made to distinguish between solutions made on the basis of different time scales that would, in principle, behave differently if the gravitational constant were variable. This study points out (implicitly) a second fundamental problem of the usual modes of determining $\ddot{L}$: only angular measures are used, and they are not all well defined (*7*) and none are sensitive to independent changes in a.

In fact, it is necessary to replace Eq. 3 with the relation

$$2\delta\dot{n}/n + 3\delta\dot{a}/a = \delta\dot{G}/G \qquad (4)$$

with the condition that one use independent partial derivatives with respect to n and a, as well as observations that are sensitive to both parameters. We have calculated numerical partial derivatives for both of these quantities independently (using appropriate variations of the earth-moon mass), and we have used these derivatives to perform a differential correction of the lunar orbit to fit 2034 laser range observations obtained at the McDonald Observatory between September 1969 and October 1976 (*8, 9*). The positions of the moon were calculated from the unpublished but widely available numerical ephemeris known as LURE2 (*10*), which was constructed with a tidal acceleration $\ddot{L} = -40$ arc sec/cy^2, following Oesterwinter and Cohen (*11*) and Van Flandern (*6*). A previously reported attempt with fewer data (*9*) gave a result whose quoted uncertainty was as large as the correction, and thus of no practical interest. Our result is a correction $\Delta\ddot{L} = +15.4 \pm 1.6$ arc sec/cy^2, resulting in a value for the tidal acceleration of

$$\ddot{L}_t = -24.6 \pm 1.6 \text{ arc sec/cy}^2$$

The value cited is that which we consider to be the best founded from an ensemble of solutions for which we used two different ephemerides, two different libration models, and data spans of 6 and 7 years. The cited uncertainty is a formal standard deviation from the adopted least-squares solution, and must therefore be regarded with extreme caution. All of the various solutions lay in the range -22 to -26 arc sec/cy^2, which suggests that a more realistic error estimate would be about ± 5 arc sec/cy^2.

In the series of solutions that gave this result, we also solved for other pertinent parameters, including the telescope and reflector coordinates, the other orbital elements, the harmonic coefficients of the lunar gravity field, and several global parameters for the rotation of the earth. All were within reasonable bounds, and the standard deviation of the postfit residuals over 7 years' data was 2.8 nsec, or 42 cm in equivalent one-way distance. We also tried to solve for $\dot{a}$ simultaneously with $\ddot{L}$, but the results were inconclusive. This was not unexpected, because the total change in the mean distance over the observed interval implied by our result given above is only about 15 cm (the level of observational noise) if the gravitational constant is really constant (*11*). These solutions did not perturb the solution for the secular acceleration significantly, but the values implied for the secular variation of G spanned a range including zero and Van Flandern's result. It will require several more years before the cosmological question of $\dot{G}$ can be resolved with any confidence.

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2. O. Calame and J. D. Mulholland, paper presented at the Symposium on Tidal Friction and Earth Rotation, Bielefeld, West Germany, 1977.
3. We follow the convention that a superior dot represents a time derivative. The relation given is imposed by our procedure, although we realize [and explained in (*2*)] that the literature is confused on this point by an ambiguity in the definition of the perturbed mean motion, which leads to different expressions for its time derivatives, by a factor of 2 in the case of $\dot{n}$.
4. In nearly all discussions, $\dot{n}$ is supposed to be constant, largely because no geophysically plausible mechanism can account for a significant variation over historic time.
5. A complete and readable history of this subject is found in P. M. Muller, "An analysis of the ancient astronomical observations with the implications for geophysics and cosmology," published privately by the author, 1975.
6. T. C. Van Flandern, *Mon. Not. R. Astron. Soc.* **170**, 333 (1975).
7. Some of the angular measures are not well defined with respect to the center of mass (CM), because they are low-precision measures of the edge of the visible disk. Some have unique systematic errors. If there is dissipation in the rotation, or if long-period effects are neglected, then the CM may appear to accelerate relative to the Watts center, used in reducing such data.
8. For discussions of the techniques required, see O. Calame, *Manuscr. Geod.* **1**, 173 (1976).
9. J. D. Mulholland, in *Scientific Applications of Lunar Laser Ranging*, J. D. Mulholland, Ed. (Reidel, Dordrecht, 1977), p. 9.
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12. While it may strike the reader as curious that we seem to say that the range data are more sensitive to angle than to distance, this is not really the case. The linear displacement along the orbit corresponding to our result is about 200 m.

