for the difficult machining of high-strength steel or titanium. The use of graphite-fiber-reinforced composites will allow greatly simplified machining procedures.

Summary

Advanced composite materials offer the potential for major savings in weight for a variety of aerospace and other structural systems. Weight savings of 10 to 50 percent have been demonstrated in comparisons of components made of these materials with corresponding metal components now used in space vehicles, aircraft, missiles, and gas turbine engines. These savings were realized through understanding of the properties and complex mechanical behavior of the basic constituents and through development of special techniques for fabricating fiber-reinforced composites.

Despite the current emphasis on boron- and graphite-fiber-reinforced epoxy composites, these materials should be considered only the first of a family or class of advanced composite materials. Various types of fiber reinforcements are either already available or rapidly becoming available, and this makes it necessary to understand the processes and economics of producing the reinforcements and matrix materials and to weigh the costs associated with the fabrication of composite structural components.

Through effective utilization of one or more of the advanced composites and an increased understanding of their inherent characteristics, improvements in performance will undoubtedly continue to be demonstrated. It seems certain that high-strength, stiff, light-weight advanced composites will come into at least limited use in the near future, their potential for general use being limited only by the eventual costs of producing them in quantity.

References and Notes

1. "Advanced composite materials," although something of a misnomer, has become a generally accepted phrase. "Improved composite materials" would be more precise.
2. For a general discussion, see G. Lubin, *Handbook of Fiberglass and Advanced Plastic Composites* (Van Nostrand, New York, 1969).
3. J. E. Ashton, J. C. Halpin, P. E. Petit, *Primer on Composite Materials: Analysis* (Technomic, Stamford, Conn., 1969).

4. I thank R. C. Tomashot, J. R. Rhodehamel, F. M. Wilson, and R. L. Rapson for their assistance in the preparation of this article.

The Evolution of Photosynthesis

Hypothesis: Photosynthetic bacteria and blue-green algae shared a common photoheterotrophic ancestor.

John M. Olson

On the basis of cell morphology, bacteria and blue-green algae form a single class of prokaryotic organisms that are characterized by the absence of a nuclear membrane, the absence of plastids, and the absence of a mitotic figure during cell division (*1*). Echlin and Morris (*2*) summarize the morphological features and the similarities in cell wall chemistry, which distinguish the bacteria and blue-green algae from eukaryotic organisms, that is, those whose cells are characterized by a well defined nucleus, presence of organelles, and division by mitosis. Of the various types of modern bacteria, the blue-green algae are obviously related most closely to the photosynthetic bacteria. Blue-green algae and photosynthetic bacteria both contain chlorophylls and carotenoids and convert light energy into chemical free energy. Based on this fundamental similarity in pigment content and function, it is postulated that present-day blue-green algae and photosynthetic bacteria have evolved from a common, chlorophyll-containing, prokaryotic ancestor.

The fundamental difference between the photosyntheses of blue-green algae and of photosynthetic bacteria is the oxygen evolution accompanying carbon dioxide fixation by the algae. The ability of the algae to produce molecular oxygen from water can be understood in terms of two photochemical steps connected in series, as first suggested by Hill and Bendall (*3*) (Fig. 1). The evidence for the series formulation is sufficiently compelling that it is now the generally accepted theory of oxygen-evolving photosynthesis (*4*).

Until 1967 it was generally thought that bacterial photosynthesis operated on the basis of one photochemical reaction analogous to system 1 in the series formulation. The inability of the bacteria to evolve oxygen was explained by the absence of the second photochemical step (system 2). It now appears that at least two species of bacteria, *Chromatium* (*5*, *6*) and *Rhodospirillum rubrum* (*7*), may carry out two photochemical reactions in parallel; one photochemical reaction center driving a cyclic electron transport chain for the production of adenosine triphosphate (ATP), and the other driving a noncyclic electron transport chain linked to substrate oxidation (Fig. 2). Thus, the fundamental requirement for oxygen evolution appears to be two reactions connected in series.

Basic Assumptions

My hypothesis for the physiology of the common ancestor and the subsequent evolutionary development of the various types of photosynthetic bacteria and algae is based on three assumptions in addition to those already mentioned.

1) The heterotrophic hypothesis. At the time the ancestral photobacterium existed, its aqueous environment contained organic compounds left over from the prebiotic phase of chemical evolution (*8*).

2) The Berkner-Marshall theory for the origin of oxygen in the atmosphere. The present amount of oxygen in the air is due to the photosynthesis of blue-green algae and eukaryotic green plants

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This article is dedicated to Louise A. Schwabe and C. B. van Niel. A condensed version was presented at the XI International Botanical Congress held in Seattle, Washington, 24 August–2 September 1969.

for the past 2 billion years or so (*9*). At the time of the ancestral photobacterium, the environment was essentially anoxygenic, and the atmosphere contained hydrogen (H_2), nitrogen (N_2), methane (CH_4), ammonia (NH_3), water (H_2O), and carbon dioxide (CO_2) (*10*).

3) The estimates of Ross and Calvin (*11*) for the maximum free energy storage in photochemical reactions of chlorophyll, 1.2 electron volts for chlorophyll a and 0.8 electron volt for bacteriochlorophyll a.

The Common Ancestor

The ancestral photobacterium is assumed to have existed earlier than 3 billion years ago when the earth was less than 2 billion years old. Several investigators (*12*) date the remnants of prokaryotic organisms at about 3 billion years ago, and the chemical evidence for chlorophyll in appreciable amounts on the earth also dates back roughly 3 billion years (*13*).

In a heterotrophic environment, the ancestral photobacterium utilized light to drive reactions for assimilation of the exogenous nutrients. Modern examples of this type of photometabolism are found among the heterotrophic photosynthetic bacteria, especially the Athiorhodaceae, and a number of algae adapted to heterotrophic conditions. For example, the blue-green alga *Chlorogloea fritschii* (*14*) photoassimilates sucrose in the absence of CO_2 after adaptation, and the green algae *Chlamydomonas mundana* (*15*) and *Chlamydobotrys* (*Pyrobotrys*) *stellata* (*16*) photoassimilate acetate with essentially no CO_2 fixation and no evolution of oxygen. Both *Chlorella* and *Scenedesmus* will photoassimilate glucose in the absence of oxygen and CO_2 (*17*). In all of the cases cited, the evidence suggests that photoassimilation requires the light-driven generation of ATP which is supplied by the operation of a cyclic electron transport system. No external electron donors or acceptors are required. "Cyclic electron transport systems for the generation of ATP appear to be ubiquitous in green plants, algae, and photosynthetic bacteria" (*6*). It is proposed that the ancestral photobacterium possessed only a cyclic electron transport system for the efficient assimilation of external nutrients (*18*).

