



The high cold biosphere - Microscope studies on the microbiology of the stratosphere

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Claims that bacteria exist in the stratosphere were made during the early 1930s and confirmed by Russian workers in the 1970s. More recently, workers in Cardiff, Sheffield and India have collaborated in sending balloons to the stratosphere to determine if a high cold biosphere exists at heights up to 41km. Using scanning electron microscopes, clumps of bacteria-like forms have been found at this height, either alone or associated with dust particles.

Vital fluorescent stains, visualized using fluorescent microscopes have been used to confirm that these clumps consist of living cells, i.e. bacteria. The obvious assumption is that these bacteria are carried to the stratosphere from Earth. However, the tropopause acts as a barrier to the free movement of particles above 17km thereby making such transfer more difficult than is generally assumed. Bacterial cell masses exceeding ten micron in diameter were found in the stratosphere. Since it seems unlikely that such large clumps could have been carried up from Earth we assume that these



Fig.1. The balloon used to carry the sampling pod to the stratosphere.

are incoming from space and that they contribute to a mixed stratospheric biosphere made up of space and Earth-derived organisms. The incoming bacteria are probably often viable, but not culturable and may make up the large population of such bacteria known, from DNA studies, to exist on Earth. It is also suggested that the transfer of bacteria from Earth to the stratosphere could



Fig.2. Sampling pod housing the cryoamplifiers carried on a (blue) carriage support.

act as means of "negative or geo-panspermia", by which space is seeded with Earth bacteria. In addition, bacteria in the high cold biosphere will be exposed to mutagenic radiation (notably UV) and the movement of bacteria from Earth to this region and back may have played a major role in the evolution of life on Earth. Similarly, bacteria incoming to Earth from space may also promiscuously exchange genes with indigenous bacteria, thereby enriching the Earth's gene pool with genes from a cosmic gene pool.

How far up into space above Earth, does life extend? Although, during the 1970s, Russian scientists did some preliminary microbial sampling of the stratosphere^[1], we still cannot be certain as to the height at which the biosphere extends above the Earth's surface. Surprisingly then, while we know a great deal about the distribution of life on the planet and how it is able to exist in extreme environments at the poles, in deep sea thermal vents, in hot springs and even deep in the earth, we simply do not know how high the biosphere extends above our heads. At what height above us does life fade away into non-biological space? This question, of course, begs another question, namely- does life in near space originate solely from Earth,

or might life be incoming to this planet from space. The idea that life came to Earth from space (so-called panspermia) has a long history. A modern version (so-called neopanspermia) suggests that not only did life on Earth originate from space, but also that microbial life is continually raining down on the planet even today. These modern versions of panspermia and neopanspermia have, despite much criticism, been enthusiastically advocated by the late Fred Hoyle and, the very much extant, Chandra Wickramasinghe of Cardiff University's Centre for Astrobiology. If microorganisms in the shape of bacteria, fungi and viruses are incoming to Earth then it should be a simple matter to send sampling devices, on rockets or balloons, to various heights above the atmosphere and to see at which point they disappear, if indeed they

ever do. Should the microbial biosphere continue from Earth into space there will then come a point when we must admit that the organisms could not have arisen from Earth and must be coming in from space. Actually, even this suggestion is debatable as it could be argued that a form of negative or geo-panspermia exists and that microorganisms are continually leaving the earth and travelling deep into space where they colonize other worlds. It comes as a surprise to find that NASA and the other space agencies have not, in recent times at least, attempted to answer the question of how high the biosphere extends.

In order to help answer this question I was lucky enough to join a team of scientists from India and the UK, who have, over the past half dozen or

