The Extent of Acetate and Ethanol Oxidation by *Euglena gracilis*

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SUMMARY: *Euglena gracilis* utilizes acetate and ethanol as energy and carbon sources. The O₂ consumption due to acetate was equivalent to a 42 % oxidation of the available acetate. CO₂ production in the presence of acetate was consistent with the assumption that acetate is simultaneously oxidized and converted to carbohydrate.

Addition of ethanol resulted in an O₂ consumption equivalent to a 39 % oxidation of this substrate. The CO₂ production due to ethanol was 0.93 μ mole per μ mole of ethanol, more than double the amount predicted if carbohydrate is the sole synthetic product of ethanol metabolism.

Conditions known to change the rate of respiration, such as composition of growth media, incubation pH, and the adaptation to acetate of ethanol-grown cells, had no effect upon the extent of ethanol and acetate oxidation.

Euglena gracilis is a member of a physiological group of protozoa termed the 'acetate flagellates', characterized by their excellent growth with acetate as the sole carbon source, and their poor utilization of glucose and related compounds (Hutner & Provasoli, 1951). Until recently, the mechanisms regulating the oxidative metabolism of the acetate flagellates had been little investigated.

Lewin (1954) described adaptive changes in the oxidation: assimilation ratio of acetate in *Chlamydomonas dysosmos*. Danforth & Wilson (1957) reported that *Euglena*, when grown upon acetate as a carbon source at pH 7.3 (Ac-grown), oxidized acetate with a higher and less pH-sensitive rate than did cells grown upon ethanol as a carbon source at pH 3.5 (Et-grown). In addition, some Et-grown cultures adapted to acetate, acquiring rates of acetate oxidation characteristic of Ac-grown cells.

A study of the extent of acetate and ethanol oxidation was undertaken for the purpose of obtaining basic information concerning the relationships of the energetic and synthetic activities of *Euglena*, and to ascertain whether conditions known to vary the rate of acetate respiration would also vary the extent of acetate oxidation.

METHODS

A streptomycin-bleached (non-photosynthetic) strain of *Euglena gracilis* var. *bacillaris*, the same as used in previous studies, was maintained in 10 ml. stock tubes on an acetate + tryptone medium at pH 7.3. Experimental cultures were grown under the two conditions listed in Table 1; Et-grown cells were grown on an ethanol + tryptone medium adjusted to pH 3.5; Ac-grown cells were grown on the above-mentioned acetate + tryptone medium at pH 7.3.

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Details of the growth procedures have been published elsewhere (Danforth & Wilson, 1957).

All cultures were incubated at 18–23° until visual inspection indicated good growth, usually within 7–14 days.

Table 1. *Composition of growth media*

	Ac-grown	Et-grown
Bacto-tryptone (Difco)	5 g./l.	5 g./l.
Na acetate	18.4 mM	0
Ethanol	0	18.4 mM
pH	7.3	3.5

The cells were then harvested and washed by centrifugation, and suspended in distilled water. A sample of this suspension was removed, fixed with ethanolic HgCl₂, and counted in a haemocytometer.

Experimental solutions of Na acetate.3H₂O and ethanol ranged from 0.8 to 120 mM; only the acetate solutions were adjusted for pH. The buffer for all determinations was an equimolar mixture of tris(hydroxy-methyl)amino-methane and phosphate yielding a final concentration of 0.011 M. Experiments were run at pH 5.5 or pH 7.3.

Determinations were conducted with a Warburg apparatus at 26.1°, having a shake rate of 120/min. and a gas phase of air. O₂ consumption was measured with 0.1 ml. of 14% NaOH or KOH in the centre well. CO₂ production was measured by the direct method (Umbreit, Burris & Stauffer, 1949) replacing the alkali in the centre well with water. The time course of CO₂ production was measured at pH 5.5, while at pH 7.3, acid was tipped into the flasks, before and at the completion of an experiment, to ascertain the total amount of CO₂ evolved.

To determine the extent of acetate and ethanol oxidation cells and buffer were added to the main compartment and 2–20 μmole substrate in 0.5 ml. were added to the side arm. The substrate was tipped into the main compartment after at least a 20 min. equilibration period, and the O₂ consumption was recorded until the respiration had returned to the endogenous level. The total amount of O₂ consumed due to the added substrate was calculated by subtracting the O₂ consumed by an endogenous control during the course of the experiment.

These values were then plotted against the amounts of substrates added, and the slope of a line of best fit, using the Least Squares method, gave a value for the average O₂ consumption per μmole of substrate. The percentage of the substrate oxidized was calculated by dividing the average O₂ consumption by the O₂ consumption expected if the substrate were 100% oxidized (3O₂:ethanol, 2O₂:acetate).

