THE SEMICONDUCTIVITY OF HEMOGLOBIN-ADSORBATE SYSTEMS

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Elliot Postow

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This is to certify that the

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presented by

Elliot Postow

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of the requirements for

Ph.D. degree in BIOPHYSICS

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Major professor

Date Sept. 20, 1968

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ABSTRACT

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By Elliot Postow

Hemoglobin may be operationally defined as a semiconductor because the temperature dependence of its conductivity follows the equation: $\sigma = \sigma_0 \exp(-E/2kT)$ where σ is the conductivity, E is the activation energy for semiconduction, σ_0 is a constant, k is Boltzmann's constant and T is the temperature. As water, ethanol or methanol are adsorbed on hemoglobin the conductivity is found to increase but its temperature dependence is of the same form. Concomitant with the increased conductivity a decreased activation energy for semiconduction is found. The pre-exponential factor in the conductivity equation is observed to remain unchanged by the adsorption process. Measurements of the dielectric constant of hemoglobin demonstrate increases caused by the adsorption of water, ethanol or methanol.

Adsorption isotherms of water, methanol, ethanol and ammonia adsorbed on hemoglobin are shown to be of the BET (Type II) form. Interpretation of the experimental results in terms of BET theory indicates that the solvent molecules are adsorbed at the polar sites which are located on the exterior surface of the protein molecule.

The dependence of the activation energy on the quantity of vapor adsorbed and the independence of the pre-exponential factor from this quantity demonstrate that the adsorbant is not acting as an impurity in the classical sense of inorganic semiconductors. Adsorption-induced decreases in the activation energy are the result of increases in the polarization relaxation energy. The energy gained when the dielectric medium relaxes, after the creation of a new charge center, is dependent upon the dielectric constant of the medium. As the amount of vapor adsorbed increases the dielectric constant increases. This increases the dielectric relaxation energy which decreases the semiconduction activation energy. Decreased activation energy results in increased conductivity.

The observed conductivity of hemoglobin with adsorbed ammonia cannot be explained by the same theory. These results more closely resemble those of the classical impurity model of semiconductivity. However, the amount of ammonia adsorbed on the hemoglobin is much greater than the amount of dopant commonly used in doped inorganic semiconductors.

A possible enzymatic model utilizing semiconductive properties of biomolecules is discussed.

THE SEMICONDUCTIVITY OF HEMOGLOBIN-ADSORBATE SYSTEMS

By

Elliot Postow

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TO MY PARENTS

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I. INTRODUCTION

Semiconductive Biological Materials

In 1941 Albert Szent-Györgi suggested that the concepts of solid state physics be applied to biological systems. He proposed the existence of conduction bands in protein structures. This interesting suggestion has since been approached from both theoretical and experimental directions in proteins as well as in several other substances of biological interest. The generality of solid state semiconduction in biochemical substances is now evident. The biological import of this property, however, is still only hypothesis.

Substances whose electrical conductivity follows:

$$\sigma(T) = \sigma_0 \exp(-E/2kT) \qquad (1)$$

where σ is the electrical conductivity; E is the activation energy for semiconduction; k is the Boltzmann constant; and T is the temperature in degrees Kelvin; are operationally defined as semiconductors. This definition eliminates the old arbitrary distinction between semiconductors and insulators while maintaining the distinction between semiconductors and metals, which exhibit a negative temperature dependence of conductivity.

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Employing the simple criterion of Equation (1), representatives of every class of biomolecules, and even complex organelles, have been shown to be semiconductors. A recent review by Gutmann and Lyons (1967) lists 116 biochemical substances, ranging from adenine to wool, upon which conductivity and semiconductivity activation energy measurements have been made. In all but three cases the activation energy for semiconduction varies between 1 and 3 eV. The fact that all of these materials are semiconductors does not, of course, suggest that the conductivity mechanism, or even that any of the parameters of conductivity, are the same in all systems. An experimental generalization which does indicate a certain uniformity of conductivity mechanisms is the similar effect of a variety of adsorbants and complexing agents with several different biochemicals. This will be discussed below.

Parameters and Mechanisms of Conductivity

An electrical current is, in the most general case, the result of the movement of several different species of charge carriers. These charge carriers may be of an electronic nature, either electrons or positive holes, or of an ionic nature, either positive or negative ions. In general, therefore, the conductivity is given by:

$$\sigma = e(\sum_h n_h \mu_h + \sum_i z_i n_i \mu_i) \qquad (2)$$

where the summation over h includes both electrons and holes;

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the summation over i includes the several species of ions present in the substance; μ is the mobility of the charge carrier indicated by its subscript; n is the density of the charge carrier indicated by its subscript; z is the valence of the appropriate ion; and e is the electronic charge. All species of charge carriers present in a substance contribute to its conductivity. An important problem in biological semiconductivity is the determination of the density and mobility of each species of charge carrier present in the material. Then the contributions of the several species of charge carrier to the total conductivity may be evaluated.

It is seen from Equation (2) that the contribution to the total conductivity of a single species of charge carrier is determined by the product of the carrier species' density and mobility. Conductivity measurements cannot, therefore, distinguish between the contributions of concentration and mobility to the product.

If the charge carriers are ionic in nature, electrolysis will occur at metal electrodes (which do not inject protons) where an electrode reaction is necessary to change from ionic carriers in the material to electronic carriers in the metal. Electronic carriers in the sample would not produce such an electrode reaction. Solid state electrolysis can, therefore, be used to distinguish between the two varieties of charge carriers. Electronic charge carriers passing through a hydrated sample for an extended period of time will decrease the amount of adsorbed water because electrolysis

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will have converted some of the water to hydrogen and oxygen. Either the increase in evolved gas or the decrease in the remanent water, in the case of a hydrated sample, can be monitored. Conductivity is a very sensitive measure of the hydration state of a protein and can therefore be used to determine the amount of water adsorbed on the protein. If ionic charge carriers pass through the sample, the conductivity will decrease as a function of time as the amount of adsorbed water decreases. Rosenberg (1962) observed hemoglobin with 7.5% adsorbed water in such an experiment. The conductivity was found to remain constant over a period of time in which ionic conductivity would have decreased one order of magnitude. It was concluded, therefore, that at 7.5% adsorbed water the conductivity of hemoglobin is at least 95% electronic.

Keratin films containing greater than 15% adsorbed water were examined by King and Medley (1949). They found hydrogen evolution sufficient to account for the conductivity as entirely ionic. Oxygen evolution was not found, but the oxygen may have been chemically sorbed onto the hemoglobin. Maričič, Pifat and Pravdič (1964a) impressed 150 V across a sample of crystalline hemoglobin with 9% adsorbed water for seven days. The evolution of gas could not be detected, thus indicating electronic conductivity. However, when hemoglobin with 15% water adsorbed was examined (Maričič and Pifat, 1966) hydrogen evolution sufficient to assign 90% of the conductivity to ionic carriers was found.

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The results described above all depend on the visual observation of hydrogen evolution. If tritiated water is used, a proportional counter can monitor the evolution of hydrogen. Hemoglobin with 30% tritiated water adsorbed was electrolized by Rosenberg (1964). The quantity of tritium detected was sufficient to account for 44% of the conductivity by an ionic mechanism. This is a lower limit on the ionic contribution to the total conductivity because exchange factors would cause the ionic contribution to be greater than that measured.

Riehl (1957) reported the activation energy of gelatin with somewhat less than 10% adsorbed water to be 1.8 eV. Drawing on the similarity between this value and the activation energy for conduction in ice, which is now known to be somewhat lower, Riehl argued for protonic conduction in the ice-like layer of water adsorbed on the protein. However, it is questionable that an ice-like array of water molecules exists at so low a hydration state, as will be discussed in a later section.

Similar results have been found in nucleic acid conductivity (Maričić and Pifat, 1966). Electronic conductivity is indicated in Na-DNA with less than 50% adsorbed water. However, when greater quantities of water are adsorbed onto the DNA the evolution of hydrogen indicates a contribution of ionic conductivity.

It appears that at low hydration states the conductivity of proteins is predominantly, if not entirely,

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electronic in nature. Ionic conductivity becomes significant when enough water is adsorbed onto the protein or nucleic acid so that hydrogen bond bridges are formed over the protein, or nucleic acid molecule. The conduction process may then proceed via water molecules without passing through the protein. The protein must, however, influence the process of charge generation because the activation energy for semiconductivity in fully hydrated proteins is not the same as the activation energy for either water or ice. 0.2-0.3 gm water are bound to each gm of dry protein in solution. Ionic conductivity becomes significant near the completion of this hydration shell.

The nature of the charge carrier has not been investigated in adsorbate systems other than water.

The dominant charge carriers in the substance may arise from an impurity or adsorbant (extrinsic), it may be indigenous to the biological material (intrinsic), or it may be injected from the electrode (in the case of electronic carriers). The inapplicability of classical impurity semiconductivity to biochemical systems, where only short range order pervails, is discussed in Section V. Suard-Sender (1965) has argued that, in proteins, an extrinsic semiconducting mechanism must apply. She reached this conclusion after calculating the energy band gap, in a two dimensional peptide network, to be 5 eV. This is significantly higher than the measured value of the semiconduction activation energy in proteins, i.e. ~2.4 eV. A possible explanation for this discrepancy is considered in Section V.

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Eley and Leslie (1963) proposed that for the hydrated protein system water acts as an electron donor. The interaction energy between impurity atoms is assumed to be inversely proportional to the distance between them. Therefore, the impurity mechanism predicts: $E = E_0 - \alpha N^{1/3}$; where N is the number of impurity atoms and α is a constant. In the intrinsic model discussed in Section II a linear dependence of activation energy on hydration is assumed. Extant data cannot definitely distinguish between these possibilities.

Eley (1967) suggests that the electrons may be injected from the metal electrodes. If this is the case then the energy required to transfer an electron from an intrinsic biochemical semiconductor to a metal electrode (p-type semiconductor) or from the metal electrode to the biochemical material (n-type semiconductor) are respectively:

$$\begin{aligned} E &= I_c - \phi \\ E &= \phi - A_c \end{aligned} \qquad (3)$$

where I_c is the solid state ionization potential of the biochemical material; A_c is the solid state electron affinity of the biochemical material; and ϕ is the work function of the metal electrode. Nine different metals have been used as electrodes by workers in the Nottingham and Michigan State laboratories. In all cases the results were independent of the electrode material. The work functions of the various metals used as electrodes differed by several electron volts.

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Therefore, the measured semiconduction activation energy must be independent of the work function of the metal electrode. If, as is suggested by Eley, the Fermi levels in the metal electrode and in the semiconductor are equal at the interface, without any bending of the energy bands in the semiconductor then we have:

$$\phi = (I_c + A_c)/2 \qquad (4)$$

Substituting Equation (4) in Equation (3) we find the activation energy to be independent of the work function ϕ. Under this condition the process of injecting electrons from a metal electrode into the sample can give rise to the measured activation energy.

