

# Examination of "Organized Elements" from the Orgueil Meteorite by Quantitative Fluorescence Microscopy

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The organized elements of the Orgueil Meteorite were examined by quantitative fluorescence microscopy. An effort was made to correlate possible morphology with the presence of the amino acid, tyrosine. Relative tyrosine levels were determined by the formation of fluorescent complexes between the tyrosine present and 1-NITROSO O 2-NAPHTHOL. The fluorescence levels produced by the tyrosine-nitroso naphthol complexes from lab cultures of microorganisms, microorganisms from peat, and the organized elements of meteorites were compared and used as an index of relative tyrosine content.

**T**HE QUESTION of extraterrestrial life has long intrigued man. Speculation upon this matter has so advanced that many indirect demonstrations of extraterrestrial life have been attempted, as also a few questioned direct demonstrations.

The indirect demonstrations are mainly based upon astronomical studies of the planet Mars, and can be set forth as follows: (A) There is speculation that the postulated Earth's primitive reducing environment ( $H_2O$ ,  $CH_4$ ,  $NH_3$ , etc.) Oparin,<sup>1</sup> Haldane<sup>2</sup> and Sagan<sup>3</sup> was similar to that of Mars. Thus, possible Martian life forms could have arisen in a manner similar to that of Earth's and followed similar evolutionary pathways up to a certain point. Since the Martian mass is less than that of Earth, the reducing environment could have drifted away at an earlier time than Earth's and was replaced by an environment different than that of Earth's. As a result Martian life forms could have arisen in a similar manner to that of Earth's, but then followed different developmental paths. (B) Dollfus,<sup>4</sup> Salisbury<sup>5</sup> and Dollfus<sup>6</sup> have attributed seasonal changes of color, delineation and polarization in the Martian dark areas to the growth of unicellular organisms. (C) Spectroscopic observations have also been sighted as possible indications of life on Mars. Sinton<sup>7,8</sup> and Colthrup<sup>9</sup> have suggested that the absorption in the infrared spectrum from the 3.4 to the 3.7 micron range is due to the presence of CH or CHO groups from plant-like material. This interpretation has recently been questioned. Kuiper<sup>10</sup> suggests that the absorption is due to plant-like material similar to Earth-type lichens, while Tikhov<sup>11</sup> suggests it is due to alpine-like plants.

These demonstrations are indirect and are obviously open to interpretation.

Direct demonstration of extraterrestrial life has been attempted by the examination of carbonaceous chondrite meteorites for organic matter, biologically crucial molecules, and possible life forms.

Organic matter was shown to be present in the carbonaceous chondrite meteorites by the early chemical investigators as Berzelius in 1834, Berthelot in 1869 and Wohler in 1858. These results were questioned at the time due to technical limitations and the problem of Earth source contamination. However, with the development of more sophisticated instrumentation and techniques, results have been obtained which indicate that the organic matter is definitely a component of the carbonaceous chondrite meteorite composition, and not Earth contamination.

Hydrocarbons, Nagy, et al.,<sup>12</sup> and Meinschein;<sup>13</sup> possible purine and pyrimidine bases, Briggs<sup>14</sup> and Calvin;<sup>15</sup> amino acids, amino sugars and sugars, Degens and Bajar,<sup>16</sup> Kaplan, Degens and Reuten<sup>17</sup> and possible porphyrins, Hodgson and Baker<sup>18</sup> have been found in extracts of the samples. The demonstrations of hydrocarbons, amino acids, amino sugars could be evidence for the abiogenic origin of the organic matter, while the possible additional presences of purines, pyrimidines and porphyrins could be evidence for a biogenic origin.

Claus and Nagy,<sup>19</sup> Fitch, et al.,<sup>20</sup> Briggs and Kitto<sup>21</sup> and Nagy, et al.,<sup>22</sup> have shown structures, "organized elements" in carbonaceous chondrites. They have suggested that the organized elements might be fossils of microorganisms or other types of structures, microspheres (droplets) abiogenically formed. These structures vary from approximately 1 to 25 microns in diameter, have symmetry, spines or horn-like structures, double walls, pores, furrows and demonstrate other possible morphological structures. Of course, when examining and interpreting specimens for possible morphology one is making a subjective evaluation, which can be questioned as to validity. In this study only "organized elements" which resemble microorganisms were examined.

This study attempts to correlate possible morphology with chemical composition, that is the presence of molecules diagnostic or suggestive of life forms. For, if structures which appear to have morphological characteristics of microorganisms, bacteria, fungi, etc. are shown to contain such compounds, proteins, amino acids, lipids, porphyrins, nucleic acids (nucleotides,

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nucleosides, purines, pyrimidines) or sugars, etc., one would have a further bit of evidence as to the nature of the "organized elements." That is whether the organized elements are fossils of microorganisms or abiogenically formed structures.

To attain this purpose, quantitative fluorescence microscopy, Hovnanian, Brennan and Botan,<sup>23</sup> and Botan and Hovnanian<sup>24</sup> was utilized. By means of this technique relative levels of concentration for fluorescent diagnostic complexes were determined.

Preliminary results are presented which assay the organized elements for the presence of the amino acid, tyrosine.

