2018

New insights into the "manna from heaven" hypothesis

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NEW INSIGHTS INTO THE "MANNA FROM HEAVEN" HYPOTHESIS

A Thesis Submitted

in Partial Fulfillment

of the Requirements for the Designation

University Honors

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May 2018

This Study by Clare Laubenthal

Entitled “New Insights into the ‘Manna from Heaven’ Hypothesis”

has been approved as meeting the thesis or project requirement for the Designation University Honors with Distinction or University Honors (select appropriate designation)

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30 April 2018 Dr. Jessica Moon, Director, University Honors Program

ACKNOWLEDGEMENTS

- Dr. Joshua Sebree
- Dr. Marek Sliwinski
- Drs. Sarah Horst and Chao He, John Hopkins University
- University of Northern Iowa College of Humanities, Arts, and Sciences
- Iowa Space Grant Consortium
- This material is based upon work supported by NASA under Award No. NNX16AL88H, administered by the Iowa Space Grant Consortium

Abstract

Titan's atmosphere is unique in that its atmospheric chemistry is thought to be similar to that of the Earth's atmosphere during the Archean Era, when bacterial life dominated the Earth[1]. Tholins form as a result of the radiation of organic gases by ultraviolet light. These molecules are considered alike to the prebiotic hazes that formed in the atmosphere of Earth during the Archean Era and which allowed for the eventual development of microbial life[2]. This study investigated whether Earth soil bacteria can grow, using laboratory created tholin analogs, or aerosols, as a nutrient source. This study is based on a microbial metabolism study performed by Carl Sagan[3], which detailed bacterial growth using Titan-analog aerosols as the sole carbon source. We built upon this study, using aerosol analogs that more closely match those of Titan coupled with modern day detection techniques and basic microbiology evaluation techniques. We have found no conclusive results for or against the findings of the original study.

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Introduction

Bacteria, the earliest known form of life, were known to have developed during the late Hadean or Early Archean Eon. The simplest type of bacteria is photosynthetic, in that they receive metabolic energy by processing sunlight. The Archean Eon's atmosphere was theorized to consist of a dense haze layer, which would have blocked sunlight, most likely preventing bacterial photosynthesis altogether. Therefore, the question arises: what did microbial life in the Archean Eon consume? The "Manna from Heaven" hypothesis proposes that tholins, consisting of complex organic molecules, could have been a viable food source for early bacteria.

The purpose of this thesis is to evaluate tholin as a possible nutrient source for bacterial life. I have modeled the methodology on a previous study, *Microbial Metabolism of Tholin,* published in 1990 by C.R. Stoker and C. Sagan.

This study was begun with the anticipation of similar results of the model study, and to confirm their findings. These results will emphasize the importance of sterilization of space craft and the validity of the "Manna from Heaven" hypothesis. Since this study will update the model study using current methodology and scientific knowledge, the results will also evaluate the study's results in regards to the advances made in astrochemistry since the study's publication. Finally, this study will add to collective knowledge of the astrochemistry field of study.

Literature Review

Tholins and their Formation

Tholins are defined as a class of molecule that is created by solar radiation in the upper atmospheric layers of some celestial bodies. The term tholin was coined by Sagan and Khare[4] in 1979 to describe the results of their astrochemical experiment. From the Greek *tholos*, meaning both "muddy" and “dome,” tholins are usually a tar-like substance consisting of complex organic molecules.

Shown in Figure 1, tholins are produced by the ultraviolet irradiation of simple gaseous molecules, primarily nitrogen and methane as well as other trace gases[5]. The energy provided by

