branch to *Clostridium* in conformance with our ferredoxin tree.

With respect to Démulin's criticism of our composite tree ([figure 5 in (1)]) it is clear in our references that we had available to us sequences of SS rRNA from *Bacillus stearothermophilus* and cytochrome *c*₃₅ from *Pseudomonas fluorescens*. This brings the number of identical species appearing on two trees to five. We regret any confusion caused by our picturing only representative sequences on those two trees. For the "plant chloroplast" branch, sequences were not available from the same species. However, comparable data in the context of major phylogenetic branches mean that the sequences and the species in which they are found are sure to be directly descended from the same gene in the first ancestor on the branch. We assumed that the green algae and vascular plants share a common chloroplast ancestor and therefore used the *c*₃₅ sequence from *Euglena* and the ferredoxin from *Scenedesmus* to locate this branch in the composite tree. In combining the ferredoxin and SS rRNA trees, we assumed that the two coccoid blue-green algae, *Aphanothecae* and *Anacystis*, shared an ancestor more recently than the time of divergence of both from *Pseudomonas*.

Our suggestion that the development of aerobic respiration preceded that of oxygen-releasing photosynthesis is not proved by sequence data. However, our reasoning does not involve arguments about whether the ancestor of *Pseudomonas* and *Anacystis* is more like one or the other organism. Most of the Rhodospirillaceae, the pseudomonads, *Escherichia*, and some species of blue-green algae such as *Nostoc* sp. strain MAC can live heterotrophically in aerobic conditions. It therefore seems reasonable to suggest that their most recent common ancestor possessed a rudimentary form of aerobic respiration. On our composite tree, we place the development of some important components of a respiratory chain slightly earlier, near the divergence of the *Bacillus* and *De- sulfovibrio* branch from the trunk of the tree.

Both of these organisms possess respiratory metabolisms. However, *De- sulfovibrio* respires anaerobically using sulfate as the terminal electron acceptor, whereas *Bacillus* respires aerobically. It is certainly possible that aerobic respiration evolved separately in all of these lines. However, that is not the simplest explanation of our composite tree. Contrary to Démulin's commonsensuse argument, Schopf (8) has pointed out that it is difficult to imagine the development of oxygen-releasing photosynthesis prior to the development in that line of a rudimentary mechanism for coping with oxygen, as oxygen is produced intracellularly in photosynthesis. This is not to say that aerobic respiration developed in anything like the present atmosphere. The high level of free oxygen in our present atmosphere is almost certainly due to oxygen-releasing photosynthesis.

Our composite tree clearly supports a symbiotic origin for the eukaryotes. It pictures the branches that contribute to the eukaryote host and organelles as distinctly separate, with each being closely related to contemporary free-living prokaryotes. Démulin states that a resolution of the question of how eukaryotes originated "will have to wait for perfectly comparable data coming from the three eukaryotic cell compartments (for example, partial sequences of the large rRNA's)." We would welcome the elucidation of additional sequence data for its value in reconstructing a highly probable evolutionary schema. However, no information is perfect. A tree based on partial rRNA sequences might well suffer all the criticisms Démulin has made here. If there is any suggestion of gene doubling in the sequences, the possibility that nonorthologous segments are being compared could be raised. One could raise questions about the accuracy of the tree in a small region and suggest that the accuracy of the overall tree is suspect. Alternative methods, whether or not sound, could be used to demonstrate the unreliability of any tree based on the segments.

In the nearly 3 years since we first presented our composite tree (9), we have found it an excellent working hypothesis with which to organize new sequence data and our ideas. We hope that it is as useful to others in the many disciplines for which it has implications.

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### References and Notes

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5. T. Hase, S. Wakabayashi, K. Wada, H. Matsu- barara, J. Biochem. 83, 761 (1978); K. G. Hutson, L. J. Rogers, B. J. Haslett, D. Boulter, R. Cam- mack, Biochem. J. 172, 465 (1978). These give the complete sequences of two ferredoxins from *Anacystis sacrum* and partial sequences of two ferredoxins from *Nostoc* sp. strain MAC, respectively. Our alignment of these sequences suggests that a single duplication occurred prior to the divergence of these blue-green algae. Taken together with the partial rRNA sequences in the other chloroplasts-blue-green algal sequences, this subtree is rooted near this duplication.
7. H. Hori and S. Osawa, Proc. Natl. Acad. Sci. U.S.A. 76, 381 (1979); H. Hori, Mol. Gen. Genet. 145, 119 (1976). From a matrix of evolution- ary distances, the authors first connect the se- quences with the smallest matrix element. They locate the point of the sequences' divergence at the midpoint of a line proportional to the matrix element. Next, they locate the next smallest matrix element and repeat the process, construct- ing the tree by successive steps. This method gives erroneous answers for branches where little evolutionary change has occurred. It is not as accurate as our method, which considers all reasonable topologies for all the branches.

25 June 1979

### Long-Term Choline Treatment of Memory-Impaired Elderly Patients

Davis *et al.* (1) and Sitaram *et al.* (2) have reported an improvement in human memory following the administration of single doses of certain cholinomimetic agents (1 mg of phystostigmine, 4 mg of arecholine, and 10 g of choline chloride). In both studies, the enhancement of memory was demonstrated in normal volunteers by means of a verbal serial learning task. These results, coupled with evidence that reduced cholinergic function may be related to the memory decline of the elderly and senile, led the authors of both reports to suggest that treatment with cholinergic agents might benefit elderly patients with memory impairment.

