
The Tricarboxylic Acid Cycle in *Nocardia Corallina*¹

OLEN BROWN and J. B. CLARK, Division of Microbiology
University of Oklahoma, Norman

Recently, evidence has accumulated for the presence of a complete Krebs tricarboxylic acid cycle in a number of bacteria, including *Escherichia coli* (Wheat and Ajl, 1954), *Xanthomonas phaseoli* (Madsen and Hocheater, 1959) and *Micrococcus sodonensis* (Perry and Evans, 1960). This cycle serves both as a source of energy and carbon skeletons for the synthesis of amino acids and other essential metabolites in the cell.

The literature contains only limited information concerning the intermediary metabolism of *Nocardia corallina*. Evidence for carbon dioxide fixation by a reversible, nucleotide dependent oxalacetic carboxylase has been presented (Baugh *et al.*, 1960). Oxidative assimilation and the endogenous metabolism have been described (Midwinter and Batt, 1960). No papers regarding terminal pathways of respiration in *Nocardia corallina* have been published.

This paper is a preliminary report, presenting evidence regarding the role of the tricarboxylic acid cycle in *Nocardia corallina*. Two morphological cell types, hyphae and coccoids, which appear in the complex life cycle (Webb and Clark, 1957) were studied. An unequivocal proof of the operation of the cycle was not attempted. However, data from two sources. Warburg experiments and spectrophotometric measurements of enzymatic activities, offer substantial criteria for evaluating this pathway in *Nocardia corallina* at two specific phases in the life cycle.

¹ This work was taken in part from a thesis presented by the senior author to the graduate faculty of the University of Oklahoma in partial fulfillment of the degree of Master of Science in Microbiology. The work was supported in part by a grant from National Institutes of Health.

MATERIALS AND METHODS

Stock cultures of *Nocardia corallina* (ATCC 4273) were maintained on nutrient agar containing one per cent fructose. Cells for experimental use were obtained by transfer from 48-hour stock culture to one-half per cent fructose agar in Kolle flasks, incubated at 29 C. Cultures were harvested at 20 hours for hyphae and at 48 hours for coccoids.

Cells for Warburg experiments were harvested in saline, centrifuged and washed three times with 0.06 M phosphate buffer, pH 6.8, and stored at 0 C in buffered phosphate solution. No cells were used which had been stored for longer than one week.

Cell-free extracts were prepared by disintegration in the cold for 20 minutes in a Mickle disintegrator (manufactured by Gomsholl, Surrey, England) with 10 per cent beads (Ballotine, No. 12.) The resulting suspension was frozen in a dry ice-acetone bath and maintained at minus 20 C until used.

Warburg techniques were those described in Umbreit *et al.*, (1945).

Aconitase and fumarase activities were measured by the method of Racker (1950).

RESULTS AND DISCUSSION

Oxygen uptake of whole cells was stimulated by all of the intermediates of the Krebs cycle which were tested, with the exceptions of citrate and alpha-ketoglutarate (Table 1). The inability of 48-hour-old coccoids to utilize citrate and alpha-ketoglutarate could be due to either a lack of the proper enzymes or to impermeability of the cells to the substrate. A demonstration of aconitase activity in cell-free extracts of 48-hour-old coccoids, by the method of Racker (1950), indicates that aconitase is present; hence, the failure of citrate to stimulate oxygen uptake is most probably due to a lack of permeability of the cell membrane to citrate. A similar condition may exist for alpha-ketoglutarate.

Fumarase, a component enzyme of the tricarboxylic acid cycle required for the conversion of l-malate to fumarate and water, was also shown to be present in cell-free extracts of 48-hour-old cells. The presence of fumarase activity is in agreement with Warburg data which indicates that oxygen uptake of whole cells is stimulated by malate.

With 48-hour-old coccoids, oxygen uptake was stimulated immediately upon tipping of the substrate. With 20-hour-old hyphae, however, a delayed stimulation of oxygen uptake resulted from the addition of citrate, alpha-ketoglutarate, succinate, or malate. Figure 1 presents typical results showing the delayed stimulation with citrate. Similar delayed stimulations of oxygen uptake have been reported in the literature. Kogut and Podoski (1953) and Barrett *et al.*, (1953), reported independently that the oxidation of citrate and other intermediates of the citric acid cycle by intact cells of *Pseudomonas* was adaptive, while the enzymes of the cycle were shown to be present in cell-free extracts of unadapted cells. Both groups of authors formulated the hypothesis that the adaptive behavior of the intact cells was due to specific, inducible permeation factors. The failure of some organisms to utilize certain intermediates of the cycle has been attributed to their inability to synthesize the appropriate permeases (Cohen and Monod, 1957). This "carrier" concept has been discussed in detail by Doudoroff (1951), and Osterhout (1952).