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Alkaloids in Whole Plant Material: Direct Analysis by Kinetic Energy Spectrometry

Abstract. *A new approach to mixture analysis has been applied to the direct detection of various alkaloids in plant materials. The method requires absolutely no sample treatment. Results are presented for cocaine, morphine, papaverine, coniine, and atropine. The signal-to-background characteristics are superior to those of conventional mass spectrometry. Sensitivity is sufficient to detect and identify between 1 and 10 nanograms of alkaloid.*

The analysis of complex mixtures is often complicated by the effort necessary in sample treatment before the components can be actually identified. We report here a new approach to mixture analysis, developed from mass spectrometry, which involves absolutely no sample treatment. We present results on the analysis of alkaloids in plant materials, exemplified by the identification of papaverine in raw opium (Fig. 1). The useful sensitivity of the technique is also shown to be better than that obtained in conventional mass spectrometry.

The basis for the technique has been described elsewhere (*1*). The sample is ionized to produce, among other things, a molecular ion of each component in the mixture. The ion of interest is mass-analyzed and allowed to react with a target gas at high kinetic energy, which causes fragmentation of the ion. By using kinetic energy analysis to identify these fragments, one can deduce the structure of the initial ion (*2*). The spectrum obtained, termed a MIKE (mass-analyzed ion kinetic energy) spectrum, closely resembles the fragmentation pattern observed in the mass spectrum of the pure compound. Thus, the MIKE technique provides a method of obtaining the mass spectrum of a given ion corresponding to a particular component of the mixture.

We have used the MIKE technique in the analysis of simple mixtures (*1, 3*). Success in the analysis of plant extracts for various alkaloids (*4*) prompted us to attempt the analysis of alkaloids from whole plant tissues (*5*).

We analyzed freshly cut poison hemlock (*Conium maculatum* L.) for the purpose of detecting the poisonous alkaloid coniine (*6*). Figure 2 illustrates the signal and background characteristics of the method: the top portion shows the background mass and MIKE spectra, and the

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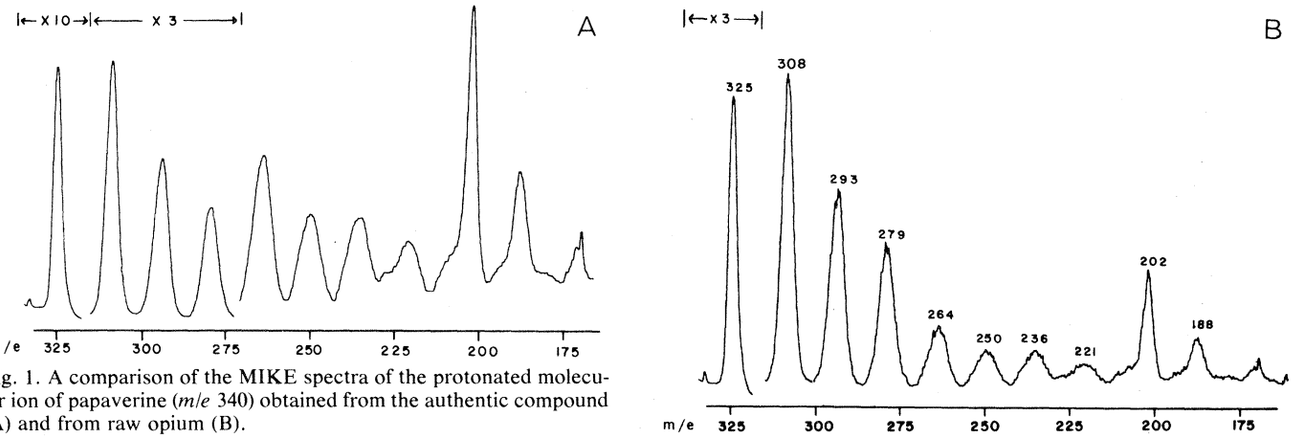