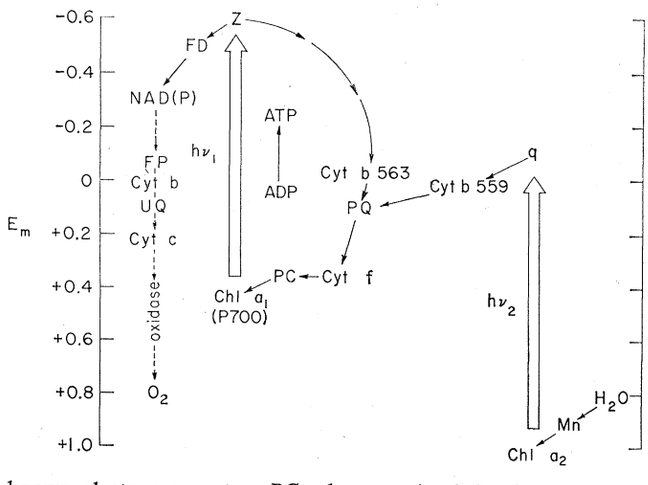
The chlorophyll serving as the reaction center and the light collector in this organism must have been closely related to chlorophyll a and bacteriochlorophylls a and b, the present-day reaction center pigments for algae and bacteria, respectively. The conversion of chlorophyll a to bacteriochlorophyll a requires the addition of one molecule of H_2O to the vinyl group on ring 1 and an intramolecular transfer of two hydrogen atoms to ring 2.

In the photosynthetic bacterium *Rhodopseudomonas spheroides*, the intermediates in the biosynthetic pathway for synthesis of bacteriochlorophyll a appear to be very similar, if not identical, to the intermediates in the synthesis of chlorophyll a in algae and higher plants (*19*). The most probable interpretation of the available data indicates that bacteriochlorophyll a is derived from chlorophyllide a in photosynthetic bacteria (*20*). If this interpretation is correct, it seems most likely that chlorophyll a evolved before bacteriochlorophyll a, and that the ancestral photobacterium utilized chlorophyll a in the photochemical reaction center which was part of the cyclic system of electron flow shown in Fig. 3.

This cyclic system is thought to have consisted of the primary electron acceptor Z, a large pool of quinone, cytochrome c, and the reaction center chlorophyll a. These components were built into the cell membrane in such a way that the quinone pool could be shared by more than one electron transport chain. The pool could also interact with exogenous reducing agents of about the same potential (that is, ~0 volt). This common pool of shared quinone was a fundamental requirement for the subsequent development of two photochemical systems in series in the algal line of evolution (*21*).

Noncyclic Electron Transport

As the concentration of nutrients in the environment decreased due to their assimilation by heterotrophic organisms, a selective advantage would have been conferred on any photobacterium that, through a few simple mutations, adapted to the utilization of some of the less reduced organic compounds left in the environment. This might well have taken place by a modification of the existing cyclic electron transport chain so as to permit the entry of electrons from exogenous donors and the reduction of exogenous organic compounds suitable for conversion to cell substance. This development is illustrated in Fig. 4.



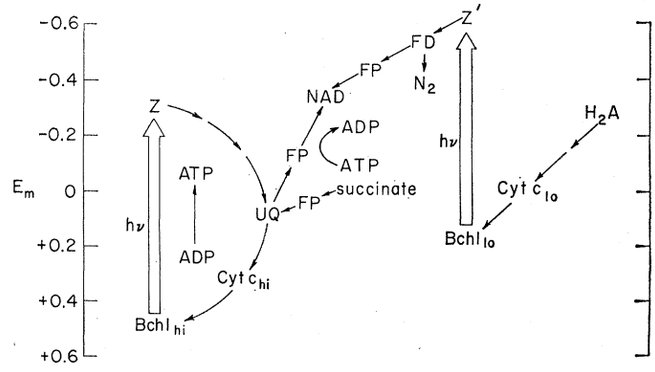


Fig. 1 (left). Electron transport schemes for oxygen-based respiration and oxygen-evolving photosynthesis. Abbreviations used are *Cyt*, cytochrome; *FP*, flavoprotein; *FD*, ferredoxin; *Z*, unknown electron acceptor; *PC*, plastocyanin; *PQ*, plastoquinone; *UQ*, ubiquinone; and *q*, unknown electron acceptor and quencher of chlorophyll fluorescence. Fig. 2 (right). Electron transport scheme for bacterial photosynthesis. The abbreviations used are the same as for Fig. 1 except for *Bchl* (bacteriochlorophyll) and H_2A (exogenous electron donor).

