the work of Poos[6] and Ten Cate,[7] who showed that constriction takes place by contraction of the sphincter alone, in contrast to the claim of Behr[8] that the normal constriction of the pupil to light takes place by an active innervation of the sphincter and simultaneous inhibition of the dilator.

Summary. The differences in pupil diameter in the dark adapted rabbit's eye can be summarized as follows: 1. It is smallest when the sympathetic is cut. This state represents an equilibrium between the elasticity of the iris and the "residual tone" of the sphincter. 2. It is slightly larger when both the sympathetic and the third nerves are cut. In this condition, elastic tissue only holds the pupil open. 3. It is still larger in the normal eye when there is an equilibrium between the "residual tone" of the sphincter on the one hand and the elasticity of the iris tissue plus the active contraction of the dilator on the other. 4. It is at its greatest possible maximum when the third nerve is cut or atropine is instilled into the normal eye. Here the "residual tone" of the dilator and the possible slight contraction of this muscle is added to the action of the elastic tissues of the iris.

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Oxidative Assimilation of Lactic Acid by *Escherichia coli.*

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Barker,[1] Geisberger,[2] and Clifton[3] have shown that the oxidation of simple substrates by washed suspensions of actively respiring, non-proliferating cells does not proceed to completion, but instead, a portion of the substrate is apparently assimilated by the cells. For example, the oxidative assimilation of acetate by *Escherichia coli* may be represented[3] as

$$2\ CH_3COOH + 3\ O_2 = (CH_2O) + 3\ CO_2 + 3\ H_2O$$

According to this equation three-fourths of the oxygen required for the complete combustion of the acetate is consumed with the

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[6] Poos, F., *Klin. Monatsbl. f. Augen.*, 1927, **78**, 874.

[7] Ten Cate, J., *Arch. Neerl. de Physiol.*, 1921, **6**, 258.

[8] Behr, *Graefe-Saemisch Handbuch der ges. Augenheilkunde*, 1924, **3**.

[1] Barker, H. A., *J. Cell. Comp. Physiol.*, 1936, **8**, 231.

[2] Geisberger, G., *Beiträge zur Kenntnis der Gattung Spirillum Ehbg.*, Dissertation, Utrecht, 1936.

[3] Clifton, C. E., *Enzymologia*, 1937, **4**, 246.

production of equivalent amounts of carbon dioxide and water, and at the same time one mol of a substance having the empirical composition of a carbohydrate is formed for each 2 mols of acetate undergoing change. In the presence of appropriate concentrations of sodium azide (NaN_3) the assimilatory process appears to be blocked, the oxidation proceeding to completion. These studies have been extended to include the oxidation of lactic acid in well washed suspensions and in actively proliferating cultures of *Esch. coli,* the rates and extent of oxygen-consumption and of carbon-dioxide production being determined at 30°C by Warburg's technic.

The oxidation of lactate (2 ml, 0.008 M, Fig. 1, A) by *Esch. coli* in a M/15 phosphate buffer of pH 7.1 proceeds at a rapid and quite constant rate until approximately two-thirds of the oxygen required for complete combustion has been consumed. Then the rate of oxidation very rapidly decreases and approaches the level of the substrate-free control. A similar behavior was noted by Cook and Stephenson.[4] In the presence of M/600 or M/400 sodium azide the oxidation proceeds nearer to completion while the theoretical amount (538 μl) of oxygen required for complete combustion of the lactate is consumed in the presence of M/200 azide before the rate of oxidation markedly decreases. These concentrations of azide have little influence on the oxygen-consumption by the substrate-free control, the highest concentration tending to decrease slightly the rate of oxygen-consumption of the blank. Sodium azide, in the concentrations employed, did not appear to exert an appreciably germicidal action under the conditions of these tests. The above results, together with an observed R.Q. of 1.0 suggest that the oxidative assimilation of lactate may be represented as

$$CH_3CHOH \cdot COOH + 2\,O_2 = (CH_2O) + 2CO_2 + 2\,H_2O$$

The influence of 3 different initial concentrations of lactic acid on the rates and extent of oxygen-consumption during the growth of *Esch. coli* in 2 ml of a synthetic medium[5] is illustrated in Fig. 1, B. In the presence of M/300 lactic acid (only source of carbon) the rate of oxygen-consumption broke abruptly and decreased to a low order of magnitude by the time that 280 μl of oxygen had been consumed. This amounts to a consumption of approximately 62% of that required for the complete oxidation of the lactate. Similar breaks in the rates of consumption were observed when 440 and 880 μl of oxygen had been consumed in M/200 and M/100 lactate

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[4] Cook, R. P., and Stephenson, M., *Biochem. J.*, 1928, **22**, 1368.

[5] Clifton, C. E., Cahen, S. F., and Morrow, G., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 40.

