These experiments show that, when the conditions of observation permit, the members of a population may display a spectrum of geotactic reactions. Some individuals give a negative response, others a positive response, and there are varying degrees in the extent to which individuals respond positively or negatively to gravity. Furthermore, it is possible to breed races of animals which perform in diametrically opposite fashion to the same physically specified stimulus conditions (*8*).

JERRY HIRSCH
Center for Advanced Study in the Behavioral Sciences, Stanford, California
L. ERLENMEYER-KIMLING
New York State Psychiatric Institute, Columbia University, New York

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8. A more extensive report of these findings is being prepared for submission to the *Journal of Comparative and Physiological Psychology*. Complete data on the behavior of the foundation population and all selected generations are available in the two doctoral dissertations on which this report is based: J. M. Weiss, Columbia Univ. (1959), and L. Erlenmeyer-Kimling, Columbia Univ. (1961). Grateful acknowledgment is expressed to T. Dobzhansky and F. J. Ryan for generously providing laboratory space and many research facilities. This research was supported by grants G-3846, G-8998, and G-13595 from the National Science Foundation.

23 May 1961

Mechanism of Inactivation of Enzyme Proteins by Ultraviolet Light

Abstract. Some quantum yields for the destruction of amino acids have been determined. The inactivation of the enzymes chymotrypsin, lysozyme, ribonuclease, and trypsin by ultraviolet light can be accounted for quantitatively by summing the products of (i) the probability that light is absorbed by a given amino acid residue, ϵ_i, and (ii) the probability that absorbed light induces a chemical change, with a quantum efficiency ϕ_i, in the residue. The principal residues involved are cystyl and tryptophanyl. Peptide bond rupture is not important. Analysis of inactivated enzymes verifies the assumption of the existence of several inactivation mechanisms.

It has been suggested that the inactivation of enzymes by ultraviolet light involves, as the primary chemical reaction, photolysis of disulfide and aromatic residues (*1*), and Setlow has had some success in calculating quantum yields for enzyme inactivation based on this assumption (*2*). For small degrees of enzyme (E) inactivation, giving rise to inactive products P, we have for the rate (*3*)

$$-\mathrm{d}(E)/\mathrm{d}t = \Phi I_{\mathrm{abs}}\epsilon_e(E)/[\epsilon_e(E) + \epsilon_p(P)] \cong \Phi I_{\mathrm{abs}}\epsilon_e(E)/\epsilon_e(E_o) \quad (1)$$

where ε_e is the molecular extinction coefficient of the enzyme and ε_p that of the products in time t, I_{abs} is the absorbed intensity, for unit path length, and Φ is the quantum yield for enzyme inactivation. If we make the assumption that an enzyme molecule can undergo inactivation by loss of identity of any one of the aromatic residues, –SS– bonds or –CONH– bonds (the other moieties do not absorb appreciably at this wavelength), we can write

$$-\mathrm{d}(E)/\mathrm{d}t \cong [I_{\mathrm{abs}}/\epsilon_e(\mathrm{E}_o)] \sum^{t} n_i\varepsilon_i(E)\phi_i \quad (2)$$

where n_i is the number of residues per molecule of enzyme of molar concentration (E), ε_i is the extinction coefficient of each residue, and ϕ_i is the quantum yield for destruction (loss of chemical identity) of each residue. Equating the rates we find that

$$\Phi_{enz} = \sum^{t} n_i\varepsilon_i\phi_i/\varepsilon_e \quad (3)$$

First we have determined the quantum yields, ϕ = reactant molecules chemically changed per quanta absorbed by reactant, for the total destruction of amino acids with appreciable absorption at 2537 A (irrespective of the multiplicity of products). These values, for acid media and under nitrogen, are listed in Table 1. For analysis, ion exchange or paper chromatography was generally used; the details are recorded elsewhere (*4*). Second, we assume that the quantum yields, ϕ_i, and extinction coefficients, ε_i, also apply to the intact protein. Although we have no way of knowing, a priori, whether ϕ_i for the amino acid side chain is the same whether free or combined, ε_i for these residues are only approximately the same for free and combined amino acids (*5*). From this information, quantum yields for enzyme inactivation, Φ, calculated from Eq. 3, are compared with the known values for chymotrypsin, lysozyme, ribonuclease, and trypsin: agreement is obtained within a factor of two to three, which is surprisingly good in view of the number of factors upon which Φ depends (*1*).

