

## Detection of microorganisms at high altitudes

AD 2003 marks the 25th year of the suggestion that interstellar grains had a biological provenance. During the period 1974–78, Fred Hoyle and one of the present authors (N.C.W.) developed the theory that grains in space had a complex organic composition, and furthermore that their inclusion in 100's of billions of comets led to transition from prebiotic matter into primitive bacterial cells<sup>1–5</sup>. The realization that comets were not the beginning, but merely a stepping stone for cosmic microbial life soon followed. It was argued that pre-existing viable bacterial cells derived from interstellar space may have become included in comets in the primitive solar system, when it was found that the extinction properties of interstellar dust matched with uncanny precision, the expected behaviour of freeze-dried bacteria<sup>6</sup>.

The extinction curve, showing the absorption and scattering of radiation from a light source by the intervening matter at different wavelengths carries the signature of the latter. Thus, theoreticians use theoretical models of dust particles to work out the predicted extinction curve and, based on the closeness of match between theory and observations, arrive at the form and composition of the intervening matter. In some cases, extra extinction in specific wavebands provides extra clues in this search.

Not long afterwards, using the above technique, it was found that observations of the infrared absorption by dust in the 2.9–3.9  $\mu\text{m}$  waveband for the galactic centre source GC-IRS7 had a distinctly bacterial signature<sup>7</sup>. Hoyle and Wickramasinghe (H–W henceforth) recognized that the 2175 Å ultraviolet extinction band may be better explained by an ensemble of biological aromatics than by spherical graphite grains as was hitherto thought<sup>8</sup>. Thus, at least two striking spectroscopic features of dust seemed to suggest strongly that living material is present everywhere in the galaxy. Other astronomical evidences, e.g. the diffuse IR bands, the complex organic composition of cometary dust, and the extended red emission in the red rectangle also served to corroborate this conclusion. But the concept of a universe replete with cosmic life was slow to gain ground<sup>9</sup> as it

means reviving the theory of 'panspermia' that normally evokes a strong emotional resistance.

### The logic of panspermia

The theory of panspermia has very ancient roots dating back to Greek times and even earlier. It was, however, first scientifically discussed by Lord Kelvin and then, at the beginning of the 20th century by Svante Arrhenius<sup>10</sup>. Arrhenius' argument that bacterial spores could be transported across the galaxy came to be bitterly contested in the 1920s. Becquerel<sup>11</sup> argued, on the basis of laboratory experiments, that bacteria could not survive space conditions, particularly exposure to ultraviolet radiation. But this and many similar early criticisms of panspermia were later shown to be flawed. For example, a thin coating of carbonaceous matter around a bacterial grain, that would inevitably form in space, would act as a screen against ultraviolet light. Protection from ionizing radiation (cosmic rays) might be more difficult, but still achievable. During an average residence time of 10 million years in a typical location in interstellar space, the cumulative radiation dose received by a bacterium is estimated as  $\sim 10^5$  rad. Although many terrestrially adapted bacterial species may not survive such a large dose, some almost certainly would. For instance, *Micrococcus radiodurans* can withstand a million rad under laboratory conditions, and there are others that are known to thrive within working nuclear reactors.

There are also uncertainties as to whether our understanding of the radiation susceptibility of microorganisms can be directly translated to interstellar conditions, in particular, whether radiation damage to bacteria is a linear process. Exposure to extremely low intensities of interstellar ionizing radiation for millions of years may be far less damaging than short pulses of high intensity radiation delivered to bacteria under laboratory conditions. In any event, bacteria or even entire ecologies of microorganisms, encased within comets or fragments of comets would be secure from cosmic radiation in space, and be safely transported

between well-separated stellar and planetary systems.