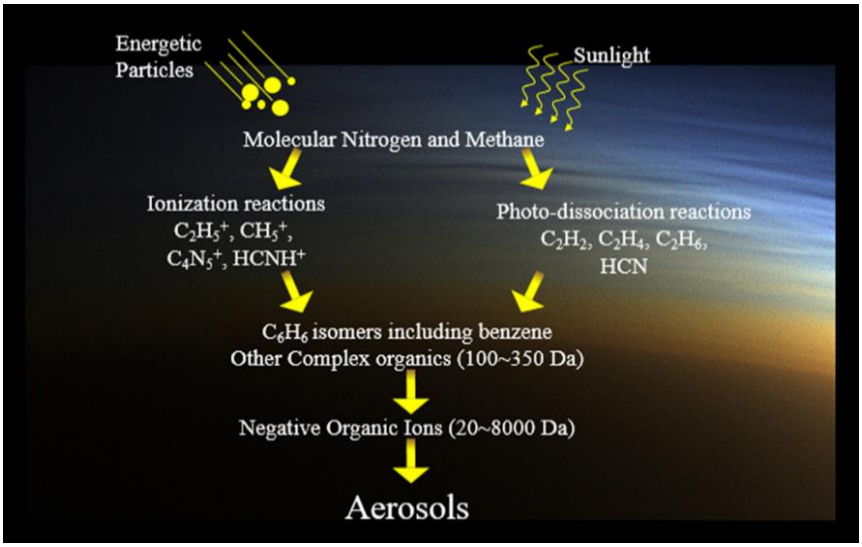

Figure 1: Simplified diagram of tholin formation[5]

the Sun’s ultraviolet light starts a series of chemical reactions that eventually results in large, complex molecules. In order for these reactions to take place, the gaseous compounds must be densely packed together. Therefore, such a process requires a dense haze layer in the upper atmosphere, which is distinguishable by a blue “halo.”

The Archean Eon

In the Archean Eon, approximately 4,000 to 2,500 million years ago, the Earth's atmosphere had the necessary conditions for tholin formation[4,6,7]. The atmosphere is known to have contained two to three times the modern level of nitrogen[7], as well as high levels of carbon monoxide, carbon dioxide, and methane[8]. The tholin formation would have taken place in an upper atmospheric level, and after formation, the heavier tholins were thought to have precipitated out of the atmosphere and fallen to the Earth's surface.

Planetary Analogs

However, without knowing the exact composition of the Earth's atmosphere at that time, the scientific community has turned to celestial bodies to fill in the gaps of information. Only two bodies in our solar system are known to have these conditions. Titan, the largest moon of Saturn, has an atmosphere composed primarily of nitrogen and methane[9,10] with trace organic compounds and a dense haze layer[11,12]. In addition, Titan is the only object in space, other than Earth, that is known to have stable liquid bodies. Titan has liquid methane lakes, as well as an active methane hydrological cycle[14], very similar to the water cycle present on Earth during the period of interest[14]. For these reasons, Titan's atmosphere is very similar to the Archean atmosphere[7] and is the subject of intense and continuous study.

As a result of the exciting New Horizons flyby in 2015, it was discovered that Pluto has an atmosphere mainly consisting of methane and nitrogen, as well as traces of other gases[16]. The upper atmosphere has the thick haze layer, shown in figure 3, necessary for tholin formation, as well as indications of tholins present on the surface of the planet[16,17]. Since these discoveries, researchers have begun to expand their methods to include Pluto as an analog to early Earth.

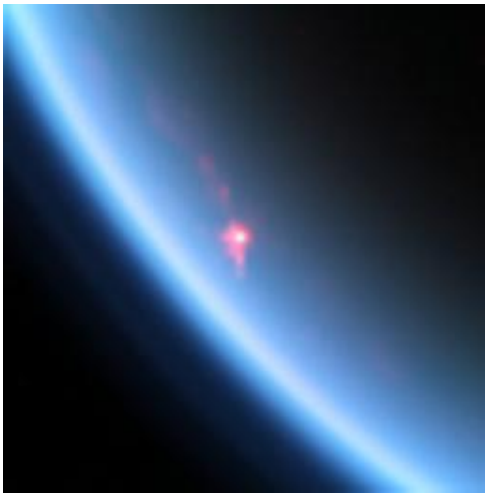

Figure 2: Titan's atmosphere has a distinctive blue halo, indicative of a dense haze layer[6]

