

Biochemistry. In the article “*crnA* encodes a nitrate transporter in *Aspergillus nidulans*” by S. E. Unkles, K. L. Hawker, C. Grieve, E. I. Campbell, P. Montague, and J. R. Kinghorn, which appeared in number 1, January 1991, of *Proc. Natl. Acad. Sci. USA* (88, 204–208), there was an error in Table 1. A nucleotide (C) was omitted from the *niiA* nucleotide sequence. The table should read as shown below.

Table 1. Homologous upstream nucleotide motifs

Gene	Position from translational initiation	Position from transcriptional initiation	Nucleotide sequence
<i>crnA</i>	–405	–218	TCG TGATCGG
<i>niiA</i>	–198	–181	---C---TC-
<i>niaD</i>	–216	ND	--T ----TT-

Data for *niiA* and *niaD* genes taken from ref. 5. Hyphens indicate identical nucleotides relative to *crnA* sequence; gaps introduced to maximize homology. ND, not determined.

Chemistry. In the article “Photoreduction of carbon dioxide by aqueous ferrous ion: An alternative to the strongly reducing atmosphere for the chemical origin of life” by Zofia Borowska and David Mauzerall, which appeared in number 18, September 1988, of *Proc. Natl. Acad. Sci. USA* (85, 6577–6580), the authors request that the main conclusion be retracted. The data on formation of formaldehyde shown in Figs. 2 and 3 and Table 1 cannot be reproduced. The error has been traced to baseline problems with the colorimetry. To resolve any ambiguity, we have repeated these experiments using ¹⁴C-labeled bicarbonate and formate. The results show that the yield of formaldehyde is less than 1% of that reported in the above article. The hydrogen yields reported in that article and in a previous paper (1) are reproducible. The data from these experiments will be published elsewhere.

1. Borowska, Z. & Mauzerall, D. (1987) *Origins Life* 17, 251–259.

Biochemistry. In the article “Expression of the bacteriopsin gene in *Halobacterium halobium* using a multicopy plasmid” by Mark P. Krebs, Thomas Hauss, Maarten P. Heyn, Uttam L. RajBhandary, and H. Gobind Khorana, which appeared in number 3, February 1991, of *Proc. Natl. Acad. Sci. USA* (88, 859–863), the authors request that the following corrections be noted. Beginning on line 13 of the Introduction, the text should read as follows: “. . . retinal in lipid or detergent vesicles. This method has been used in most of the studies reported (2). bR has also been regenerated in *Schizosaccharomyces pombe* expressing the *bop* gene (40). In the second” In *Materials and Methods*, under “Transformation and Characterization of Transformants” (p. 859), the sucrose concentration was given as 75% (wt/wt); it should be 75% (wt/vol). On page 863, left column, the sentence beginning on line 43 should read as follows: “The *bop* promoter is likely to be located within the 390-bp region upstream of the transcriptional start site.”

40. Hildebrandt, V., Ramezani-Rad, M., Swida, U., Wrede, P., Grzesiek, S., Primke, M. & Buldt, G. (1989) *FEBS Lett.* 243, 137–140.

Photoreduction of carbon dioxide by aqueous ferrous ion: An alternative to the strongly reducing atmosphere for the chemical origin of life

(photochemistry/Archaen oceans/Banded Iron Formations/hydrogen/formaldehyde)

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ABSTRACT We have shown that ferrous ion at neutral pH photoreduces water to hydrogen with a high quantum yield on excitation with near-ultraviolet light. This simple system also efficiently reduces carbon dioxide (bicarbonate ions) to formaldehyde. Overall, these reactions offer a solution to a dilemma confronting the standard or Oparin-Urey model of the origin of life. If carbon dioxide was the main form of carbon on the primitive earth, the ferrous photoreaction would have provided the reduced carbon necessary to form amino acids and other biogenetic molecules. We believe this system may have been the progenitor to the biological photosynthetic systems.

