Search for Organized Elements in Carbonaceous Chondrites

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The possible occurrence of life forms in meteorites, as reported by Claus and Nagy (*1*), has aroused considerable interest and discussion (*2–11*). Because of the far-reaching implications of these findings, we have sought to confirm them. To date, our efforts have been largely unsuccessful.

In our first study (*5*) we found only three classes of particles that seemed to match the characteristics of the "organized elements": troilite and magnetite globules; sulfur and hydrocarbon droplets; and what appeared to be mineral grains. Later it became evident that some particles we have regarded as mineral grains were classified as organized elements by Nagy *et al.* However, two serious discrepancies remained: (i) particles of even as simple a morphology as Fig. 3 of (*7*) were present in our samples in far lower abundance than the ~1680 per milligram reported by Claus and Nagy; (ii) particles of more highly structured morphology [see, for example, *1*, Figs. 2, 3, 4; *7*, Figs. 1, 4, 5] had not been seen in our samples.

Part of the discrepancy might well be due to subjective differences in morphological judgment. To resolve this question with as little recourse to subjective judgment as possible, we conducted a systematic search, based on four generic properties of the organized elements (*1, 7*)—namely, (i) fluorescence in ultraviolet light; (ii) biological staining; (iii) insolubility in hydrofluoric acid; and (iv) density less than 2.4 g/cm^3.

In order to preclude sampling errors, we obtained from the U.S. National Museum (*12*) a sample of the same Orgueil stone studied by Nagy *et al.* For some experiments, we used sample No. 223 from the Muséum National d'Histoire Naturelle in Paris (*13*). In the preparation of slides for microscopic examination, we followed the technique of Claus and Nagy, crushing the material as gently as possible to prevent damage to fragile microstructures.

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Systematic Search for Organized Elements

Ultraviolet fluorescence. As already noted (*5*), our attempts to locate organized elements by their ultraviolet fluorescence had been unsuccessful. In an area where 39 organized elements should have been present, according to the abundance figures of Claus and Nagy, 15 fluorescent particles greater than 2 μ in diameter were found. However, these particles were quite irregular in shape, both under ultraviolet and under bright-field illumination. Many had a refractive index close to that of glycerin; some resembled fluorescent mineral grains; others resembled the thin wrinkled scraps of translucent, sheetlike material described by Briggs and Kitto (*4*) (Fig. 1, *a–d*). The similarity of the former material to Wilson's (*14*) hydrocarbon polymer synthesized by a Miller-Urey reaction has already been pointed out (*4*). Regardless of the nature of this material, it would seem that the majority of the fluorescent particles do not have the morphology of organized elements. Conversely, if the organized elements were present at levels of ~1680 per milligram, then none of the 39 expected in the area scanned manifested its presence by ultraviolet fluorescence.

Biological staining. One of the most significant properties reported for the organized elements is their positive reaction in the Feulgen test (*1, 7*), which has been regarded as an indication of the presence of DNA (*1, 2, 7*). However, one factor that must be taken into account in any interpretation of the Feulgen test is its innate lack of specificity. The color reaction in the Feulgen test is produced by the familiar Schiff's reagent, which gives a red color with aldehydes (as well as with some ketones, some unsaturated compounds, and most oxidants). Intact DNA does not contain any Schiff-reactive groups, but if the DNA is partially hydrolyzed by dilute HCl, deoxyribose phosphate groups are exposed, and the aldehyde form of deoxyribose is then free to give a typical aldehyde color reaction with Schiff's reagent (*15, 16*). What makes this test rather specific for DNA in biological tissues is the circumstance that almost all other Schiff-reactive groups are either absent initially or are removed from the tissues during fixing or HCl-hydrolysis (*15, 16*). With unfamiliar samples, it is essential to determine, by means of proper controls, whether any interfering materials are present that will react directly with Schiff's reagent. Such substances would give a false positive Feulgen reaction, simulating the presence of DNA.

As controls for the staining reactions on the Orgueil meteorite, rat spleen and kimberlite were used. Kimberlite, the diamond-bearing ultrabasic rock from South Africa, was chosen because of its mineralogical similarity to the Orgueil meteorite, and also because of its deep-seated origin, which would seem to lessen the likelihood of indigenous biological activity. Two series of staining experiments were conducted: a standard Feulgen test in which the samples were hydrolyzed with 1*N* HCl at 60°C for 1 hour, then treated with Schiff's reagent (Fig. 2, *a–c*), and direct treatment with Schiff's reagent, without preliminary acid hydrolysis (Fig. 2, *d–f*). In the first series, all three samples gave a positive reaction, and although the color was somewhat lacking in brilliance in the case of Orgueil and the kimberlite, well over half the particles stained. In the second series, the nuclei of the rat spleen cells did not stain at all, as expected, but the Orgueil meteorite and the kimberlite stained at least as well as before. Clearly, some Schiff-reactive groups are present in kimberlite and the meteorite, and the development of a magenta color in the Feulgen reaction is in this instance not specific for DNA.

We also failed to detect DNA by the methyl green reaction (*15*), which proceeds by a different mechanism.

