SYMPOSIUM ON AUTOTROPHY[1]

IV. Some Thoughts on the Energetics of Chemosynthesis

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Clayton (7) has explained the modern concept of the means whereby photosynthetic organisms obtain the supplies of adenosine triphosphate (ATP) and reduced pyridine nucleotides necessary for the operation of the Calvin cycle that is responsible for the reductive incorporation of carbon dioxide into their cellular materials. In both plants and photosynthetic bacteria, the really fundamental part played by light is the generation of ATP by photophosphorylation, the generation of reduced pyridine nucleotides being the result either of a process ancillary to photophosphorylation (in green plants) or of a process totally unconnected with it (in photosynthetic bacteria). The accent on photophosphorylation by Arnon and his school somewhat strengthens a definition of autotrophs that I happened to make some time ago (10), namely, that they are "organisms that do not use organic compounds as primary sources of energy." This definition seems to me to be better than one referred to by Umbreit (21), based on "rays of the sun," since it subsumes green plants, photosynthetic bacteria, and chemosynthetic bacteria, all of which are, in common parlance and by dictionary definition, autotrophs. I shall devote most of my remarks to a consideration of some of the problems the chemosynthetic autotrophs face in obtaining an energy supply from inorganic sources since this aspect of their metabolism is, to me at any rate, the most fascinating one.

On general biochemical grounds we should expect the *energy dynamo* of these organisms to be, as it is in other organisms, a cytochrome system coupled to a phosphorylating system generating ATP, the whole dynamo being driven by oxidation of the inorganic substrate. Is it true, then, that such systems are to be found in the chemosynthetic autotrophs?

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It is certainly true of *Nitrobacter*. The direct participation of a cytochrome system in nitrite oxidation by *Nitrobacter* has been demonstrated (11), one of the cytochromes concerned has been characterized (5), and cell-free cytochrome-containing fractions have been shown to phosphorylate while oxidizing nitrite (1, 13). A curious point about this oxidation is that the E_0' (pH 7) of the nitrite-nitrate system (+0.35 v) is appreciably higher than that of the cytochrome *c* of *Nitrobacter*, which has an E_0' of +0.25 v (5). It is therefore just possible that, to oxidize nitrite, *Nitrobacter* may be compelled to modify it in some way, perhaps by synthesizing some such compound as adenyl nitrite, so as to lower the redox potential of the nitrite to one more compatible with reduction of the cytochrome by nitrite. It is perhaps significant here that Aleem and Nason (1) found it impossible to uncouple phosphorylation and nitrite oxidation in cell-free preparations of *Nitrobacter*, although normal uncoupling agents merely suppress nitrite oxidation in whole cells (6). Nitrite oxidation by *Nitrobacter* may well prove to be a more complex process than it appears to be at first sight. Similar difficulties, i.e., those involved in dealing with a substrate with a redox potential appreciably above that of a normal cytochrome system, confront the ferrobacilli and thiobacilli utilizing the ferrous-ferric oxidation as a source of energy, since the E_0' of this system at pH 7 (which is presumably the pH of the microenvironment of the enzymes effecting the oxidation) is almost identical (+0.32 v) with that of the nitrite-nitrate system. Ferrous iron oxidation, too, may not be quite as simple as it appears to be.

When we turn to the other chemosynthetic autotrophs we find ourselves, fortunately, traversing much less difficult terrain. The substrates used by the sulfur-oxidizing thiobacilli all have redox potentials below that of cytochrome *c*. Phosphorylation coupled with substrate oxidation was, of course, demonstrated in the thiobacilli before it was demonstrated in any other auto-

troph (22) and has since been amply confirmed. The participation of cytochromes in substrate oxidation by these organisms is also well documented (4, 16, 20). *Nitrosomonas* is a somewhat more complex case but does not present any real problem. It carries out a multistep oxidation of ammonium ions in which one intermediate remains unidentified; we may, however, assume with probably no great error that the intermediate is at an oxidation level in the region of that of hyponitrite ($H_2N_2O_2$) and that some unstable compound such as HNO is actually involved. If we make these assumptions, then the E_0' values of the steps in ammonium oxidation can be calculated (2) as follows:

