

## Masses Staining Positive for DNA-Isolated from the Stratosphere at a Height of 41 km

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### Abstract

Clumps of stratospheric material with individual diameters of 10-30 micrometers were isolated in a sterile manner from a height of 41 km. The masses showed no obvious morphology, but stained positive for DNA and are therefore considered to be unequivocally biological. We suggest that the masses are arriving to the stratosphere from space and not upcoming from Earth; evidence is provided to support this view.

**Keywords:** Membrane staining; Stratosphere sampling; Earth biology

### Introduction

During the early years of this century, researchers at the University of Cardiff, working in collaboration with Indian workers, isolated from the stratosphere (at a height of 41 km) a particle mass which exceeded 10 micron in size and which stained positive for living matter (using a fluorescent carbocyanine stain) [1]; the particles were assumed to be bacteria. The senior author and co-workers, used Live/Dead stain to confirm the presence of such masses, and showed that they contain live and dead particles [2]. They also isolated, on growth media, bacteria and fungi from these stratosphere samples [2,3] and provided further evidence for the presence of bacteria in the stratosphere [2,4,5]. Confirmation of the presence of cultureable bacteria was then provided by a number of other workers [6-8].

Some of the data from the above mentioned research was not evaluated at the time. Since recent studies have demonstrated the presence of unusual biological entities in the stratosphere [9-11] it appears an opportune moment to reassess some of this neglected data. The aim of the present paper is therefore to discuss some of this earlier data in relation to these new findings relating to the presence, and origin, of the biological entities currently being isolated from the stratosphere.

### Materials and Methods

#### Stratosphere sampling

The stratospheric air samples were obtained from a height of 40-41 km using a cryosampler attached to a balloon. Strenuous efforts were made to avoid contamination from the balloon and of the sampling probe during the balloon's ascent into space, further details of which are reported elsewhere. The balloon carrying a cryosampler payload was launched on 21 January, 2001 from Hyderabad, India. The cryogenic sampler comprised a 16-probe assembly. Each probe had a volume of 0.35 l and was made of high vacuum grade stainless steel. It was capable of holding a vacuum at 10<sup>-6</sup> mb and a pressure of 600b. The temperature cycling ability of the probes was tested between -246°C and 140°C. To minimise contamination, only the minimum required electron-beam welds were made and the interior was electroplated. The probes and manifolds were cleaned with acetone and four washes of demineralised water; the complete assembly was steam baked and finally heated with infrared lamps to 140°C. To prevent collection of out gassed substances from the gondola, a 2 m intake tube (sterilised

as above) formed a part of the payload assembly and the assembly was tethered to the balloon by a sterilised 100 m line. The probe mouth was covered during balloon ascent and had a metallic (Nupro) valve, which could be remotely operated, from the ground.

#### Membrane staining

The micropore filters on which material from the stratosphere was deposited were aseptically cut in a Class 2 cabinet. Samples were then transferred onto new glass microscope slides which had been washed, and polished and then placed in a sterile petri dish. DAPI (10 µl, 0.1 mg/ml in SDW) was added to each sample. The stain was left to develop under low light for 15 min. A cover slip was then placed on the filter piece and the sample was observed using an epifluorescence microscope; images were captured using sigma scan software. Controls using known bacteria were also included in order to confirm that the staining process worked. In the case of For DiOC<sub>6</sub> staining, 1 µl of a stock solution of 0.1 mg per ml in ethanol diluted with sdw to give a 1:100 solution.

### Results and Discussion

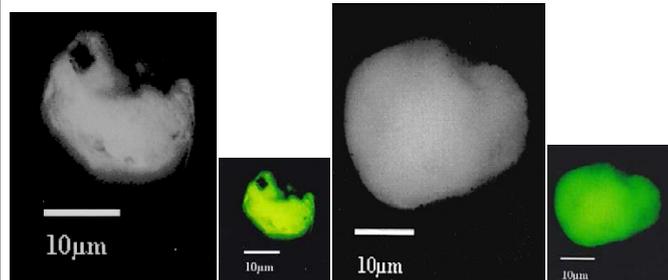
Figures 1 and 2 show masses isolated from the stratosphere at a height of 41 km and associated images following staining with the DNA-specific stain DAPI. Both masses appear to be amorphous and do not possess evidence of cell walls or appendages which would aid their identification as individual organisms. DAPI staining shows that all of the masses contain uniformly-distributed DNA; as a result, we conclude that they are biological. Figure 2 shows the top part breaking down into particles. Another amorphous mass, isolated from 41 km, is shown in Figure 3; positive staining with DiOC<sub>6</sub> shows this mass is also biological (DiOC<sub>6</sub> is a fluorescent stain used to stain endoplasmic reticulum, vesicles, membranes and mitochondria). It is noticeable that the masses are not made up of individual components of the size of