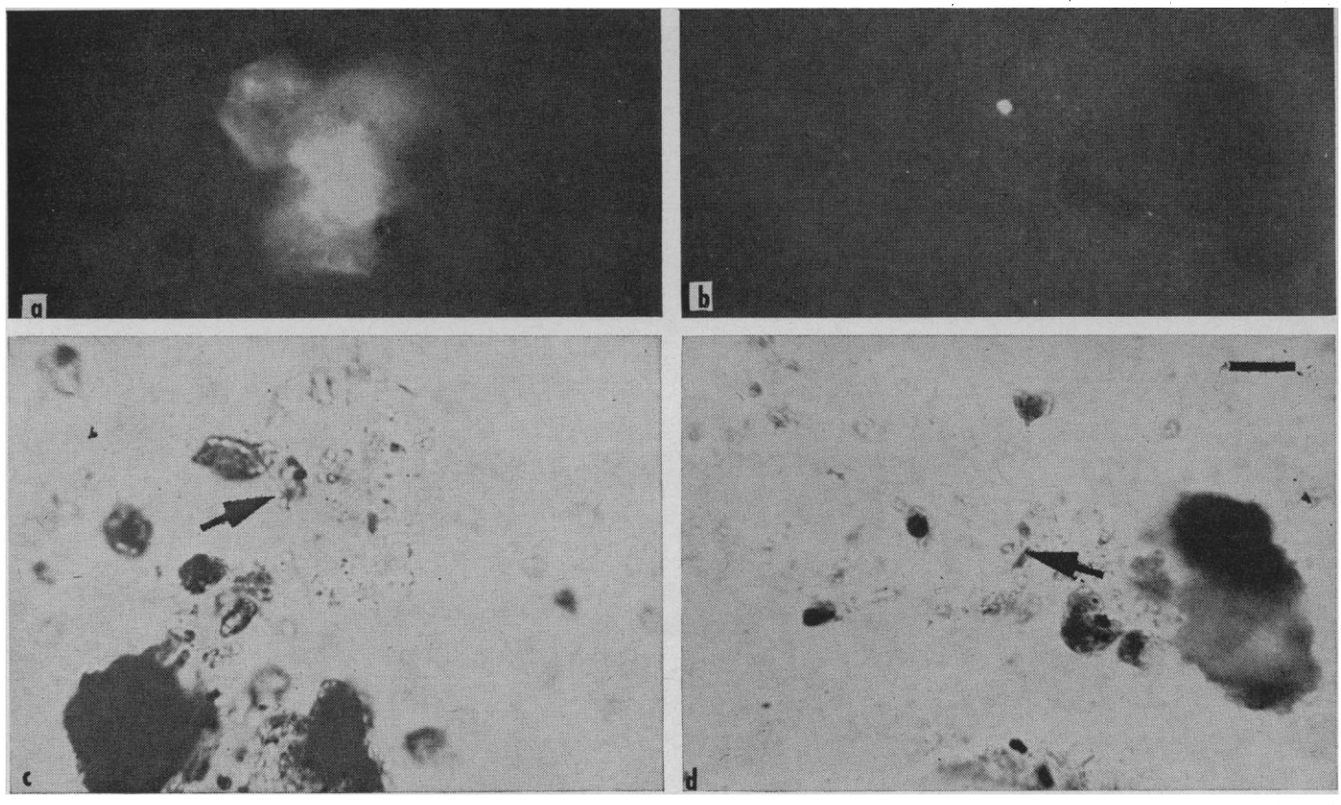

Fig. 1. Two typical fluorescent particles in the Orgueil meteorite (glycerin mount). (*a* and *b*) Under ultraviolet fluorescence; (*c* and *d*) under bright-field illumination. (Scale, 20 μ)

Again, the staining persisted in both kimberlite and the Orgueil meteorite even after a perchloric acid treatment that should have removed any DNA present.

The nature of the reactive material is not known at present. It may be an organic surface coating on the mineral grains, or it may actually be the hydrated silicate itself which comprises the bulk of the Orgueil meteorite and the kimberlite. The staining seemed to be diminished slightly after prolonged extraction with solvents but the results were not conclusive. Attempts to block the reactive groups were also unsuccessful.

Two conclusions from the staining experiments can be stated with certainty.

1) Owing to the presence of interfering groups, it is not possible to decide by staining reactions whether DNA is present in the meteorite.

2) In our samples of the Orgueil meteorite there exists no significant concentration of particles that differ from the remainder of the meteoritic particles in *both* morphology and response to biological stains.

The effect of acids. Nagy and his associates stated that treatment for 15 minutes with boiling concentrated hydrofluoric acid dissolved all but a few of the mineral grains in Orgueil and Ivuna material, whereas it left "spotted, bleached-out pellicles still morphologically identifiable as remnants of the organized elements." Urey (*11*) reports that treatment with concentrated hydrofluoric acid at 80°C for 8 hours left "many outlines with apparently unresolved cell walls of about the sizes of the organized elements. They looked like small pellicles with the interiors destroyed. They were present in very great numbers—some six or ten in one field of the high-power microscope."

We treated several 20- to 30-mg portions of the Orgueil meteorite according to the procedures of Nagy *et al.* and of Urey. In the former case, a copious residue remained, comprising 49 percent, by weight, of the original sample; the residue, with Urey's procedure, was only slightly smaller. Since the carbon content of the Orgueil meteorite is only 3.1 percent, most of the residue must have been inorganic. X-ray diffraction indicated that the residue consisted mainly of magnesium fluoride and troilite. The persistence of MgF_2 is not surprising, since the magnesium content of the Orgueil meteorite is high (9.5 percent), while the (calculated) solubility of MgF_2 is only 25 mg/lit. in concentrated hydrofluoric acid and 73 mg/lit. in water.

Microscopic examination indeed showed particles not unlike those described by Nagy and his associates and by Urey. In addition to a large number of spotted brownish particles of irregular shape, a smaller number of colorless particles of low refractility, rounded or irregular in shape, were present (Fig. 3, *a* and *b*). As already pointed out by Nagy *et al.*, the colorless particles were difficult to recognize in water or glycerol mounts, except under phase contrast illumination (Fig. 3*c*). To determine whether any organic particles were present, it was necessary to remove the inorganic matter, which, as indicated by the weight of the residue, still comprised the bulk of the material. Treatment with cold 6*N* HCl did indeed reduce the residue to ~3 percent, leaving nothing but a brown, shapeless pulp, amorphous as seen on x-ray examination and lacking any distinct features in its infrared spectrum (*17*). However, HCl destroys some types of organic matter as well as inorganic matter (*18*), and the residues from both types of digestion by hydrofluoric

acid were therefore allowed to stand in contact with mixed-bed ion exchange resin for 24 hours. This treatment is known to dissolve many inorganic salts of low solubility (*19*) but should leave even the most delicate organic structures wholly unaffected. The colorless particles in the residue disappeared almost entirely during this treatment, thus revealing their inorganic nature. X-ray examination of the residue still showed lines of troilite, a greatly weakened MgF_2 pattern, and a few unidentified lines. The refractive index of MgF_2 is low (1.378 to 1.390), and it would seem that the acid-resistant pellicles (*7, 11*) may in fact have been nothing but MgF_2 pseudomorphs after silicate grains.

