crust. Continuous observation of gas quality at a location geochemically sensitive to stresses at depth could therefore be meaningful for earthquake prediction.

RYUICHI SUGISAKI
Department of Earth Sciences, Nagoya University, Chikusa, Nagoya, Japan

References and Notes

1. M. A. Sdovsky, I. L. Nesesov, S. K. Nigatullaev, L. A. Latynina, A. A. Lukk, A. M. Semenov, I. G. Simbireva, V. I. Ulmov, *Tectonophysics* **14**, 295 (1972).
2. R. Sugisaki, H. Anno, M. Adachi, H. Ui, *Geochem. J.* **14**, 101 (1980); H. Wakita, Y. Nakamura, I. Kita, N. Fujii, K. Notsu, *Science* **210**, 188 (1980).
3. R. Sugisaki, *Nature (London)* **275**, 209 (1978).
4. T. W. Robinson, *Trans. Am. Geophys. Union* **20**, 656 (1939).
5. R. Sugisaki, *Bull. Am. Assoc. Pet. Geol.* **48**, 85 (1964).
6. I. M. Longman, *J. Geophys. Res.* **64**, 235 (1959); T. Mikumo and M. Kato, *Disaster Prev. Res. Inst. Kyoto Univ. Bull.* **26**, 71 (1976).
7. K. Honda, K. Kurita, Y. Hamano, *Abstr. Pap. Geochem. Soc. 152* (1979).
8. According to H. Yamamoto and A. Masuda (in preparation), rare earth elements in the mineral water show an La-depleted pattern normalized by chondrite. This suggests that the water originates at depth.
9. T. Rikitake, *Tectonophysics* **26**, 1 (1975).
10. I am grateful to A. Yamamoto for providing the computer program for calculating the earth tide, to R. Shichi and M. Furumoto for providing extensometer data at Inuyama and for valuable comments, and to Y. Isobe for technical assistance.

15 December 1980

Homogeneous Fluorescent Immunoassay

Abstract. *A fluorescent immunoassay based on the correlation of fluctuations in particle number measures the amount of tagged species bound to micrometer-sized beads and is insensitive to background fluorescence. Without separation steps, a competitive assay can resolve 1 nanogram of gentamicin per milliliter from a total sample volume of only 10 microliters.*

New trends in instrumentation in clinical chemistry (*1*), such as automated immunoassays, are simplifying clinical procedures. An example is an immunoassay that works with homogeneous samples; the technique does not require the separation of bound from free unknown and is insensitive to background sources naturally present in the sample. We developed a fluorescent immunoassay (*2*) and report data that demonstrate the sensitivity of this assay without the conventional separation steps.

The fluorescence from a small sampling volume ($\sim 10^{-6}$ ml) is detected by a photomultiplier. The detected signal fluctuates in time because of fluctuations in the number of fluorescent particles in the sampling volume. A microprocessor calculates the temporal autocorrelation function of this fluctuating signal. To improve the sampling statistics, the sampling volume is periodically scanned through the specimen, and the microprocessor computes the correlation function at a time equal to the scanning period. This ensures that each sampling volume is periodically measured with the same period for all sampling volumes. Fluctuations in the detected signal that are not correlated over this scanning period contribute only to the baseline of the correlation function. However, a fluctuation in the signal from a specific sampling volume due to a fluctuation in fluorescent particle numbers that persists beyond the scanning period drives the correlation function above its baseline. The microprocessor determines the correlation peak height S above the baseline averaged over the number of sampling intervals.

In the competitive assay the unknown (U) competes with a tagged mimic (U*) for a limited number of binding sites ($\bar{U}$) that are attached to beads suspended in the sample. Our technique is not limited to such competitive assays. For example, a sandwich assay is possible in which the complexes $\bar{U}$*-U-$\bar{U}$ are formed on the bead. The usual procedure would be to separate out the beads by centrifugation and measure the bead fluorescence. To eliminate the separation steps requires a measurement highly sensitive to the fluorescence bound to beads and less sensitive to the fluorescence free in solution resulting from the unbound tagged molecules and the natural fluorescence of the specimen.

