Table 1. Molecular penetration relative to water obtained experimentally for squid axon membrane, Exp, compared with that calculated for a membrane with rigid pores of 4.25 Angstrom units, $(P_M/P_W)_4$, or with monolayer islands composed of five molecules, $(P_M/P_W)_M$. The mean molecular radius, r, is in Angstrom units.

r*	$(P_M/P_W)_4$*	Exp.*	$(P_M/P_W)_M$
		Water	
1.5			
		Methanol	
1.83	0.62	0.65±0.03	0.62
		Ethylene glycol	
2.24	0.28	0.28±0.03	0.28
		Glycerol	
2.77	0.1	0.04±0.02	0.076

* From Villegas and Barnola (*11*).

cause an increase, ΔS, in the surface pressure, S. Both U_M and the work of entry will be increased, since they are functions of S. As measured in long chain alcohols at values of S above 15 dy/cm, U_M increases by 2.5 cal for each dyne increment in surface pressure (*13*); moreover, the work done will be increased from Sa to $(S + \Delta S/2)a$.

It remains to define the dependence of ΔS on a/m. For convenience one may use the linear relation (which gives a constant ratio) for liquid condensed stearate, namely, 5.65 dy/Å^2, or for solid condensed stearate, 66.7 dy/Å^2 (*14*). The particular values at this stage are not important since, by an appropriate selection of m, ΔS can be given a range of values pertinent to experimental data. Thus, with 5.65, $m = 5$ will give the same range of ΔS as $m = 60$ for 66.7. An intermediate value will be taken for S, namely, 15 dy/cm, and n will be taken as 16, as for stearate.

The basic equation for calculating relative permeabilities thus becomes

$$\ln \frac{p_1}{p_2} = n \frac{\Delta U_{M2} - \Delta U_{M1}}{RT} + S \frac{(a_2 - a_1)}{kT} + \frac{(\Delta S_2 a_2 - \Delta S_1 a_1)}{2kT} \quad (3)$$

Substituting the constants that have been given, we have, at 25°C,

$$\ln \frac{p_1}{p_2} = 40 \frac{(\Delta S_2 - \Delta S_1)}{592} + 15 \frac{(a_2 - a_1)}{4 \cdot 10^{-14}} + \frac{(\Delta S_2 a_2 - \Delta S_1 a_1)}{8 \cdot 10^{-14}}$$

in which $\Delta S = 1.1a$, in dy/cm when a is in Å^2. $a = \pi r^2$ and r, the mean molecular radius, is in cm for a in the last two terms of the equation. The term 40 follows from $n = 16$ and $\Delta U_M = 2.5\Delta S$, as discussed above.

With the data and the relationships given, the permeabilities to molecules such as studied with the giant axon of squid (*9*) have been calculated; these are compared in Table 1 with the experimental data and with the figures calculated assuming rigid pores of 4.25 Å radius (*9*). The monolayer results are about as good as those obtained by rigid-pore theory.

The present calculations show that certain characteristics of monolayers can provide an alternative to rigid-pore theory for accounting for membrane permeability to polar molecules. They are necessary but do not suffice to establish the validity of the original assumptions. More information, such as the effect of temperature and of agents that alter S (*6*), as well as the behavior of other lipoidal monolayers, may provide additional tests of the proposed model, and perhaps a more circumscribed picture of the situation in living membranes (*15*).

