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TEMPERATURE EFFECTS ON BIOLOGICALLY
INTERESTING MEMBRANE SYSTEMS

W. Drost-Hansen

Miami University

Prepared for:

Office of Saline Water

August 1972

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MEMBRANE SYSTEMS

United States Department of the Interior



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**TEMPERATURE EFFECTS ON BIOLOGICALLY INTERESTING
MEMBRANE SYSTEMS**

August 1972

Drost-Hansen, W.

OSW R & D
Progress Report No. 790

**University of Miami
Coral Gables, Florida 33124**

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The overall objective of this study was to investigate the role of water structure, especially the structure of water near interfaces, in the properties and functioning of membranes, particularly biological membranes. It was found conceptually important to study membranes ranging from the most simple types to complex, living membranes. It was suspected and, in turn, supported by the results of the experiments, that the structuring of water in or near membranes appears to play an important and, at times dominating role in determining functional properties of membranes, regardless of the level of morphological complexity or the operational intricacies of the membranes.

***Membranes, Biological Membranes, Water Structure, *Interfaces,
*Membrane Processes**

Details of illustrations in
this document may be better
studied on microfiche.

***Ilani Membranes, *Millipore Membranes, *Liquid Membranes,
*Vycor Membranes, *Bioelectric Potentials, *Alga Valonia**

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RESEARCH AND DEVELOPMENT PROGRESS REPORT NO. 790
Int-OSW-RDPR- 72-790

August 1972

TEMPERATURE EFFECTS ON BIOLOGICALLY INTERESTING
MEMBRANE SYSTEMS

By W. Drost-Hansen, University of Miami, Coral
Gables, Florida, for Office of Saline Water, J. W.
O'Meara, Director; W. Sherman Gillam, Assistant
Director, Research; Sidney Johnson, Chief, Applied
Science Division

Grant No. 14-01-0001-1649

UNITED STATES DEPARTMENT OF THE INTERIOR . Rogers C. B. Morton, Secretary
James R. Smith, Assistant Secretary for Water and Power Resources

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FOREWORD

This is one of a continuing series of reports designed to present accounts of progress in saline water conversion and the economics of its application. Such data are expected to contribute to the long-range development of economical processes applicable to low-cost demineralization of sea and other saline water.

Except for minor editing, the data herein are as contained in a report submitted by the contractor. The data and conclusions given in the report are essentially those of the contractor and are not necessarily endorsed by the Department of the Interior.

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I. INTRODUCTION

A. Overall Objectives

The overall objective of the present study was to investigate the role of water structure (especially the structure of water near interfaces) in the properties and functioning of membranes, particularly biological membranes. It was found conceptually important to study membranes ranging from the most simple types to complex, living membranes. It was suspected and, in turn, supported by the results of the experiments, that the structuring of water in or near membranes appears to play an important (and, at times dominating) role in determining functional properties of membranes, regardless of the level of morphological complexity or the operational intricacies of the membranes.

Much of the work to be described in this report has already been published in the periodical literature, or in Monographs, Conference Proceedings, etc., but a number of experimental details are reported here for the first time. However, a certain amount of the experimental results obtained will require further study before the information can be usefully presented and exploited.

B. Staff

Over the past five years, the following staff has participated in various aspects of the experimental and theoretical work. These include:

Dr. Paul Cratin, present address: Chairman, Department of Chemistry, Central Michigan University, Mt. Pleasant, Michigan.

Dr. David Rands, Professor of Chemistry, Southern Illinois University, Edwardsville, Illinois.

Mr. Larry Korson, Laboratory Manager, LABORATORY FOR WATER RESEARCH, Department of Chemistry, University of Miami, Coral Gables, Florida.

Dr. Anita Thorhaug (Bader), present address: Department of Microbiology, School of Medicine, University of Miami, Miami, Florida.

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Mrs. Rhonda Waldinger, Department of Chemistry, Tallahassee Community College, Tallahassee, Florida, 32304.

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Mr. Ronald White, Research Associate.

Mrs. Elaine Kelly; graduate student.

II. EXPERIMENTAL STUDIES

As mentioned in the Introduction, it was deemed important to study membranes ranging in complexity from the conceptually simplest to those with the complexities of living organisms. Outlined below is a brief discussion of work done on each of a number of membrane systems.

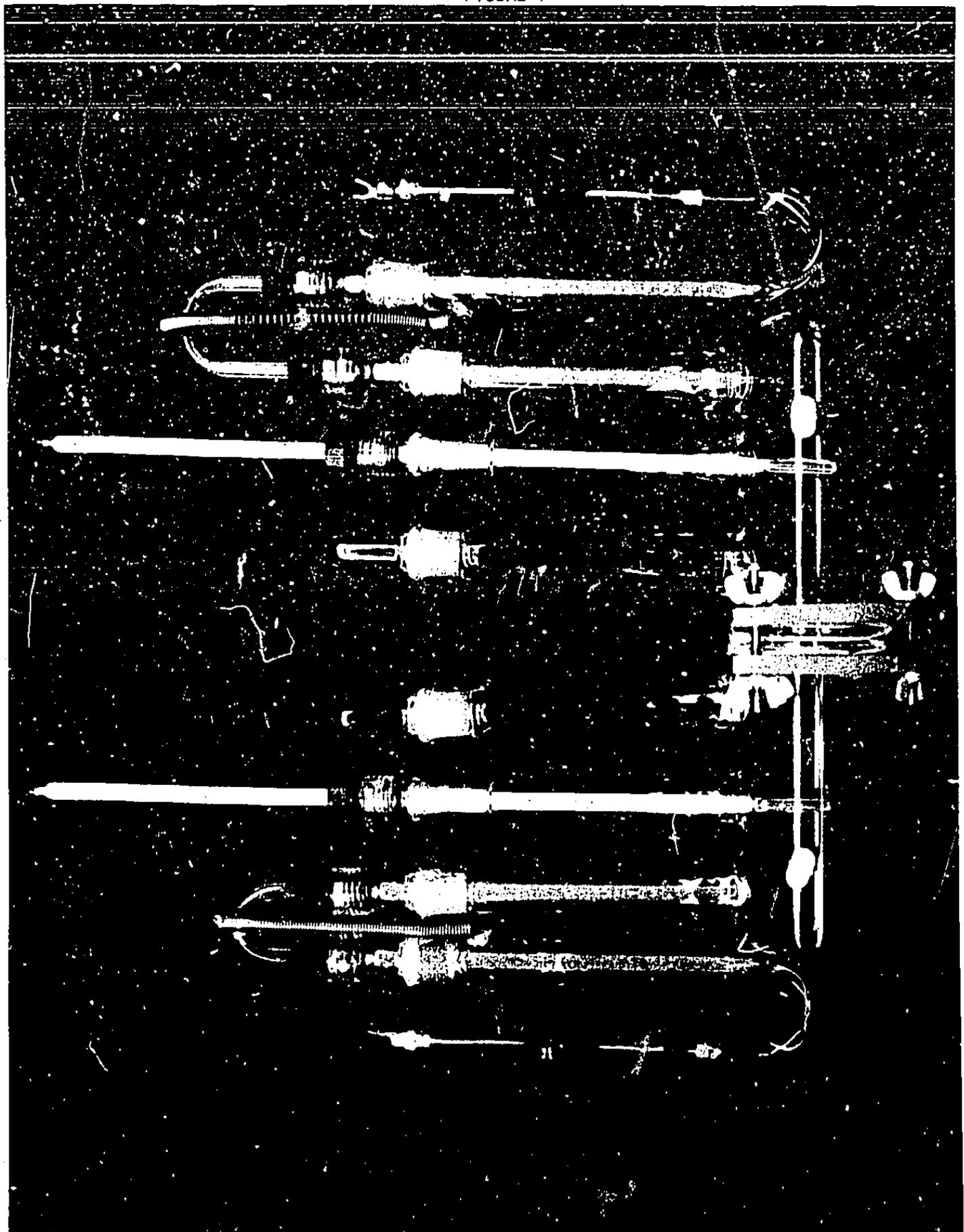
A. Ilani Membranes

Ilani, and Ilani and co-workers (1965; 1966; 1968) have described a unique membrane system, consisting of a porous matrix saturated with an organic, water-insoluble liquid. In the experiments to be described here, the membrane material consisted of Millipore filters; the organic phase was toluene. Conceptually, the membrane is, in part (and only in part) a liquid membrane, conveniently "stood up vertically" to allow the organic, immiscible liquid to separate two aqueous, bulk phases. In part, the membrane system is of interest in connection with cellular physiology, as it contains (unfortunately, rather intimately mixed) aspects of the cellulosic membrane walls of many plants and a "hydrophobic constituent" resembling the lipid part of bimolecular lipid films. The porous matrix itself - the cellulose matrix - possesses continuous, liquid-filled pores; originally, these pores are filled with toluene; however, water slowly penetrates into the pores and apparently displaces part of the toluene.

1. Concentration potential measurements

The membranes were prepared from Millipore filters soaked in toluene. The filters, in turn, were clamped between two half-cells. A photograph of the experimental cell is shown in Figure 1. Each half cell (electrode chamber) could be stirred individually by a magnetic stirring bar, operated by submersible magnetic stirrers. The entire setup was carefully thermostated in a temperature bath. Potentials were measured and recorded by use of either a Keithly Model 630 or Model 660A Potentiometer. Temperature was monitored, either by a quartz crystal thermometer, or merely with mercury-in-glass thermometers. When continuous recordings of potentials were made as a function of temperature (using an X-Y recorder) the X axis was used to display the temperature, usually by means of the output from a thermistor thermometer. Heating rates were adjusted empirically to be sufficiently slow to insure near-equilibrium (and close to steady-state) conditions. Thus, only minor temperature differences are expected between the recorded bath temperature and the temperature within each cell compartment. Furthermore, because of the symmetry of the two half-cells (with essentially equal amounts of stirring in either half-cell) only very minimal temperature differences (say, less than 0.05°C) occurred across the membrane between the two half-cells. Thus, the occurrence of thermoelectric effects may therefore be ruled out completely. Bias due to asymmetry in the silver/silver chloride electrodes employed in the potential measurements was usually corrected for (or ignored, if of sufficiently small, constant magnitude). The preparation of silver/

FIGURE I



silver chloride electrodes followed standard procedures [Ives and Janz (1961)].

Typical examples of observed potential vs. temperature curves are shown in Figures 2,3,4, and 5.

Figure 2 shows a typical run in which a toluene saturated filter separated 0.100 and 1.000 molar potassium chloride solutions. It is seen that the potential is approximately equal to that predicted from theory. Using the Nernst equation, and neglecting differences in temperature dependencies of the activity coefficients for the salt at the two different concentrations, the expected temperature coefficient is indicated by the three dotted lines (merely parallel displacements, with slope corresponding to $T_1/(T_1 + \Delta T)$). From Figure 2, it is obvious that (a) the theoretical temperature coefficient is observed only (approximately) over the range from 30 to 55 or 58°; (b) the observed temperature coefficient is notably smaller between 16° and (approximately) 28°; and (c) a notable change in slope occurs in the vicinity from 28 to 31°. (A second anomaly is seen also in the vicinity of 60°).

Figure 3 shows a similar run. In this experiment, the temperature was increased and then decreased. It is seen that the change in slope at 30° is somewhat smaller in this case, especially for the ascending temperature curve. The effect, however, is still observable, especially as reflected in the cooling curve. A third experiment is shown in Figure 4. The change in this case is dramatic in the vicinity of 29 to 30°. The curve was obtained during cooling.

Several experiments were made with considerably larger concentration differences. Thus, Figure 5 shows the temperature dependence of the concentration potential obtained, with a membrane separating 15 millimolar and 15 micromolar potassium chloride solutions. Again, a notable change in the temperature coefficient is observed between 28 and 30° C.

Many experiments have been performed with this type of system. The results of such concentration potential measurements as a function of temperature, have often, but not invariably, revealed the existence of thermal anomalies near 30°. An element of non-reproducibility exists. This may probably be explained, in part, in terms of changes in the membrane, due to dissolution and transfer of toluene from the membrane, and the movement and adsorption of water onto the cellulose material. This will be discussed briefly in the section on bi-ionic potentials.

2. Bi-ionic potential measurements

Using the same apparatus as employed for the concentration potentials, measurements have been made of bi-ionic potentials across the toluene saturated membrane filters (the Ilanl membranes). Bi-ionic potentials are frequently used for studies of relative rates of transfer of two ions of the same charge. Thus, normally, measurements are made with identical concentrations (and close to identical ion

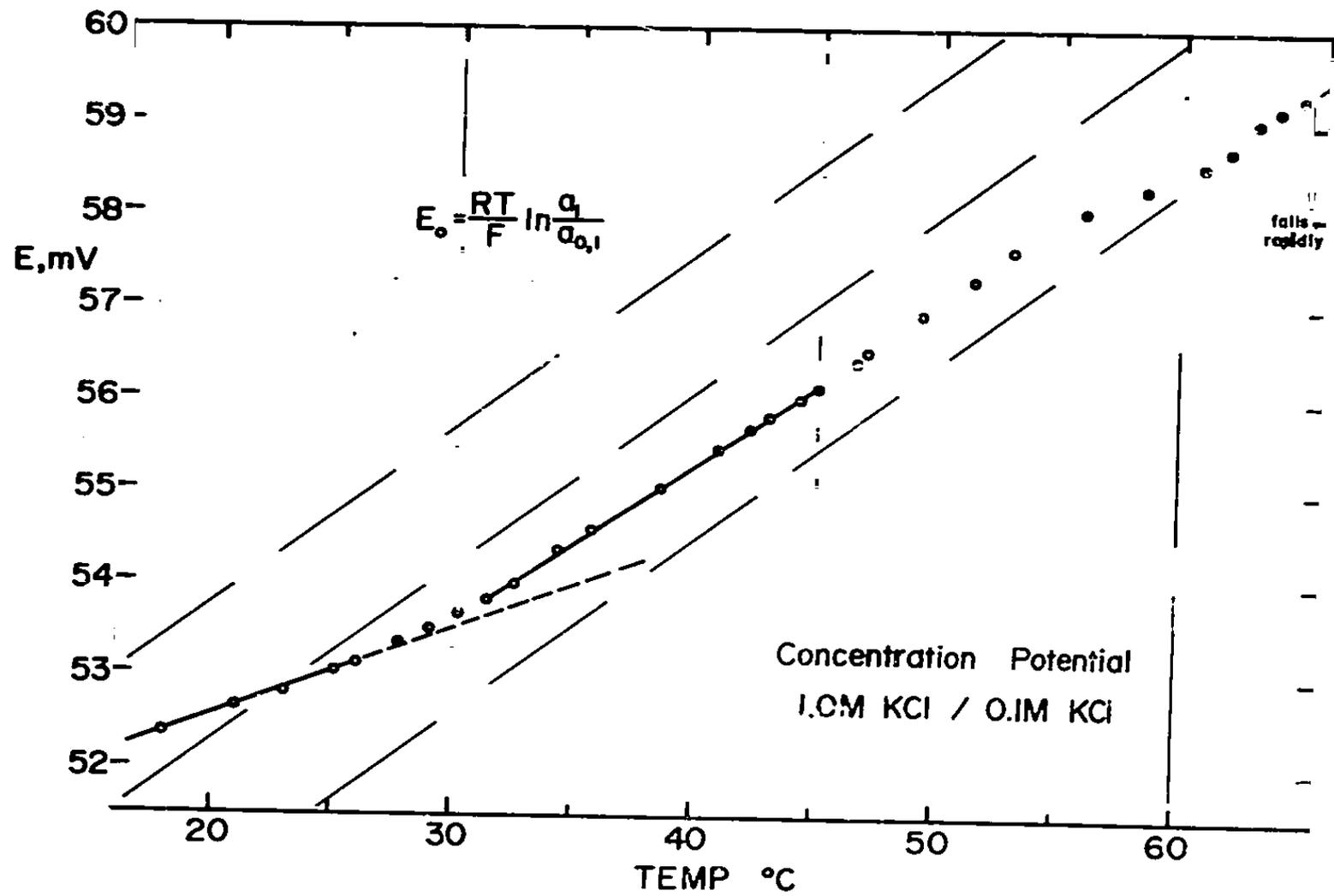


FIGURE 2

FIGURE 3

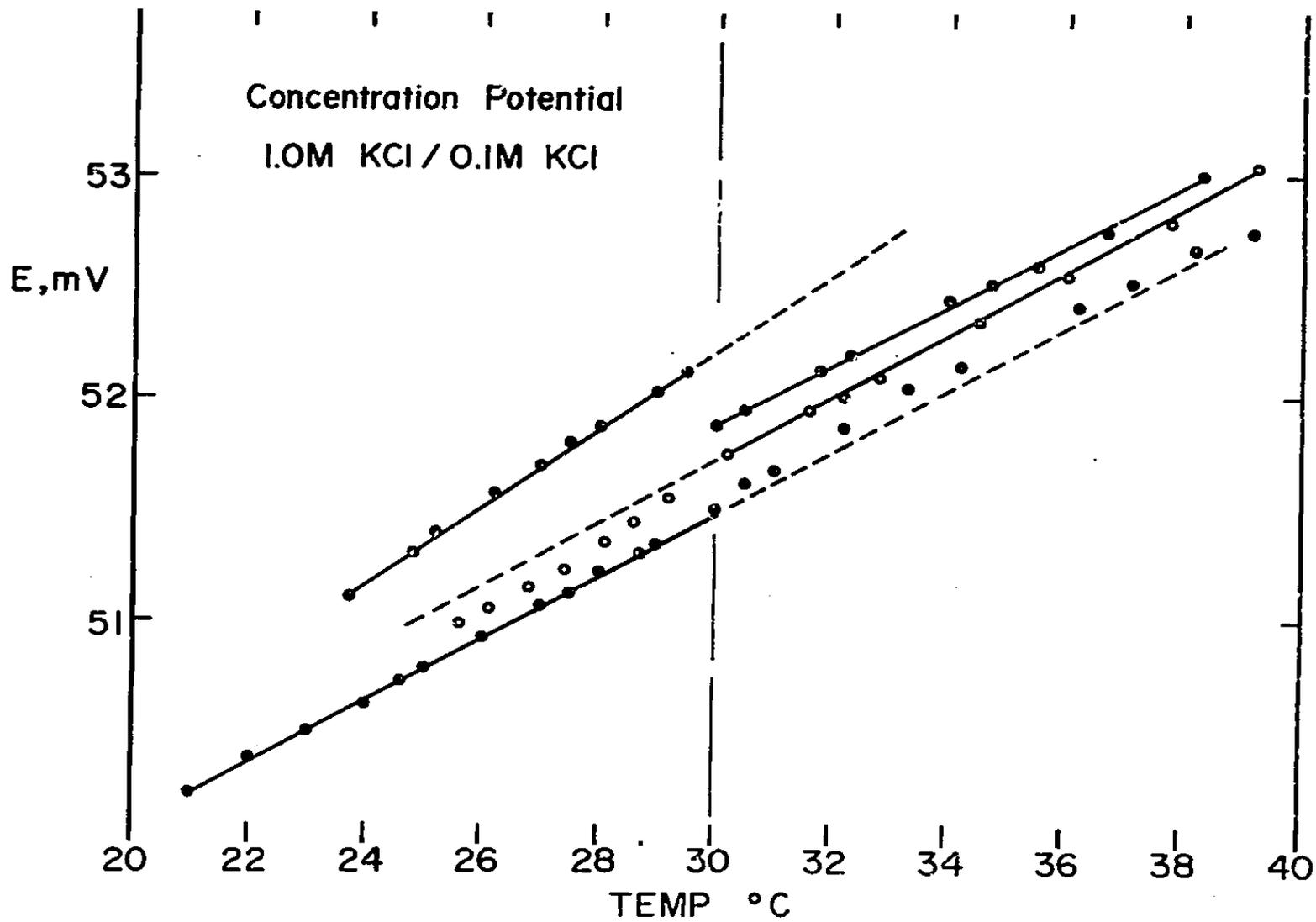
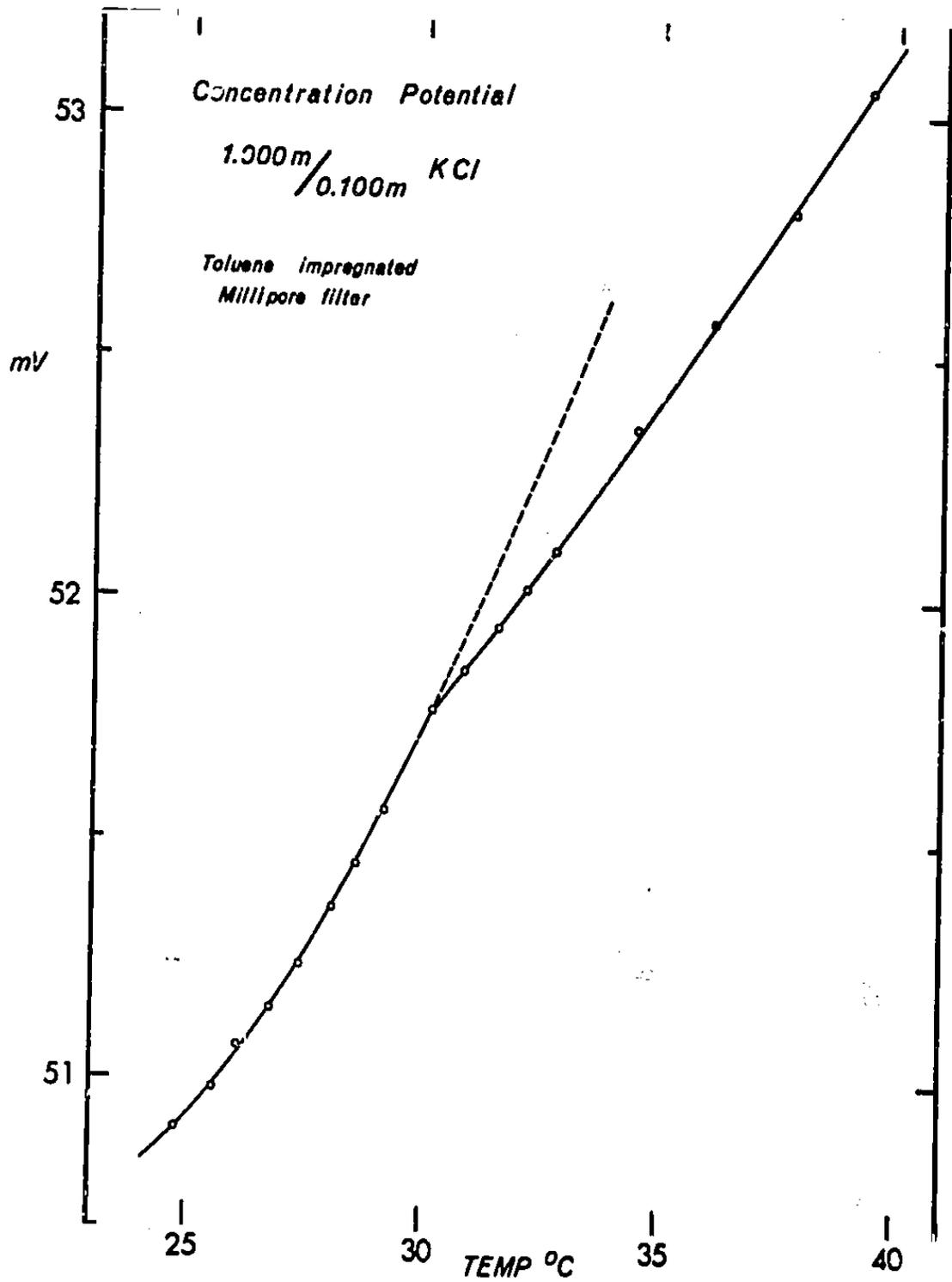


