Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich
125/1, 61–72, März 1980

Bacterial respiration, with special reference to *Paracoccus denitrificans*

by F. R. WHATLEY

Introduction

Bacteria capable of oxidative metabolism (aerobic bacteria) resemble mitochondria in that they couple ATP synthesis to the transfer of electrons along an electron transport chain from a reduced donor substance to an oxidised acceptor, characteristically molecular oxygen. The ability of bacteria and mitochondria to make ATP appears to be dependent on proton pumping, with the return of the expelled protons through a membrane-bound *ATPase,* and the spatial arrangement of the electron transport intermediates in the energy transducing membranes is of crucial importance.

In spite of their overall similarity, the details of the electron transport chain in most aerobic bacteria is significantly different from those in mitochondria. Often the bacteria have branched chains with a choice of ways of transferring electrons through different cytochromes to oxygen e.g. the cytochrome *o* and *d* systems of *Escherichia coli,* with low and high affinities for oxygen respectively. One is reminded here of *Arum spadix* mitochondria, which have a branched chain to oxygen through cytochrome *b* or cytochrome aa_3, but mitochondria with branched chains are very unusual. Other aerobic bacteria can substitute an alternative electron acceptor for oxygen, the use of nitrate in denitrifying bacteria being an example.

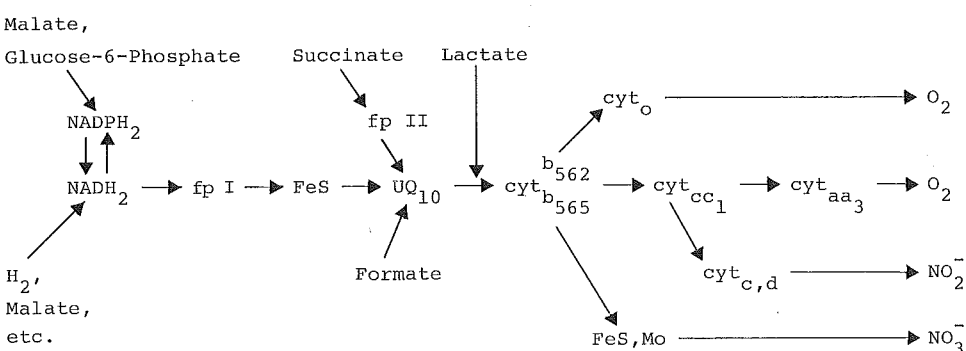
In a number of photosynthetic bacteria of the purple non-sulphur group, including *Rhodopseudomonas spheroides,* an alternative terminal acceptor can be generated in the absence of oxygen by a photoreaction within the organism. Under aerobic conditions *Rhodopseudomonas spheroides* operates an electron transport chain which is similar in many respects to the normal mitochondrial chain. It appears to be an obligate aerobe in the sense that it cannot ferment anaerobically. However, in the absence of oxygen a photosynthetic electron transport chain is set up which uses many of the intermediates of the aerobic electron transport chain and which differs from it essentially only in

the electron donor and electron acceptor at the beginning and the end of the chain respectively.

Paracoccus as an obligate aerobe

Paracoccus denitrificans is interesting because it has a mitochondrial type of electron transport chain and the details of the oxidative phosphorylation mechanism appear very similar in *Paracoccus* and in a typical mitochondrion. *Paracoccus* has great nutritional adaptability in its use of a wide range of electron donors and carbon substrates, but unlike facultative aerobes, e.g. *Escherichia coli,* it cannot ferment any of them and is obligatorily dependent on an oxidative type of metabolism.