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References and Notes

1. H. Davson and J. F. Danielli, *The Permeability of Natural Membranes* (Cambridge University Press, London, 1943).
2. J. D. Robertson, in *Biophysics of Physiological and Pharmacological Actions*, A. M. Shanes, Ed. (AAAS Publ. No. 69, American Association for the Advancement of Science, Washington, 1961), p. 63.
3. R. Hober, *Physical Chemistry of Cells and Tissues* (Blakeston, Philadelphia, 1945).
4. R. J. Archer and V. K. La Mer, *J. Phys. Chem.* **59**, 200 (1955).
5. J. C. Skou, *Biochim. Biophys. Acta* **30**, 625 (1958); ——— *Acta Pharmacol. Toxicol.* **10**. 317 (1954); J. A. Clements and K. M. Wilson, *Proc. Natl. Acad. Sci. U.S.* **48**, 1008 (1962).
6. A. M. Shanes and N. L. Gershfeld, *J. Gen. Physiol.* **44**, 345 (1960); N. L. Gershfeld and A. M. Shanes, *Science* **129**, 1427 (1959).
7. G. T. Barnes and V. K. La Mer, in *Retardation of Evaporation by Monolayers*, V. K. La Mer, Ed. (Academic Press, New York, 1962), p. 9.
8. M. Blank, *ibid.*, p. 75.
9. R. Villegas and F. V. Barnola, *J. Gen. Physiol.* **44**, 963 (1961).
10. A. M. Shanes, *Pharmacol. Rev.* **10**, 59 (1958); J. M. Tobias, in *Modern Trends in Physiology and Biochemistry*, E. S. G. Barron, Ed. (Academic Press, New York, 1952), p. 291.
11. R. Villegas and G. M. Villegas, *J. Gen. Physiol.* **43**, 73 (1960).
12. A. M. Shanes and M. D. Berman, *J. Pharmacol. Exptl. Therap.* **125**, 316 (1959).
13. M. Blank and V. K. La Mer, in *Retardation of Evaporation by Monolayers*, V. K. La Mer, Ed. (Academic Press, New York, 1962), p. 59.
14. V. K. La Mer and M. L. Robbins, *J. Phys. Chem.* **62**, 1291 (1958).
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Gamma Irradiation of Polypeptides: Transformation of Amino Acids

Abstract. *The formation of aspartic acid from glutamic acid, of aspartic and glutamic acids from proline, of α-amino-n-butyric acid from methionine, of aspartic acid from histidine, of dihydroxyphenylalanine from tyrosine, of tyrosine and dihydroxyphenylalanine from phenylalanine, of alanine from cysteine, and of glycine from alanine was observed when aqueous solutions of these amino acids in the form of peptides or polyamino acids were irradiated. When poly-L-glutamic acid or poly-L-proline was irradiated in the presence of C^{14}-labeled $NaHCO_3$, the radioactive carbon was fixed by the aspartic and the glutamic acid.*

In their paper on the effect of gamma radiation on the amino acid content of insulin, Drake *et al.* (*1*) reported that two amino acids, threonine and alanine, increase in amounts while all others are destroyed. If amino acid transformations occur as a result of the irradiation this observation could be adequately explained. We would like to present data here that show the frequency and extensiveness of such conversions.

The peptide (1 to 2 mg) in 1 ml of 0.1N borate buffer (pH 8.3) was placed in a pyrex tube, flushed with helium, and evacuated. The exposure to helium and subsequent evacuation were repeated three times before the tube was sealed and irradiated by a Co^{60} source (approximately 0.125 ×

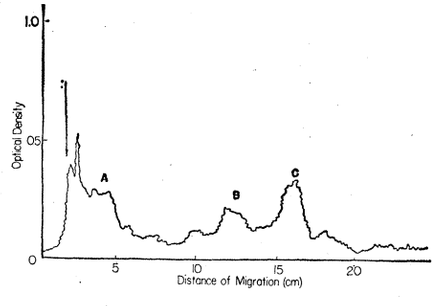

Fig. 1. Fixation of CO_2 during transformation of amino acids. Approximately 5 μc of C^{14}-labeled CO_2 and 7 mg of poly-L-proline in 2 ml of 0.1N borate buffer pH 8.3 were irradiated with 8×10^6 r. After hydrolysis the mixture was chromatographed; the secondary butanol—formic acid solvent (*3*) system was used. A radioautogram was prepared and translated into the above curve by means of a densitometer. Arrow, point of application; *A*, ninhydrin-negative unknown; *B*, aspartic acid; *C*, glutamic acid.