FIGURE 4



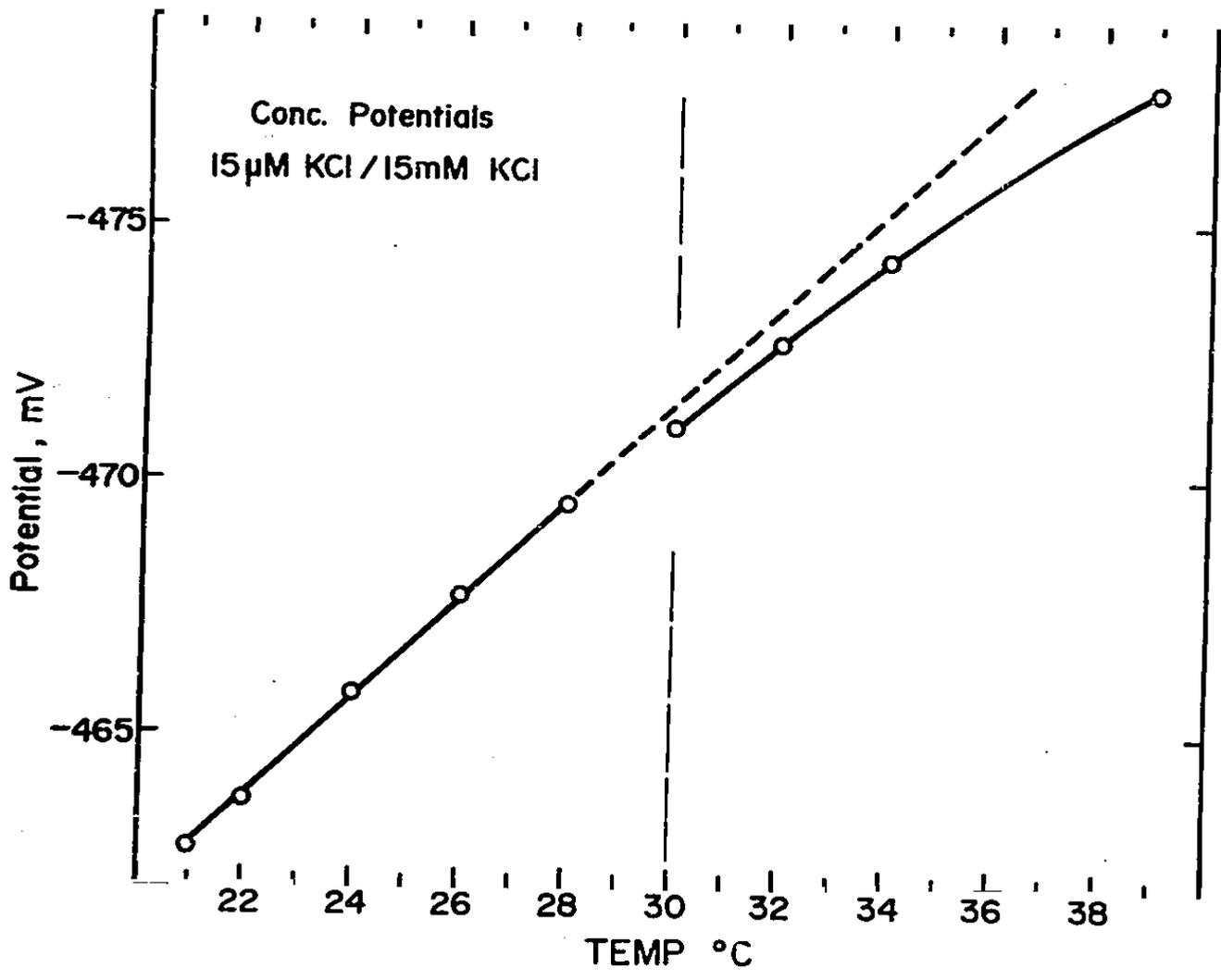


FIGURE 5

-R-

activities) on either side of the membrane. The potential established depends on the ion mobilities within the membrane. Under steady-state conditions, it is assumed that the bi-ionic potential can be expressed by the formula in Equation (1), where:

$$E_{BI} = \frac{RT}{F} \ln \frac{U_+^{(1)} + U_-}{U_+^{(2)} + U_-} \quad (1)$$

where $U_+^{(1)}$ and $U_+^{(2)}$ are the mobilities of cations 1 and 2, respectively, and U_- is the mobility of the (common) anion. For cation selective membranes (membranes containing fixed, anionic sites), the mobility of the anion is small, and the expression for the potential reduces to equation (2).

$$E_{BI} = \frac{RT}{F} \ln (U_+^{(1)} / U_+^{(2)}) \quad (2)$$

Figure 6 shows an example of a bi-ionic potential, as a function of temperature. The membrane separates two bulk phases, 1.00 molal in potassium chloride and sodium chloride, respectively. (The curve was obtained during cooling of the system). A notable change in temperature coefficient is observed at approximately 30°. If, in Equation (2), the relative ion mobilities of the two cations are assumed temperature independent, or possessing identical temperature dependencies, the temperature-vs.-potential curve is indicated by the dotted line. It is seen in this case that the "theoretical" temperature coefficient is observed only in the region below approximately 30°. Above this temperature, the temperature coefficient is far too high. Measurements have also been made of bi-ionic potentials at considerably lower concentrations. Figure 7 shows the potentials observed between 0.015 molar potassium chloride vs. 0.015 molar sodium chloride solution (Run 103). It is seen that a small but definite anomaly occurs in the vicinity of 28° C.

While a large number of experiments have been made to determine the temperature effects on bi-ionic potentials, the system, unfortunately, lacks reproducibility. The difficulties are ascribed, in part, to the gradual loss or redistribution of toluene and to the invasion of the membrane by water which subsequently may react with the cellulose matrix. The effects of water on the cellulose matrix are presently poorly understood. The initial condition of the membrane plays an important role. Thus, it appears certain that the relative humidity under which the membranes are initially stored, notably influences the properties of the membranes subsequently observed. Figure 8 shows consecutive "cycling" of a membrane system around 30°. The repeated heating and cooling cycles lead to gradual changes in the observed bi-ionic potentials (although the net effect is only of the order of 2% changes in the potential over several cycles). (Note that the total temperature range studied here is far too small to allow any statements to be made as to the occurrence of a thermal anomaly.

FIGURE 6

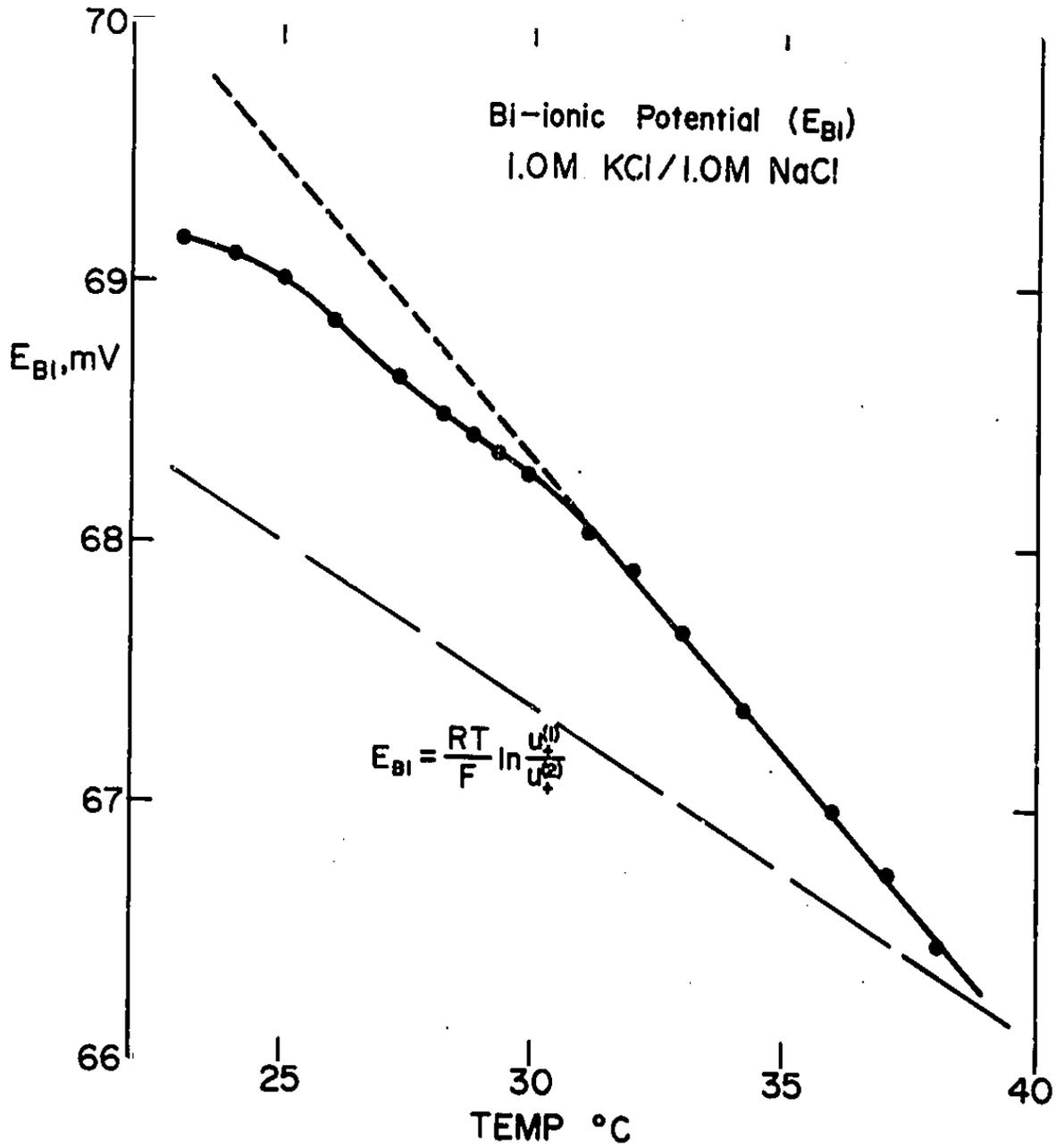


FIGURE 7

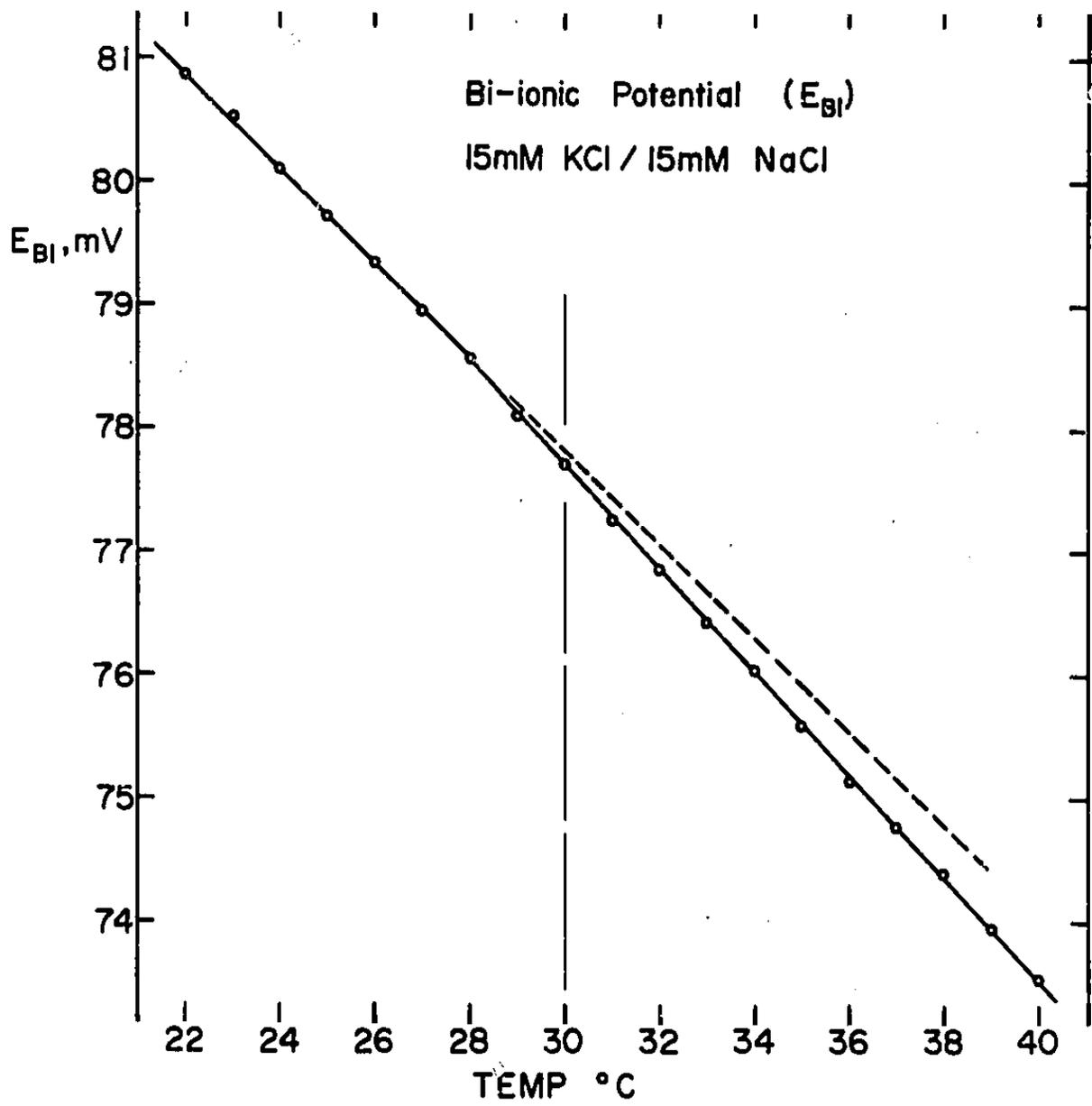
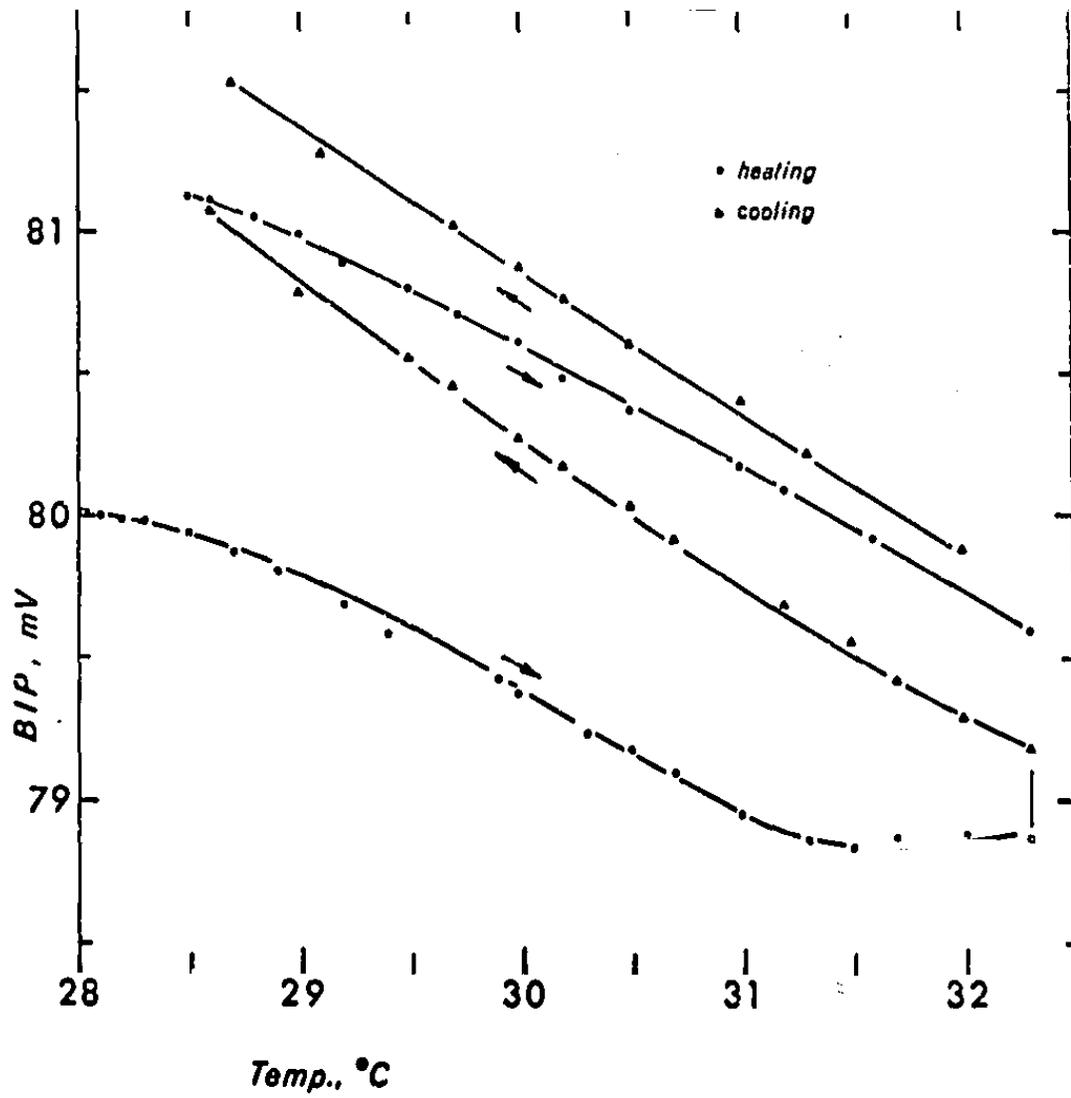


FIGURE 8



3. Summary of potential measurements

Although the reproducibility, at times, was less than satisfactory, there appears little doubt that anomalous changes occurred in the membrane potentials as functions of temperature in the vicinity of 30°. Other experiments suggested somewhat similar changes near both 45° and near 60°. The mechanism of these changes is difficult to determine. As discussed previously (Drost-Hansen, 1969), it appears certain that no thermal anomalies occur in the properties (and hence the structure) of bulk water or bulk, aqueous solutions (of low molecular weight electrolytes). The changes observed in many systems, including the membranes discussed in this report, are most likely due to changes in the water in or adjacent to the membrane. The exact site of these changes is not known. The changes may represent structural changes in the vicinal layers, possibly identifiable, in part, with the innermost "stagnant" (unstirred) layers, vicinal to the outside of the membranes. In the alternative, the changes may reflect differences in the state of aggregation of the dissolved water in the toluene-cellulose matrix, or water contained in the pores of the membrane. Such changes are certainly expected to influence the state of hydration and mobilities of dissolved as well as "bound" ions in the composite membrane material.

4. Conductance measurements

Surface conductance continues to be a somewhat ill-defined concept. In order to initiate studies on surface conduction, data on the surface conductivity of porous Vycor were used in connection with other data reported in the literature (primarily by Schuffe, who has worked mostly with narrow capillaries). The analysis is based on a relatively crude model presented by the Principal Investigator some years ago (1959). This analysis is briefly indicated below and one aspect is elaborated on in some detail. Unfortunately, surface conductances are difficult to measure with any degree of accuracy or precision. Indeed, vast ranges of apparent values have been reported by different authors. Thus, Overbeek (in Kruyt's volumes on colloid chemistry, 1949; 1952) has collected an interesting table of surface conductances from which it is apparent that vastly different values have been reported under what, presumably, are identical circumstances. Thus, McBain and various co-workers; Urban and co-workers; Fricke and Curtis, and also Wigja have reported data for the surface conductivity of Pyrex glass in contact with 10^{-3} molar KCl solution. The values reported ranged from 0.4×10^{-9} ohms $^{-1}$ to 100×10^{-9} ohms $^{-1}$. Furthermore, surface conductivity for pauci- or multi-molecular adsorbed layers is apparently subject to enormous variations. In fact, the surface conductance under such circumstances appears to be highly non-linear. Thus, for water adsorbed on wool fibers (water contents expressed as moisture adsorbed, x-grams of water per m grams of solid); the surface conductivity increases proportional to the "loading" (x/m) to the 15th power! Even the surface conductivity of porous Vycor changes in a very non-linear fashion as the result of adsorption of ammonia from the vapor phase. Thus, an increase of loading by less than a factor of 3 (from 9 milligrams/gram Vycor to

24 milligrams/gram solid Vycor) results in an increase in surface conductivity from $2 \cdot 10^{-15}$ to $2 \cdot 10^{-12}$ ohms⁻¹; in other words, a thousand-fold increase. Interestingly enough, the increase in surface conductance of Vycor due to water is far less pronounced.

