PROGRESS TOWARDS THE VINDICATION OF PANSPERMIA*

N.C. Wickramasinghe[1], M Wainwright[2], I V. Narlikar[3], P Rajaratnnm[4], M.J. Harris[1] and D. Lloyd[5]

[l]Cardiff Centre for Astrobiology, Cardiff University, 2 North Road, Cardiff CT10 3DY, UK;
[2]Department of Molecular Biology and Biotechnology, University of Sheffield Sheffield S10 2TN, UK;
[3]Inter-University Centre for Astronomy and Astrophysics, Post Bag 4, Ganeshkhind, Pune 41 l 007 India;
[4]Indian Space Research Organisation, Antariksh Bhavan, New Bel Road, Bangalore 560 094 India;
[5]Cardiff School of Biological Sciences, Cardiff University, P.O. Box 915, Cardiff CF10 3TL, Wales, UK;

*Cardiff Preprint published in *Astrophysics and Space Science*, **283**, 403-413 (2003)

Abstract

Theories of panspermia are rapidly coming into vogue, with the possibility of the transfer of viable bacterial cells from one planetary abode to another being generally accepted as inevitable. The panspermia models of Hoyle and Wickramasinghe require the transfer of viable bacterial cells from interstellar dust to comets and back into interplanetary and interstellar space. In such a cycle a viable fraction of as little as 10^{-18} at the inception of a newly formed comet/planet system suffices for cometary panspermia to dominate over competing processes for the origin and transfer of life. The well-attested survival attributes of microbes under extreme conditions, which have recently been discovered, gives credence to the panspermia hypothesis. The prediction of the theory that comets bring microbes onto the Earth at the present time is testable if aseptic collections of stratospheric air above the tropopause can be obtained. We describe a recent collection of this kind and report microbiological analysis that shows the existence of viable cells at 41km, falling to Earth at the rate of a few tonnes per day over the entire globe. Some of these cells have been cultured in the laboratory and found to include microorganisms that are not too different from related species on the Earth. This is in fact what the Hoyle-Wickramasinghe theory predicts. The weight of evidence goes against the more conservative explanation that organisms are being lofted to the high atmosphere from the ground.

1. Introduction

*.....Microbiology may be said to have had its beginnings in the nineteen-forties. A new world of the most astonishing complexity began then to be revealed. In retrospect I find it remarkable that microbiologists did not at once recognise that the world into which they had penetrated had of necessity to be of cosmic order. 1 suspect that the cosmic quality of microbiology will seem as obvious to future generations as the Sun being the centre of the solar system seems obvious to the present generation.....*Fred Hoyle (1916-2001)

When Fred Hoyle (1982) made this prophetic statement at the end of a public lecture in Cardiff on 15 April 1980, theories of panspermia were still regarded with suspicion. No amount of reasoned argument, no matter how rigorous or compelling could deflect the hardened skeptic from the conventional wisdom of a purely terrestrial origin of life. Twenty two years on the situation is rather different. Panspermia theories are now being discussed as a serious possibility for the origin of life on Earth, and indeed the same ideas that were hotly contested in 1980 are now sliding almost imperceptibly into the realms of orthodox science. In recent years the limits of microbial life on the Earth have expanded to encompass an extraordinarily wide range of habitats: geothermal vents, the ocean floor, radioactive dumps and Antarctic soil, eight kilometers underneath the Earth's crust, to name but a few. The long-term survivability of bacteria has also been extended from 25-40 million years (Cano and Borucki, 1995) to a quarter of a billion years in the case of a bacterium entrapped in a salt crystal (Vreeland *et al*, 2001). Such properties, particularly in the case of extremophiles, are coming to be regarded as being of crucial relevance to astrobiology (Cowan and Grady, 2000).

The theory of panspermia (Hoyle and Wickramasinghe, 1983) does not address the question of a first origin of life, but only argues for its continuation once an origin is achieved. Starting from the premise that a *de novo* origin of life involves superastronomical improbabilities two well-attested empirical facts are invoked to justify its case. Firstly, as stated earlier, microbes under appropriate conditions, have an almost indefinite persistence and viability. Secondly, given the right conditions and environments microbes can replicate exponentially.