Fig. 1. A comparison of the MIKE spectra of the protonated molecular ion of papaverine (*m/e* 340) obtained from the authentic compound (A) and from raw opium (B).

bottom portion shows these spectra after the sample has been introduced. The protonated alkaloid of interest, mass-to-charge ratio (*m/e*) 128, has not increased relative to the background in the mass spectrum, and yet its presence is readily detected from the MIKE spectrum. Figure 3 illustrates the fragmentation pattern of coniine and serves to confirm the presence of the alkaloid. The presence of what are apparently the two major alkaloids in this plant, γ-coniceine (*m/e* 126) and conhydrine (*m/e* 144), is indicated from the mass spectrum. The analysis of coniine therefore serves to point out the usefulness of the MIKE technique in the identification of minor components in very complex mixtures and demonstrates that it is not necessary to analyze pure compounds in order that positive identifications be made.

To demonstrate further the scope of the technique, we attempted to identify a variety of alkaloids from different plant materials. One system chosen was raw opium from *Papaver somniferum* L. (*7*). Morphine (*m/e* 268) was readily identified; an authentic sample was also analyzed, and the two spectra matched exactly. The amount of morphine introduced into the instrument was estimated to be ~ 50 μg. This identification prompted us to study another opium alkaloid, papaverine, which was at least ten times less abundant in the sample. Figure 1 compares the MIKE spectrum of the molecular ion (*m/e* 340) for the plant material and reference papaverine and shows the signal-to-noise characteristics obtained in the analysis. From these data, we estimate the sensitivity of the technique to be currently on the order of 1 to 10 ng (*8*).

We expanded the variety of plants studied by identifying cocaine in *Erythroxylon coca* L. leaves and atropine in *Datura stramonium* L. seeds. The parent ions of both components

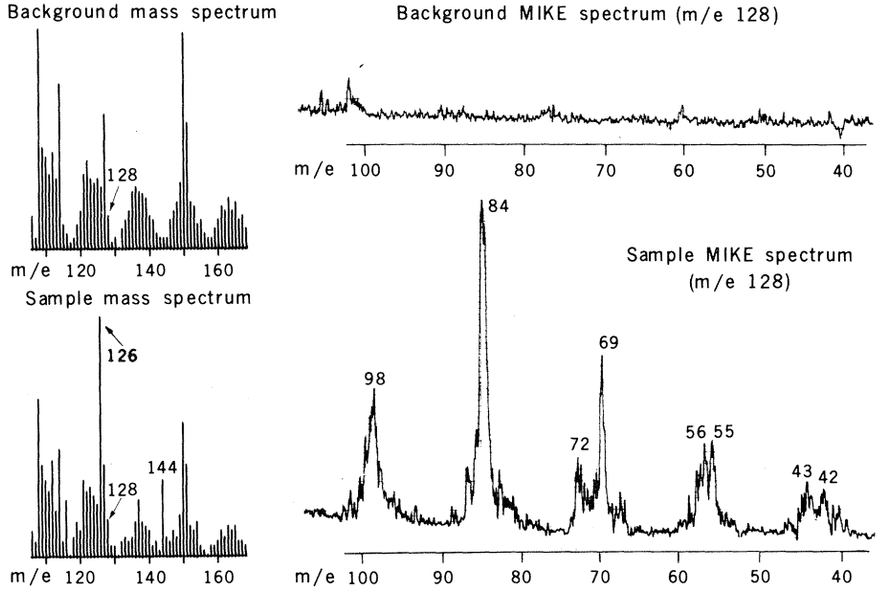

Fig. 2. A comparison of background mass and MIKE spectra (top portion) with those obtained upon sample introduction (bottom portion) for *Conium maculatum* L. Energy spectra are taken on the ion of *m/e* 128, corresponding to the molecular ion of coniine, in the presence of other major components of the plant, for example, *m/e* 126 and 144.