RESULTS

Euglena has a low, but measurable, rate of respiration when incubated in the absence of substrate. The respiration rate increases and remains constant for some time when an oxidizable substrate is added to the cells. After the respira-

tion rate returns to the endogenous level, all the substrate is assumed to be removed from the medium. Figure 1 illustrates this behaviour in the case of Ac-grown cells, incubated with varying amounts of acetate.

Fig. 1

Fig. 2

Fig. 1. O_2 consumption of Ac-grown cells on acetate. 0, 10, 20, 60 μ mole of acetate added at zero time. Cells incubated at pH 5.5.

Fig. 2. Relationship between total O_2 consumption of *Euglena* and amount of substrate added. $\circ$, assuming endogenous respiration suppressed by substrate; $\times$, assuming endogenous respiration continues during substrate oxidation. Data for acetate include 13 determinations from 4 separate experiments; for ethanol, 11 determinations from 3 experiments.

In order to calculate the efficiency of substrate metabolism by *Euglena*, it is necessary to determine whether the endogenous metabolism is inhibited during substrate oxidation, as has been reported for other organisms (Barker, 1936; Syrett, 1951). If the endogenous metabolism is suppressed by the presence of exogenous substrate, all the oxygen consumed during the presence of substrate must be attributed to substrate oxidation, while if the endogenous metabolism continues unchanged, the endogenous oxygen consumption must be subtracted from the total consumption in order to determine the oxygen consumption due to the substrate. Figure 2 presents data bearing on this problem. In this figure the same data are plotted in two ways: first on the assumption that the endogenous respiration is suppressed in the presence of substrate, and also on the assumption that the endogenous respiration continues unchanged. In the case of ethanol oxidation, both assumptions result in a constant ratio of oxygen consumption to substrate, while for acetate only the assumption that endogenous respiration continues yields such a constant ratio.

As Syrett (1951) has pointed out, under certain conditions both assumptions concerning the behaviour of the endogenous respiration in the presence of substrate may produce a constant ratio of oxygen consumption:substrate consumption, and in such cases, this criterion is of no value in distinguishing between the two alternatives. This is clearly the case for ethanol oxidation by *Euglena*. In the case of acetate, on the other hand, only the assumption

that endogenous respiration continues during substrate oxidation gives a constant oxygen:substrate ratio.

It is concluded, therefore, that endogenous metabolism continues unchanged during oxidation of exogenous acetate. In the case of ethanol, no direct evidence supports such a conclusion, but since it seems likely that the two substrates would have similar effects upon the endogenous metabolism, it is assumed that neither acetate nor ethanol suppresses the endogenous metabolism.

Fig. 8. The extent of acetate oxidation by *Euglena*. Values of a single class (e.g. Ac-grown cells at pH 7.3 with 10 μ mole of acetate) averaged together. Slope is 18.8 μ l. O_2 : μ mole acetate, equivalent to a 42% oxidation of acetate.

In Fig. 3, the O_2 consumption due to acetate, in a series of 44 trials, is plotted against the initial amounts of acetate added. Experiments were conducted with Et-grown and Ac-grown cells, incubated at pH 5.5 and 7.3. Cell concentrations ranged from 1.5×10^6 to 10×10^6 cells per flask. Acetate amounts ranged from 0.8 to 20 μ mole. The slope of the line of best fit is 18.8 μ l. O_2 per μ mole of acetate added, and is equivalent to the oxidation of 42% of the available acetate.

Adaptation to acetate had no effect upon the extent of acetate oxidation. Figure 4 illustrates the events during the adaptation to acetate of cells from an 8-day-old culture of Et-grown cells. The rate of acetate respiration increased 44%, but the extent of acetate oxidation was no different from that found in the absence of adaptation.

The time course of CO₂ production, determined at pH 5.5, was linear (except during adaptation), and the respiratory quotient of acetate oxidation was 1.01 ± 0.08 , independent of the experimental conditions. The results of 15 trials, at pH 5.5 and 7.3, of Ac-grown and Et-grown cells, are tabulated in Table 2. Adaptation to acetate, which occurred in 7 of the 15 trials, had no effect upon the respiratory quotient.

Table 2. *The respiratory quotient of Euglena on acetate*

Growth substrate	Acetate (mm)	Respiratory quotient	
		pH 5.5	pH 7.3
Ethanol	30	1.00	—
	30*	1.10	—
	10*	1.06†	—
	30*	0.75	1.06†
	30*	—	0.90†
	30*	—	0.96†
	30	0.98†	0.91†
Acetate	30	1.00	—
	30	1.17	1.35†
	30	1.06†	1.04†
Average		1.01	1.04
Average (all values)		$1.02 \pm 0.09†$	(9 %)

* Adaptation.

