The search for living cells in stratospheric samples

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ABSTRACT

Air samples are to be collected at various altitudes in the stratosphere using balloons flown from Hyderabad, India. The samples will be passed through sterile micropore filters, after which the filters will be analysed using voltage sensitive lipophilic dyes to detect the presence of either active or non-active cells. Organisms detected in this manner will be studied using static mass spectroscopy to establish isotopic ratios $^{13}C/^{12}C$ and D/H, which would distinguish between terrestrial and extraterrestrial cells.

Keywords: Panspermia, cometary dust, stratosphere, microorganisms

1. INTRODUCTION

The idea that microbial life occurs in great profusion on a cosmic scale has been developed in its various aspects by Hoyle and Wickramasinghe over the past two decades[1] . The concept of panspermia which implies the dispersal of life throughout the Universe had its roots in classical Greece and could be traced back to Aristarchus of Samos in the 3rd Century BC. During the nineteenth century this idea had many illustrious proponents, including Helmholtz, Kelvin, Tyndall and later Svante Arrhenius. Although panspermia did not enter the mainstream of scientific thought for a long time, the situation seems to have changed dramatically over the past few years. The idea that prebiotic matter needed for life's origins came from comets[2] has been accepted for close upon a decade, and recently even the prospect of comets seeding the inner planets with fully-fledged microbial life is entering the realm of respectable scientific discussion. These developments have undoubtedly been prompted by the advent of new observations and facts spanning a broad range of scientific disciplines.

It has been known for many years that spectroscopic signatures of interstellar and cometary grains are at the very least consistent with the presence of bacterial material[1,3,4]. Isotopic evidence for life on the Earth older than 3.9 billion years[5] puts back the presumptive origin of terrestrial life to a geological epoch that was characterised by intense cometary bombardment, thus giving additional credibility to the idea that life itself was introduced from outside. Furthermore there is a growing body of evidence that suggests the presence of fossilised microorganisms in carbonaceous meteorites[6] and perhaps also in a rock originating from Mars (ALH84001)[4,7].

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The idea of microbial life arriving at the Earth from space at the present time is one that merits the most serious attention of the scientific community. Relevant experimental techniques in microbiology have advanced to a point where it is now possible to identify even a single living cell using fluorescent dyes[8,9]. In addition there have been remarkable advances in methods available for sterilising equipment and examining samples in a contaminant free manner. Some preliminary evidence for the detection of living cells in the upper atmosphere had been reported long ago[10,11], but for some inexplicable reason, this early work was not pursued to a logical conclusion. Balloon flights continued to be used[9] for the collection of stratospheric aerosols, but no attempt was subsequently made to search for microbial content. From 1974 onwards Brownlee et al[12] deployed NASA-U2 aircraft to collect stratospheric particulates. The collection procedure was in essence the fly-paper technique, where surfaces coated with thick films of silicone oil were exposed to the relative air flow. Extraterrestrial particulates trapped in the silicone film were separated from terrestrial contaminants using isotopic criteria, similar to ones that we propose to use. These so-called 'Brownlee particles' have consistently shown evidence of carbonaceous material with a high organic content, and in one published instance an object was found that possessed the morphology and structure of an iron-oxidising bacterium[13]. Subsequently Clemmett et al[14] have found evidence of aromatic and aliphatic hydrocarbons of high molecular weight in eight Brownlee particles of proven cometary origin. Whether some or all of the cometary organics is derived biogenically is a matter of paramount importance to resolve.

In an attempt to address this question, we are in the process of planning for the collection of dust samples at various levels in the stratosphere using high-altitude balloons flown from Hyderabad, India. We present here an outline of our proposed program and of the techniques to be used for counting the number of cells, alive or dormant.

2. ESTIMATES OF NUMBER DENSITY OF CELLS

If the flux of cells over the whole Earth is known, we can estimate the steady-state number density at any altitude h. This is accomplished by first calculating the terminal velocities, w, of particles in a standard atmosphere at various heights h, as done by Kastin[15], and then using an equation of continuity to give a number density $N \propto w^{-1}$.