A limited number of conductance studies have been made on Hani membranes. Figure 9 shows the resistance of such a membrane as a function of temperature. The resistance was measured at approximately 1500 Hz, using a Wayne-Kerr Autobalance Universal Bridge, Model B641. The curve shows the results for one complete cycle of heating and cooling. Some hysteresis is observed (probably attributable, in part, to different rates of solution of the toluene). It is seen that a small but very real, anomalous change in resistance vs. temperature occurs in the vicinity of 30°. Because of the difficulties encountered with the long-term stability of toluene saturated cellulose matrices, these experiments were terminated and conductance measurements continued on simpler types of membranes. These are described in Section II.B. ("Millipore Membrane Studies").

The anomalous temperature dependencies observed for both concentration potentials and bi-ionic potentials must be due to some change in the ion mobilities within the membrane. This, of course, is eminently consistent with the observed changes in membrane resistances (see below). Furthermore, it should be noted that the anomalies appear independent of the concentration of the electrolytes used: thus, anomalies have been observed in the concentration potential measurements between 15 millimolar and 15 micromolar solutions and up to concentrations of 1 and 0.1 molar, respectively. This also is consistent with the observations which have been made repeatedly that the anomalous changes in vicinal water structure appear almost independent of electrolyte (and non-electrolyte) concentration, and in fact, relatively independent of whether or not the water is adjacent to a hydrophilic or a hydrophobic material. The latter is the so-called "paradoxical effect". For a discussion of this, see the papers by Drost-Hansen (1971, 1972).

5. Experimental notes

In connection with the concentration potential and bi-ionic potential measurements, two types of salt bridges have been employed. For temperatures below approximately 40°, the conventional potassium chloride-agar bridge has been used. However, because of the sol-gel transformation of agar, a different type of salt bridge has been used for the measurements at higher temperatures. The type of salt bridge devised is sketched in Figure 10. As seen from this illustration, the salt bridge is shaped in the form of an H, where the salt bridge itself extends from point A to point B. The solution in the salt bridge proper is two molar potassium chloride. The "membranes" at points A and B are made of leached, porous Vycor. These "plugs" have moderately low resistance; the material itself is highly porous and rather inert. In each salt bridge, the remainder of the bridge (from Point B through Point C) is filled with the (toluene saturated) liquid containing the appropriate electrolyte solution (from the respective chamber of the

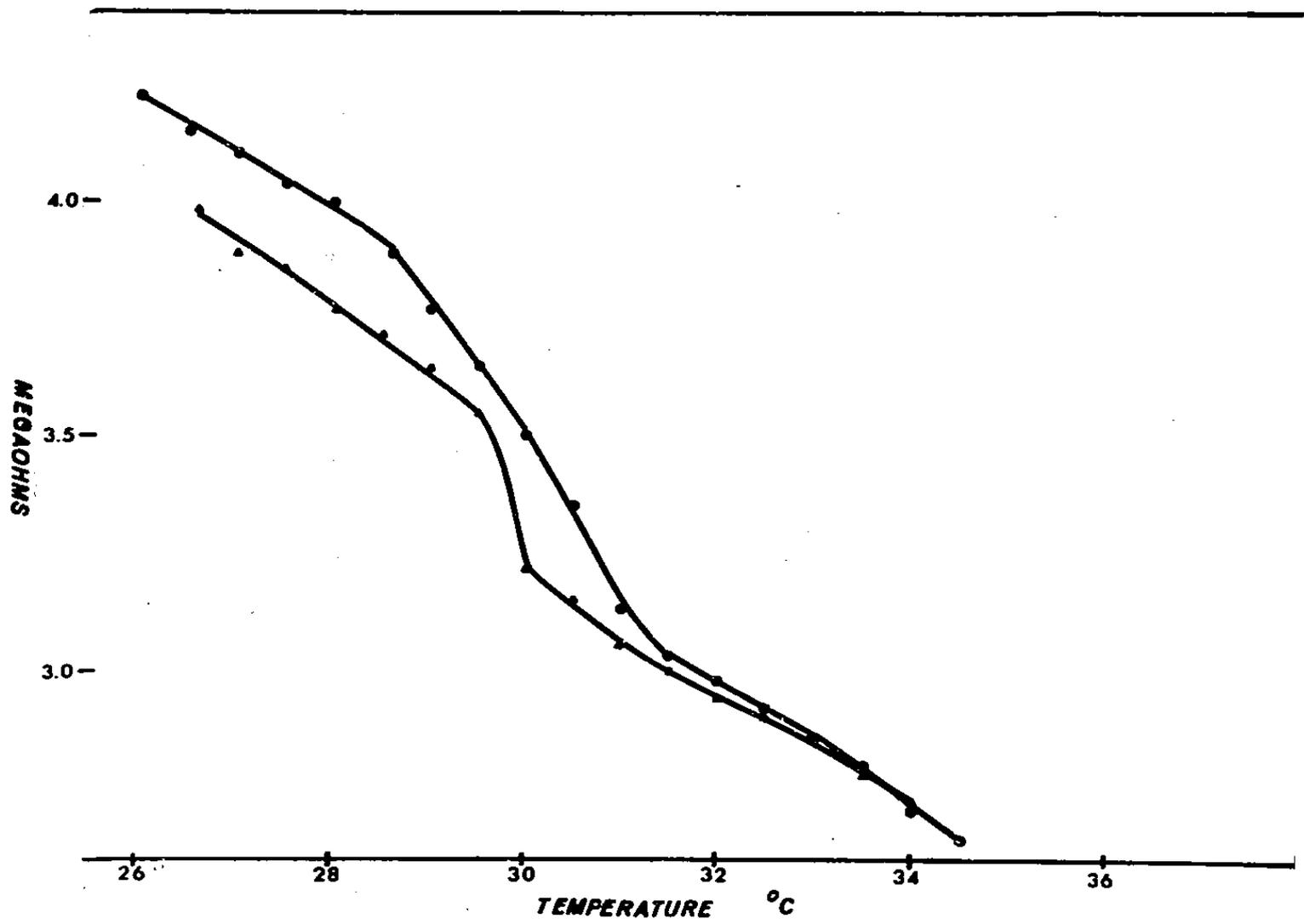
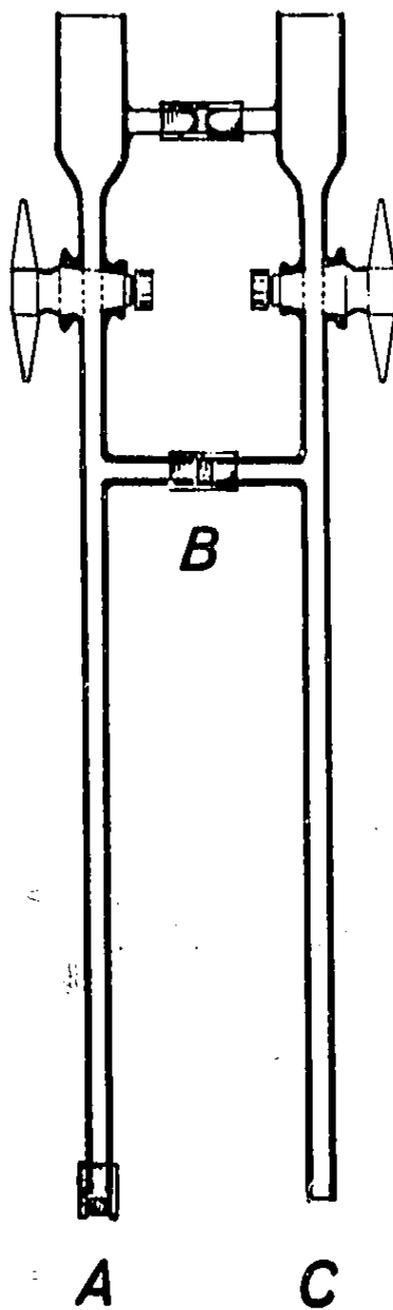


FIGURE 9

FIGURE 10



Porous Vycor Plug Salt Bridges

diffusion half cell). The average pore diameter of the leached Vycor is approximately 40 Å. However, because the total void space is around 28%, it is estimated that the resistance of these porous Vycor plugs (saturated with two molar potassium chloride) is only of the order of 20 ohms/millimeter length of the plug: (outside diameter, 7 millimeters). Thus, the total resistance of the entire salt bridge does not greatly exceed that of the equivalent, two molar potassium chloride alone. The salt bridge has far greater stability than the traditional agar salt bridge. However, problems were encountered due to the diffusion of the toluene, both into the porous plug itself and, particularly, into the Tygon sleeve, resulting in swelling of the Tygon tubing. In later modifications of this type of salt bridge, heat-shrinkable Teflon has been successfully used instead of the Tygon, but sufficient information was not obtained in this connection to yield reliable general conclusions.

The frequency dependence of the observed resistance was anomalous. Following standard practice in conductance studies, the resistance was plotted as a function of the reciprocal square root frequency. The total range covered was from about 500 Hz to 25,000 Hz. A typical example is shown in Figure 11. It is noteworthy that the frequency dependence is far from linear. As was observed in the potential measurements on llani membranes, the resistance also changed with time, probably due to losses of toluene. The specific nature of the frequency dependence has not been studied in detail, although such a study might be most rewarding (compare Coster and Simons, 1970).

B. Millipore Membranes (Hydrophobic and Hydrophilic)

It is eminently reasonable to expect that most membrane properties can be attributed to two factors: pore size (and pore size distribution) and surface free energy. How these two variables affect the transport of water and aqueous solutions through membranes is discussed briefly.

For the studies on the Millipore Membranes, a special membrane cell was devised. The cell is shown in Figure 12; it consists of modular units fabricated of Lucite. The current-carrying electrodes were made of platinum gauze, epoxied onto the end plates of the cell. The platinum gauze electrodes (of well-defined geometry) were firmly attached to sealed-off wires connected through an insulated glass tube to the body of the cell. The main cell is provided with a number of ports, allowing for renewal of solutions and measurements of temperature (with mercury thermometers or thermistor probes). The same ports could also be used for silver/silver chloride electrodes for potential measurements. Finally, provisions were made for a submersible Gelger counter for diffusion studies; (this part of the experiment has not been completed). The geometry of the cell could be changed, at least to the extent of varying the separation between electrodes or changing relative volumes on either side of the membrane by adding or taking away modular units ("spacers"). The membrane was clamped between the central portions of the cell. Throughout, rubber o-rings were used between each modular

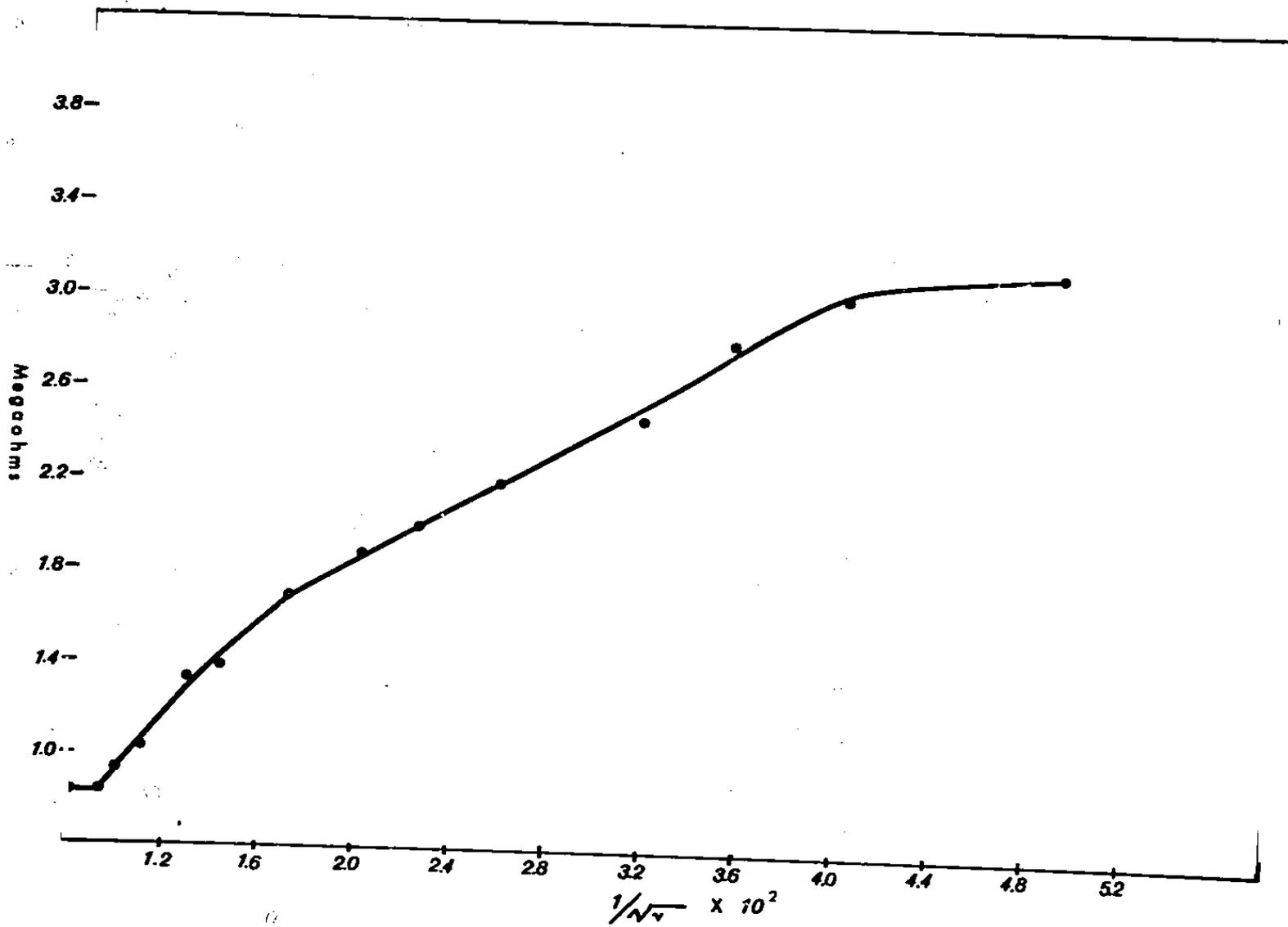
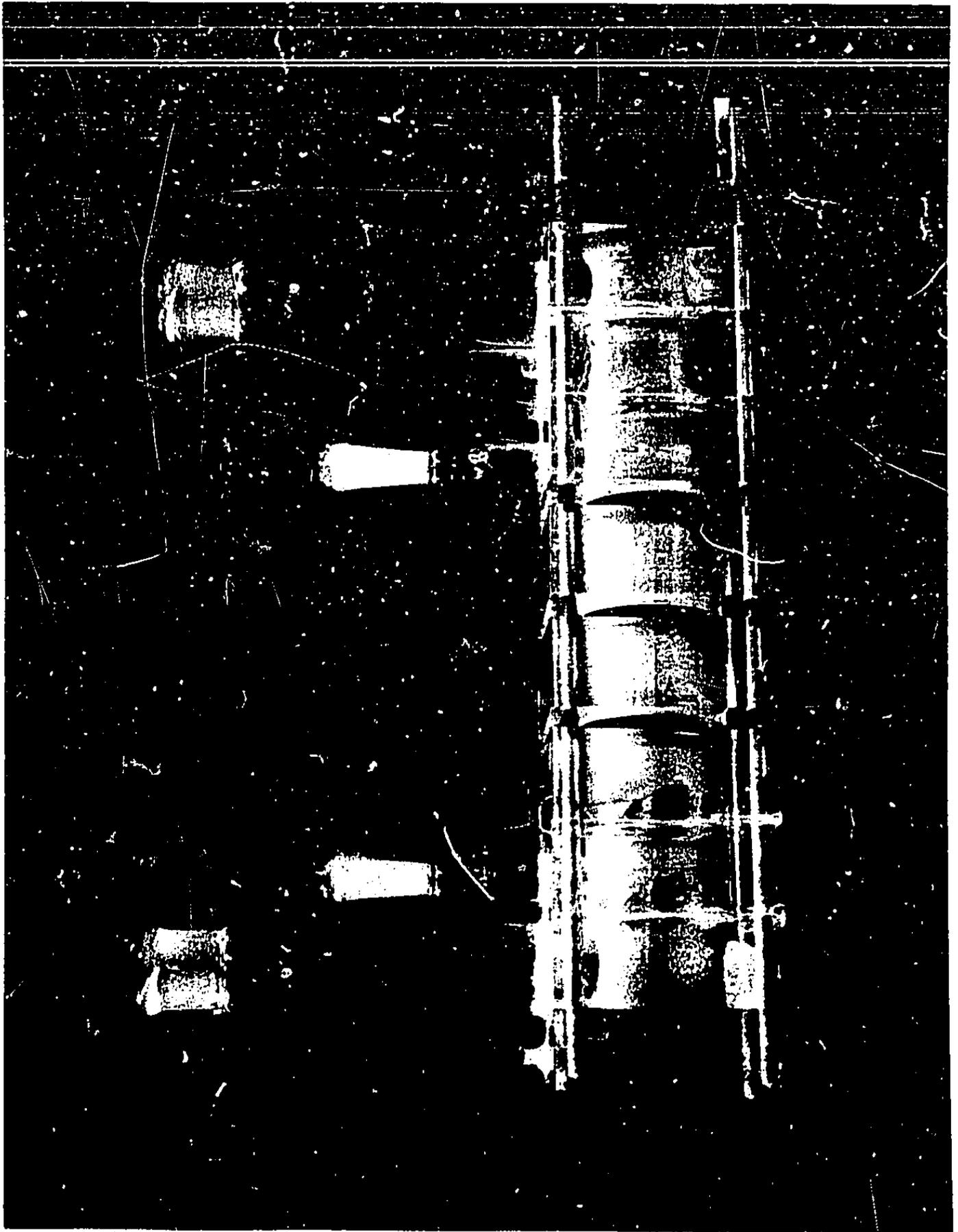


FIGURE 11

FIGURE 12



segment. These segments, in turn, are clamped by means of four support rods. The assembly is water-tight and during operation, the body of the cell is completely immersed in a water or an oil bath with only the access ports extending above the level in the bath.

Early in the study, it became obvious that conductance (and capacitance) measurements on membrane systems could furnish some rather specific information about the mobility of water in these systems. Unlike potential measurements, however, conductances do not lend themselves to rigorous, thermodynamic interpretation. Hence, while a theoretical interpretation may be hampered by a lack of knowledge about the role played by water structure in membrane transport, this study has shown that conductance (and capacitance) measurements offer sensitive ways of probing changes in the "environment".

Additional conductance studies on "llani" membranes were first carried out because of the substantial quantity of work which had already been done on these membranes. As discussed above, the llani membrane consists of a solid matrix - a Millipore filter - supporting a liquid organic phase (in our experiments, toluene). In each of the cells used in these studies, it was found that many variables adversely affected the experimental measurements, and these effects had to be eliminated before any confidence could be placed in the data obtained. This is especially true when data are gathered near the temperature where thermal anomalies have been noted.

The table below lists some of the factors and the special precautions taken to eliminate (or at least minimize) their effects:

| <u>FACTOR</u> | <u>SPECIAL PRECAUTION</u> |
|---------------------------|--|
| 1) Hydraulic permeability | Equalization of liquid levels in both compartments. |
| 2) Osmotic permeability | Equalization of osmotic pressures in both compartments with suitable reagents. |
| 3) Solubility | All solutions used in these studies were saturated with toluene prior to filling of cells. |
| 4) "Aging" effects | The determination of the effects of aging became an important aspect of the total investigation, including attempts to elucidate hysteresis effects. |
| 5) Mixing | Both half cells were stirred continuously. |
| 6) Stagnant layers | No special effort was made to determine the effects of the thickness of the stagnant layers. |

Figure 13 (Graph 46) is a reproduction of a typical run in which the conductance (and, more often than not, also capacitance) was monitored as a function of temperature. (This curve is especially interesting because it represents the first set of measurements that could be reproduced consistently with a high degree of precision). The system is composed of an I1ani membrane (Hydrophobic Millipore filter saturated with toluene) separating identical 0.01 M KCl solutions saturated with toluene.

At low temperatures, the conductance is small - a phenomenon indicating that the membrane acts as an effective insulator between the two conducting solutions. At somewhat higher temperatures, the conductance increases slowly at first, then very rapidly - the curve passing through a point of inflection of 32.8°C - and, at about 34°C , assumes a more or less constant rate of increase to near 60°C . At this temperature, the conductance rises extremely fast with temperature. It is important to note the three temperature regions where points of inflection occur in the curve (or where the curve exhibits an abrupt change): 30° , 45° (a very subtle, but real inflection) and 60°C - temperatures at which many aqueous systems have shown the occurrence of thermal anomalies!

It should be pointed out, however, that subsequent experiments demonstrated the existence of another factor - one which frequently overrides any "anomalous" thermal behavior - but only at the initial point of inflection. It was found that the position of the initial inflection point depended on a number of variables:

- 1) The initial temperature of the system
- 2) The rate of heating, and
- 3) The nature and history of the membrane.