† Acid-tipped method.

‡ Average deviation.

The extent of ethanol oxidation was determined at pH 5.5 and 7.3, using Et-grown and Ac-grown cells. Cell concentrations ranged from 2.0×10^6 to 7.0×10^6 cells per flask, and the initial amounts of ethanol ranged from 2.5 to 30 μ mole. In Fig. 5, the O₂ consumption due to ethanol is plotted against the initial amounts of ethanol. The slope of the line of best fit is equivalent to a 39 % oxidation of the ethanol added to the cells.

The time course of CO₂ production, in the presence of ethanol at pH 5.5, was linear with both Et-grown and Ac-grown *Euglena*. The results of 12 trials, at low and high pH, using Et-grown and Ac-grown cells, presented in Table 3, yield 0.33 ± 0.04 as the respiratory quotient for ethanol oxidation, independent of the experimental conditions.

Since there is no direct evidence that the endogenous metabolism continues during the oxidation of ethanol, the data for ethanol oxidation have also been calculated on the assumption that endogenous respiration is suppressed in the presence of exogenous ethanol. Under this hypothesis, the pertinent values become: μ l. O₂ per μ mole ethanol, 32.5 ± 1.2 ; respiratory quotient on ethanol, 0.40 ± 0.004 .

DISCUSSION

There is a certain circularity in concluding that the endogenous metabolism continues during acetate oxidation because this assumption gives constant oxygen:substrate ratios and then using data corrected on the basis of this conclusion to demonstrate that oxygen:acetate ratios do not change with

Fig. 4

Fig. 5

Fig. 4. Effect of adaptation to acetate on extent of acetate oxidation. Et-grown cells, incubated at pH 7.3 with 0, 10, 15, 20, 40 μ mole of acetate. Average μ l. O_2 due to acetate: μ mole acetate (for 10, 15, 20 μ mole acetate) is 18.8.

Fig. 5. The extent of ethanol oxidation by *Euglena*. Slope is 26.3 μ l. O_2 : μ mole ethanol, equivalent to a 39 % oxidation of ethanol.

Table 3. *The respiratory quotient of Euglena on ethanol*

Growth substrate	Ethanol (mm)	Respiratory quotient	
		pH 5.5	pH 7.3
Ethanol	30	0.29*	0.23*
	30	0.41	—
	5	0.28	—
	30	—	0.32
	30	—	0.32
	30	—	0.32
Acetate	3	0.31	—
	30	0.34	—
	30	0.34*	0.40*
	30	—	0.34
	30	—	0.37
Average		0.33	0.33
Average (all values)		0.33 $\pm$ 0.04†	(12 %)

* Acid-tipped method.

† Average deviation.

environmental conditions. In our opinion, recognition of this circularity does not seriously weaken the conclusions. If the extent of oxidation of acetate actually varied, there is no reason to believe that any simple assumption concerning the endogenous respiration should give constant ratios. Moreover, it is apparent from Fig. 2 that the variation in oxygen:substrate ratios introduced by using the 'wrong' assumption concerning the endogenous respiration is chiefly between results obtained with different amounts of acetate. Similar results in

this respect were obtained regardless of the past history of the cells or the pH of incubation. Such variations are irrelevant to the conclusion that neither growth medium nor pH alters the efficiency of acetate metabolism by *Euglena*.

Euglena incubated on acetate consumed 0.84 μ mole of O_2 for each μ mole of acetate utilized, equivalent to a 42 % oxidation of this substrate, independent of the experimental conditions. Similar values have been reported for other organisms. Barker (1936) observed at 50 % oxidation of acetate with *Prototheca zopfii*, a colourless alga. Using a differential calorimetric technique Blum, Podolsky & Hutchens (1951) found that *Chilomonas paramecium* oxidized 40 % of the available acetate. Cirillo's data (1956) indicate that *Polytoma uvella*, a phytoflagellate, oxidized 46 % of the available acetate.

Assuming that all the acetate not oxidized was assimilated by the cells, the oxidation: assimilation ratio of acetate was 0.72 for *Euglena*. The respiratory quotient of acetate was 1.0, independent of the experimental conditions; 0.84 μ mole of CO_2 was produced for each μ mole of acetate utilized. If all the acetate not oxidized were converted into a carbohydrate-like material, the empirical equation for the process would then be:

This equation satisfies the theoretical conditions for the simultaneous oxidation of acetate and its conversion to carbohydrate, and the respiratory quotient and percentage oxidation experimentally observed. The results are thus consistent with the simultaneous oxidation and conversion to carbohydrate of acetate, but cannot be considered proof that such is the case.