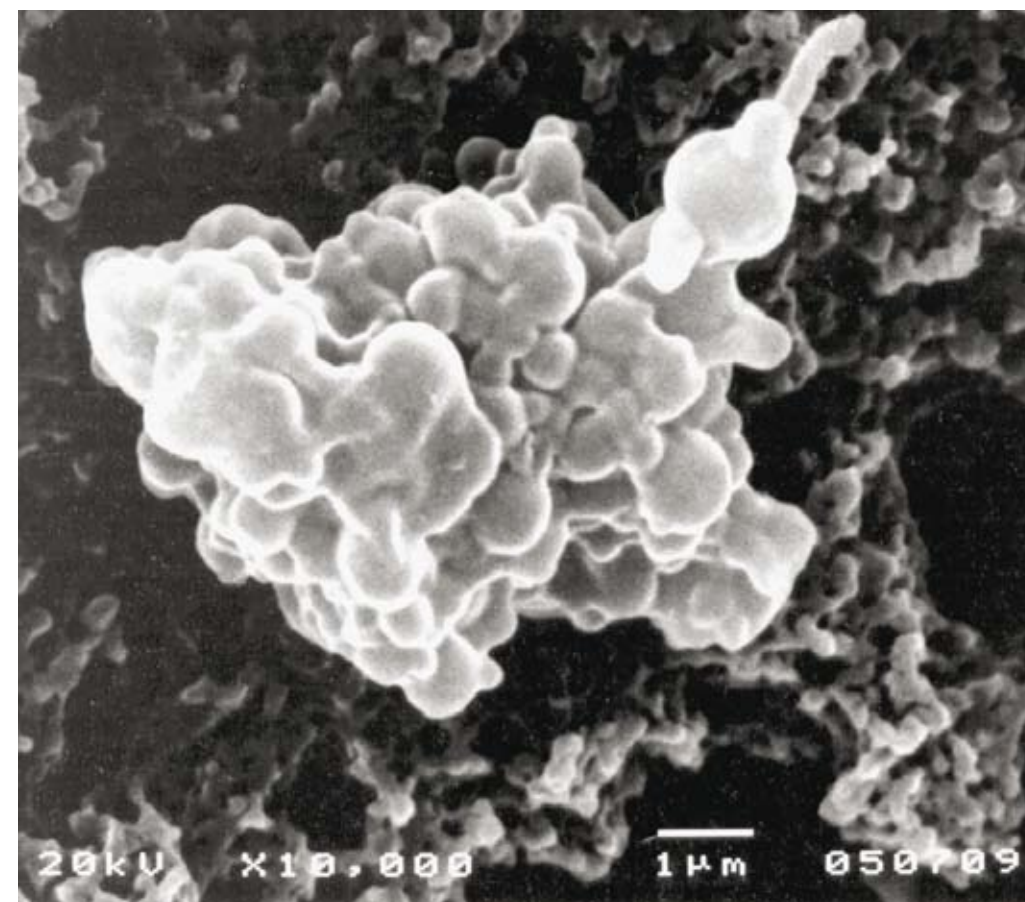


Fig.3. A scanning electron micrograph of a clump of bacteria (10µ plus across) found in stratospheric air sampled at a height of 24.6-27.9km (from Harris et al.^[3]).

more years, tried to answer this question by using balloon-carried atmospheric samplers^[2-8]. These huge helium balloons (Figure 1) carry a sampling pod (Figure 2) which lifts a number of so-called cryosamplers into the high atmosphere. These consist of high purity steel tubes, into which large volumes of air is collected, at any chosen height, as the balloon crosses over India at a maximum height of 41km (around 25 miles); a region I have termed the “high cold biosphere”.

The stratosphere is the region of the Earth's atmosphere that is found above the tropopause, which exists at an average height of around 17km. The stratosphere is stratified, with warmer layers high up and cooler layers farther down; in this respect it is the exact opposite of the troposphere. The stratosphere exists to a height of around 50km, where the temperature is around 27°K (-3°C), falling to around -20 to -30°C at around 41km; as we shall see this is the height at which most of recent sampling of the stratosphere has been conducted. These temperatures compare with -80 to -90 for the coldest Antarctic values;

the Antarctic is however, much warmer than the stratosphere in summer, at around 5-15°C.

As early as the turn of the nineteenth century, it was shown even common bacteria (as exposed to specialized so-called psychrophiles) could survive at a temperature of -252°C. Since however, microorganisms may exist in particulate or soot-masses, they may never be exposed to such extremely low temperatures during their stay in the stratosphere; this is due to the fact that such particle masses act as solar collectors and thereby increase in temperature when exposed to the sun. There is no regular turbulence in the stratosphere and air movement is largely horizontal. The ozone layer occurs at the top of the stratosphere at around 50 km. The region is also rich in sulphuric acid and is exposed to large amounts of UV. Again, the fact that microorganisms may reside within alkaline particulate masses, may reduce the toxic effects of stratospheric acids and ozone and these inorganic masses may also shield bacteria from the damaging effects of both UV and ionizing radiation.

