Biological Formation of Molecular Hydrogen

A "hydrogen valve" facilitates regulation of anaerobic energy metabolism in many microorganisms.

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The metabolic versatility of microorganisms is particularly striking in respect to alternative mechanisms for obtaining growth energy. Many microorganisms derive energy by the same type of process characteristically used by complex heterotrophic forms, that is, from oxidation of metabolites, with molecular oxygen serving as the ultimate, and obligatory, oxidant. On the other hand, many organisms normally obtain energy through less-efficient anaerobic processes in which oxygen does not participate. In an anaerobic pattern observed in a limited number of microorganisms, an inorganic anion such as nitrate or sulfate is the terminal oxidant, while in more frequently encountered variations the ultimate electron acceptor is an organic compound derived from degradation of the energy source (for example, carbohydrates). Between the two extremes noted are a number of organisms, the facultative anaerobes, which have the capacity to grow either as aerobes or anaerobes; these are of particular interest as experimental systems in connection with problems of regulation of cell metabolism.

In heterotrophic organisms, the anaerobic mode of growth poses special problems for the cell with respect to the disposition of electrons from energy-yielding oxidation reactions. This is particularly so when the overall adenosine triphosphate (ATP) requirement for biosynthetic activity can be satisfied only by degradation of a relatively large quantity of an organic compound that serves as the energy source. Accordingly, various kinds of specific controls are necessary to regulate electron flow in the metabolism of strict and facultative anaerobes. One of these is reflected by the ability of many such organisms to dispose of "excess" electrons (e) in the form of molecular hydrogen (H_2) through the activity of hydrogenases which, in effect, catalyze the reaction:

$$2e + 2H^+ \longrightarrow H_2.$$

Advances in our understanding of hydrogenase action, electron-transport reactions, and control mechanisms stimulated the present reevaluation of the significance of H_2 formation in the anaerobic metabolism of heterotrophic and photosynthetic microorganisms.

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Categories of Hydrogen Producers

Ability to produce H_2 has been observed in a relatively large number of microbial species, including significantly different taxonomic and physiological types. Representative organisms can be separated into four categories, each with prominent group characteristics (Table 1). This classification is admittedly arbitrary from a number of standpoints, but it is useful as a basis for further discussion.

Organisms in category I are all heterotrophic anaerobes whose growth is inhibited by molecular oxygen; they also resemble each other in that, as far as is known, they do not contain electron carriers of the cytochrome type. Keto acids, notably pyruvate, or reduced two-carbon compounds are the primary electron donors for H_2 formation by most organisms of category I; in some species, these or closely related donors may be generated by fermentation (anaerobic decomposition) of amino acids. The *Micrococcus* species indicated are further distinguished by ability to produce H_2 from purines or pyrimidines.

Category II comprises many heterotrophic facultative anaerobes that typically contain cytochromes and evolve H_2 from formate. The immediate precursor of formate is pyruvate, an important intermediate in the fermentation of carbohydrates and certain amino acids.

Desulfovibrio desulfuricans is in a class by itself in that it is a heterotrophic strict anaerobe that contains a cytochrome. This organism normally uses sulfate as the terminal oxidant for energy-yielding, cytochrome-linked anaerobic oxidations (for example, of lactate), but certain strains can liberate H_2 from pyruvate and formate when sulfate is absent.

Category IV consists of photosynthetic microorganisms, both bacteria and algae, which can produce H_2 by a light-dependent mechanism. Formerly it was usually assumed that the immediate electron-donor was a "photoreductant" created by interaction of light with the photochemical apparatus, but more recent studies indicate that the donor is reduced pyridine nucleotide generated by the oxidation of either organic or inorganic compounds. Certain photosynthetic organisms are also able to produce H_2 in darkness from substrates such as pyruvate and formate, presumably by mechanisms similar to those occurring in categories I to III.

Hydrogen Formation by Heterotrophs

Strict anaerobes. Except for organisms with rather specialized types of metabolism (such as, *Clostridium kluyveri, Diplococcus glycinophilus, Methanobacterium omelianskii*), most bacteria typical of category I produce pyruvate as an intermediate in the fermentation of the energy source. Pyruvate is, in large measure, further degraded by an energy-yielding "phosphoroclastic" cleavage:

$$CH_3COCOOH + H_3PO_4 \longrightarrow CH_3COOPO_3H_2 + CO_2 + H_2$$

The mechanism of this reaction is still not well understood, but it is known that free formate is not an intermediate and that electrons released during the oxidative decarboxylation of pyruvate

are liberated in the form of H_2 (*1–3*).