## MATERIALS AND METHODS

### *Meteorite Sample*

A sample of the Orgueil Meteorite was obtained from the Museum of Science, Boston, Massachusetts, through the courtesy of Mr. John Patterson, Director of the Charles Hayden Planetarium. The sample consisted of approximately 900 mgs of the meteorite in a small tightly stoppered glass vial. The vial has been known to be unopened for the last 26 years. The previous history of the specimen is not clear, but it was known to be a part of the Jackson Meteorite collection, specimen number 68 which was donated to the museum with the entire Jackson collection. The specimen consists of small friable grey-black particles which vary in size from approximately ½ mm to 5 mm in diameter.

### *Reagent and Glassware Preparation*

(a) Glassware used in the study was soaked in detergent solution, rinsed, immersed over night in cleaning solution (chromic acid) and then rinsed with distilled water which was membrane filter sterilized.

(b) The reagents used in the study were prepared with sterile distilled water and were then sterilized by membrane filtration.

### *Bacterial, Fungal, Yeast and Lichen Smear Preparations*

(a) Cultures of *B. globigii*, Baker's yeast and *Neurospora crassa* were grown on their respective media for 1-7 days at room temperature.

(b) A loop of the growth was suspended in sterile distilled water, smeared, on sterile chemically clean glass slides, allowed to air dry, and heat fixed. A lichen preparation was made by scrapping samples off of tree bark and suspending them in sterile distilled water, then suspension was treated as previously described.

(c) The smears were divided into two groups, one a control group and the other group was stained for tyrosine. The smears were kept in petri dishes until ready for further manipulations.

(d) The controls were sealed with coverslips which were attached with "Duco" cement. The other slides, after staining were also sealed in the same manner.

(e) The sealed slides were then examined with the Leitz fluorescence microscope (to determine color of their fluorescence auto and induced). The Avco quantitative fluorescence microscope was then used to determine the level of their fluorescence.

### *Peat Smear Preparations*

(a) Samples of interglacial peat dated between 38,000 and 100,000 years old were kindly supplied by Dr. E. Barghorn of Harvard University.

(b) Core samples of the interior of the sample were obtained using a sterile borer. An interior part of the core was cut off with a sterile chemically clean knife. Approximately 30 mgs were ground in a sterile chemically clean mortar and then suspended in sterile distilled water. The suspension was then smeared, air dried and heat fixed. All the manipulations were carried out in a rigid type glove box.

(c) The smears were temporarily protected with coverslips and examined for fluorescence of the mineral matter and biological structures (cells of bacteria, fungi, plants, etc.) with a Leitz fluorescence microscope. A tungsten (visible) and a U.V. (Osram 200 W) light source was used for the examination. The rationale for this procedure was to locate areas of interest (biological structures) and determine the color of the autofluorescence. This was necessary in order to distinguish between the autofluorescence of the mineral matter and the biological structures and the induced fluorescence which would be produced after staining. The induced fluorescence is caused by the formation of a fluorescent complex (a condensation reaction between the staining reagent and tyrosine).

(d) After the Leitz microscope examination, the areas of the slide not protected by the coverslip were cut off and the smear replaced in the glove box. The coverslip was removed, and the smear stained for tyrosine. After the staining procedure the coverslips were cemented on with Duco cement and the specimen was examined with the Avco quantitative fluorescence microscope.

### *Meteorite Sample Smear Preparation*

(a) 30 mg. samples of the Orgueil Meteorite were ground and treated as described in the Peat Smear Preparation section.

(b) The staining and microscopic observation of the meteorite smears was carried out in the same manner as described in the Peat Smear Preparation.

(c) The "organized elements" and the mineral matter of the meteorite sample were handled in the same manner as the biological structures were treated in the Peat Smear Preparation.

(d) Samples of the minerals, Olivine, Amphibole and Magnetite were ground in a mortar and handled as described in the Peat Smear Preparation. The minerals were used as controls to see if they were receptive to the tyrosine staining procedure.

### *Mitotic Figure Preparations*

(a) Mitotic figure preparations were obtained from onion root tips, squashes, and examined in an unstained and stained state (tyrosine stain).

### *Photography*

(a) Photographs of the specimens were taken from the T.V. screen using a Polaroid camera with type 47 Polaroid film.

### *Tyrosine Staining Procedures*

(a) The tyrosine fluorescence staining solution is a modification of the procedure developed by Unden-

friend and Cooper<sup>25</sup> and Waalkes and Udenfriend.<sup>26</sup>

(b) Solutions A and B were prepared. Solution A was a 0.1 per cent 1 nitroso 2-naphthol in 95 per cent ETOH. Solution B was 127.5 mls of a 1 to 5 (v/v) nitric acid plus 2.5 mls of a 2.5 per cent aqueous NaNO<sub>2</sub>.

(c) Smears were immersed into a mixture of equal parts of Solution A and B and incubated at 55°C for 30 minutes.

(d) At the termination of the incubation period the staining mixture was poured off and the slides were cooled to room temperature.

(e) After cooling the smears were washed in ethylene dichloride twice (5 minutes each wash). The unreacted nitroso naphthol was removed as were such other possible compounds as p-hydroxyphenylacetic acid, P-hydroxyphenylpyruvic acid, etc. which might condense with the nitroso naphthol, Udenfriend.<sup>27</sup> The smears were then air dried and sealed with a coverslip and examined with u.v. light at 365 m $\mu$ .