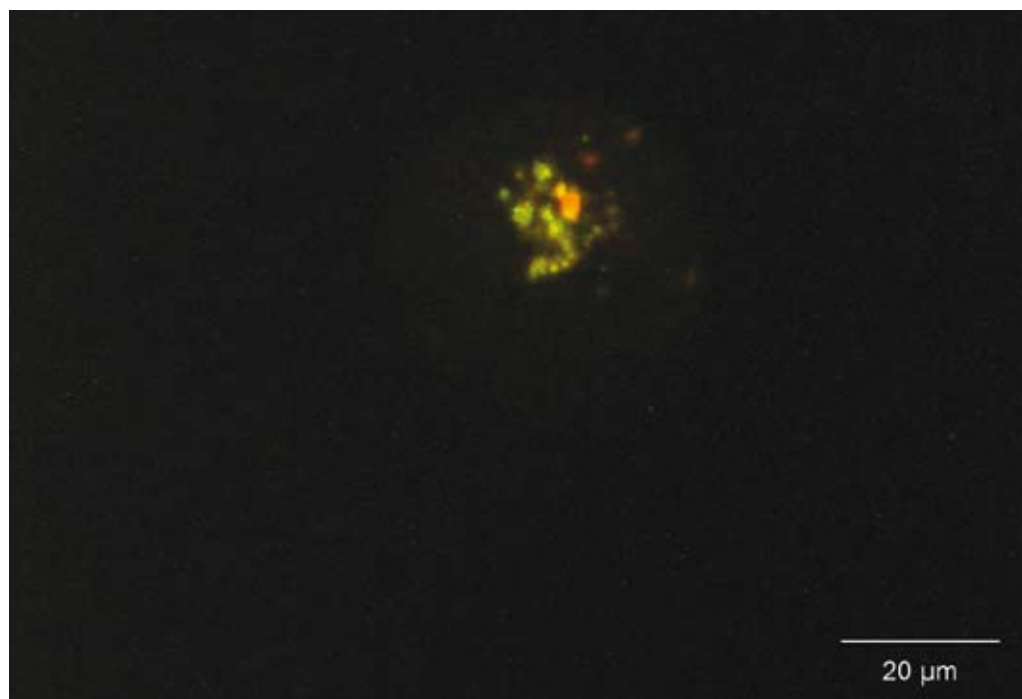


Fig. 4. Use of Live /Dead stain to detect bacteria in samples from the stratosphere (red and green staining respectively shows dead and living cells).



Fig.5. A transmission electron micrograph of a red rain cell (kindly provided by C.Wickramasinghe, Cardiff University). Scale bar 300nm.

Do Microorganisms Exist in the Stratosphere?

During the 1970s Russian workers found both bacteria and fungi in the stratosphere, at heights of 60-70km. No organisms were, however, isolated above this height. This team employed a meteorological rocket carrying a pre-sterilized stratospheric sampler, housed in the nose cone; this was heated to red hot (and thereby sterilized) on its way to the stratosphere^[1]. Here, an explosive charge separated the sampler from the rocket which then sampled the stratosphere, as it descended. Before exiting the stratosphere, the sampler was sealed and, on return to Earth, was incubated, without ever being opened. This allowed the authors to conclude that everything growing inside the sampler had originated from the stratosphere. Fungi (*Circinella muscae*, *Aspergillus niger* and *Penicillium notatum*) were isolated from a height of 48-77km and bacteria of the genera, *Micrococcus* and *Mycobacterium*. They also found that pigmented microbes could withstand the high vacuum and high levels of ultraviolet radiation likely to be present in the stratosphere. These

Russian scientists did not however, comment on the possible mechanisms by which the organism they isolated reached the stratosphere, i.e. they did not indicate whether they considered that these microorganisms were coming in from space, or exiting from Earth.

More recently, air samples were collected using the balloon-cryosampler set-up mentioned above. The system was sterilized and contamination from the balloon was avoided. On return to Earth, the air in the cryosampler tubes is passed through microbiological filters with ultrasmall pores (0.2 micron) which filter out any bacteria and fungi; in addition the tubes are washed out with sterile buffer, which is then similarly filtered; as a result, the pristine white membranes become covered with stratospheric dust. Throughout the whole sampling process and the subsequent microbiological processing due attention is paid to the avoidance of contamination. So that we can be certain that any microorganisms that arrive on the filters are obtained from the stratosphere. The micropore filters were studied using a variety of microscope techniques in order to determine whether any microorganisms were obtained from the stratosphere.