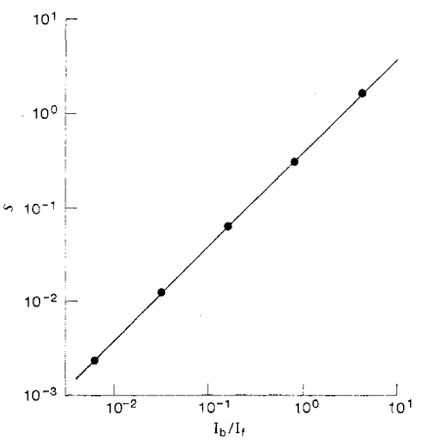

Fig. 1. The correlation peak height S in arbitrary units is plotted as a function of I_b/I_f for Dow fluorescent spheres plus rhodamine. If S were sensitive to the rhodamine in the measured range of I_b/I_f, S would deviate from the 45° diagonal and settle to a constant value determined by the fixed concentration of rhodamine.

Consider a homogeneous sample with two fluorescent species: one consists of free molecules, each with one unit of fluorescence, and the other suspended beads, with n units of fluorescence (n tagged molecules bound to the bead). If the average fluorescent free molecules and beads per sampling volume are N_f and N_b, respectively, the contribution to S from each species (*2*) is given by

$$S_f = i^2 N_f$$
$$S_b = i^2 N_b n^2 \quad (1)$$

with

$$S = S_f + S_b \quad (2)$$

and i a constant having the units of intensity. The average fluorescent intensities from each of the two species are

$$I_f = iN_f$$
$$I_b = iN_b n \quad (3)$$

with

$$I = I_f + I_b \quad (4)$$

In this model we have assumed that the fluorescent efficiencies of bound and unbound molecules are equal.

Under appropriate conditions there are two reasons why S is dominated by S_b. First, bright fluorescent objects contribute more strongly to S than they do to I. This is seen by forming the following ratios from Eqs. 1 and 3,

$$\frac{I_b}{I_f} = \frac{N_b n}{N_f}$$
$$\frac{S_b}{S_f} = \frac{N_b n^2}{N_f} = \left(\frac{I_b}{I_f}\right) n \quad (5)$$

From Eq. 5 we see that if n is large enough, it is possible for $S_b \gg S_f$ even with $I_f > I_b$. For example, suppose the bead structure and reagent concentrations are such that an assay is performed with n varying between 10^3 and 10^4. To determine S_b with 1 percent accuracy from a measurement of S, we want $S_b/S_f \geq 100$. From Eq. 5 we see that at the low end in the range of n, we would still be able to tolerate $I_f = 10\ I_b$.

The second reason for the relative insensitivity of S to the free fluorescence is the relative diffusion rates of large and small particles. Since the fluorescent intensity fluctuations are due to particle number fluctuations, these fluctuations come and go as the particles diffuse in and out of the sampling volume. Because large particles diffuse slowly, large-particle fluctuations will persist longer and thus contribute more strongly to the cor-

0036-8075/81/0612-1266$00.50/0

relation function S than small particle fluctuations. In other words, in Eqs. 1 and 2 there should be size-dependent multiplicative factors associated with S_f and S_b that describe the relative amount by which the bead fluorescence correlates more efficiently than the free fluorescence.

The fact that S is dominated by the bead fluorescence is the most significant feature of this assay because it allows the elimination of separation steps. This can be illustrated by data from two systems.

First, we synthesized a test system of fluorescent latex spheres (Dow; 0.46 μm in diameter) and rhodamine dye. We made five samples with the same concentration of dye but with serially lower concentrations of fluorescent spheres. Each dilution reduced the bead concentration, and hence I_b/I_f, by one-fifth. Figure 1 shows S plotted as a function of I_b/I_f. From Eqs. 1 and 2 we expect S (and I_b/I_f where I_f is constant) to decrease in direct proportion to N_b only when $S \simeq S_b \gg S_f$. From Fig. 1 we see that this is true even for $I_b/I_f \simeq 5 \times 10^{-3}$.

For a clinically significant test we used the Bio-Rad gentamicin fluorescent kit (Bio-Rad Laboratories, Richmond, California). This is a competitive assay with a fixed concentration of tagged gentamicin [G*] and a variable concentration of untagged gentamicin [G] competing for a fixed number of antibody sites on beads (G + G* in excess). As [G] is increased, the fluorescent brightness of the beads will decrease; that is, n will decrease. From Eqs. 1 and 2 we see that $S^{1/2} \propto n$ as long as S is insensitive to the free fluorescence. Samples were made from the Bio-Rad kit by mixing the G*, G, and bead reagents together and incubating for at least 30 minutes. The free fluorescence is due to the unbound G* in addition to extra dye that Bio-Rad adds to the G* tracer to make that reagent obviously yellow.

