600 million years as indicated by Ronov (*8*) and the volume of sedimentary rocks has been reasonably stable as implied by Garrels and Mackenzie's model, the reservoir of reduced organic carbon has been large throughout the later part of geologic time. This does not require that atmospheric oxygen has been strictly constant; the other oxygen sinks may be important controls and might fluctuate independently. It does imply, however, that there is no need to call on photodissociation of water or other potential sources for atmospheric oxygen. We can thank green plants.

PAUL ENOS

Department of Geology,
State University of New York at
Binghamton, Binghamton 13901

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4. B. Mason, *Principles of Geochemistry* (Wiley, New York, ed. 3, 1966), p. 208; data from F. A. Pareth, *Quart. J. Roy. Meterol. Soc.* **63**, 433 (1937).
5. P. E. Cloud, *Amer. J. Sci.* **272**, 541 (1972). The values used were essentially those of Rubey (*3*). This test is not very sensitive to atmospheric oxygen which constitutes only 2 percent of the total considered.
6. W. S. Broecker, *Science* **168**, 1537 (1970).
7. R. M. Garrels and F. T. Mackenzie, *ibid.* **163**, 570 (1969).
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I did not say (*1*), nor do I see that I implied, that most or effectively all atmospheric oxygen did not originate by photosynthesis. In fact my concern with the great mass of reduced carbon implies a belief that this was the main origin. However, the continual appearance of new reduced material from outside the photosynthetic cycle causes a problem if there is no other process involved, and analogous problems intrude themselves (*1*) in regulation and at the time of origin of an oxidizing atmosphere. Enos (*2*) does not reach these problems.

LEIGH VAN VALEN

Commission on Evolutionary Biology,
University of Chicago, Chicago, Illinois

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Origin of Mitochondria

The origin of eukaryotic organelles is an intriguing phylogenetic question both because of the antiquity of divergence of eukaryotes and prokaryotes and because of the enormous structural and functional differences between these grades of organization. It is refreshing to find a review (*1*) that opposes the widely publicized notion of the endosymbiotic origin of eukaryotic organelles (*2*). We feel, however, that some of the principles of systematics have been ignored by both those in favor of and against the endosymbiosis hypothesis.

According to Raff and Mahler, the time lag between the 1.8-billion-year-old oxidized red beds (an indication of free O_2 in the atmosphere) and the oldest probable eukaryote fossils (Beck Springs Dolomite, 1.2 to 1.4 billion years ago) discredits the endosymbiosis hypothesis. We find nothing in the hypothesis itself that requires that "eukaryotes acquired aerobic symbionts as free O_2 began to appear." The general biochemical opportunism of prokaryotes (witness their present metabolic diversity) suggests that they would quickly evolve to utilize this O_2. That such aerobic prokaryotes would immediately thereafter enter into an endosymbiotic relationship with biochemically more restricted protoeukaryotes, or that they would immediately begin to evolve into such organisms, however, is not clear. Whether endosymbiotic or not, eukaryotes would follow rather than precede the release of O_2 into the atmosphere. That they may have followed the release of sufficient O_2 by some 4×10^8 years seems not in conflict with either hypothesis. On the other hand, given the difficulties of distinguishing small protoeukaryotes from prokaryotes in the early part of the fossil record, it is possible that the two events were more nearly simultaneous than Raff and Mahler indicate.

More to the point is that eukaryotic cells are not just anaerobic cells with mitochondria (*1, 3*). If it can be shown that an aerobic pathway in the cytoplasm is a primitive feature of eukaryotes inherited directly from their prokaryote ancestors, then the endosymbiotic origin of mitochondria is less likely. A homologous aerobic pathway present both in prokaryotes and in the cytoplasm of eukaryotes (and therefore encoded by the nuclear genome) would be inherited by the eukaryote directly from the aerobic prokaryotic common ancestor of both. The protoeukaryote itself would therefore have been aerobic; as such, it would not need an endosymbiont to provide it with oxidative respiratory functions. Some of the data presented by Raff and Mahler support the primitive aerobiosis of eukaryotic cells, whereas other data can be explained equally well by either hypothesis.

Raff and Mahler did not explain what they mean by bacterial and mammalian superoxide dismutases being "different." If the difference indicates that the loci encoding these enzymes are not homologous and therefore have no common ancestry, this is entirely consistent with the endosymbiosis hypothesis. If, on the other hand, the enzymes are homologous, and "different" only indicates that they have a phylogenetically distant common ancestry and have diverged since, this would not be consistent with endosymbiosis, but then the significance would be in the similarity rather than, as Raff and Mahler stated, in the dissimilarity.

That yeasts ultimately have an absolute requirement for O_2 in their requirement for oleate and steroids (which can only be synthesized aerobically) does not per se refute the endosymbiosis hypothesis, but need only mean that their potential for anaerobic growth arose secondarily after the establishment of aerobic respiration, regardless of whether the latter was by an endosymbiotic event or by progressive evolution. On the other hand, Raff and Mahler's point that steroids are made aerobically in some prokaryotes and in the cytoplasm of all eukaryotes suggests (if the pathways are homologous) that eukaryotes have always been aerobic and have inherited nuclear genes for that biosynthetic pathway from their prokaryotic ancestors. Similarly, that advanced prokaryotes and all eukaryotes utilize the same aerobic pathway to make monounsaturated fatty acids is an important point for establishing the primitive aerobiosis of eukaryotes.

