

toluidine. Moreover, uptake of [2-¹⁴C]uracil (Schwarz/Mann; specific activity of 62 mCi/mmole) (6) was blocked by proflavine hemisulfate ($1.9 \times 10^{-5}M$; Sigma), a known inhibitor of RNA synthesis (7), but was not blocked by *p*-toluidine ($4.7 \times 10^{-5}M$). Thin-layer chromatography of benzene extracts of supernatants from cultures labeled with *o*-[U-¹⁴C]toluidine (California Bionuclear Corporation; specific activity of 13.7 mCi/mmole) showed no formation of other ¹⁴C-labeled compounds. Stepwise degradation of cells labeled with *o*-[¹⁴C]toluidine according to the method of Pelroy *et al.* (8) did not reveal specific labeling of any particular cell fraction.

None of these lines of investigation gave any clear-cut indication of the metabolic process affected by *p*-toluidine. However, lack of any immediate effect and the gradual loss of viability suggest that the metabolic process affected is a slow one approaching the generation time.

In general, the blue-green algae are much more sensitive to aniline and *p*-toluidine than the bacteria and other algae (Table 1). In the case of three blue-green algae the sensitivity to *p*-toluidine was remarkable. The data herein demonstrate the extreme sensitivity of strain PR6 (growth inhibition by *p*-toluidine and aniline at 1 μ g per disk in the algal lawn assays and 50 ppb in liquid culture). In view of the lack of information on the concentrations and fate of anilines in the environment, it is of interest to note recent reports of aniline and substituted anilines in water-soluble extracts of fuel oils (2), industrial effluents (9), and soils as degradation products of herbicides (10, 11). Aniline residues are also known to be precursors of azobenzenes (11). Games and Hites (9) reported that the concentration of aniline in the final effluent of a dye-manufacturing plant was 10 to 96 ppb, well within the toxicity range of organisms such as strain PR6. Clearly, more information is needed about the biological effects of these compounds in order to better understand their impact on the environment.

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Assembly of Greek Marble Inscriptions by Isotopic Methods

Abstract. *Classical Greek inscriptions cut in marble, whose association as original stelai by archeological methods was debatable, were selected for study. Using traditional geological techniques and determinations of the per mil increments in carbon-13 and oxygen-18, it was determined that fragments could be positively assigned to three stelai, but that fragments from three other stelai had been incorrectly associated.*

The provenance of marble used in classical Greece for inscriptions, sculpture, and other monuments is still a leading problem in archeology, despite at least 100 years of research and debate in the field (1). In describing inscriptions, archeologists commonly give a statement of provenance along with the dimensions and some physical attributes of the stone. The epigraphic literature, however, is full of cases where different authors describe the same stone in contradictory or confusing terms. For example, sample IG I², 732 has been called "weise," "Hymettian," and "Pentelic" in separate publications (2, p. 72). In an attempt to develop a descriptive terminology, without implying provenance, the use of petrographic attributes has been suggested (2). These attributes include both the structural elements present—that is, foliation and lineations—as well as other physical properties such as color, grain size, and the presence of accessory minerals.

In addition to these observations, which can be made with only a hand lens, isotopic ratios of oxygen and carbon have been found useful (3). System-

atic differences in these isotopic ratios were found in samples collected from the principal quarries of classical Greece, and these different values could be seen to characterize each quarry area. Samples from ancient quarries of Paros, Naxos, Penteli, and Hymettus were analyzed and the results plotted as the per mil increment in ¹³C ($\delta^{13}C$) against the per mil increment in ¹⁸O ($\delta^{18}O$) [figure 1 in (3)]. Using isotopic ratios, a provenance can be inferred for a particular classical Greek marble; a sample of only 10 to 20 mg is needed for this type of analysis (4).

In addition to provenance, isotopic ratios of stone fragments bearing different inscriptions can help decide whether the pieces belonged to the same stele; a small difference in isotopic ratios would suggest that the fragments were originally parts of the same material. Although we have not yet determined the variation within a particular block of marble or the possible effects of slight surficial weathering, our data indicate that surficial samples from the same marble block may have a difference of up to 0.4 per mil in both $\delta^{18}O$ and $\delta^{13}C$. If two samples



Fig. 1. Sample EM 8682, oriented with the top to the viewer. The stone is 18 cm thick. Color variation in bluish grays makes up the planes of layering, dipping to the right for EM 8682 (shown) and to the left for EM 8680 (not shown).

have a variation greater than this for either isotope it is unlikely that they come from the same marble unit. The limited data available suggest that the actual variations within a particular marble unit may be around ± 0.1 per mil, close to the experimental accuracy (5).

