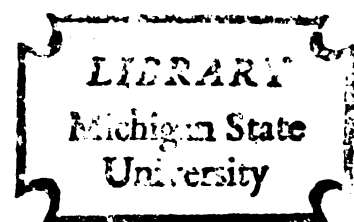


THE SEMICONDUCTIVE PROPERTIES OF
LIPIDS AND THEIR RELATION TO THE
ELECTRICAL CONDUCTIVITY OF
LIPID BILAYERS

Thesis for the Degree of Ph. D.
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GORDON LEE JENDRASIAK
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thesis entitled

The Semiconductive Properties of Lipids and their
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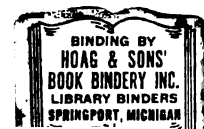
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ABSTRACT

THE SEMICONDUCTIVE PROPERTIES OF LIPIDS AND THEIR RELATION TO THE ELECTRICAL CONDUCTIVITY OF LIPID BILAYERS

by Gordon Lee Jendrasiak

A study was made showing that lipid films in the dry state behave as electrical semiconductors with high activation energies. For phospholipids, these activation energies range from 4.8 to 6.3 e.v. Upon hydration, the activation energies decrease whereas the conductivities increase. The dry conductivity values of $> 10^{-16} \Omega^{-1} \text{cm}^{-1}$ increase upon hydration to values as high as $10^{-5} \Omega^{-1} \text{cm}^{-1}$ for certain lipids. The hydrated lipid activation energy values range from 1.0 to 2.8 e.v. An effect parallel to that of water is found with exposure of lipids to iodine vapor; here the activation energies range from 2.7 to 3.2 e.v. The combination of water and iodine vapors gives unexpected results.

With the adsorption of either water or iodine, the dielectric constant, frequency dispersion and dissipation factor of the lipids increased over the dry state values. For egg lecithin, it is shown that the increase in dielectric constant, upon hydration or iodination, can account for the increase in electrical conductivity. In this respect, the lipids follow a macroscopic model which has been proposed for organic semiconductors. The model is discussed.

Lipid bilayers were found to exhibit an increase of 10^3 - 10^5 in their electrical conductivity with exposure of the bilayer to iodine solution. No concomitant increase in the D.C. dielectric constant of the bilayer was observed; the sensitivity of the method, however, was such as to probably preclude observation of the small expected increase.

Spectroscopic evidence was obtained indicating that lipids form charge-transfer complexes with iodine. Such complexing occurred in organic solvents, in water dispersions and in the solid state. The type of complexing depended on the nature of the lipid. This charge transfer evidence is taken as suggestive of a possible electronic conduction mechanism, not only in the lipid films, but also in the lipid bilayers.

THE SEMICONDUCTIVE PROPERTIES OF LIPIDS AND THEIR
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OF LIPID BILAYERS

By

Gordon Lee Jendrasiak

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To My Wife

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I. INTRODUCTION

Beginning with the suggestion by Szent-Györgi¹ that biological macromolecules may behave as semiconductors in their biological function, a large amount of work has been done on the semiconductive properties of these materials. Proteins,² nucleic acids,³ dried chloroplasts,⁴ dried rods⁵ and mitochondria⁶ have all been found to exhibit semiconductive behavior. Perhaps because of their seemingly inert biological character, lipids have not been studied to any extent, until recently. Lee and Lowry⁷ did work on the effect of moisture on waxes wherein moisture was found to increase the electrical conductivity and the dielectric constant. The increase in conductivity was greater, the greater the amount of adsorbed water. This work, however, was done with the purpose of studying the electrical insulation properties of the waxes.

Other early work was that done on the electrical conductivity of soap-water systems as a function of temperature.⁸ Later workers noted an anomalous dielectric dispersion of lecithin in mineral oil.⁹

Recently Chapman and Leslie¹⁰ and Rosenberg and Jendrasiak¹¹ have reported on the semiconductive behavior of lipids. Somewhat earlier, Wobschall and Norton¹² reported on the electrical conductivity of steroids.

The present work was undertaken to answer the following questions: (1) Do thin lipid films exhibit semiconductive behavior? (2) How does hydration effect this behavior? (3) What is the relationship between the electrical conductivity and the moisture adsorption? These questions were all investigated and the results are discussed.

In view of the recent interest shown in the electrical properties of lipid bilayers, as model systems for biological membranes, it was of interest to see if the results of the semiconductive work could be applied to the electrical conductivity of lipid bilayers. This was done and has led to the charge-transfer investigation as discussed in this thesis.

II. THEORETICAL BACKGROUND

Materials are said to exhibit electrical semiconductivity if they obey the relationship

$$\sigma = \sigma_0 e^{-E/kT}$$

where σ is the electrical conductivity at some temperature $T(^{\circ}\text{K})$; σ_0 is the electrical conductivity at $T \rightarrow \infty$ $^{\circ}\text{K}$; k is Boltzmann's constant and E is the activation energy of the conduction process.

Some workers prefer to use the relationship

$$\sigma = \sigma_0 e^{-E/2kT}$$

wherein the E is identified with an energy band gap. This form of the expression assumes intrinsic conductivity in the material and an energy band model is often assumed to hold.

Unfortunately because of the difficulty of purifying biological materials and the lack of information on the structure of the material (very often it is in the non-crystalline amorphous state) no generally acceptable models of the electrical conductivity of biological materials have been reported. Measurements of the mobility of the charge carrier, which should provide information enabling formulation of such a model, have been unsuccessful

in biological materials; such measurements have been made, however, in certain organic semiconductors. The writer¹³ has attempted, unsuccessfully, to obtain D.C. Hall effect and magnetoresistance effect measurements on proteins, nucleic acids and lipids; Trukhan,¹⁴ however, claims to have obtained mobility measurements for proteins and nucleic acids. His measurements were made at microwave frequencies and whether the results so obtained can be applied to the D.C. case is problematical. It does appear that in an amorphous film of biological macromolecules, the intermolecular and "interparticle" barriers might well set a limiting value to the charge carrier mobility. If this were the case, it is not unreasonable to expect the microwave and D.C. mobility values to differ; the charge carrier under the influence of the D.C. field travels the entire sample dimension, possibly across many of these barriers. The charge carrier under the influence of a microwave field, on the other hand, presumably vibrates at the microwave frequency and the actual path length travelled due to the electric field may be quite short (about 3.4×10^{-8} cm for $E = 100 \frac{\text{V}}{\text{cm}}$ and $\mu = 2 \frac{\text{cm}^2}{\text{volt-sec}}$ for each $\frac{1}{2}$ cycle).

Two models have been suggested for electrical conduction in biological macromolecules: (1) The energy band model and (2) the hopping model. Until further information is forthcoming on the structure of the biological material being measured and until reasonably certain mobility measurements are obtained, little can

be done in choosing which model is more appropriate, and the subject is of necessity, highly empirical.

The work of this report will concern itself with the electrical properties of lipids. The structures of the lipids used in this study are shown in Appendix 1, page 124. Note the presence of only saturated fatty acid chains in synthetic lecithin whereas egg lecithin contains roughly 50% unsaturated fatty acid chains. The unsaturated fatty acid is presumably in the α position of the molecule.

Lecithin acts as an internally neutralized compound¹⁵ with an isoelectric point of 7.5. The choline residue has a strong basicity. It is hypothesized that the best representation for lecithin is that gives in Appendix 1. On the alkaline side of the pH scale, the acidic phosphoric acid hydrogen apparently reacts with the strongly basic choline. The buffering capacity of lecithin is very small. It should be mentioned that the actual lecithin structure is still apparently far from being settled.

Oxidized cholesterol or oxycholesterol as it is often called is the resulting product of cholesterol oxidation. The structure has not been elucidated. Actually oxycholesterol is believed to be a substance of varying composition. Some workers feel that the principle oxidation products are 7-ketocholesterol and the epimeric 7-hydroxycholesterols.¹⁶ The presence of a keto group may give the oxycholesterol charge donor properties. The oxidized cholesterol is apparently insoluble in water.

Cholesterol palmitate is chemically an ester of cholesterol. On heating, the cholesteryl esters exhibit the property of changing to a turbid liquid state before melting to a clear liquid at a higher temperature. This is a general property of the natural cholesteryl esters as well as the synthetic ones. The turbid liquid state has properties actually intermediate between a solid and a liquid phase. The name given to this state is the mesomorphic state. Two forms of this state occur: the smectic form, wherein the liquid does not exhibit a normal flow but rather gliding movements in one plane, and the nematic in which the liquid flows readily but has a thread-like structure and a comparatively low viscosity. The cholesteryl esters are not soluble in water.

By definition, lipids have little or no solubility in water; on the other hand, nearly all lipids have some affinity for water. The tendency of lipids not to dissolve in water is due to the much greater mutual attraction of water molecules to themselves rather than to the hydrocarbon portion of the lipid. The interaction of lipids and water depends on the balance between the hydrophilic and hydrophobic portions of the lipid molecule.

Lecithin, when exposed to water, swells to give a paracrystalline aqueous phase which appears in the form of elongated cylinders (myelin forms). When lecithin is completely anhydrous or contains less than 5% water, it

appears as a birefringent system formed of assembled parts having their optical axes randomly directed.¹⁷ This disordered crystalline state exists even for a homogeneous lecithin. X-ray studies indicate that the lecithins exist as bimolecular leaflets with the hydrocarbon chains parallel and the charged head groups opposing one another.¹⁸ Water is apparently adsorbed between the polar heads thereby increasing the spacing between the leaflets.¹⁹ Continued uptake of water vapor expands the gap between the bimolecular leaflets until finally a colloidal solution is obtained at saturation; the leaflets are then detached from one another.

When a concentration of 5% water is reached, at least for egg lecithin, a paracrystalline phase is obtained, exhibiting short range order. Above 12% water adsorption, a single, homogeneous, translucent and rather fluid phase is obtained. When the percentage of water adsorbed reaches 45%, the mixture becomes turbid. Vacuoles of water appear which are dispersed in the paracrystalline phase. When 55% water adsorption is reached, the lamellar phase appears in the form of myelin figures on anisotropic droplets. More than about 80% adsorbed water is needed in order for the elements of the paracrystalline phase to be scattered and to float in the water phase. Note that in all these cases, however, the lecithin exists in the bilayer form with hydrocarbon chains internal to the bilayer and polar groups external.

Evidence has been presented that lecithin is micellar down to concentrations as low as 10^{-5} gm/ml.²⁰ The micelles (for egg lecithin) are thought to have a disc-like structure with a disc thickness of about $70\text{\AA}$ ⁰ i.e., the thickness of a lipid bilayer. The micelles vary in size, depending on lecithin concentration and other experimental conditions; in all cases, however, thicknesses corresponding to the bilayer case are observed.

Lipids are soluble in many organic solvents, but here the structural arrangement of the lipids seems to be in question. Lecithin dissolved in a non-polar solvent does not change the dielectric constant of the solvent; the lecithin behaves as if its polar groups were neutralized.²¹ This suppression of the dipole moment has been explained by the formation of molecular associations, in agreement with the results of molecular weight measurements. These results are thought by some to indicate some sort of aggregate structure with the polar groups directed toward the interior and the hydrocarbon chains toward the exterior. Whether such a structure is present and in what solvents it exists is a question for further research to answer.

III. SEMICONDUCTIVE BEHAVIOR OF LIPIDS

Theory

The model used for the study of the electrical behavior of lipids in the solid state is essentially that put forth by Rosenberg.²² One starts with the operational definition of a semiconductor

$$\sigma(T) = \sigma_0 \exp(-E_A/2kT) \quad (1.)$$

where the symbols have the meanings mentioned previously

Now $\sigma(T) = n(T) \cdot \mu(T) \cdot e$, where $n(T)$ is the number of charge carriers (per cm^3) present at temperature T , $\mu(T)$ is the mobility of the charge carrier at T and e is the charge on the electron.

Then

$$\sigma(T) = e \sigma_0 e^{-nE_A/kT} \mu_0 e^{-\mu E_A/kT}$$

where ${}^nE_A$ and ${}^\mu E_A$ are the activation energies for the generation of charge carriers and for the mobility of the charge carrier, respectively. Here a one charge carrier model is being assumed. It has been found for anthracene as well as for inorganic materials that $\mu \propto \frac{1}{T^n}$ where $n = 1-1.5$ therefore in what follows it will be assumed that μ for the lipids is a slowly varying function of the temperature in comparison to n ; this implies that E_A is the

activation energy for charge carrier generation. At the moment there is no real justification for this assumption other than the anthracene and inorganic work.

Now it has been observed that hydration increases the conductivity of the material and for the lower values of the per cent water adsorbed (m), the conductivity follows the relationship:

$$\sigma(m) = \sigma_{\text{DRY}} \exp(\alpha m) \quad (2.)$$

For high values of m , saturation occurs and the conductivity no longer increases.

If one plots Equation (1.) for various hydration states of the material, it is found that σ_0 has the same value for the various hydration states whereas E_A decreases with increasing hydration of the sample.

Since σ_0 is the same no matter what the hydration state, we may replace σ_{DRY} in (2.) by (1.) and obtain

$$\sigma(m, T) = \sigma_0 \exp [-(E_{\text{DRY}} - \gamma m)/2kT] \quad (3.)$$

or

$$\sigma(m, T) = \sigma_0 \exp (-E_{\text{WET}}/2kT) \quad (4.)$$

where $E_{\text{WET}} = E_{\text{DRY}} - \gamma m$ and $\gamma = 2kT\alpha$ (5.)

The above derivation is based on the empirical Equations (1.) and (2.). Let us now consider our biological material as a continuous medium of static dielectric constant κ . The work that must be done to remove a charge from

one molecule of the material and place it on another molecule of the material an effectively infinite distance away, will be equal to the ionization energy (I) minus the electron affinity (A) of the molecule, minus the charge stabilization due to the relaxation of the dielectric media, in a sphere of radius R, around the two new charge points. In the dry state this work is given by

$$E_{\text{DRY}} = I - A - (e^2/R)(1-1/\kappa) \quad (6.)$$

When a material of high dielectric constant is adsorbed by the proteins, the effective dielectric constant of the biological material increases. For a given value of hydration, m, the dielectric constant is κ' (where $\kappa' > \kappa$) and (5.)

becomes
$$E_{\text{WET}} = I - A - (e^2/R)(1-1/\kappa') \quad (7.)$$

where e is the charge on the electron.

Eliminating (I-A) between (6.) and (7.) produces

$$E_{\text{WET}} = E_{\text{DRY}} - (e^2/R)(1/\kappa - 1/\kappa') \quad (8.)$$

It is of course assumed that (I-A) is the same for the material in the dry and hydrated states. Comparing Equations 5. and 8., m is related to the effective dielectric constant

by
$$\gamma m = (e^2/R)(1/\kappa - 1/\kappa') \quad (9.)$$

Solving this equation for κ gives

$$\kappa' = \kappa / (1 - \kappa \chi m) \quad \text{where } \chi = R\gamma / e^2 \quad (10.)$$

This equation predicts an increase in the effective dielectric constant for the material as the water (or other

polar adsorbate) increases in amount. This relationship has been verified for haemoglobin²² and collagen.²³ Presumably it would apply to any situation wherein the dielectric constant of the material in question is changed.

We may now rewrite Equation (3.) as

$$\sigma(T, \kappa') = \sigma_0 \exp \left(\frac{-E_{\text{DRY}}}{2kT} \right) \exp \left[\left(\frac{e^2}{2kTR} \right) \left(\frac{1}{\kappa} - \frac{1}{\kappa'} \right) \right] \quad (11.)$$

Notice that as κ' gets to be large, $\frac{1}{\kappa'} \ll \frac{1}{\kappa}$ and the second exponential term in (11.) becomes constant. Thus, a saturation effect for σ is predicted.

Also from (8.) we can predict as a minimum value for the activation energy:

$$\text{minimum } E_{\text{WET}} = E_{\text{DRY}} - \frac{e^2}{R\kappa}, \quad \text{when } \frac{1}{\kappa'} \ll \frac{1}{\kappa}$$

This model has been applied to proteins and nucleic acids. The work of this paper has, in part, been an attempt to apply it to lipids.

Experimental Details

The lipids used for the semiconductivity portion of this work were as follows: Cholesterol Palmitate obtained from Nutritional Biochemicals Corporation; DL - Alpha Lecithin (synthetic- β , γ -Dipalmitoyl - DL - α - Lecithin) also obtained from Nutritional Biochemicals Corporation; egg lecithin (chromatographically pure) obtained from General Biochemicals. All of these materials were used without further purification.

The solutions used for forming the thin films were made by dissolving the synthetic lecithin and the cholesterol palmitate in chloroform (spectro grade) whereas the egg lecithin was dissolved in n-decane (reagent grade). The cholesterol palmitate solution was approximately 0.16 M while the synthetic lecithin was about 0.02 M. The egg lecithin solution was a 1% (weight of lecithin: volume of decane) solution.

The quartz plates upon which the films were formed were Suprasil #1 obtained from Engelhard Industries, Inc. They were one (1) inch in diameter and one-sixteenth $\left(\frac{1}{16}\right)$ inches thick. The electrodes were vacuum deposited on the quartz plates by the Bendix Corporation, Research Laboratories.

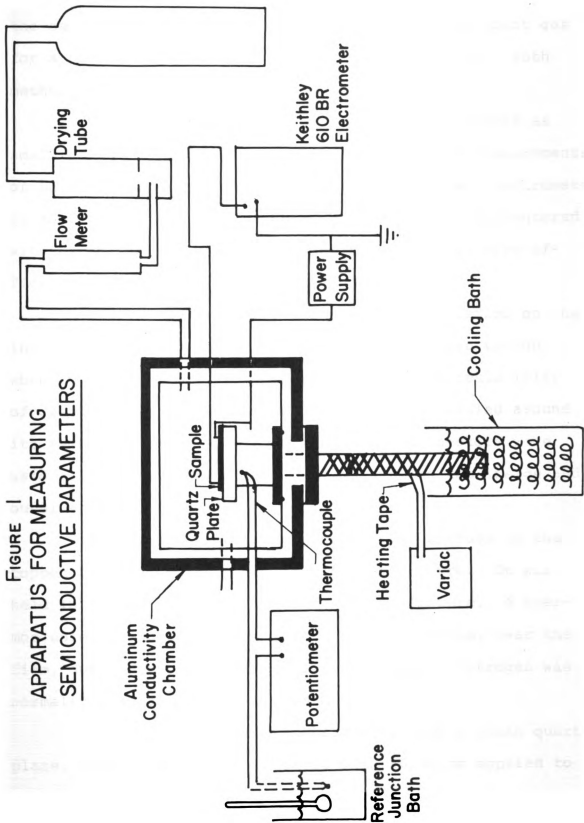
The pattern, which was centered on the plates, consisted of two electrodes, each 0.5 cm square, separated by 0.5 cm. The electrodes were formed by first vacuum depositing nichrome on the plates and then gold on the nichrome. Electrical contact to the gold was then made by indium soldering wires to the gold.

The films were formed by depositing, by means of a syringe, several drops of the particular lipid solution on the plate between the gold electrodes and in contact with the electrodes. The lipid solution did not touch the indium solder joints. After the film was deposited, it was placed in the test chamber where electrical contact was made and then dried with helium gas, normally for 24 hours. At this

time no current could be observed passing through the sample and this state was then taken as the operational "dry state." It should be mentioned that this "dry state" could always be attained with the lecithin but could not be attained with cholesterol palmitate. Even after heating the cholesterol palmitate (40-50°C) for several days with the chamber subject to vacuum pumping, a measurable current was still obtained.

Although calculations of the film thickness were not made for all the films, a typical cholesterol palmitate film might be about 10 μ thick; a typical synthetic lecithin film was about 50 μ thick; an egg lecithin film had a typical thickness of 40 μ . These values are representative of course; the film thickness varied from film to film depending upon the amount of lipid solution put down on the quartz. In general, the films probably ranged from 10 to 100 μ in thickness.

With the film in the chamber, the chamber was placed in the circuit shown in Figure 1. Normally voltages from 1 volt to 20 volts were applied to the sample. To bring the sample to various hydration states, after drying, a variety of salt solutions could be sealed in the chamber. Beakers of water were normally sealed in the chamber to bring the sample to the fully hydrated state; an alternative method was to use the helium as a carrier gas and pass it through a gas generator bottle of water and then on to



the sample. After the sample was exposed to the moist gas for a number of hours, the chamber was sealed off. Both methods gave equivalent conductivity values.

The Keithley electrometer can detect currents as small as 10^{-15} a and this value set the limit for measurements of the dry state conductivity. A vibrating reed electrometer is somewhat more sensitive but the difficulties encountered with using it (due to the response time) made it more efficacious to use the Keithley instrument.

The aluminum chamber was covered with teflon on the inside so as to prevent leakage currents. Clean teflon, when fully hydrated, still exhibits a surface resistivity of greater than $10^{17} \Omega\text{-cm}$. The chamber was sealed around its removable cover with an O-ring. BNC connectors were used to establish electrical contact between the inside and outside of the chamber.

The quartz plate made good thermal contact to the copper bar used for controlling the temperature. It was held in contact with the bar by a teflon fixture. A thermocouple made good contact with the quartz plate, near the film, due to the same teflon fixture. Liquid nitrogen was normally used as the cooling bath material.

It should be mentioned here that with a clean quartz plate, heating the plate to 70°C , with 20 volts applied to the electrodes, produced no measurable current; thus, the quartz itself, should make no contribution to the current measured for the dry state activation energies.

With a clean quartz plate in the chamber and the atmosphere fully hydrated, the current across the quartz plate at 20v is between 0.01 to 0.001 that of the fully hydrated lecithin film at the same temperature; it is therefore felt that the leakage current across the hydrated quartz plate does not significantly contribute to the current being measured for fully hydrated lecithin.

It should be noted that with time and with the synergistic action of iodine and high and low temperatures along with the abrasive cleaning action, the gold may "flake off" from the nichrome or the nichrome may come off the quartz. The usual cleaning procedure was to remove the lipid with choloroform; the quartz plate was then cleaned in turn with distilled water, acetone and ethanol, by means of a cotton swab. The plate was then air-dried.