It is evident from the bulk and the high mineral content of the residue that persistence of a "pellicle" after treatment with hydrofluoric acid is not a sufficient criterion of organic composition. After removal of MgF_2, no structures resembling organized elements seemed to remain.

Density separations. The organized elements are said to concentrate in the fraction of density between 2.3 and 2.4 (*7*). We had previously examined a fraction of density ≤ 2.31 and found it to contain numerous yellowish-to-brownish grains, similar to those that made up the bulk of the meteorite (*5*). X-ray examination showed primarily the lines of epsomite ($MgSO_4 \cdot 7H_2O$, $\rho = 1.636$ g/cm^3). The Orgueil meteorite contains about 15 percent of this mineral. After the epsomite had been dissolved in water, there was only a small residue. X-ray diffraction showed

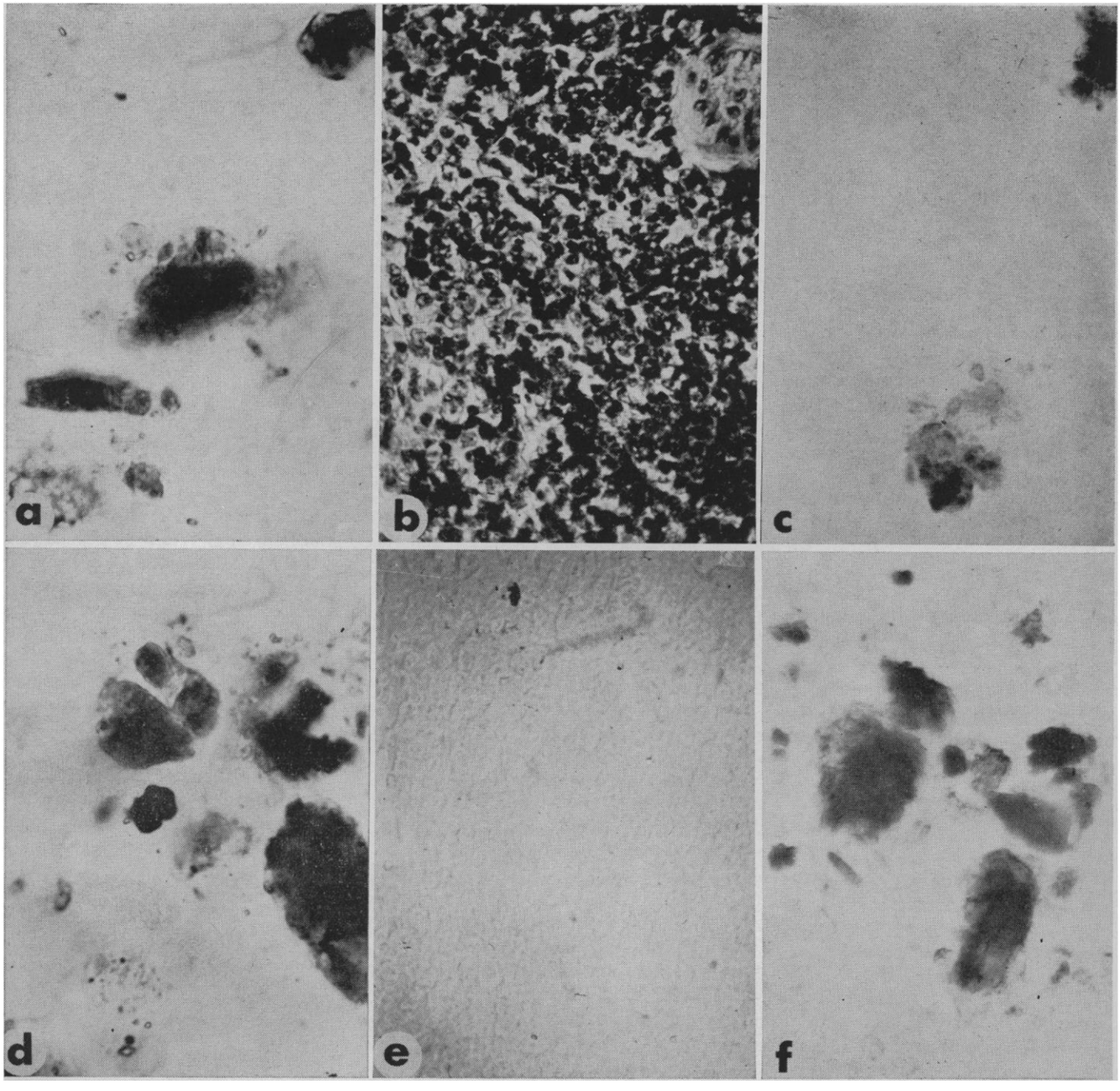

Fig. 2. (*a*) Orgueil meteorite, (*b*) rat spleen, and (*c*) kimberlite stained with Feulgen reaction. All three samples show staining though the color is less brilliant in *a* and *c* than in *b*. Treatment with Schiff's reagent only gives no staining of the DNA in rat spleen (*e*), but stains Orgueil meteorite (*d*) and kimberlite (*f*) as intensely as the Feulgen reaction.

