The Evolution of Oxygen As a Biosynthetic Reagent*

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ABSTRACT The biosynthesis of certain cell constituents: monounsaturated fatty acids, tyrosine, and nicotinic acid, is oxygen-dependent in many higher organisms. The same compounds can be synthesized by different, oxygen-independent pathways in lower organisms. The general outlines of these pathways are described and the importance of the compounds synthesized is discussed. An examination of the distribution of these pathways among living organisms reveals that oxygen-dependent pathways replaced the "anaerobic" pathways at different branch points on the evolutionary tree. Other groups of compounds are discussed, which are not distributed as widely among living organisms, but are found in all higher organisms. These compounds have specialized functions and their biosynthesis requires molecular oxygen. The oxygen-dependent portions of the biosynthetic pathways leading to porphyrins, quinone coenzymes, carotenoids, sterols, and polyunsaturated fatty acids are summarized. The distribution and functions of these compounds are also considered and an attempt is made to place them in the framework of evolution. While sterols and polyunsaturated fatty acids are found exclusively in the higher Protista and multicellular organisms, carotenoids, porphyrins, and quinones are also found in bacteria. The possibility of oxygen-independent mechanisms for their biosynthesis is discussed.

The appearance of oxygen in the earth's atmosphere was an event of profound significance for the evolution of life. On the one hand, it provided a means for converting organic substrates into molecules of much lower energy content than could be achieved by anaerobic processes, resulting in an efficient utilization of these materials for energy production; and, on the other hand, it provided a biochemical reagent whose versatility and importance can only be appreciated by a careful examination of biosynthetic reactions as well as degradative reactions. The statement that all the higher forms of life are

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aerobic, may at first glance seem commonplace, and may be ascribed to the greater efficiency of energy production, to the development of respiratory organelles within the cells, and to the development of cells, tissues, and organ systems for the transport of oxygen to all parts of the body. I should like to develop the theme that oxygen, functioning as a biosynthetic reagent, must have played an important role in the evolution of these subcellular organelles and tissues.

In the areas of catabolism and anabolism, the anaerobic way of life represents the primordial condition. The evolution of life in air can be seen to involve both increasing efficiency and specialization. The basic building blocks of cells: proteins, nucleic acids, polysaccharides, most lipids and the monomeric units from which these polymers are synthesized can all be produced in the absence of molecular oxygen. As far as we know, the same amino acids, nucleotides, and many of the same sugars are present in both anaerobic and aerobic organisms, and there is no reason to believe that the mechanisms of synthesis of the macromolecules formed from these monomeric units differ in any fundamental way in lower and higher forms of life.[1]

As life evolved towards aerobiosis, certain new types of molecules became essential, for example, components of electron transport systems and the respiratory pigments. As will be shown later, new oxygen-dependent reactions were developed in response to this need. First, however, I should like to discuss three biochemical pathways in which oxygen-dependent mechanisms in higher organisms produce the same end-products as those produced anaerobically in lower organisms.

Monounsaturated Fatty Acids

An oxygen-dependent mechanism for the biosynthesis of unsaturated fatty acids in higher organisms was originally sought when Andreasen and Stier pointed out in 1954 that oleic acid is required for the growth of yeast under anaerobic conditions (1).

A requirement for molecular oxygen and TPNH was first demonstrated with a particulate preparation from yeast, which converted the coenzyme A derivatives of long-chain fatty acids to the corresponding Δ^9-monounsaturated fatty acids (2). Studies on another particulate enzyme preparation obtained from *Mycobacterium phlei* have revealed further requirements for Fe^{++} and a flavin, either FAD or FMN (3). Requirements for TPNH and molecular oxygen are characteristic of mixed function oxidases or hydroxylases, enzymes which catalyze the incorporation of one atom of atmospheric oxygen into

[1] Cohen has cautioned against an uncritical acceptance of the unity of macromolecular biosynthesis in lower and higher forms of life (77).

various substrates (4). The other atom is presumed to be reduced to water. An hydroxylated intermediate has been sought in both the *M. phlei* and yeast fatty acid desaturation reaction, but no oxygenated intermediate has been isolated and the detailed mechanism is still not understood. Desaturation of long-chain fatty acids occurs in animals and the reaction appears also to be oxygen-dependent (5).

