

lize in a diffusing and opaque array of microcrystals, precluding optical measurements.

3) They are not inert chemically, especially at the high temperatures (1000 to 2000 K) that are obtainable in the diamond cell and that are necessary for many geophysical problems.

We measured the profile of the ruby luminescence lines up to 150 kbar in solid helium and could see no measurable broadening that would indicate pressure inhomogeneities within the cell. Although this must be checked at higher pressures, it is an indication that helium behaves as a rather "soft" crystal and may well counter the first factor. Concerning the second factor, we could verify (Fig. 2a) that the presence of helium microcrystals does not prevent optical measurements in the cell. Diffusion on grain boundaries is kept at a low level because of the low refractive index of the solid. In other measurements we found it straightforward to crystallize helium in the cell, as a single crystal with no diffusion at all. This would certainly be true at all temperatures, in contrast to metastable ethanol-methanol mixtures. With respect to the third factor, helium is known to be remarkably inert and would probably be the best pressure-transmitting medium, in that respect, for solid-state studies (9).

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Interplanetary Dust: Trace Element Analysis of Individual Particles by Neutron Activation

Abstract. Although micrometeorites of cometary origin are thought to be the dominant component of interplanetary dust, it has never been possible to positively identify such micrometer-sized particles. Two such particles have been identified as definitely micrometeorites since their abundances of volatile and nonvolatile trace elements closely match those of primitive solar system material.

Micrometeorites are micrometer-sized interplanetary dust particles that enter the earth's atmosphere without melting (1). They are collected from the stratosphere by U-2 aircraft (2) and are being studied because a significant fraction may be particles from comets (3). Comets are volatile-rich bodies which may be well-preserved planetesimals that formed at very low temperatures and have been stored at a great distance from the sun, probably for most of the age of the solar system.

We report here the first use of highly sensitive neutron activation analysis techniques to determine trace element abundances of individual micrometeorites. The two particles analyzed [U2-13A1 (70 by 35 μm) and U2-14A6 (42 by 28 μm)] are among the largest of more than 400 extraterrestrial particles that have been collected during 5 years of U-2 flights and are very close to the largest size (for their composition type) that can enter the atmosphere without suffering melting or partial vaporization. Prior to activation analysis, both particles were subjected to routine morphological and qualitative elemental analysis in optical microscopes and scanning electron mi-

croscopes (SEM) with energy-dispersive x-ray (EDX) analysis. In the SEM work, it was necessary to sputter-coat the particles with a 100-Å layer of Pd. The diffraction pattern for particle U2-14A6 (made with a small Debye-Scherrer camera) indicates that the bulk of the particle is composed of amorphous or poorly ordered materials. The only strong lines were from magnetite, a minor phase in the particle. Quantitative EDX analysis of particle U2-13A1 (Table 1) represents an average for the top few micrometers of the particle (about 10 percent of the particle mass) (4).

Both particles are black, opaque aggregates of micrometer- and submicrometer-sized grains. Particle U2-13A1 is porous both because of fine-scale voids between individual constituent grains and because of large open vugs as big as 15 μm across. It is more porous than the most porous meteorites and probably has a density of $< 2 \text{ g cm}^{-3}$. Qualitatively, both of the particles have essentially chondritic compositions and may be classed as chondritic aggregate interplanetary dust particles, the most common type of micrometeorite (2).

The results of neutron activation analyses (5) for the two particles are shown in Table 2. Because there is some uncertainty in estimating the weight of these particles, the observed elemental contents are reported as such without converting into concentrations. The concentration of these elements in C1 chondrites (a group of primitive, chemically undifferentiated meteorites that best represent the cosmic or solar system abundance of chemical elements) is also given (6). Relative abundances of all the elements except Au in the stratospheric particles are very similar to those of C1 chondrites (7). Relative to C1 chondrites, the mean abundances of six nonvolatile elements (Fe, Ni, Cr, Co, Sc, and Ir) in these two particles are 7.33×10^{-8} and 10.5×10^{-8} . If these particles are of C1 composition, then the corresponding weights of 0.07 and 0.11 μg are in good agreement with those inferred from the particle size. The abundance patterns of the particles closely match those of primitive C1, C2, and C3 carbo-

Table 1. Surface composition of particulate matter collected from the stratosphere by EDX analysis in an SEM. Values represent element/silicon ratios (in percent by weight). Particle U2-13A3 is a 7- μm chunk of material which fell from the main mass of particle U2-13A1 during handling.