Changing any of these variables resulted in a shift of the position of the initial point of inflection. The cause of this was determined in the following manner: conductance measurements were made on systems identical to that described for Figure 13 (Graph 46) except that the temperature was held constant and conductance recorded as a function of time (the abscissa). The curve obtained was S-shaped (as in Graph 46) but the conductance reached a maximum value and remained essentially constant. Subsequent isothermal experiments demonstrated that the position of the inflection point could be shifted merely by changing the temperature. To confirm this in a slightly different manner, an experiment was performed in a system identical to that described above; Figure 14 (Graph 53). The system was heated from 24° to 46.5°C . The characteristic S-shaped curve was obtained with an inflection point near 37°C ; above this temperature, the conductance leveled off and remained constant with time. From this information it may be inferred that the rapid rise in conductance with temperature - or with time under isothermal conditions - is due to the penetration into and through the membrane by the aqueous solutions. This information can be utilized to account for the entire S-shaped curve - irrespective of whether the curve

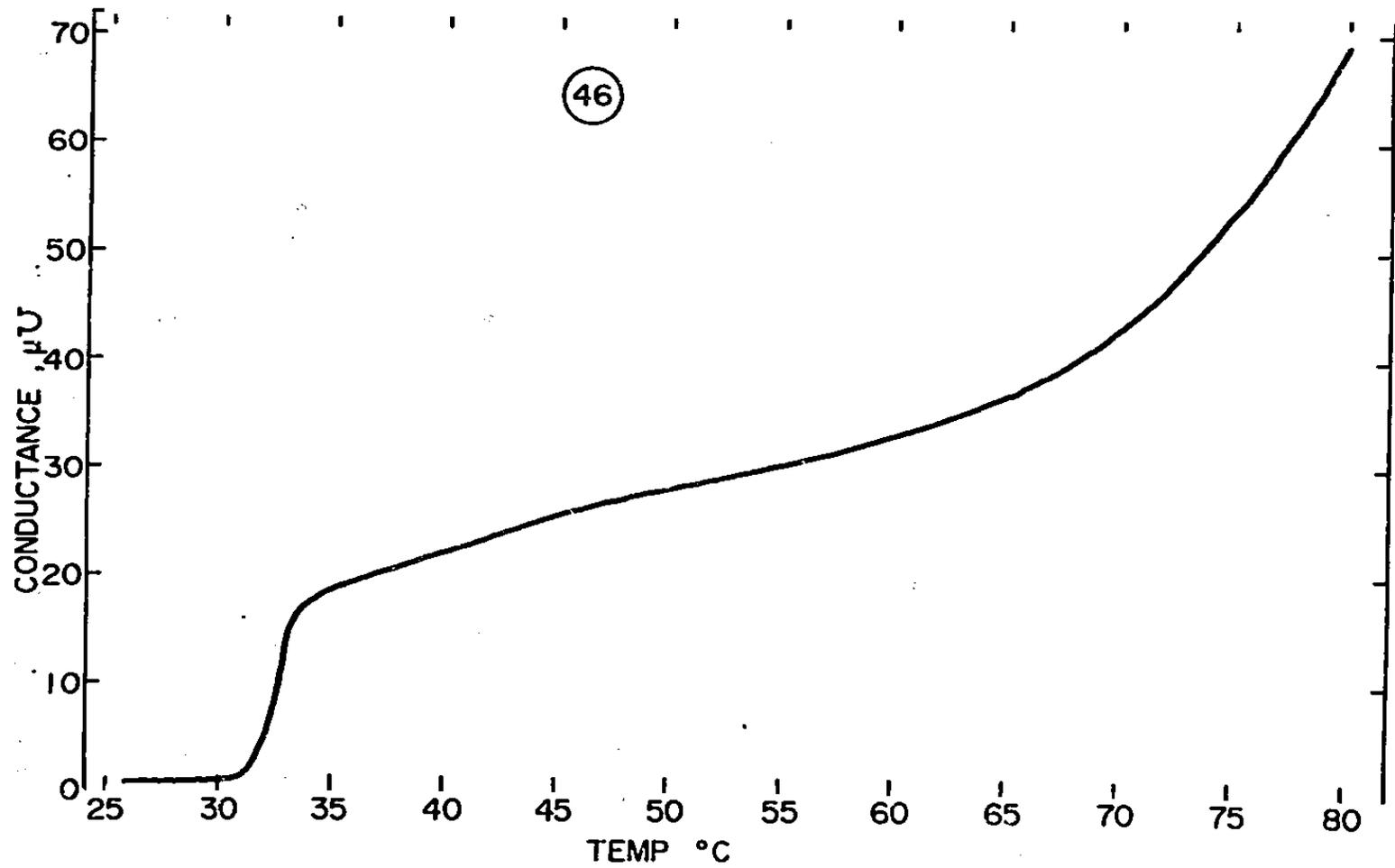


FIGURE 13

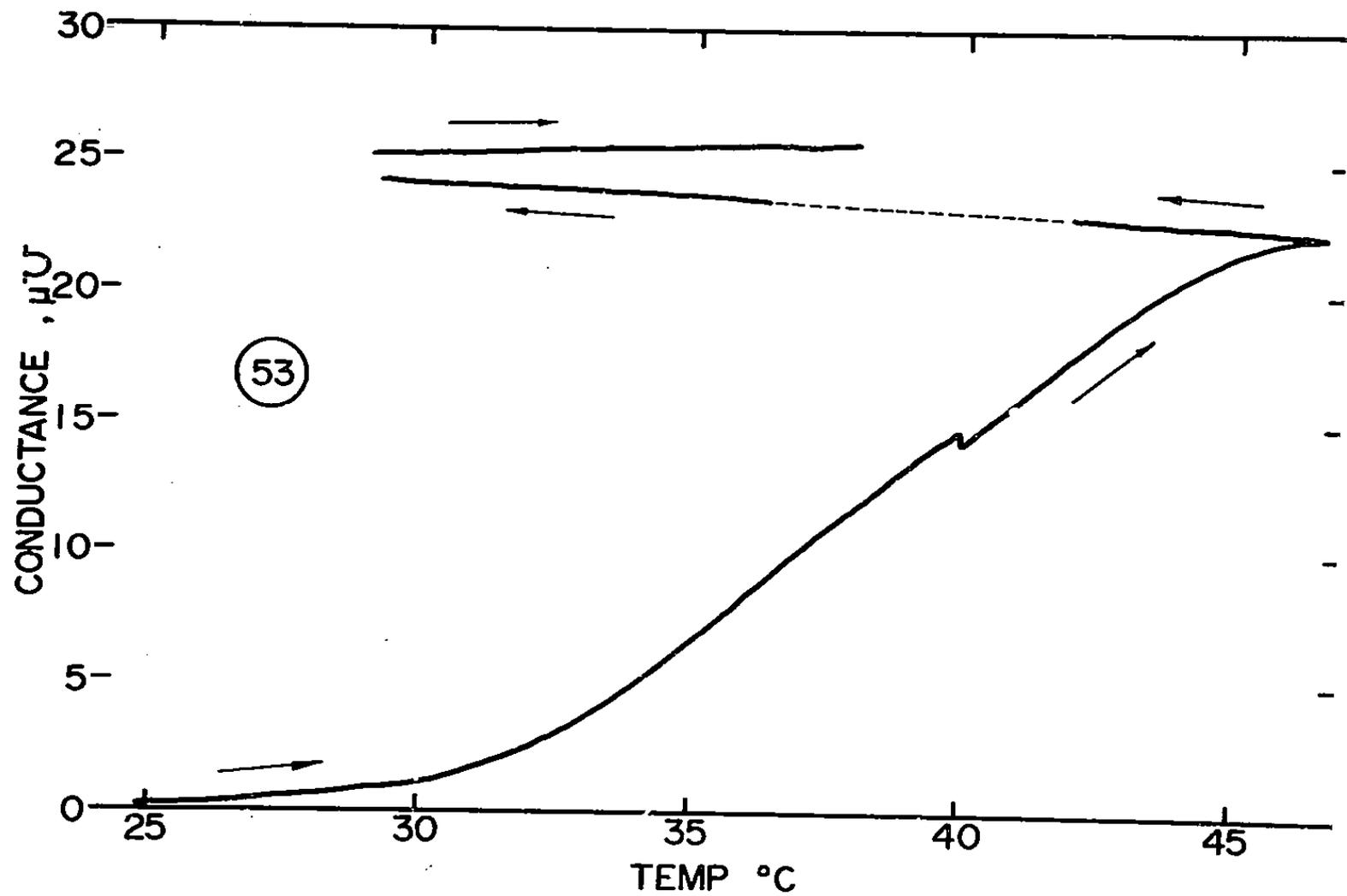


FIGURE 14

53

described represents an isothermal run or was obtained under conditions of changing temperature. At the beginning of the experimental run, the membrane, a non-conductor, insulates the two conducting solutions from one another (hence low conductance). With time, these solutions are drawn by capillary action (primarily, and by the presence of, any incidental pressure head) into the pores. As each pore is filled with conducting (aqueous) solution, it forms an electrolytic bridge between the liquids. The conductance, then, gives a measure of the number and areas of channels open between the two solutions. Thus, the rate of change of conductance gives a measure of the rate of permeation of the liquid phase into the membrane. At the inflection point, this rate is maximum. Above the point of inflection, the conductance will begin to rise more slowly because most of the pores have now been filled. If the run is carried out isothermally, the conductance will reach a maximum value and remain constant, as all channels are open and no factor operates to change the conductance further. This is observed experimentally! If, however, the temperature continues to rise, the conductance will likewise increase - not because there remains more channels to be filled (it is obvious that they are completely filled with solution) but because of the direct effect of temperature on the pore conductance! It is not surprising, then, that any "ordinary" thermal anomaly will be completely obscured by the comparatively huge changes in conductance in this region. To illustrate the point, consider Figure 15 (Graph 59): this system is identical in every respect to that described in Figure 13 (Graph 46) with one exception: the membrane contained no toluene. Note that the 30° C inflection is absent, but another occurs at 45° C; this large break would mask any subtle changes due to a thermal anomaly. Hence, it must be pointed out that the overall picture needs to be examined in the light of these findings - not merely a small segment of an experimental run.

During the present study, several curves were obtained which exhibited behavior which cannot be explained completely and satisfactorily. Figure 16 (Graph 57) is an experimental curve which should have been nearly identical to Figure 13 (Graph 46) as all experimental conditions - with the exception of heating rate - were the same. In this case, the curve passes through a maximum (after the inflection point), then decreases - albeit slightly - in spite of increasing temperature. The curve finally becomes constant after heating ceases. When the heater is again turned on (point "A"), the conductance increases once more, as expected.

Figure 17 (Graph 58) shows an identical case, except that at point "A", a cooling was initiated. As expected, the conductance decreases with decreasing temperature - but note the strangely abrupt manner in which this takes place. The reason for the maximum followed by decreasing conductance with increasing temperature is unknown, but the frequent occurrence of this phenomenon suggests that it is not an artifact, due to errant equipment or technique.

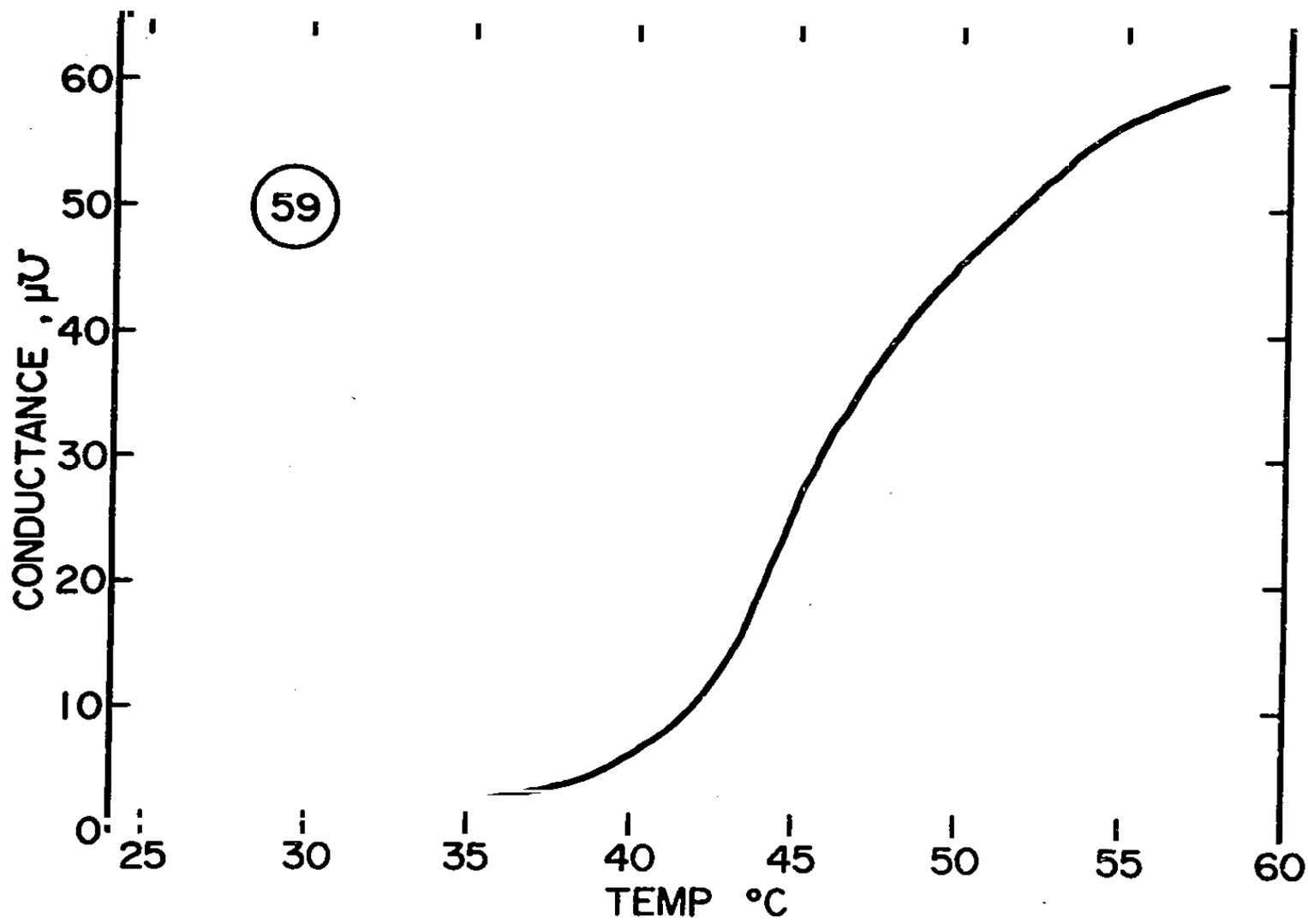


FIGURE 15

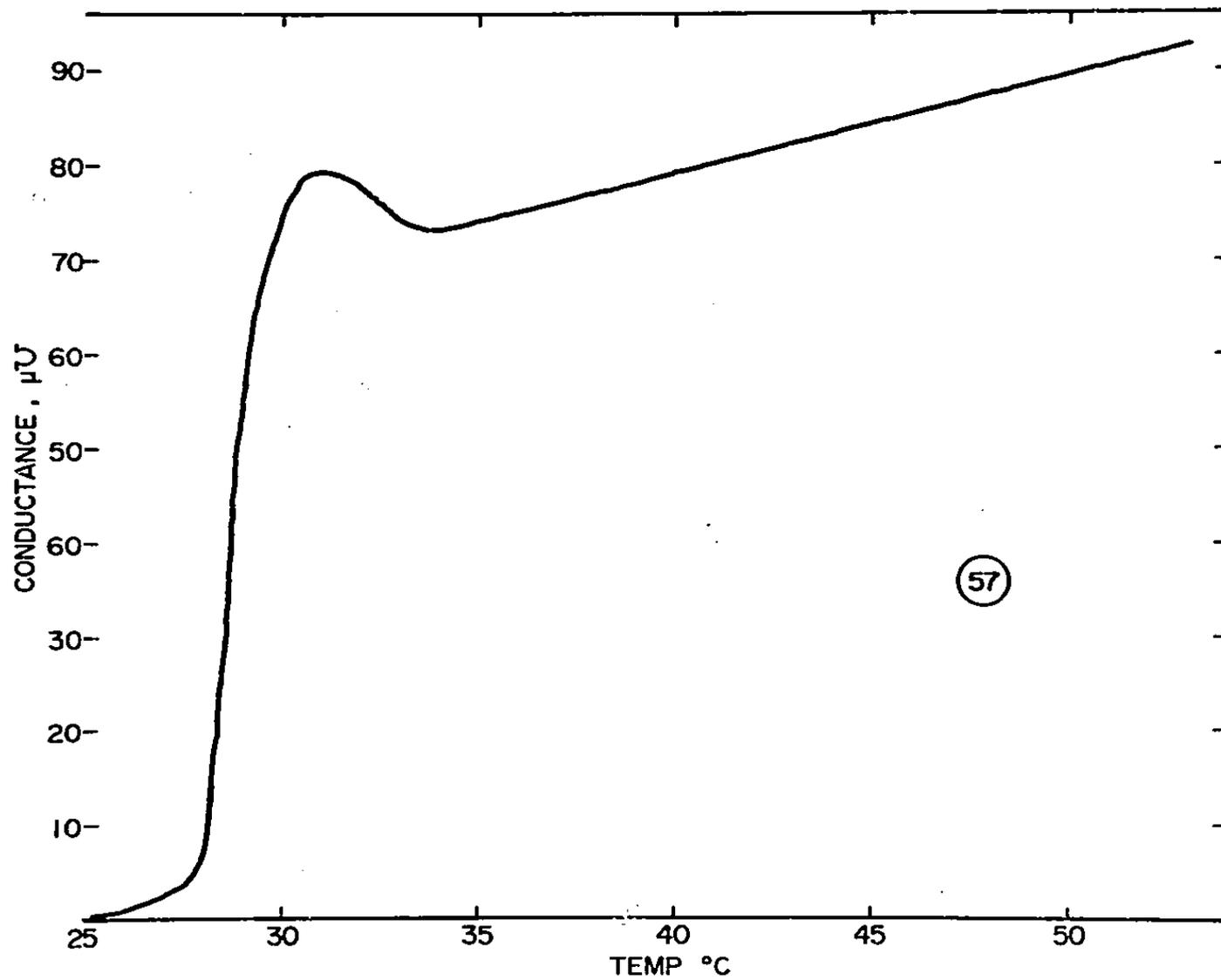


FIGURE 16

57

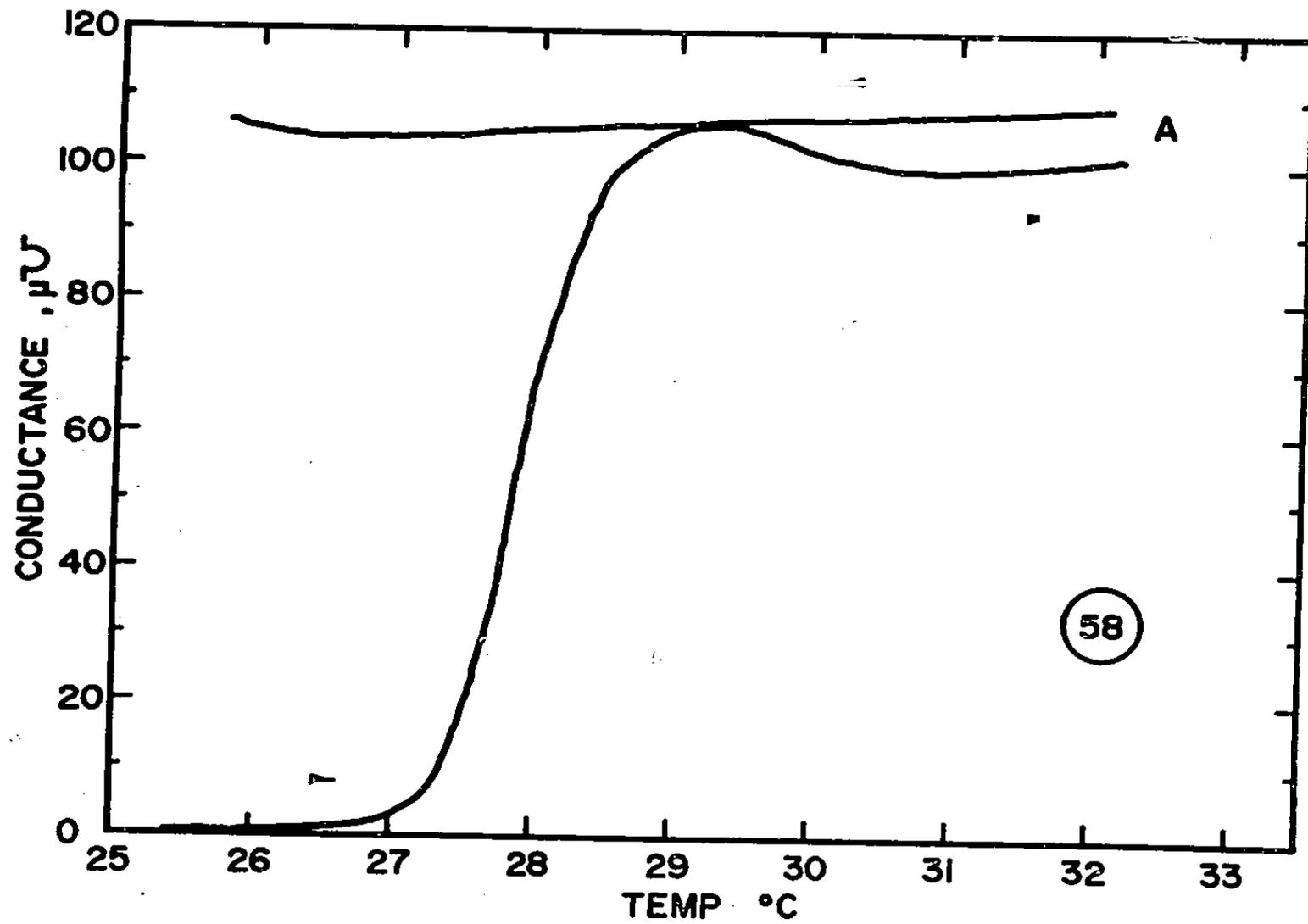


FIGURE 17

In order to determine the effect of a liquid phase on membrane behavior, a number of experiments were performed with and without toluene. Membranes made of Teflon gave identical responses with and without toluene; the conductance failed to change in the temperature interval from 24 to 60° C. This is most likely due to the inability of the water to "wet" a surface of such low surface free energy (or to penetrate it). On the other hand, mixed ester and nylon Millipore filters showed some hydrophobic character, for instance hysteresis effects, when saturated with toluene, but none when toluene was absent. Apparently, the hydrophilic character of these membranes allows for instantaneous wetting when these membranes are first placed in contact with the solutions, and penetration is complete before the first data point can be obtained. Graphically, this corresponds to a conductance long past the inflection point; physically, all the channels are filled and the sole effect observed is the reversible changes due to temperature on conductance.

