the crude nature of the experiments we are ignoring the fact that the isotopic composition of dissolved oxygen in the ocean is altered by biological activity (*9*). Also, some sulfur and sulfide oxidation occurs by biological means in the ocean and inorganically on the continents and in the atmosphere under nonoceanic conditions.

However, the results are encouraging enough to consider as a working hypothesis that the reduction of sulfate and oxidation of sulfides (and probably sulfur) exert the primary control on the oxygen isotope composition of oceanic sulfate.

Should further research confirm this hypothesis, then there are some very important geochemical implications. The total oxygen tied up as sulfate in the oceans is about 2.6×10^{15} metric tons (*4*). Inorganic isotopic equilibrium between this sulfate and ocean water ought to be established in a period of about 250,000 years. If oxidation-reduction turnover of the sulfate is responsible for preventing the establishment of isotopic equilibrium, then approximately 7.8×10^{14} metric tons of elemental oxygen (30 percent of the sulfate oxygen) passes through the sulfur cycle over a time period certainly less than 250,000 years and probably less than 50,000 years. This represents more than half the oxygen found in the present atmosphere and suggests that the sulfur cycle could be one of the important factors regulating the oxygen balance in the ocean-atmosphere system.

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References and Notes

1. R. V. Teis, *Geochemistry USSR English Transl.* **1956**, No. 3, p. 257 (1957).
2. T. C. Hoering and J. W. Kennedy, *J. Am. Chem. Soc.* **79**, 56 (1957).
3. A rough estimate of the maximum analytical uncertainty in my determinations would be about 25 percent, while the discrepancy with Teis is almost two orders of magnitude. Without more complete information on the experimental and analytical techniques used by Teis it is not possible to speculate on the source of this discrepancy. I find my two experiments at 25°C to be particularly persuasive in that one ran for a little more than a year (8930 hours) while the other ran for a little less than 2 years (16,600 hours), and they both yielded essentially the same rate. It should be noted that my *p*H measurements were all made at room temperature.
4. W. T. Holser and I. R. Kaplan, *Chem. Geol.* **1**, 93 (1966).
5. D. A. Northrup and R. N. Clayton, *J. Geol.* **74**, 174 (1966).
6. $\delta O^{18} = \left(\frac{O^{18}/O^{16} \text{ sample}}{O^{18}/O^{16} \text{ standard}} - 1 \right) \times 1000.$
The standard used in this report is the O^{18}/O^{16} ratio of standard mean ocean water (SMOW) as defined by Craig. Sulfates were run on carbon dioxide gas produced by carbon combustion of barium sulfate, with an adaptation of the technique described by R. N. Clayton and S. Epstein [*J. Geol.* **66**, 352 (1958)].
7. J. R. O'Neil and R. N. Clayton, in *Isotopic and Cosmic Chemistry*, H. Craig, S. L. Miller, G. J. Wasserburg, Eds. (North-Holland, New York, 1964), p. 157.
8. C. E. Junge and T. G. Ryan, *Roy. Meteorol. Soc.* **84**, 46 (1964).
9. N. M. Rakestraw, D. P. Rudd, M. Dole, *J. Am. Chem. Soc.* **73**, 2976 (1951).
10. Shell Development Co. (a division of Shell Oil Co.), Exploration and Production Research Div., Houston, Texas. EPR publication No. 484.

20 February 1967

Living Relative of the Microfossil Kakabekia

Abstract. *A living, ammonia-obligate, umbellate form, similar to the Precambrian microfossil* Kakabekia umbellata *Barghoorn, has been isolated from two soil specimens collected at Harlech, Wales. This organism is amenable to culture on agar and in broth. The two soil specimens are similar in that they differ from a typical clay loam in high content of carbon, hydrogen, and organic nitrogen and low levels of sodium, potassium, and titanium. In all other constituents, such as calcium, magnesium, and iron, they are quite dissimilar.* Kakabekia*-like forms can be grown in glucose-ammonia media with the latter as the sole source of nitrogen, but they can also be grown on peptone and silicate in glucose-free media. Ammonia is necessary, and growth is always slow without glucose. The fission process was not observed, but the enlargement and differentiation of a preumbellate structure into its "mature" form, followed by disintegration (senescence) of this stage, was seen. An ontogeny is proposed in which the stalk and basal bulb of the complete umbellate structure are assumed to be part of the reproductive apparatus.*

During an investigation of biological behavior in exotic and harsh environments, the ammonia-tolerant microflora in soils of different origins was studied. Ammonia tolerance is remarkably widespread (*1*), but even more remarkable was the appearance, in soil specimens from Wales, of a small microorganism exceptional for its complex structural differentiation. This form did not fall into ordinary categories such as bacteria, algae, and so forth, and its affinities remained totally obscure until *Kakabekia umbellata* Barghoorn (a Precambrian microfossil) was described by Barghoorn and Tyler (*2*).