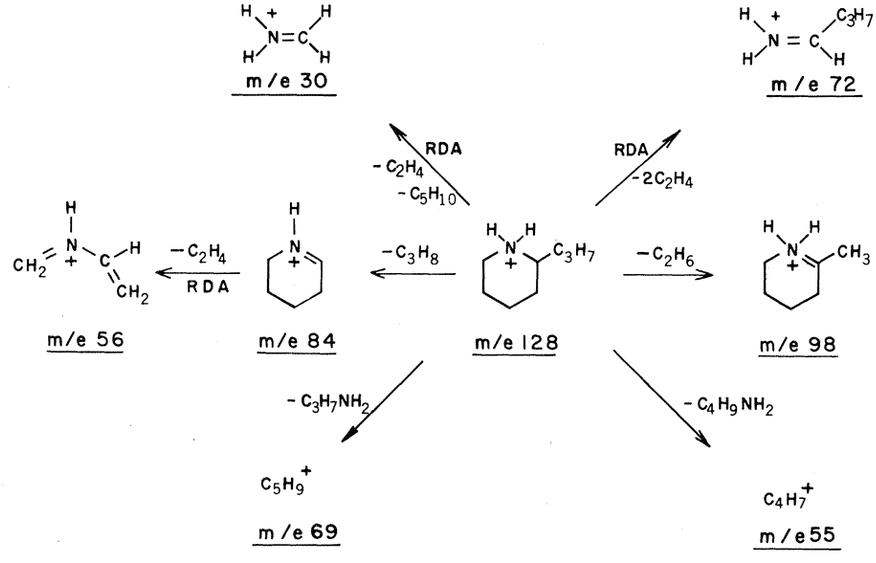

Fig. 3. Fragmentation pattern of coniine.

were readily identified on the basis of exact matches of the energy spectra with those of the authentic compound spectra, and the major fragmentations observed are those expected (*9*). These data clearly demonstrate the capabilities of the MIKE technique for the direct analysis of plant materials.

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5. Plant tissues were ground under liquid nitrogen to facilitate cell breakage and inserted into the ion source by means of the direct insertion probe. We carried out the chemical ionization with isobutane reagent gas. Source temperatures were maintained between 240° and 300°C, and supplementary probe heating (to 200°C) was needed only in the analysis of atropine. Nitrogen was used as the target gas at an indicated pressure of 2×10^{-5} torr.
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7. The CI mass spectrum of opium yields ions that are characteristic of the individual alkaloids [S. Zitrin and J. Yinon, *Anal. Lett.* **10**, 253 (1977)]. This procedure is less reliable than that reported here and is not generally applicable to components of complex mixtures.
8. Recent results have extended MIKE's detection limit to 10^{-10} to 10^{-11} g (G. A. McClusky, R. W. Kondrat, R. G. Cooks, in preparation).
9. The most abundant ions in the cocaine spectrum are due to loss of 32 (16 percent), 106 (28 percent), 122 (100 percent), and 138 (12 percent). The corresponding ions in atropine are due to loss of 32 (100 percent), 94 (31 percent), 148 (30 percent), and 166 (56 percent).
10. We thank Profs. T. L. Kruger and J. W. Amy for valuable discussions. This work was supported by the National Science Foundation.