Although *Paracoccus* may use nitrate or nitrite instead of oxygen its energy metabolism remains essentially aerobic even in the absence of oxygen. Cells of *Paracoccus* grown with adequate aeration in the presence of nitrate are unable to use nitrate as the terminal electron acceptor because the synthesis of the respiratory (dissimilatory) nitrate reductase is repressed by oxygen. However, cells grown anaerobically with nitrate acquire nitrate and nitrite reductases as induced enzymes but can still use oxygen because the cytochrome oxidase is a constitutive feature of the cell [1]. Other facultative aerobic bacteria e.g. *Escherichia coli* may also be able to adapt to the use of nitrate as an anaerobic alternative to oxygen. When cells of *Paracoccus* or *Escherichia* which have been previously adapted to nitrate are provided with nitrate and oxygen at the same time they use oxygen in preference to nitrate, but as soon as all the oxygen is used they immediately switch to the use of nitrate [2]. The nature of the switching mechanism whereby the electrons are channelled to the appropriate acceptor is not certain, but the energetic advantage of using oxygen rather than nitrate or nitrite in terms of ATP synthesis is clear from Fig. 1. The



fp = flavoproteins; FeS = non-heme iron containing electron carrier;
UQ = Ubiquinone; other abbreviations have their usual meaning.

Fig. 1 Possibilities for Electron flow along the Respiratory Chain of *Paracoccus denitrificans.*

switch may depend on the redox state of the cytochrome *b* in the organism. Attempts to examine the redox state of *Paracoccus denitrificans* are complicated by the existence of a rather high background absorption in the cytochrome *b* region and by the possibility that only a part of the cytochrome *b* is reactive. In the case of the mutant *E. coli* K12, which reduces nitrate but cannot use nitrite as terminal acceptor, the spectroscopic evidence is much clearer and is consistent with the view that electrons can only pass to nitrate when the cytochrome *b* is largely in the reduced state (i. e. the "affinity" of reduced cytochrome *b* for the iron sulphur centres in nitrate reductase is low). Under aerobic conditions reduced cytochrome *b* does not accumulate because it is rapidly reoxidised by cytochrome *c*, which in turn is maintained in the oxidised state by the operation of cytochrome aa_3. Although this simple kinetic model is adequate, alternative explanations for the switch from oxygen to nitrate are possible.

Paracoccus can grow either autotrophically with hydrogen and carbon dioxide or heterotrophically with a large variety of carbon compounds and it does this whether the terminal electron acceptor is oxygen, nitrate, nitrite or nitrous oxide. *Paracoccus* fixes carbon dioxide by the operation of the reductive pentose cycle [3]. It can use hydrogen gas to supply the NADH and also as the source of the electrons which are passed along the electron transport chain to oxygen or alternative acceptors to supply the ATP needed for the reductive pentose cycle. When formate or methanol is offered as the sole carbon and energy source it is oxidised to carbon dioxide, which is again fixed by the reductive pentose cycle; the hydrogen atoms provided by the substrate again supply the NADH, as well as the electrons passed along the electron transport chain to oxygen or nitrate to supply the ATP. The incorporation of the carbon of methanol by the fixation of carbon dioxide is more expensive energetically than if the methanol were incorporated directly as in other bacteria, but QUAYLE has suggested that *Paracoccus* gains an advantage in using this energetically less efficient mode of metabolism by having the capability of growing on one-carbon compounds using enzymes for which it already has the code [4].

Under heterotrophic conditions *Paracoccus* can use a wide variety of compounds as carbon and energy sources. Its versatility is indicated in the original formal description of *Paracoccus* [5], which states that it could utilise 64 different organic compounds as a sole carbon source out of a total 143 different organic compounds offered. Physiological experiments, feeding tests and enzyme assays of cell free extracts of *Paracoccus* have indicated the pathways by which some of these compounds are metabolised and these have been recently reviewed [6]. Glucose is metabolised via the ENTNER-DOUDOROFF pathway or by the hexose monophosphate pathway, or by a combination of the two. The EMBDEN-MEYERHOF glycolytic pathway via fructose-1.6-*bis*-phosphate is absent. Pyruvate is oxidised to carbon dioxide via the tricarboxylic acid (TCA) cycle, as also are succinate and malate, although both of these di-

carboxylic acids must first be converted to pyruvate through the operation of the malic enzyme. When acetate is the sole carbon and energy source it is metabolised by the glyoxylate cycle, which runs in parallel with the TCA cycle. When glycolate is the sole carbon and energy source the β-hydroxyaspartate pathway operates. This pathway, which is unique to *Paracoccus,* brings about the direct condensation of 2C to a 4C dicarboxylic acid, which is further metabolised through the TCA cycle. This great flexibility of *P. denitrificans* is in contrast to the very restricted list of carbon compounds that can normally be handled by mitochondria. However many aerobic bacteria can utilise quite a wide range of carbon substrates.

