

for interpreting stratigraphic data and for use in prediction is p_{ij} . The experimental situation is such that $x = n_{ij}$ and N_{ij} are known and p_{ij} must be inferred. Exact knowledge of p_{ij} is prohibited by the limitation of finite sampling. However, statistical techniques can be applied to estimate p_{ij} and obtain a confidence interval.

To obtain information about p_{ij} we introduce the maximum-likelihood estimator $\hat{p}_{ij} = x/N_{ij}$. The density function of $\hat{p}_{ij}$ is

$$f(\hat{p}_{ij}; p_{ij}) = \frac{N_{ij}!}{(N_{ij}\hat{p}_{ij})!(N_{ij} - N_{ij}\hat{p}_{ij})!} p_{ij}^{N_{ij}\hat{p}_{ij}} (1 - p_{ij})^{(N_{ij} - N_{ij}\hat{p}_{ij})} \quad (3)$$

$\hat{p}_{ij} = 0, 1/N_{ij}, 2/N_{ij}, \dots, 1; i \neq j$

The value of the estimator $\hat{p}_{ij}$ obtained from N_{ij} repetitions of the experiments is $\hat{p}'_{ij} = n_{ij}/N_{ij}$. These values of the estimator are recognized to be the matrix elements of Eq. 1. The usual methods for calculating the confidence intervals for the binomial distribution make use of the fact that the distribution function of Eq. 2 can be approximated by the normal distribution for large numbers of repetitions (for large N_{ij}) when the distribution possesses a minimum amount of skewness ($p_{ij} \approx 1/2$). However, typically the data available to the stratigrapher do not satisfy this approximation. Thus, we have formulated a technique for calculating confidence intervals that are applicable even when the number of repetitions of the experiment is small and the distribution is highly skewed. To be specific, we have a method for calculating the value of the lower end point p_{ij}^l and the upper end point p_{ij}^u of the confidence interval such that $P(p_{ij}^l \leq p_{ij} \leq p_{ij}^u) = .90$. The conditions that the p_{ij}^l and p_{ij}^u must satisfy are

$$\sum_{\hat{p}_{ij} = \hat{p}_{ij}^l}^1 f(\hat{p}_{ij}; p_{ij}^l) = 0.05 \quad (4a)$$

$$\sum_{\hat{p}_{ij} = 0}^{\hat{p}_{ij}^u} f(\hat{p}_{ij}; p_{ij}^u) = 0.05 \quad (4b)$$

To perform the necessary summations, we make use of the relation

$$\sum_{s=a}^n \frac{n!}{s!(n-s)!} p^s (1-p)^{n-s} = Q(F_{v_1, v_2}) \quad (5)$$

where $v_1 \approx 2(n-a+1)$, $v_2 = 2a$ and $p = a/[a + (n-a+1)F]$. The $Q(F_{v_1, v_2})$ are related to the incomplete beta functions (4).

The results of the statistical analysis describing the ordering of events i and j are conveniently summarized in Fig.

1b. The actual probability of the ordering p_{ij} and its observed estimator p'_{ij} lie within the confidence interval between p_{ij}^l and p_{ij}^u . The width of this interval, $\delta_{ij} = p_{ij}^u - p_{ij}^l$, is represented by the distance between the points p_{ij}^l and p_{ij}^u .

The values of p_{ij}^l calculated from Eq. 4a are collected in Table 1 as a function of N_{ij} and n_{ij} for $N_{ij} = 1, 2, \dots, 16$. Similarly, values of p_{ij}^u are calculated from Eq. 4b. However, it is more instructive to consider the width of the confidence interval δ_{ij} , which is presented in Table 2 as a function of N_{ij} and n_{ij} . For fixed values of the ratio $\hat{p}'_{ij}$, δ_{ij} is a monotonic decreasing function of N_{ij} , and for large N_{ij} it decreases as $1/N_{ij}^{1/2}$. This slow decrease in the width of the confidence interval with increasing N_{ij} must be weighed by the stratigrapher against the time and money to be invested in measuring, collecting, and studying samples from the stratigraphic sections.