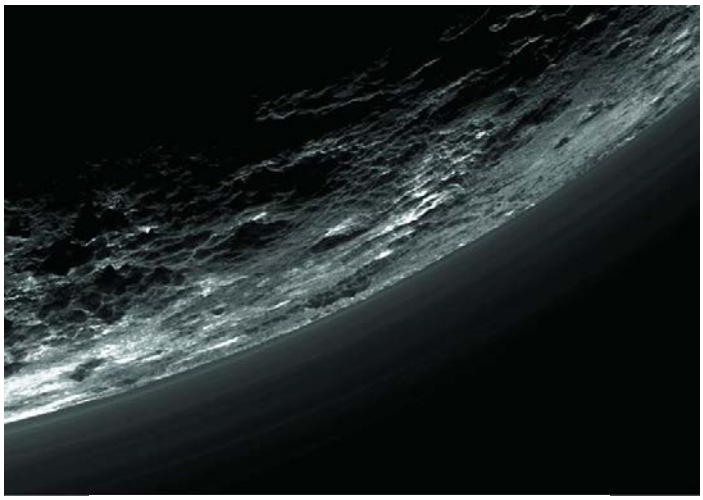

Figure 3: Pluto's atmosphere has several distinctive haze layers[14]

The "Manna from Heaven" Hypothesis

While how life first developed on Earth cannot be fully explained, single-celled life is thought to have developed during either the late Hadean Eon or the early Archean Eon[17]. However, during the Archean Eon, the atmosphere was very dense[4,7]. As a result, very little sunlight would have been able to reach the surface. Therefore, the first life forms on Earth would have been dependent on the surrounding prebiotic chemicals, which would have consisted of the precipitated tholins from the upper atmosphere. Thus, we have the "Manna from Heaven" hypothesis.

The "Manna from Heaven" hypothesis was first tested by C.R. Stoker in *Microbial Metabolism of Tholin*[3]. In this study, tholin analogs, or aerosols, were produced by introducing electricity to gas samples containing equimolar amounts of methane and ammonia, as well as 2.5% water vapor. Stoker extracted bacteria from a wide variety of environments, making sure to exclude any photoautotrophic organisms, and were successful in growing some samples on tholin media. Stoker were able to isolate aerobic, anaerobic, and facultatively anaerobic bacteria which were

able to use aerosols as a sole carbon source, including strains of Actinomyces, Clostridium, Pseudomonas, Bacillus, Acinetobacter, Paracoccus, and Alcaligenes[3].

In order to test the "Manna from Heaven" hypothesis, bacteria taken from soil will be inoculated using Titan and Pluto-analog aerosols as the sole nutrient source. The methodology was modeled after the Stoker study *Microbial Metabolism of Tholin*[3], published in 1990. Our study reevaluated the Stocker study using current knowledge of tholin formation and modern methodology.

Methodology:

Production of Aerosols

Man-made tholins, called aerosols, can be created in laboratories using an advanced version of the classic Urey-Miller experiment, shown in Figure 4. In the Urey-Miller experiment, energy, in the form of an electric spark via plasma coils, is applied to a mixture of gases and the resulting precipitate is collected. The University of Northern Iowa's Astrochemistry Laboratory has the experimental setup shown in Figure 5. This is essentially a larger, more complex version of the classic Urey-Miller experiment, which allows for reactions to occur at high pressure with little chance of contamination. The desired gases are pumped into the mixing chamber and allowed to homogenize over at least 4 hours. Then, the gas mixture is pumped into the reaction chamber by a Mass Flow Controller (Alicat, MC2SLPM), which allows for a constant, steady flow of gas. The homogenous mixture is then exposed to