This fossil was found in a chert deposit about 2×10^9 years of age in the Gunflint range of southern Ontario. Deposition at this site was apparently associated with a change, in the microhabitat at least, from reducing to oxidizing conditions. Barghoorn and Tyler noted that the affinities of *K. umbellata* could not readily be assigned ". . . to a living counterpart, provided any exists." When petrographic sections containing *Kakabekia* were compared with the living microorganism from Welsh soil, cultured under ammonia, the two organisms were found to be similar in size and structural detail (*3*). Within the respective morphological ranges of fossil and living populations, there were individuals that were essentially identical. At points of closest correspondence, the following description fits both forms: an umbellate form 5 to 10 μ in diameter with a centrally attached stalk, about 5 to 15 μ long, that has a terminal swelling or enlargement. The umbrella or crown is more or less polygonal and heavily rimmed, with five

Table 1. Chemical composition of soil samples tested for *Kakabekia*-like forms. Organic constituents were analyzed by combustion; the principal inorganic, calculated as oxides, by spectrography; and the trace inorganic, by spectrography. Strontium, zinc, and molybdenum were not detected.

Constituent (mg/g dry wt.)	Soil H-1 Harlech (castle wall)	Soil H-2 Harlech (courtyard)	Soil T-1 Tarrytown (orchard)
Organic			
C	106.1	46.0	24.8
H	15.3	6.7	5.5
N	9.2	9.2	5.0
Principal inorganic			
SiO_2	766.	779.	797.
Fe_2O_3	11.4	71.5	42.9
Al_2O_3	5.7	3.8	3.8
K_2O	4.8	9.6	24.0
CaO	1.4	56.0	14.0
TiO_2	1.1	2.9	4.3
MnO	1.0	2.6	1.0
P_2O_5	0.9	1.8	1.8
MgO	.7	6.8	6.8
Na_2O	.4	1.3	7.8
Trace inorganic			
Ba	0.10	0.20	0.20
Rb	.10	.20	.30
Cr	.08	.08	.08
Cu	.02	.80	.20
Ni	.02	.08	.04
B	.01	.03	.03
Zr	.00	.03	.20
V	.00	.08	.10
Pb	.00	.10	.04

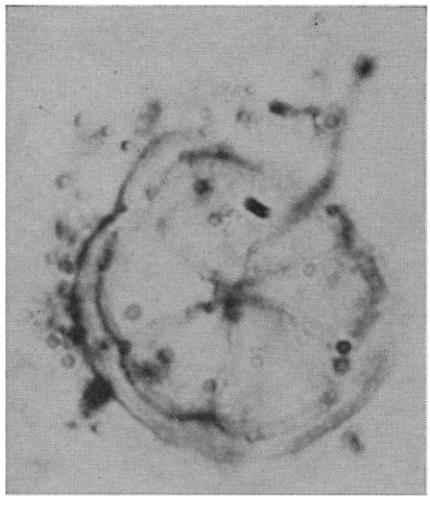

Fig. 1 (left, top). Typical *Kakabekia*-like form grown on nutrient agar in a mixture of ammonia and air (50:50). (× 950)

to eight parts. Partitions radiate from the center of the crown and suggest the ribs of an umbrella or spokes of a wheel. The stalk is often straight, but sometimes it appears twisted or "hawser-like" (Fig. 1). There are variations in both populations; the living forms (Fig. 2) often have a circular crown and sometimes have no stalk. Nevertheless, the similarities have warranted the designation "*Kakabekia*-like" for the living form. Furthermore, a relation between *Kakabekia umbellata* Barghoorn and our similar form was indicated by the presence of alga-like bodies, morphologically identical to modern blue-green algae (such as the Oscillatoriaceae), both as microfossils in Gunflint chert and as part of the living microflora of the Harlech soil samples. Normal-appearing microflora in our cultures also minimizes the likelihood that the *Kakabekia*-like forms merely result from the action of ammonia on common soil organisms.