Figure 2 shows $S^{1/2}$ plotted as a function of [G] for two concentration ranges. The curves are the standard curves for gentamicin determination from our correlation technique with homogeneous samples. These curves coincide with the standard curve supplied by Bio-Rad, in that a given dilution of the standard results in the same change in the measured signal. Furthermore, the midpoint of curve B is translated to higher concentrations by a factor of 10. Finally, the assay can resolve gentamicin at 1 ng/ml, ($2.6 \times 10^{-9} M$) from a total specimen volume of only 10 μl.

All points in Figs. 1 and 2 are mean values from several measurements on the same sample. The standard deviation

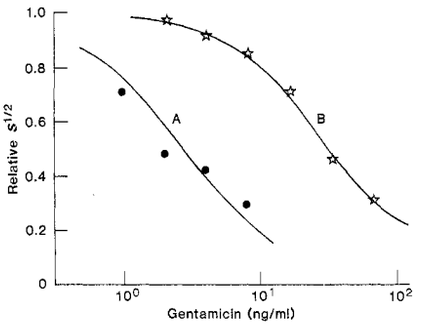

Fig. 2. Relative values of $S^{1/2}$ are plotted as a function of gentamicin concentration without separation. The $S^{1/2}$ was normalized by the measured value from a sample with a fixed concentration of gentamicin tracer and zero concentration of untagged gentamicin. The concentrations of gentamicin are those of the actual measured samples. (The concentrations of the gentamicin standards supplied with the kit before dilution range from 2 to 16 μg/ml, comparable to concentrations in serums.) Curve B gives data for samples with ten times the concentrations of G, G*, and beads relative to curve A.

relative to the mean values of S for a given sample decreases with the inverse of the square root of the total sampling time. The scatter of points on curve A (Fig. 2) is due to shorter tests than for the samples of curve B; a typical time for measuring a single mean value on curve B was 20 minutes. The settling of the beads during the long experiments adversely affects the deviation. We used commercially available bead reagents, but our signal-to-noise ratio would improve if we used a bead with a greater number of active sites and a density close to that of water to reduce the settling.

Our tests demonstrate the feasibility of this fluorescent immunoassay with homogeneous samples. This technique may simplify the clinical procedures for both fluorescent and radioimmunoassays (*1*). There is an additional advantage: without the separation steps, this technique in principle can follow the kinetics of the antibody-antigen reaction.

JONATHAN BRIGGS
VIRGIL B. ELINGS
DAVID F. NICOLI
Department of Physics, University of California, Santa Barbara 93106

References and Notes

1. R. J. Elin, *Science* **210**, 286 (1980).
2. D. F. Nicoli, J. Briggs, V. B. Elings, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4904 (1980).
3. We thank D. Riege for comments on the gentamicin assay and bead reagents and M. Weissman for giving us the fluorescent Dow spheres.

3 February 1981

Fly Ash–Derived Strontium as an Index to Monitor Deposition from Coal-Fired Power Plants

Abstract. *The combustion of western U.S. coals releases significant amounts of strontium, which is relatively enriched in the fine particles of fly ash. Fly ash–derived strontium is readily absorbed by agronomic and native plant species when incorporated in soil. The strontium-87 to strontium-86 ratios of fly ash and soils were significantly different, but similar ratios were found in fly ash and plants treated with fly ash. A technique for measuring and monitoring deposition from coal-fired power plants is inferred from the enhanced plant uptake of fly ash strontium and the similarity in the isotopic ratios of fly ash and treated plants.*

In view of the nation's increasing demand for coal and the serious concerns over associated environmental impacts, knowledge of the environmental pathways of coal combustion residues is essential. Various attempts to monitor the deposition of particulate matter emitted from coal combustion onto soils and vegetation have had only limited success, mainly because of the difficulty of distinguishing between deposited and natural background materials (*1*). The possibility that strontium uptake in native vegetation (*2*) and soils (*3*) might be used as a monitor of power plant effluents has been suggested.

Coals from the western United States contain on the average greater concentrations of strontium than their eastern and midwestern counterparts (*4*). The strontium content of western coal ranges from 93 to 500 parts per million (ppm), averaging about 260 ppm. Lignitic coals, which are abundant in the West, have the highest average strontium content (300 ppm) (*5*). The average strontium content of fly ash from several U.S. sources was estimated at 1334 ppm (*6*), and it usually increases as the particle size decreases (*7*, *8*). Relative to typical soils (300 ppm of strontium) (*9*), emitted fly ash is thus highly enriched in strontium, and, depending upon the recipient soil and the amount of fly ash deposited, the strontium content of soil will increase (*8*).

Greenhouse experiments have demonstrated that fly ash–derived strontium is

0036-8075/81/0612-1267$00.50/0