#### Microscope Manipulations and Standardization

(a) The primary filter of the quantitative fluorescence microscope system, Hovnanian, Brennan and Botan<sup>23</sup> was removed and the monochromator set at 450 m $\mu$ . The condenser was adjusted as was the alignment of the microscope and the photomultiplier probe to obtain a light output reading, 65,000 millimicrolumens.

(b) Each time a smear was examined the light output was checked to be sure that the level of the light (65,000 m $\mu$  lumens) exciting the smear was the same for each set of determinations.

(c) The magnification factor of the quantitative fluorescence microscope used in these studies was approximately 3700 X.

## RESULTS

Fluorescence, both auto and induced was found in the meteorite, peat, bacterial, fungal, yeast and algal smears.

The autofluorescence seen was white, blue, pink, red, and yellow. The induced fluorescence (due to the formation of fluorescent complexes) was yellow-green. The fluorescence peak for the tyrosine fluorescent complex was in the 570 m $\mu$  waveband. Autofluorescence of the specimens examined was not seen in the yellow-green of the 570 m $\mu$  waveband.

The following figures were representative photographs of the results obtained using the Avco quantitative fluorescence microscope when examining stained and unstained specimens.

Figure 1, *B. globigii* smears from lab cultures, unstained gave a fluorescence reading of 70 units, while the stained smears read 350 units.

Figure 2, Baker's yeast (*Sacchromyces cervisiae*) from a mixed lab preparation unstained read 130 units, while the stained read 550 units.

Figure 3, *Neurospora crassa* from a lab culture read unstained 30 units and stained 120 units.

Figures 4 and 5 was a Lichen genus and species un-

known. The unstained algae fluoresced 220 units, the hyphae 120 units. The stained algae fluoresced 250 units, while the hyphae fluoresced 150 units.

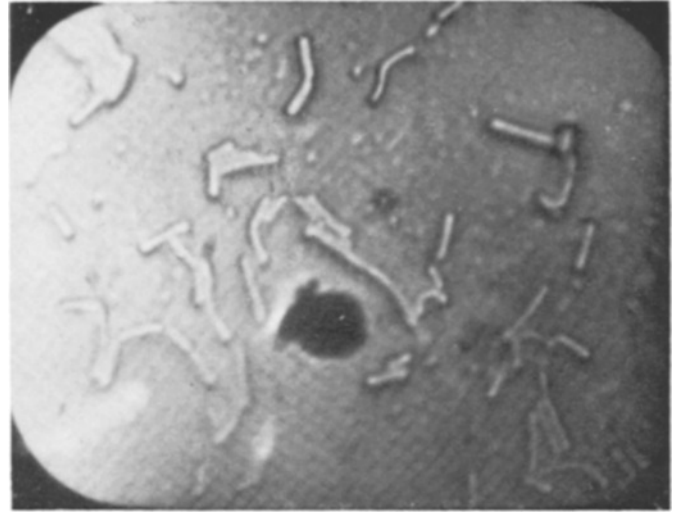


Fig. 1. *B. globigii* smear, lab culture.

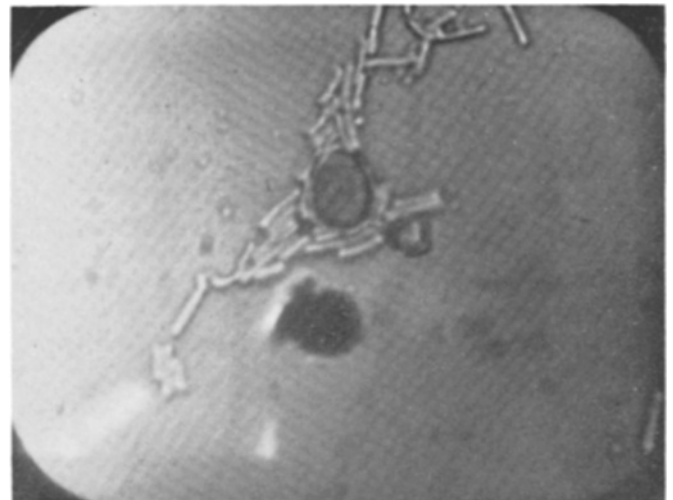


Fig. 2. Baker's yeast, mixed lab culture.

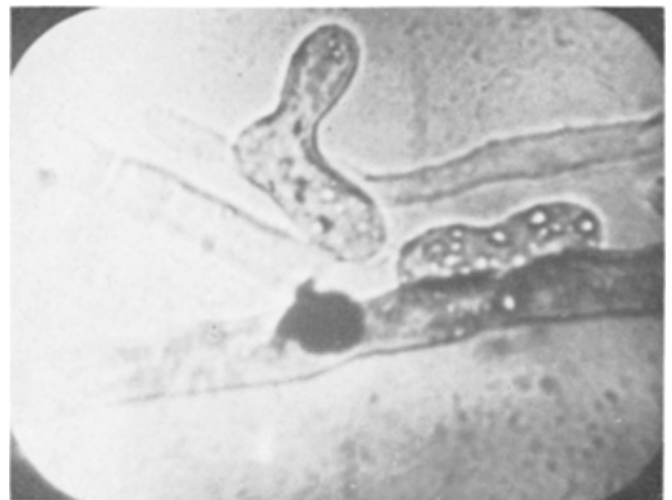


Fig. 3. *Neurospora crassa*, lab culture.