Inscriptions stored in the Epigraphical Museum and Agora Museum, Athens, the association of which has been debated by archeologists, were selected for study. All stones were first examined with a hand lens and their geological features noted: foliation, lineations, grain size, accessory minerals, and color. Isotopic ratios for oxygen and carbon were determined for six stelai; these data are reported in the conventional δ notation relative to the PDB (Pee Dee belemnite) standard. Samples are identified in standard epigraphic notation: EM numbers are from the Epigraphical Museum; I from the Agora Museum, and IG from *Inscriptiones Graecae*.

EM 4598/EM 7442 (IG II², 739). Both have similar structures comprised of (i) very weak foliation of aligned and flattened calcite crystals and some mica-rich planes which dip at a low angle to the bottom, and (ii) weak lineation by aligned mica flakes running top to bottom. The grain size is about 1 mm, with larger grains up to 4 mm.

The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values (Table 1) for both pieces are very close, differing by 0.2 and 0.1 per mil, respectively. The isotopic ratios are not diagnostic for the well-known classical quarries of Mount Hymettus or Mount Penteli but suggest an origin in unknown quarries in the upper part of Mount Penteli (6). Both the geological structures and the isotopic ratios permit the pieces to be associated (7).

EM 8682 (IG II², 2937)/EM 8680 (IG

Table 1. Values of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (relative to PDB) for inscriptions.

Inscriptions	$\delta^{13}\text{C}$ (per mil)	$\delta^{18}\text{O}$ (per mil)
EM 4598/EM 7442	2.29/2.51	-5.13/-5.00
EM 8682/EM 8680/I 5810	2.21/2.29/2.61	-1.74/-1.82/-1.54
EM 7981/EM 8005	2.37/2.43	-3.92/-3.93
EM 13354A/EM 13354	2.97/2.60	-7.94/-8.24
EM 2685/EM 6958	1.53/2.70	-3.41/-5.79
EM 12892/EM 13393	2.22/2.16	-4.83/-2.41

II², 2423)/I 5810 (8). All three are composed of fine-grained, bluish gray marble, with grains averaging $\frac{1}{4}$ to $\frac{1}{2}$ mm in diameter. A well-developed color layering dips about 20° to the right in the case of EM 8682 (Fig. 1), and about 10° to the left in both EM 8680 and I 5810. The reversed direction of dip in EM 8682 compared to EM 8680 and I 5810 makes it highly unlikely that the first piece was ever part of the same stele as the second and third.

The isotopic ratios of all three pieces are fairly close. The difference in both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ is 0.1 per mil between the EM pieces and about 0.3 per mil between I 5810 and the EM pieces. The isotopic ratios allow the association of the three stones.

A reasonable deduction from these data is that two separate stelai were made at about the same time from marble obtained from the same quarry; perhaps the same quarried slab was split to make the two stelai. The isotopic ratios suggest that the marble is Hymettian (9).

EM 7981/EM 8005/EM 12910. All have similar geological structures: a very weak foliation plane consisting of flattened marble grains, $\frac{1}{4}$ to 1 mm in diameter, averaging about $\frac{1}{2}$ mm and with no apparent lineations present on the face. Each has an irregular plane of fracture with a delineation shown by slickensides

and with no apparent accessory minerals. The geological structures allow the association of all three pieces.

Isotopic ratios were not determined for EM 12910, but they are virtually identical for EM 7981 and EM 8005 and permit the two pieces to be associated. The isotopic compositions are not diagnostic for the known Pentelic or Hymettian quarries.

The treatment of the backs of these inscriptions shows the fallacy of using the nature of the working as a criterion for reassembling a stele (Fig. 2). The geological and isotopic data present a strong case for associating the stones, yet the workings on the backs of EM 7981 and EM 8005 are very different. Sample EM 8005 alone has two distinctly different types of working (10).