The closer p_{ij} approaches 1 the more certain the relation $\tau_i < \tau_j$ between events i and j becomes. Hence for stratigraphic correlation it is desirable to have a parameter which expresses how closely p_{ij} approaches 1. Two necessary conditions for p_{ij} to approach 1 are: (i) $\hat{p}'_{ij}$ must approach 1 and (ii) $1 - p_{ij}^l$ must approach 0. Thus we can define a single reliability parameter ($R = \hat{p}' / (1 - p_{ij}^l)$), which incorporates both of these conditions. The most reliable sequence of events is that set of events which maximizes R between each pair of events. For sequences with the same number of events or stratigraphic levels,

the most reliable is that which has the largest geometric average value of R . The largest values of the geometric average of R correspond to sequences with a small number of levels. As the number of levels increases, this reliability parameter tends to decrease. Depending on the application, the stratigrapher must weigh the usefulness of a fine stratigraphic subdivision with a large number of levels against the increased reliability obtained by considering a smaller number of levels.

JOHN R. SOUTHAM

WILLIAM W. HAY

Division of Marine Geology and Geophysics, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida 33149

THOMAS R. WORSLEY

Department of Oceanography, University of Washington, Seattle 98195

References and Notes

1. International Subcommission on Stratigraphic Classification, *Lethaia* **5**, 283 (1972).
2. A. B. Shaw, *Time in Stratigraphy* (McGraw-Hill, New York, 1964); J. A. Jeletzky, *J. Paleontol.* **39**, 135 (1965); A. E. Cockbain, *ibid.* **40**, 206 (1966); W. W. Hay and P. Cepek, *Am. Assoc. Pet. Geol. Bull.* **53**, 721 (1969); R. B. McCammon, *Bull. Geol. Inst. Univ. Upps.* **2**, 49 (1970); J. E. Hazel, *Geol. Soc. Am. Bull.* **81**, 3237 (1970).
3. W. W. Hay, *Eclogae Geol. Helv.* **65**, 255 (1972).
4. M. Abramowitz and I. A. Stegun, *Handbook of Mathematical Functions* (Government Printing Office, Washington, D.C., 1972).
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Molecular Geometry in an Excited Electronic State and a Preresonance Raman Effect

Abstract. *Observations of Raman spectra of various molecules at different exciting laser wavelengths lead to an empirical rule. If a Raman line becomes stronger when the exciting frequency is brought closer to the frequency of an electronic band, this means that the equilibrium conformation of the molecule is distorted along the normal coordinate for the Raman line in the transition from the ground to the excited electronic state.*

In examining the Raman spectra of various molecules, we have observed that some Raman lines become more intense as the exciting frequency approaches the absorption frequency corresponding to an excited electronic state. Some of the results of such examinations are given in Table 1, where the amount, ξ , of the intensity enhancement caused by changing the exciting

laser beam from 5145 Å to 2573 or 3511 Å is given for several Raman lines. The 5145-Å and 3511-Å laser beams were obtained with a Coherent Radiation model 52G argon ion laser, and the 2573-Å beam was obtained with the same laser by converting the fundamental power at 5145 Å in a nonlinear optical crystal, ammonium dihydrogen phosphate.

The ammonia molecule, NH_3 , is pyramidal in its ground electronic state, $\tilde{X}$, and planar (1) in its lowest excited electronic state, $\tilde{A}$ (at 2168 Å). Therefore, on going from $\tilde{X}$ to $\tilde{A}$, the molecule should experience an umbrella motion. Let us now examine the Raman spectrum of ammonia. The Raman line corresponding to the ammonia umbrella vibration appears at 1053 cm^{-1} in the liquid and at about 940 cm^{-1} as a doublet (because of the inversion) in the gas. This is a rather weak Raman line of ammonia. However, when the exciting light is changed from green (5145 Å) to ultraviolet (3511 Å)—that is, when the exciting frequency is brought nearer to the $\tilde{A} \leftarrow \tilde{X}$ absorption band—the intensity of the Raman line corresponding to the umbrella vibration is enhanced more than that of any other line in Table 1.

The lowest-frequency absorption band ($\tilde{A} \leftarrow \tilde{X}$) of methylamine,

CH_3NH_2 , is found at about 2200 Å. An examination of the vibrational fine structure (2) of this band indicates that the amino group is planar in $\tilde{A}$ and pyramidal in $\tilde{X}$. Therefore, the greatest change in the molecular geometry on going from $\tilde{X}$ to $\tilde{A}$ is expected to take place along the amino wagging coordinate.

