THE EFFECTS OF GASEOUS ANESTHETIC AGENTS AND WATER VAPOR ON THE ELECTRICAL CONDUCTIVITY OF LIPID COATED PROTEINS

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ABSTRACT

THE EFFECTS OF GASEOUS ANESTHETIC AGENTS AND WATER VAPOR ON THE ELECTRICAL CONDUCTIVITY OF LIPID COATED PROTEINS

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When chloroform and water are mixed in a single container, and their vapors are passed into a chamber containing a dry protein coated with a petrolatum film, the electrical conductance of the sample is found to rise by approximately six orders of magnitude in two hours. If, however, the liquids are placed in separate vessels, vaporized, and the vapors passed into the chamber, the conductance of the dry, petrolatum coated protein only increases by about two orders of magnitude.

This finding is explained by two hypotheses which are tested, and subsequently one is presented as being better in suitably explaining the data. One hypothesis explains the current increase as caused by a union between water and chloroform molecules which is able to penetrate the coating. The other suggests that the effect is dependent only on the partial pressure of water with the chloroform affecting the petrolatum layer in some way, possibly by changing the viscosity, to allow the water to diffuse to the protein. This second hypothesis is advanced as the more tenable.

These large conductance increases are caused by the adsorption of water on to the protein which is known to be a semiconductor. This is thought to change the bulk dielectric constant of the material and result in a lowering of the energy ("activation energy") needed to separate the positive protein ion and the electron. Several experiments show that the larger the dielectric constant of the adsorbed material, the larger will be the conductance increase.

A postulated mechanism of anesthetic agents is that they act to

bring water into the insulating myelin which surrounds the nerve cell. A change in the insulating properties of the myelin could easily change the electrical characteristics of the action potential; in particular, it would lead to a more rapid attenuation(internodal)of the action potential. These attenuations would then be the initial steps in a train of events ending in narcosis.

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By

Michael Robert Powell

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To My Wife and Parents

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HISTORICAL

Anesthesia has been defined as a loss of consciousness and an insensibility to pain; it is a state in which reflex action is lost, and a sleep from which one can not be awakened. The methods and drugs for inducing this state are many and varied. They comprise examples from almost every class of chemical compound with examples of almost every functional group. The trenchant idea of this statement is best exemplified in the case of xenon which has been shown to produce narcosis in man at 0.7 atm. (1). This material is not known, or expected, to have any chemical reactivity in physiological conditions.

For all of our knowledge of drugs and "materia medica", the mode of action is as yet unknown at the cellular and the molecular level for all but a few of them. Narcotic agents were some of the first drugs to be investigated in a rational and systematic way.

General anesthesia was independently discovered in 1845 by Morton and Long in the United States. Ether, chloroform and nitrous oxide were the agents known at that time for inducing narcosis in a person. The first theory of anesthesia proposed was that of Bibra and Harless in 1847. They suggested that brain lipids were dissolved by the anesthetic agents and deposited in the liver. A test of this showed that it might be the case as more lipid material was found in the liver following anesthesia than before.

The most widely known of the theories was that of Overton (2) and Meyer (3) who, independently, noted the relationship between anesthetic potency and the oil-water partition coefficient. The most potent anesthetics were those which had large coefficients. This tendency has, in recent times, been used to explain the transport of anesthetic agents

to the brain.

Other theories have been advanced but to a much lesser degree. The surface tension theory of Taube (4) in 1904 had many exceptions, notably chloroform, to the rule that agents which lower the surface tension of water also produce the anesthetic state. Changes in membrane permeability [King, 1930 (5), Winterstein, 1915 (6), Clements and Wilson, 1964 (7)] and changes in the colloid character of the cellular protein [Bernard, 1875 (8), Bancroft, 1931 (9), Ebbecke, 1936 (10)] have also been submitted as explanations of anesthetic action.

The thermodynamic theories of Ferguson in 1939 (11), Butler in 1948 (12), Brink and Posternak in 1948 (13) and Mullins in 1954 (14) have shown that the anesthetic compounds have the same thermodynamic activity at a given level of anesthesia (usually defined for the third stage of the third plane). While the researchers have shown this to be true, they have not been able to advance a convincing and proven argument as to why this concentration of drugs results in narcosis. Thoughts have generally developed around the increased impedance of the membrane to the passage of ions, or the displacement of aqueous material from the non-aqueous phase.

In 1961, papers by Pauling (15) and Miller (16) appeared which treated narcosis as an interaction of the narcotic agent with the water (or fluid) of the brain. Small microcrystals of water, stabilized by the anesthetic drug, were presumed to interfere with the flow of ions necessary for electrogenesis, or they were thought to block active sites of enzymes in some way. Both theories placed most of the effect at the synapses. The temperature dependence predicted by both theories has been checked in anesthetized goldfish and found to be that which was expected (17).

INTRODUCTION

This study has been initiated to unlock the "molecular door", as it were, and to push it open a small way. It started with a simple idea: That electrical current loss in nerve systems can result in an attenuation of the signal if the time of action and the current path were of sufficient length. This loss could come from leakage of current through a previously insulating membrane. Basic testing of this principle is the subject of this thesis.

In this study, the phospholipid membranes which surround the nerve axons were thought to be the point of attack by the anesthetic agents. The myelin of the Schwann cells in the so-called "myelinated nerves" acts passively as an electrical insulator to prevent the rapid attenuation of the action potential as it passes from node to node. Even in the axons not possessing nodes, a thinner but substantial covering of neurolemma determines the spread of the electrotonic currents thought responsible for the initiation of electrogenesis.

If in a nerve fiber with core radius r and specific resistance σ, a sheath of thickness $\pi \gg r$ and specific resistance $\bar{\sigma}$ is wound, it can be shown that i, the current flowing longitudinally in the fiber, will vary with the distance x as

$$i_{(x)} = i_o e^{-\alpha x}$$

where

$$\alpha = \left[\frac{\beta + 1}{\beta} \quad \frac{2\sigma}{\pi \bar{\sigma} r} \right]^{1/2}$$

and β is the ratio of the resistance of a unit length of core to that of a unit length of the outside sheath.[18] For the potential at any point inside the core, a similar expression is true, namely,

$$v(x) = v_o e^{-\alpha x}.$$

Thus, it can be seen that reducing the resistivity of the insulating sheath results in a reduction of the height of the "action potential" at a distance from the point where electrogenesis is occurring. The propropagation of the nerve impulses would especially be affected in the fibers where saltatory conduction was occurring.

This change in the resistivity of the myelin could be caused by the introduction of water into the phospholipid-protein layers. Small amounts of water, as later will be shown, will markedly change the resistivity of proteins and other biological materials.

Semiconduction, the increase of conductance with increasing temperature (as opposed to conductors whose resistance increases with increasing temperature), has been shown to occur in many biological materials [hemoglobin (19), serum albumin (20), cytochrome C (21), wool (22), gelatin (23), chloroplasts (24), rods of the retina (25), and collagen (26)]. The increase of conductance with increasing hydration was first studied extensively by Murphey and Walker (27), and it has been recently reviewed by Eley (28) and Rosenberg (29). It has been shown by Maričič, et al. (30) and Rosenberg (31, 32) that at low percentages of adsorbed water, conduction is caused by electronic rather than ionic processes. This means that water bridges, water channels, or the movement of ions is not necessary.

In a theory advanced by Rosenberg (33), the increase in conductance of a biological semiconductor with hydration is caused by a change in the bulk dielectric constant of the material. In the study he showed that the activation energy for semiconduction decreased with added water; this resulted in greatly increased conductances with only slightly hydrated

proteins.

From Baxter's equation, found to hold for a protein (wool)

$$i = i_o \exp(-E/2kT),$$

and King and Medley's equation

$$i = I_{dry} \exp(\alpha m),$$

a combined equation can be written:

$$i = i_o \exp[(-E_{dry} + \alpha m 2kT)/2kT].$$

The justification for this comes from the observation that while the slope changes with hydration (in a plot of log i vs. 1/T), the intercept (which is i_o) does not. Making the substitution $\beta = \alpha 2kT$,

$$i = i_o \exp[(-E_{dry} + \beta m)/2kT],$$

we get

$$i = i_o \exp[-E_{wet}/2kT]$$

by letting

$$E_{wet} = E_{dry} - \beta m.$$

The energy needed to separate an electron from a positive molecule, and place it on another like, dry molecule is

$$E_{dry} = I - A - e\phi,$$

where I and A are the "ionization potential" and the "electron affinity", respectively, and ϕ is the electrostatic potential on an electron from the induced or oriented dipoles. This ϕ reduces the energy to separate the charges because the electron now finds itself in a more favorable environment, and this lowers its energy. If the medium is treated as a continuum (34), ϕ is given by

$$\phi = [e/r][1-1/K]$$

where r is the radius of polarization, and K is the dielectric constant of the dry protein. If the dielectric constant of the medium changes,

as by the addition of water, ϕ is now

$$\phi' = [e/r][1 - 1/K'].$$

We could now write

$$E_{wet} = I - A - e\phi'.$$

Subtracting E_d from E_w gives

$$E_w = -e[\phi' - \phi] + E_d$$

$$= [-e^2/r][(1/K) - (1/K')] + E_d.$$

Recalling that $E_w = E_d - \beta m$, then

$$-\beta m = [-e^2/r][(1/K) - (1/K')]$$

and

$$i = i_o \exp-\left\{ E_d-(e^2/r)[(1/K) - (1/K')]\right\} /2kT$$

or

$$i = i_o \exp[-E_d/2kT]\cdot\exp\left\{ [e^2/2kTr][(1/K)-(1/K')] \right\}.$$

The change in the dielectric constant K' leads to an increase in the current i which can pass through a protein under a given potential. If this occurs in the normally resistive myelin, it can be seen that this resistance would drop and the neurolemmal covering would become less insulating.