The results of these experimental findings were used to determine quantitatively the effect of temperature on the rate of liquid penetration into the membrane. It has already been suggested that the maximum penetration rate corresponds to the point of inflection of the conductance vs. time plot; this rate is inversely proportional to the time required to reach this point, τ . Assume that the intrusion of liquid into the membrane may be represented by the standard rate expression:

$$\frac{d \ln \tau}{dT} = - \frac{\Delta E_a^\ddagger}{RT^2}$$

in which ΔE_a^\ddagger is the apparent energy of activation of membrane penetration, T temperature (°K) and R the gas constant. Thus, if ΔE_a^\ddagger is constant in the temperature interval studied, integration yields the usual Arrhenius equation:

$$\log \tau = \frac{\Delta E_a^\ddagger}{2.3RT} + \text{Constant}$$

A plot of $\log \tau$ vs. $1/T$ is linear with slope equal to $\Delta E_a^\ddagger / 2.3R$. A number of specific cases will be considered.

Hydrophobic Millipore membranes exhibited consistently reproducible behavior within a given batch, but reproducibility among different batches was virtually nonexistent. The trouble was traced to a wetting agent, used in the production of the filters; each batch contained a concentration of agent, different from every other batch. The wetting agent served to lower the surface free energy of the substrate and thereby reduced the barrier to penetration. Figure 18 gives a $\log \tau$ vs. $1/T$ plot of data obtained on a (truly) hydrophobic Millipore filter, i.e., in which no wetting agent was employed. The apparent energy of activation is huge - 30,000 calories per mole - a number exceeding some chemical bond energies! Figure 19 shows $\log \tau$ vs. $1/T$ plots for two sets of data obtained on the same batch of Millipore hydrophobic membranes containing traces of the wetting agent - one set for each of the conductance cells used. The slopes of these curves are equal; hence, the apparent energies of activation are also identical - 14,000 calories

FIGURE 18

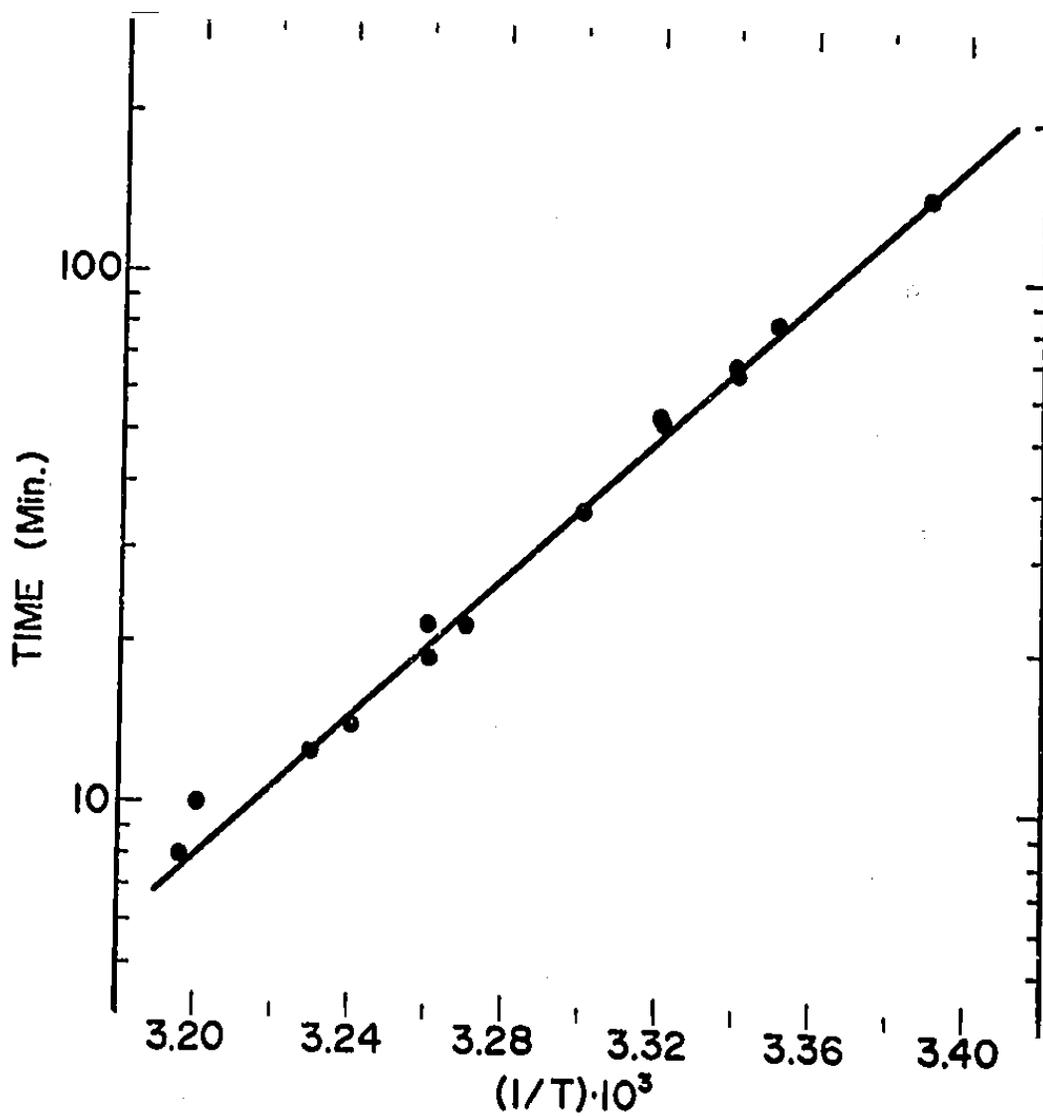
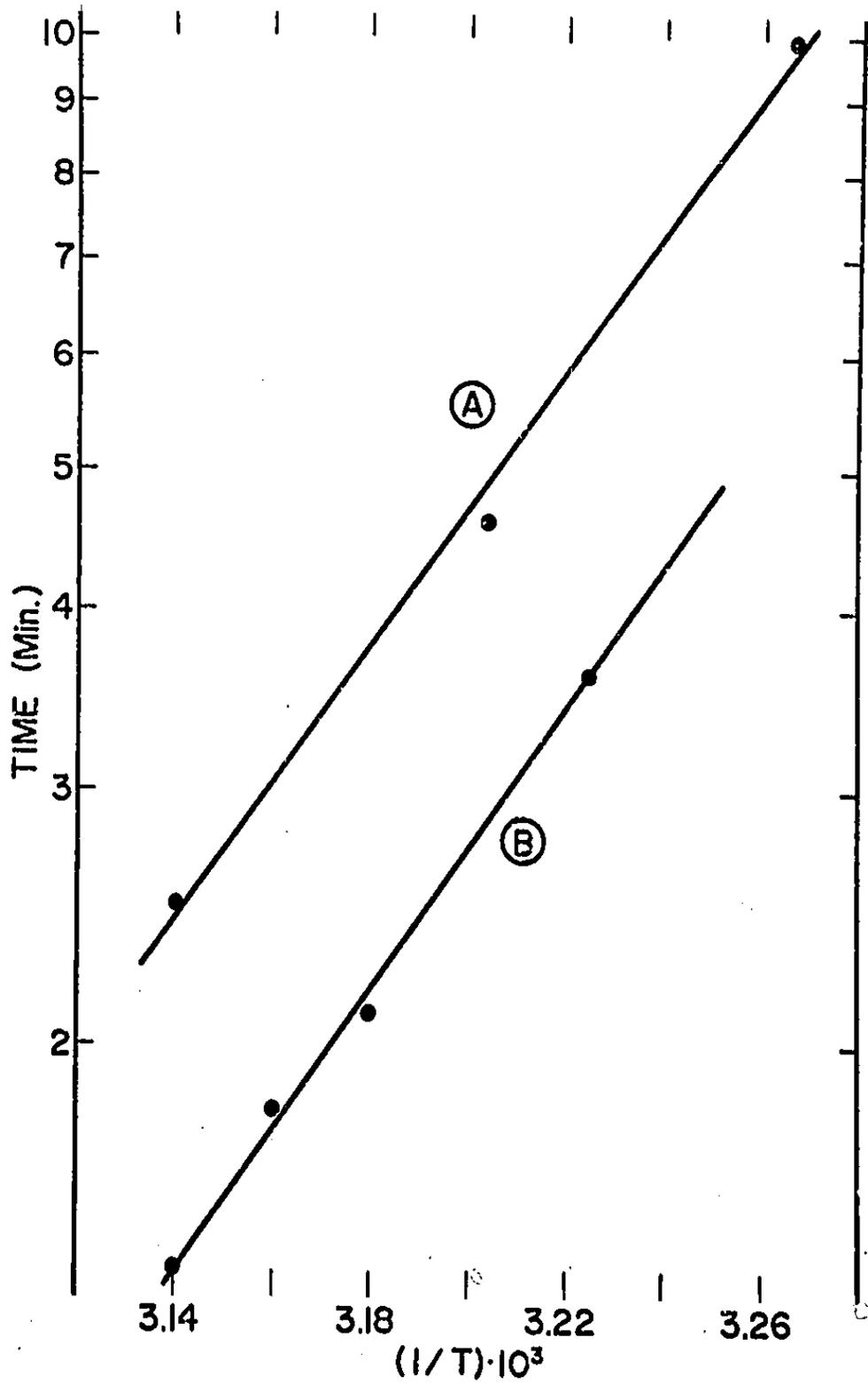


FIGURE 19



per mole. Thus, it is seen that quantities of surface active impurities too small to be detected by classical analytical methods, may cause the demise of "normal expectation" in surface chemical experiments of this type. It should be mentioned that the intercepts of these plots are different; It is left for speculation that the entropy of activation (related to the intercept) is likely a function of the specific details of the membrane system: its geometry, etc. No explanation will be attempted for this phenomenon, nor for the exceedingly high values for ΔE_a^\ddagger of the truly hydrophobic membrane.

C. Porous Vycor Membranes

Preliminary studies have been made of the conductance in porous Vycor. The main object was to determine the extent of discrete ion size effects on the conductive processes in porous Vycor and the possible role of the water in the narrow pores of this material. The Vycor matrix has an average pore diameter of approximately 40 Å. It was felt that careful studies of the energetics of the transport processes in this material might reveal something about the interaction between the state of water in the narrow pores of the Vycor and the state of hydration of the ions during passage through the Vycor membrane.

Measurements have been made over extended temperature ranges (at fairly closely spaced temperature intervals about 1 or 2° C) of 10^{-2} molar solutions of all alkali chlorides and the chlorides of some quaternary ammonium compounds. These data are still being worked on. One difficulty encountered is the lack of comparable bulk conductance data, measured at closely spaced temperature intervals, in order to obtain accurate, relative apparent energy of activation data for comparison purposes.

To date, it appears that the energies of activation for conduction of lithium and sodium chloride (10^{-2} molar solutions) are nearly identical and somewhat larger (but only by a small amount) than the energies of activation for the conduction of potassium, rubidium, and cesium chlorides. For the latter three salts, the apparent energies of activation appear nearly identical.

It had been expected, that since the porous Vycor possesses an extremely high surface-to-volume ratio, thermal anomalies were likely to be observed. This, however, was not confirmed. No indications were found for anomalies (near 30°) in the conductance of the alkali ions through the porous Vycor matrix. However, measurements with the larger quaternary ammonium ions did suggest that changes occurred in the vicinity of 30°. It is speculated that this effect is related primarily to changes in the hydration of the larger quaternary ammonium ion, rather than in the nature of the water in the pores of the Vycor. Before these data can be published, however, additional confirmatory studies will be required.

Figure 20 outlines in some detail the conceptual model that has been employed in a preliminary fashion to account for the conductance of solutions in porous Vycor. In this treatment, it is assumed that the pores are cylindrical and of uniform size; furthermore, it is assumed that conduction takes place via two different paths: through the bulk phase and over the surface; hence, each pore may be treated as consisting of two parallel conductors.

For parallel conductors, the total resistance of a system is given by the expression:

$$(1) \quad \frac{1}{R_t} = \frac{1}{R_b} + \frac{1}{R_s}$$

where b and s refer to the bulk and surface, and R_t is the total resistance. The resistance of the solution is given by the standard expression:

$$(2) \quad R_B = \frac{P_B \cdot L}{A_B}$$

In which P_B is the specific resistance; L and A_B refer to the length and cross-sectional area of the liquid column.

In like manner, a relationship may be written for the surface resistance:

$$(3) \quad R_S = \frac{P_S \cdot L}{A_S}$$

Now the resistance is simply the reciprocal of the conductance, so that substitutions can be made in the equations:

$$(4) \quad C_t = C_B + C_S$$

and

$$(5) \quad C_t = \frac{A_B}{P_B L} + \frac{A_S}{P_S L}$$

From our model, the following relationships are evident:

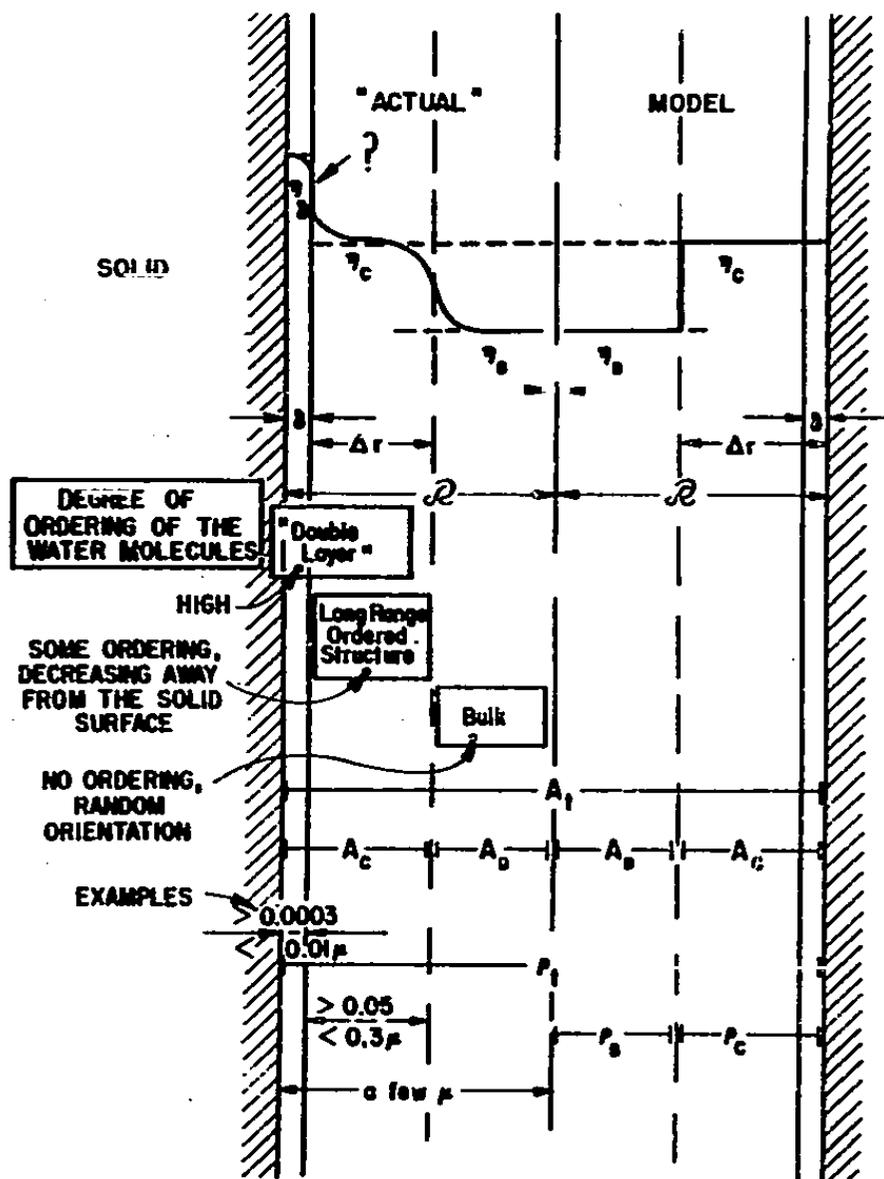
$$(6) \quad A_B = \pi r^2$$

$$(7) \quad A_S = 2\pi rL$$

so that expression (4) becomes:

$$(8) \quad C_t = \frac{\pi r^2}{P_B L} + \frac{2\pi r}{P_S}$$

Equation (8) can be used to find both the specific resistance of the bulk and surface phases provided that C_t , r, and L are known. For our purposes, it is necessary to know the contribution of both C_B and C_S over several orders of magnitude of pore size. This was accomplished in the following way:



LEGEND

- η_b = VISCOSITY OF BULK LIQUID
- η_c = " " " LONG RANGE ORDERED STRUCTURE
- η_s = " " " "CLASSICAL" DOUBLE LAYER
- δ = THICKNESS OF "CLASSICAL" DOUBLE LAYER
- Δr = " " " LONG RANGE ORDERED STRUCTURE
- R = RADIUS OF CAPILLARY
- A_t = TOTAL CROSS-SECTIONAL AREA
- A_s = CROSS-SECTIONAL AREA OF BULK LIQUID
- A_c = " " " LONG RANGE ORDERED STRUCTURE
- p_b = CONDUCTIVITY OF BULK LIQUID
- p_t = " " " ENTIRE CAPILLARY
- p_c = " " " LONG RANGE ORDERED STRUCTURE

FIGURE 20

Differentiating (8) with respect to "r" gives:

$$(9) \quad \frac{dC_+}{dr} = \frac{2\pi r}{P_B L} + \frac{2\pi}{P_S}$$

Multiplying (9) by (r/C₊) we obtain:

$$(10) \quad \frac{d \log C_+}{d \log r} = \left[\frac{2\pi r^2}{P_B L} + \frac{2\pi r}{P_S} \right] / C_+$$

which simplifies to:

$$(11) \quad \frac{d \log C_+}{d \log r} = \frac{2C_B + C_S}{C_B + C_S}$$

In principle, a log-log plot of C₊ vs. r can now be used to determine the contribution of each conductance. Of more immediate use, however, is the application of (11) to test the validity of the model proposed. This may be done by finding the limiting slopes of the log-log plot:

As r → 0, r² → 0 much faster, so that:

$$(12) \quad \frac{(d \log C_+)}{(d \log r)}_{r \rightarrow 0} = \frac{C_S}{C_S} = 1$$

As r → ∞,

$$(13) \quad \frac{d(\log C_+)}{d(\log r)} = \frac{2C_B}{C_B} = 2$$

The experimental values observed for the slopes when r is small (i.e., 40 Å) is found to be 1.1 and when r is large (i.e., in a large capillary) the slope is found to be 2.1 - both values in reasonable agreement with the proposed model. Because of this agreement, additional work was carried out to determine both temperature and pore size effects on the conductivity of various salt solutions in order that energies of activation could be calculated, and quantitative information about ion discrimination in various membranes could be obtained. This aspect is still being pursued.

D. Liquid Membranes (Butanol)

Schulman and co-workers (see Rosano et al., 1961) measured the flux of electrolytes and of water across a system consisting of:

solution (I)/n-butanol/solution (II).

