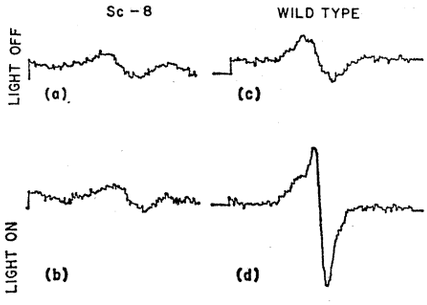

Fig. 3. (*a*) Electron paramagnetic resonance spectrum from a suspension of a "CO_2" mutant (No. 8) in the absence of illumination. (*b*) Spectrum from the same suspension under white light illumination, illustrating lack of *R* signal present in a wild-type suspension under illumination (*d*). (*c*) Spectrum of *S* signal from wild-type cells; the larger amplitude reflects the greater Hill activity of wild type (see Table 1). All tracings are made with suspensions containing 5×10^8 cells per milliliter and with identical instrument parameters.

EPR behavior. Washed chloroplasts show a decreased *S* signal (*2, 4, 11*), and this may be correlated with the decline in Hill activity brought about by such treatment. However, hydrogen-adapted wild-type *Scenedesmus*, stabilized (*6*) with 3(3,4-dichlorophenyl)-1-1-dimethylurea (DCMU) possess a prominent *S* signal despite the fact that they are evolving no oxygen (*12*). Thus, the block imposed by DCMU occurs nearer the point of oxygen release than that brought about by manganese deficiency or by any of the mutants yet examined, and agrees with an earlier conclusion of Bishop (*13*).

The semiquinone of plastoquinone has been suggested as the organic free radical responsible for the slow signal (*9, 14*). Mutant 11 contains plastoquinone in normal amounts, and manganese deficiency does not alter the content of plastoquinone in wild-type or mutant cells. It is possible that manganese deficiency, thorough washing of chloroplasts, or whatever change it is that produces the "O_2" mutants, while not removing or preventing the formation of quinone, interferes with its oxidation or reduction or both, and therefore prevents the formation of a steady-state semiquinone, which is the free radical observable by EPR spectroscopy (*15*). Perhaps some water-soluble compound, dependent on manganese for its synthesis, is a necessary link in the electron pathway. The structural integrity of the intact system may also have been altered, since treatment of *Chlorella* with ultrasound abolishes the *S* signal, although the cell material was not fractionated (*16*).

The *R* signal has been assumed to be a free radical form of chlorophyll or of some component intimately and invariably associated with the primary light acceptor. Its absence in cells that have large amounts of the known photosynthetic pigments implies that an electron acceptor or donor which may not yet be identified is required for the production of the *R* signal. The *R* signal may be due to the photooxidized form of a pigment absorbing at about 700 mμ (P700) which, although it has not been isolated or further characterized, is believed to act as the terminal energy collector in photosynthesis by accepting energy from an excited state of chlorophyll (*17*). One possible explanation for the lack of *R* signal in mutant 8 is that it lacks P700. Another possibility is that the structure of the chloroplast is faulty, and that the electron evident as the *R* signal is not in this mutant, separated from the chlorophyll molecule.

We have reported the separation of the two typical photoinduced EPR signals in algae by the use of mutant strains blocked in different portions of the photosynthetic cycle, and made some guesses on the possible explanation for the observations. When the explanations can be offered on a solid basis, they should contribute to the understanding of the overall process (*18*).