Further experimentation is required to affirm the presence of enzymes of the Krebs cycle in cell-free extracts of 20-hour-old hyphae. However, as already mentioned, aconitase activity was demonstrated in 48-hour-old coccoids which were impermeable to citrate.

TABLE 1. RESPIRATORY DATA OBTAINED FROM 20-HOUR-OLD HYPHAE

| Substrate | Endogenous QO ₂ | Exogenous QO ₂ | Percent Stimulation of Endogenous QO ₂ |
|---------------------|-------------------------------|------------------------------|---|
| Acetate | 18.15 | 32.67 | 179.9 |
| Citrate | 8.33 | 3.94 | 32.1 |
| Alpha-ketoglutarate | 8.82 | 4.48 | 50.8 |
| Succinate | 8.66 | 11.61 | 122.3 |
| Pyruvate | 11.21 | 4.79 | 42.7 |
| Malate | 10.51 | 5.18 | 49.2 |

Respiratory Data Obtained From 48-Hour-Old Coccoids

| Substrate | Endogenous QO ₂ | Exogenous QO ₂ | Percent Stimulation of Endogenous QO ₂ |
|---------------------|-------------------------------|------------------------------|---|
| Acetate | 16.66 | 27.25 | 163.4 |
| Citrate | 12.21 | 0.46 | 3.9 |
| Alpha-ketoglutarate | 12.24 | 0.03 | 0.0 |
| Succinate | 12.66 | 4.82 | 38.0 |
| Pyruvate | 14.88 | 3.88 | 26.0 |
| Malate | 13.94 | 4.54 | 32.6 |

Warburg flasks contained: 0.1 ml of 0.1 M substrate, 3.3 mg (dry weight) cells, 0.2 ml KOH in center-well, and 2.7 ml of 0.06 M phosphate buffer, pH 6.8. All substrates were neutralized with NaOH before addition. Temperature, 30 C.

SUMMARY

The following data are presented regarding the operation of the tricarboxylic acid cycle in *Nocardia corallina*: 1) Oxygen uptake of 48-hour-old coccoids is stimulated by addition of pyruvate, acetate, succinate, and malate. 2) Citrate and alpha-ketoglutarate do not stimulate oxygen uptake; however, aconitase activity is present in cell-free extracts, indicating that the lack of stimulation is most probably due to impermeability. The reason for the failure of alpha-ketoglutarate to stimulate oxygen uptake was not determined. 3) Oxygen uptake of 20-hour-old hyphae was stimulated by all of the above named intermediates.

In addition, evidence regarding the formation of specific, adaptive permeases for citrate, alpha-ketoglutarate, succinate and malate by 20-hour-old hyphae is presented.

