

Search for Micro-Organisms in Outer Space

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Introduction

How did life appear on the Earth for the first time? Is it peculiar to Earth or is it widespread in the universe?

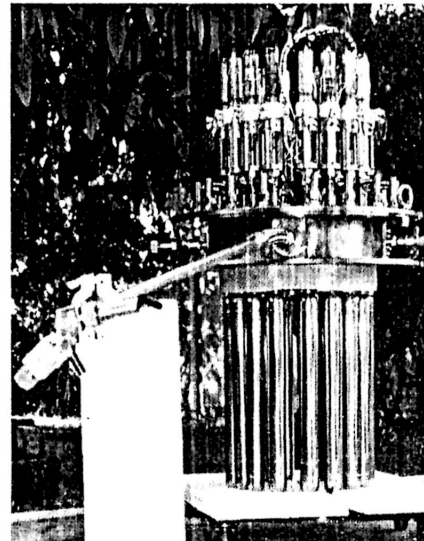
What are our chances of detecting life of extra-terrestrial origin?

Questions like these are widely asked of the astronomer as well as the biologist. The astronomer sounds very optimistic by stating that the Earth is but one of the nine known planets around the Sun, which is but one out of more than a hundred billion stars going round in the Milky Way, which is but one of billions of galaxies in this apparently boundless universe. So why should life appear only here on the Earth? Surely there must be millions (if not billions) of extraterrestrial civilizations more advanced than us in the Galaxy. The biologist may cast a damper

on this enthusiasm by pointing out that even if each such star having a planet around it is taken as a 'trial' for this experiment that we call 'life', the chance of life appearing out of nothing (that is, from a non-living environment) may be so small that perhaps all trials but one (the one here on the Earth) have failed. In that case we may well be alone in the universe!

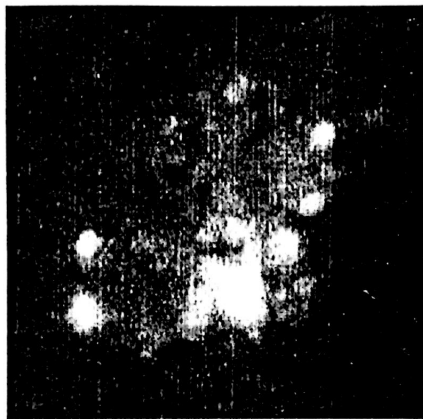
Rather than play a theoretical game of probabilities it may instead be worth searching for extraterrestrial life without any preconditions. Maybe it is well advanced with high technology at its beck and call. Or, maybe it is in a primitive microbial form. Of course the search strategy will depend on what we are looking for.

The programme called the Search for Extra-Terrestrial Intelligence (SETI) for example has set ambitious targets to look for very advanced civilizations through



The payload attached to the balloon flown on January 21, 2001

exchanges of radio signals carrying information. For this programme we need very large and efficient antennas to send and receive radio messages. Perhaps it may be easier to detect such advanced cultures through their radio messages than to look for very primitive life forms like cells or viruses and bacteria. Yet both



Shining visible cells found on filters in the Cardiff analysis

approaches are essential to our quest for life in the universe.

In this article I will briefly review a modest but pioneering attempt of the latter kind.

The panspermia hypothesis revived

It may indeed seem difficult if not impossible to search this vast universe to find microorganisms, which cannot be detected without a sophisticated microscope. Yet, there is a possibility that makes the task tantalizingly easier, a possibility that depends on the viability of a very original and remarkable hypothesis. The hypothesis was proposed by Fred Hoyle and Chandra Wickramasinghe from the UK in the mid-1970s.

The basic tenet of this hypothesis is that microorganisms are widely present in the vast interstellar space just as gas and dust are known to be present there. The presence of various atoms and molecules in the interstellar space can be inferred from the extinction they produce of light passing through them. In principle, each atom or molecule interacts in its own characteristic manner with light waves of different wavelengths. Thus in some cases the visible wavelengths may be absorbed much more than the infrared ones. In some cases there may be a sudden increase in the absorption of waves of specific wavelengths. The long and short of it is that studies of extinction of

light at different wavelengths can yield information about the composition of the intervening dust.

