process itself generates no significant heat or other pollutants. Overall, the dialytic battery should be a very clean process for power generation.

In evaluating an infant technology, one should probably begin on an optimistic note to avoid discarding ideas prematurely. But we do wish to sound several warnings. First, domestic river flow is not nearly sufficient to satisfy current energy demand in the United States. The dialytic battery should be considered an adjunct to efforts in other directions, or for smaller-scale use in special situations and perhaps in less developed areas of the world. Second, power generation will not be the most effective use of fresh water where the water itself is in demand for industrial, agricultural, or personal use. Third, the cost of components of the dialytic battery will increase along with that of energy, although not necessarily in direct proportion. In conclusion, large-scale energy conversion by the dialytic battery may become practical, but only with major advances in the manufacture of ion exchange membranes and with careful optimization of operating conditions.

JOHN N. WEINSTEIN

Laboratory of Theoretical Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

FRANK B. LEITZ

Bureau of Reclamation, Department of the Interior, Denver, Colorado 80225

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7. G. Murphy [*Ind. Eng. Chem.* **50**, 1181 (1958)] considered a variation in which mixing of brackish and sea waters could be used directly, without electrodes, to desalinate water. K. H. Meyer and W. Straus [*Helv. Chim. Acta* **23**, 795 (1940)] had studied membrane arrays in another context.
8. (i) Minor ionic constituents can be assigned experimentally determined transference numbers; (ii) osmotic and electroosmotic water fluxes can be analyzed in the framework of irreversible thermodynamics; (iii) the effect of concentration polarization due to current passage can be approximated most simply by substituting for Eq. 1 the expression

$$V^{o}_{\text{stack}} = 2N\,\frac{\alpha RT}{\mathcal{F}}\ln\left(\frac{c_s-\delta_s I/2A\mathcal{D}}{c_r+\delta_r I/2A\mathcal{D}}\right)^{0.917}$$

where $\mathcal{D}$ is the NaCl diffusion coefficient, δ is an average unstirred layer thickness, and the other symbols are as defined later in the text; the exponent relates activity and concentration ratios in the range 0.001 to 0.6M; and (iv) changes in concentration along each channel can be handled by integration or by choice of reasonable averages.
9. G. L. Wick and J. D. Isaacs, "Salinity power," *Scripps Inst. Oceanogr. Inst. Mar. Resour. Ref. 75-9* (1975); S. Loeb, *Science* **189**, 654 (1975); R. S. Norman, *ibid.*, p. 655. Hypersaline bodies of water could be used in conjunction with either brackish or sea water. Such special situations would permit higher mass transfer rates through the dilute compartments and would solve in part the problem of water pretreatment, but would also require much more selective membranes.
10. In terms of conventional cation transport numbers τ_+ [for example, see N. Lakshminarayanaiah, *Transport Phenomena in Membranes* (Academic Press, New York, 1969, p. 6)], we have $\alpha = \tau_+^c - \tau_+^a$. If all current passing through the anion exchanger were carried by Cl^- and all current through the cation exchanger by Na^+, then α would be unity; if neither membrane were selective, then α would be zero.
11. Electrode reactions could be chosen to yield useful products, but the first law of thermodynamics dictates that any energy so used would represent an inefficiency in power generation. Electrode cost must be considered in planning stack size.
12. F. Leitz, "High temperature electrodialysis—Phase I," *U.S. Dep. Inter. Off. Saline Water Res. Dev. Rep. No. 912* (1974).
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14. *Hydroelectric Plant Construction Cost and Annual Production Expenses, Sixteenth Annual Supplement—1972* (Federal Power Commission, Washington, D.C., 1975).
15. First, current densities in the dialytic battery are an order of magnitude lower than in electrodialysis. Second, in the dialytic battery, polarization reduces concentration differences across the membrane instead of developing the alkaline, salt-rich layer responsible for scale formation. Finally, a leaky membrane, even one grossly torn, removes only the potential of a single cell pair rather than contaminating the product from the entire stack.
16. We assume straight-line amortization at 5 percent interest, 90 percent operating time, and running costs small by comparison.
17. Resistances were obtained by an a-c method in 0.1M NaCl; measurements of membrane resistance between solutions of pertinent unequal concentration are planned. The parameter α, determined from concentration potentials, varied only a few percent over the range of concentration pairs used.
18. We thank H. Viklund of Ionics, Inc., for his assistance in taking data and B. Bunow, J. Isaacs, and K. S. Spiegler for their advice on the manuscript. Experiments were performed at Ionics, Inc., Watertown, Mass. A preliminary version of the theoretical analysis was presented in San Francisco, September 1974, before the Salinity Power Study Group sponsored by the Scripps Institution of Oceanography and Oregon State University.

