

4. A. S. Todd, *J. Pathol. Bacteriol.* **78**, 281 (1959); T. Astrum, in *Proteases and Biological Control*, E. Reich, D. B. Rifkin, E. Shaw, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1975), p. 343.
5. N. W. Seeds, A. G. Gilman, T. Amano, M. W. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **66**, 160 (1970).
6. B. Hamprecht, *Int. Rev. Cytol.* **49**, 99 (1977).
7. J. E. Bottenstein and G. H. Sato, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 514 (1979).
8. P. R. Becherer and J. T. Wachsmann, *J. Cell Physiol.* **104**, 47 (1980).
9. A. Krystosek, in preparation.
10. Anuran and chick sensory ganglia neurons explanted into culture dishes extend axons along the substratum [R. G. Harrison, *J. Exp. Zool.* **9**, 787 (1910); D. Bray, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 905 (1970); M. A. Luduena, *Dev. Biol.* **33**, 268 (1973); P. C. Letourneau, *ibid.* **44**, 77 (1975)]. Elongation occurs at the growth cone, which rapidly extends and retracts long microspikes. Axonal growth cones can also be seen in the developing nervous system by using histological techniques [S. Ramon y Cajal, *Studies on Vertebrate Neurogenesis*, L. Guth, Transl. (Thomas, Springfield, Ill., 1960)]. Growth of neurites from neuroblastoma cells is qualitatively similar, in terms of the cellular processes involved, to that described for primary neurons (5).
11. G. A. Dunn, *J. Comp. Neurol.* **143**, 491 (1971).
12. Fibroblasts of the European field vole (*Microtus agrestis*) transformed by the Schmidt-Ruppin strain of avian sarcoma virus were the kind gift of R. L. Erikson. Properties of the cells are described in Y. C. Chen, M. J. Hayman, P. K. Vogt, *Cell* **11**, 513 (1977); M. S. Collett and R. L. Erikson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2021 (1978); and S. E. Nielson and T. T. Puck, *ibid.* **77**, 985 (1980).
13. W. E. Laug, P. A. Jones, C. A. Nye, W. F. Benedict, *Biochem. Biophys. Res. Commun.* **68**, 114 (1976).
14. J. P. Quigley, *J. Cell Biol.* **71**, 472 (1976).
15. P. H. Black, *Adv. Cancer Res.* **32**, 76 (1980).
16. K. M. Yamada, B. S. Spooner, N. K. Wessells, *Proc. Natl. Acad. Sci. U.S.A.* **66**, 1206 (1970).
17. J.-D. Vassalli, J. Hamilton, E. Reich, *Cell* **8**, 271 (1976).
18. J. C. Unkeless, A. Tobia, L. Ossowski, J. P. Quigley, D. B. Rifkin, E. Reich, *J. Exp. Med.* **137**, 85 (1973).
19. A. G. Ware, M. M. Guest, W. H. Seegers, *Arch. Biochem.* **13**, 231 (1967).
20. M. W. Mosesson, *Biochim. Biophys. Acta* **57**, 204 (1962).
21. Casein agarose and fibrin agarose overlay assays were previously used to detect PA [A. R. Goldberg, *Cell* **2**, 95 (1974); P. Jones, W. Benedict, S. Strickland, E. Reich, *ibid.* **5**, 323 (1975)]. Agarose overlay thickness was approximately 400 μm in the center of the plate, increasing to 2000 μm at the edges (2.3 ml of substrate per 60-mm dish). Typical development times for lytic zones around transformed cell colonies were 12 to 16 hours at 37°C. More than 24 hours was needed to detect fibrinolysis by single NG108 cells. With the fibrin slide method clots had an average thickness of 15 μm and could vary above and below this value on the same cover slip. Thickness of the overlay is decreased when the clot is dehydrated for microscopic viewing. Partial fibrinolysis was detected after as little as 30 minutes, and incubations of 2½ to 3 hours were used for the examples shown in Fig. 1.

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Asbestos Surface Charge Heterogeneity and Biological Effects

To accomplish the goal of Schiller *et al.* ["to minimize the undesirable occupational hazards" of amphibole particles (1, p. 1530)], it is important to relate biological effects to the reported surface charge heterogeneity, which is responsible for a smaller net surface charge for blocky amphibole cleavage fragments than for elongated asbestos fibers. The impact that long-term *in vivo* leaching has on net surface charge and surface charge heterogeneity should be considered when one is relating the results of Schiller *et al.* to long-term biological effects.