The "considerable amount of evolutionary divergence" between bacterial and mitochondrial cytochromes noted by Raff and Mahler has no bearing on the question of mitochondrial origins. Considerable divergence would be expected by both the endosymbiotic and nonendosymbiotic hypotheses.

Raff and Mahler say that "the widespread occurrence of cytochromes in anaerobic bacteria" is significant for their argument. Traits that would be present in both ancestral anaerobic and aerobic prokaryotes do not, however, indicate that the protoeukaryote was aerobic. Since cytochromes are present in most extant anaerobic prokaryotes as well as in all aerobes, cytochromes would also be present in an anaerobic protoeukaryote. Presence of structural genes for cytochromes in the host would provide a reasonable explanation for the loss of such genes from the endosymbiont: they would be duplicated functions and would easily be lost as unnecessary. The data on the relative divergence of bacterial and eukaryotic cytochromes c, as presented by Raff and Mahler, only show that, if endosymbiosis occurred, it occurred before the divergence of the eukaryotic kingdoms.

Although we do not accept the endosymbiosis hypothesis, we think that many of the arguments given against it are as irrelevant as much of the data proposed in favor of it. That eukaryotic cells have always been aerobic, as suggested by Cohen and by Raff and Mahler, is, however, a forceful argument against endosymbiosis.

Under either hypothesis, the greatest amount of evolution has been in the nuclear genome. The ultrastructural and biochemical features in which mitochondria resemble bacteria are mainly, from a phylogenetic viewpoint, retained primitive states and therefore contain little phyletic information (*4*). As such, they carry no weight in establishing the endosymbiosis hypothesis as more than possible. None of these features is inconsistent with the nonendosymbiosis hypothesis (*5*).

THOMAS UZZELL
Academy of Natural Sciences,
Philadelphia, Pennsylvania 19103
CHRIS SPOLSKY
Department of Microbiology, Yale
University, New Haven, Connecticut

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9 January 1973

We have read the comments by Uzzell and Spolsky (*1*) with considerable interest and are gratified not only because they find our point of view "refreshing" but especially since they must have found it sufficiently persuasive so as to "not accept the endosymbiosis hypothesis" themselves. We regret that they feel that we have ignored some of the principles of systematics, but on reexamination do not think their case sufficiently strong as to warrant any major modification of our proposal (*2*). Without wishing to engage in polemics, we take issue with the following points specifically raised by Uzzell and Spolsky.

We are well aware of the pitfalls of arguing from negative data in paleontology (or elsewhere). Our discussion of the Precambrian fossil record was intended primarily to show that the present evidence indicates an ancient and intimate association of eukaryotes with free oxygen, and that eukaryotes probably arose only after free oxygen became available in the atmosphere.

Uzzell and Spolsky reiterate the view that "eukaryotic cells are not just anaerobic cells with mitochondria." They rightly point out that the aerobic nature of the ancestral protoeukaryotic cell would make the acquisition of an aerobic symbiont unnecessary and weaken the logical basis of the endosymbiont theory. Examination of our article will convince the reader that this is precisely the point upon which this part of our argument rests.

A considerable body of biochemical evidence supports our contention that the eukaryotic extramitochondrial cytoplasm is primitively adapted to the use of free oxygen. We have noted four major types of adaptation:

1) The presence of enzymes such as superoxide dismutase in the cytoplasm to protect cellular components from autooxidation.

2) The requirements of the few anaerobic eukaryotes for certain products of aerobic, eukaryotic metabolism.

3) The synthesis of steroids and unsaturated fatty acids in the eukaryotic cytoplasm by exclusively aerobic pathways.

4) The presence of cytochomes, differing from those of the mitochondria, functioning in mixed function oxidase reactions in the cytoplasm.

Uzzell and Spolsky quibble with our use of the word "different" in our discussion of bacterial and eukaryotic superoxide dismutases. We were referring to enzymic properties (*3*), not to sequence or genetic data which do not yet seem to be available. Therefore, we cannot discuss homology. However, given that superoxide dismutase is vital to aerobic life, we predict a very high degree of divergence (difference) between the eukaryotic and bacterial enzymes since this enzyme must have originated at least 1.8 billion years ago with the appearance of free oxygen.

Cytochromes are widely distributed among anaerobic bacteria. We see little reason to doubt that the ancestral protoeukaryote also had available a cytochrome system capable of eventual evolution to being able to utilize free oxygen. Thus, acquisition of such a system from an aerobic endosymbiont was obviated.

We are surprised that Uzzell and Spolsky feel that divergence of bacterial and mitochondrial cytochromes has "no bearing on the question of mitochondrial origins." We, in accord with others interested in this question, are aware that mitochondria possess several prokaryotic characteristics; this similarity indicates a certain evolutionary conservativeness of these organelles. Thus, if the symbiotic hypothesis were valid, one would expect the cytochromes [which are slowly evolving proteins (*4*)] to show a significant relatedness.

Last, we are particularly puzzled by Uzzell and Spolsky's proposal that "presence of structural genes for cytochromes in the host would provide a reasonable explanation for the loss of such genes from the endosymbiont. . . ." What evolutionary advantage do they envisage for the acquisition of respiratory endosymbionts by a cell already in possession of a cytochrome system?

R. A. RAFF
Department of Zoology,
Indiana University, Bloomington 47401
H. R. MAHLER
Department of Chemistry,
Indiana University

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