In that the current distribution in a nerve is a function of the myelin resistance in an exponential way, and the resistance of the biological materials in the myelin is a function of the amount of adsorbed water in an exponential way, the incorporation of only a small amount of water into the nerve insulation would be expected to change the electrical characteristics of this nerve by an enormous amount.

Using a microbalance to measure the adsorbed dielectric (water and vapors of organic liquids), measurements were made simultaneously of the weight gain and the electrical conductance. It was found that compounds

with low dielectric constants (1-10) caused current increases of three to four orders of magnitude over the dry state conductance, while compounds with larger (25-80) dielectric constants brought about current increases of from five to ten orders of magnitude above the dry state value. Water was, by far, the agent which caused the greatest increase in conductance.

While water is able to effect large current increases in proteins when it is adsorbed, the necessary prerequisite is, of course, adsorption. If a material cannot adsorb a given compound , then there will be no increase in the conductance when it is exposed to the vapors. [This was seen in many experiments where organics which were nonpolar were exposed to hemoglobin, a soluble protein and one with its polar amino acid side chains on the exterior. The nonpolar organics did not adsorb to the polar protein and no current increase was noted.] A protein coated with a very nonpolar substance would not be expected to adsorb water; its conductance would not increase when placed in an atmosphere of water vapor. However, if water vapor and organic vapor were present simultaneously, it was thought that current increases would result in the biological materials if water could now penetrate the coating.

In the preliminary investigations of a molecular theory of general gaseous anesthesia, tests were made only in the vapor phase. It was found that different effects were observed if the organic liquids were mixed with the water and vaporized as opposed to the mixing of these compounds in the vapor phase. In time, two hypotheses were developed to explain this effect. The first hypothesis supposed that the water and chloroform were forming an "association" or "union" of some type, perhaps by hydrogen bonding or by dipole-induced dipole effects. This formed, in effect, a "molecular sandwich" which presented its more hydrocarbon face to the

lipid and thus "trucked" the more polar water through this hydrophobic coating. The second hypothesis explained the effect as being a change in the partial pressures of water and chloroform. The chloroform was thought to modify the lipid coating in some way, perhaps by a change in its viscosity or by the presentation of more polar sites in the coating, such that the water was now ableto penetrate to the protein and be adsorbed on to it.

While associations in the liquid phase have been postulated for many years, little is known of these associations in the vapor phase with the exception of hydrogen-bonded complexes. Munson and Tyndall[35] reported associations of alkali metal ions with water, and Munson and Hoselitz[36] found associations of alkali metal ions with the gases xenon and krypton. These studies were made at pressures of less than one mm. Hg. From studies of the mobility, it was determined that the ratio of water to ion was about 6:1 and rare gas to ion at about 3:1. These associations were most likely of the charge-dipole (ion, water, respectively) and charge-induced dipole (ion, rare gas, respectively) types.

Associations of the type investigated here might possibly be of the dipole-dipole or dipole-induced dipole types of H-bonded. Examples of dipole-induced dipole compounds, or Van der Waal compounds, are seen in the inert gas chlathrates[37]. As is known, the polarizability of a molecule, the tendency to form induced dipoles, is expressed in the form of the molar refractivity or the Van der Waal's constants. Polarizability accounts for many physical phenomena observed, and many relation-

ships between the anesthetic potency of compounds and physical parameters have been reported on in the past[15, 16, 38, 39, 40]. In general, it is noted that the more polarizable a molecule, the lower the partial pressure needed for anesthesia (third plane of the third stage) must be.

In more biological systems (e.g., living dogs), complexes of the rare gases have been noted[41, 42]. These were found while investigating anesthesia caused by the rare gases. They have some importance in the transport of the gases to the various parts of the body during narcosis.

EXPERIMENTAL...

The proteins used in this study were bovine hemoglobin and bovine collagen. The hemoglobin was twice recrystallized, salt free and 100% electrophoretically pure; no further purification steps were taken. The collagen was in the form of strips (Ethicon Corporation, Somerville, New Jersey) with the dimensions 2cm long, 0.2 wide, and 5×10^{-3} cm. thick. The collagen was extracted from the Achilles tendon and reconstituted in the form of a tape or strip.

The organic liquids were all of reagent grade and had been dehydrated by standing several days over calcium chloride or anhydrous sodium sulfate. The chloroform was distilled over sodium metal in a nitrogen atmosphere for one of the tests; no difference was noted from chloroform dried only with calcium chloride so the procedure was not repeated in subsequent trials. The stabilizing alcohol in the chloroform was removed by the calcium chloride.

The petrolatum used in making the films was commercial "petroleum jelly" ("Vaseline"). No steps were taken to purify it further. The paraffin was the commercial form used in home canning to cover foods for sterilization.

Nitrous oxide, reagent grade, was obtained from the Matheson Company.

The flow gases used were nitrogen and helium. Both were dry and of high quality. Tests made using either gas showed no difference in effect. Thus, it was concluded, the effect was not a function of the carrier gas.

The conductance as a function of the weight of adsorbed compound was measured in the equipment shown in figure I. The main component was a Cahn electrobalance; the chamber was sealed and the vapors were led in through ports. The samples consisted of hemoglobin powder which was

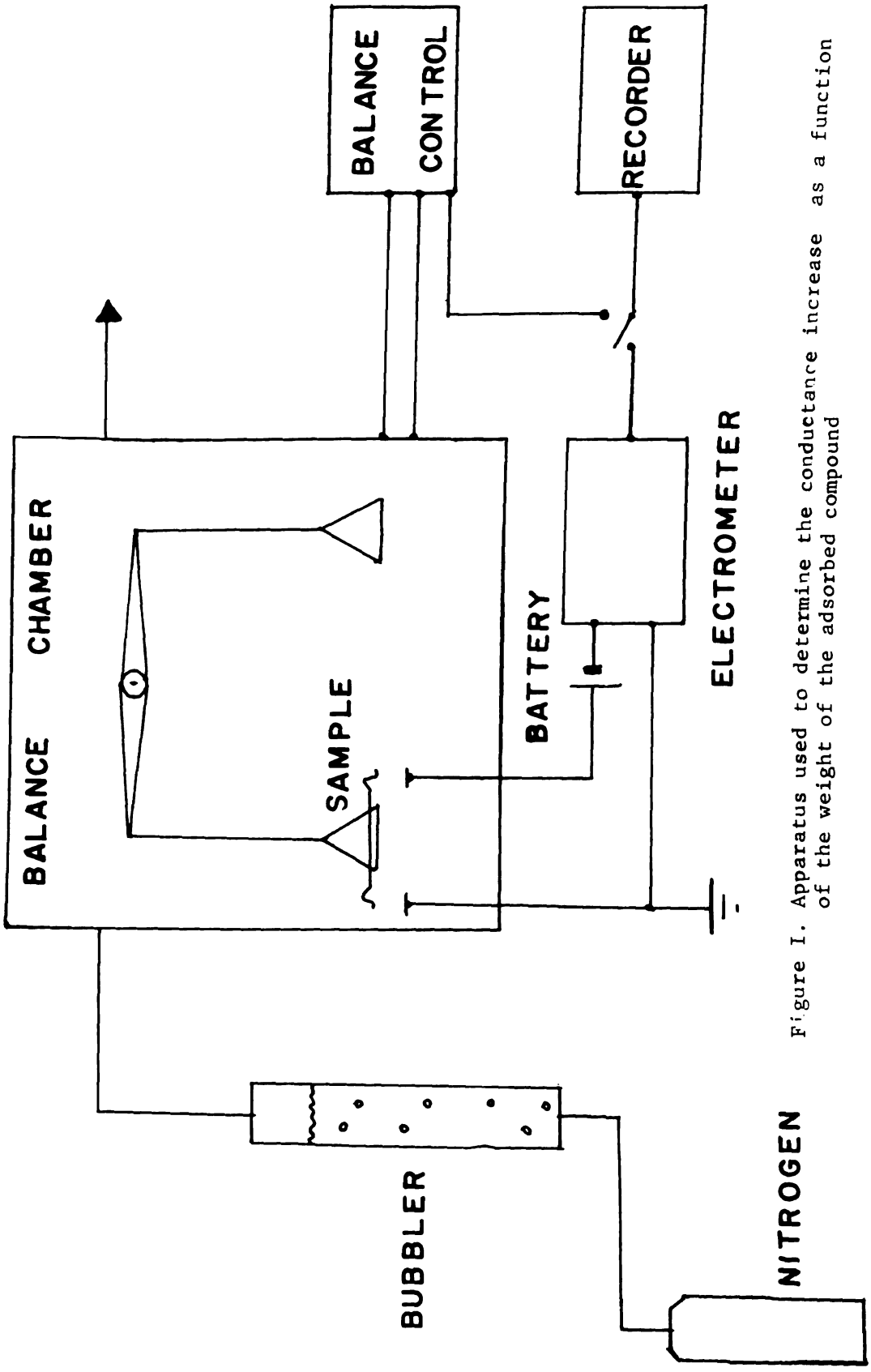

Figure I. Apparatus used to determine the conductance increase as a function of the weight of the adsorbed compound

pressed into pellets; gold leaf electrodes, attached with conducting epoxy, made contact to aluminum electrodes on the floor of the weighing chamber. By lowering the balance arm, the electrical leads on the sample made contact with the electrodes of the balance and the conductance could then be measured with an electrometer. Essentially simultaneous measurements could then be made of the current and the sample weight.

The increase in current with adsorbed compounds (water and the vapors of organic liquids) is plotted in Figure II. It should be noted that only those compounds with large dielectric constants gave large increases in the current. (The figures in parenthesis refer to the dielectric constant at 25°C.)

The apparatus for detecting the current increases with anesthetics and water was constructed of glass tubing with short rubber connections. Gas (nitrogen, helium, or nitrous oxide) leaving the cylinder passed through flow meters which were graduated to read flows of from 10 to 150 cc./min., Figure III.