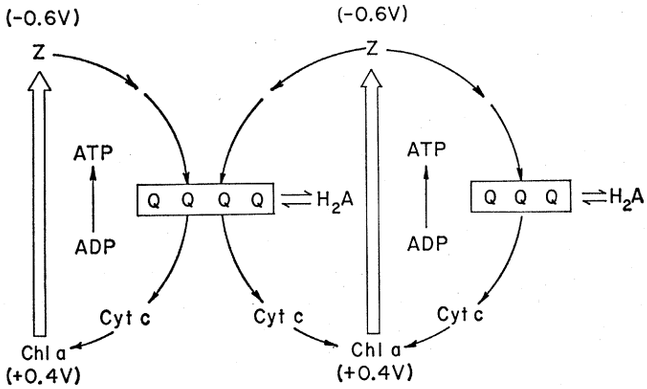
Evolution of Light-Harvesting Systems

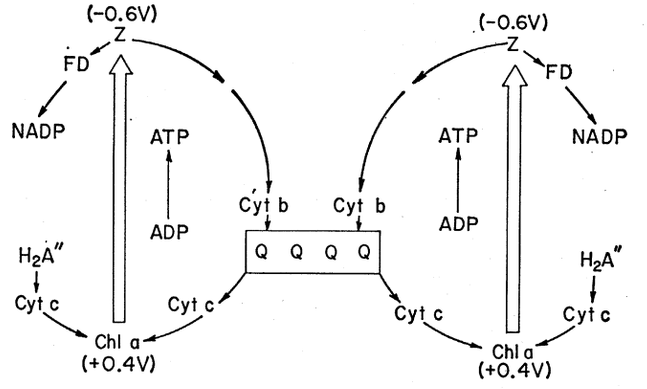
Concurrent with the development of the electron transport system was the development of a more efficient light-harvesting system for driving the reaction centers of the evolving photobacteria. The light-collecting chlorophyll a was probably contained in a chlorophyll-protein complex similar to those found in present-day bacteria, algae and higher plants (*22*). Such a complex would have had a subunit weight of ~40,000 and five molecules of chlorophyll a embedded in each subunit. The chlorophyll-protein complexes would necessarily have been built into the bacterial membrane along with the electron transport components. If any carotenoid were present in the ancestral photobacterium, it might well have been neurosporene (*23*) or lycopene (*24*). The size of the photosynthetic unit may have increased by the addition of more light-collecting chlorophyll per reaction center. Also the development of specialized internal membranous structures (that is, chromatophores, vesicles, or thylakoids) would have greatly increased the pigment content of each photoorganism and its ability to trap the light. At the same time such a development in a densely populated habitat at least 10 meters below the surface of the water would have led to mutual shading and a competition for the available light in the blue region of the spectrum. In such a situation there would have been a strong selective advantage for a mutational switch from chlorophyll a to bacteriochlorophyll a for the underlying bacteria. The advantage of moving from ~ 440 nm (blue band of chlorophyll a) to ~ 370 and 590 nm (violet and orange bands of bacteriochlorophyll) was gained at the sacrifice of ~ 0.4 electron volt in the maximum free energy stored in the primary photoact, and prevented the bacteriochlorophyll-containing line from ever developing the capacity to evolve oxygen.

Evolution of Photosynthetic Bacteria

The first organisms to synthesize bacteriochlorophyll a instead of chlorophyll a probably organized the bacteriochlorophyll a into a chlorophyll-protein complex similar to the chlorophyll a–protein produced prior to the switch. It might be expected therefore that the bacteriochlorophyll absorption spectrum in vivo would show only a single far-red peak at about 800 nm (*25*) in addition to the orange band at about 600 nm. As the blue-green algae developed the capacity to evolve oxygen, and sufficient oxygen accumulated in the atmosphere to form a protective ozone layer, some photosynthetic bacteria moved up toward the surface of the water where a much broader spectral range of sunlight could penetrate. From these bacteria two lines of development emerged.

One line of bacteria gradually modified the association of bacteriochlorophyll with protein so as to produce more highly aggregated states of bacteriochlorophyll. This led to the generation in the spectral region between 800 and 900 nm of new absorption bands which are characteristic of present-day purple bacteria (Thiorhodaceae and Athiorhodaceae) (*26*). This movement of the peak of bacteriochlorophyll absorption toward the infrared has culminated in strains of photosynthetic bacteria which have further mutated from the synthesis of bacteriochlorophyll a to bacteriochlorophyll b, the precise structure of which is still unknown. In *Rhodopseudomonas viridis,* a heterotroph, and in *Thiococcus* sp., an autotrophic sulfur bacterium, the far-red absorption band of bacteriochlorophyll b in vivo is located at about 1.03 micrometers (*26, 27*). These photosynthetic bacteria take advantage of a small spectral window between 0.97 and 1.19 micrometers in the absorption





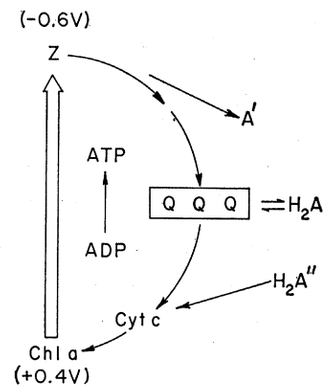


Fig. 3 (left above). Cyclic electron transport scheme for the hypothetical ancestral photobacterium. Symbol *Q* stands for quinone. Other abbreviations as in Figs. 1 and 2.

Fig. 4 (right above). Modification of cyclic electron transport system to enable noncyclic electron flow from exogenous donor H_2A'' to exogenous acceptor A′. It is also possible that A′ could have been reduced by reverse electron flow from H_2A.

Fig. 5 (left). Further modification of cyclic and noncyclic electron transport in the algal line of photosynthetic organisms. H_2A'' represents a class of moderately strong reductants such as hydrazine.

spectrum of water (*28*). The other evolutionary line of photosynthetic bacteria developed an entirely new group of chlorophylls as accessory pigments to bacteriochlorophyll a. These accessory pigments, known as chlorobium chlorophylls (*26*), are the characteristic major light-collecting pigment of the green bacteria (Chlorobacteriaceae). This development permitted the green bacteria to utilize the light in the spectral region between 700 and 800 nm and thereby avoid shading by either purple bacteria or algae (*29*).

Since all present-day photosynthetic bacteria so far studied have at least two c-type cytochromes which appear to function independently in separate electron transport chains, it is assumed that independent cyclic and noncyclic electron transport chains are the rule in photosynthetic bacteria (*6*) (Fig. 2). In an autotrophic sulfur bacterium such as *Chromatium*, the cytochrome c in the noncyclic system has a redox potential about 0.3 volt lower than that of the cytochrome c in the cyclic system. It seems reasonable to think that the development of a low potential electron transport system from exogenous donors such as sulfide, elemental sulfur, thiosulfate and sulfite might be necessary for the light-driven reduction of a powerful reducing agent such as ferredoxin which plays a key role in both nitrogen fixation and CO_2 fixation. It is therefore suggested that the bacterial counterpart of the noncyclic electron transport shown in Fig. 4, gradually shifted in the negative direction of the redox scale in a series of small mutational steps in response to an evolutionary pressure for more and more powerful reductants in environments lacking H_2.

Evolution of Blue-Green Algae

Those photosynthetic organisms which retained chlorophyll a (that is, the blue-green algal line) were able to adapt their noncyclic electron transport systems to the reduction of ferredoxin without substantial modification of the photochemical reaction utilized for the basic cyclic system, because of the 1.2 electron volts available in the photoact. Thus as the supply of exogenous organic substrates was exhausted, the algal prototypes developed the enzymes for a system of CO_2 fixation based on ATP and nicotinamide-adenine dinucleotide (NADP) (reduced by means of ferredoxin).