it to consist of the layer-lattice silicate (Orgueil LM), which is the principal constituent of the meteorite (*20*). Unlike previously separated samples of this mineral, which were so thoroughly intergrown with magnetite as to defy any attempt at separation, this fraction was almost free of magnetite. Evidently the silicate particles entrained in the epsomite are of higher mineralogical purity than the remainder, and it is not unlikely that such yellowish silicate particles, lacking the usual opaque magnetite inclusions, were classified as organized elements by Nagy *et al.*

When another sample of meteorite, from which the epsomite, sulfur, and soluble organic compounds had been removed by extraction with water, cyclohexane, chloroform, and methanol, was put through a density separation procedure, only a minute trace of material of $\rho \leq 2.4$ remained. An amount somewhat larger but still comprising less than 1 percent of the material had a density less than 2.5. Like the epsomite-entrained material, its x-ray pattern revealed only Orgueil LM with small amounts of magnetite, though this fraction was not as pure in this respect as the former sample. If the organized elements had indeed been concentrated in this fraction, one would have expected to find them at levels of $\sim 1.7 \times 10^5$ per milligram. However, microscopic examination failed to disclose a significant level of organized elements.

Highly-Structured Organized Elements

In these searches we again failed to find any highly-structured organized elements resembling those reported by Claus and Nagy [*1* (Figs. 2, 3, 4); *7* (Figs. 1, 4, 5)]. Regardless of whether external morphology, ultraviolet fluorescence, biological staining, or insolubility in acids was used as the criterion, no such particles were detected in our samples. In order that this discrepancy might be resolved, Nagy and Claus invited us to study their samples in their laboratory in return for our samples, which they had described earlier (*7*).

Their material did indeed contain a few particles of striking morphology; we had not found, nor did they find, such particles in our material. Four such particles are shown in Figs. 4–6, together with several terrestrial contaminants which they resemble.

The particle in Fig 4*a* resembles a furnace ash sphere (*21*). The particle in Fig. 5*a*, reportedly stained with the Feulgen reaction, resembles *Juniperus* or *Taxus* pollen, as was suggested independently by O. C. Durham, P. H. Gregory, and B. B. Siegel. Two other brightly stained particles from the same slide resembled starch grains, and we cannot rule out the possibility that the particle in Fig. 5*a* is in fact a starch grain (compare it with Fig. 5*b*). The organized element in (*2*), Figs 2 and 3, also seems to bear some resemblance to *Juniperus* pollen, although Pearson (*9*) suggested a resemblance to *Corylus, Betula, Myrcia,* and *Alnus,* whereas Gregory (*6*) mentioned a fungus spore as a further possibility.

Two more particles, classified by Claus as type 2 organized elements with double wall and spiny surface, are illustrated in Fig. 6, *a–d*. A particle of strikingly similar morphology and color is shown in Fig. 6, *e* and *f*. It is a common ragweed pollen grain. There has been some disagreement about the size of the particles in Fig. 6, *a–d*. Claus originally reported a diameter of 10 to 12 μ, exclusive of spines, and later revised this figure to 14.5 μ (*22*). We measured the particles of Fig. 6, *c* and *d*, and obtained a value of 18 μ, inclusive of spines.

Urey (*11*) reports 15.0 to 15.5 μ, exclusive of spines, and maintains that this value is at variance with the accepted size for ragweed pollen, since "Wodehouse [*23*] records that ragweed pollen grains vary from approximately 17 to 24 μ in diameter." Actually, Wodehouse's measurements *include* spines, hence his values and Urey's and Claus's cannot be compared. Our value of 18 μ, on the other hand, lies well within the size range (16.5 to 19.2 μ) for the two most common varieties of *Ambrosia* (*A. elatior* L. and *A. trifida* L.) (*23*). Although it may not be possible to prove by morphological criteria alone whether or not the organized elements shown in Fig. 6, *a–d*, are pollen grains of *Ambro-*