Figures 6 and 7 were specimens from peat. Figure 6 was bacterial-like structures that fluoresced stained 20 units. Figure 7 was plant material stained read 30, units. Both Figures 6 and 7 unstained did not fluoresce.



Fig. 4. Lichen.



Fig. 5. Lichen.

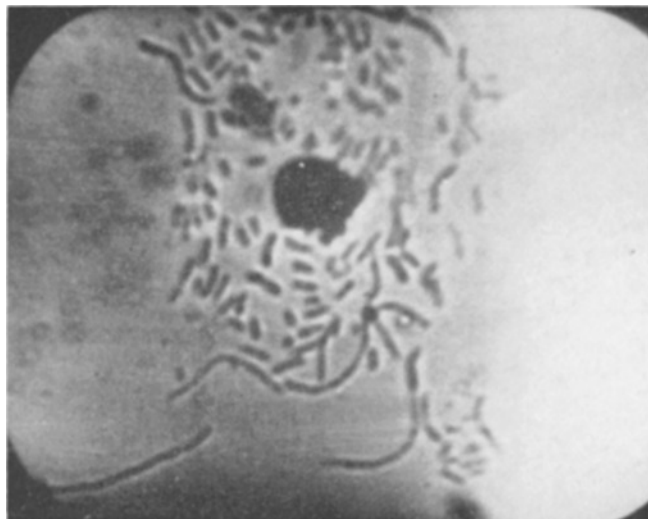


Fig. 6. Bacterial-like structures from peat.

Figures 8 and 9 were specimens from the meteorite material. Figure 8 was a fungal-like structure, had no measurable fluorescence unstained. Figure 9 was a yeast-like structure with no measurable fluorescence unstained. Both figures fluoresced 20 units when stained.

Figures 10, 11, 12 were taken at three depths of focus. The specimens were from the meteorite material. The structures were spore like and appear to have spines, figure 10 bottom focus, 11 middle focus and 12 top focus. The stained fluorescence level was 33 units, while the unstained was not fluorescent.

Figure 13 were granules found in the meteorite material, unstained fluorescence (yellow) varies from 1-5 units, depending on the size of the spheres, also included is an "organized element," which is yeast like. Figure 14 was a sphere of unstained amorphous sulfur which fluoresced from 1 to 10 units, depending on the size of the spheres.

Figures 15a, 15b, 15c, 15d were taken at four depths of focus of the same specimen. Figure 15e represents an increase in magnification from approximately 3700 X to 5000 X of the same specimen. The unstained speci-

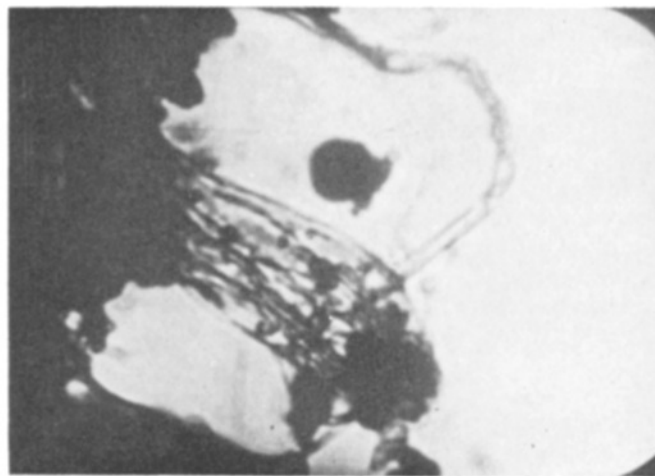


Fig. 7. Plant material from peat.

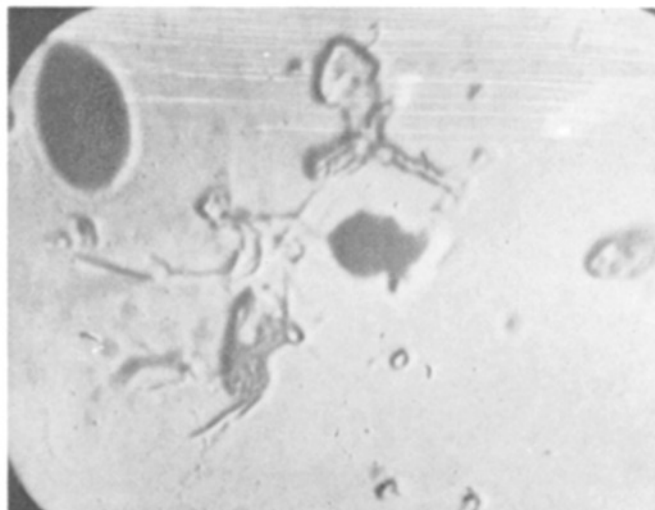


Fig. 8. Fungal-like structures from meteorite.