At the inception of the solar system, 100 billion cometary objects condensed near the present-day orbits of the outermost planets, and in doing so, mopped up tens of earth masses of interstellar dust. The main logic of panspermia stems from the fact that less than one in  $10^{18}$  interstellar grains in the form of viable bacteria reaching such a new comet-forming environment is all that is needed to re-establish a cosmic biological system. Exponential amplification of microbes would take place in the interiors of radioactively heated comets that maintain liquid conditions for millions of years.

The history of molecular astronomy shows that initially astronomers were reluctant to accept the existence of molecules in giant gas clouds in the interstellar space, the accepted paradigm of the 1950s being that only neutral hydrogen atoms can exist there. Fred Hoyle, who had proposed the existence of molecular clouds found so much opposition to his ideas that he wrote it up as part of his science fiction novel *The Black Cloud* (which, of course, became immensely popular!). In the 1960s, however, millimetre wavelength astronomy became well-established and the existence of such clouds was confirmed beyond any doubt. Thus, astrochemistry is now a flourishing subject. In the case of astrobiology too, astronomers did not initially take kindly to the adulteration of their subject with an alien discipline, i.e. biology; likewise biologists felt uncomfortable with the prospect of astronomers encroaching onto their territory. Here again, history seems to be about to repeat itself.

Several modern developments, in many different branches of science, point in the direction of a cosmic connection for life. The first relates to the dating of the oldest life on the earth. Mojzsis *et al.*<sup>12</sup> used carbon isotope measurements in sedimentary rocks to detect evidence of microbial life on the earth before 3.83 Gyr ago. This was at the end of a period of late heavy bombardment of the earth by comets and meteorites (the Hadean Epoch), when *in situ* development of a primordial soup would have been impossible to achieve. Mojzsis *et al.*<sup>13</sup> have also found

evidence of a possible Hadean ocean as early as 4.3 Gyr ago, and Nisbet and Sleep<sup>14</sup> have argued that bombardment was so severe as to periodically evaporate such oceans, converting them entirely into steam.

Whilst *in situ*, prebiotic evolution may be hard put to produce the desired results under such harsh conditions, the cometary collisions may themselves have injected life onto the earth on several occasions during the period 4.3–3.83 Gyr ago. It is an interesting possibility that bacterial phyla that survived under such episodes of recurrent evaporation were thermophiles, which are indeed to be found at the base of the phylogenetic tree. These were simply the lifeforms that happened to survive the harsh prevailing conditions out of a very much larger set that came in.

### The H–W hypothesis

The H–W panspermia theory requires life to have been introduced to earth for the first time by comets some 4 b.y. ago, with an ongoing incidence of microorganisms continuing to the present day. Recent discoveries of organic molecules and fragile structures within the Mars meteorite ALH84001 have gone in the direction of supporting the idea that microbial life could indeed be transferred in viable form between objects within the solar system<sup>15,16</sup>. We also briefly examine this theory in the light of the commonly accepted view that life originated here on the earth.

Panspermia models would require that all the higher members of the tree of life came in the form of genetic components to be assembled in response to the changing physical conditions on the earth. The perceived evolution in the phylogenetic tree of terrestrial life (within its branches, Archaea, Bacteria and Eukarya) then becomes an artefact of the re-assembly process of the cosmically-derived genes. This point of view is consistent with recent assertions that recent biochemical evolution amounts to little more than ‘tinkering with the available equipment, adapting existing organs (and biochemical processes) to new purposes’<sup>14</sup>. H–W have maintained over many years that little of great innovative significance in biology ever happened on the earth. The changing physical environment of the earth provided the assembly conditions for the grand tapestry of cosmic

life. Terrestrial life developed rather like the fitting together of a gigantic child’s jig-saw puzzle<sup>17</sup>. The H–W criticism of the standard hypothesis of terrestrial life starting *in situ*, namely the improbability of the first origin of life has been re-iterated by persons of no less stature than Francis Crick and Margulis. For instance, Margulis<sup>18</sup> has stated that to proceed ‘... from a bacterium to people is less of a step than to go from a mixture of amino acids to that bacterium’. As H–W pointed out, such bacterial life already existed on the earth at the crack of dawn in its history. With the development of humans from bacteria taking a full 4 b.y., one faces an obvious dilemma, namely with the age of the earth being around 4.6 b.y., there is insufficient time available for the evolution of bacteria to have occurred.