Sometime in the course of evolution, the algal prototype interposed cytochrome b as a carrier, presumably but not necessarily between the primary acceptor Z and the quinone pool. The reason for supposing that the evolution of cytochrome b came after the divergence of the photosynthetic bacteria that contain bacteriochlorophyll is that there appear to be no b-type cytochromes in either the Thiorhodaceae or the Chlorobacteriaceae (*6*). (However, members of the Athiorhodaceae such as *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides* do appear to contain b-type cytochromes. It is not entirely clear whether the b-type cytochromes function in both respiratory and photosynthetic electron flow in these organisms.) Figure 5 summarizes the supposed structure of the photosynthetic electron transport pathways in the algal prototype.

Although the photosynthetic bacteria were limited either to heterotrophic environments or to highly reducing environments, the algal prototype was able to fix CO_2 with exogenous electron donors (H_2A'' in Fig. 5) that were less powerful reductants than those required by the bacteria. This was permitted by the extra 0.4 electron volt stored in the photoact with chlorophyll a. Eventually some algal prototypes found themselves in environments where the supply of exogenous electron donor was gradually being used up. Under these conditions there was a powerful selective advantage for mutants that could utilize weaker electron donors in place of the former donors. The evolutionary pressure for the utilization of ever weaker electron donors gradually forced one reaction center toward ever higher redox potentials in order to be able to extract electrons from the new donors (*30*). In this manner system 2 evolved from system 1 in a long series of small mutational steps. From the very beginning of the differentiation between the two photosystems they were linked through the common pool of quinone (*21*) (Fig. 5). Thus, the effective utilization of two photochemical reactions in series for the production of a strong reductant (for example, ferredoxin)

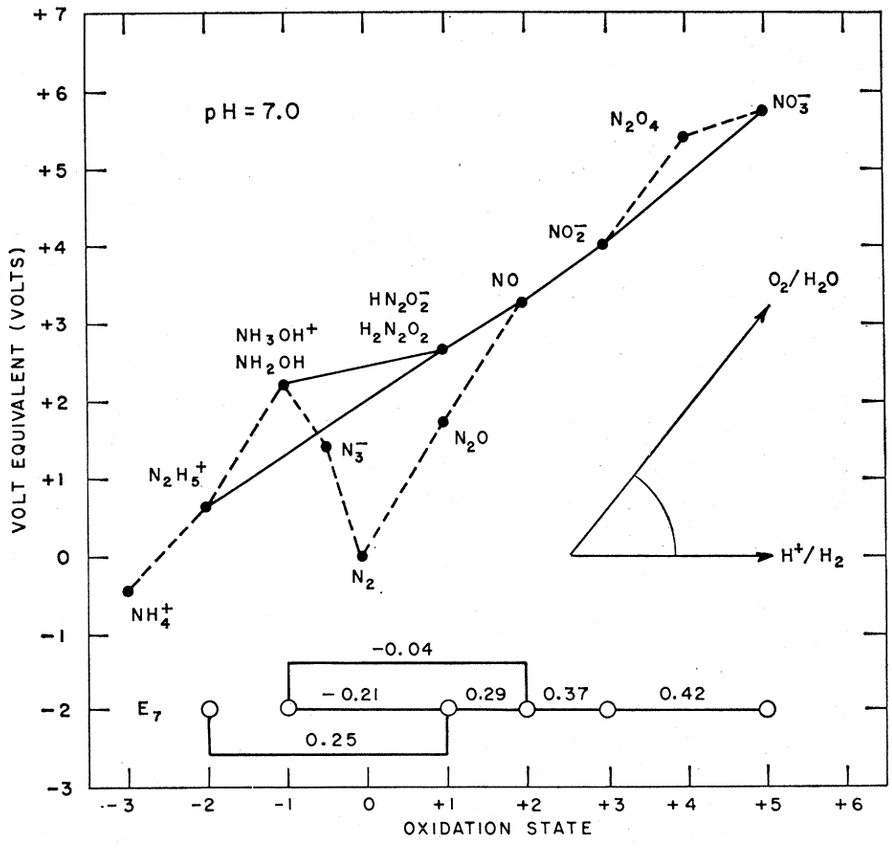


Fig. 6. Oxidation state diagram for nitrogen at *p*H 7.0, after Phillips and Williams (*30*). The energy of formation (ΔG) in electron volts per N atom minus ΔG for the reaction $H^+ + e^- \rightarrow \frac{1}{2}H_2$ is shown for each oxidation state. The slope of each line connecting two oxidation states is equal to $E_7 - E_7$ (H^+/H_2) for that reaction. Redox potentials (E_7) for transitions between oxidation states are shown in the lower part of the figure, after Latimer (*32*).

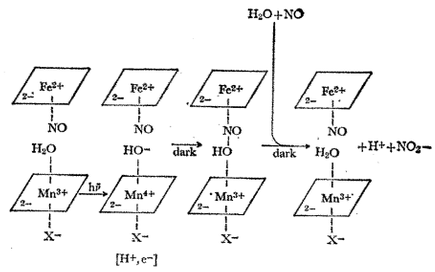


Fig. 7. Oxidation of NO to NO_2^-. The X^- denotes a negatively charged group on the enzyme.

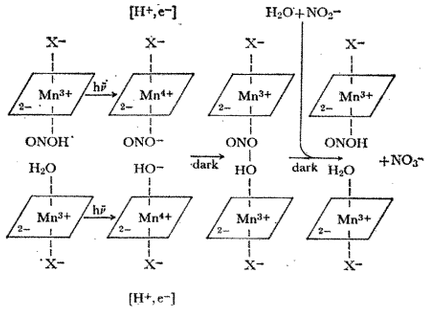


Fig. 8. Oxidation of NO_2^- to NO_3^-.

from electron donors of relatively high potential was achieved by these algal prototypes long before they were able to extract electrons from H_2O.

Electron Donors for System 2

The first electron donors utilized by the protoalgae may have been hydrazine and hydroxylamine (*31*). The oxidation potentials for converting hydrazine or hydroxylamine to nitric oxide are about + 0.25 and − 0.04 volt, respectively, at *p*H 7.0 (*32*) (Fig. 6; Table 1). It is also possible that some photosynthetic organisms were able to use both $N_2H_5^+$ and NH_4^+ as electron donors with the formation of either N_2 or N_2O as the by-product, since the redox potentials are −0.75 volt ($N_2H_5^+/N_2$), −0.28 volt (NH_4^+/N_2), +0.13 volt (NH_4^+/N_2O), and +0.16 volt ($N_2H_5^+/N_2O$). However, neither N_2 nor N_2O could be utilized as an electron donor after the NH_4^+ or $N_2H_5^+$ were used up, because the oxidation potentials (+1.3 volt and +1.2 volt, respectively) would have been much too high for the protoalgae. If, however, the protoalgae were able to convert $N_2H_5^+$ to NO enzymically, the NO could have been later utilized as an electron donor since its oxidation potential (+0.37 volt) is only 0.12 volt higher than that for oxidizing $N_2H_5^+$ to NO. Only the protoalgae which produced NO would have been in a position to develop the capacity to oxidize the NO further to NO_2^-.