Net surface charge, as presented in terms of electrophoretic mobility (μ), is expressed in (1) as a function of basal (μ_1) and lateral (μ_2) electrophoretic mobilities and the aspect ratio for a cylindrical particle (γ , length divided by diameter)

$$\mu = \frac{\mu_1 + 2\mu_2\gamma}{2\gamma + 1} \quad (1)$$

Adsorption studies with amphibole particles have shown that in neutral aqueous media μ_1 is positive because of cation layers and μ_2 is negative because of silica ribbons (2). By statistically fitting empirical data to Eq. 1, Schiller *et al.* quantified μ_1 and μ_2 for amphibole particles.

In relating charges on fragments and fibers to long-term biological effects, a complicating factor is *in vivo* leaching

(3), which for amphiboles would mainly decrease the positive basal charge by preferential dissolution of hydrated surface cations (4). For amphibole edge leaching, cation removal changes μ_1 to $\mu_1^*(t)$, where t is time; as μ_2 is essentially determined by less soluble silica, its value is relatively constant. The net electrophoretic mobility for leached fibers [$\mu^*(t)$] is thus a function of leaching time and is approximated by

$$\mu^*(t) = \frac{\mu_1^*(t) + 2\mu_2\gamma}{2\gamma + 1} \quad (2)$$

To simulate the rate of change for μ_1 in *in vivo*, we leached fibers of the amphibole crocidolite for various times using Tyrode's (physiological buffer) solution (5). From measurements of $\mu^*(t)$ for fibers having a γ of 5, I estimate that $\mu_1^*(t)$ would equal zero after 33 days and that on further leaching, both edges and faces would become negatively charged. Since similar leaching would occur *in vivo*, its impact on the net surface charge and surface charge heterogeneity should be considered when one is relating the results of Schiller *et al.* to long-term biological effects. In addition to leaching, adsorption onto fibers of materials such as surfactants (5) would also affect the fiber surface charge *in vivo*.

Long-term leaching *in vivo* could obliterate fiber edge and face charge differences. Blocky cleavage fragments and elongated fibers would then display

a net surface charge comparable to that for homogeneous materials such as silica, quartz, and glass and independent of γ . Certain fibers having homogeneous surfaces, such as glass fibers, are similar to crocidolite in that fibers with large values of γ induce a higher incidence of malignant pleural mesenchymal neoplasms than those with small values (6). As the net surface charge is independent of γ for fibers having homogeneous surfaces, factors other than fiber edge and face charge heterogeneity would account for the observed variation in biological activity.

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References and Notes

1. J. E. Schiller, S. L. Payne, S. E. Khalafalla, *Science* **209**, 1530 (1980).
2. N. A. Prasad and F. D. Pooley, *J. Appl. Chem. Biotechnol.* **23**, 675 (1973).
3. A. Holmes and A. Morgan, *Nature (London)* **215**, 441 (1967); A. Morgan, A. Holmes, C. Gold, *Environ. Res.* **4**, 558 (1971).
4. J. Ralston and J. A. Kitchener, *J. Colloid Interface Sci.* **50**, 242 (1975); I. Choi and R. W. Smith, *ibid.* **40**, 253 (1972).
5. W. G. Light and E. T. Wei, *Nature (London)* **265**, 537 (1977); *Environ. Res.* **13**, 135 (1977); in *The In Vitro Effects of Mineral Dusts*, R. C. Brown, Ed. (Academic Press, London, 1980), p. 139.
6. M. F. Stanton, M. Layard, A. Tegeris, E. Miller, M. May, E. Kent, *J. Natl. Cancer Inst.* **58**, 587 (1977); M. F. Stanton and C. Wrench, *ibid.* **48**, 797 (1972).
7. I thank W. C. Cooper, E. T. Wei, E. T. Peters, and A. E. Rogers for reviewing the manuscript.

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The comments by Light raise an interesting area for further study, namely, to determine the relationship between electrophoretic mobility (μ) and aspect ratio (γ) for amphibole fragments and fibers that have been suspended in biological fluids. However, there is no basis for assuming that in biological systems basal electrophoretic mobility (μ_1) varies with time and lateral electrophoretic mobility (μ_2) is constant. One could test this hypothesis by obtaining data on μ and γ for leached fibers and fragments by the method that we used; μ data from fibers alone are not sufficient to permit one to determine the effects of leaching independently on μ_1 and μ_2 . Equation 5 that we derived (1) shows that μ is a function of γ and charge densities on different regions of the particles. It is necessary to measure γ along with μ in order to evaluate μ_1 and μ_2 .

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References

1. J. E. Schiller *et al.*, *Science* **209**, 1530 (1980).

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