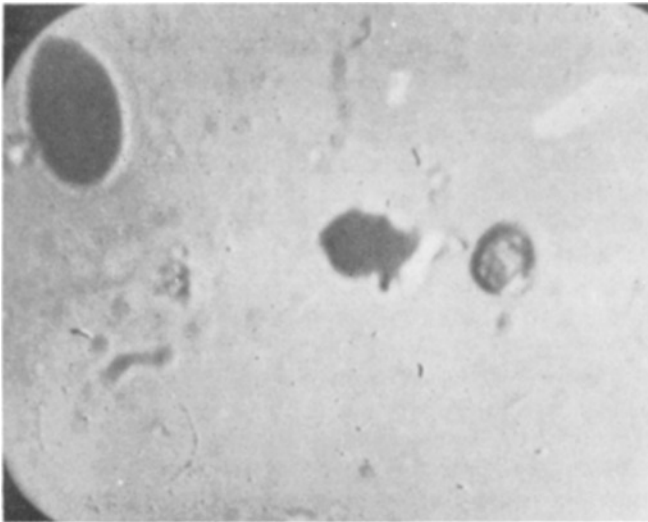


Fig. 9. Yeast-like structure from meteorite.

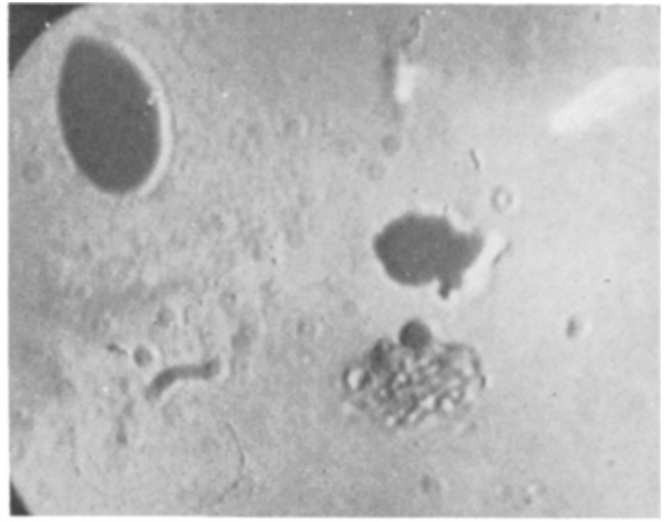


Fig. 12. Spore-like structure from meteorite, bottom focus.

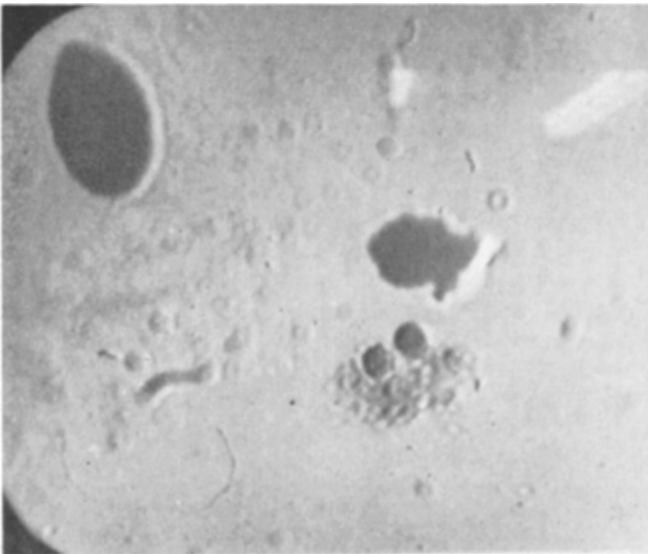


Fig. 10. Spore-like structure from meteorite, top focus.

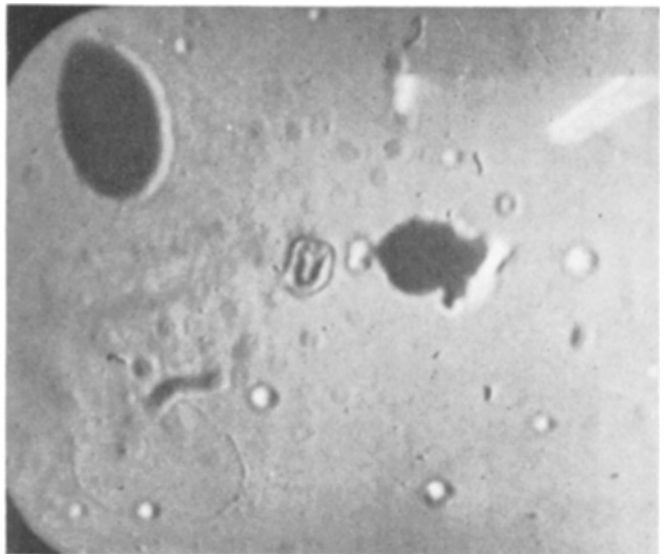


Fig. 13. Granules and yeast-like structure from meteorite.

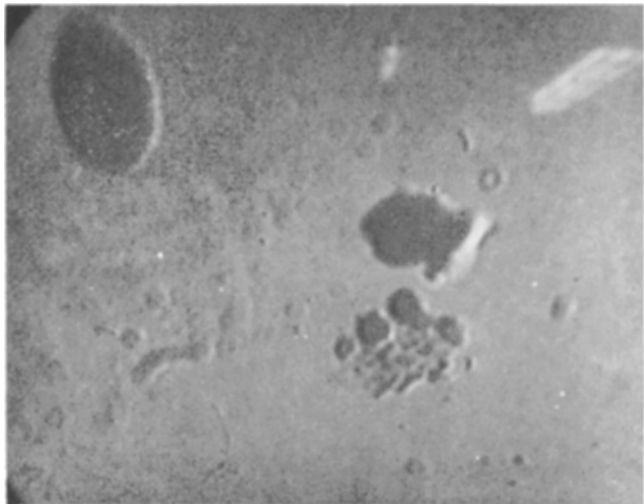


Fig. 11. Spore-like structure from meteorite, middle focus.