In the Raman spectrum of methylamine, the two lines at 952 and 1178 cm^{-1} show the greatest resonance effect when the exciting light is changed from 5145 to 2573 Å. These lines are assigned to two coupled modes of amino wagging and methyl rocking (3), so that both involve the amino wagging mode. Note that the intensity enhancement of the CN stretching Raman line at 1044 cm^{-1} when the exciting light is changed from 5145 to 2537 Å is only of the same order of magnitude as that of the CH_3 degenerate deformation line.

This is in contrast with the case of formamide, HCONH_2 . Here, the line at 1315 cm^{-1} assigned to the CN stretching vibration (4) becomes markedly stronger when the exciting beam is changed from 5145 Å to 2573 Å. The next greatest intensity enhancement is observed for the carbonyl stretching line at 1672 cm^{-1} . Then how about the excited state geometry of this molecule? In the transition from $\tilde{X}$ to $\tilde{A}$ (corresponding to the 1717-Å band of formamide vapor) one of the lone-pair electrons on the nitrogen atom is brought up onto the antibonding π^* orbital of the carbonyl group (5), and therefore the CN bond is expected to become shorter and the CO bond longer. The next lowest excited electronic state, $\tilde{B}$, is a $\pi \rightarrow \pi^*$ state (corresponding to the 1570-Å band). Here, the CO bond is also expected to be longer than that in $\tilde{X}$.

The next example is *cis*-dichloroethylene, ClCH=CHCl . This compound shows the lowest-frequency electronic band ($\tilde{A} \leftarrow \tilde{X}$) in the region from about 2000 to 2300 Å. As in the case of ethylene, it is likely that the molecule has a twisted conformation around the CC bond in equilibrium in the excited state (6), with the result that the CC bond is longer than in the ground electronic state. Correspondingly, in the Raman spectrum of this compound, the intensity enhancement is greatest for the lines assigned to the CC stretching vibration (1579 cm^{-1}) and the torsional oscillation (401 cm^{-1}).

Finally, among the Raman lines of propargyl alcohol, $\text{HC}\equiv\text{CCH}_2\text{OH}$, two at 217 and 552 cm^{-1} show prominent intensity increases as the frequency of the exciting light increases. These lines are assigned to vibrational modes in which in-plane CCC and CCO bending take place (7). On the basis of our knowledge of the acetylene molecule (8), it is likely that the CCC part of propargyl alcohol is not linear but is bent in equilibrium in the lowest excited state.

Thus, we consider that we have evidence for a general rule: If a Raman line becomes stronger when the exciting frequency is brought closer to the frequency of an electronic band $\tilde{A} \leftarrow \tilde{X}$, then the equilibrium conformation of the molecule is distorted along the normal coordinate for the Raman line in the transition from the ground state ($\tilde{X}$) to the excited state ($\tilde{A}$). The theoretical background of this rule will be discussed in detail elsewhere (9).

Table 1. Preresonance Raman effects. The intensity enhancement is defined by $\xi(3511 \text{ Å}) = [I\lambda_s^4/I_s\lambda_s^4]_{3511}/[I\lambda_s^4/I_s\lambda_s^4]_{5145}$ or $\xi(2573 \text{ Å}) = [I\lambda_s^4/I_s\lambda_s^4]_{2573}/[I\lambda_s^4/I_s\lambda_s^4]_{5145}$, where I and λ are the intensity and wavelength of the Raman scattering excited by the laser beam, whose wavelength is given in angstroms as a suffix, and I_s and λ_s are the intensity and wavelength of the Raman line used as an internal intensity standard. Standard lines are indicated by parentheses in the ξ column. Letters in parentheses in the assignments specify the symmetry types of the vibrations.