The water and the organic liquids were held and vaporized in gas washing bottles. The gas was dispersed into small bubbles by a fine glass frit before entering the liquid. The depth of the liquid was always at least two inches deep. The bottles were thermostated at 20°C. in a water bath. This temperature, being lower than the ambient, prevented the vapors from condensing in the glass tubes.

The gas flow was slow enough so that saturation of the carrier gas by the organic vapors was expected to occur. This was checked in the case of water with an Aminco electric hygrometer and found to be true; the actual relative humidity being about 93%.

The sample chamber was constructed of brass and lined with Teflon,

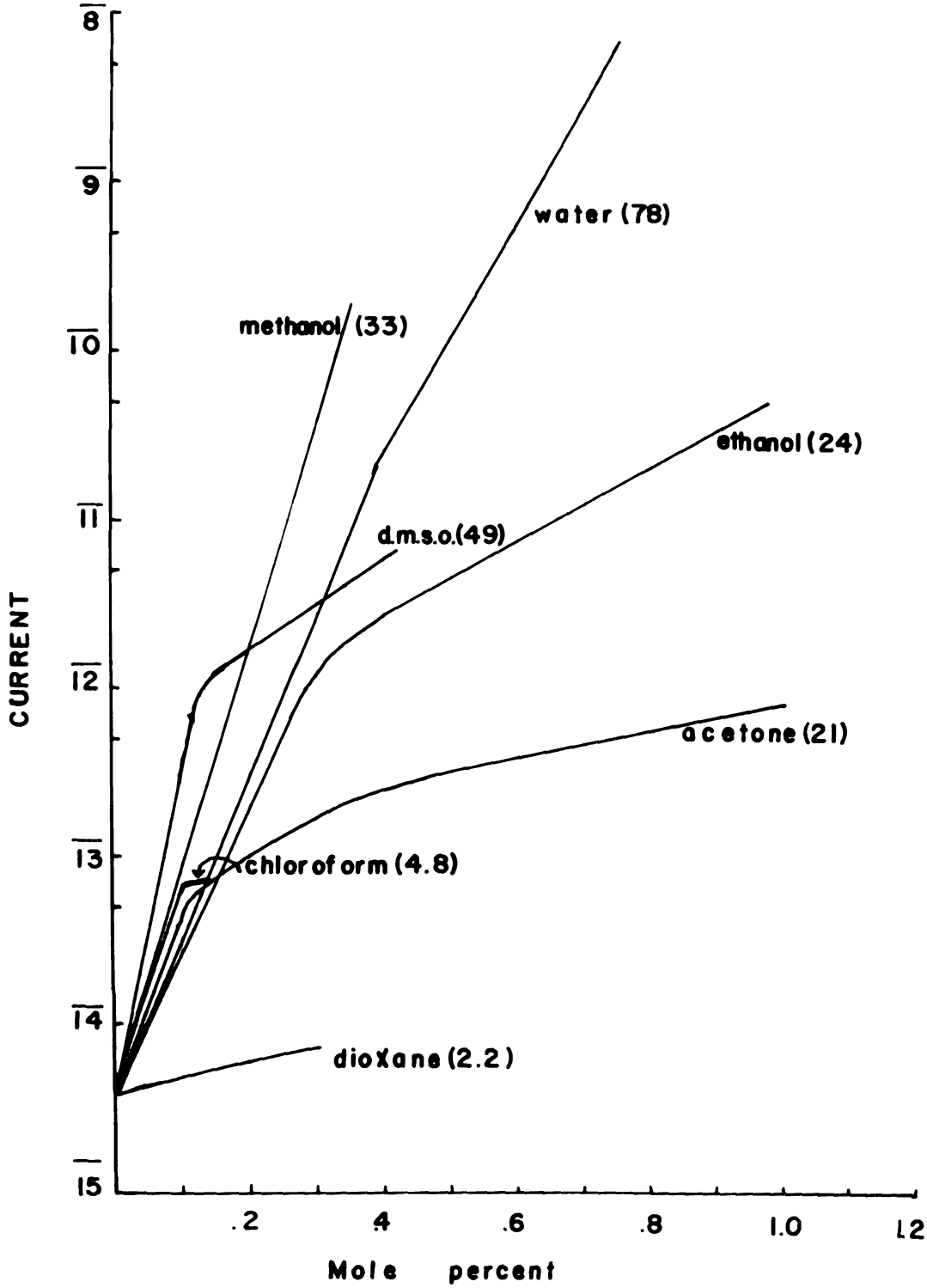

Figure II. Log Current vs. Mole Percent of an adsorbed compound of a given dielectric constant

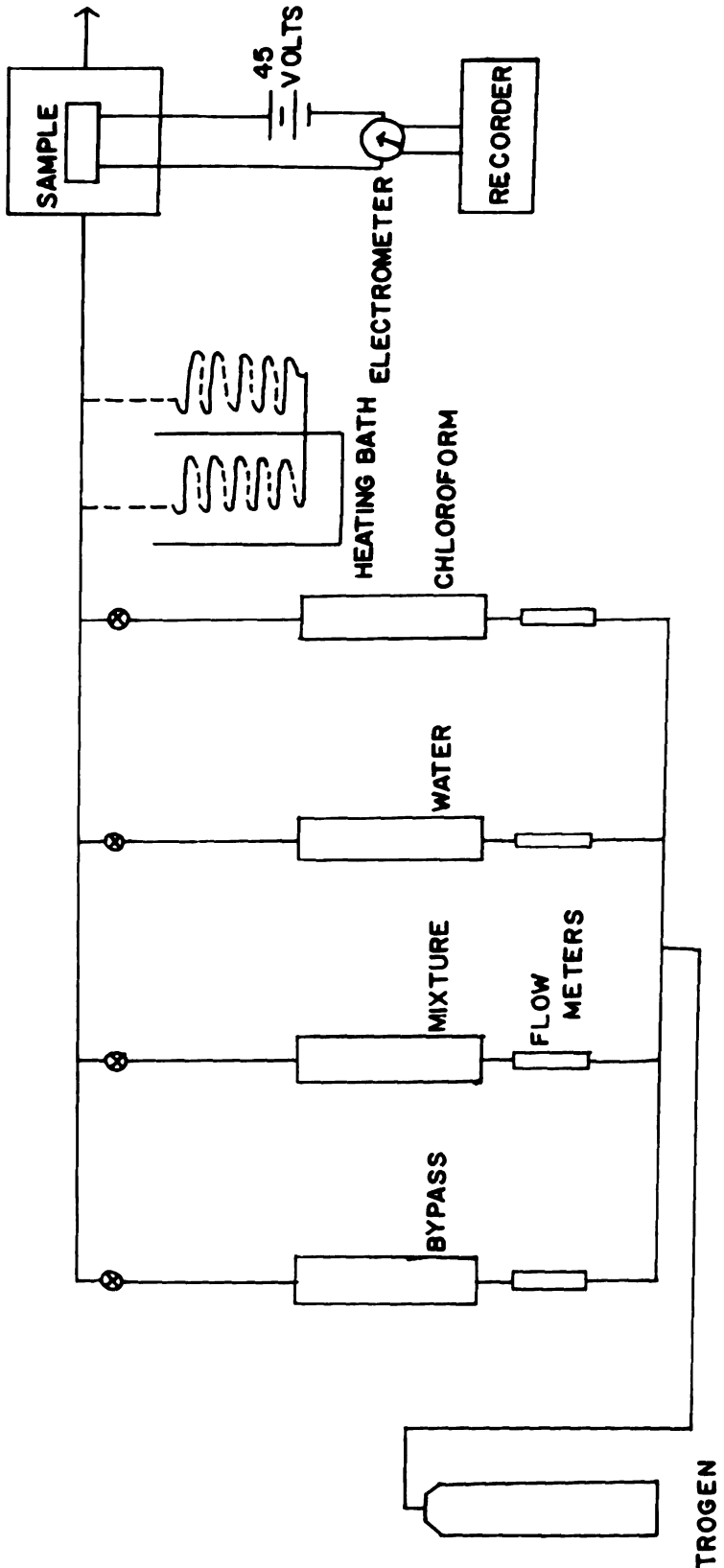

Figure III. Apparatus used to determine the effect of gaseous anesthetic agents and water on petrolatum coated collagen

and the flow gas entered and exited by ports on the opposite sides. The sample of collagen tape was suspended by clip leads in the center of the chamber and these leads also acted as the electrical connections. The potential was applied by a regulated direct current source or by batteries depending on what potential was desired. The electrometer measurements were recorded on a strip chart recorder which plotted current vs. time.

The collagen samples were prepared by soaking them in a saturated solution of petrolatum in chloroform for at least one hour and then air dried at room temperature. Immersion in the solution for only a few minutes did not result in a water resistant coating. Most likely the petrolatum was slow in diffusing into the protein strip or adsorbing onto it. The samples often needed an extra drop of the petrolatum solution after they were affixed to the leads. Apparently some of the coating could be removed in the transfer and clipping process and had to be replaced.

The paraffin solution was made by dissolving paraffin in chloroform. As this material is very soluble in chloroform, a saturated solution was found to be too concentrated and the films resulting from it too thick. Thus it was necessary to dilute and test each sample. This was performed on a trial and error basis as the number of samples prepared with this material were so few that a standard procedure was never developed.

RESULTS

When pure, dry nitrogen was passed into the sample chamber containing a strip of uncoated collagen, the current was seen to fall to about 10^{-13} amperes with an applied potential of 45 volts. This was taken as the current of the dry sample. If the nitrogen was bubbled through water before entering the chamber, the current rose by about six orders of magnitude, curve 1, Figure IV. When chloroform vapors were added in place of those of the water, the current rose by about three orders of magnitude, curve 2. This is in agreement with the values found by measuring the current and weight increases on the balance, Figure II. Mixtures of water vapor and chloroform vapor gave currents similar to those of vapors from the mixed liquids, curves 3 and 4.

