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The effects of pentachlorophenol on the electrical conductivity of lipid bilayer membranes

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Title: The Effects of Pentachlorophenol On The Electrical Conductivity

of Lipid Bilayer Membranes.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

Kwan Hsu, Chairman

Pavel K. Smejtek

Makoto Takeo

Robert L. Casteel

The effects of pentachlorophenol (PCP), a widely used pesticide,
on the electrical characteristics of lipid bilayer membranes has been
studied. When a small amount of PCP (even at a concentration of a few
micromoles per liter) is present in the electrolytic solution surround-
ing the membrane, the electrical conductivity of the membrane signifi-
cantly increases. The present work was concerned with detailed measure-
ments of the changes in the conductivity caused by PCP under chemically
controlled conditions. The experimental results were analyzed to de-
termine the permeant species in the membrane, and an attempt was made
to correlate the data with existing models of membrane transport.

THE EFFECTS OF PENTACHLOROPHENOL CN THE

ELECTRICAL CONDUCTIVITY OF LIPID BILAYER MEMBRANES

by

WILLIAM HARVEY PERMAN

A thesis submitted in partial fulfillment of the
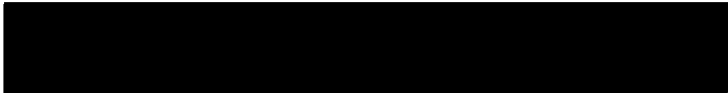requirements for the degree of

MASTER OF SCIENCE
in
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Portland State University
1974

TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of

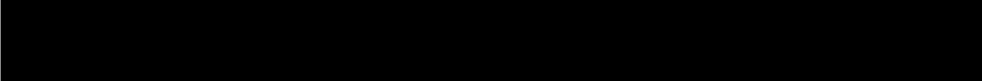William Harvey Perman presented August 9, 1974.

Kwan Hsu, Chairman

Pavel K. Smejtek

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Mark Gurevitch, Head, Department of Physics

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August 9, 1974

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CHAPTER 1

INTRODUCTION

Living systems depend heavily upon membranes to provide the necessary physical and biochemical compartmentalization. The cells of higher organisms have several different membranes, each with a specific function. The plasma or outer membrane of the cell acts as an envelope and a permeation barrier. It holds the cytoplasm within the cell and selectively allows nutrients to enter and waste products to leave the cell. Organelle membranes serve as regulators of protein synthesis as well as sites for energy transduction within the cell. The most important organelle is the mitochondrion, as the oxidative phosphorylation of ADP to ATP occurs on the mitochondrial membrane.

Lipids are the major components in biological membranes (1). They form a cohesive matrix which is responsible for the gross properties of the membrane (2). Phospholipids are the most abundant lipids found in membranes of higher plants and animals (1). Their basic structure is shown in Figure 1. They are essentially phosphoglycerides with a phosphate group attached to the third carbon of the three carbon glycerol backbone, with two fatty acids (RCOOH) esterfied to the other two glycerol carbons. The "R" groups are comprised of saturated and unsaturated carbon atoms and are not polar. The phosphate group along with its functional "X" group are polar, and form a

polar 'head' on the lipid molecule. As a result, phosphoglycerides are the most polar of all lipids.

The most common lipids found in the membranes of higher plants and animals are phosphatidyl ethanolamine (cephalin), and phosphatidyl choline (lecithin) (1). The "X" group of lecithin, the lipid used in the present work, is a choline group $N(CH_3)_3$ (Fig. 2). Lecithin is uncharged at neutral pH due to the positive charge of the choline group which compensates the negative charge on the phosphate group.

The polar heads of the lipids are attracted toward polar solvents such as water. The hydrocarbon tails are insoluble in water, and instead prefer a non-polar environment. This amphiphatic character of the lipid molecules is responsible for the formation of artificial lipid membranes. If a glob of a natural lipid, such as lecithin in the solvent decane, is placed on the orifice of a septum immersed in an aqueous medium, the lipid spontaneously thins and forms a bimolecular membrane. This lipid bilayer is similar to the natural lipid membrane in thickness, water permeability, capacitance, dielectric breakdown voltage, and mechanical properties such as surface tension (3) (see Table I).

Many compounds have been added to the artificial membrane system in attempts to modify the lipid properties. Thus far, the artificial membranes and modifying agents have been shown to be useful as experimental models for at least five basic types of biological membranes (5). They are the plasma membrane of the red blood cell, the nerve membrane of axons, the cristae membrane of mitochondria, the thykaloid
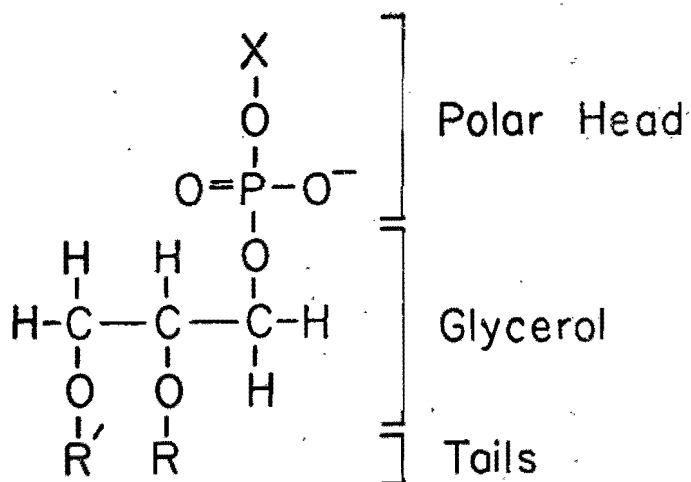
Figure 1. Basic structure of phosphoglycerides.
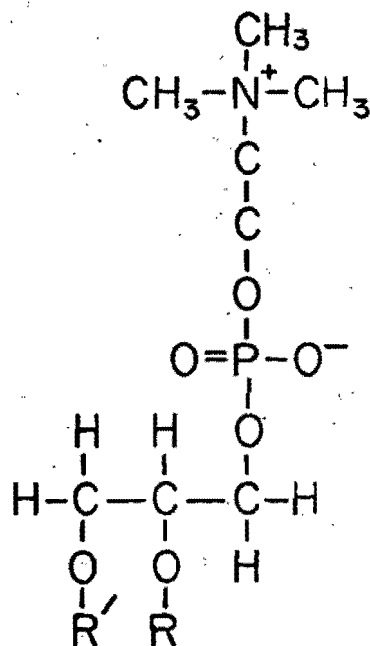
Figure 2. Structure of Lecithin.

membrane of chloroplasts, and the rod membrane of the retina.

TABLE I

COMPARISON OF INTRINSIC PROPERTIES BETWEEN
ARTIFICIAL LIPID MEMBRANES
AND BIOMEMBRANES (4)

Property	Artificial Membrane	Biomembrane
Thickness ($\overset{\circ}{A}$)	50-70	50-150
Mechanical Properties	Relatively unstable	Stable
Surface Tension (dynes/cm)	2-0.1	0.5 to almost 0
Capacitance ($\mu F/cm^2$)	0.33-1	0.5-2
Dielectric breakdown voltage (mV)	80-500	80-500
Electrical Resistance ($\Omega \cdot cm^2$)	about 10^8	less than 10^5
Permeability (cm/sec) Water (tracer) Water (osmotic)	1×10^{-3} 1×10^{-3}	$4\text{-}40 \times 10^{-4}$ More than tracer
Ionic Selectivity	Little, depending on head groups	Considerable

A specific group of additives dramatically affects the permeability of the lipid membranes, and in some cases affects the ion selectivity. "Uncouplers" comprise one class of this group. These compounds prevent or uncouple the oxidative phosphorylation of ADP to ATP on the mitochondrial membrane. Many compounds from this class are used as pesticides and herbicides. Since it was found that these compounds (6) increase the electrical conductivity of lipid membranes, it is generally believed that electrical measurements on these membranes may reveal more detailed information about changes in ionic transport associated with the presence of pesticides in the membrane system.

Pentachlorophenol (PCP) is an uncoupler and acts as a weak acid. This means that when neutral molecules of the uncoupler (HA) are present in aqueous solution the molecules dissociate into anions (A^-) and protons (H^+) as shown in Figure 3. The degree of dissociation depends upon the pK of the solution and the characteristic dissociation constant.
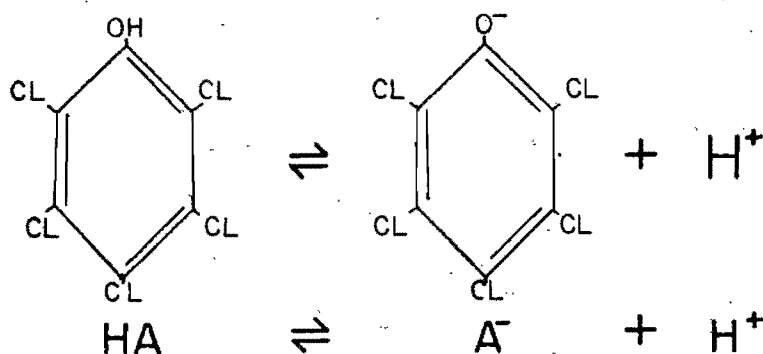
Figure 3. The weak acid uncoupler PCP (HA) dissociates into anions (A^-) and protons (H^+).

It was first proposed that uncouplers such as PCP made the membrane selectively permeable to either the H^+ or the OH^- ions (7). However, this approach cannot explain the conductivity maximum occurring near or at the pK of the uncoupler. It is also energetically difficult, due to a large change of polarization energy, for an ion of small radius such as a proton to go from a high dielectric medium such as water to a low dielectric medium such as the membrane. However, ions whose charge is delocalized over a large area have less difficulty permeating the membrane. On the basis of the above argument, it has been proposed that the protons are "carried" across the membrane by the uncoupler molecules. Several carrier models have been formulated, some of which are contradictory. Thus, one part of the problem is to establish the species of the transport ions in the presence of a mem-

brane modifier and the mechanism of charge transport. The present work makes an attempt to answer some of the above questions for the pesticide pentachlorophenol. Since movement of ions across the membrane constitutes a current, we therefore can monitor changes in the membrane transport processes by measuring changes in the membrane conductivity due to the change of the pH of the solution bathing the membrane, the applied voltage, and the uncoupler concentration. All experiments were performed with lecithin-cholesterol membranes using PCP as the uncoupler.

