the estimation of non-protein nitrogen in blood; the same method could, of course, be used for other nitrogen estimations. The procedure given below is recommended on account of its simplicity; some methods which are described have reached a stage of complexity such that they are scarcely practicable in clinical work. Attention is directed to (1) the use of a metal bath in combustion, (2) the method of making the fluid alkaline after combustion. The difficulty in estimations on alcoholic extracts of blood is that the fatty compounds are very resistant to combustion; in estimations on fat-free materials, *e.g.* urea solutions or urine, a paraffin bath at 180–190° can be used in place of the metal bath.

Mix 5 cc. serum with 25 cc. methylated spirit in a flask, which is then corked and allowed to stand for a time. Filter and place two portions of 10 cc. in large test-tubes (8 × 1 inch; *A* in Fig. 1); add 4 drops caprylic alcohol and 2 drops nitrogen-free sulphuric acid. Place the test-tubes as nearly vertical as possible in a beaker of water; keep the water boiling until the alcohol is evaporated and the residue begins to blacken. Throughout the process of combustion it is very important to avoid as far as possible the spread of unoxidised material up the sides of the tube. Add 3 cc. of the following “sulphate mixture”:

Water	200 cc.
Nitrogen-free sulphuric acid	100 cc.
Potassium sulphate	21 g.
Copper sulphate	5 g.

(Some potassium sulphate crystallises out when the mixture cools.)

Hold the test-tube in a paper holder and boil until the water is evaporated; rapid shaking is necessary to prevent bumping. When the liquid thickens and chars and acid fumes appear, lay the tube in a metal bath (“compo” tubing heated over a Bunsen burner in an enamelled bowl). If all water has not been driven off, the tube is apt to crack when laid on the molten metal. The tube is left in the bath for 15 minutes or so, and then examined for unoxidised material on the sides. This may be removed (1) by very vigorous

boiling of the acid over a free flame; the condensing acid vapours then wash down the sides of the tube; (2) by tilting the tube so that the hot acid runs nearly up to the brim; (3) by washing down with a little water when cool; this last is not a very effectual method. Tubes must always be heated thoroughly over a free flame before being placed on the metal bath. When combustion is complete the tube is allowed to cool, and 8–10 cc. water are added.

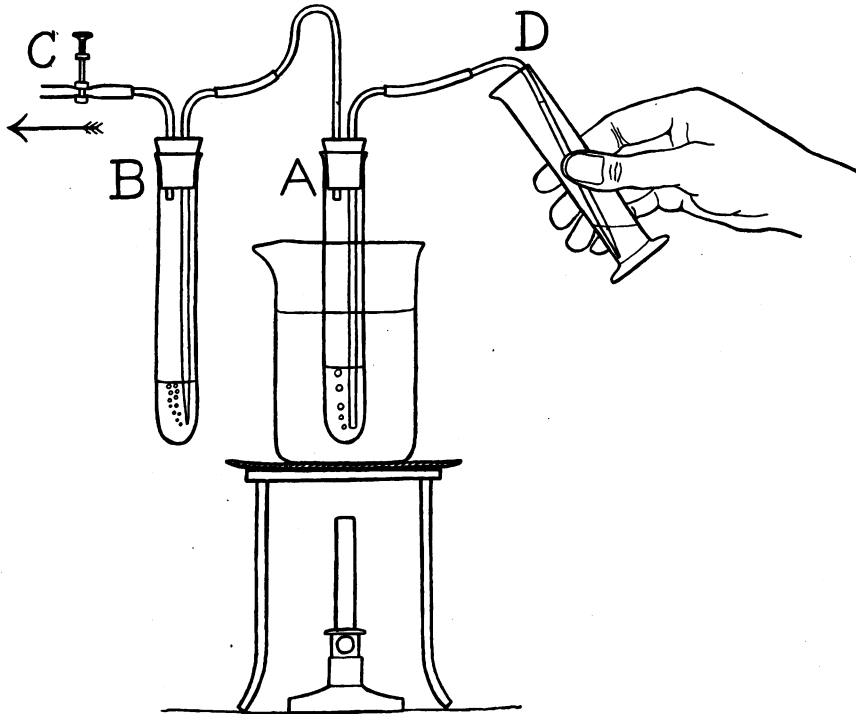


Fig. 1.

The distillation apparatus is shown in Fig. 1, and need not be described in detail; no rubber discs, perforated tubes, bulbs filled with wool, special burners, or condensers, are used. If sufficient water pressure is available, two sets of apparatus containing the duplicate samples are connected to one pump by means of a Y-piece. Two test-tubes (*A* and *B*) of size 8 × 1 inch are held in position by clamps which are not shown in the figure. The tube entering *B* is drawn to a not very fine point; the tube leaving *B* is connected to a water pump fitted with a safety bottle and, preferably, with a mercury manometer. *B* contains 15 cc. 0.01 *N* H₂SO₄ and seven drops 0.2 % methyl red in alcohol.

When the tubes are in position, screw up clamp *C*, turn the pump full on, and loosen the clamp until a gentle stream of air is drawn through apparatus. A glass tube *D* is connected by rubber tubing to the inlet tube of *A*, and dipped into 10 cc. 40 % caustic soda in a cylinder; the soda is thus drawn