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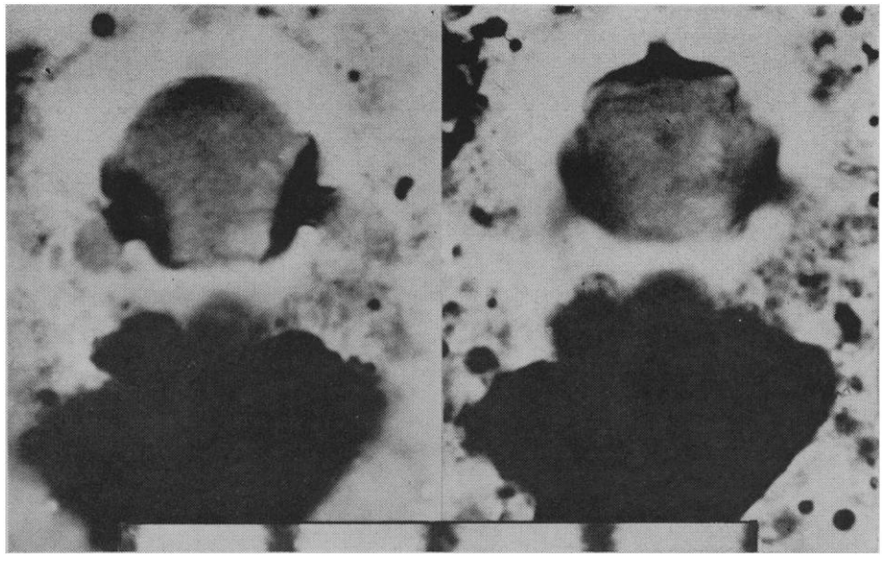
29 April 1963

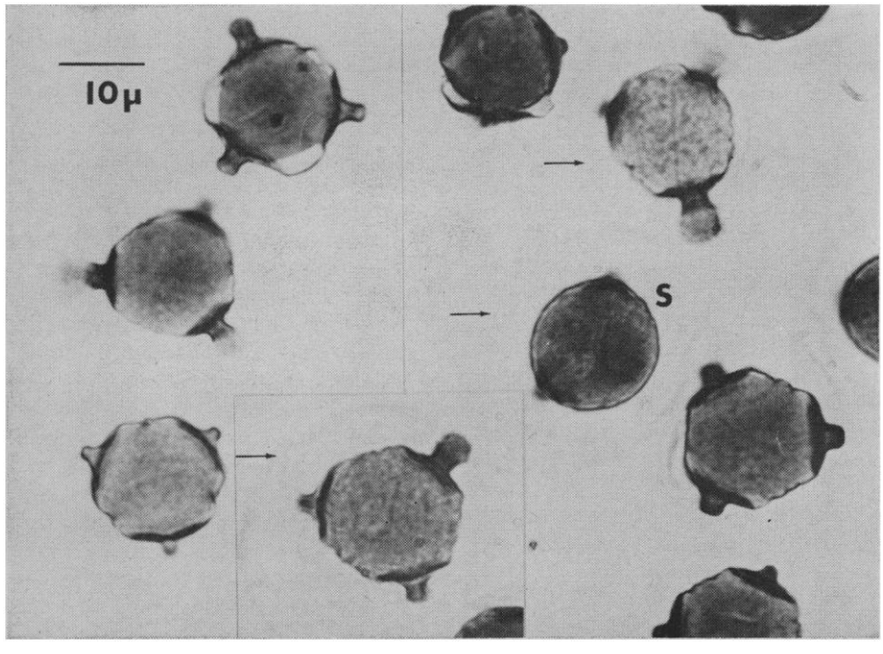
Organized Element: Possible Indentification in Orgueil Meteorite

Abstract. *Ragweed pollen stained by the Gridley method becomes distorted so that it resembles Claus and Nagy's Type 5 organized element, a particle found in a Gridley-stained preparation of the Orgueil carbonaceous chondrite.*

One of the most striking apparently biogenic particles reported from carbonaceous chondrites is the "Type-5 organized element" (*1*). It is hexagonal, with tubular protrusions extending from alternate walls, and is surrounded by a clear halo. It seems to be quite rare. Claus and Nagy (*2*) found only two such particles (along with several fragments) in Orgueil, and four in Tonk. One of these particles has been illustrated in the literature (*1–3*); this is the only particle which we were able to examine personally and to photograph in Dr. Claus' laboratory (Fig. 1). It appears on a slide stained with the Gridley method, and is orange-brown in color.