EM 13354/EM 13354A. Both have similar geological features. The foliation and lineations are very weak; a foliation may be almost parallel to the faces, and a weak mineral orientation of calcite grains runs from top left to bottom right at an angle of about 100° to the lettering. The grain size is about $\frac{1}{2}$ mm, with many grains up to $\frac{3}{4}$ and 1 mm. Altered pyrite, as iron hydroxides, is common. Each stone has a different thickness, but the backs slope up noticeably toward the top, implying that the stele might originally have been cut with an upward ta-

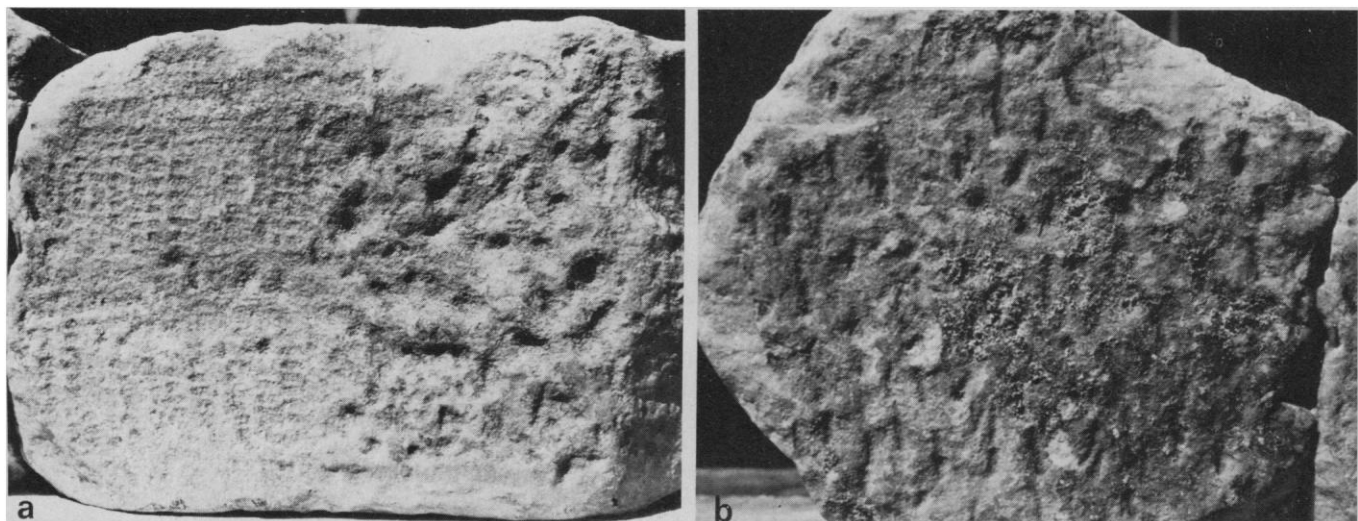


Fig. 2. Samples EM 8005 (a) and EM 7981 (b). The two pieces can be associated by geological and isotopic criteria, yet three different kinds of working are apparent on their backs. The join between the pieces in (b) is 15 cm long.

per. The geological structures allow the association of the two pieces.

The isotopic ratios are moderately close: the difference is 0.4 per mil for $\delta^{13}\text{C}$ and 0.3 per mil for $\delta^{18}\text{O}$. The isotopic ratios are diagnostic for Pentelic marble and allow the association of the stones (11).

EM 2685 (IG II², 185)/EM 6958 (IG II², 121). Geological features in both inscriptions are similar. Prominent fold axes on the backs run from top to bottom, parallel to the lettering (Fig. 3). The grain size in both is about 1/2 to 1 mm; accessory micas are present. Sample EM 2685 is white to dark gray, and EM 6958 is white to yellowish white. Because these colors have been affected by weathering, we cannot know how much credence to give to this criterion.

Although the geological features allow an association, the isotopic ratios are sufficiently different that it is impossible for these stones ever to have been part of the same stele. The difference is 1.2 per mil in $\delta^{13}\text{C}$ and 2.4 per mil in $\delta^{18}\text{O}$. The isotopic values suggest that EM 2685 came from quarries on Mount Hymettus and EM 6958 from Mount Pentelikon (12).

EM 12892/EM 13393. Geological features of the two are similar. Foliation is very weak and appears to be almost flat, dipping about 10° or less to the bottom. Mica is abundant in both and forms a lineation, as seen on the face that runs from top to bottom. The grain size is generally 1/4 to 1/2 mm but reaches a maximum of 1 mm. Both pieces are white to gray in color, but EM 12892 shows more severe weathering effects. The isotopic data do not allow the inscriptions to be associated (13).