Dry state activation energy runs were made by heating the sample to some temperature significantly below its melting point and then allowing it to cool back to room temperature. The cooling rate was kept at about 5C° per 2-3 mins. A stopwatch was used to check the cooling rate. The current was read on the Keithley 610 BR at 5C° intervals.

For the hydrated specimens, a similar procedure was employed. The sample was cooled at almost 5C° per 2-3 mins. and this current again read at 5C° intervals

during cooling. Spot checks of this procedure by taking measurements during the heating portion of the curve for the hydrated specimen revealed no difference in the slope of the activation energy curves so obtained.

The conductivity of samples was obtained by measuring the area of the film and weighing the quartz plate with and without the lipid film present. A density of 0.9 gm/cm^3 was used in the calculation for all the lipids. No allowance was made for adsorbed water, however, the conductivities so obtained are probably good to within a factor of 2. This was felt to be sufficiently accurate in view of the exploratory nature of the investigation.

Where it was desired to expose the sample to iodine vapor, two small beakers, containing iodine crystals, were sealed in the chamber with the sample. After about 24 hours a steady state equilibrium seemed to be established and measurements could then be made. The iodine used was re-sublimed, obtained from Mallinckrodt Chemical Works.

The dielectric measurements were made by spreading egg lecithin on a circular disk of porous metal and placing another disk of the same material over it. Metal-to-metal contact was prevented by small pieces of teflon (4 mil) placed between the plates at their periphery. The "sandwich" was then placed in a Balsbaugh Laboratorie Model LD.3 chamber for measuring capacitance. The chamber contained 2 metal plates, 1 of which was mounted on a micrometer screw.

The sandwich was placed between the plates and its thickness could be measured by means of the micrometer.

The chamber was then connected to a General Radio Company Capacitance Measuring Assembly, Type 1610. The capacitance bridge was a type 716-C and its null detector was a type 1232-A. The guard circuit was not used.

In this case, also, to obtain the effects of adsorbate on the sample, water or iodine crystals were sealed in the chamber with the sample. Helium gas was again used for drying purposes.

Dispersion measurements were made between 30 c.p.s. and 10^5 c.p.s.

These measurements were very difficult to perform. To get a uniform layer of lipid spread over the porous metal plate was difficult and the layer eventually obtained was probably of varying thickness. Because of the rapid oxidation of egg lecithin it was felt that heating would degrade the sample so this was not attempted even though a more uniform sample might have been obtained.

In order to get electrical contact between the micrometer plate and the porous metal, a certain amount of pressure had to be applied to the sample by means of the micrometer. Since the lipid is quite plastic, it was never certain just how the sample was effected by the pressure exerted by the micrometer screw. In addition, it was never certain whether the same pressure was being applied to the

sample, each time the micrometer fixture made contact with the metal-lipid sandwich, since the pressure was subjectively determined by the experimenter.

Upon sample hydration, the situation became much worse. Phospholipids are known to swell upon moisture adsorption and myelin-like projections develop. Even at the lowest hydration level used (R.H.: 14.5%), a measurement could not be made of the capacitance. The capacitance measurements were made at states intermediate between the "dry" state and the equilibrium state characteristic of an R.H. of 14.5%. Since the sample was not at equilibrium in these states, the measurements were not considered to be of high precision. If the sample were examined it was seen that the lipid had exuded out around the edges of the porous metal plates. Moreover, any attempt to establish electrical contact to the sample by means of the micrometer caused a further loss of lipid from between the plates. Nevertheless, since it was mainly desired to show that the dielectric constant greatly increased upon hydration, it is felt that the results obtained do indicate this.

The last measurements made in this portion of the work involved the amount of water adsorbed by a lipid film, at various relative humidities, and measurements of the electrical conductivity at these humidities. For this purpose a Cahn electrobalance was used. The balance mechanism itself was placed in a plexiglass chamber through which dry

helium was passed in order to dry out the sample. The chamber was then sealed off. The electrical connections were made through various connectors sealed into the plexiglass. A rubber gasket was placed around the removable front of the chamber. The egg lecithin film was placed down in the usual manner but now a small piece of 4 mil thick teflon tape was used as substrate. It was necessary to use teflon rather than quartz since the film then would have been only a small fraction of the weight of quartz and balancing would have been difficult. The teflon with its lipid film was then placed on the balance pan. Previous work had indicated that the electrical conductivity and activation energy of synthetic lecithin were unchanged if it were placed on a teflon substrate rather than quartz. A second egg lecithin film was now placed on the quartz plate mentioned earlier and this plate was placed in the plexiglass chamber very close to the film on teflon. The electrical current was read from the film on quartz whereas the moisture adsorbed was determined for the film on teflon. Very little error was probably due to this arrangement.

Previous to the weighing operation, the teflon tape was thoroughly dried and weighed. It was then exposed to a fully hydrated helium atmosphere (R.H. ~ 95%) and weighed again. The moisture adsorbed by the teflon tape itself was then assumed to be a linear function of the relative humidity between the dry state (0 R.H.) and 100% R.H. These values

were then corrected for the area occupied by the lipid film, since it was assumed that the teflon adsorbed no water where it was covered by the lipid film. These corrected values were then subtracted from the actual weights of the moisture adsorbed by the lipid, as measured by the electrobalance.

It should be mentioned here that helium appeared to be a more efficient drying agent than nitrogen gas.

Experimental Results

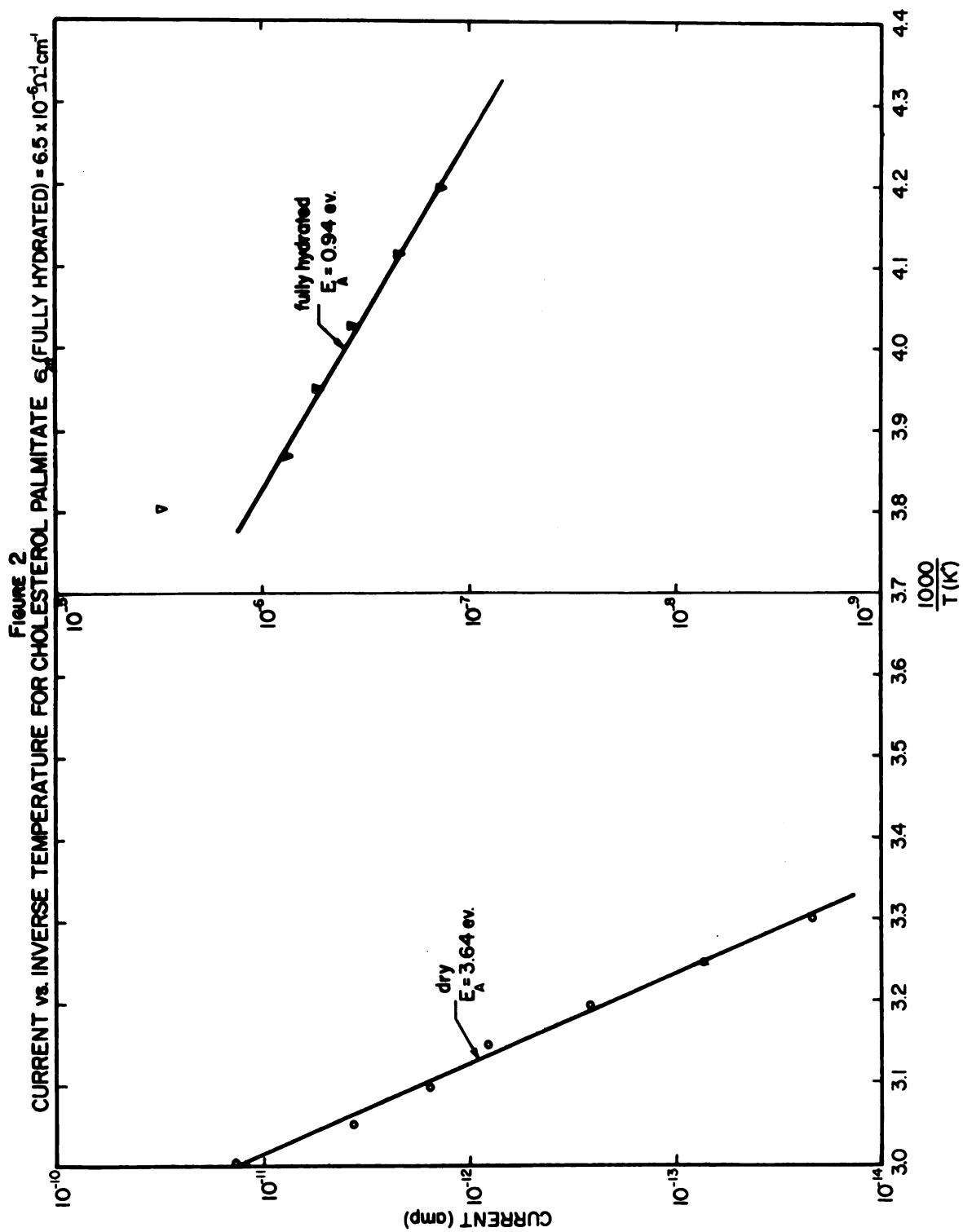
Typical values of the semiconduction activation energies and room temperature conductivity values are collected in Table 1. Figures 2, 3 and 4 are representative plots of the log of the current versus the inverse temperature for the lipids used. From the slope of such plots, activation energy values were calculated. Synthetic lecithin showed extremely large activation energies (E_A) in the dry state and the room temperature current was below the sensitivity of the electrometer (10^{-15} a). The E_A values for cholesterol palmitate may represent values for the slightly hydrated state as will be discussed presently. No dry activation energy values were obtainable for egg lecithin since its softening point ($\approx 60^\circ\text{C}$) was quite low.

When exposed to an ambient atmosphere, at a high relative humidity (95% R.H.) of helium gas, the conductivity of all three lipids increased by 9-11 orders of magnitude, and the E_A 's decreased concomitantly to a range

Table 1. Activation energies and conductivities of lipid films[†]

Substance	Dry			Fully Hydrated			Fully Hydrated and With Iodine		
	E_A (e.v.)	σ_{25}^*	E_A (e.v.)	σ_{25}^*	E_A (e.v.)	σ_{25}^*	E_A (e.v.)	σ_{25}^*	
Synthetic Lecithin	4.8-6.3	$< 0^{-16}$	1.7-2.8	10^{-5}	---	---	---	---	
Egg Lecithin	---	$< 10^{-16}$	2.5-2.7	10^{-7}	2.7-3.2	10^{-7}	1.9-2.1	10^{-7}	
Cholesterol Palmitate	2.9-3.5	10^{-15}	1.0-1.9	10^{-6}	---	---	---	---	

* σ_{25} ($\Omega^{-1} \text{cm.}^{-1}$)[†] From $\sigma(T) = \sigma_0 e^{-E_A/2kT}$



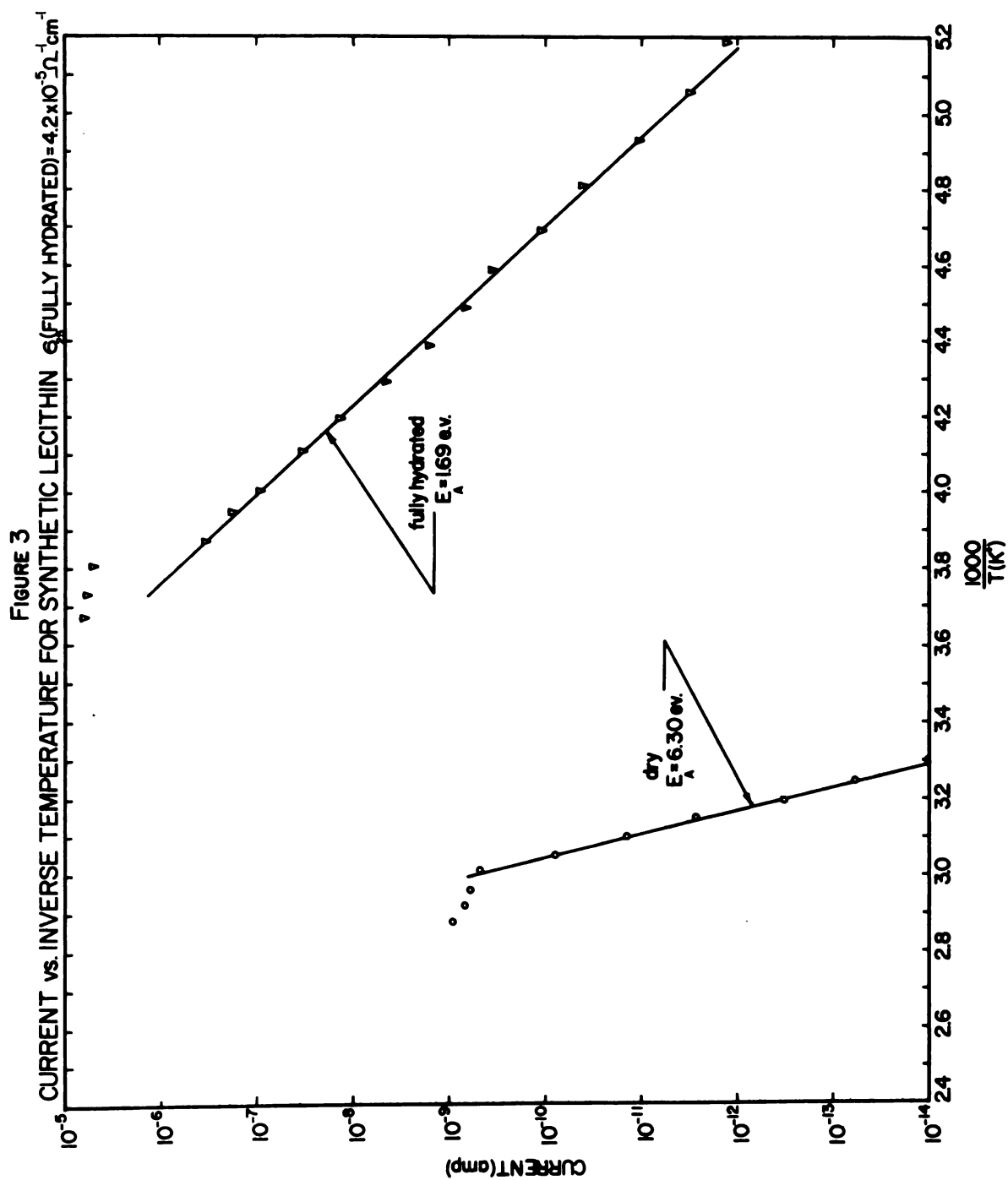
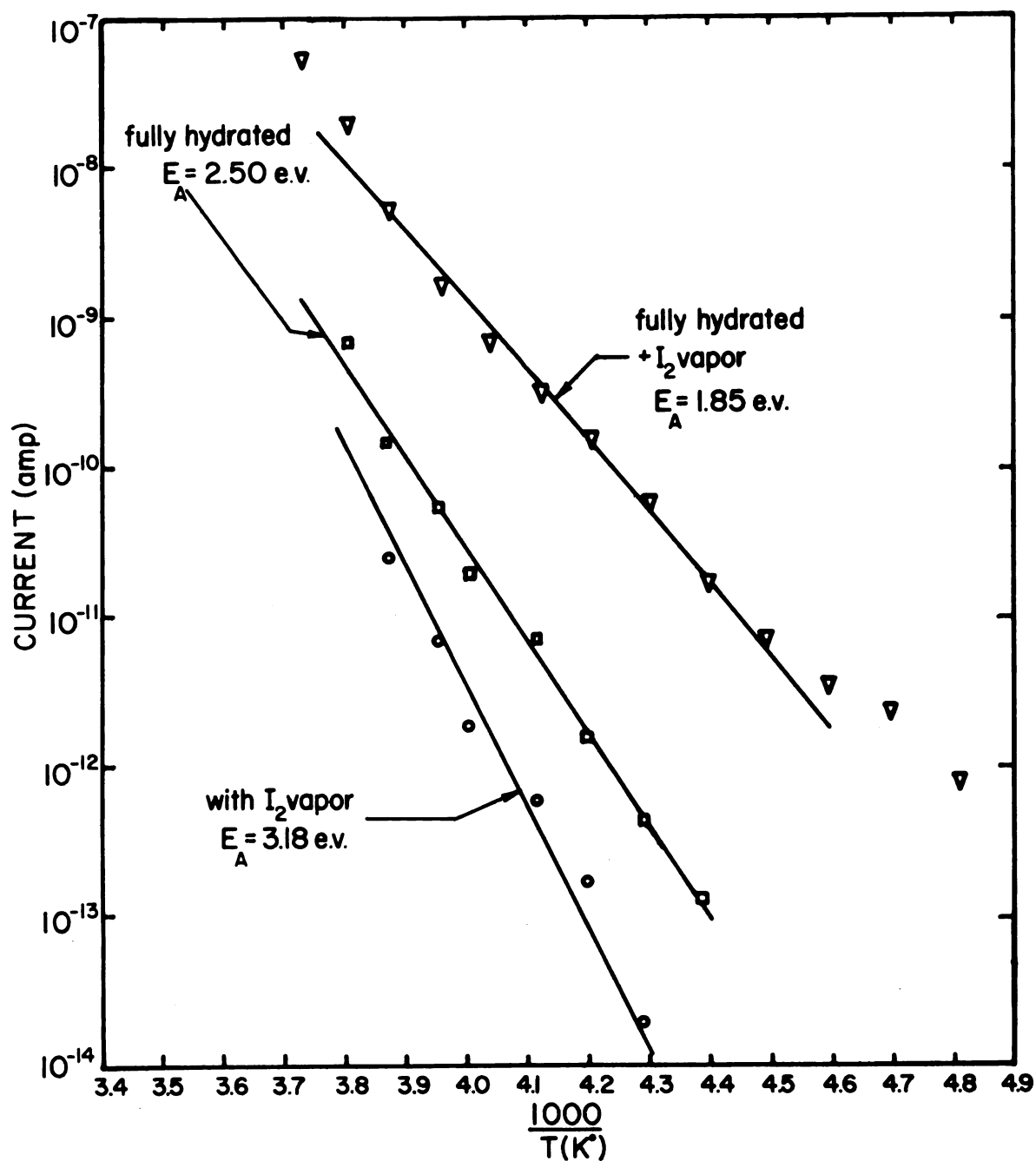


FIGURE 4
CURRENT vs. INVERSE TEMPERATURE FOR EGG LECITHIN
 σ_{25} (FULLY HYDRATED) = $2.5 \times 10^{-7} \Omega^{-1} \text{cm}^{-1}$



of values between 1.0 e.v. and 2.8 e.v. This decrease is similar to the results obtained with crystalline proteins and DNA.

If dry films of egg lecithin are exposed to iodine vapor, the conductivity changes parallel the hydration effect. This effect seems to be at least partially reversible in that passing dry helium gas over the iodinated sample decreases the current.

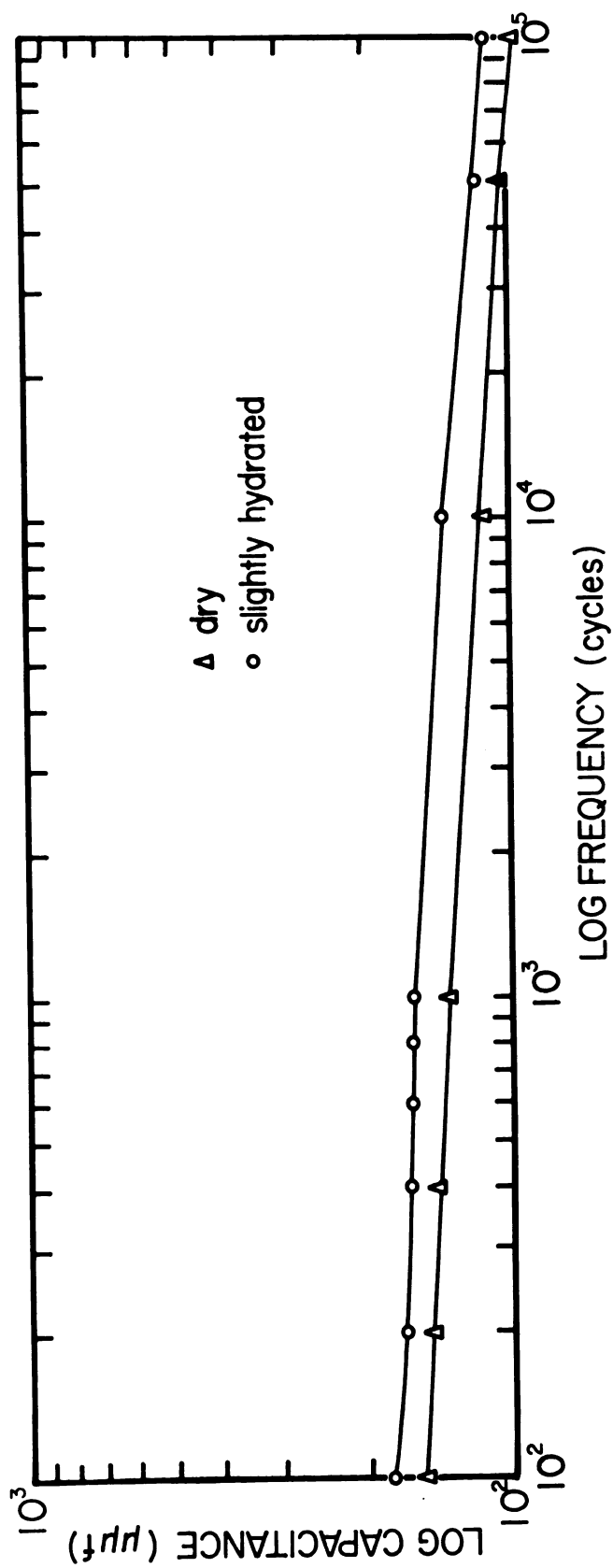
Of some interest is the observation that the conductivities of the fully hydrated and the iodinated egg lecithin films are the same; the activation energy values for the iodinated cases, however, are significantly higher than for the hydrated cases. This may indicate different adsorption sites for iodine and water. The difference in activation energy values is somewhat puzzling, however, in view of the similarity of room temperature conductivity values.