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Photoproduction of Molecular Hydrogen by a Plant-Algal Symbiotic System

Abstract. *The rapidly growing water fern* Azolla, *which contains a nitrogen-fixing blue-green algal symbiont, has been studied as a possible system for photoproduction of molecular hydrogen. When this plant is grown on a combined nitrogen supply, photochemically generated hydrogen can be diverted through the algal nitrogenase system, which serves as a source of molecular hydrogen generated from water. This symbiosis has several advantages as a possible biological energy conversion system.*

In the past few years, hydrogen has been considered as a renewable nonpolluting fuel, and this consideration has led to reports about its possible biological production by photosynthetic systems (*1*, *2*). Two known systems for photohydrogen production, the anaerobically adapted green algal system of Franck and Gaffron (*3*) and the photosynthetic bacterial system of Gest and Kamen (*4*), require an exogenous electron donor and hence are not independent of a substrate other than water. Suitably constructed enzymatic systems containing chloroplasts can evolve hydrogen from water, but these are labile and, at present, have limited utility (*5*).

Benemann and Weare's report (*6*) that the nitrogen-fixing blue-green alga *Anabena cylindrica* could evolve hydrogen from water opened up the possibility of finding similar nitrogenase-containing photochemical systems that are stable and useful for biological hydrogen production. The water fern *Azolla*, a rapidly growing plant that contains a nitrogen-fixing blue-green algal symbiont *Anabena azolla* within its leaf cavity (*7*), offers several advantages as a hydrogen-producing system.

Plants were grown in shallow layers of a nitrogen-free or nitrate-containing Hoagland solution (*8*) in 3-liter Fernbach flasks under constant illumination in air at 25°C. Approximately 30 g of fresh plant tissue was collected in 2 weeks from each flask, depending on inoculum size. Samples of individual plants or extracts were incubated in the light in 15-ml Erlenmeyer flasks fitted with screw caps containing

Table 1. Acetylene reduction and hydrogen evolution of *Azolla* plants grown on nitrogen-free and nitrate media. All values are nanomoles of gas formed per hour per gram of fresh plant tissue. Approximately 2 mg of nitrogen and 0.4 mg of total chlorophyll are contained in 1 g of tissue. When added, acetylene was 10 percent of the gas phase. The assay medium was the same as the growth medium.

Gas phase	Evolution (nmole hour^{-1} g^{-1})	
	Ethylene	Hydrogen
Nitrogen-fixing plants		
Argon	900	130
Argon, dark control	0	0
Argon (80 percent), O_2 (20 percent)	320	90
Nitrogen	460	0
Air	500	0
Nitrate-grown plants		
Argon	500	500
Argon, dark	0	0
Argon (80 percent), O_2 (20 percent)	340	300
Nitrogen	500	120
Air	340	120

Table 2. Effect of carbon monoxide on acetylene reduction and hydrogen evolution. When added, acetylene was 10 percent of the gas phase.

Gas phase	Evolution (nmole hour^{-1} g^{-1})	
	Ethylene	Hydrogen
Nitrogen-fixing plants		
Argon	1200	250
Argon (90 percent), CO (10 percent)	0	250
Nitrate-grown plants		
Argon	200	170
Argon (90 percent), CO (10 percent)	0	170
Algal-free *Azolla**	0	0

*Four-hour assay.

rubber septa for removal and injection of appropriate gas atmospheres. Acetylene reduction to ethylene [a measure of nitrogenase (nitric-oxide reductase, E.C. 1.7.99.2) activity] and hydrogen evolution were assayed gas chromatographically with Poropack R (*9*) and molecular sieve 5A columns, respectively, at 55°C (*5, 10*).