Using their previous experience of such diagnostics that had given useful information about the nature of interstellar dust, Hoyle and Wickramasinghe found that the extinction produced in the infrared source GC-IRS 7 as obtained by David Allen and Dayal Wickramasinghe, *did not match the pattern of any inorganic grain combination. In fact, Wickramasinghe found that the curve fitted very well that expected for extinction produced by E-Coli bacteria.*

This interpretation came as a great revelation to these two scientists and set them thinking further along the lines of the above hypothesis. First, can bacteria survive in space, what with bombardment from harmful x-rays, gamma-rays and ultraviolet radiation? Second, is there any mechanism available for these to be transported to the Earth? For, the second part of the Hoyle-Wickramasinghe hypothesis is that life on the Earth was seeded by such inputs from outside and the comets visiting inner parts of the solar system carry these microorganisms from far afar, from where the comets are supposed to originate. These comets sometimes brush the Earth's atmosphere with their tails. Such contacts provide opportunities for the bacteria and viruses carried on the comets to be passed on to the Earth's atmosphere, from where they descend to the ground level.

This hypothesis was a revival of the panspermia concept introduced by Svante Arrhenius, in 1903, several decades earlier. Arrhenius had suggested that living cells can be carried across space over large distances being propelled by radiation pressure. That idea as well as its revival in the form of the Hoyle-Wickramasinghe hypothesis

received considerable flak from biologists as well as astronomers. How can life survive the harmful x and gamma ray radiation? How can life form in the interstellar space?

Lately the opposition to the panspermia theories has mellowed somewhat, with the realization that bacteria can be much hardier than earlier imagined, and with the possibility of meteoritic material carrying fossilized life from Mars to the Earth as might have happened in the example of the Alan Hills Meteorite. It thus became a feasible proposition to test the Hoyle-Wickramasinghe hypothesis by examining the Earth's atmosphere at great heights for live microorganisms.

The ISRO cryosampler balloon experiment

Independently of the Hoyle-Wickramasinghe hypothesis, and in fact predating it by a decade or so, experiments were performed by NASA to sample air at high altitudes by sending balloon and rocket borne payloads. The experiments claimed to have found living cells at heights upto around 70 km. In 1979 S.V. Lysenko in the USSR also reported finding fungi and bacteria at heights 48-77 km by using a rocket-borne payload. However, the biological protocol in these set ups was not such as to guarantee that there had been no contamination in the air samples examined. Therefore, the conclusions were largely ignored. Nor were there any follow-up experiments to resolve any doubts on this count.

After the Hoyle-Wickramasinghe hypothesis was proposed, it was felt by some of us in India that a balloon-borne payload can be sent up with sufficient biological controls, so as to achieve credible results. The Indian Space Research Organization (ISRO) had developed in the course of its atmospheric studies, sufficient

expertise to collect air samples at balloon-accessible altitudes under sterilized conditions so as to analyze them for chemical composition. So in principle it was possible to conduct such an experiment.

In practice, there was need to have a collaborative effort to which several different institutions could contribute. The experiment that was proposed to ISRO under my leadership therefore included participation from institutions in India and the UK. Mr P. Rajaratnam from ISRO, Dr S. Ramadurai from the Tata Institute of Fundamental Research (TIFR), Dr S. Shivaji and Dr G.S.N. Reddy from the Centre for Cellular and Molecular Biology (CCMB) and Dr P.M. Bhargava from Anveshna, all from India, and Professor Chandra Wickramasinghe from the Centre for Astrobiology (CAB), Professor David Lloyd and Ms Melanie Harris from the School of Pure and Applied Biology, all from Cardiff University and Dr Milton Wainwright from Department of Biology and Biotechnology of Sheffield University in the UK participated in the effort.

After brainstorming meetings the infrastructure of the payload was decided and it was mostly fabricated in the ISRO labs. The balloon itself was provided by TIFR and was flown from its Balloon Facility in Hyderabad on January 21, 2001.

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, were 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

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

The samples so collected were then passed on for analysis to CCMB in India and to CAB in Cardiff, there being duplicates of probes at each of four selected height ranges: 19-20 km, 24-28 km, 29-39 km and 39-41 km approximately. Under aseptic conditions the air in each probe was passed through two filters of sizes 0.45 and 0.22 micron in succession. These filters were then searched for living matter.