Claus and Nagy pointed out that this particle "is entirely dissimilar in its morphology to known terrestrial form[s]" (*1*), and suggested that it be recognized as an extraterrestrial genus, *Daidaphore berzelii* (*2*). The tubular projections were stated to contain up to 50 filaments (*2*). Mueller, on the other hand, has suggested that this particle is a limonite pseudomorph after troilite (*4*). Orange, hexagonal mineral grains do occur in Type 1 carbonaceous





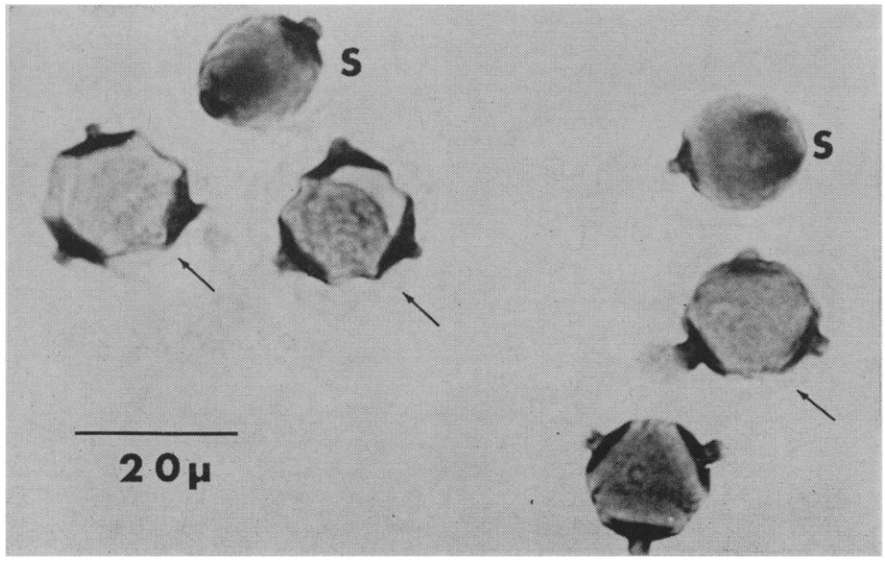


Fig. 1 (top). Two views of a type 5 organized element, discovered by Claus and Nagy (*1*) in a preparation of Orgueil meteorite stained by Gridley method and mounted in Canada balsam. Note halo and brush-like protrusions. The vertical lines at bottom of picure are 10 μ rulings of a stage micrometer, photographed at the same magnification. Figs. 2 and 3. False ragweed (*Franseria acanthicarpa*) (middle) and short ragweed (*Ambrosia elatior*) pollen (bottom), stained by Gridley method and mounted in Permount. The distorted central portion of the pollen grains, altered by the staining procedure, is an oblate spheroid, flattened at germinal pores, which when seen in polar view appears hexagonal and in equatorial view, oval (*S*). Arrows point to halo, formed by detached exine.

chondrites but do not have tubular projections or a clear halo.

Since the organized elements were reported to stain with various biological stains (*1*), and since several of the type-2 organized elements appear to be ragweed (*5*) or other (*6, 7*) pollen grains, we examined the staining properties of various pollen grains. Siegel (*8*) had previously suggested, on the basis of the trigonal symmetry of the type-5 element, that it might be a distorted pollen grain. The distortion might have been caused by the chromic acid of the Gridley staining procedure, since it is known to have a "violent and disruptive effect" on cells (*9*). An unexpected finding was that ragweed pollen grains stained with the Gridley method have an appearance strikingly similar to the type-5 organized element.

Pollen from giant, short, western, southern, slender, and false ragweed (*Ambrosia trifida, A. elatior, A. psilostachya, A. bidentata, Franseria tenuifolia,* and *F. acanthicarpa*) was suspended in Mayer's egg albumin and spread on microscope slides. The naturally shed pollen was untreated except for that from *A. psilostachya* and *A. bidentata* which was obtained from dried flowers and then had been washed with acetone and carbon tetrachloride (*10*). The slides were either air-dried, or fixed in alcohol; they were then treated with the various reagents used in the Gridley staining procedure in order to determine the effect of each solution alone and in various combinations, on the pollen grains. The Gridley stain, originally developed to demonstrate fungi in tissue sections, consists of treatment with 4 percent chromic acid for 1 hour followed by reaction with Schiff's reagent and staining with aldehyde fuchsin and metanil yellow (*11*).