Anaerobic microorganisms also synthesize monounsaturated fatty acids and it is obvious that the mechanism just described is unavailable to them. In searching for an alternative mechanism, it was found that these organisms cannot desaturate long-chain fatty acids (6). This was the case in a number of bacteria, including some facultative anaerobes (7). With these facultative organisms, it was also learned that the presence of oxygen in the atmosphere did not influence the mechanism of formation of unsaturated fatty acids or the extent of unsaturated fatty acid synthesis. A potential mechanism was revealed when it was shown that medium chain-length fatty acids, for example octanoic and decanoic acids, were converted by growing clostridia into both long-chain saturated and unsaturated fatty acids (6). The precursors were found to be incorporated intact and to form the methyl terminal portion of the end-product acids (8). Since this is an anaerobic pathway, we need not go into the details of the mechanism. The hypothesis that the formation of the double bond probably involves a β, γ-desaturation of a β-hydroxy fatty acid intermediate has recently been confirmed in experiments with cell-free systems from *E. coli* (9). There is another important difference between the so called aerobic and anaerobic pathways. The anaerobic pathway in bacteria usually leads to the formation of the Δ^9-C_{16} acid, palmitoleic acid, and the Δ^{11}-C_{18} acid, *cis*-vaccenic acid, whereas the aerobic mechanism leads to the formation of the Δ^9-isomers in both cases. We think this is important because the Δ^9-C_{18} acid, oleic acid, is the starting point for the formation of the polyunsaturated acids, which will be discussed later.

There is no clear-cut phylogenetic division between organisms having the anaerobic pathway and those carrying out an oxidative desaturation. Most eubacteria studied thus far have the anaerobic pathway. This is true for aerobic pseudomonads and facultative anaerobes, *e.g. E. coli*, as well as strict anaerobes. We stress this point because the term anaerobic pathway has led to the false impression that it is found only in anaerobic organisms. The oxygen-dependent desaturation is used by many different types of organisms, from actinomycetes and myxobacteria, to fungi and protozoa, on up to the vertebrates (10). The recent finding that several eubacteria also carry out an oxidative desaturation has made the boundary somewhat fuzzy (11). The formation of monounsaturated fatty acids in plants is also oxygen-dependent but the mechanism is still unclear (10).

Tyrosine

In the next example of evolution away from an anaerobic pathway, mammals appear to have lost completely the ability to synthesize the benzene ring and have come to rely entirely on exogenous sources. An oxygen-dependent reaction converts phenylalanine to tyrosine (12). A pteridine, dihydrobiopterin, is thought to function as an electron donor in the reaction (13). TPNH also participates by maintaining the pteridine in the reduced state. One atom of oxygen per molecule is incorporated para to the alanine side chain, the other atom of oxygen is reduced to water (14). The physiological importance of this reaction is dramatically illustrated by the genetically determined disorder known as phenylketonuria. In the absence of the enzyme, phenylalanine is converted to phenylpyruvic acid, an abnormal metabolite. Retarded mental development is regularly associated with this metabolic defect.

In animal tissues the hydroxylation reaction takes place even when there is an abundant supply of tyrosine in the diet. Perhaps two other functions are of greater importance. One, the further oxidation of tyrosine to homogentisic acid, eventually leads to disposal of the aromatic ring system. The other, by way of dihydroxyphenylalanine, leads to important end-products of aromatic metabolism, noradrenalin, melanin, and thyroxine. These compounds have specialized functions, and are found only in higher organisms.