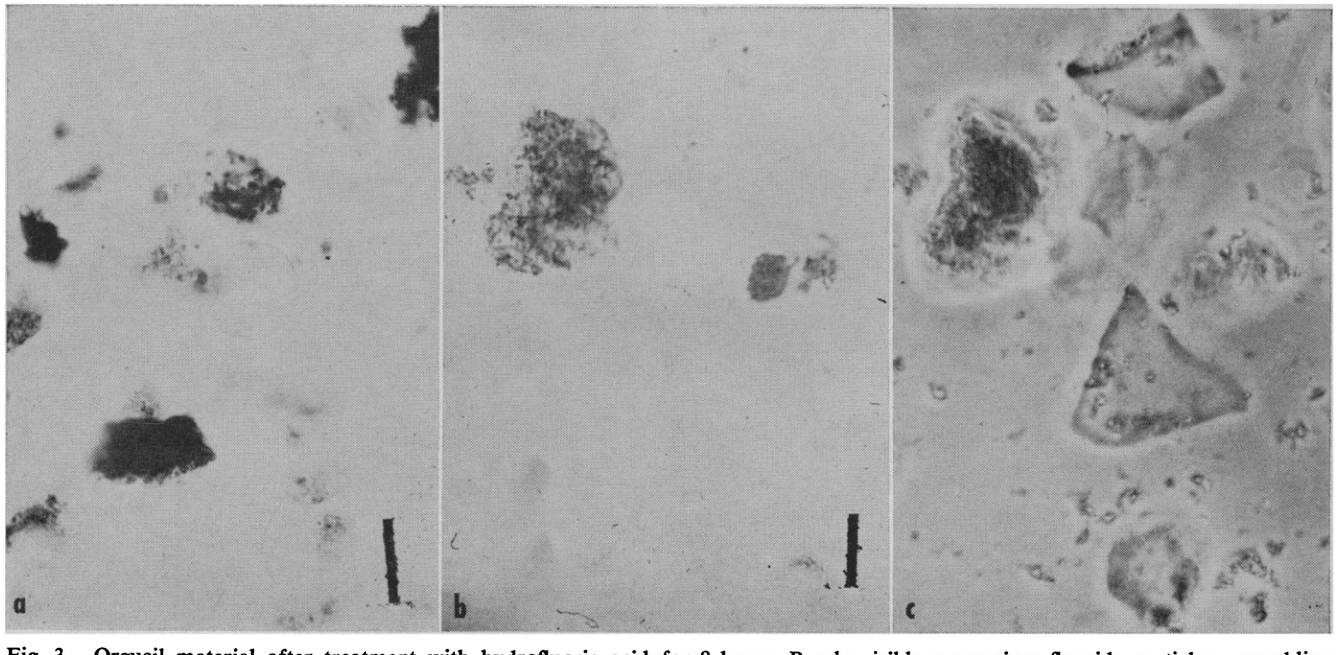

Fig. 3. Orgueil material after treatment with hydrofluoric acid for 8 hours. Barely visible magnesium fluoride particles, resembling organic pellicles, remain (*a* and *b*), but their inorganic nature is proved by their disappearance after treatment with ion exchange resin. *c*, Same field as *b* under phase-contrast illumination. (Scale 20 μ)

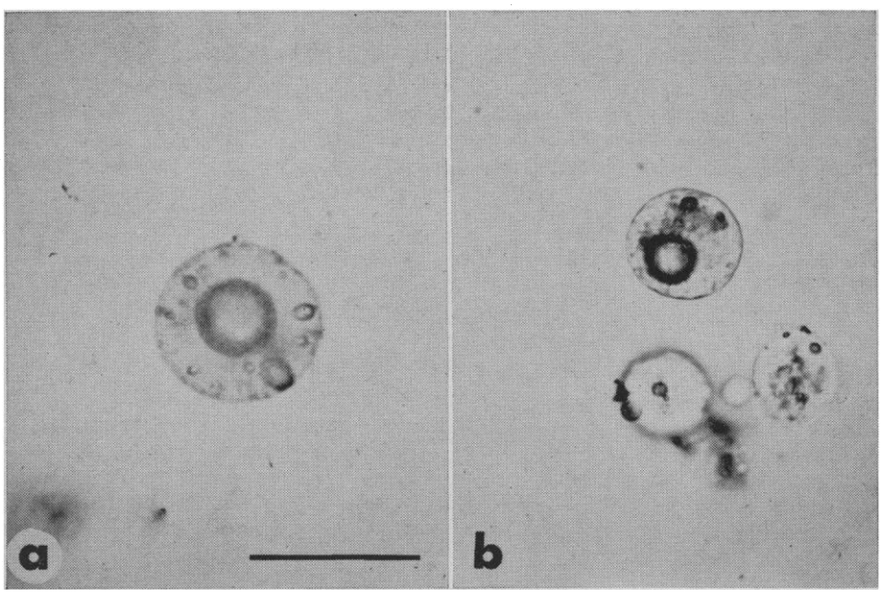

Fig. 4. (*a*) Organized element, resembling *Thecamoeba,* from preparation of Claus and Nagy. (*b*) Furnace ash sphere from pollen survey slide. Similar particles were found in a fly ash sample (*27*) and in dust samples from the American Museum of Natural History in New York. (Scale, 20 μ)

sia or related species, the resemblance is perhaps worthy of note.

Two other particles illustrated by Nagy *et al.* (*7,* Fig. 1; *1,* Fig. 1), although superficially similar to the particles in Fig. 6, seem to be much smaller (diameters of 12 and 11 μ, respectively, according to their measurements). It is therefore unlikely that they are *Ambrosia* pollen. However, these particles seem to be very rare, and it will therefore be necessary to prove that they are not terrestrial contaminants.

We do not mean to imply that *all* highly-structured organized elements are terrestrial contaminants. We had an opportunity to examine the type 5 organized element (*1,* Fig. 4), and we have thus far failed to find a satisfactory morphological match for it. Bernard B. Siegel (*24*) suggested that it might be a distorted pollen grain of the genus *Betula,* whereas P. H. Gregory suggested pollen of *Corylus,* the Myrtaceae, or Onogaraceae as further possibilities. The distortion might have been introduced during the preparation of the slide, which was mounted in egg white; stained according to the method of Gridley [this involves, typically, consecutive treatments with 1*N* chromic acid; Schiff's reagent; water; 70-percent, 80-percent, 90-percent, and absolute alcohol; alcohol-xylene; and xylene], and finally mounted in Canada balsam. It is not known to what extent such treatment would change the shape of pollen grains, and the nature of this particle thus remains an open question.

Thin Sections

To permit the detection of organized elements under conditions least prone to contamination, we obtained several plastic-impregnated thin sections of the Orgueil meteorite. The sections were prepared by the Rév Laboratories, under exactly the same conditions as those under which the sections of Nagy *et al.* had been prepared. However, our sections were furnished without cover glass, in order that the surface might be examined directly. For microscopic examination, a cover glass was attached with ethylene glycol (ethlyene glycol had also been used as a cutting and grinding lubricant during preparation of the sections). As a control, a thin section was prepared from a blank piece of the impregnating plastic (epoxy resin).

As expected, rounded yellowish grains identical to those in the crushed material were found to be present, though in much smaller numbers than 1700 per milligram. At that abundance, in a section 20 μ thick, some 10^4 organized elements per square centimeter would be expected. Actually, no more that a few hundred per square centimeter seemed to be present. None of these fluoresced—a finding in accord with our earlier findings on crushed material. Similar particles were also present in a thin section of kimberlite, so a biological origin of these structures seems unlikely.