Oxidative phosphorylation

The electron transport chain resides in the boundary membrane (plasmamembrane) of the bacterium. It therefore appeared attractive to make preparations of bacterial cell membranes and to study their ability to carry out oxidative phosphorylation. In 1960 I tried to do this for *Paracoccus (Micrococcus) denitrificans* and was able to make preparations which would oxidise succinate with oxygen or nitrate as electron acceptor. When these preparations were supplied with ADP and inorganic phosphate, together with a glucose + hexokinase trapping system to pick up any ATP formed, glucose-6-phosphate was shown to accumulate and this was taken to show the formation of ATP at the expense of succinate oxidation. Attempts to purify the system by cleaning up the membrane fragments led to inconsistent results. Eventually it became clear that ATP synthesis depended on cytoplasmic components as well as on membrane particles. It was finally established in experiments with Dr. B. GRANT that glucose was actually the substrate being used for phosphorylation in these experiments and that the use of succinate was not consistently required; moreover the phosphorylation was completely insensitive to dinitrophenol. Armed with this information it became clear that the ATP was being formed from glucose via substrate phosphorylation.

What then was the function in these experiments of the membrane fragments, which appeared to be essential for the ATP formation? It is suggested that the electron transport system in the membranes was necessary for the reoxidation of the NADH formed in the preliminary oxidative stages of the fermentation, as indicated in Fig. 2. Either nitrate or oxygen could serve as electron acceptor in this reoxidation system and electrons would be passed along the electron transport chain. ATP formation would presumably not occur because the chain was largely uncoupled. If this description is correct it would be expected that pyruvate and lactate dehydrogenase added to the extract might well replace oxygen or nitrate by providing an alternative means of reoxidising NADH. That this is so is shown by the results in Table 1. It is apparent that lactate dehydrogenase (NAD-dependent) is absent from *Paracoccus* and that its absence is responsible for the inability of this bacterium to grow

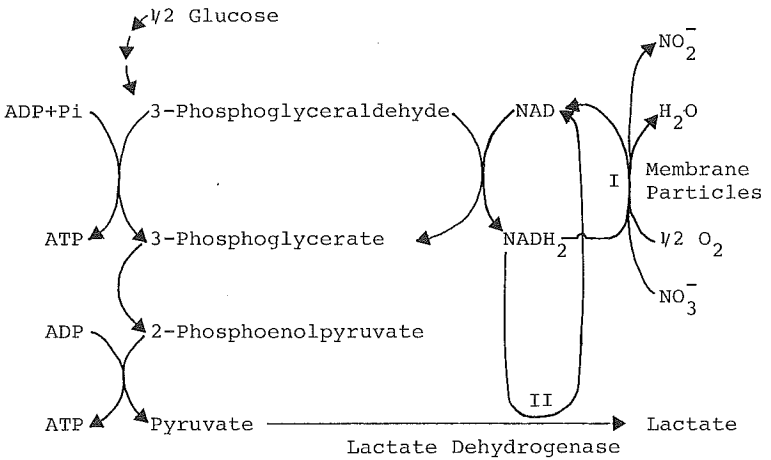


Fig. 2 Substrate Level Phosphorylation driven by Oxidation on Membrane Particles of *Paracoccus denitrificans* (I) or by Lactate Dehydrogenase (II).

Table 1 The influence of various electron acceptors on ATP formation by extracts of *Paracoccus denitrificans**

Electron Acceptor	μ moles ATP formed		
	I	II	III
none	0.8	1.4	1.0
NO_3^- (anaerobic)	6.9	6.0	7.1
O_2 (air)	9.1	7.1	7.5
pyruvate + lactate dehydrogenase (anaerobic)	8.4	8.0	7.1

* Data of B. R. GRANT and F. R. WHATLEY for three separate experiments, I–III.

anaerobically by fermentation. When it is offered lactate, *Paracoccus* uses the enzyme lactate, cytochrome *b* oxido-reductase, which is similar to the enzyme found in yeast, and does not go through NAD.

