LI. A NOTE ON THE ESTIMATION OF THE CARBON-CONTENT OF SOLUTIONS.

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THE method to be described in this note was evolved to meet a special case, namely, the estimation of very small quantities of a carbon-containing compound in pure solution. In the first of a series of papers on inositol [Needham, 1923], the causes which led to the working out of the method are discussed, and it will be sufficient to say here that it was desired to know the carbon-content of amounts of solution containing as little as 20 mg. or less of *i*-inositol. But the method has also proved applicable to any solution of unknown carbon-content, and has been used successfully by other workers in the laboratory in dealing with bacterial culture media, etc. [Stephenson and Whetham 1923].

The method is not a wet combustion in the usual sense, for that would imply oxidation with chromic or sulphuric acid and weighing the gas evolved, as in the method of Tangl and Kereszty [1911]. Rather it is a combustion from solution, for an accurately measured out quantity of the liquid is evaporated from the receptacle in which the combustion is to take place. A wet combustion in the strict sense would not have been so useful in the case of inositol, because of its great stability towards mineral acids, but in some cases would be no doubt preferable. The sulphuric or chromic acid methods appear to possess no advantage in the matter of time and although the use of a silica tube is avoided, yet a special and somewhat complicated piece of apparatus is necessary for the carrying out of the decomposition.

The central point of the apparatus is a quartz combustion tube filled in the usual manner with rolls of copper gauze and a quantity of copper oxide in wire-form. The boat is of silica and may vary in size; into it is run from an accurate micro-burette a known amount of the solution to be estimated. Throughout the process of combustion, which is over in 20 minutes, a stream of oxygen is passed through the apparatus. A wash-bottle of concentrated sulphuric acid, towers containing soda-lime and sticks of dry potassium hydroxide, and a baryta wash-bottle to indicate the presence of any trace of carbon dioxide come between the oxygen-source and the furnace. On the other side of the furnace the gases first pass through a bulb to collect the condensed water, furnished at the bottom with a tap so that after every estimation the water produced can be run off, and no risk incurred of its

absorbing carbon dioxide in subsequent experiments. True, it could absorb a small amount during the combustion which produced it, but this danger is the less, as, having just been condensed from the state of vapour, it is hot. Next, the gases pass through a bulb containing sulphuric acid, and a U-tube filled with calcium chloride. Between these and a long cooling coil there is placed a screen about five feet square, made of wood, and covered with thick asbestos on both sides. After the cooling coil comes a 2-way tap which can direct the gas either through an index-bubbler or through the absorption apparatus. This, shown in greater detail in the appended figure, is an ordinary

Diagram of the absorption-bulb in the combustion method.

Total internal capacity of bulb =	32.5 cc.
Space occupied by tubes =	4.0 cc.
" " " baryta =	5.0 cc.
" " " acid =	1.0 cc.
	10.0 cc.
	10.0 cc.
	22.5 cc.

Value employed in solubility correction

10.0 cc.
22.5 cc.

large thin glass test-tube, drawn out and rounded, and is attached to a Haldane gas measuring apparatus, made exactly as described by Haldane [1920]. The tube connecting with the furnace has another tap through which the gases are led on down the tube *R* to the bottom of the absorption bulb, through the rubber cork *V*. *U* is a collar of rubber tubing to permit of a firm hold for the crucible-tongs when the bulb is shaken in the process of evolving the gas. Exactly 5 cc. of saturated baryta are placed in the bottom of the flask as shown in the diagram so that the tube *R* dips down into it. The tube *Q*, containing 1 cc. of a saturated solution of tartaric acid, which connects with the measuring burette, is pierced by an aperture just underneath the

cork *V*, large enough to permit an easy shaking out of the tartaric acid, and sufficiently small to prevent any escaping before the proper time. The carbon dioxide, then, produced by the combustion of the carbon-containing compound, is dried, cooled, and caught by baryta in such a manner that it can subsequently be driven off and measured. Since it is one of the heavier gases, it tends to collect at the bottom of the cooling coil, but it is readily washed out from there by a rapid stream of oxygen passed through at the conclusion of the combustion.

First, the blank of the whole apparatus was determined. In all cases whether blank or not, the procedure was exactly the same. The results here given are corrected for solubility of CO_2 in the saturated barium tartrate solution, and reduced to N.T.P. Haldane describes the corrections in full.

Blank experiments (empty boat, or boat with distilled water).

Exp. No. IV	12	0.082 cc. of gas evolved		
	13	0.070	"	"
	14	0.075	"	"
	16	0.090	"	"
	18	0.102	"	"
	19	0.091	"	"
	20	0.084	"	"
	21	0.095	"	"
	22	0.088	"	"

Average = 0.081

This blank value was redetermined from time to time, and grew more constant as the apparatus was used. Its high figure cannot be accounted for by traces of CO_2 or by the "reagent blank" (no oxygen stream or heating) for the latter is very constant at 0.015. If oxygen is passed through without heating the combustion tube, but keeping all the other conditions constant, the blank is still at 0.015, so the most probable supposition is that small quantities of carbon monoxide from the oxygen cylinder are burnt in the tube and appear as carbon dioxide. An iodine pentoxide tube would have avoided this, but the constancy of the blank was thought to render it unnecessary.

The next thing that was done was to place in the tube accurately measured out amounts of a solution of known strength of some pure carbon compound and carry out combustions on them. Inositol was chosen.

0.1 cc. of a 0.5 % solution of inositol (equivalent to 0.5 mg. inositol); gas evolved in cc. 0.333, 0.391, 0.408, 0.370, 0.382; average = 0.377; calculated value = 0.374.

0.2 cc. of a 0.5 % solution of inositol (equivalent to 1 mg. inositol); gas evolved in cc. 0.761, 0.718, 0.716; average = 0.730; calculated value = 0.758.

The micro-combustion method was then checked against the evaporation of the final solution, first in a porcelain basin, and then in a platinum dish; followed by weighing the residue before and after incineration. The figures of a typical example are as follows:

From 5 cc. of solution, there were evolved $\left\{ \begin{array}{l} 0.310 \text{ cc. of gas.} \\ 0.326 \quad \quad \quad \text{,,} \end{array} \right.$

The solution therefore contained 9.19 mg. of inositol %.

Weight of platinum dish	24.5300 g.
" " after evaporation to dryness	24.6020 g.
" " after incineration	24.5928 g.
Weight of organic content	9.2 mg.

This method has been used to obtain preliminary data concerning the inositol content of the tissues of rabbits: the figures are given in the other paper.

As against the process of evaporating to dryness and drying to constant weight, it possesses great advantage in the matter of time. From first to last the method of combustion from solution requires only a little over half an hour, whereas the alternative procedure demands a very much longer wait.

It is to be hoped that the method above described will prove useful in the future to workers desiring to estimate the carbon-content of quantities of liquid containing less than 20 mg. of the carbon-containing substance per 100 cc.

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