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Cyanine Dye Structural and Voltage-Induced Variations in Photo-Voltages of Bilayer Membranes

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Summary. Flash illumination alters the voltage across bilayer lipid membranes in the presence of certain cyanine dyes. The waveforms of the photo-voltage vary systematically with dye structure and imposed transmembrane voltage. Experimental results are reported for 27 positively charged cyanine dyes, primarily oxazole derivatives, using lecithin/oxidized cholesterol bilayer membranes and 10-mM sodium chloride solutions. Several dyes do not induce any photo-voltages. Examples are 3,3′ diethyl 9 ethyl 2,2′ oxacarbocyanine iodide, 3,3′ diethyl 2 oxa 2′ thiacyanine iodide, and 3,3′ dimethyl 2,2′ indocarbocyanine iodide. Several dyes, when added to one side of the membranes, induce monophasic waveforms. Examples are 3,3′ dimethyl 2,2′ oxacarbocyanine chloride, and 3,4,3′,4′ tetramethyl 2,2′ oxazalinocarbocyanine iodide. Other dyes induce a photo-voltage only if transmembrane voltages are imposed. These waveforms are biphasic with some dyes (3,3′ diethyl 2,2′ oxacarbocyanine iodide, for example) and monophasic with other dyes (3,3′ dibutyl 2,2′ oxacarbocyanine iodide, for example).

The photo-voltage waveforms are explained by models that consider the movement of charged dye molecules within the membrane, following optical excitation. The dye movements are probably induced through charge rearrangements in the dye associated with long-lived triplet states, isomerization, or through excimer formation. These results provide information on the location and orientation of the dye molecules within bilayer membranes. The variations which occur in the waveforms with applied voltage indicate that these membranes are fluid in the direction perpendicular to the membrane plane.

Cyanine dyes are being used in increasing numbers of laboratories as fluorometric probes for monitoring membrane potential in a variety of biological and artificial systems (Cohen et al., 1974; Laris & Pershadsingh, 1974; Sims et al., 1974; Adamich, Laris & Sweeney, 1976; Laris, Pershadsingh & Johnstone, 1976; Salama & Morad, 1976; Tasaki & Warashina, 1976; Waggoner, 1976). Quantitative theories explaining the time course and magnitude of the fluorescence changes resulting from transmembrane voltage variations are lacking, although plausible mechanisms are described in several of the above papers. Ullrich and Kuhn (1969 and

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1972) first reported that voltage transients are induced across bilayer lipid membranes by a cyanine dye and illumination. Huebner (1975) reported that 8 of the 10 cyanine dyes, under the conditions then studied, induce these photo-voltages. The purpose of this paper is to describe the experimental results for 27 cyanine dyes, to report that variations result in the photo-voltage waveforms from imposed transmembrane voltages, and to analyze a theoretical model which describes the photo-voltage waveforms observed. These studies provide unique data relating to (i) the location and movement of charged dye molecules in artificial membranes (Huebner, 1977), and (ii) the physical processes which occur in the dye molecules and membranes as a result of light absorption.

Materials and Methods

Bilayer lipid membranes were prepared at a 1.3-mm diameter hole in a Teflon inner cup, which was held inside a pyrex glass outer cup. Both cups were filled with 0.01 M NaCl. The syringe method of forming membranes was used, in which the membrane-forming solution was ejected against the Teflon cup just below the hole. A polyethylene (Intramedic) tube which covered and extended ~ 1 cm beyond the syringe needle was used to wipe the membrane forming solution over the hole as the solution flowed upwards on the cup. The membrane forming solution was a 1:1 by weight solution of lecithin (chromatographically pure egg L-α-lecithin or phosphatidylcholine, Koch-Light Laboratories, Ltd.) and oxidized cholesterol in decane. Membrane illumination was provided by a xenon flash tube in a General Radio Corporation type 1538-A stroboscope with a P4 capacitor assembly, which was modified to lower the discharge path impedance. The peak illumination intensity was estimated by the method previously described (Huebner, 1975) to be about 5×10^9 lux at the membrane, with the 1/3 peak intensity points separated in time by 7 μsec.

The dyes were normally added to the inner cup solution 15 min after the membrane was formed, by using an Oxford Sampler repeating micropipetting system and mixed aqueous/ethanol solutions of the dyes. The percent ethanol in the inner cup solution is given below. The input resistance of the membrane electrometer was reduced to 10^9 or 10^7 ohms by precision resistors. A voltage was applied through the precision resistors to adjust the transmembrane voltage. The membrane resistance values reported below were obtained by using the average current value required to produce plus and minus 60 mV transmembrane voltages. The photo-voltage waveforms and resistance values were recorded 15 min after the dye addition. Electric noise from the stirring motor required that the stirring magnets be stopped during measurements. The temperature was regulated only by building air conditioning, but did not vary outside the range of 22 to 26 °C. Other details of the apparatus and procedures are as previously described (Tien & Huebner, 1973; Huebner, 1975).

Results

The resistance of the membranes used in this work were always greater than 3×10^9 ohms before dye addition. All dyes lowered the membrane resistance. Examples of the dye structure, nomenclature and abbreviations

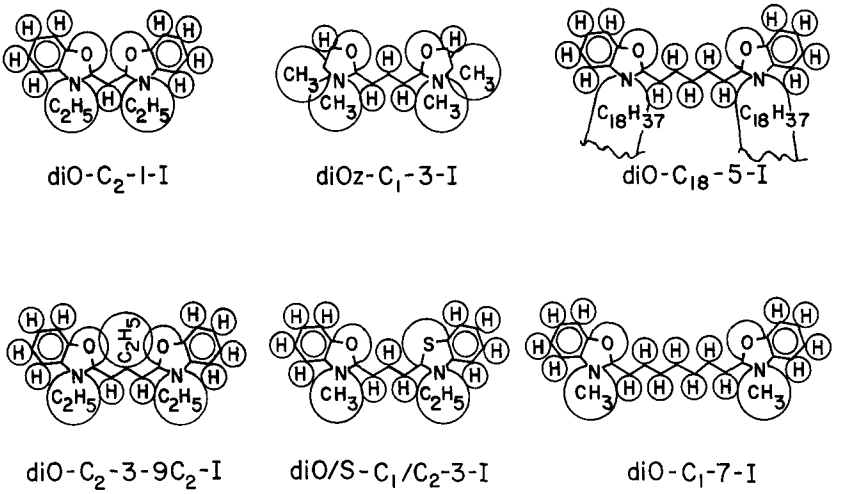

Fig. 1. Six dye structures, illustrating abbreviations used in this work. All dyes are shown in the planar "all-trans" configuration; anions, double bonds, and electrical charges are not shown. Some overlapping of the van der Waals circles may be observed. The illustrated dyes are 3,3′ diethyl 2,2′ oxacyanine iodide, abbreviated as diO-C_2-1-I; 3,3′,4,4′ tetramethyl 2,2′ oxazalinocarbocyanine iodide, or diOz-C_1-3-I; 3,3′ dioctadecyl 2,2′ oxadicarbocyanine iodide, or diO-C_{18}-5-I; 3,3′ diethyl 9 ethyl 2,2′ oxacarbocyanine iodide, or diO-C_2-3-9C_2-I; 3 methyl 3′ ethyl 2,2′ oxathiacarbocyanine iodide, or diO/S-C_1/C_2-3-I; and 3,3′ dimethyl 2,2′ oxatricarbocyanine iodide, or diO-C_1-7-I. The dye, 3,3′ dimethyl 2,2′ indocarbocyanine iodide, not shown, is abbreviated as diI-C_1-3-I. It differs from the dye diO-C_1-3-I in that $(CH_3)C(CH_3)$ is substituted for the oxygen atoms

used in this paper are given in Fig. 1. The data obtained are summarized in Table 1. The photo-voltage waveform amplitudes were reproducible to within about 5 % or 0.05 mV (whichever is larger) from flash to flash on each membrane, and to within about 20 % from membrane to membrane with the same dye and dye concentration. The resistance values were generally reproducible to within 20 % from membrane to membrane. Each entry in Table 1 represents average results for a minimum of 3 membranes. Typically 5 membranes were evaluated for each dye concentration reported.

The polarity of the imposed transmembrane voltages and photo-voltages described here are established by noting that a positive transmembrane voltage excursion results from transporting positive charge through the membrane toward the inner cup solution. Similarly, a positive membrane voltage will produce an electric field (a vector, defined as the magnitude and direction of force on a unit positive charge) which cause mobile positive charges to move within the membrane in the direction from

Table 1. The properties of lecithin/oxidized cholesterol bilayer membranes in the presence of cyanine dyes

Dye (source[a])	Dye conc. (μM/liter)	Ethanol conc. (%)	Resistance		Photo-voltage amplitude in mV, (risetime in ms) at			Notes[b, c]
			Memb. (ohms)	Shunt (ohms)	+60 mV	0 mV	−60 mV	
diO-C_2-1-I (KL 7835)	2	0.5	8×10^8	10^9	0	0	0	
	10	2	3×10^8	10^9	0	0	0	
	20	5	1.5×10^8	10^9	0	0	0	
	100	2.5	5×10^7	10^9	0	0	0	
diO/S-C_2-1-I (KL 7857)	20	0.4	3×10^8	10^9	0	0	0	
	100	1.6	3×10^7	10^7	0	0	0	
diOz-C_1-3-I (NK 307)	30	0	2×10^9	10^9	+0.15 (~400)	+0.05 (−)	0	
	100	0	8×10^8	10^9	+0.35 (~400)	+0.1 (−)	+0.05 (−)	*See* Fig. 2
diO-C_1-3-Cl (NK 354)	3	0.5	2.5×10^8	10^9	+0.7 (165)	+0.25 (25)	+0.2; −0.1 (10; ~450)	*See* Fig. 2
	5	1	1.5×10^8	10^9	+1.3 (80)	+0.75 (10)	+0.7; −0.05 (7; −)	
	20	0	6×10^7	10^9	+1.5 (60)	+1.1 (5)	+1.15; −0.2 (5; ~450)	*See* Fig. 2
diO-C_1-3-I (AW & KL 7871)	1	0.5	5×10^8	10^9	+0.6 (200)	+0.25 (10)	+0.2; −0.1 (8; ~200)	
	20	0.7	1×10^8	10^9	+1.6 (55)	+1.2 (6)	+0.9; −0.4 (4; ~200)	
	40	1	5×10^7	10^9	+1.75 (30)	+1.2 (6)	+1.0; −1.0 (4; 170)	
diO-C_2-3-I (AW & KL 7834)	10	0.5	2×10^8	10^9	−0.2; +0.4 (20; ~300)	+0.05 (−)	+0.2; −0.4 (20; ~300)	*See* Fig. 3
	20	1	3×10^7	10^9	−0.8; +0.1 (10; ~300)	−0.1; +0.5 (−)	+0.5; −0.7 (12; 210)	