The results of many studies indicate close similarity in the nature and sequence of the catalysts constituting the terminal portion of the hydrogen-evolving systems of organisms of category I. In this regard, the clostridia have been examined more thoroughly than have other bacteria in this group; for present purposes they will be considered typical representatives. Early observations made with a clostridial hydrogenase revealed many striking parallels between the ability of cell-free preparations to produce H_2 from pyruvate on the one hand and from hydrosulfite on the other; this led to the suggestion that a common carrier was required for electron transfer to the hydrogenase (*4*). Methyl viologen, a one-electron dye of low redox-potential ($E'_0 = -446$ mv), effectively replaced the unknown factor (with hydrosulfite as electron donor), which evidently was readily lost during the initial steps in purification of the hydrogenase (*4, 5*). In contrast, a number of known electron-transfer cofactors, including pyridine nucleotides and flavins, were ineffective.

Subsequent investigations showed that the natural factor which mediates electron flow from pyruvate, or hydrosulfite, to hydrogenase in *Clostridium pasteurianum* is an iron-containing protein designated ferredoxin (*6*). Similar iron proteins have been isolated from various clostridia and other organisms and show a spectrum of differences in composition and other properties (*7–9*). The foregoing and related developments permit the construction of a general scheme for production of H_2 by organisms of category I (Fig. 1).

Similarity of ferredoxin function in the hydrogen metabolism of strict anaerobes of different physiological types is indicated by the fact that, at least in some instances, the "specific" ferredoxins are functionally interchangeable in cell-free experimental systems (*10*). It should be emphasized, however, that ferredoxins can also act as electron carriers in processes that do not culminate in formation of H_2, as in reduction of pyridine nucleotides by pyruvate (*11*) and light-dependent reduction of nicotinamide adenine dinucleotide phosphate by green-plant chloroplasts (*7*). Accordingly, carriers of the ferredoxin type are also found in organisms that do not normally produce H_2.

The "strict anaerobe" *Desulfovibrio desulfuricans* might superficially be regarded as belonging to category I, but analysis of its metabolic pattern suggests that it shows even greater affinities with the coli-aerogenes bacteria of category II. It is, in fact, becoming increasingly clear that overall designations such as "facultative" and "anaerobic" lack precise biochemical meaning, and it seems advisable to use such terms only for rough, operational classifications. There is still some doubt as to the occurrence and possible participation of a ferredoxin in the metabolism of *D. desulfuricans*. Accumulating evidence indicates that the cytochrome c_3 ($E'_0 = -205$ mv), characteristically found in this organism (*12*), may fulfill certain electron-transfer functions attributed to ferredoxin in other types of anaerobes.

Facultative anaerobes. Pyruvate is also produced as a key fermentative intermediate in the facultative anaerobes (category II) and is subsequently metabolized through various alternative routes, which include the following type of "clastic" reaction:

$$CH_3COCOOH + H_3PO_4 \longrightarrow CH_3COOPO_3H_2 + HCOOH$$

Although the "clostridial" and "coli-type" (*13*) phosphoroclastic reactions show gross overall similarity, they clearly differ in certain fundamental aspects that remain to be further elucidated.

In bacteria of category II, formate produced by the phosphoroclastic reaction is, in large part, converted to H_2 and CO_2 by an additional sequence of anaerobic reactions catalyzed by the "formic hydrogenlyase enzyme complex." This complex comprises at least two enzymes, a soluble formate dehydrogenase and particulate hydrogenase, and two unidentified intermediary electron-carriers designated X_1 and X_2 (*14, 15*) (Fig. 2).

An active "hydrogenlyase," with properties closely corresponding to those of the natural complex, is readily reconstructed by mixing complementary portions of the system obtained from non-gas-producing coliform variants (*15*). Recent studies (*16*) provide strong circumstantial evidence for the identity of carrier X_2 with a newly discovered (*17*) *c*-type cytochrome of low redox-potential ($E'_0 = -225$ mv). The low-potential cytochrome is produced by coliform bacteria only during growth under anaerobic conditions (*18*), that is, under conditions favorable for development of the hydrogenlyase system. Accordingly, X_1 may well have the function of a cytochrome-*c* reductase or, in the case of *D. desulfuricans*, a cytochrome-c_3 reductase (*19*).

Efforts so far to detect ferredoxin in facultative anaerobes have given negative results (*20*), and several other lines of evidence attest to qualitative differences in the hydrogen-evolving sys-

Table 1. "Classification" of microorganisms evolving H_2.

