[CONTRIBUTION FROM THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE[1]]

An Improved Carbon-14 Wet Combustion Technique

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A modified wet oxidation procedure for organic material and its application to the analysis of C^{14}-labeled substances is reported.

This paper describes a modification of the apparatus and procedure of Lindenbaum, Schubert and Armstrong[2] for the wet combustion of carbon-containing materials used in tracer studies.

The use of barium hydroxide as absorbent for the carbon dioxide produced in the combustion of a substance containing a labeled carbon atom involves advantages as well as disadvantages. The former include the fact that the blanks usually are very low.

On the other hand some shortcomings are allied with barium hydroxide. It is not satisfactory when it is necessary to dilute a highly radioactive material with carrier. Also the barium carbonate obtained is not homogeneous when there is discrimination between carbon isotopes as found recently by Armstrong and collaborators[3] in the wet combustion of carbon-14 labeled urea and derivatives.

Furthermore, the precipitate of barium carbonate formed inside the gas inlet tube of the absorption device described by Lindenbaum, *et al.*, is not easily removed quantitatively. While the error introduced thereby is relatively minor, it may not be negligible when small samples are analyzed.

Therefore, an improved apparatus and procedure were developed which materially aided in the expeditious analyses for carbon and isotopic carbon content of numerous samples of biological material and pure labeled compounds.

Experimental

Apparatus.—When the exact procedure of Lindenbaum, *et al.*, was followed with barium hydroxide as absorbent, the blanks amounted to 2–3 mg. which was probably due to the diffusion of a mist of sulfur trioxide. To obviate this, a glass wool plug was inserted in the side-arm between the combustion tube and absorber. This reduced the blanks from an average of 5 to 3 mg. of barium carbonate, when using sodium hydroxide as the absorbent. A similar arrangement was used by Harper and co-workers.[4]

The all-glass absorption tube, shown in Fig. 1, was built from a 34/45 standard taper joint (Corning No. 6590). The male joint with a drip tip was closed to give a tube H of 70 ml. capacity. A fritted gas dispersing tube J (Corning No. 39533-C) in the center and a stopcock G on the side of the female joint completed the absorber.

The tube was filled with 35 ml. of a 1% sodium hydroxide solution (prepared by diluting 32 ml. of a 50% (wt./vol.) sodium hydroxide solution to two liters) by inserting the tube at F into K, opening the 3-way stopcock on the filling device to connect with the alkali supply and applying a slight vacuum at G. The vacuum was released by turning the 3-way stopcock connecting the tube to carbon dioxide-free air *via* L and the Caroxite containing tube M. Before use the filling device was washed through L and K to eliminate any carbonate on K.

After a combustion the gas dispersing tube assembly (female joint) was removed and washed out by adapting the washing device N to joint F, filling N with water, and alternately applying pressure and suction with the rubber bulb while manipulating stopcock P.

