fects of age structure (*2, 3, 9*) and of time delays in the population's regulatory processes (*18, 19*). We think that the simple qualitative insights provided by $T_R(Y)$ will continue to be a reliable guide in these more complicated situations, in which the exact analysis will necessarily be numerical.

The main points that emerge from this analysis are:

1) For a population harvested for sustained yield in a randomly fluctuating environment, the relative variability in the population magnitude, and thence in the yield, increases systematically as the harvesting effort increases. That is, the predictability of the catch tends to decrease as the catching effort increases, particularly when overexploitation has resulted in the population's being kept below the MSY level. This appears to be a feature of many fisheries over the past 30 years (*20*) and of some whaling industries (*21*).

2) These effects are relatively more pronounced under a harvesting strategy that seeks to keep the yield constant (a strategy of constant quotas), than under a strategy of constant effort (for example, a fixed total number of fishing hours). Although this conclusion is based on oversimplified models, it is likely to remain true in more sophisticated and realistic studies. If so, it holds implications for the laws regulating fisheries and other harvested populations.

3) We have assumed that the policy aim is to maximize Y. As Clark (*4, 5*; see also *22*) has emphasized, in practice the aim will often rather be to maximize the present value (PV) of discounted net economic revenue. Unless there is a combination of high harvesting costs and a low discount rate, the sustained population value will be below the MSY point. It follows that, in a simple analysis, the characteristic return time will typically be longer, and the population fluctuations relatively more severe, if PV rather than Y is maximized. However, a more realistic accounting of the economic costs of harvesting is likely to introduce feedback mechanisms which help to stabilize the system (*4, 5, 19, 22*).

4) In general, given the environmental unpredictabilities of the real world, stability considerations suggest that it is usually undesirable to use nonfeedback control policies (such as MSY) to manage natural resources.