Since there are no clearly efficacious treatments currently available for age-related memory impairment (3), we find the data in (1) and (2) to be encouraging and agree that the potential use of cholinergic agents in senility should be investigated. However, the likelihood of suc-
cess in this endeavor must be tempered by two considerations. First, cholinergic agents might not improve cholinergic function in elderly patients whose cholinergic deficits are due to a structural loss of brain tissue, as would be the case in patients with Alzheimer's disease or multi-infarct dementia. Second, the demonstration of improved memory with a single dose of physostigmine or choline chloride does not necessarily imply that long-term treatment would continue to produce such improvement. The homeostatic process of the brain may act to reverse a temporary increase in acetylcholine availability.

The above considerations may account for the fact that several clinical trials with choline in elderly subjects have failed to demonstrate the expected improvement in memory or other aspects of cognition. Boyd et al. (4) treated seven patients who were severely impaired with Alzheimer's disease with 5 g of choline chloride per day for two weeks and then 10 g per day for 2 weeks. No significant cognitive or clinical changes were produced. In a placebo-controlled study with eight normal elderly subjects, Mohs et al. (5) found no improvement in memory storage, retrieval, mood, or social functioning after treatment for 7 days with 16 g of choline chloride per day. The memory tests in this study were similar to those used by Davis et al. (1) in their study of the effects of physostigmine on normal volunteers. A study conducted in our laboratory (6) also failed to confirm a memory enhancement with long-term choline chloride treatment. Fourteen elderly outpatients suffering from mild to moderate cognitive impairment received choline chloride treatment for 4 weeks. The dosage was gradually increased during the first 2 weeks, with maximum doses of 12, 16, or 20 g per day, depending on individual patient tolerance; dosage at the highest level of tolerance was maintained during the final 2-week period. Of 26 cognitive test measures, including both memory and performance tasks, none showed statistically significant improvement after treatment. There were also no significant changes in mood or behavior. Finally, Etienne et al. (7) reported no improvement in three patients with Alzheimer's disease after they were treated for 1 month with up to 8 g of choline bitartrate per day, and in a placebo-controlled crossover study, Smith et al. (8) found no cognitive changes in ten Alzheimer's patients treated with 9 g of choline bitartrate per day for 2 weeks.

It is thus apparent that preliminary trials of long-term choline treatment in the elderly have failed to demonstrate the improvement in memory reported for younger, cognitively unimpaired adults treated with a single dose of a cholinergic agent. Although several of the cited studies were not placebo-controlled, it is not likely that investigator bias or placebo effects would produce negative rather than positive results. Another possibility is that there is a narrow effective dose range because of a curvilinear or biphasic dose-response function. However, a wide dose range has been used (5 to 20 g per day), and there is apparently no evidence supporting a presynaptic biphasic effect. It remains possible, however, that a subgroup of individuals may respond to choline; such individuals might have an underlying cholinergic deficiency but retain sufficient functioning presynaptic neurons.

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References and Notes
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9. Supported by PHS grant 1RO1 MH 25990.
29 September 1978; revised 9 April 1979

Ferris et al. correctly point out some serious obstacles to the development and use of long-term cholinomimetic treatments for age-related memory deficits. Following our report of enhancement in long-term memory functioning with physostigmine we investigated the effects of choline chloride on memory. Doses of both 8 and 16 g of choline chloride per day, administered in a double-blind crossover design, did not significantly affect memory functioning in the same young normal people who were previously improved by physostigmine (1, 2). Similarly, neither 8 nor 16 g of choline chloride per day, administered under double-blind conditions, significantly enhanced memory functioning in elderly subjects with age-related memory impairments (3, 4). Patients with Alzheimer's disease have also received 2 to 16 g of choline chloride per day in a double-blind crossover study extending 56 days, and also derived no benefit from choline chloride treatment. In contrast, low doses of physostigmine (0.25 to 0.50 mg) administered to elderly demented and nondemented subjects significantly improved their ability to store information into long-term memory (2).

These data indicate that if choline chloride, or presumably lecithin, has an effect on memory it is quite subtle, possibly affecting only certain kinds of memory traces (5), and may be apparent only in subgroups of people, although choline probably increases striatal cholinergic activity. These results raise the question of whether the use of acetylcholine precursors can increase cholinergic activity in hippocampal and cortical cholinergic synapses.

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References and Notes
19 June 1979

Cardiopulmonary Changes in Kittens During Sleep

Baker and McGinty (1) asserted in their title that their animal model showed "cardiopulmonary failure" and that this had important implications for the sudden infant death syndrome (SIDS). Whether the heart is primarily involved in SIDS is a matter of intense interest to physicians, scientists, and even lay groups associated with this tragic problem, the foremost cause of death in infants during the first year of life. I submit that Baker and McGinty have based their
Long-term choline treatment of memory-impaired elderly patients
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Science 205 (4410), 1039-1040.
DOI: 10.1126/science.472728