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, $(NH_2)_2CO$, 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*

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12. Supported by grants from the Mitsubishi Foundation and the Ministry of Education of Japan.

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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 normal 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

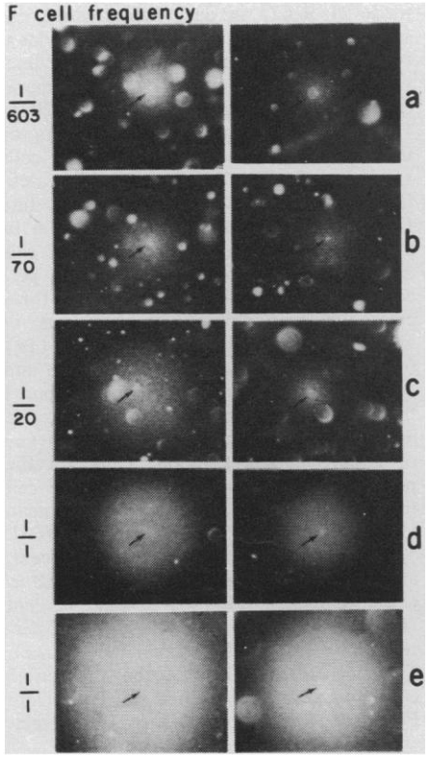


Fig. 1. Dark-field photomicrographs (× 200) of single cell immunodiffusion reactions (*3–5*). In each photomicrograph, the arrow at the center of the galaxy-like pericellular immunoprecipitate denotes the erythrocyte ghost remaining after disruption of cells and release of hemoglobin into agarose saturated 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.

(*p*H 8.4) (*10*) and 9 percent (by volume) rabbit antiserum reactive solely with HbF. Portions (8 $\mu$l) of individual hemolyzates, prepared by freezing and thawing freshly drawn whole blood and diluted to contain known concentrations (30 to 100 mg/ml) of total hemoglobin, were allowed to diffuse from wells ~2.5 mm wide for 3 days at 24°C. The diameter of the immunoprecipitate surrounding each well was then measured and the concentration of HbF determined (*7*) by reference to a standard line obtained with 8-$\mu$l portions of solutions containing 0.05 to 1.0 mg of HbF per milliliter; the correlation coefficient for regression of immunoprecipitate areas on known concentrations of HbF was 0.995. Our standards contained whole blood hemolyzate from an HPFH homozygote, who exhibits only HbF (*9, 11*). With such standards, the concentration of HbF was gauged by spectrophotometry of cyanomethemoglobin derivatives without recourse to the more elaborate procedures (*12*) required when mixtures of HbF and HbA in less precisely known proportions are employed (*13*) for standardization.

Erythrocytes from the HPFH homozygote and from his heterozygous mother were also used for standardization of the microscopic immunodiffusion analysis of F cells. This standardization involved (i) determination of mean diameters of pericellular immunoprecipitates (Fig. 1) for the HPFH heterozygote and homozygote; (ii) estimation of mean corpuscular hemoglobin (MCH) (*14*) for each HPFH subject; and (iii) measurement, by the ratio of phenylalanine to isoleucine (*12*), of the percentage of HbF present in the HPFH heterozygote (*15*). The resulting two-point standard line obtained when reaction diameter was plotted against the logarithm of the HbF concentration (in picograms per cell) was exactly parallel to, and nearly coincident with, a three-point standard line obtained with the antibody dilution method of Gitlin *et al.* (*4*). In the latter procedure, erythrocytes from the HPFH homozygote were reacted with different dilutions of antiserum to HbF.

Allowing for the imprecision in estimating the frequency of F cells and measuring the sometimes poorly defined boundaries of pericellular immunoprecipitates (*16*), Fig. 2 shows that the F cell contribution of HbF is adequate to account for all of the HbF present in whole blood hemolyzates during adult life. Since the percentage of HbF within F cells is restricted to a comparatively narrow range (~ 14 to 28 percent of total cellular hemoglobin) in all subjects, it is not surprising that the approximately 30-fold range of hemolyzate HbF should be matched by a corresponding approximately 20-fold range in F cell frequencies. Thus, the individual with the least HbF (~ 0.03 percent) in whole blood hemolyzate (*11*) had the lowest number of F cells (1 per 603 erythrocytes), while those with the greatest percentage of hemolyzate HbF (~ 0.5 to 0.7 percent) had the greatest abundance of F cells (1/20 to 1/62). This roughly linear relation between the quantity of HbF in whole blood hemolyzate and the frequency of F cells also exists in conditions where the percentage of HbF is increased (*17*). For example, among women in the 23rd and 31st weeks of pregnancy and among adults with a variety of types of leukemia, there is usually a slight to moderate increase in whole blood HbF; this increase is invariably accompanied by a proportional increase in F cell frequency (*17*). In other words, the increase in HbF in these situations seems to develop from an amplification of cells which are present as a kind of atavism in all of us.