CHAPTER 2

EXPERIMENTAL METHODS

I. LIPIDS

The lipid used to form the membranes was egg yolk lecithin ex-
tracted and purified by Dr. K. Hsu using the procedure developed by
Singleton (8). The lecithin was stored in chloroform sealed under
nitrogen in ampules containing about 5 ml each of the solution at the
concentration of 25 mg/ml. The ampules were kept in the freezer (about
-15°C) until needed. The contents of each ampule were filtered using a
0.45μ millipore filter before use to remove the fine alumina particles
which had come down with the lecithin fractions from the column.
Purity of the lecithin was tested by thin-layer chromatography. Only
one spot was observed in iodine vapor, and the R_f value was comparable
to that of commercially prepared lecithin. Two stock solutions were
prepared. One contained lecithin only in a 1% (w/v) n-decane solution.
The other contained lecithin and cholesterol mixture (1:1) by weight,
in n-decane solution with the same lecithin concentration as before.
These solutions were kept under nitrogen and stored in the freezer
until needed. Small portions of the stock solution were used to pre-
pare the membranes of the day. These solutions were kept in the re-
frigerator during the day, and stored under nitrogen in the freezer
at night. Lipid solutions kept in this manner were found to last four
weeks without losing their ability to make stable membranes. The

cholesterol was a gift from Dr. McClure and was of high purity. The addition of cholesterol to the lecithin solution gave the membranes greater stability without changing their intrinsic conductivities.

II. ELECTROLYTE

The electrolyte used was 0.1 M/L potassium chloride. This established a large excess indifferent electrolyte. The solutions were buffered with a potassium-citrate-borate buffer system in a pH range of 3 to 11. Composition of the buffer solutions was chosen according to Liberman (9), with 0.2 M/L of potassium phosphate, 0.2 M/L potassium citrate, and 0.05 M/L boric acid. The solutions were titrated to the desired pH using either hydrochloric acid or potassium hydroxide. The pH of the solutions was determined with a Corning model 7 pH meter. The solutions were kept in the refrigerator and were brought to room temperature before use.

III. PENTACHLOROPHENOL

The pentachlorophenol (PCP) was obtained from the Aldrich Chemical Company, and was 99+% pure. A stock solution of PCP at 1mM/L was prepared in the above buffer at a pH of 7.0, and was stored in the refrigerator to retard decay. In order to obtain the desired PCP concentrations, appropriate amounts of the stock solution were pipetted into buffer solution, and then titrated to pH and final volume. The amount of PCP in each solution was then measured spectrophotometrically within at least a week after it was prepared.

IV. NONACTIN

The nonactin (NON) was a gift from the Squibb Company. A stock
solution of NON was prepared at 0.1mM/L in ethyl alcohol, and stored
in the refrigerator under nitrogen to retard decay. Experimental
solutions containing PCP and NON were prepared new each day and were
adjusted to give a final NON concentration of 3µM/L. The alcohol con-
tent in the solutions never exceeded 3% of the total volume.

V. CIRCUIT

A diagram of the electric circuit used is shown in Figure 4. A
Keithly model 417 picoammeter was used to measure current, and a
Princeton Applied Research electrometer, model 135 was used to measure
both current and voltage. Corning calomel electrodes were used to
measure the current and membrane potentials and were protected from
contamination by using saturated agar bridges. When the membrane re-
sistance approached that of the calomel electrodes, which is about
10k, the calomel electrodes were replaced with Ag/AgCl electrodes whose
resistance was of the order of 10 ohms.

VI. ARTIFICIAL SYSTEM

A cylindrical teflon cup with an orifice about half-way down its
length, was situated in a rectangular plastic holder. Solution added
to this system was free to equalize through the orifice in the cup.
A membrane across the orifice divides the solution into two compart-
ments which are electrically insulated except through the membrane.
Two cups were used: one had a diameter of 1.79 mm, the other a diameter

POWER SUPPLY

MEMBRANE

PICOAMMETER

ELECTRODE

CUP
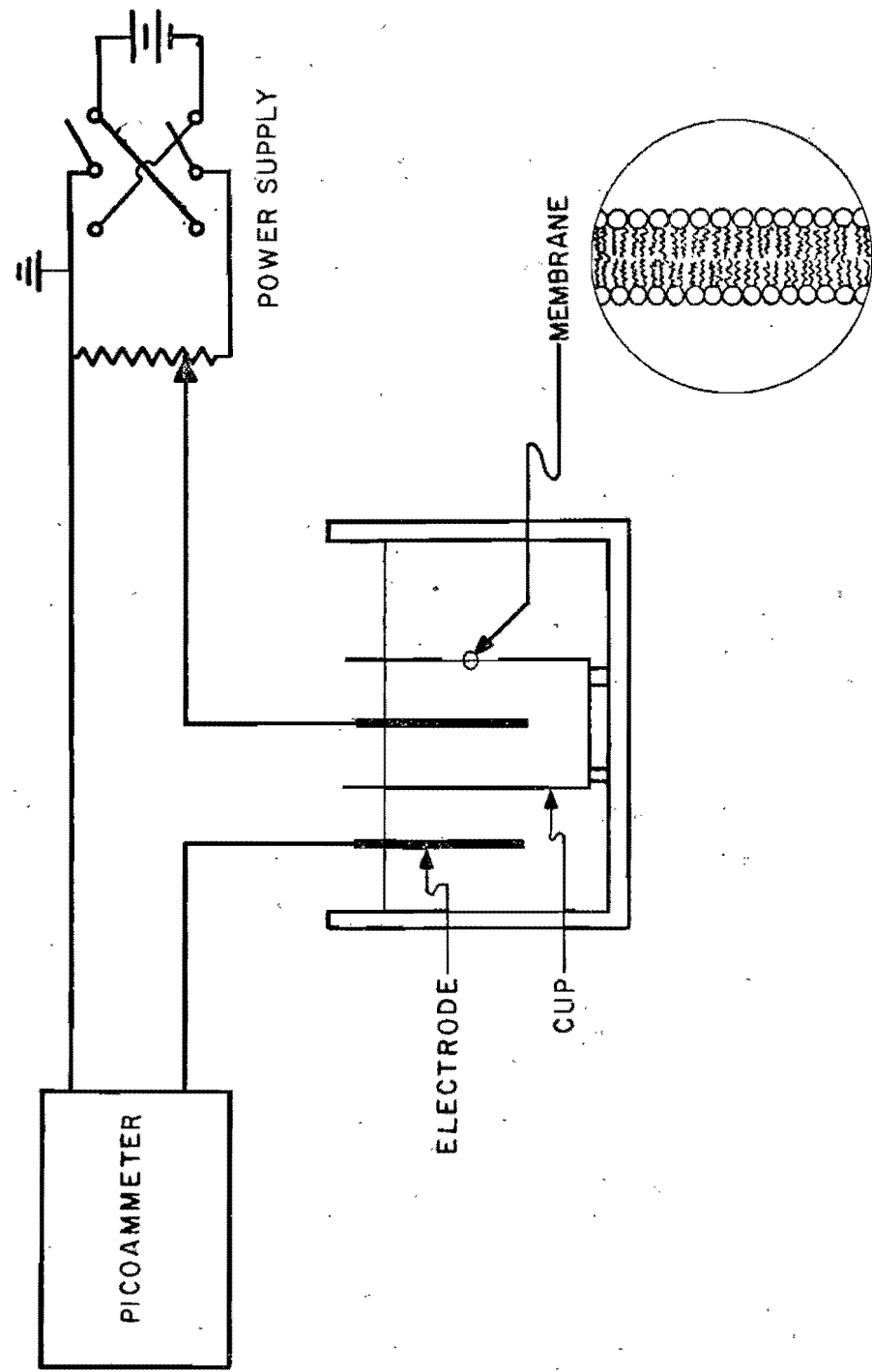
Figure 4. Circuit diagram.

of 1.95 mm. The cups were cleaned before each use either by boiling in an alcohol-sodium hydroxide solution, or by a thorough alcohol washing; then rinsed repeatedly with distilled water and allowed to dry. For a more durable membrane, the orifice was provided with a lecithin coat. This was done by painting the orifice with the lecithin solution several times, allowing the lipid solution to dry between each application. This lecithin coat serves as a "base" for the membrane. When the base was well formed, the cup was placed inside the holder and solution was added. The solution level was adjusted such that it was just a little above the orifice.

In general, the solution around the membrane was not stirred. However, during the concentration gradient studies the inner compartment, to which the PCP was added, was stirred for a short period of time to insure proper mixing.

VII. MEASUREMENTS

Stability of the membrane was judged from the variation of current with time when a voltage of 34 mv was applied across the membrane. When the variation was within 5% within 50 minutes, the relationship between current and voltage was investigated. The applied voltage was varied from 0 to 210 mv in steps, and the current was recorded. Conductivity data were calculated typically at 30 and 50 minutes after membrane formation at the applied voltage at 34 mv, taking into account the potential due to the electrodes. If the current reading at 34 mv showed a 10% difference after the I-V curve was taken, the data were not used.

CHAPTER 3

EXPERIMENTAL RESULTS

The membrane current behaves ohmically for small voltages up to

50 mv or less, and increases more rapidly from voltages of 80 to 210

mv when no PCP is present (Fig. 5). There is no indication of satura-

tion processes as these curves continue upward until the membrane

breaks down. Measurements of membrane conductivity for various values

of pH indicate a slight effect of pH upon membrane conductivity (Fig. 6).

The membrane conductivity is changed considerably by the addition

of pentachlorophenol in different environments.

I. CONDUCTIVITY AS A FUNCTION OF PH

The amount of dissociated uncoupler in solution depends upon the

pH. In this experiment, the amount of PCP added to the solution has

been kept constant at $50\mu M/L$. This value was chosen on the basis of

preliminary studies which indicated that the conductivity begins to

saturate at a concentration of about 3 to $50\mu M/L$ of PCP.