If a sample, previously coated with petrolatum, was used in place of the uncoated one, different effects were observed. When water vapor was added, the current rise was very slight, curve 1, Figure V; chloroform caused a greater rise, presumably because it was adsorbed into the petrolatum coat and could diffuse into the protein, curve 2. The chloroform current was approximately the same for the coated and the uncoated samples. If the vapors from water and chloroform were allowed to mix before entering the chamber, it was found that the current rise was less than that of the pure chloroform vapor, curve 3. In none of the experiments was there an indication that current rises, with only the mixture of pure vapors, were very large. In fact, the current obtained was that of the diluted chloroform vapor.

If chloroform and water were mixed together in the same gas washing bottle and the carrier gas passed through, a new effect was noted. Unlike the small current rise effected by the mixed vapors, the current rise from

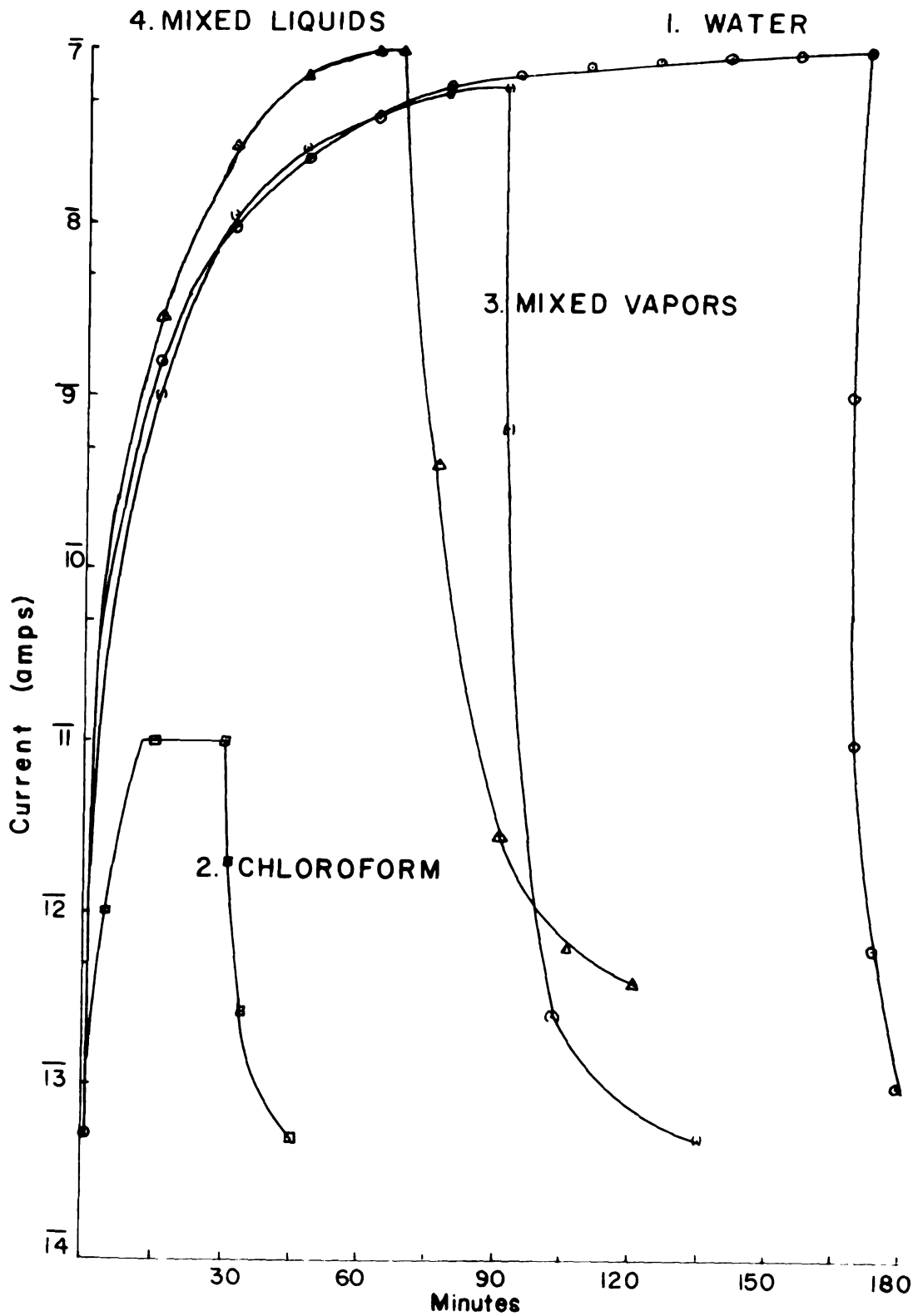

Figure IV. Log Current vs. Time for uncoated collagen exposed to the indicated vapors

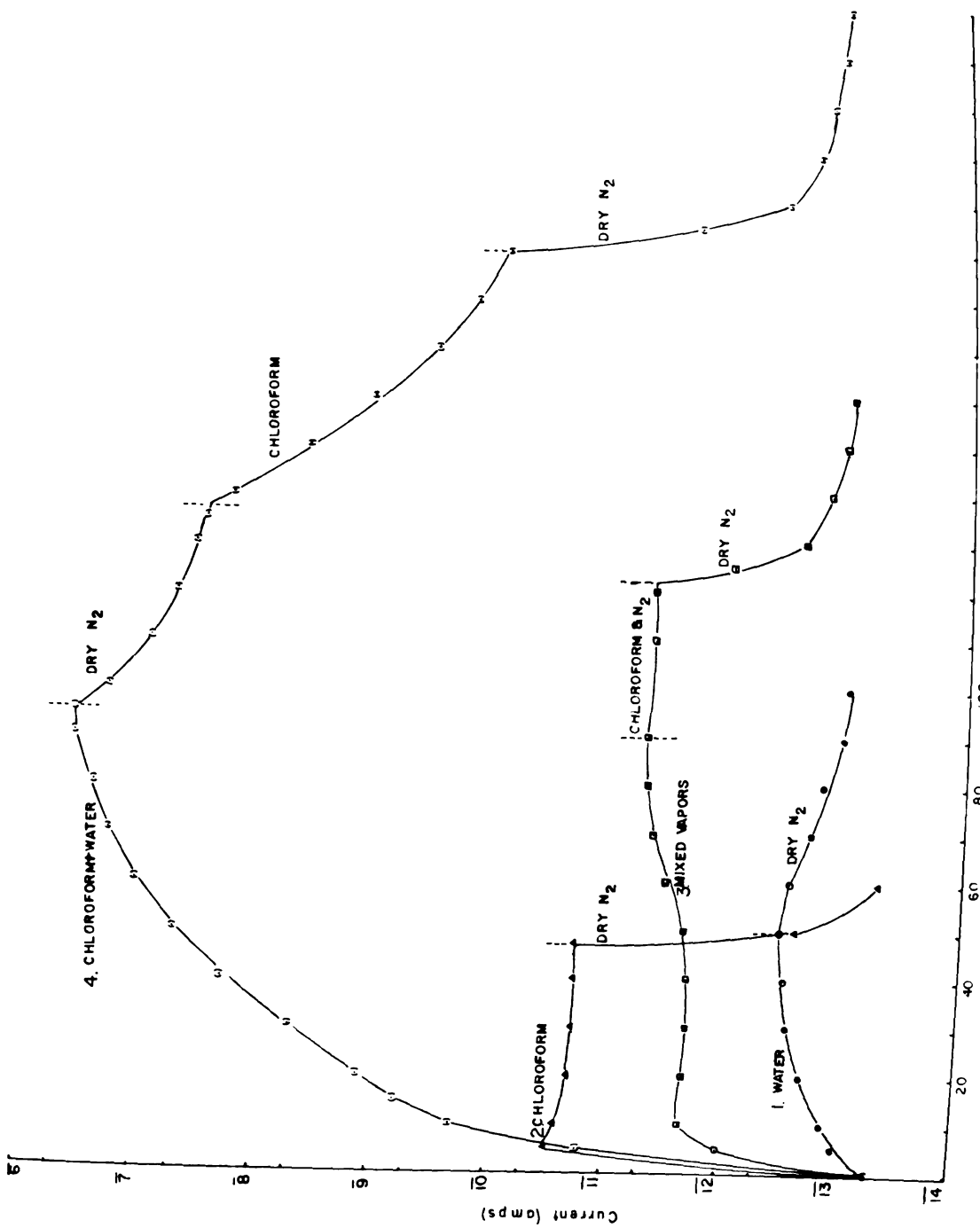

Figure V. Log Current vs. Time for coated collagen exposed to the indicated vapors

the mixed liquids was about six orders of magnitude, curve 4.

The sample could always be dried and returned to its initial current simply by passing dry nitrogen into the chamber when the sample was the uncoated one. However, if a coated sample was used, it was found that it could not be easily dried after it had been exposed to the vapors from the mixed liquids, and the conductance had risen to a high value. In this case, it was found necessary to pass chloroform vapors into the chamber until the current dropped to the "chloroform level", and then to complete the drying with the pure nitrogen.

These reported effects were reversible and repeatable over many cycles of "hydration" and "dehydration", and at no time did it appear that the petrolatum coating was being damaged or being removed by the vapors. Following each cycle, water vapor was passed into the chamber and the current would not rise above the expected small amount of one order of magnitude above the dry state. This was interpreted as implying the integrity of the coating.

A. Possibility of a Critical Concentration:

It was thought that a particular concentration of agents were coming into the sample chamber when the flow gas passed through the liquids mixed in the washing bottle. When extra water vapor or extra chloroform vapor was added to the flow stream (through a small "bleeder" flow), the change in the current was small (the "bleeder" with nitrogen only acted to produce a slight dilution effect on the current levels).

If the first hypothesis, of the "association", was probable, changing the concentration of the vapors coming into the chamber would not greatly affect the equilibrium current levels. This was true because the "association", which was postulated to be responsible for the penetration of the

petrolatum coating, would still be present in the same concentration.