diO-C_3-3-I (AW)	10	0.3	1×10^8	10^9	−0.5; +0.8 (30; 380)	0	+0.4; −0.8 (30; 330)	*See* Fig. 3
	20	0.5	2×10^7	10^9	−1.2; +0.4 (20; 340)	0	+1.4; −0.7 (20; 330)	
	50	1	1×10^7	10^9	−2.0; +0.05 (10; −)	0	+2.1; −0.1 (10; −)	
	100	3	3×10^6	10^9	−2.0 (7)	0	+2.3 (8)	
diO-C_4-3-I (AW)	1	0	2.5×10^7	10^9	+2.6 (175)	+0.1 (~175)	−2.0 (170)	*See* Fig. 4 1 μM < T_c < 6 μM
diO-C_5-3-I (AW)	0.15	0.3	1×10^8	10^9	+1.3 (160)	0	−1.6 (160)	
	3	1	4×10^6	10^7	+2.0 (36)	0	−2.1 (38)	
diO-C_6-3-I (AW)	0.1	0.3	3×10^7	10^9	+1.8 (80)	0	−1.8 (80)	0.1 μM < T_c < 0.4 μM $p\sim5\%$
diO-C_8-3-I (AW)	0.1	0.3	6×10^7	10^9	+1.5 (40)	0	−1.5 (40)	*See* Fig. 4 0.1 μM < T_c < 0.4 μM $p\sim10\%$
diO-C_{10}-3-I (AW)	0.4	0.2	5×10^7	10^9	+1.1 (42)	0	−1.2 (48)	$p=23\%$ 0.4 μM < T_c < 1.5 μM
diO-C_{14}-3-I (AW)	1	0.5	2×10^8	10^9	+1.3 (100)	0	−1.3 (110)	1 μM < T_c < 4 μM $p=33\%$

[a] The sources of the dyes are identified by the following abbreviations: AW, Professor Allan Waggoner, Chemistry Department, Amherst College; KL, Koch-Light Laboratories, Ltd., Buckinghamshire, England; and NK, The Japanese Research Institute for Photosensitizing Dyes Co., Lts., Okayama, Japan. The numbers following these letter abbreviations are catalog numbers.

[b] T_c indicates the transition concentration, the dye concentration at which the type of waveform attributed to dye complexes began to appear.

[c] p is equal to $(A_i - A_o)/A_i$, where A_i is the waveform amplitude with the incident light polarized so that the electric vector is in the membrane plane, and A_o is the amplitude with the electric vector 45° out of the membrane plane. The waveform risetimes did not change with polarization.

Table 1 (continued)

Dye (source[a])	Dye conc. (μM/liter)	Ethanol conc. (%)	Resistance		Photo-voltage amplitude in mV, (risetime in ms) at			Notes[b, c]
			Memb. (ohms)	Shunt (ohms)	+60 mV	0 mV	−60 mV	
diO-C_{18}-3-I (AW)	0.6	0.3	3×10^9	10^9	+0.05 (−)	0	−0.05 (−)	
	2	0.8	1×10^8	10^9	+0.9 (130)	0	−0.9 (130)	*See* Fig. 4 $p=35\%$
diO-C_2-3-9C_2-I (KL 7824)	40	0.5	1×10^8	10^9	0	0	0	
	Sat	1.5	2×10^7	10^7	0	0	0	
diO/S-C_1/C_2-3-I (KL 7877)	20	0.4	2×10^8	10^9	−0.1 (~50)	0	+0.1 (~50)	
	60	1.2	3×10^7	10^7	−0.15 (~20)	0	+0.3 (20)	
	100	2	2×10^7	10^7	−0.25 (10)	0	+0.4 (10)	
diO/S-C_2-3-I (NK 720 & KL 7837)	20	0.4	2×10^8	10^9	−0.05 (−)	0	+0.05 (−)	
	60	1.2	2×10^7	10^7	−0.1 (−)	+0.05 (−)	+0.2 (~10)	
	100	2	2×10^7	10^7	−0.15 (~5)	+0.05 (−)	+0.4 (10)	
diI-C_1-3-I (NK 513)	80	4	4×10^6	10^7	0	0	0	
diO-C_1-5-I (AW)	1	0.5	2×10^8	10^9	+0.05 (−)	+0.05 (−)	+0.05 (−)	
	10	4	2×10^7	10^9	−0.05 (−)	+0.1 (~1)	+0.2 (~1)	
	20	2	2×10^7	10^9	−0.1 (~1)	+0.15 (~1)	+0.25 (~1)	

diO-C_2-5-I (AW)	10	0.3	2×10^7	10^7	-0.1 (~3)	0	$+0.1$ (~3)	*See* Fig. 3
	20	0.2	10^7	10^7	-0.15 (3)	0	$+0.15$ (3)	$T_c\sim60$ μM
diO-C_3-5-I (AW)	20	0.2	10^7	10^7	-0.05 ($-$)	0	$+0.05$ ($-$)	
diO-C_4-5-I (AW)	30	0.3	3×10^6	10^7	-0.1 (~10)	0	$+0.1$ (~10)	30 μM $< T_c <$ 100 μM
diO-C_5-5-I (AW)	40	0.5	3×10^6	10^7	-0.05 ($-$)	0	$+0.05$ ($-$)	*See* Fig. 4
diO-C_{18}-5-I (AW)	10	1	2.5×10^6	10^7	-0.1 (~1)	0	$+0.1$ (~1)	
	30	3	2×10^6	10^7	-0.4 (1)	0	$+0.35$ (1)	
diI-C_1-5-I (NK 529)	80	4	5×10^6	10^7	0	0	0	
diO-C_1-7-I (NK 199)	2	0	2×10^8	10^9	-0.05 ($-$)	0	$+0.05$ ($-$)	This dye discolors in less than 1 hr in solution.
	20	0	2×10^7	10^9	-0.05 ($\lesssim20$)	0	$+0.05$ ($\lesssim20$)	
diO-C_2-7-I (NK 1511)	2	0.5	4×10^7	10^9	-0.05 ($-$)	0	$+0.05$ ($-$)	This dye discolors in a few hours in solution.
	20	1	1×10^6	10^9	-0.05 ($-$)	0	$+0.05$ ($-$)	

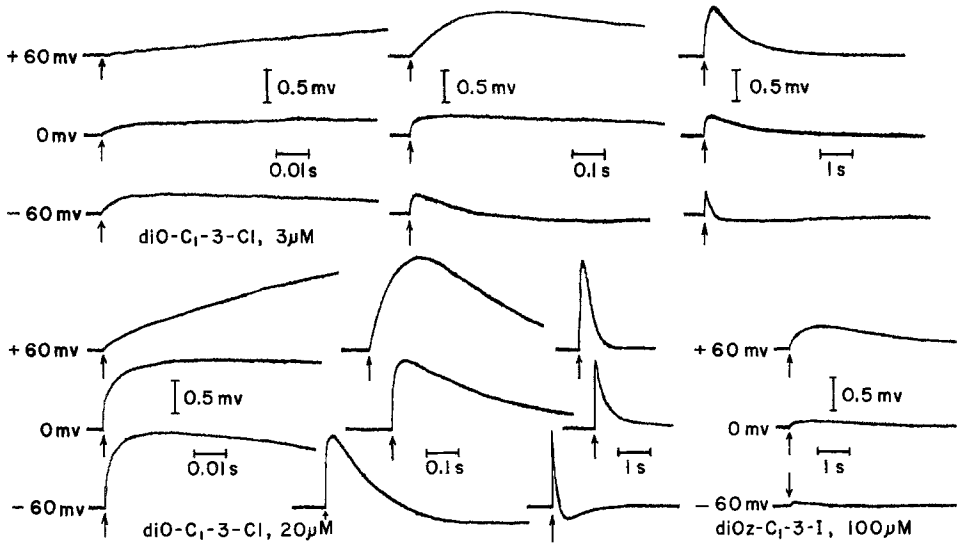

Fig. 2. Photo-voltage waveforms resulting from flash illumination, using methyl derivative dyes added to the positive side of the membranes. Successive traces were recorded at various oscilloscope sweep speeds with the transmembrane voltage adjusted to +60, 0, and −60 mV, as indicated for diO-C_1-3-Cl at 3 and 20 μM. The traces for diOz-C_1-3-I at 100 μM at +60, 0, and −60 mV, are shown for one sweep speed. Vertical arrows identify the time of the 7 μsec flash. A 10^9 ohm shunt resistor was used for all traces

the inner cup solution toward the outer cup solution. The photo-voltage risetime is defined here as the time required after the flash illumination for the waveform to reach 80% of its maximum amplitude. In the case of biphasic waveforms, the amplitudes of both voltage excursions are listed, as measured from the preillumination (base line) voltage value. Two risetime values are given for biphasic waveforms.