This system was chosen by Schulman as an example of a conceptually simple "oil membrane". The experimental setup used by Rosano et al. is shown in Figure 21. It is important to insure good stirring of all three phases: the two aqueous and the overlaying butanol, to minimize bulk phase diffusion processes. Under proper conditions, the rate

Keithley Electrometer

Ground

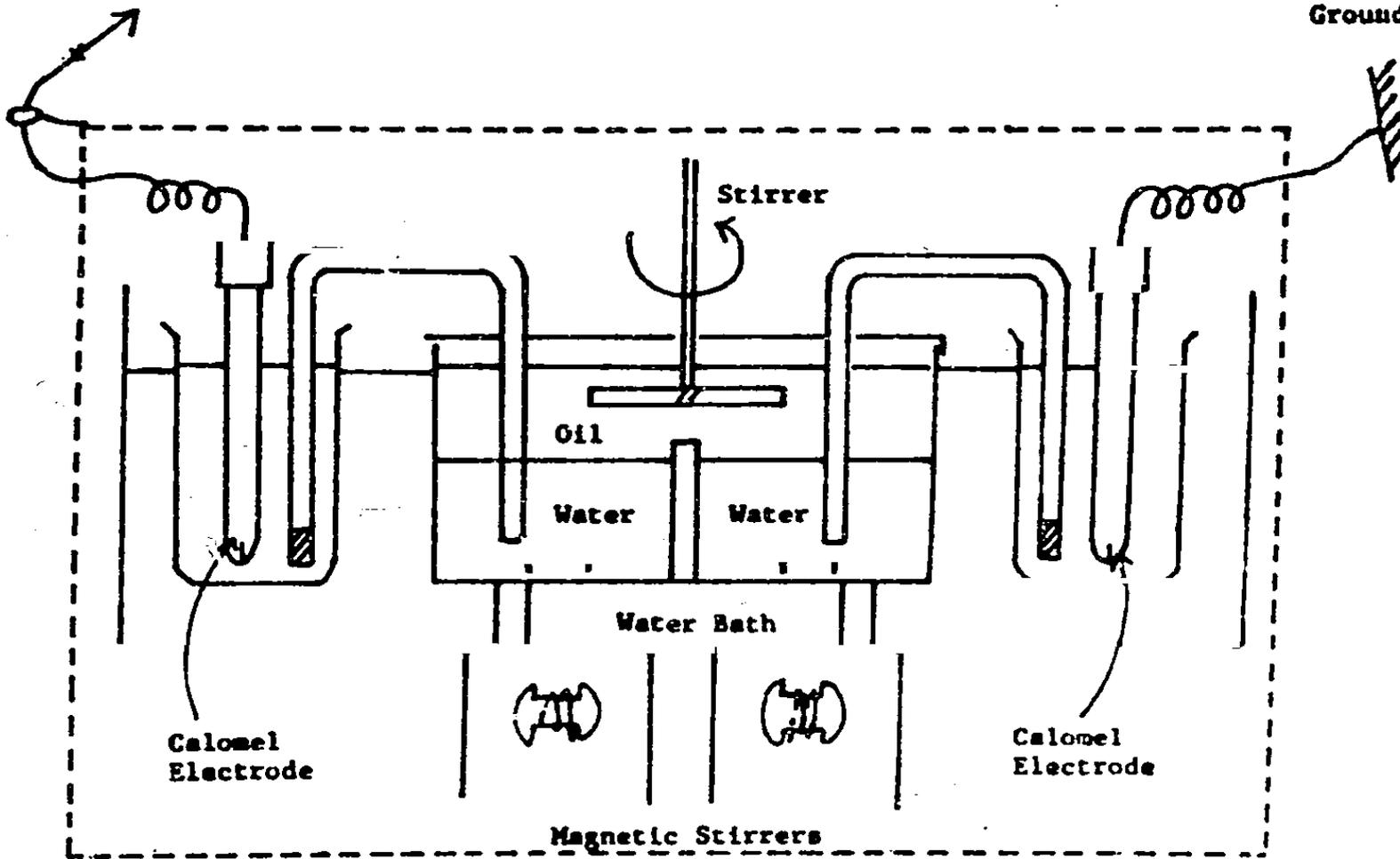


FIGURE 21

determining steps are essentially located at the two water/butanol interfaces. While conceptually simple, the system in actual practice is somewhat complex. The mutual solubility of water and butanol is relatively high. In Schulman's laboratory, the experimental setup was used mostly in a semi-quantitative fashion (or at least without attempts to obtain highly accurate data); some results are shown in Figure 22. Later, the Principal Investigator redrew the data by Rosano et al., suggesting that a notable change occurs near 30° which was overlooked by Schulman and his co-workers; see Figures 23 and 24.

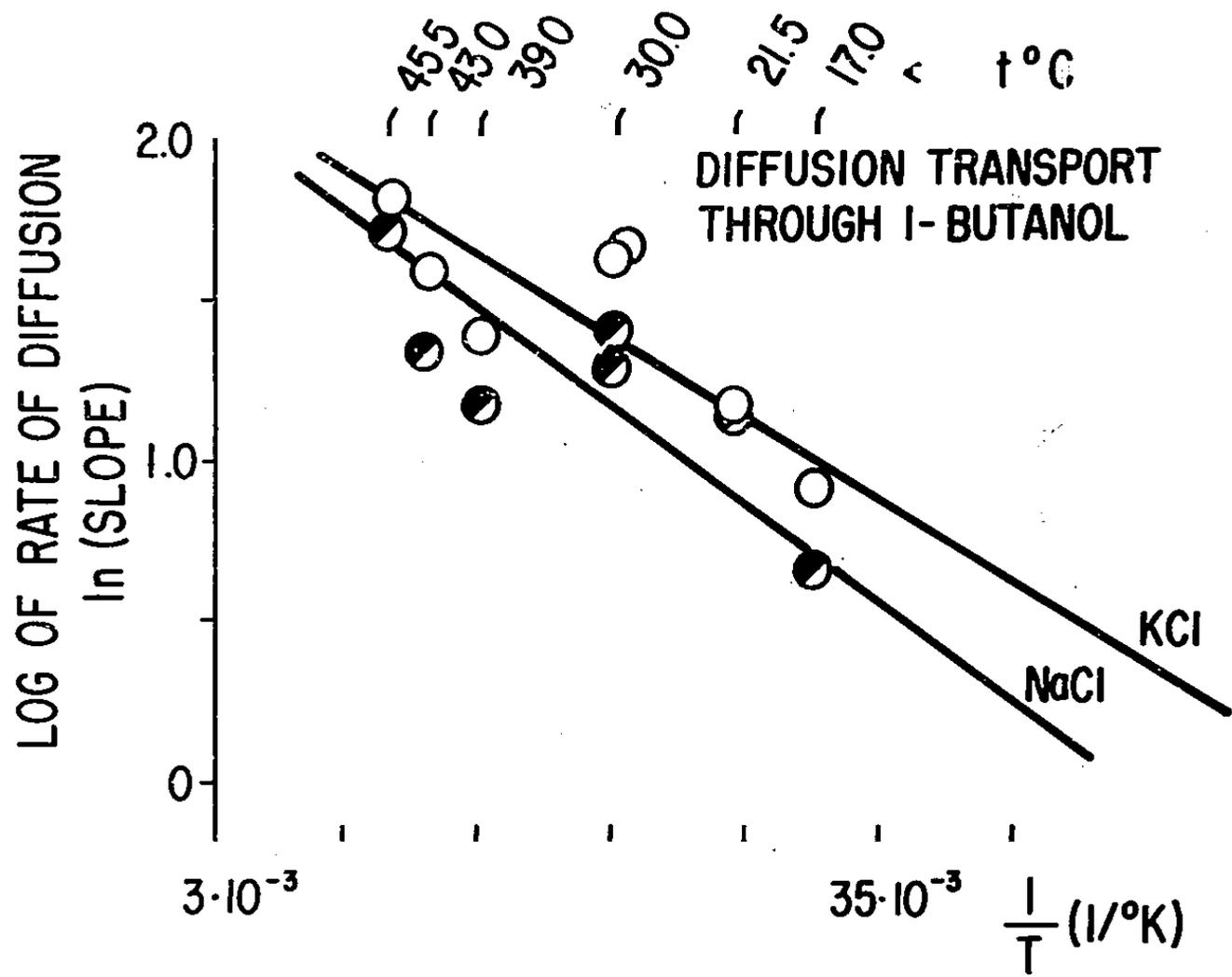
Ting and co-workers have investigated the same system. These authors were interested in modeling the plasma membrane; the energetics observed for the crossing of electrolytes over the water/butanol interface is comparable to that observed for crossing the plasma membrane.

The original apparatus employed by Schulman and co-workers was extremely simple. A considerably more complex version was developed in the Principal Investigator's laboratory, to allow better definition of area and dynamic conditions prevailing at the water/butanol interfaces. Agitation was effected by mechanical stirrers placed in the butanol phase, while a liquid circulation system was used to renew the aqueous electrolyte at each interface in the two separate compartments. The volume of the new system was adjusted so as to minimize the total amount of butanol, compared to the total amount of electrolyte solution. Thus, only relatively small changes in net concentration of electrolytes in the two different compartments were incurred over any period of time (long enough to make a run).

Experimentally, the system was utilized in a dynamic mode where the temperature of the bath was changed continuously at a reasonably constant rate of change. Because of the internal circulation, the electrolyte compartments were assumed to be nearly at the temperature of the bath and the difference in temperature between the two half cells negligible. Silver/silver chloride electrodes were used with salt bridges, provided with porous Vycor plugs. The potentials were measured with a Keithly 660A Electrometer. The output from the electrometer was recorded on an X-Y recorder, the X-axis of which was driven by the output from a thermistor thermometer.

The results of these experiments repeatedly indicated anomalous, abrupt changes in the potentials between, for instance, 1 molar and 10⁻² molar potassium chloride solutions, separated by butanol. The observed variations in potential (of a total potential of about 100 millivolts) was several millivolts as the temperature was increased over, say, 10° C. The changes observed were often remarkably sharp, mostly occurring somewhere between 15.5 to 19.5° C - often near 16°. Similar anomalies were observed also in the vicinity of 30°. Some of the data obtained were reported in a Ph.D. Dissertation from the Principal Investigator's Laboratory (by Dr. Anitra Thorhaug (Bader), 1969).

FIGURE 22



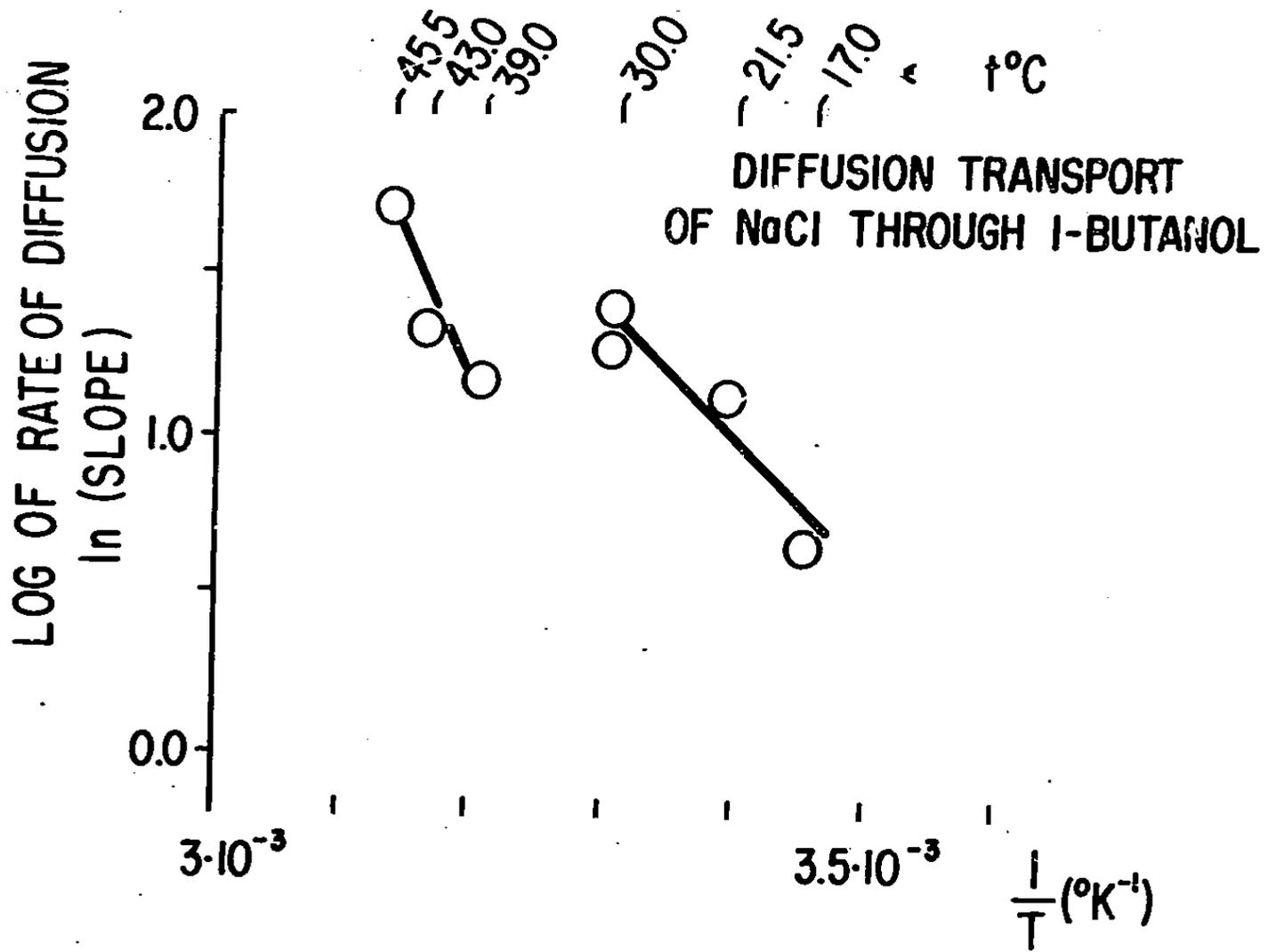


FIGURE 23

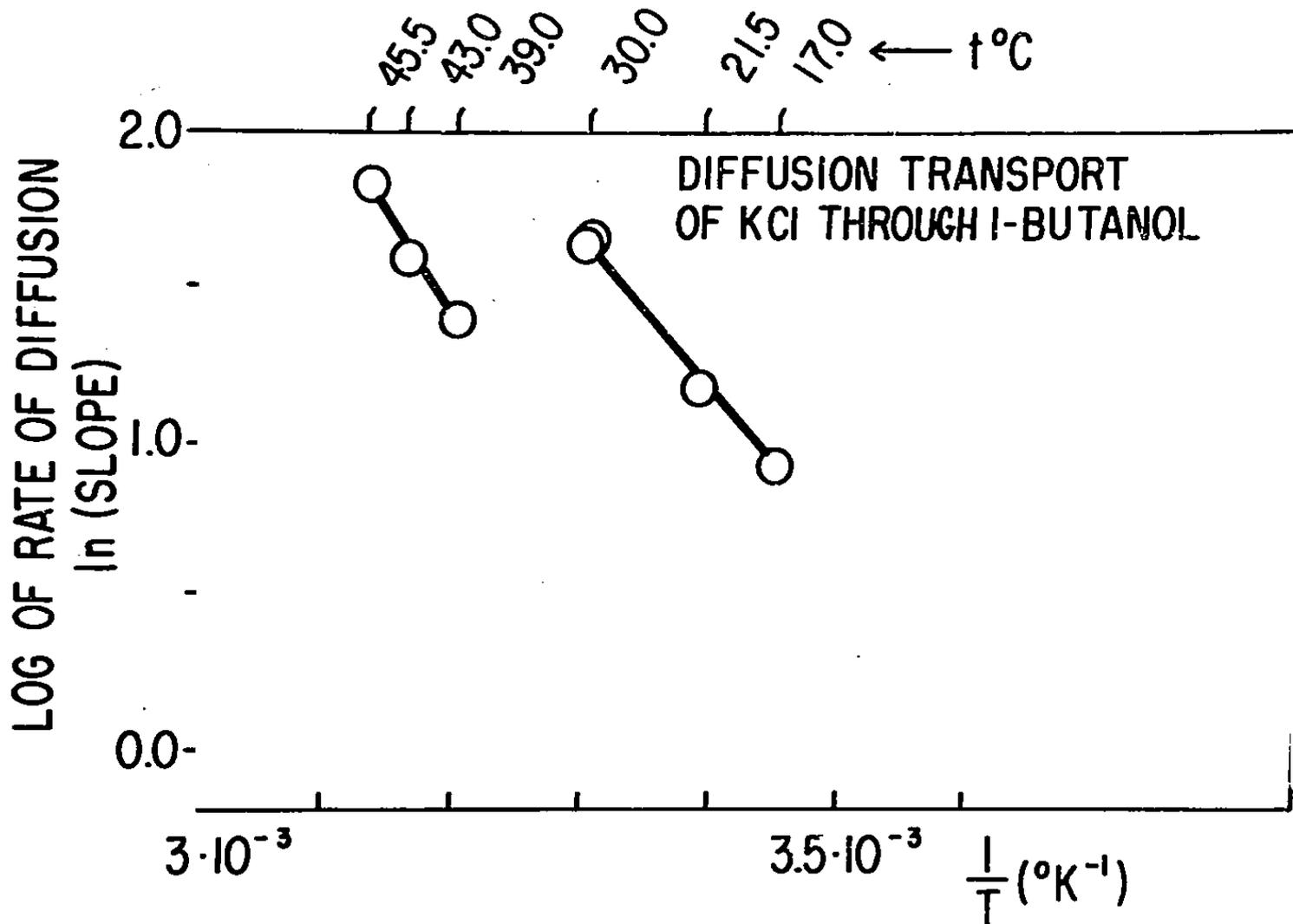


FIGURE 24

E. Bioelectric Potentials In the Giant Alga, Valonia

In connection with the Principal Investigator's general studies of water near interfaces, membrane potential measurements were performed on the giant alga, Valonia. In the systematic search for information about the role of water structure in various membrane systems, the Valonia represented the most complex type of membrane studied. This study was supported by a number of different agencies, including the Office of Saline Water. A very preliminary report (mostly as the result of studies supported by NSF) was published in Nature (Drost-Hansen and Thorhaug, 1967). A considerably more detailed report of these studies appeared in a doctoral dissertation from the University of Miami (A. Thorhaug, 1969). A fairly comprehensive report of these studies was reported later (A. Thorhaug, 1971). The essential observation here - as with the model membranes described previously in this report - was the occurrence of abrupt changes in the bioelectric potentials near 15 and 30° C. Between these two temperatures, the potential observed (for three species of Valonia) has practically zero temperature coefficient. In all three species, the temperature coefficient was numerically very large outside of the temperature interval between 15 and 30°; at lower temperatures, the sign of the temperature coefficient depended on the species studied.

III. THEORETICAL AND CONCEPTUAL STUDIES

Many of the conclusions which have been reached from the experimental studies summarized above, together with those derived from a study of the literature, have been published in a recent paper in the treatise on Chemistry of the Cell Interface (H.D. Brown, Editor, Part B, article entitled "Structure and Properties of Water at Biological Interfaces", by W. Drost-Hansen, 1971, Academic Press, New York, pg. 1-184). From this article, the section on membranes is quoted in toto.

The general problem of morphology, structure, and functioning of cellular membranes is outside the scope of the present chapter. We are concerned here only with the role which water may play in membrane stability and functioning. However, in order to appreciate this problem it is necessary to have some acquaintance with the nature of cellular membranes, and the reader is referred to the monographs edited by Järnefelt (1968), Kavanau (1965), D. Chapman (1968), New York Heart Association (1968), several of the symposia published by the Society for Experimental Biology (see S. E. B., 1965), Schlögl (1964), Clark and Nachmansohn (1954), Kleinzeller and Kotyk (1961), Schoffeniels (1967), Snell *et al.* (1970), and particularly Lakshminarayanaiah (1969). The last-mentioned volume (dealing especially with transport phenomena in membranes) contains an extensive bibliography, including an excellent annotated bibliography of recent work; see also the earlier work of Davson and Danielli (1943). Among other reviews of biological membrane structure, see "Recent Progress in Surface Science" edited by Danielli *et al.* (1964, 1970) and also the selection of papers by Brunton and Park (1968). Two symposia on membrane properties deserve special mention: "Membrane Phenomena" (see Faraday Society, 1956) and "Biological Membranes: Recent Progress" (New York Academy of Sciences, 1966). See also "Symposium on Cell Membrane Biophysics" (1968).

In terms of transport processes the thermal anomalies may be seen as manifestations of water structure changes, resulting in vastly different mobilities of solutes (ions and low molecular weight nonelectrolytes) in the vicinal water in membranes. Again, a change in the vicinal water structure may, in turn, result in a change in the conformation of the substrate. In terms of the holist theory, the thermal anomalies are manifestations of changes in the thermodynamic activities of the solute as the

vicinal water structure suddenly changes. It is also possible, of course, that the influence of the changes of the water structure simultaneously affect both thermodynamic equilibrium and transport properties. Thus, by itself, the occurrence of thermal anomalies does not distinguish between active and passive transport, on the one hand, and change of the cell water *in toto*, on the other hand.

A recent study of membranes by dielectric measurements will illustrate the likely (or perhaps maximal) extent of water structuring in membranes. Coster and Simons (1970) have used a Wayne-Kerr conductance bridge to study the capacitance changes in membranes. The main result from this study was the discovery of layers of water near lipid membranes with properties notably different from the properties of bulk water. The apparent thickness of the water layers adjacent to the membrane appeared to approach 40,000 Å! Such a layer (4 μ thick) would obviously have a very pronounced effect on the overall electrical properties of all types of cells if these do, indeed, have characteristics similar to the lipid membranes proposed initially by Danielli and Davson (1935). However, Coster and Simons (1970) call attention to the need to examine carefully some of the underlying assumptions required to extract the value for the thickness of the changed water layer from the experimental data; it is to be hoped that this type of study will continue as it appears to offer a very promising approach to the understanding of vicinal water in biological systems.

More than anyone else, it is probably Stein (1967) who has contributed the most systematic analysis of membrane functioning in terms of the underlying molecular processes, especially transport theories. Stein seems particularly attuned to the possible structural roles of water in the morphology and functioning of membranes.

3. Examples of Thermal Anomalies in Membranes

Remarkably sharp anomalies in membrane properties have been observed by Dalton and Smart (1967). It is interesting that in these studies one of the characteristic temperatures—at which an abrupt change in the energy of activation for the conduction through the toad bladder membrane occurs—is very close to 28°C (28.2 \pm 0.4°C). It seems reasonable to propose that this change is a manifestation of a change in water structure associated with the vicinal water of the membrane. However, a second (and often very abrupt) anomaly occurs in the vicinity of 37.2°C. The origin of this anomaly is far more difficult to explain within the framework of what has been discussed in the present paper as this temperature does not coincide with any of the known thermal anomalies due to water

structure changes. However, as pointed out in Section V,C an anomaly near 37°C could be due to a transition in the lipids of the membrane.