According to H–W cosmic life, with its fullest range of evolution-potential, is a fact that needs to be in some way reconciled with cosmology. If we assume an ultimate origin, then all the resources of all the stars in all the galaxies in the observable universe would be needed to accomplish this near miraculous feat. But once life originates then its continued existence and dispersal is assured by the well-attested survival properties of bacterial cells. The overall logic of this ongoing process is summarized in Figure 1.

Besides the radiation resistance of bacteria that we have alluded to, the limits of microbial life on our planet have expanded to encompass an extraordinarily wide range of habitats. Microbes are found in geothermal vents, the ocean floor, in radioactive dumps and in the Antarctic soil. Microorganisms have been recovered from depths of 8 km

beneath the earth’s crust, and laboratory studies have shown that bacteria can survive pressures at ocean depths of thousands of kilometres or more. The long-term survivability of bacteria has also been extended from the range of 25 to 40 m.y.<sup>19</sup> to a quarter of a billion years in the case of a bacterium entrapped in a salt crystal<sup>20</sup>. Direct proof of the survival of bacteria exposed to radiation in the near-earth environment has also been demonstrated using NASA’s Long Exposure Facility.

What is now required is a direct demonstration that viable microbes exist within cometary material and that they are being transferred to the earth. Studies of cometary dust in the stratosphere have been initiated and may soon provide such evidence. While such *in situ* space experiments are a long-term objective of space science, definitive results from this quarter may be at least a decade away. In the mean time, there exists collective evidence that comes close to demonstrating the reality of panspermia.

We describe here an experiment sponsored by the Indian Space Research Organization (ISRO) consisting of a collection of samples of air from varying heights in the stratosphere followed by their microbiological analysis under aseptic conditions. This was a multidisciplinary effort in which several scientists from different institutions in India and the UK have participated at various stages. The Indian side includes J. V. Narlikar from IUCAA as the leader of the group with P. Rajratnam from ISRO, S. Ramadurai from the Tata Institute of Fundamental Research, Mumbai, S. Shivaji and G. S. N. Reddy from the Centre for

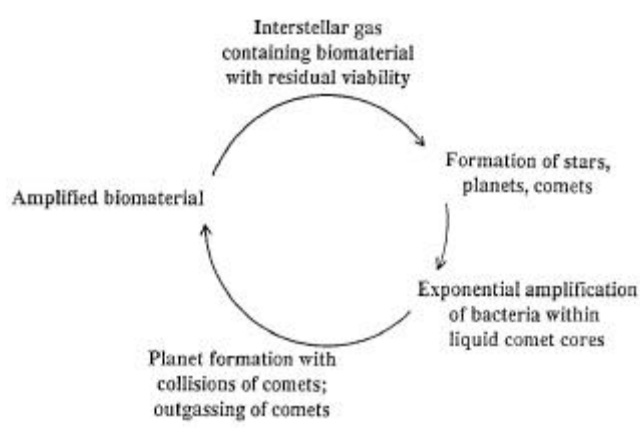


Figure 1. Cosmic life cycle.

Cellular and Molecular Biology, Hyderabad and P. M. Bhargava from Anveshna, Hyderabad. The UK side was led by N. C. Wickramasinghe and included David Lloyd and Melony Harris from the School of Pure and Applied Biology, Cardiff University and M. Wainwright from the Department of Molecular Biology and Biotechnology, University of Sheffield.