10^6 r/hr. The peptide was hydrolysed with 6*N* HCl in evacuated tubes for 22 hours at 110°C. The resulting hydrolyzate was taken to dryness in a vacuum over NaOH pellets, dissolved in sodium citrate buffer of *p*H 2.2, and analyzed in a Spinco amino acid analyzer according to the method of Moore, Spackman, and Stein (*2*). Amino acids formed from other amino acids were identified by cochromatography on the column and also on paper with Hausmann's solvent system (see *3*).

Table 1 shows that glutamic acid is transformed into aspartic acid, proline into glutamic and aspartic acid, methionine into α-amino-*n*-butyric acid, histidine into aspartic acid, tyrosine into dihydroxyphenylalanine (DOPA), phenylalanine into tyrosine and dihydroxyphenylalanine, cysteine into alanine, and alanine into glycine. These data are in agreement with those of Rowbottom (*4*) who found that irradiation of tyrosine in aqueous solution produces dihydroxyphenylalanine and of Vermeil and Lefort (*5*) who irradiated phenylalanine and obtained *o*-, *m*-, and *p*-hydroxyphenylalanine. They also complement the findings of Grant *et al.* (*6*), of Markakis and Tappel (*7*) who reported that cystine can be changed into alanine, and those of Kopoldova *et al.* (*8*) who detected α-amino-*n*-butyric acid, as well as threonine, alanine, aspartic acid, and serine, among the decomposition products of methionine.

Table 1. Amino acid transformaton as a result of gamma irradiation. The amounts of amino acids are given in micromoles (irradiated in 1 ml of buffer).

	Poly-L-glutamic acid			
Dose (10^6 r)	0	5.83	8.03	
Glutamic acid	5.6	2.8	0.95	
Aspartic acid	0	0.56	0.76	
	Poly-L-proline			
Dose (10^6 r)	0	3.11	7.65	9.83
Proline	15.7	8.6	3.6	2.3
Glutamic acid	0	1.4	1.0	1.1
Aspartic acid	0	0.11	0.18	0.22
	Glycyl-L-methionine			
Dose (10^6 r)	0	4.72	7.19	
Glycine	2.9	1.6	1.2	
Methionine*	1.9	0.91	0.38	
α-Aminobutyric acid	0	0.70	0.93	
	Glycyl-L-histidine			
Dose (10^6 r)	0	3.62	4.97	7.18
Glycine	5.6	2.4	1.9	0.58
Histidine	5.1	0.78	0	0
Aspartic acid	0	0.44	0.44	0.96
	L-leucyl-L-tyrosine			
Dose (10^6 r)	0	3.62	5.35	6.43
Leucine	3.4	2.3	1.6	0.72
Tyrosine	3.4	2.4	1.0	0.42
DOPA	0	0	0.17	0.18
	DL-phenylalanylglycine			
Dose (10^6 r)	0	2.41	5.28	7.01
Glycine	6.7	6.6	5.6	5.5
Phenylalanine	6.9	1.8	0.45	0
Tyrosine	0	0.04	0.29	0.20
DOPA†	0	0.08	0.15	0.16
	Glutathione			
Dose (10^6 r)	0	1.07	6.22	
Glutamic acid	3.9	3.7	2.8	
Glycine	3.9	4.2	3.2	
Cystine*	3.4	2.6	0.85	
Alanine	0	0.51	1.2	
	L-alanyl-L-leucine			
Dose (10^6 r)	0	3.33	5.82	10.26
Alanine	5.9	3.0	2.11	0.60
Leucine	5.9	1.3	0.85	0.54
Glycine	0	0	0.15	0.41

* Methionine and cystine are partially destroyed during acid hydrolysis. † Small peaks for meta and ortho hydroxyphenylalanine were also observed.