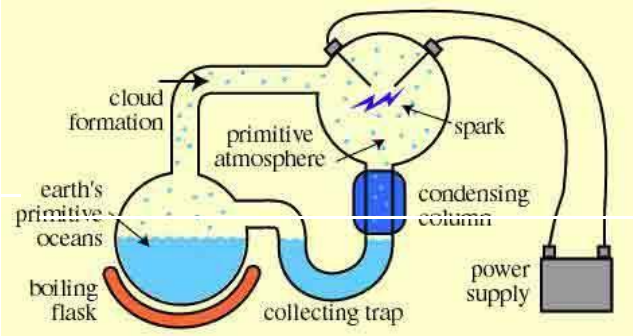

Figure 4: Basic Urey-Miller concept

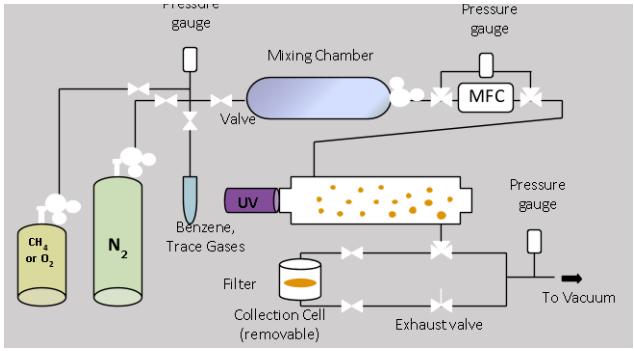

Figure 5: UNI's Astrochemistry Laboratory experimental setup

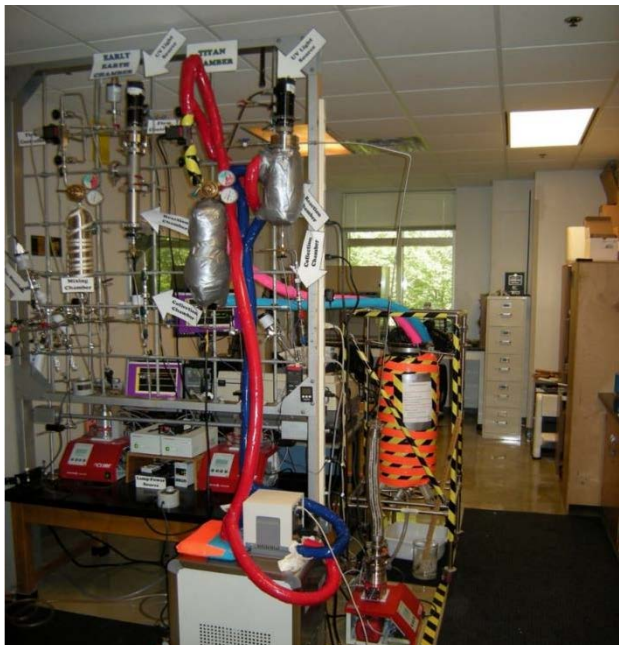

Figure 6: Astrochemistry Laboratory atmospheric chambers and collection system

ultraviolet radiation by a deuterium lamp (Hamamatsu, L11798) with a spectral distribution of 115 nm to 400 nm. This results in a more realistic analog to tholin formation, as the deuterium lamp is a closer analog to solar radiation than Tesla coils or plasma. The gases react and form heavier compounds, which are then precipitated out and collected. The entire setup is under vacuum pressure and closed off to outside contamination.

In addition, Sarah M. Hörst and Chao He at the Department of Earth Sciences at John Hopkins University are in collaboration with UNI's Astrochemistry Laboratory. Aerosol samples used in this study were taken from both institutions.

Creating Viable Aerosol Media

Our study was modeled on the Stoker paper from 1980, which described the creation of a solid aerosol medium, similar to agar plates, but using silica as the solidifying agent. However, as the preparation of this medium is an outdated procedure and multiple attempts saw no success in solidifying as described, the procedure was discarded in favor of a liquid suspension medium.

To determine the optimal concentration of aerosol in which to grow bacteria, a variety of concentrations were tested. A soil sample from the UNI Prairie Trail near the Biology Research Complex in Cedar Falls, Iowa was collected, weight approximately 0.25 mg, was mixed into 25 mL water until homogenous. The sample was then centrifuged at 500 rpm to separate soil and other debris from the bacterial cells.

Aerosol samples of varying composition were suspended in sterile water and diluted to 5 g/L, 1 g/L, and 0.1 g/L. These samples were inoculated by transferring 50 µL of supernatant from the soil sample to the aerosol samples. The inoculated samples were grown at 37 ^{o}C for 24 hours in a shaker and 50 µL were plated.

An aerosol concentration of 1 g/L was determined to be the best concentration for further testing, as it grew the most bacteria.

Collection of Soil Samples

Five soil samples, weighing approximately 0.25 mg, were collected from the UNI Prairie Trail near the Biology Research Complex in Cedar Falls, Iowa. These samples were each placed into 25 mL water until homogenous. After centrifuging at 500 rpm, five 50 µL samples of supernatant were plated out on LB-agarose plates, then grown for 24 hours at 37 ºC. Each bacterial species present in these results were transferred to LB-agarose slants, grown under the same conditions, and stored at 4 ºC.