### Collection of stratospheric samples over Hyderabad, India

Air samples were collected over Hyderabad, India on 21 January 2001 in four height ranges: 19–20, 24–28, 29–39 and 39–41 km. The collection involved the deployment of balloon-borne cryosamplers of the type described by Lal *et al.*<sup>21</sup>. The cryosampler comprised 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 avoid 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, was electropolished and the finished cryoprobes were cleaned in an ultrasonic bath and gassed out in vacuum at 400°C.

Prior to assembly and launch, the probes and all their components were again thoroughly sterilized. They were flushed with acetone and were heat and steam sterilized to temperatures of 180°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 sterilized 100 m long rope. As a further precaution against the possibility of collecting any traces of outgassed material from the balloon surface, a sterilized intake tube 2 m long formed an integral part of the cryosampler ensemble.

The payload manifold is shown in Figure 2.

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 41 km. The cryosampler manifold, once the probes were filled was parachuted back to ground.

### Biological analysis

In order to test the feasibility of collection of air samples aseptically and to test the rDNA sequencing as well as other procedures for identification of the microorganisms, a preliminary analysis of one of the probes having air sample collected from the altitude range of 10–36 km was conducted by Shivaji and Reddy along with Bhargava. This sample had been collected in a balloon flight launched from Hyderabad on 29 April 1999. All procedures were carried out in a sterile equipment. The sample air was passed through a 0.45 micron filter and

then through a 0.22 micron filter. The existing air was passed through a calibrated flowmeter. Each filter was placed in a nutrient agar plate; no growth occurred after 7 days incubation at 25°C. The filter was then transferred to a blood agar plate and incubated at the above temperature for 11 days, when six distinct colonies were seen growing from the 0.45 micron, but not the 0.22 micron filter. The colonies were sub-cultured and maintained on nutrient agar. The cultures have been deposited in the MTCC Type Culture Collection at the Institute of Microbial Technology, Chandigarh, India.

Based on the morphological, physiological and biochemical characteristics and additionally on the basis of the 16S rDNA sequencing, the isolates were identified as *Pseudomonas stutzeri*. These samples were collected from a range of heights, including altitudes as low as 10 km where contamination from the earth occurs. As a result, while such experiments gave us a wealth of practical experience for future balloon flights, they provided little in the way of convincing evidence for the existence of microorganisms in the stratosphere. These studies



**Figure 2.** Evacuated and sterilized cryoprobes. (Left) Assembled in a manifold ready for immersion in liquid Ne for launch. (Top right) Heat sterilization under vacuum in an infrared set-up at 140°C. (Bottom right) Steam sterilization procedure.

did however, emphasize the need for samples to be collected at heights well above, where terrestrial contamination is even remotely possible.

Returning to the 2001 flight, we discuss here laboratory analysis that was conducted in the UK relating to only two probes:

*Probe A:* Collection between 30 and 39 km altitude, a total quantity amounting to 38.4 l of air at NTP.

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

The air from the exit valve of each probe was passed in a sterile system in a microflow cabinet sequentially through a 0.45  $\mu\text{m}$  and a 0.22  $\mu\text{m}$  micropore cellulose nitrate filter. The filter diameter was 47 mm. When the cylinders are brought to ground, the air is at a pressure of some 200 atmospheres. The valves are slowly opened in a sterile system, no suction pump or similar device is needed.

The microbiological aspects of the analysis that are summarized in this section are described in more detail by Harris *et al.*<sup>22</sup> and Wainwright *et al.*<sup>23</sup>. Harris *et al.*<sup>22</sup> reported the discovery of clumps of cocci-shaped, sub-micron-sized particles of overall average radius 3.0  $\mu\text{m}$  from isolates of filters. The clumps were identified first using a scanning electron microscope and subsequently by epifluorescence microscopy. The latter technique involved the use of a membrane-potential-sensitive dye (a cationic carbocyanine) with fluorescence interpreted as revealing the presence of viable cells. Such fluorescent spots are seen, for example, in the left panel of Figure 3. A similar procedure using the nucleic acid stain acridine orange was also found to reveal the presence of clumps of cells containing nucleic acid, vide the right panel which was from an isolate from 39 and 41 km (see right panel of Figure 3). Initial attempts to culture the coccoid cells were unsuccessful and they were deemed to be viable, but non-cultureable bacteria.