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References and Notes

1. M. B. Schaefer, *Inter-Am. Trop. Tuna Comm. Bull.* **1** (No. 2), 27 (1954).
2. R. J. H. Beverton and S. J. Holt, *Fish. Invest. Minist. Agric. Fish. Food (G.B.) Ser. II Salmon Freshwater Fish.* **19**, 1 (1957).
3. D. H. Cushing, *Marine Ecology and Fisheries* (Cambridge Univ. Press, Cambridge, 1975).
4. C. W. Clark, *Mathematical Bioeconomics* (Wiley, New York, 1976); *Science* **181**, 630 (1973).
5. The background papers for the Food and Agriculture Organization Scientific Consultation on Marine Mammals, Bergen, Norway, 31 August to 9 September 1976, contain extensive reviews of harvesting theory. One major recommendation is that the effects of environmental randomness be explored for fisheries models [C. W. Clark, *FAO Document ACMRR/MM/SC/65* (1976)].
6. R. M. May and R. H. MacArthur, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1109 (1972); J. Roughgarden, *Am. Nat.* **109**, 713 (1975).
7. R. M. May, *Stability and Complexity in Model Ecosystems* (Princeton Univ. Press, Princeton, N.J., ed. 2, 1975).
8. S. J. Holt, *FAO Document ACMRR/MM/IV/4* (1975); *FAO Document ACMRR/MM/EC/29* (1975).
9. J. R. Beddington, in preparation.
10. R. M. May, G. R. Conway, M. P. Hassell, T. R. E. Southwood, *J. Anim. Ecol.* **43**, 747 (1974). T_R is essentially the reciprocal of the real part of the dominant eigenvalue. T_R is further defined and discussed in J. R. Beddington, C. A. Free, J. H. Lawton [*ibid.* **45**, 791 (1976)] and D. Ludwig [*SIAM (Soc. Ind. Appl. Math.) Rev.* **17**, 605 (1975)].
11. This formula is
$$\frac{T_R(Y)}{T_R(0)} = \frac{2}{1 \pm \sqrt{1 - Y/Y_{MSY}}}$$
The + sign applies if the sustained yield is obtained by maintaining N^* above the MSY point; the − sign pertains if N^* is below the MSY point.
12. A. R. Kiester and R. Barakat, *Theor. Popul. Biol.* **6**, 199 (1974); H. C. Tuckwell, *ibid.* **5**, 345 (1974); M. Feldman and J. Roughgarden, *ibid.* **7**, 197 (1975); N. Keiding, *ibid.* **8**, 49 (1975).
13. H. S. Horn, *Ecology* **49**, 776 (1968).
14. When white noise in a differential equation is obtained by first letting the noise correlation time tend to zero, and then letting the time step tend to zero, the Ito calculus is appropriate; if the limits are taken in the opposite order, the Stratonovich calculus applies [(*7*), pp. 203–205 and 229–231; R. M. Capocelli and L. M. Ricciardi, *Theor. Popul. Biol.* **5**, 28 (1974); (*12*)]. We use the Ito calculus on the grounds that Eq. 5 is an approximation to an age-structured fish population, with population growth taking place in discrete time steps; however, our general conclusions are not dependent on the choice between the two calculi.
15. The result is
$$f(n) = \beta[\Gamma(\alpha + 1)]^{-1}(\beta n)^{\alpha} e^{-\beta n}$$
where
$$\alpha = 2(r_0 - E - \sigma^2)/\sigma^2$$
and
$$\beta = 2r_0/(K\sigma^2)$$
Hence the average yield is
$$\langle Y \rangle = (KE/r_0)(r_0 - E - \tfrac{1}{2}\sigma^2)$$
and the CV of Y is
$$CV_Y = \left(\frac{\tfrac{1}{2}\sigma^2}{r_0 - E - \tfrac{1}{2}\sigma^2}\right)^{1/2}$$
16. F. Brauer and D. A. Sanchez, *Theor. Popul. Biol.* **8**, 12 (1975).
17. A yield curve that is modified to allow for limitation of effort (curve c in Fig. 1) is
$$Y = ZN/(N + \epsilon K)$$
where ϵ and Z are constants. Using this in Eq. 1 gives the dynamic equation
$$dN/dt = rN(1 - N/K) - ZN/(N + \epsilon K)$$
It is a routine algebraic exercise to find the equilibrium point for this system, and to show that T_R as a function of Y under this harvesting strategy is
$$\frac{T_R(Y)}{T_R(0)} = \frac{(1 + 2\epsilon \pm \rho)}{(1 \pm \rho)(\epsilon \pm \rho)}$$
with
$$\rho \equiv \sqrt{1 - Y/Y_{MSY}}$$
As in (*11*) the + signs apply if N^* is above the MSY value, the − signs if it is below. In the limit $\epsilon \to 0$, we recover Eqs. 6 and 7; in the limit $\epsilon \to \infty$ (and $Z/\epsilon K \to E$), we recover Eqs. 1 and 4; curve c of Fig. 2 shows the intermediate case $\epsilon = 0.5$.
18. F. Brauer, *Math Biosci.*, in press.
19. C. W. Clark, *J. Math. Biol.*, in press.
20. M. B. Schaefer, *Inter-Am. Trop. Tuna Comm. Bull.* **2**, 245 (1957); R. C. A. Bannister, *Rapp. P. V. Reun. Cons. Int. Explor. Mer*, in press.
21. For example, R. Gambell [*FAO Document ACMRR/MM/EC/9* (1975)] and T. Kasuya and N. Miyazaki [*FAO Document ACMRR/MM/EC/25* (1975)].
22. R. M. May, *Nature (London)* **263**, 91 (1976).
23. We thank C. W. Clark, S. J. Holt, and an anonymous reviewer for helpful comments and the King's College Research Centre, King's College, Cambridge, for its hospitality. This work is supported by grant DEB 75-10464 from the National Science Foundation.

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Pesticide Uptake into Membranes Measured by Fluorescence Quenching

Abstract. *Pesticides that contain chlorine have been shown to quench the fluorescence of carbazole-labeled phospholipids. Incorporation of these carbazole-labeled phospholipids into model membranes provides a system that allows the rapid determination of the uptake rates of chlorinated hydrocarbons into model membranes. This technique can be used in the determination of diffusion rates and partition coefficients of chlorine-containing organic compounds in model membrane systems, and hence may provide a method by which the bioaccumulation potential of synthetic chlorine-containing compounds can be estimated.*

Chlorinated hydrocarbons have played a valuable role in the control of insect-born diseases, and in increasing agricultural production through the control of crop-damaging insects (*1*). As a result, these molecules have been widely dispersed in the biosphere. Unfortunately, chlorinated hydrocarbons such as DDT and the PCB's (*2*) are persistent in the environment and accumulate in the food chain. Bioaccumulation of chlorinated hydrocarbons necessarily involves transport into and across cell membranes. Since many chlorinated hydrocarbons have very low water solubilities they are associated with particulate mat-