Hoyle and Wickramasinghe sought over many years to identify interstellar and cometary dust with bacteria and their degradation products. Indeed the first identification of organic dust in space was made by Wickramasinghe (1974), and the first suggestion of cometary dust being organic, was made by Wickramasinghe

and Vanysek (1975). An organic characterisation of the dust is now universally accepted although our original papers are rarely cited. The complex organic character of cometary dust including molecules that have a biological relevance is used nowadays to argue that cometary injections were important for bringing the organic primordial soup to Earth, although a purely terrestrial scheme of origination is preferred thereafter. The alternative biological interpretation of cosmic dust (cometary and interstellar) was based on infrared spectroscopy, and required a third of the carbon in interstellar space to be tied up in the form particles that resembled bacteria and their degradation products (Hoyle, Wickramasinghe and Al-Mufti, 1984). Occam's razor cannot be appropriately used to discard this possibility so long as the overall efficiency of any competing inorganic process leading to a similar result is unresolved.

2. Cometary panspermia

Comets are known to have formed in the early stages of the condensation of the solar system. Cometary panspermia requires some small fraction of microorganisms present in the interstellar cloud from which the sun and planets formed to have retained viability, or to be capable of being reactivated after being incorporated within newly-formed comets of the early solar system. The fraction could be exceedingly small. The present-day Oort cloud contains some 100 billion individual comets and their total mass is comparable to the combined masses of the outer planets Uranus and Neptune. With one percent of the mass of the initial comet cloud being made up of interstellar dust the total number of "graveyard bacteria" accommodated in a single comet would be ~ 10^{28}. A viable fraction as small as one part in 10^{18} would still yield some 10 billion bacteria for each newly formed comet. If replication can occur within the comet, the previous history of all interstellar destructive processes becomes irrelevant, because of the enormous capacity of even a single viable microbe to increase its number.

Water is a major component of comets, but it had been thought for a long time that this could only exist be in the form of ice. However, Hoyle and Wickramasinghe (1985) argued that the cloud from which the solar system formed contained radioactive isotopes, including ^{26}Al with a half-life of three-quarters of a million years. These isotopes were present because a nearby supernova dispersing such nuclides had exploded at the time the solar system formed.

Radioactive heat sources served to maintain a warm liquid interior in each one of a 100 billion comets for the major part of a million years, and this was ample time for the minute surviving fraction of interstellar microbes to replicate and fill a

substantial fraction of the volume of a comet. Cometary activity in the outer regions of the solar system 4 billion years ago, including collisions between cometary bodies, would have led to the expulsion of a fraction of regenerated bacteria back into interstellar space. A fraction of comets would also be deflected into the inner regions of the solar system, thus carrying microorganisms onto the Earth and other inner planets. The first life on the Earth 4 billion years ago would, according to this model, have been brought by comets. Because comets have continued to interact with the Earth throughout the past 4 billion years, a strong prediction of the model is that cometary injections of microbial life must be an ongoing process.

3. Ongoing incidence of bacteria onto the Earth

The best way to test this theory would be to look for microorganisms being injected to the Earth at the present time. Is there any evidence for a population of newly-introduced microorganisms in the high stratosphere? The extension of the biosphere upwards to include various levels of the atmosphere has been under discussion intermittently for many years, particularly in relation to the transport of pathogenic microorganisms from one part of the globe to another (McCarthy, 2001). The occurrence of microorganisms in the cumulous clouds is not in dispute, nor is their role in nucleating atmospheric ice crystals (Jayaweera and Flanagan, 1982; Bigg, 1983). But their origin in all such cases is most likely terrestrial, microbes carried in wind and air currents.

Convection currents lead to mixing of ground level particulates in the air that can be carried relatively easily into the troposphere, but temperature inversions beyond 15 km lead to barriers through which very few aerosols can penetrate. Whenever rare events such as volcanic eruptions loft particles above 30 km, particles larger than a few microns fall back quickly to the ground under gravity. The isothermal temperature regime between 15 and 25 km effectively stops the ascent of particulates, and the rapidly rising ambient temperature gradient at higher levels makes the upper stratosphere almost impervious to the transport of aerosols from the ground.

The above considerations did not, however, prevent earlier investigators from probing the stratosphere for microorganisms, particularly in the years immediately prior to the space age. Such studies carried out during the 1960's have been reviewed in some detail by Bruch (1967). Bacteria as well as fungi were claimed to be found in samples collected over the altitude range 18-39 km, but these results were generally dismissed as being contaminants. Such a dismissal may possibly

have been justified particularly in view of the primitive nature of the sterilization and identification protocols that were used at the time. For example, these early investigators had no access to the techniques of molecular biology.