Manganese

From a survey of the redox potentials of the various photosynthetic c-type cytochromes including cytochrome f (*33*), it appears as if a cytochrome c could have mediated the transfer of electrons from hydrazine (H_2A'') (Fig. 5). However, in order to extract an electron from nitric oxide (+0.37 volt), a more powerful oxidant than Fe(III) cytochrome c would have been an advantage. A simple way to raise the potential of the cytochrome would have been to substitute another transition metal for iron in the porphyrin. Since manganese is an essential component of system 2 in all modern plants (*34*), it is reasonable to postulate that manganese was one of the transition metals which was successfully substituted for Fe during the evolution of photosystem 2. Loach and Calvin (*35*, *36*) have shown in a model compound, manganese hematoporphyrin 9, that the most stable form is the one in which Mn is in the 3 + -oxidation state. Although Mn(II) hematoporphyrin is a strong reductant ($E_7 = -0.34$ volt), Mn(IV) hematoporphyrin is a powerful oxidant. If the redox potential for Mn (IV) / Mn (III) hematoporphyrin couple at *p*H 7.0 is calculated according to equation 5 of Loach and Calvin (*35*), the value of + 0.97 volt is obtained. It seems reasonable to postulate, therefore, that Mn(IV) was substituted for Fe(III) in the "cytochrome" that reacts with exogenous electron donors, and thereby the redox potential was raised sufficiently for effective oxidation of NO to NO_2^- (Fig. 7). Also favoring the proposal shown in Fig. 7 is the fact that NO is like CO in forming complexes with transition metals (*37*). Concomitantly, the redox potential of the reaction center chlorophyll a_2 would have also been increased in order to oxidize the manganese-porphyrin complex.

Table 1. Redox potentials of hypothetical reactions.

Reaction	E_7 (volt)
1) $NH_2OH \rightarrow NO + 3H^+ + 3e^-$ $NH_3OH^+ \rightarrow NO + 4H^+ + 3e^-$	−0.04
2) $N_2H_5^+ + 2H_2O \rightarrow 2NO + 9H^+ + 8e^-$	+0.25
3) $NO + H_2O \rightarrow NO_2^- + 2H^+ + e^-$	+0.37
4) $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$	+0.42
5) $2NO_2^- + H_2O \rightarrow NO_3^- + NO_2 + 2H^+ + 3e^-$	+0.58
6) $NO_2 + 2H_2O \rightarrow NO_2^- + O_2 + 4H^+ + 3e^-$	+0.79
5 + 6) $NO_2^- + 3H_2O \rightarrow NO_3^- + O_2 + 6H^+ + 6e^-$	+0.69
7) $2H_2O \rightarrow O_2 + 4H^+ + 4e^-$	+0.82

Further Evolution of System 2

After the supply of nitric oxide was depleted and sufficient nitrite had accumulated in the aqueous environment, the stage was set for the next step in the evolution of algal photosynthesis—the oxidation of nitrite to nitrate. This is a two-electron oxidation and it would have required the utilization of 2 quanta of light. (The formation of an intermediate corresponding to NO_2 or N_2O_4 at this stage is ruled out by the excessively high oxidation potential required.) A hypothetical two-electron mechanism involving two manganese-porphyrin complexes is shown in Fig. 8 for the oxidation of nitrite to peroxynitrous acid which spontaneously rearranges to nitric acid. Such a structure could have resulted from a gene duplication for the manganese-porphyrin enzyme shown in Fig. 7.

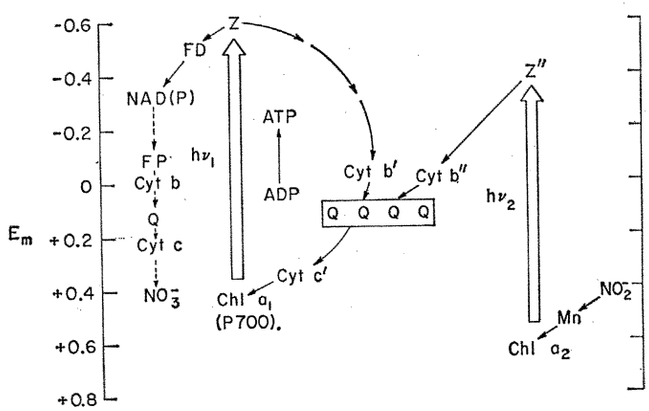


Fig. 9 (left). Electron transport scheme for nitrate respiration and nitrite-based photosynthesis. Abbreviations are explained in Fig. 1.

Origin of the Respiratory Electron Transport Chain

After algal photosynthesis had evolved to the point where organisms could utilize sunlight for oxidizing nitrite to nitrate, there would have been a selective advantage under some circumstances to an organism which could reverse the process at night and thereby couple ATP formation to the oxidation of cell reserves by nitrate. The cyclic electron transport system (Fig. 5) could have been adapted to this function by one mutation to link reduced pyridine nucleotide to cytochrome b and another mutation to facilitate the oxidation of cytochrome c by nitrate. In the prokaryotic algae respiratory electron transfer and cyclic photosynthetic electron transfer shared the same electron transport chain (*38*), since specialized organelles (that is, chloroplasts and mitochondria) did not evolve until after the algae had developed the capacity to produce oxygen (*9*). The structure and function of both photosynthetic and respiratory electron transport chains are summarized in Fig. 9.

Oxygen Evolution

Nitrate is the end of the line for the oxidation of nitrogen compounds in photosynthesis (Fig. 6). The possibility of further oxidation of nitrate to peroxynitrate (NO_4^-) is eliminated by the excessively high oxidation potential required (<1.4 volts). Likewise the formation of any other free peroxide such as H_2O_2 is prohibited by the redox requirement.

