Western New York Branch

University of Rochester Medical School, April 17, 1926.

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A sensitive method for measuring carbon dioxide.

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The most sensitive method hitherto used for measuring the carbon dioxide output of tissues *in vitro* is the method of Tashiro,[1] depending upon the detection of a crystal of barium carbonate in a solution of barium hydroxide under the microscope. It was found that 1×10^{-7} of a gram of carbon dioxide gave a visible precipitate in 10 minutes. An unknown amount of carbon dioxide was measured by finding such a dilution that a visible precipitate was just formed in 10 minutes. This method has not been found useful in practice in the hands of others.

The next most sensitive method is the indicator method of Osterhout,[2] which in the hands of Parker[3] has been found adequate for the detection of 1×10^{-6} grams. It takes, however, 20×10^{-6} grams to produce the standard color change in the indicator. If this change takes place in 20 minutes, Parker finds himself able to detect a perceptible color change after 1 minute, *i. e.*, from the effect of 1×10^{-6} grams.

At the Cleveland meeting of the Federation, December, 1925, the writer demonstrated a carbon dioxide method based upon measurements of the conductivity of barium hydroxide, with which it is possible to detect 1×10^{-7} of a gram of carbon diox-

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1 Tashiro, S., *Am. J. Physiol.*, 1913, xxxii, 107.

2 Osterhout, W. J. V., *J. Gen. Physiol.*, 1918, i, 17.

3 Parker, G. H., *J. Gen. Physiol.*, 1925, vii, 641.

ide. The method was designed for the estimation of the carbon dioxide output from a stimulated nerve. Owing to an unavoidable delay in completing the experiments on nerves it has seemed advisable, in response to requests from other workers, to publish a brief description of the method, which is not new in principle. It was pointed out to me at Cleveland that it had been used by Spoehr[4] for relatively large amounts of CO_2.

Conductivity measurements are made in 7 cc. of a solution of barium hydroxide 0.00475 M. This solution is contained in a tube 10 cm. long and 12 mm. outside diameter, supplied with electrodes 5.7 cm. apart. Air is introduced into this tube from the respiration chamber and passes through the solution in a row of bubbles. The bubbles emerge from the surface of the solution into a bubbling chamber and return through two valves to the respiration chamber again. Circulation of the air is accomplished by a rising and falling mercury surface placed between the two valves. The valves are made of tapering glass tubes about 18 mm. long and 4 mm. in diameter, filled with mercury, sealed off and carefully ground into their seats until air tight. The mercury surface is made to rise and fall by a levelling bulb actuated by an automobile windshield cleaner. The mercury surface is covered with oil to avoid mercury vapor in the apparatus. It is important to keep the rate of circulation of air constant throughout an experiment. This rate is recorded by the addition of a side arm on the respiratory chamber over which is fitted a small rubber balloon. The balloon is enclosed in a glass bulb, one outlet from which goes to a volume recorder, which writes on a drum. The excursion of the volume recorder follows faithfully the movements of the mercury surface. Carbon dioxide is carefully excluded from the air space between the balloon and the volume recorder. The respiration chamber may be of any convenient shape, preferably as small as possible. In my apparatus it is about the size of an ordinary 6 inch test tube, closed at the top by a ground glass stopper and communicating with the absorption chamber by a narrow tube at its base. The whole circuit is, therefore, of glass with the exception of the balloon, which may be omitted except when needed as a pressure equalizer in special experiments. Stop cocks are sealed into the circuit on either side of the valves to assist in regulating the air

[4] Spoehr, H. A., *J. Ind. and Eng. Chem.*, 1924, xvi, 128.

content of the apparatus, introducing particular gases according to needs and in drying the valves (by a current of air) in case they become moist. The temperature of the water bath is regulated to ±0.001° C. The conductivity is measured by a Leeds Northrup 470 cm. slide wire, a resistance box of Curtis coils, using an audio oscillator as a source of current. With this arrangement the accuracy depends upon the ability to maintain a constant base line rather than upon the accuracy with which the conductivity is measured. Aside from the rigid exclusion of outside air from the apparatus and general cleanliness, the regularity of the base line depends largely upon smooth working of the valves and the regularity of pumping. The conductivity change is never quite zero, even in the absence of tissue in the respiration chamber, and a small correction must be applied for absolute values. This may depend upon a reaction between the solution and the glass. A similar objection applies to the colorimetric method.

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On the phase reversal of the lipoid-aqueous systems in the bacterial cell wall.

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In previous communications, Mellon[1, 2] has shown that the principle of ion antagonism is applicable to bacteria. Under the conditions of the experiment with strain N.D.—67 the principle operated in a Na:Ca ratio as high as 100 or 150 to 1. This strain, which is quite stable in H_2O and NaCl solutions, is quickly precipitated by $CaCl_2$ solutions in strengths as dilute as 10^{-6} cm. The physico-chemical mechanism suggested was reversal of the aqueous-lipoid system in the wall of the cell whereby $CaCl_2$

[1] Mellon, Ralph R., *J. Med. Res.*, 1922, lxiii, 345.

[2] Mellon, Ralph R., Hastings, W. S., and Anastasia, C., *J. Immunol.*, 1924, ix, 365.