This was indeed the situation until early February 2002, when one of us (M.W.) succeeded serendipitously in obtaining cultures from isolates of air filters. Using a soft potato dextrose agar medium (PDA) and taking every conceivable precaution against contamination, the following cultures of microorganisms were grown:

(i) The coccus (spherical bacterium, often growing in clumps) 99.8% similar (as

determined by 16S rRNA analysis) to *Staphylococcus pasteurii*.

(ii) The bacillus (rods), 100% similar (as determined by 16S rRNA analysis) to *Bacillus simplex*.

(iii) A fungus identified as *Engyodontium album* (Limber) de Hoog.

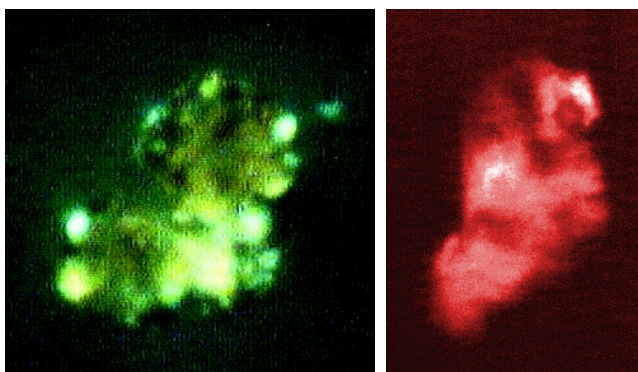
Figure 4 shows images of (i) and (ii) with a light microscope. Figure 5 shows images using an SEM, the top left image being from an earlier work<sup>22</sup>.

None of the above, (i), (ii) or (iii), is a common contaminant, nor has it been used in laboratories in which this work was done. Furthermore, the lack of any growth on the control membranes, not exposed to stratospheric air that were placed in the same media under the same conditions, gives us confidence to assert that the organisms were collected from the stratosphere. The control probe was one in the cryoprobe ensemble, sterilized together with the rest. It was not exposed to ambient air in flight. It went up and came down unexposed. To rule out any process contamination at any stage, phosphate buffered solution was introduced into this probe: and the probe was then subjected to vibration for 48 h. Thereafter, the contents in the probe were extracted employing a sterile syringe and filtered through the 0.7/0.4/0.2 micron membranes stacked in the filtering device. The membranes were then analysed in a like manner, as the probes in the main sample. The end result was that no microorganisms were detected on the membranes, thus establishing that the probes were sterile. Incidentally, this also established that the procedure employed in the secondary process of probe

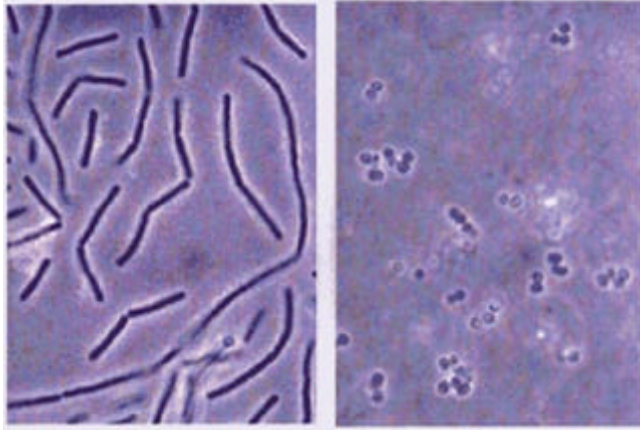
washout, to extract microorganisms stuck to the inner walls, has also been contamination-free.