All of the photo-voltage waveforms described in this paper were modified by imposed transmembrane voltages. Four distinct photo-voltage waveform shapes were observed to result from the dyes studied here. In addition, several dyes did not induce any photo-voltages. Three of the waveform shapes observed are illustrated in Figs. 2, 3, and 4. The fourth shape was observed when certain of the dyes were present in an amount exceeding a certain concentration, identified as the transition concentration in Table 1. This fourth shape of the photo-voltage waveform included a part that appeared as a discontinuity at sweep speeds below 100 μs/division, and is illustrated for dyes diO-C_{18}-3-I and diO-C_4-5-I in Fig. 5. The amplitude of this portion of the waveform decreases at less than a linear rate with decreases in the light intensity. The appearance of this fourth shape of

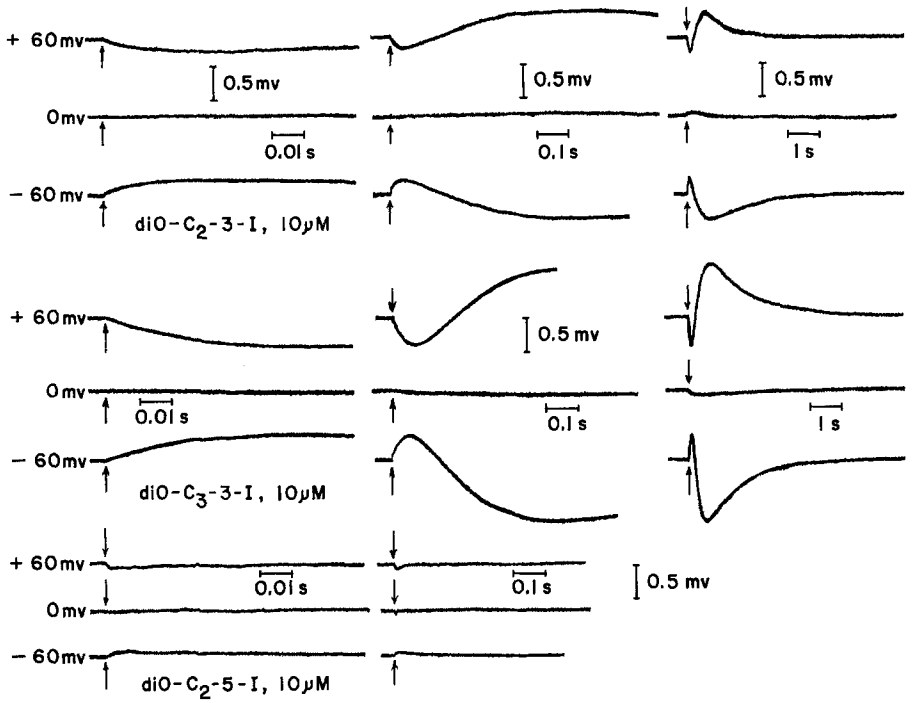

Fig. 3. Photo-voltage waveforms resulting from flash illumination, using ethyl and propyl derivative dyes added to the positive side of the membrane. Vertical arrows identify the time of the 7 μsec flash. A 10^9 ohm shunt resistor was used with diO-C_2-3-I and diO-C_3-3-I, and a 10^7 ohm shunt resistor was used with diO-C_2-5-I

waveform has not been carefully studied. Most of the data taken with these dyes was obtained at dye concentrations below which this fourth waveform shape occurs. The transition concentration seemed not to be strictly reproducible, although it may be that it varies with the amount of ethanol in the test solutions. Reduced amounts of the dye diO-C_{18}-3-I in the membrane forming solution induced only the slowly rising part of the waveform, as shown in Fig. 4 for the case where dye is added to the aqueous solution. The photo-voltage waveform amplitude induced by adding diO-C_{18}-3-I to the membrane forming solution typically decreased by 5 % every 5 min after the membrane had gone black. This decrease is attributed to the loss of dye from the membrane to the aqueous solutions, since similar decreases were not observed when the dye was added to the aqueous solution. The rate of change in the waveform amplitude was not altered by illuminating the membrane with 250 flashes during a 5-min period. The photo-voltage amplitude for membranes with diO-C_{18}-3-I added to the

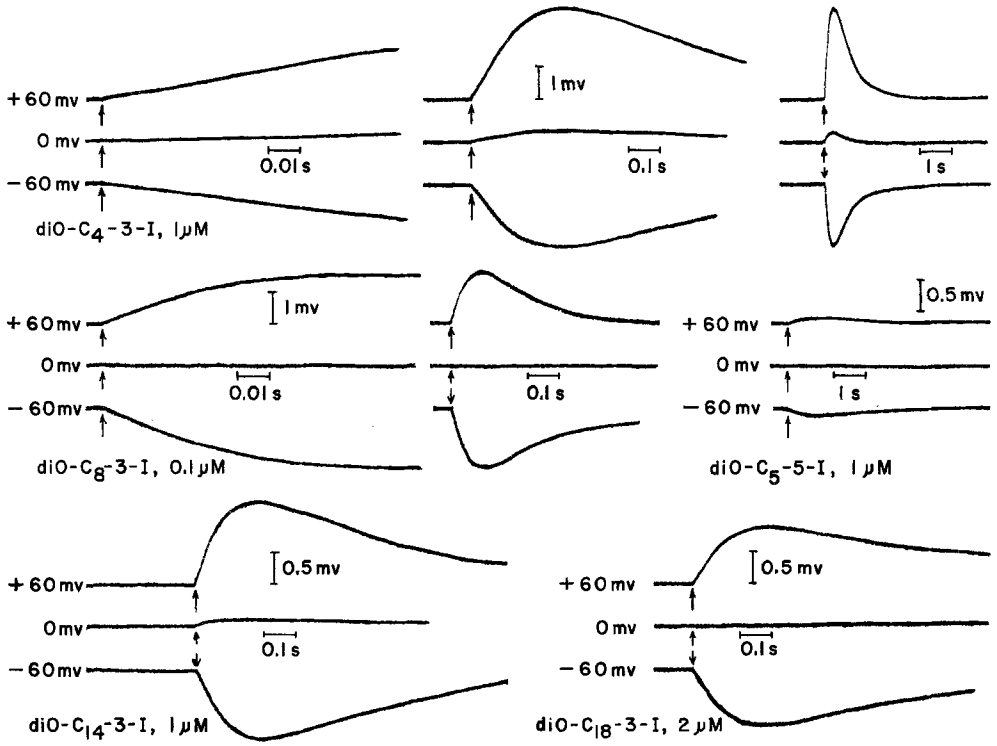

Fig. 4. Photo-voltage waveforms resulting from flash illumination, using dyes with butyl or longer alkyl groups, the dyes being added to the positive side of the membrane. Vertical arrows identify the time of the 7 μsec flash. A 10^9 ohm shunt resistor was used on all traces

membrane forming solution, and membranes in the presence of diO-C_1-3-I did not vary when the partial pressure of oxygen was varied from near 0% (by bubbling nitrogen in the solutions for 20 min before forming the membranes) to near 100% (by bubbling oxygen in the solutions for 20 min before forming the membranes). The photo-voltage amplitudes were also not altered by repeated illumination (more than 100 flashes) under these conditions.

Figure 5 also illustrates the resulting waveforms when the membrane resistance was reduced by the addition of an antibiotic, and when diO-C_1-3-I was added to both sides of the membrane at 2.5 μM. Adding diO-C_1-3-I to both solutions at 10 μM concentrations produced photo-effects with amplitudes of only about 0.1 mV, at +60 mV and −60 mV.

The reduction of the light intensity by neutral density grey filters resulted in proportional (i.e., linear, to within the limits given above) reductions in the waveform amplitudes, provided the dye concentration did

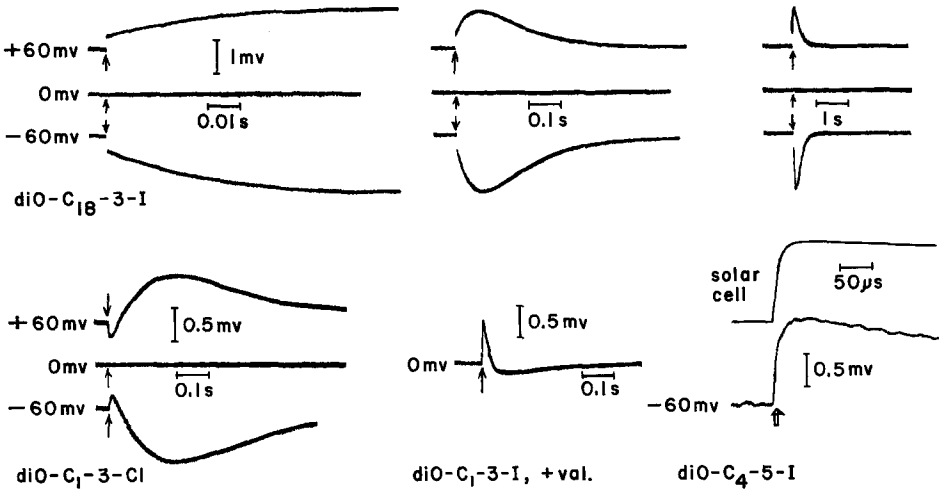

Fig. 5. Photo-voltage waveforms resulting from flash illumination, using the following membrane-dye arrangements. diO-C_{18}-3-I was added to the membrane forming solution. diO-C_1-3-Cl was added to both sides of the membrane to 2.5 μM. diO-C_1-3-I and valinomycin were both added to the positive side of the membrane at 10 and 0.5 μM, respectively. diO-C_4-5-I at 100 μM was added to the positive side of the membrane, and the waveform was recorded simultaneously with the solar cell voltage variation. A 10^9 ohm shunt resistor was used on all traces

not exceed the transition concentration. The use of cut-off filters from Sargent-Welch Scientific Co.'s Planck's Constant Apparatus (Cat. No. 2120) demonstrated that the effective radiation for inducing the photo-electric effects is between 450 and 570 nM for the diO-C_n-3-I dyes. The 455-nM filter, which has a transmittance of 90% at 480 nM, reduced the photo-voltage amplitude by about 10% for these dyes. A 1-cm aqueous solution of diO-C_1-3-I at 10 μM in the light path reduced the photo-voltage amplitude for dye diO-C_1-3-I by about 95%. This demonstrates that the main monomer absorption peak of the dye is producing the photo-electric effects. The 1 cm of 10 μM diO-C_1-3-I dye in the light path reduced the photo-voltage amplitude for diO-C_6-3-I (at +60 mV) by about 90%, and for diO-C_{18}-3-I (at +60 mV) from 80 to 90%. Rotation of the plane of polarization of the incident light altered the amplitude of the photo-voltage waveforms for some dyes, as identified in Table 1.

Fe^{2+} and Fe^{3+} ions (from chloride salts) in 100 μM concentrations separately and collectively reduced the photo-voltage amplitudes from membranes prepared with diO-C_{18}-3-I incorporated. The effects of these ions on photo-voltages induced when diO-C_1-3-I was added to the aqueous

solution has been previously described (Huebner, 1975). In no case has the photo-voltage amplitude been increased by these ions.

Theory

Introduction

This section includes a summary of the study of light flash-induced photo-voltage waveforms in bilayer membranes and the development of an equivalent circuit model of membranes in the presence of cyanine dyes.