In the hydration region where electronic charge carriers are believed to dominate, several mechanisms of conductivity can be considered. Charge carriers may be localized on a given site for long periods of time and then, in a short period of time, 'hop' or 'tunnel' to a second site where again they remain for some period of time. A second model visualizes the charge carriers drifting in a conduction band to which they have been thermally excited from either the valence band or an impurity center. These several models are depicted in Figure 1. Typical values of the mobility, the average velocity of a carrier in the direction of an electric field of unit strength, will vary with the model chosen. Hopping or tunneling models predict low mobilities, <1 $cm^2/(volt\text{-}sec)$, while in systems in which the band

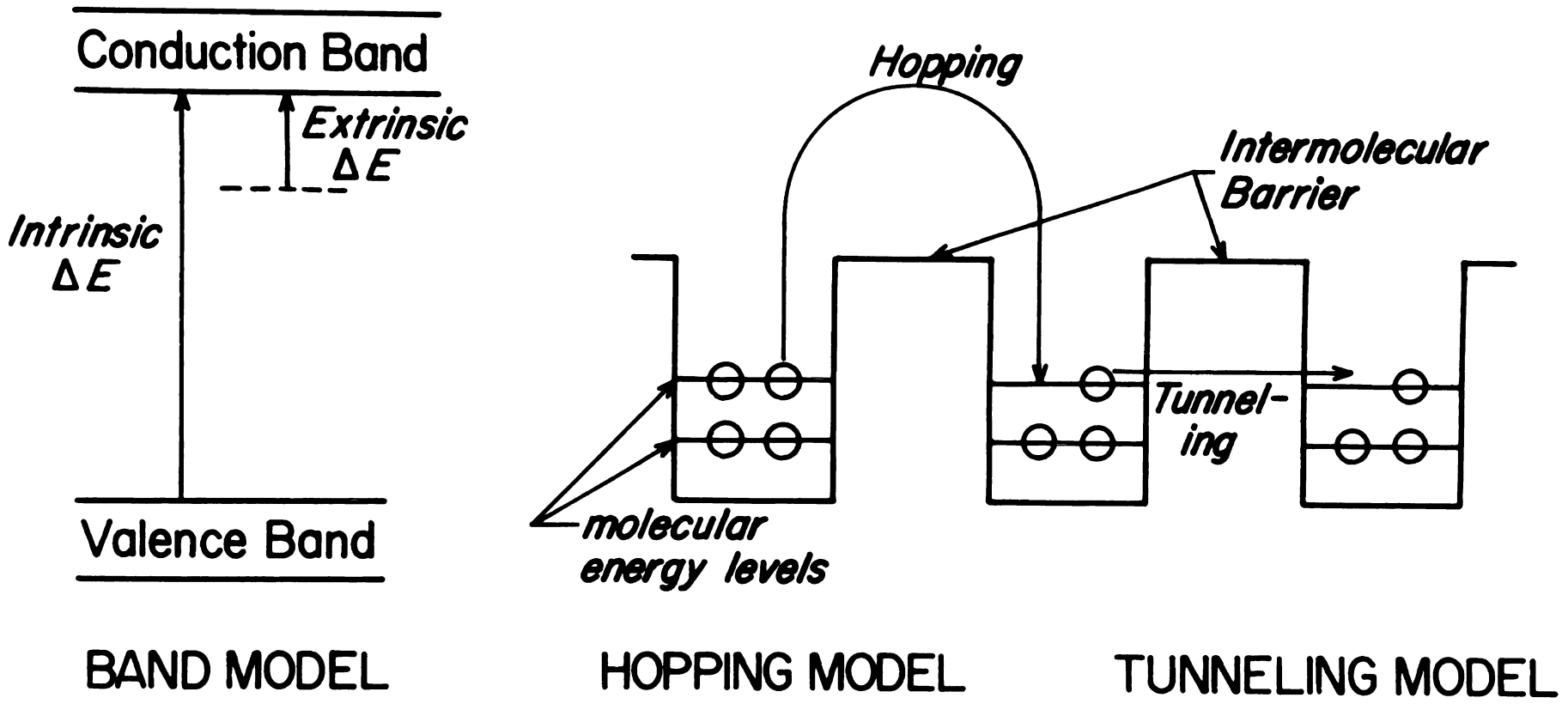

Figure 1. Band, hopping and tunneling models of semiconduction.

model is applicable higher mobilities, >1 cm^2/(volt-sec), are expected. Mobilities in the band model are very dependent on the band width. As the band width decreases the expected mobility likewise decreases. Eley (1967) has shown that an electron possessing a mean free path of 70 Å in a band of 0.01 eV will have a mobility of ~0.2 cm^2/(volt-sec). It is possible that mobility measurements can determine which model is appropriate to describe protein semiconductivity.

Conductivity measures the product of mobility and carrier density, as can be seen in Equation (2), and is therefore insufficient to determine the mobility. If, however, the density of conducting states is estimated, an indication of the mobility may be found from:

$$\sigma = \mu e N_0 \exp(-E/2kT) \qquad (5)$$

where N_0 is the effective density of conducting states. (It has been assumed in Equation (5) that mobility is not an activated process. This is not true in the case of a hopping model of conductivity.) However, this method of obtaining a value for the mobility of the charges cannot be used because reasonable estimates of the density of conducting states, N_0, do not exist for biomolecules.

Mobilities can be determined from experiments in which a pulse of charge carriers is created at a temporal and spatial point. The charges then drift, in an electric field, to a second point there they are monitored and the time recorded. If the charges are photo-created the method

is essentially that of LeBlanc (1959) and Kepler (1960). This method of mobility determination was attempted in β-carotene (Bonniface, 1968). Most biochemicals are not photoconductors (Liang and Scalco (1964), reported photoconduction in DNA but it can be demonstrated that this was probably the result of a bolometer effect) therefore, this method of mobility determination is rarely applicable.

An alternative method of carrier injection is via an electron beam. Recently Delany and Hirsch (1968) have used the electron bombardment technique to determine carrier mobilities in anthracene crystals. Using 10-60 keV electrons they obtained values for the drift mobilities of electrons and holes as 0.92 and 0.38 $cm^2/(volt\text{-}sec)$ respectively. These values compare favorably with the uv flash measurements of Kepler (1960). This method has not been applied to biochemical materials.

A third method of mobility determination is by use of the Hall effect or magnetoresistance which, in the case of inorganic semiconductors, is the simplest means. The Hall mobility is not necessarily the same as the drift mobility (which is measured by the charge injection methods discussed above). Traps do not reduce the Hall mobility but they do reduce the drift mobility. A Hall effect is produced when a transverse magnetic field is impressed across a conductor or semiconductor. A potential difference which is perpendicular to both the direction of the current and the direction of the impressed magnetic field is then produced.

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The mobilities of ionic carriers are too small to be detected by the Hall effect. The absence of a Hall effect does not, however, indicate ionic conductivity because electrons may also possess mobilities too small to be detected. Observation of a Hall effect indicates the electronic nature of the carrier. The sign of the Hall effect indicates the sign of the dominant carrier, i.e. electrons or holes. Although attempts have been made (Jendrasiak, Leffler and Rosenberg, 1967) dc Hall voltages or magnetoresistance effects have not been observed in proteins or lipids.

In the above discussion it was assumed that neither the magnetic field nor the applied voltage was time dependent. Although usually true this is not a condition of the Hall effect. Hermann and Ham (1965) have developed a system where both the magnetic field and the applied potential are time dependent. The sample is rotated in a static magnetic field thus simulating an alternating magnetic field. Recently, Hermann and Ham (1967) have used this technique to determine an ac Hall mobility for poly (n-vinyl-carbozole) -iodine, a donor-acceptor complex, as 0.5 cm^2/volt-sec. This method, although most promising, has not yet furnished mobility measurements for any biomolecules.

A field in the microwave region, 10^{10} Hz, is impressed across the sample in the method of Trukhan (1966). At these high frequencies the capacitative effects of intergranular spaces are of no consequence. This effect

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is discussed in Section V. Trukhan investigated hemoglobin and DNA in both the dry and hydrated states. Measurements were made in the light as well as in darkness. These results are summarized in Table 1. Illumination, as is expected, does not appreciably change the mobility of carriers in hemoglobin, which is about 2 cm^2/volt-sec or in DNA which is less than 1 cm^2/volt-sec. Trukhan finds that hydrating hemoglobin changes it from a p-type semiconductor, dominated by hole conduction, to an n-type semiconductor, in which the majority of the charge carriers are electrons. The effect of denaturation, in both hemoglobin and DNA, is to decrease the mobility to values which then could not be detected by the apparatus. As the data of Trukhan is the first report of protein, or nucleic acid, mobility measurements, corroboration is necessary.

The conductivity properties of biochemical substances are only beginning to be understood. Basic questions concerning the origin, nature and mobility of the dominant charge carriers are still unresolved.

Adsorption Isotherms

Several types of adsorption isotherms have been reported. Brunauer (1945) has considered the six principal shapes as illustrated in Figure 2. Type I is the familiar Langmuir isotherm which may be roughly characterized by a monotonic approach to a limiting adsorption which presumably corresponds to the adsorption of a single complete monolayer.

Table 1. Microwave Hall mobilities of hemoglobin and DNA.

Table 1. Microwave Hall mobilities of hemoglobin and DNA.

Substance	Illumination	Hydration State	Mobility (cm^2/volt-sec)	Carrier Species
Hemoglobin	dark	0.8 moles/gm	2 ± 50%	electrons
Hemoglobin	light	0.8 moles/gm	3.2 ± 40%	electrons
Hemoglobin	dark	heated to 90°C	2 ± 60%	holes
Denatured Hemoglobin	dark	heated to 90°C	0	-----
DNA	dark	normal	0.5 ± 40%	holes
DNA	weak ultra-violet	normal	0.85 ± 50%	holes
Denatured DNA	dark	dry	0	------

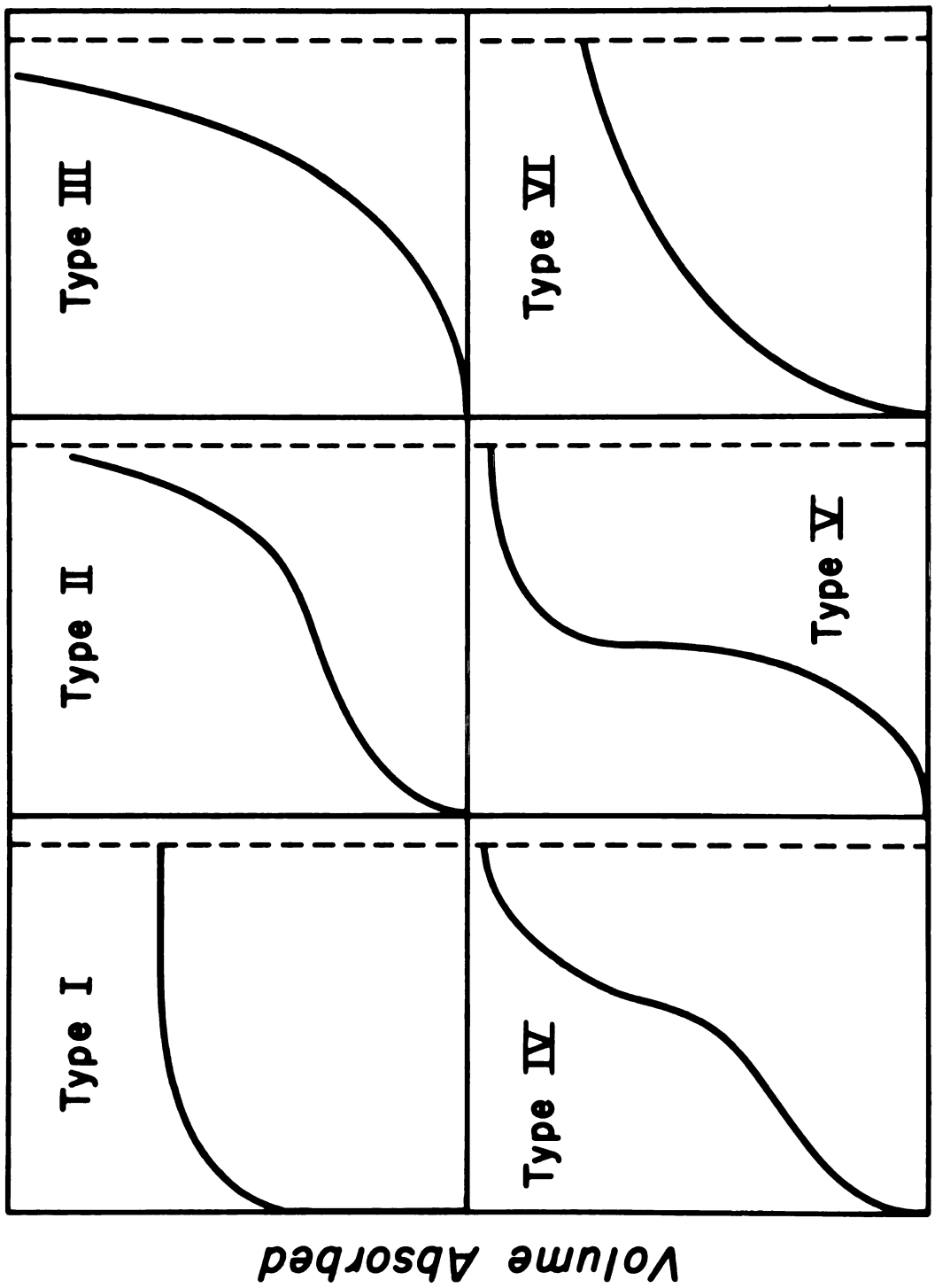

Figure 2. Six different isotherms describing physical adsorption processes. P_0 is the vapor pressure at the temperature of the experiment.