Element	Particle		Bulk C1 (6)
	U2-13A1	U2-13A3	
Na	0.25*	0.10	0.098
Mg	0.84	0.87	0.890
Al	0.11	0.12	0.099
S	0.86	0.28	0.53
Ca	0.11	0.10	0.10
Ti	0.006		0.005
Cr	0.023	0.025	0.019
Mn	0.030	0.024	0.017
Fe	2.97	2.12	1.72
Ni	0.15	0.11	0.091

*"Laboratory weathering" is probably responsible for the high Na value (Na tends to migrate toward the surface of the particle) and for antlerlike growths. A peculiar aspect of particle U2-13A1 was the growth of antlerlike fibers during a 2-year storage period in the laboratory. The horn-shaped structures were not seen in the original photos taken soon after collection.

Table 2. Abundances of trace elements in two particles collected from the stratosphere. Uncertainties quoted are based on 2σ counting statistics for both samples and standards (σ is the standard deviation).

Element	In particle		In C1 chondrites (g/g)	Abundance relative to C1 chondrites	
	U2-13A1 (g)	U2-14A6 (g)		Particle U2-13A1 ($\times 10^{-8}$ g)	Particle U2-14A6 ($\times 10^{-8}$ g)
Fe	$(1.20 \pm 0.03) \times 10^{-8}$	$(2.28 \pm 0.04) \times 10^{-8}$	1.71×10^{-1}	7.02	13.3
Ni	$(9.18 \pm 1.13) \times 10^{-10}$	$(7.32 \pm 0.95) \times 10^{-10}$	1.03×10^{-2}	8.91	7.11
Na	$(4.86 \pm 0.12) \times 10^{-10}$	$(3.20 \pm 0.17) \times 10^{-10}$	5.11×10^{-3}	9.51	6.25
Cr	$(1.76 \pm 0.03) \times 10^{-10}$	$(3.11 \pm 0.03) \times 10^{-10}$	2.25×10^{-3}	7.84	13.8
Zn	$(4.89 \pm 1.80) \times 10^{-11}$	$(1.07 \pm 0.18) \times 10^{-10}$	3.03×10^{-4}	16.1	35.3
Co	$(3.82 \pm 0.06) \times 10^{-11}$	$(2.83 \pm 0.05) \times 10^{-11}$	4.83×10^{-4}	7.90	5.85
Sc	$(2.90 \pm 0.22) \times 10^{-13}$	$(5.68 \pm 0.22) \times 10^{-13}$	5.10×10^{-6}	5.69	11.1
Au	$(3.03 \pm 0.09) \times 10^{-13}$	$(6.39 \pm 0.12) \times 10^{-13}$	1.52×10^{-7}	199	420
Ir	$(3.42 \pm 0.92) \times 10^{-14}$	$(6.11 \pm 0.79) \times 10^{-14}$	5.14×10^{-7}	6.65	11.9

naceous chondrites (8) (Fig. 1). Although we cannot tell from the neutron activation results alone whether these stratospheric particles resemble C1 or the fine-grained, volatile-rich materials of C2 or C3 chondrites, we can eliminate ordinary chondrites as well as the high-temperature chondrules in the C2 and C3 chondrites as the source material. Ordinary chondrites, which account for about 80 percent of the stony meteorites seen to fall, are depleted in Zn by a factor of 8 relative to C1 chondrites (6). Heating experiments on primitive chondrites show substantial loss of Zn taking place at temperatures $> 600^\circ\text{C}$ (9). Similarly, volatilization studies have established that loss of S occurs at about 900°C (10). Retention of volatiles such as Na, Zn, and S indicates only minimal thermal alteration, although the maximum temperature reached is difficult to accurately es-

timated because the duration of heating during entry into the atmosphere is only about 10 seconds.

Both of the interplanetary dust particles appear to be very primitive, undifferentiated solar system materials with high volatile content. It is remarkable that the analyses of two $50\text{-}\mu\text{m}$ chunks of interplanetary dust should agree with the cosmic abundances for all elements, whereas analyses of similar volumes of the fine-grained "matrix" in C1 meteorites show large depletions (greater than a factor of 2) in Na, S, Ca, Ti, and Mg and the "matrix" in C2 meteorites shows large depletions in Ca (11).