For the formation of aspartic acid from glutamic acid one could either postulate decarboxylation of the gamma-carboxyl group of glutamic acid by oxidation of the gamma carbon atom or splitting between the beta and gamma carbons with subsequent fixation of CO_2 on the beta carbon atom (*9*). To test these hypotheses, poly-L-glutamic acid was irradiated in the presence of $NaHCO_3$ labeled with C^{14}. After hydrolysis and chromatography, a radioautogram indicated activity in the position for aspartic acid thus suggesting that the second possibility is plausible. The occurrence of the C^{14}-labeled CO_2 in the glutamic acid itself might be explained by a recarboxylation of a decarboxylated glutamic acid free radical. A third unidentified, ninhydrin negative, radioactive product was also observed. Similarly, gamma irradiation of poly-L-proline (*10*) in the presence of C^{14}-labeled $NaHCO_3$ yielded labeled glutamic and aspartic acid, Fig. 1 (*11*).

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References and Notes

1. M. F. Drake, J. W. Giffee, D. A. Johnson, V. Loenig, *J. Am. Chem. Soc.* **79**, 1395 (1957).
2. S. Moore, D. H. Spackman, W. H. Stein, *Anal. Chem.* **30**, 1185 (1958).
3. W. J. Hausmann, *J. Am. Chem. Soc.* **79**, 3181 (1952).
4. J. Rowbottom, *J. Biol. Chem.* **212**, 877 (1955).
5. C. Vermeil, and M. Lefort, *Compt. Rend.* **244**, 889 (1957).
6. D. W. Grant, S. N. Mason, M. A. Lind, *Nature* **192**, 352 (1961).
7. P. Markakis, and A. L. Tappel, *J. Am. Chem. Soc.* **82**, 1613 (1960).
8. J. Kopoldova, J. Kolousek, A. Barbicky, J. Liebster, *Nature* **182**, 1074 (1958).
9. A suggestive discussion with Dr. B. Tolbert, University of Colorado, is gratefully acknowledged.
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Siphonophores and the Deep Scattering Layer

Abstract. *Bathyscaphe dives in the San Diego Trough have revealed a close spatial relation between siphonophores and the deep scattering layer as recorded by precision depth recording echo-sounders. Measurements of gas bubbles within the flotation structures of* Nanomia bijuga *captured in a closing net in an ascended scattering layer indicate that these are very close to the resonant size for 12-kcy/sec sound. Because such organisms are capable of making prolonged vertical migrations, and are widespread geographically, they are very probably the major cause of stratified zones of scattering throughout the oceans of the world.*

Despite intensive study, the question of the causes of ubiquitous, mid-depth zones of oceanic, sonic reverberation generically known as the deep scattering layer (DSL), has evaded a satisfactory answer. The general interpretation of the acoustical evidence is that diminutive fishes with gas-filled swim bladders are the principal scatterers. More frequently, though, net hauls taken through scattering layers at their daytime depths return virtually empty or with the catch dominated by euphausiids. These hauls have not demonstrated that the populations of these small shrimps are large enough to account for measured reverberation volumes (*1*, *2*).

Recent observations from the U.S. Navy bathyscaphe *Trieste* have revealed that colonial hydrozoan jellyfish known as siphonophores are probably the primary cause of the deep scattering layer. Six dives were made from January to October 1962 off San Diego, site of the discovery of the deep scattering layer (*3*, *4*). Scattering conditions were recorded either on an EDO depth-finding system or Precision Depth Recorders (PDR), or both, from surface ships while the *Trieste* was ascending. Typically (*5*, *6*) the layer was located between 260 and 440 m during daylight hours and usually displayed an upper and lower component. Observations from the bathyscaphe permitted general identification of organisms thought capable of scattering 12-kcy/sec sound. Whenever possible, counts were made, and by computing the angle of view and the maximum range at which the various types of organisms could be recognized, the volume of water containing a given number of