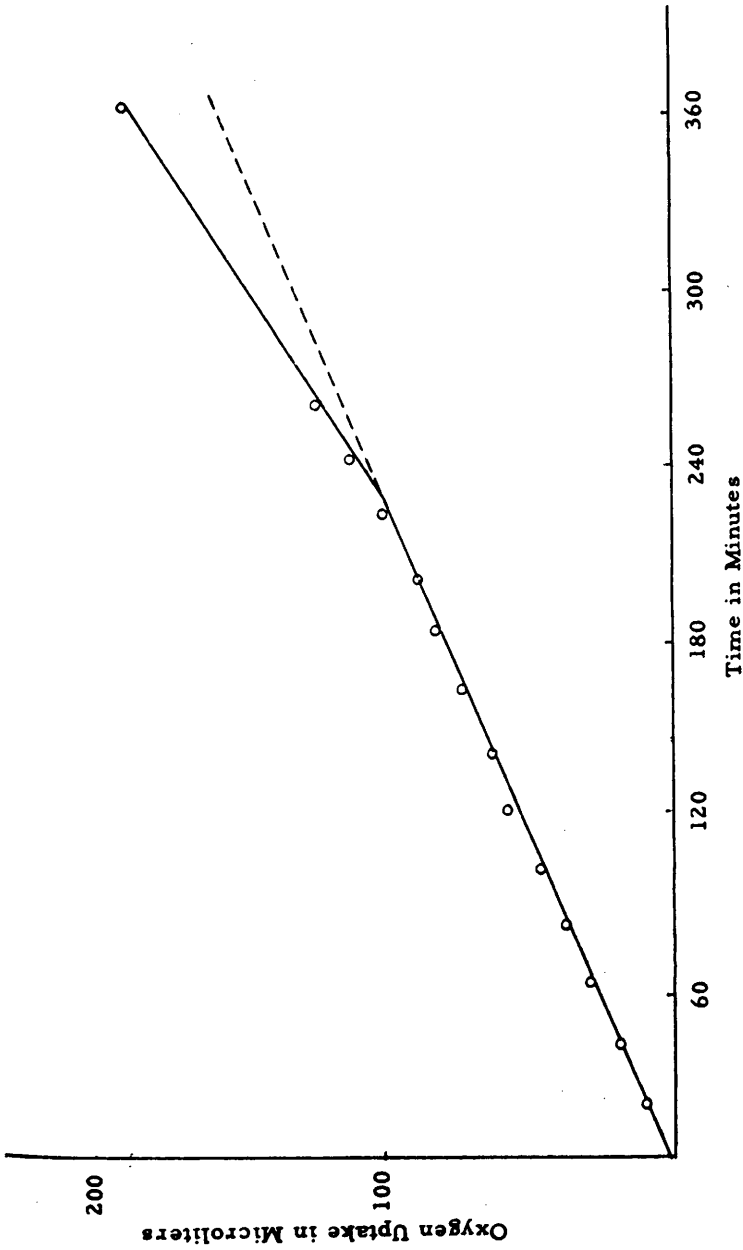


Figure 1. Delayed stimulation of oxygen uptake by citrate with 20-hour-old cells. Substrate tipped at 120 minutes.

LITERATURE CITED

- Barret, J. T., A. D. Larson and R. E. Kallis. 1953. The nature of the adaptive lag of *Pseudomonas fluorescens* toward citrate. *J. Bacteriol.* 65: 187-192.
- Baugh, C. E., G. W. Claus and C. H. Werkman. 1960. Hetrotrophic fixation of Carbon dioxide by extracts of *Nocardia corallina*. *Arch. Biochem. Biophys.* 86: 255-259.
- Cohen, G. N. and J. Monod. 1957. Bacterial permeases. *Bacteriol. Rev.* 21: 169-191.
- Doudoroff, M. 1951. The problem of the direct utilization of disaccharides by certain microorganisms. *Phosphorous Metabol.*, Edited by W. D. McElroy and B. Glass. Johns Hopkins Press, Baltimore.
- Kogut, M. and E. P. Podoski. 1953. Oxidative pathways in a fluorescent pseudomonas. *Biochem. J.* 55: 800-811.
- Madsen, N. B. and R. N. Hocheester. 1959. The tricarboxylic acid and glyoxylate cycles in *Xanthomonas phaseoli*. *Can. J. Microbiol.* 5: 1-8.
- Midwinter, G. G. and R. D. Batt. 1960. Endogenous respiration and oxidative assimilation in *Nocardia corallina*. *J. Bacteriol.* 79: 9-17.
- Osterhout, W. J. V. 1952. The mechanism of accululation in living cells. *J. Gen. Physiol.* 35: 579-594.
- Perry, J. J. and J. B. Evans. 1960. Oxidative metabolism of lactate and acetate by *Micrococcus sodonansis*. *J. Bacteriol.* 79: 113-118.
- Racker, E. 1950. Spectrophotometric measurements of the enzymatic formation of fumaric and cis-aconitic acids. *Biochem. Biophys. Acta.* 4: 211-214.
- Umbreit, W. W., R. H. Burris and J. F. Stauffer. 1945. *Manometric techniques*, Burgess Publishing Co., Minneapolis.
- Webb, R. B., and J. B. Clark. 1957. Cytogenetic study of *Nocardia corallina*, *J. Bacteriol.* 74: 31-42.
- Wheat, R. W. and S. F. Ajl. 1954. Component enzymatic reactions of the tricarboxylic acid cycle in *Escherichia coli*. *Arch. Biochem. Biophys.* 49: 17-18.
-