Microorganism	Electron donor(s) for formation of H_2	Reference cited*
Category I: Heterotrophic anaerobes, strict		
Clostridium butylicum	Pyruvate	*1*
C. pasteurianum	Pyruvate	*52*
C. kluyveri	Ethanol (Acetaldehyde?)	*53*
C. tetanomorphum	Pyruvate†	*54*
Diplococcus glycinophilus	Glycine	*55*
Peptostreptococcus elsdenii	Pyruvate	*56*
Micrococcus lactilyticus	Pyruvate	*57*
(Veillonella gazogenes)	α-Ketoglutarate	
	Purines	
M. aerogenes	Pyruvate	*58*
	Purines	
	Pyrimidines	
Butyribacterium rettgeri (G3)	Pyruvate	*59*
Methanobacterium omelianskii	Acetaldehyde‡	*60*
(Methanobacillus omelianskii)	Ethanol‡	
Category II: Heterotrophic anaerobes, facultative		
Escherichia coli and related bacteria	Formate	*30*
Aeromonas hydrophila	Formate	*23, 61*
Bacillus macerans	Formate	*62*
B. polymyxa	Formate	*50*
Category III: Heterotrophic anaerobes, strict (cytochromes)		
Desulfovibrio desulfuricans	Pyruvate§	*63*
	Formate§	
Category IV: Photosynthetic ‖		
Nonsulfur purple bacteria	Organic compounds	*39, 45*
Sulfur purple bacteria	Organic compounds	*64*
	Thiosulfate	*36, 65*
Anaerobically "adapted" algae	Reduced pyridine nucleotide	*43, 66*

* Selected references only. † *C. tetanomorphum* and several other organisms in category I produce H_2 during catabolism of certain amino acids (for example, cysteine, serine, threonine, glutamate); α-keto acid intermediates presumably are the immediate electron donors. ‡ In the absence of carbon dioxide. § In the absence of sulfate. ‖ Only light-dependent formation of H_2 is considered here, and it is assumed that reduced pyridine nucleotide is the "immediate" electron donor. Several photosynthetic microorganisms produce H_2 in darkness (either endogenously or from substrates such as formate and pyruvate), apparently through mechanisms similar to those characteristic of categories I–III (*45, 47, 67*).

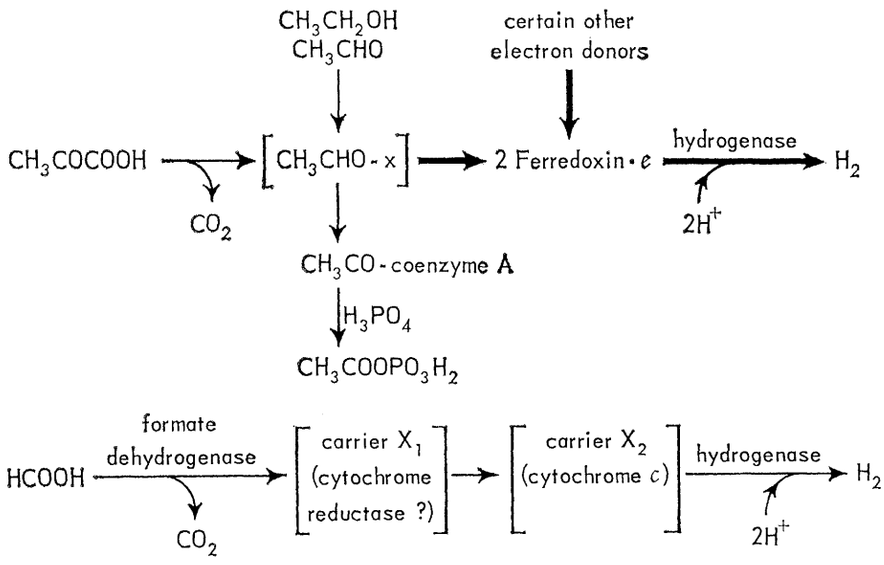

Fig. 1 (upper). General scheme for production of hydrogen by clostridia and related organisms. Broad arrows indicate electron-transfer reactions directly related to the H_2 formation. The chemical nature of the intermediate in brackets remains uncertain; it may be hydroxyethylthiamin diphosphate or a related compound (*3*). Fig. 2 (lower). The formic hydrogenlyase enzyme complex. Horizontal arrows indicate the direction of electron transfer. Low-potential *c*-type cytochromes in facultative anaerobes and cytochrome c_3 in the strict anaerobe *Desulfovibrio desulfuricans* participate in electron transfer to hydrogenase; these heme proteins thereby have functions analogous to that of ferredoxin in H_2 formation in the clostridial-type process.