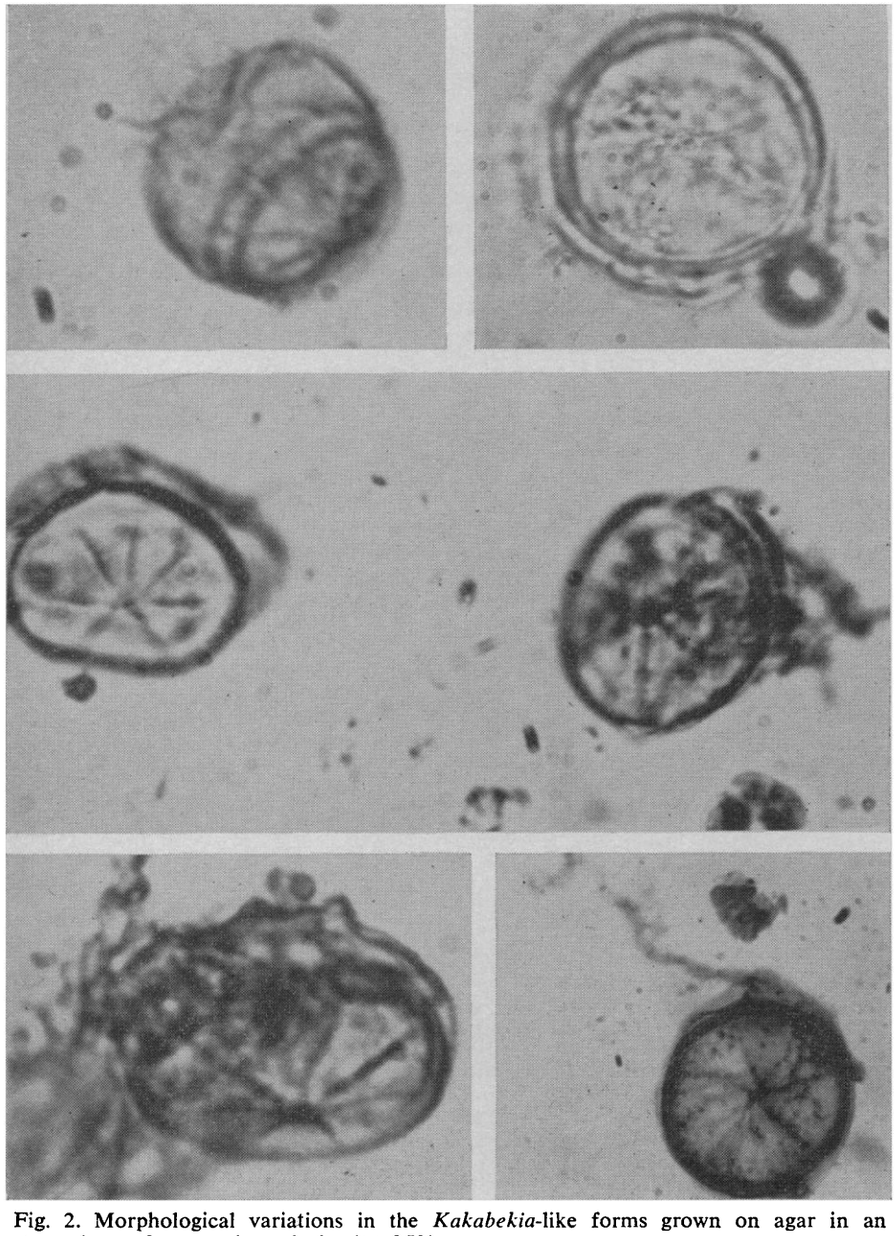

Fig. 2. Morphological variations in the *Kakabekia*-like forms grown on agar in an atmosphere of ammonia and air. (× 950)