Azolla plants can be grown on nitrate. Under these conditions the plants still contain algal symbionts and show nitrogenase activity, although upon repeated transfer on nitrate such activity eventually declines. Nitrogen-fixing plants and plants grown on nitrate media were compared (Table 1) with respect to nitrogenase and hydrogen-evolving activity. Whereas plants grown on nitrate evolve molecular hydrogen at rates comparable to their total nitrogenase activity, plants grown under nitrogen-fixing conditions evolve hydrogen at only a small fraction of the rate of nitrogen fixation. In both plant samples, nitrogenase activity and hydrogen evolution were light-dependent and were greatest in inert atmospheres. Hydrogen evolution under these conditions remained constant for at least 24 hours. In some plant samples grown on nitrate, hydrogen evolution actually exceeded total nitrogenase activity measured as acetylene reduction.

It seemed possible that at least some of the hydrogen evolved by plants grown on nitrate could be derived from a larger pool of endogenous reserves no longer needed for nitrogen reduction. However, attempts to increase the pools and enhance hydrogen evolution by various means, such as preincubation with carbohydrates, were unsuccessful. Furthermore, hydrogen evolution by both plant types was insensitive to carbon monoxide, indicating that the major source of hydrogen was the algal nitrogenase, and not some other hydrogenase activity elaborated in the symbiotic system. Plants freed of algae by prolonged growth in nitrate media containing antibiotics did not reduce acetylene or evolve hydrogen (Table 2).

Low concentrations ($10^{-5}M$) of DCMU [3′-(3,4-dichlorophenyl)-1′,1′-dimethylurea], an inhibitor of oxygen evolution (photosystem II), inhibited acetylene reduction and hydrogen evolution to a similar extent in both nitrogen-fixing and nitrate-grown plants. Considering that the intact plants had to transport the inhibitor, the inhibition was very rapid and did not require prior incubation to deplete endogenous reserves, as is common with many algal systems (*11*) (Table 3). These data indicate that a major part of the electrons for hydrogen evolution by this system are derived from water by utilizing photosystem II.

The blue-green algae in leaves of *Azolla* do not occur as either individual cells or free chains of cells, but as spherical bundles or packets of chains surrounded by a membrane-like structure (*8, 12*). These packets are readily observed in preparations of crushed leaves and can be isolated from leaf homogenates after vacuum infiltration and incubation of leaves with a mixture of pectinase (polygalacturonase, E.C. 3.2.1.15) and cellulase (E.C. 3.2.1.4). About 10 percent of the total nitrogenase activity can be recovered in leaf homogenates, and these preparations also yield hydrogen in a manner characteristic of the plants from which they are derived. Homogenates from nitrate-grown plants also display greater hydrogen-evolving activity than preparations from plants grown under nitrogen-fixing conditions (Table 4).

Hydrogen evolution is a side or partial reaction of the nitrogenase-catalyzed reduction of nitrogen to nitrate (*13, 14*). Photochemically generated reductants diverted through photosynthetic nitrogen-fixing systems can serve as electron donors to hydrogen, provided the nitrogenase complex, which is unstable in the presence of oxygen, can be suitably protected. This protection must be achieved in some physiological manner in the blue-green algae, which can produce both hydrogen and oxygen photosynthetically. In *Azolla* plants growing on nitrate, the algal nitrogenase is no longer essential for the plants' nitrogen supply, and hence a greater part of the nitrogenase activity can be utilized for hydrogen evolution from photoreductant. Furthermore, the algal packets are not directly exposed to exogenous plant nutrients because neither nitrate nor ammonia inhibits nitrogenase activity in these plants. Hence, the *Azolla–Anabena azolla* symbiosis is particularly attractive as a system for biological hydrogen production.