Type II isotherms, generally referred to as BET isotherms (Brunauer, Emmett and Teller, 1938), are very common in the case of physical adsorption. The BET isotherm corresponds to multilayer formation. Prior to the theoretical contribution of Brunauer et al. it was the practice to take a point at the knee of the curve as the point of completion of a monolayer. Surface areas calculated using the knee point method were found to be consistent with those obtained by using adsorbates which give a BET adsorption isotherm. Type III is relatively rare and is characterized by a heat of adsorption equal to or less than the heat of liquefaction of the adsorbate. Both Types IV and V are the result of capillary condensation phenomena in that they level off before saturation pressure is attained. They often exhibit pronounced hysteresis effects. Type VI is descriptive of chemisorption and usually referred to as a Freundlich isotherm. This process is described by the equation:

$$x = mp^{1/n} \tag{6}$$

where x is the weight increase caused by adsorption; p is the pressure of the adsorbate; m and n are constants, n always being greater than unity.

Isotherms for the adsorption of water, methanol, ethanol, and ammonia, on hemoglobin as illustrated in Figures 18-21 are of the BET type. A careful analysis of this type of isotherm according to the method developed

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by Brunauer et al. (1938) can yield both the surface area of the monolayer and the heat of adsorption of the first layer of adsorbed molecules. This is developed in Section II.

Hemoglobin

Hemoglobin, the most important respiratory pigment in higher vertebrates, is not found in solution but is found in a highly concentrated form in the erythrocytes. The overall shape of the molecule resembles a spheroid with a length of 64 Å, a width of 55 Å and a height of 50 Å. It is a tetramer of molecular weight 64,450 consisting of two different types of chains known as α- and β-chains. The α-chains contain 141 residues each and the β-chains 146 residues each. The configuration of each of the chains is very similar to that of myoglobin. Hemoglobin molecules are packed in a pseudo-face-centered-cubic crystal. Contact between neighboring tetramers exists only at a few points. Water fills the volume between molecules in the fully hydrated crystal. A 2.8 Å resolution model of horse oxyhemoglobin has recently been constructed from 100,000 x-ray reflections of three isomophous replacements (Perutz et al. 1968a,b).

In hemoglobin the non-polar cavities of the chains shelter the hemes. Excluding the covalent bond between iron and histidine, there are about sixty cases where atoms of the globin are within 4 Å of heme atoms. In all

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but three cases, one in the α-chain and two in the β-chain, the interaction is non-polar (Perutz et al., 1968b). The three polar contacts are exposed to water. A relative invariance is found in the residues in contact with the heme group. Only two exceptions are known. This would indicate that nearly all of these residues are necessary for the functioning of the hemoglobin molecule.

The interaction between unlike chains is predominantly non-polar. The few hydrogen bonds found between α- and β-chains are all exposed to water. Contact between like chains may possibly occur through salt bridges.

An internal cavity lined with polar residues, especially serine and threonine, extends all the way down the molecular dyad axis. The shape of this cavity can be represented by two boxes each 25 Å deep, along the axis of the molecular dyad, 20 Å long and 8-10 Å wide. These two spaces separate like chains.

Polar residues are located either on the exterior of the molecule or in the large internal cavity along the dyad axis. In both cases they are in contact with water. Exception is made in the case of an occasional serine or threonine whose hydroxyl group is hydrogen bonded to a carbonyl group within the same α-helix. Large non-polar groups may occupy the interior of the chain, be situated in the surface crevices of the subunits, or reside at the boundary between unlike subunits. The surface crevices minimize the contact of non-polar groups with water.

Two non-polar side chains, a cysteine and a leucine, are however found to protrude into the surrounding water.

Perutz et al. (1965) suggest that the presence of a single, non-hydrogen-bonded group with a large dipole moment in the interior of a hemoglobin subunit would be sufficient to make the tertiary structure unstable. Mutations which cause the replacement of an interior non-polar residue by a polar residue would therefore probably be lethal. No such replacements have been observed in any of the several abnormal human hemoglobins thus far. The change in free energy arising from the introduction of a polar group in the interior of a hemoglobin subunit has been estimated by Perutz (1965) as 3,500 cal./mole. This figure may be a bit high but it does indicate that the instability created by a single uncompensated polar group may be of the same magnitude as the weak bond energies which stabilize tertiary configuration.

Clearly the general distribution of side chains in hemoglobin is in accord with the principles of protein structure formulated by Kauzmann (1959). The free energies of protein molecules are minimized if their exteriors are polar and their interiors non-polar, as in soap micelles. Groups carrying a net charge, or strong dipoles, produce strong potential fields around them. This effect can be mitigated by placing these groups in an environment of high dielectric constant, i.e. water on the exterior of the molecule. If hydrophobic groups are found on the

exterior surface of the molecule, they tend to immobilize the water molecules in their vicinity reducing the entropy of the system. Tanford (1962) has estimated the increase in unitary free energy caused by exposing one mole of non-polar side chains to water as: tryptophan 3, tyrosine 2.9, phenylalanine 2.65, leucine 2.4, and valine 1.7 kilocalories. The packing of non-polar side-chains in such a manner that they are not in contact with water adds additional stability to the hemoglobin molecule. This is mainly the result of entropy changes connected with alterations of the water structure around the side chains (Nemethy and Scheraga, 1962).

Klotz (1960) has proposed that the stability of hydrophobic interaction arises from the ice-like water structure formed around exposed hydrophobic side chains. This is similar to the ice-like structures formed about argon or methane in water. The creation of polar hydrates produces a negative enthalpy change. It is therefore argued that the stability of hydrophobic interactions is the result of this favorable enthalpy change. As indicated above, only two non-polar side-chains extend from hemoglobin. Structured water, other than that bound to polar groups, has not been observed on the surface of protein molecules. Thus, ice-like structures, at best, provide only a small degree of structural stability to hemoglobin.

Reduced iron, in the ferrous state, is in the center of a square planer heme group. It is further coordinated

to one strong field ligand, histidine, and one weak field ligand, water. The introduction of one strong, nitrogenous, ligand to a heme group facilitates the introduction of a second strong ligand. It is only because the heme is protein bound, i.e. in a medium of low dielectric strength of the surrounding globin, that such a complex as reduced hemoglobin can exist at all. π-bonding substances, e.g. 0_2, CO or CN^-, readily substitute for the water at the sixth coordinate position. The binding of 0_2 to heme does not cause the oxidation of iron to the ferric state, methemoglobin, something readily accomplished by oxidizing agents. Again it is the hydrophobic environment of the heme group, as provided by the globin portion of hemoglobin, which is believed to permit the reversible oxygenation of hemoglobin.

Each of the subunits is capable of binding one molecule of oxygen. The binding constants for the addition of each successive molecule of oxygen to the hemoglobin tetramer is different. The ratio of the four stepwise constants is approximately 1 : 4 : 24 : 9. Addition of the first molecule of oxygen produces a conformation transition, allosteric effect, which favors the association of a second molecule of oxygen at a second heme group in the tetramer etc. Oxygenation dependent changes in the orientation of the α- and β-chains with respect to each other have been found by Perutz (1968b).

II. THEORY

Biological Semiconductivity

At constant temperature the conductivity of a protein will increase with hydration as found by King and Medley (1949), Eley and Spivey (1960) and Rosenberg (1962). In the region of hydration where conductivity is believed to be principally electronic in nature, a good fit to the observed data is provided by:

$$\sigma(m) = \sigma_D \exp(\alpha m) \qquad (7)$$

where σ_D is the dry state conductivity; m is the % water adsorbed onto the protein; and α is a constant. Cutomary usage is to state m in weight % of water adsorbed. However if several adsorbates are to be compared, as is the case in Sections IV and V, it is more meaningful to state m in terms of mole %. This change serves only to alter the value of the constant α.

In the region where ionic conductivity is believed to dominate, a saturation of conductivity with respect to hydration is observed and Equation (7) does not apply.

Dry proteins obey the operational definition of semiconductors, Equation (1), i.e.:

$$\sigma_D = \sigma_0 \exp(-E_D/2kT) \tag{8}$$

where σ_D is the conductivity of the dry protein; and E_D is the activation energy of the dry protein. Similarly hydrated proteins are operational semiconductors because they obey the relation:

$$\sigma = \sigma_0 \exp(-E_H/2kT) \tag{9}$$

where E_H is the activation energy of the hydrated sample. The pre-exponential factor, σ_0, does not change with hydration of hemoglobin. This is perhaps atypical of biochemical systems and will be discussed in Section V.

Combining Equations (8) and (9) we obtain:

$$\sigma(T,m) = \sigma_0 \exp\left(\frac{-E_D}{2kT} + \alpha m\right) \tag{10}$$

Equating Equation (9) with Equation (10) we observe that:

$$E_H = E_D - 2kT\alpha m \tag{11}$$

Some basic concepts of solid state theory may provide a degree of physical insight to better understand these results. As a first approximation the hydrated crystalline protein is considered to be represented by a continuous medium which can be described by a single dielectric constant κ. In this medium the work necessary to relocate a charge from a neutral portion of the protein molecule to a previously neutral portion of another, or

distant part of the same molecule, can be calculated. If the charge is moved a considerable distance, then the Coulomb interaction between the charges may be neglected and the charges are essentially free. The energy required for such a process is:

$$E_D = I_g - A_g - 2P \tag{12}$$

where I_g is the gas state ionization potential of the substance; A_g is the gas state electron affinity; and P is the stabilization resulting from polarization relaxation at each site of ionization (Lyons, 1957). The polarization stabilization is the result of the relaxation of the dielectric media in a spherical region around each of the two newly created charges and is given by:

$$P = \frac{e^2}{2R} (1 - 1/\kappa) \tag{13}$$

where R is the radius of the spherical region in which the relaxation occurs; and κ is the effective dielectric constant of the medium considered as a bulk property. Combining Equations (12) and (13) we obtain:

$$E_D = I_g - A_g - \frac{e^2}{R} (1- 1/\kappa) \tag{14}$$

Hydrated protein possesses a higher effective dielectric constant κ'. Since hydration cannot alter either the gas state ionization potential or the gas

state electron affinity of the protein we may write for the hydrated protein:

$$E_D = I_g - A_g - \frac{e^2}{R}(1 - 1/\kappa') \qquad (15)$$

Eliminating $(I_g - A_g)$ between Equations (14) and (15) yields:

$$E_H = E_D - \frac{e^2}{R}(1/\kappa - 1/\kappa') \qquad (16)$$

Comparing Equations (11) and (16) we obtain:

$$2kT\alpha m = \frac{e^2}{R}(1/\kappa - 1/\kappa') \qquad (17)$$

Introducing this result into Equation (10) yields:

$$\sigma(T,\kappa') = \sigma_0 \exp\frac{-E_D}{2kT} \cdot \exp\frac{e^2}{2kTR}(1/\kappa - 1/\kappa') \qquad (18)$$

(Rosenberg, 1962a).