Within the experimental error of the measurements, it could not be conclusively said that no change actually occurred. When water was added to the vapor from the mixed liquids, the current rose slightly. If it were not for the experimental error inherent in the samples, this could be taken as evidence for the second hypothesis, that of the chloroform modifying the lipid coating.

B. Duplication of Vapor Ratios and Concentrations:

When the ratios of water vapor to chloroform vapor were duplicated by adjusting the nitrogen flow through each of the pure liquids, the large current increase was not seen even though the effluent contained the molecules in the proper ratio. The chloroform vapors were also passed into rapidly flowing water so that the effluent contained the vapors in their normal vapor pressures. However, the concentration of chloroform in the water was never allowed to build up. It was found that the large current rise with time was not observed until the water flow was stopped, and the concentration of chloroform in the water reached saturation (Figure VI).

However, it was later found that the chloroform was dissolving in the rapidly flowing water. The effect of the current rise could be explained by hypothesis two (chloroform modifies the petrolatum coating) in that the concentration of the chloroform has decreased. Hypothesis one would explain this observation by saying that the chloroform concentration in the water was not sufficient to form any of the postulated "association".

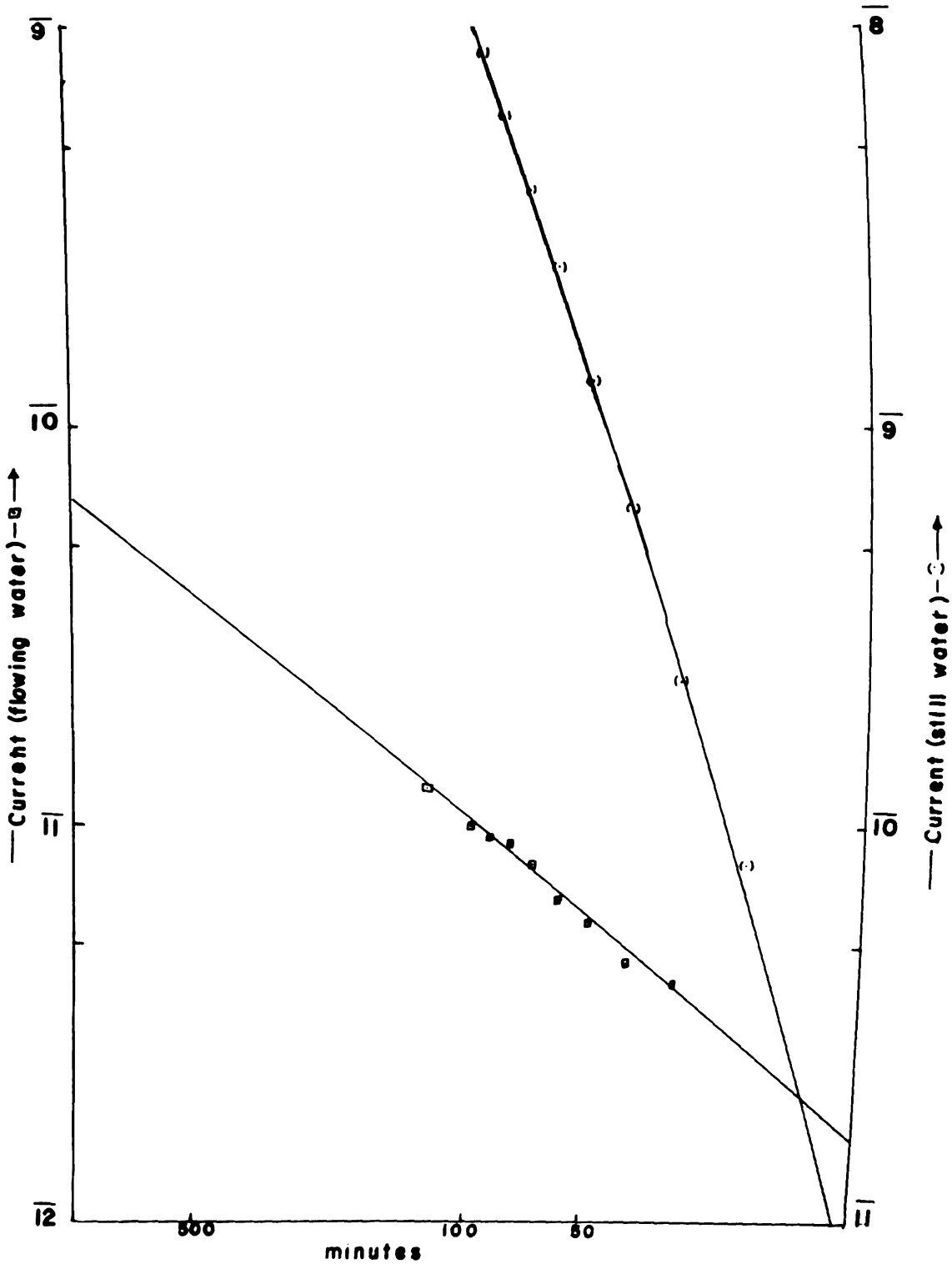

Figure VI. Log Current vs. log Time for coated collagen with flowing and still water

Varying the ratios of the components from pure chloroform to pure water, Table I, never gave an effect larger than the pure chloroform current, and no resemblance of the effect found from the vapors of the mixed liquids. (It should be noted, though, that the pressures of the liquids never got above one-half their saturated vapor pressures. This means that if the modification of the petrolatum layer took place at a pressure of chloroform greater than one-half the saturated vapor pressure, this effect of the current increase would not be noted in this experiment.) Conclusive evidence in favor of either of the two hypotheses could not come from this data.

C. Effect of Polar Adsorbents

A column of silica gel (SiO_2) was put in the flow train at a point just before the vapors entered the chamber. The nitrogen gas carrier was passed into the washing tower containing the chloroform-water mixture, and these vapors then passed through the column. Heating was noted in the tube as the vapors adsorbed on to the solid agent; this, of course, was the heat of adsorption. After a few minutes the tube cooled to room temperature indicating that all the adsorption sites were filled. The sample current then began to rise in the coated collagen to the low level seen when the mixed vapors were employed. The adsorption and desorption of the chloroform and water apparently resulted in a different state than they were in when they first entered the tube. Removal of the tube resulted in a rapid rise of the current to a high level characteristic of the mixed liquids (Figure VII). In terms of the first postulate, this experiment shows that the "complex" dissociates when adsorbed onto the polar protein. This would be expected in that,

TABLE I

Equilibrium Currents at Various Ratios of Mixed Vapors

Flow Meter Setting	cc./min.	Calculated Pressure	Equilibrium Current
1) 10 $CHCl_3$	86	100 mm. Hg	1.4×10^{-11}
10 nitrogen	86	---	
2) 15 H_2O	149	20 mm.	3×10^{-12}
4 $CHCl_3$	29	32 mm.	
3) 6 H_2O	45	5.5 mm.	9×10^{-11}
15 $CHCl_3$	149	123 mm.	
4) 7 H_2O	54	22.5 mm.	7×10^{-13}
7 nitrogen	54	---	
5) 7 H_2O	54	12.5 mm.	5×10^{-11}
7 $CHCl_3$	54	100 mm.	

$P_o(H_2O)^{25^o}$ = 25 mm. Hg

$P_o(CHCl_3)^{25^o}$ = 200 mm. Hg

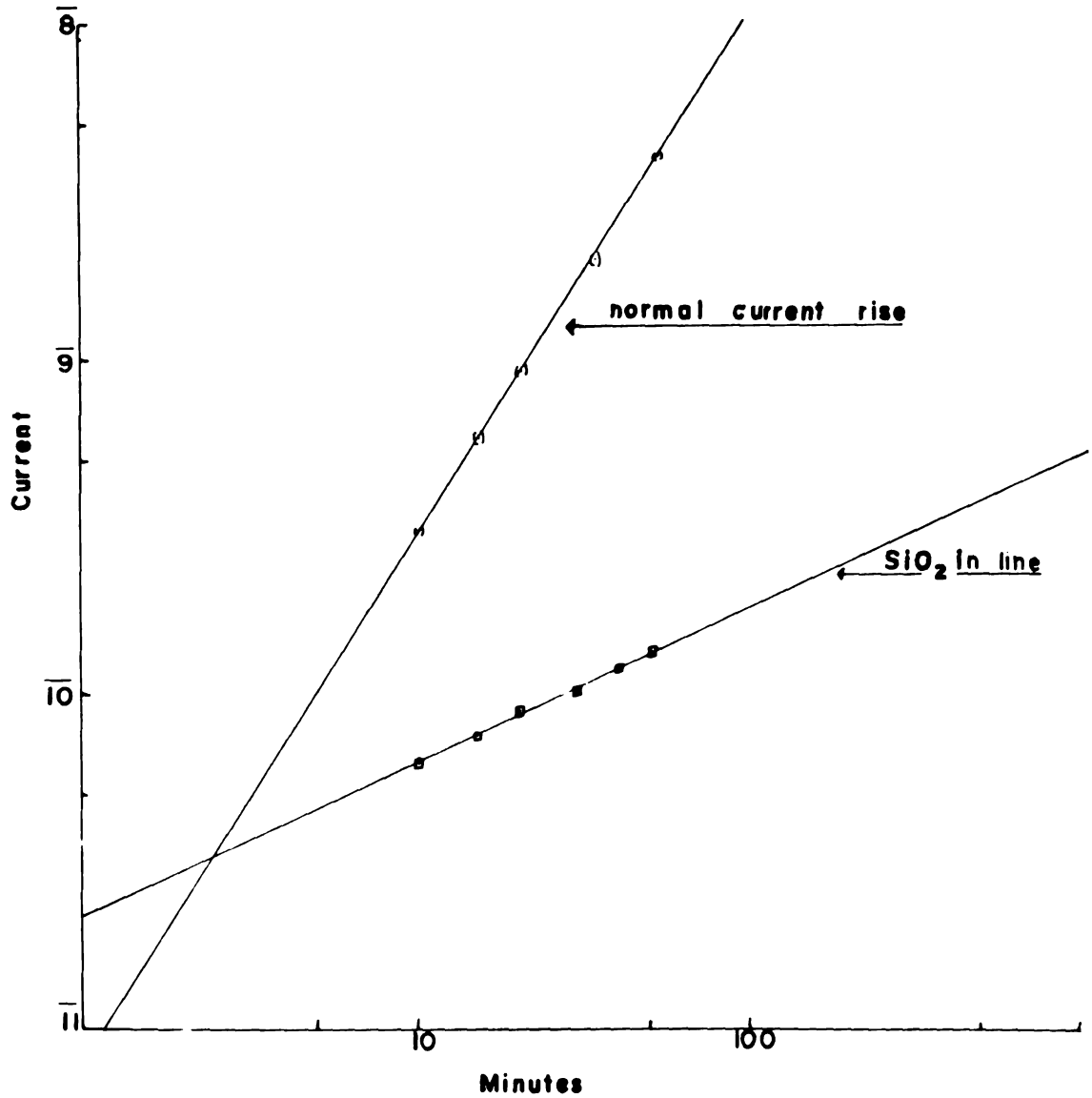

Figure VII. Log Current vs. log Time for coated collagen with the vapors passing through a tube of silica gel