However, it is unlikely that *Paracoccus* uses substrate phosphorylation alone for growth, and this conclusion was confirmed by growing the organism on ^{14}C-glucose or malate and carrying out a simple balance sheet of the fate of the carbon. When growth on either of these radioactive substrates is completed, examination of the cell material produced and of the medium in which the cells were grown reveals that approximately 60% of the carbon is present as ^{14}C in the cells, 35% is accounted for as CO_2 and approximately 5% remains in the medium as unchanged glucose or malate. No fermentation products accumulate in the medium. These results indicate a high growth yield (the detailed studies of STOUTHAMER confirm the high yield [7]) and are consistent with the

occurrence of oxidative phosphorylation as the main ATP source. This suggested that it would be worthwhile looking for oxidative phosphorylation activity in membrane particles. I therefore asked P. JOHN to attempt to demonstrate this activity and he was successful. By lysozyme treatment of *Paracoccus*, followed by osmotic lysis, he prepared electron transport particles capable of oxidative phosphorylation [8] and showed they could make ATP with oxygen or nitrate and with very respectable P:2e ratios. He later showed that the particles making ATP have an inside-out orientation and that they are capable of a high degree of respiratory control [9]. The *Paracoccus* particles very closely resemble mitochondria in the details of their respiratory control behaviour.

Aerobically grown *Paracoccus* contains an electron transport chain which in its components and functional organisation more closely resembles the inner mitochondrial membrane than the electron transport chain described for any other bacterium [6, 10]. The electron transport chain includes an energy-dependent nicotinamide-nucleotide-transhydrogenase activity, and a number of NADH dehydrogenase activities and the ability to show reversed electron-flow in the presence of excess ATP. The chain is sensitive to low concentrations of rotenone and piericidin A and on examination by EPR at 77° K it shows characteristic g = 1.94 signals, indicating the presence of up to four iron/sulphur (FeS) centres. The signal is decreased when iron is limiting. As in mitochondria the electron transport is sensitive to low concentrations of antimycin A. The sole functional quinone of the respiratory chain is ubiquinone-10. There are at least two kinetically and spectroscopically distinguishable b-type cytochromes and the terminal oxidase is cytochrome aa_3. Cytochrome *o* is also present. The amount of cytochrome *o* increases when the cells are adapted to nitrate and it has also been reported that the amount of cytochrome aa_3 diminishes on growth with nitrate. Kinetic data showing that cytochrome *o* acts as a terminal oxidase is so far lacking (unlike *E. coli* and some primitive mitochondria) but the importance of this observation is unclear. Electrons are fed into the electron transport chain at the level of NADP, NAD or UQ_{10} through, for example, malic enzyme, malate dehydrogenase or succinate dehydrogenase. As in mitochondria the oxidation of succinate is inhibited by thenoyltrifluoroacetone and by carboxin. The cytochrome oxidase of *Paracoccus* has fewer subunits than the normal aa_3 of mitochondria [11] but there is evidence that copper is present in the cytochrome oxidases of both *Paracoccus* and mitochondria in addition to the normal two haem groups.

In addition to its inability to ferment substrates, which permits an uncomplicated look at aerobic metabolism, a great advantage of working with *Paracoccus* is the ease with which subcellular particles capable of oxidative phosphorylation can be isolated from it. In oxygen electrode experiments, phosphorylating particles from the plasmamembrane of *Paracoccus* show a mitochondrial type of respiratory control [9]. The addition of ADP stimulates the rate of electron flow until it has all been converted to ATP; thereafter the rate of respiration returns to the initial state IV. The stimulated rate also returns to

state IV on the addition of venturicidin, which specifically inhibits the ATP synthesising system.