The pH range covered was from 3 to 11, the data presented in

Figure 7. The error bars represent the standard deviation; the points

themselves represent the mean value of several different membranes

formed on the two different cups. The maximum conductivity occurs at

a pH of about 5.3, which is very close to the pK of the uncoupler,

which Beilstein quotes as 5.26 (10). Another notable feature is that
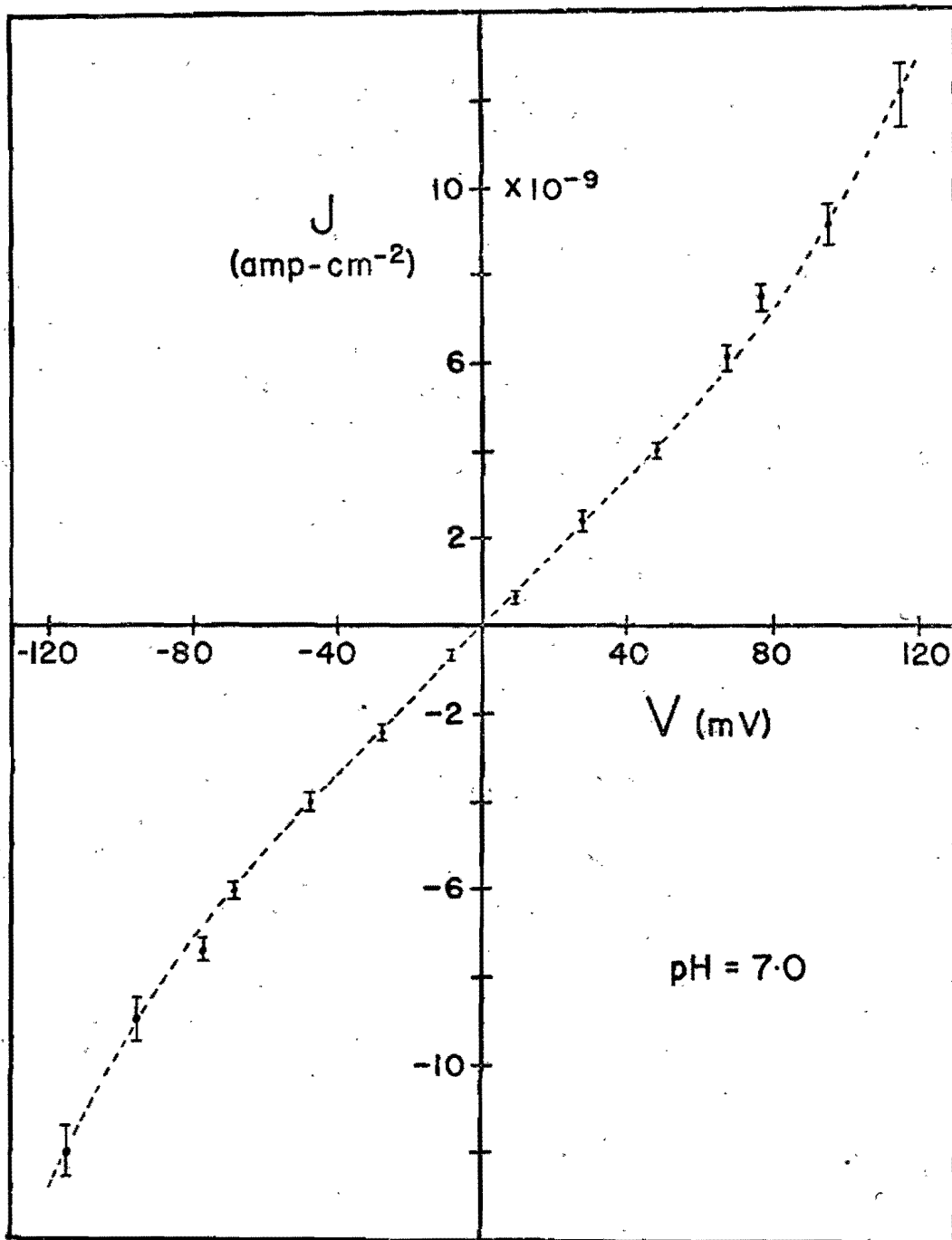
Figure 5. Current versus voltage for membrane system without PCP.
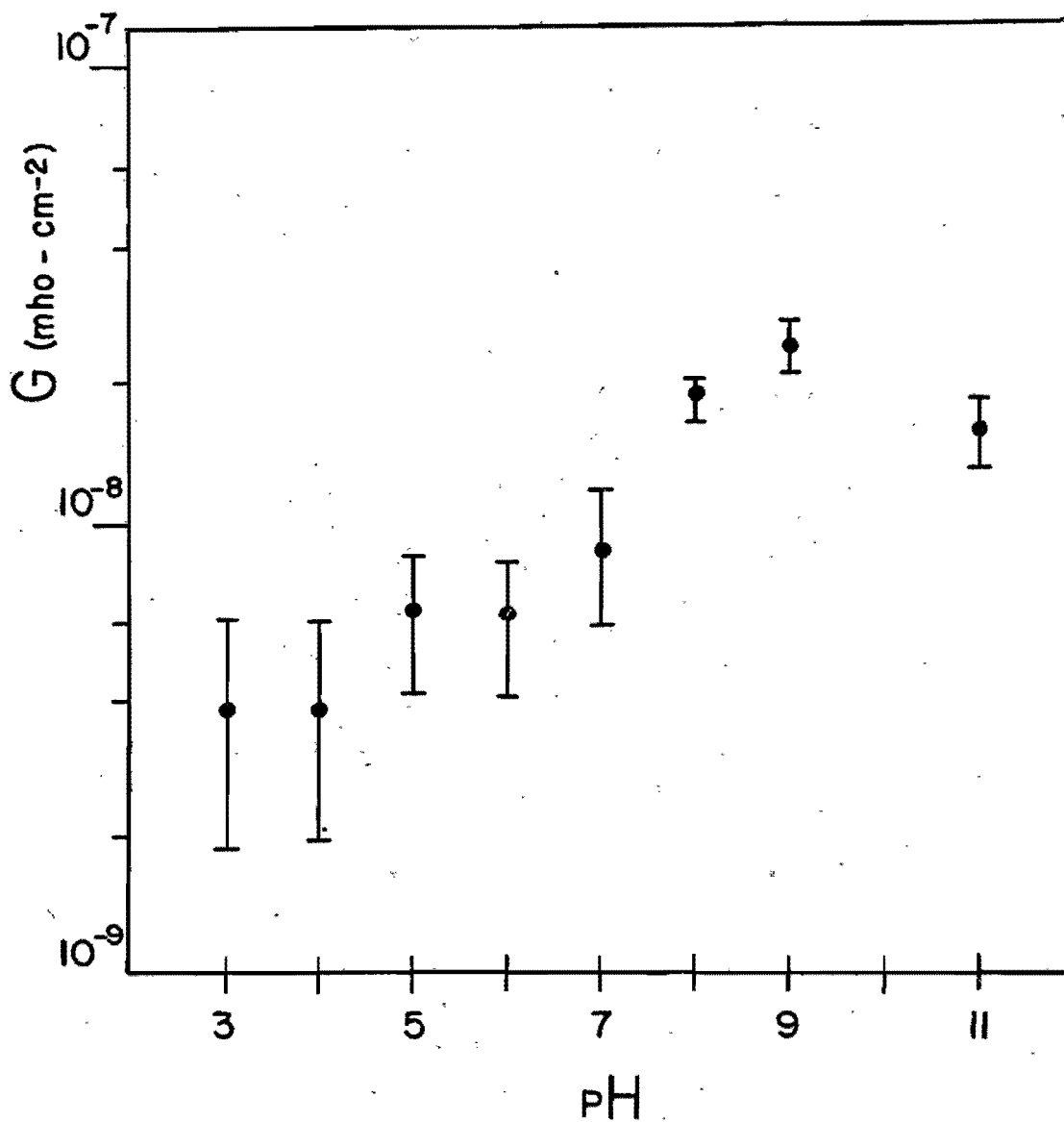
<u>Figure 6</u>. Conductivity versus pH for membrane system without PCP.

PCP has increased the conductivity of the membrane a thousand-fold at pH 5.3, as compared with the system without the pesticide.

II. CURRENT AS A FUNCTION OF VOLTAGE

Figure 8 shows an example of a typical current-voltage relationship for 50µM/L PCP at pH 7. The current increases more rapidly at higher voltage. In the presence of PCP the membrane conductivity curves show a greater curvature. The relationship between curvature of the current-voltage characteristics and pH for a constant amount of PCP can be seen in Figure 9. In comparing the curvature of the current-voltage relationship, all current values were normalized at the highest voltage used. The analysis indicates that the curvature is greater at both high and low pH, and it is smaller near the pK of the uncoupler.