once the water-chloroform "complex" penetrates the petrolatum coating, the water apparently can not pass out. Only by adding chloroform vapor to the chamber is it possible to remove this water. In the light of the first hypothesis, this would be explained by saying that the "complex" is again formed on the protein beneath the petrolatum layer. Then, because of the concentration gradient of more "complex" on the inside of this coating, the water-chloroform can diffuse out.

However, further studies using cobalt chloride "indicating silica gel" showed that the water was not passing through the tube as originally expected. The heating that was noted was caused entirely by the adsorption of chloroform (heat of adsorption) and not by the water as first supposed. The water entered the chamber only after the tube had been removed. Hypothesis one was not substantiated by this experiment.

D. Effect of Heating the Vapors

Passage of the vapors from the mixture of liquids through a heated tube produced little change in the rate or level of current increase. For temperatures between room temperature and that of boiling water, a glass U-tube containing copper turnings was placed in the flow train. The internal temperature of the gas was monitored with a thermocouple probe. A copper coil was also used with the same results. For higher temperatures, the copper coil was heated with a Cal-cord and temperatures up to 220 ^{o}C. were measured. The residence time in this tube was approximately one minute, and the rate of gas flow was sufficient to insure turbulent mixing and proper heat exchange. This flow rate was approximately 100 cc./min. If hypothesis 1 is correct, the effect is not found unless the <u>liquids</u> are in contact--the intimate contact of <u>only</u> the vapors

is not sufficient. Thus, if the hypothesized association had broken up in the heated tube, it would not be expected to reform. The temperature tests indicate an apparent stability; this is incompatible with present knowledge of the stability of hydrogen-bonded or dipole-induced dipole bonded compounds. (It is difficult to make a definite statement, since reproducibility was not possible from sample to sample to a degree sufficient for a quantitative study. The most which can be determined is seen on plots of the log time vs. log current. [This is from the equation $I = C \cdot t^m$ where I is the current, C is the constant of proportionality, t is the time, and m is an exponent. Taking the log of both sides results in the following equation: $\log(I) = \log(C) + m \log(t)$.] If a series of these log-log plots were made while the temperature is varied from run to run, a decreasing slope is noted as the temperature increases. This is by no means unequivocal as can be seen from Figure VIII.) A series of curves at increasing temperatures does not always yield lines of decreasing slope. From this data it must be concluded that the "unions" postulated could not exist or they would have been broken up by the heating.

Hypothesis two could explain the change in slopes, if they indeed were not caused by experimental error, by a slight thermal decomposition of the chloroform. This would change the pressure (or concentration) of chloroform, thus affect its ability to modify the lipid coating. The major evidence of these results indicates that hypothesis one is incorrect.

Trials were made using organic liquids other than chloroform. The increase of current with time in the coated samples was noted with many, particularly those which are good anesthetic agents. Experiments were

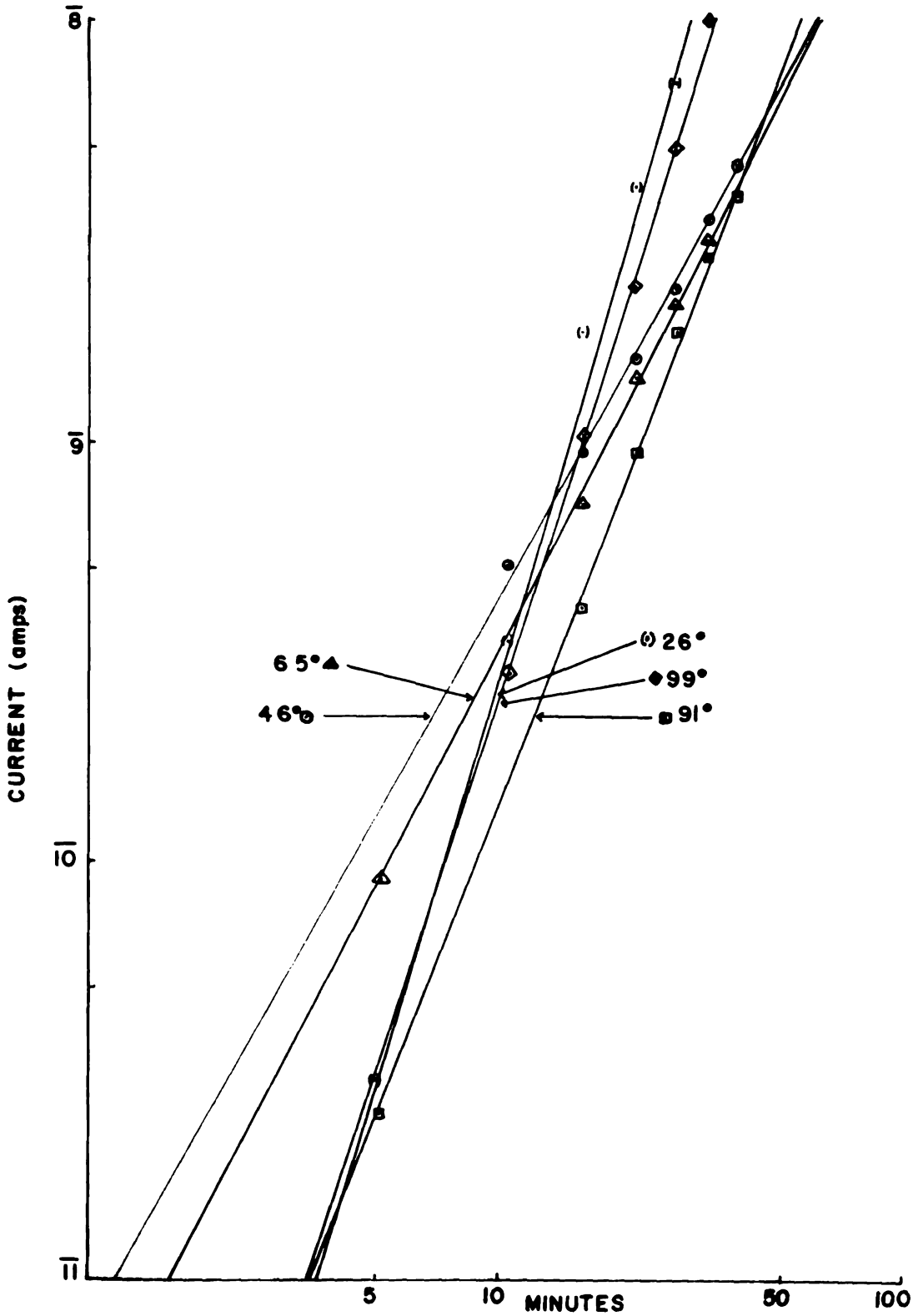

Figure VIII. Log Current vs. log Time for coated collagen with the vapors passing through a heated tube

made using diethyl ether, trichloromethane, dichloromethane, tetrachloromethane, cyclohexane, isopropanol, and ethanol. Of these, only the ether and the halogen substituted methanes were capable of producing large current increases when the liquids were mixed with water. Plots of the current increases with time are shown in figures IX and X.

E. Effect of Dissolved Compounds

With the possibility that dissolved ions or proteins in the water of the bubble tower might seriously affect the appearance of the current rise, a 5% sodium chloride solution (in water, weight-volume) was used in conjunction with the chloroform in place of the previously used distilled water. There was no appreciable difference in the log time-log current plots when compared with the controls. The same rapid rise in current was noted when a solution of hemoglobin in water over chloroform was used. When concentrated solutions of sodium chloride, potassium thiocyanide, and ammonium chloride were used, the current rise was present but was diminished in slope; this was most likely caused by a lowering of the activity and vapor pressure of the water. The slope decreased as the activity decreased. This is reported in Table II.

This presents evidence in favor of hypothesis two. When the chloroform concentration is a constant, lowering of the vapor of water results in a reduction of the current rise. Since the same amount of chloroform is present in all cases, the only variable is the pressure of water (which in uncoated systems determines the current rise).