tems of strict and facultative anaerobes. Most significant in this respect is the consistently observed failure of components isolated from strict anaerobes to interact with those from facultative bacteria. For example, the formate dehydrogenase of coliform organisms will not couple with the hydrogenase in crude clostridial extracts (active in producing H_2 from pyruvate) to give a "model" formic hydrogenlyase system (*14*). Similarly, the formate dehydrogenase of *Clostridium acidi-urici*, a strict anaerobe which does not produce H_2, does not couple with *C. pasteurianum* hydrogenase (*21*). These observations point to an obligatory association between the formate dehydrogenase of the hydrogenlyase complex and cytochromes of low redox-potential. Direct evidence (*19*, *22*) for participation of cytochrome c_3 as an electron carrier in the hydrogenlyase complex of *D. desulfuricans* adds further weight to the conclusion that the terminal portion of the complex consists of a low-potential cytochrome which interacts with the hydrogenase of the organism in question. There is reason to believe that *D. desulfuricans* may be a transitional organism in the sense that it contains both a "coli-type" hydrogenlyase system and a "clostridial-type" phosphoroclastic reaction, but the evidence is still fragmentary.

The existence of numerous, and possibly transitional, forms of gas-producing organisms within the facultative group provides the opportunity to evaluate the hypothesis that *c*-type cytochromes with a low potential, rather than ferredoxins, are electron carriers in the productions of H_2 from formate. Results of current studies (*23*) with *Aeromonas hydrophila*, an organism with the morphology of a *Pseudomonas* and certain metabolic features of coli-aerogenes bacteria, support the hypothesis in that the organism forms a low-potential cytochrome *c* and shows formic hydrogenlyase activity when grown anaerobically. On the other hand, preliminary experiments with *Bacillus macerans* suggest that in certain kinds of facultative bacteria the transfer of electrons to hydrogenase may involve an alternative electron carrier. *Bacillus macerans* produces cytochromes aerobically, but is unable to do so anaerobically; as in other facultative anaerobes, the bacterium also seems to be devoid of ferredoxin (*23*). Nevertheless, anaerobically grown cells of *B. macerans* actively break down formate, producing hydrogen.

Control of Anaerobic Electron Flow

Little is known of the specific factors governing the formation of hydrogen-evolving systems in obligate anaerobes, except that an adequate supply of iron is required during growth. As with coli-aerogenes bacteria (*24*), clostridia (*25*) and related organisms grown in iron-deficient media do not produce appreciable quantities of H_2. Accordingly, fermentations by cells deprived of iron are characterized by substantial alterations in the balance of end products. In typical saccharolytic clostridia, absence of functional hydrogen-evolving systems leads to a marked shift from the usual metabolic pattern to fermentations of the "homolactic" type (*25*). With coli-aerogenes bacteria, iron deficiency causes similar changes and, in addition, an accumulation of formate in the medium; iron-deficient cells show little or no hydrogenase activity and are incapable of converting formate to H_2 and CO_2 (*24*). Thus, loss of the "hydrogen valve" restricts these bacteria to more conventional fermentations of the kind observed in certain organisms incapable of producing H_2; pyruvate now acts as a major terminal electron-acceptor. From the reaction sequences already discussed, it can be deduced that iron deficiency produces these effects by restricting the biosynthesis of ferredoxin, the hemin of cytochrome *c*, and—in some organisms—of hydrogenase itself.

In facultative anaerobes, biosynthesis of enzymes used in terminal anaerobic electron-transport is usually repressed during growth in the presence of molecular oxygen (*26*); on removal of O_2, synthesis of such enzymes becomes derepressed. Thus it would seem that when facultative anaerobes are deprived of the "favored" terminal electron-acceptor, oxygen, they respond by producing enzymes necessary for alternative mechanisms of anaerobic disposition of electrons. On the other hand, in the presence of O_2 the aerobic citric acid–cycle system (or a modification thereof) is induced, and the cell economizes by repressing formation of the "anaerobic" electron-acceptor enzymes (*27*). The phenomenon of oxygen repression thus acts as a slow "coarse"-control mechanism and supplements the rapid "fine"-regulatory control of the feedback-inhibition type exerted through the classical Pasteur effect (*28*). In conformity with the foregoing, certain of

the catalysts required for hydrogen formation are also subject to oxygen repression (*29, 30*). The sensitivity of the formic hydrogenlyase system to oxygen repression suggests that this complex, like other terminal systems of anaerobic electron-transfer, normally performs important functions in anaerobic energy metabolism.