Photoelectric effects in bilayer membranes were discovered by Tien (1968), and have subsequently been widely investigated (for a review, *see* Tien, 1974). The time constants of the processes producing the photo-voltage waveforms are generally only observable by using brief light flashes to initiate the effects (Ullrich & Kuhn, 1969; Kobamoto & Tien, 1971; Huebner & Tien, 1972). The long time constants (some over 1 sec) of the light-induced charge transport processes are of interest since they are of comparable length to the time constants observed with the flash illumination of biological membranes (Brown & Murakami, 1964; Bulychev et al., 1976).

Two general types of physical phenomena have been considered as being responsible for producing flash-induced photo-electric effects in bilayer lipid membranes: light-induced redox reactions, and photo-ionic effects. The light-induced redox reactions have been studied in detail for biological (i.e., visual, photo-synthetic and related) pigments in H.T. Tien's laboratory. Three distinct light-activated processes for transporting charges across bilayer membranes have been identified (Huebner & Tien, 1972). The fastest process, which has a characteristic time of less than a microsecond, is believed to transport an electron from the pigment across the membrane to an electron acceptor (such as Fe^{3+}). A slower process, probably resulting from charges being transported in association with the reduction of the photo-oxidized pigment, has a characteristic time in the millisecond range (Huebner & Tien, 1973). In membranes prepared from chloroplast extracts, the slowest light-induced charge transport process is driven by the electrochemical gradient of protons (Huebner & Tien, 1972). This process is probably ionic in nature. The time constant for this component is often longer than the RC time constant of the membrane. The photo-voltage waveforms can be described mathematically by considering that the membrane capacitance integrates the net transmembrane current, including the current which flows through the membrane resistance. Recall that

$$V_m = Q/C_m = (1/C_m)\int dQ = (1/C_m)\int (dQ/dt)\, dt = (1/C_m)\int I\, dt,$$

where I is the sum of all currents altering the charge on the capacitor, C_m. In the absence of the slowest component, a fraction of a sec after the light flash, current flowing through the membrane resistor discharges the photo-voltage with the characteristic RC time constant of the membrane (Huebner & Tien, 1973). This RC discharge of the photo-voltage, induced by the pigments and electron acceptors added to opposite sides of the membrane, provides the primary evidence that electric charge is transported directly through the membrane.

Two distinctly different types of photo-effects have been observed with cyanine dyes absorbed in bilayer membranes (Huebner, 1975). One type, called the "slow effect" occurs with dyes which have quinoline groups. The risetime and discharge time of the induced photo-voltage waveforms are several powers of ten longer than the RC time constant of the membrane. This effect has not been studied further since its discovery, and additional experimental investigations should precede a further discussion of it. This paper is a result of a further study of the "fast effect", which, so far, is known to be induced by symmetric (and some nearly symmetric) oxazole, thiazole, selenazole, and oxazalino derivative cyanine dyes. The risetime and discharge times of the fast effects are both less than the RC time constant of the membrane, provided the dyes or some other agents do not significantly reduce the membrane resistance.

Ullrich and Kuhn (1969 and 1972) and Trissel and Läuger (1972), referring to Ullrich and Kuhn's data, considered that the cyanine dye, diO-C_1-3-I, undergoes a light-induced redox reaction with a closely associated, but unidentified, electron donor adsorbed to the same surface of the membrane as the dye. Huebner (1975) proposed that the fast effects were induced by photo-ionic effects, caused by light-induced charge rearrangements within the dye molecules, resulting in a physical movement of the dye within the membrane interface.

Equivalent Circuit Model

It is clear from the experiments with diO-C_1-3-I, for example, that the dye and illumination cause an electric displacement toward the dye containing solution. An electric displacement is defined here as a vector equal to the electrical charge e times distance, and it is positive in the direction of movement of positive charge. An electric displacement toward the dye containing solution may be created by a positive charge moving

from the membrane toward that solution, or a negative charge moving from that solution into the membrane. The purpose of this section is to consider the shape of the photo-voltage waveforms expected to result from various illumination-induced electrical displacements in the membrane dye system.

The equivalent circuit models developed here presume that optical excitation results in charge rearrangements within some dye molecules, which results in making the dye temporally more hydrophilic. That is, optical excitation alters the dye's equilibrium position in the membrane, possibly by localizing the dye's delocalized charge (Weinstein, Apfelderfer & Berg, 1973). Modifying the dye by optical excitation results in molecular rearrangements in the membrane interface, which result in net electric displacements. The photo-voltage waveforms developed are seen to depend upon the sign, magnitude, and speed of these net electric displacements.

Three different arrangements of the dye are considered: (i) dye molecules absorb into one membrane surface, (ii) dye molecules absorb into both membrane surfaces, and (iii) dye molecules permeate the membrane, but are not absorbed (to an appreciable extent) in either membrane surface.

Bilayer lipid membranes in the absence of modifying agents have an equivalent circuit of a resistor in parallel with a capacitor, as illustrated in Fig. 6*A*. The membrane resistance R_m and capacitance C_m are given by:

$$R_m = \rho l/A, \quad \text{and} \quad C_m = \varepsilon A/l \tag{1}$$

where the membrane has an effective cross sectional area A, thickness l, resistivity ρ, and dielectric constant ε. The interior hydrophobic core (an oil-like film) of the membrane constitutes the capacitor dielectric. The aqueous solutions are highly conducting by comparison. Electric displacements within this dielectric will produce changes in the transmembrane voltage if the displacements have a vector component normal to the membrane plane.

The transmembrane voltage, V_m or V_{12}, is related to the electric field (**E**, a vector) within the membrane dielectric by:

$$V_m = V_{12} = -\int_1^2 \mathbf{E} \cdot d\mathbf{l}.$$

That is, the transmembrane voltage is equal to the negative line integral of the scaler product of the electric field and displacement ($d\mathbf{l}$) vectors. Displacement of electric charges within the dielectric creates (or alters) electric fields within the dielectric, which alters the transmembrane voltage. Conversely, the imposition of a transmembrane voltage produces an electric field within the dielectric, and thus a force on all electrically charged

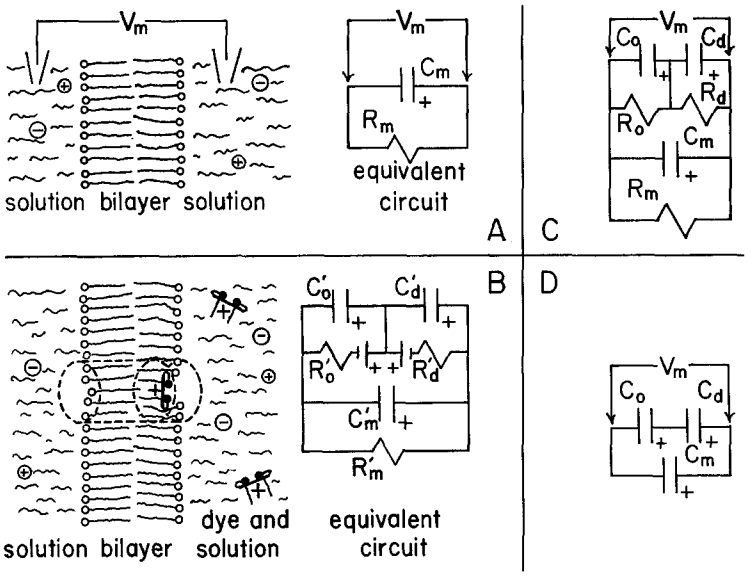

Fig. 6. (*A*): An unmodified bilayer lipid membrane and equivalent circuit schematic diagram. (*B*): A schematic circuit diagram of a bilayer membrane with dye molecules adsorbed into one surface. Cylindrical sections are shown cut through the membrane around each dye adsorption site. (*C*): The equivalent circuit resulting from combining identical elements from *B*. (*D*): The equivalent circuit shown in *C*, valid for a time interval following the flash, providing the interval is less than any of the time constants, $R_m C_m$, $R_o C_o$, and $R_d C_d$. The letter symbols used are defined in the text. The right hand capacitor plates are identified as the positive plates, as discussed in the text

objects within the membrane. The magnitude of the force will depend upon the voltage gradient (i.e., the electric field strength) where the charge is, if the charge is localized. The force on molecules with delocalized charges may be determined by integrating the product of the charge distribution and electric field over the molecule. It is through this force that transmembrane voltages effect changes in the position of dye molecules in membranes. As will be seen, by altering the position of the dye molecules in membranes, the transmembrane voltages alter the waveforms induced by the charged dyes and illumination.

a) Dye molecules in one membrane surface. The dye molecules considered in this section absorb into one membrane surface, but do not cross (permeate) the membrane. The forces pulling these dye molecules into the membrane core and the forces pulling the dye into the water must be balanced in order for the dye to occupy the surface. That is to say, these unexcited dye molecules occupy ground state equilibrium positions in the

membrane, near the surface. The confinement of the positive charge (i.e., its localization) in the excited state alters the forces acting on the dye molecules. Owing to the high dielectric constant of water, localizing the dye's charge results in an enhanced dielectric force which pulls the dye molecules toward the water, creating a displacement of positive charge toward the adjacent aqueous solution. That is to say, the dye molecules raised to an excited state are not in equilibrium at the same location as the unexcited dye molecules, owing to their modified charge structure. The dye molecules repositioning in the membrane produce an electric displacement which is observable as a transmembrane voltage change (a photo-voltage). A transmembrane voltage change of the opposite polarity occurs as the dye molecules de-excite and return to their pre-illumination configuration and their ground state equilibrium positions in the membrane.

An electric circuit equivalent of the membrane-dye system can be constructed by assuming that n individual dye molecules are absorbed in identical configurations and separated spacially. The model presented here considers that only one type of dye molecule or complex is involved, but this model can be generalized to consider different types of dyes absorbed to the same membrane by a straight-forward addition of the associated electric circuit components. The dye molecules are isolated from the bulk of the membrane at the site of each absorbed dye molecule by constructing imaginary cylinders, with the cylindrical cross section being just large enough to include the dye molecule (*see* Fig. 6*A–B*). The portion of the membrane outside these cylinders retains the bulk properties of the membrane. The electric circuit equivalent of one of the cylindrical sections includes C'_d and R'_d, the capacitance and resistance of the end of the section with the absorbed dye molecule, and C'_0 and R'_0, the capacitance and resistance of the other end. The batteries in the equivalent circuit membrane model have equal voltage, and charge the inner plates of C'_d and C'_o positively. The positive charge inside the physical membrane model is due to the adsorbed dye cations. Electric field lines extending from those cations through the regions of the membrane associated with C'_o and C'_d to the counter ions in the aqueous solutions produce the electric potentials in the physical model. The model developed here considers that illumination transports the charged dye across the capacitor C'_d. It is the objective of this section to derive the relation between this transported charge and the experimentally observable variations in the membrane voltage, V_m.