The quantum yield for the formation of hydrogen on irradiating aqueous ferrous ion at neutral pH with near-ultraviolet light is surprisingly high, ≥ 0.3 (1). A concerted reaction mechanism was proposed to explain these results. The photooxidation of ferrous ion in the Archaen oceans has been proposed as an explanation of the Banded Iron Formations (2) and supporting evidence has been obtained (3-5). We were interested in the relevance of this reaction to the hypothesis that early photosynthesis could involve the formation of hydrogen (6). We have suggested (1) that this source of hydrogen may also contribute to the solution of an increasingly vexing problem concerning the origin of essential constituents for the chemical origin of life. The comprehensive data of Stribling and Miller (7) clearly show that the yield of amino acids from a spark discharge decreases continuously as the reduction level of carbon decreases from -4 (methane) through -2 (carbon monoxide) and drops to insignificance on approaching +4 (carbon dioxide). However, geochemical models increasingly favor the emission of carbon dioxide, not methane, from degassing of the early earth (8-10), although there is no geological evidence available on this important matter. A continuous and large-scale production of reducing equivalents is thus needed as an alternative standard model of the origin of life. It is possible that hydrogen formation from irradiated aqueous ferrous ion at neutral pH or, more likely, the highly reactive intermediate reducing equivalents, served to reduce the carbon dioxide. Getoff (11, 12) reported that UV light induces photochemical reduction of carbon dioxide in aqueous solutions of ferrous ion leading to the formation of several organic compounds. Ackerman *et al.* (13) have shown that in the presence of transition metal salts aqueous saturated solutions of carbon dioxide were reduced to formate and formaldehyde and then, in low yield, to methanol and methane when irradiated with UV light of 254 nm. Joe *et al.*[†] report that formate and formaldehyde were produced on irradiating solid ferrous carbonate suspended in water. Most of the above work was

exploratory and detailed yields were not measured. Our quantitative data indicate that irradiating aqueous ferrous ion at neutral pH with near-UV light (306-390 nm) reduces bicarbonate solutions to formaldehyde in good yield.

MATERIALS AND METHODS

Solutions. The stock solutions of aqueous 0.2 M FeSO₄ were prepared from analytically pure reagent containing <0.02% Fe³⁺ as described (1). The 3-ml samples (total volume) were prepared by syringing aliquots of double-distilled water, 0.2 M FeSO₄, and 1 M NaOH or 1 M H₂SO₄ (the latter two for adjustment of pH) into the Pyrex glass photolysis cell. All solutions were purged with helium gas prior to syringing into the Pyrex cell that was kept under a constant flow of helium gas (4-8 ppm of O₂) of 10 ml/min. The level of oxygen was monitored during the entire experiment. The photolysis cell (5 cm × 2.2 cm × 1 cm) had four arms. Two arms were used to flow the helium gas through the cell. The other two arms, tightly plugged with septum plugs (6.5 mm × 8 mm from Anspec), were used for syringing samples with only a small leakage of air and for insertion of a microelectrode that allowed constant monitoring of pH.

Irradiation. The irradiation of samples was performed with a Hg/Xe lamp of 200 W held in a housing with parabolic reflector (Photon Technology 02-A1000). The distance between the lamp and the cell was 12 cm. A 5-cm-path-length quartz cell filled with 1% aqueous CuSO₄ was interposed between the lamp and the cell to remove infrared from the light beam. The solution was circulated from a reservoir with a peristaltic pump. The short-wavelength UV light emitted by the lamp was cut off at 306 nm with a Pyrex filter. The intensity of the light used in these experiments was 40 mW·cm⁻², unless stated otherwise. The incident wavelengths were 300-650 nm of which only the 300- to 400-nm range are active. To change the light intensity, screen filters were interposed between the lamp and the photolysis cell. The light intensity was measured by ferric oxalate actinometry (1, 14). The sensitivity range of this chemical actinometer (<300-450 nm) matches the active wavelengths of the light source. The amount of molecular hydrogen produced was measured with a zirconium oxide electrode (6) with a detection limit of 300 pmol of H₂ per min.