However the evolution of a continuous nitrite-nitrate cycle in which nitrite was oxidized to nitrate during the day by photosynthesis and nitrate was reduced to nitrite during the night by respiration presumably led to a steady amount of nitrite in the aqueous environment of the protoalgae over a period of time sufficiently long to permit another doubling in the manganese-porphyrin enzyme. This mutation gave rise to an enzyme with four manganese-porphyrin complexes (Fig. 10). Two of these were specialized for binding H_2O and the other two for binding HNO_2. The oxidation of one HNO_2 to HNO_3 continued in this new enzyme as in the old (Fig. 8); however, it is postulated that the other HNO_2 was oxidized to nitrogen dioxide (NO_2), which, having an unpaired electron, would have been bound tightly to the manganese-porphyrin complex.

As the nitrate diffused away from the enzyme, the nitrogen dioxide might have shifted so that its two oxygen atoms were in coordination with two manganese atoms and also were hydrogen-bonded to the two water molecules coordinated to the other two manganese atoms (Fig. 10). The two bound water molecules were oxidized to two hydroxyl radicals which were stabilized by the hydrogen bonding to the nitrogen dioxide. Oxidation of the NO_2 to the nitronium cation (NO_2^+) provides the hypothetical intermediate for oxidizing the stabilized OH radicals to O_2. In this reaction the nitronium cation is reduced to nitrite. The redox potential for the overall six-electron reaction is $+0.69$ volt as compared to $+0.42$ volt for the oxidation of nitrite to nitrate and $+0.82$ volt for the oxidation of water to oxygen (Table 1). This most primitive mechanism of oxygen evolution thus involved the oxidation of one NO_2^- to NO_3^- for each O_2 molecule formed.

As the oxidation potential of system 2 reached the vicinity of $+1.0$ volt, the bound HNO_2 may have been coordinated to two manganese atoms (Fig. 11) and oxidized to NO_2 ($E_7 = +0.87$ volt) without the formation of any nitrate. The nitrite would then serve as a catalyst regenerated at the end of the reaction, and water alone would serve as the electron donor of photosynthesis.

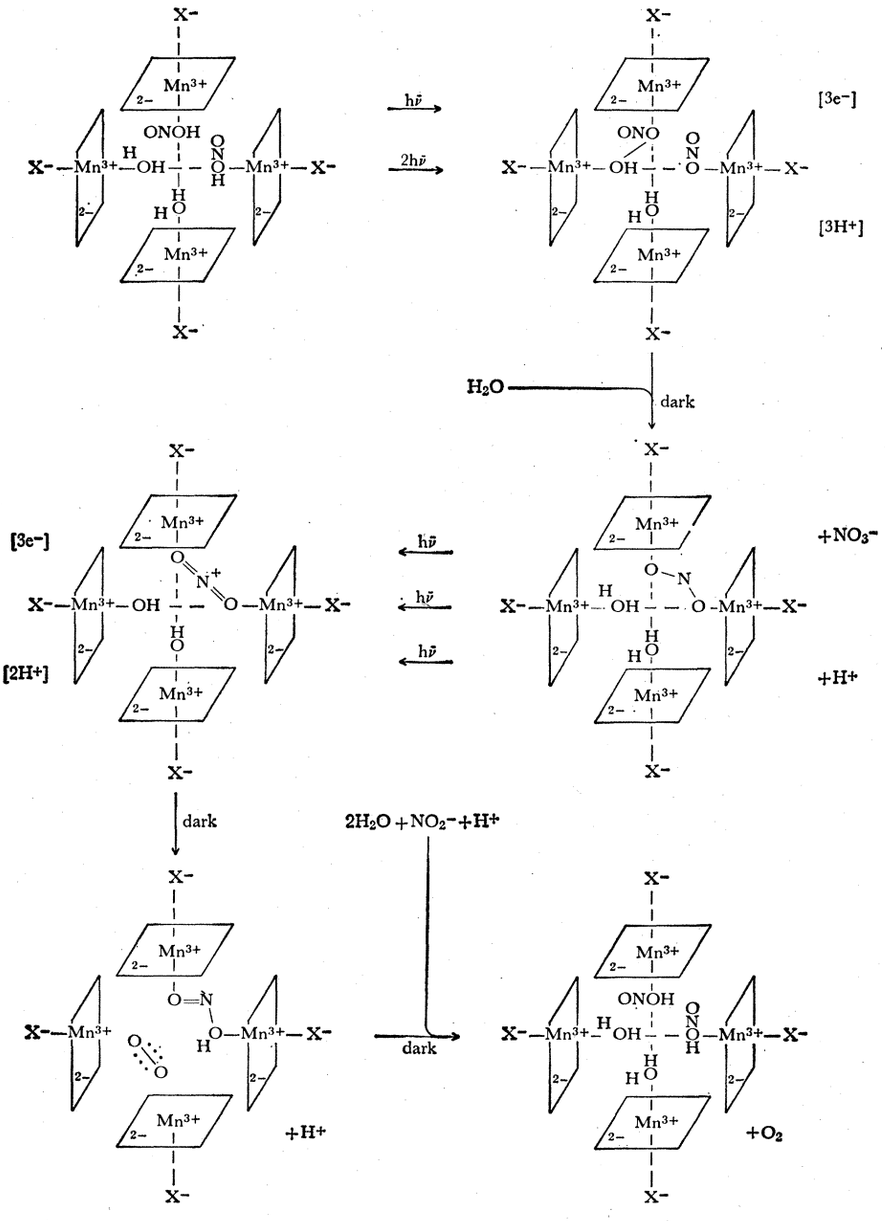


Fig. 10. Oxidation of NO_2^- and H_2O to NO_3^- and O_2.

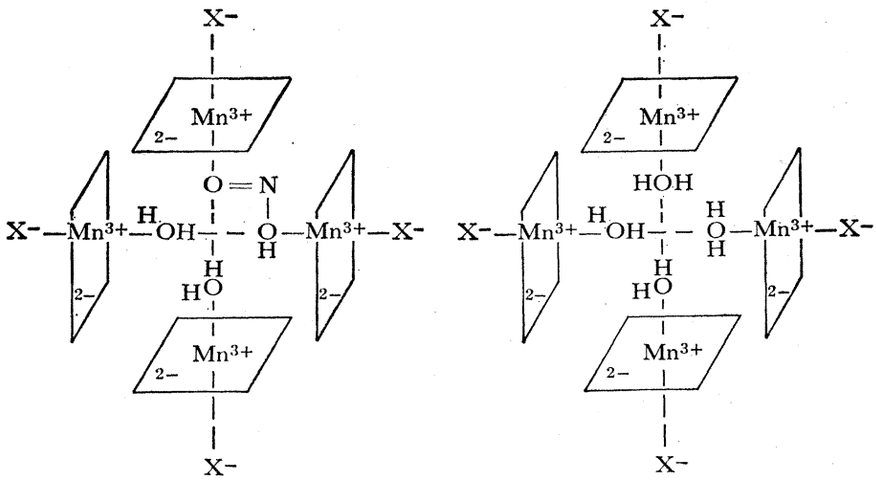


Fig. 11 (left). Initial state of the O_2-evolving enzyme with HNO_2 as a catalyst. Fig. 12 (right). Initial state of the O_2-evolving enzyme in modern plants.