While derepression by anaerobiosis is a prerequisite for biosynthesis of components of the hydrogenlyase complex, other factors may have significant influence. Studies (*31*) with a mutant of *Escherichia coli* unable to synthesize hemin (*32*) suggest that synthesis of the cytochrome *c* plays a pivotal role in development of the overall system. When the mutant is grown anaerobically in the presence of iron and in the absence of hemin, the cells show greatly diminished hydrogenase and no hydrogenlyase activity. Under similar nutritional circumstances, only the formate dehydrogenase component is significantly derepressed when cultures are shifted from aerobic to anaerobic growth conditions. Consequent addition of hemin (after 3 to 4 hours) causes the prompt appearance of cytochrome *c*, hydrogenase, and hydrogenlyase activity. On the other hand, if hemin is added to growing cells during the early stages of derepression, a lag of several hours (characteristic of wild-type *E. coli*) is observed. These observations (Fig. 3) suggest that the mutant is capable of making the apoenzyme of the cytochrome during the derepression phase, and that addition of hemin permits rapid completion of synthesis of cytochrome. The cytochrome can then serve as a carrier in the transfer of electrons to hydrogenase.

Control of biosynthesis of the dehydrogenase component of the hydrogenlyase complex is more difficult to analyze due to the presence of two kinds of formate dehydrogenase activity. *Escherichia coli* possesses a particulate (membrane-bound) enzyme system which oxidizes formate with oxygen to CO_2 and H_2O; methylene blue also serves as an effective electron-acceptor for formate oxidation (*33*). Development of this activity is favored by aerobic growth conditions. Anaerobically, dehydrogenase activity with methylene blue occurs (in reduced amount) and, in addition, a soluble formate dehydrogenase which shows high activity with the one-electron dye, benzyl viologen ($E'_0 = -359$ mv). The soluble enzyme

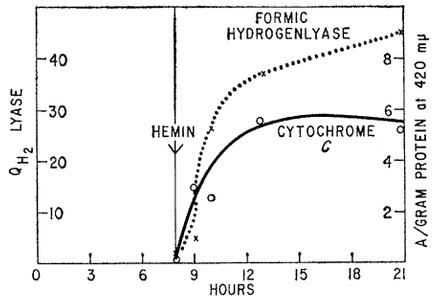

Fig. 3. Synthesis of low-potential cytochrome-*c* and formic hydrogenlyase activity in a heminless mutant of *Escherichia coli*; after Gray and Wimpenny (*31*). The cells were grown anaerobically until derepression was complete. At the time indicated by the arrow, hemin (4 μg/ml) was added. Q_{H_2} lyase: microliters of H_2 produced per hour per milligram of cells (dry weight) with formate as substrate. Right-hand axis: amount of cytochrome *c*, measured in terms of absorbancy (*A*) at 420 mμ per gram of bacterial protein (from the soluble fraction of disrupted cells).

has been identified as the dehydrogenase that functions in the hydrogenlyase complex (*14, 15, 34*). Although it seems unlikely, the possibility that there is only one formate dehydrogenase, the properties of which are altered by binding with the cytoplasmic membrane, cannot be completely dismissed.

The extent of H_2 production during heterotrophic fermentation processes is also strongly influenced by events at the electron-transfer level. Particularly important in this respect is potential competition for electrons among alternative terminal acceptors. In certain instances (as in bacteria of the coli-aerogenes group and clostridia), several acceptors may be reduced, and the amounts of the fermentation end products then reflect the balance among the different electron-transfer pathways. In general, the yield of H_2 is directly related to the state of reduction of the fermented energy source, and tends to be inversely related to the total quantity of reduced end products which accumulate in the medium. These considerations were the basis of an earlier suggestion (*30*) that the magnitude of hydrogenase activity might be the crucial factor regulating the disposition of electrons into available alternative pathways. This interpretation is supported by the results of experiments with intact cells of clostridia and coli-aerogenes bacteria. For example, when the hydrogen-evolving system is inhibited by carbon monoxide, fermentation of glucose by *Clostridium butyricum* is characterized by production of lactate rather than H_2, CO_2, and fatty acids (*35*); similar shifts in fermentation patterns caused by growth in iron-deficient media have already been mentioned.