In this model the effect of hydration, or more generally adsorption, is to increase the conductivity of the protein by increasing the effective dielectric constant of the medium. This serves to increase the polarization relaxation energy which then decreases the activation energy for semiconduction.

Adsorption Isotherms

BET theory extends Langmuir's approach to multilayer adsorption. It is assumed that the Langmuir equation

applies to each adsorbed layer. Furthermore it is postulated that the heat of adsorption for the first layer E_1 may have some special value, whereas for all succeeding layers the heat of adsorption is equal to the heat of vaporization of the liquid adsorbate, L, i.e. $E_2 = E_3 \ldots = E_i = L$. It is also assumed that the average time of sojourn of a molecule on each of the layers, excluding the first, is the same. The average time a molecule remains in a given surface layer is identical with the reciprocal of the frequency of oscillation perpendicular to that surface. This development further assumes that evaporation, or condensation, can occur only from, or on, exposed surfaces. The model is then one in which the surface of the adsorbent can be divided into a portion, S_0, which is uncovered, a portion, S_1, which is covered by a single layer of adsorbed molecules, a portion, S_2, which is covered by two layers, etc. Equilibrium demands that the amount of each type of surface reaches a steady value with respect to the next deeper level. Then for the first molecular layer we have:

$$a_1 p S_0 = S_1 b_1 \exp(-E_1/RT) \qquad (19)$$

where a_1 is a constant given by kinetic theory; p is the vapor pressure of the adsorbate species; b_1 is a constant which depends on the frequency of oscillations (perpendicular to the surface) of the molecules in the first layer. Similarly for the second and succeeding layers of adsorbed molecules we may write:

$$a_2 p S_1 = S_2 b_2 \exp(-E_2/RT) \tag{20}$$

$$a_i p S_{i-1} = S_i b_i \exp(-E_i/RT) \tag{21}$$

where $a_2/b_2 = a_i/b_i$. Setting $\frac{a_i p \exp(E_i/RT)}{b_i}$ equal to a new constant y, we may rewrite Equations (20) and (21) as:

$$S_i = y^{i-1} S_1 \tag{22}$$

Combining Equations (19) and (22) we obtain:

$$S_i = y^{i-1} \frac{a_1 p}{b_1} S_0 \exp(E_1/RT) \tag{23}$$

By defining a new constant C as $\frac{a_1 b_i}{a_i b_1} \exp\{(E_1 - E_i)/RT\}$, this may be rewritten as:

$$S_i = C y^i S_0 \tag{24}$$

The total number of molecules adsorbed is:

$$z = z_m (S_1 + 2S_2 + 3S_3 + \ldots iS_i + \ldots) \tag{25}$$

where z_m is the total number of molecules adsorbed in a square cm of complete monolayer. The total number of molecules adsorbed per cm^2 is given by:

$$\left\{ \frac{z}{\sum_{i=0}^{\infty} S_i} \right\} = z_m \left\{ \frac{\sum_{i=0}^{\infty} iS_i}{\sum_{i=0}^{\infty} S_i} \right\} \tag{26}$$

If the surface area of one gram of the adsorbent is A cm^2, then the total number of molecules adsorbed on one gram of adsorbent is $A_z / \sum_{i=0}^{\infty} S_i$. The corresponding number of molecules adsorbed in a completed monolayer is A_{z_m}. If x is the adsorption per gram of adsorbent at a partial pressure p and x_m is the corresponding term for a monolayer, then from Equation (26) we obtain:

$$\frac{x}{x_m} = \left\{ \frac{A_z / \sum_{i=0}^{\infty} S_i}{A_{z_m}} \right\} = \left\{ \frac{z_m \sum_{i=1}^{\infty} iS_i}{z_m \sum_{i=0}^{\infty} S_i} \right\} \tag{27}$$

Substituting Equation (24) in Equation (27), we obtain:

$$\frac{x}{x_m} = \frac{CS_0 \sum_{i=1}^{\infty} iy^i}{S_0 + CS_0 \sum_{i=1}^{\infty} y^i} \tag{28}$$

Both sums are infinite geometric progressions. Rewriting therefore yields:

$$\frac{x}{x_m} = \frac{CS_0 y/(1 - y)^2}{S_0\{1 + Cy/(1 - y)\}} \tag{29}$$

which may be rewritten as:

$$\frac{x}{x_m} = \frac{Cy}{(1 - y)(1 - y + Cy)} \tag{30}$$

At saturation the amount adsorbed on a free surface is infinite. Then at $p = p_0$ $x \to \infty$ or $y = 1$. When $y = p/p_0$ we obtain the familiar BET equation (Brunauer, Emmett and Teller, 1938)

$$\frac{p}{x(p_0 - p)} = \frac{1}{x_m C} + \left(\frac{C-1}{x_m C}\right)\frac{p}{p_0} \tag{31}$$

It is assumed that $\frac{a_1 b_2}{a_2 b_1} \approx 1$ which simplifies the expression for C to:

$$C = \exp\{(E_1 - L)/RT\} \tag{32}$$

According to Equation (31) a plot of $p/x(p_0 - p)$ vs. p/p_0 should yield a straight line of slope $(C - 1)/x_m C$ and intercept $1/x_m C$. Thus from the slope and the intercept it is possible to obtain both the monolayer adsorption (X_m) per gram of adsorbent and, if the latent heat of

condensation (L) is known, the heat of adsorption of the monolayer (E_1).

Equation (31) cannot be used to describe the adsorption isotherm at relative pressures (p/p_0) above 0.35. This has been explained (Gregg, 1961) as arising from the effect of narrow pores in limiting the thickness of the film. The BET model implicitly assumes that upon condensation, in any layer after the first, the molecule gives up its full latent heat of liquefaction. In the case of a molecule condensing into a liquid it will have a coordination number, number of nearest neighbors, of 12. But in the absence of horizontal neighbors, as is often the case in physical adsorption, the coordination number is much less than 12 (Hirst, 1948). When this is true the heat evolved should be only a fraction of the latent heat of liquefaction. Halsey (1948), therefore, believes BET theory capable of explaining only monolayer adsorption.

III. EXPERIMENTAL METHODS

Sample Preparation

The protein used in this study was dialyzed (salt free), twice recrystallized bovine hemoglobin processed by Servac and obtained from Gallard-Schlesinger. It was used without further purification. A compacted tablet was formed by pressing the hemoglobin crystallites in a die which had been teflon coated to avoid sticking. Pressures of 10^3Kgm per cm^2 were applied to the die with a pneumatic press. In order to avoid localized heating which serves to denature the hemoglobin the pressure was increased slowly. Denaturation could be determined by visual observation of the compacted table as denatured portions of the hemoglobin are of a very much darker color than is native hemoglobin.

Conductivity Measurements

Compacted tablets having a surface area of 4 cm^2 and a thickness of 0.03 - 0.05 cm were fastened between solid metal electrodes with the aid of spring clips. Stainless steel, copper, brass or tin oxide coated glass electrodes were used at various times with no discernable difference in the conductive properties of the hemoglobin

sample. Teflon was used for insulation throughout. One electrode was in contact with a mercury pool which was, in turn, connected to a stout copper rod. The rod which projects out of the chamber was used as a thermal sink. The apparatus is illustrated in Figure 3.

A copper-constantan thermocouple, inserted in the mercury pool, was used to monitor the temperature. The sample completed an electrical circuit between a battery and a vacuum tube electrometer (Keithley model 610BR). At times higher voltages were supplied with a regulated dc power supply (Keithley model 230).

The sample was dried by heating it to 95°C for a minimum of 12 hours. Heating was in a dry nitrogen atmosphere. This procedure was found to be entirely equivalent, as far as electrical measurements are concerned, to drying in vacuum. After the sample was cooled to room temperature, a fixed partial pressure of an adsorbent was introduced into the chamber.

Saturated salt solutions were used to establish various relative humidities in the sealed chamber. At constant temperature, 24 ± 1°C in the case of these experiments, a saturated salt solution will reach equilibrium with the atmosphere above it at some relative humidity. The equilibrium relative humidity will depend on the salt chosen as seen in Table 2 (O'Brein, 1948). The saturated salt solution fixes the relative humidity in the sealed chamber. At this fixed relative humidity the hemoglobin

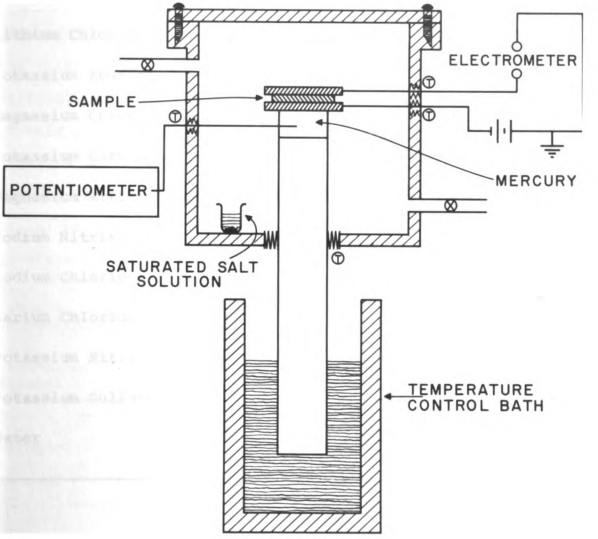


Figure 3. Schematic diagram of the apparatus used to measure conductivity and semiconduction activation energy. The sample makes thermal contact with the temperature control rod via a cup of mercury. All insulation indicated by the symbol (T) is of teflon.

Table 2. Equilibrium relative humidity over some saturated salt solutions.

Salt	Equilibrium Relative Humidity at 24°C
Lithium Chloride	12
Potassium Acetate	23
Magnesium Chloride	33
Potassium Carbonate	44
Magnesium Nitrate	54
Sodium Nitrite	65
Sodium Chloride	76
Barium Chloride	88
Potassium Nitrate	92
Potassium Sulfate	97
Water	100

sample adsorbs a given quantity of water which was determined with an electro-microbalance. A relatively long period of time, on the order of days, was required to establish an equilibrium between the saturated salt solution, the atmosphere within the chamber, and the sample.

The atmosphere in the conductivity chamber was regulated to various partial pressures of ethanol or methanol by slowly exchanging the nitrogen atmosphere in the chamber for the nitrogen atmosphere over a thermostatically controlled reservoir of the desired alcohol. If this process is continued for 12 hours, the partial pressure of the alcohol in the conductivity chamber very closely approximates the equilibrium vapor pressure of the alcohol at the temperature of the alcohol reservoir as given in Table 3.