The anaerobic pathway to tyrosine is the well known shikimic acid pathway, in which the hydroxyl group of tyrosine is introduced at an early stage and can be traced back to an hydroxyl group of glucose. The anaerobic route to tyrosine is one of the two branches diverging at the prephenic acid stage; the other branch gives rise to phenylalanine (15).

The shikimic acid pathway is widely distributed in nature. It has been found in facultative anaerobes (16, 17), fungi (18), *Euglena*, and is used by higher plants (19).

Nicotinic Acid

The third example of dual pathways leading to the same end-products is that of nicotinic acid, a part of the universal pyridine nucleotide coenzymes. In fungi and animals, nicotinic acid is obtained by an oxygen-dependent degradation of tryptophan. Oxygen participates in the first reaction of this sequence, the opening of the indole ring catalyzed by tryptophan pyrrolase (20). As demonstrated by Hayaishi *et al.*, one mole of oxygen enters into each mole of tryptophan (21). The first product, formylkynurenine, undergoes hydrolysis to kynurenine. Oxygen next enters at the hydroxylation of kynurenine to yield 3-hydroxykynurenine, a reaction similar in many respects to the hydroxylation

of phenylalanine (22). Finally, the benzene ring of 3-hydroxyanthranilic acid is cleaved by another oxygenase. The last process provides nicotinic acid ribotide by a suitable rearrangement to quinolinic acid followed by addition of ribose phosphate (23). Two atoms of oxygen are consumed in the reaction catalyzed by 3-hydroxyanthranilic acid oxidase (24).

Degradation of tryptophan in animals also provides several excretory products, including xanthurenic and quinolinic acids, as well as the precursors for ommochromes, the characteristic visual pigments of insects (25).

Of the anaerobic pathway to nicotinic acid, we can say little. The reactions have not been studied in strict anaerobes, but many anaerobes do not require an exogenous supply of this vitamin. Ortega and Brown have found that C^{14}-labeled glycerol and succinic acid serve as precursors of nicotinic acid in resting *E. coli* (26). Similarly, in plants, tryptophan does not serve as a precursor of nicotinic acid, but such small molecules as glycerol, succinic acid, and aspartic acid have been found to serve as precursors of the pyridine moiety of nicotine (27, 28) and the pyridine nucleus of ricinine, a related compound (29, 30). No closer relationship has been established between the bacterial and plant pathways to nicotinic acid and its derivatives.

Oxygen-Dependent Biosyntheses of Specialized Components of Higher Organisms

In the three pairs of pathways discussed so far, all organisms have to have at least one member of the pair, usually to the exclusion of the second. The next group of biosynthetic pathways is characteristically absent in most anaerobic microorganisms. Each aerobic pathway makes its appearance at some branch point on the evolutionary tree, usually when a need for the compound synthesized arises. We have referred to these compounds as specialized compounds, in distinction to the universal components, tyrosine, monounsaturated fatty acids, and nicotinic acid (31).

Porphyrins

Though porphyrins make their appearance early in evolution, they are not found in the strictly anaerobic clostridia, the microaerophilic lactobacilli, or in the facultatively anaerobic streptococci or pneumococci (32). Not all anaerobes are devoid of heme pigments. Some sulfate-reducing and denitrifying bacteria, which perform a so called anaerobic respiration, are known to be rich in cytochromes (32). In addition many photosynthetic bacteria are well endowed with hemoproteins of both the cytochrome and chlorophyll types when grown under anaerobic conditions. The association of porphyrins, in the form of cytochromes, catalases, hemoglobin, and myoglobin, with respiration, is familiar to all students of biology.