Table 1. Estimation of quantum yields for enzyme inactivation from quantum yields for amino acid destruction (2537 A).

Amino acid, ϕ	Enzyme							
	Chymotrypsin ϵ_e = 23,000		Lysozyme ϵ_e = 18,000		Ribonuclease ϵ_e = 4,400		Trypsin ϵ_e = 15,500	
	n_i	$n_i\epsilon_i\phi_i$	n_i	$n_i\epsilon_i\phi_i$	n_i	$n_i\epsilon_i\phi_i$	n_i	$n_i\epsilon_i\phi_i$
Cystine, 0.13	5	175	5	175	4	140	6	210
Histidine, <0.03	2	<0.015	1	0.007	4	<0.03	1	0.007
Phenylalanine, 0.013	6	11	3	5	3	5	3	5
Tryptophan, 0.004	7	80	8	92	0		4	46
Tyrosine, 0.0020	4	3	3	2	6	4	4	3
—CONH— as in acetylalanine, 0.05	~200	~1	~130	~1	~130	~1	~200	~1
	Φ *for enzymes*							
Calculated	0.01		0.01		0.30		0.01	
Known	0.005		0.024		0.027		0.105	

By chemical analysis of hydrolyzates of ultraviolet-inactivated enzymes, it was also found that about one tryptophan residue per enzyme molecule is destroyed in chymotrypsin and trypsin and that two to three disulfide linkages are broken in trypsin, 1.7 in ribonuclease, and none in chymotrypsin. No phenylalanine, tyrosine, lysine, and/or histidine or arginine residues were changed; with a calculated inactivation well over 99.999 percent, some of these were altered, however. No titratable amino groups appeared during inactivation of the nonproteolytic enzymes, which indicates no ruptured –CONH– (compare *6*). Histidine does not appear to be the vulnerable group with ultraviolet light; this residue is primarily involved in photosensitized inactivation with visible light, incidentally (*7*).

That breakage of hydrogen bonds is also involved in the inactivation-denaturation of enzymes has been demonstrated elsewhere and involved a study of the influence of temperature on Φ (*1*).

A. D. MCLAREN
R. A. LUSE
College of Agriculture, University of California, Berkeley

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15 May 1961

Separation Rate and Neighbor Diffusivity

Abstract. Separation rates of neighboring pieces of orange peel floating on the sea were measured under fresh breeze conditions. When Stommel's equation for neighbor diffusivity (F) was applied to the data it became apparent that the F value increased by an order of magnitude whenever the time adrift increased by this amount. This is a result of the fact that the increase in spacing distance is squared while the time adrift is not. It is recommended that a standard time of 1 second be used whenever Stommel's equation is applied in neighbor diffusivity problems. Recomputation of data from the literature showed that neighbor diffusivity varied between 0.08 and 1 cm²/sec while time adrift varied between 10 and 10^8 seconds. Further study of separation rates, as parameters of surface turbulence, is recommended.

Turbulence is one of the most important factors influencing aquatic ecology and quantitative measurements of it are needed. Hutchinson (*1*) has reviewed the theoretical treatment of turbulent energy relationships, stating that, if all the energy in the largest eddy of a system is considered, turbulent energy varies directly with the size of the space under consideration; thus if l is the linear dimension, average turbulent energy will be proportional to $l^{2/3}$, average eddy viscosity to $l^{4/3}$, and average velocity to $l^{1/3}$. Hutchinson also summarized the work of Richardson and Stommel on neighbor diffusivity showing that this parameter is analagous to eddy diffusivity in transport equations of the Fickian type, and reporting that neighbor diffusivity, computed from separation rates of wet pieces of paper floating on the sea, varied as the 4/3 power of the distance separating neighbor pairs. This observation would seem to recommend the neighbor diffusivity as a measure of turbulent energy and to substantiate the theoretical relationship between energy and the linear dimensions of the space under consideration. Olson and Ichiye (*2*) have published a graph showing that the neighbor diffusivity (F), when computed with Stommel's (*3*) equation (*4*):