Reduction of CO₂. Three-milliliter samples for studies of the reduction of CO₂ were prepared by injecting into the photolysis cell aliquots of deaerated water and 0.2 M FeSO₄ and bringing the pH to 6.0 with 1 M NaOH. Subsequently an aliquot of 0.2 M NaHCO₃ was injected and the desired pH

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[†]Joe, H., Kuma, K., Paplawski, W., Rea, B. & Arrhenius, G. (1986) Fifth Meeting of the International Society for the Study of the Origin of Life and the 8th International Conference on the Origin of Life, July 21-25, 1986, Berkeley, CA, pp. 191-192 (abstr.).

was obtained by adding 1 M NaOH or 1 M H₂SO₄. The cell was kept under a constant flow of helium gas of 10 ml·min⁻¹. In all experiments gas equilibration was obtained in less than 2 min. On irradiation hydrogen production was observed with the characteristic liquid-gaseous equilibration time of 30 sec. Experiments were conducted with magnetically stirred and with unstirred samples, the latter to minimize loss of volatile products such as formaldehyde. The unstirred samples typically formed ≈80% as much H₂ as the stirred sample. At various time intervals, samples of 0.5 ml were withdrawn from the photolysis cell with a gas-tight syringe and were used to assay formate or formaldehyde. The absorption coefficient at 320–360 nm for ferrous ion at pH 6.0–7.2 is 3 ± 2. It is difficult to be more precise as even 0.1% of oxidation by traces of O₂ will lead to greater absorption by ferric hydroxide species.

Formate Formation. Formation of formate was measured by a simplification of a method using formate dehydrogenase (15). Samples (0.5 ml) were incubated for 15 min at 37°C with 0.01 unit of formate dehydrogenase dissolved in 0.5 ml of 20 mM potassium phosphate at pH 7.4 and 0.5 ml of 10 mM NAD⁺. The fluorescence of cooled samples was excited at 350 nm and read at 450 nm. The detection limit was 10⁻⁵ M formate in the original sample.

Formaldehyde Formation. Formation of formaldehyde was measured by the method of Feigl and Oesper (16). Each 0.5-ml aliquot was mixed immediately after withdrawal with 2 ml of 6 M H₂SO₄ and with 5 mg of chromotropic acid. Samples were heated for 15 min in a water bath at 60°C. After cooling to room temperature, the absorption was read at 570 nm (Zeiss spectrophotometer model PMQ-II). ε = 3.8 × 10⁴.

Chemicals. All chemicals were of analytical grade or better. FeSO₄·7H₂O, NaHCO₃, sodium formate, and chromotropic acid were from Fluka. Formaldehyde was from Merck. Formate dehydrogenase, potassium phosphate, and NAD were from Sigma. Helium gas containing 4–8 ppm O₂ was from Matheson.

RESULTS

We have shown (1) that the rate of production of hydrogen is linear in concentration of ferrous ion between 10⁻¹ and 10⁻² M. We have now extended this linear range to between 10⁻² and 10⁻⁴ M (Fig. 1). The pH profile remains the same at these low concentrations: increasing at pH >5.5 and leveling off at pH 8 (data not shown). The reaction is linear in light intensity and responds to wavelengths between 300 and 390 nm (1).

The addition of bicarbonate ion to the aqueous ferrous ion decreases the rate of hydrogen formation and produces formaldehyde (Fig. 2) on irradiation. The production of formaldehyde resembles that of hydrogen in two respects. Wavelengths >310 nm are sufficient since the reaction occurs in a Pyrex cell. A pH value >5.5 is necessary, presumably to form the active (Fe²⁺)_n(OH⁻)_m species (1). It differs, however, in that formaldehyde production stops at pH >7.5. This probably reflects the inactivity of carbonate ion, but the inherent complexity of this four-electron reduction requires further study to dissect its mechanism.