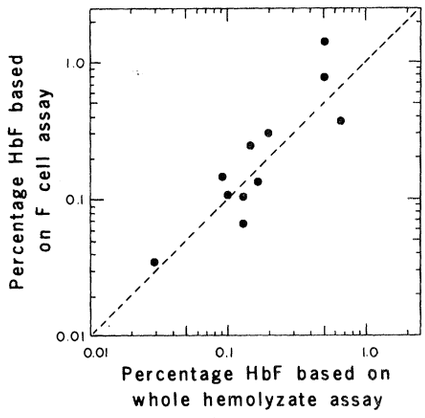


Fig. 2. Percentage of HbF contributed by F cells alone plotted against percentage of HbF measured in hemolyzates of whole blood for 11 normal adults (8 men and 3 women). Measurements were made in duplicate under the conditions noted in the text or in the legend to Fig. 1. Microscopic measurements of many reaction diameters for each subject were made at × 625 in dark field by one observer who was unaware of the individual results depicted on the abscissa. Ordinal percentages were derived by (i) enumeration of F cell frequency (range, 1/20 to 1/603); (ii) estimation of diameters of immunoprecipitates surrounding F cells (for example, Fig. 1, a to c); (iii) calculation, by reference to a standard line (*11*), of average HbF per F cell (4.6 to 9.0 pg per cell); (iv) conversion of this quantity to a percentage (14 to 28 percent) following estimation (from erythrocyte and hemoglobin concentrations in whole blood) of the average MCH (*14*) for all erythrocytes (30 to 37 pg); and (v) expression of the total HbF contributed by F cells as the product of the percentage of HbF per F cell times the frequency of F cells in whole blood. Thus, for the point closest to the dashed line, the F cell frequency was 1 in 148 erythrocytes; mean HbF per F cell, 5.1 pg; overall MCH, 32 pg, and HbF per F cell, 15.9 percent; and contribution of HbF from F cells = (15.9 percent) (1/148) = 0.11 percent (compare with the estimate of 0.10 percent HbF in whole blood hemolyzate). The dashed line denotes the theoretical concordance attained when the HbF from F cells accounts for all the HbF detectable in whole blood hemolyzates.

The mechanism underlying these findings is uncertain; we do not know whether F cells arise from the same clones of erythroid stem cells generation after generation or represent regulatory accidents which consistently but randomly appear in different stem cell lines in each erythroid generation. Whatever the explanation, the results are of fundamental interest for they point to the need for cell-based analyses of other fetal proteins which reappear in the course of malignant transformation (*18*).

The finding (*17*) that the production of F cells is sharply increased during a period of pregnancy has potential therapeutic significance for the management of sickle cell anemia, for it is known (*19*) that sickling and its effects are attenuated by whole blood concentrations of HbF higher than 5 to 10 percent. Since the quantity of HbF (14 to 28 percent) within F cells already exceeds the concentration which can prevent sickling, it is possible that the selective enrichment of F cells could ameliorate sickle disease.

SAMUEL H. BOYER
THOMAS K. BELDING
LOUISE MARGOLET
ANDREA N. NOYES

*Division of Medical Genetics and Clayton Laboratories, Department of Medicine, Johns Hopkins University and Hospital, Baltimore, Maryland 21205*

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15. Ratios of phenylalanine to isoleucine for the HPFH heterozygote were measured in hydrolyzates obtained directly from washed erythrocytes. This procedure differs from that previously reported (*12*) insofar as the hemolyzates used here were not otherwise fractionated. The validity of our procedure, when applied to the HbF-rich hemolyzate from the HPFH heterozygote (88 percent HbF during an episode of iron-deficiency anemia; MCH, 14.4 pg per cell) who is also heterozygous for thalassemia, was established by a parallel finding of nearly perfect agreement between observed (3.84) and expected (3.75) phenylalanine/isoleucine ratios when erythrocytes from the HPFH homozygote (100 percent HbF; MCH, 23.7 pg per cell) were examined in the same way.
16. Our resolution of the diameter of each pericellular immunoprecipitate (Fig. 1) was abetted by repeated changes in focal plane, a maneuver not easily illustrated. Although many reaction diameters still remained hard to define, two different observers (S.H.B. and T.K.B.), looking at unidentified preparations, repeatedly obtained similar measurements; furthermore, the mean reaction diameter of F cells from any one subject was distinctive and remained grossly constant in day-to-day sampling. In any case, the crude nature of diameter estimates does not affect our conclusions. For example, among six subjects whose F cell contributions of HbF lie above the dashed line in Fig. 2, arbitrary twofold depreciation of mean pericellular diameters would only lead to a closer fit to that line. Accordingly, diameter estimates, while the least reliable of our measurements, weigh no more heavily in their errors than the other forms of measurement used.
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11 October 1974