Inoculating Aerosol Samples

A bacterial mixture was created from the stored bacterial samples. 25 mL of sterile water was inoculated via loop with all of the stored samples. The sample was vortexed and 50 uL of the sample was plated on a LB-agar plate. The plate was cultured at 37 ºC for approximately 24 hours and the colonies were counted. This was used to determine the initial colony forming units (CFUs) present when the aerosol samples were inoculated.

Aerosol samples were tested regularly for sterility. A 1 mL sample of 1 g/L aerosol was inoculated with 10 µL of the bacterial mixture. An equal volume sample of sterile water was inoculated identically for a negative control. A 1 mL sample of standard LB liquid medium was also inoculated identically for a positive control. These samples were grown in a shaker at 30 ºC for approximately 24 hours. 50 µL of the samples were plated on LB-agar plates and grown at 37 ºC for approximately 24 hours, then the CFUs were counted.

NMR Testing

The aerosol samples that were determined to have a high cell count were collected and centrifuged at 1000 rpm to pellet the cells. The cells were then diluted with sterile water and lysed with a Baasen Biosonik probe sonicator at 50% using six 10 second exposures separated by one-minute chills on ice. The same volume of the identical uninoculated aerosol medium was collected for comparison. The samples were dried using a vacuum pump and then diluted with Dimethyl sulfoxide (DMSO). The samples were analyzed using an Nuclear magnetic resonance (NMR) spectroscopy.

Results and Discussion

Preliminary Results

Samples	CFUs from tholin culture	CFUs from sterile water
A	TNTC	TNTC
B	TNTC	TNTC
C	TNTC	120
D	18	120
E	0	45
F	113	120
G	13	120
H	94	45
I	TNTC	45

Table 1: Bacterial growth comparisons, aerosol media vs sterile water

Bacterial growth counts are shown in Table 2. TNTC (too numerous to count) notes either too many colony forming units (CFUs) to count or a spreading bacterial growth. In our preliminary results, we were not able to determine any difference in bacterial growth between the negative control and the aerosol samples. The LB medium, not shown here due to too few samples completed, consistently had a higher bacterial count than either the negative control and the aerosol

samples. This suggests that the bacteria are not actually using aerosols as their nutrient source and are instead simply existing in solution, not multiplying, or are self-metabolizing.

These findings disagree with the original study, which stated that a wide variety of bacteria were able to metabolize aerosols as their sole nutrient source, including strict aerobes, strict anaerobes, and facultative anaerobes. However, since Stoker neglected to include a negative control, it is possible that the need for such a control was simply overlooked.

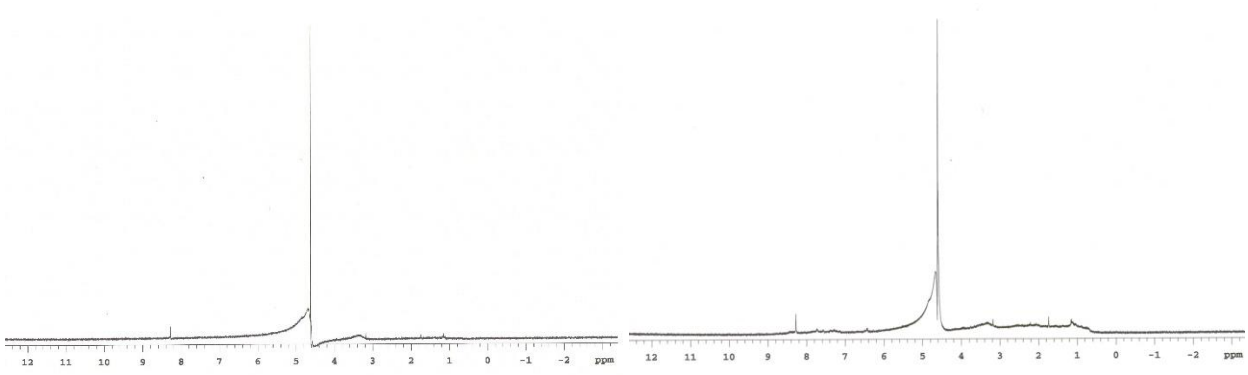

Figure 7: Preliminary NMR scans: lysed cells (left) and undigested aerosol (right)

In the initial scans shown in Figure 7, the large peaks at 4.8 ppm in each scan is due to the contamination of water in the sample. This error can be corrected in future work. These results simply show that the procedure for the NMR analysis of lysed cells is a success and can be used when continuing this project.