How do we distinguish bacteria from inorganic artifacts when using the scanning electron microscope?

Microscope studies on stratospheric samples

The filters were initially examined using the scanning electron microscope (in some cases the samples were gold-coated, in others, an environmental scanner was used and no coating was applied). Figure 3 shows that clumps of

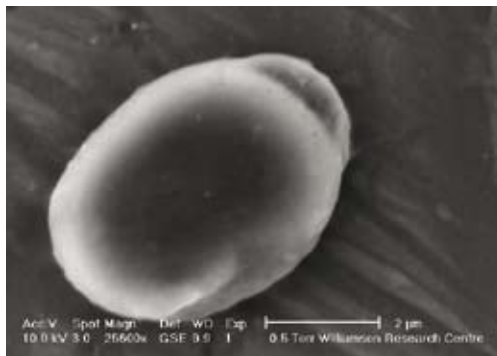


Fig.6. Scanning electron micrograph of red rain cell.

what look like biological cells were found on the membranes sampled from various heights in the stratosphere. These clumps are of approximately the right size and shape to be bacteria. Had this microphotograph originated from, say, a soil sample then most microbiologists would assume the clumps shown were indeed a mass of bacterial cells; further tests however, were needed on the stratospheric samples in order to confirm this. The sample filter membranes were next exposed to a variety of cell-specific fluorescent stains and examined using a fluorescent microscope. The stains used fluoresce under this microscope, the colour produced depending on the stain used. In

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the same way the biological component stained by the stains employed varies, so that some detect DNA, while others specifically detect living cells. The so-called Live/Dead stain was particularly useful since it stains living cells green, while dead cells stain red. Using such stains we were able to confirm that the cell-like masses on the membranes were indeed living, biological entities, i.e. bacteria (Figure 4). As a result of these studies, we were able to show that clumps of bacteria exist in the

stratosphere at heights up to 41km. Subsequently two bacteria (*S. pasteurii* and *B. simplex*) and a fungus called *Engyodontium album* were grown from the samples obtained at 41km and follow on work showed that samples taken from this height contained a range of bacteria (mainly species of *Bacillus*), including some novel species.

Further scanning electron microscopy work showed the presence of other bacteria-like particles including ultra-small of diameter less than 1 micron. How can we determine if such very small bacteria are in fact bacteria? This is a major problem since the atmospheric samples deposited on the membranes were rich in cosmic dust particles, some of which have the superficial appearance of bacteria. How then do we distinguish bacteria from inorganic artifacts when using the scanning electron microscope? Firstly, we go on size and morphology; bacteria have size range from around 0.1 to 1 micron in size and are usually coccid or rod shaped. They also frequently possess appendages such as flagellae. The stratospheric particles, which we consider to be ultrasmall bacteria, are seen to possess appendages called fimbriae, minute hair-like projections which attach the organism to natural surfaces. The presence of such appendages provides prima facie evidence that the particles are bacteria. An alternative approach which can be used to differentiate organisms from inert particles is to determine the elemental composition of the particles. By using Energy Dispersive X-ray Analysis (EDX), we can measure the elements present in the particles being viewed under the scanning electron microscope. Bacteria will contain carbon, nitrogen and phosphorus, but generally, not large amounts of elements like calcium, silicon or metal elements like iron. Of course some bacteria may accumulate such elements, but unless they have characteristic bacteria-like structures, these forms will unfortunately be discarded by our approach. A final means of differentiating bacteria from inorganic dust involves the use of what we term the “electron beam destruction method”.

Here, we apply the electron beam of the scanning electron microscope to the particles for a relatively long period; the inorganic particles resist this treatment, while organic particles (presumed to be bacteria) are holed by the beam. It is important to note that not all bacteria-like particles found in the stratosphere are in fact bacteria. As a result, confirmatory tests, like these, must be applied before any bacteria-like particle is referred to as being a bacterium.

