Dear Brig,

Thanks for the paper. I am now back from a wonderful Astrobiology Conference in Pushchino, Russia and I will now provide comments. Feel free to put this on-line if you wish. I stand behind every word, even though some may not be happy with what I have written. Prior discoveries should not be ignored.

Meteorite Contamination and "The Dog that Didn't Bark"

Richard B. Hoover, Moscow, Russia, June 13, 2016

In Sir Arthur Conan Doyle's story *Silver Blaze*, Sherlock Holmes solved the theft of a prize racehorse by concluding from the fact that the guard dog didn't bark, hence "the midnight visitor was someone the dog knew well." This absence of an expected fact ("negative fact") is typically ignored when considering the question of bio-contamination of carbonaceous meteorites. Since the early 1960's when Fitch and Anders argued that the "organized elements" found in the Orgueil and Ivuna meteorites were all the result of contamination by deformed grains of pollen. This universal contamination hypothesis was more recently advanced by Andy Steele, based on a fragment of the Murchison meteorite that I had received from the Field Museum (located in Chicago, Illinois and not Australia as he claims). The high nitrogen content (~8-10 % atomic) of these filaments and their behavior under the electron beam during Energy Dispersive X-ray Spectroscopy at 15 keV, led me to conclude that these clearly biological remains were modern fungal contaminants. Optical microscopy revealed pale blue filaments of unidentified fungi that had contaminated the exterior of the fusion crust and invaded old cracks in this fragment of stone. I communicated these discoveries to David McKay at NASA/JSC and he indicated Andy Steel would find this of interest and might be able to identify this fungal contaminant. At his request I sent a sample of this contaminated piece of Murchison to Dr. Steele with plans for a joint paper, in the event that he should able to successfully identify this fungal contaminant. To my great astonishment he did not identify the fungal filaments, but he did publish a paper indicating that he had discovered modern fungal contamination in a Murchison meteorite sample that I had provided to him. (http://www.lpi.usra.edu/meetings/LPSC99/pdf/1293.pdf)

This paper may have led readers to conclude the microfossils (discovered independently in separate samples of the Murchison and Ivuna carbonaceous meteorite by me and by Academician Alexei Yu. Rozanov at the Paleontological Institute of the Russian Academy of Sciences in Moscow) were merely modern biological contaminants. The suggestion by Dr. Steele that all meteorites are easily contaminated may have been intended to cast doubt on the validity of all studies of indigenous microfossils in carbonaceous meteorites. It has come to be widely accepted by many in the scientific community of the United States that:

1. Carbonaceous meteorites are heavily contaminated by modern terrestrial microorganisms.

2. Carbonaceous meteorites are primitive, pristine, proto-solar nebula condensates that contain a valid record of organics that formed over 4.5 billion years ago.

Viewed together, however, these two statements are in obviously contradiction. If carbonaceous meteorites are contaminated by terrestrial biology, then they are not pristine and can really provide no useful information about organics on either the meteorite parent body or the early solar system. Clearly, that it would be impossible to separate ancient organic molecules from those introduced as recent biological contaminants and none of the papers concerning meteorite organics have suggested any effort being made to perform this task. Furthermore, the meteoriticists who have published papers about amino acids, nucleobases, fatty acids, sugar acids or any other organics in carbonaceous meteorites have never suggested that any of their samples are contaminated by modern biology, as it would mean the results of their careful research would have absolutely no validity.

But meteoriticists have uncovered many "negative facts" or "Barking Dogs" that they have failed to recognize or acknowledge. These negative facts clearly establish that even though the meteorite fusion crust and old cracks could be contaminated and should be avoided, the interior regimes of these stones are not contaminated by modern terrestrial biology.

1. <u>Only 8 of the 20 life-critical protein Amino Acids are present in carbonaceous meteorites</u>. The enigma of the missing amino acids as <u>Mike Engel and Stephen Macko pointed out</u> in 2003 constitutes a "barking dog" that shows the Murchison meteorite is not heavily contaminated by modern microbes as Steele claimed.

All living organisms contain all 20 protein amino acids and all 23 proteinogenic amino acids. It is impossible to contaminate a meteorite with fungi or bacteria without also introducing all of these life-critical biomolecules.