Addition of ethanol to *Euglena* resulted in the consumption of 1.17 μ mole of O_2 for each μ mole of ethanol utilized, equivalent to the complete oxidation of 39 % of this substrate, independent of the experimental conditions. Again assuming that all the substrate not oxidized was assimilated by the cells, the oxidation: assimilation ratio of ethanol was 0.64. If all the ethanol not oxidized were converted into a carbohydrate-like material, the theoretical equation for this simultaneous oxidation and synthesis may be represented as (based on the O_2 :ethanol ratios)

The respiratory quotient predicted by this equation is 0.15, while the experimentally determined respiratory quotient for ethanol oxidation was 0.33. Instead of 0.17 μ mole of CO_2 being produced for each μ mole of ethanol utilized, it was found that 0.39 μ mole of CO_2 was produced. The results do not agree with the assumption that carbohydrate is the sole synthetic product of ethanol metabolism.

Lewin (1954) reported an adaptation of *Chlamydomonas dysosmos* apparently involving their ability to assimilate acetate. The cells oxidized acetate at once, but a 60 min. exposure period was necessary before assimilation began.

Although it is not pointed out in the text, the data of Tanenbaum (1956, figs. 1, 2) indicate that ethanol-grown *Acetobacter* also adapt to acetate. O_2 consumption and CO_2 production due to acetate reached maximum rates only

after a lag of about 10 min. On ethanol, CO_2 production was similarly delayed, but O_2 consumption began immediately at its maximum rate. This suggests that, in this case, ethanol is promptly oxidized to the level of acetate, but acetate cannot be oxidized without a brief adaptation period. In *Euglena*, the extent of oxidation of both ethanol and acetate was constant under all experimental conditions. There is evidence (Danforth & Wilson, 1957) that the adaptive change in the rate of acetate metabolism of *Euglena* involves an increased permeability to acetate ions. Thus, it would appear that, in three different micro-organisms which show adaptive changes in acetate metabolism, three distinct adaptive mechanisms are involved.

Conditions markedly affecting the rate of substrate oxidation in *Euglena*, such as adaptation to acetate, altered pH and lowered substrate concentration, had no effect upon the extent of oxidation. The oxidation: assimilation ratios of these substrates did not vary in response to environmental changes. It is reasonable to conclude that under these experimental conditions, the oxidative metabolism of *Euglena* is not regulated by changes in the extent to which a substrate is oxidized or incorporated. It is possible that other conditions might alter these oxidation-assimilation ratios. Cirillo (1956) reported a 15% increase in the rate of acetate respiration and the extent of total oxidation in the presence of NH_4Cl by *Polytoma*. The effect of an exogenous nitrogen source on the extent of acetate and ethanol oxidation has not been studied in *Euglena*.

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REFERENCES

- BARKER, H. A. (1936). The oxidative metabolism of the colorless alga, *Prototheca zopfii*. *J. cell. comp. Physiol.* 8, 231.
- BLUM, J. J., PODOLSKY, B. & HUTCHENS, J. O. (1951). Heat production in *Chilomonas*. *J. cell. comp. Physiol.* 37, 403.
- CIRILLO, V. P. (1956). Induced enzyme synthesis in the phytoflagellate, *Polytoma*. *J. Protozool.* 3, 69.
- DANFORTH, W. F. & WILSON, B. W. (1957). Adaptive changes in the acetate metabolism of *Euglena*. *J. Protozool.* 4, 52.
- HUTNER, S. H. & PROVASOLI, L. (1951). In *Biochemistry and Physiology of Protozoa*, Vol. 1, p. 27, ed. by Hutner, S. H. and Lwoff, A. New York: Academic Press Inc.
- LEWIN, R. A. (1954). The utilization of acetate by wild-type and mutant *Chlamydomonas dysosmos*. *J. gen. Microbiol.* 11, 459.
- SYRETT, P. J. (1951). The effect of cyanide on the respiration and the oxidative assimilation of glucose by *Chlorella vulgaris*. *Ann. Bot., Lond.* 15, 473.
- TANENBAUM, S. W. (1956). The metabolism of *Acetobacter peroxidans*, I. Oxidative enzymes. *Biochim. biophys. Acta*, 21, 335.
- UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1949). *Manometric Techniques and Tissue Metabolism*. Minneapolis: Burgess Publishing Co.

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