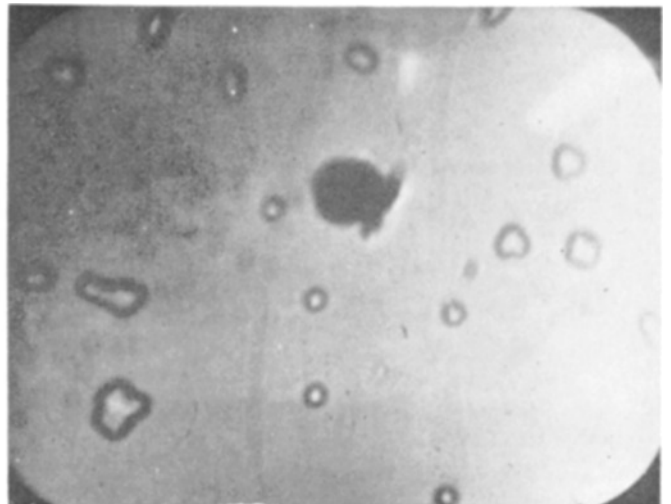


Fig. 14. Amphorus sulfur spheres.

men did not fluoresce, while the stained fluoresced 28 units.

The large cell-like structure in Figures 15a to 15e

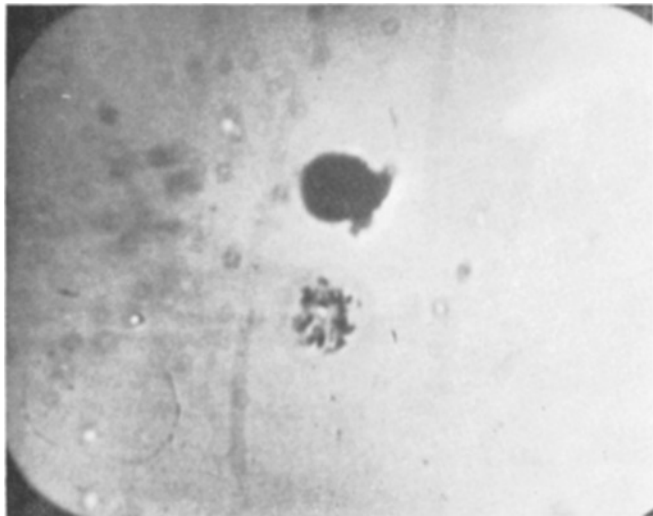


Fig. 15a. Cell-like structure from meteorite, top focus.

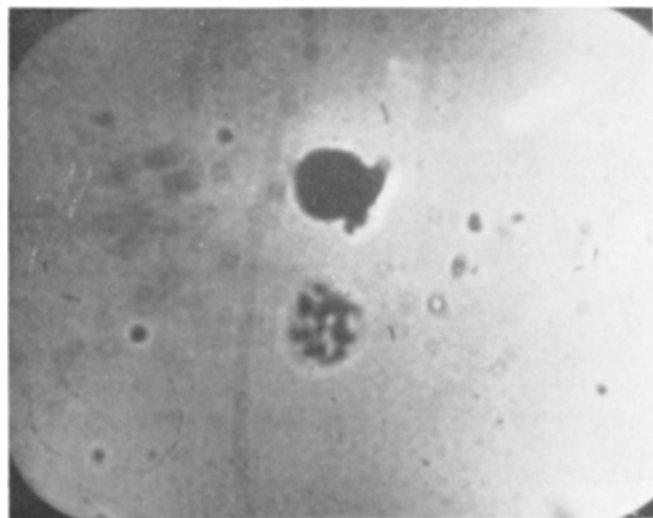


Fig. 15b. Cell-like structure from meteorite, middle focus.

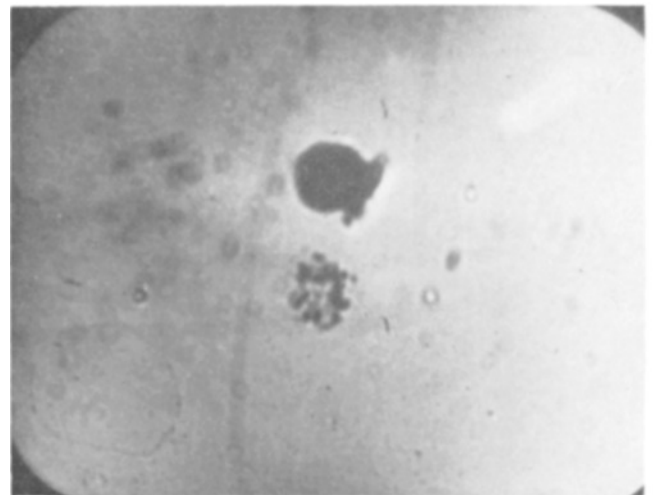


Fig. 15c. Cell-like structure from meteorite, lower middle focus.

appear to resemble a cell that was undergoing a mitotic division. The dark masses might be chromosomes. Figure 16 was an onion root tip mitotic figure. Unstained it fluoresced 30 units, while stained it fluoresced 900 units.

Table I is a compilation of the fluorescence levels obtained and the colors of fluorescence in an unstained and stained state when the specimens were excited by the ultraviolet and near ultraviolet light.