Phosphorylating particles are of necessity inside out vesicles, having an orientation which is the reverse of that of the plasmamembrane in the intact cell. Preparations of right-side-out vesicles are impermeable to NADH and to ADP. Right-side-out particles can be made to oxidise NADH by treatment with a preparation of bee venom which contains mellitin [12]; the latter compound makes "holes" in the membrane by attacking the phospholipids and permits access of NADH to the internal electron transport system, although at the same time it apparently prevents ATP synthesis by making the membranes freely permeable to protons. If *Paracoccus* is grown on a succinate/NO_3 medium a high percentage of the vesicles obtained by a standard preparation technique (lysozyme treatment followed by osmotic lysis) are inside-out, whereas if the cells are grown aerobically on a glucose medium a high percentage of the vesicles are right-side-out. If a mixed preparation is poured through a column made from agarose to which ADP molecules have been bound, the right-side-out particles pass straight through, whereas the inside-out particles become bound to the immobilised ADP [13]. Washing with water or buffer does not release the inside-out bound particles but treatment with a solution of Na-ADP does release them and they then pass through the column. The status of the two fractions can then be readily assessed using the bee venom assay. If the fraction is right-side-out it shows a large enhancement of oxidative activity (with NADH as substrate) after treatment with bee venom, whereas if it is insideout it shows little enhancement. The possibility of mixed sidedness due to migration of membrane components during preparation of particles cannot be completely ruled out.

In cells grown anaerobically with nitrate, phosphorylating particles synthesise ATP with NADH as donor and nitrate as acceptor, whereas succinate cannot give any phosphorylation when the reduction of nitrate can proceed only as far as nitrite (see Fig. 1). It has been shown that outward proton translocation takes place in whole cells oxidising endogenous substrates. When particles capable of synthesising ATP with NADH are isolated they normally lose most or all of the soluble nitrite reductase during the preparation. Crude preparations of broken cells (not particle preparations) appear to be able to make small amounts of ATP using succinate or hydrogen or NADH as electron donors, with nitrite as terminal acceptor. Nitrite is toxic to whole cells when present above a rather low concentration. This is one reason why it is difficult to grow cells anaerobically with nitrite in place of nitrate. The formation of ATP with nitrite as terminal acceptor is therefore subject to potential inhibition by even moderate concentrations of nitrite.

Other aerobic bacteria

The majority of aerobic bacteria have several mitochondrial features in common [10, 14] (Fig. 3 and Fig. 4). These include the NADH-NADPH trans-

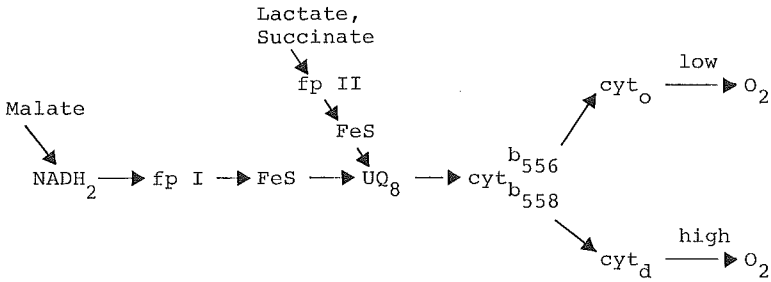


Fig. 3 Possibilities for Electron flow along the Respiratory Chain of *Escherichia coli.*

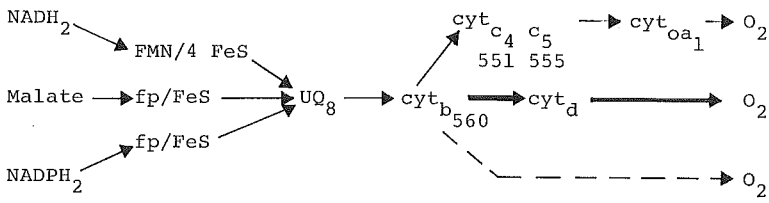


Fig. 4 Possibilities for Electron flow along the Respiratory Chain of *Azotobacter vinelandii* (the significance of the highly branched chain is discussed in ref. 14).