Also of interest is the apparent synergistic effect of iodine and moisture. A fully iodinated specimen showed an activation energy of about 3.2 e.v. If now the sample was fully hydrated, the conductivity remained the same, however, the activation energy dropped to about 1.9 e.v., a value lower than either the hydrated or iodinated case values. Again, this behavior might be indicative of a difference in adsorption sites for iodine and for water.

It should be noted that for any one sample, the activation energy values were reproducible, from run to run, to within about 0.2 e.v.

If lipids follow the model discussed in the beginning of this section, an increase of dielectric constant should occur upon hydration of the material. This increase, in turn, would then account for the decrease in activation energy concomitant with the increase in electrical conductivity. Moreover, one might expect the dielectric constant values to show a frequency dispersion, upon hydration. Since iodine vapor also decreases the activation energy of lipids, over the dry state value, as well as increases the conductivity, one might well look for similar behavior with iodine vapor exposure of the lipid. Figure 5 displays the capacitance of egg lecithin as a function of the frequency of the applied voltage. The difference between the "dry" and "slightly hydrated" samples, used in plotting Figure 5, was that the slightly hydrated sample was helium dried for several days; measurements were then made on the sample. The sample was then dried overnight, under vacuum at 35°C; in this condition, the sample was then placed in the capacitance bridge and again a set of measurements was made. The sample in this state was designated as dry. It is evident, however, that the dry sample displays almost as much dispersion as it did in the "slightly hydrated" state. Its capacitance, over the frequency range studied, is less however.

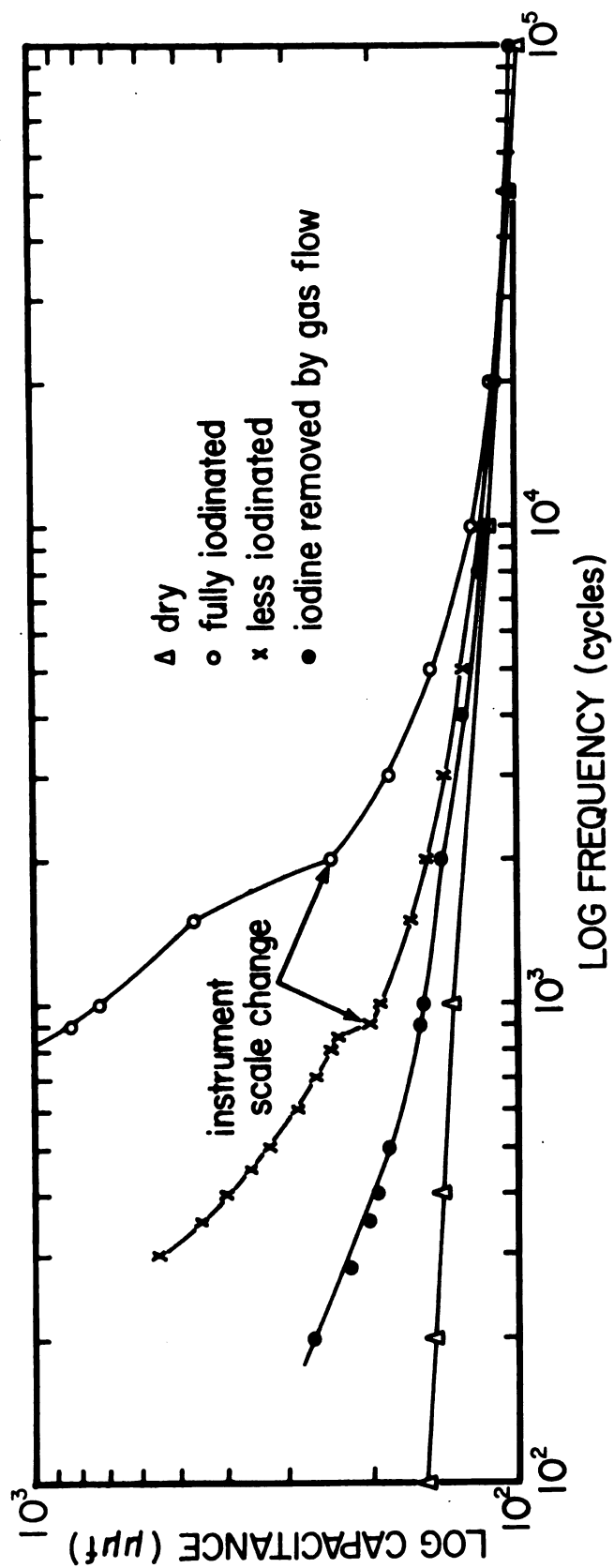
FIGURE 5
CAPACITANCE vs. FREQUENCY OF APPLIED VOLTAGE FOR EGG LECITHIN
EFFECT OF MOISTURE



Although plots of the capacitance vs. frequency could not be obtained, for egg lecithin, at a series of known humidity values, on several occasions it was possible to monitor the capacitance while the sample was just starting to become hydrated. This enabled observations to be made before the sample degraded to the extent that meaningful measurements could not be made. These observations all revealed a large increase in capacitance at low frequencies (< 1500 c.p.s.) and a large dispersion. Although quantitative measurements could not be made, the expected increase in capacitance and dispersion was observed.

Figure 6 shows the same sample as displayed in Figure 5 in the dry condition. This sample was then allowed to adsorb various amounts of iodine. The plot designated as "fully iodinated" illustrates the capacitive behavior after the sample was exposed to iodine vapor for 24 hours. At this time, the dissipation factor (at frequencies below 1 kc) had attained a value so high that meaningful measurements of it, as well as the capacitance, could not be made on the bridge. The iodine crystals were then removed from the chamber and the chamber resealed. The values of the capacitance for the fully iodinated case were obtained by making the measurements immediately after resealing. At this time no values of capacitance could be read for frequencies < 900 c.p.s. since the dissipation factor was off scale for the bridge. The very large dispersion obtained is evident from the plot.

FIGURE 6
CAPACITANCE vs. FREQUENCY OF APPLIED VOLTAGE FOR EGG LECITHIN
EFFECT OF IODINE VAPOR



The plot shown as "less iodinated" shows the values of capacitance, obtained with the sample just mentioned, but after waiting for 24 hours. Apparently some iodine had now been desorbed and values of the capacitance at frequencies as low as 300 c.p.s. could be read. A considerable dispersion is still evident.

Finally, the sample designated as "least iodinated" was obtained by exposing the "less iodinated" sample to a flow of helium gas for 4 days. At this time, it appeared that very little iodine could be further removed by the helium and the dispersion curve shown was then obtained. It is clear that a large dispersion still remains but it is also clear that the dispersion is approaching that displayed by the "dry" sample. This indicates that the sample may not have degraded extensively due to the iodine exposure.

Although the data are subject to certain criticisms, as will be discussed shortly, the increase in capacitance (and presumably therefore in dielectric constant) and dispersion is seemingly valid. These increases occur both for hydration and iodine vapor exposure and are consistent with the macroscopic model²² discussed earlier in this report.

One can make some "rough" calculations of the dielectric constant for the various hydrated and iodinated states: The porous metal plates used to "sandwich" the lipid had a 3/4" diameter and a thickness of 4 mils was

assumed for the lipid; because of measurement difficulties, the actual value might have been somewhat greater. The results are shown in Table 2.

It is of interest here that although the dielectric constant values (κ) for the slightly hydrated and least iodinated samples (at 1 kc) are essentially the same, the dispersion exhibited by the least-iodinated sample is much greater. This can be seen from Figures 5 and 6.

Low frequency dielectric constants for dry lipids should probably have values of 2 to 3. As can be seen, the driest sample used seems to have values within this range although the amount of dispersion shown indicates it is probably somewhat hydrated.

Note, also that for frequencies of 10 kc and above, there exists practically no dispersion for the iodinated sample as compared to the dry sample. Since the dispersion occurs at relatively low frequencies, it may be that orientational polarization is responsible for the dispersion. The iodine and water apparently both enhance such polarization since the dispersion occurs for both these vapors.

Table 3 displays the dissipation factor data. The dissipation factor, of course, says nothing about the type of loss mechanism operative in the lipid. As can be seen in the table, the dissipation factor increases, in all cases, with a decrease in frequency of the applied voltage. For both the dry and hydrated samples, the increase is

Table 2. Dielectric constants of egg lecithin at various frequencies and for various ambient atmospheres

Sample	Frequency (c.p.s.)					
	50	200	500	1 kc	10 kc	100 kc
Dry	3.5	3.1	2.9	2.8	2.4	2.0
Slightly Hydrated	4.1	3.5	3.4	3.3	2.8	2.3
Least Iodinated	---	5.5	3.8	3.2	2.4	2.0
Less Iodinated	---	---	6.8	4.0	2.5	2.1
Fully Iodinated	---	---	---	15	2.6	2.1

Table 3. Dissipation factors of egg lecithin

Frequency (c.p.s.)	SAMPLE				
	Dry	Slightly Hydrated	Least Iodinated	Less Iodinated	Fully Iodinated
50	0.13	0.23	---	---	---
100	0.081	0.15	---	---	---
200	0.066	0.086	0.61	---	---
280	---	---	0.48	---	---
300	---	---	---	1.3	---
350	---	---	0.40	1.1	---
400	0.063	0.061	0.36	0.99	---
450	---	---	---	0.89	---
500	---	---	0.31	0.81	---
600	---	0.054	---	0.71	---
700	---	---	---	0.64	---
800	---	0.053	---	0.58	---
850	---	---	---	0.56	---
900	---	---	0.28	0.47	1.9
1 kc	0.065	0.054	0.20	0.44	1.8
1.5	---	---	---	0.33	1.3
2	---	---	0.15	0.27	0.67
3	---	---	---	0.21	0.47
4	---	---	0.12	---	---
5	---	---	---	0.16	0.31
8	---	---	0.088	---	---
10 kc	0.060	0.083	0.092	0.11	0.18
20	---	---	---	0.081	0.11
50	0.040	0.063	0.051	0.048	0.074
100 kc	0.024	0.052	0.041	0.043	0.054

about the same even though the dissipation factor values are higher for the hydrated sample. This is consistent with the fact that the two dispersion curves are approximately congruent, even though displaced from one another. A tremendous dissipation factor was noted upon further hydration. No meaningful values could be obtained, however. The dissipation factor behavior, upon iodination, parallels that upon hydration. Again, the enormous increase in dissipation factor at the low frequencies is evident.

The macroscopic model predicts not only an increase in conductivity upon hydration but also a saturation behavior for this increase. Figure 7 illustrates the weight percentage moisture adsorbed, on an egg lecithin film, at various relative humidities, as obtained with the electrobalance. Figure 8 is a plot of these weight percentages vs. the conductivity of the egg lecithin at each relative humidity. To the best of the present writer's knowledge, the only previous work done along these lines is that of Elworthy.¹⁹ He measured the weight percentage moisture adsorbed for both egg and synthetic lecithin, in the powdered form. The plot shown in Figure 7 agrees reasonably well with that of Elworthy.

Figure 8 clearly displays a saturation effect of the current with increased moisture adsorption. This is the same behavior as was found with proteins. For lipids, the saturation effect occurs at about a 20% weight percentage

FIGURE 7
PERCENT H_2O ADSORBED vs. R.H. FOR EGG LECITHIN

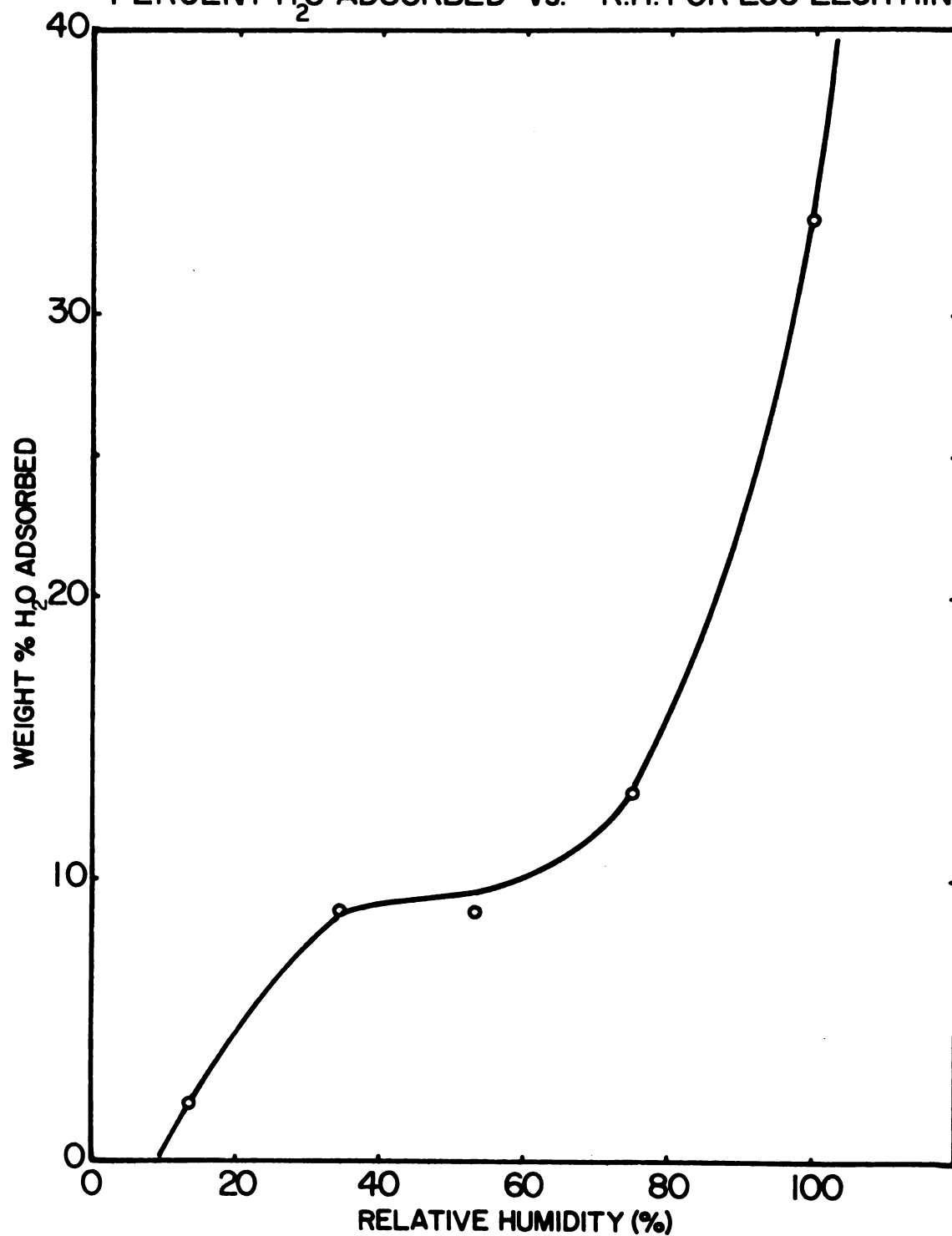
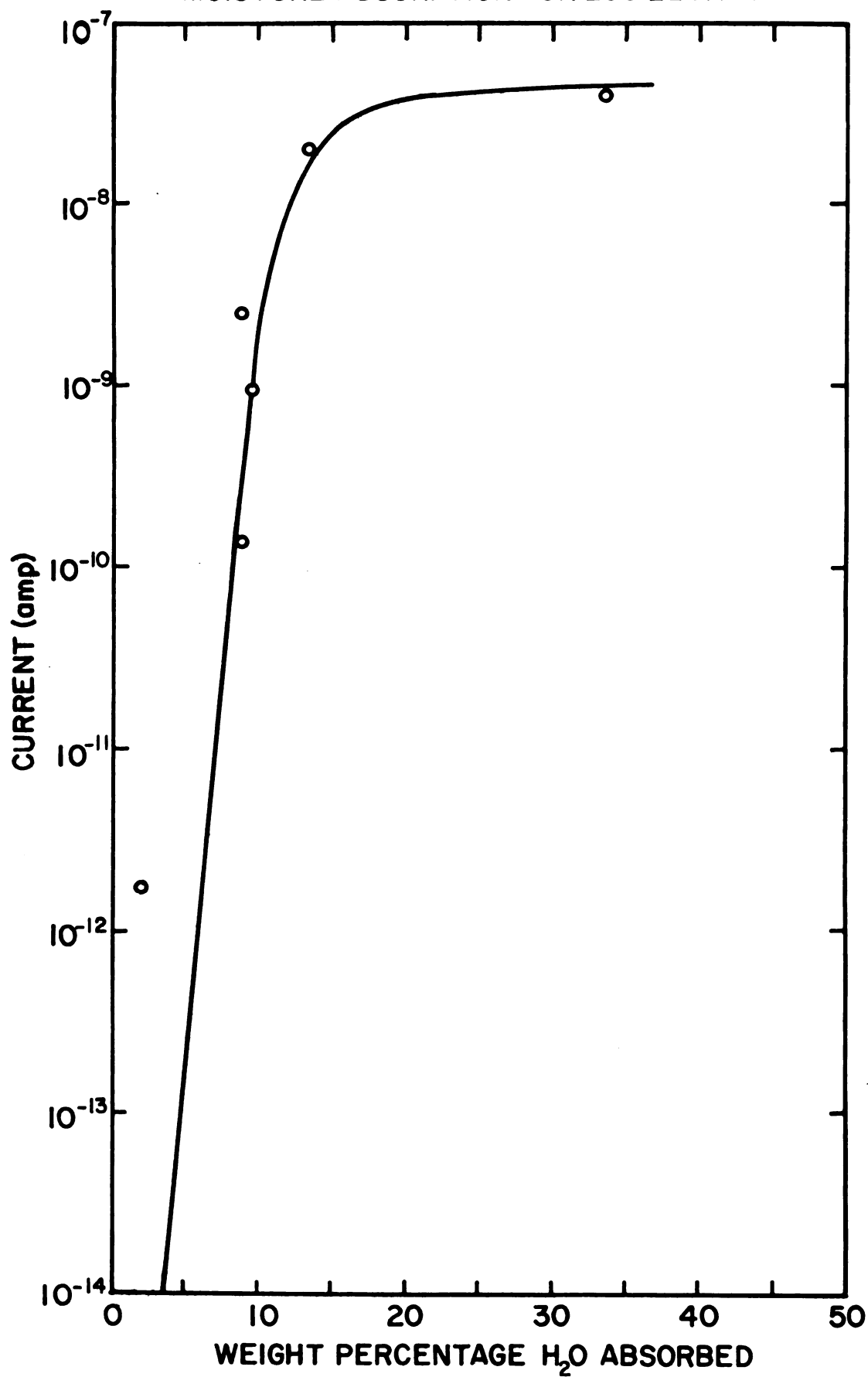


FIGURE 8
CURRENT vs. WEIGHT PERCENTAGE
MOISTURE ADSORPTION FOR EGG LECITHIN



of adsorbed water. Notice that for this 20% water adsorption, the current has increased by approximately ten orders of magnitude over its dry state value (obtained by extrapolating the plot of Figure 8 to 0% moisture adsorption). This large current increase can be explained by the increase in dielectric constant, upon hydration of the lipid.

At this point it is of interest to make some calculations from the model, using the experimental values obtained.

Consider Equation (11.) which indicates that saturation of the conductivity (or current) occurs when $\frac{1}{\kappa'} \ll \frac{1}{\kappa}$ then assuming $\kappa = 3$ for the egg lecithin (dry) and a saturation current increase of 10^{10} as indicated above

$$\exp \left[\frac{e^2}{2kTR\kappa} \right] = 10^{10}$$

from which we get $R \approx 4 \text{ \AA}$, a reasonable value for the radius of polarization. Then from Figure 8, $\alpha \approx 1.0/\text{per cent adsorbed H}_2\text{O}$.

$$\text{Now } \gamma = 2kT\alpha \text{ and for } T = 297^\circ\text{K}$$

$$\gamma = 7.58 \times 10^{-14} \text{ erg/per cent H}_2\text{O adsorbed}$$

$$\text{Now, } \kappa' = \kappa / \left[1 - \kappa \left(\frac{R\gamma}{e^2} \right) \right]$$

therefore, at saturation

$$\kappa' \approx 14 \qquad \text{for egg lecithin}$$

From Figure 8, in order to obtain a 20% weight adsorption of water on a lecithin film, a relative humidity of about 85% is necessary.

This concludes the results obtained for the semi-conductive portion of this work.