After nearly three decades of inactivity on this front, Narlikar *et al* (2000) sought to repeat the experiments of the 1960's using the best available modern techniques. This included rigorous sterilisation protocols, combined with state-of-the-art balloon experimentation technology that had been successfully used in India for research in atmospheric physics as well as cosmic ray and infrared astronomy. The scientific argument for repeating the old experiments was because of the importance of testing panspennia theories (Hoyle and Wickramasinghe, 1983) on the one hand, and the desirability of defining an upper limit of the terrestrial biosphere on the other.

4. Collection of stratospheric samples over Hyderabad, India

The present report relates to air samples collected over Hyderabad, India on 21 January 2001 at various heights upto 41 km. The collection involved the deployment of balloon-borne cryosamplers of the type described by Lal *et al* (1996). The cryosampler comprised of a 16-probe manifold, each probe made of stainless steel capable of withstanding pressures in the range 10^{-6} mb (ultravacuum) to 200 b.

At every stage in the design and construction of the cryosampler instrument the most stringent precautions were taken to aviod any risk of contamination. When selecting the material for the cryoprobes the best quality stainless steel (SS 304L) was chosen and exhaustive tests were carried out to ensure there were no cracks or porosity in the finished product. Only electron beam welding was deployed in construction and the number of electron beam welds was limited to the absolute minimum of two for each probe. The interior of the cryoprobes, apart from being machined to the highest degree of surface finish, were electropolished, and the finished cryoprobes were cleaned in an ultrasonic bath and gassed out in vacuum at 400 degrees C.

Prior to assembly and launch the probes and all their components were again thoroughly sterilised. They were flushed with acetone and were heat and steam sterilised to temperatures of 180^{o}C for several hours. The entrance to each probe was fitted with a metallic (Nupro) valve which was motor driven to open and shut on ground telecommand. The payload trailed at a shallow angle of elevation behind the balloon gondola, being tethered by a sterilised 100m - long rope. As a further

precaution against the possibility of collecting any traces of outgassed material from the balloon surface, a sterilised intake tube 2m long formed an integral part of the cryosampler ensemble. The manifold of probes ready for launch and two stages of pre-launch sterilisation are shown in the panels of Fig. 1.

Figure 1: Evacuated and sterilised cryoprobes: *Left*: Assembled in a manifold ready for immersion in liquid Ne for launch. *Top right*: heat sterilisation under vacuum in an infrared setup at 140 degrees Celsius. *Bottom right*: steam sterilisation procedure.

Throughout the flight the probes remained immersed in liquid Ne so as to create a cryopump effect, allowing ambient air to be admitted when the valves were open. Air was collected into a sequence of probes during ascent, the highest altitude reached being 41km. The cryosampler manifold, once the probes were filled, was parachuted back to ground. The probes were stored at -80^{o}C until the laboratory work began. Half of the probes were retained in India, where analysis continues, and half were sent to Cardiff.

We discuss here laboratory analysis that relates to only two probes:

Probe A: Collection between 30 - 39 km altitude, a total quantity amounting to 38.4 litres of air at NTP

Probe B: Collection between 40 and 41 km altitude, a total quantity amounting to 18.5 litre of air at NTP.

5. Procedures for extraction particulate component from the air

Two procedures were used to extract aerosols aseptically from the probes:

Procedure 1: The air from the exit valve of each probe was passed in a sterile system in a microflow cabinet sequentially through a 0.45μm and a 0.22 μm micropore cellulose nitrate filter. (The filter diameter was 47 mm.)

Procedure 2: Following the completion of Procedure 1, the probes were injected with sterile phosphate buffer pH7.3 solution, agitated for several hours in a shaker to dislodge particles adhered to the walls, and the liquid syringed out and passed sequentially through three filters: (i) 0.7μm glass microfiche filter, (ii) 0.45μm cellulose acetate filter, and (iii) 0.2μm cellulose acetate filter.

Most of the aerosols are expected to have been retrieved in Procedure 1.

6. Results of examining the samples

The microbiological aspects of the analysis summarised in this section are described in more detail by Harris *et al* (2001) and Wainwright *et al* (2002). Harris *et al* (2001) reported the discovery of clumps of cocci shaped sub-micron sized particles of overall average radius 3.0μm from isolates of filters that were recovered from the probes using Procedure 1. The clumps were identified first using a scanning electron microscope and subsequently an epiflourescence microscope. The latter technique used a membrane-potential-sensitive dye (a cationic carbocyanine) with fluorescence interpreted as revealing the presence of viable cells (See example in the left panel of Fig. 2).

