9241

An Improved Thunberg Technique for Bacterial Oxidations.

FRANK H. JOHNSON.* (Introduced by E. Newton Harvey.)
From the Laboratory of General Physiology, Princeton University.

Although more accurate methods of measuring redox potentials in connection with bacterial oxidations are now available,[1,2] the Thunberg technique,[3] which has been extended by Quastel[4,5] and others, will no doubt continue to find wide application. It is especially useful where a large number of determinations are to be made, because of its greater simplicity and less time-consuming nature than the electrometric methods. At the same time, however, there are important sources of error. The purpose of this paper is to describe an improved method which either totally eliminates or greatly reduces the errors associated with the Thunberg technique as it has generally been used.

In a recent critical study, Yudkin[6,7] points out 6 factors which may interact to alter the reduction time of methylene blue by bacteria, when the suspension is evacuated and placed in a water bath. These are: (1) the temperature lag effect, (2) presence of residual oxygen, (3) gradual poisoning of the enzyme by methylene blue, (4) affinity of the enzyme for methylene blue, (5) presence of hydrogen donators in the suspension, and (6) the products of the action of the bacteria on the substrate which can act as hydrogen donators. The evacuation has nearly always been carried out for less than 2 minutes, with a water aspirator. Since Harvey[8] has shown that the rate of reduction of methylene blue by dehydrogenases in milk is proportional to the reduction in oxygen tension, the question naturally arises concerning the efficacy of one or 2 minutes' evacuation. This point was tested by luminous bacteria, which constitute a very good indicator for the presence of oxygen,[9] for their light dims at a partial pressure of about 2 mm. of mercury[10] and remains visible at 10^{-5} atmospheres.[11]

---

* Eli Lilly and Company Research Fellow.

1 Korr, I. M., *J. Cell. Comp. Physiol.*, 1935, **6**, 181.

2 Yudkin, J., *Biochem. J.*, 1935, **29**, 1130.

3 Thunberg, T., *Skand. Arch. f. Physiol.*, 1920, **40**, 1.

4 Quastel, J. H., and Whetham, M. D., *Biochem. J.*, 1924, **18**, 519.

5 Quastel, J. H., *Erg. Enz. Fors.*, 1932, **1**, 209.

6 Yudkin, J., *Biochem. J.*, 1933, **27**, 1849.

7 Yudkin, J., *Biochem. J.*, 1934, **28**, 1454.

8 Harvey, E. Newton, *J. Gen. Physiol.*, 1919, **1**, 415.

9 Hill, S. E., *Science*, 1928, **67**, 374.

10 Shoup, C. S., *J. Gen. Physiol.*, 1929, **13**, 27.

11 Harvey, E. N., and Morrison, T. F., *J. Gen. Physiol.*, 1923, **6**, 13.

To 3 standard-type Thunberg tubes, each containing 1 cc. of buffer solution in the side arm, were added 9 cc., 6 cc., and 3 cc., respectively, of a very dilute suspension of luminous bacteria in phosphate buffer, pH = 7.30. They were connected with a Hyvac pump and exhausted continuously. A mercury manometer in the system indicated that a vacuum approximately equal to the vapor pressure of water was quickly reached. But the time for the complete removal of the oxygen, as judged by the complete extinction of luminescence, was 19 minutes for the first tube, 26 for the second, and 36 for the third. A 10 cc. portion of the same suspension in a test tube indicated a dimming time without evacuation (due only to the using up of oxygen by the respiration of the organisms) of approximately 36 minutes. Furthermore, when the buffer was

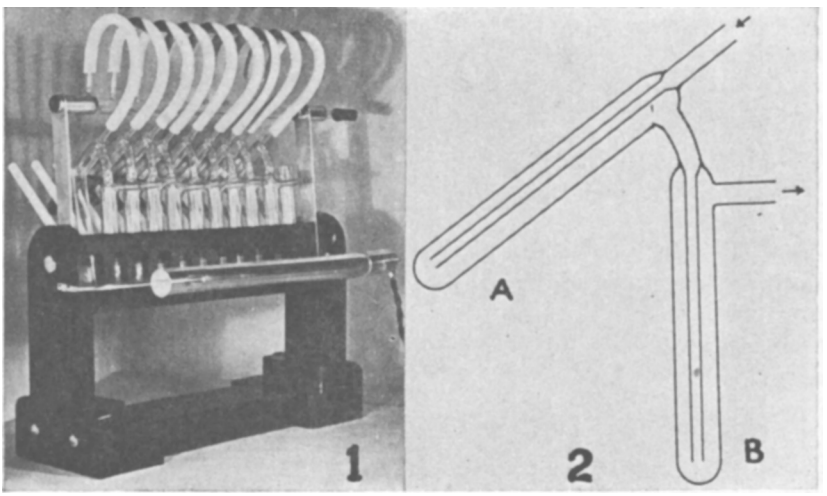

FIG. 1.

Apparatus for simultaneous deaeration of a series of the tubes illustrated in Fig. 2. The manifold is attached to the rack so that the whole unit can be rotated on its long axis near its base, thus mixing the substrate plus methylene blue solutions of all tubes with the bacteria at the same moment. When restored to the upright position, decolorization of the dye is observed, from the opposite side, against the long electric light globe (held within the split brass tube) as a source of illumination. For *perfect* deaeration lead tubing should be used entirely in conducting the gas to the vessels, but thick rubber tubing as illustrated has been found quite effective and easy to manipulate. The manifold can be slipped out of the brass holders to remove or replace vessels. The latter are loaded while tilted to the rear. The dowls prevent the rear arms of the vessels from swinging sideways during tilting.