The experimental procedures used in this research allow variations in V_m to be observed following a light flash. The dynamic or "AC" voltage variations in this equivalent circuit do not depend upon the static or "DC"

voltages of internal parts, so the variations in V_m will not be altered by setting the battery voltages to zero. This allows the battery symbols to be taken out of the schematic diagram without altering the expected variations in V_m.

The membrane may be divided into n identical segments with each segment including one adsorbed dye molecule and the surrounding area of the unmodified membrane. R'_m and C'_m are the resistance and capacitance of the unmodified surrounding region of the membrane associated with each of the n adsorbed dye molecules. The dynamic performance of the membrane as a whole will be the same as the dynamic performance of the individual parallel circuits shown in Fig. 6*B*. The membrane voltage variations expected can then be determined by analyzing the equivalent circuit shown in Fig. 6*C*, where from well-known laws of electric circuits it follows that:

$$\begin{aligned} R_m &= R'_m/n; \quad & C_m &= n C'_m; \quad & R_o &= R'_o/n; \\ C_o &= n C'_o; \quad & R_d &= R'_d/n; \quad & C_d &= n C'_d. \end{aligned} \tag{2}$$

The charge transported across the capacitor C_d in this analysis is identified as $Q(t)$. As will be seen, the time variation of $Q(t)$ provides specific information about the molecular processes occurring in the membrane following light absorption.

Note that from Eqs. (1) and (2), $R_m C_m = \rho \varepsilon$, and $R'_o C'_o = R_o C_o$. Assuming that the absorbed dye molecules do not alter the bulk properties of the membrane in the end of the cylinders opposite the dye absorption site, it follows that $R_m C_m = R_o C_o = \rho \varepsilon$. This assumption is not required in this analysis, but it provides a convenient estimate of the value of $R_o C_o$. The time constant $R_m C_m$ is a convenient reference, as it is readily determined experimentally.

It is informative to consider this equivalent circuit model in several approximations. First, for times much less than $R_m C_m$ and $R_d C_d$, the charge transported by all resistors is ignorable, so the charge $Q(t)$ generated by the dye molecules on capacitor C_d is confined (for this time) to the right plates of capacitors C_d and C_m. The convention used here is that charge on the right-hand capacitor plates is positive; so for example, $+Q_d$ is the amount of positive charge on the right-hand plate of capacitor C_d. It follows then that:

$$Q_d + Q_m = Q(t) \tag{3}$$

and

$$-Q_m - Q_o = 0. \tag{4}$$

The transmembrane voltage, however, is

$$V_m(t) = Q_m/C_m = Q_o/C_o + Q_d/C_d. \tag{5}$$

Combining Eqs. (3), (4), and (5) to eliminate Q_m, Q_o, and Q_d,

$$V_m(t) = \frac{Q(t)}{C_m C_d(1/C_m + 1/C_d + 1/C_o)} \tag{6}$$

is obtained. Thus, for times less than $R_m C_m$ and $R_d C_d$ the transmembrane voltage varies linearly with $Q(t)$. This is an important result. Increases as well as decreases in $Q(t)$ can be directly observed, limited only by the fidelity of the membrane voltage electrometer and recording oscilloscope, and an RC time constant specified below.

$Q(t)$ will return to a zero value as the dye molecules de-excite and return to their ground state equilibrium positions in the membrane. In this model, this is accomplished by C_d discharging through R_d. It is found experimentally for the fast effect or F group of cyanine dyes (Huebner, 1975) that the photo-voltage waveforms from flash illumination discharge substantially faster than $R_m C_m$. Thus, for these dyes $R_d C_d$ must be less than $R_m C_m$, and the approximation where the charge transported by R_m and R_o may be ignored with respect to the charge transported by R_d is now considered. This is equivalent to considering the circuit shown in Fig. 7*A*, which may be transformed to the other equivalent circuits shown there. The charge $Q(t)$ generated on C_d will appear in the equivalent circuit on capacitor C_e and will decay in time as:

$$Q_e = Q(t)e(-t/R_d C_e). \tag{7}$$

Since C_e is the equivalent of C_d and C'_e, the charge Q_e is divided between C_d and C'_e such that $Q_d + Q'_e = Q_e$, and $V'_e = Q'_e/C'_e = V_d = Q_d/C_d$. The charge

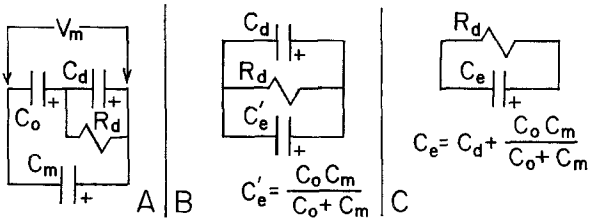

Fig. 7. The equivalent circuits in the approximation that $R_m C_m$ and $R_o C_o$ are large compared to $R_d C_d$. The three circuits shown are all equivalent, although in *B* and *C* the transmembrane voltage is not available externally to the circuit elements

$+Q'_e$ is the same charge that resides on the right plate of capacitor C_m (since capacitors in series have the same charge as their series combination) and this is the charge Q_m. Arranging these expressions to eliminate Q_e, Q'_e and Q_d,

$$V_m(t) = Q_m/C_m = \frac{C_o Q(t)}{C_d C_o + C_d C_m + C_o C_m} e - t \Big/ \left(R_d \left[C_d + \frac{C_o C_m}{C_o + C_m} \right] \right) \quad (8)$$

is obtained. Thus, in this approximation, an exponential decay of the voltage resulting from flash excitation with a time constant that can be much faster than $R_m C_m$ is predicted.

It is also useful to consider the case where the effective membrane resistance is low. This situation may be achieved experimentally by adding a shunt resistor R_s, or by using dyes or other chemicals which intentionally or inadvertently reduce R_m. If a shunt resistor is added, the resistor R_m in Fig. 6 must be replaced with R_e, where $R_e = R_m R_s/(R_m + R_s)$. The current $i_e = V_m/R_e = Q_m/R_e C_m$ will then discharge C_m through R_e. Assuming that the currents carried by R_o and R_d are small compared to i_e (that is, assuming the value of R_s used is such that the time constant $R_e C_m$ is much less than $R_o C_o$ and $R_d C_d$), the following equations may be written. The total electrical charge on the right-hand plates of C_d and C_m as a function of time following the light flash gives:

$$Q_d + Q_m = Q(t) - \int (Q_m/R_e C_m)\, dt. \quad (9)$$

The charge on the left-hand plates of C_m and C_o gives:

$$-Q_m - Q_o = \int (Q_m/R_e C_m)\, dt. \quad (10)$$

Note that the integrals are $\int i_e\, dt$, and account for the current flowing through the resistor R_e. The charge on the right-hand plate of C_o and the left-hand plate of C_d gives:

$$Q_o - Q_d = -Q(t). \quad (11)$$

Eqs. (5) and (11) may be combined to give:

$$Q_d = \frac{C_o C_d}{C_o + C_d} \left(\frac{Q_m}{C_m} + \frac{Q(t)}{C_o} \right).$$

Differentiating this equation and Eq. (9) with respect to time, and eliminating $\dot{Q}_d$ between them yields:

$$\dot{Q}_m + \frac{Q_m}{T} = \dot{Q}(t) \frac{R_e C_m}{T} \left(1 - \frac{C_d}{C_o + C_d} \right) \quad (12)$$

where $T = R_e\left(C_m + \frac{C_o C_d}{C_o + C_d}\right)$. (The dot over a quantity here represents its derivative with respect to time.) Equation (12) may be simplified by multiplying it by $e^{t/T}$, and integrating over time. The left side of Eq. (12) becomes:

$$\int \left(\dot{Q}_m + \frac{Q_m}{T}\right) e^{t/T} dt = \int \left(\dot{Q}_m e^{t/T} + \frac{Q_m}{T} e^{t/T}\right) dt = \int \frac{d}{dt}(Q_m e^{t/T})\, dt$$
$$= \int d(Q_m e^{t/T}) = Q_m e^{t/T}.$$

Multiplying Eq. (12) by $e^{-t/T}$ then yields:

$$Q_m = e^{-t/T} \frac{R_e C_m}{T} \left(1 - \frac{C_d}{C_o + C_d}\right) \left[\frac{QB}{\tau} + \int \dot{Q}(t) e^{t/T} dt\right] \tag{13}$$

where QB/τ is a constant of integration.

Equation (13) may be solved if $Q(t)$ is known. It is convenient to assume that $Q(t) = Q e^{-t/\tau}$, where τ may be considered the lifetime of the dye's excited state. This assumption incorrectly represents the initial portion of the waveform by giving $Q(t)$ the amplitude Q at $t = 0$. This should cause no difficulty since the relation betwen $Q(t)$ and the initial portion of the waveform was derived above [*see* Eq. (6)]. This assumption is probably also incorrect because the dye molecules in rearranging the membrane interface after excitation are also modifying their own environment. Thus, a first order rate process for the dye de-excitation is probably not adequate, but it is useful for predicting approximations of the waveforms. Using this assumption, Eq. (13) yields:

$$V_m = Q_m / C_m = R_e Q \left(1 - \frac{C_d}{C_o + C_d}\right) \left[\frac{B e^{-t/T}}{\tau T} - \frac{e^{-t/\tau} - e^{-t/T}}{\tau - T}\right]. \tag{14}$$

At $t = 0$, the second term in the time-dependent part (inside the square bracket) is zero, while the sign of the first term depends upon B. The initial positive value of the experimental photo-voltage waveforms indicates that B is positive. The first term then is greater than zero for all times greater than zero, but decreases in time as $e^{-t/T}$. The rate of change and the magnitude of the second term depends upon the values of T and τ, but for all cases (i.e., $T < \tau$, $T = \tau$ and $T > \tau$) this term is zero at $t = 0$. It then becomes negative, and subsequently returns to zero as t increases. Thus, when $R_e C_m \ll R_o C_o$ and $R_d C_d$, this theory predicts that a biphasic photo-voltage waveform may result, depending on the values of B, T, and τ.