hydrogenases, a number of NADH dehydrogenases (required to feed electrons into the electron transport chain), succinate dehydrogenase, FeS proteins, components of the tricarboxylic acid cycle and the complete membrane-bound proton translocating ATPase system. In *Paracoccus* and in *Escherichia coli* the ATPase is sensitive to 7-chloro-4-nitrobenzo-2-oxo-1:3 diazole as it is in mitochondria, and the inhibition in each is reversed by dithiothreitol; tyrosine is implicated at the active centre of this enzyme ([14], p. 78). In Gram positive aerobes like *Bacillus megaterium,* the quinone present in the electron transport chain is a naphthoquinone; in the Gram negative aerobes the quinone is a ubiquinone based on 2:3 dimethoxy-5-methylbenzoquinone. The isoprenoid side chain may contain between 7 and 10 isoprene units. In *Paracoccus denitrificans* UQ_{10} is present, as in mitochondria. Other aerobic bacteria with UQ_{10} are *Pseudomonas denitrificans, Acetobacter xylinum, Achromobacter cycloclastes, Rhodopseudomonas spheroides* and *Rhodospirillum rubrum.* UQ_8 is present in *Escherichia coli* and *Azotobacter vinelandii.* The mitochondria of some protozoa, fungi and algae contain UQ_7, UQ_8 and UQ_9.

The sequence of cytochromes in the electron transport, which in *Paracoccus nitrificans* is characteristically "mitochondrial" in detail, is highly variable in the aerobic bacteria. Cytochrome *aa*$_3$, with two haem groups, is present in *Paracoccus denitrificans* as a constitutive enzyme although the amount of cy-

tochrome aa_3 is reduced to one-third when the bacterium is grown with nitrate instead of oxygen. A characteristic cytochrome aa_3 is present in *Mycobacterium phlei, Sarcina lutea, Bacillus megaterium* and *Rhodopseudomonas spheroides.* In *Azotobacter vinelandii* and *Haemophilus parainfluenzae* the terminal cytochrome oxidase is cytochrome aa_2, with cytochrome *o* also present. Cytochrome *a* is absent from *Escherichia coli, Staphylococcus aureus, Acetobacter suboxidans* and *Rhodospirillum rubrum.* In these latter organisms cytochrome *o* can act as an alternative terminal oxidase. In *Azotobacter suboxidans* cytochrome *o* is the only terminal oxidase whereas an alternative acceptor e.g. cytochrome *d* in *Escherichia* or the light generated acceptor in *Rhodospirillum* is found in others. Cytochrome *o* also occurs in a number of yeasts and in a few parasitic protozoa, e.g. *Trypanosoma mega.*

Cytochromes *b* are widely present in aerobic bacteria. In a few cases it is easy to show that there are two spectroscopically recognisable forms. In *Paracoccus* NADH and succinate reduce cytochrome b_{560} (see Fig. 1), dithionite in addition reduces cytochrome b_{566}. The amount of the cytochromes *b* goes up three-fold when *Paracoccus* is grown on nitrate and cytochrome *o* (a modified cytochrome *b*) also increases markedly under these conditions. The *spectroscopic* examination of cytochromes *b* in kinetic experiments is complicated in many cases. *E. coli* has now been shown to have two cytochromes *b.* Cytochrome *b* is widely distributed in aerobic bacteria, though it has not always been shown to be present in more than one form.

Cytochrome *c* is present as cytochrome *c* and c_1 in *Paracoccus denitrificans, Mycobacterium phlei, Bacillus subtilis, Micrococcus lutea* and many other bacteria. There is however no easily detectable cytochrome *c* in *E. coli, Staphylococcus aureus,* and *Aerobacter aerogenes,* although fourth order derivative spectroscopy suggests that cytochrome *c* may be present in small amounts in some of these organisms.