III. CONDUCTIVITY AS A FUNCTION OF PCP CONCENTRATION

These measurements were made to indicate the processes of charge carrier formation. Figure 10 shows the data for several different values of pH. Near the pK of the uncoupler the conductivity varies as the square of the PCP concentration in solution (a slope of 2 on the log-log plot). As the pH becomes more basic the concentration dependence becomes linear, until at the pH of 8.7 there is no apparent dependence of conductivity upon concentration. Again these curves show that the conductivity tends to saturate at PCP concentrations of 30 to 50µM/L.
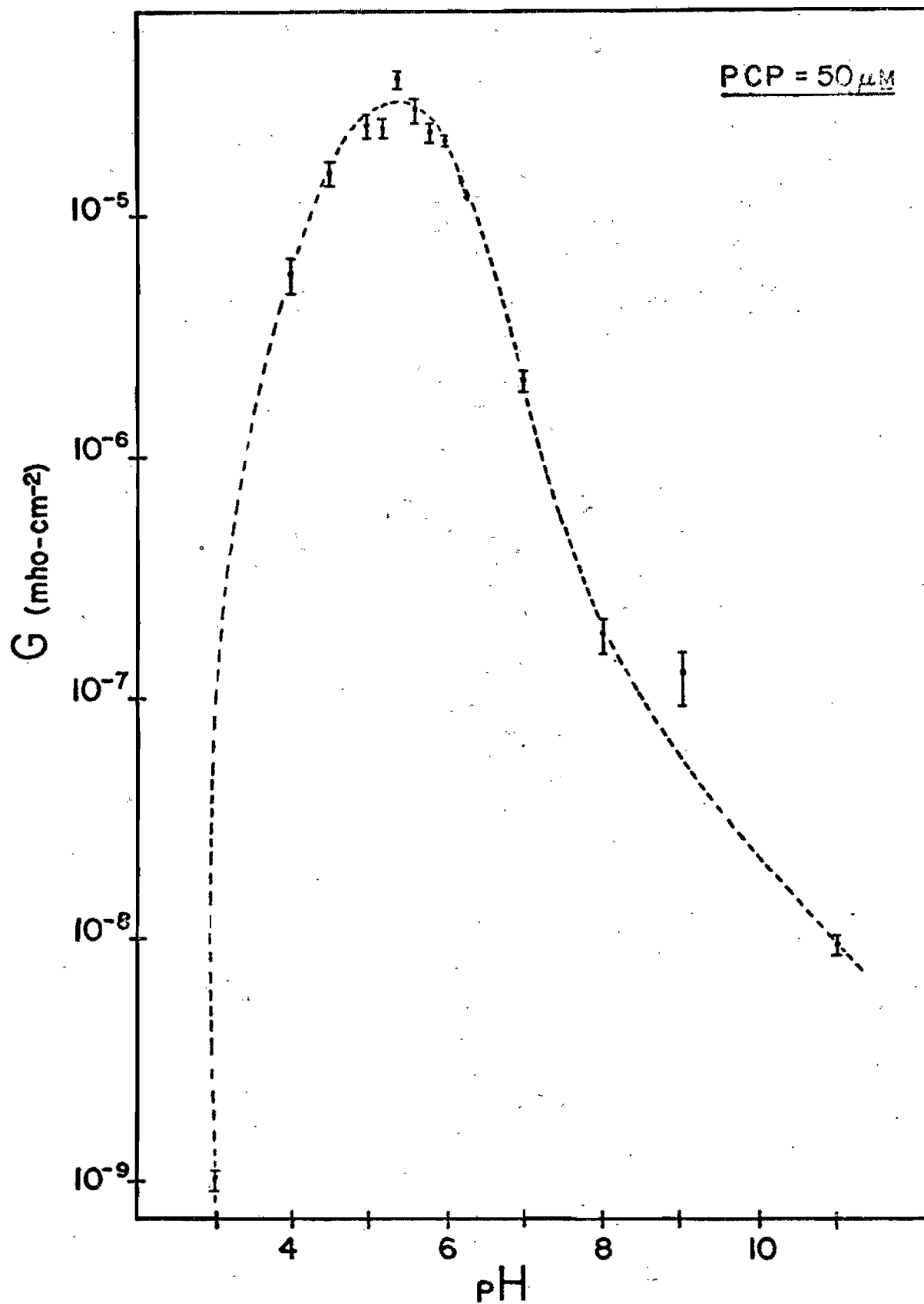
Figure 7. Conductivity versus pH for PCP system. The maximum occurs at pH 5.4.
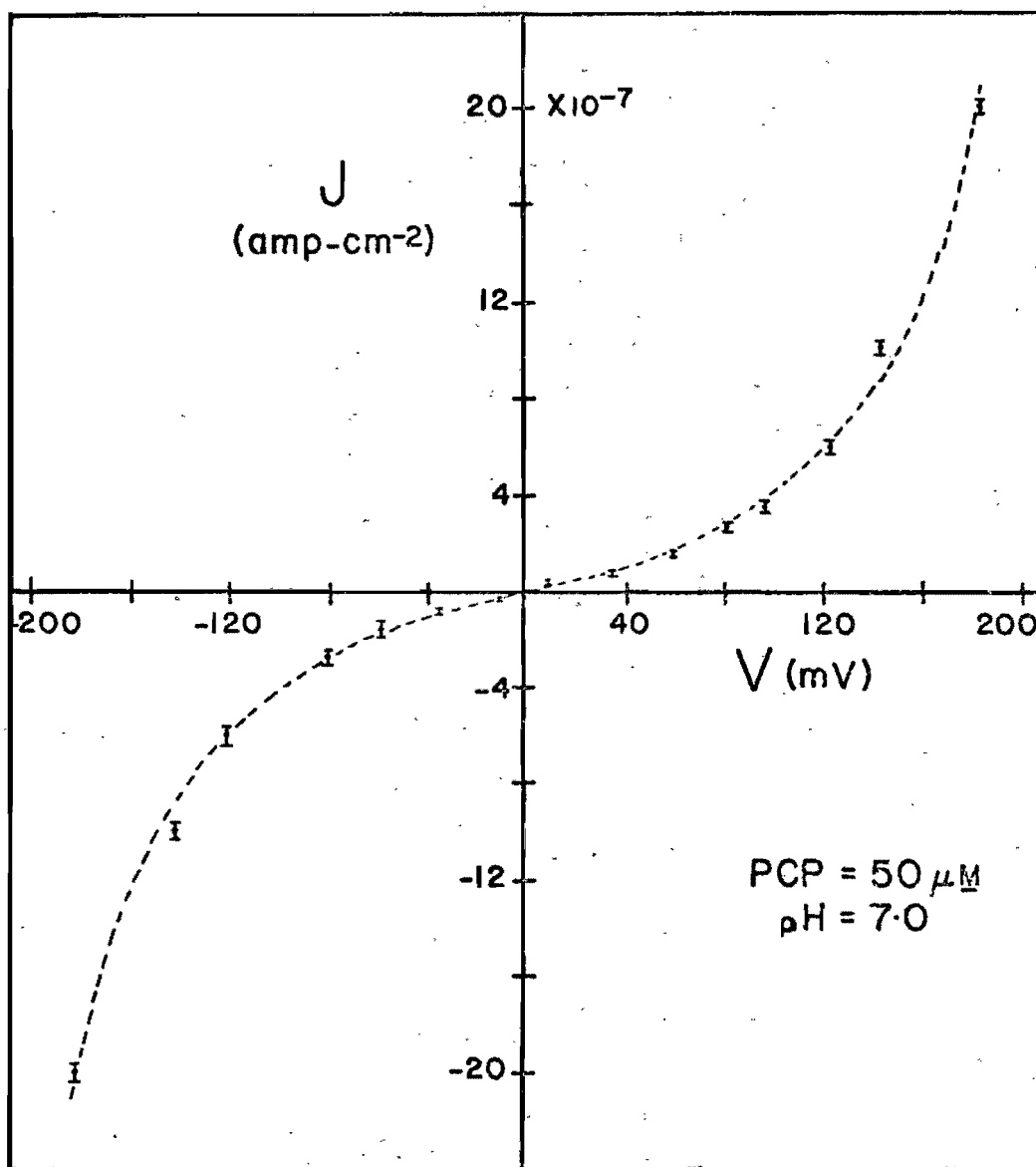
Figure 8. Current versus voltage at pH 7 for PCP system.
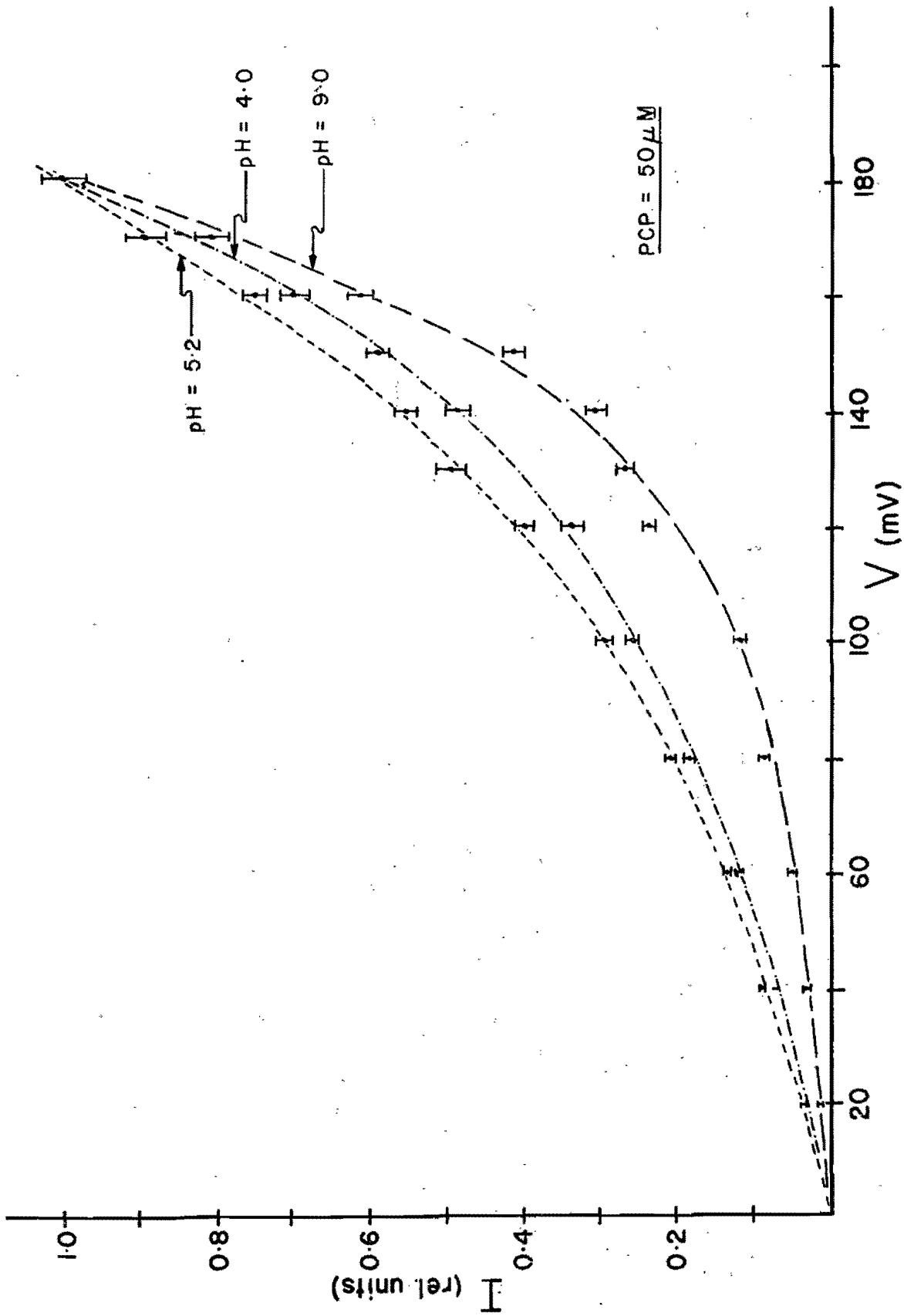
Figure 9. Relationship between curvature and pH for PCP system.
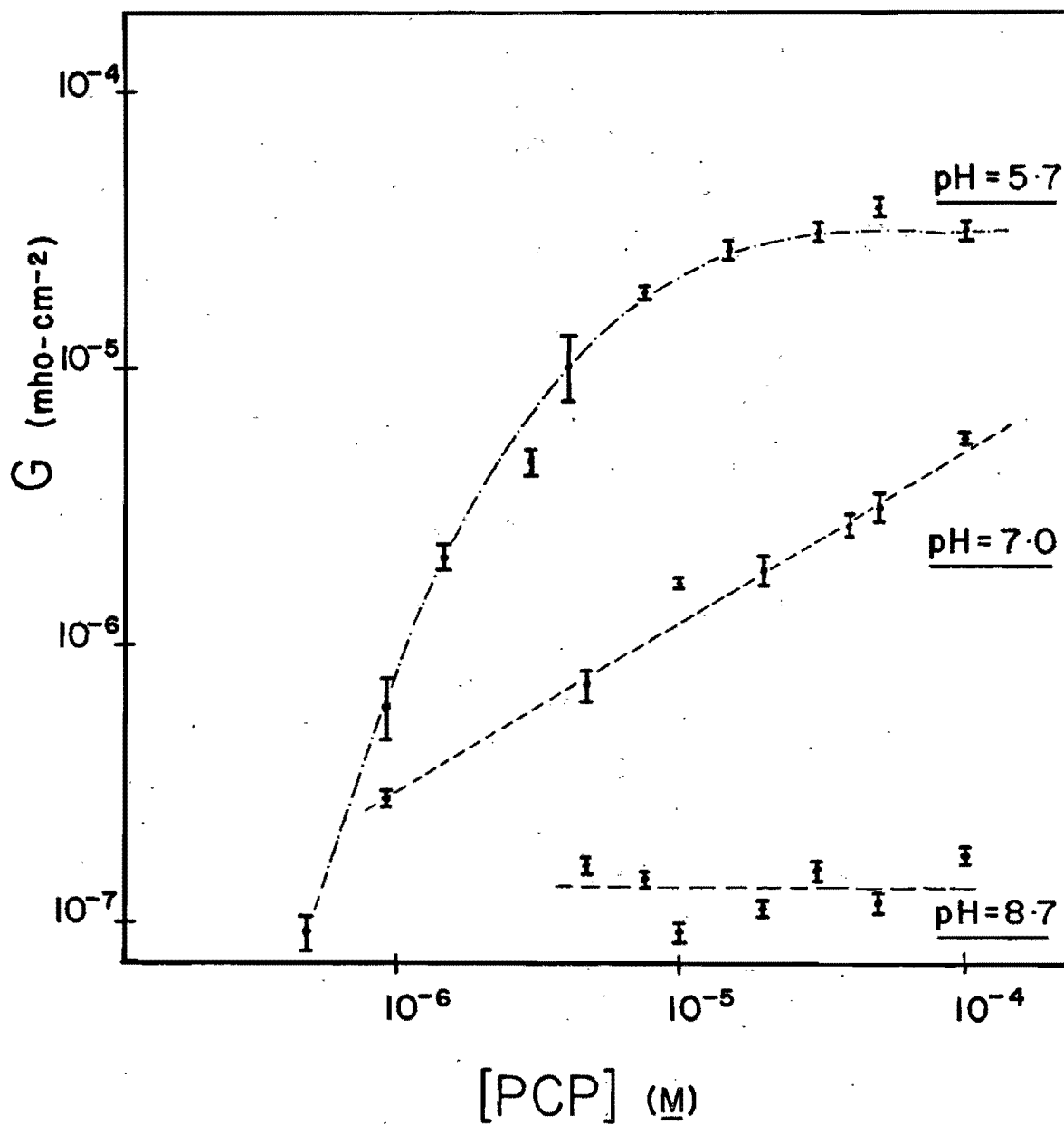
Figure 10. Relationship between conductivity and uncoupler concentration at several different values of pH. Quadratic dependence disappears as pH is increased..