Several interesting classes of particles were observed that had not been seen in crushed material. These included (i) bluish, highly refractile grains of irregular-to-rounded outline; (ii) filamentous and tubular structures, from 10 to 20 μ in diameter and ranging up to several hundred microns in length; (iii) translucent spheroids, 10 to 100 μ in diameter, pale yellow, grayish, or colorless, with finely sculptured or wrinkled surfaces; and (iv) yellow, spheroidal particles.

Particles of the first three classes seemed to be contaminants or artifacts, as they also appeared in the blank plastic. Class i particles were corundum grains ground into the plastic during preparation of the section, while class ii particles, which fluoresced strongly in ultraviolet, seemed to be fibers, either originally present in the plastic or picked up during preparation of the sections. Class iii particles (Fig. 7*a*) were particularly suggestive of organized elements and frequently fluoresced slightly (bluish or yellowish) in ultraviolet. However, these particles were usually located on the surface of the section (Fig. 7*b*). They were especially numerous at the edge of the section, or wherever the plastic had worn down completely during grinding. Some particles were not only located on the surface but also extended beyond the fusion crust (Fig. 7, *c* and *d*), clearly showing their extraneous origin. Attempts to remove them by means of a micromanipulator showed that they were firmly attached to the surface and had nearly the same mechanical strength as the epoxy resin itself. It seems that these spheres were formed from the embedding plastic during grinding, perhaps by reaction with the ethylene glycol lubricant. This is conclusively proved by their presence in the blank plastic section.

The particles of class iv occurred in only one of the four sections and appeared to consist of sulfur. As far as is known, sulfur does not occur in globular form in the Orgueil meteorite, and the sulfur globules, too, seem to

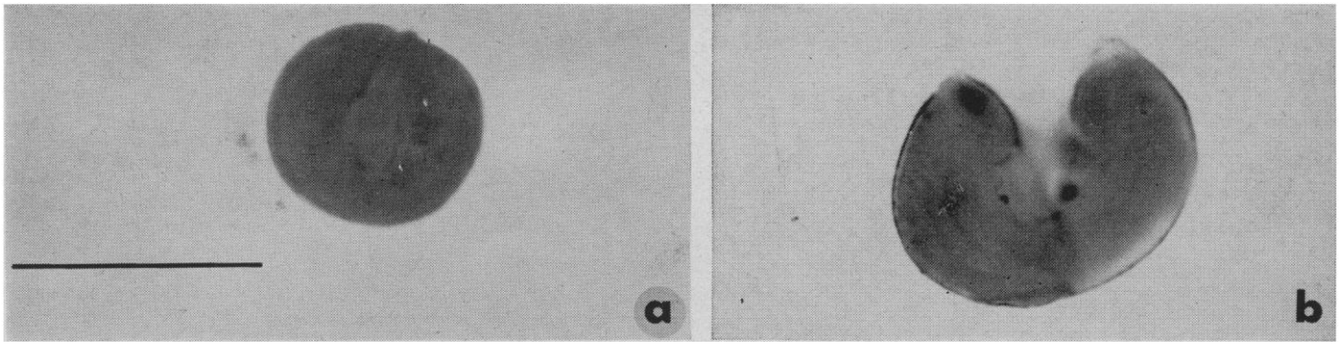

Fig. 5. (*a*) Organized element from preparation of Claus and Nagy. (*b*) Starch grain, stained with the periodic acid-Schiff reaction. (Scale, 20 μ)