Vibrational frequency (cm^{-1})	Assignment	$\xi(3511 \text{ Å})$	$\xi(2573 \text{ Å})$
<i>Ammonia (gas)</i>			
933 } 968 }	Umbrella vibration	10.6 9.5	
3220	Overtone of degenerate deformation	2.3	
3334	Symmetric stretching	(1.0)	
<i>Ammonia (liquid)</i>			
1053	Umbrella vibration	3.16 ± 0.43	
1616	Degenerate deformation	(1.00)	
<i>Methylamine (aqueous solution)</i>			
952	CH_3 rocking + NH_2 wagging		1.9 ₂
1044	CN stretching		0.9 ₁
1178	NH_2 wagging + CH_3 rocking		3.2 ₀
1358	NH_2 twisting		1.1 ₅
1438	CH_3 symmetric deformation		1.0 ₃
1476	CH_3 degenerate deformation		(1.00)
1612	NH_2 scissoring		1.6 ₉
<i>Formamide (aqueous solution)</i>			
1095	NH_2 rocking		(1.00)
1315	CN stretching		1.7 ₄
1393	CH in-plane deformation		1.1 ₅
1598	NH_2 scissoring		0.9 ₃
1672	CO stretching		1.2 ₇
2896	CH stretching		0.9 ₅
<i>cis-Dichloroethylene</i>			
401	Torsion (A_2)	1.59 ± 0.16	
561	CCl deformation (B_1)	1.24 ± 0.16	
707	CCl stretching (A_1)	(1.00)	
1178	CH in-plane deformation (A_1)	1.25 ± 0.13	
1579	CC stretching (A_1)	1.67 ± 0.20	
<i>Propargyl alcohol</i>			
217	CCC in-plane deformation (A')	1.48 ± 0.14	
308	CCC out of plane deformation (A'')	1.23 ± 0.12	
552	CCO in-plane deformation (A')	1.69 ± 0.22	
911	CC stretching (A')	(1.00)	
1034	CO stretching (A')	0.88 ± 0.09	

The rule should be valid not only for Raman lines corresponding to totally symmetric vibrations but also for those corresponding to non-totally symmetric vibrations (10).

This rule should be useful for predicting molecular geometries in excited electronic states. For example, we found that in the Raman spectrum of urea, $(\text{NH}_2)_2\text{CO}$, in aqueous solution a line at 1470 cm^{-1} , assigned to the antisymmetric NCN stretching vibration (11), becomes markedly stronger when the exciting light is changed from 5145 to 2573 Å. On the basis of the empirical rule, we predict that in the lowest excited electronic state $\tilde{A}$ of this molecule there is a distortion along the antisymmetric NCN stretching coordinate. This means that we are predicting a double-minimum potential function in $\tilde{A}$.

The rule would also be useful as an additional means of characterizing Raman lines of more complicated molecules. In the Raman spectra of proteins and nucleic acids, the biologically interesting environment of a chromophore group is often reflected in the intensities (rather than the frequencies) of its Raman lines. Our rule would be helpful in understanding such a situation.

AKIKO Y. HIRAKAWA
MASAMICHI TSUBOI

Faculty of Pharmaceutical Sciences,
University of Tokyo, Hongo,
Bunkyo-ku, Tokyo, Japan

References and Notes

1. A. E. Douglas, *Discuss. Faraday Soc.* No. 35 (1963), p. 158.
2. M. Tsuboi, A. Y. Hirakawa, H. Kawashima, *J. Mol. Spectrosc.* **29**, 216 (1969).
3. A. Y. Hirakawa, M. Tsuboi, T. Shimanouchi, *J. Chem. Phys.* **57**, 1236 (1972); unpublished results.
4. I. Suzuki, *Bull. Chem. Soc. Jap.* **33**, 1359 (1960). This assignment is also supported by unpublished results of M. Tsuboi and I. Suzuki on the effect of ^{15}N substitution.
5. S. Nagakura, *Mol. Phys.* **3**, 105 (1960).
6. A. J. Merer and L. Schoonveld, *Can. J. Phys.* **47**, 1731 (1969); P. D. Foo and K. K. Innes, *J. Chem. Phys.* **60**, 4582 (1974).
7. R. A. Nyquist, *Spectrochim. Acta Part A* **27**, 2513 (1971).
8. C. K. Ingold and G. W. King, *J. Chem. Soc.* (1953), pp. 2725 and 2745; K. K. Innes, *J. Chem. Phys.* **22**, 863 (1954).
9. M. Tsuboi and A. Y. Hirakawa, in preparation. A brief description was given by M. Tsuboi at the 4th International Conference on Raman Spectroscopy, 25 to 29 August 1974, Brunswick, Maine.
10. M. Tsuboi and A. Y. Hirakawa, in *Jasco Report* (Jasco Co., Hachijai, Tokyo, 1972), vol. 9, No. 6.
11. A. Yamaguchi, *Nippon Kagaku Zasshi* **78**, 1319 (1957); ———, T. Miyazawa, T. Shimanouchi, S. Mizushima, *Spectrochim. Acta* **10**, 170 (1957).
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Fetal Hemoglobin Restriction to a Few Erythrocytes (F Cells) in Normal Human Adults

Abstract. During adult life, the quantity of fetal hemoglobin (HbF) present in F cells—that is, rare erythrocytes which are reactive with rabbit antiserum to human HbF during microscopic immunodiffusion—is sufficient to account for all of the small quantity (less than 0.7 percent) of HbF normally present in whole blood. Thus, erythrocytes are normally heterogeneous with respect to the presence of HbF.