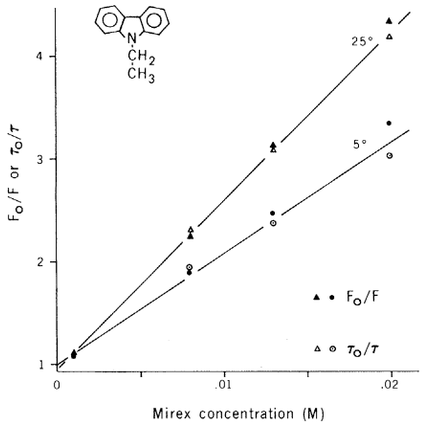
ter in the hydrosphere. Therefore, the biological uptake of chlorinated hydrocarbons by cell membranes probably involves the exchange of chlorinated hydrocarbons from the particulates to which they are adsorbed.

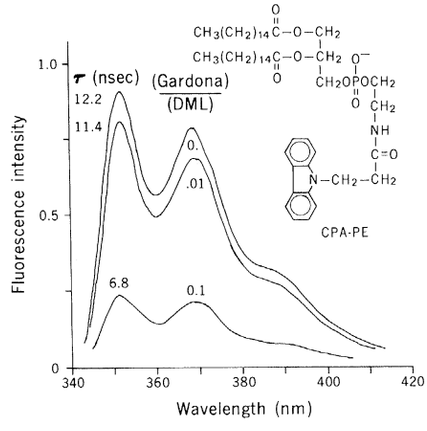
We have found that the fluorescence of carbazole-labeled phospholipids is quenched efficiently by a number of chlorinated hydrocarbons which have markedly different chemical structures. Using carbazole-labeled model membrane systems and the fluorescence quenching technique, we have been able to determine both diffusion rates and partition coefficients for chlorinated hydrocarbons (*3*, *4*). Such measurements are critical if we are to understand why these chlorine-containing organic compounds accumulate in living tissue.

The fluorescence quantum yield and the fluorescence lifetime of *N*-ethyl carbazole in the presence of mirex (an insecticide used to kill fire ants) is presented in Fig. 1. Similar quenching data were obtained when lindane, gardona, or DDE (*2*) (see Table 1), or carbon tetrachloride, endrin, methoxychlor (*3*), or methylmercuric chloride (*5*) were used to quench the fluorescence of *N*-ethyl carbazole. The fluorescence emission spectrum of *N*-ethyl carbazole is unaltered during quenching. This fact, in addition to the equivalent fractional decrease in both fluorescence lifetime and yields, indicates that the observed quenching is due to collisional encounters between the carbazole moiety and the quencher, and that complex formation is not significant (see Table 1). Thus, this quenching method provides information on the diffusional rates and the local concentrations of the chlorinated hydrocarbons.

Carbazole-labeled phospholipid was synthesized by covalently attaching 9-carbazole propionic acid to phosphatidyl ethanolamine through an amide linkage (*6*). This synthesis was accomplished by reacting 9-carbazole propionyl chloride with the phospholipid (see legend to Fig. 2), with preparative thin-layer chromatography being used for purification. The product *N*-(9-carbazopropionyl)-phosphatidyl ethanolamine, dipalmitoyl (CPA-PE), migrated as a single spot in two different chromatographic solvents, and the structure was confirmed by proton nuclear magnetic resonance (NMR) at 270 Mhz.

Phospholipid vesicles which contained 1 percent CPA-PE were prepared as outlined in Fig. 2. Addition of chlorinated hydrocarbon to these labeled phospholipid vesicles decreases the quantum yield for the CPA-PE labeled vesicles without changing the fluorescence emission spectrum. Also, Fig. 2 shows the fluorescence lifetimes of the CPA-PE labeled vesicles. At high gardona concentrations the decrease in yield is larger than the decrease in lifetime, indicating an apparent static component in the quenching. This component is probably a result of the high concentration of gardona in the vicinity of the fluorophore. Similar results are seen in homogeneous solution when the concentration of quencher is sufficiently high that the fluorophore is likely to be adjacent to a quencher at the moment of excitation (*7*). Thus the fluorescent properties of carba-