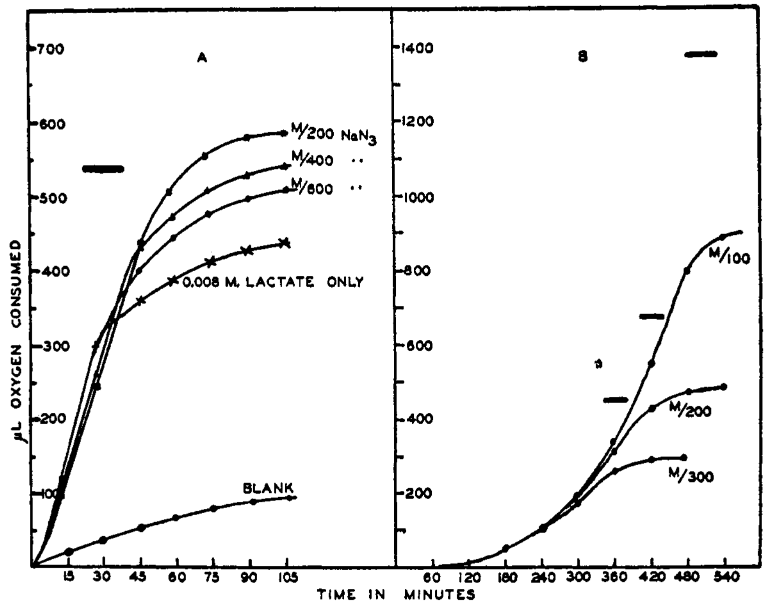

Fig. 1.

A. The influence of different concentrations of sodium azide on the oxidation of 2 ml of 0.008 M lactate by *Esch. coli.*

B. The influence of different initial concentrations of lactate on the rate and extent of oxygen-consumption in cultures of *Esch. coli.* The heavy horizontal lines above the curves represent the theoretical values for the oxygen required for complete combustion of the different amounts of lactate, and are placed immediately above the times at which the substrate is assumed to have been consumed.

medium, respectively, corresponding to an oxygen-consumption of approximately 65% of the theoretical. The rates of carbon dioxide production, and the total amounts of carbon dioxide produced were equal to the corresponding values for oxygen-consumption. The maximal numbers of bacteria developed in these cultures were 59, 81, and 160 × 10^7 per ml in the M/300, M/200, and M/100 lactate cultures, respectively.

Typical values of the amounts of oxygen consumed per ml per hour and per cell per minute together with the numbers of viable bacteria at the end of each interval of time, as observed in a 0.004 M lactate medium, are presented in Table I.

The total oxygen consumed in the experiment outlined in Table I is approximately equal to 66% of that required for the complete combustion of the lactic acid to carbon dioxide and water. In all of the cultures studied not only did the rates of oxygen-consumption and of carbon dioxide production markedly decrease when approximately two-thirds of the lactate had been oxidized, but there was also a marked decrease in the growth-rate at the same time.

TABLE I.

Influence of the Age of a Culture on the Rates of Oxygen-consumption in a 0.004 M Lactate, Inorganic-medium Culture of *Escherichia coli.*

		μl O_2 consumed by culture	
Time, min.	No. of bacteria per ml at end of time interval	per ml per hr	per cell per min.
0-210	12×10^7	6.0	—
210-300	23 "	28.0	1.84×10^{-9}
300-360	36 "	26.0	1.49 "
360-420	54 "	42.5	1.60 "
420-480	72 "	47.5	1.26 "
480-540	77 "	22.5	0.5 "
540-660	80 "	10.0	0.1 "
	Total oxygen consumed	182.5 μl	

This decrease became apparent when approximately 60% of the theoretical amounts of oxygen required for complete combustion had been consumed. The rates of oxygen-consumption observed after 66% of the theoretical amount had been consumed could be accounted for on the assumption that the cells were utilizing reserve cellular material.

The observations reported in this paper lend further support to the hypothesis advanced by Clifton, *et al.*,[5] and by Hershey and Bronfenbrenner[6] that, when oxygen is available in excess, the growth rate and maximal population developed under favorable conditions is primarily controlled by the available foodstuff rather than by any accumulation of inhibitory products. Furthermore, it appears that the equation suggested for the oxidative assimilation of lactic acid by washed suspensions of *Esch. coli* may equally well apply to the assimilation of lactic acid in actively proliferating cultures of this bacterium. Also, the addition of sodium azide to the culture medium proved to be inhibitory to growth, thus suggesting that assimilatory processes are again blocked by the action of the azide. These, and other results to be reported later, suggest that respiration and assimilation are closely connected processes and that the respiration of heterotrophic bacteria may well be represented by the general equation:

Foodstuff $+ O_2 =$ Assimilated material $+ CO_2 + H_2O$; much as $CO_2 + H_2O +$ light $= (CH_2O) + O_2$ represents the assimilatory process in the green plant. In the former oxidative type of assimilation, carbon dioxide and water might be regarded as the waste products just as oxygen is the waste product of the assimilatory process in the plant kingdom.

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[6] Hershey, A. D., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 556.