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Chick Embryonic Skin as a Rapid Organ Culture Assay for Cellular Neoplasia

Abstract. *We used chick embryonic skin (CES) in organ culture to assess the neoplastic potential of a variety of cultured human and nonhuman cell lines. Cells derived from cancer tissues grew in CES and formed tumors in nude mice while cells derived from normal tissues grew in neither system. The CES proved to be more sensitive than the nude mouse when used to assay SV40 transformed human cells; each of four such lines grew in CES while only one of the four lines grew and formed tumors in nude mice. In addition, the patterns of invasion by inoculated cells can be easily studied in the CES. These results suggest that CES in organ culture offers an inexpensive, rapid, and reliable alternative to the nude mouse as a tumorigenicity test.*

Tumor formation at the site of inoculation has been the ultimate criterion for the transformed phenotype of tissue culture cells (*1*). For human cells in culture, tumorigenicity tests in vivo in xenogeneic hosts have been developed and include systems such as the nude mouse (*2*), the antithymocyte serum (ATS)-treated newborn hamster (*3*), the ATS-treated mouse (*4*), and the antithymocyte globulin (ATG)-treated nonhuman primate (*5*). While the formation of a tumor in these systems defines the transformed phenotype, the lack of tumor formation could be due to nonimmune rejection mechanisms (*6*), an insufficient number of cells inoculated (*7*), or other unknown factors (*8*). These models in vivo also have the disadvantages of maintenance and upkeep of animals, the need for immunosuppression, and occasionally lengthy times for tumor formation.

Because of these considerations and our preliminary findings that differences in sensitivity to tumor formation exist among various animal models, we were interested in developing a method for tumorigenicity testing that would be inexpensive, rapid, and reliable. Previous work suggested that the invasiveness of cells into chick embryonic skin (CES) might be related to malignancy (*9*). We found that human tumor cell lines did show extensive invasion in CES; however, we also found that normal fibroblasts such as WI-38 showed invasion as well. In attempts to find a marker for neoplasia other than invasion we considered the fact that tumor formation in xenogeneic models results from proliferation of inoculated tissue culture cells. We reasoned that CES in organ culture inoculated with cells would closely mimic the subcutaneous inoculation of cells in an animal, and that growth of the inoculated cells might then be considered equivalent to tumor formation in an animal. Further support for this idea came from early experiments in which the human tumor cell lines WiDr (*10*) and HT-1080 (*11*) formed macroscopic tumors in nude mice 2 weeks after inoculation, whereas the human diploid fibroblast line WI-38 (*12*) did not. Three days after inoculation we could find viable and actively mitotic WiDr and HT-1080 cells by microscopic examination of inoculation sites. In contrast, WI-38 cells were necrotic 1 day after inoculation with no mitoses and by 3 days no WI-38 cells were distinguishable. Thus, in vivo, the tumorigenicity of inoculated cells could be predicted by the presence of actively growing cells 3 days after inoculation.

Figure 1 illustrates the results when cells are inoculated onto CES. Figure 1A shows a fragment of CES inoculated only with medium after 3 days in culture. The skin was viable throughout its depth and showed the ability of the agar base to maintain tissue structure. Figure 1B shows a fragment of CES cultured for 3 days with WiDr cells. The skin substrate was viable and clusters of epithelioid cells with nuclei much larger than the chick tissue cells were seen. The cell clusters showed vigorous mitotic activity and invasion, and had the same histological appearance as tumors formed by the injection of WiDr cells into nude mice. The HT-1080 cells gave similar results. Another culture of CES with WiDr was minced and explanted into a 25-cm^2 flask (Costar). The resulting monolayer had a mixed pattern with many round colonies of large, epithelioid cells against a background of small fibroblasts. The epithelioid colonies had the same morphology as WiDr cells inoculated alone into parallel flasks. Poliovirus inoculated onto the mixed monolayer lysed only the epithelioid cells, indicating that they were of primate and not avian origin. As another test to demonstrate that the epithelioid cells were WiDr, the mixed monolayer was challenged with a medium containing only galactose as a sugar source, because chick cells cannot survive in such a medium (*13*). Most of the background fibroblasts died after 3 days while the epithelial colonies survived. In control experiments, chick cells derived from 9-day-old embryos cultured alone died in this medium while WiDr cells cultured alone survived. When WI-38 cells were cultured on CES for 3 days they showed no evidence of mitoses in the inoculated cells; in some cases the WI-38 cells became necrotic. Thus, the ability of inoculated cells to proliferate in the CES correlated with proliferation and tumorigenicity in vivo.

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