Locations that have been sampled and examined for *Kakabekia*-like forms include loam soils from western Europe, the Dakotas, New York, and Pennsylvania; peats from northern England; sands from Florida; and laterites from the Guianas, Curaçao, and other Caribbean locations. Sampling involved only the upper 1 to 3 cm of the A horizon. In no case was there any indication of a *Kakabekia*-like form when these soils were cultured for 30 days at 25°C on 2 percent agar containing 1.0 percent Bacto-peptone plus 0.5 percent beef extract under an atmosphere of 50 percent ammonia in air. Two samples from Harlech, Wales, yielded *Kakabekia*-like organisms under these and similar cultural conditions. The first sample (H-1) was collected in August 1964 at the inside base of the wall of Harlech Castle; the second (H-2), collected in August 1965, was taken from the center of the castle courtyard. After 6 to 7 months' storage under air-dry conditions, H-1 yielded far more umbellate forms than H-2; however, H-1 fell markedly in yield during storage. On nutrient agar under an atmosphere of ammonia and air (50 : 50), sample H-1 yielded 70 to 150 organisms per square centimeter when tested after 6 months and only 10 to 15 per square centimeter 1 year later. Sample H-2 yielded only 5 to 10 individuals per square centimeter when tested 7 months after collection. For comparison, samples of local sandy loam soil from Tarrytown, New York (T-1) were cultured under the same conditions, but no *Kakabekia*-like organisms were found.

The physical and chemical characterization of soils that yield *Kakabekia*-like forms has not been completed, but their major chemical features are given in Table 1. Sample T-1 agrees well in composition with various loams (*4*) and has, therefore, been used as a reference sample. On this basis, H-1 and H-2 are grossly alike in containing

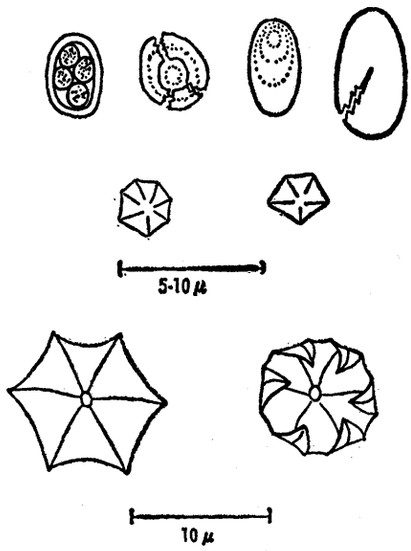

Fig. 3. Recognition problems in the examination of cultures for *Kakabekia*-like forms. Diagrammatic representations of confusing soil structures (top row); crystals formed under ammonia (middle row); and typical and in-folded *Kakabekia*-like organisms (bottom row).

more C, H, N, and Al and less K_2O, TiO_2, Na_2O, and Rb than T-1 contains. All three samples contain similar amounts of Cr. Many elements that were lower in H-1 than in T-1, (Fe, Ca, and Cu, for example) were higher in H-2 than in this reference sample. Conceivably, the differences shared by H-1 and H-2 relative to T-1 may have qualitative significance and the differences between H-1 and H-2 quantitative significance with respect to the yield of *Kakabekia*-like forms.

Both H-1 and H-2 contain objects, similar in size to the *Kakabekia*-like forms, that could cause confusion (Fig. 3). These objects include structures resembling tetraspores of unicellular algae or sporangia of ascogenous yeasts (top row, left); smooth polished granules of quartz that may have a striking degree of radial or concentric structural detail as a result of cracking or differential solution (top row, right); and crystals that form only in the presence of ammonia (middle row). Confusion is heightened by the tendency of highly pointed umbellate crowns of *Kakabekia*-like forms to become folded (bottom row).

Cultivation of *Kakabekia*-like forms from H-1 and H-2 has been the object of concerted efforts. Inorganic nutrients, amino acids, and vitamins, separately; Eagle-Hanks medium; and complex organic fluids (including blood, milk, coconut milk, and urine) were all tested. Liquid culture media, both shaken and stagnant, were compared with semisolid media; and differential sterilization of sample H-1 was also attempted (Table 2). Like the NH_3-tolerant green algae and diatoms, *Kakabekia*-like forms were more heat- and radiation-sensitive than blue-green algae or bacteria were.

In broth culture, the time required for appearance of *Kakabekia*-like forms from sample H-2 was a sensitive indicator of nutritional balance. Without ammonia (that is, aerobic culture in nutrient broth) no forms had appeared after 75 days. In NH_4OH, no forms were observed in the absence of peptone, even when silicate and glucose were provided. In 5*M* and 10*M* NH_4OH, responses to peptone, silicate, and glucose were similiar: at low concentrations of peptone (1 g/liter) organisms were observed either with silicate (1 g/liter) plus glucose or with 10 g of silicate per liter without glucose, but they appeared only after many weeks of incubation. At high concentrations of peptone, *Kakabekia*-like forms appeared rapidly when glucose was present, irrespective of silicate, but they appeared only slowly when given 10 g of silicate per liter in the absence of glucose. Possibly the low vigor of this sample and the requirement for peptone, a complex source of nitrogen, are related.