Table 3. Effect of DCMU on acetylene reduction and hydrogen evolution. One-gram samples were removed from a large plant culture and assayed; $10^{-5}M$ DCMU was then added and samples removed periodically for assays. Cultures were maintained in light throughout the experiments. All assays were in argon or 90 percent argon–10 percent acetylene, in light.

Conditions	Evolution (nmole hour^{-1} g^{-1})	
	Ethylene	Hydrogen
Nitrogen-fixing plants		
Control, no DMCU	1000	300
DCMU (10^{-5} *M*)		
4 hours	300	170
8 hours	220	120
16 hours	80	90
Nitrate-grown plants		
Control, no DCMU	500	500
DCMU (10^{-5} *M*)		
4 hours	220	260
8 hours	200	140
16 hours	40	90

Table 4. Comparison of acetylene reduction and hydrogen evolution of plants and homogenates of *Azolla*. All values are nanomoles per hour per gram of fresh tissue. Homogenates were prepared by vacuum infiltration of 4 g of plant tissue in 20 ml of 0.15*M* citrate-phosphate buffer, *p*H 6.5, which contained 0.1*M* thioethanol, 100 mg of cellulase (Onozuka), and 100 mg of pectinase (Sigma). This treatment was followed by incubation for 2 hours at 25°C in an argon atmosphere. The gelatinous digest was recovered by filtration, washed with buffer, and assayed.

Conditions	Evolution (nmole hour^{-1} g^{-1})	
	Ethylene	Hydrogen
Plants		
Nitrogen-fixing	1200	360
Nitrate-grown	800	760
Homogenates		
Nitrogen-fixing	50	15
Nitrate-grown	50	180

J. W. NEWTON
Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

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Brain Acetylcholine: Control by Dietary Choline

Abstract. *Acetylcholine concentrations in whole rat brain or in various brain regions and free choline concentrations in blood serum and brain vary with dietary choline consumption. The increases in brain acetylcholine after treatment with physostigmine (an inhibitor of acetylcholinesterase) or after consumption of a diet high in choline are additive, suggesting that choline acts by increasing acetylcholine synthesis.*

The availability of amino acid precursors is an important factor controlling the rates at which neurons in rat brain synthesize such monoamine neurotransmitters as serotonin (*1*) and the catecholamines (*2*). We have shown that the administration of a single intraperitoneal dose of choline can cause sequential elevations in brain choline and acetylcholine (ACh) concentrations in rats (*3*). Haubrich *et al.* failed to detect increased brain ACh in rats given choline intravenously and killed by decapitation. However, in a subsequent study they did observe that brain ACh increased in guinea pigs receiving choline by intracarotid perfusion (*4*). Moreover, Nagler *et al.* have reported significant decreases of ACh content in the brains of weanling rats deprived of choline for 5 days (*5*). These observations have been taken as evidence that pharmacologic or pathologic changes in choline availability can also influence the synthesis of its neurotransmitter product, ACh, in rat brain (*3*). A significant fraction of plasma and, therefore, brain choline is of dietary origin (*6*). We show that physiologic variations of choline content in the diet (that is, variations within the range that omnivorous animals or humans might consume from day to day) are associated with parallel changes in brain ACh concentrations, especially within the caudate nucleus. These observations suggest that the amounts of ACh stored in terminals of central cholinergic neurons may not be constant. Thus, the number of ACh molecules released when these neurons fire may vary with nutritional status and may be altered by administering or withholding choline.

Adult male Sprague-Dawley rats were given free access to food and water and maintained in group cages in a room kept at 20° to 22°C; light (Vita-Lite, 33 μw/cm^2) was furnished between 8 a.m. and 8 p.m. daily. The animals were killed between 9 and 11 a.m. at the end of the experimental period. Food and water consumption was measured daily. In experiments requiring injections, animals received 1 ml per kilogram of body weight intraperitoneally; control and treated animals were injected and killed alternately to minimize the effects of possible rhythms (*7*). Animals whose brains were assayed for choline and ACh were killed by a 3.5-second exposure of the head to microwave radiation in the waveguide of a modified Litton microwave oven (Medical Engineering Consultants) to protect brain choline and ACh from enzyme-mediated postmortem changes (*8*). Brains were excised and frozen until assayed. Choline and ACh were extracted (*3*) and assayed (*9*). Animals whose serums were assayed to determine choline concentrations were killed by decapitation; blood was collected from the cervical wound. Data were analyzed according to Student's *t*-test, linear regression analysis, or two-way analysis of variance.