TABLE II

MEMBRANE POTENTIAL (Vm) FOR PCP
CONCENTRATION GRADIENTS
AND PH GRADIENTS

MEMBRANE #	(date)	PCP* (μM/L)	Vm (mV)	pH**	Vm (mV)
1	(12-20-73)	25	0	-	-
2	(12-20-73)	35	0	-	-
3	(12-21-73)	35	-4	-	-
4	(1-6-74)	5	5	-	-
5	(1-12-74)	70	14	-	-
6	(1-12-74)	70	10 to 25	-	-
7	(2-8-74)	-	-	6.6	25
8	(2-10-74)	-	-	6.3	35
9	(2-10-74)	-	-	"	33
10	(2-11-74)	-	-	"	35
11	(2-15-74)	-	-	"	34
12	(2-16-74)	-	-	6.56	-47
13	(2-16-74)	-	-	"	-47
14	(2-16-74)	-	-	"	-54
15	(2-18-74)	-	-	"	-53
16	(2-23-74)	-	-	"	-48
17	(2-23-74)	-	-	4.84	57
18	(2-28-74)	-	-	"	60
19	(2-28-74)	-	-	"	60
20	(3-1-74)	-	-	"	57
21	(3-1-74)	-	-	"	57
22	(3-1-74)	-	-	"	60

* Initial PCP concentration 5μM/L in both compartments

** Initial pH 5.7 in both compartments

IV.   PRESENCE OF NONACTIN IN THE PCP SYSTEM

The deviation from the expected dependence of conductivity upon
concentration of uncoupler, and the assymmetry of the conductivity
versus pH curves indicate the probability of charging effects on the
membrane at high pH.   It was proposed that a negatively charged mem-
brane surface would enhance the conductivity of the positively charged
carrier complex (11).   This was tested by means of an antibiotic, non-
actin, which forms a positively charged carrier complex with potassium
ions.   The amount of nonactin used was 3 M/L.   This gave a base con-
ductivity at least a factor of ten higher than that of the PCP system,
which insured that the membrane conductivity due to the PCP could be
neglected.

The data for pH 6 and pH 7 are shown in Figures 11 and 12.   In
both cases, the membrane conductivity increased markedly with the in-
crease of the uncoupler concentration.