As expected, the partial inhibition of hydrogen formation by bicarbonate ion increases with decreasing concentration of ferrous ion and decreases with decreasing concentration of bicarbonate ion. Thus bicarbonate ion, or carbon dioxide, competes for reducing equivalents that otherwise form hydrogen. However, the amount of formaldehyde formed in a given time is ≈8% of the hydrogen formed, in terms of reducing equivalents, whereas the inhibition of hydrogen is ≈23% (Fig. 2). The discrepancy occurs because the measurements refer to the steady state, where the formation and further reaction of formaldehyde are balanced. Thus more hydrogen is used than appears as formaldehyde. This was

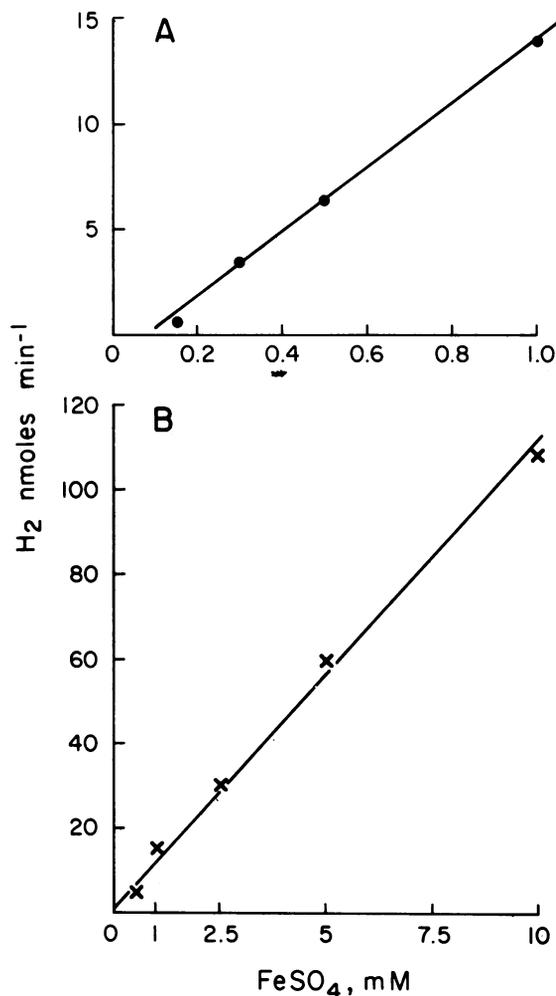


FIG. 1. Effect of low (10⁻⁴ to 10⁻³ M) (A) and medium (10⁻³ to 10⁻² M) (B) concentration of Fe²⁺ ion on hydrogen formation in magnetically stirred samples of aqueous FeSO₄ at pH 7.2, irradiated with near-UV light.

directly measured by illuminating a 10⁻⁴ M solution of formaldehyde in 0.1 M ferrous ion at pH 6.8. The half-life of the formaldehyde was ≈1 min under these standard conditions. Thus its reaction is rapid and can account for the extra loss of hydrogen formation.

Formate should be an intermediate in the reduction of carbon dioxide to formaldehyde, but we were unable to measure it at the limit of detection, 10⁻⁵ M. This concentration corresponds to ≈30 nmol in the cell and to the amount of formaldehyde we do detect (Fig. 2). Thus the failure may well be caused by the inadequate sensitivity of the analytic method. In fact, formate is readily reduced to formaldehyde under the conditions that reduce carbon dioxide (Fig. 3). About 5% of the formate was converted to formaldehyde in the steady state. Thus the concentration of formate from reducing carbon dioxide under these conditions will never be high.

Although the formation of hydrogen was linear with light intensity, irrespective of the presence of bicarbonate ion, the formation of formaldehyde from bicarbonate was supralinear (ref. 1 and Table 1). The limited data suggest the rate depends on the square of the light intensity and is not surprising given the requirement of a four-electron reduction process.

A 1:10 dilution of the bicarbonate and ferrous ion solutions decreased the formation of formaldehyde only by a factor of 4. Thus the dependence on concentration is less than linear.

