Nannobacteria: Size Limits and Evidence

The term "nannobacteria" has recently been applied to objects found in geological specimens from Mars and Earth. In their report "Search for past life on Mars: Possible relic biogenic activity in martian meteorite ALH84001" (16 Aug. 1996, p. 924), David S. McKay *et al.* describe "[n]umerous ovoids, about [0.1 micrometer] in diameter" in the meteorite that appear "similar in size and shape to nannobacteria in travertine and limestone" (p. 928). And in their report "Bacteria as mediators of copper sulfide enrichment during weathering" (24 May 1996, p. 1153), Richard H. Sillitoe *et al.* describe "0.03- to 0.05-[micrometer] nannobacteria" in chalcocite from northern Chile (p. 1154).

Is there a lower size limit for microorganisms? There are both experimental and theoretical answers to this question. The diameter of the smallest known microorganism is about 0.34 micrometer (*1*), as found in bacteria that can be grown in culture and in marine picoplankton, which cannot be grown in culture, but can be identified by ribosomal RNA sequence analysis (*2*).

Early (1967) theoretical estimates of a minimal diameter for a living, spherical cell yielded values of about 0.10 micrometer (*3*). The theoretical minimum diameter of a cell, calculated from the size of macromolecular components now known to be necessary and sufficient for a living cell (*4*, *5*), is about 0.14 micrometer. Thus, designation of an Earth object with a diameter of less than 0.14 micrometer as a life form would require exceptional data. In the absence of such data, terms like "nannobacteria" should be avoided.

For samples from Mars one could argue that life may have evolved using different components and processes from those on Earth, so this lower size limit for life may not apply. Even in this case, however, an oval object with a diameter less than 0.20 micrometer would contain only about 100 million atoms (*6*). It is difficult to imagine how such a small number of atoms could carry out all the information storage, metabolic and assembly pathways, and replication processes needed for a living system. But a scientist should never say "Never."

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The "nannobacteria" seen by McKay *et al.* are the same size as an average ribosome, smaller than most viruses, and, if they contain both a cell wall and a membrane, would have no internal volume. McKay *et al.* make reference to a paper by Folk in which nannobacteria on terrestrial carbonate deposits were described (*1*). In both papers, the interpretation that these objects were bacteria was based entirely on structural observations determined by scanning electron microscopy and was not supported with microbiological data. One is thus left in the position of trying to support evidence for bacterial fossils on structural grounds alone. Given this constraint, the structures should as much as possible be consistent with the physical and chemical needs for supporting metabolism. The structures described in these papers, however, do not meet these requirements and, in fact, their size may argue against the case.

Most of the bacteria known to biologists are in the size range of 0.2 to 2 micrometers in diameter (*2*). Even the "ultramicrobacteria" (*3*) that are difficult to culture and hard to prove as viable retain sizes of approximately 0.2 micrometer in diameter.

The lower limit for metabolically active life is defined by the chemical reality of Avogadro's number—if solution chemistry is to drive metabolism, then concentrations of reactants must be adequate to allow chemistry to occur. If volumes are so small that no molecules are present at the required concentrations, then metabolism may be impossible. That is, if reactions are to operate in the micromolar to millimolar range, then cells should have a diameter of 0.1 micrometer and larger (*4*).

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In his letter "In defense of nannobacteria" (22 Nov., p. 1288), Robert L. Folk argues for the existence of nannobacteria as small as 0.01 micrometer. This proposition can be shown both by measurements of bacteria in natural environments (even those living under strong starvation) and by theoretical considerations to be impossible.

If we consider the minimum amount of DNA, ribosomes, enzymes, lipids, and so on that make up an organism, we can calculate a theoretical minimum diameter of about 0.3 micrometer for a living cell.

We have measured several thousands of aquatic bacteria by different techniques, such as epifluorescence microscopy (EFM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM), and we have also determined mass and DNA content of single bacteria (with TEM and EFM, respectively) by using electron energy, high-resolution cameras, and advanced image analysis techniques (*1*). All our measurements in lake, sediment, river, soil, snow, and rainwater samples by EFM confirmed that 0.2 micrometer was the smallest bacterial diameter. There are DNA containing particles, mostly viruses, smaller than 0.2 micrometer in the aquatic environment, and some viruses can be as large as 0.2 micrometer (*2*). Both theoretical considerations and measurements, however, show that the smallest bacterium we can imagine has a volume of 0.005 square micrometer. If it is coccoid, the diameter is 0.2 micrometer.

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Response: I appreciate the theoretical arguments of the biological community that the so-called "nannobacteria" are far below the calculated lower limits of life *as we know it*, but there are unquestionably biological-looking objects in a great many minerals, and despite their minute size they are clearly not artifacts (*1*). Virus particles, which have about 1/100 the volume of most nannobacteria, would not be classed as "inorganic mineral formations," and yet they function virulently as quasibionts.

I have cultured nannobacteria on stubs of metallic aluminum in tap water, and I recently found that the mucus-like nannobacterial globs fluoresce strongly in ultraviolet light, signifying that they contain organic molecules. No fluorescence is observed on bare parts of the stub or on the container, so they seem to be metabolizing the aluminum.

It is heartening to see that a few scientists in the medical community are now recognizing nannobacteria of the same size and morphology (*2*) that I find in the mineralogical world. In buckshot-style reconnaisance, I have found them in the human intestinal tract, on human hair, and even in human teeth and dental plaque. For those who are curious, nannobacteria can be captured in abundance by evaporating water from a faucet or seawater to dryness and examining the residue under the SEM at a magnification of 50,000 or more (salt must be back-dissolved out for best results). Good hunting, you biologists!

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Corrections and Clarifications

An article by Dennis Normile about a report by Japan's committee on fiscal reform (News & Comment, 13 June, p. 1642) incorrectly described its recommendations regarding the International Thermonuclear Experimental Reactor (ITER) project. The committee has recommended that Japan not proceed with an invitation to host the project during an upcoming 3-year period of special fiscal reform. The report does not mention a date for the start of construction.

In the 18th line of the caption for table 1 (p. 1901) of the report "The activity and size of the nucleus of Comet Hale-Bopp (C/1995 O1)" by H. A. Weaver *et al.* (28 Mar., p. 1900), "ro^{-3} kg s^{-1}" should have been "10^{-3} kg s^{-1}." The formula in reference 6 of the same report (p. 1903) was incorrect. The correct formula appears below.

$$d_N = [(2.99 \times 10^8)\, 10^{0.2(m_{sun} - m_{comet} + 5 \log r\Delta + 0.035\phi)}] / A_p^{0.5}$$

Letters to the Editor

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