Conclusions. In many of the inscriptions examined, we discovered that the ancient quarrymen took advantage of the fact that marble tends to split most easily along its foliation planes as well as along its more prominent lineations. Thus any inscription with a foliation plane parallel to its face and a lineation perpendicular to the lettering direction, running from top to bottom, was oriented that way by the quarryman for ease of cutting. Most of the stelai have the same orientation; all that can be said about two separate inscriptions with similar foliation and lineation features is that their geological structures permit an association. Other geological features such as color, banding, grain size, and accessory minerals should also be examined. If all geological features match, the isotopic ratios of the inscriptions should be determined before they are associated as parts of the same original stele.



Fig. 3. Sample EM 6958. A strong lineation by fold axes that run from top to bottom and parallel to the direction of lettering on the face is seen on the back. The stone is 22 cm wide.

In any particular stele, the variation in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values should be less than about 0.4 per mil. If the differences are greater, the stones could not have been part of the same original. We recommend that whenever there is doubt about the association of stones, both a detailed examination of the geological features and an isotopic analysis be carried out. An association is justified only if both agree. The techniques described here as well as our conclusions are equally applicable to fragmentary marble sculpture.

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10. Sample EM 12910 was associated with the other two pieces (IG II², 1654) by E. Schweigert [*Hesperia* 7, 268 (1938)]. The association was rejected by W. E. Thompson [*ibid.* 39, 56 (1970)], primarily on the basis of the spacing of the letters.
11. These two stones, which, according to P. Peppas-Delmouzou, director of the Epigraphical Museum, were found together and identified by workers in marble at the National Archaeological Museum of Greece as being from the same stele, were associated by M. T. Mitsos [*Archaeol. Ephemeris* (1965); p. 134]. Later, O. W. Reinmuth [*The Ephebic Inscriptions of the Fourth Century, B.C.* (Leiden, 1971), pp. 1–4] referred to them as "the earliest known ephebic inscription." In favor of the disassociation of the two pieces are A. G. Woodhead, [*Suppl. Epigr. Graecum* 78, 23 (1968)], D. Lewis [*Classical Rev.* 23, 254 (1973)], and (at great length) F. W. Mitchel [*Z. Papyrol. Epigr.* 19, 233 (1975)]. Mitsos replied to his critics in *Archaeol. Ephemeris Chron.* (1975), p. 39.
12. A. Wilhelm's association of the two pieces was communicated to J. Kirchner, who recorded Wilhelm's opinion in his commentary to IG II² 185. M. J. Osborne [*Annu. Br. Sch. Athens* 66, 323 (1971)] rejected the association.
13. The two stones from the north slope of the Acropolis were tentatively associated by R. S. Stroud [*Hesperia* 40, 174 (1971)].
14. The original idea for this study came from W. K. Pritchett, who also supplied the epigraphic references. Procedures and conclusions are our own responsibility. We thank J. E. Noakes and B. K. Sen Gupta for critically reviewing the manuscript. We acknowledge support from the American Philosophical Society, Johnson Fund grant 1270 (to N.H.), and NSF grant DES 74-13268 (to D.B.W.). We are also indebted to P. Peppas-Delmouzou for her kind cooperation and assistance.

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Rabies Viruses Increase in Virulence When Propagated in Neuroblastoma Cell Culture

Abstract. Several strains of attenuated rabies virus lacking the capacity to kill adult mice acquired a high lethal potential for mice after one to five serial passages in murine or human neuroblastoma cells. The virulence acquired after passage in neuroblastoma cells is a stable genetic trait retained during subsequent passage of viruses in nonneuroblastoma cell systems.

Myriad types of animal-pathogenic viruses, including rabies virus, have been reduced in virulence by passage in cell cultures. Diverse cell culture systems, often selected serendipitously, have been used, and the attenuation obtained has usually required many serial passages. Flury high egg passage (HEP) rabies virus, widely used as a live virus

veterinary vaccine, was attenuated, as indicated by loss of lethal potential for adult mice, after 174 passages in chick embryos (1). This virus regained pathogenic potential for adult mice after passage in newborn mice (1) but has never been reported to revert to virulence after passage in cell culture.

Enhancement of virus virulence after