Carbonaceous chondrites are of unique value because it is believed that their compositions approximate that of the solar nebula. They are, however, very rare and only five of the 3000 meteorites that have been examined are clas-

sified as C1 chondrites. Of recovered meteorites that were observed to fall, only 2.6 percent are C1 and C2 chondrites. The results in this report, if generalized to cover other similar micrometeorites, indicate that the major fraction of extraterrestrial bodies that enter the earth's atmosphere are carbonaceous chondrite-like objects. The average elemental composition of interplanetary dust falling onto the moon is also a primitive undifferentiated material very similar to carbonaceous chondrite-like objects (12). Carbonaceous chondrites are rare among the recovered meteorites because of their extreme fragility; most fist-sized pieces disintegrate during entry through the atmosphere. Textural and morphological investigations indicate that many interplanetary particles are distinctly different from carbonaceous chondrites, even though their elemental compositions are approximately the same (13). The solar nebula apparently produced a variety of cometary and asteroidal materials with the same solar elemental abundance but with differing mineralogical, textural, and isotopic compositions.

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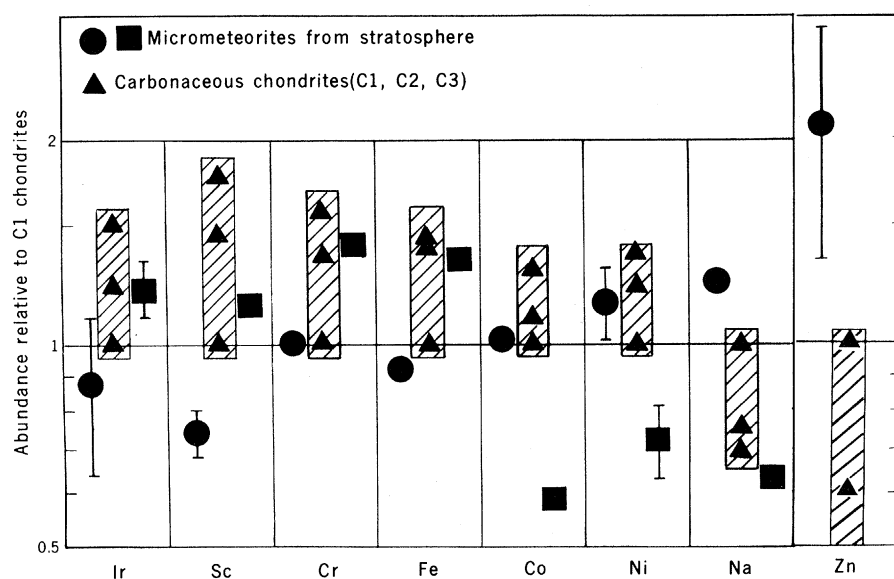


Fig. 1. Abundances of trace elements in the two stratospheric particles relative to C1 chondrites. There is an excellent match in the abundance pattern between the stratospheric particles and C1 chondrites. The presence of volatile elements such as Na and Zn in cosmic proportions with other nonvolatile elements rules out ordinary chondrites as well as high-temperature chondrules in C2 and C3 chondrites as the source material for these particles.

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Opiate (Enkephalin) Receptors of Neuroblastoma Cells: Occurrence in Clusters on the Cell Surface

Abstract. A bioactive, fluorescent derivative of enkephalin, Tyr-D-Ala-Gly-Phe-Leu-Lys-rhodamine, was used to determine the distribution of opiate receptors in living neuroblastoma cells. The receptors appeared in clusters on the cell surface, and no internalization was detected. No specific fluorescence or clusters were observed in the presence of [D-Ala², Leu⁵]enkephalin or at 4°C, and the clusters were much reduced under ionic conditions (that is, with 100 millimolars sodium) that specifically decrease the binding of opiate agonists.

The enkephalins are two naturally occurring pentapeptides, Tyr-Gly-Gly-Phe-Met (Met-enkephalin) and Tyr-Gly-Gly-Phe-Leu (Leu-enkephalin), with morphine-like activities (1, 2). Met-enkephalin and Leu-enkephalin interact with opiate receptors in brain membrane preparations (3) and with specific cell surface receptors in cultured neuroblastoma cells (4, 5). These peptides have been widely implicated as having neurotransmitter or neuromodulator functions at the synaptic junction.