In some bacteria, notably clostridia, the control systems that balance anaerobic electron-transfer seem to include the reutilization of evolved H_2. This is suggested by the fact that preparations of clostridial hydrogenase can reduce pyridine nucleotides with H_2 by a ferredoxin-dependent process (*11*). Since reduced pyridine nucleotides are required for fatty-acid formation and other reductions, a reversible hydrogenase system provides, in principle, a delicate control valve for regulating electron flow. The clostridia and metabolically similar anaerobes apparently must cope with special problems in controlling energy metabolism; this is perhaps primarily due to the circumstance that fermentative generation of a limited amount of ATP in such organisms requires "disposal" of a comparatively large number of electrons.

Photoproduction of Hydrogen

The photosynthetic bacterium *Rhodospirillum rubrum* does not produce molecular hydrogen during anaerobic photosynthetic growth on organic compounds, such as malate, when an ammonium salt is the nitrogen source and is not growth-limiting. If the medium is altered so that the ammonium salt is limiting, H_2 is evolved after the nitrogen source is exhausted (*36*); this fact and results of experiments (*36*) with metabolic inhibitors indicate that NH_4^+, or a derivative metabolite, represses formation of one or more protein components of the hydrogen-evolving system. Derepression of the system occurs during a 1- to 2-hour interval when the cells are rapidly photometabolizing organic substrates in the absence of significant growth.

When the source of nitrogen is glutamic acid, however, H_2 is continuously evolved as a major product (*37*); free ammonia is not detectable during growth in such media. Resting (nongrowing) cells derived from glutamate cultures immediately produce H_2 when illuminated in the presence of utilizable organic compounds, such as certain intermediates of the citric acid cycle (*37,*

Table 2. Roles of H_2 formation in anaerobic metabolism.

Category	Energy source* (anaerobic)	Coupling of H_2 evolution with energy-yielding reactions	Postulated role of H_2 evolution
		Clostridium pasteurianum†	
I	Fermentation	Intimate	Disposal of electrons from energy-yielding oxidations
		Escherichia coli†	
II	Fermentation	Close but not obligatory	Promotion of energy-yielding oxidations through removal of a product (formate)
		Desulfovibrio desulfuricans † §	
III	Oxidation of organic compounds with $SO_4^=$ as electron acceptor	As in I or II	As in I or II
		Rhodospirillum rubrum	
IV	Light	Indirect	Regulation of reducing power and energy "pools"

* Under "normal" growth conditions. † Representative organisms. § In this organism, formation of H_2 represents an alternative metabolic pattern observed in the absence of the "normal" terminal acceptor (such as $SO_4^=$); both the clostridial and coli-aerogenes types of H_2-evolving systems may be present, with functions similar to those noted above.

38). Under suitable conditions, resting cells of *R. rubrum* will in fact photometabolize acetate and dicarboxylic acids, forming H_2 and CO_2 in quantities closely approximating those expected from complete decomposition of the substrate (*39*):

Acetate, $C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 4H_2$

Succinate, $C_4H_6O_4 + 4H_2O \rightarrow 4CO_2 + 7H_2$

Fumarate, $C_4H_4O_4 + 4H_2O \rightarrow 4CO_2 + 6H_2$

L-Malate, $C_4H_6O_5 + 3H_2O \rightarrow 4CO_2 + 6H_2$

These remarkable metabolic conversions appear to occur by the extensive operation of an anaerobic citric acid cycle coupled with a light-dependent process which brings about reoxidation of reduced pyridine nucleotide (generated by the oxidative steps of the cycle) by liberation of H_2 (*39*, *40*). Despite other possible alternatives, we regard the following representation (*41*, *42*) of the hydrogen-evolving reaction as most consistent with the physiological and biochemical facts available:

$$\begin{array}{c} PNH + H^+ \rightarrow PN^+ + H_2 \\ \uparrow \\ X\sim,\ X\sim P,\ ATP \\ \uparrow \\ \text{light} \end{array}$$

where PNH and PN^+ represent the reduced and oxidized forms of pyridine nucleotide, respectively; and where $X\sim$ and $X\sim P$ symbolize energy-rich intermediates associated with photophosphorylation. This formulation is supported by recent demonstration (*43*) of light-dependent evolution of H_2 from reduced pyridine nucleotide by cell-free extracts of anaerobically adapted *Chlamydomonas eugametos*. Although light-dependent production of H_2 may well serve the same general function in photosynthetic bacteria and adapted algae, it is quite possible, and perhaps likely, that the electron-transfer carriers and pathways concerned are significantly different. Further work is required with both systems to define unambiguously a possible role of iron proteins. In this connection, light-dependent H_2 production has been observed (*8*) in a model cell-free system composed of green-plant chloroplasts and crude hydrogenase preparations from heterotrophic strict anaerobes; evaluation of the significance of this artificial model must obviously await further knowledge of the natural systems.