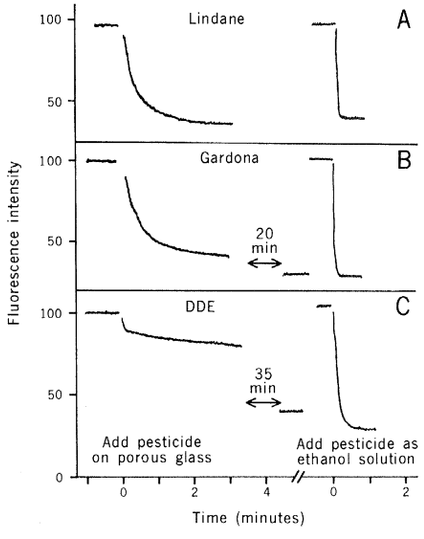


Fig. 1 (top left). Quenching of *N*-ethyl carbazole by mirex as observed by both fluorescence lifetimes and yields. The solvent used was cyclohexane. See Table 1 for more details. Fig. 2 (top right). Fluorescence emission spectra of CPA-PE labeled DML vesicles containing gardona. Phospholipid vesicles were prepared by mixing chloroform solutions of DML and CPA-PE (mole ratio 100 to 1), drying them under a nitrogen flow, suspending the lipids in buffer, subjecting them to sonication for about 15 minutes at 37°C, and then centrifuging at 20,000*g* for 90 minutes. The molar ratio of gardona to DML, prior to sonication, is indicated along with the fluorescence lifetime of each preparation. The buffer was 0.01*M* tris, 0.05*M* KCl, *p*H = 7.5; the concentration of DML was 1 mg/ml; 5°C; excitation wavelength, 333 nm; excitation and emission band-passes 8 and 2 nm, respectively. *N*-(9-Carbazopropionyl)-phosphatidyl ethanolamine, dipalmitoyl (CPA-PE) was synthesized from 9-carbazole propionyl chloride and dipalmitoyl-L-α-phosphatidyl ethanolamine in dry benzene (*6*). The acyl chloride of 9-carbazole propionic acid was prepared by refluxing this material in dry benzene containing an equivalent amount (on a molar basis) of thionyl chloride. The final product, CPA-PE, was purified by preparative thin-layer chromatography on silica, with ethyl ether, benzene, ethanol, acetic acid, and water (40:40:20:8 : 4 by volume) being used as the solvent system. The isolated material migrated as a single spot, as observed by phosphate staining, charring with dichromate in H_2SO_4, and silica plates with a fluorescent indicator, in both the solvent system used for purification and in chloroform, methanol, and water (65 : 25 : 4 by volume). The structure of this phosphate and carbazole-containing product was confirmed as CPA-PE by proton NMR at 270 Mhz. The NMR spectrum was taken in hexadeuterobenzene and showed aromatic multiplets at δ = 8.02, 8.05, and 7.49 parts per million (ppm) relative to tetramethyl silane. These are similar to *N*-ethyl carbazole resonances at δ = 8.11, 8.08, and 7.46 ppm. In addition, resonances at δ = 0.91 (terminal methyl resonance), and δ = 1.55 (methylene resonances) were found, which are similar to the resonances found in DML (δ = 0.94 and 1.51 ppm, respectively). Fig. 3 (bottom right). Pesticide uptake by lipid bilayers as observed by quenching of CPA-PE. Dipalmitoyl-L-α-lecithin (DPL) vesicles were labeled with CPA-PE by sonication (see Fig. 2). At the indicated time, pesticide was added as either a concentrated ethanol solution or as a dry coating on porous glass beads (300 m^2/g). The amounts of pesticide per gram of porous glass were 84, 50, and 40 mg for lindane, gardona, and DDE, respectively. A quantity of coated beads (about 10 mg) adequate to yield a final molar ratio of DPL to pesticide of 10 to 1 was added to 10 ml of the DPL vesicles, 1.0 mg of DPL per milliliter of buffer. The fluorescence intensity was recorded at 350 nm while the solution was continually stirred. An approximate 10 percent decrease in fluorescence intensity is observed at the time uncoated porous glass beads are added. This effect is due to the light scattered by the beads.

zole-labeled phospholipid provide a sensitive probe that reports on the presence of chlorinated hydrocarbons in membranes.