$$F = \overline{(l_1 - l_0)^2}/2T$$

increases by approximately one order of magnitude whenever $(l_1 - l_0)$ increases by an order of magnitude. They summarized data for parsnip pieces, mimeograph paper, dye spots, drift cards, and drift bottles, in which $\overline{(l_1 - l_0)}$ varied between 10 and 10^8 cm. I became interested in investigating neighbor diffusivity as a possible parameter of surface turbulence in aquatic environments, and carried out some experiments at the Alligator Harbor Marine Laboratory of the Florida State University (Tallahassee) during December 1959 (*5*). I used five pieces of orange peel which were deposited at the end of the laboratory pier, as close together as possible, and were allowed to drift ashore. Their time adrift was measured, and their distance apart in the direction perpendicular to the direction of the drift. Thus the shape of the shore line did not influence the measure of separation rate. From eight sets of data obtained under conditions of fresh breeze I obtained an average separation rate of 0.8 cm/sec. The averages from individual sets of data ranged from 0.24 to 1.2 cm/sec. If this value of 0.8 cm/sec is regarded as a reliable measure of the rate at which neighbor pairs separate when floating on the sea and I compute the neighbor diffusivity, using Stommel's equation, assuming a constant separation rate of 0.8 cm/sec operating for various times adrift, I obtain the values in column 3 of Table 1. In columns 4 and 5 I have entered data computed from the graph of Olson and Ichiye. This table shows that most of the increase of F with increasing values of $\overline{(l_1 - l_0)}$ simply results from the fact that $\overline{(l_1 - l_0)}$ is squared in Stommel's equation, while T is not.

In private communications, Olson and Ichiye kindly provided me with sample sets of data, computations, and opinions which enabled me to verify that I have not misinterpreted the data in their graph. Column 5 in Table 1 shows that the average separation rates of neighbor pairs throughout this wide range of times adrift are all reasonably close to my experimental value of 0.8 cm/sec. If one wishes to employ an equation such as Stommel derived for neighbor diffusivity, then a standard time interval, preferably 1 second, should be used in the computation. If this is done, the F values obtained from the data in column 5 of Table 1 will range from 0.08 to 1 cm²/sec. It appears that the purported proportionality of neighbor diffusivity with distance of spacing ($F = kl^{4/3}$) (*6*) is a result of mathematical treatment that disappears when one uses a standard time interval in Stommel's equation. The theoretical relationship between turbulent energy and the linear dimension of the space under consideration is not confirmed by these data. If this relationship were valid, the separation rates in column 5 of Table 1 should be of the order of 100 cm/sec when $\overline{l_1 - l_0}$ equals 10^6 cm, that is, they should vary as the cube root of the linear dimension. The data in Table 1 suggest that the separation rate and neighbor diffusivity are relatively independent of the linear dimension of the space under consideration. Olson and Ichiye emphasized the fact that the data they compiled did not meet the requirements made by Stommel in his derivation of the theoretical relationship. This fact may be responsible for the discrepancy between theoretical and observed separation rates. The most significant feature of these data is the relatively constant average

Table 1. Neighbor diffusivity values, computed from Stommel's equation, compared with the rate of separation of neighbor pairs.

Time adrift (sec)	$\overline{(l_1-l_0)}$ assuming separation rate of 0.8 cm/sec	Computed neighbor diffusivity $\frac{\overline{(l_1-l_0)^2}}{2T}$	Neighbor diffusivity from graph of Olson and Ichiye	Separation rate (cm/sec) computed from graph of Olson and Ichiye $\frac{\overline{(l_1-l_0)}}{T}$
10	8	3.2	3 (*6*)	0.77
10^2	80	32	15 (*6*)	0.55
10^4	8×10^3	3.2×10^3	0.8×10^3	0.40
10^6	8×10^5	3.2×10^5	6×10^5	1.1
10^8	8×10^7	3.2×10^7	2×10^8	1.4