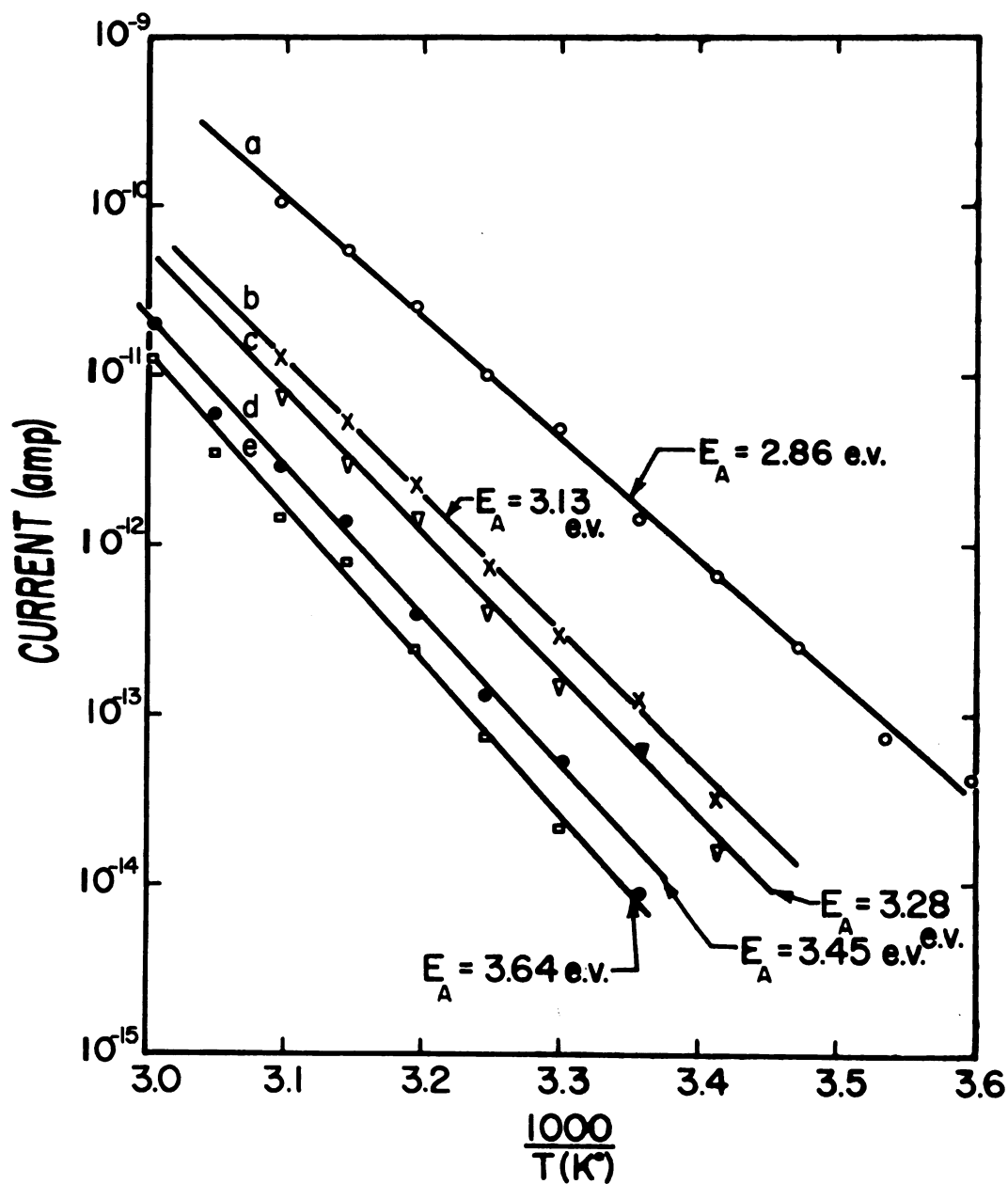
Discussion of Experimental Results

What follows will be comments on the experimental results just mentioned.

The cholesterol palmitate E_A values for the dry state activation energies probably represent slightly hydrated states of the material. Even after repeated vacuum pumping and raising the sample temperature to 40° C, for a number of days, a measurable current was still observed. The current did decrease with time of sample drying, however.

Activation energy runs were made on one sample at various stages of drying. The results are shown in Figure 9. Although all of the stages represent relatively dry states of the sample, the increase in activation energy values with increased drying is evident. Plot a indicates the status of the cholesterol palmitate sample after helium drying for several days; at this point, it appeared that further helium drying would have little effect on the sample. The current at this time was $\approx 10^{-12}$ a and the activation energy was 2.86 e.v. The chamber was now pumped, overnight, with a vacuum pump and the current dropped to 8×10^{-14} a; at this stage, the activation energy was 3.13 e.v.

FIGURE 9
CURRENT vs. INVERSE TEMPERATURE FOR CHOLESTEROL
PALMITATE AT VARIOUS STAGES OF DRYNESS



Several activation energy runs increased it further to 3.28 e.v.; apparently, the rise in sample temperature to 50°C, during the runs, aided in the drying. The activation energy runs were made while the pumping was proceeding. Another night of vacuum pumping raised the activation energy to 3.45 e.v. and further increases in temperature to 50°C raised it to 3.64 e.v. Because of time limitations, the experiment was discontinued at this time.

What would have been desirable would have been to plot a family of activation energy plots for all the lipids; these plots would represent sample states from the driest obtainable up to the fully hydrated state, including various intermediate states between dry and fully hydrated. It was not found possible to do this with the lipid films, however. The method used was to pass moist helium over the sample, fully hydrating it; at this point activation energy runs were made. Dry helium was then allowed to flow through the chamber, decreasing the electric current. A new hydration state was thereupon established and the chamber sealed off. This state was less than fully hydrated but not anywhere near dry, as indicated by the current values. If now an activation energy run were attempted, apparently moisture inside the chamber condensed on the sample as the temperature was lowered. This, in turn, caused a current increase. By the time a current decrease, due to the semiconductive mechanism, set in, further decrease of temperature was not attainable.

Thus a meaningful activation energy plot for the particular hydration state in question was impossible. The gradual decrease in activation energy with increasing hydration, however, is illustrated to some extent in Figure 9 even though here all the states studied were presumably near the "dry" state. Nevertheless, Figure 9 does provide a graphic illustration of Equations (4.) and (5.) which were discussed in the theoretical portion of this work.

Activation energy values for egg lecithin films in the dry state were unobtainable. Egg lecithin softens at 60°C. It was felt that a temperature of 50°C was the maximum to which the sample, safely, could be subjected. At this temperature, the current increase from the room temperature value would not have allowed a sufficient number of points to be obtained for a meaningful activation energy plot.

The current increase in egg lecithin, due to iodine vapor exposure, was not completely reversible. Apparently, because of the amount of iodine adsorbed by the chamber itself, as well as by the lipid, it was not possible to completely remove all of the iodine from the system with the helium gas. Thus the sample never fully returned to the initial dry state.

It should be mentioned here that in all activation energy runs, the conductivity returned essentially to its initial room temperature value after restoration of

thermal equilibrium. An exception was the cholesterol palmitate which has already been mentioned. This return of the initial conductivity indicated that no specimen degradation had occurred, due to heating or cooling.

For any one lipid, the activation energy range, as can be seen in Table 1, is rather large. This may reflect differences in film morphology from film to film. It was difficult to consistently lay down the same amount of lipid solution for each sample, with the syringe. This film variation might also be evidenced by the fact that the current varied from film to film, often by as much as an order of magnitude.

Additionally, the lipids may be chemically altered upon atmospheric exposure and the large activation energy ranges may reflect differences in oxidation states of the lipids. The egg lecithin, for example, is known to contain unsaturated fatty acid chains and these chains are thought to undergo oxidation with only a short amount of air exposure. The egg lecithin activation energy values, however, seemed to be more reproducible from sample to sample than the values of activation energy, from sample to sample, for synthetic lecithin samples. Synthetic lecithin has only saturated fatty acid chains and, therefore, should be more resistant to oxidation.

To obtain more precise values for the conductivities and activation energies would require much more controlled

sample preparation conditions. Such conditions were not within the scope of this work at the time of sample preparation.

The values of the activation energies appeared to be independent of the rate of heating or cooling, provided the same rate was used throughout an individual run. As a matter of fact, if the rate were changed during the run, straight lines could be drawn through the points obtained with the same heating or cooling rate; although the lines would be displaced from one another, they were, nevertheless, parallel i.e., they had the same slope.

Of the three lipids studied, cholesterol palmitate exhibited the most irreproducible behavior. On a number of occasions, plots of log of the current versus the inverse temperature (for the fully hydrated case) were obtained, which were curved rather than straight lines. It is possible that this type of behavior is due to phase changes in the sample; cholesterol palmitate does display a so-called liquid crystalline phase. Similar non-linear behavior has been observed by other workers for steroids.¹²

For the synthetic lecithin exposed to iodine vapor, the results are somewhat ambiguous: In one case, the dry sample, after being exposed to iodine vapor for several days, displayed a current just readable on the electrometer, i.e., 10^{-14} a. This increase could well have arisen from moisture leakage into the chamber. In a second case, however, the

current value started at 10^{-12} a (presumably due to moisture adsorption when placing iodine in the chamber). After 5 days of iodine vapor exposure, the current rose to 10^{-10} a before the test was discontinued. It certainly appears that the iodine vapor does not act as rapidly on synthetic lecithin as on egg lecithin. This result is consistent with the spectroscopic work which will be discussed later in this thesis.

The rise in conductivity just mentioned could also have been due to moisture leakage into the chamber over the five or six day test period. The fact that the current did not exceed 10^{-14} a in the first case just mentioned was taken as evidence that the iodine, itself, did not provide an electrical leakage path between the gold electrodes on the quartz plate. To test this idea further, however, a quartz plate with no lipid film was exposed to the iodine vapor. After 1 day of exposure, the current appeared to stabilize at 10^{-11} a and remained there for the next three days. An activation energy run was then made on the plate: the current rose to 10^{-10} a at 5°C and remained constant down to -50°C . After thermal equilibrium was re-established, the current was $\sim 10^{-10}$ a where it remained for the next 4 days. At this time, it was found that the iodine had caused the gold electrodes to flake off from the nichrome underlay. This behavior just discussed seemed to indicate that with a lipid film present on the quartz plate, the iodine vapor

acting on quartz did not play a significant part in the activation energy measurements. It may well be that with a lipid film (typically 50μ thick), covering the quartz plate between the electrodes and also covering a good portion of the electrode area itself, a complete leakage path on quartz cannot be established by the iodine. For the bare quartz plate, such a path may have been established in the $\frac{1}{2}$ cm. length between the electrodes and this might have accounted for the relatively high current.

A few words should be said about polarization effects in the samples: For synthetic lecithin, at least, the current versus voltage behavior seems to be ohmic from 1 to 100 v (provided the film is laid down properly): if the lipid film does not lie smoothly over the gold electrodes or if the film touched the indium solder joints, polarization effects manifest themselves as the appearance of a current upon removal of the applied voltage. This current was reversed in direction from that observed with applied voltage present and, moreover, was of the same order of magnitude.

In one synthetic lecithin film, it was noticed that the film seemed to have a very rough appearance near the electrodes. Upon varying the applied voltage from 1 to 1000 v the value of the equilibrium current was the same for all voltages. Immediately upon raising the voltage, the current rose to a value in keeping with Ohm's Law; within minutes, however,

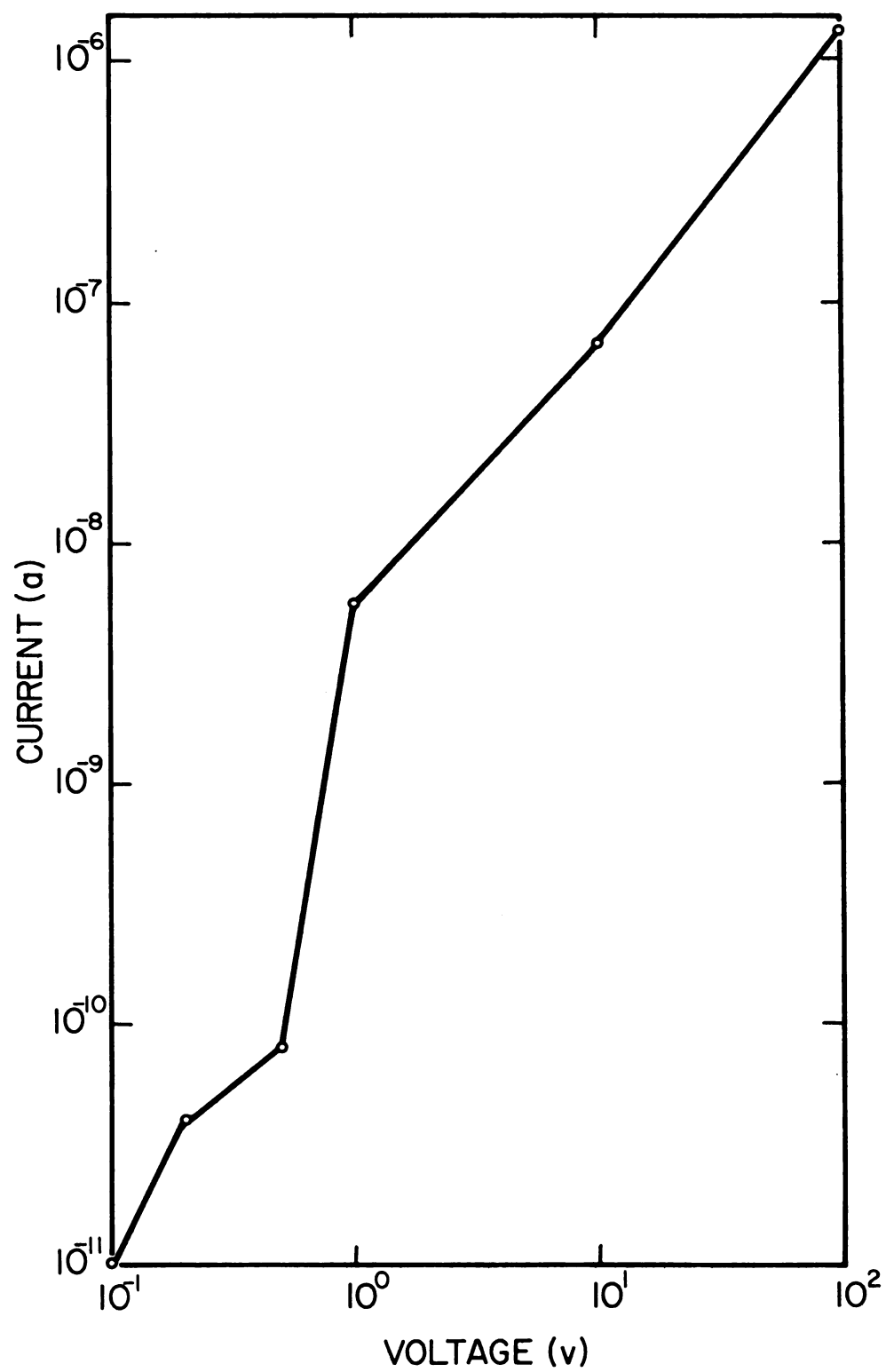
it dropped to a value of the same order of magnitude that was attained at the previous voltage. Shorting the leads together for several hours did not do more than drop the current by about an order of magnitude. This might indicate that some sort of electrochemical reaction was occurring at the electrodes. The current behavior for a synthetic lecithin film, as the voltage is varied, is shown in Figure 10. Normally, voltages of 1 to 20 v were used for all the measurements discussed in this thesis.

With iodinated samples, a decrease in current with time was occasionally noted after exposure to iodine vapor. This might be an indication of ionic current existing in the sample. No currents were observable, with iodinated samples, upon removal of the applied voltage.

Whether polarization effects might be involved in the variability of the activation energy, from sample to sample, should be considered.

As mentioned in the experimental result section of this work, the capacitance measurements of the lipids are subject to great experimental difficulties. Even with the lowest relative humidity used ($\approx 14.5\%$), the moisture adsorption by the lipid was apparently sufficient to cause the lipid to swell. This swelling, in turn, caused the lipid to flow out from between the porous metal plates; it will be remembered that the metal plates are under pressure due to the micrometer fixture. At this time no readings

FIGURE 10
Current vs Applied Voltage for Synthetic Lecithin
at 95 Percent Relative Humidity



were obtainable on the instruments. Although one might criticize the observations on the hydrated samples because of the liquid-crystalline behavior of the lipid, the same criticism is not applicable to the iodinated lipid. In this case, the samples seemed to maintain their integrity. This seems to be borne out in Figure 6 wherein removal of much of the iodine tends to return the sample to its initial condition, before iodination. If sufficient time were allowed to remove the iodine, the sample would probably have returned to its "dry" state condition.

It should be mentioned that in Figure 6, both the "fully iodinated" as well as the "less iodinated" plots display apparent discontinuities. The discontinuities occur between 1500 and 2000 c.p.s. for the fully iodinated curve and between 850 and 900 c.p.s. for the less iodinated one. The discontinuities are not actually the result of capacitance changes in the samples but rather are due to the changing of scales on the General Radio Corporation instrument used. At both the 1500 c.p.s. and 850 c.p.s. points, it was necessary, in order to obtain a null point on the bridge, to have the range selector control at a value lower than advised by the manufacturer. Although using the instrument in this manner might alter the absolute values of the capacitance readings obtained from the actual values, nevertheless the general shape of the curves would not be changed; especially, the large dispersion is still valid. The capacitance points,

whose absolute values could be questioned, are those at frequencies lower than the points of discontinuity.

The percentage moisture adsorption measurements also presented some problems. The biggest problem was that once a particular salt solution was sealed in the plexiglass chamber, rezeroing and recalibration of the electrobalance were not possible without breaking the seal of the chamber. This opening of the chamber, of course, would invalidate the measurement. It was hoped that by allowing the electrobalance to operate for several days prior to the measurement and by zeroing and calibrating the instrument just before the chamber was sealed, the amount of drift in the instrument over the next few days would be minimal. Preliminary checking did indicate that the electrobalance zero and calibration did not change significantly after the first several days of operation. An error from this drifting is nevertheless present and thus the absolute values for the moisture adsorbed are biased to some extent, especially at the low relative humidity states. The general shape of the curves of Figures 8 and 9, and specifically the saturation effect, are valid, however.

Conclusions

1. Lipids behave as electrical semiconductors in the dry, hydrated and iodinated states.

2. The activation energies for phospholipids in the dry state are exceedingly high (4.8 to 6.3 e.v.).

3. When hydrated, the electrical conductivity of the lipids increases by a factor of 10^9 or greater; concomitant with this increase is a decrease in activation energy.

4. This decrease in activation energy can be explained by using Equation (5.).

5. The dielectric constant of the lipid increases upon hydration.

6. The decrease in activation energy can be related to the dielectric constant increase by Equation (8.).

7. Conclusion 3, 4, 5 and 6 can be also applied when the dry lipid is iodinated.

8. For both hydrated and iodinated phospholipids, the activation energies are high (1.7 to 3.2 e.v.).

9. The current versus per cent moisture adsorbed, for lipids, displays a saturation effect as predicted by Equation (11.).

IV. LIPID BILAYERS

Theory

One of the central problems of modern biology is the relationship between the structure and function of biological membranes. Because of its bimolecular structure, it is quite difficult to make measurements on the membrane itself and, therefore, it would be advantageous to have a model membrane with which to work. One very promising approach in this direction has been the recent development of lipid bilayers.^{24,25,26} As it turns out, the combination of properties commonly found in natural membranes is a combination essentially unknown in any other relevant structure with the exception of these lipid bilayers.

The bilayers are formed by placing a small amount of a solution, consisting of lipid dissolved in an organic solvent, over an orifice. The orifice is surrounded on both its sides by an aqueous solution electrolytic in nature. distilled water is also used. Upon placing the lipid solution over the orifice, a rainbow-like pattern of reflected light is observed when viewing the orifice. At this time the lipid structure in the orifice is much thicker than its final value; it is also of much higher electrical resistance than when it thins down. The transition from this bulk solution condition, to that of the stable bilayer structure,

occurs quite rapidly (on the order of seconds) depending upon the experimental conditions. This process appears to be a time phase transition due to solvent evaporation and is analogous to the formation of "soap bubbles."

Upon thinning to its bilayer structure ($50\text{--}100\text{ \AA}^0$ thick), the membrane appears black by reflected light. This is due to the fact that light passes from the outside of the bilayer (i.e., the aqueous solution) to the front surface of the structure where reflection occurs; some light, however, is transmitted through the bilayer to its back surface where reflection again occurs. The light reflected from the front surface and that from the back surface are 180° out of phase; this is because in one case light is reflected from lipid back into solution whereas in the other case it is reflected from solution back into lipid. The phase reversal is due to the differences in indices of refraction for the solution and for the lipid. Destructive interference can then occur provided the difference in path length, between the two reflected rays of light, is small compared to the wavelength of light. This of course is the case when the bilayer structure forms (thickness $< 100\text{ \AA}^0$) and hence its black appearance.

Various optical and electrical measurements can now be performed on the membrane. In addition, the effect of changes in the solution surrounding the bilayer can be studied with respect to their influence on bilayer properties.

The thickness of the membranes is determined both optically and by capacitance measurements. It varies from 50 $\text{\AA}$ to 100 $\text{\AA}$ depending on experimental conditions and the nature of the lipid used. Because of these measured thickness values and the known lengths of the lipid molecules, the structure is generally thought to be bilayer with the hydrocarbon portions of the lipid interior to the structure and the polar portions exterior, exposed to the aqueous environment. The evidence indicates that the hydrocarbon interior of the bilayer is liquid-like; it appears that in order to form stable bilayers, it is necessary that the hydrocarbon portion of the lipids used be liquid-like at the temperature of the experiment. Note, for example, that egg lecithin, which contains unsaturated fatty acids, forms stable bilayers at 35°C; synthetic lecithin, on the other hand, which contains saturated fatty acids only, forms stable bilayers²⁷ only at temperatures of about 60°C. However, bilayers formed from oxidized cholesterol²⁶ have been used in the work of this thesis as well as by other workers. These bilayers are exceedingly stable and what the prerequisite of a liquid-like interior means for these bilayers is not known.

It should be kept in mind at this point that the primary evidence that the membrane structure is bilayer is a dimensional argument based on membrane thickness and lipid dimensions.

In addition to the liquid-like state of the hydrocarbon chains, another important condition for stable membrane formation appears to be the net electrostatic charge of the lipid molecules. Some workers feel that a net charge of zero may be necessary for membrane formation.²⁵

Some idea of the size of the bilayer structure can be obtained from the following: the openings upon which the membranes are formed are typically 1-2 mm in diameter; it is quite difficult to form stable membranes, by the brush technique, on larger openings. Since the bilayer is 70 ⁰ Å thick, the number of molecules present in the structure is of the order of 10^{13} . How many of these molecules are lipid molecules and how many are organic solvent molecules is not known. Also, it should be noted here that during bilayer formation, as the solvent evaporates and the structure thins down, the bulk of the lipid molecules accumulate in a torus around the opening. This torus is presumably much thicker than the bilayer and what the nature of the torus-bilayer interface is, is not known. How this interface and how the torus itself contribute to the properties of the bilayer also is not known.

A high index of refraction, $n = 1.66$, has been calculated for some bilayers i.e., egg phosphatidyl choline bilayers²⁵ (egg lecithin). Since the lipid, used to form the bilayers, has an index of refraction of > 1.5 , it appears that the molecules of the bilayer are highly oriented. It appears, moreover, because of the high index of refraction

value, that the lipid content of the membrane is high whereas the organic solvent content is low. The refractive indices of natural cellular and subcellular organelle membranes are also high, compared to water.