As demonstrated in this chapter, many data from the biochemical and biological literature show evidence of thermal anomalies, but the anomalies have frequently been overlooked or ignored by the authors themselves. A striking example of the recognition that not all functional relationships are straight lines (or, at best, simple smooth curves) is reported in the discussion "Cellular Dynamics" (Peachey, 1968). When Booi (see Peachey, 1968) became aware of the data on the anomalous phospholipid membrane resistances as a function of temperature, as presented by T. E. Thompson (1964; also see Drost-Hansen and Thorhaug, 1967), he reported an observation which he had previously considered too unimportant to publish, namely, that in the range from 17.5° to 27.5°C the permeability of onion scale was relatively temperature independent. However, below this temperature the water permeability dropped, apparently significantly, and above 27.5° the permeability increased rapidly. Booi conjectured (undoubtedly correctly) that these "breaks" might be related to the anomalies reported by Thompson for the electrical resistance of the phospholipid membranes as a function of the temperature.

Siegel (1969) has studied the excretion of β -cyanin by beet roots as a function of temperature in an oxidizing environment. An Arrhenius plot of the β -cyanin rate of discharge versus reciprocal absolute temperature shows two distinct limiting curves—changing from one curve segment to another at 60°C. The difference in the apparent energies of activation is considerable (93 kcal/mole below 60°C and 18 kcal/mole above 60°C).

Among the anomalous, abrupt changes in membrane properties reviewed by Drost-Hansen and Thorhaug (1967) were the rates of diffusion of sodium and potassium chloride across a "butanol membrane" (data from Rosano *et al.*, 1961). Mention was also made of the temperature dependence of the resistance of a phospholipid bimolecular membrane studied by T. E. Thompson (1964) and the highly anomalous conductance of a barium stearate multilayer membrane studied by Nelson, and Blei (1966). Additional data on collodion-potassium oleate membranes were reported by Nelson, and these frequently exhibited maxima near 15°C in bi-ionic potentials. Particularly interesting results were obtained (using 1 M solutions of KCl and NaCl separated by the membrane) at high temperatures. The results, reversible below 50°C, suggest that significant, anomalous changes occur in these membranes as a function of temperature.

Some interesting results obtained by differential scanning calorimetry were discussed by Stein (1968). The study by Stein involves spectroscopic and calorimetric measurements of biological membrane materials, as well

as of some simple aqueous systems, particularly lipid-water systems. The results on the materials from the membrane of *Mycoplasma laidlawii* are especially interesting as they clearly show several transitions near the temperatures discussed in the present article and here ascribed to the changes in vicinal water structure. It is not presently possible to correlate all of the observations made by Stein with the changes discussed in the present chapter; however, the study is important in demonstrating some possible advantages of differential calorimetry over other tools such as high-resolution NMR or optical rotation dispersion studies. Incidentally, Stein concludes from this study that his data can be interpreted most readily in terms of the membrane bilayer hypothesis, but Stein does not emphasize the role of water structure changes in this connection.

Chaudhry and Mishra (1969) measured the diffusion of ^{24}Na across atrial wall segments from the rat heart. The measurements were made with equal sodium ion concentrations on both sides of the wall of the tissue studied. The authors observed transitions in the diffusion versus temperature at 15°, 32°, and 42°C. Because these values are close to those described by the present author, suggesting the occurrence of higher-order phase transitions in water near interfaces, Chaudhry and Mishra observe that this may be taken as evidence for the diffusion of the sodium ion through water-filled pores. While the experiments by Chaudhry and Mishra are remarkable, caution must be exercised in the interpretation. Thus, conceivably the transport through the membrane might still take place via mechanisms other than diffusion in water-filled pores; the notable temperature effects might be due to structural effects in the layers of water adjacent to the membrane rather than in the membrane itself. However, the study by Chaudhry and Mishra certainly deserves careful consideration and emphasizes the utility of further detailed studies along these lines.

4. Permeability Studies

In an impressive series of studies, Wright and Diamond (1969; also see Diamond and Wright, 1969) have studied the membrane permeability of various nonelectrolytes. The study is not only a profound and extensive, comparative study of various permeability coefficients for a large number of nonelectrolytes, but it is based on a surprisingly simple approach—the nonelectrolyte movement is monitored through the attendant osmotic flow through the membrane, giving rise to a streaming potential.

In connection with osmotic flow, it is important to consider the existence of an unstirred layer; this was discussed in some detail by Wright and Diamond. They correctly point out that the unstirred layer may play a

dominant and, in fact, destructive influence on the interpretation of data aimed at calculating activation energies of permeation (from permeability measurements at different temperatures), solute-solvent interactions in membrane permeation, and comparisons of measured and observed reflection coefficient values in connection with different kinetic models of membrane structure and permeation processes. However, in the study of the rabbit gall bladder membrane by Wright and Diamond the effect of the unstirred layer played a considerably less crucial role. According to these authors, the unstirred layer effect is simply to shift the observed reflection coefficients since the method is essentially a comparative method. Nonetheless, it is obvious that the effect can hardly be completely neglected since the unstirred layer will, if structured, obscure differences between the structured elements of water in the permeated membrane material and in the unstirred layer.

Many nonelectrolytes may be diffused through cell membranes, but vast differences are observed in the permeation rates. Thus, Wright and Diamond (1969) note that molecules of approximately the same size and molecular weight and diffusion coefficients in bulk solution may differ by as much as a factor of 10^3 in the rate of diffusion through various membranes.

Wright and Diamond measured the permeability of some sixty nonelectrolytes and correlated the permeabilities with the oil-water partition coefficient (and with the ethyl ether-water partition coefficient). Similar studies have been carried out by Collander (see, for instance, Stein, 1967). Phenomenologically, the data from these studies (as well as many others; see the two foregoing works for references) have provided a large amount of information. However, it appears very difficult to elucidate systematically the relation between the nature of the nonelectrolyte and its permeability. Obviously, size alone will play an important role as will the presence of functional groups, steric aspects, etc. Yet, undoubtedly superimposed on these facets is the probable importance of the hydration of the nonelectrolytes, the hydration of the membrane matrix, and the detailed structure of the vicinal water of the membranes. Unfortunately, it will no doubt be some time before these relationships will begin to be understood quantitatively. Wright and Diamond refer to "anomalous" and "normal" permeability components; there is little doubt that the detailed nature of the matrix will influence relative permeabilities of the solutes. It is interesting that in spite of all the information and its correlation, for instance, with lipid solubility, it is still impossible to make significant statements regarding the likelihood that permeation (especially for water) is primarily via a continuous phase (water-filled pore) or a solvent process in the membrane material.

Among the several significant facts noted by Wright and Diamond is the possible important effects of unstirred layers on tracer permeability when compared to osmotic permeabilities. The difference was tentatively identified with momentum transfer between solute and water molecules. The articles by Wright and Diamond (1969; Diamond and Wright, 1969) should be consulted for details in connection with this discussion; the authors point out that:

All these properties of water in narrow channels are intermediate between properties of bulk water and of ice, indicating that water near a charged surface assumes a more ordered, ice-like structure. Most of this experimental work has been in channels with diameters in the range of 100-1000 Å, whereas anomalous non-electrolyte permeation in the gall bladder disappears for solutes with more than about three carbon atoms (hydrated diameters larger than about 5 or 6 Å).

Further permeability studies have been published by Wright and Prather (1970; also, see, Prather and Wright, 1970). Finally, it should be noted that Wright and Diamond (1969) analyzed the available data in terms of molecular models, taking into account the various types of forces affecting the nonelectrolytes, such as the permanent dipoles, induced dipoles, van der Waals forces, including short-range repulsive forces and inductive effects.

5. *Unstirred Layers*

For some interesting measurements and, particularly, some interesting speculations regarding the effects of unstirred layers (in connection with transport numbers), see the article by Barry and Hope (1969). These authors are not concerned with the possible structuring of any vicinal water in or adjacent to the cell wall and cell membranes, but draw attention to the charge accumulation effects which may occur in such regions due to superimposed electric fields.

The thickness of unstirred layers is usually taken to be more than a few microns and often as much as 20-50 μ (see, for instance, Curran). For a discussion of the effect of unstirred layers on the determination of apparent permeability coefficients, see the discussion by Goldup *et al.* (1970, p. 244). Recently, another study has dealt with the problem of the effects of the unstirred layer. Green and Otori (1970) have studied by a direct optical method the thickness of the unstirred layer of fluid adjacent to two solid interfaces. The surfaces observed were, respectively, the posterior surface of the rabbit cornea and a glass surface (a contact lens). The procedure was to study the movement of various types of small, discrete light-scattering particles, such as polystyrene (less than 0.25 μ in

diameter) or carmine particles. The thickness of the layer in which there was no notable movement of the suspended particles (other than that which could be ascribed to diffusion) was determined optically. In the unstirred case, the layer thickness appeared to depend on the nature of the solid material, being about 350 μ thick on the cornea and 150 μ thick on the contact lens. The thickness was also measured with vigorous stirring, which reduced the stagnant layers to 65 μ and less than 20 μ , respectively. It is unfortunate that not quantitative estimates were presented for the shear rates. Thus, it is relatively uninformative to know the motion in the cell was achieved by stirring at 400 rpm with a Teflon-coated magnetic stirring bar. However, even without a quantitative estimate of the degree of agitation at the interface, it does appear as if very notable thicknesses must be allowed for in diffusion studies at membranes.

6. Comparison of Membrane Matrix Effects

In connection with the structural characteristics of the cellular plasma membrane, Schultz and Asunmaa (1970) have studied the possibility of ordered structures of water. The article reviews some of the evidence available for ordering of water near solid surfaces in general and near membranes in particular. The authors appear to adopt the notion of a well-defined thickness of very highly structured water. One of the most interesting observations from the studies of these authors is the suggestion that "It has been demonstrated that the characteristics of ordered water in a porous glass desalination membrane are very similar to those in a cellulose acetate desalination membrane. This result is very surprising and further experimental work is required to see if the same is true in other strongly hydrophilic membranes."

The present author does not necessarily subscribe to the notion of a very sharply defined, structured layer near any solid interface nor to the experimental foundation on which the proposed similarity in water structure between the highly different substrates has been based. At the same time, however, as discussed elsewhere in the present chapter, there is evidence that the detailed chemical nature of the substrate may play only a secondary role. In other words, the proposal by Schultz and Asunmaa does agree qualitatively with the observation that similar, ordered structures appear to be induced by the proximity to different solid interfaces, as evidenced by the occurrence of thermal anomalies at the same temperatures, regardless of the nature of the substrate.

a. *Spectroscopic Studies.* Only relatively few systematic studies have been made on membranes by spectroscopic means. However, Zundel (1969) has recently published a monograph devoted to the study of hydration and intermolecular interactions with polyelectrolyte membranes by

infrared spectroscopy. Although this book deals with physicochemically well-defined types of membrane materials, it is very likely that the general approach will become a model for spectral studies of other types of membrane materials, including biological materials. As could be expected, a large fraction of this study is devoted to the spectroscopic properties of the water of such membranes and membrane materials. It should be stressed, however, that the approach, while sound and extremely fundamental, does not allow for more sophisticated aspects, such as modified or extensive hydration structures. This is quite natural, of course, considering the relatively insensitive technique used, namely, the spectral changes primarily effected through the hydrogen bond with its attendant large bandwidth. Those aspects that result from the ion hydration or the specific polymeric contributions from the membrane substrate will not readily reflect the details of "secondary hydration structure" effects (see the discussion of Sidorova's studies, Section V,B,6,h).

b. *Study by Resing and Neihof.* Resing and Neihof (1970) studied the nature of adsorbed water on bacterial cell walls by an NMR technique. These authors worked with a "representative bacterium" (*Bacillus megaterium*) over a wide temperature range with isolated cell walls containing approximately 33% water. The authors found no evidence that the water of these cell walls was "ice-like" nor did they find that the mobility of the water was of the order of magnitude expected for "solid-state like" water (in the temperature range in which the bacterium grows). Specifically, the authors found that "the distribution function necessary to fit the cell wall relaxation time data is so broad that it reaches, with appreciable amplitude, from the liquid value to the value in ice. Nevertheless, the median is much closer to the jump time for liquid water than for ice. The conclusion is clearly that the water in cell walls is not 'ice-like' in terms of mobility."

A number of comments are appropriate regarding the study by Resing and Neihof (1970). These authors did not discover any notable reduction in the mobility of the water near the membrane surface. On the other hand, as discussed in the section dealing with NMR studies by Glasel, it has been clearly shown that some macromolecules do not appear to possess extensive hydration structures. Again, notable differences are frequently observed between the properties of water in living organisms and in dead organisms (and, incidentally, between dormant and active states). The argument by Resing and Neihof, that removing some of the water (from "at least 90% of the volume") to 33% water (by lyophilizing and rehumidifying) should not necessarily be expected to result in a water structure resembling that in the living organism. However, it might still be argued that since most any solid interface apparently tends to induce

vicinal water structures, some kind of structure should still have been observed—even though it might have no relation to the original water structure of the living bacterium. It seems at this time that the study by Resing and Neihof is more an anomaly than a general finding. As such, the study deserves careful consideration but the results certainly cannot readily form the basis of more generalized statements about absence of ordered water structure near biological interfaces!

The membrane samples used by Resing and Neihof (1970) were prepared by storing the specimens in an evacuated desiccator over saturated sodium acetate solutions, corresponding to a relative humidity of 76% (at 20° C). Such drying may significantly influence the nature of the adsorbed macromolecules. Wetzel and co-workers (1969), for example, have shown that pronounced changes in the UV absorption spectrum and the diroism of oriented films of calf thymus DNA occur as a function of relative humidity. Particularly, these authors found that notable hysteresis effects were observed in the range of relative humidities from 0 to 65% and called attention to the structural changes proposed by Falk and co-workers (1962, 1963) in the structure of DNA in the range from 55 to 75% relative humidity. Other authors have demonstrated changes in configuration in the range from 75 to 92% relative humidity in DNA. Thus, the absence of evidence for structuring at the cell walls studied by Resing and Neihof (in the range of water contents employed by these authors) does not rule out the possibility that structuring may indeed occur in the living cell.

7. Studies by Good

An important contribution to the understanding of the state of water in membranes has come from the extensive studies by Good (see also Coldman) on the hemolysis of mammalian erythrocytes. For over a decade Good has studied the details of hemolysis of erythrocytes as a function of temperature in the presence and absence of various pharmacons. For this study, Good and co-workers have based their approach entirely on the kinetics of the hemolysis of the erythrocyte membranes. The results have been cast in the form of the Eyring rate equation and the results interpreted in terms of the apparent enthalpies, entropies, and free energies of activation (and the activation equilibrium constant K).

The most obvious results obtained by Good and Coldman is the impressive degree of linearity between the apparent entropy of activation as a function of the apparent enthalpy of activation. Stressed by several authors as well as elsewhere in this paper, the detailed information about the apparent enthalpies of activation and the entropies of activation are

far more revealing than merely the apparent free energy of activation (ΔG^\ddagger). The linear relationships between ΔS^\ddagger and ΔH^\ddagger is often encountered in chemical kinetics. Good points out that the proportionality constant in the expression

$$\Delta H^\ddagger = \Delta H_0^\ddagger + T_c \Delta S^\ddagger$$

can usually be related to the influence of the solvent on the kinetic process in question (see the detailed discussion of the enthalpy-entropy compensation phenomenon in Section V,B,3,d).^{*} Coldman and Good (1968b) interpret the compensation phenomenon as implying that the hydration of the membrane plays the dominant, controlling role in the hemolysis of most of the erythrocyte cells (with the exception of cells from cattle and dogs). The authors further go on to note that the value for the apparent entropy of activation (ΔS^\ddagger , extrapolated to 0°K) is practically equal to the standard entropy of water and suggest that the process appears to take place in a wholly ordered water structure environment. This is interesting in connection with the recent NMR studies discussed in Section III,F. Coldman and Good (1968a) summarize some of the pertinent conclusions:

It is concluded from these results that linearity between the Arrhenius activation parameters depends more on cell membrane hydration than on any other single factor.

Furthermore,

It has recently been postulated that the cell membrane contains an interconnected hydrogen-bonded framework—a hydrate continuum—that permeates the ordered lipoprotein structure (16) and it has also been proposed that changes in the configuration of the lipids may determine the water content of the membrane structure (17). More recently a model has been put forward (18) which assigns to water an important role as an integrated structural component of the membrane protein, and which provides also for extensive cell surface hydration. There are, therefore, grounds for supposing that ordered water of hydration is just as important a constituent of the cell membrane as it is of the intracellular phase, and it may be that cell swelling in a hypotonic medium is not due merely to an increase in volume of the intracellular phase, but also to an increase in volume of the membrane.

In a subsequent series of papers, Good and co-workers studied the effects of various pharmacons on the hemolysis of erythrocytes. Particular interest attached to the study of the effects of the barbiturates [also, see, the article by Tracey (1968)]. Again, it was found by Good and co-workers that the kinetics conform with the compensation law; that is,

^{*} Note that in Section V,B,3,d we were concerned mostly with equilibrium properties, whereas Good's studies are of rate processes.

that the apparent entropy of activation is linearly related to the apparent enthalpy of activation and thus reveals the likely importance of hydration effects on the mechanism of the process. Furthermore, Coldman and Good (1969) note that the results suggest that the nonpolar side chains of the barbituates play an important role through the attendant hydration phenomenon and relate this observation in terms of the pharmacological activity. Specifically, with regard to hydrophobic hydration (referred to by Good and co-workers as "apolar hydration"), the authors note that:

... it is well known (21), however, and its occurrence depends on the structure of the solute and the absence of a direct interaction between solute and water; the solute behaves essentially as an inert support that maintains the first layer of surrounding water molecules in tetrahedral configuration, thus favoring the formation of pentagonal polyhedral hydrogen-bonded structures or clathrate cages of water. The capacity of water to form such cages is almost limitless, and studies with alkyl-substituted ammonium salts show that entities as large as the tetraisobutyl group (22) and the benzene ring (23) can be enclosed by water in this way.

Finally, in a separate study, Coldman and Good (1969) studied the hydrational effects of leptazol and concluded "... that the convulsions induced by leptazol, insulin hypoglycemia and electric-shock treatment may depend on the disruption of cerebral hydration structure."

In conclusion, there is little doubt that the studies by Good and co-workers will become classics in their approach to the understanding of the kinetic behavior of biological systems (through careful studies of temperature effects) and through the information which may be obtained from such studies regarding the role played by the solvent—the water.

8. Other Studies on Erythrocytes

Controversy continues as to whether or not the permeation of water through red cells is through individual pores (by a flow mechanism perhaps resembling Poiseuille flow) or through some "solubility matrix effect" (implying the absence of discrete pores). Recently, Solomon and co-workers (see Vieira *et al.*, 1970) studied the hydraulic permeability of erythrocytes of humans and of dogs as a function of temperature. Based on their measurements, the authors concluded that the product, $L_p \times \eta_w$, was constant over the temperature range studied. This, in turn, was interpreted as supporting the view that over the temperature range of interest (5–39°C), temperature induced no restraints on the equivalent pores. Though it could not be ascertained that Poiseuille flow did, indeed, take place in such small pores, speculations were advanced that at least for the dog erythrocyte membrane, the diffusion of water occurred through a

diffusion mechanism similar to that occurring in free solution. Figure 17A shows the normalized hydraulic conductivity coefficient for canine erythrocytes, as shown by Solomon and co-workers. Figure 17B shows the same data redrawn by the present author. It is immediately obvious

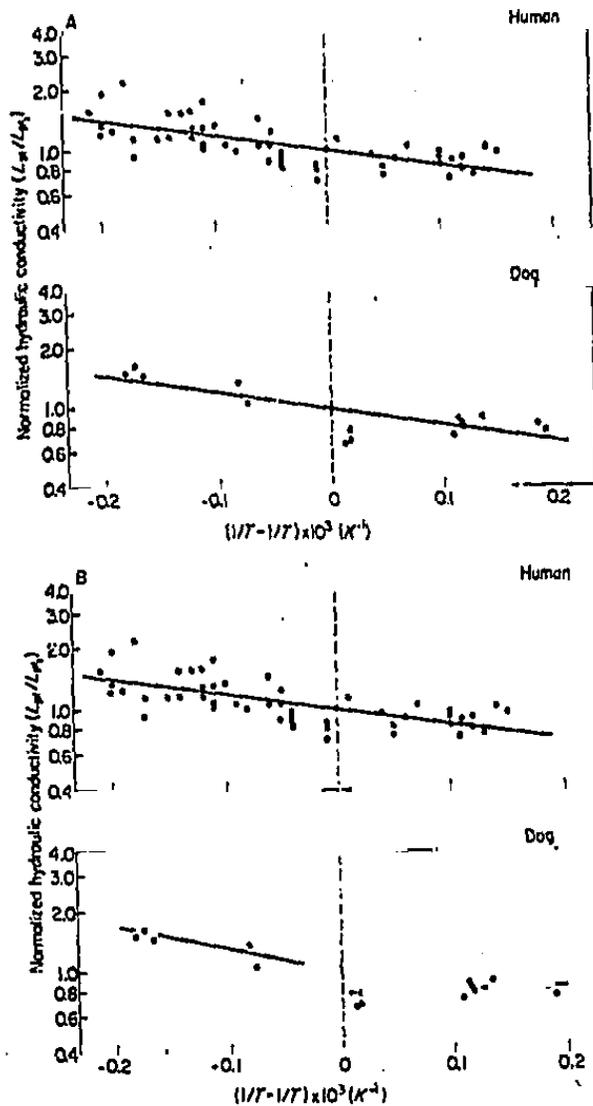


FIG. 17. (A) Normalized hydraulic conductivity coefficient for erythrocytes. (Solomon and co-workers, see Vieira *et al.*, 1970, with permission.) (B) Same data points as in Fig. 17A but curve redrawn by present author.

that the points below approximately 27°C are more-or-less temperature independent, and thus the product of the hydraulic permeability coefficient and the viscosity of the bulk water will not be temperature independent as required for Solomon's interpretation. The difference in the two sets of curves (A and B) comes, of course, from the different ways of assessing experimental errors: Solomon and co-workers merely drew a straight line, fitted by a least-square best fit, through all the data points obtained without any regard for trends. The redrawn illustration (Fig. 17B), however, becomes just as probable when the possibility of a structural change near 30°C has been accepted.

