A New Apparatus for the Determination of Carbon in Biological Materials by Wet Combustion

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In the course of studies upon the metabolism of carbon sources used in media for the growth of mould fungi (Hockenhull, 1946), a method of analysis for carbon by wet combustion due to McCready & Hassid (1942) was modified so that the evolved carbon dioxide could be collected in baryta in a special spiral bubbler after Pearce & Robertson (1936). This apparatus, while giving satisfactory results for quantities of carbon up to 10 mg., had the following disadvantages: (i) neoprene tubing (being more resistant to acid attack than rubber) had to be used for connecting the condenser and the three bubblers used; (ii) the normal type bubblers used for scrubbing (cf. *E* and *F* on Fig. 1) were awkward to refill; (iii) the apparatus took up too much horizontal space; (iv) although the spiral bubbler was potentially highly efficient, individual ones varied greatly in their performance. Fig. 1 illustrates a modified apparatus, made entirely in Pyrex glass.

Method of operation

Preparation of the apparatus. The joints below *F* and tap *T*₁ were lubricated with syrupy phosphoric acid and the joints above *F* with water. Taps *T*₂ and *T*₃ were treated with vaseline or tap grease. All the bubblers contained beads (6–7 mm.). The bubbler *E* contained conc. H_2SO_4 ; *F*, water; *G*, 25 ml. of 0.1 N-Ba(OH)₂ and *H*, 5 ml. of 0.1 N-Ba(OH)₂ with water and beads to fill sufficiently. A little *sec.*-octanol may be added to *G* and *H* to prevent persistent foaming.

Method of analysis. The samples should contain about 10 mg. of C. It should be dry, or in not more than 0.2 ml. of aqueous solution, in which case about 200 mg. of KIO_3 must be added. It is placed in *A* (capacity 25 ml.) and the apparatus is connected up. A brisk stream of CO_2 -free air (more than 100 ml./min.) is sucked through. Van Slyke-Folch combustion mixture (4 ml.) (McCready & Hassid, 1942) is added from *C*. *A* is heated so that the contents boil in 2 min., the flame being removed if evolution of I_2 begins. The reaction mixture is maintained at the boil, while 2.5–3.0 l. of CO_2 -free air pass through the apparatus and carry the CO_2 formed into the alkali.

When the process has been completed, suction is stopped and the apparatus equilibrated with the atmosphere, taking care not to allow siphoning back of the contents of the bubblers.

Fig. 1. Apparatus for determination of carbon by wet combustion: *A*, combustion flask 25 ml.; *B*, jointed tube carrying tap funnel; *C*, *D*, spiral water condenser; *E*, bubbler with conc. H_2SO_4 ; *F*, bubbler with water; *G* and *H*, bubblers with standard alkali for CO_2 adsorption. *T*₁, *T*₂ and *T*₃, taps. All in Pyrex glass; standard ground joints, all B 14.

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H and *G* are removed and their contents are run off at T_1 and T_2 into a covered flask. *H* and *G* are washed out twice with CO_2 -free water and the washings added to the rest of the alkali. The residual $\text{Ba}(\text{OH})_2$ is titrated with 0.1 N-HCl, using thymolphthalein as indicator. Each determination takes about 30 min.

More dilute alkali and HCl are used for smaller quantities of sample.

Accuracy of method

Five 0.5 ml. portions of sucrose solution, each containing 10.8 mg. of carbon, were dried at 90° before analysis. The results were 11.0, 10.9, 10.8, 11.3 and 10.6 mg. (mean 10.92 mg., s.d. 0.23 mg.). Five determinations of carbon content were carried out upon potassium hydrogen phthalate (A.R.) using approx. 10 mg. of dry solid, giving a mean of 47.0 % (s.d. 0.8 %, calculated 47.1 %). Five determinations

were carried out without prior drying down upon 0.2 ml. of a solution containing approx. 10 mg./ml. of potassium hydrogen phthalate; the results gave a mean of 47.6 % (s.d. 1.3 %; calculated by direct titration 47.2 %). Determinations carried out on mould metabolism solutions, after drying down, and upon dried mycelial felts have given the same order of reproducibility at approx. 5 mg. carbon/sample (s.d. 2.1 and 1.9 % respectively).

SUMMARY

A new apparatus for the determination of carbon in biological materials by wet combination is described.

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Polarographic Determination of Lead in Urine

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Most of the methods that have been proposed in recent years for the determination of small amounts of lead are based on colorimetry and the use of dithizone (Tompsett & Anderson, 1935; Kerch, 1940). For microdeterminations of metals the methods of polarographic analysis are attractive since they are simultaneously qualitative and quantitative, and the diffusion currents from which values are obtained can be measured accurately and reproducibly. Since extraneous lead is the only substance which can contribute to the reagent blank, the latter is generally smaller than would be found in a colorimetric method, which may also be less specific.

Procedures for the polarographic determination of lead in urine have been described by Bombach & Cholak (1941), Reed & Gant (1942) and Weber (1947). Bombach & Cholak (1941) introduced a stage of electrolytic separation, and each of the three methods involved dry ashing at high temperatures; with the methods described below it has been possible to reduce the time taken for each determination, to avoid dry ashing, and to use less specialized equipment than is demanded by the above authors, while considerably improved accuracy and sensitivity are attained in a special modification.

Lead may be determined polarographically in a variety of conditions (Kolthoff & Lingane, 1941);

when dissolved in acid citrate or tartrate solutions (Suchy, 1931; Shakhov, 1939) the conditions are especially suitable since the half-wave potential is smaller in acid than in neutral or alkaline solution. Since the lead 'wave' is found at a lower applied potential in acid media, there is a greater chance of any other electro-reducible substances giving their waves after lead, in which case their presence will not interfere. The author has found that the diffusion current of lead may be readily and accurately determined in 0.2 M-dipotassium hydrogen citrate (pH 5.2), and that in this medium the waves are not in any way distorted by the formation of maxima; the half-wave potential was found to be -0.49 V. (measured against the Standard Calomel Electrode).

In the present work, the lowest concentration of lead in the polarograph cell which gives a readily measurable diffusion current has been found to be of the order of $500 \mu\text{g./l.}$ Most values reported in the literature for the urinary excretion of lead by normal subjects are below $100 \mu\text{g./l.}$ (Kerch, 1940; Tompsett & Anderson, 1935); a process for concentration of the metal is therefore necessary. Also, since it is possible that urine may contain polarographically active substances which might interfere with the determination of lead, a partial isolation of the lead has been incorporated in the final method. This