

Comment on E. Broda's Recent Publications

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Summary. E. Broda's recent argument against our concept that nitrate respiration antedated oxygen respiration is criticized.

Key words: Nitrate Respiration/Fermentation/Energy Metabolism/Evolution

E. Broda recently published an excellent monograph (1975a) on the evolution of energy metabolism and an original review (1975b) on the history of inorganic nitrogen in the biosphere in which he repeatedly criticized our concept (Egami, 1974) that nitrate respiration antedated oxygen respiration. His criticism is based on the following suppositions: (1) The atmosphere before the appearance of blue-green algae was anoxygenic. In an anoxygenic atmosphere nitrate could not be produced. (2) Nitrate reduction observed in strict anaerobes such an *Clostridium perfringens* has no physiological significance and nitrate is nothing but an incidental electron acceptor.

The physiochemical or geochemical evidence supporting his first statement is not convincing. The existence of nitrate in aqueous inclusions of a nepheline-basalt (Sugawara et al., 1944, 1949) and in lunar fines (Hintenberger et al., 1970) provide evidence of the accumulation of nitrate under anoxygenic atmospheres.

Even based upon the thermochemical equation cited in his review (1975b)

$$N_2 + H_2O + 2.5 O_2 = 2NO_3 + 2H^+; \Delta G'_O = -15.1 \text{ Kcal}$$

(Standard conditions, pH=7)

the possibility cannot be excluded that a physiologically significant amount (10^{-7} N) of nitrate accumulated in the primeval sea in contact with an atmosphere with a minute amount of free oxygen (dioxygen).

Moreover nitrate formed by thunderstorms or other energy

sources was dissolved in the primeval sea and could have accumulated to levels even above the thermodynamic equilibrium concentration. Thus the formation and accumulation of nitrate in the primeval sea cannot be ruled out.

His second supposition is not supported by experimental evidence at all. The observation that nitrate can stimulate the growth and the energy-yielding processes of *Clostridium perfringens* (Hasen & Hall, 1975) and the existence of a specific enzyme, "nitrate reductase", in the organism (Chiba & Ishimoto, 1973) completely undermine this argument.

So we continue to insist that one of the major evolutionary pathways from fermentation to oxygen respiration is as follows:

Fermentation... \rightarrow Nitrate Fermentation \rightarrow Nitrate Respiration \rightarrow Oxygen Respiration

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Note Added in Proof. Formation of NO_2 even in a reduced atmosphere consisting of H_2 , N_2 and CO under UV irradiation was reported by G. Winnewisser et al. (1974). Reference: Winnewisser, G., Mezger, P.G., Breuer, H. (1974). Fortschr.d.Chem.Forsch. 44, 69. Berlin, Heidelberg, New York: Springer.