In such a model the resulting relative density of cells as a function of altitude is plotted in Fig. 1 for various values of the radius. The normalisation in this plot is arbitrarily set to a value of 1 cell per 10 litres of air at an altitude of 30 km, a figure that is consistent with claims from older measurements[10], but one which as we shall see is likely to be a generous underestimate. It is known that the daily input of extraterrestrial material worldwide is upward of some 50 tonnes [16]. If a fraction of say 0.1% of this is comprised of bacterial particles of average radius ~ 0.3μm, which enters at the altitude of 30 km at speeds of 3.3×10^{-2} cm/s (ref.15) the number density of cells at 30 km is about 1 per litre, some ten times our normalisation value. Frank and Sigwarth [17] have argued in recent years that 'small icy comets' provide a mass input of water-ice of about 30,000 tonnes per day. If these so-called 'small icy comets' consist in reality of assemblies of separate cometary grains, as has been proposed by Hoyle and Wickramasinghe [18], and if 1/3 of the total mass is in the form of organics, as for the case of comet Halley [3], the number densities of cells could be several orders of magnitude higher than the values contemplated above. In such a case we could confidently expect our experiments to yield statistically significant counts of cells. We note also that the number density of 1 per 10 litres obtained in the old experiments could turn out to be a gross *underestimate*, because they would only have taken account of culturable and active cells. Recent work on bacterial populations in a wide range of terrestrial samples have shown that for every culturable cell there could be millions of more dormant cells that could be detected only through their nucleic acid content. If such cells are included then tens of thousands of cells per litre might be found at stratospheric altitudes.

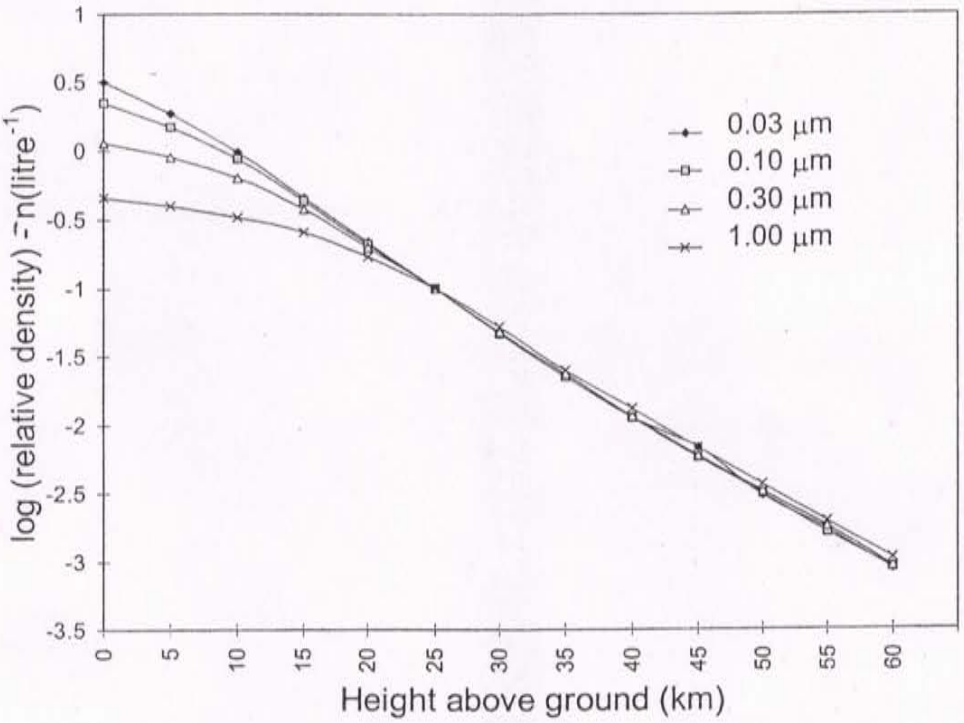

Figure 1. Normalised number density profiles for spherical bacteria of various radii falling through the atmosphere. The normalisation is to 1 cell per 10 litres at a height of 30km

3. EXPERIMENTAL TECHNIQUES

The primary goal in the experiment will be to collect samples of air at different heights in the stratosphere under perfectly sterile conditions. With expected bacterial population densities in the range of say 1 - 10^6 per litre it would be sensible to maximise the volume of collected air subject to experimental constraints. We aim to collect of the order of 10 - 100 litres of air at STP at each altitude, and for this purpose plan to use a cryopump of the type devised by Shyamlal et al [19]. Perfectly sterilised steel containers are vacuum baked and evacuated to almost zero pressure in a sterile atmosphere. A number of such containers will be flown on a balloon using the TIFR balloon launching facility. The containers will be cooled to liquid neon temperatures in flight and connected to bellow-sealed stainless steel valves, which could be operated remotely from ground command. When the valve of a container is opened the surrounding air will rush in, and after collecting the requisite amount of air the container will be hermetically sealed. This procedure is to be repeated at several heights up to a maximum of approximately 40 km, leading to recovery of air samples at different altitudes in the stratosphere.