F. Physical Chemical Tests

As another test of the first hypothesis, attempts were made to find another component in the chloroform-water mixture using classical techniques of physical chemistry. Infra-red spectra were taken by placing

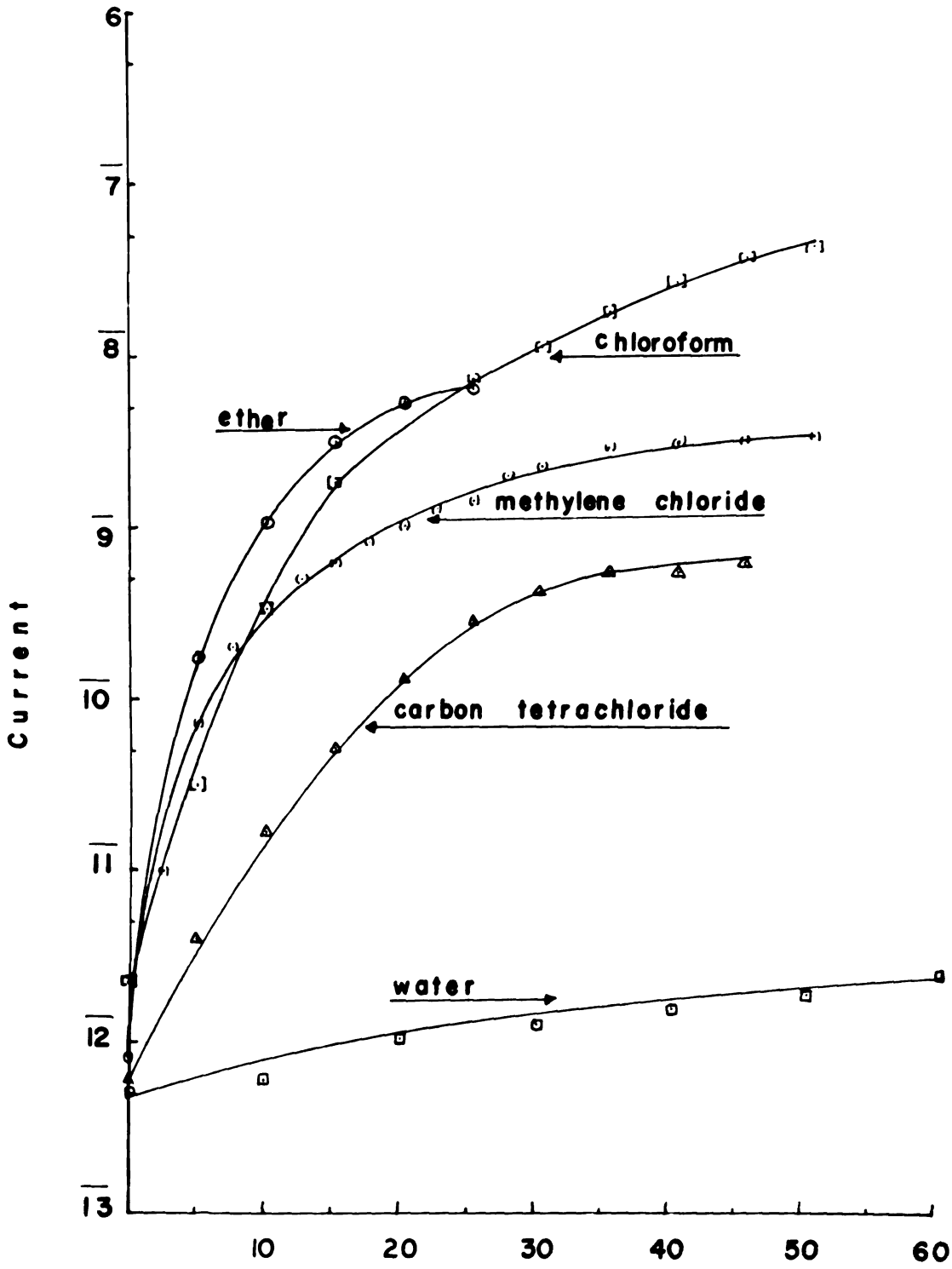

Figure IX. Log Current vs. Time for coated collagen exposed to the vapors of water and water + anesthetic agents

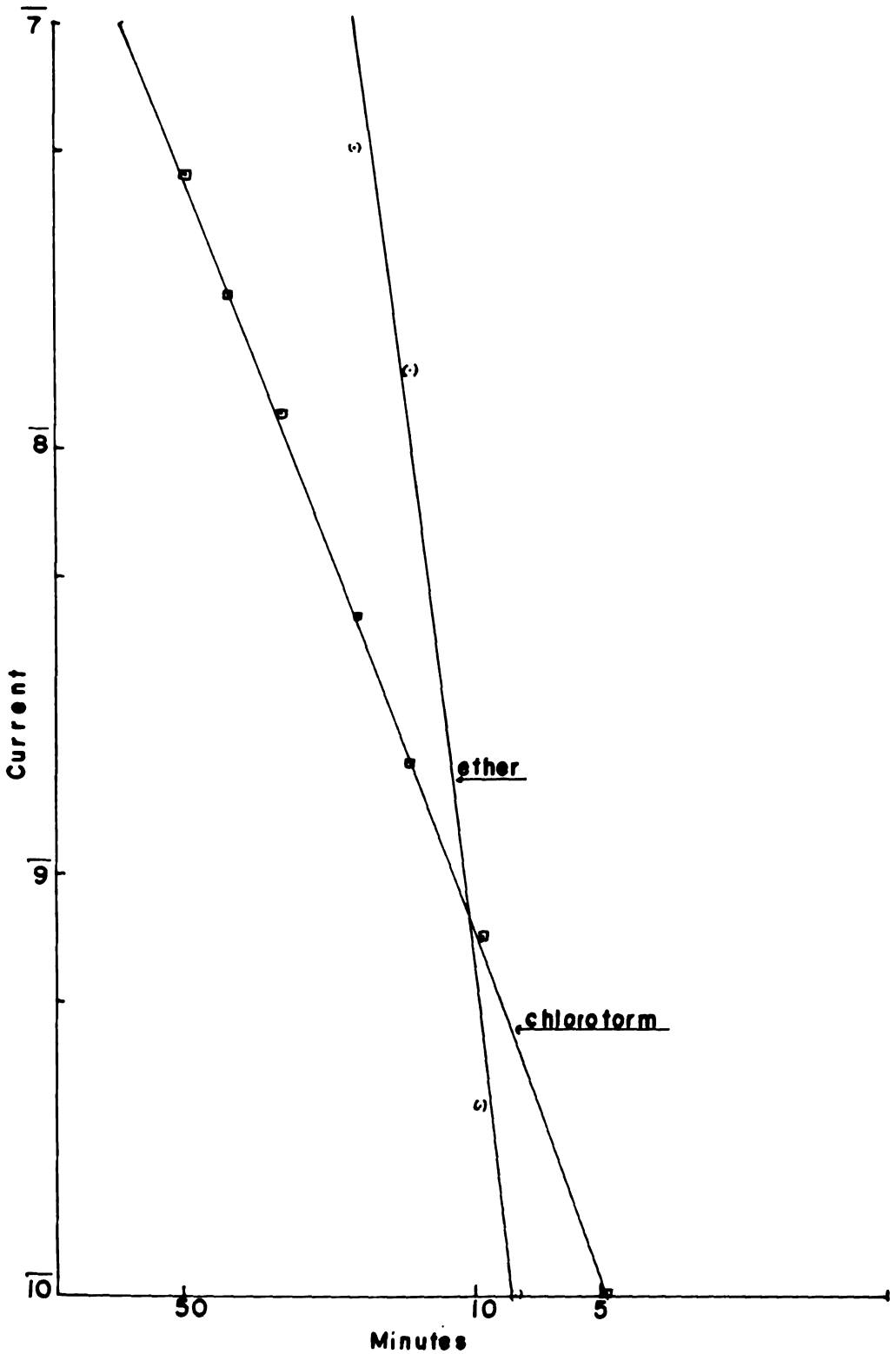

Figure X. Log Current vs. log Time for coated collagen exposed to the vapors of chloroform + water and ether + water

TABLE II

Slope of a Plot of Log Current vs. Log Time as a Function of the Relative Humidity (activity) of the Water

Slope	Relative Humidity*
2.7	100%
1.9	80%
1.4	70%
0.9	57%

*From a National Bureau of Standards Table

the samples in a sandwich cell of IRTRAN. All tests were made in the liquid phase and the path lengths varied from very thin films to those of 0.025 mm. Samples of water-saturated chloroform and chloroform-saturated water were checked for the presence of absorption peaks different from those of the pure liquid. In no case was a new band detected--even when the instrument was set on slow scan. The presence of a band between 3,200 and 3,500 $cm.^{-1}$ was expected to indicate a shift of the -OH stretching frequency to one of lower energy. As gas cells were not available, this could not be tested in the vapor phase. No detection of the -OH...O= stretch is evidence against the first hypothesis, that of an "association". However, if the union was by forces other than hydrogen bonding, the stretch would not be noted. In view of the thermal decomposition data, the bond would have to be a strong one; no such bond was indicated from the IR data.

Similar mixtures of chloroform-saturated water and water-saturated chloroform were injected into a Beckman GC-2 vapor phase chromatograph. The coil was filled with a polar packing and kept at $40^{o}C.$ during elution with nitrogen. The sample was resolved completely into its components--water and chloroform--with no trace of a third new component which would indicate the presence of a compound with different mobility in the column. Again, this must be taken as evidence against hypothesis

G. Duplication of Exact Partial Pressures

If, indeed, the effect was caused entirely by the partial pressures of the chloroform and water, an absolute duplication of the vapor pressures (rather than the approximations as discussed before) should give it even when separated washing bottles were used to hold the liquids.

The composition of the azeotrope formed when water and chloroform

are vaporized is approximately eight molecules of chloroform and one molecule of water. The vapor pressure of chloroform at 25^{o} is 200mm. and the vapor pressure of water at 25^{o} is 25mm. The molecular ratio predicted when a mixture of the liquids is made (assuming ideality) together is 8:1. It will be noticed that this is approximately that of the azeotrope.

To adjust the vapors in the proper ratio, it is only needed to notice that the pressure of each component of the "mixed liquids" is the saturated vapor pressure; the pressure of the components in the "mixed vapors" is one-half the saturated vapor pressure. By adding a bleeder flow of dry nitrogen equal to the flow passing through the mixed liquids, a pressure of one-half the saturated vapor pressure for each component is made. By switching the flows so that they now passed through the separate washing bottles and then combined, the pressure of the liquids (in the now "mixed vapors" experiment) is one-half the saturated vapor pressure. This is shown in Figure XI. No change in the slope of the line is seen. When the flow rates are adjusted such that the bleeder flow is reduced, and the pressures of the other components increase, the rapid current rise is noted. The change in the effect of current rise as a function of the pressure of the components (resulting possibly in a modification of the lipid layer) is shown by this experiment, and it is thus concluded that hypothesis two is the more tenable.