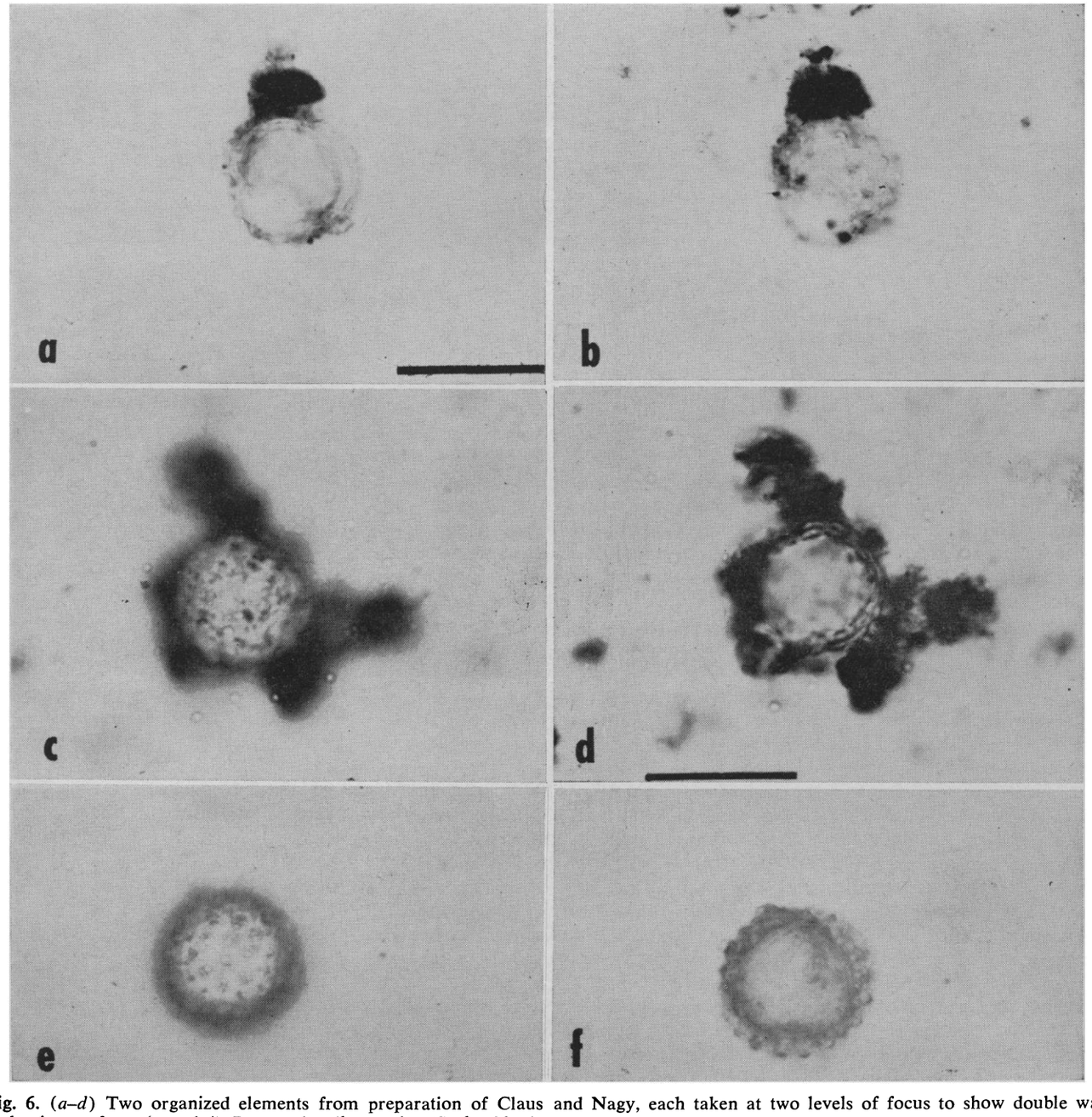

Fig. 6. (*a–d*) Two organized elements from preparation of Claus and Nagy, each taken at two levels of focus to show double wall and spiny surface. (*e* and *f*) Ragweed pollen grain. (Scale, 20 μ)

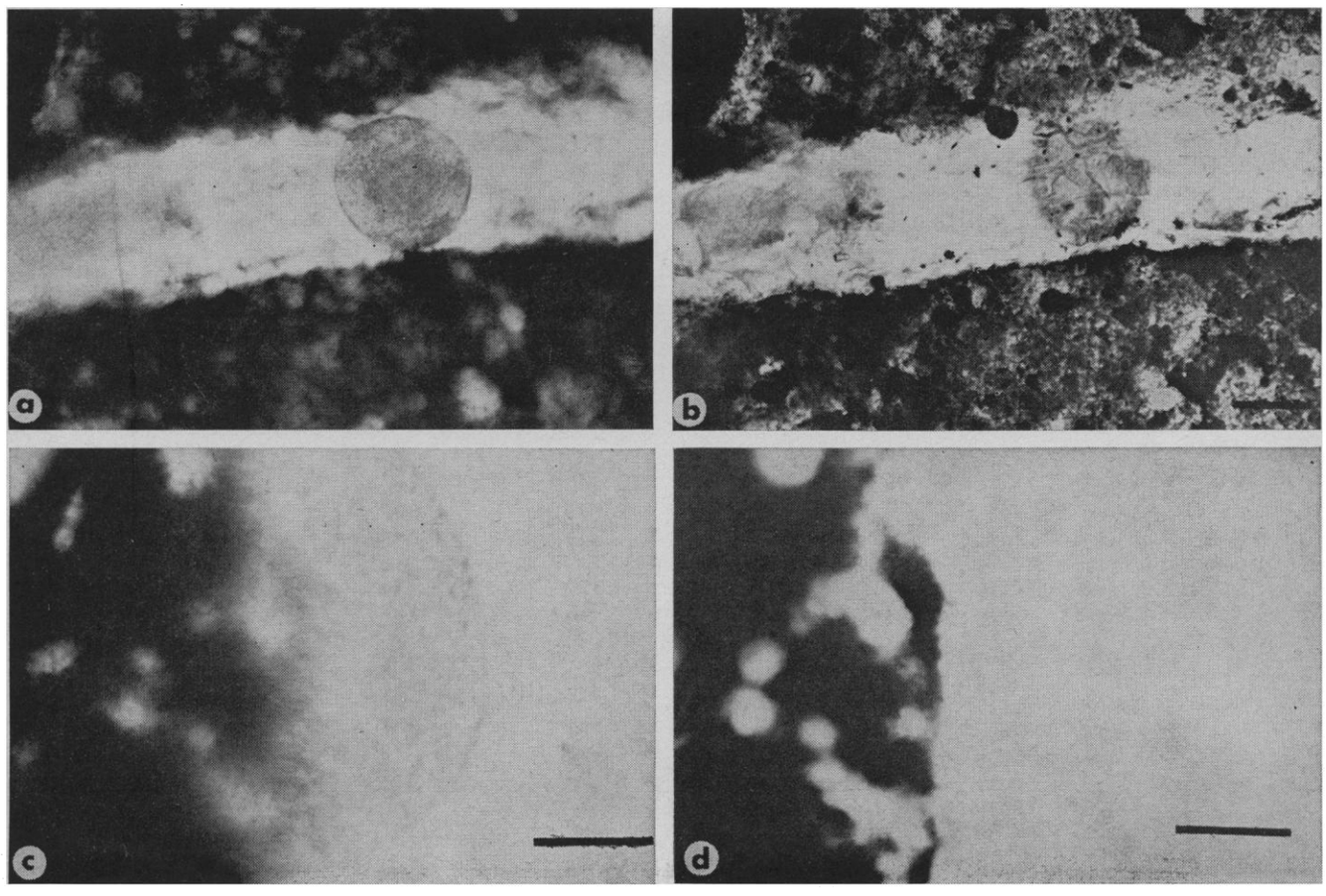

Fig. 7. (*a* and *b*) Spherical particles on the surface of the thin section, at two different levels of focus. (*c* and *d*) Another spheroidal particle, extending beyond the fusion crust. All these particles appear to be artifacts produced during grinding of the section. (Scale, 20 μ)

be artifacts produced during grinding.