In the future, aerosol sample spiked with carbon-13, an isotope that is easily detected using NMR analysis, can be used as media. The NMR analysis can be used to determine definitely whether or not bacteria are metabolizing the aerosol sample.

Procedural Development

The model study, *Microbial Metabolism of Tholin* by C.R. Stoker, detailed a "weaning" process of bacteria from soil to aerosol samples. This involved a number of stepwise growth procedures where the concentration of soil of the growth medium decreased and the aerosol concentration increased. The purpose of this was to slowly encourage the bacteria that were able to digest aerosols to metabolize the aerosol instead of the soil. Due to the time constraints of our project, it was decided to skip this step. In hindsight, this decision could have resulted in the differences in results between this study and the model study. In addition, the silica plates used to solidify the aerosol medium would not work as described in the model study. When attempted, the plates either did not solidify at all or were too soggy to use as media. Eventually, this part of the procedure was dropped and the liquid media described in the procedure was adopted.

When determining the best aerosol concentration for bacterial growth, 4 g/L cultures regularly produced less growth than the cultures of a lesser concentration. In addition, the aerosol samples received are very small, averaging at 22.60 milligrams. Therefore, it was decided to exclude this concentration from the bacterial growth study.

Since the negative control was inoculation of sterile water, which was not included in the original Stoker paper, it was determined that the 0.1 g/L aerosol sample was too close to the negative control. Therefore, it was decided to exclude this concentration from the bacterial growth study. However, it is important to mention that bacterial growth was present in the 0.1 g/L aerosol concentration, as other laboratories that might want to replicate this study might not have access to as much aerosol.

While the model paper selected specific bacterial species that were thought to be well suited for growth in aerosol media, it was decided to extract a wide variety of bacterial species from local soil samples. As the samples used in our procedure were compositionally different from those in the model study, this was done to ensure growth of at least one species in the aerosol media.

Conclusion

This study has evaluated the "Manna from Heaven" hypothesis, which proposes that tholins, consisting of complex organic molecules, could have been a viable nutrient source for early bacteria. This study has been modeled on the methodology of a previous study, *Microbial Metabolism of Tholin,* published in 1990 by C.R. Stoker and C. Sagan. The anticipated results of this study were predicted to be similar to those of the model study and to confirm their findings, which were that many species of bacteria were able to grow using tholin analogs as a nutrient source.

These results will comment on the importance of sterilization of space craft and the validity of the "Manna from Heaven" hypothesis. Since this study will update the model study using current methodology and scientific knowledge, the results will also evaluate the study's results in regards to the advances made in astrochemistry since the study's publication.

The ability of bacteria to grow in tholin analogs was revaluated by comparing bacterial growth in aerosol culture to a negative and positive control. Our preliminary results show no significant difference between aerosol and sterile water bacteria counts. This could be attributed to three reasons. The first is the change in aerosol composition due to the advancement in the field of astrochemistry. As a community, we know much more about the process of tholin formation and are, therefore, able to make aerosols that are much closer analogs to tholin. Second, the procedure had to be modified according the project limitations. This modification could have significantly changed the results. Third, the model study did not include a negative control, which means that Stoker did not explore all explanations of bacterial growth.

However, it must be mentioned that this study's sample size is small and must be expanded on before declaring any conclusions with confidence. In addition, these results should not be applied to aerosols made by other methods, as different methods can change the aerosol composition.

If these results do remain with further experimentation, it could disprove the model study, *Microbial Metabolism of Tholin.* It could also call into question the "Manna from Heaven" hypothesis and suggest that an alternate nutrient source was consumed by the bacteria during the Archean Eon. In addition, these results builds upon the current knowledge base of and will assist other research in the astrochemistry field.

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