$$NH_4^+ + H_2O = NH_2OH + 3H^+ + 2\,e^- \quad +0.89\text{ v} \qquad (1)$$

$$NH_2OH = NO^- + 3H^+ + 2e^- \quad -0.11\text{ v} \qquad (2)$$

$$NO^- + H_2O = NO_2^- + 2H^+ + 2\,e^- \quad +0.23\text{ v} \qquad (3)$$

The first of these steps, which is known to be the actual reaction used by *Nitrosomonas* (8, 9), could scarcely be coupled to a cytochrome since it has an E_0' above that of the oxygen electrode. Therefore, to make the reaction proceed from left to right, either the hydroxylamine concentration must be kept low by rapid utilization of hydroxylamine in the second step or the ammonium ions must be activated in some way by combination with a carrier as suggested by J. H. Anderson (*private communication*). Either explanation would cover the observed fact that the reaction does not take place with disintegrated cells, in which all reactions are slowed down and many coupling mechanisms necessarily destroyed. Although both the second and the third steps are theoretically capable of bringing about cytochrome reduction and although insufficient is known about the cytochromes of *Nitrosomonas* (17) to allow for any firm conclusions, there are yet some grounds for believing (12) that the second step is the one used by *Nitrosomonas* for cytochrome reduction. No theoretical problem whatever arises over the reduction of cytochromes, with consequent oxidation of hydrogen, by the hydrogen bacteria. A pyridine-nucleotide-coupled hydrogenase (14) in the presence of hydrogen would immediately supply the appropriate cytochrome reductant, i.e., reduced pyridine nucleotide.

There are thus no major difficulties in envisaging the operation of phosphorylating cytochrome systems in the chemosynthetic autotrophs and thus envisaging a supply of ATP for the Calvin cycle known to occur in some of these organisms (3, 13, 15, 18, 19) and probably occurring in all. When we turn, however, to the question of the supply of reduced pyridine nucleotide, also essential for running the Calvin cycle, the outlook is much more obscure.

The E_0' of pyridine nucleotides at pH 7 is about -0.27 v. Once again there is no difficulty in envisaging the reduction of pyridine nucleotides by the substrates of the thiobacilli or the hydrogen bacteria. Nor is the E_0' of the second step in *Nitrosomonas* too alarmingly discrepant from the desired value, especially as we do not really know whether the formulation of this step is precisely correct. But with *Nitrobacter*, and with ferrobacilli and thiobacilli oxidizing ferrous iron, the problems are enormous. Here, in the oxidations of nitrite and of ferrous iron, we have systems with E_0' values of more than $+0.3$ v so coupled with pyridine nucleotide systems with E_0' values of almost -0.3 v that the latter systems actually become reduced. The 0.6 v that separates the coupled systems represents an energy gap of some 27 kcal; in other words, this is the order of energy that must be fed into the coupled systems, buffered at pH 7, if pyridine nucleotides are to be reduced at the expense of oxidation of nitrite or ferrous iron. This is, of course, reflected by the well known fact that nitrate reductase (prepared from heterotrophic organisms) has an equilibrium almost entirely in favor of triphosphopyridine nucleotide oxidation and nitrate reduction. How this equilibrium is altered in *Nitrobacter*, and the similar equilibrium involving ferrous ions altered in ferrobacilli and thiobacilli, we do not know. Perhaps nitrite and ferrous iron are activated in some way as was suggested for cytochrome reduction by these ions; perhaps there are active transport mechanisms for removing nitrate or ferric iron from the site of pyridine nucleotide reduction as soon as these ions are formed; or perhaps reduced pyridine nucleotides are similarly removed. Whatever the mechanism there are few more fascinating problems in the field of autotrophic biochemistry than this: How do *Nitrobacter* and the ferrobacilli manage to reduce, as they must of necessity reduce, di- and triphosphopyridine nucleotide? The problem

resolves itself, in the last analysis, into the much wider one: How do *Nitrobacter* and the ferrobacilli manage to work at all?

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