The chromic acid treatment removed the outer part of the exine and apparently caused swelling of the remaining exine. This resulted in some increase in diameter and loss of much of the spiny surface detail of the ragweed pollen grains. The pollen cell was shrunken and the intine was collapsed around it. The intine usually remained attached to the germinal pores. In pollen grains seen in polar view, the intine appeared hexagonal in outline with short tubular appendages attached to germinal pores protruding from alternating surfaces (Figs. 2, 3, and 4*b*). The portion of the intine from which the appendages protruded was apparently thicker so that these walls were smoother, more distinct, and thicker. In air-dried smears, the germinal pores frequently were ruptured, and the cell cytoplasm exuded in brush-like or worm-like ribbons up to 20 μ long. Frequently such protrusions extended through two or even all three of the germinal pores. In alcohol-fixed slides, such protrusions were quite rare, although the appearance of the intine and cell was otherwise like that in air-dried preparations.

The exine did not appear to become stained with any of the reagents; the faint yellow color on the surface of the pollen grains was probably due to staining by metanil yellow of the egg albumin coating the pollen grain. The intine had a magenta color when reacted with Schiff's reagent after treatment with chromic acid; no staining was observed if this treatment was omitted.

Aldehyde fuchsin caused slight purple staining of the intine with or without chromic acid treatment. Metanil yellow produced yellow-brown staining of the cytoplasm but little staining of other components of the pollen grain. However, the intine had a brownish purple color after it was exposed to the complete Gridley stain procedure although it did not appear to become stained when treated with metanil yellow alone. The vacuoles and cell nucleus did not appear to become stained with the reagents used.

All six species of ragweed had a similar appearance after application of the Gridley stain, except for some differences primarily in the shape of the exine. *Ambrosia elatior, A. trifida,* and *F. acanthicarpa* resembled most closely the type-5 organized element. Minor differences were evident in different preparations of the same batch of pollen. It is likely that minor variations in the staining procedure will slightly alter the final appearance of the stained pollen grains.

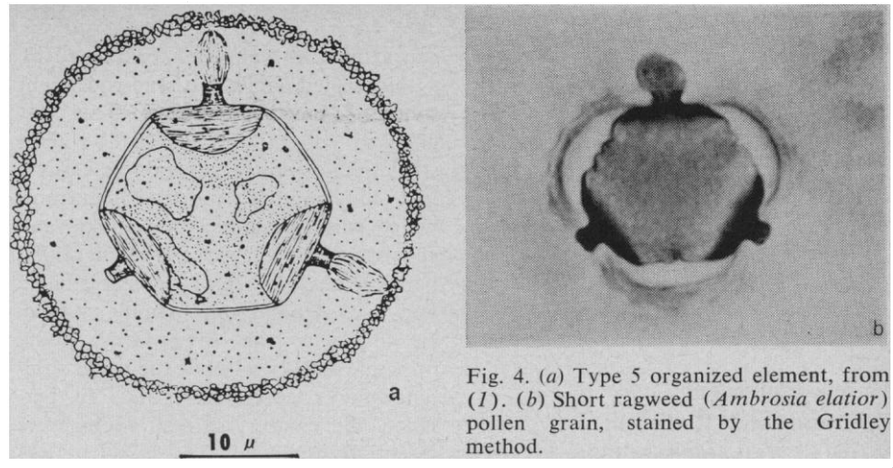


Fig. 4. (*a*) Type 5 organized element, from (*1*). (*b*) Short ragweed (*Ambrosia elatior*) pollen grain, stained by the Gridley method.