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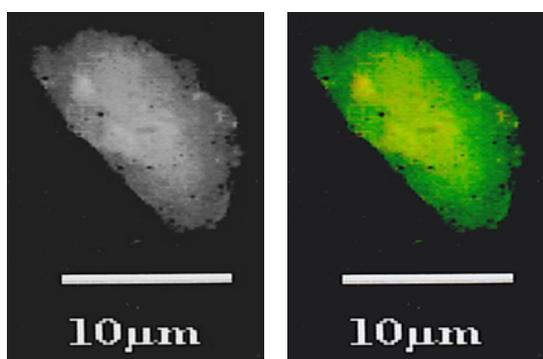
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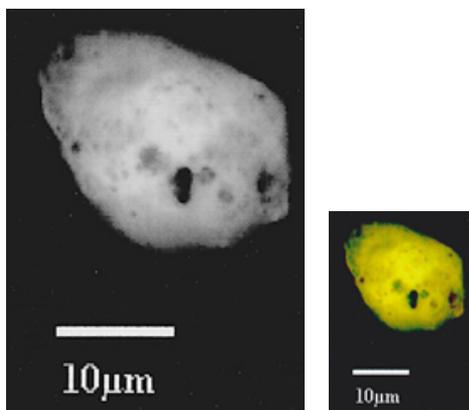
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**Figure 1:** Particle masses isolated from the stratosphere, stained with DAPI.



**Figure 2:** Particle mass isolated from the stratosphere, stained with DAPI, showing edges breaking into nanoparticles.



**Figure 3:** Particle mass isolated from the stratosphere, stained with DiOC<sub>6</sub>.

bacteria (around 1 micron) instead, the irregular, particulate edge of the masses (Figure 2) suggests that they are made up of nanoparticles, such as nanobacteria or even viruses. Unfortunately, the degree of magnification used and the resultant resolution does not allow us to provide any further information on this point. All of the biological masses shown exceed 10 microns in size, the largest, shown in Figure 1B exceeding 30 microns. These dimensions are significant because it is generally accepted that particles bigger than 5 microns cannot cross from the Earth through the tropopause and into the stratosphere [12,13], let alone be elevated to a height of 41 km. It is possible however, that larger particles could be elevated into the lower stratosphere, by a very large volcanic eruption. The sampling trip was however, made 2

years after the previous last volcanic eruption, so any particles from this source would have deposited back to Earth before they could be sampled by the cryosampler. It is highly significant that no pollen, grass, algae or fungal spores (some of which are similar in size to the observed masses) was seen on the membranes on which was deposited material from 41 km. It is impossible to believe that there exists a mechanism which could transfer the fluorescent-staining masses from Earth to 41 km without also delivering examples of such Earth biology.

An alternative explanation is that the masses are made up of biological particles which are small enough to be carried in to the stratosphere at a height of 41 km or above, where they were re-aggregated and compressed into the masses seen in Figures 1 and 2. Such masses comprised of terrestrial biological material might then be posited to have been sampled by the cryosampler on the journey back to Earth. Because of the exceedingly low ambient densities prevailing in the stratosphere the operation of such a particle-compression mechanism appears to be exceedingly unlikely. It is possible, however, that a much larger cometary bolide, containing both nanometre and micrometre-sized biological entities (viruses and bacteria), disintegrated in the upper stratosphere and recondensed into the amorphous DNA imbued structures shown in Figures 1-3. This could well be similar to the reconstitution of calcite and carbon dust from the material of an exploding meteorite described by Corte et al. [14].

In the case of the samples discussed in the present paper care was taken at every stage of their collection and processing to avoid contamination. We also checked some possible, common contaminants, such as dandruff, skin and laboratory dust and none were similar in appearance to the masses shown in the above figures.

On the balance of probabilities, we conclude therefore that the masses were sampled as they were incoming to Earth from space. Recent studies have shown that naked DNA can survive re-entry from space into the atmosphere of Earth [15,16]. It is probable however that, instead of travelling to Earth in a naked unprotected state, the incoming masses found here were shielded from UV and other extremes, by cosmic dust or by being embedded in small ice particles.

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