Trace amounts of fetal hemoglobin (HbF) persist in nearly all normal human adults (1). In this report we show that the bulk of this HbF, rather than being uniformly dispersed among all erythrocytes, as it is in heterozygotes (2) for hereditary persistence of fetal hemoglobin (HPFH), is confined to a few erythrocytes.

Our evidence depends on the detection in normal adults of rare erythrocytes—termed F cells (3)—which develop microscopic pericellular immunoprecipitates (4, 5) on reaction with antibodies to HbF (6). Representative F cell immunoprecipitates are illustrated in Fig. 1, a to c, and contrasted in Fig. 1, d and e, with immunoprecipitates surrounding erythrocytes from a heterozygote and a homozygote for HPFH. The assertion that F cells contain the bulk of HbF present in whole blood rests on the rough concordance observed (Fig. 2), in each of 11 nor-

mal adults, between the percentage of HbF found by macroscopic immunodiffusion assay (7) of whole blood hemolyzate and the percentage contributed by F cells alone (Fig. 2). All of these analyses depended on the preparation (3) of rabbit antiserum which, after appropriate immunoadsorption, was reactive only with HbF. Antiserum specificity was verified by (i) formation of a solitary HbF-centered arc following immunoelectrophoresis of umbilical cord blood hemolyzate containing both HbF and adult hemoglobin (HbA); (ii) an immunodiffusion reaction of identity with cord blood and with purified (8) HbF derived from an HPFH homozygote who entirely lacks (9) adult hemoglobins; and (iii) nonreactivity with a hemoglobin-free mixture of erythrocyte proteins.

Percentages of HbF in whole blood hemolyzates were obtained with immunodiffusion gels (7) prepared at 37°C from a mixture containing 1.5 percent (weight to volume) agarose (Sea Plaque, Rockland, Maine) in sterile borate-buffered saline solution

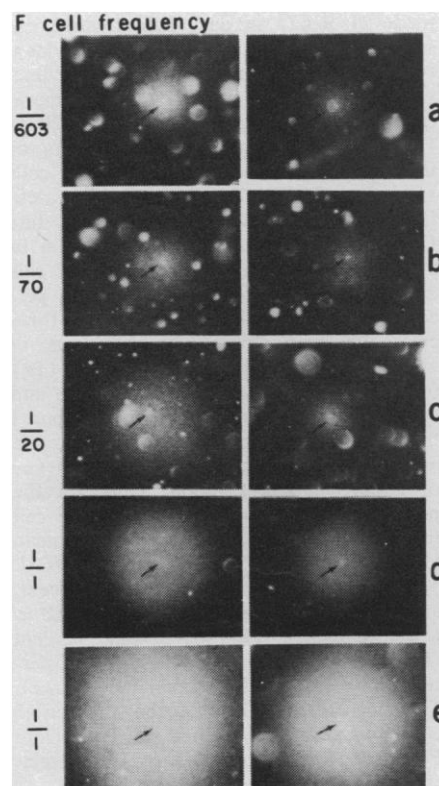


Fig. 1. Dark-field photomicrographs ($\times 200$) of single cell immunodiffusion reactions (3–5). In each photomicrograph, the arrow at the center of the galaxy-like pericellular immunoprecipitate denotes the erythrocyte ghost remaining after disruption of cells and release of hemoglobin into agarose saturated with rabbit antiserum to human HbF (3). The various-sized globes, particularly evident in (a) to (c), represent not erythrocyte ghosts but out-of-focus refractile bubbles and debris. At the microscope, these artifacts are not easily confused with either ghosts or immune reactions. (a to c) Reacting erythrocytes (arrows) are F cells from three different normal human adults; (d) erythrocytes from a woman heterozygous for HPFH (mean, 12.7 pg of HbF per cell); and (e) erythrocytes from her homozygous son (mean, 23.7 pg of HbF per cell). In each row, two reactions are shown for each individual. Frequency of reacting cells among all erythrocytes in whole blood is given for each subject on the left.