To most microbiologists, the fact that these isolates have essentially the same characteristics as terrestrial microorganisms is a problem, since one would assume that non-terrestrial microbes would have evolved at different rates elsewhere. This finding is, however, fully consistent with panspermia theories in which organisms on earth are derived from cometary organisms that transit through the stratosphere. The main features of bacterial genotypes are derived, according to this theory, through a process of cosmic evolution and they are being constantly replenished from space.

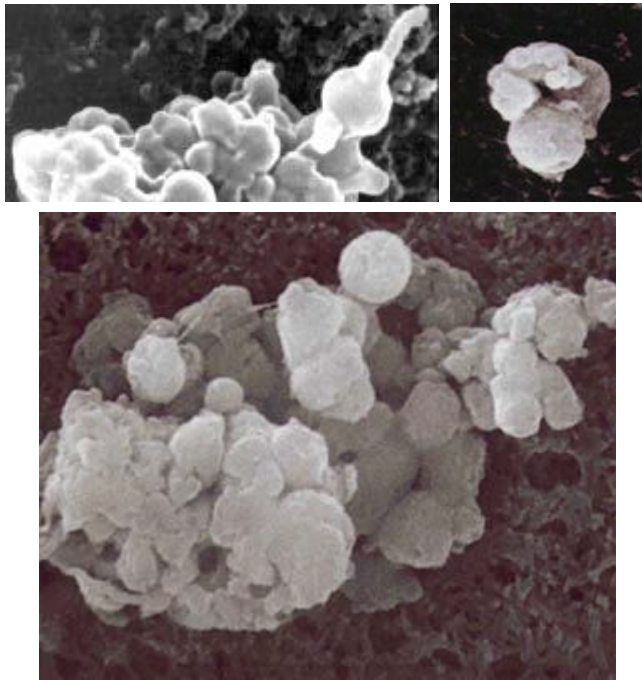
With instrumental and laboratory contamination excluded at all stages of the experiment, two options remain. First, one might think that the organisms obtained from the stratosphere were carried from the ground in a volcanic eruption or in an exceptional meteorological event. The other possibility is that they arrived 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 20 January 2001, and calculations along the lines carried out by Colbeck<sup>24</sup> suggest that steady infall would drain out particles of 3  $\mu\text{m}$  radius in a matter of weeks. A similar objection applies to rare meteorological events, assuming our collections on 20 January 2001 gave us representative stratospheric samples at 39–41 km. No process that is purely terrestrial can sustain the high densities of bacterial clusters as we shall derive in the arguments in the following section.



**Figure 3.** (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 detects membrane potential and viability of cells and the latter the presence of nucleic acid.



**Figure 4.** Cultures of *B. simplex* (left) and *S. pasteurii* (right) grown on LB medium after isolation using soft PDA from stratospheric samples at 41 km ( $\times 1000$  using phase contrast microscope).



**Figure 5.** (Top left) Cocci and rod identified with SEM from membranes at 41 km 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.

What about debris from man-made spacecrafts that litters the atmosphere? Since the amount of such material is very small (estimates are available from space agencies), the chance of our organisms being derived from such debris is very low.

Finally, could we have picked up the bacteria sticking to the balloon itself? This possibility too can be discounted because there is a long tethering rope (around 100 m) connecting the balloon to

the payload and normally in a windy situation (as prevailing at the time), the payload traverses a different part of space to that which is swept by the balloon. [Those who are mathematically minded may like to examine this issue in the rest-frame of the balloon!]

Such findings emphasize the need to exchange samples between laboratories so that they can be independently checked. It should be pointed out however, that the microbiological studies described

above are based on sampling episodes and as such are open to the vagaries of the non-uniform distribution of culturable microorganisms. As a result, workers in one laboratory may, by chance, culture microorganisms from stratosphere-air-derived samples, while workers elsewhere do not. Such discrepancies of experimental replication do not in themselves indicate that positive findings necessarily result from contamination, and merely that on one occasion, but not in another, culturable organisms were present on the membrane and thereby available for culturing. This point is emphasized by Figure 3, where the cells are seen to be present in isolated clumps, rather than being evenly spread over the surface of the filter.