In 1982, they discovered that the chiral amino acids in the Murchison CM2 carbonaceous meteorites exhibited an enantiomeric excess (Engel, M. H. and Nagy, B, "Distribution and Enantiomeric Composition of Amino Acids in the Murchison Meteorite." Nature **1982**, 296, 837-840.) Several meteoriticists had quickly dismissed this result and argued that this finding had to be due to terrestrial bio-contamination. However, in 1997, Engel and Macko demonstrated that the individual amino acid enantiomers in Murchison are enriched in the stable isotope ¹⁵N relative to their terrestrial counterparts (Engel, M. H. and Macko, S. A. "Isotopic Evidence for Extraterrestrial Non-Racemic Amino Acids in the Murchison Meteorite." Nature **1997** 265-268.)

This result proved that these amino acids were indeed extraterrestrial invalidating the contamination hypothesis.

It is also well known that over long time periods, homochiral amino acids begin to become racemic after the death of terrestrial organisms---giving rise to the field of amino acid geochronometry. Furthermore, many of the same (more unstable) amino acids that are absent in the carbonaceous meteorites are also missing from the bones of Hadrosaurs and other ancient fossils. This suggests that the microfossils Hoover, Rozanov and others have found in Murchison could be the remains of ancient cyanobacteria and other microorganisms that inhabited the

Murchison parent body with partial racemization loss of a subset of their protein amino acids taking place over a time span of several millions of years.

2. Only 3 of the 5 life-critical nucleobases are present in Carbonaceous meteorites (Cytosine and Thymine and missing).

3. Life-critical sugars Ribose and Deoxyribose and not found in Carbonaceous Meteorites.

The missing sugars and nucleobases provide additional proof that these stones are not contaminated by modern bacteria----as it would be impossible to have bacteria that contain no DNA. These sugars are present in every living organism on Earth but they <u>have never been</u> <u>detected in meteorites</u>.

In their recent paper "Enantiomer Excesses of Rare and Common Sugar Derivatives in Carbonaceous Meteorites" (PNAS, Early Edition, June ??, 2016) it is very unfortunate that Cooper and Rios make some misleading claims that misrepresent important well-established fact. The second paragraph states *"To date, most chiral meteoritic compounds are reported to be racemic mixtures, i.e. their D and L enantiomers are equal in abundance (3)."*

Many other researchers (Burton, Callahan, Cronin, Glavin, Kvenvolden, Martins, Pizzaello and others) produced data confirming the validity of the initial reports of Engel, Nagy and Macko. It is a mystery why Cooper and Rios failed to reference their pioneering papers about the enantiomeric excess and the missing protein amino acids in the Murchison. It is now well established that many different groups (CI, CM CB, and CH) of carbonaceous meteorites contain extraterrestrial amino acids, nucleobases and that many of these life-critical biomolecules exhibit an enantiomeric excess of *L*-amino acids and *D*-sugar derivatives.

Cooper and Rios used extremely sensitive GC-MS methods to measure enantiomeric excesses in common and rare sugar acids in order to show that their Murchison sample has not been contaminated by modern terrestrial bacteria. However, they fail to note that they did not find ribose of deoxyribose which should have been present if the meteorite was contaminated. During the past two decades of research, I have identified modern terrestrial biological contaminants on the fusion crust and in old cracks within only one Murchison sample, which I then provided to Andy Steele. I have never observed any recognizable biological contaminants in freshly fractured interior surfaces of any carbonaceous meteorite I have studied. This conclusion is based on EDS measurements showing Nitrogen content of possible biological remains being wellbelow 0.5% instrument detection limit, which is well below the value of 2-20% atomic Nitrogen content of all living and recently dead organisms This paper fails to give credit where credit is due. The authors also ignores the absence of facts that provide evidence that conclusively rules out the possibility that the interior surfaces of these carbonaceous meteorites are contaminated by modern terrestrial biology. If they or anyone else can explain how a meteorite could be contaminated with modern bacteria and not also contain all 20 protein amino acids, DNA/RNA nucleobases and sugars, ATP, NADH, pigments, etc., I will be eager to listen.