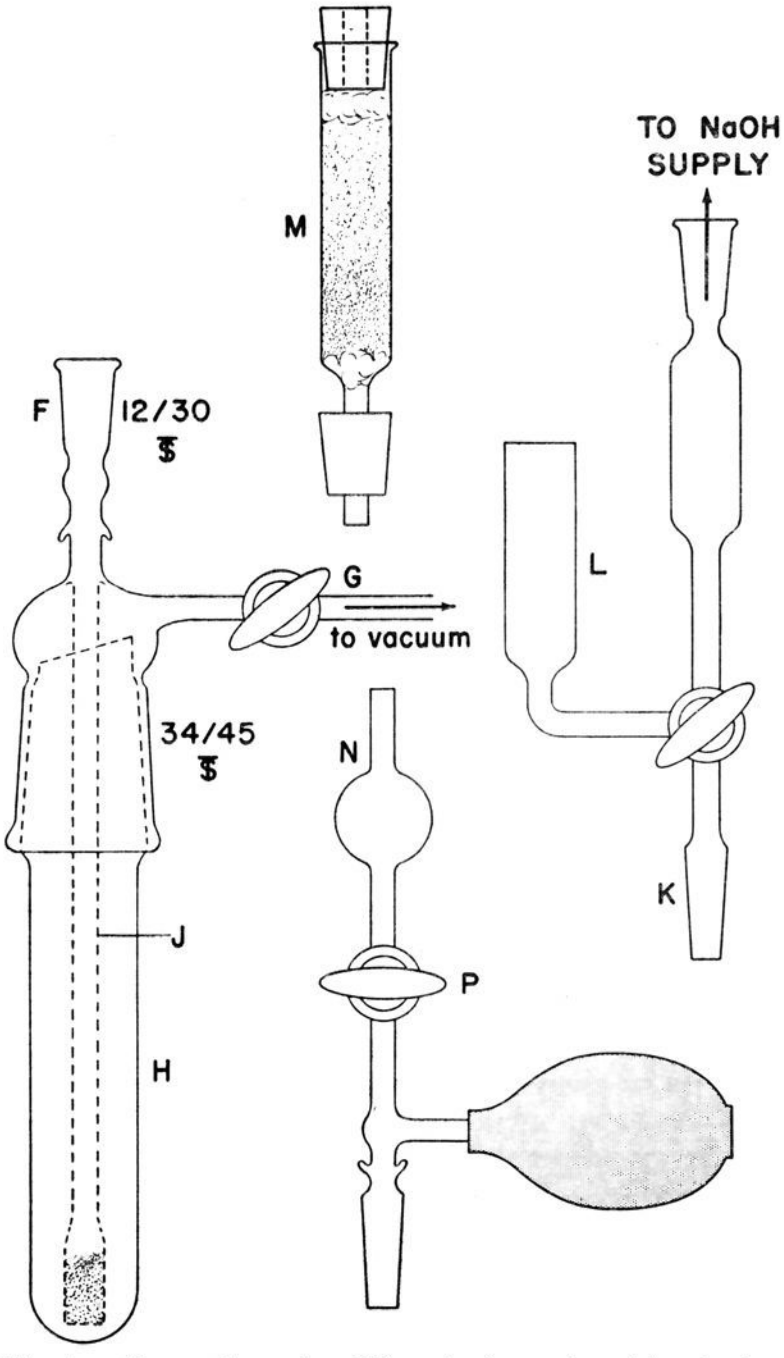

Fig. 1.—Absorption tube, filling device and washing device

Procedure.—The combustion fluid was prepared as previously described[2] except that after the period of heating the mixture was allowed to cool in a vacuum of 15–20 cm. This effected a better removal of dissolved gases and gave constant blanks.

Combustions were carried out simultaneously in two sets of apparatus, a complete duplicate run (including the weighing of the samples) requiring 35 to 45 minutes. In our hands this modified procedure permitted the combustion of 50% more samples per day than the method[2] described previously. A trained operator could readily handle three combustions at the same time if the equipment were available.

The sample was weighed into the combustion tube, about 300 mg. of potassium iodate added, and the apparatus assembled. Organic matter in aqueous solutions such as urine or bile was burned after the careful addition in an ice-

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(1) National Institutes of Health, Public Health Service, Federal Security Agency.

(2) A. Lindenbaum, J. Schubert and W. D. Armstrong, *Anal. Chem.*, **20**, 1120 (1948).

(3) W. D. Armstrong, L. Singer, S. H. Zbarsky and B. Dunshee, *Science*, **112**, 531 (1950).

(4) P. V. Harper, Jr., W. D. Neal, Jr., and G. R. Rogers, *J. Lab. Clin. Med.*, **36**, 321 (1950).