Porphyrin biosynthesis, as it occurs in vertebrates, can be divided into two

phases. The early steps in the sequence: the condensation of succinic acid and glycine to yield α-amino-β-ketoadipic acid, its conversion to δ-aminolevulinic acid, the formation of the pyrrole ring, and the condensations which lead to the tetrapyrrole structure of the porphyrinogens (33) are all anaerobic reactions. The subsequent reactions leading from coproporphyrinogen to protoporphyrinogen and the further transformation of the hexahydroporphyrin structure to that of porphyrin are catalyzed by mitochondrial enzymes and require molecular oxygen. In the reaction studied by Sano and Granick, two propionate side chains of coproporphyrinogen are converted to vinyl groups by an oxidative decarboxylation. No other oxidant, including flavins, cytochrome *c*, or artificial electron acceptors, is effective in replacing molecular oxygen (34). That this requirement for molecular oxygen may also exist at the microbial level is indicated by the finding that *Bacillus cereus* and *Staph. aureus* accumulate coproporphyrinogen under anaerobic conditions (35).

If oxygen participates as a reagent in these reactions, we are faced with the problem of porphyrin synthesis in the anaerobic microorganisms which have cytochromes and chlorophyll. Several years ago we suggested the possibility that an oxidant produced by the photosynthetic process can fulfill the biosynthetic role of molecular oxygen in the case of the photosynthetic bacteria, which, unlike higher plants, do not produce oxygen (31). It is well known that both reductants and oxidants are produced in the photolysis of water (36).

Even if this hypothesis is eventually substantiated it is inadequate to explain the presence in anaerobic sulfate-reducing and denitrifying bacteria of cytochromes in which the heme moiety has vinyl side chains. Perhaps, sulfate, nitrate, or their metabolites serve in place of oxygen in these reactions as well as in respiration. Lemberg has suggested that the vinyl side chains, which are linked to cysteine residues of protein in these cytochromes, could be produced anaerobically by reaction of the cystine disulfide bond with the propionate side chains:

$$-CH_2CH_2COOH + R-S-S-R \rightarrow -\underset{\displaystyle S\\ \displaystyle R}{CH}-CH_3 + CO_2 + RSH \quad (37).$$

Lemberg's proposal has recently received some experimental support from the work of Sano *et al.*, who showed that free cysteine reacts with protoporphyrinogen during autoxidation of the latter in an acid medium, and also under anaerobic conditions. The yield in the absence of oxygen was not as great (38). This non-enzymic reaction yielded a product which, like *c*-type cytochromes, had a thiol ether linkage to the α-carbon atom of the vinyl side chains.

The control of tetrapyrrole biosynthesis has recently been reviewed by Lascelles (39). Several points are worth making in the context of this discussion. Although oxygen is required for the conversion of coproporphyrinogen to porphyrin, there are other steps in the synthesis of heme, that are inhibited by oxygen. Ferrochelatase, the pig liver mitochondrial enzyme system that catalyzes the insertion of ferrous ions into protoporphyrin, is one example (40). Another oxygen-sensitive reaction is the conversion of porphobilinogen to uroporphyrinogen (41). These effects may explain the finding that heme formation in avian erythrocytes (42), *Aerobacter aerogenes* (43), *Pseudomonas fluorescens* (44), and other microorganisms (39) is optimal at low to moderate oxygen concentrations but falls off at higher concentrations. On the other hand, it is well known that many microorganisms have markedly reduced cytochrome levels in the *absence* of oxygen; *e.g.*, yeast (45, 46), *Bacillus cereus* (47), and *A. aerogenes* (43). Thus oxygen controls heme synthesis both by its participation as a reagent and by indirect effects on enzyme activities and possibly on protein synthesis itself (48).

Quinone Coenzymes

The problem of quinone coenzyme biosynthesis appears to be an extension of the problem of aromatic biosynthesis, on the one hand, and isoprene biosynthesis, on the other. The steps leading to the polyisoprenoid side chain are similar to those leading to the synthesis of squalene, a precursor of sterols. These steps, for reasons we shall discuss later, are assumed to be independent of molecular oxygen.