The oxygen thus formed, would have a tendency to oxidize any nitrite in the environment of the algae, and therefore a selective advantage would accrue to an organism that could dispense with nitrite for photosynthesis. It is postulated that a mutation to convert the nitrite-binding manganese-porphyrin complexes shown in Fig. 11 to water-binding sites as shown in Fig. 12 essentially completed the evolution of the oxygen mechanism to its present structure in which two pairs of water molecules are bound to four manganese-porphyrin complexes with two hydrogen bonds between the two pairs. Four quanta are required to oxidize the four H_2O molecules to four hydroxyl radicals tightly bound to the manganese atoms of the enzyme. Because the hydrogen bonding is maintained throughout the four steps of the reaction, each of the intermediates ($3H_2O \cdot OH$, $2H_2O \cdot 2OH$, $H_2O \cdot 3OH$) is stable. The final complex of four hydroxyl radicals collapses in a dismutation reaction to form one molecule of oxygen and two molecules of water.

This theory of oxygen evolution thus leads to a four-electron mechanism that requires four molecules of water and produces one molecule of oxygen by means of an enzyme that contains at least four manganese-porphyrin complexes. From the redox potential values for manganese hematoporphyrin (*35*), it appears reasonable to assume that the oxidation potential of the hypothetical manganese-porphyrin complex [Mn(IV)/Mn(III)] exceeds that for O_2/H_2O by about 0.2 volt.

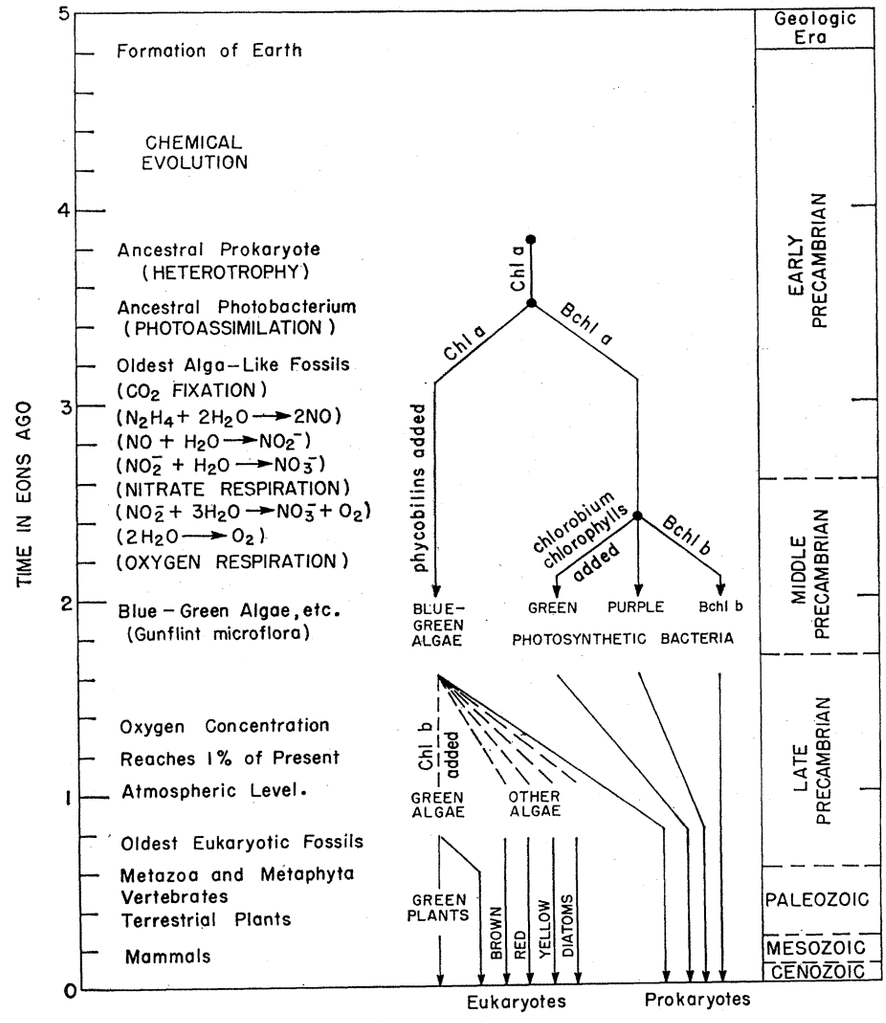


Fig. 13. Summary of evolutionary theory.

Reaction Center of System 2

Loach and Calvin (*39*) have suggested that manganese pheophorbide a might be the "special chlorophyll" of system 2. Such a reaction center pigment would need an absorption band at about 670 nm in vivo. Mn(III) methyl pheophorbide a dissolved in 20 percent ethanol in water at *p*H 7.85 has a moderately strong absorption band close to 665 nm, and thus could qualify as a candidate for the reaction center pigment for system 2. Cheniae and Martin (*40*) have indicated the existence of two functionally different pools of manganese on the oxidizing side of the reaction center of system 2. The size of the combined pool in the blue-green alga *Anacystis* is estimated to be 6 to 12 atoms of manganese per reaction center, whereas in the green alga *Scenedesmus* the amount is 5 to 8, and in spinach chloroplasts it is 5 to 6 (*41*). The larger pool of manganese may correspond to the four manganese-porphyrin complexes in the hypothetical oxygen-evolving enzyme, and the smaller pool of manganese might correspond to one or more molecules of manganese pheophorbide in the photochemical reaction center of system 2.

In manganese-deficient organisms, added manganous ion, Mn(II) is bound either in light or darkness. Activation of the bound manganese for the evolution of oxygen, however, requires light absorbed by system 2 (*42*). This light-dependent activation is consistent with the idea that Mn(II) must be oxidized to Mn(III) in order to function either in the oxygen-evolving enzyme or in the "special chlorophyll" of system 2.

It should be emphasized that there is as yet no convincing experimental evidence either for or against the idea that manganese functions in a manganese-porphyrin complex in photosynthesis.