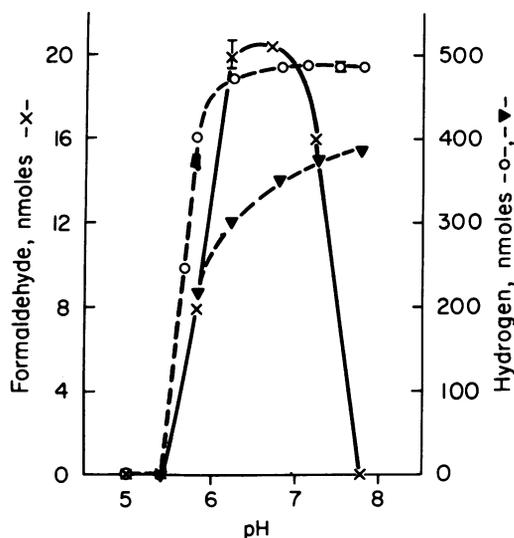


FIG. 2. pH dependence of hydrogen (\circ , ∇) and formaldehyde (\times) production on irradiating 0.1 M FeSO_4 with (\times , ∇) and without (\circ) 0.1 M NaHCO_3 with near-UV light. Samples (3-ml) of various pH values were irradiated for 5 min without stirring. The total quantities of H_2 and of formaldehyde were determined. Error bars are shown on two of the points, \times at pH 6.3 and \circ at pH 7.5.

DISCUSSION

The suggestion (2) that the Banded Iron Formations were the result of photooxidation of ferrous ion and not of oxidation by free oxygen has now received additional support (3–5, 17, 18). Our finding of the high quantum yield for this reaction (1) is explained by the concerted mechanism of this reaction and requires that the calculation of Francois (18) be corrected. This will increase the photochemical contribution to the formation of the deposits and further weaken a requirement for the presence of oxygen during this early period (3.5–2 gigayears). The measurement of hydrogen formation at a ferrous ion concentration of 10^{-4} M (Fig. 1) brings the reaction to within the range estimated for the concentration of ferrous ion in these Archaen basins (19). It is possible that the apparent lag in hydrogen formation at $<10^{-4}$ M (Fig. 1) reflects the aggregation of the $\text{Fe}^{+2}\text{OH}^-$ species proposed by our model (1). However, the data in this region are close to

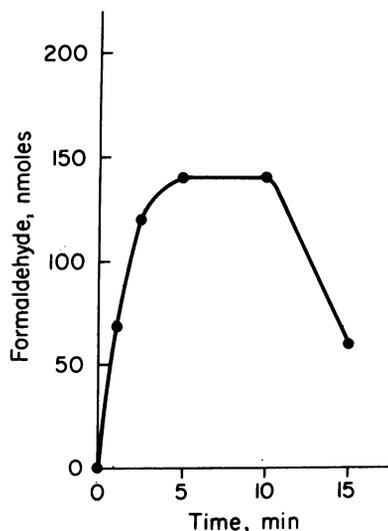


FIG. 3. Formation of formaldehyde as a function of time of irradiation with near-UV light of solutions of 1 mM sodium formate in the presence of 0.1 M FeSO_4 , pH 6.8.

Table 1. Effect of the intensity of the near UV light on production of hydrogen and formaldehyde from ferrous sulfate with and without sodium bicarbonate

Intensity, $\text{mW}\cdot\text{cm}^{-2}$	Time of irradiation, min	Hydrogen, nmol		Formaldehyde, nmol
		0.1 M FeSO_4	0.1 M FeSO_4 + 0.1 M NaHCO_3	
3	2	50	14 ± 0.3	0 ± 1.5
	5		38	0
	10		74	0
	15		112	0
16	2	200	64	2.3
	5		160	3.2
	10		314	2.9
	15		470	0
40	2	485	136	17.9
	5		340	20.5
	10		678	19.0
	15		1008	12.0