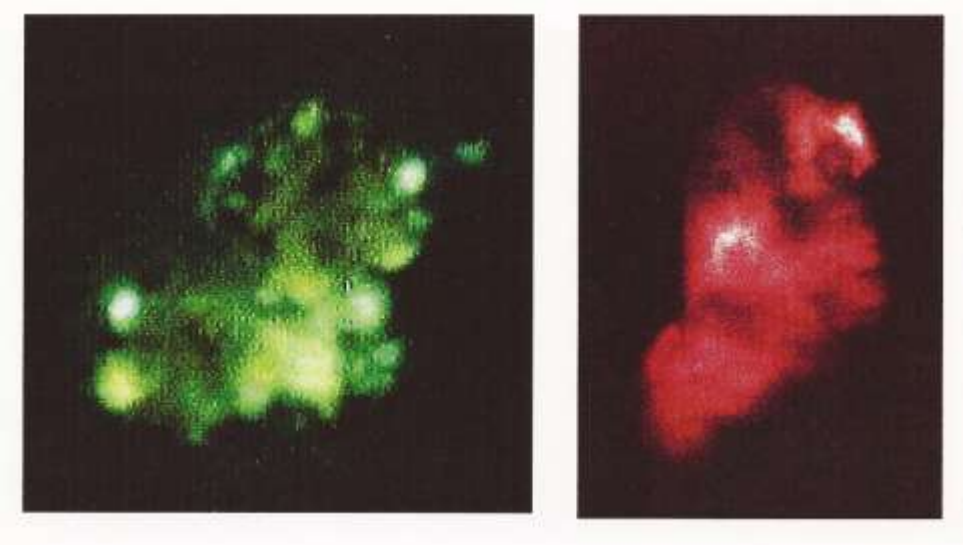

Figure 2: Left: Clump of cells from a stratospheric isolate fluorescing after staining with carbocyanine dye; Right clump of cells from 39 km fluorescing after staining with acridine. The former detecting membrane potential and viability of cells, the latter the presence of nucleic acid.

A similar procedure using the nucleic acid stain acridine orange was also found to reveal the presence of clumps of cells containing nucleic acid as shown for example in the right panel which was from an isolate from 39km. (Right panel of Fig. 2). Both these detection methods yielded an average of one clump of viable cells per 5mm x 5mm ($25mm^2$) of filter area. With a total filter area close to 2000 mm^2 the entire membrane would have contained ~80 clumps of viable cells, which must therefore represent the main bacterial content of the air collected from a height of 41km. The NTP concentration of clumps is therefore ~ 80/18.5 = 4.3 per litre. Converting this to the ambient conditions at 41 km ($P=2.9x10^{-3}$b, T=253K eg. Allen, 1967) we arrive at a local clump density of $1.4x10^{-5}$ per litre. We estimate an average mass for a porous 3 μm radius clump to be 3×10^{-11}g. The settling speed of such a particle at 41km has been calculated by Colbeck (2001) to be 0.18 cm/s at 41km. Using these values and taking the average surface area of the Earth to be $5x10^{18}$ cm^2 we obtain an infall rate of:

$\sim 1.4x10^{-5} \times 3x10^{-11} \times 5x10^{18} \times 0.18 = 3.78x10^{2}$ g/s = 3 tonnes per day

over the entire globe. Whatever the source of the clumps might be, such an infall or fallback rate from 41 km would seem inescapable.

With an average of $2.4x10^{-9}$ g of bacteria (deemed viable) collected per filter it would indeed be a little surprising to find that they are all non-culturable. This was

indeed the situation until early February 2002, when one of us (MW) succeeded serendipitously in obtaining cultures from isolates derived from both procedures 1 and 2.

Using a soft potato dextrose agar medium (PDA) and taking every conceivable precaution against contamination the following cultures of microorganisms were grown:

(a) The coccus (spherical bacterium, often growing in clumps) 99.8% similar (as determined by 16S RNA analysis) to *Staphylococcus pasteuri*
(b) The bacillus (rods), 100% similar (as determined by 16S RNA analysis) to *Bacillus simplex*
(c) A fungus identified as *Engyodontium albus* (Limber) de Hoog

Figure 3 shows images of (a) and (b) with a light microscope. Figure 4 shows images using an SEM, the top left image being from earlier work (Harris *et al*, 2001).