V.   PCP CONCENTRATION GRADIENTS ACROSS THE MEMBRANE

It was expected that a concentration gradient across the mem-
brane would produce a gradient of charge carriers and thus generate
a potential across the membrane.   The experimental results indicate
that even for large PCP gradients (as much as 30 M/L) no significant
membrane potential occurs (see Table II).   The small membrane potentials
seen resulted from the addition of PCP to the electrolyte.   However,
large membrane potential resulted from a pH gradient across the mem-
brane when the PCP concentration was equal on both sides of the membrane.
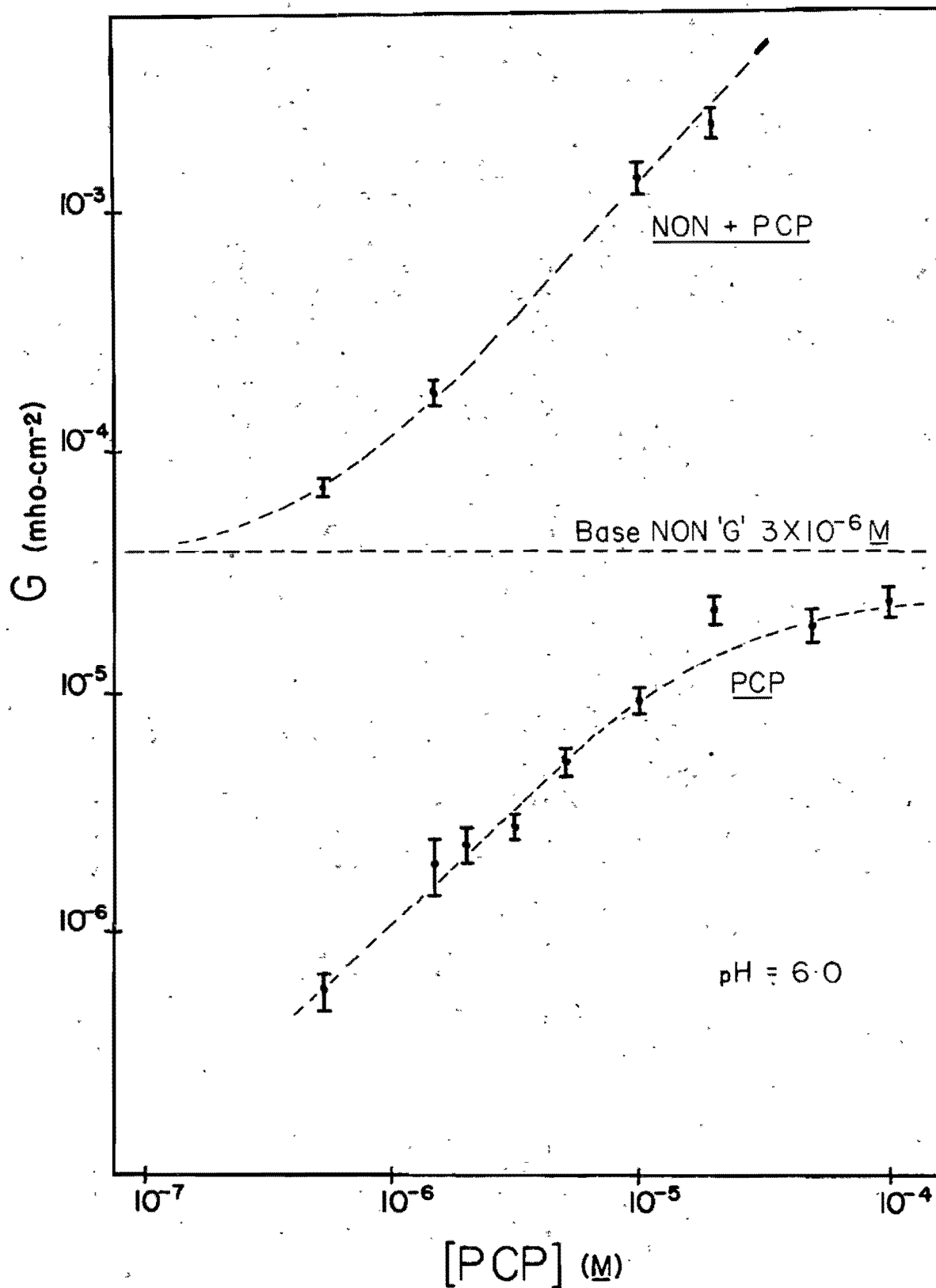
Figure 11. Relationship between conductivity and uncoupler concentration for a constant amount of nonactin at pH 6. Previous PCP data are also shown.
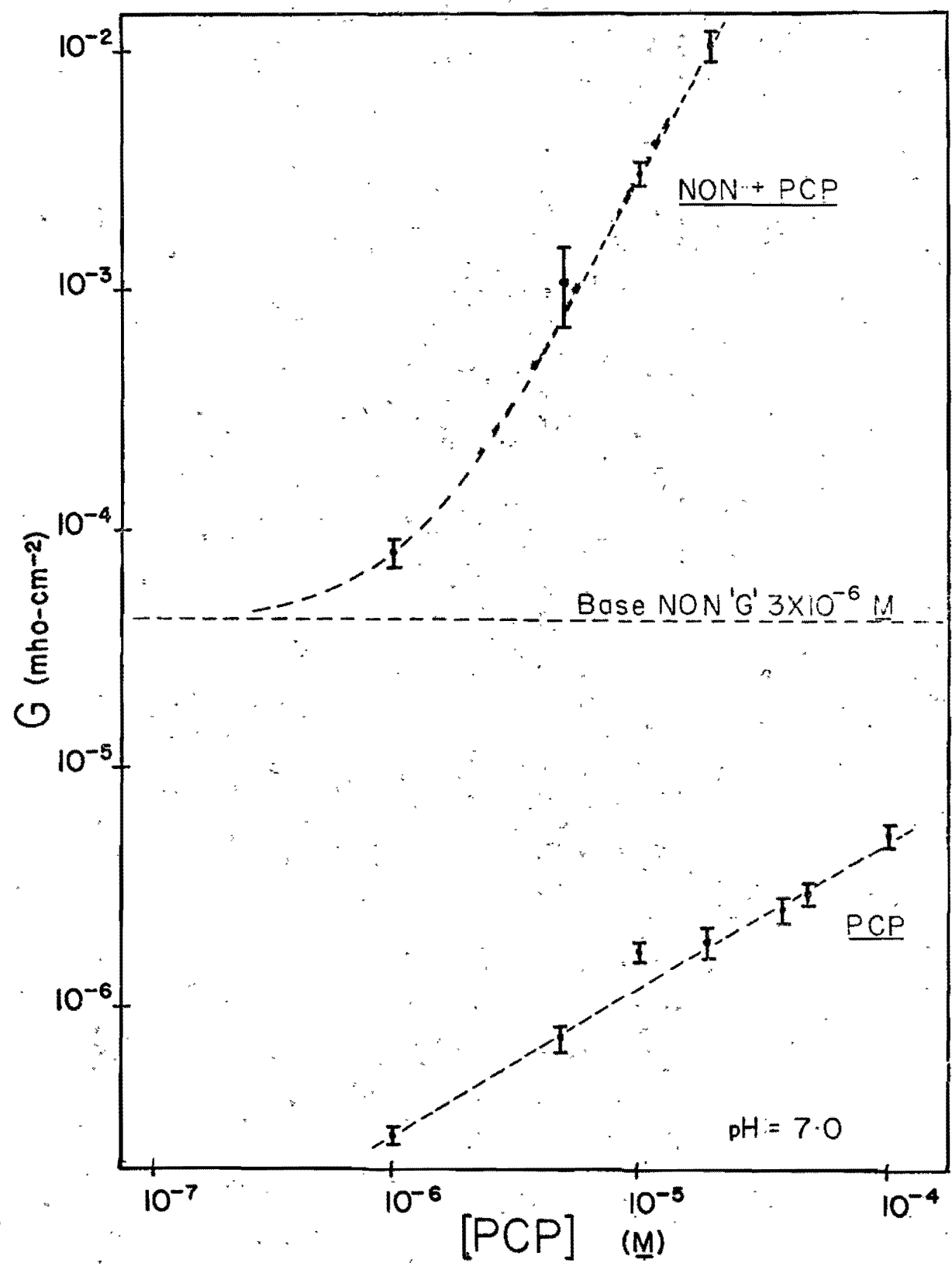
Figure 12. Relationship between conductivity and uncoupler concentration for a constant amount of nonactin at pH 7. Previous PCP data are also shown.

CHAPTER 4

DISCUSSION

There are indications in the literature (11) that according to
their effect on the conductivity of lipid membranes, uncouplers can be
divided into at least two classes, each with a different mechanism of
carrier transport across the membrane.

For the first class the membrane conductance is directly propor-
tional to the anionic form of the weak acid uncoupler. The conductivity
maximum occurs on the alkaline side of the pK, and the current-voltage
curves saturate. The properties of these uncouplers are best explained
in a model developed by LeBlanc (12) for carbonylcyanide m-chlorophenyl-
hydrazone (CCCP). The model assumes that the uncoupler HA and its an-
ion A^- are the only permeant species in the membrane. It can explain
the current saturation and the conductivity maximum in alkaline pH by
taking into account the effect of 'unstirred layers' near the surface
of the membrane.

The conductivity of the second class of uncouplers is proportional
to the square of the uncoupler concentration in aqueous solution. The
maximum conductance occurs at or very near the pK of the uncoupler and
the current-voltage curves do not saturate.

Finkelstein (7) developed a model based on Liberman's data and
postulated the existence on an uncoupler 'dimer' complex, HA_2^-. This
membrane permeable charged species is formed by the combination of

neutral molecular HA with the negative ion A^-, a product of dissociation of HA.

.Several important features of the present experimental results such as the maximum conductivity near the pK of PCP (Fig. 7), the dependence of conductivity on the PCP concentration (Fig. 10), and the curvature of the current-voltage characteristics (Fig. 8), suggest the applicability of the latter model to the present membrane-uncoupler system.

The model is based upon the assumption that the following reactions occur in the aqueous solution:

$$HA = H^+ + A^- \qquad\qquad K_1 = \frac{[H^+][A^-]}{[HA]} \qquad\qquad (1)$$

$$HA_2^- = HA + A^- \qquad\qquad K_2 = \frac{[HA][A^-]}{[HA_2^-]} \qquad\qquad (2)$$
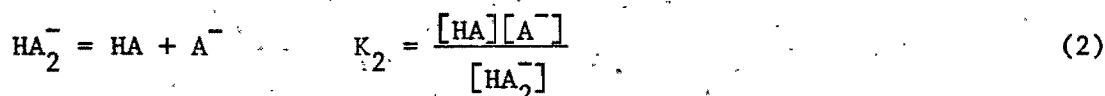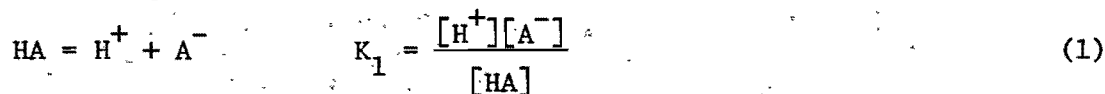
where the brackets denote the concentration of the species in the aqueous solution. Equation (1) is the statement that the uncoupler acts as a weak acid in solution, and equation (2) assumes that the neutral HA molecule combines with an A^- anion to form a charged dimer complex. The concentration of the dimers is assumed to be much less than that of either the A^- or the HA species. Therefore, the total concentration of uncoupler $[HA_T]$, may be approximated by the sum of the HA and A^- concentrations:

$$[HA_T] = [HA] + [A^-] \qquad\qquad (3)$$

Using the above three equations, an expression for the concentration of the dimers in terms of the initial total amount of uncoupler added to the solution $[HA_T]$, the pH of the solution, can be derived. The dimer concentration is derived as follows from equation (1) to (3):

$$[HA_2^-] = \frac{K_1}{K_2} [HA_T]^2 \frac{[H^+]}{(K_1 + [H^+])^2} \qquad\qquad (4)$$

The dimers are assumed to be the major permeant charged species in the membrane. If it is also assumed that the rate limiting step in the transport is the movement of the dimer molecule through the membrane, indicated by an absence of saturation in the current-voltage characteristics, and the density of dimers in the membrane is proportional to that in aqueous phase, the membrane conductance, G, will be directly proportional to the dimer concentration in the electrolyte solution. That is:

$$G \; \alpha \; [HA_2^-]^* \qquad\qquad (5)$$

where (*) denotes the dimer concentration in the membrane. The conductivity can then be related to the uncoupler concentration and the solution pH by substitution of equation (4) into equation (5):

$$G = b \; \frac{K_1}{K_2} \; [HA_T]^2 \; \frac{[H^+]}{(K_1 + [H^+])^2} \qquad\qquad (6)$$

where b is a constant, proportional to the product of the mobility of HA_2^- in the membrane and the partition coefficient of HA_2^- between water and the membrane. For a given uncoupler concentration equation (6) predicts a maximum in the conductivity when the proton concentration is equal to the dissociation constant of the uncoupler, that is pH equal to pK. This is indeed in agreement with the observed result for pentachlorophenol (Fig. 7).