The above collection procedure is expected to yield tens of litres of air at equivalent STP for each altitude. The air samples will be passed through sterile micropore filters that retain any incipient organisms that are present. The filters can then be treated with fluorescent dyes. Dyes such as ethidium bromide, acridine orange, propidium iodide and fluorescein diacetate have been widely used for this purpose [8,9]. Dyes that are sensitive to membrane potential could not only detect bacterial content but also distinguish between viable, non-viable and dormant cells. We propose to use a cyanine dye, which is cationic, and oxonol dye which is anionic, both of which are commercially available. Such fluorescent dyes could be easily displayed and photographed and even the presence of a single organism in a sample would be detectable by this technique. If we have active organisms the application of cationic dyes will result in the appearance of

fluorescent coloured spots, the number of fluorescent spots defining the number of active cells [8]. On the other hand if the cells collected are 'dormant' or dead, treatment with anionic dyes will also result in fluorescent, but differently coloured spots. Thus using cationic as well as anionic dyes differential counts of 'active' and 'dormant' cells can be made.

4. TERRESTRIAL CONTAMINATION

Once cells have been detected from stratospheric samples one has next to decide whether these are of extraterrestrial or terrestrial origin. A significant terrestrial component would indeed be expected, particularly at the lower altitudes since aeroplanes and man-made vehicles reach altitudes as high as 15 km. And terrestrial microorganisms could be carried to even greater heights in rare events such as volcanic eruptions. We propose to model the density profiles expected for such terrestrially originating microorganisms, that might serve as a first order discriminant of terrestrial *vs* extraterrestrial cells. It might be expected that terrestrial contaminants will follow a somewhat different density profile from extraterrestrial cells falling downwards through the atmosphere.

More definite indications of an extraterrestrial origin would follow from determinations of carbon and hydrogen isotopic ratios. Determinations of parallel stable isotopic compositions $\delta^{13}C$ and δD by static mass spectroscopy will be made to a high level of precision ($\pm 0.3\%$) from the analysis of sub-micromolar samples [20]. Since cometary and terrestrial isotopic ratios are different, this procedure could test the possibility that some of the recovered cells are of extraterrestrial origin. A positive result will constitute unequivocal proof for the detection of extraterrestrial cells. The work will require collaboration with Professor C.T. Pillinger's group at the Planetary Sciences Research Institute of the Open University at Milton Keynes, UK.

Another important technique would be to test the optical activity of the amino acids derived from cells collected in the sterile microporous filter. This technique is very simple and might constitute a further independent test of extraterrestrial origin, if a D/L ratio significantly different from the average for terrestrial microoganisms is found. The argument being that there is no *a priori* reason to expect the same distribution of optical activity as exists in the amino acids of terrestrial cells.

5. DEPLOYMENT OF HUMAN RESOURCES

The Indian-based collaborators of this report will be responsible for the over-all coordination of the research programme, and specifically the logistics of the balloon flight and the sample collection techniques.

The Cardiff-based team will be responsible for microbiological detections, isotopic analyses as well as theoretical modelling and data analysis.

6. CONCLUDING REMARKS

An outline of an international experimental and theoretical programme for obtaining unambiguous evidence for *panspermia* has been sketched above. The steady-state number density of cells at various altitudes can be estimated if the total mass influx of cells, particle sizes and an appropriate atmosphere model is prescribed. Under the most favourable set of conditions one might expect upwards of ten thousand extraterrestrial cells per litre (not all culturable) at an altitude of 30km, and this is well within the limits of present-day detection techniques. We propose to use perfectly sterile equipment and containers to recover air samples at various altitudes, up to a maximum of 40km. The samples will be passed through sterile micropore filters and membrane sensitive fluorescent dyes will be use to count both 'active' and 'dormant' cells. The isotopic abundances of C and H combined with the D/L ratio of amino acids in the cells will serve to distinguish between cells of extraterrestrial and terrestrial origin.

Once the presence of extraterrestrial cells is established the next step would be to collect adequate numbers of 'active' cells that might be cultured in suitable nutrient media. Cells of extraterrestrial (or cometary) origin could then be examined microscopically, classified and placed within the context of terrestrial microbiology. A major milestone in our attempts to trace life's origins in the Universe would then have been reached.

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