The electron transport chain in mitochondria is very sensitive to rotenone, which is thought to inhibit the NADH/ubiquinone step. That of *Paracoccus denitrificans* and *Mycobacterium flavum* is rather less sensitive. The electron chains in most bacteria, including *Mycobacterium phlei,* are quite insensitive to added rotenone [6]. This pattern of sensitivity is difficult to explain. So is the enormous difference of sensitivity to antimycin *a,* which in mitochondria readily inhibits electron transport at the level of cytochrome *b. Paracoccus denitrificans* and *Mycobacterium flavum* are quite sensitive to antimycin *a, Mycobacterium phlei* and *Micrococcus lutea* are insensitive and *Azotobacter vinelandii* is sensitive only to very high concentrations of antimycin *a.* Since these observations depend on the access of the inhibitor to the appropriate site the interpretation of these data could be very complicated [6].

The number of protons pumped by the electron transport chain appears to differ in different aerobic bacteria. The published values may well depend on the details of the experimental procedures used and the precise conditions of growth of the organism. Based on an oxygen pulse technique in which the

amount of ADP accumulated is related to the amount of oxygen given in a single dose, the H^+/O ratio in mitochondria is 8 when NADPH is offered as substrate and similar values have been obtained for whole cells of *Paracoccus* and for *Paracoccus* particles. When cells of *Paracoccus denitrificans, Hydrogenomonas eutropha* and *Bacillus subtilis* have been starved of substrate and are then given an oxygen pulse they have given a H^+/O ratio of 8, implying the endogenous accumulation of NADPH or its equivalent. The H^+/O ratios from *Escherichia coli* with malate as substrate are reported to be 4 and with succinate to be 2. *Bacillus megaterium* has been reported to give a ratio of 4 and *Acetobacter suboxidans* and *Kurthia zopfii* seem only to give a ratio of 2. The efficiency of coupling of ATP synthesis to oxygen used (P/O ratio) is reported as between 1 and 2 for several bacteria but never as high as the 3 characteristic of mitochondria. A P/O ratio with NADH of 1.5 has been reported for *Paracoccus denitrificans,* 0.9 for *Pseudomonas saccharophila,* 1.0 for *Azotobacter vinelandii,* 1.2 for *Mycobacterium phlei,* and 1.8 for *Nitrobacter agilis.* By contrast a P/O ratio of only 0.3 has been reported for *E. coli.* The P/O ratios with succinate (2 in mitochondria) are much less and at best approximate to 0.5 in those cases where it has been measured, e. g. *Paracoccus denitrificans, Escherichia coli, Pseudomonas saccharophila* [10].

The step between cytochrome *c* and oxygen via cytochrome aa_3 can be made to operate using TMPD (tetramethyl-p-phenylene diamine) as an electron donor to react directly with cytochrome *c* without going through the rest of the electron transport chain. *Paracoccus denitrificans* yields no ADP coupled to this step, neither does *Pseudomonas saccharophila.* However the literature indicates that *Azotobacter vinelandii, Mycobacterium phlei* and *Nitrobacter agilis* all give detectable amounts of ADP in this step, as do mitochondria (P/O = 1).

Respiratory control in *Paracoccus* appears to be imposed at the level of NADH oxidation and it has not been shown with succinate. For any other bacteria the demonstration of respiratory control is less convincing. However indications of an effect of ADP on the rate of electron transport have been noted in preparations from several bacteria and with *Nitrobacter winogradskyi* the addition of ADP is reported to double the rate of electron transport and the rate falls to the original when the ADP is used up. Occasionally *Pseudomonas denitrificans* has been reported as showing a cycle of respiratory control.

The subunit structure of ATPase has been examined in some detail in *Escherichia coli* and some other facultative aerobes. Studies of selected mutants of *E. coli* indicate that as in mitochondria the ATPase consists of α, β, γ, δ, and ε subunits. In addition there are good indications that the subunits are arranged within the membrane in a configuration like those in mitochondria as well as in chloroplasts [14]. It is likely that ATPase will be shown to have an essentially similar structure in all groups of aerobic organisms when their ATPases are examined in detail.