Our next task was to try and determine the origin of the microflora of the high cold biosphere; did they arrive at this height from Earth or from space? The common assumption would be that



Fig.7. Cosmic dust from a stratospheric sample causing discolouration of micropore filter (kindly provided by C.Wickramasinghe, Cardiff University).

any microbes found in the stratosphere would have been ejected from Earth. Such transfer could certainly be achieved as the result of volcanic eruptions. However, the samples we used were obtained two years after the last major volcanic eruption on Earth, plenty of time for any particles, the size of bacteria, to have sedimented from the stratosphere. In fact, it seems very unlikely that bacteria routinely reach the stratosphere from Earth. This is because the tropopause acts as an effective barrier (at around 17km) to the passage of particles (but not gases) from Earth

into the stratosphere. Bacteria might however, be lofted into this region by unknown mechanism or by electrostatic processes and so-called blue lightning^[9-11]. Such processes will however, only be capable of transporting very small particles of less than 1 micron in diameter. It should be remembered that we have found clumps of bacteria in the stratosphere of diameters exceeding 10 microns (Figures 3 and 4). It seems extremely unlikely that such clumps could have been carried from Earth to heights up to 41km. As a result, we conclude that there exists in the high cold biosphere a mixed population of bacteria, made up of bacteria small enough to be carried (by some as yet unknown mechanism) to 41km (these we assume are bacteria which we isolate), and a population made up of clumps of bacteria which could not have possibly been carried up from Earth to the stratosphere. These we suggest must come from space and are not routinely isolated on our bacterial growth media. Perhaps these unculturable “space” bacteria make up most of the viable, but unculturable bacteria which we know exist in soils, and other environments on Earth. Although we cannot culture these bacteria, we know from the use of fluorescent staining that they are viable, and, as a result, may grow and exchange genetic information with Earth bacteria.

The high cold biosphere is an extreme environment and any organism present will be exposed to low temperatures, acids, and high levels of UV. Bacteria need not however, grow in this environment, but may merely survive, as “extremophiles”. The presence of cosmic dust will help protect bacteria from these extremes while passing through the stratosphere.

The High Cold Biosphere and Evolution

The existence of a stratospheric biosphere may have had an important effect on the evolution of life on Earth. As has already been mentioned, space-derived bacteria might exchange their

genes with the indigenous Earth microflora. In addition, any bacteria transferred from Earth to the stratosphere will be exposed to high levels of mutagenic UV radiation and other forms of radiation. This will induce mutations in bacteria passing into the stratosphere. Such mutagenesis will be far greater than that which occurs on Earth, where the amount of UV is reduced by the atmosphere and clouds. The high cold biosphere may therefore act as a huge mutation- generator, a vast laboratory where new bacterial genomes are created in bacteria which then return to Earth and promiscuously transfer new information to the non-exposed bacteria. This process could occur on numerous occasions, with bacteria being continually returned to the stratosphere for a new dose of mutagenic radiation. While the acquisition of an atmosphere and cloud layer was absolutely essential for the development of life on Earth, it could be argued that such a protective layer reduced the level of bacterial mutagenesis; the transfer of bacteria from Earth to the stratosphere however, circumvented these protective effects of Earth's atmosphere.

The Enigma of Red Rain

The above mentioned microscope techniques were invaluable when we came to investigate another atmospheric biological phenomenon, namely the so-called Red Rain of Kerala^[9]. Although this recent example of red rain fell in 2001 across the state of Kerala in India; red rains, and associated red snows have been recorded since antiquity. We were fortunate to obtain a sample of the red rain from Kerala, and by using a variety of microscope techniques confirmed that its redness is due to the presence of biological cells. By using scanning and transmission electron microscopy, we were able to determine the morphology of these cells. Of particular note is the blood cell-like appearance of many of the red rain cells when viewed using a scanning electron microscope. This is however, an artifact of the techniques employed, which causes the cells to implode; true red rain cells are egg

shaped (Figures 5,6). Initial reports claimed that the red rain cells lacked DNA; however using a variety of DNA-specific fluorescent stains, we were able to show that this is not the case and that red rain cells do typically contain DNA. Studies by us, using the transmission electron microscope show that red rain cells also contain daughter cells (Figure 5). Studies by Louis and co-workers^[12] appear to show that red rain cells have some unusual biological properties. It is not clear however, from where these cells originate; do they originate from space or are they carried up from the oceans on Earth and then fall back as rain? It seems highly unlikely however, that cells of this size could be carried from Earth to the stratosphere; answers to the questions, about the exact nature and origin of red rain must therefore await further studies.

In conclusion, it is hoped that other scientists, including those working for NASA who are likely to have the knowhow and resources to study the biology of the stratosphere and beyond, will take an interest in the high cold biosphere. In particular, we need to know how far the biosphere extends above our heads and whether or not microorganisms are continually raining down on us from space in cosmic dust (Figure 7).

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