The thin sections were scanned under ultraviolet illumination, since the organized elements are reported to show a characteristic greenish-yellow, green, or pink fluorescence that distinguishes them from the minerals having a bluish fluorescence (*7*). No fluorescent particles were found, however, that did not fall in one or the other category of contaminants or artifacts discussed above. Numerous pink particles were seen, but in the vast majority of cases their pink color was not true fluorescence. Ultraviolet-fluorescence microscopes use dark-field illumination, with a high-pressure mercury arc as the light source. The filters used to cut out visible light invariably have an appreciable transmittance in the red portion of the spectrum, so that any birefringent or highly refractile particles appear red when viewed with ultraviolet light in the fluorescence microscope. When a filter that absorbed red light but was transparent to ultraviolet was placed over the light source, the pink color disappeared, showing conclusively that it was not true fluorescence. Without additional tests of this kind it is impossible to determine whether or not the organized element reported to have a pink fluorescence (*7*, Fig. 5) was merely a birefringent mineral grain.

The other organized element from a thin section (*7*, Fig. 4) appears to be opaque, to judge from examination of the original section as well as the published photograph. Previously it had been emphasized that all organized elements in crushed preparations were transparent (*1*, *7*). Moreover, it is not certain that the spines illustrated in the drawing, spaced 0.3 μ apart, are real. This spacing is appreciably smaller than the practical limit of resolution of the microscope, even when allowance is made for the fact that the particles must be viewed through a layer of optically imperfect magnesium sulfate. Some surface structure seems to be present in this particle, but it is probably similar to that of the spherical magnetite particles which the particle otherwise resembles.

Although more than 90 percent of the apparent organized elements in the thin section are undoubtedly artifacts, contaminants, or mineral grains, it is entirely possible that a few of the structures are indeed of extraterrestrial biological origin. Our work shows, however, that even thin sections can abound in artifacts, and that extensive controls and independent tests are required before a given particle can be accepted as indigenous to the meteorite. Sectioning of a plastic-impregnated specimen on the microtome (*25*) might give more conclusive results, as it would eliminate the grinding step.

Discussion

On the basis of these observations it would seem that the organized elements of Claus and Nagy might best be divided into two classes: particles of simple and of highly-structured morphology, respectively. Particles of the first class are definitely indigenous to the meteorite, although their numbers seem to have been overestimated by

Nagy *et al.* As noted, their morphology is simple, and they appear to lack all the other properties suggestive of a biological origin that were previously attributed to them: fluorescence in ultraviolet, biological staining, and insolubility in acids. The density of most of these particles is high, indicating a predominantly inorganic composition. The case for a biological origin thus rests entirely on their featureless morphology. It would seem that further, independent criteria must be developed before a biological origin of these particles can be safely postulated.

Particles of the second class have a complex morphology; some fluoresce in ultraviolet; many take biological stains; others reveal their organic composition by their low density or their insolubility in acids. All these properties speak strongly for a biological origin. However, these particles are exceedingly rare. They have not been found in our samples of the Orgueil meteorite. We cannot completely rule out the possibility that this discrepancy is due to sampling error, although some of our material came from the very stone studied by Claus and Nagy. None of these particles have been conclusively identified in thin sections, and the particles in thin sections most nearly resembling them appear to be artifacts. Many of them show a morphological resemblance to common airborne contaminants. None of the particles of this class seem to have been observed by two or more independent workers; neither Staplin (*10*) nor Palik (*8*) mention particles of the type described by Nagy *et al.*, although they do report other highly structured particles. Thus, the particles of the second class pose a problem exactly opposite to that posed by those of the first class: although it is almost certain that they are of biological origin, it is very unlikely, in the majority of cases, that they are indigenous to the meteorite. It is unfortunate that Nagy and his associates fail to make a distinction between these two classes and thus give the impression that the striking properties of a few particles of the second class (for which contamination was not ruled out) also pertain to the much more numerous, probably indigenous, particles of the first class.

We believe that the problem of organized elements merits further investigation. However, our work shows that the problem is a much more difficult one than has been implied in previous publications. The particles of the first class are in a morphological no-man's-land, and to establish their possible biological origin, new techniques and new criteria will have to be developed. As for particles of the second class, proof must be given that they are not terrestrial contaminants. This, like all negative proof, may be very difficult to obtain, unless present techniques are improved very greatly (*26*).

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27. The fly ash sample was obtained through the courtesy of Drs. P. H. Gregory and H. E. Crossley.

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Electron Microscope Studies of Ribosomal Clusters Synthesizing Hemoglobin

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Ribosomes are RNA-protein particles which have a diameter of approximately 230 A in the electron microscope. For many years it has been believed that they are the site of protein synthesis. However, in studies with rabbit reticulocytes, we have been able to show that the site of hemoglobin synthesis in vivo is not the single ribosome but rather a cluster of ribosomal particles, which we have called a "polyribosome" or simply a polysome (*1*). Somewhat similar observations have also been made by Gierer (*2*). In this paper we describe electron-microscopic studies of this protein-synthesizing structure.

The isolation of this larger unit in protein synthesis rests heavily on recognizing that it is extremely fragile and is easily broken up by mechanical agitation or by enzymatic activity. By using reticulocytes, one can gently lyse the cells in hypotonic buffer and thereby obtain a lysate which has in it all of the components of the hemoglobin-synthesizing cell without having subjected the cell contents to degradation from shear because of too much manipulation. The existence of the polysome was first demonstrated by incubating the cells in vivo with a mixture of C^{14}-labeled amino acids and then lysing the cells. One ml of this lysate was layered on top of a 15 to 30 percent sucrose density gradient in a SW 25 Spinco ultracentrifuge tube and then spun for approximately 2 hours. After this, the tube was punctured and fractions were collected and analyzed. The fractions obtained in this way were kept for electron-microscopic studies. As shown in Fig. 1, the optical density shows two peaks, one of which occurs with a sedimentation constant of 76 S and is associated with the single ribosomal units.

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