The D.C. electrical resistance of the membranes has been reported to have values of 10^5 to $10^8 \Omega\text{-cm}^2$. The capacitance values have been reported to be 0.1 to $0.5 \mu\text{f/cm}^2$. The bilayer dielectric constant has been found to be about 3 and is apparently constant over the frequency range of 5×10^{-3} to 10^7 c.p.s. It has been concluded that the capacitance and conductance of phospholipid bilayers are controlled almost entirely by the hydrocarbon portion of the lipids and that the polar groups make a negligible contribution.²⁸ The resistance of the bilayers has been reported as being ohmic up to about 200 mv.

The dielectric strength of the bilayers is about 3×10^5 v/cm and exceeds that of polyethylene or porcelain.

For comparison purposes: many natural membranes have been reported as having capacitance values of about $1 \mu\text{f/cm}^2$. The electrical resistance values of many, but not all, natural membranes have been reported to lie in the range 10^3 to $10^5 \Omega\text{-cm}^2$. Natural membranes display ohmic behavior up to about 200 mv. High dielectric strengths are characteristic of many natural membranes; membrane potentials of about 100 mv across $100 \text{ }^0\text{A}$ are observed.

The bilayers have a large permeability coefficient²⁵ for water, $0.16 \mu/\text{min-atm}$. Values of from 0.1 to 3.0 are

observed for natural membranes. The fact that the bilayers exhibit a large electrical resistance coupled with a relatively large water permeability coefficient is perhaps somewhat surprising. The low dielectric constant (≈ 3) and the lack of a frequency dispersion for the dielectric constant indicate that water is probably not present to any great extent in the bilayer structure.

It has generally been assumed that electrical conductivity through the bilayers occurs via an ionic (perhaps protonic) mechanism.²⁰ Experimental verification of this assumption has not been made by direct measurements such as radioactive tracer measurements. Indirect measurements do indicate that a non-electronic mechanism may be at least partly involved in bilayer conductance.^{25,29}

In this connection a very interesting point arises: if one takes the resistivity (ρ) value for fully hydrated egg lecithin ($10^7 \Omega\text{-cm}$), as obtained in the first part of this work, and inserts it into the formula $R = \rho \frac{L}{A}$ and, if L and A are given typical bilayer dimensions, one obtains an R of 10^2 to $10^3 \Omega$! Yet the measured R is usually 10^9 to $10^{10} \Omega$. This of course assumes that the lipid in the bilayer is fully hydrated, which it is apparently not. Nevertheless, this apparent discrepancy between the two sets of values is great. One possible explanation, of course, is that the work with the thin lipid films was done with gold electrodes which passed both ionic and electronic current. The liquid electrodes used with the bilayers passed ionic current only.

In order to understand what follows, it is necessary to consider an experiment performed by Kallmann and Pope:³⁰ these workers did experiments indicating that charge carriers can be injected from certain liquid electrodes into anthracene crystals. Since the conductivity of thin ($\approx 10 \mu$) single crystals of anthracene was measured by the use of liquid electrodes, the situation had a certain similarity to the conductivity measurements of lipid bilayers wherein liquid electrodes were also used. With a 1 M NaI solution as one electrode and a 1 M NaI solution saturated with iodine as the other electrode, the above workers found that if the iodine containing electrode were positive, the current was some 80 times what it was with no iodine present. It was proposed that the electrodes injected positive holes into the anthracene. When an electron acceptor like I_2 was used in solution as an electrode, formation of an activated complex between the iodine and the anthracene was postulated. The complex presumably dissociated into an anthracene molecule containing a "hole" and the negatively ionized iodide ion. It was calculated that the energy balance was favorable for such a reaction. It has been suggested by others³¹ that the reaction is due to formation of a charge-transfer complex.

Now, to evaluate the experimental work to follow, the following line of argument should be considered. (1) In view of the work done on proteins and nucleic acids, it was felt that at least part of the current in the hydrated lipid films

(Part III of this work) was electronic; measurements are presently underway to test this idea but it was adopted as a working hypothesis. (2) In view of the large difference in conductivities between films and bilayers, it was felt that perhaps the bilayers were not hydrated; dielectric work supports this. (3) Because of the Kallmann-Pope work, it was felt that iodine might increase the bilayer conductivity. (4) If this increase occurred, the same line of reasoning might be used to explain it as was used for the anthracene, i.e., a complex between the iodine and lipid. (4) If this charge-transfer complex could be shown to exist, electronic conduction would be suggested since all known charge-transfer mechanisms are electronic in nature, rather than ionic.

This line of argument now was tested experimentally.

Experimental Details

The egg lecithin used was that mentioned in Part III of this work. In addition, cholesterol (Eastman Kodak Corporation) was oxidized²⁶ and used for bilayer formation. The bilayers were formed by the "brush technique" over a circular opening in a teflon cup. The opening was approximately 1.2 mm in diameter and the cup wall thickness was thinned down to about 80 mils at the opening.

The teflon cup was then mounted in a glass chamber. A glass coil was placed in the chamber, around the teflon

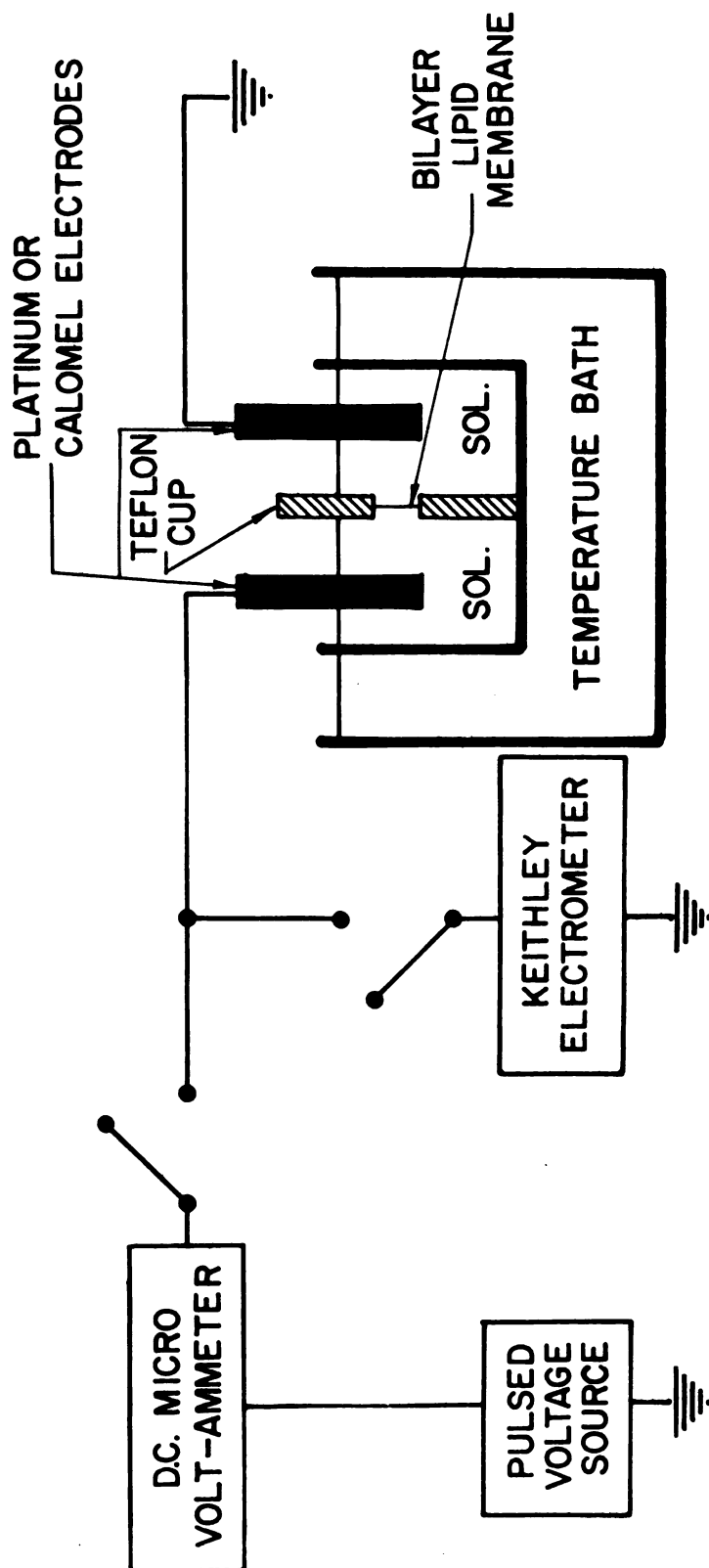
cup, and connected to a Haake type Fe circulating thermostatic bath. Solution was then poured into the glass chamber and the teflon cup. The temperature was usually measured with a mercury thermometer for the solution in the teflon cup. Oxidized cholesterol bilayers were made at room temperature whereas egg lecithin bilayers were formed at temperatures of approximately 34.5°C.

The electrodes used were either calomel or platinum. Polarization effects with the platinum electrodes only become evident when the current exceeds 10^{-7} a. The experimental arrangement for the conductivity measurements is shown in Figure 11.

The solutions used for bilayer formation were egg lecithin in normal decane (1% weight: volume) and oxidized cholesterol solution (octane) as described by Tien.²⁶ All reagents were practical grade. A number 2 sable hair brush was dipped into the solution and a drop of the solution was brushed over the opening in the teflon cup. The opening was observed with a Bausch & Lomb Stereo Zoom microscope at a magnification of 30X. The light source was a Unitron microscope illuminator.

Immediately upon placing the drop of lipid solution over the opening, a colored pattern could be observed with the microscope. At this time, with 50 mv from the pulsed voltage source, applied to the membrane, no current could be read on the Micro Volt-ammeter (Hewlett Packard 425). The colored pattern undergoes various changes until finally

FIGURE 11
Set-up for Measuring Electrical Conductivity Parameters of Lipid Bilayers



a small black spot could be observed (usually at the bottom of the pattern) which then rapidly spread until the greater part of the opening became black. Actually, at this time, there was a slight greyish appearance to the bilayer due to a small amount of reflected light. The current could then be read and since the voltage applied to the bilayer was known, the bilayer resistance could be determined.

The combination of electrodes plus solution usually displayed a resistance of 10^4 to $5 \times 10^4 \Omega$ when electrolytes were used; for double-distilled water, the resistance was 10^6 to $10^7 \Omega$.

Bilayers made from oxidized cholesterol formed quite rapidly (1-2 min.) and were very stable. Egg lecithin bilayers also formed rapidly, however, they tended to break, before thinning down to the bilayer structure, much more often than the oxidized cholesterol structures. The area of the opening in the teflon cup, occupied by the lipid bilayer, was often much smaller than the actual area of the opening, for egg lecithin bilayers. Indeed, it was quite difficult to obtain bilayers occupying more than 50% of the opening area.

The iodine solution used in this work was a saturated (room temperature) solution of iodine crystals (re-sublimed) in double-distilled water.

The cleanliness of the apparatus was of paramount importance. If proper precautions were not taken, bilayers would not form or, if they did form, were very unstable.

The cleaning procedure was to soak the entire assembly (glass chamber, teflon cup and glass coil) in a water solution of Haemo-Sol. The components were then thoroughly rinsed in distilled water. The teflon cup was then usually placed in ethanol and then rinsed again with distilled water. If stable bilayers would not form during the course of an experiment (as often happened) the entire procedure would be repeated.

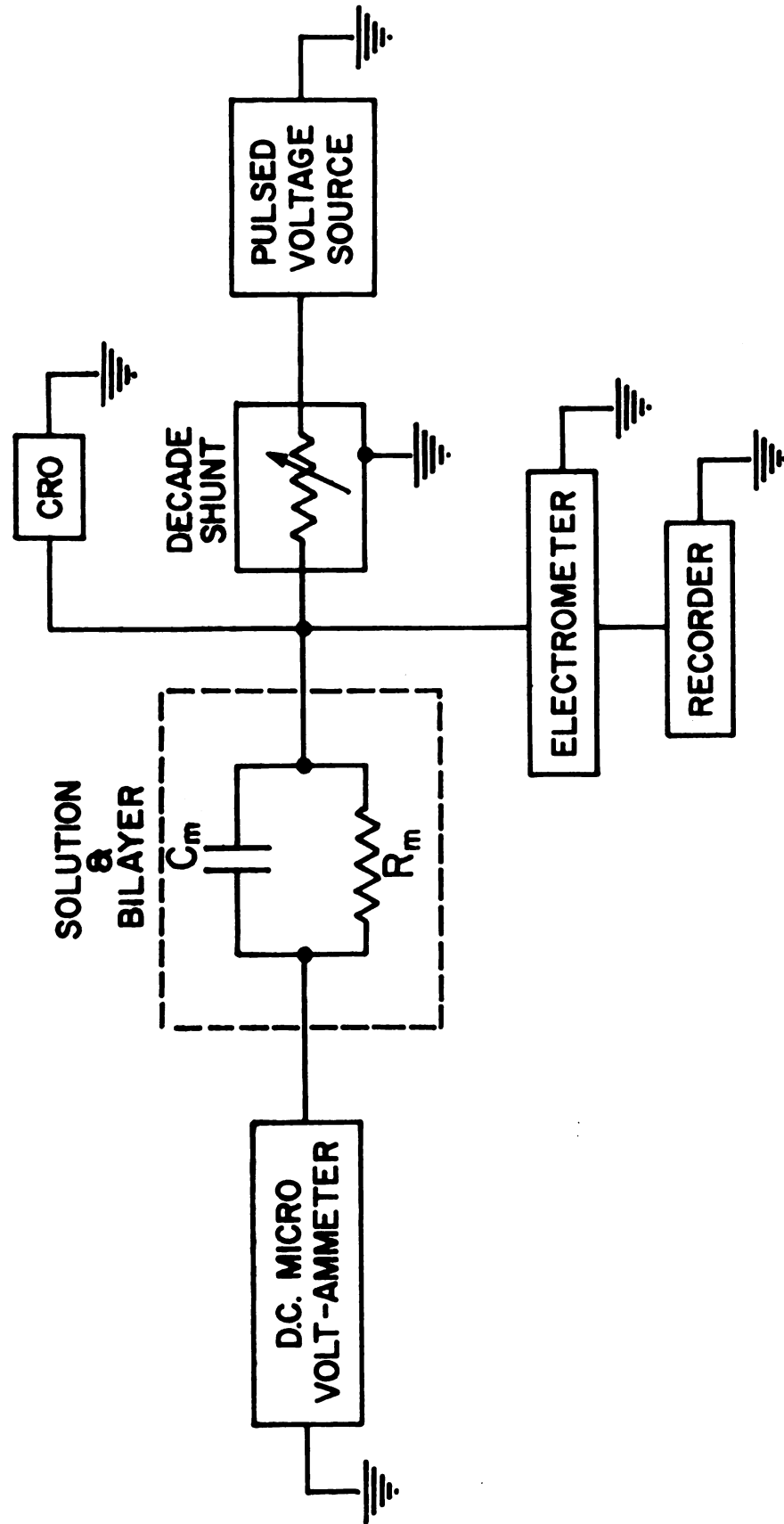
The brushes had to be properly trimmed in order to get stable bilayers. During the course of the work, they had to be periodically soaked in ethanol in order to remove excess lipid which accumulated on the hairs.

The bilayer containing chamber was not electrically shielded, therefore, during the electrical measurements, the experimenter had to remain relatively quiet so as to prevent capacitive pick-up.

For the bilayer capacitance measurements, the circuit shown in Figure 12 was used. A Bausch & Lomb VOM 5 recorder was used to obtain the capacitance values, for bilayers having resistances in the $10^9 \Omega$ - $10^{10} \Omega$ range. When the iodinated bilayers were studied, the bilayer resistance was too low to give a reasonable decay time measurement on the VOM 5 and, therefore, the oscilloscope (Tektronix 502 A) was used.

The bilayers were charged by the application of a voltage from the pulsed voltage source. The decay time for discharge of the bilayer was then measured from the known recorder chart speed or from the oscilloscope with a known

FIGURE 12
Set-up for Measuring Bilayer Capacitance



sweep speed. With the scope, a Polaroid camera was used to obtain a record of the voltage decay. From the decay time value, the known membrane resistance R_m and the resistance in the decade shunt box, the membrane capacitance, C_m , could be determined.

Experimental Results

The experimental results for the lipid bilayer conductivity measurements are summarized in Table 4. In all cases studied, the addition of iodine to the solution surrounding the bilayer lowers the electrical resistance. The effect occurs no matter whether the iodine is present inside the teflon cup, only, outside the cup, only, or both inside and outside the cup. Moreover, if the bilayer is formed with iodine already present in the solution in the cup and glass chamber, its electrical resistance is lowered from what it would be were it formed in the solution without the presence of iodine. The effect is a function of iodine concentration but no quantitative relationship was established; indeed, the amount of iodine necessary to lower the bilayer resistance a given amount seemed to vary among membranes of the same lipid.

The results shown in Table 4 are not meant to be precisely quantitative, however, if sufficient iodine were present in the solution surrounding the bilayer, the bilayer resistance decreased to about 10^5 - $10^6 \Omega$, in all cases studied.

Table 4. Electrical resistance of lipid bilayers

Substance	Resistance			
	In H ₂ O	In NaCl-H ₂ O sol. (0.1N)	In NaI-H ₂ O sol. (0.1N)	In I ₂ -H ₂ O sol. (saturated)
Egg Lecithin	10^9 - $10^{10} \Omega$	$10^9 \Omega$	10^6 - $10^7 \Omega$	$10^6 \Omega$
Oxodized Cholesterol	$10^9 \Omega^*$	$10^9 \Omega$	$10^7 \Omega$	$10^6 \Omega^*$

*Same values for platinum or calomel electrodes.

†One part saturated iodine solution: 4 parts 0.1N NaI solution.

**Solution formed by making saturated iodine solution 0.1N with respect to NaI.

Limiting resistance for 0.1 N NaI sol. : $10^3 \Omega$

Limiting resistance for distilled H₂O : $10^5 \Omega$

The effect was symmetrical with respect to the polarity of the applied voltage. This symmetry existed even if the iodine were present on only one side of the bilayer, as well as when it was present on both sides. The lowered resistance values indicated in Table 4 are not all to be taken as minimum values; in the case of distilled water surrounding the bilayer, for example, the resistance of the water itself may be higher than the resulting bilayer resistance thus setting a lower limit to the measurable bilayer resistance.

Note that membranes formed in pure water or in 0.1 N NaCl solution all had high resistance values, 10^9 - $10^{10} \Omega$. With membranes formed in 0.1 N NaI, however, the resistance values were lowered considerably. This is consistent with the results of other workers^{29,32} who find a lowering of resistance by about 2 to 3 orders of magnitude, for egg lecithin bilayers, when Cl^- is replaced by I^- in the solution surrounding the bilayers. This process was found to be reversible by these workers; reversibility was not studied in the work of this thesis.

Since iodine in water solutions display the presence of I_3^- , which in turn suggests the presence of I^- , the question becomes whether iodine is lowering the bilayer resistance, or whether I^- is responsible for the lowering. Still another possibility is that both substances lower the resistance but, perhaps, by different mechanisms.

Note the following, however: the addition of iodine to the NaI solution surrounding the bilayer always lowered the electrical resistance of the bilayer, no matter what its value before the iodine addition. No bilayer values less than $10^5 \Omega$, however, were observed. Since the number of I^- ions added to the solution by the addition of iodine is small ($< 10^{-5}$ M) compared to the number already present in a 0.1 N NaI solution, it appears that the iodine effect is independent of any I^- effect.

An additional experiment was done to test this hypothesis: Iodine is present in saturated water solution in a concentration of 10^{-4} M; the amount present in the solution surrounding the bilayer was often 10^{-5} M. At these concentrations the resistance was lowered by 3 to 5 orders of magnitude. If I^- were present in these cases, it would be present in concentrations no greater than 10^{-5} M and probably much less.

Now, if the bilayers are formed in 10^{-4} M NaI solution, the resistance is decreased, at most, by 1.0 to 1.5 orders of magnitude from the resistance in pure water. This experiment indicates that the iodine is much more effective in lowering the bilayer resistance than is I^- . It suggests, moreover, that traces of iodine present in NaI solution, rather than the I^- ions, may actually be responsible for the lowering of the bilayer resistance. Spectroscopic evidence, obtained with oxidized cholesterol, seems to support this idea, as will be discussed presently.

Note also that both oxidized cholesterol and egg lecithin bilayers, formed in 0.1 N NaCl solutions, exhibited high resistance values, $\approx 10^9 \Omega$. The presence of iodine, in the solution, however, lowered the resistance values to $10^6 \Omega$ or less. Thus the iodine effect occurs even in the presence of a different halide.

The effect of pH on egg lecithin bilayers is shown in Table 5.

Table 5. Effect of pH on bilayer resistance

pH	Resistance (Ω)
5.0 (H_2O)	10^{10}
5.0 (Iodine in H_2O , sat.)	10^6
7.3 (0.02 N Histidine in H_2O)	10^9
8.0 (0.1N $NaHCO_3$ in H_2O)	10^8
8.2 (1N $NaHCO_3$ in sat. iodine sol.)	10^6

Since the resistance appears to decrease as the pH increases, it is possible that protonic conduction through the bilayer is not the dominant charge carrying mechanism. Since the iodine effect takes place at pH values of 5.0 and 8.2, it appears that the lowering of the bilayer resistance by iodine is pH independent; moreover, it is

indicated that the lowering of the bilayer resistance by iodine does not involve a predominantly protonic mechanism.

The results for the capacitance measurements of 24 egg lecithin bilayers are shown in Table 6. The average capacitance per unit area, for the bilayers, is $0.26 \mu\text{f}/\text{cm}^2$. This value is significantly lower than the values of 0.33 to $0.38 \mu\text{f}/\text{cm}^2$ obtained by other investigators.^{29,33} Note, however, that these other workers made their capacitance measurements for bilayers surrounded by KCl solution. This may account for some of the difference between their results and the work of this thesis. Note the apparent lack of agreement between the estimated bilayer area and the total resistance measured as well as the capacitance. A typical recorder trace of the voltage decay curve of a bilayer is shown in Figure 13. The standard deviation for the data is $0.089 \mu\text{f}/\text{cm}^2$.