In rat tissues, the ring systems of phenylalanine and tyrosine serve as precursors of the quinone moiety of coenzyme Q. The aerobic hydroxylation of phenylalanine followed by conversion of tyrosine to homogentisic acid has been proposed as an oxidative route to the formation of the quinone system (49). The finding that coenzyme Q synthesis is inhibited in yeast under anaerobic conditions also supports an oxygen-dependent pathway (50). Recent work has implicated *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid as intermediates between tyrosine and coenzyme Q in rats and yeast (51). Extremely interesting is the finding that *p*-hydroxybenzoic acid is also a probable intermediate in the shikimic acid pathway leading to coenzyme Q in *E. coli* (52). Cox and Gibson have also provided evidence which indicates that the naphthoquinones arise from the shikimic acid pathway by way of 3,4-dihydroxybenzaldehyde (52). Whether these final steps leading to quinone formation in bacteria are aerobic or anaerobic is not known at this time. There is disagreement in the literature concerning the presence of coenzyme Q in anaerobic *E. coli* (53, 54).

Functionally, quinone coenzymes are associated with respiration and oxidative phosphorylation and their distribution in nature parallels that of

respiratory and photosynthetic systems. In addition to a wide variety of higher organisms they have been found in many bacteria, with the exception of clostridia and lactobacilli (53, 54). Two recent reports indicate the presence of quinones in certain anaerobic species; *e.g.*, bacteroides and *Veillonella alcalescens* (55, 56)

Carotenoids

Carotenoid biosynthesis also branches off from the polyisoprenoid pathway. The condensation of two geranyl-geraniol units yields a 40-carbon compound, which is either lycopersene or phytoene (57). In analogy with squalene biosynthesis, these reactions are very probably oxygen-independent. Indeed, phytoene is known to accumulate in certain carotenoid-synthesizing microorganisms under anaerobic conditions. The introduction of four additional double bonds to form lycopene, however, is probably oxygen-dependent. Suzue has shown that extracts of *Staph. aureus* are capable of converting phytoene to δ-carotene, a cyclization product of lycopene, only in the presence of oxygen (58). Since the cyclization of lycopene to form β-carotene has been reported to take place under anaerobic conditions (59), we tentatively assign the oxygen-dependent portion of the pathway to the formation of lycopene. Varma and Chichester have provided additional support for this hypothesis with their finding that tomato homogenates require oxygen for the conversion of isopentenyl pyrophosphate to lycopene (60). Rilling has recently reported that potassium ferricyanide can replace oxygen as an electron acceptor for this process in a species of mycobacterium (61).

As is the case with porphyrins, carotenoids are not found in anaerobic clostridia and lactobacilli, but they are found in strictly anaerobic photosynthetic bacteria. These organisms synthesize carotenoids under anaerobic conditions only in the presence of light. In the absence of light, oxygen is necessary for carotenoid synthesis (62). These facts strengthen the argument that a photolytically produced oxidant participates in biosynthetic reactions in these primitive organisms. Animals lack the ability to synthesize carotenoids *de novo*, so that all their carotenoids are derived from plant and microbial products (57).

A variety of carotenoids (xanthophylls) contain oxygen functions, and most of these appear to be derived from carotenes during the late stages of biosynthesis. These reactions are also light- or oxygen-dependent. In a number of cases the oxygen of the carotenoid structure has been shown to arise from $^{18}O_2$ rather than from $H_2^{18}O$ (63, 64).

Sterols

I should now like to turn to the biosynthesis of two classes of compounds, sterols and polyunsaturated fatty acids, whose members are found only in

higher organisms. Stanier and van Niel have advocated an important and useful division among the protists, a group which includes bacteria, fungi, protozoa, and algae. Those cells in which the cytoplasmic membrane is the only bounding element which can be structurally defined have been designated procaryotic. This group includes the bacteria and the blue-green algae. The eucaryotic organisms are those which possess such membrane-limited organelles as nuclei, mitochondria, endoplasmic reticulum, and chloroplasts. This group includes all the other algae, the fungi, and the protozoa (65). It is probably no accident that sterols and polyunsaturated fatty acids distribute themselves almost precisely along the same lines with one exception, the presence of polyunsaturated fatty acids in the blue-green algae (10). Polyunsaturated fatty acids have been found in no other procaryotic organisms and sterols have been found only in the eucaryotic organisms.