Table 3. Vapor pressure of alcohols as a function of temperature.

Alcohol	Temperature		
	0°C	15°C	24°C
Methanol	30 mm	76 mm	130 mm
Ethanol	12 mm	33 mm	56 mm

Mixed streams of nitrogen and anhydrous ammonia were introduced into the dry conductivity chamber. By altering the relative flow rates of the two gases the concentration of ammonia in the chamber could be regulated.

The conductivity of the sample was monitored throughout the adsorption process. After the conductivity was found to be constant, the chamber, in the case of alcohol adsorption, was sealed. As the temperature of the sample was altered, by cooling or heating the copper rod, simultaneous measurements of temperature and current were made using the thermocouple-potentiometer and the electrometer. It is not desirable to use an equilibrium method of determining the semiconduction activation energy of samples with adsorbed vapors because during the interval required to establish thermal equilibrium the adsorption equilibrium will be altered. If equilibrium conductivity measurements are made, the adsorbtion state of the sample will change between conductivity measurements in which case each conductivity measurement is descriptive of a sample at a different adsorption state. The reproducibility with which the system can be recycled, when dynamic measurements are made, indicates that the adsorption state of the system is not significantly changed when the sample temperature is changed rapidly, i.e. 1.5°C per minute.

Dielectric Measurements

Compacted tablets, 3.5 cm diameter 0.02 - 0.05 cm thick, are measured in a stainless steel dielectric cell (Balsbaugh Laboratories model LD-3). The high electrode is connected to a micrometer drive. Electrode separation, therefore, may be accurately determined. The ground

electrode, 2.5 cm diameter, is guarded. Insulation throughout the chamber is of teflon. Regulation of the relative vapor pressure of the adsorbates in the dielectric chamber was effected in the same manner as in the conductivity chamber.

Capacitance measurements in the frequency range of 30 Hz - 100 KHz were made with a General Radio model 1610-B Capacitance Measuring Assembly. The assembly consists of:

1. The Type 716 Capacitance Bridge, A Schering Bridge which is direct reading in capacitance from 30 Hz to 100 KHz, and in dissipation factor at 100 Hz, 1 KHz, 10 KHz and 100 KHz.

2. The Type 716-P4 Guard Circuit, which permits measurements using a guard electrode with the dielectric cell. This is sometimes called a Wagner-ground circuit.

3. The Type 1302-A Oscillator which has a frequency range of 10 Hz to 100 KHz.

4. The Type 1231-B Amplifier and Null Detector, a combination solid state amplifier and sensitive visual null detector.

A 100 pF capacitor was placed in parallel with the sample capacitance. Figure 4 provides a schematic illustration of the capacitance measuring assembly including the dielectric cell. The direct reading method of determining capacitance was used throughout. In this mode, capacitance of the magnitude measured, could be determined to an

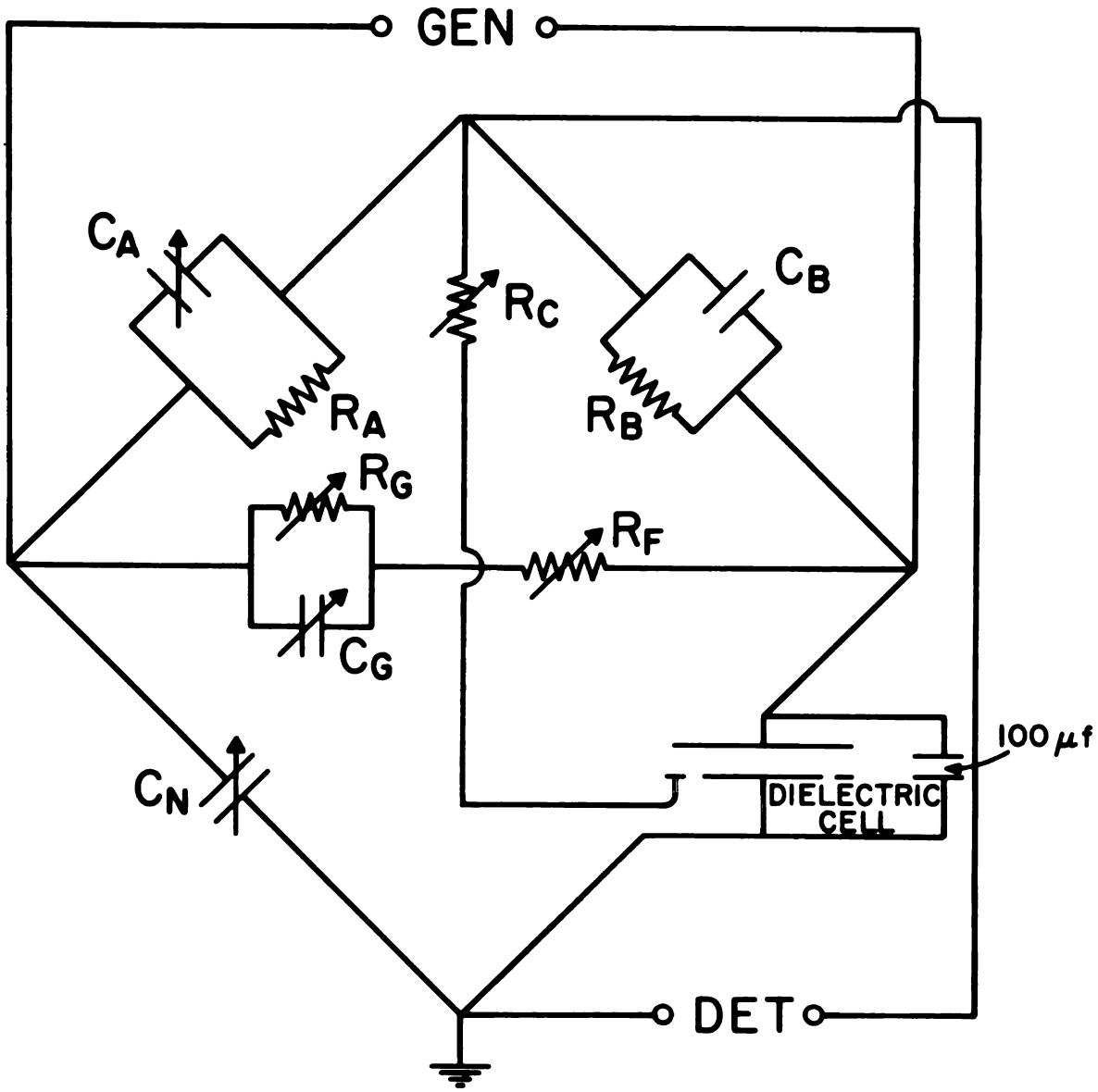


Figure 4. Schematic diagram of the capacitance bridge assembly. The Schering bridge consists of the variable air capacitors C_A and C_N, the fixed capacitor C_B and the fixed resistors R_A and R_B. The guard on the lower electrode is connected to the Wagner ground circuit which consists of three variable resistors, R_C, R_F and R_G and a variable capacitor C_G.

accuracy of ± 0.8 pF. Dissipation factors could be determined to an accuracy of ± 2%.

Some extremely low frequency, 0.1 - 10 Hz, measurements of capacitance were made with a guarded bridge designed by Nakajima and Saito (1958). The assembly, built by the Ando Electric Co. Ltd. of Tokyo, Japan consists of:

1. The Type ULO-5 Oscillator, a multi-wave form oscillator operating in the range of 8×10^{-4} - 1.2×10^{3} Hz.

2. The Type TR-4 Bridge consisting of a variable condenser and a conductance shifter. A Wagner potential balancing circuit is also included.

3. The Type EC-3 Null Detector which is a directly coupled dc amplified with 100 megohm input resistance.

The capacitance of the sample was measured in a vacuum, 10^{-2} torr, by enclosing the dielectric sample chamber in an outer brass chamber which was evacuated with a mechanical pump. All insulation in the outer chamber was of teflon and all leads were shielded. The outer chamber was at ground potential.

Adsorption Isotherm Measurements

A portion of a compacted tablet weighing ~30 mgm was placed on a Cahn electrobalance (Model RG). The electrobalance was placed in a glass vacuum chamber and connected to a recorder (Bausch and Lomb Model VOM-5). The sample was weighed on a Mettler balance under laboratory atmospheric conditions and then reweighed on the electrobalance

when the balance chamber was at atmospheric humidity. Counter balancing a portion of the sample weight increases the accuracy with which adsorption induced weight increases can be determined. After the sample chamber had been evacuated and the sample heated to 95°C for a minimum of 12 hours, a "dry reading" was obtained. From these three measurements the dry weight of the sample was obtained.

Buoyancy corrections were calculated both for the determination of the dry weight and for the weight of the adsorbed vapors. In all cases, excepting that of ammonia adsorption, buoyancy corrections were small when compared to the weight of the quantities being determined. Ammonia results presented in Section IV have been corrected for buoyancy. Calibration measurements, made on counter balanced pans with no sample, showed that water and ethanol vapors affected the system while methanol and ammonia had little effect. In the Cahn electrobalance some electronic apparatus is included in the balance chamber and is exposed to the vapors introduced for adsorption studies. The negative balance corrections for water and ethanol adsorption studies have been added to the results presented in Section IV.

In the cases of water, ethanol and methanol the liquid was placed in the liquid reservoir as illustrated in Figure 5. After degassing, a small amount of the vapor over the liquid was introduced into the evacuated balance