Summary

The characteristic branched chain found in aerobic bacteria serves to differentiate them from normal mitochondria, although there are a few special cases of branched chains in plant mitochondria e. g. *Arum spadix* and ripening fruit in the climacteric. Typical examples are represented in Fig. 1 *(Paracoccus),* Fig. 3 *(Escherichia)* and Fig. 4 *(Azotobacter).* A study of the energy metabolism of aerobic bacteria reveals the overall similarity of oxidative phosphorylation and electron transport processes with mitochondria but important differences in detail. Such studies show the value of comparative biochemistry and have given us important new clues about biological energetics. The great similarity of the electron transport chains of *Paracoccus denitrificans* (and of *Rhodopseudomonas spheroides*) and of mitochondria have led us to speculate on the biochemical alteration and conservation to be expected during the transition from bacterial endosymbiont to mitochondrial organelle envisaged by the theory of serial endosymbiosis [15]. In addition the inside-out particles of *Paracoccus* have already shown their experimental value in studies of substrate uptake by a proton symport mechanism and in tests of the validity of probes of mitochondrial energy metabolism, since many of the embarrassing problems of access of the probes to the active site are thereby removed. Studies of bacterial respiration are interesting in their own right but also because they can help our understanding of mitochondria.

References

[1] KLUYVER, A. J. (1965): In The Microbe's Contribution to Biology. (KLUYVER, A. J. and VAN NIEL, C. B. eds.) p. 93–129. Harvard University Press.

[2] JOHN, P. (1977): Aerobic and anaerobic bacterial respiration monitored by electrodes. J. Gen. Microbiol. *98,* 231–238.

[3] KORNBERG, H. L., COLLINS, J. F. and BIGLEY, D. (1960): The influence of growth substrates on metabolic pathways in *Micrococcus denitrificans.* Biochim. Biophys. Acta *39,* 9–24.

[4] COX, R. B. and QUAYLE, J. R. (1975): The autotrophic growth of *Micrococcus denitrificans* on methanol. Biochem. J. *150,* 569–571.

[5] DAVIS, D. H., DOUDOROFF, M., STANIER, R. Y. and MANDEL, M. (1969): Proposal to reject the genus *Hydrogenomonas:* taxonomic implications. Int. J. Syst. Bacteriol. *19,* 375–390.

[6] JOHN, P. and WHATLEY, F. R. (1977): The Bioenergetics of *Paracoccus denitrificans.* Biochem. Biophys. Acta *463,* 129–153.

[7] STOUTHAMER, A. H. (1976): Yield Studies in Microorganisms. Meadowfield Press, Shildon, U. K.

[8] JOHN, P. and WHATLEY, F. R. (1970): Oxidative phosphorylation coupled to oxygen uptake and nitrate reduction in *Micrococcus denitrificans.* Biochim. Biophys. Acta *216,* 342–352.

[9] JOHN, P. and HAMILTON, W. A. (1970): Respiratory control in membrane particles from *Micrococcus denitrificans.* FEBS Letters *10,* 246–248.

[10] JOHN, P. and WHATLEY, F. R. (1977): *Paracoccus denitrificans* Davis *(Micrococcus denitrificans* Beijerinck) as a mitochondrion. Adv. Bot. Res. *4,* 51–115.

[11] LUDWIG, B.: Personal communication at this meeting.

[12] BURNELL, J. N., JOHN, P., and WHATLEY, F. R. (1975): The reversibility of active sulphate transport in membrane vesicles of *Paracoccus denitrificans.* Biochem. J. *150,* 527–536.

[13] FERGUSON, S. J., KELL, D. B. and JOHN, P. (1978): Resolution of *Paracoccus denitrificans* membrane vesicles of differing configuration using affinity chromatography. FEMS Microbiol. Letters *3,* 151–154.
[14] HADDOCK, B. A. and JONES, C. W. (1977): Bacterial respiration. Bacteriological Reviews *41,* 47–99.
[15] JOHN, P. and WHATLEY, F. R. (1975): *Paracoccus denitrificans* and the evolutionary origin of the mitochondrion. Nature (Lond.) *254,* 495–498.

Address of the author:
Prof. Dr. F. R. WHATLEY, School of Botany, University of Oxford, South Parks Road, Oxford OX1 3RA, UK.