In summarizing the biosynthesis of the quinone coenzymes and the carotenoids, I have already alluded to an anaerobic phase in the biosynthesis of sterols. The condensation of isopentenylpyrophosphate to yield farnesylpyrophosphate and the condensation of two of these 15-carbon units to yield squalene are known to occur under anaerobic conditions. This has been demonstrated in yeast and with animal tissue enzymes (66, 67). From the cyclization of squalene on to the synthesis of cholesterol, there are at least five separate steps, all catalyzed by particulate enzymes, in which molecular oxygen is utilized, the cyclization of squalene to lanosterol (68, 69), the first step in the removal of each of three methyl groups from lanosterol (70), and the double bond shift in ring B of zymosterol (71).

While the cyclization of squalene could conceivably occur in the absence of oxygen, aerobic cyclization possesses the advantage of placing an hydroxyl group derived from molecular oxygen in the 3-position of cholesterol (68, 69). This functional group is of importance in a number of subsequent transformations of cholesterol. The necessity for oxygen in the elimination of the methyl groups of lanosterol as CO_2 is easier to rationalize, since dehydrogenation is excluded as an initiating reaction on fully substituted carbon atoms.

Oxygen plays an essential role in the further metabolism of cholesterol to the bile acids and steroid hormones. Hayano has recently reviewed the numerous oxygenases participating in these processes (72). Oxygen enters the 7α-, 12α-, and 16α-positions of the sterol nucleus and also participates in the degradation of the cholesterol side chain during the formation of the bile acids. In the formation of the steroid hormones, oxygen has been implicated in the degradation of the cholesterol side chain to yield pregnenolone (72), a precursor of progesterone. This product in turn is converted by way of other oxygen-dependent pathways to the androgens and, from these, to the estrogens. Formation of the estrogens requires oxidative removal of the angular C-19 methyl group as a prelude to aromatization of ring A (72). The biogenesis of

the corticosteroids in the adrenal cortex is replete with hydroxylation reactions involving the 11β-, 17α-, 18-, and 21-positions.

As structural components of cells, sterols appear to play a major role in the eucaryotic cytoplasmic membrane and endoplasmic reticulum (73). The importance of the steroid hormones for integration of many of the activities of tissues and organs in animals could not be justly discussed in a short review.

Polyunsaturated Fatty Acids

The comparative biochemistry of polyunsaturated fatty acid synthesis is another area of biosynthesis that has recently developed along interesting lines, since there are marked differences from one phylogenetic group to the next. Some generalizations can be made. First, the starting point for the insertion of additional double bonds is usually the Δ^{9}-C_{18} compound, oleic acid. Second, the insertion of additional double bonds into monoenoic acids is an oxygen-dependent process in all systems in which it has been studied (10). Third, this process does not occur in any species of bacteria investigated to date (10). From here on the pattern is broken up along evolutionary lines. Fungi and green plants are capable of synthesizing α-linolenic acid ($\Delta^{9, 12, 15}$-C_{18}), whereas ciliates synthesize γ-linolenic acid ($\Delta^{6, 9, 12}$-C_{18}). Vertebrates are blocked in the introduction of a second double bond into oleic acid. They require a source of linoleic acid ($\Delta^{9, 12}$-C_{18}) in the diet, which they then convert to polyunsaturated fatty acids of the γ-linolenic type (*e.g.* arachidonic acid) (10).

The α-linolenic acid of green plants is found in the photosynthetic apparatus in the form of mono- and digalactosyl glycerides (10). Lipids of this type have not been found in animal tissues. Animal polyunsaturated fatty acids are also found in association with intracellular organelles, particularly the mitochondrial membrane. Recent work of Fleischer, Green, and their associates has demonstrated the importance of these lipids for electron transport in isolated mitochondria (74).