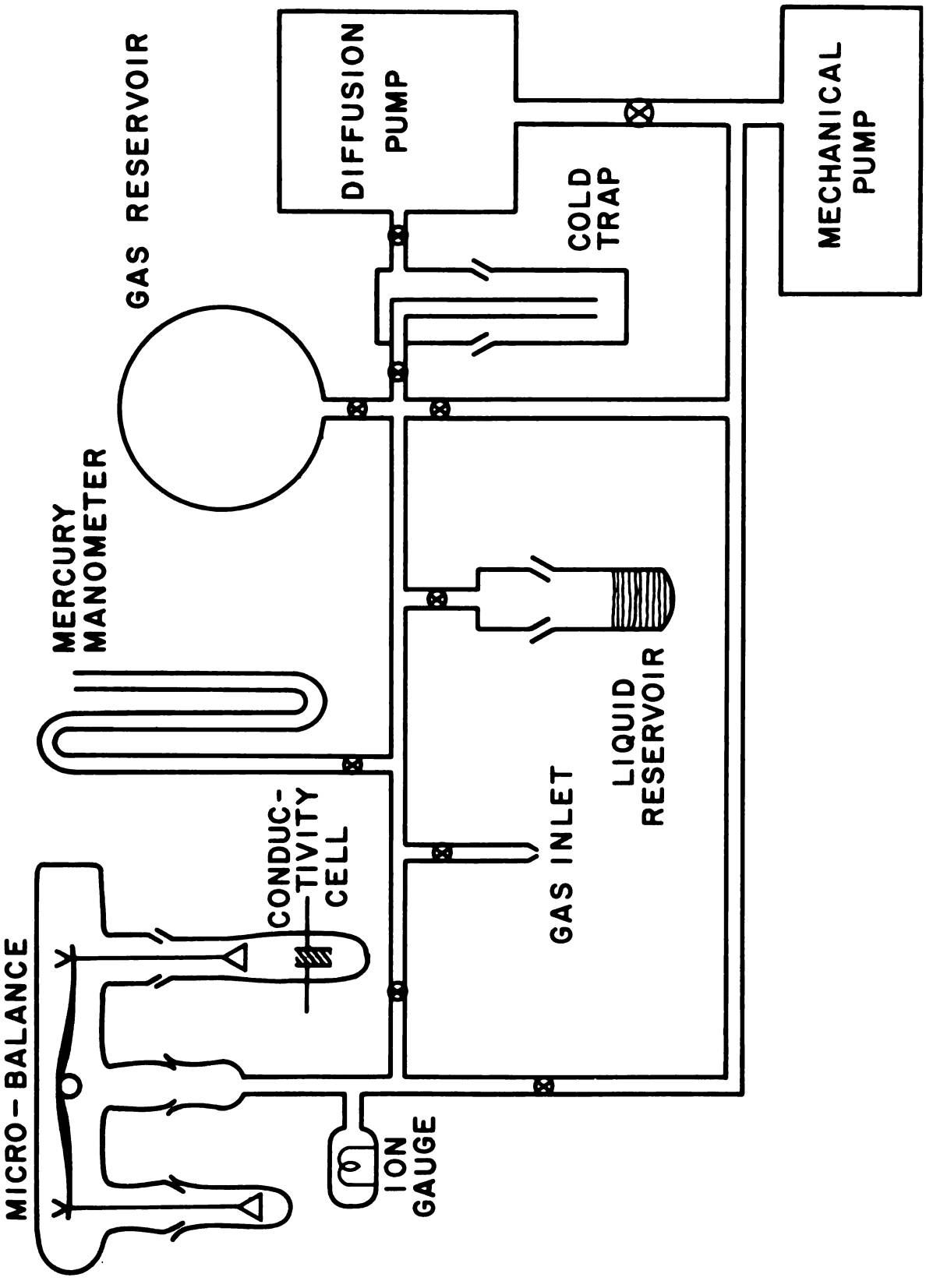


Figure 5. Schematic diagram of vacuum micro-balance apparatus.

chamber. Ammonia was introduced into the system by attaching a cylinder of anhydrous ammonia to the gas inlet as illustrated in Figure 5. The vapor pressure was determined with the aid of a mercury manometer. Adsorption isotherms for water, methanol and ethanol were determined when the sample was at room temperature, 24°C. In the case of ammonia adsorption, the sample was kept at 0°C in order to decrease p_0 for ammonia from 9.6 atmospheres at 24°C to 4.2 atmospheres at 0°C.

The sensitivity of this arrangement could allow a precision of weighing of 5×10^{-6} gm which is less than 0.1% of the sample weight. This is about 1% of the weight change from one adsorption state to the next. By far the greatest experimental error, in the present apparatus, resides in the measurement of the partial pressure of the vapor.

IV. EXPERIMENTAL RESULTS

Conductivity Measurements

Most conductivity measurements were made with a 45 V battery impressed across the sample. However, when the activation energy of dry hemoglobin was to be determined a significantly greater voltage (450 V or 600 V) was impressed across the sample. This was necessary in order to extend to lower temperatures the range over which the conductivity could be determined experimentally. (Current can not be read effectively below 10^{-14} amp. with a vacuum tube electrometer. Although a vibrating style of electrometer is capable of measuring significantly smaller currents, it is unsuited for use in the dynamic determination of activation energies because of its long response time. As was discussed above it was necessary to make dynamic determinations of the activation energy.)

Before conductivities and activation energies determined at different applied voltages are to be compared, it is necessary to demonstrate the ohmic nature of the system. In Figure 6 the log of the current is plotted against the log of the impressed voltage for a sample of hemoglobin in an atmosphere equilibrated at 20% relative humidity. Between 2 and 600 V the sample closely approximates ohmic

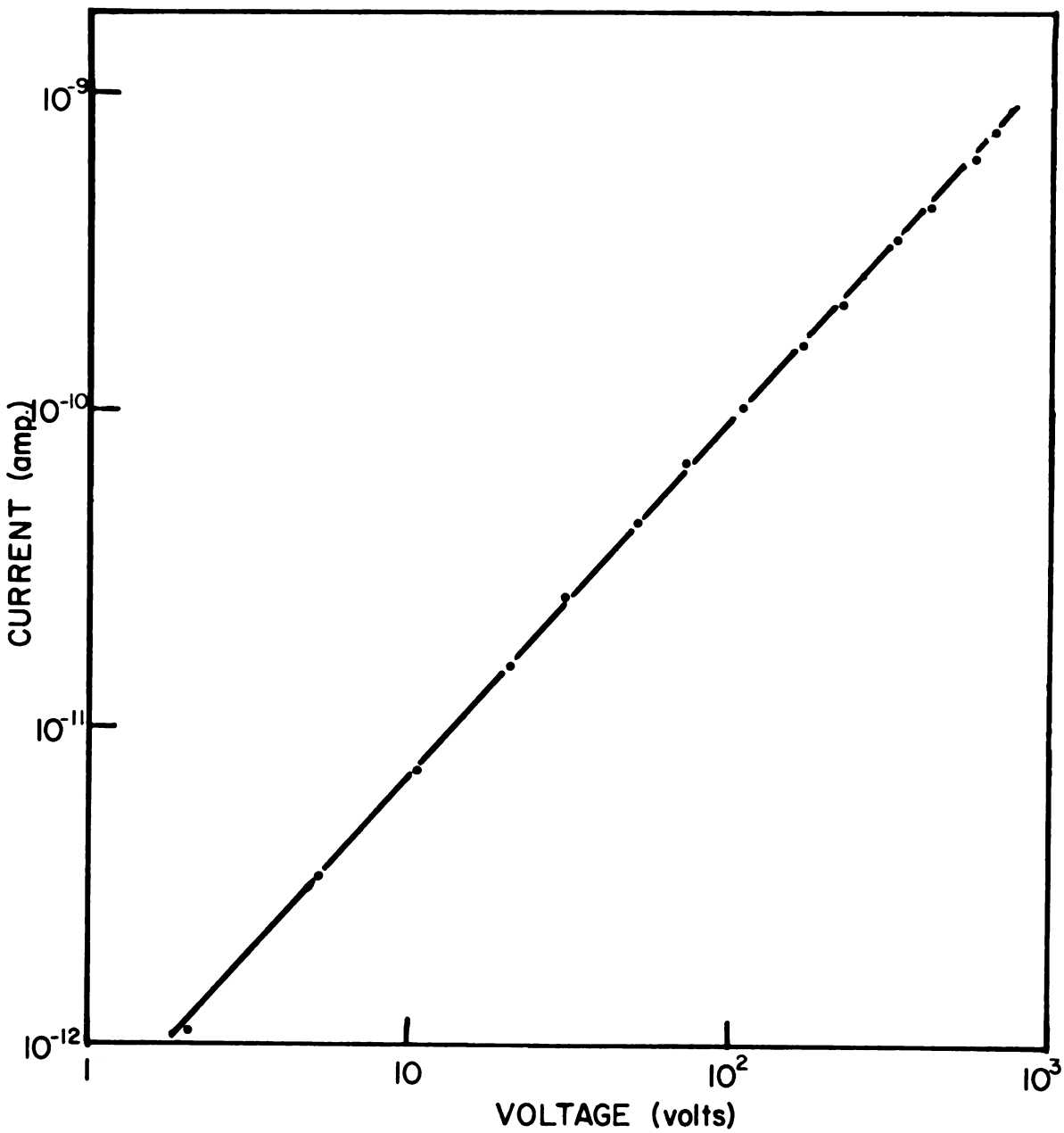


Figure 6. Ohm's law plot for hydrated hemoglobin.

behavior (i.e. the slope is 49° instead of the 45° predicted by Ohm's law).

The temperature dependence of the conductivity of hemoglobin has been found to follow Equation (1) which operationally defines semiconductors. Similarly, the temperature dependence of the conductivity of hemoglobin with various quantities of adsorbed water, ethanol, methanol or ammonia follows an equation of the same form. A plot of log σ against 1/T will yield the two constants σ_o and E. A series of such plots for hemoglobin at various states of hydration is illustrated in Figure 7. The values of σ_o and E obtained from these graphs is given in Table 4. If σ_o does not vary with hydration then when the log of the conductivity, at constant temperature, is plotted against the activation energy for each of the variously hydrated samples of hemoglobin a straight line should be obtained. This is illustrated in Figure 8. This graph should have and does have a slope of 1/2kT which, in this case, is 20 eV^{-1}. From Figure 8 and from Table 4 we can see that hydrating hemoglobin does not alter the pre-exponential factor, σ_o, but it does effect a change in the semiconduction activation energy.

The activation energy of a compacted tablet of hemoglobin in equilibrium with 30, 76, and 130 mm of methanol can be obtained from the data illustrated in Figure 9. Values of 1.75, 1.45 and 1.20 eV respectively were found to be typical of several runs under the same vapor pressure. In Figure 10 the log of the relative conductivity, at 300° K, is plotted against the activation energy for each of the

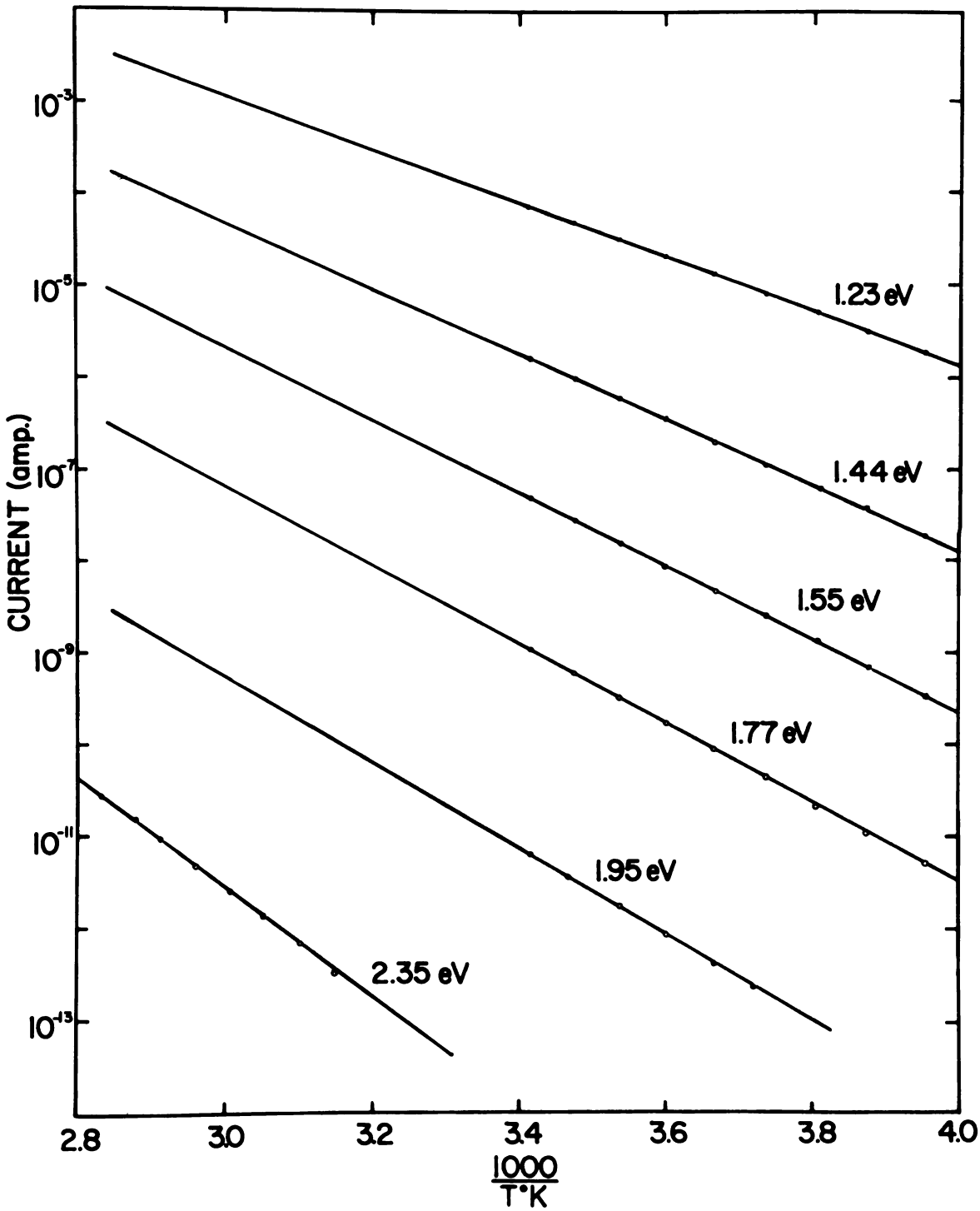


Figure 7. The semiconduction activation energy of hemoglobin at several different hydration states.