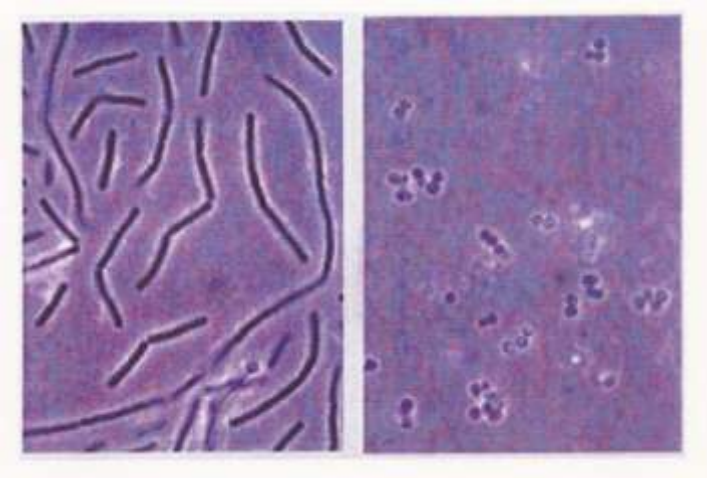

Figure 3: Cultures of *B. Simplex* (Left) and *S. Pasteuri* (Right) grown on LB medium after isolation using soft PDA from stratospheric samples at 41 km (x1000 using phase contrast microscope).

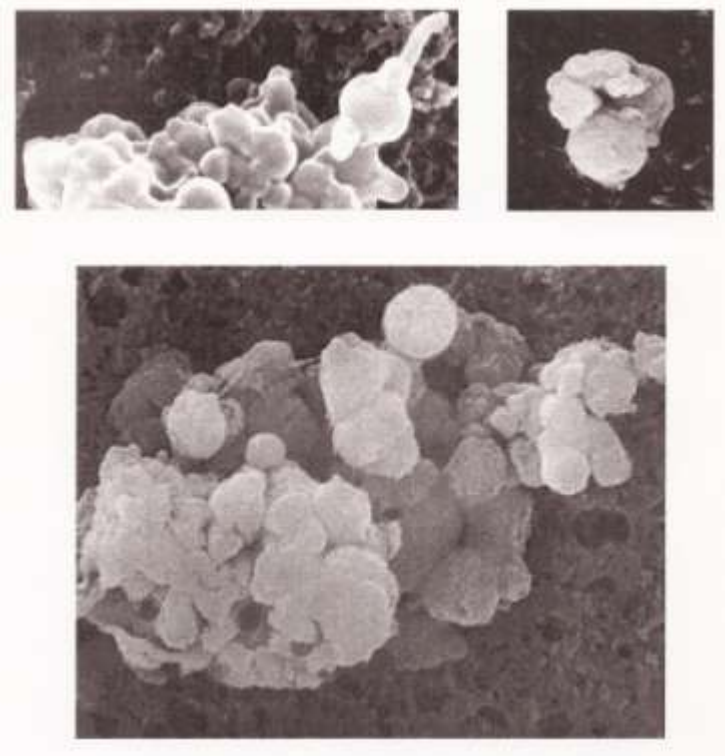

Figure 4: *Top left:* cocci and rod identified with SEM from membranes at 41km prior to culturing.
Top right: Growing cocci taken from the surface of soft PDA medium.
Bottom: Dividing cocci taken from the surface of soft PDA medium.

None of the above, (a), (b) or (c), are common contaminants, nor had they been used in the laboratories in which this work was done. Furthermore the lack of any growth on the control membranes, that were treated in the same way but not exposed to stratospheric air, gives us confidence to assert that the organisms originated in the stratosphere. The fact that these are similar to terrestrial microbes is no problem; it is fully consistent with panspermia theories in which Earth organisms are derived from cometary organisms that transit through the stratosphere.

7. Discussion

The first unequivocal recovery of any culturable microorganisms from 41 km in the stratosphere using modern aseptic collection protocols and molecular identification criteria must surely be deemed of historic importance. With instrumental and laboratory contamination excluded at all stages of the experiment two options remain. Firstly, one might think that they were carried from the ground

in a volcanic eruption or in an exceptional meteorological event. The other is that they arrive from space. A volcanic origin is ruled out for the simple reason that there was no volcanic eruption recorded in a two-year run-up to the balloon launch date on January 20, 2001, and for reasons already stated a settling rate at 0.18cm/s from 41 km as calculated by Colbeck would drain out particles of 3 μm radius in a matter of weeks. A similar objection applies to rare meteorological events. Assuming our collections on January 20, 2001 gave us representative stratospheric samples at 41km no process that is purely terrestrial can sustain the high densities of bacterial clusters as are implied.