If the bilayer behavior follows the model outlined in the semiconductive theory portion of this report, the presence of iodine should increase the dielectric constant and therefore the capacitance of the bilayer. If one uses Equation 11. and solves it so as to find the change in κ necessary to account for the observed increase of 3 orders of magnitude in the bilayer conductivity, an increase in κ of about 0.3 is necessary for the observed effect. If the bilayer is assumed to have a κ (with no iodine present) of 2, this increase in dielectric constant represents a

Table 6. Capacitance of egg lecithin bilayers

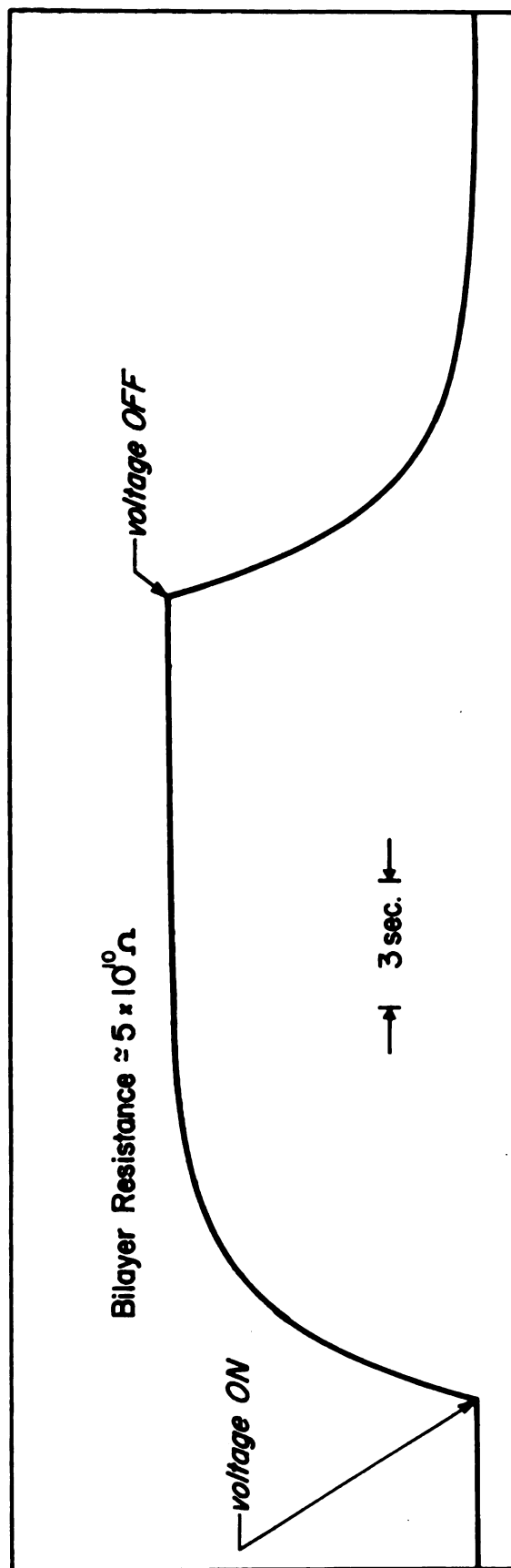
Membrane No.	R_m ($10^{10} \Omega$)	Estimated Bi-layer Area (% of hole area)	Measured Capacitance ($10^{-9}f$)	Capacitance per Unit Area ($\mu f/cm^2$)
1	2.4	10	0.82	0.51
2 (initial)	1.2	75	1.6	0.14
2 (after 15 min.)	1.6	75	2.7	0.25
3	1.2	25	1.6	0.44
4	0.44	85	2.1	0.17
5	1.6	70	2.2	0.22
6	1.3	33	1.7	0.34
7	1.3	65	2.5	0.26
8	1.6	75	2.5	0.23
9	0.46	75	2.8	0.35
10	2.4	75	2.3	0.21
11	1.6	33	1.7	0.36
12	1.4	33	1.4	0.29
13	1.3	75	1.9	0.17
14	1.4	60	2.0	0.23
15	2.4	33	1.6	0.32
16	0.57	75	2.3	0.21
17	2.4	50	2.1	0.28
18	2.4	80	2.6	0.22
19	4.9	75	2.1	0.19
20	2.4	85	2.6	0.20
21	2.4	60	1.8	0.20
22	1.0	85	2.8	0.23
23	4.9	33	1.6	0.30
24	4.9	50	1.8	0.25

Average C = $0.26 \mu f/cm^2$

Range of C/area = $0.37 \mu f/cm^2$

Standard Deviation = $0.089 \mu f/cm^2$

FIGURE 13
Voltage Decay Across Egg Lecithin Bilayer

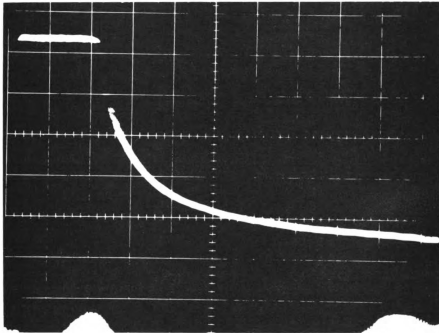


capacitance change of about 15%. No difference between the decay time of a bilayer (without iodine present) and one with iodine present could be detected. A good illustration of this is shown in Figure 14. This photograph shows two oscilloscope traces, superimposed on one another. One trace is for a bilayer (egg lecithin) having a resistance of $10^9 \Omega$ in 0.1 N NaCl; the second trace is the decay curve for the same membrane after iodine was added to the NaCl solution and the membrane resistance was $10^8 \Omega$. No difference in decay time can be discerned except for a thickening of the trace due to the superposition of the two traces. Figure 15 displays two separate voltage decay traces. The one trace is for a bilayer of $10^9 \Omega$ in NaCl; the other is for the same bilayer after the addition of iodine. The resistance had now dropped to $10^7 \Omega$. Again no difference in decay times can be detected. After a number of such attempts at measuring capacitance changes, it was concluded that any change in bilayer capacitance, upon addition of iodine to the surrounding solution, was not detectable by the experimental set-up. The expected increase in dielectric constant upon iodine addition requires a more sensitive capacitance measurement than could be obtained by a decay time measurement.

Discussion of Experimental Results

The observation that iodine lowers the bilayer resistance to about the same extent whether NaI is present or not is of some interest. The presence of I^- ions in water

FIGURE 14
Voltage Decay Across Egg Lecithin Bilayer
Before and After Iodine Addition

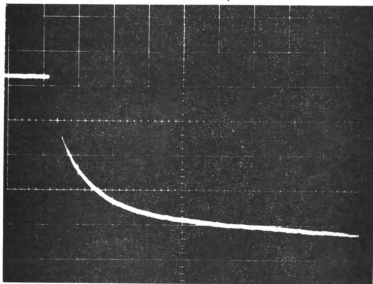


$R_m = 1 \times 10^9 \Omega$ before iodine addition

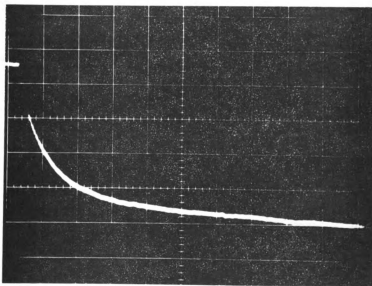
$R_m = 1 \times 10^8 \Omega$ after iodine addition

1cm = 0.1 msec

FIGURE 15
Voltage Decay Across Egg Lecithin Bilayer
Before and After Iodine Addition



Without Iodine : $R_m = 5 \times 10^9 \Omega$ 1 cm = 0.1 msec



With Iodine : $R_m = 5 \times 10^7 \Omega$ 1 cm = 0.1 msec

is known to increase the solubility of iodine due to I_3^- formation. Thus, the presence of NaI in the concentration used should increase the number of I_3^- ions present in the iodine solution (this was shown spectroscopically). Since the resistance lowering does not seem to be much effected, this is indicative of the I_3^- ions themselves not being directly involved in the increased conductivity of the bilayers.

The amounts of iodine, present in the solution, necessary to produce a given bilayer resistance, as shown in Table 4, are not to be taken as minimal; the values chosen were convenient values at the time of experimentation. Amounts of iodine much less than those indicated in the table were, at times, observed to produce the same resistance lowering. Since these amounts did not always produce the maximum lowering, however, the values of iodine concentration as indicated in Table 4 were chosen since these values always produced the lowering indicated in Table 4. As mentioned, bilayer resistances of less than $10^5 \Omega$ were not observed.

The capacitance measurements should be much more reproducible than the conductivity measurements. The capacitance of the bilayers, was found by other workers, to be directly proportional to bilayer area; such was not the case for the resistance where often smaller bilayer areas produced smaller measured resistance values of the bilayer than

did larger areas. A direct relationship between bilayer capacitance and bilayer area was not found as can be seen from Table 6. At the time of these capacitance measurements, however, a calibrated eyepiece was not available for the microscope; the area estimation of the bilayers was, therefore, quite subjective and probably subject to large errors. Later studies with a calibrated eyepiece indicated that the areas as estimated in Table 6 are probably larger than the actual areas. This would tend to make the capacitance per unit area values, shown in the table, larger than indicated. On the other hand, for the smaller bilayers at least, the capacitance of the lipid torus became a significant part of the measured capacitance. This can be seen when it is realized that the capacitance of the lipid-torus structure is probably less than 15% of the capacitance of the bilayer, on a per unit area basis; for small bilayers, however, the torus area was often greater than 90% of the whole area. If the measurements of the smallest bilayers were corrected for this, their capacitance values per unit area were much closer to the values of the larger membranes. A strong effort was made to make measurements on bilayers occupying more than 50% of the hole area; for every such stable bilayer obtained, however, perhaps as many as ten unsatisfactory bilayers would be obtained.

It thus appears that the capacitance values as shown in Table 4 are lower than the actual values because

of the overestimation of bilayer area. The fact that the capacitance of the lipid non-bilayer structure was not taken account of would tend somewhat to compensate for this error, but would probably not do so significantly except for the smallest bilayers.

For all the bilayers having resistance values of 10^9 - $10^{10} \Omega$, a $10^9 \Omega$ resistor was used in the decade shunt box. The decay time was measured from the recorder trace using a chart speed of 20 in/min. The voltage obtained from the pulsed voltage source was fixed at 50 mv. The circuit was checked by replacing the glass chamber assembly by a parallel combination of a 10^{-9} farad capacitor with a $10^{10} \Omega$ resistor. With this set-up, the measured decay time was in error from the calculated value by about 25%. This error arose presumably because of the response time of the recorder as well as because of the capacitance of the leads and circuit components. The recorder response time prevented measurement of the capacitance of the chamber assembly with no bilayer present.

The capacitance value of the non-bilayer lipid structure is obtained as follows: The hole is covered with a lipid solution in the usual manner; every now and then, such a structure does not thin down to the bilayer form nor does it break. Its resistance is beyond the sensitivity of the instruments ($> 10^{12} \Omega$) and it is referred to as a "glob." Its capacitance per unit area is about 15% or less that of the bilayer of the lipid, for the same area.

If the recorder is replaced by an oscilloscope (at the output of the electrometer), in order to measure fast decay times, the A.C. noise is too large to make such a measurement practical. The slow response time of the recorder filters out such noise, whereas the oscilloscope, because of its wide band-pass, allows the noise to pass and obscure the signal. Thus the CRO was placed as shown in Figure 12.

Since the input resistance of the CRO was $\approx 10^7 \Omega$, a $10^5 \Omega$ resistor was used in the decade shunt box. If distilled water were used for surrounding the bilayer, no difference in decay times could be detected for the system with or without a bilayer! Yet such differences were readily detectable with the recorder and a $10^9 \Omega$ resistor in the shunt box. If a model system, was used, consisting of a 10^{-9} f capacitor in parallel with a $10^{10} \Omega$ resistor, the measured decay time was only 10% higher than the calculated decay time. It was then decided to substitute 0.1N NaCl solution for the distilled water. The impedance of this system was normally $< 50,000 \Omega$ yet its decay time was the same as for the system with distilled water present. Now, however, if bilayers were formed, an increased decay time was apparent. This indicated that the Cl^- ions perhaps increased the bilayer decay time. The reasons for the above behavior are not clear. In any event, the bilayer capacitance with and without the presence

of iodine could now be measured, as shown in Figures 14 and 15. No difference could be detected.

Very recent work³⁴ by others, however, indicates that the capacitance (and therefore presumably the dielectric constant) of egg lecithin bilayers does increase upon exposure to iodine. For bilayers ($R_m \approx 10^7 \Omega\text{-cm}^2$) formed in a solution of 0.5 M Na_2SO_4 , a capacitance of $0.33 \frac{\mu\text{f}}{\text{cm}^2}$ was found; with 0.89 mM of I_2 and 0.59 mM of I^- present in the solution surrounding the bilayer, the capacitance rose to $0.51 \frac{\mu\text{f}}{\text{cm}^2}$. With 1.3 mM of I_2 present in the solution of Na_2SO_4 , but no I^- present, the R_m was $= 10^2\text{-}10^3 \Omega$ the value predicted, for the egg lecithin bilayer (exposed to iodine), on page 23. The measurement of this low value was obtained by the utilization of an A.C. method; in addition the solution (surrounding the bilayer) impedance was lowered below that for the solution, used by the writer of this thesis, through the addition of Na_2SO_4 . The authors,³⁴ moreover, conclude that the electrical properties of lipid bilayers, in I^- solutions, depend on the presence of minute amounts of I_2 .

Conclusions

1. Iodine, present in the solution surrounding lipid bilayers, lowers the bilayer resistance by 3 to 5 orders of magnitude. This resistance lowering parallels that exhibited by anthracene.³⁰

2. Recent work³⁴ indicates that, with the proper experimental conditions, the resistance of an egg lecithin bilayer is lowered, due to iodine, to a value of an order of magnitude of $10^2 \Omega$; this is the value predicted for the bilayer if the conductivity value of a fully iodinated egg lecithin film is used, as shown on page 23.

3. NaI also lowers the bilayer resistance, however, iodine produces the same magnitude of lowering at a much lower concentration than does NaI; this suggests that the two substances may act via different mechanisms or, alternatively, that traces of iodine may be present in the NaI. Recent work,³⁴ indicates that traces of iodine may account for the resistance lowering.

4. An increase in dielectric constant of the bilayer, due to the presence of iodine, was not observed. The expected increase, however, was probably below the level of experimental sensitivity.

5. Recent work,³⁴ by other workers, verifies an increase in bilayer capacitance of the order of magnitude expected for an increase in dielectric constant, as calculated on page 71, for bilayers exposed to iodine.

V. SPECTROSCOPIC STUDIES

Theory

As mentioned in Parts III and IV of this work, the nature of the interaction between iodine and lipid, both in the solid state films and in the bilayers, is of primary importance. It was hypothesized, early in the course of this work, that a charge-transfer type interaction might be involved; this provided the motivation for Part V of this work.

Charge-transfer reactions, producing donor-acceptor complexes, have been the subject of much interest in many fields, including modern biology.³⁵ The complex arises from a weak interaction of certain organic substances, functioning as electron donors, with other substances which act as electron acceptors. In many cases, these complexes are so unstable that they cannot be isolated in the pure state, at ordinary temperatures; they exist only in solutions in equilibrium with their components. The bonding forces are usually much weaker than those involved in covalent bonding. The degree to which electron transfer, from the donor to the acceptor component, takes place,³⁶ is much less than ordinarily occurs when chemical compounds are formed.

The complexes do display differences in their physical properties from those of the pure components; the absorption spectra is one of the most studied of these properties.

The substances which serve as donors are often put into two classes: One group includes the π donors; the electrons available for sharing are those contained in the π molecular orbitals and the resulting complex is called a π complex. The acceptor is considered not to make a deep penetration into the π orbitals. The second category consists of substances in which there are nonbonded electrons (lone pairs) available for coordination; these form n-complexes.

The acceptor and donor substances include a wide variety of materials, both organic and inorganic.

The description of the donor-acceptor type molecular complex, which is now generally accepted, is that presented by Mulliken.³⁶ For a 1:1 donor-acceptor complex in the ground state, the complex is described in terms of the ground state wave function ψ_N

$$\psi_N \cong a \psi_0 (D,A) + b \psi_1 (D^+-A^-)$$

where D represents the donor and A the acceptor. $\psi_0 (D,A)$ is the wave function of the state where there is no donor-acceptor interaction.

$\psi_1 (D^+-A^-)$ is the wave function for the state where charge is transferred from D to A.

If $a^2 \gg b^2$, the molecular complex is regarded as a resonance hybrid receiving a major contribution from a

no-bond form and a minor contribution from a dative form in which an electron has been transferred from the donor to the acceptor. The covalent bond of D^+-A^- is sometimes described as an intermolecular semi-polar bond³⁷ although it need not necessarily be interatomic in nature.

The complex in the excited state E is described by

$$\psi_E \approx a^* \psi_1 (D^+-A^-) - b^* \psi_0 (D,A)$$

where $a^* \approx a$, $b^* \approx b$ and $a^2 \gg b^2$. The excited state is largely dative in character and often can be obtained by the absorption of either visible or ultraviolet light. The transition $N \rightarrow E$, which accompanies the absorption of light of appropriate wave length, corresponds to the transfer of an electron from the donor to the acceptor. The corresponding spectrum of the complex, which is considered to be characteristic of the complex as a whole, is called an intermolecular charge-transfer spectrum. The charge-transfer absorption bands of donor-acceptor complexes frequently appear in the visible rather than the ultraviolet region of the spectrum. This band may often be one not found in the spectra of either of the components of the complex.

The charge-transfer transition energy should be a function, both of the ionization potential of the donor I_p , and the electron affinity of the acceptor, E_A . The frequency ν_{CT} of the charge-transfer absorption maximum has been proposed to obey the relationship

$$h\nu_{CT} = I_p - E_A - W$$

where h is Plank's constant and W is the dissociation energy of the charge-transfer excited state. As a result of a more detailed analysis, it is felt that the actual expression may be non-linear and that the above mentioned expression may represent but a segment of the non-linear curve.

For the work of this thesis the spectrophotometric determination of the equilibrium constant K and the extinction coefficient ϵ_{DA} is of importance. The determination is usually made at the maximum of the charge-transfer (CT) band of the donor-acceptor complex (DA) in solution. Then,

$$D + A \rightleftharpoons DA \text{ and } K \equiv \frac{[DA]}{[D][A]} \quad (12.)$$

where $[D]$, $[A]$ and $[DA]$ are the concentrations of donor, acceptor and complex, respectively.

The usual procedure is to measure the optical density d , at the complex absorption maximum, for a series of solutions of varying donor concentration. The donor is present in large excess of the acceptor in all solutions. If only the complex absorbs significantly at the wave length of the measurement, the optical density is related to the concentration of complex and the cell path length ℓ by,

$$d = \epsilon_{DA} \ell [DA] \quad (13.)$$

If $[D]_i$ and $[A]_i$ refer to initial concentrations, before complexing occurs, Equation 12. may be rewritten as,

$$K = [DA] / [D]_i \{ [A]_i - [DA] \} \quad (14.)$$

since $[D]_i \gg [A]_i$

Then,

$$\frac{[A]_i \ell}{d} = \frac{1}{\epsilon_{DA}} + \frac{1}{K\epsilon_{DA}} \left\{ \frac{1}{[D]_i} \right\} \quad (15.)$$

Plots of the values of $\frac{[A]_i \ell}{d}$ versus $\frac{1}{[D]_i}$, for solutions of 1:1 complexes, normally give straight lines; from the slopes and intercepts of these lines, K and ϵ_{DA} are determined.

At a given wavelength, the optical density of a solution containing donor, acceptor and a 1:1 complex is defined by

$$d = \log \frac{I_0}{I} = \epsilon_D [D] \ell + \epsilon_A [A] \ell + \epsilon_{DA} [DA] \ell \quad (16.)$$

where I_0 and I are the intensities of the incident and emergent light.

In many cases, only a fraction of the donor or acceptor is complexed, even in the presence of a large excess of the other component. In such cases, the K and ϵ_{DA} of the complex are evaluated simultaneously, as mentioned above. A somewhat more generally useful adaptation of this procedure, however is that due to Ketelaar;³⁸ it is used in the work of this thesis. In practice, the spectroscopic measurements are usually restricted to solutions in which the donor is present in large excess of the acceptor; customarily, an acceptor-free solution which contains the donor at the same concentration as in the solution of the complex, is used as a blank. Under these conditions, the measured optical density d' is given by

$$d' = d - \epsilon_D \ell \Sigma D = [A] \epsilon_A \ell + [DA] \epsilon_{DA} \ell \quad (17.)$$

where $\Sigma D \gg \Sigma A$ and $\Sigma D - [DA] \approx \Sigma D$

The apparent extinction coefficient of the acceptor in the donor solution is defined as

$$\epsilon_a = \frac{d'}{\ell \Sigma A} \quad (18.)$$

From Equations 17. and 18.

$$\frac{1}{\epsilon_a - \epsilon_A} = \frac{1}{K[D](\epsilon_{DA} - \epsilon_A)} + \frac{1}{\epsilon_{DA} - \epsilon_A} \quad (19.)$$

the Ketelaar Equation where

$$\Sigma D = [D] + [DA] \text{ and } \Sigma A = [A] + [DA].$$

When this equation is applicable, a straight line is obtained by plotting values of $\frac{1}{\epsilon_a - \epsilon_A}$ versus $\frac{1}{[D]}$. The extinction coefficient of the complex is estimated from the intercept of this line and K is calculated from the slope and intercept.

Since the formation of donor-acceptor complexes is accompanied by electron transfer, from donor to acceptor, changes may occur in the electrical and magnetic properties of the components when they are complexed. Iodine is subject to electronic polarization when it interacts with non-polar or low polarity solvents.³⁹ In other cases, when one or both of the components is polar, the dipole moment of the complex may be larger or smaller than the sum of the moments of the components. When both components are non-polar, the observed dipole moment apparently results

entirely from the dative form (D^+-A^-) which contributes to the structure of the ground state; the nonbonded form has no moment. An increase in dipole moment, upon complexing of lipid and iodine, would according to the model outlined in Part III of this thesis, provide the mechanism for the increase in conductivity of the lipid films upon iodine exposure; the same complexing might be responsible for the increased conductivity observed in the lipid bilayers, perhaps again due to the mechanism outlined in the model of Part III. The detection of a charge-transfer complex between lipid and iodine and the concomitant increase in dielectric constant of the lipid, due to this complexing, are thus seen to be key points in the logic of this thesis.