Figure 8. The variation of conductivity with semiconduction activation energy for hemoglobin at various hydration states.

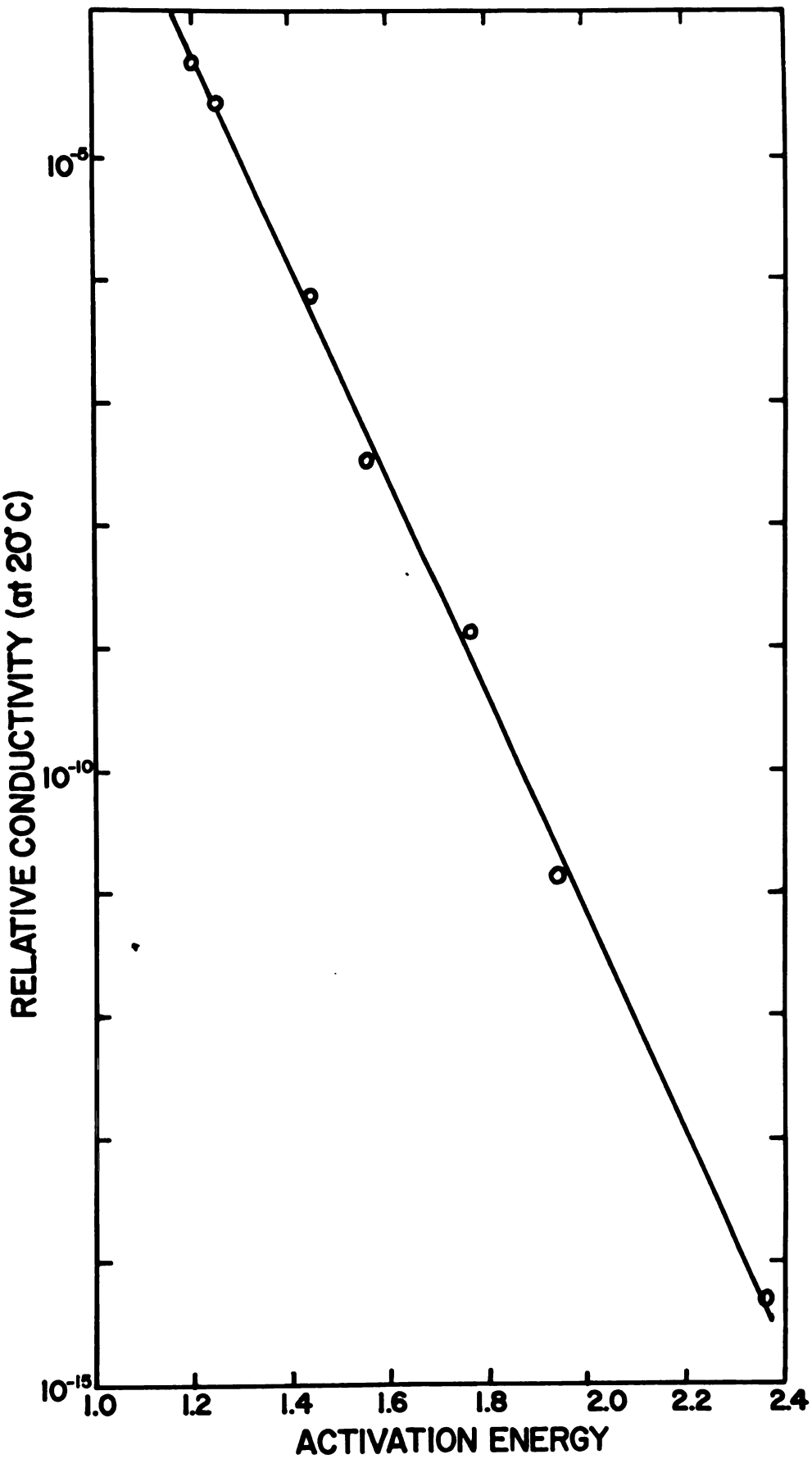

RELATIVE CONDUCTIVITY (at 20°C)
10⁻⁵
10⁻¹⁰
10⁻¹⁵
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
ACTIVATION ENERGY

Figure 8.

Figure 9. The semiconduction activation energy of hemoglobin in equilibrium with various partial pressures of methanol. Curve A is at 130 mm, B is at 76 mm and C is at 30 mm of methanol. Curve D is taken from data on dry hemoglobin.

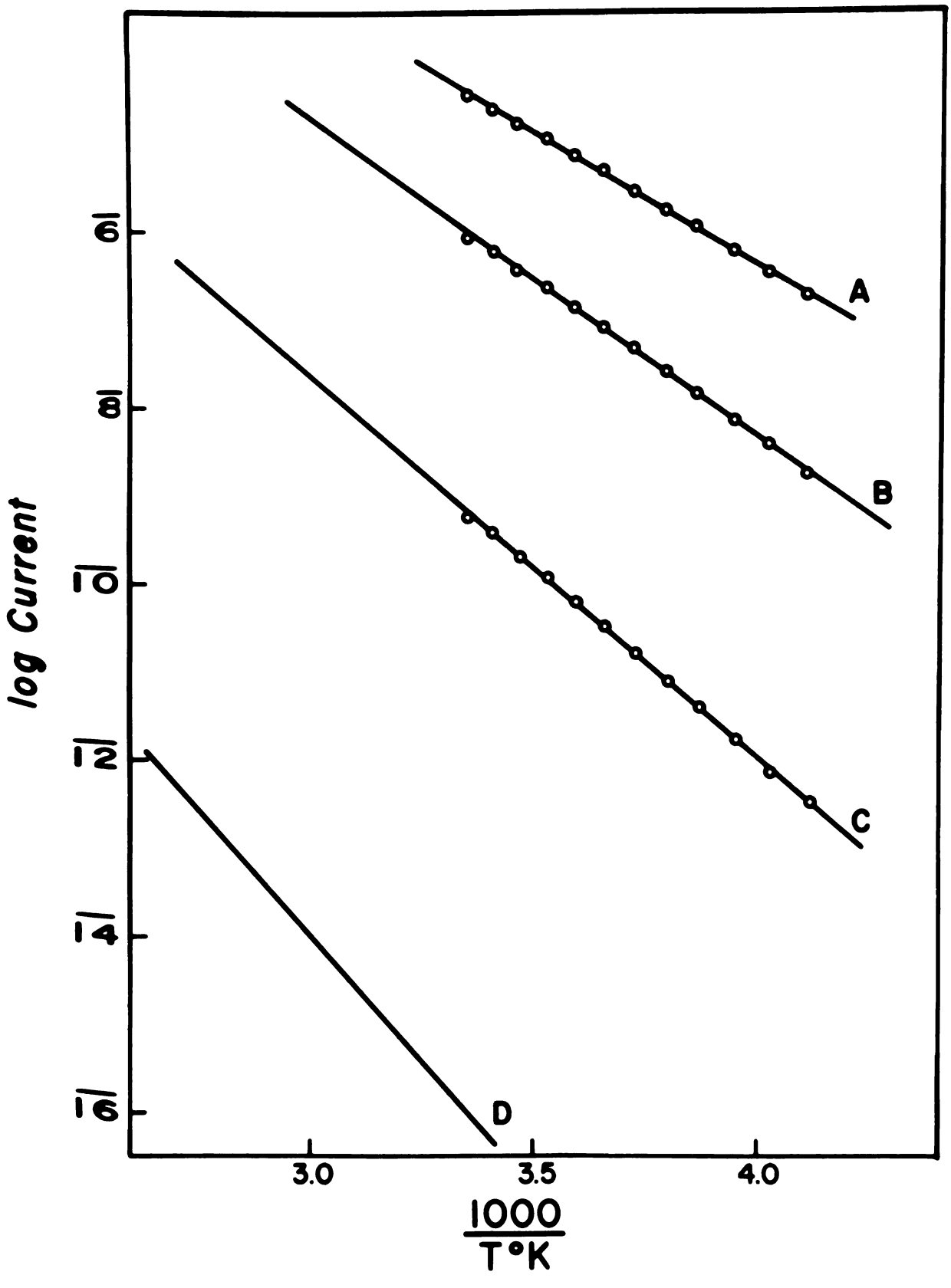


Figure 9.

Figure 10. The variation of conductivity with semiconduction activation energy for hemoglobin with various quantities of methanol adsorbed.

