rect associations are given equally well for both groups.

To check this conclusion experiment 3 was performed. Subjects were presented with single items, one at a time for 3 seconds each with a 5-second interval between them. Only one trial was given. Subjects in one group had to "spell" each item and subjects in the other group had to "pronounce" each item as it was presented. There were ten subjects in each condition. After a 1-minute rest, subjects were given a 2-minute free-recall period. Again the results were clear. The spelling group recalled a mean of 3.0 items (S.D., 1.8), whereas the pronouncing group recalled a mean of 5.5 (S.D., 1.7). A test of significance yielded a t of 3.20, which is significant since $p < .01$. The difference is accounted for primarily in the greater number of no-response errors by the spelling group, but these subjects also made many more errors in which the first letter only was correct than did those in the pronouncing group.

The spelling-out procedure makes it more difficult to learn items. The main question has been whether an association between two items develops instantaneously or gradually. For purposes of exploring this question it is not desirable to use the spelling-out procedure. Whatever accentuates the difficulty of learning items is not desirable. As to why spelling-out leads to difficulty one can only speculate that it leads to a fragmentation into parts of what in the pronouncing method is more of a unitary whole. Perhaps the trace is less available because it has a somewhat attentuated unity character.

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11 March 1963

Membrane Permeability: Monolayer Relationships

Abstract. *A model of permeation of living membranes is proposed in which penetration by polar molecules takes place through islands composed of limited numbers of lipoidal molecules in a state comparable to that of certain compressed monolayers. These islands are visualized as scattered within a rigid, relatively impervious matrix. Relationships for penetration of monolayers by gases have been applied to this membrane model. Calculations on this basis demonstrate that the permeabilities relative to water are described at least as well by this model as by that assuming rigid pores of 4.25 Å radius.*

It has been postulated on theoretical grounds (*1*), and experimental evidence appears to be offered by modern electron microscopy (*2*), that the living membrane is a bimolecular leaflet of lipid, the aqueous surfaces of which are bounded by protein. The permeability characteristics of living membranes suggest that the lipoidal layers are the primary barriers to diffusion (*3*). Moreover, the penetrability of fatty monolayers is susceptible to change through alterations in the tightness of packing expressed as surface pressure (*4*); this result is of interest in view of the demonstration that the permeability changes in living membranes brought about by "stabilizing" (local anesthetics, alcohols, "inert gases") and "labilizing" (veratrum alkaloids) drugs are closely correlated with changes in monolayer packing induced by pharmacologically effective concentrations of these drugs (*5, 6*).

According to Archer and La Mer (*4*) and Barnes and La Mer (*7*), monolayer penetrability to water, p (the inverse of their monolayer resistance, r), may be expressed as an exponential function of the experimental activation energy, U; a frequency coefficient, C, can be lumped with energy and entropy terms which are assumed to undergo little change, thereby giving what is considered to be a constant C'. Therefore, if comparison is to be made of the penetrabilities of 2 different molecules, 1 and 2, one may write

$$p_1/p_2 = \exp (U_2 - U_1)/RT \qquad (1)$$

where R is the gas constant and T the temperature. Among the energy terms composing U, are U_M, in cal/mole, for the interaction of the individual methylene groups (CH_2) with each other, and the work of passage of a molecule of cross-sectional area, a, through a monolayer of surface pressure, S, in dy/cm; this work is Sa. Taking account of these specific terms, one may rewrite Eq. 1 as follows:

$$\ln \frac{p_1}{p_2} = n \frac{(U_{M2} - U_{M1})}{RT} + S\frac{(a_2 - a_1)}{kT} \qquad (2)$$

in which n is the number of CH_2 groups and k the Boltzmann constant. The U_M of monolayers is regarded as unaffected by a, hence the term containing it is ordinarily neglected in comparisons of monolayer penetration by different molecules (*8*).

The highest value of S obtainable for aliphatic molecules about 18 carbon atoms (25Å) long is of the order of 40 dy/cm. Even at this high surface pressure, Eq. 2 leads to permeabilities relative to water that are too large compared to experimental figures. Thus, if the radius of a molecule of H_2O is 1.5Å, and those of a molecule of methanol, ethylene glycol, and glycerol, respectively, are 1.83, 2.24, and 2.77Å (*9*), the relative permeabilities by computation are 0.71, 0.43, and 0.19, compared to the experimental values in the giant axon of squid of 0.65, 0.28, and 0.04 (*9*). The computations, as for the rigid-pore model calculations (*9*), ignore hydration energies.

Equation 2 can predict permeability better by considering the regions where permeation occurs as only a part of the surface of living membranes—in fact, islands composed of a limited number of lipoidal molecules in a relatively rigid, impervious matrix. Restricted regions have been proposed for ion penetration in excitable membranes (*10*). This concept also provides a basis for the fact that the ratio of the penetration rate of water in monolayers (*8*), to that in the squid giant axon membrane (*9, 11*), is approximately 1000 : 1. For simplicity, only one of the membrane lipid layers is regarded as governing permeation; attention has been called to several studies which suggest that this is the case for ions (*12*).

By limiting the number, m, of membrane molecules at the site of entry, and by fixing the area available to them, p is made more sensitive to the size of a penetrating molecule. This is because the passage of a molecule through an island decreases the area per membrane molecule by the factor a/m (a being the cross-sectional area of the penetrating molecule), which in turn will

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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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 ×

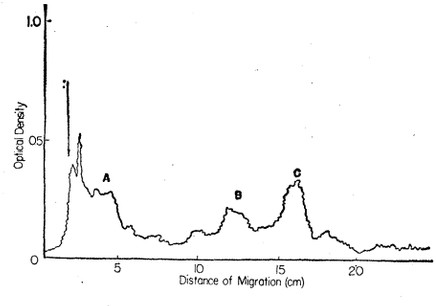

Fig. 1. Fixation of CO_2 during transformation of amino acids. Approximately 5 μc of C^{14}-labeled CO_2 and 7 mg of poly-L-proline in 2 ml of 0.1N borate buffer pH 8.3 were irradiated with 8×10^6 r. After hydrolysis the mixture was chromatographed; the secondary butanol—formic acid solvent (*3*) system 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.