Since pesticide uptake often occurs on particulate materials, we chose pesticide-coated porous glass, with a surface area ratio of 300 m^2/g, as our experimental system for studying particle-mediated transport. These glass beads were coated with pesticide by immersing the beads in a solution of pesticide with ethanol as the solvent. Evaporation of ethanol results in the glass beads becoming coated with pesticide. The amounts of pesticide were adequate to coat approximately 25 percent of the surface area with a monolayer of pesticide (see legend to Fig. 3).

Pesticide-coated beads also quenched the fluorescence of CPA-PE labeled vesicles. Figure 3 shows the decrease in quantum yield observed when CPA-PE labeled vesicles are mixed with glass beads coated with DDE, lindane, or gardona. In each case the total amount of quenching agrees with the amount observed when an equivalent amount of pesticide is added by way of a concentrated ethanol solution. These data indicate that the uptake of lindane, gardona, and DDE are complete in about 3, 20, and 60 minutes, respectively.

To determine whether the porous glass particulates disrupt lipid bilayers, we assayed the integrity of the phospholipid vesicles by two methods, and found no disruptive effects due to porous glass. The first assay measured the release of trapped umbelliferyl phosphate (UP) (*8*). Release of UP is measured by hydrolysis to the highly fluorescent product umbelliferone. The hydrolysis is carried out with alkaline phosphatase in the external volume. This assay indicated that no disruption of the vesicles was caused by contact with the porous glass. The second assay utilized splitting of the choline resonances by the shift reagent Eu^{3+} as measured by proton NMR at 270 Mhz. When the ratio of dimyristoyl-L-α-lecithin (DML) to Eu^{3+} is 1.7 to 1 (5 mg of DML per milliliter of D_2O), an approximate 25-hertz upfield shift of the outer choline resonance is observed. Contact of these model membranes did not change this splitting between the inner and outer choline resonances. Such splitting is eliminated on disruption of the vesicles (*9*). These experiments show that the glass beads do not change the structure or integrity of phospholipid vesicles, but the pesticides do quench the fluorescence of the CPA-PE label. Using this technique we are able to measure accurately the uptake rates and concentrations of chlorinated hydrocarbons in phospholipid vesicles. Presumably, similar measurements will be possible in cell membranes.

The method described herein, which can be used to measure both transport rates and partition coefficients (*4*) in phospholipid systems, demonstrates that fluorescence methods can be used to follow particle-mediated transport of toxic materials into phospholipid vesicles. This method has promise for determining dose-response criteria for particle-mediated transport of carcinogenic hydrocarbons as well as chlorinated hydrocarbons.

Table 1. Quenching of *N*-ethyl carbazole by chlorinated hydrocarbons. Quenching of fluorescence is possible by a variety of mechanisms (*10*). Dynamic quenching provides information on the collisional frequency of the fluorophore with the quencher. This frequency is a function of the diffusional rate of the quencher and its local concentration around the fluorophore. Collisional or dynamic quenching is described by the Stern-Volmer equation (*11*),

$$F_0/F = 1 + k\tau_0[Q] \quad (1)$$

where F_0 and F are the fluorescence yields in the absence and presence of the quencher, respectively, $[Q]$ is the quencher concentration, τ_0 is the lifetime of the excited state in the absence of the quencher, and k is the bimolecular quenching constant. This constant is given by the Smoluchowski equation (*12*),

$$k = \gamma 4\pi\, \sigma_{pq} N(D_p + D_q)/1000 \quad (2)$$

where σ_{pq} is the sum of the molecular radii of the probe and quencher, D_p and D_q are the diffusion coefficients of the probe and quencher, respectively, N is the Avogadro number, and γ is the quenching efficiency, or the fraction of collisions between probe and quencher that are effective in quenching. Quenching of fluorescence is also possible by nondiffusional processes in which the fluorophore and quencher form a nonfluorescent complex prior to excitation, thereby preventing a fraction of the fluorophores from being observed fluorometrically. The uncomplexed fluorophores are unperturbed: hence their lifetime is equal to τ_0. Collisional quenching, being a rate process which depopulates the excited state, reduces the observed lifetime in proportion to the reduction in yield. Therefore, experimental proof of dynamic quenching lies in the verification of

