

per milliliter, only a factor of 10 less than the titer of SFFV<sub>AP-L</sub> stocks prepared by helper-virus rescue and assayed under similar conditions.

Although the results in Fig. 2 show no evidence that recombination has occurred between the defective M-MuLV and SFFV in the  $\psi$ -2 cells to yield a replicating helper virus, it is possible that a low titer of such a virus may be amplified in the mouse and be responsible for the results obtained. This, however, does not appear to be the case since grossly diseased spleens from mice infected with SFFV<sub>AP-L</sub>( $\psi$ -2) showed no evidence of helper virus-encoded gene products (for example, see Fig. 3C) and gave negative results for reverse transcriptase. Furthermore, cell-free homogenates from these spleens were unable to transfer replicating virus to NIH 3T3 cells or to induce disease when injected into other mice.

These studies show that the envelope gene of SFFV is capable of producing a malignant transformation by conferring a strong proliferative potential on a relatively few progenitor cells. Affected cell populations containing transforming DNA expanded out of control and caused a fulminating disease. Previous work in vitro with helper-deficient virus preparations suggested that SFFV alone can transform hematopoietic cells (18), an observation that is extended by our experiments in vivo. It is now clear that the oncogenic potential of SFFV can be realized without the spread of virus and recruitment of cells.

The present studies demonstrate that under conditions in which sufficient target cells are accessible in vivo, a nonreplicating retrovirus vector containing a transforming gene can be introduced into animals by direct inoculation. Previously reported experiments have shown that nontransforming genes, such as the hypoxanthine phosphoribosyltransferase (HPRT) gene and a drug resistance gene (Neo<sup>R</sup>), can be efficiently introduced into hematopoietic cells of mice by delivery of the genes first to bone marrow cells in vitro and then engrafting them in vivo (2, 3). Williams *et al.* (3) have been successful in applying this technique in combination with helper-free retroviral vectors. Their methods of introducing genes into mice, as well as those described in this report, may have application to studies of various types of transforming genes.

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#### References and Notes

1. A. Joyner *et al.*, *Nature (London)* **305**, 556 (1983).
2. A. D. Miller *et al.*, *Science* **225**, 630 (1984).
3. D. A. Williams *et al.*, *Nature (London)* **310**, 476 (1984).
4. R. Mann, R. C. Mulligan, D. Baltimore, *Cell* **33**, 153 (1983).
5. R. D. Cone and R. C. Mulligan, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6349 (1984); S. Watanabe and H. Temin, *Mol. Cell. Biol.* **3**, 2241 (1983).
6. S. Ruscetti and L. Wolff, *Curr. Top. Microbiol. Immunol.* **112**, 21 (1984).
7. L. Wolff, E. Scolnick, S. Ruscetti, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4718 (1983); S. Clark and T. Mak, *ibid.*, p. 5037; H. Amanuma *et al.*, *ibid.*, p. 3913.
8. D. L. Linemeyer *et al.*, *J. Virol.* **43**, 223 (1982).
9. J. Kamnichik *et al.*, *ibid.* **44**, 922 (1982).
10. L. Wolff *et al.*, *ibid.* **53**, 570 (1985); W. D. Hankins, J. Kamnichik, J. Luna, in *Globin Gene Expression and Hematopoietic Differentiation*, G. Stamatoyannopoulos and A. W. Nienhuis, Eds. (Liss, New York, 1983), pp. 245-261.
11. P. T. Tambourin and F. Wendling, *Nature (London)* **324**, 230 (1971).
12. P. J. Southern and P. Berg, *J. Mol. Appl. Genet.* **1**, 327 (1982).
13.  $\psi$ -2 cells from D. Williams and R. Mulligan were kindly provided through W. F. Anderson and P. P. Kantoff at the National Institutes of Health.
14. D. Baltimore, *Nature (London)* **226**, 1209 (1970); H. M. Temin and S. Mizutani, *ibid.*, p. 1211.
15. W. D. Hankins *et al.*, *ibid.* **276**, 506 (1978).
16. T. A. Kost, M. J. Koury, S. B. Krantz, *Virology* **108**, 309 (1981); H. J. Seidel and L. Kreja, *Exp. Hematol.* **10**, 459 (1982).
17. H. Hara and M. Ogawa, *Am. J. Hematol.* **1**, 453 (1976).
18. W. D. Hankins and S. B. Krantz, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5287 (1980).
19. A. A. Axelrad and R. A. Steeves, *Virology* **24**, 513 (1964).
20. D. L. Linemeyer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1401 (1981).
21. S. Chattopadhyay *et al.*, *Nature (London)* **295**, 25 (1982).
22. E. Southern, *J. Mol. Biol.* **98**, 503 (1975); T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982), pp. 382-389.
23. S. Ruscetti *et al.*, *J. Virol.* **30**, 787 (1979).
24. F. L. Graham and A. J. van der Eb, *Virology* **52**, 456 (1973).
25. We thank J. F. Mushinski, D. Lowy, and P. Tambourin for their critical reading of this manuscript, and M. Millison and V. Rogers for editorial assistance.