In photosynthetic bacteria, maximum photoproduction of H_2 occurs under conditions in which the cell might be expected to generate ATP, or its precursors, and reduced pyridine nucleotide in excess of the demands of the biosynthetic apparatus (*39*, *41*). Accordingly, light-dependent H_2 formation in such organisms may be viewed as the manifestation of a regulatory system which aids in maintaining these important substances at concentrations consistent with the overall rate of biosynthetic activity (*39*, *40–42*). This interpretation is supported by studies (*39*) with certain inhibitors of light-induced phosphorylation, such as antimycin A and redox dyes. Addition of such compounds to illuminated intact cells of *Rhodospirillum rubrum* completely abolishes H_2 formation and also usually induces the cells to resort to anaerobic fermentation of endogenous reserves to fatty acids. Thus, interference with the photophosphorylation mechanism leads to inhibition of H_2 evolution and causes the illuminated cells to behave as though in darkness. This interesting phenomenon indicates that photophosphorylation may normally inhibit fermentation and has been described (*44*) as a "photosynthetic Pasteur effect." In addition to antimycin A and redox dyes, certain uncouplers of oxidative phosphorylation in mitochondria, such as pentachlorophenol and *m*-chlorocarbonyl cyanide phenylhydrazone, are also potent inhibitors of photoproduction of H_2 by *R. rubrum* (*42*).

H_2 Formation and N_2 Reduction

The hydrogen-evolving system of photosynthetic purple bacteria is subject to several regulatory controls which interlock with nitrogen metabolism. In addition to the repression caused by ammonium salts, hydrogen evolution by active cells is effectively (and reversibly) inhibited by molecular nitrogen (*38*), which can be utilized as the sole nitrogen source for photosynthetic growth by all types of photosynthetic bacteria (*45*, *46*). Results of experiments with *R. rubrum* indicate that N_2 probably inhibits H_2 formation by two mechanisms. Since N_2 represents a quantitatively important "sink" for metabolic reducing power, competition between the hydrogenase and "nitrogenase" systems for electrons from a common donor could account for inhibition (*47*). Parenthetically, it may be added that the extent of such competition would be expected to vary in different anaerobic organisms that fix

nitrogen, depending on the "reduction pressure" exerted on a common electron donor—reduced ferredoxin in some organisms (*48*). Molecular nitrogen also manifests a "catalytic" effect on the hydrogen-evolving system of photosynthetic bacteria; thus, in short-term experiments the amount of N_2 actually reduced is considerably less than the predicted quantity (*49*). The "catalytic" action of N_2 suggests the possibility that evolution of H_2 requires a protein catalyst whose activity can be controlled through an "allosteric transition" caused specifically by the nitrogen molecule. According to this notion (*41*), the signal for cessation of energy-dependent H_2 evolution from organic compounds by the anaerobic citric acid cycle is the appearance of a readily utilizable nitrogen source which satisfies the requirements for immediate endergonic synthesis of proteins and other macromolecules.

Certain purple bacteria, such as *R. rubrum*, are facultative anaerobes in that they can grow either photosynthetically in the absence of oxygen, or as typical aerobic heterotrophs in darkness; *R. rubrum* is incapable, however, of fixing substantial amounts of N_2 under the latter conditions (*49*). This and other observations (*50, 51*) suggest that oxygen may repress biosynthesis of catalysts concerned with both H_2 evolution and N_2 fixation in facultative anaerobes.

Significance of H_2 Evolution

Transfer of electrons to hydrogenase, with consequent formation of H_2, is evidently related in varying degree to the primary reactions of energy metabolism in different organisms. In clostridia and related bacteria, production of H_2 is intimately linked with the energy-yielding cleavage of pyruvate to acetyl phosphate and CO_2. On the other hand, in heterotrophic facultative anaerobes the precursor of H_2 (formate) is a product of an analogous pyruvate cleavage; in these organisms, decomposition of formate to H_2 and CO_2 by the hydrogenlyase complex may well represent a means of "pulling" the energy-yielding breakdown of pyruvate. In the photosynthetic microorganisms of category IV, H_2 formation is not essential for operation of the energy-generating photophosphorylation system, but rather reflects an alternative scheme of electron flow which apparently serves regulatory functions. Our views on the roles of H_2 production in anaerobic metabolism are summarized in Table 2, which is offered as a first approximation and guide.