Cultured neuroblastoma cells contain large numbers of opiate (enkephalin) receptors and have been extremely useful in elucidating the cellular and molecular actions of opiates and opioid peptides (4, 5). Neuroblastoma-glioma hybrid cells (strain NG108-15) and N4TG1 neuroblastoma cells have about 100,000 and 20,000 enkephalin-binding sites per cell, respectively (4, 5). The interaction of enkephalin with receptors in neuroblastoma cells has been studied in detail by using ¹²⁵I-labeled derivatives of the metabolically stable enkephalin analog [D-Ala², D-Leu⁵]enkephalin (5).

Image-intensified fluorescence microscopy has proved useful in visualizing the patterns and the mobility of receptors for epidermal growth factor, insulin, and α_2 -macroglobulin in intact cells (6). In the present studies, a bioactive, fluorescent analog of enkephalin was used to visualize enkephalin receptors in neuroblastoma cells. The receptors appeared to form

clusters on the cell surface, and no internalization was detected.

The Tyr-D-Ala-Gly-Phe-Leu-Lys-rhodamine was synthesized by conventional procedures, derivatization being achieved by reaction of the blocked hexapeptide with tetramethylrhodamine isothiocyanate followed by removal of protecting groups; final purification was by thin-layer chromatography (7). The rhodamine peptide inhibits the binding of ¹²⁵I-labeled [D-Ala², D-Leu⁵]enkephalin to brain membrane preparations and NG108 cells with apparent IC₅₀ values (2) of about 5.9 and 5.5 nM, respectively. Cells of strain NG108-15 were grown in Dulbecco's MEM containing HAT and 10 percent FBS; whereas cells of strain N4TG1 were grown in Dulbecco's MEM containing only 10 percent FBS. At confluence, the cells were removed from the flask by incubating them with 1 mM EDTA for 5 minutes at 37°C. The detached cells were washed three times with 50 mM tris-HCl, pH 7.7, containing 0.25M sucrose, 2 mM MgCl₂, and 5 mM glucose; they were then suspended in the same buffer and incubated with the rhodamine-labeled enkephalin analog before being examined by fluorescence microscopy (8). The cells were not fixed for these studies. The results with cells of strain NG108 are shown in Fig. 1.

Many patchy areas of bright fluorescence, spreading over the cell surface, were detected with 5×10^{-8} M rhodamine derivative (Fig. 1A). These clus-

ters were not seen in the presence of 5×10^{-6} M [D-Ala², Leu⁵]enkephalin (Fig. 1B), or with other cell lines (for example, 3T3 fibroblasts, KB epidermoides) that lack opiate receptors. Whereas enkephalin suppressed completely the appearance of clusters, a few clusters could be detected in the presence of high concentrations (10^{-4} M) of naloxone, and a slightly greater number were visible with 10^{-4} M morphine than with naloxone. These findings are consistent with the findings that both naloxone and morphine have very much lower affinities than enkephalin for receptors in neuroblastoma cells, and that the affinity for morphine is lower than that for naloxone (4, 5). At 4°C no discrete clusters could be discerned, even after incubation for 2 hours (Fig. 1C). In the presence of 0.1M NaCl, only a few patches were detected (Fig. 1D), and the brightness and number of patches were diminished substantially (compared with Fig. 1A). Very similar phenomena were observed with N4TG1 cells (data not shown), except that there were fewer patches compared to NG108-15 cells.

These results are consistent with the known characteristics of opiate receptors, and with our previous studies (5) of the interaction of ¹²⁵I-labeled [D-Ala², D-Leu⁵]enkephalin with receptors in neuroblastoma cells. The binding was reduced in the presence of sodium and at 4°C, even after prolonged periods of incubation. The fluorescent clusters probably represent the specific binding sites (receptors) for enkephalins. The non-specific binding of rhodamine-enkephalin, although weak, is uniformly distributed over the cell, making it difficult to determine whether some enkephalin receptors are distributed diffusely as well as in patches. We do not know whether the receptors occur naturally in patches or whether their distribution is altered as a result of binding; the binding (5) of enkephalins to NG108 cells is a relatively slow process, even at 37°C, and the appearance of patches coincides closely with the increase in binding as determined with ¹²⁵I-labeled analogs. Thus a redistribution of occupied receptors into patches may occur more rapidly than binding, making the latter process rate-limiting.

In contrast to epidermal growth factor (EGF) and insulin receptors on fibroblasts (6), where fluorescence labeling shows that the receptors are initially distributed uniformly and quickly form patches that are subsequently internalized (6), the enkephalin-labeled receptor patches appeared only slowly and were not internalized. The visible patch-