The alternative extraterrestrial origin (Hoyle and Wickramasinghe, 1981, 2000), although controversial, is more attractive as an explanation of our findings. The bacterial material, cultured in the present experiment, and detected earlier through fluorescence microscopy, can be regarded as forming part of the 100 tonnes/day input of cometary material known to reach the Earth. Critics of panspermia may argue that 3 μm radius particles get burnt through frictional heating and end up as meteors. Some fraction may do so, but the rest would not. Survival depends on many factors such as angle of entry and mode of deposition in the very high stratosphere. Several modes of entry can be considered that permit intact injection into the stratosphere, possibly starting off as larger aggregates released from comets that disintegrate into a cascade of slow-moving smaller clumps at heights above 270km where frictional heating would be negligible. Evidence for such disintegrations have been available for many years (Bigg, 1983), and more recent studies of Brownlee particles collected using U2 aircraft have also shown the survivability of extremely fragile organic structures (Clemett, *et al*, 1993).

Acknowledgement.
We thank Professor lan Colbeck for a personal communication and advice. We are most grateful to Professor K. Kasturirangan, Chairman Space Commisson of India and Indian Space Research Organisation (ISRO) for making available ISRO's state-of-the-art cryogenic sampler payload together with avionics and special sterilisation systems, specifically developed by ISRO for this investigation, and for his sustained guidance and encouragement.

References:

Allen, C.W., 1967. *Astrophysical Quantities*, (London Athlone Press)
Bigg, E.K.,1983. In *Fundamental Studies and the Future of Science*. pp.38-51. (Ed.

C. Wickramasinghe), University College Cardiff Press.
Bruch, C.W.,1967. *Symp Soc Gen Microbiol* **17**,345-373
Cano, R.I. and Borucki, M., 1995, *Science*, **268**, 1060-1064
Clemett, S.I., Maechling, C.R., Zare, R.N., Swan, P.D., and Walker, R.M., 1993, *Science* **263**, 721-725.
Colbeck, I., (1998). In *Physical and Chemical Properties of Aerosols* (Ed. I.Colbeck), London, Blackie.
Cowan, D and Grady, M., 2000, *Microbiology Today*, **27**, 174-177
Harris, M.J., Wickramasinghe,N.C., Lloyd,D., Narlikar,J.V., Rajaratnam,P., Turner,M.P.,Al-Mufti,S., Wallis,M.K., Ramadurai,S.& Hoyle, F. 2001., The detection of living cells in stratospheric samples. *Proc SPIE Conf 4495* (In Press).
Hoyle, F., 1981. *The relation of biology to astronomy* (University College Cardiff Press, 1981)
Hoyle, F. and Wickramasinghe, N.C., 1981. In *Comets and the Origin of Life* (ed. C. Ponnamperuma) pp 227-239, D. Reidel
Hoyle, F. and Wickramasinghe, N.C., 1985. *Living Comets* (University College, Cardiff Press)
Hoyle, F. & Wickramasinghe, N.C., 2000. *Astronomical Origins of Life: Steps towards Panspermia*. Kluwer Academic Press.
Hoyle, F., Wickramasinghe, N.C. and Al-Mufti, S., 1984. *Astrophys.Sp.Sci.*, **98**, 234
Jayaweera, K. & Flanagan, P.,1982. *Geophys Res Lett* **9**, 94-97.
Kasten, F., 1968. *J.appl. Meteorol* **7**, 944-947.
La1,S., Archarya,Y.B., Patra,P.K., Rajaratnam,P.,Subbarya,B.H. & Venkataramani,S., 1996. *Indian J Radio Space Phys* **25**,1-7.
McCarthy, M., 2001. *Lancet*, **358**, 478.
Narlikar, J.V., Ramadurai, S., Bhargava, Pushpa, Damle, S.V., Wickramasinghe, N.C., Lloyd, D., Hoyle, F. and Wallis, D.H., 1998. *Proc. SPIE Conf 3441*, 301-305
Vreeland, R.H., Rosenzweig, W.D. and Powers, D., 2000. *Nature***, 407**, 897-900
Wainwright, M., Wickramasinghe, N.C., Narlikar, J.V. and Rajaratnam, P., 2002. Submitted to *Microbiology*
Wickramasinghe, N.C., 1974. *Nature*, 252, 462
Wickramasinghe, N.C. and Vanysek, V., 1975. *Astrophys.Sp.Sci*, 33, L19