In initial studies (Fig. 1) groups of eight to ten rats consumed the diet deficient in choline (Nutritional Biochemicals) with or without added choline (0.183 or 1.83 percent) for 11 days (*10*). Five to seven rats from each group were killed by microwave irradiation, and their brains were dissected into various regions and assayed for choline and ACh; the remaining animals were killed by decapitation and their serums were assayed for choline. Serum choline concentrations were proportional to mean dietary choline intake (r = .91); the average serum choline concentrations for rats consuming an average of 0, 20, or 129 mg of choline daily were: 8.0, 15.3, and 32.6 nmole/ml, respectively. The ACh concentration in the caudate nucleus was 28 percent greater in rats consuming 20 mg of choline per day and 45 percent greater in rats consuming 129 mg of choline per day than in animals deprived of choline. The ACh concentration also was significantly increased in other brain regions of rats ingesting the larger amount of choline. Similar results were obtained by adding choline to the drinking water (*11*). In general, the consumption of large doses of choline depressed food intake (Fig. 1 and Table 1); however, the lower choline dose, which also elevated caudate ACh concentrations, had no effect on food consumption.

To determine whether the choline-induced rise in brain ACh reflected accelerated synthesis or decreased breakdown of the neurotransmitter, the effects of dietary choline were compared in animals that were or were not also treated with physostigmine, an inhibitor of brain acetylcholinesterase. Groups of rats consumed a diet deficient in choline (Bio-Serv, Inc.) and drank distilled water with or without added choline chloride [1.5 or 15 mg/ml (Table 1, cerebrum animals); 15 mg/ml (Table 1, caudate animals)] for 7 days. At the end of this period, half of the animals in each group were injected with physostigmine salicylate (1 mg/ml intraperitoneally, dissolved in saline); the other half received only saline. Animals were killed by microwave irradiation 20 minutes after injection,

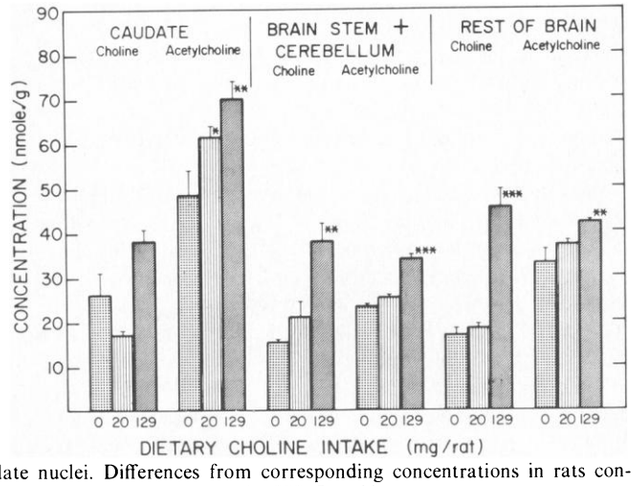

Fig. 1. Effect of dietary choline content on choline and ACh concentrations in various brain regions. Rats (weighing 90 g) consumed diets having an average of 0, 20, or 129 mg of choline per day for 11 days, thereby consuming 12 ± 1, 11 ± 1, and 7 ± 1 g of food per day, respectively. All groups consumed 17 to 19 ml of water per day. Bars represent mean concentrations, and vertical lines represent the standard error of the mean. "Rest of brain" refers to the cerebrum minus the caudate nuclei. Differences from corresponding concentrations in rats consuming no choline are indicated by *, $P < .05$; **, $P < .01$; ***, $P < .001$.