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## Acid Rain: Statistical Analysis of Ionic Correlations Questioned

In analyzing data on the chemical composition of rainwater, Gorham *et al.* (1) claim that hydrogen ions are correlated "much more closely with sulfate than with nitrate" and also that the H<sup>+</sup> correlation with the sum of SO<sub>4</sub><sup>2-</sup> and NO<sub>3</sub><sup>-</sup> is slightly less than that between H<sup>+</sup> and SO<sub>4</sub><sup>2-</sup> alone. We wish to point out several shortcomings in this analysis and thereby to suggest that less sweeping conclusions are appropriate.

First, for acid-base systems that are weakly buffered, such as rainwater, the relation between (H<sup>+</sup>) and (anion) (2) is inherently nonlinear unless (H<sup>+</sup>)  $\gg$  (other cations) (3). Rainwater often fails to satisfy this inequality, so that linear regression is inappropriate. In dilute systems (SO<sub>4</sub><sup>2-</sup>) and (NO<sub>3</sub><sup>-</sup>) increase in proportion to the amount of each acid added to a fixed volume of water; however, (H<sup>+</sup>) may or may not increase in proportion depending on the presence of base (for example, NH<sub>3</sub>) with which it can react. Indeed, this nonlinear functional relation of (H<sup>+</sup>) and (anion) is well known as the classical titration curve of a weak base titrated by a strong acid. No data are presented in the report by Gorham *et al.* (1) which can be tested against this fundamental chemical principle.

Second, it is a fact that both HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>, when added to rainwater, cause the pH to drop and that a given concentration (in equivalents per liter) of either acid will cause an identical drop in pH. The lower correlation of (H<sup>+</sup>) with (NO<sub>3</sub><sup>-</sup>) and other statistical calculations seem to be used by Gorham *et al.* to suggest that SO<sub>2</sub>, as a precursor of H<sub>2</sub>SO<sub>4</sub> in the atmosphere, is more likely

to produce rainwater acidity than NO<sub>x</sub>, the precursor of atmospheric HNO<sub>3</sub>. Gorham *et al.* further claim that their "analysis also supports the focus of emission control upon SO<sub>2</sub> . . ." We suggest that it is prudent to consider both H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> as important contributors of acidity to rainwater. The possibility exists that NO<sub>x</sub> sources may be located close to sources of basic materials that might neutralize some of the HNO<sub>3</sub> that is formed. However, if NO<sub>x</sub> were not emitted, these basic materials would be available to react with the H<sub>2</sub>SO<sub>4</sub> formed from SO<sub>2</sub>. Control of sources of HNO<sub>3</sub> would appear to be as important to rainwater pH as control of sources of H<sub>2</sub>SO<sub>4</sub> on an equivalent-for-equivalent basis.

Third, the value of a correlation coefficient is limited by the individual variance of the variable as well as by the number of observations used. This is especially true when two or more correlations are compared. It is customary to report confidence limits about correlation coefficients so that their reliability is apparent. From the work of Gorham *et al.*, it is impossible to tell how confident one can be in calling  $r^2 = 0.85$  statistically significant, let alone in concluding that the difference between an  $r^2$  of 0.85 and 0.83 has any meaning whatever. In other words, if the linear correlations were indeed meaningful, the difference between the H<sup>+</sup>/SO<sub>4</sub><sup>2-</sup>  $r^2 = 0.85$  and the H<sup>+</sup>/NO<sub>3</sub><sup>-</sup>  $r^2 = 0.63$  really may not be significant and the difference between the H<sup>+</sup>/SO<sub>4</sub><sup>2-</sup> relation and the H<sup>+</sup>/(SO<sub>4</sub><sup>2-</sup> + NO<sub>3</sub><sup>-</sup>) may not even pass a hypothesis test at the 50 percent level. What might

have been more meaningful than a discussion of residuals would be the use of direct bivariate regression, which would have shed light on the possibility that ( $\text{SO}_4^{2-}$ ) and ( $\text{NO}_3^-$ ) are equally prone to association with ( $\text{H}^+$ ).

Fourth, it is common practice in multivariate analysis to standardize (autoscale or regularize) variables to avoid inadvertent variable weighting. Thus, by not standardizing the 40 sites before K-means clustering, Gorham *et al.* are heavily weighting the sites with higher concentrations. Is this plausible? Would their conclusions change if they gave equal importance to the chemistry at all sites?