It is difficult to be certain of the size of the type-5 organized element. Measurements made from published drawings (Fig. 4*a*; *1, 2*) indicate that the distance from one hexagonal face to the one opposite is 15 to 16 μ and that the diameter of the halo is about 33 to 35 μ. Measurements of our own photomicrographs (Fig. 1) of one type-5 organized element gave 12 μ and 19 to 21 μ for these two dimensions. Measurement of 10 randomly selected pollen grains in an air-dried, Gridley-stained preparation of *A. elatior* gave a range of 12.9 to 14.5 μ and 17.4 to 19.9 μ, respectively. Corresponding dimensions of *A. trifida* were 13.3 to 14.9 μ and 18.7 to 21.8 μ. Occasional grains were somewhat larger or smaller. The size depends somewhat on the amount of exuded cytoplasm; the grains with the more prominent appendages tend to be smaller. The dimensions of the type-5 organized element appear to be within the range of those of various ragweed pollen grains stained with the Gridley method.

Other pollen grains were also examined after staining with the Gridley method. Alcohol-fixed preparations of white birch and black birch (*Betula populifolia, B. lenta*) and ironwood (*Ostrya virginiana*) had a somewhat hexagonal appearance but appendages were not present, and the intine near germinal pores was quite thick. Red cedar and mountain cedar (*Juniperus virginiana* and *J. sabinoides*) retained a spherical shape with central polyhedral markings. The appearance was quite similar to some of the type-2 organized elements (*2*, Fig. 4, p. 15).

The Orgueil preparation, in which the original type-5 organized element of Claus and Nagy was found, was definitely stained with the Gridley method. Although we cannot state with certainty that this particle is in fact a distorted ragweed pollen grain, it resembles Gridley-stained ragweed pollen in size, color, and morphology.

This supports the previous contention that morphology alone is not an adequate criterion for proving the extraterrestrial origin of suspected life forms in meteorites (*5*). Gregory has emphasized that one person can be familiar with only a fraction of the morphological diversity of plant spores (*7*). Even common pollen grains may become so altered that they are unrecognizable when treated in an unfamiliar way.

In the present instance, the resemblance of the type-5 organized element to a common air-borne pollen grain, treated according to a relatively standard biological staining procedure, has gone unnoticed for more than a year. Regardless of its true identity, one can no longer say that the type-5 organized element is entirely dissimilar in its morphology to known terrestrial forms (*12*).

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29 March 1963

Muscle-Equivalent Environmental Radiation Meter of Extreme Sensitivity

Abstract. *A 16.5 liter spherical ion chamber was constructed of conducting muscle-equivalent plastic and filled to 760 mm-Hg with a muscle-equivalent gas. The use of the chamber for measurements of natural environmental backgrounds was made quite feasible with the aid of the recently developed Shonka vibrating quartz fiber electrometer. This instrument is routinely operable at the extremely high sensitivity of better than 5000 divisions per volt. This system, therefore, has made possible reproducible measurements of absorbed dose-rates of fractions of a micro-rad per hour without any need for the usual corrections for wall-effect, stopping power, and so forth.*

The recent development by one of us (*1*) of a vibrating quartz fiber electrometer has made possible the construction of an uncomplicated, unpressurized, portable environmental radiation meter of extreme sensitivity.

The electrometer is routinely operable at the high sensitivity of better than 5000 divisions per volt. The sensitivity and balance adjustments may be made in the presence of a d-c signal on the fiber. The electrometer has an inherent capacitance of 1 to 2 picofarads and a rapid response with no detectable anistropy. Thus the instrument is an ideal detector for null measurements. A 3-lb transistorized power supply capable of operating the system continuously for 150 hours is in use for field measurements.

A 16.5-liter pseudosphere with walls 6 mm thick was welded from six molded sections of conducting muscle-equivalent plastic (*2*). The entire assembly contains no metal, and has a polyethylene guard-ring insulator and a molded polystyrene insulator supporting a thin central collecting rod, usually run at plus 80 volts and terminated by a thin-walled, hollow, tissue-equivalent sphere 4 cm in diameter. This was filled to 760 mm-Hg at 15°C with a stable, nonexplosive gas, muscle equivalent for photons and fast neutrons, which was formulated by Shonka, and consists of 41.11 percent neon, 39.59 percent ethylene, 16.17 percent ethane, and 3.13 percent nitrogen by volume. In this way the Bragg-Gray cavity principle applies without the usual stopping power corrections, and, furthermore, the cavity size restrictions were essentially removed.

