Are these stratospheric nanoparticles bacteria?

In order to determine if micro-organisms can be found in the stratosphere, balloon-transported cryosamplers have been used to sample the stratosphere at a height of 41 km. The cryosampler cylinders were washed out with phosphate buffer which was then passed through 0·2 μm cellulose nitrate filters to trap any particles, including micro-organisms. The membranes were then gold-coated and examined using scanning electron microscopy. At all stages of the preparation of the cryosampler, balloon flight and subsequent laboratory examination, every effort was made to maintain sterility. Further details of the sampling procedure are reported elsewhere (Harris *et al.*, 2002; Wainwright *et al.*, 2003).

Nanoparticles were occasionally observed on the membranes. The obvious question then arose, could some of these particles be bacteria? The existence of very small bacteria (now referred to as nanobacteria) has long been recognized but largely overlooked (Wainwright, 1999). However, the finding of very small bacteria-like structures in an Allan Hills meteorite (McKay *et al.*, 1996), despite being controversial, has once again focused attention on nanobacteria.

The surface of the membranes on which the stratospheric wash-out was deposited was seen to be covered with a variety of particulates not seen on control filters. Fig. 1(a) shows one of a large variety of such particulate masses. The initial impression is that this mass is a clump

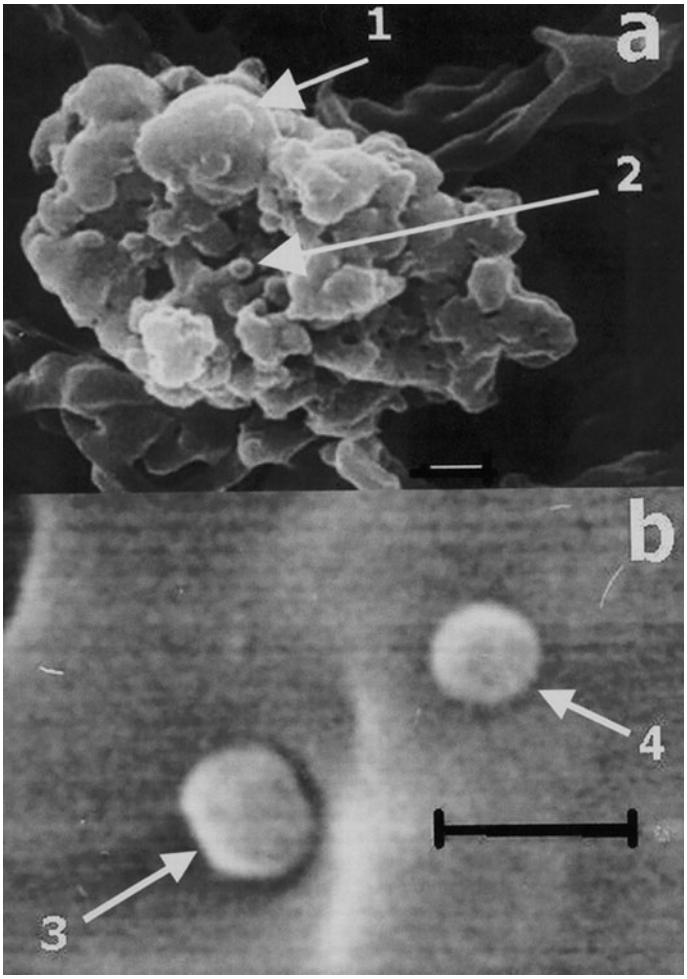

Fig. 1. (a) Particulate mass sampled at a height of 41 km. (b) Two bacteria-like nanoparticles possessing what appear to be fimbriae. Bar, 0·3 μm.

of bacteria overlaying inorganic dust. The largest cocci-like particles are of the order of 1 μm in diameter (Fig. 1a, 1), while numerous sub-micron cocci-like structures can also be seen (Fig. 1a, 2). Such masses could act as a protective carrier for stratospheric micro-organisms, as any micro-organism in the centre of the mass would be protected from the damaging effects of UV light. But do such masses contain bacteria, or are they simply a mixture of inorganic particles? One approach to answering this question would be to employ EDAX (Energy Dispersive Analysis of X-rays) analysis.