The model (eq. 6) also predicts that if the pH is held constant the conductivity should vary as the square of the uncoupler concentration. As follows from our experimental data, this quadratic dependence is observed for low concentrations of PCP (up to 10μM/L), and is typical for low pH values (Fig. 10). The model in this present state cannot account for the variance of the concentration dependence with pH,

and the observed saturation of the concentration of membrane conduct-
ivity.

Similar effects were observed by Liberman and Topaly (9) for tetra-
chloro-2-trifloromethylbenzimidazole (TTFB), for which the dependence
of conductivity upon uncoupler concentration was found to be linear
at high pH. Also Bielawski (13) and Hopfer (14) who both investi-
gated the effects of dinitrophenol (DNP) found that the conductivity
did not depend upon the square of the uncoupler concentration, but was
somewhere in between a linear and quadratic dependence, in apparent
contradiction with the above model.

McLaughlin (11) was able to resolve the conflict in the existing
data by proposing that the anions of the uncoupler adsorbed on the
surface of the membrane, and charged it negatively. If the surface
potential is taken into account, the concentration of the permeant
HA_2 species in the membrane can be related to the HA_2 concentration in
the solution immediately adjacent to the membrane by:

$$[HA_2^-]^* = K[HA_2^-] \exp (F\Psi/RT) \tag{7}$$

where the asterisk (*) denotes concentration of HA_2^- within the mem-
brane, F is the Faraday, Ψ is the potential due to surface charge on
the membrane, and K is the membrane-aqueous phase partition coefficient
for the dimer. Thus, the conductivity of the membrane in the presence
of surface charge is:

$$G = bK \frac{K_1}{K_2} [HA_T]^2 \frac{[H^+]}{(K_1+[H^+])^2} \exp (F\Psi/RT) \tag{8}$$

The deviation in the uncoupler concentration from the above quad-
ratic dependence at high pH can be explained by changes in the mem-
brane surface charge (11). The adsorption of the A^- ion to the mem-

brane surface would depress the conductance of negatively charged

carriers, and enhance the conductance of positively charged carriers.

The idea of using the positively charged nonactin potassium complex to

probe the negative surface charge on the membrane has been exploited

by McLaughlin (11). The membrane conductivity due to this complex is

given by:

$$G = b' \; [K^+][NON] \; \exp \; (-F\Psi/RT) = G_o \; \exp \; (-F\Psi/RT) \qquad (9)$$

where b' is a constant which includes the membrane-solution partition

coefficient for the complex and the association constant for the com-

plex formation; and Ψ is the surface potential of the membrane (11).

In the experiment when nonactin was added to the PCP system, the con-

ductivity increased with increased PCP concentration (see Figures 11

and 12). If the membrane was not charged in the presence of penta-

chlorophenol, the conductance of the nonactin-PCP system would be the

sum of the conductivity due to each component alone. However, if the

membrane was charged we can use equation (8) to find the numerical value

of the surface potential on the membrane at each uncoupler concentra-

tion. If G_o is the conductivity at zero charging potential, which is

experimentally the base conductivity of the nonactin complex in the

absence of PCP, we can then obtain a numerical value for exp $(-F\Psi/RT)$

and the membrane charging potential, Ψ, by finding the ratio of G to

G_o for each data point. Tables III and IV show the analysis of the

data for the PCP-NON system at pH 6 and 7.

If we now correct the previous data of the conductivity versus un-

coupler concentration for these pH's by multiplying the original data

points by the appropriate value of exp $(-F\Psi/RT)$, we find that the

TABLE III

ANALYSIS OF NON-PCP DATA TO DETERMINE
MEMBRANE CHARGING AT PH 6.0

PCP (μM/L)	G/G_o	Ψ (mV)	G' (mho $\cdot$ cm^{-2})	G^* (mho $\cdot$ cm^{-2})
0.5	1.77	-14.8	5.9×10^{-7}	1.0×10^{-6}
1.5	4.19	-37.0	2.0×10^{-6}	8.4×10^{-6}
10.0	36.3	-92.8	9.5×10^{-6}	3.4×10^{-4}
20.0	55.2	-103.8	2.2×10^{-5}	1.2×10^{-3}

TABLE IV

ANALYSIS OF NON-PCP DATA TO DETERMINE
MEMBRANE CHARGING AT PH 7.0

PCP (μM/L)	G/G_o	Ψ (mV)	G' (mho $\cdot$ cm^{-2})	G^* (mho $\cdot$ cm^{-2})
1.0	1.95	-17.3	2.9×10^{-7}	5.7×10^{-7}
5.0	25.6	-83.8	1.0×10^{-6}	2.6×10^{-5}
10.0	74.4	-111.3	1.7×10^{-6}	1.3×10^{-4}
20.0	256	-143.4	1.9×10^{-6}	4.9×10^{-4}

G^* is the original conductivity (G') multiplied by the appropriate exp
$(-F\Psi/RT)$ to correct the conductivity for the adsorbed charge.

corrected data fit the predicted quadratic relationship of conductivity upon uncoupler concentration, as shown in Figures 13 and 14.

Additional information about the membrane transport processes can be obtained from the membrane potential data. According to the above model, if the permeant charged species are the postulated dimers HA_2^-, then the membrane potential, Vm, is determined by:

$$Vm = -\frac{RT}{F} \ln \frac{[HA_2^-]_1}{[HA_2^-]_2} \qquad (10)$$

where $[HA_2^-]_2$ and $[HA_2^-]_1$ are the concentrations of dimers on opposite sides of the membrane. Using equation (4) one can show that membrane potentials can be generated by concentration gradients of PCP, in contradiction with the experimental results (see Appendix A).

This disagreement with the observations can be removed if it is assumed that in addition to the HA_2^- dimers, the membrane is permeable to neutral molecules of pentachlorophenol, HA. It follows from equations (10), (1) and (2) and the assumption for constant concentration of neutral molecules across the membrane that:

$$Vm = -\frac{RT}{F} \ln \frac{[H^+]_2}{[H^+]_1} \qquad (11)$$

(see Appendix B). Thus, the membrane potential is produced only by the pH gradient and not by the gradient of PCP, which was found in the experiment. It follows from equation (11) that even though the transported species are negatively charged dimers, the membrane potential is the same as if it were produced by the transport of protons. The pH studies done with PCP, Table II, are in agreement with equation (11).