It is also useful to consider the effects of forces which result from imposing a transmembrane voltage on membranes. A positive transmembrane voltage will produce a force on positively charged dye molecules which are absorbed on the inner cup side (side 1) of the membrane which will move the dye molecule further into the membrane. A negative voltage will produce a force which will move these dye molecules further out of the membrane toward the water. The distance a dye molecule is moved by a transmembrane voltage will depend upon the strength of the force produced relative to the other forces on the molecule. Several different types of effects may be expected from repositioning impermeant dye molecules in membranes. These include: (i) variations in absorption and emission spectra and excited state lifetimes, resulting from modifications in the dye molecules' environment, (ii) variation in the number of dye molecules absorbed in the membrane surface, and (iii) variation in the rate excited dye molecules reposition after being optically excited and after returning to the ground state.

b) Dye molecules in both membrane surfaces. It is useful to consider an equivalent circuit model for the case where dye molecules are absorbed to both sides of the membrane. This situation may arise experimentally when impermeable dyes are added to both sides of the membrane, or by using dyes which permeate the membrane. It is convenient to consider that n_1 dye molecules occupy the right-hand side of the membrane (the inner cup solution side), and n_2 dye molecules occupy the left-hand side, so that:

$$\begin{aligned} &n=n_1+n_2; \quad C_{d1}=n_1 C'_d; \quad R_{d1}=R'_d/n_1; \\ &C_{o1}=n_1 C_o; \quad R_{o1}=R'_o/n_1; \quad C_{d2}=n_2 C'_d; \\ &R_{d2}=R'_d/n_2; \quad C_{o2}=n_2 C'_o; \quad \text{and} \quad R_{o2}=R'_o/n_2. \end{aligned} \tag{15}$$

The resulting electric circuit equivalent of this membrane is shown in Fig. 8. The dye molecules on both sides of the membrane are not required to be identical, but this analysis does require that the same values of C'_d, C'_o, R'_o, and R'_d result from both dyes.

As in section *a*, the following equations may be written:

$$V_m=\frac{Q_m}{C_m}=\frac{Q_{d1}}{C_{d1}}+\frac{Q_{o1}}{C_{o1}}=\frac{Q_{d2}}{C_{d2}}+\frac{Q_{o2}}{C_{o2}}; \tag{16}$$

$$Q_{o2}+Q_{d1}+Q_m=Q_1(t); \tag{17}$$

$$Q_{d2}-Q_{o2}=-Q_2(t); \tag{18}$$

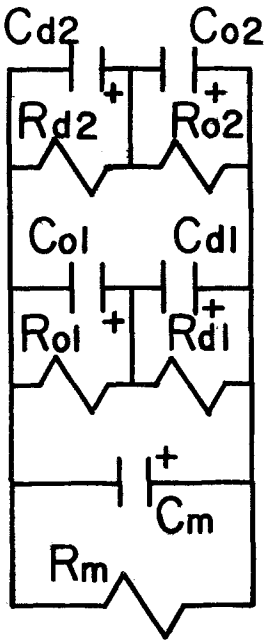

Fig. 8. The equivalent circuit for the case where dye molecules are absorbed in both surfaces (*1* and *2*) of the membrane

$$Q_{o1} - Q_{d1} = -Q_1(t); \quad \text{and} \tag{19}$$

$$-Q_{d2} - Q_{o1} - Q_m = Q_2(t), \tag{20}$$

where $Q_1(t)$ and $Q_2(t)$ are the charges transported to the outside plates of capacitors C_{d1} and C_{d2} by the absorbed dyes and illumination. Writing V_m as

$$V_m = \frac{n_1}{n}\left(\frac{Q_{d1}}{C_{d1}} + \frac{Q_{o1}}{C_{o1}}\right) + \frac{n_2}{n}\left(\frac{Q_{d2}}{C_{d2}} + \frac{Q_{o2}}{C_{o2}}\right),$$

and using Eqs. (17) through (20) to eliminate Q_{o1}, Q_{o2}, Q_{d1}, Q_{d2}, and Q_m,

$$V_m = \frac{Q_1(t) - Q_2(t)}{C_d C_m \left(\frac{1}{C_m} + \frac{1}{C_d} + \frac{1}{C_o}\right)} \tag{21}$$

is obtained, where $C_d = n C'_d$, and $C_o = n C'_o$. Equation (21) may be compared to Eq. (6), in that Eq. (21) shows that for times less than the RC time constants, the membrane voltage varies linearly with the differential charge

transported, $Q_1(t)-Q_2(t)$. If the dyes are identical and situated the same way in a membrane, but $n_1=2n_2$ for example, then a photo-voltage proportional to $1/2Q_1(t)$ will be observed. On the other hand, if the dyes are either different in structure or are situated differently in the membrane (as a result of a transmembrane voltage, for example), a photo-voltage proportional to the difference of $Q_1(t)$ and $Q_2(t)$ will result.

Transmembrane voltages may thus be expected to modify the photo-voltage waveforms by two types of interactions. First, both the ground and excited state positions that a dye molecule will occupy in the membrane will be modified by the voltage. This will occur directly from the electric force on charged dye molecules. It may also occur for electrically neutral dye molecules, if charged species in the membrane are rearranged. A buoyancy analogy is useful to consider here. A modified microenvironment seems certain to result for dye molecules in membranes containing charged molecules as a result of imposed voltage. Thus, the quantities in the equations developed which depend upon the dye's microenvironment will vary with transmembrane voltage. These quantities include R'_o, C'_o, R'_d, C'_d, and τ.

The second type of effect that the transmembrane voltage may have is to alter the net electrochemical potential of the dye at the two membrane surfaces relative to the bulk solution. For impermeant dye species, this will alter the number of dye molecules absorbed on the membrane adjacent to the dye containing solution. Thus n, which is defined as part of R_o, C_o, R_d and C_d, will be a function of the transmembrane voltage. For dye molecules which permeate the membrane, the number of dye molecules absorbed in both surfaces will be altered by the transmembrane voltage as dye molecules pass through the membrane. Thus n_1 and n_2 in Eq. (15) will also be a function of the transmembrane voltage.

c) Permeant dye molecules in the membrane. The presence of charged dye molecules which permeate the membrane will reduce the membrane resistance by providing an alternate conductance mechanism. Figure 9 shows the equivalent circuit of the membrane, shunt resistor, and external battery. The voltage across R_m is:

$$V_m = V_a R_m/(R_m+R_s) \cong V_a R_m/R_s \tag{22}$$

where the approximation is valid when R_m is much less than R_s. Since V_a and R_s are constant during and following illumination, variations in V_m result from variations in R_m. The point here is that optically exciting the dye molecules, causing a localization of their charge, will temporarily increase

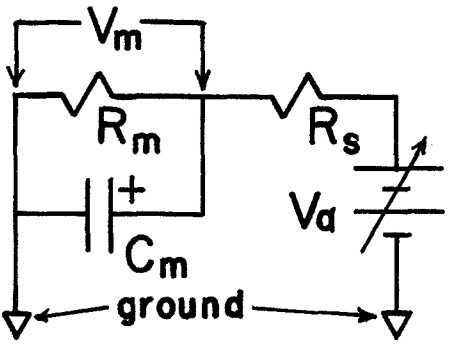

Fig. 9. The equivalent circuit for the case where dye molecules permeate the membrane, and a transmembrane voltage is imposed through a shunt resistor, R_s

the membrane resistance and therefore the membrane voltage, as the dye molecules with localized charges are less able to permeate the membrane.

Discussion

Comparison of Theory and Experiment

The three basic photo-voltage waveform shapes obtained experimentally, as illustrated in Figs. 2–4, compare respectively to the waveforms predicted by theory sections *a*, *b*, and *c*. It is possible to rationalize these results by considering the dye structures and the hydrophilic and lipophilic forces which will act on these dyes when in a membrane. It is convenient to consider the carbocyanine dye series first. In this series the hydrophilic and chromophoric parts of the dye molecules are fixed, while the lipophilic parts of the molecules are systematically altered.

The three dyes, diOz-C_1-3-I, diO-C_1-3-C1 and diO-C_1-3-I, are relatively insoluble in the membrane, as judged from the high membrane resistance values in Table 1. Of these three dyes, diOz-C_1-3-I gives the highest resistance value and induces positive monophasic waveforms at +60, 0, and −60 mV. This is consistent with the view that this dye absorbs into the positive membrane surface or interface, but does not permeate the membrane. That is, the hydrophilic forces holding the dye in the membrane surface are sufficient to resist the lipophilic forces pulling the dye into the membrane core. Upon illumination, the positively charged dye molecules move toward the positive solution, generating the rising portion of the photo-voltage, as described by Eq. (6). As the dye molecules de-excite and

return to their previous position in the interface, the membrane voltage returns to the baseline (the pre-illumination value), as described by Eq. (8).

The waveform variations with imposed transmembrane voltage result from electric forces altering the equilibrium position of the absorbed dye molecules. The dielectric "image" force on a charge in a low dielectric constant medium, a distance h from a high dielectric constant medium, varies approximately as one over the square of $2h$. Increasing h with a positive voltage will reduce the force resulting from charge localization. This causes the excited dye molecule to move toward the solution more slowly initially, thus increasing the waveform risetime. Positive voltages also increase the waveform amplitude, a result of increasing both the distance over which the dye molecules move after being optically excited and the number of dye molecules absorbed. Negative voltages have the opposite effects, decreasing the waveform risetime and amplitude.

The resistance values and waveform amplitudes for membranes in the presence of dyes diO-C_1-3-Cl and diO-C_1-3-I apparently differ as a result of different anion dissociation constants. These dyes give lower membrane resistance values than diOz-C_1-3-I, apparently as a result of the benzene rings making them more soluble in the membrane core. These two dyes induce positive monophasic waveforms at $+60$ and 0 mV, and biphasic waveforms at -60 mV, using a 10^9 ohm shunt resistor. Biphasic waveforms are also obtained with diOz-C_1-3-I (100 μM on the positive side of the membrane only) at -60 mV, when using a 10^8 ohm shunt resistor. The waveform is again monophasic when a 10^7 ohm resistor is used.