## DISCUSSION

The question as to the nature of the "organized elements" of the meteorites is far from settled. Three schools of thought are presently expounded.

One group suggests that the "organized elements" might be spherules, microchondrules, or condensation droplets (an inorganic matrix with organic matter attached to it). The second group suggests that the "organized elements" might be fossil microorganisms or their casts. The third suggests that the "organized elements" are contaminants. Evidence for any of these three situations is far from conclusive.

Although the case for contamination in many of the Orgueil specimens has been eliminated, Anders and his associates have shown that Orgueil specimen number 9419 is contaminated (*Science* 146:1157-1161, 1964). Therefore, contamination should always be considered and guarded against.

As stated in the "Introduction," compounds of biological significance have been extracted from the meteorites as for example, amino acids, lipids, carbohydrates, amino sugars and hydrocarbons. The question still unanswered is—were these compounds and the "organized elements" produced by an abiogenic process, or by means of a biogenic one.

Some of the principle objections to the organic matter and "organized elements" in the meteorite being of biological origin are the lack of chlorophyll, pheophytins, cytochromes, porphyrins, purines, pyrimidines and the lack of optical rotation.

The absence or a low level of the biologically im-

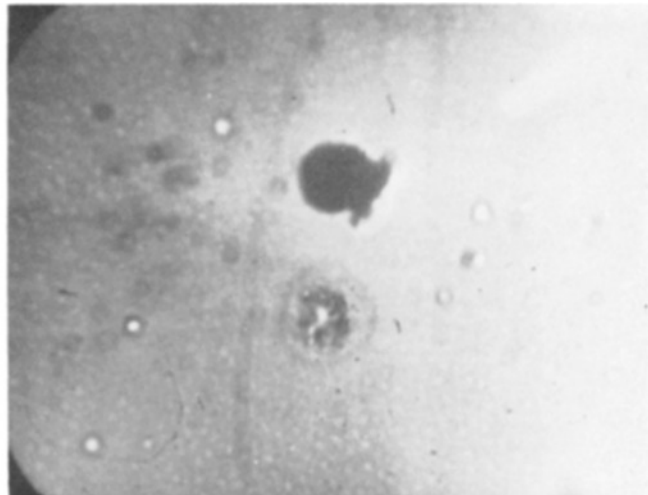


Fig. 15d. Cell-like structure from meteorite, bottom focus.

portant molecules as cytochromes and porphyrins (a possible degradation product of cytochromes), an essential component of an organism respiratory or electron transfer system might be explained on the following basis. It is assumed by most investigators that a reducing atmosphere was present when most Earth's organisms evolved, Oparin,<sup>1</sup> Haldane,<sup>2</sup> Sagan.<sup>3</sup> A similar reducing environment is postulated for the environment of the Orgueil Meteorite's parent body, Nagy, et al.<sup>28</sup> As such it is postulated that the early organisms were heterotrophic anaerobes and then evolved into other respiratory forms, McElroy and Seliger<sup>29</sup> and Botan.<sup>30</sup> Under such conditions cytochrome

TABLE I. FLUORESCENCE LEVELS AND COLORS OF STAINED AND UNSTAINED SPECIMENS

Specimen	Unstained		Stained	
	Level	Color	Level	Color
<i>B. globigii</i>	70 units	blue-white	350 units	yellow-green
Baker's yeast	130 units	blue-white	550 units	yellow-green
Lichen				
a) algae	220 units	red	250 units	yellow-green
b) hyphae	120 units	yellow	150 units	yellow-green
<i>N. crassa</i>	30 units	yellow	120 units	yellow-green
Peat specimens				
a) bact. mass	---	---	20 units	yellow-green
b) plant material	---	---	30 units	yellow-green
Meteorite Specimen				
a) fungal-like	---	---	18 units	yellow-green
b) yeast-like	---	---	20 units	yellow-green
c) spore-like	---	---	33 units	yellow-green
d) fungal-like	---	---	28 units	yellow-green
e) granules	1 - 5	yellow	1-5 units	yellow
f) sulfur gran.	1 - 10	yellow	1-10 units	yellow
g) lg. cell-like	---	---	28 units	yellow-green
Minerals				
a) olivine	1 - 3	pink	1-3 units	pink
b) amphibole	1 - 4	pink	1-4 units	pink
c) magnetite	1 - 5	yellow-black	1-5 units	yellow-black
Mitotic Figure				
a) onion root tip preparation	30	blue-white	900 units	yellow-green

enzymes or other aerobic respiratory enzymes were not present or were present in low levels.

The situation is seen in the present day anaerobic heterotrophic organisms which display a lack of cytochromes, or an incomplete assortment of them, Burrows.<sup>31</sup> Therefore, the lack of or the low level of cytochromes or porphyrins in meteorite material would not be a serious objection. Likewise the lack of, or the low level of chlorophylls, pheophytin (a degradation product of chlorophyll) and porphyrin (also a possible degradation product of chlorophyll) would not be a serious objection, since heterotrophic anaerobes usually are non-photosynthetic. Additionally it should be noted that Hodgson and Baker<sup>18</sup> have reported the possible presence of porphyrins at a level of 0.01 p.p.m.