Biological results

Without going into technical details, it may be stated that the biology groups in Cardiff and Sheffield found positive evidence



Halley's comet with its long tail. Do such cometary tails carry bacteria?

sterilized 100m - long rope. As a further precaution against the possibility of collecting any traces of outgassed material from the balloon surface, a sterilized intake tube 2m long formed an integral part of the cryo-sampler ensemble.

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

for living matter even at the heights of 41 km. David Lloyd and Melanie Harris in Cardiff found that when the filters were coated with a special cationic dye and subjected to ultra-violet light, they showed certain spots undergoing fluorescence. This is a standard technique for biologists to identify viable (living) cells. Similarly, coating with certain anionic dyes leads to the discovery of dead cells. The former were of interest to the experiment and their existence could be confirmed by observations through an

epifluorescence microscope. The numbers of such 'shining spots' were fewer in samples at higher altitudes than in samples at lower ones. This is entirely consistent with the hypothesis that there is a shower of living matter from outside and these microorganisms are steadily sinking to lower altitudes because of the Earth's gravity.

Another confirmatory evidence came from the biology lab at Sheffield, where Milton Wainwright succeeded in (serendipitously) detecting and growing bacteria on some of the filters. He discovered that the normal culturing media did not succeed in culturing the bacteria he had found, but a medium known as the potato-dextrose agar did succeed in growing them. He found that the microorganisms grown were of the following variety:

- a) The coccus (spherical in shape growing in clumps) 99.8% similar, as determined by the 16S RNA analysis, to *Staphylococcus pasteurii*
- b) Rodlike bacillus 100% similar as determined by the 16S RNA analysis to *Bacillus simplex* and
- c) A fungus identified as *Engyodontium albus* (Limber) de Hoog.

None of the above are common contaminants, nor had they been

used in the labs where this work was done. Furthermore, 'control filters' that had not been exposed to stratospheric air but were otherwise treated in the same way did not reveal these microorganisms on them. Although a very large fraction of terrestrial bacteria are of the non culturable type, those few that are culturable naturally are in far greater abundance and thus are likely to be picked up as a contaminant. The fact that the bacteria found are not culturable therefore lends further credibility to the conclusion that they are indeed from the stratospheric air.

Concluding remarks

These findings certainly make one take the panspermia hypothesis more seriously than has hitherto been done. Even granting that the cells and microorganisms are from the stratospheric air at 41 kilometres, the following doubts can be raised regarding our findings. Firstly, could these microorganisms have been lifted from the Earth? Atmospheric disturbances, upward air-currents, storms etc do churn layers of air above heights not accessible to aircrafts. There are occasional volcanic eruptions that blow matter high into space. Could we have picked up living matter from such events? The answer is that had the matter been collected from below 25 km, this

might have been the case, but there is no evidence to date of terrestrial matter being lifted to greater heights than this. Even volcanic ash stays at this height only for a few weeks and then sinks down to ground level. It may be mentioned that there had been no such eruption at least two years prior to the date of the experiment (21 January 2001).

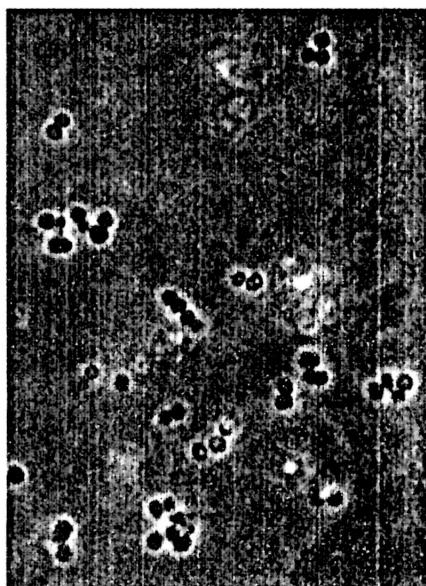
What about debris from man-made spacecrafts that litters the atmosphere? When one checks with the amount estimated to be lying around (there are such estimates available from space agencies), one finds that the chance of our picking up such material on a random sampling basis is extremely small.

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

So, we feel confident that what has been detected is from a height of 41 kilometres and it could not have got there from below. Did it get there from a comet? This may be further checked by doing an isotopic analysis of the samples to see if the abundances of various isotopes are different from those on the Earth and closer to what is found in a comet. There is of course need to carry out further experiments with greater efficiency in which specific questions can be addressed.

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

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Types of microorganisms (a) and (b) found in the analysis conducted at Sheffield, U.K.