bath of one volume of oleum, as described elsewhere.[5] After evacuation to 2 cm. by a water-pump (one pump for each assembly) connected to side-arm G, 10–15 ml. of combustion fluid (the amount depending on the weight of the sample) was added. The stopcock G was closed when the evolution of gas was fairly rapid. The tube was heated with a small flame at a rate which would raise the temperature to the boiling point after approximately seven minutes (a stop watch was used routinely to time the combustions). Gentle boiling was then maintained for three minutes. The fumes, appearing in the later stages of the combustion, cleared after about one minute, and the apparatus was again slowly evacuated. Carbon dioxide-free air was then admitted by cautiously opening stopcock C (*cf.* Fig. 1, reference 2). After pressure equilibration air was drawn through the apparatus for five minutes by applying slight suction at G, C being open. At the end of the five minute period, the absorption tube was disconnected and the carbonate solution rinsed into an erlenmeyer flask for direct precipitation, or into a volumetric flask if aliquots were to be taken. Two milliliters of a 20% ammonium chloride solution was added to neutralize partially the hydroxyl ions. Where required, dilution with unlabeled sodium carbonate was carried out at this stage. The solutions were warmed to 60–70° and barium carbonate precipitated by the addition of a slight excess (1.5 ml. per 15 mg. of carbon) of a barium chloride solution (350 g. $BaCl_2 \cdot 2H_2O$/l.) from a buret. The precipitate was allowed to settle for a few hours (or overnight), then was filtered in a suitable size sintered glass crucible of medium porosity, washed with hot water and dried at 140–150°.

An alternative precipitation procedure avoiding the transfer of radioactive solutions was carried out by adding the ammonium chloride solution to the absorber tube at the end of a combustion. Barium carbonate was then precipitated in the same tube by addition of a solution of barium chloride and centrifuged.

Aliquots of the barium carbonate were plated on aluminum disks as described by Calvin, *et al.*[6] The method is also amenable to variations; for example, the carbonate resulting from the combustion can be precipitated directly on plates in a centrifuge type of arrangement, thereby avoiding entirely the separate precipitation of the solid, as reported by Hutchens, *et al.*[7] Incidentally, the combustion procedure of these authors is not applicable to some organic compounds which are incompletely oxidized by a combustion fluid not containing iodic acid.[8–10]

Results.—Blanks amounted to 3–4 mg. (0.015–0.02 mM.) of barium carbonate. Where a blank of such a magnitude would be objectionable, carbonate-free sodium hydroxide solution, prepared by diluting sodium ethylate with carbonate-free water, should be used as absorbing solution.

The combustion of 10 samples of benzoic acid (theoretical carbon content 68.85%) gave the carbon percentages of 68.1, 66.9, 69.4, 69.0, 68.9, 69.1, 69.3 and 69.0% which averages 68.8%. Similarly the combustion of 4 samples of 2-acetylaminofluorene (theoretical carbon content 80.69%) yielded C = 81.0, 81.0, 81.3, and 80.4% with an average of 80.9%.

The analysis of 4 samples of ω-methyl labeled 2-acetylaminofluorene for radioactivity showed an activity of 69.4, 69.9, 70.6 and 68.7 counts/min./γ *i.e.*, an average of 69.7 counts/min./γ. Similarly an average of 4,150 ± 70 counts/min./γ was found when the procedure was followed with 9-labeled 2-acetylaminofluorene. In these analyses of high activity samples the material was diluted 4- to 16-fold before combustion with inactive 2-acetylaminofluorene. Aliquots of the sodium carbonate solution produced by the combustion were again diluted with unlabeled carbonate to bring the activity of the barium carbonate plates within the useful range of the counting system.

The results obtained in the combustion of biological materials using this apparatus and procedure are described elsewhere.[5,11] Recoveries of radioactivity ranged from 95 to 107% of the dose after oral administration of the labeled compound to rats.

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[5] J. H. Weisburger, E. K. Weisburger and H. P. Morris, *J. Natl. Cancer Inst.*, **11**, 797 (1950).

[6] M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. F. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 118.

[7] T. T. Hutchens, C. K. Claycomb, W. J. Cathey and J. T. Van Bruggen, *Nucleonics*, **7**, 41 (1950).

[8] Reference 6, p. 94.

[9] R. M. McCready and W. Z. Hassid, *Ind. Eng. Chem., Anal. Ed.*, **14**, 525 (1942).

[10] D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).

[11] H. P. Morris, J. H. Weisburger and E. K. Weisburger, *Cancer Research*, **10**, 620 (1950); and papers to be published.