Two separate and distinct arrangements are responsible for the various biphasic waveforms. These two arrangements are considered in the theory section *a*, Eq. (14), and in section *b*. The first arrangement occurs when the effective membrane resistance is low so that $R_e C_m$ is less than $R_d C_d$. Then, following flash illumination, the voltage is returned to the baseline by current flowing through the low membrane resistance before the dye molecules de-excite. This produces a biphasic waveform as the de-exciting dye molecules still move back into the membrane interface, driving the membrane voltage below the baseline. Waveforms which are probably produced by this arrangement are shown in Fig. 2*a* of Huebner (1975) where a 10^7 ohm shunt resistor was used, and Fig. 5 of this paper where an antibiotic was used to reduce the membrane resistance.

The second arrangement which produces biphasic wave-forms is dye molecules being in both membrane interfaces. No photo-voltages result at 0 mV when diO-C_1-3-I is added in equal concentrations to both sides of the membrane (*see* Fig. 5). This is predicted by Eq. (21), as $Q_1(t) = Q_2(t)$ which

yields $V_m(t) = 0$. At $+60$ mV the dye absorbed to the positive interface (side 1) of the membrane is pushed into the membrane by the electric force, while the dye absorbed to the negative interface (side 2) is pushed out of the membrane. Thus $Q_1(t)$ will vary in time as the $+60$ mV waveform when this dye is only on the positive side of the membrane, while $Q_2(t)$ will vary as the -60 mV waveform. The net photo-voltage waveform then may be constructed by subtracting the -60 mV waveform trace from the $+60$ mV waveform trace when the dye is present on only the positive side of the membrane. This is as expected from Eq. (21).

Dye molecules placed only on side 1 of the membrane may populate side 2 by permeating the membrane. The population of permeant dye molecules in interface 2 (n_2) will increase with increased ability of the dye to permeate the membrane, and will decrease with the dye's increased ability to go into the aqueous solution on side 2. Mr. John Baker, in our lab, has shown that the negative part of the biphasic response at -60 mV can be increased in amplitude by using a hydrostatic pressure to bulge the membrane into a hemisphere sticking into the inner cup. At $+60$ mV under these conditions, a small negative excursion occurs on the photo-voltage waveform before the larger positive excursion. This is consistent with the view that diO-C_1-3-I permeates these bilayers, but in this geometry is unable to effectively diffuse away from surface 2, thus resulting in an increased n_2. The biphasic response still occurs at -60 mV when the hydrostatic pressure is reversed, the waveform being identical to the waveform for a planar membrane.

A biphasic type of photo-voltage waveform is also observed for diO-C_1-3-I (*see* Ullrich & Kuhn, 1969 or 1972) with illumination periods of $\gtrsim 1$ sec. Those results, which we reproduced, are in agreement with the model described here. When the light is turned on, the dye molecules absorbed to the positive membrane surface move toward the positive solution, producing the positive voltage transient. Individual dye molecules will de-excite and recycle as a steady-state condition is reached. Current through the membrane resistor, however, will return the membrane voltage to the baseline with the membrane RC time constant. A negative voltage transient will result from the dye de-exciting and returning to their ground state equilibrium position in the membrane after the light is turned off. These results indicate that both mechanisms for producing biphasic wave-forms operate under some conditions in these membranes.

Increasing the length of the alkyl groups on the dye molecules will increase the strength of the lipophilic forces acting on the dye in the membrane interface. The waveforms and membrane resistance values for the diO-C_2-3-I and diO-C_3-3-I dyes are consistent with the view that these

dyes primarily occupy both membrane surfaces. Thus, the theory section *b* and Eq. (21) will apply to the wave-forms. The increased length of the alkyl groups apparently facilitates the movement of dye through the membrane core, but the hydrophilic forces are still sufficient to keep the adsorbed dye near the membrane surfaces most of the time. The increased lipophilic force probably also helps reduce the loss of the dye to the aqueous solution on side 2 of the membrane.

The increased lipophilic forces on the dyes diO-C_n-3-I for $n \geqq 4$ exceed the hydrophilic forces so the absorbed dye primarily occupies the membrane core. Consequently, Eq. (22) applies to the waveform. For $n \geqq 8$ polarization effects are observed (*see* Table 1). Unfortunately only the oxacarbocyanine dyes produce large enough waveform amplitudes for the polarization effects to be observed by the techniques used here. The results for diO-C_{18}-3-I agree with the orientation found by fluorescence techniques (Yguerabide & Stryer, 1971). Both the fluorescence and waveform polarization data can be explained by assuming that the fatty acid groups of the membrane lipids are oriented perpendicular to the membrane plane, and that as the dye alkyl groups are increased in length the dyes' alkyl groups increasingly take on that same orientation, which increasingly orients the dye absorption moment in the membrane plane.

No photo-voltages result from the dye diO-C_2-3-9C_2-I. This may result from the 9 ethyl group crowding the molecule, preventing it from being in the planar "trans" configuration shown in Fig. 1 (*see* Henrichs & Gross, 1976). However, preliminary experiments (meaning that one membrane was evaluated in the presence of each dye) indicate that the dyes diS-C_2-3-9C_1-I and diSe-C_2-3-9C_1-Br induce photo-voltage wave-forms similar to those induced by diO-C_2-5-I (*see* Fig. 3). Those waveform amplitudes are too small to justify much analysis here, but Henrichs and Gross did find different relative populations of the "cis" and "trans" conformational isomers for these three dyes.

Other preliminary results using dis-C_n-3-I, diS-C_n-5-I, and diSe-C_n-3-I series dyes agree with the model proposed above. Those results are generally that smaller amplitude photo-voltage waveforms and lower membrane resistance values result at comparable dye concentrations. This is consistent with the view that the hydrophilic forces are progressively weaker for the thiazole, salenizole and indolenine dyes.

There may be several reasons for the smaller amplitude waveforms from the diO-C_n-1-I, diO-C_n-5-I, and diO-C_n-7-I dyes, all relating to a lack of significant charge redistribution upon optical excitation in the membrane environment. The diO-C_n-1-I dyes may not be long enough to permit an

effective charge distribution. The diO-C_n-5-I and diO-C_n-7-I dyes may not allow enough charge localization to produce a significant change in the hydrophilic force, making movement of these dyes in the membrane interface slower than the diO-C_n-3-I dyes. The membrane resistance is low in the presence of diO-C_n-5-I and diO-C_n-7-I dyes. The experimental method of observing the voltage waveforms employed here is not well suited to detecting motion of charged molecules that require times for motion that are long compared to the membrane $R_m C_m$ time. Monitoring the current flow while using a low impedance voltage clamp may help circumvent this difficulty.

Comparison of the Possible Mechanisms

The assumptions required to account for the photo-voltage waveforms with various mechanisms are considered in this section. The procedure used is to explain how a particular mechanism could produce the photo-voltages, and then to comment on how that mechanism fits the data available. The discussion will generally assume that a dye concentration gradient exists across the membrane. The following five mechanisms of producing photo-electric effects in membranes are offered for consideration. They will be discussed in turn.

Photo-Ionic Effects:

1) Light-induced charge rearrangements in the dye.
2) Light-induced changes in dye aggregation.
3) Light-induced decomposition of the dye.

Other,

4) Light-induced redox reactions in the membrane.
5) Light-induced heating of the membrane.

1) Light-Induced Charge Rearrangements in the Dye. This mechanism assumes that light absorption temporarily modifies the electric charge structure of some of the dye molecules in the membrane, thereby modifying the dye's solubility in the membrane. The physical processes which could produce the charge rearrangements required are believed to include long-lived excited (presumably triplet) states, and isomerization or conformational changes. This mechanism requires that the dye's solubility in the membrane remain modified for a time on the order of the photo-voltage risetime. This time is several powers of ten times longer than the observed lifetime of fluorescent states of these cyanine dyes (diO-C_2-n-I and diS-C_2-n-I for $n=1$, 3 and 5) in solution (Roth & Craig, 1974). McCartin

(1965), however, reports metastable sterioisomer lifetime for diS-C_2-3-I in ethanol in the msec range. Ehrlich describes transient absorption changes with relaxation lifetimes of about 5 msec for diS-C_2-3-pts$^-$ in gelatin (1975*a*), and for about 30 msec in emulsions of silver bromoiodide crystals (1975*b*). These results, and studies with other cyanine dyes (Buettner, 1967), support the view that the lifetime of light-modified dye states are increased when the dye is bound in a more rigid microstructure, which presumably includes bilayer membranes. In fact, Chance and Baltscheffsky (1975) find a transient absorbance change in the 100-msec range for a merocyanine dye in the chromatophore membrane of *Rhodospirillum rubrum.*

The details of how charge rearrangements can account for the observed waveforms was considered above. Calculations of the charge rearrangements expected upon optical excitation of several cyanine dyes have been published (Weinstein, Apfelderfer & Berg, 1973). Rough calculations based on their data indicate that these charge rearrangements would be sufficient to induce photo-voltage waveforms with the risetimes observed. It seems important to specifically point out here that modifying the dye structure through isomerization must necessarily rearrange the dye molecules' charge. It also seems worth noting that space-filling models of four of the five dyes which do not produce photo-effects (diO-C_2-1-I, diO/S-C_2-1-I, diO-C_2-3-9C_2-I and diI-C_1-3-I) are somewhat sterically hindered from making transitions between the "trans" form shown in Fig. 1 and the "mono-cis" form (*see* Ehrlich, 1975*a*). It is also interesting to note that diO-C_1-3-I in the "trans" form has both oxygen atoms on the same side of the molecule, so they could both be in contact with water while the lipophilic parts of the molecule remain in the membrane. This is not possible in the "mono-cis" form. Thus, isomerization may be expected to produce changes in the solubility of the dye in the membrane from steric effects as well as from charge rearrangements.

Molecular rearrangements (which may result from long-lived excited states, isomerizaton or conformational changes) which make the dye more hydrophilic appear to be capable of producing the photo-voltage waveforms observed. The experiments performed to date, however, do not provide an unambigious basis for identifying the causative mechanism. As will be seen below, another mechanism (excimer formation) may also account for the photo-electric effects.