Conclusion

After a mechanism for oxidizing water to oxygen was perfected in the algae some 2 billion years ago (*43*), the amount of oxygen in the aqueous environment gradually rose. At some point those organisms that could utilize molecular oxygen for respiration would have had a distinct advantage over those utilizing nitrate. The respiratory chain for nitrate respiration (Fig. 9) was presumably modified for respiration of oxygen by the evolution of a cytochrome oxidase. With the creation of an aerobic environment by the blue-green algae and the development of efficient respiratory mechanisms, the evolution of eukaryotic forms and life as we know it today became possible (*9, 10, 44*).

Summary

A common ancestor for photosynthetic bacteria and blue-green algae existed earlier than 3 billion years ago (Fig. 13). This ancestral photosynthetic prokaryote utilized chlorophyll a in a photochemical reaction center which was part of a cyclic system of electron flow through a large pool of quinone that was shared by more than one electron transport chain. The cyclic flow of electrons was coupled to phosphorylation which in turn was coupled to assimilation of exogenous organic compounds. The photosynthesis consisted of a photoassimilation similar to that of acetate exhibited today by some algae.

The first series of mutations enabled the development of a noncyclic electron transport chain in addition to the original cyclic chain. This permitted the reduction of substances with potentials below that of the quinone pool with electrons supplied by substances with potentials above that of the quinone pool.

Concurrent with the evolution of the electron transport system, the development of more efficient light-harvesting systems to drive the reaction centers took place. Eventually, mutual shading of competing organisms gave an advantage to the mutational switch from chlorophyll a to bacteriochlorophyll a for the underlying bacteria. This advantage was gained at the loss of ~ 0.4 electron volt in the free energy stored in the primary photoact and prevented the bacteriochlorophyll-containing line from ever developing the capacity to evolve oxygen.

Those photosynthetic organisms that retained chlorophyll a were able to reduce ferredoxin without substantial modification of the photochemical reaction suitable for the basic cyclic system, because of the 1.2 electron volts available in the photoact. This permitted the development of a system of CO_2 fixation based on ATP and NADP (reduced by ferredoxin). These organisms also evolved cytochrome b as a carrier.

As the supply of the exogenous electron donor for the reduction of ferredoxin diminished, there was an advantage for those organisms which could utilize weaker reducing agents in place of the original electron donors. This was accomplished by modification of the photochemical reaction in a noncyclic system so as to form a stronger oxidant and a weaker reductant in the photoact. The weaker reductant delivered its electrons to the quinone pool, and the stronger oxidant was able to extract electrons from the weak reducing agents that remained in the environment. This was the first step in the evolution of system 2. The original noncyclic system continued to receive electrons from the quinone pool and to deliver them to ferredoxin as does system 1 in a modern oxygen-evolving organism.

The original exogenous electron donors for the algal line of photosynthetic organisms were hydrazine and hydroxylamine which were oxidized to nitric oxide. After the evolution of a manganese-porphyrin enzyme, the algae could oxidize the NO to NO_2^-, and later the NO_2^- to NO_3^-. A respiratory chain for nitrate reduction evolved by a modification of the cyclic electron transport chain.

The final steps in the evolution of algal photosynthesis were modifications of system 2 that enabled the oxidation of water itself. This permitted the development of efficient respiratory mechanisms based on oxygen and the evolution of eukaryotic organisms (*45*).

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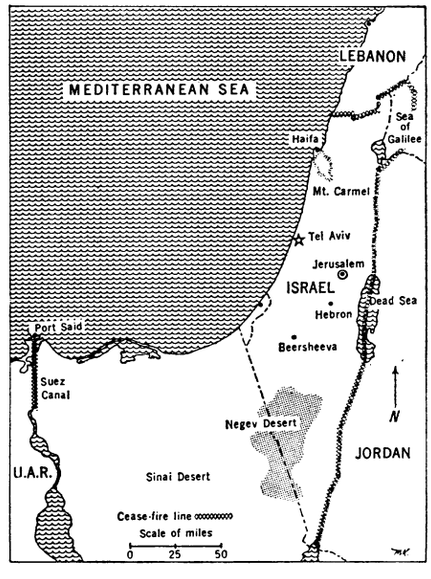
46. The work at Brookhaven National Laboratory was supported by the U.S. Atomic Energy Commission. I am indebted to my colleagues. Geoffrey Hind, Robert Heath, and Philip Thornber for many stimulating discussions. Thanks are also due to Prof. E. S. Barghoorn for his helpful comments on Fig. 13.

ISRAEL



Research and Education Booming in a Nation at War

Israel. I was with several Israelis during a tour in mid-March of scientific and educational institutions here when a news broadcast reported the downing of four Egyptian MIG's. My companions were exultant. Then one of them gasped and said, "Damn it, I forgot to take the chicken out of the freezer." On another occasion, I visited an American physicist who went to work a few months ago in a laboratory in Jerusalem. "Why?" I asked. He explained that he is Jewish and has long been interested in Israel. He continued, "From the professional point of view, it's a small country and you can take an idea of your own and really carry it through. Besides," he said, "I really think my wife and children are safer here than they were back in Washington, D.C. The city streets are absolutely safe at any hour." Having heard that at least half of all research and development in Israel is now in the military area, I asked him whether the Israelis were interested in his extensive military research experience in the United States. "No," he said, "military research here is very self-contained, and they're very security conscious. I wouldn't mind, but they're not interested in me."

The two encounters were a bit jarring, but coming as they did early in a 2-week tour, were appropriate introductions to the numerous incongruities of this tiny, peculiar, and haunted country. Is Israel a land of scientific strength? The answer is that it is strong for its size, but it is a *very* small country. With a population of 2.8 million, it turns out more scientific papers than all of Latin America or Africa. Figures compiled in 1964 show that it roughly ranked with Great Britain and Japan in the number of scientists and engineers per 10,000 of population—10.7 (which is less than half the figures for the U.S. and Sweden). With 1/1400 of the world's population, it has been calculated, Israel produces 1/200 of the scientific papers. Extraordinary. But the fact is that all of Israel contains about 3500 scientists and engineers. They are heavily represented among Jews of European, Russian, and American origin, but not so often among the so-called "Oriental" Jews, who now comprise over half the population, which somewhat explains why there are not even more scientists and engineers in Israel's population.

Small, but rendered strong for its size through its use of scientific skills—well, yes and no. After more than a decade of talk and planning about "science-based" industry, Israel's