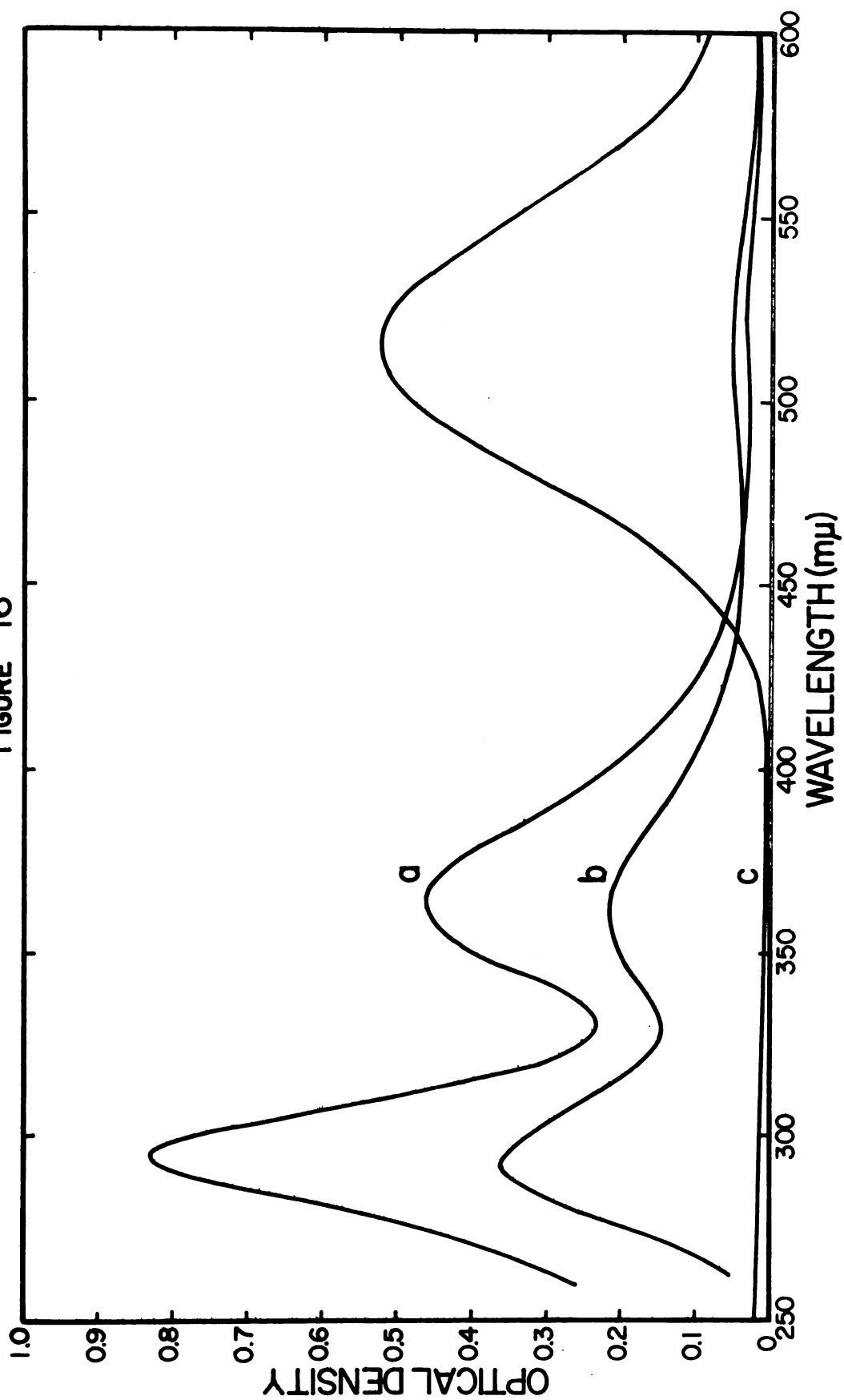
Experimental Details and Results

All measurements were taken with a Cary model 15 recording spectrophotometer. The lipids used were those mentioned in other sections of this work. Spectro grade carbon tetrachloride was used.

Figure 16 displays the following spectra: (a) the complex of egg lecithin with iodine; (b) the complex of synthetic lecithin with iodine; and (c) iodine in carbon tetrachloride. The lecithins themselves exhibit essentially no absorption above $250\text{ m}\mu$. The iodine exhibits the expected $520\text{ m}\mu$ peak. When iodine in CCl_4 was added to a solution of a lecithin in CCl_4 , the violet color of the iodine in CCl_4 changed to a brownish color; this was

Figure 16. Absorption spectra of lecithin and iodine in carbon tetrachloride. (a) Egg lecithin 1.18×10^{-4} M. (b) Synthetic lecithin 6.21×10^{-3} M. (c) Iodine 5.63×10^{-5} M. (Iodine concentration same in a, b, and c.)

FIGURE 16



suggestive of a charge-transfer interaction. For both lecithins two new absorption bands appeared in the regions of 366 $m\mu$ and 294 $m\mu$; concomitant with this appearance, the iodine band at 520 $m\mu$ decreased in intensity. The concentrations used were as indicated in the legend of Figure 16 and a carbon tetrachloride solution was used as a blank.

By varying the concentrations of iodine and lecithin, it appeared that the lipid and iodine complexed in a ratio of 2:1; this was true for both lecithins. The determination was made by the method of continuous variation.⁴⁰

Note especially that the optical density values indicate that the equilibrium constant for the egg lecithin interaction is greater than for the synthetic lecithin interaction with iodine. It was necessary to use a much smaller concentration of egg lecithin than of synthetic lecithin, for the same iodine concentrations, so as to display both absorption spectra on the same scale.

Since the lipid bilayers are approximately 70⁰Å thick, it was not feasible to study spectroscopically their interaction with iodine. As an approximation to the bilayer interaction with iodine, dispersed egg lecithin in water was chosen. As mentioned in Part II of this work, egg lecithin dispersed in water has a micellar structure which, in terms of thickness and arrangement of the polar and non-polar parts of the lipids, approximates the bilayer structure. It was felt that the iodine interaction with these

micelles would not be greatly different from its interaction with bilayers.

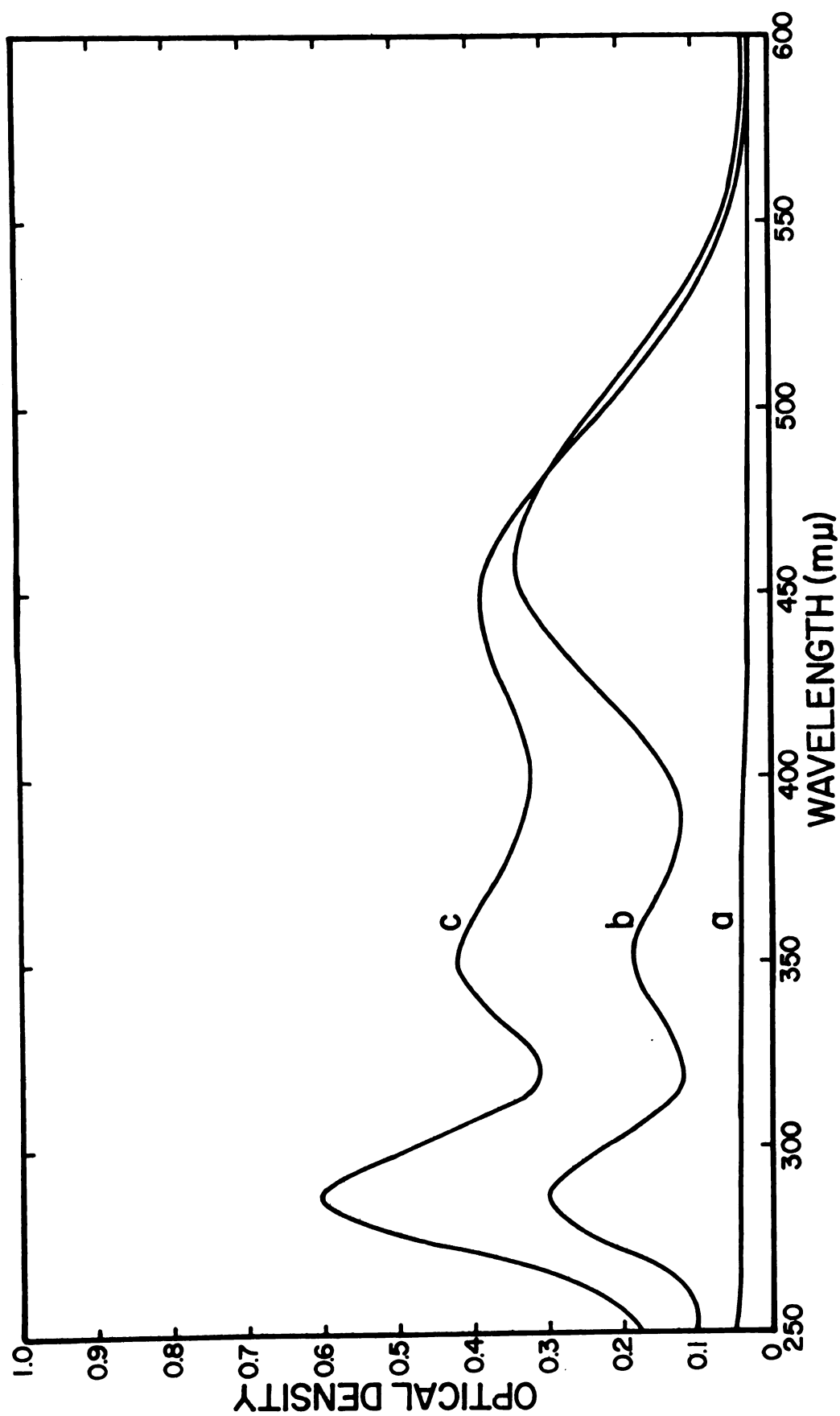
A saturated iodine solution was formed as mentioned in Part IV. An egg lecithin dispersion was formed by adding 0.013 gm egg lecithin to 100 ml of double distilled water and shaking vigorously for several minutes. The dispersion was then allowed to settle for one or two hours. The resulting liquid was quite clear.

The pertinent spectra are shown in Figure 17. A saturated iodine solution, diluted 2:1, displays absorption peaks at 351 $m\mu$ and 287 $m\mu$ as well as the solvated molecular iodine band ($\lambda_{MAX} = 460 m\mu$). Egg lecithin in water (dispersed as mentioned) exhibits essentially no absorption between 250 and 600 $m\mu$. If now one part of the clear lecithin dispersion and one part of the diluted iodine solution were mixed, the spectrum shown in Curve C, Figure 17, resulted. Note the decrease in intensity of the molecular iodine band, relative to the 351 and 287 $m\mu$ bands which increased in intensity. Since the concentration of egg lecithin in water was not accurately known (after settling), a comparison, in terms of equilibrium constants, could not be made between the iodine-lipid interaction in water and in CCl_4 . It does appear, however, that the K for the lipid-iodine interaction in CCl_4 is greater than the K for the same interaction in water, as seen from the optical densities.

The spectra of thin films of egg lecithin were also studied. The films were formed by evaporating the chloroform

Figure 17. Absorption spectrum of egg lecithin and iodine in water. (a) Egg lecithin dispersed in water 1.53×10^{-5} M. (b) Iodine in water approximately 4×10^{-4} M. (c) Egg lecithin and iodine in water (concentrations same as in a and b).

FIGURE 17



from a few drops of lecithin in a chloroform solution. The solution was approximately 0.02 M. The films were formed on one of the flat surfaces of a 5 cm quartz cell. Since quartz was also the substrate used for the electrical measurements of egg lecithin films, the results of the electrical and spectroscopic studies may be correlated. The films were dried with helium gas and then a crystal of iodine was sealed in the cell and allowed to sublime. The free iodine was then evacuated from the cell at low temperature.

The spectra of such a film are shown in Figure 18. Egg lecithin again displays essentially no absorption between 250 and 600 $m\mu$ (Curve a). Upon exposure of the film to iodine vapor, two bands appear, one at 370 $m\mu$ and the other at 295 $m\mu$ (Curve b). Although somewhat broadened, the bands are similar to those obtained for the egg lecithin-iodine interaction in water. As in the aqueous system, the shorter wavelength band is more intense than the longer wavelength one. If moisture was allowed to enter the cell after the iodine had sublimed, the spectra became enormously broad and interpretation became impossible.

The spectrum of the oxidized cholesterol-iodine interaction is shown in Figure 19. Here the spectra were obtained by placing 1 cm, cuvettes of oxidized cholesterol in CCl_4 and iodine in CCl_4 , in the reference chamber of the spectrometer. The other chamber contained a cuvette of carbon tetrachloride as well as the cuvette of the oxidized

Figure 18. Absorption spectrum of egg lecithin film exposed to iodine vapor (film is on quartz). (a) Egg lecithin film on quartz. (b) Egg lecithin film, exposed to iodine vapor, on quartz.

FIGURE 18

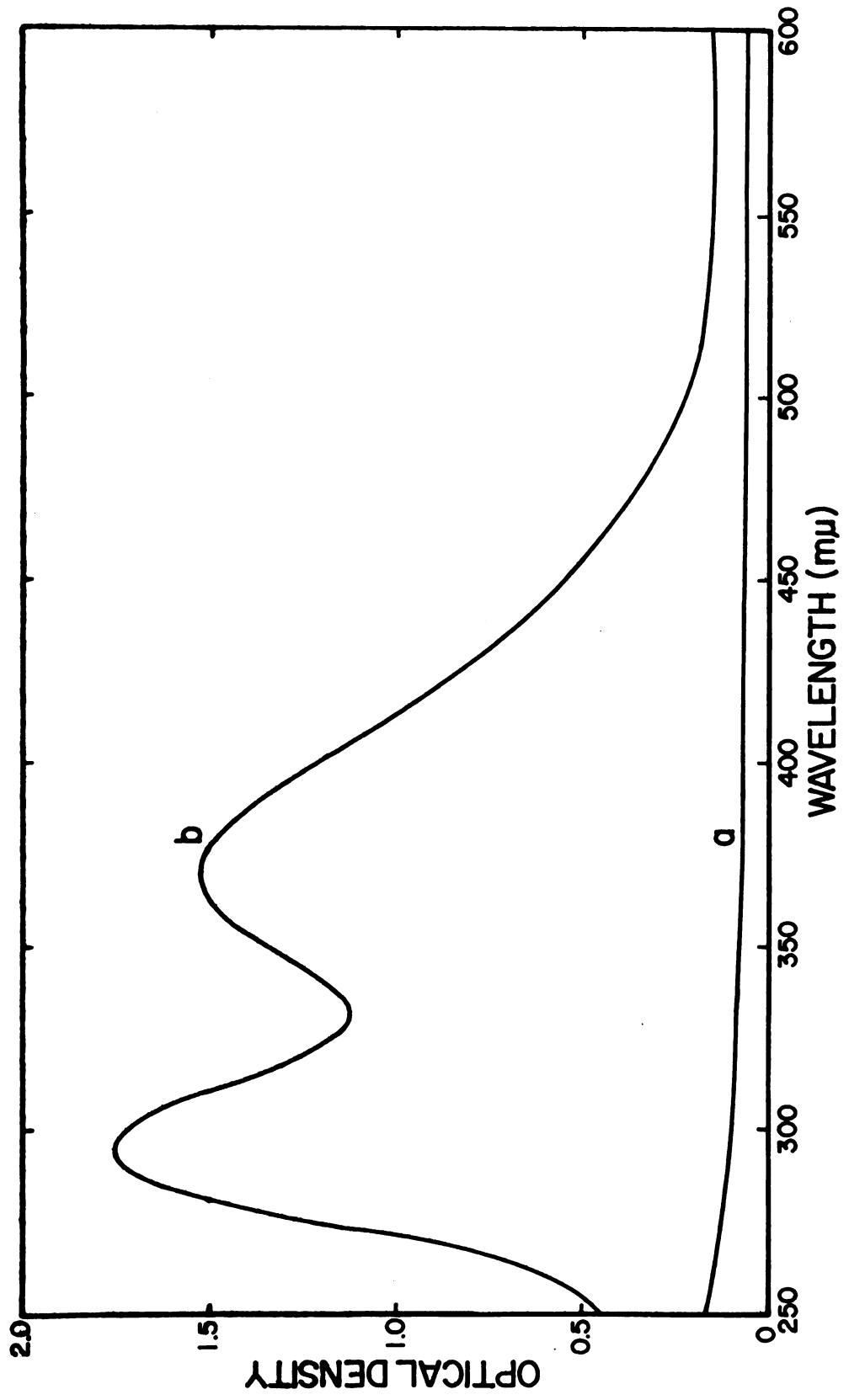
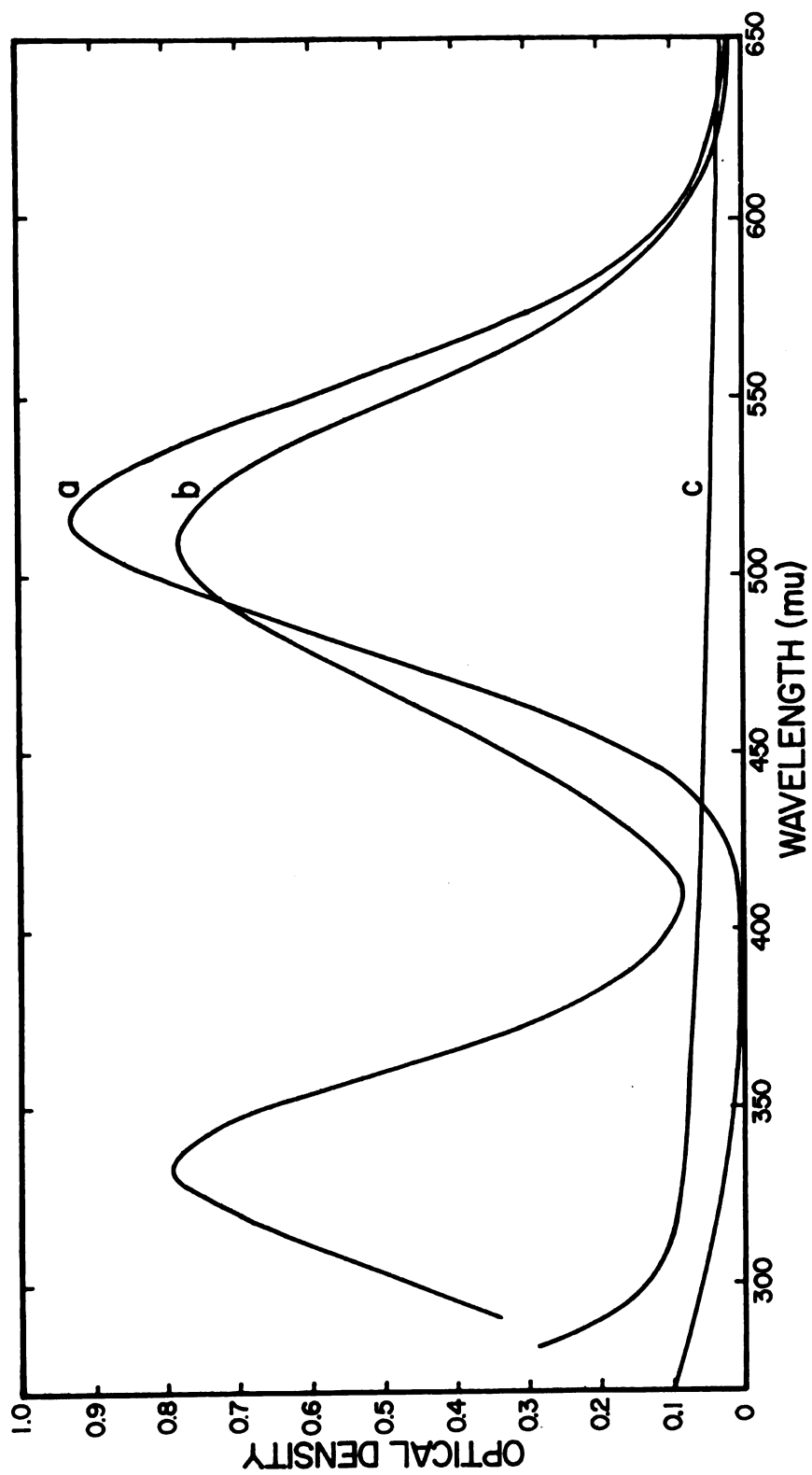


Figure 19. Absorption spectrum of oxidized cholesterol and iodine in carbon tetrachloride. (a) Iodine 11.3×10^{-4} M. (b) Oxidized cholesterol 1.28×10^{-1} M and iodine 11.3×10^{-4} M. (In b, the O.D. shown is twice the experimental O.D.) (c) Oxidized cholesterol 1.28×10^{-1} M.

FIGURE 19



cholesterol and idoine in CCl_4 . Note the shifted iodine band at 509 $\text{m}\mu$ in Curve b and the appearance of a new band at 332 $\text{m}\mu$. Neither oxidized cholesterol or iodine absorb at 332 $\text{m}\mu$. This band was thus considered to be a charge-transfer band. Since the spectrum was obtained by balancing the solution of the complex against the free donor and free acceptor, the spectrum so obtained represents primarily the charge-transfer complex. ($\epsilon_{518} = 935 \frac{\text{liter}}{\text{mole-cm}}$.)