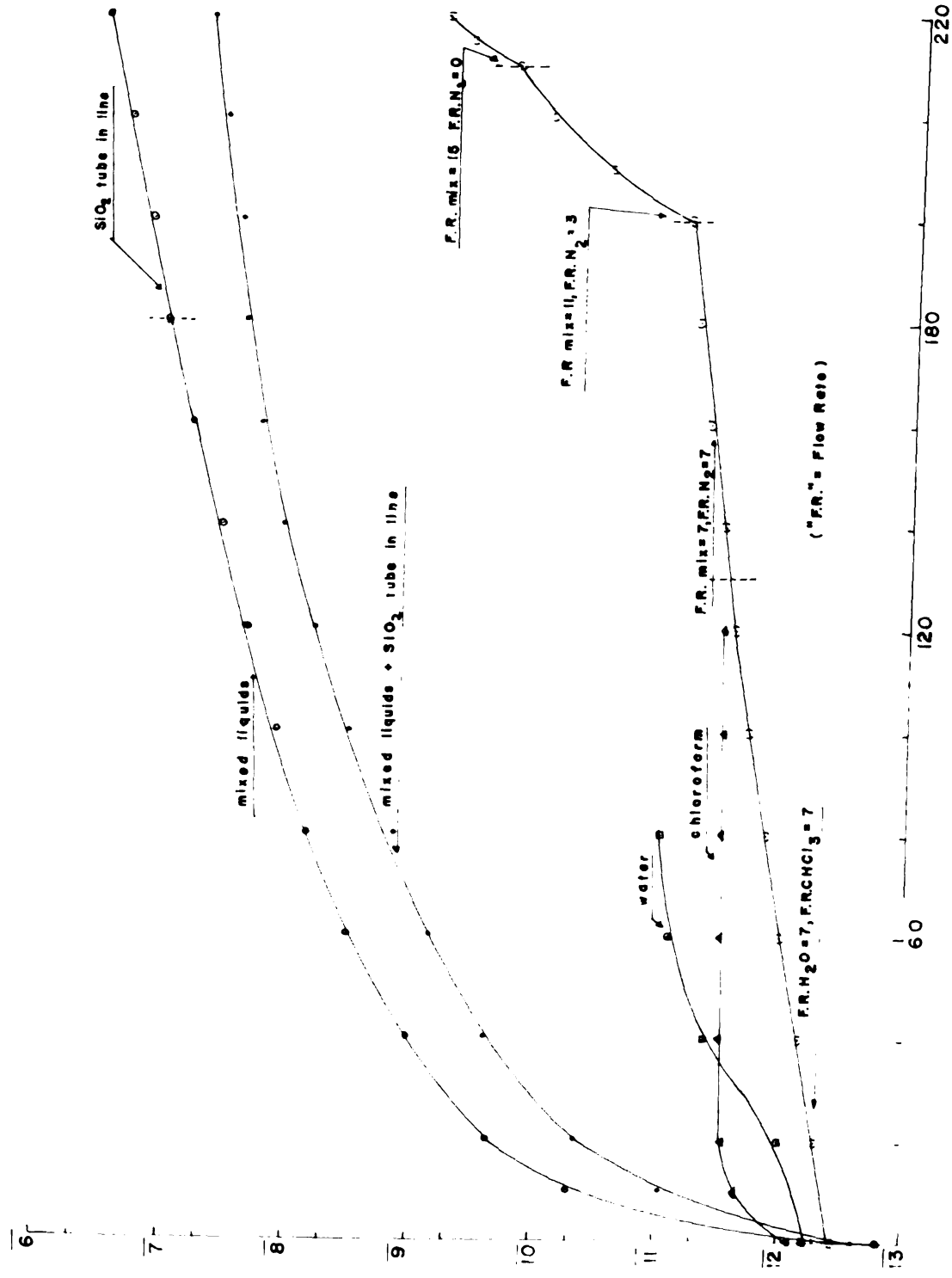

Figure XI. Log Current vs. Time for coated collagen exposed to the vapors of chloroform + water at various concentrations and with a silica gel tube in the flow line

DISCUSSION

The overall impression which one is able to derive from the series of experiments reported here is that of the penetration of a hydrophobic layer by a water molecule when it is in some way "assisted" by molecules of organic compounds. The mechanism of the "assistance" has been, by no means, conclusively demonstrated. However, the cause of the rapid current rise has been distinguished in the form of the two hypotheses. The first explained the effect as one of "association" of water and chloroform which was better able to penetrate the hydrophobic lipid coating. The second hypothesis, and the one proved to be more probable was that of a pronounced effect on the current rise by the pressures of the components, water and chloroform, which acted in a synergistic fashion to aid in the penetration of the water.

The observation that conductance increases are effected by the adsorption of high dielectric constant materials has been demonstrated by means of the simultaneous weight-current measurements on the microbalance. The same current increases are noted in the sample chambers containing the coated and uncoated protein samples. There can be little doubt that the water is being adsorbed on the proteins, be they coated or uncoated.

The original thought behind the hypothesis that a union existed between water and chloroform was the method for the penetration of the hydrophobic coating. Any molecule or collection of molecules containing water must not appear too "waterlike" (meaning it must not be as polar as water) to a hydrophobic coating if adsorption is expected to occur on the protein. This follows from the premise that to get to the protein, it must first dissolve in the petrolatum. Most

likely, the associations would be composed of at least two organic molecules and a water molecule; this might be thought of as a molecular sandwich complex. If sufficient concentrations of both the organic and the water were present, as in the liquids of the washing bottles, the associations would be kinetically favorable. The reaction would occur as:

$$n\ H_2O + m\ CHCl_3 = [(H_2O)_n\text{-}(CHCl_3)m]$$

$$K = \frac{[(H_2O)_n\text{-}(CHCl_3)m]}{[(H_2O)]^n\ [(CHCl_3)]^m}$$

and would proceed further to the right as the concentrations of water and chloroform are increased; this is given by the equilibrium constant. The complex as postulated by hypothesis one, however, was shown to be untenable by a series of experiments in which the expected results did not occur.

The second hypothesis explained the results as an effect of the pressures of the water and the organic compounds on the entrance of water into the hydrophobic coating. This was shown to be probable. Apparently, once the pressure (concentration) of chloroform is at a certain amount, the water is able to penetrate the coating and increase the current carrying ability of the protein by a method already explained.

From experiments done on other biological semiconductors (hemoglobin), it could be calculated that the difference in equilibrium current for a relative humidity of 50% and one of 100% was about four orders of magnitude. This was the difference noted between the current when the water-chloroform mixture contained water at 50% relative humidity and when the water-chloroform mixture contained water at 100% relative humidity. Thus, it is apparent that, once the water enters the protein, the current increase could be predicted as if the sample was not coated at all.

The question remains of how the water penetrates the petrolatum coating. It is seen that the water alone, at a relative humidity of 100%, penetrates the coating but very slowly. The final solution to this problem has not yet been worked out. It is possible that the introduction of polar groups into the coating might lower the free energy of the water which would enter, and stabilize, to a small degree, the water's presence. These polar groups come, of course, from the compound which has dissolved in the petrolatum. It is noted in the course of the investigation that not all organic compounds have the same effect of allowing the water to penetrate. This difference could possibly be caused by a difference of concentration of the organic compound in the ambient nitrogen of the chamber, or it could be caused by a difference in the solubility of the organic vapor in the petrolatum. In general, those compounds which were good gaseous anesthetics acted to allow the water penetration; nitrous oxide, however, was not found to be successful.

Further work relating the vapor pressures and solubility of these compounds in petrolatum and other lipids will have to be performed at a later time.

A second method for the penetration of water would be a simple change in the viscosity of the petrolatum. This has never been measured but has been observed visually. The entrance of water into the coating appears to be diffusion controlled, that is, the current at equilibrium is always the same, but the time needed to reach equilibrium is different. In the case of the drying of the samples, a combination of chloroform and dry nitrogen will dry the sample in about two hours whereas dry nitrogen alone will take about twelve to fifteen. It is apparent in

all cases that the water is not totally restricted, but only restricted if a short span of time is considered.

The diffusion of water through the coating is governed by the concentration gradient and the diffusion coefficient, or as expressed by Fick's Law:

$$dC/dt = -DA\ dc/dx,$$

where C is the amount of water, dc/dx is the concentration difference between the protein and the ambient, A is the area through which the diffusing is done, and D is the "diffusion coefficient" of water in petrolatum. The diffusion coefficient, in turn, is given approximately by the Stokes-Einstein Equation

$$D_{12} = kT/6\pi\eta_1 r_2,$$

where D_{12} is the diffusion coefficient of molecule (2) in solvent (1), η_1 is the viscosity of solvent (1), and r_2 is the radius of the diffusing molecule (2).

It can be seen that the lowering of the viscosity of the petrolatum coating by the chloroform would indeed change the penetration rate of water to the protein and affect the time needed to change the dry sample's conductivity.

By means of these organic compounds, it is possible that water is brought into the protein-phospholipid membranes which surround and make up the nerve. As has been shown, water greatly lowers the resistance of a hydrophobic coated protein; this was the model system developed to simulate, in a small way, the molecular structure of the neurolemma.

The great change in resistance can be expected to affect the internodal electrical characteristics of the nerve axon, and perhaps also, the synaptic junctions. A total cessation of nerve activity is not expected to occur

in clinical doses of anesthetic drugs, but, instead, a reduction in "spike" velocity and "spike" height such that the total effect is one of a large change on the nervous system leading to narcosis.

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