CONCLUDING REMARKS

Oparin (75) and J. B. S. Haldane (76) postulated the origin of life during the period in which the earth's atmosphere was anaerobic. In addition to citing evidence for an anaerobic atmosphere preceding the present aerobic era, they also noted that fermentative metabolic processes in living organisms of widely different phylogenetic groups are very similar, while oxidative processes tend to be more diverse. They believed that this fact also argued for a prior origin of anaerobic organisms.

The biosynthetic pathways discussed in this paper also support their postulate (Table I). Eubacteria, the simplest organisms morphologically, and perhaps the most closely related to the primitive forms of life, possess anaerobic

mechanisms for synthesizing monounsaturated fatty acids, tyrosine, and nicotinic acid. On the other end of the scale, vertebrates have aerobic mechanisms for the synthesis of these compounds. In each case, the aerobic mechanism appears to have evolved at a different branch point in the phylogenetic sequence. The oxidative pathway to nicotinic acid was adopted by the ancestors of present-day fungi as well as by those of the vertebrates. The oxidative pathway to tyrosine appears to have been adopted only by animals,

TABLE I

	Unsaturated fatty acid synthesis	Nicotinic acid synthesis	Tyrosine synthesis	Porphyrins	Quinone coenzymes	Carotenoids	Sterols	Polyunsaturated fatty acids
Animals (mammals)	0	0	0	+	+	+*	+	+*
Protozoa	0	N?	0?	+	+	+‡	+	+
Higher plants	0	N	N	+	+	+	+	+
Algae	0	N?	N?	+	+	+	±§	+
Fungi	0	0	N	+	+	+	+	+
Yeast	0	0?	N	+	+	+	+	+
Bacteria		N	N‖			±	−	−
Actinomycetales	0			+	+			
Pseudomonadales	N			+	+			
Eubacteriales	N¶			±**	±‡‡			

O = oxygen-dependent; N = anaerobic; + = present; − = absent

* Derived from dietary sources.
‡ In phytoflagellates (78).
§ Not in blue-green algae.
‖ Some pseudomonads can oxidize phenylalanine to tyrosine, but they may have the shikimic acid pathway for aromatic biosynthesis as well.
¶ With some exceptions including a bacillus, a micrococcus, and a species of corynebacteria.
** With the exception of the clostridia, lactobacilli, and some streptococci.
‡‡ Found in most bacteria, with the possible exception of clostridia and lactobacilli. Two recent reports indicate their presence in certain anaerobic species, *e.g.* several bacteroides and *Veillonella alcalescens*.

while the aerobic desaturation of fatty acids appears in all present-day groups of higher organisms.

Furthermore, a number of oxygen-dependent biosynthetic reactions only appear in higher organisms, and these reactions are on pathways leading to such compounds of specialized function as sterols and polyunsaturated fatty acids. Thus, oxygen, in cooperation with certain enzymes, provided living systems with materials needed for life at a higher, more organized stage of evolution.

Carotenoids, porphyrins, and quinones occupy an indeterminate position between the two groups of compounds summarized above. Although they are usually thought of as specialized compounds involved in photosynthesis and

respiration, all are found in certain anaerobic organisms as well as higher organisms. In photosynthetic bacteria a combination of light and anaerobic conditions results in the synthesis of carotenoid pigments and porphyrins. Porphyrins and quinones are also found in a few species of non-photosynthetic, obligate anaerobes. Apparently, as is the case with monounsaturated fatty acids, oxygen-dependent pathways for the synthesis of carotenoids, porphyrins, and quinones evolved very early in the aerobic era. A number of biosynthetic problems concerning both the anaerobic and aerobic biosynthesis of these compounds will have to be solved before a coherent scheme for their biochemical evolution can be formulated.

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