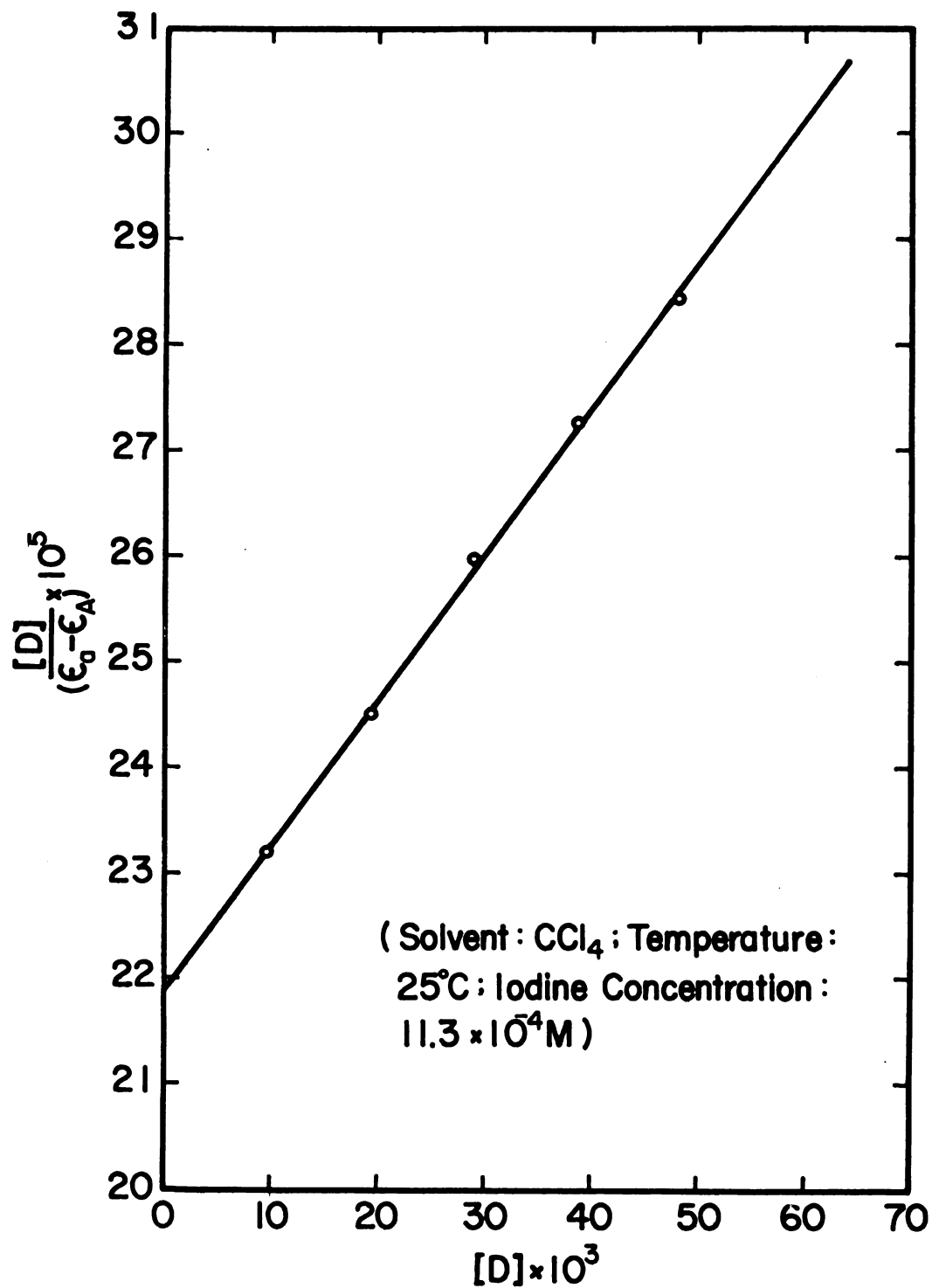
It was found, by varying the concentrations of oxidized cholesterol and iodine in CCl_4 , that the complex was apparently a 1:1 lipid-iodine complex. By use of the Ketelaar equation at the λ_{MAX} of the complex, the equilibrium constant was estimated. A donor concentration range of 9.68×10^{-3} to 48.4×10^{-3} M was used with a fixed iodine concentration of 11.3×10^{-4} M. The K value so determined was approximately $6.26 \frac{\text{liter}}{\text{mole}}$. A plot of the Ketelaar equation is shown in Figure 20 ($\epsilon_{509} = 800$; $\epsilon_{332} = 795$).

It was not attempted to study the iodine-oxidized cholesterol interaction in water because of the difficulty of getting a dispersion of oxidized cholesterol in water. Also, oxidized cholesterol thin films could not be obtained and, therefore, no solid state spectra were obtainable for this lipid-iodine interaction.

Discussion of Experimental Results

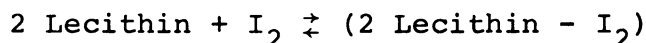
The appearance of the two new bands for egg lecithin in CCl_4 , water and in the solid state is attributed to the

FIGURE 20
PLOT OF THE KETELAAR EQUATION FOR COMPLEX
OF OXIDIZED CHOLESTEROL (D) AND IODINE (A)

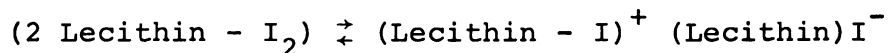


formation of I_3^- ions. This is consistent with the decrease in intensity of the iodine band. It is possible that the resulting I_3^- ions could be complexed to the lipid.⁴¹

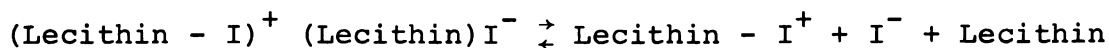
Because of the appearance of the I_3^- bands and because the lipid-iodine complex is apparently a 2:1 complex, the following reaction is postulated between lipid and iodine:



The right-hand side of this equation would represent the so-called "outer complex" as suggested by Mulliken.⁴² This outer complex would then rearrange so as to produce the following reaction:



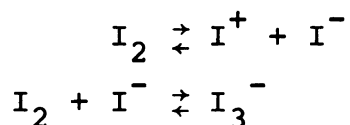
The right-hand side of this equation would represent some sort of "inner complex" according to Mulliken's scheme. The inner complex is essentially an ionic structure and dissociates as



The I^- ions would immediately combine with free I_2 (this reaction is highly favored) to give I_3^- ions. These I_3^- ions would then be responsible for the two spectral bands observed around 294 m μ and 366 m μ . A similar scheme has been used to explain the interaction of β -carotene with iodine⁴³ (another explanation⁴⁴ has recently been given, however) and the interaction of the aromatic amines, amino acids and proteins with iodine.⁴⁵

Referring back to Figure 16 it can be seen that the result, indicating that K for the iodine-egg lecithin reaction is greater than K for the iodine-synthetic lecithin reaction, is consistent with the electrical results of Part III. There it was found that iodine increased the conductivity of egg lecithin films to a greater extent than it did for synthetic lecithin films.

With respect to the I_3^- ion, it should be mentioned that it has been suggested that the formation of this ion, in water, is due to impurities. The reaction is supposedly:



It is also worth noting that if NaI is added to a solution of iodine in water, the molecular iodine band disappears and the two I_3^- bands increase greatly in intensity. This result should be kept in mind when the mechanism of the resistance decrease, in the lipid bilayers, is considered for iodine-NaI solutions surrounding the bilayers.

The complex between oxidized cholesterol and iodine is apparently an outer complex of the form (cholesterol - I_2). This suggests that the lecithins have stronger donor properties than does oxidized cholesterol. It is of some interest that the apparent charge-transfer band at 332 $m\mu$ was obtained for both newly opened bottles of cholesterol as well as oxidized cholesterol.²⁶ The intensity, of course, is

less with the newly opened cholesterol. It appears that some oxidation is present even in the fresh cholesterol. Only the oxidized cholesterol,²⁶ however, was able to form bilayers.

All the spectra recorded in this section were run within one-half hour of formation of the lipid solutions. It was found that the optical density of the lipid solutions did change with time.

Conclusions

1. Iodine forms a charge transfer complex with lecithins. This complex may be of the inner-outer variety proposed by Mulliken. The equilibrium constant for the complexing with egg lecithin is greater than for the complexing with synthetic lecithin (by about a factor of 10), in CCl_4 .

2. The complex formation is evidenced by the appearance of I_3^- bands in the absorption spectra ($\lambda_{\text{MAX}} \approx 350 \text{ m}\mu$ and $\lambda_{\text{MAX}} \approx 290 \text{ m}\mu$). For egg lecithin, these bands occur for the material dissolved in CCl_4 , dispersed in water or made into a solid state film.

3. Iodine complexes with oxidized cholesterol as an outer complex. The complex is evidenced by the appearance of an absorption band with a λ_{MAX} of $332 \text{ m}\mu$. The equilibrium constant for the complex formation is $6.26 \frac{\text{liter}}{\text{mole}}$.

VI. SPECULATIONS

It is of some interest, here, to consider the activation energies of lipids. If the activation energy range for fully hydrated phospholipids is 1.7 to 2.7 e.v., the expression $n_0 e^{-E_A/kT}$ gives an exceedingly low value for the number of charge carriers present in the material. This is assuming that E_A represents the activation energy for charge carrier generation, only. Perhaps E_A in Equation 1. (page 9) is actually made up of an E_A^n and an E_A^μ , as mentioned previously. In the dry state, then, E_A would equal $(E_A^n + E_A^\mu)$. Upon hydration, E_A would be lowered due to a decrease in E_A^n rather than E_A^μ . The decrease in E_A^n would be explained by the model in the theory of Part III. E_A^μ , however, would not significantly change upon hydration. Thus, the rather high activation energies for phospholipids, in comparison to other biological materials, would be ascribed to a constant value of E_A^μ plus a value of E_A^n which depended upon hydration (or iodination).

Alternatively, it could be assumed that E_A^μ is effected by hydration (or iodination) but other work⁴⁶ makes this assumption somewhat tenuous. The results indicate that the ambient vapor induced, bulk conductivity effects are phenomena involving the production of

charge carriers. For the case of anthracene crystals exposed to iodine vapor, the drift mobility of the charge carrier did not change before and after iodine exposure; the conductivity did increase significantly, however, with iodine exposure.

Regardless of the activation energy, it would be of interest to see if the phospholipid films exhibited photoconductivity. Dry lecithins do have their main absorption bands in the region of 2000-2500⁰Å; this corresponds to an energy of 5-6 e.v. If photoconductivity were present, it might be valuable to interpret the absorption of optical energy in terms of the contributions to the activation energy, just mentioned. Similar thinking would apply to the hydrated or iodinated lecithin films. Here the activation energy range would correspond to light of wavelength 4600 to 6200⁰Å.

The nature of the charge carrier in lipids as well as the possibility of surface conduction should be investigated. Hall and magnetoresistance effect measurements, along with solid state electrolysis studies, are presently being undertaken. Such measurements would determine the nature of the charge carrier as well as its mobility; unfortunately, the apparatus sensitivities are still too low to obtain meaningful measurements.

The possibility of surface conduction is present, especially for the hydrated films. It might be fruitful to compare the electrical conductivity of lipid films

with lipids in the "sandwich-cell" configuration. This might also say something about orientation effects; it is known that the orientation of a deposited film is effected by its substrate.⁴⁷ What has been said for hydration applies equally well to iodine exposure of the lipids.

The dielectric dispersion of the lipids merits further study. What the mechanism of the lipid dielectric dispersion is, is not known. The fact that a large dispersion occurs at low frequencies indicates that perhaps large structures of the films, such as liquid crystalline regions, may perhaps be aligning themselves with the electric field. The alignment of liquid crystals by electric fields is known.⁴⁸ It should be noted, however, that this same low frequency dispersion is observed with proteins and inorganic substances; in these materials, a liquid crystalline phase is not present. Why this same low-frequency dispersion should also occur, when the lipid films are exposed to iodine, is somewhat puzzling; iodine is not known to increase the liquid crystalline behavior of lipids. Presumably, both water and iodine adsorption occur at the polar regions of the lipid molecules. The lipid bilayers apparently then become detached from one another. Since the bilayers are symmetrical, however, it is not obvious how this would increase the dielectric constant of the material.

What should also be investigated further is the apparent synergistic action of water and iodine upon lipids. It is difficult to conceive of how hydration, of a lipid

film exposed to iodine, decreases the activation energy, yet, does not increase the room temperature conductivity.

Since the work of this thesis indicates that iodine forms a charge-transfer complex with lipids, it would be of interest to determine more about the complex. It seems reasonable to expect iodine to react, in the case of lecithin, at the α carbon position; possibly it interacts with O^- of the phosphorus group. In the case of oxidized cholesterol, the reaction could well occur with a keto group. Also, in both cases, the iodine may attach itself across a double bond in an actual chemical reaction; this mechanism might be expected to be less important than charge-transfer formation, however. This is indicated by the reversibility of the iodination of the lipid films as seen in Figure 6.

Since the formation of a charge transfer complex with the lipids apparently occurs for the lipid bilayer case, the nature of this complex becomes of even greater importance. Because the formation of this complex could either create electronic charge carriers in the bilayer or possibly alter the bilayer structure, the question of the nature of the charge carriers through the bilayer needs answering. If the carriers are ionic, the complex formation could alter the bilayer permeability to these ions; if they are electronic, the complex could furnish additional carriers for the conduction process. Quite possibly both conduction mechanisms are involved. In any event, radioactive tracer experiments should determine the nature of the conduction process.

It should be noted that whenever bilayer conductivity measurements are made, the conductivity through the lipid bilayer-torus system is being measured. The measurement includes not only current through the bilayer proper but also current through the lipid-teflon interface, current through the torus-bilayer interface as well as any current through the lipid torus itself. As mentioned previously the bilayer resistance values are quite irreproducible. Some workers⁴⁹ feel that this variability comes about because of current leakage paths around the bilayer. These workers speculate that the highest resistance values observed are probably characteristic of the bilayer itself, whereas, the lower values arise from "conducting channels" at the rim of the teflon hole.

It may be of value, here, to calculate the resistance of a bilayer-torus system assuming that the bilayer conductance is very, very small compared to the torus conductance. If the torus is assumed to occupy about 25% of the hole area in the teflon and if its thickness is assumed to be roughly twice that of the cup, at its thinnest part, a resistance of about $10^9 \Omega$ is obtained. This assumes the torus to have the conductivity of fully hydrated egg lecithin (i.e., $10^{-7} \Omega^{-1} \text{-cm}^{-1}$). Since the egg lecithin in the torus probably exists in bilayer form and since it may have water channels in it, this approach may not be too unreasonable. This approach also assumes that conductivity through the torus occurs as it does in the films.

Another way of looking at this idea is to speculate that in the thin films, the bilayers act as high resistance "barriers" to the current in the same way that crystallite interfaces may act as current barriers in inorganic material. The current in thin films then may go around and over the bilayers. Now if a bilayer is formed in a teflon hole, its resistance would be high since now the current could only travel via the barrier. The more leakage paths there were surrounding the bilayer area, the lower would be the measured resistance of the bilayer. Since the leakage paths would be in parallel to the bilayer, they would determine the resistance measured; conversely since the bilayer is essentially non-hydrated but of high capacitance, its capacitance would be in series with that of the leakage paths and would determine the measured capacitance for the system.

The iodine, then, might serve two functions: it could further increase the number of leakage paths in the material surrounding the bilayer. It would probably not increase the conductivity of the torus, per se, since iodine vapor did not greatly increase the conductivity of hydrated egg lecithin films. The iodine could also increase the conductivity of the bilayer itself; now the conduction might occur both via the bilayer as well as through leakage paths. The bilayer capacitance would now be altered, either due to a charge transfer mechanism or else due to a change in structure. This structural change might allow hydration

of the bilayer and, thus, the dielectric constant increase could be explained.

An experimental approach which might shed some light on the conductivity dependence on lipid structure would be one in which the current is measured in a direction perpendicular to that in which it is measured in the bilayer structure. In the bilayer, the current presumably moves in the direction of the fatty acid chains of the lecithin; it would be of interest to measure the current perpendicular to the chains. One such experiment has been done⁵⁰ with calcium stearate which, in some respects, is not too different from lecithin. Multilayers of this material were formed between cracked glass slides and placed between two liquid electrodes. The current now travels in a direction normal to the axis of orientation of the long-chain acids. The resulting conductance was $10^{-4} \Omega^{-1} \text{-cm}^{-1}$. If lecithin were placed in this configuration, would its conductance now be 10^{-6} - $10^{-8} \Omega^{-1} \text{-cm}^{-1}$ as was found for the films? If it were, this might suggest an interesting dependence of the current upon the orientation of the lecithin molecules.

In this same vein, it should be mentioned that the lipid bilayers are apparently not hydrated.

This is deduced from the facts that the dielectric constant of the bilayer is low (≈ 3) and no dispersion is observed over a large frequency range. Why the bilayer is not hydrated is somewhat puzzling. Since the interior of the bilayer is presumably hydrocarbon-like it may be that the water remains at the hydrophilic boundary of the bilayer; yet, as mentioned before, the bilayer exhibits a reasonably high permeability coefficient, for water. Perhaps the water exists in a bound form i.e., an ice-like form. This structure might exclude ions and conduction might occur by a protonic mechanism.

Regarding the charge carriers, it is instructive to consider Table 7. This table itemizes possible mobility values for the charge carriers in the bilayer; these values are assumed values, and from the given value of resistance calculated for the bilayer, the number of charge carriers per bilayer is determined. The n (charge carrier density/cm³) was determined from $\sigma = ne\mu$ where μ is an assumed mobility and σ is calculated from the membrane current and dimensions.

One can see from the table that for reasonable mobility values, the number of charge carriers in a given bilayer is quite small. The calculation assumes, of course, that the major portion of the current is carried through the bilayer.

Table 7. Number of charge carriers in lipid bilayer for various mobilities of charge carrier

$\mu \left(\frac{\text{cm}^2}{\text{volt-sec}} \right)$	$n', \left(\text{no. of charge carriers per bilayer} \right)$
1	6×10^{-4}
10	6×10^{-5}
0.1	6×10^{-3}
10^{-2}	6×10^{-2}
10^{-3}	6×10^{-1}
10^{-4}	6
10^{-5}	6×10
10^{-6}	6×10^2
10^{-7}	6×10^3
10^{-8}	6×10^4

If now one does the same type of calculation for an egg lecithin film, one obtains the results of Table 8.

Table 8. Number of charge carriers in lipid film for various mobilities of charge carrier

$\mu \left(\frac{\text{cm}^2}{\text{volt-sec}} \right)$	$n' \left(\text{no. of charge carriers per film} \right)$
1	1.6×10^9
10	1.6×10^8
0.1	1.6×10^{10}
10^{-2}	1.6×10^{11}
10^{-3}	1.6×10^{12}
10^{-4}	1.6×10^{13}
10^{-5}	1.6×10^{14}
10^{-6}	1.6×10^{15}
10^{-7}	1.6×10^{16}
10^{-8}	1.6×10^{17}

If a representative mobility is chosen, say $\mu = 10^{-4}$, it turns out that there are 2 charge carriers per 10^5 lipid molecules in the film; in the bilayer case there are 6 charge carriers per 10^{13} lipid molecules. It is questionable, however, if the charge carrier mobilities are the same in the film and in the bilayer.

It might be of interest at this point to speculate on the biological implications of this work. It is known that thyroxine and iodine both effect the mitochondrial structure, as evidenced by a change in water permeability and swelling of the structure.

The physiological result is an apparent uncoupling of oxidative phosphorylation. The electron transport process has been theorized to involve electronic conduction. Thus, the effect of iodine itself, or as a constituent of a hormone, could be linked to a physiological process involving electronic conduction. Such speculations could be generalized to all bodily processes effected by iodine containing hormones.

VII. SUMMARY OF CONCLUSIONS

Following is a list of conclusions from the whole of this thesis:

1. Lipids do obey the operational definition of semiconductivity; in particular, phospholipids display exceptionally large activation energies in comparison to other biological materials (4.8 to 6.3 e.v.).

2. Hydrated lipids also follow the operational definition of semiconductivity; the activation energies are considerably lower than the dry state values, but again, the phospholipids display values high compared to other biological materials (1.7 to 2.8 e.v.).

3. The conductivity of hydrated lipids increases enormously (by a factor of more than 10^9) over the conductivity of dry lipids. The increase can be accounted for by the lowering of the activation energies as predicted by Equation 3.

4. The dielectric constant of the hydrated lipids increases over its dry state value. This increase is especially evident at low frequencies (less than 1000 c.p.s.). Conclusion 3 can be explained by this increase as is expected from Equation 11. The meaning of the dispersion of the hydrated lipids is not known.

5. Iodine vapor increases both the electrical conductivity and the dielectric constant of the lipids; concomitant with this increase is a decrease in activation energy. What has been said in Conclusions 3 and 4 applies here, also.

6. The increase in the dielectric constant of the lipids, upon iodine exposure, is due to the formation of a charge-transfer complex between lipids and iodine.

7. The dielectric constant of the lipid, exposed to iodine vapor, displays a large low frequency dispersion; the reason for this dispersion is not known.

8. The presence of iodine, in the solution surrounding lipid bilayers, decreases the bilayer resistance by 3 to 5 orders of magnitude. This result is consistent with the work of other investigators. No disymmetry in the effect was found with respect to the applied voltage.

9. The presence of NaI in the solution also lowers the bilayer resistance. This lowering may be independent of the lowering by iodine. It is suggested that it may be accounted for by traces of iodine impurity in the NaI solution.

10. Lipids dissolved in organic solvents, dispersed in water or in the form of solid state films, display absorption spectra indicating formation of a charge-transfer complex. The complex of the lipid with iodine is different for the lecithins than for oxidized

cholesterol, as revealed by the spectra, being an inner complex in the former case and an outer complex in the latter case.

11. Because iodine increases electrical conductivity of the lipid films by increasing the dielectric constant of the lipid, it is suggested that the same process may occur in the bilayer.

12. Insofar as Conclusions 10 and 11 are valid, this suggests that at least part of the electrical conduction of lipid bilayers may proceed via an electronic mechanism since all presently known charge-transfer, conductivity processes are electronic in nature.

In summary, it may be said that the work of this thesis has posed many more questions than it has answered. Insofar as the lipid bilayer is a valid model for a cellular membrane, the ideas invoked in this work may be applicable to biological systems.

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APPENDIX

Structures of Lipids

CC(C)CCCC(C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C

CHOLESTEROL