The data obtained by Solomon and co-workers for the hydraulic conductivity coefficient in human erythrocytes may or may not show the same effect, namely, that below 27°C the hydraulic conductivity is essentially temperature independent but increases slightly above this temperature. However, in the case of the experiments with the human erythrocytes, the experimental scatter is too large to draw any conclusions with certainty. The large scatter in the data in the latter case by itself does obviously not prove the converse hypothesis, namely, that the least-square best fit adopted by Solomon and co-workers is the proper analytical functional representation of the temperature dependence of the hydraulic conductivity. For a discussion of the pore concept in membranes, see also Solomon (1968).

The studies by Solomon and co-workers have in the past contributed significantly to our understanding of membrane functioning and particularly to the problem of the mechanism of solvent and solute transport across membranes. The example discussed here is in no way meant to belittle the signal contributions of Solomon and co-workers, but rather to call attention to the dangers inherent in overlooking the importance of trends versus experimental errors in experimental data.

The possible existence of actual water-filled "pores" in cell membranes (of lipidic nature) compared to other possible mechanisms for transport, especially for water, has been discussed by several authors. In addition to the contributions by Solomon *et al.*, see also the article by Ilani and Tzivoni (1968) who suggest that the water, at least in the simple hydrophobic membranes studied by these authors (prepared by impregnating filter matrices with toluene or other organic liquids), does not possess actual "open pores"—hardly a surprising conclusion. The inferences from this study were tentatively considered in connection with the general cell membrane. In this connection, see also the studies by Ting *et al.* (1966). These authors have considered the possible existence of "soft ice" at the interface between butanol and an aqueous salt solution. The butanol was chosen as an experimentally convenient and conceptually reasonably

simple model system of a lipid by Schulman and co-workers (see Rosano *et al.*, 1961). Ting *et al.* note, incidentally, that at least for rubidium, the Arrhenius plot of the rate of transfer of the ion across the interface suggests a break at 15°C. Compare in this connection the note by Drost-Hansen and Thorhaug (1967). Among the many other studies concerned with the problem of the possible existence of water-filled pores in membranes, attention should be drawn to the study by Gutknecht (1968) who concluded that, at least for *Valonia*, it appears unlikely that the protoplast contained specific, water-filled pores.

9. Bangham's Studies

S. M. Johnson and Bangham (1969) have studied the effects of anesthetic agents on the phospholipid membranes; Bangham and co-workers (1965a,b, 1966) have contributed greatly to the understanding of membrane properties and processes (as well as anesthesia) over several years. Johnson and Bangham (1969) specifically studied the permeability to potassium ions of 4% phosphatidic acid—96% phosphatidylcholine liposomes. Experiments were performed in the presence and absence of anesthetic agents, including ether, chloroform, and *n*-butanol; the effect of valinomycin was also studied. The rate data obtained were exploited in terms of apparent enthalpies of activation in an Arrhenius equation. Among the results obtained were (1) the observation that a cation permeability barrier was located at the water-lipid interface, (2) "the anesthetics increased the freedom of movement of groups in the lipid molecules near the interface," (3) the increase in permeability of K^+ in the presence of valinomycin was due to an entropy increase in the activated state, approximately $35 \text{ cal} \times \text{mole}^{-1} \times \text{deg}^{-1}$, and (4) "the increased freedom of movement in the interface when the anesthetic was present allowed the valinomycin to adopt a more favorable orientation in the interface for the exchange of K^+ ."

The study by Johnson and Bangham is a relatively straightforward application of an Arrhenius equation to the experimental rate data observed. However, the study is a typical example of the dangers which result from neglecting obvious anomalies, such as trends in experimental data. Figure 18A (Johnson and Bangham, 1969, p. 93, Fig. 3) shows the liposome permeability in the presence of chloroform. Valinomycin was present in a mole ratio of 1:10⁶ (lipid). Characteristically, the trends in the data were ignored. Figure 18B shows the same data as reported in the original article, but with the straight lines deleted and more realistic curves drawn in. It is seen that, indeed, rather abrupt anomalies do occur, and these reflect vastly different slopes, i.e., very different activation parameters. Hence,

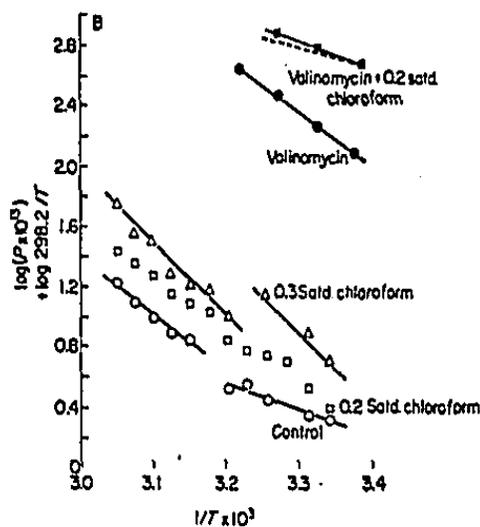
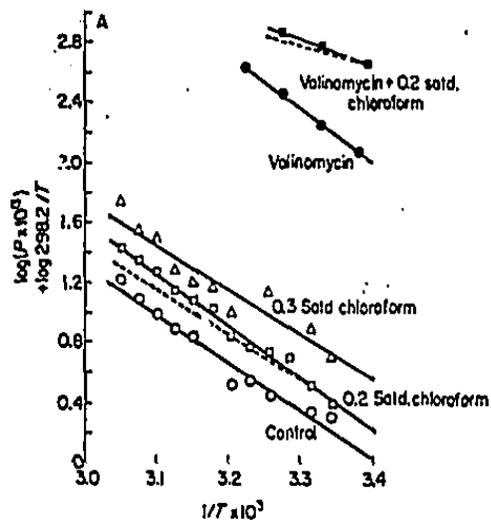


FIG. 18. (A) Liposome permeability; Arrhenius plot. (S. M. Johnson and Bangham, 1969, with permission.) (B) Same data points as shown in Fig. 18A but with curves redrawn by the present author (note—~~one curve deleted for clarity~~).

although the present author does not take issue with the general attempt to interpret molecular happenings in terms of a simple kinetic rate expression, he does take issue with the frequently practiced custom of ignoring realistic error limits and the forcing of all experimental data to

fit straight lines in order to obtain, by "brute force methods," data for the apparent activation parameters.

In connection with the study by Johnson and Bangham, notice in Fig. 18B that the failure to produce a reasonably smooth curve (i.e., free of thermal anomalies) increases as the concentration of chloroform increases. A tempting suggestion here is that the chloroform stabilizes vicinal water structures through clathrate hydrate formation—the more pronounced this structure is, the more abrupt the attendant thermal transitions will be (compare the studies by Nelson and Blei, 1960). Note also, however, that chloroform is readily soluble in the lipid.

There is little doubt that the curves shown in Fig. 18B fit the experimental data considerably better than the straight lines proposed by Bangham and Johnson. However, it should be noted in Fig. 18B that the anomalies do not appear to occur near 30° and 45°C as would be expected, but rather tend to show an anomaly in the vicinity of 38°C. In this connection, compare the discussion of the phase transitions of the lipids and particularly the studies by Chapman and co-workers, Sections V,C;1 and 2, and the studies by Stein, Section V,D,3.

10. Cellulosic Membranes

The problem of water in and adjacent to cellulose has been a subject of a great deal of research because of the obvious industrial importance, as well as the role cellulose plays in understanding the physiology of plants; many of the temperature-sensitive phenomena observed in plant growth are likely related to the water-cellulose interaction. In this section, however, the stress is primarily on the simplified system of isolated cellulose and water.

Notable contributions in this field have come from studies such as those by Goring and co-workers in Canada, by Forslind in Stockholm and by the researchers at the Swedish Forest Research Laboratory in Sweden. Before discussing these contributions, attention is called to results of Haase and Steinert (1959), discussed by the present author (Drost-Hansen, 1969b). Haase and Steinert observed notable anomalies in the permeability of treated cellulose membranes and in the apparent heat of transport across cellulosic membranes, near 32° and 45°C. The authors noted that no evidence of anomalies had been reported for the cellulose itself at these temperatures. [It should be noted, however, that in subsequent studies by Haase and de Greiff (1965), the effect was not reproduced. In this connection, see the discussion by Kerr (1970), by the present author (Drost-Hansen, 1969b), and in Section VI,J.]

Martin-Löf and Söremark (1969a,b,c) have studied thermal transitions

in cellulose utilizing various techniques. These authors have observed abrupt anomalies at a number of discrete temperatures (in some cases, near 0°, 35°, and 60°C); most of the studies were by some type of dilatometry. However, additional experiments were carried out, for instance, with D-glucose, where measurements were made of the optical rotation. This quantity showed a minimum near 28°C and a maximum near 47°C. These results should be compared with the study by Kendrew and Moelwyn-Hughes from 1940 (quoted by Drost-Hansen, 1967a): these authors studied the optical rotation of several different reducing sugars. In all of the available data, it appears that a thermal anomaly occurs in the vicinity of 15°C.

Finally, it is of interest also to note that the studies by Martin-Löf and Söremark also revealed anomalies near 30°C in the NMR linewidth in hemicellulose. The reader is referred for details to the extensive reports by these authors. It should be mentioned that the authors do not necessarily consider the anomalies as manifestations of higher-order (cooperative) phase transitions, but tentatively suggest the possibility (Martin-Löf and Söremark, 1969) that the anomalies may owe their existence to the sudden onset of rotational modes of individual water molecules.

Martin-Löf and Söremark note that Wahba has added both infrared spectroscopy evidence and optical refraction data to suggest the occurrence of transitions in the water-cellulose system; anomalies were also noted by Back who used a sonic pulse technique and by Kubat using a torsion pendulum.

The earlier studies by Ramiah and Goring (1965) were also concerned with water-cellulose interactions. Again, anomalous changes were observed in the expansion of water-swollen materials (cellulose, hemicellulose, lignin). The authors described the changes and perturbations in the water structure caused by the hydrophilic surfaces of the woody macromolecules. For some details and references, the reader is referred to the paper by the present author (Drost-Hansen, 1969b) or the original papers by Goring and co-workers.

Gary-Bobo and Solomon (1971) have studied transport across cellulose acetate membranes of varying porosity. The authors chose these membranes to "gain further insight into the nature of water-membrane interactions," and employed measurements of hydraulic conductivity and diffusion coefficients (using tritiated water) as a function of temperature. One of the aims of the study was to distinguish between influences due to geometry (such as tortuosity, etc.) and the effects of the water-membrane interactions. Previously, Solomon has advocated that viscous flow through membranes with even very small equivalent pore radii is essentially "elastic"; Gary-Bobo and Solomon claim also that "all the experimental re-

sults can be accounted for in terms of known properties of free water and no anomalous behavior of water needs to be postulated." However, Gary-Bobo and Solomon do point to the importance in the diffusion process of the water-membrane interactions, even across membranes with large equivalent pore radii. The choice of cellulose acetate appears somewhat unfortunate for a study of water-membrane interactions for the very reason the authors stress as being of particular interest. The authors quote Franks (1965) stating that since "hydroxyl groups do not alter water structure much, if at all, the behavior of the water in the membrane might be expected to be similar to that of water in bulk." It would seem that, in order to study water-membrane interactions, it would have been better to choose a hydrophobic membrane or one of a less obvious hydrophilic nature. However, compare in this connection, also, the statement by Tait and Franks (1971) that the hydroxyl groups certainly are "sensed" by the aqueous environment, since water appears able to distinguish between α - and β -methyl pyranosides. In spite of the hydrophilic nature of the membrane material, Gary-Bobo and Solomon demonstrated water-membrane interactions by the notable differences in the observed energies of activation for diffusion in the different membrane materials. Thus, in the membrane with the smallest pore radii, the apparent energy of activation 7.8 kcal/gram mole, compared to 4.8 kcal/gram mole for self-diffusion in water (the value at 20° obtained by Wang, 1965). Gary-Bobo and Solomon also stress the notable difference in the small-pored membranes between the apparent energies of activation for diffusion compared to viscous flow; they suggest that notably different mechanisms are involved and that, in this connection, "viscous flow is a relative motion of portions of a liquid, diffusion is a relative motion of its constituents." It is interesting to speculate that, were it possible to measure viscosity over a very wide range of shear rates, the "limiting value" for the apparent energy of activation for infinitely small shear rates might approach that observed for diffusion! As mentioned briefly in Section VI, J, it is not inconceivable that the viscosity of vicinal water may be shear-rate dependent. Forslund (1968) has previously claimed that water is non-Newtonian. At the same time, the studies by R. J. Miller (1968) failed to demonstrate the existence of a definite critical shear stress; thus, the water at least does not actually "gel" under the conditions of the studies by Miller and co-workers (who worked mostly with clay matrices).

11. Diffusion Studies

A vast amount of literature exists on the diffusion of various solutes (of both low and very high molecular weight) in water through various

porous materials. However, only a very limited number of studies have been reported where the particle size and the pore diameters do not differ greatly and the dimensions are still large enough to justify *a priori* the use of classic hydrodynamics (Poiseuille flow). Recently, Uzelac and Cussler (1970) have studied the diffusion of monodispersed spherical latex particles (diameter 910 Å) through Millipore filters. These filters had nominal pore diameters of 2200, 3000, 4500, and 12,000 Å. The results of the study are of obvious interest to biophysics as a model for the study, for instance, of red cells passing through vascular capillaries or large macromolecules passing through discrete pores in various types of membranes. Many of the results from this study are important with respect to a theoretical interpretation in terms of the movement of an inert sphere diffusing in a continuum liquid. The authors claim that the temperature dependence is that which would be expected on the basis of the simple expression used by the authors, although an inspection of the data may throw some doubt on the validity of the conclusion. The authors give the expression for the diffusion coefficient (D) as:

$$D = \frac{kT}{6\pi\eta a k_1(a/R)} \quad (1)$$

where $k_1(a/R)$ is a tabulated function. Admittedly, whereas the diffusion coefficients themselves differ by as much as 60%, the product $a\eta/kT$ differs by only 10%. The ratio of this coefficient at 25° and 45°C, respectively, is less than 1 for the larger pore size (0.45 μ). Whether or not significance can be attached to this, the authors point out that the limiting value of the diffusion coefficient for the ratio a/R (particle diameter to pore diameter) = 0 is about 10 times larger than the Stokes-Einstein value. The authors claim that this result is not an experimental artifact although they are unable to give an interpretation for the observations. They also call attention to some other studies which have reported enhanced diffusion coefficients—much larger than those predicted by the Stokes-Einstein equation. It is in this connection that it is of interest to consider the possibility that part of the liquid structure in the pores of the Millipore matrix may be disordered, as proposed recently by the present author (Drost-Hansen, 1969b). Some rather tenuous evidence for this has already been noted, based on data for the diffusion coefficient of a number of gases in aqueous suspensions, although there is apparently no general agreement on this point. However, if, indeed, a disordered zone may also exist in reasonably small pores, a greatly enhanced diffusion coefficient might be expected if the viscosity of less structured liquid is lower than that of the ordinary bulk water (and the highly ordered, vicinal water).

12. Active Transport

Electrolyte pumps, required to explain active transport, have been discussed in inordinate detail over the past several decades. The differences in the electrolyte contents between interstitial fluid and cell fluid is remarkable. These different electrolyte solutions are separated by the cell membranes, and the various equilibria (or, rather, steady-state processes) are normally considered to require metabolically derived energy. Apparently, in all active transport processes, the source of energy for separating the different electrolytes is derived from ATP. To "explain" active transport in classic terms, various "carriers" are required; these are usually assumed to be proteins. In this section are discussed (if only briefly) some aspects of an alternative view of active transport. A more detailed discussion of this alternative explanation is presented in Section V,G, while some selected aspects of water structure, ATP, and the ATPase problem will be treated later in a separate paper (Drost-Hansen, 1971; in preparation).

Changes in the structure of the intracellular water is a possible cause of transmembrane movements against a "total stoichiometric" concentration gradient. Changes in "available" ions (i.e., changes in "effective concentrations") have been termed changes in "solubility." This, however, seems a poor terminology, since the total number of ions in solution may remain unchanged; instead, what is changed is the activity, of the ions (and other solutes). An inspection of even the simplest forms of the Debye-Hückel expression for the activities of ions reveals that notable changes are effected through a change in the dielectric constant of the solvent.

All cells are considered capable of active transport. In this section, we restrict the discussion to plant cells, based on the extensive writings by Stadelmann with some comments in terms of the water structuring discussed in this chapter. In a recent review, Stadelmann (1970) calls attention to the criteria proposed by Sitte (1969) for active transport. Three criteria must be simultaneously obeyed to consider a movement of ions truly to be of the nature of an active transport: "(1) the process uses energy, (2) there is a stoichiometric relation between the amount of ATP used up and the amount of substance transported, (3) the energy supplied from ATP is used directly for the transport of the substance under consideration."

Difficulties have arisen in the past in attempts to demonstrate the reality of active transport for lack of suitable "trans-membrane carriers"; these "transport proteins" have been termed "permeases," "trans-locases," "transfer locases," etc. Such carriers should have some stoichiometric relationship to the number of ions transported. It is possible that one advantage of the alternate theory—that ions are transported merely in a

gradient of ion activity (due to the different aqueous environments)—is the fact that no stoichiometric relationship is necessarily required. The amount (and possibly the rate) of transport may instead be determined exclusively by the changes in activity of the ions and the water molecules (hence, changes in "apparent solubility," in current terminology). Certainly, of the three criteria discussed above, requirement (2) may play the key role in settling the question of active transport versus a movement in an activity gradient. It is likely, in fact, almost certain, that both mechanisms will require energy [requirement (1)], and it will probably be difficult to prove or disprove any one-to-one correspondence between the energy used and the energy directly supplied from ATP for the transport process under consideration. It is also possible that the actual transport may depend on, or be facilitated by, one of the "transport proteins." However, the transport proteins act merely as the vehicle for the transported solute. Effectively, this may reduce the energy barrier to be overcome in the process of moving the ion across the membrane, but it bears no relation to the energetics that determine why the ion is moved against the stoichiometric concentration gradient.

As discussed above, the alternative to active transport is the existence of solute activity gradients, caused by the different aqueous environments within the cells. Relaxation measurements may prove to be the most direct way of obtaining further information regarding the structural characteristics of the intracellular water. If the dielectric properties of the intracellular water were known, and particularly if it were possible to obtain information about the variation in effective dielectric constant as a function of electrolyte and nonelectrolyte contents and as a function of the proximity to the membranes and surfaces of the various organelles, it might be possible to calculate actual ion activities, using suitably modified, classic solution theory. In view of the above, the current work in the author's laboratory is now being directed toward measuring directly the dielectric properties of water near interfaces, especially in and adjacent to membranes, and eventually of intact biological systems. Thus, while it may be a decade or more before a detailed understanding will have been reached regarding the structural characteristics of vicinal water, empirical values for effective dielectric constants (and especially their dependence on distance from the interface—if such information can be obtained) may prove useful in estimating actual activity gradients in cellular systems.

IV. SUMMARY

The studies reported here have shown that the structure of water near many interfaces - including the water in and adjacent to a large variety of membranes - possesses unusual structural characteristics. While the specific structures have not been clearly delineated, it appears likely that they have the attributes of stabilized entities of the type envisioned in the mixture model for water (such as high-pressure ice polymorphs, clathrate cages, etc.). These vicinally stable structures display anomalous thermal properties at a number of discrete, rather narrow temperature intervals. These transitions significantly affect the properties and functioning of some membranes. The effects are observed with both simple, physico-chemically well-defined membranes, as well as with biological membranes. The vicinal water structures appear characterized by long-range ordering and by (exceedingly) low energetics.

V. RECOMMENDATIONS

In view of the findings of this research, it is recommended that careful studies be continued on the nature and role of structured water near interfaces, particularly in and adjacent to membranes. Because of the highly non-linear effects observed, temperature changes of only a few degrees may notably enhance or diminish the functioning of some membrane systems (in biological membrane systems, the changes may be completely dominant, often leading to irreversible cessation of normal functioning). The possible effects of these water structure changes on the functioning of desalination membranes is recommended.

VI. PUBLICATIONS

A large number of papers have resulted from the support received by the Office of Saline Water for the study of the structure and properties of water, particularly water near membranes (and other types of interfaces). Some of these papers are listed below.

Drost-Hansen, W., Thermal anomalies in aqueous systems - manifestations of interfacial phenomena?, Chem. Phys. Let., 2, 647-652 (1968).

Drost-Hansen, W., On the structure of water near solid interfaces and the possible existence of long range order, Ind. Eng. Chem., 61, 10-47 (1969).

Drost-Hansen, W., Role of water structure in cell-wall interactions, Fed. Proc., 30 (5), 1539-1548 (1971).

Drost-Hansen, W., Effects of pressure on the structure of water in various aqueous systems. Paper presented at the Society for Experimental Biologists' Symposium #26, on "The Effects of Pressure on Organisms", Bangor, Wales, United Kingdom, September 6-10, 1971. To be published in the SEB Proceedings, 1972.

Drost-Hansen, W., Molecular aspects of aqueous interfacial structure. Paper presented at the Air/Sea Interface Chemistry Symposium, January 31-February 2, 1972, Ft. Lauderdale, Florida. (Abstract published in the Bulletin, American Meteorological Society; paper to be published in J. Geophys. Res., August 1972.

Drost-Hansen, W., Water at biological interfaces. Paper presented at the XVth Solvay Conference on "Electrostatic Interactions and the Structure of Water", Brussels, Belgium, June 1972. Paper to be published in the Conference Proceedings.

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