Recently, the presence of viable, but non-culturable bacteria in the stratosphere has been confirmed (by M.W.). In these studies, a viable staining technique has been employed which differs from that initially used by workers at Cardiff. This has provided independent confirmation of the presence of viable, but non-culturable bacteria at a height of 41 km. The control for the viable staining test involved the same procedures being applied to pristine filters not exposed to stratospheric air. Null results were obtained for all the controls.

The argument that the high degree of sequence homology with modern terrestrial counterparts that were found for the three microorganisms that were cultured<sup>23</sup> makes a terrestrial origin more likely should be viewed with caution. Similar identities have been found in the case of a 40 m.y.-old bacterium trapped in amber that has been revived<sup>19</sup>, and for a halogenic bacterium trapped in a Permian salt crystal for over 250 m.y. (ref. 20). The expected divergence between modern and ancient bacteria is not found in these interesting results – again consistent with an ongoing space incidence, and confirming our results.

### The infall hypothesis

The alternative hypothesis of extraterrestrial origin (refs 9 and 17), although controversial, is more attractive as an explanation of our findings. The bacterial material, cultured in the Sheffield 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  $\mu\text{m}$  radius particles get burnt through frictional heating and end up as meteors. Some fractions may do, but others 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 which disintegrate into a cascade of slow-moving smaller clumps at heights above 270 km where frictional heating would be negligible. Evidence for such disintegrations has been available for many years<sup>25</sup>, and more recent studies of Brownlee particles collected using U2 aircraft have also shown the survivability of extremely fragile organic structures<sup>26</sup>.

Based on the cells detected on some filters (cf. Figure 3), a crude estimate can be made of the amount of such material falling in at such heights.

Both these detection methods yielded an average of one clump of viable cells per 5 mm  $\times$  5 mm (25 mm<sup>2</sup>) of filter area. With a total filter area close to 2000 mm<sup>2</sup>, the entire membrane would have contained  $\sim$  80 clumps of viable cells, which must therefore represent the main bacterial content of the air collected from a height of 39–41 km. The NTP concentration of clumps is therefore  $\sim$  80/18.5 = 4.3 per litre. Converting this to the ambient conditions at 39–41 km, ( $P = 2.9 \times 10^{-3}$  b,  $T = 253$  K, see ref. 27), we arrive at a local clump density of  $1.4 \times 10^{-2}$  per litre. We estimate an average mass for a porous 3  $\mu\text{m}$  radius clump to be  $3 \times 10^{-11}$  g. The settling speed of such a particle at this height has been calculated by Colbeck<sup>24</sup> to be  $\sim$  0.18 cm/s. Using these values and taking the average surface area of the earth to be  $5 \times 10^{18}$  cm<sup>2</sup>, we obtain an infall rate of:

$$\begin{aligned} &\sim 1.4 \times 10^{-5} \times 3 \times 10^{-11} \\ &\quad \times 5 \times 10^{18} \times 0.18 \\ &= 3.78 \times 10^2 \text{ g/s} \\ &\approx 3 \text{ tonnes per day} \end{aligned}$$

over the entire globe. Whatever the source of the clumps might be, such an infall or fallback rate from 39 to 41 km would seem inescapable. With an average of  $2.4 \times 10^{-9}$  g of bacteria (deemed viable) collected per filter, it would indeed be a little surprising to find that they are *all* non-cultureable.

A further (again, somewhat crude) check can be made on the infall hypothesis by referring to the work of Kasten<sup>28</sup> who has shown that the number density  $N$  of particles in a state of steady infall at height  $h$  goes as  $w^{-1}$ , where  $w$  is the terminal velocity for that size of particle at that height. Using this result one expects an exponential *growth* of  $N$  as the height *decreases*. A comparison of the number of viable cells estimated for probes at heights 25 and 40 km suggests that  $N$  for the former height is some 13–14 times larger than that for the latter height. Kasten's calculated curve (see Figure 6) for particles of this size shows this factor to be  $\sim$  10. Given the crudeness of the estimates, they are consistent with the infall hypothesis.