The lack of, or the low level of purines and pyrimidines is still a vexing matter. For these compounds are definite requirements of life as we know it (components of nucleic acids) and are quite geochemically stable. Thus they should not completely disappear in the sample which is biogenically produced. It should be noted at this point that if the hydrocarbons, amino acids, sugars, etc. were produced abiogenically there should have been some purine or pyrimidine like compounds produced. For Oro<sup>32</sup> has reported in his discharge experiments, compounds which absorb in the U.V. Compound A which was formed has a maximum absorption at 257 m $\mu$  and a minimum at 232 m $\mu$ , and Compound B maximum at 262 m $\mu$  and a minimum at 234 m $\mu$  in 0.1N HCl. These two compounds have a marked similarity to isocytosine and adenine. Thus, the absence of the purines and pyrimidines in the meteorite might be due to the fact that we are not extracting them or just getting very low levels of them by the classical extraction techniques. Also Briggs<sup>14</sup> and Nagy, et al.,<sup>33</sup> have reported the possibility of these compounds being present in meteorite samples.

The problem of optical activity for the meteorite material has been elucidated by Nagy, et al.<sup>34</sup> In their procedure, extracts of the meteorite material were saponified, fractionated, and the fractions examined for optical activity. A small but well reproducible laevo-

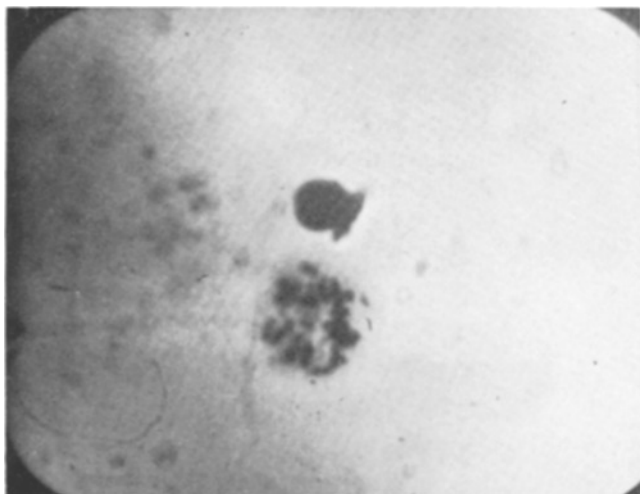


Fig. 15e. Cell-like structure from meteorite, middle focus, magnification increased to approximately 5000x.

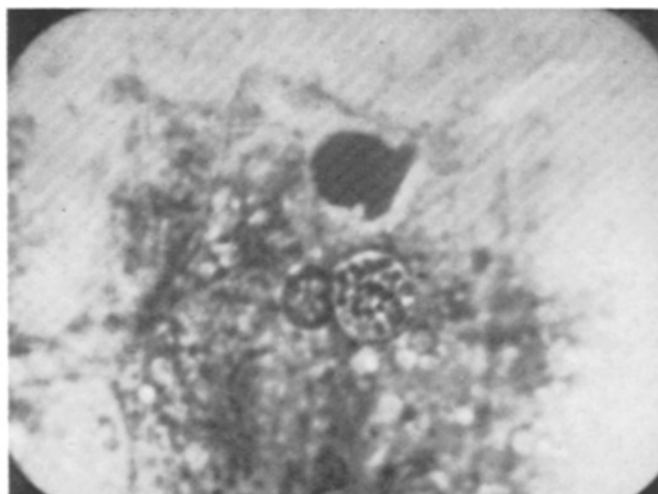


Fig. 16. Mitotic figure from onion root tip.

rotation was reported. Nagy, et al.,<sup>34</sup> attributed the failure of previous investigations to demonstrate optical activity to the use of instrumentation lacking in sufficient sensitivity and the use of unfractionated samples.

In this study, "organized elements" which had marked morphological characteristics resembling cells or organisms as we know them on Earth were examined. In these structures the amino acid, tyrosine, was demonstrated by the formation of fluorescent complexes. The level of fluorescence of the "organized elements" was compared to that of lab cultured organisms (present day organisms) and organisms obtained from peat 38,000 to 100,000 years old. It was observed that the fluorescence level of the "organized elements" was about an order of magnitude lower than that of the present day organisms. This indicates a low level in tyrosine in the specimens. It is postulated that the low tyrosine levels indicate that the "organized elements" found in the meteorite are not Earth source but inherent components of the meteorite. It is also postulated that the presence of tyrosine at such low levels is a significant bit of evidence in the favor of the "organized elements" being biological structures.

Figures 15a, 15b, 15c, 15d and 15e are quite interesting as they resemble a structure undergoing a mitotic division. Figure 16 is a mitotic figure from an onion root tip which can be used for the purpose of comparison. The size of the mitotic figures of the onion root tip and the possible mitotic figure of the meteorite specimen is almost identical. But when comparing the fluorescence we see that the onion mitotic figure is approximately 30 times as great. This would indicate that the tyrosine content of the onion mitotic figure is much greater than that of the possible meteorite mitotic figure. One might explain this difference as due to the age of the meteorite material versus that of the modern onion material. During the process of aging, one could expect a degradation of the tyrosine. Thus, one might postulate that the meteorite possible mitotic figure is not a modern contaminant but an inherent component of the meteorite.

If the structure demonstrated is truly a mitotic figure and is truly an inherent native component of the meteorite, then the possibility of this "organized element" being a life form is strongly supported.

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