Formation of H_2 occurs as a major physiological process only in hydrogenase-containing organisms which display anaerobic energy-metabolism patterns of the following kinds: (i) The supply of energy is limited and obtained by fermentation in the absence of "external" electron acceptors. In such organisms (clostridia, for example) the bulk of the fermentable substrate is consumed for the purpose of obtaining energy; extensive accumulation of reduced organic products is typical and cell yields are relatively low. (ii) Energy is obtained from anaerobic oxidations with an inorganic terminal oxidant other than molecular oxygen. In this pattern, formation of H_2 presumably signifies the operation of alternative energy-yielding mechanisms of the kind described for categories I and II, under conditions of deficient supply of the inorganic terminal oxidant; *Desulfovibrio desulfuricans* and *Methanobacterium omelianskii* may be considered as examples. (iii) Energy supply and reducing power are potentially available in excess, relative to the overall rate of biosynthesis. This situation is encountered in photosynthetic bacteria growing on organic carbon sources with saturating illumination; reduced organic by-products are not produced in significant amounts and cell yields are remarkably high.

Conclusion

From a general standpoint, the formation of molecular hydrogen can be considered a device for disposal of electrons released in metabolic oxidations. We presume that this means of performing anaerobic oxidations is of ancient origin and that the hydrogen-evolving system of strict anaerobes represents a primitive form of cytochrome oxidase, which in aerobes effects the terminal step of respiration, namely the disposal of electrons by combination with molecular oxygen. We further assume that the original pattern of reactions leading to H_2 production has become modified in various ways (with respect to both mechanisms and functions) during the course of biochemical evolution, and we believe that this point of view suggests profitable approaches for clarifying a number of problems in the intermediary metabolism of microorganisms which produce or utilize H_2. Of special general importance in this connection is the basic problem of defining more precisely the fundamental elements in the regulatory control of anaerobic energy metabolism. Among the more specific aspects awaiting further elucidation are: the relations between formation of H_2 and use of H_2 as a primary reductant for biosynthetic purposes; the various forms of direct and indirect interactions between hydrogenase and N_2 reduction systems; and the transitional stages between anaerobic and aerobic energy-metabolism patterns of facultative organisms.

References and Notes

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Science and Antiscience

We have yet to achieve a unifying view on which to base a conception of the nature of man.

Walter Russell Brain

I very much appreciate the privilege of addressing you and of attending this meeting as the representative of the Council of the British Association for the Advancement of Science. A few months ago, as president of that Association, I delivered an address on "Science and Behaviour." That is a large subject, and I did not attempt to do more than deal with it in outline. I realized that I left many important questions unanswered, and some, indeed, unasked. This, together with some reactions to my address in the English press and elsewhere, made me welcome this opportunity of carrying some of my ideas further.

Lord Brain is Consulting Physician to London Hospital and Maida Vale Hospital for Nervous Diseases, London. This is the text of an address presented 27 December at the Montreal meeting of the AAAS.

In my presidential address I drew attention to our collective failure to foresee the consequences of much recent and current scientific work, and I stressed the need for more education in science. Tonight, however, I am concerned with more subtle, and therefore less obvious, obstacles to the acceptance of scientific ideas, for I want to inquire into the nature of current prejudices against science and scientists. By prejudices I mean emotional attitudes more positive and active than mere ignorance, even though, as I hope to show, some of them are the outcome of ignorance. No doubt these prejudices vary in force from one country to another, and in individual countries from one stratum of society to another. They need to be taken seriously, because scientists constitute a minority of all populations, and in democratic societies the practical uses of their achievements depend to a considerable extent upon their acceptance by the majority. And in that majority there are intelligent people whose education has given them little or no knowledge of science. Some of these are suspicious of a culture which they do not understand. Since these intelligent people are often also influential, they tend to propagate their suspicions among those who listen to what they say. My object in this address is, first, to try to remove some at least of the unreasonable suspicions which the nonscientists may harbor about science, and to make scientists themselves aware of them.

But it would be a mistake to suppose that all the hostility to science is due to either ignorance or prejudice. Science seems to many to present a complex challenge to other ways of thought, which, though not perhaps actually older, were well established for centuries before science grew out of its infancy. Part of this challenge, however, comes, not from science itself, but from philosophical ideas which science is thought to support; but part of it is more fundamental because it is the challenge of new facts about man and the world, which science is revealing, and of novel situations which call for decisions about action.