# Vaterite: A Mineralization Product of the Hard Tissues of a Marine Organism (Ascidiacea)


Abstract. *X-ray diffraction and infrared absorption spectra show that the spicules of the common tropical ascidian,* Herdmania momus, *are mineralized with vaterite. These are the first strictly marine organisms known to normally precipitate vaterite. The biomineralization of vaterite may constitute another link between the urochordates and vertebrates. The vaterite of ascidian spicules immersed in natural seawater remains mineralogically unchanged for 1 year, which indicates that vaterite may be preserved transiently in marine sediments.*


Vaterite ($CaCO_3$) is a metastable polymorph of crystalline calcium carbonate. It is easily precipitated in the laboratory, but it is rare in nature as it usually converts to one of the more stable carbonate phases, aragonite or calcite, and possibly to monohydrocalcite ($CaCO_3 \cdot H_2O$) (*1*). Vaterite is precipitated in nature by a variety of organisms, both normally and under pathologic conditions (*2*, *3*). It has been found also in artificially induced skeletal repair and in laboratory cultures under abnormal conditions (*4*, *5*). In the sea vaterite is synthesized by two fish species: *Acipenser sturio*, a sturgeon, which migrates occasionally from rivers to marine waters, and *Gadus morrhua*, a cod, which deposits vaterite under pathologic conditions (*3*).

Here we report the normal skeletal mineralization by vaterite of a sessile, benthic marine animal, the tunicate, *Herdmania momus* (Savigny, 1816) (Urochordata: Ascidiacea), and comment on its possible evolutionary and sedimentary implications. *Herdmania momus* is a highly variable species (or species complex), widely distributed in warmer seas. It is most abundant in nearshore waters 0 to 30 m in depth, but occasionally occurs below 500 m (*6*). Its mineralized hard parts consist of fusiform spicules, fringed by rows of

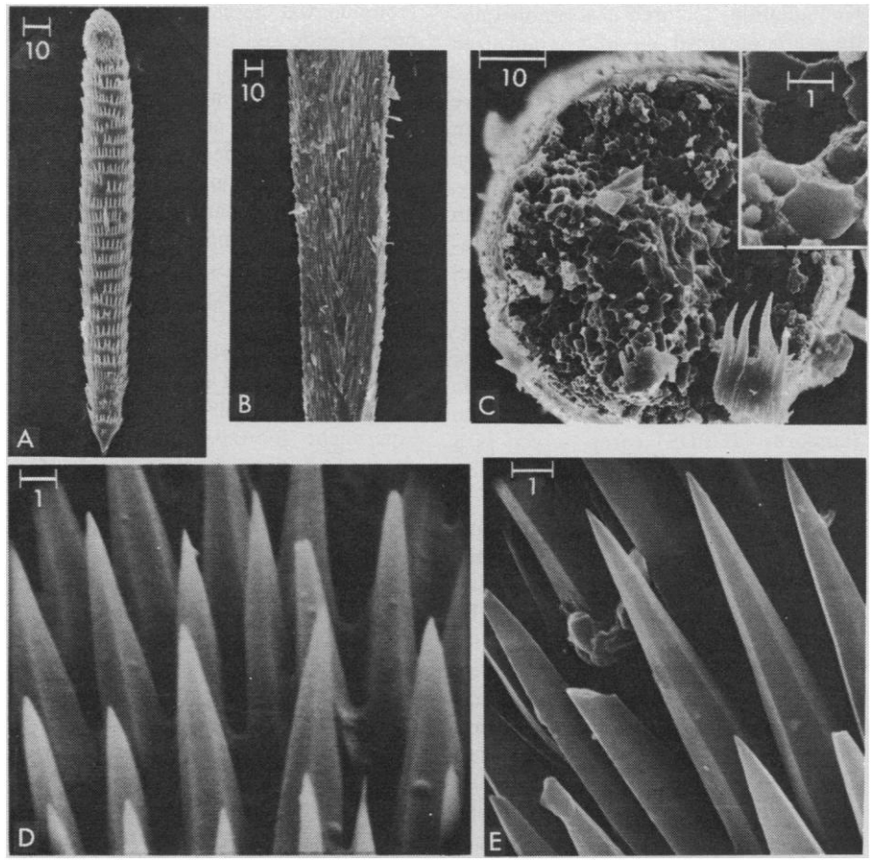


Fig. 1. Scanning electron micrographs showing spicule morphology (A); crystal fabric of the spicule core as seen in a lengthwise cut (B) and in cross section (C); arrangement and crystal habits of fringing spines (D); and crystal shapes of fringing spines of a spicule after exposure to seawater for 1 year; note the patchy, crystal coatings (E).