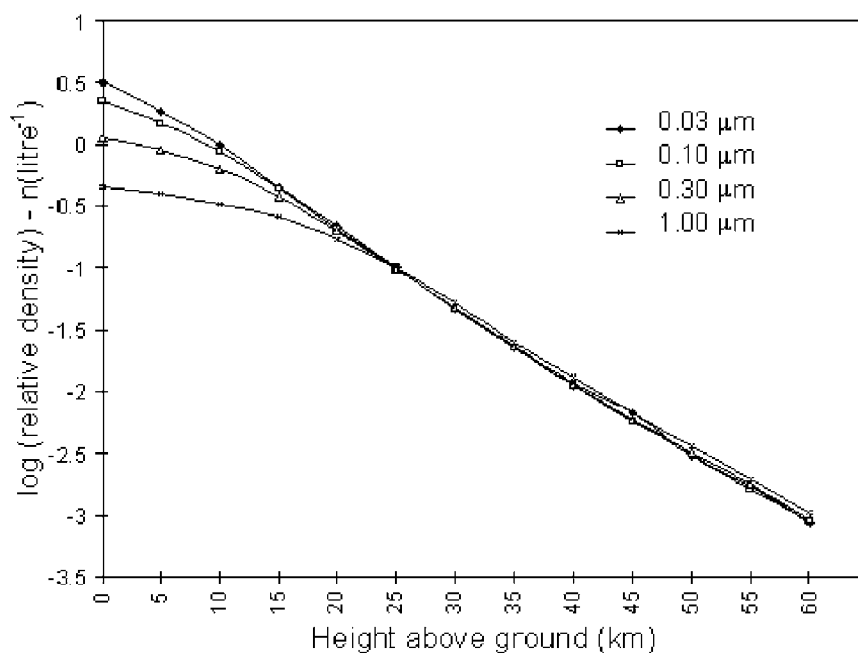
### Concluding remarks

In conclusion, we feel confident that we have detected viable, but non-cultureable bacteria and have cultured two bacteria and a fungus from the stratosphere (at a height of 41 km). Although some unknown mechanism by which microorganisms can be transported from the earth to this height may exist, based on current knowledge it appears that this is unlikely and the observed microorganisms have a non-terrestrial origin. Although we are

convinced that the precautions taken during sample collection and analysis rule out the likelihood of contamination, we do recognize the need for further experiments in order to unequivocally confirm the validity of our position.

Future studies by us will concentrate on the use of isotopic analysis of the stratosphere-derived samples to determine if the abundances of various isotopes differ from those of microorganisms found on earth. In addition, we will attempt to undertake a similar balloon experiment within a few weeks to months of a major cometary passage or a meteor shower, in the hope of detecting a significant rise in the number of microorganisms in the collected air samples; such a finding would point to a cometary origin for these organisms.

We, of course, recognize the need to design further experiments in which specific questions can be answered with greater efficiency and certainty. Can, for example, the samples be analysed *in situ*, without leaving the probes? Can we improve the data on which the calculations made above are based? Can satellites be used to examine air at much greater heights, where the expected numbers according to the Kasten's result are very small? The enormous significance of the question that we are trying to answer points to



**Figure 6.** Normalized number density profiles for spherical bacteria of various radii falling through the atmosphere. The normalization is to 1 cell per 10 l at a height of 30 km.

the need for such experiments to be undertaken, even taking into account the considerable problems they pose for current technology.

Fred Hoyle, who pioneered this idea, as he did many other radical ideas in astronomy was alive when our preliminary findings emerged. He passed away on 20 August 2001. This article is dedicated to his memory.

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