The more vigorous H-1 provides *Kakabekia*-like forms rapidly in glucose-ammonia-peptone broth and even shows activity when provided with glucose and ammonia only. In 5*M* to 10*M* ammonia, the organism appears within 10 days and increases in number to about 300 individuals per cubic centimeter. The increase in number is linear, attains a maximum, and then declines. These features will be considered in relation to ontogeny. More complex media that contained blood, urine, coconut milk, or milk were at best no improvement over glucose-ammonia-peptone and, in some cases, were highly inhibitory.

Kakabekia-like forms were not found in samples of dry soil, nor was their morphology typical in less than about 10 days under the best culture conditions thus far devised. The preumbellate stage of this organism is quite different in organization. Between the 5th and 10th day of culture on glucose-peptone agar a number of objects were observed that might constitute a preumbellate developmental series (Fig. 4). One of these (far right) is easily recognized as an antecedent of the umbellate form; the others are arranged speculatively. All of these structures have in common an external wall or ring

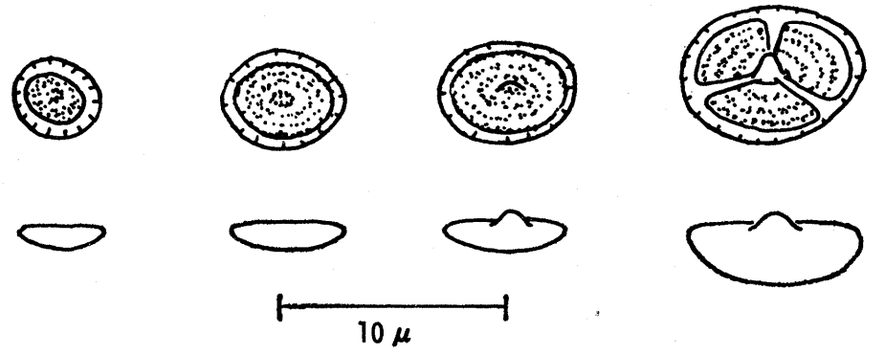

Fig. 4. Diagrammatic representation of a possible ontogenetic series.

Table 2. Effects of heat and γ-radiation (Co^{60}) treatments on the yield of *Kakabekia*-like forms and other microorganisms from soil sample H-1. Organisms found, +; not found, 0.

Treatment	Organims found				
	Kakabekia-like (No. per cm^2)	Diatoms	Blue-green algae	Green algae	Bacteria
None	8	+	+	+	+
None	11	+	+	+	+
125°C, 1 hour	6	+	+	+	+
6 hours	0	0	+	0	+
24 hours	0	0	0	0	0
Co^{60} (10^5 rads)	0	0	0	0	+

that remains more or less constant in thickness.

This implies that morphogenesis proceeds by intercalation of materials into this ring, rather than by a "stretching" process. Previously, this ring was thought to be siliceous in umbellate specimens (*3*). No intermediate between the tripartite stage and the "mature" forms with six to eight parts has been observed; however, these preumbellate forms are rarely seen in cultures in which umbellate structures are relatively abundant. (The tripartite structure has never been found with the more mature stages.) After prolonged cultivation, the count of intact umbellate forms began to decline, eventually falling to zero, and the mature form disintegrated after several weeks in culture. Specimens showing ring and spoke structures in the process of disintegration have been seen (Fig. 5). Because of the absence of a logarithmic phase in growth curves and the failure, during many hours of visual observation, to note any signs of a fission process, we conclude that the ontogeny of this organism is complex and involves a distinct reproductive phase. This phase may have gone unrecognized because of the organism's small size and a structure that renders it difficult to distinguish from the larger motile bacteria. Even the earliest presumptive developmental stage (Fig. 4) could not in itself be identified with the later tripartite form were it not for the suggestive intermediate structures. A possible ontogeny for these *Kakabekia*-like forms is:

Small (about 1 μ) heavy-walled ellipsoid form (cyst, zygospore, zoospore?)
↓
Enlargement 5 to 10 times and radial segmentation (mitosis or meiosis?)
↓
Stalk (stipe) "primordium"
↓
Mature umbellate form with stalk and bulb
⟶ gametogenesis or sporogenesis
↓
Senescence and degeneration

The material within the spokes or septa of the umbrella gave a positive reaction with both Feulgen and acetoorcein stains, and the stalk was sometimes "cellular" in appearance. No organized nuclear structure has been observed, however. Conceivably, genetic material from the umbrella migrates into the stalk, aggregates in the basal bulb, and is released as a reproductive stage.