2) Light-Induced Changes in Dye Aggregation. Two basic variations of this mechanism are considered: that dimers in the membrane are dissociated by illumination, and that excited dye molecules associate to form

dimers (excimers) in the membrane. The first variation of this mechanism would increase the concentration of dye monomers in the membrane above the equilibrium concentration by the disassociation of dimers upon illumination, thereby causing some dye monomers to leave the membrane, creating the electric displacement and the rising part of the photo-voltage waveform. After the dye returns to the ground state, dimers would reform, causing the membrane concentration of monomers to fall below the equilibrium concentration, causing additional dye to be absorbed from the solution, discharging the photo-voltage waveform.

The five dyes which do not produce photo-voltage waveforms (diO-C_2-1-I, diO/S-C_2-1-I, diO-C_2-3-9C_2-I, diI-C_1-3-I and diI-C_1-5-I) provide support for this model. The oxacyanine ("-1-") dyes do not form dimers as readily as the oxacarbocyanine ("-3-") dyes (*see* West & Pearce, 1965). The "-9C_2-" group of the diO-C_2-3-9C_2-I dye and the methyl parts of the indo groups of the diI dyes sterically hinder dimer formation.

Diffusion of the excess monomers out of the membrane would be expected to take longer if the dye were pushed further into the membrane, as is expected to be the case at +60 mV, thereby providing an explanation of the variation of the risetime with applied voltage.

Data which does not support this dimer photo-disruption mechanism includes the fact that the diO-C_n-3-I dyes produce photo-effects when they are illuminated with white light, but not when the region of the spectrum immediately near the monomer absorption peak ($\sim$480 nM) is filtered out. The solvent shifts for these dyes from water to organic solvents is typically less than 10 nM. Thus, these dyes produce photo-effects when illuminated at the monomer absorption peak, but not when illuminated with all other regions of the visible spectrum, which should include the absorption peak of any dimers (*see* West & Carroll, 1966). In experiments performed by Ms. Rebecca Stalvey in our laboratory, no absorption of phosphatidyle choline suspensions including diO-C_1-3-I (at concentrations used in this work) was observed which could be attributed to dimers. This is consistent with the view that no dimers form in the membrane at the concentration of dye studied here. Finally, the dyes which are expected to form dimers more readily (i.e., the dicarbo- and tricarbocyanine dyes) produce photo-voltages with less amplitude than the dyes which form dimers less readily (i.e., the carbocyanine dyes).

The second variation of this mechanism could produce the photo-voltage waveforms by dimers forming with, say one excited dye molecule and an unexcited dye molecule, which upon association become less soluble in the membrane than the monomers. These excimers would transport their

positive charge out of the membrane, causing the rising part of the photo-voltage, while reducing the dye concentration in the membrane. Dye absorbing from the solution in response to the reduced dye concentration in the membrane would discharge the photo-voltage.

It seems reasonable to assume that the five dyes which do not produce photo-effects may not form excimers for reasons considered above relating to dimer formation, but perhaps the carbocyanine dyes will form excimers more readily than the dicarbocyanine or tricarbocyanine dyes. The long risetimes may be explained by the time required for the excimers to form and move out of the membrane. Applied voltages would be expected to vary the risetime, as explained above. Increased concentrations of the dye should increase the risetime and amplitude of the photo-voltage, as the dye molecules could form excimers more rapidly at higher concentrations. This is observed, although part of the increased risetime at higher dye concentrations may be due to lower membrane resistance.

3) Light-Induced Decomposition of the Dye. Light-induced decomposition of the dye could produce a more hydrophilic cation (Byers, Gross & Henrichs, 1976), which would be pulled by dielectric image forces toward the adjacent aqueous solution, creating the electric displacement required for the rising part of the photo-voltage. The applied voltage would modify the dye's position before decomposition, and thereby modify the waveform risetime. The dye in the membrane lost to decomposition could be replaced by diffusion of dye from the aqueous solution, transporting charge back into the membrane, discharging the photo-voltage. The millisecond risetime of the waveforms would be accounted for by the time required for the decomposition reaction to be completed and for the products to separate.

This mechanism does not seem capable, without additional assumptions, of explaining why five dyes (diO-C_2-1-I, diO/S-C_2-1-I, diO-C_2-3-9C_2-I, diI-C_1-3-I and diI-C_1-5-I) do not produce any photo-effects, while the remaining dyes do. The amplitude of the photo-voltage for dyes diO-C_2-3-I, diO-C_2-5-I and diO-C_2-7-I at +60 mV, for example, vary directly with the dye's photo-stability as reported by Sims et al. (1974). That is, the dye that is the most stable against photo-decomposition produces the largest photo-effect, while the least stable dye produces the smallest photo-effect. The lower membrane resistance for the more lipophilic dyes may account for part of this difference, but more of the lipophilic dye is expected to be absorbed into the membrane, and more dye adsorbed in the membrane would make the photo-voltage larger if other factors were equal. In addition, the more lipophilic dyes should be absorbed further inside the membrane,

which would produce a larger electric displacement per charge moving out to the aqueous solutions.

The variations in the photo-voltage waveform amplitude with repeated flash illumination when diO-C_{18}-3-I was incorporated in the membrane does not support this mechanism. In this case, the membrane cannot absorb additional dye from the aqueous solution, so dye lost to photo-decomposition will not be replaced from the solution. The fact that the photo-voltage amplitude is not decreased additionally by hundreds of flashes indicates that the loss of dye to photo-decomposition of a single flash is small. It may be argued in this case that dye lost to photo-decomposition in the membrane could be replaced by diffusion of dye into the membrane from the torus; however, photo-decomposition should also occur in the torus. Assuming that equal percentages of the dye photo-decomposes in the membrane and torus, and that the membrane and torus remain in equilibrium with respect to dye adsorption, the torus should not alter the amount of dye in the membrane.

The same logic can be applied to the case where dye is added to the aqueous solution. If the dye photo-decomposes in the membrane, it should also photo-decompose in the aqueous solutions. Assuming that equal percentages of the dye photo-decomposes in both the membrane and solution, and assuming that the membrane-solution system remains in equilibrium with respect to dye adsorption, then the dye lost to photodecomposition in the membrane would not be replaced from the solution. One obvious difficulty with these arguments is that the light intensity at various places inside the solution, and the quantum efficiency of the light flash-induced decomposition in the membrane, torus, and solution are not known.

Further, if the light-induced decomposition is assumed to be due to photo-oxidation, then the photo-voltage amplitude should be increased with increased oxygen partial pressure, and with dyes that form dimers more readily (*see* Byers, Gross & Henrichs, 1976). These two factors should also produce greater variation in the waveform amplitude with repeated illumination. The experimental results are that variations in the oxygen pressure does not vary the photo-voltage amplitudes. Finally, the dyes which dimerize the least (*see* West & Pearch, 1965) also produce the largest photo-voltage amplitudes, which is inconsistent with the photo-oxidization mechanism.

4) Redox Reactions within the Membrane. The transfer of an electron from an electron donor further out in solution to a photo-excited dye

molecule in the membrane surface would produce an electric displacement, causing the photo-voltage to rise (Trissel & Läuger, 1972; Ullrich & Kuhn, 1972). Reversing the reaction would transfer the charge back, discharging the photo-voltage. Both time constants would be independent of the membrane RC time constant.

It is unclear what the electron donor is in this mechanism, but the choices are limited to the water, the membrane, another dye molecule of the same species, the dye's anion (which can be bromide, chloride or iodide), some contaminant (which must occur in these dyes from a number of independent sources), or some combination of these. This mechanism does not seem capable, without a number of additional assumptions, of explaining why five of the dyes do not produce any photo-effects, while the remaining dyes do. It does not seem to be able to account for the variations in the risetime with applied voltage. Finally, the experiments with $FeCl_2$ and $FeCl_3$ (which could presumably donate or accept an electron from the dyes) are not consistent with this model.

5) Light-Induced Heating of the Membrane. Considering only 3 of the 27 dyes described in this paper is sufficient to prove that membrane heating cannot be responsible for these photo-effects. The dyes diO-C_1-3-I, diO-C_2-3-I and diO-C_2-3-9C_2-I are virtually identical in their optical absorption spectra. They all adsorb into the membrane, as judged from the membrane resistance in the dye's presence. These three dyes differ only in the substitution of 2 ethyl groups for 2 methyl groups, and the substitution of 1 ethyl group for a hydrogen atom. Yet diO-C_1-3-I induced a monophasic photo-voltage at $+60$, 0, and -60 mV, while diO-C_2-3-I does not induce a photo-voltage at 0 mV, but induces a biphasic photo-voltage at $+60$ and -60 mV, and diO-C_2-3-9C_2-I does not induce any photo-voltages. The only materials which can absorb the light used inside the membrane test cell are the dyes. These dyes, since their absorption is nearly the same, should produce the same heating effects. Yet, the photo-voltages produced are distinctly different. Thus, it is clear that heating of the membrane cannot be responsible for these photo-effects.

Conclusion

The photo-voltage waveforms which result from flash illumination vary systematically with dye structure and imposed transmembrane voltage. The equivalent circuit model presented here is consistent with the waveforms observed. The model supposes that the photo-voltages result from physical

movements of charged dye molecules which are absorbed in the membrane. Details of the mechanism(s) which produce these electric displacements remain uncertain, although the data presented here is consistent with either a charge rearrangement, isomerization, or an excimer formation mechanism.

Previous models for explaining photo-voltages in membranes require the intermolecular movement of electrons (Trissel & Läuger, 1972; Ullrich & Kuhn, 1972; Huebner & Tien, 1973; Tien & Huebner, 1973; Hong & Mauzerall, 1974; Tien, 1974). The results presented here demonstrate that intermolecular electron movements are not an exclusive requirement for obtaining photo-voltages, but that photo-voltages may also result from intramolecular movement of electrons in molecules that are too small to span the membrane.

Detailed calculations of the forces rearranging the membrane constituents following flash illumination should ultimately be possible for whichever mechanism is responsible. In fact, those calculations may provide a method of determining the responsible mechanism. Attempts at those calculations are presently hindered by a lack of precise knowledge of (i) the dye molecules' charge distributions in the membrane interface, (ii) the dielectric constants and viscosity (i.e., the structure) of the membrane interface where the dye molecules are absorbed, and (iii) the nature of the aqueous phase adjacent to the membrane interface where the dye is absorbed (*see* Wang & Bruner, 1977). There are numerous lipid systems and thousands of dyes available for the type of investigations reported here. It seems hopeful that the experimental methods discussed in this paper will contribute toward an improved understanding of the interfacial region of membranes.

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