The current-voltage characteristics of many membrane-uncoupler systems have been found to be non-linear (11). It is of considerable interest to establish the origin of this nonlinearity. In our case, the
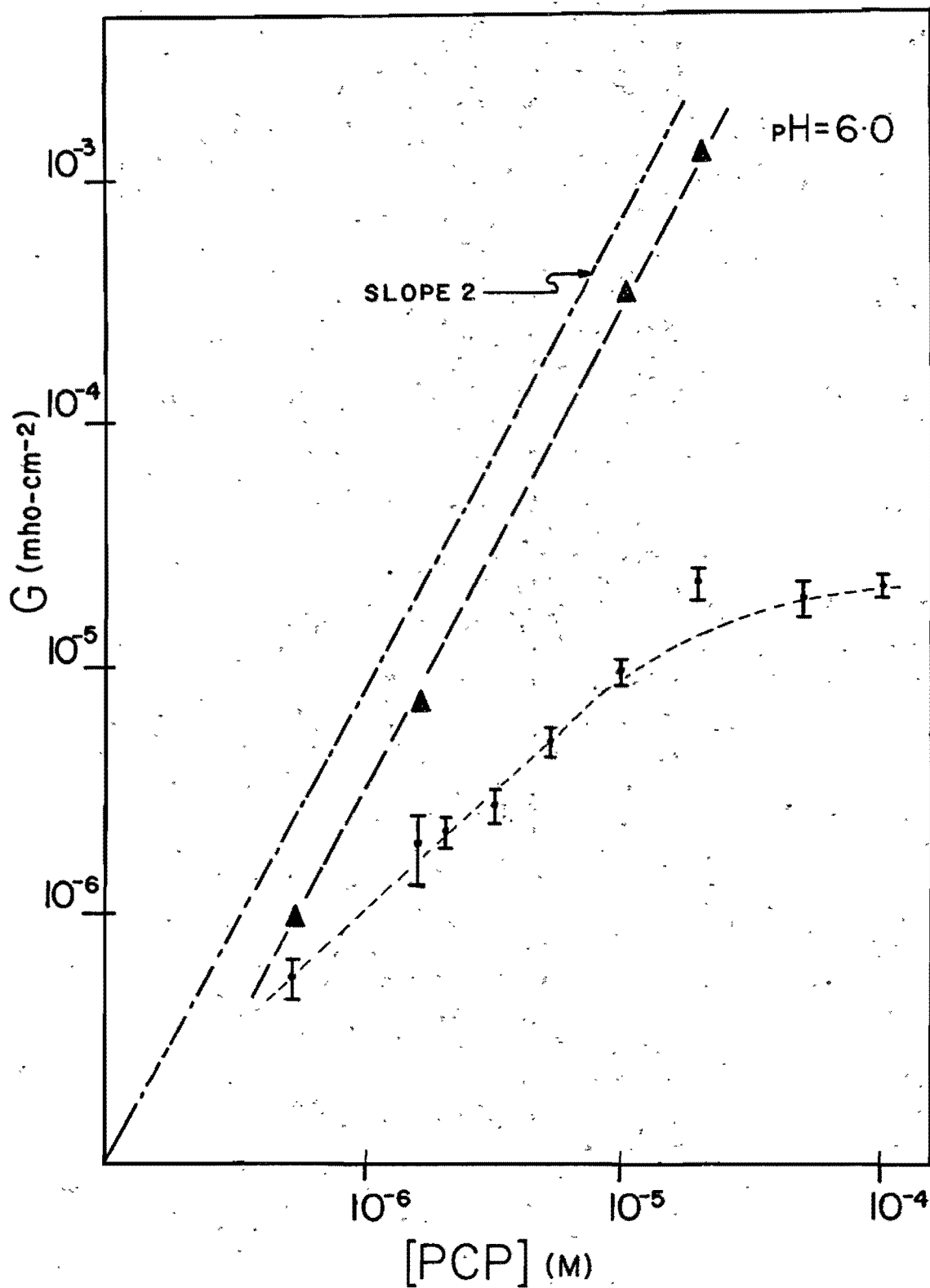
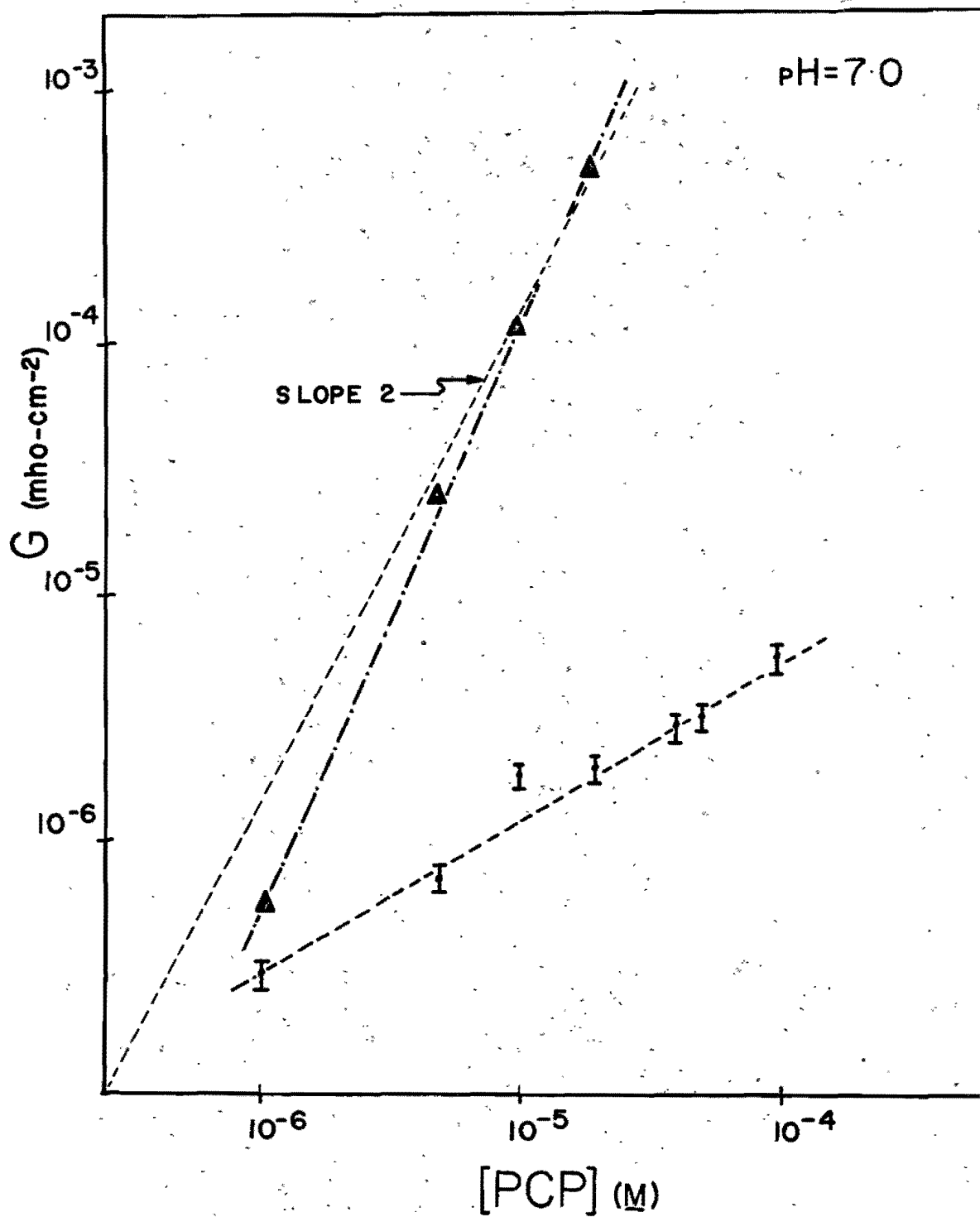Figure 13. Corrected conductivity versus uncoupler concentration data for pH 6. (▲)

Figure 14. Corrected conductivity versus uncoupler concentration data for pH 7. (▲)

current increases at a faster rate than the voltage, giving rise to a
superlinear current-voltage curve (Figure 8). There are two transport
models which are able to explain the upward curvature of the current-
voltage curves. In one model the membrane is considered to be a po-
tential energy barrier for the transported ions. According to Hall
et al (15) the current density for this type of transport is given by:

$$J = A \frac{C_1 \exp\left(\frac{ZeV}{2KT}\right) - C_2 \exp\left(\frac{-ZeV}{2KT}\right)}{\int_{-1}^{1} \exp\left(\frac{w(y)}{KT} - \frac{ZeV}{2KT} y\right) dy} \qquad (12)$$

where C_1 and C_2 are concentrations of the transported species at the
right and left membrane interfaces, Z is the ion valence, V the applied
potential, w the position dependent potential energy, and y the dimen-
sionless distance ($y = 2x/d$) from the central plateau of the membrane,
and d the membrane thickness. This model is best able to predict the
membrane current-voltage curvature when the membrane barrier is approx-
imated by a barrier of trapezoidal form.

The second model proposed to explain the current-voltage dependence
is based on Eyring's kinetic treatment of the membrane (16). The mem-
brane is again considered as a barrier; however, the transportation is
in the form of "jumping" from one side of the barrier to the other.
In this case, the net current is proportional to the difference of the
ion fluxes from left to right, and right to left. The current density
can be written as:

$$J \propto C_1 \exp\left(-\frac{wo}{KT} - \frac{ZeV}{2KT}\right) - C_2 \exp\left(-\frac{wo}{KT} + \frac{ZeV}{2KT}\right) \qquad (13)$$

where wo is the barrier height in the central plateau region of the
membrane. Bruner (17) modified this model by proposing "binding sites"
on the membrane interfaces which must be available in order for the

permeant species to "jump" across the membrane barrier.

Comparison of the experimental current-voltage curves with those predicted from the above models indicate that the second model, with the modification proposed by Bruner, is the most adequate (18). Thus, the nonlinearity of the current-voltage curves, and therefore, the voltage dependent conductivity induced in the membrane in the presence of pentachlorophenol has a simple physical explanation. The effect of saturation in the conductivity with increased uncoupler concentrations (Figure 10) can be explained by a limited number of binding sites available at the membrane interface.

In summary, the ion transport in the presence of PCP begins with the formation of the dimer complex in the aqueous solution immediately adjacent to the membrane. These dimers then partition between the aqueous solution and the membrane, possibly to binding sites on the membrane. The charged complexes are driven across the membrane by the applied electric field. At the other membrane interface they are either released or dissociated; the neutral molecules may then diffuse back through the membrane and become available for further binding with the anions of the uncoupler. As the current-voltage curves indicate, the rate-limiting step in the transport process is the electrodiffusion of the dimer complex from one side of the membrane barrier to the other.

CHAPTER 5

CONCLUSIONS

The effects of the uncoupler pentachlorophenol upon the electrical characteristics of lipid bilayer membranes has been studied. It has been determined that the conductivity depends upon the square of the uncoupler concentration, until saturation is reached at about 50μM/L. The conductivity depends upon the solution pH for a fixed uncoupler concentration, the maximum occurring at a pH equal to the pK of the uncoupler. A membrane potential does not occur in the presence of a PCP gradient, but does appear if a gradient of pH is formed across the membrane. The current-voltage characteristics of the membrane do not saturate, with the greatest curvature at both high and low pH, the least curvature at the pK of the uncoupler. On the basis of the above data the mechanism for charge transport can be explained by the assumption that the neutral uncoupler molecules act as carriers for the dissociated uncoupler anions, the permeant species being the HA_2^- dimer.

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APPENDIX A.

If the dimer concentrations on either side of the membrane are not equal, then a potential should develop across the membrane according to:

$$Vm = -\frac{RT}{F} \ln \frac{[HA_2^-]_1}{[HA_2^-]_2} \qquad (10)$$

where $[HA_2]_1$ and $[HA_2^-]_2$ are the dimer concentrations on opposite sides of the membrane. We can relate these dimer concentrations to the total uncoupler concentration by:

$$[HA_2^-] = \frac{K_1}{K_2} [HA_T]^2 \cdot \frac{[H^+]}{(K_1 + [H^+])^2} = K[HA_T]^2 \qquad (4)$$

Thus, if we substitute this expression of the dimer concentration into equation (10) we obtain:

$$Vm = -\frac{RT}{F} \ln \frac{K[HA_T]_1^2}{K[HA_T]_2^2} \quad \text{or} \quad Vm = -\frac{RT}{F} \ln \frac{[HA_T]_1^2}{[HA_T]_2^2}$$

According to this expression a potential should develop across the membrane in the presence of a gradient in the uncoupler concentration.

APPENDIX B

In order to derive equation (11), let us first start with equation
(10):

$$Vm = -\frac{RT}{F} \ln \frac{[HA_2^-]_1}{[HA_2^-]_2} \tag{10}$$

Now if we use equation (2) and substitute the expressions for $[HA_2^-]$ into
equation (10), we obtain:

$$Vm = -\frac{RT}{F} \ln \frac{[HA_1]_1[A^-]_1}{[HA_2]_2[A^-]_2} \cdot \frac{K_2}{K_2} \tag{A}$$

If we now substitute the values of A^- as defined by equation (1), we
obtain:

$$Vm = -\frac{RT}{F} \ln \frac{[HA]_1^2/[H^+]_1}{[HA]_2^2/[H^+]_2} \tag{B}$$

And since the uncoupler concentrations are equal on each side of the
membrane equation (B) becomes:

$$Vm = -\frac{RT}{F} \ln \frac{[H^+]_2}{[H^+]_1} \tag{11}$$