Unfortunately, this technique cannot be used to detect organic carbon and any results are complicated by the likely presence of surface mineral coatings. We were therefore restricted to carefully scrutinizing the surface of the membranes in the hope of finding isolated cocci-like particles that possessed some characteristic that would make them clearly identifiable as bacteria.

Fig. 1(b) shows two such isolated nanoparticles, both slightly larger than 0·1 μm in diameter and clearly resembling very small cocci. The lowermost particle appears to be slightly constricted in the middle (Fig. 1b, 3), suggesting that it is in the process of dividing, a characteristic not associated with inorganic particles. The possibility that these particles are bacteria is further strengthened by the presence of what appear to be faintly discernible fimbriae, forming at the junction of the particle and the underlying membrane (Fig. 1b, 4). While such fimbriae are frequently observed when bacteria are grown on membranes, it would seem extremely unlikely that inorganic particles would, by chance, produce fimbriae-like structures only at the point where the particle and membrane meet. Similar nanoparticles, possessing what appear to be fimbriae, were reported by Bigg (1984) in samples obtained from the upper atmosphere. Using transmission electron microscopy, Bigg (1984) studied samples from heights above 25 km and observed thin, rod-shaped bacteria-like particles which were approximately 0·5 μm in diameter and appeared to possess a double membrane.

Although our observations [as well as those of Bigg (1984)] remain tentative, they provide suggestive evidence for the presence of nanobacteria at a height of 41 km in the stratosphere. While such evidence is not conclusive it is important because it provides an incentive to direct future stratospheric sampling towards the specific goal of finding nanobacteria; a goal that would probably have been overlooked without such preliminary evidence. We recognize that better evidence is required for definitive conclusions to be drawn and we would welcome comments from readers on the current evidence and ways in which future work could further investigate the presence of bacteria, including sub-micron forms, in the stratosphere.

Acknowledgements

Thanks are due to Dr Melanie Harris for initial contributions to this work.

M. Wainwright,[1]
N. C. Wickramasinghe,[2] J. V. Narlikar[3] and P. Rajaratnam[4]

[1]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, UK

[2]Cardiff Centre for Astrobiology, Cardiff University, 2 North Road, Cardiff CF10 3DW, UK

[3]Inter-University Centre for Astronomy and Astrophysics, Post Bag 4, Ganshkhind, Pune, 411 007, India

[4]Indian Space Agency Research Organization Antariksh Bhavan, New Bel Road, Bangalore, 560 094, India

Correspondence: M. Wainwright (m.wainwright@sheffield.ac.uk)

Bigg, E. K. (1984). Particles in the upper atmosphere. In *Fundamental Studies and the Future of Science*, pp. 38–51. Edited by C. Wickramasinghe. Cardiff: University of Cardiff Press.

Harris, M. J., Wickramasinghe, N. C., Lloyd, D. & 7 other authors (2002). Detection of living cells in stratospheric samples. In *Proceedings of SPIE. Instruments, Methods, and Missions for Astrobiology IV*, vol. 4495, pp. 192–198. Edited by R. B. Hoover, G. V. Levin, R. R. Paepe & A. Y. Rozanov. Bellingham, WA, USA: International Society for Optical Engineering.

McKay, D. S., Gibson, E. K., Jr, Thomas-Keprta, K. L., Vali, H., Romanek, C. S., Clemett, S. J., Chiller, X. D., Maechling, C. R. & Zare, R. N. (1996). Search for past life on Mars: possible relic of biogenic activity in Martian meteorite ALH84001. *Science* **273**, 924–930.

Wainwright, M. (1999). Nanobacteria and associated 'elementary bodies' in human disease and cancer. *Microbiology* **145**, 2623–2624.

Wainwright, M., Wickramasinghe, N. C., Narlikar, J. V. & Rajaratnam, P. (2003). Microorganisms cultured from stratospheric air samples obtained at 41 km. *FEMS Microbiol Lett* **218**, 161–165.

DOI 10.1099/mic.0.26907-0