This ontogenetic picture differs from that proposed by Barghoorn and Tyler (*2*), which was based on arrangement of their fossil specimens. It is, essentially, the inverse of their sequence which begins with the basal bulb as the direct progenitor of the umbellate stage. Both ontogenetic sequences are subject to the same limitations on actual observation of developmental processes in pure culture or in the field. It can be hoped that the "living fossil" will eventually resolve this problem.

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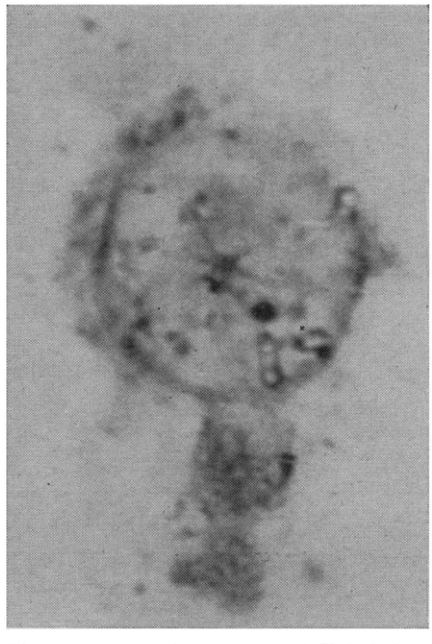

Fig. 5. Degenerating (senescent) *Kakabekia*-like form from a 6-week-old culture. (× 1250)

References and Notes

1. S. M. Siegel and C. Giumarro, *Icarus* **4**, 37 (1965); ———, *Proc. Nat. Acad. Sci. U.S.* **55**, 349 (1965); S. M. Siegel and Olive Daly, *Plant Physiol.* **41**, 1218 (1966); and reports under contract NASw-767 with NASA (Semiannu. Rep. 1965–66).
2. E. S. Barghoorn and S. A. Tyler, *Science* **147**, 563 (1965).
3. S. M. Siegel and C. Giumarro, *Proc. Nat. Acad. Sci. U.S.* **55**, 359 (1965).
4. F. E. Bear, Ed., *Chemistry of the Soil* (Reinhold, ed. 2, New York, 1964).
5. Work carried out under contract NASw-767 with NASA. We thank Professor Elso Barghoorn and his colleagues for their encouragement and cooperation.

20 February 1967

Mitochondrial-Satellite and Circular DNA Filaments in Yeast

Abstract. *Mitochondrial DNA of* Saccharomyces cerevisiae *contains a satellite DNA (density, 1.682) that appears to exist as open-ended filaments at least 5 microns long. DNA from intact cells contains circular filaments whose lengths vary from 0.5 to 7 microns, with a great majority at 1.95 microns. The circular DNA has a density similar to that of the major nuclear peak (1.697). When heat-denatured mitochondrial-satellite DNA is renatured, it cross-links to form a molecule that is larger than the native molecule. The formation of cross-links results in hypersharpening of the density profiles in cesium chloride and also leads to failure to pass Millipore filter paper.*

Genetic and cytological evidence (*1*) of the presence of DNA that is not physically integrated into the nuclear genome has recently been confirmed; the evidence has been extended to the extraction and purification of organelle-specific DNA from chloroplasts (*2*) and mitochondria (*3*, *4*). This type of DNA is of relatively small molecular size. The DNA isolated from mitochondria of several higher organisms is circular (*5*, *6*). DNA obtained from yeast mitochondria is lighter in density than nuclear DNA. Our new data suggest that mitochondrial-satellite DNA is not circular but in a linear form about 5 μ long. Circles of varying sizes, however, have been detected in DNA either extracted from intact yeast cells or obtained from mitochondrial preparations, but they are present in the heavier DNA, of nuclear density, that is present in both preparations. Mitochondrial-satellite DNA that had been denatured and renatured has also been examined by electron microscopy; it proved to be extensively cross-linked and to contain denatured portions of the molecule; consequently it manifests hypersharpening of its profile in CsCl-gradient centrifugation and does not pass Millipore filters.

A strain of commercial (Red Star brand) *Saccharomyces cerevisiae* was aerated to stationary phase in a medium consisting of 2-percent Difco