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#### References

1. E. Gorham, F. B. Martin, J. T. Litzau, *Science* 225, 407 (1984).
2. Parentheses denote concentration in equivalents per liter.
3. J. J. Morgan, in *Atmospheric Chemistry*, E. D. Goldberg, Ed. (Dahlem Konferenzen, Berlin, 1982), pp. 17-40.

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Charlson and Kowalski make four points, to which we respond sequentially.

1) They claim that linear regression is inappropriate. However, in the legend to figure 1 of (1) we stated that "scatter plots indicate linearity of regression." An attempt to fit a curvilinear model to the data (annual averages) would produce higher order coefficients not significantly different from zero.

2) They suggest that it is prudent to consider both  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$  as important contributors to acidity in rainwater (see also their third point). As they remark (although they provide no evi-

dence),  $\text{NO}_x$  sources may be located closer to sources of basic materials than  $\text{SO}_2$  sources, and these basic materials could potentially neutralize more  $\text{H}_2\text{SO}_4$  if production of  $\text{HNO}_3$  were reduced. We agree. However, we suggested (1) (equally without evidence) that emissions proportionally richer in  $\text{NO}_x$  than in  $\text{SO}_2$  may also be richer in basic materials, so that they are inherently less capable of producing acids. Control measures might reduce both acidic and basic components. We also suggested that  $\text{NO}_x$  or  $\text{HNO}_3$  may react more effectively than  $\text{SO}_2$  or  $\text{H}_2\text{SO}_4$  with basic materials in the atmosphere, so that the potential for acid production of  $\text{NO}_x$  may be reduced more than that of  $\text{SO}_2$  by natural means. Whether such basic materials would react equally well with  $\text{SO}_2$  and  $\text{H}_2\text{SO}_4$  if  $\text{NO}_x$  concentrations were lowered remains to be determined.

3) The significance of our statistical correlations is questioned. However, from this work it is entirely possible to tell how confident one can be in calling  $r^2 = 0.85$  statistically significant. The only information required is the sample size,  $n = 82$ , as specified in our report. If one uses transformed  $z$  scores for  $r = 0.92$ , with a standard error of  $1/(n-3)^{1/2}$ , reference to the tables in any book on statistical methods allows one to calculate that  $r = 0.92$  is significantly different from zero ( $P < 10^{-8}$ ). Likewise, calculation of  $z$  statistics from the given data shows that the difference between  $r^2$  values of 0.85 and 0.63 is highly significant ( $p < 0.002$ ). Charlson and Kowalski miss the point of our comparison of  $r^2(\text{H}^+/\text{SO}_4^{2-}) = 0.85$  with  $r^2(\text{H}^+/\text{SO}_4^{2-} + \text{NO}_3^-) = 0.83$ . We reiterate, if  $\text{SO}_2$  and  $\text{NO}_x$  are equally prone to produce  $\text{H}^+$  and can do so independently, one would expect  $\text{H}^+$  to correlate much better with ( $\text{SO}_4^{2-} + \text{NO}_3^-$ ) than with  $\text{SO}_4^{2-}$  alone. It does not! We see no reason to withdraw our claim (1) (based also on examination of Studentized re-

siduals) that statistical analysis indicates that "much of the correlation between  $\text{H}^+$  and  $\text{NO}_3^-$  over the broad geographic range examined here is probably due to their strong mutual correlation with  $\text{SO}_4^{2-}$ ."

4) Charlson and Kowalski claim that we should have standardized variables before clustering sites. Our use of unstandardized variables in the cluster analysis was a matter of choice and understanding of the outcome, which in this case was easily interpretable. Clustering in more than three groups (not reported because of space constraints) revealed that the outer ring of sites low in pollutant ions split into an eastern group high in sea-spray ions and a western group low in those ions.

In conclusion, we claimed (1) merely that our analysis "supports" the present focus of emission control upon  $\text{SO}_2$ , for which there are several prior reasons. Perhaps the phrasing "is compatible with" would be preferable to Charlson and Kowalski. They do not dispute our other major points: (i) the need for detailed investigation of the abundance of  $\text{NH}_4^+$  and  $\text{Ca}^{2+}$  in relation to the proportions of  $\text{SO}_2$  and  $\text{NO}_x$  in urban-industrial emissions and of how these cations interact with  $\text{SO}_2$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{NO}_x$ , and  $\text{HNO}_3$  and (ii) the need to lower target loadings of wet  $\text{SO}_4^{2-}$  deposition from 20 to between 14 and 16  $\text{kg ha}^{-1} \text{ year}^{-1}$  if sensitive aquatic ecosystems are to be protected.

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#### References

1. E. Gorham, F. B. Martin, J. T. Litzau, *Science* 225, 407 (1984).

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