Calibration of the chamber was carried out with a 1.11-mg radium needle (certified by the National Research Council of Canada) in 0.5 mm of platinum, with usual checks for saturation, scattering, and inverse-square law behaviors. An extremely precise and accurate determination of the ratio of muscle gas to air (designated "W")—the energy to create an ion pair—was made for us by W. P. Jesse of St. Procopius College, Lisle, Illinois. The volume and capacitance of the chamber were measured to better than 0.5 percent. The ionization rate calculated for our muscle chamber was compared with the ionization actually obtained. The agreement was good enough to assure that the ratio of electron stopping powers for our wall and gas is essentially unity.

The portability of the system and its extraordinary sensitivity of 0.33 mv/sec for 1 μrad/hour, enabled us to make measurements of environmental background in a skiff on Lake Michigan, on top of a ranger-type tower 40 m high, and at various land sites and in buildings within the Chicago area.

A value of 4.2 μrad/hour for the cosmic ray ionization at sea level was derived from the measurements made on Lake Michigan; the lake presumably shielded the device from terrestrial radiation and was itself assumed free of appreciable radioactivity. Corrections were made for the height of Lake Michigan (about 580 feet) and for radon content in the air, but not for geomagnetic latitude. When one considers the greater response of our chamber to low-energy radiation, the value is in reasonable agreement with those determined by other investigators. Solon (*3*), Burch (*4*), Neber (*5*), and Hess (*6*) obtained values of 3.8, 3.3, 4.7, and 3.4 μrad/hour, respectively.

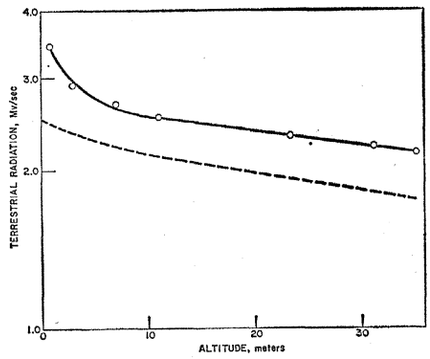

Fig. 1. Air attenuation of terrestrial radiation. Solid line, experimental data; broken line, theoretical, after Hultquist (*8*) and O'Brien (*9*).

Daily measurements in a first-floor laboratory over a period of months yielded a mean of 6.8 μrad/hour with a coefficient of variation of better than 0.5 percent.

The total radiation at points outside the building corresponded to about 14 to 15 μrad/hour, which is a shade higher than the values of 12 to 13 μrad/hour reported for this area by the Health and Safety Laboratory of the Atomic Energy Commission (*3*). The material in our building walls evidently attenuates the outdoor radiation considerably.

Measurements were carried out over a period of months in an Iron Room with an 8-inch wall. The mean of very reproducible readings was 1mv/sec, corresponding to about 3 μrad/hr. Thus the cosmic ray attentuation by the Iron Room is about 67 percent, which agrees very well with measurements made by F. W. Spiers (*7*) in Leeds, England.

Figure 1 displays the data obtained on the tower. Each point is the mean of about 15 measurements made at intervals which were weeks apart. The coefficient of variation of the data at each altitude is less than 4 percent. The ordinate has been reduced to net terrestrial radiation by subtraction of the cosmic ray contributions. The attenuation by air of terrestrial radiation has been calculated both by Hultquist (*8*) and by O'Brien *et al.* (*9*) on the basis of assumed concentrations of U, Th, and K. After filtration of the soft components by the first 10 m of air there seems to be substantial agreement between the observed and calculated attenuation factors.

The Shonka electrometer with its extreme sensitivity has made possible a system for the reproducible