$$F_0/F = \tau_0/\tau \quad (3)$$

where τ is the lifetime in the presence of the quencher (*13*). The bimolecular quenching constants were determined from fluorescence lifetime measurements of solutions of equivalent fluorophore concentration but varying quencher concentration. Lifetimes were measured using the phase shift technique (*14*) with a modulation frequency of 10 Mhz [25°C; excitation wavelength, 333; emission filter, 0–52 (Corning)]. All fluorescence measurements were made with either an SLM Instruments spectrofluorometer or their cross-correlation, phase-modulation lifetime instrument. Absolute ethanol and cyclohexane were used as solvents; $\tau_0 = 13.8 \pm 0.4$ nsec in absolute ethanol, 14.4 ± 0.2 nsec in cyclohexane. Quenching efficiences were calculated according to Eq. 2 and the Stokes-Einstein equation for evaluation of the diffusion coefficients of the probe and quencher.

Solvent	k ($10^9 M^{-1}$ sec^{-1})	γ Efficiency
	Mirex	
Ethanol	8.8	1.42
Cyclohexane	11.2	1.46
	Lindane	
Ethanol	1.2	0.20
Cyclohexane	1.6	0.21
	DDE	
Ethanol	2.6	0.43
Cyclohexane	1.1	0.14
	Gardona	
Ethanol	5.4	0.88
Cyclohexane	6.1	0.81

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References and Notes

1. O. T. Zimmerman, *DDT, Killer of Killers* (Industrial Research Service, Dover, N.H., 1946).
2. The abbreviations used are: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; PCB, polychlorobiphenyl; and DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene.
3. J. R. Lakowicz and D. Hogen, *Adv. Exp. Med. Biol.*, in press.
4. J. R. Lakowicz, D. Hogen, G. Omann, in preparation. As indicated in Fig. 2, there is an approximate 10 percent decrease in fluorescence lifetime and yield for a 0.01-mole fraction of gardona in DML vesicles. Thus, if one assumes an experimental accuracy of 2 percent, one pesticide molecule per 500 phospholipid molecules should be detectable. 3-Amino-9-ethyl carbazole has a fluorescence lifetime near 27 nsec and is also sensitive to quenching by chlorinated hydrocarbons. The increased lifetime should provide even greater sensitivity to low chlorinated hydrocarbon concentrations. An additional advantage of this quenching technique is that it allows the amount of pesticide in a membrane to be measured without separation of the water and membrane phases. This permits membrane uptake rates to be measured, even when these rates are nearly as fast as the solution mixing times. We have used this method to determine the partition coefficients of chlorinated hydrocarbons between the membrane and aqueous phases. For example, CCl_4 concentrates 540-fold in DML vesicles at 31°C (*2*), and lindane 3000-fold at 36°C. Using present instrumentation we estimate partition coefficients from 10 to 10^5 could be determined. Increased sensitivity, possibly by photon-counting techniques, may increase this range to 10^8.
5. S. Rakow and J. R. Lakowicz, in preparation.
6. J. R. Lakowicz and D. Hogen, in preparation.
7. G. K. Rollefson and H. Boaz, *J. Phys. Colloid Chem.* **52**, 518 (1948); H. Boaz and G. K. Rollefson, *J. Am. Chem. Soc.* **72**, 6436 (1950); I. M. Frank and S. J. Vavilov, *Z. Phys.* **69**, 100 (1931).
8. H. R. Six, *Biochemistry* **13**, 4050 (1974).
9. R. Lawaczeck, M. Kainosho, S. I. Chan, *Biochim. Biophys. Acta* **443**, 313 (1976).
10. J. B. Birks, *Photophysics of Aromatic Molecules* (Wiley-Interscience, New York, 1970), pp. 403–517.
11. O. Stern and M. Volmer, *Z. Phys.* **20**, 183 (1919).
12. M. Smoluchowski, *Z. Phys. Chem.* **92**, 129 (1917).
13. W. M. Vaughan and G. Weber, *Biochemistry* **9**, 464 (1970).
14. R. D. Spencer and G. Weber, *Ann. N.Y. Acad. Sci.* **158**, 361 (1969).
15. We thank the Freshwater Biological Research Foundation, and especially its founder R. Gray, Sr., for assistance. We also acknowledge the support of the American Heart Association and NIH grants 5R01-Am18101-3 and 1R01-ES-01283-01A1, and thank J. M. Wood and D. Hogen for constructive criticism of the manuscript.

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