Solutions (3 ml) of pH 6.8 were irradiated without stirring for the indicated times. Amount of hydrogen was derived from the integral of the electrode signal from the gas phase, and formaldehyde was measured from a solution containing 0.1 M FeSO_4 and 0.1 M NaHCO_3 in aliquots withdrawn at that time.

the present sensitivity of the method. Concentrations of ferrous ion below several millimolar do not produce sufficient formaldehyde to be detected at the present limit of sensitivity. However, the less than linear decrease of yield with decreased ferrous–bicarbonate ion concentration argues favorably that the reaction occurs at lower levels. More sensitive measurements (e.g., fluorescent derivatives or radiocarbon labeling) are required to confirm this view. The situation is similar for the determination of formate ion, a likely intermediate. The rapid disappearance of formaldehyde when added to the irradiated, neutral ferrous ion solution indicates that it may well be reduced to methanol and to methane. Evidence for their formation under somewhat similar conditions exists (11, 13). There is evidence for the reduction of nitrogen to ammonia in a presumed thermal reaction with ferrous hydroxide at the very specific pH of 8.6 (20). The present photoreaction may reduce nitrogen, but the presence of traces of ammonia in the ferrous salt and interferences of the iron salts in colorimetric reactions prevented a clear conclusion.

The present evidence is sufficient to show that the prevalence of carbon dioxide over methane on the early earth is an insufficient argument to invalidate the Oparin–Urey scheme of the chemical origin of life. The efficient reduction of carbon dioxide to formaldehyde by neutral aqueous ferrous ion when irradiated with near ultraviolet light will provide the necessary reduced carbon to form the amino acids, purines, pyrimidines, etc. In fact the presence of appreciable amounts of formaldehyde will contribute to many of these reactions, as well as open channels to the formation of sugars. The fixation of nitrogen, if in fact it occurs, would supply the necessary ammonia in a continual, steady-state manner.

The Banded Iron Formations themselves allow an estimate of the reducing power available at that time (2.5 gigayears). The reducing equivalents in the Hamersley basin alone amount to 3×10^{11} electrons per year (19) or 0.3% of the present fixation of carbon by photosynthesis. The total reducing power of that basin, 10^{18} equivalents, could reduce about one-third of the present total oceanic carbon dioxide, itself ≈ 100 times that now present in the atmosphere (21). Thus the photochemical formation of the Banded Iron Formations could have reduced a considerable fraction, if not all, of the available carbon in the Archaen times. Our crude estimate of the small optical cross section of the photoactive

species ($\approx 3 \times 10^{-6} \text{ \AA}^2$) still implies that the lifetime of the ferrous ion in the upper levels of the primitive ocean would only be $\approx 10^6$ sec or 1 week. This estimation assumes the present solar output between 300 and 400 nm. The early sun was cooler but may have had a much larger output in the UV region (22). Thus the rate of photooxidation will be limited by the supply of ferrous ions from upwelling currents and river runoff to the shallow seas.

The composition of iron oxides of the Banded Iron Formation in the Hamersley basin is about one-half magnetite (Fe_3O_4) and one-half hematite (Fe_2O_3). In an aggregate of ferrous hydroxide the photooxidation may be self-limiting since the ferric ion is a powerful quencher of the reaction (1). However, in the more dilute Archaean ocean, near neutral pH, the highly insoluble ferric hydroxide or magnetite would rapidly precipitate, leaving the ferrous ion to undergo efficient photooxidation. The reduced carbon formed by the reduction of carbon dioxide could sediment, possibly as cellular debris since possible microfossils have been found in these formations (23). The ferric oxides would oxidize this carbon to carbon dioxide and become (partially) reduced to hematite. In this way both the prevalence of hematite and the relative absence of carbon in these formations could be explained. A bacterium has been found that utilizes precisely this reaction as an energy source (24). It reduces ferric oxide to hematite with acetate. Thus considerable carbon cycling may have occurred in these Archaean basins.

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