FIG. 2.

Vessel for separate deaeration of substrate plus methylene blue solutions (in arm A) and the bacterial suspension (in arm B). The solutions and the suspension, respectively, are washed down by the final addition of a buffer solution. After use, the vessels are readily cleaned by cleaning solution followed by running water.

emptied from the side arms to the suspension in the evacuated tubes, a bright flash of light occurred, showing the presence of dissolved oxygen. This light faded rapidly, but went completely out only after several minutes. It was not restored by shaking the tubes, which were now practically rid of all oxygen.

These facts clearly indicate that even with prolonged evacuation, the final removal of the oxygen depends on the respiration of the organisms, and indeed, under ordinary conditions, the latter is probably by far the more important factor. In order to insure complete anaerobiosis, without depending on respiration to use up the oxygen, deaeration by a stream of pure hydrogen or pure nitrogen is the most effective means, and by using for this purpose a type of tube introduced by Harvey[12] and suggested by him for use in this connection, the bacterial suspension can be deaerated separately from methylene blue and substrate solutions. (Fig. 2.) Separate deaeration followed by anaerobic mixing eliminates possible errors in reduction rate arising from the following causes: (1) the aerobic metabolism of the substrate by the bacteria, before the complete removal of oxygen, (2) the influence of different substrates in stimulating respiration to different degrees, and hence to different rates of oxygen removal, (3) substrates that cause little or no increase in oxygen consumption, but which act as hydrogen donators anaerobically,[13] (4) substrates that actually inhibit oxygen consumption, but which may reduce methylene blue anaerobically (alpha methylglucoside; see below), (5) substances which inhibit aerobic metabolism of substrates, but which are without effect on anaerobic dehydrogenases,[14] and (6) substances which may inhibit anaerobic dehydrogenase activity without appreciably affecting aerobic respiration.[14] In addition, separate deaeration, by shortening the reduction time, minimizes the toxic action of the dye, and since it can be carried out at a constant temperature completely avoids the temperature lag effect.

Taking advantage of these principles, the apparatus pictured in Fig. 1 was constructed. With this arrangement a series of tubes can be completely deaerated with hydrogen purified over hot platinized asbestos, or nitrogen purified over hot copper, within a very few minutes. The substrate and dye solutions in the rear arms can then be added to the bacterial suspension in the front arms at exactly the same time. The data in Table I indicate how this method can avoid some fundamental errors.

12 Harvey, E. Newton, *Biol. Bull.*, 1926, **51**, 89.

13 Cook, R. P., *Biochem. J.*, 1930, **24**, 1538.

14 Keilin, D., *Proc. Roy. Soc. Lond.* (B), 1930, **106**, 418.

TABLE I.

Time for 90% reduction of methylene blue (1:50,000) by a phosphate buffer suspension of luminous bacteria (*Achromobacter Fischeri*), with and without added substrate.

Suspension	Substrate	Reduction time		
		Deaerated by evacuation	Deaerated by hydrogen	Deaerated by nitrogen
Unwashed	—	27′	7′	7′ 15″
Washed 4 times	—	>3 days	>6 hr.	>6 hr.
,, ,, ,,	M/10 glucose	27′	8′	8′
,, ,, ,,	M/10 alpha methyl glucoside	>3 days*	29′	29′

*Barely perceptible decolorization in 6 hrs, that amounted to approximately 30% reduction over a period of 3 days.

It is a matter of considerable importance that the usual technique of evacuation gave only a vague evidence for the dehydrogenation of alpha methylglucoside. This substance has been found to inhibit aerobic respiration of washed cells[15] and would thus lengthen the time for removal of oxygen. Yet the new method shows that it is readily dehydrogenated. In addition to use with bacterial suspensions, this method should work equally well with any tissue or tissue preparation which can be delivered with a pipette.

9242

Effect of Testosterone on Somatic Growth.

C. S. MCEUEN, H. SELYE AND J. B. COLLIP.

From the Department of Biochemistry, McGill University, Montreal, Canada.

It has been shown by Steinach and Holzknecht[1] that ovarian hormones inhibit growth. They found that implantation of ovaries into male guinea pigs or rats decreased their growth rate in every case if the transplant took. Later, these findings were confirmed by Bugbee and Simond,[2] and Spencer, *et al.*,[3, 4] using oestrin preparations. Spencer, *et al.*,[5] came to the conclusion that the growth in-

15 Johnson, Frank H., *J. Cell. Comp. Physiol.*, 1936, **8**, 439.

1 Steinach, E., and Holzknecht, G., *Archiv. f. Entwcklngsmechanik d. Organ.*, 1916, **42**, 490.

2 Bugbee, E. P., and Simond, A. E., *Endocrinol.*, 1926, **10**, 360.

3 Spencer, J., Gustavson, R. G., and D'Amour, F. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 500.

4 Spencer, J., D'Amour, F. E., and Gustavson, R. G., *Am. J. Anat.*, 1932, **50**, 129.

5 Spencer, J., D'Amour, F. E., and Gustavson, R. G., *Endocrinol.*, 1932, **16**, 647.