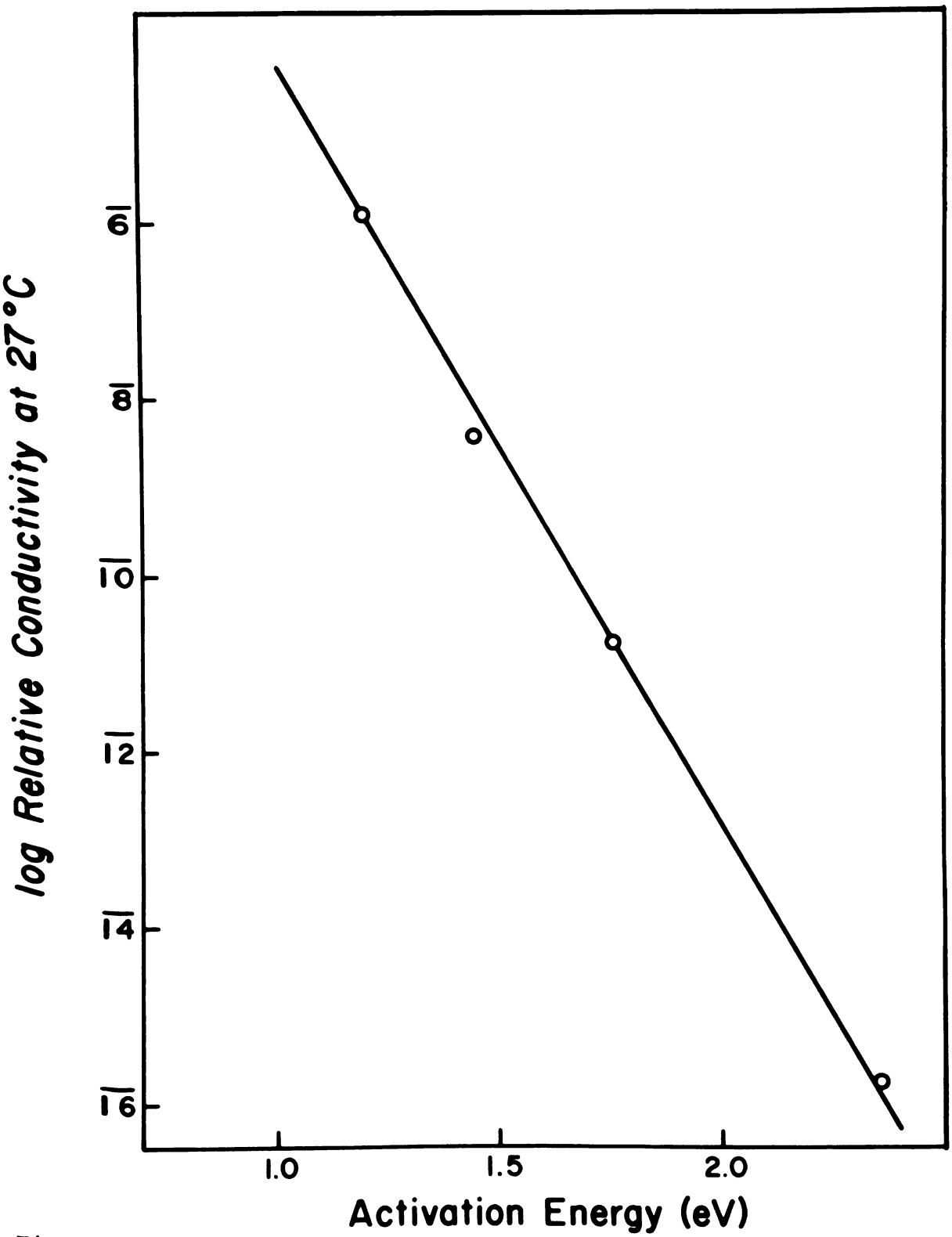

log Relative Conductivity at 27°C
6
8
10
12
14
16
1.0
1.5
2.0
Activation Energy (eV)

Figure 10.

Figure 11. The semiconduction activation energy of hemoglobin in equilibrium with various partial pressures of ethanol. Curve A is at 56 mm, B is at 33 mm, C is at 12 mm and D is with no ethanol present.

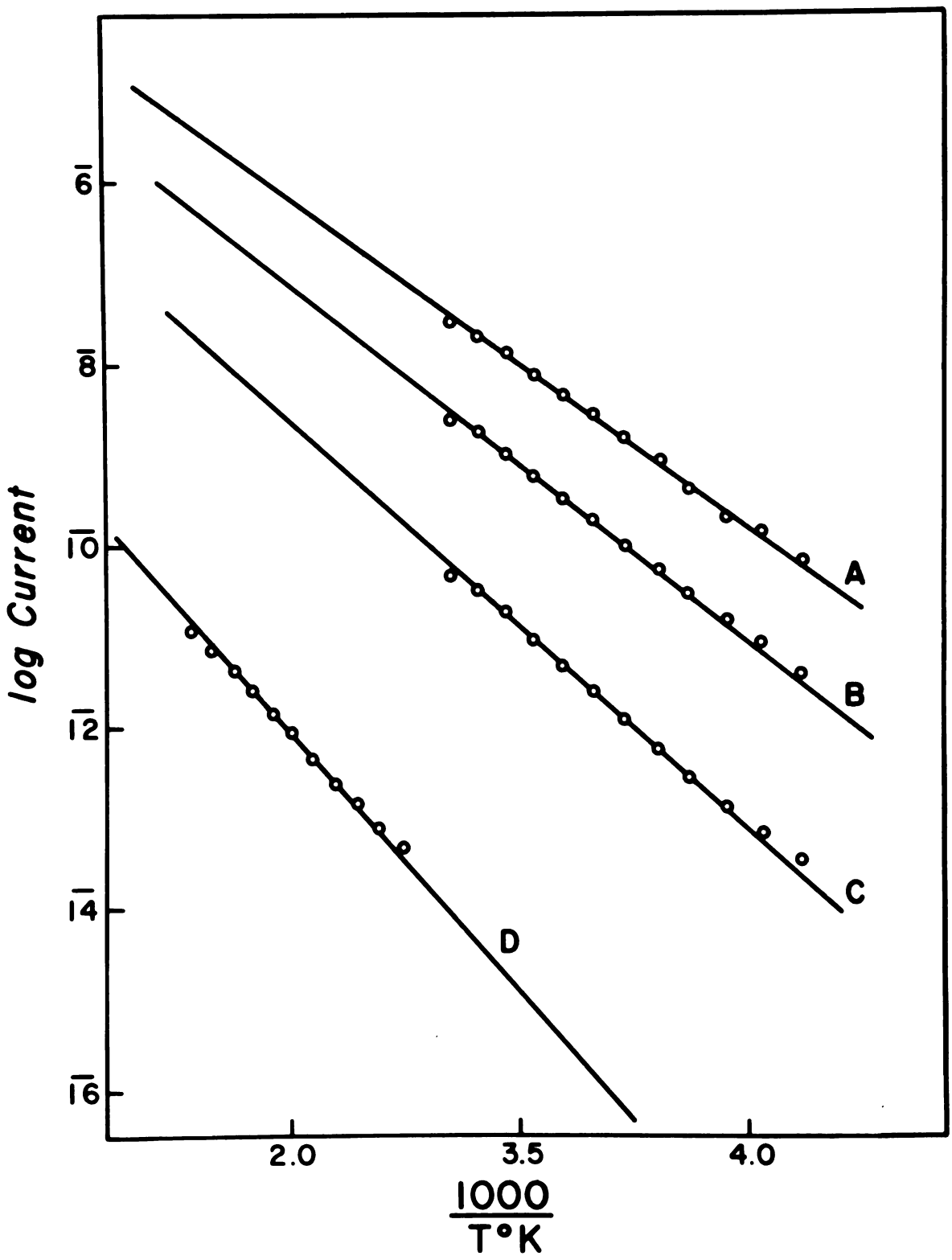


Figure 11.

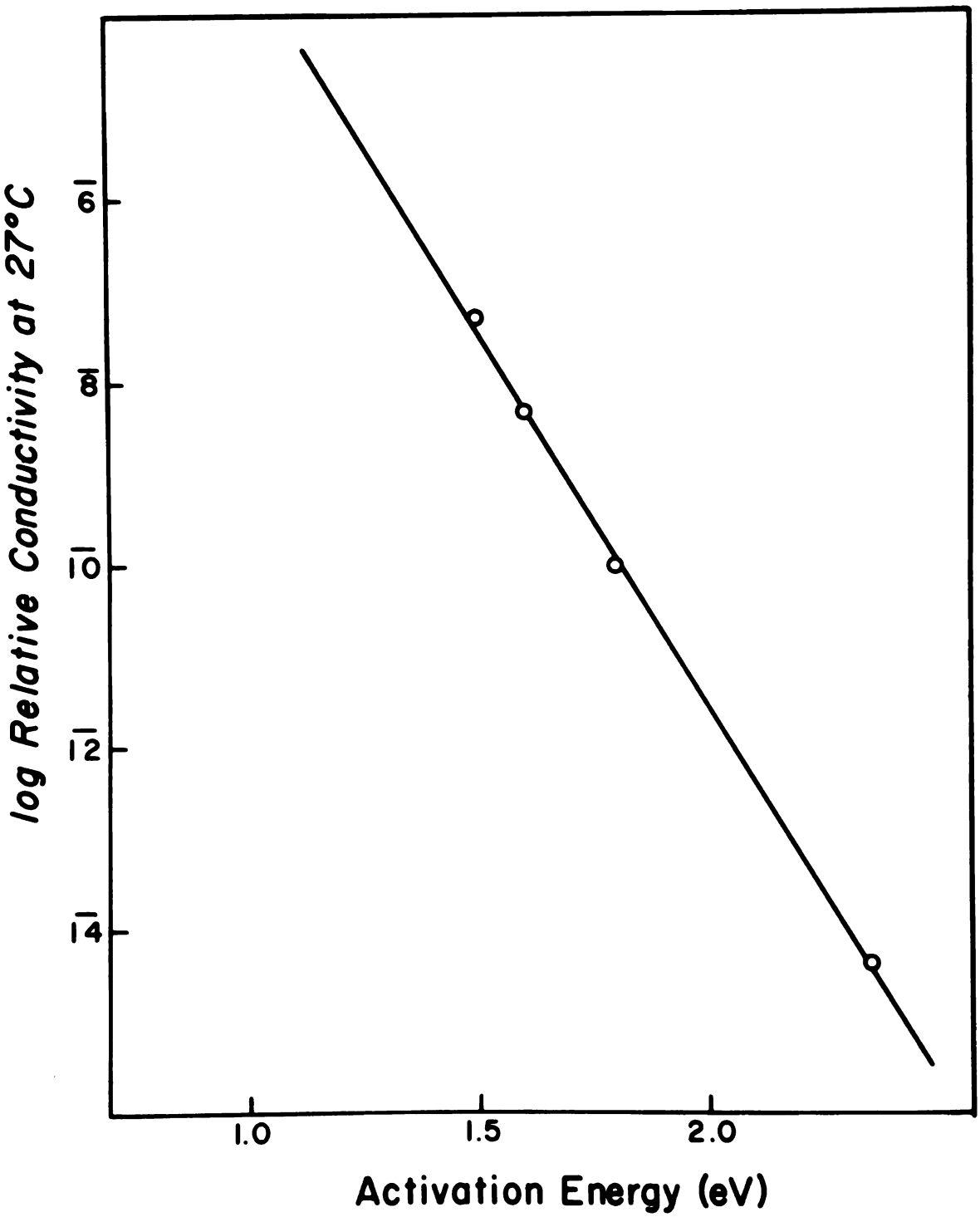


Figure 12. The variation of conductivity with semiconduction activation energy for hemoglobin with various quantities of ethanol adsorbed.

Table 4. Conductivity of hemoglobin with adsorbed water, methanol, and ethanol.

p/p_o in %	Activation Energy (eV)	σ_o (Ohm-cm)$^{-1}$
0 water	2.35	60
12 water	1.95	60
33 water	1.77	180
53 water	1.55	49
75 water	1.44	140
90 water	1.23	84
21 methanol	1.75	30
59 methanol	1.45	100
∿100 methanol	1.20	30
23 ethanol	1.8	70
58 ethanol	1.6	25
∿100 ethanol	1.5	35

methanol adsorption states. Again a straight line of slope 20 eV^{-1} is obtained. This demonstrates that the adsorption of methanol on hemoglobin decreases the activation energy but does not alter the pre-exponential factor, σ_o, in the conductivity equation.

Figure 11 illustrates the temperature dependence of the conductivity of dry hemoglobin as well as hemoglobin in equilibrium with 12, 33, and 56 mm of ethanol. The activation energies have been calculated and are presented in Table 4. The pre-exponential factor, σ_o, is again seen to remain constant, under the adsorption of ethanol. In Figure 12 when the log of the conductivity, at constant temperature, is plotted against the activation energy a slope of 20 eV^{-1} is again found. Typical values of the activation energy and pre-exponential factor, σ_o, for hemoglobin with various quantities of adsorbed water, methanol and ethanol are presented in Table 4. The pre-exponential factor is an extrapolation and should be taken only to indicate the order of magnitude.

Ammonia appears to affect the conductivity in a different manner than do the vapors considered above. The adsorption of different but unknown quantities of ammonia decreases the conductivity of hemoglobin but the activation energy remains constant at 0.8 eV. As the conductivity increases the value of σ_o also increases. Figure 13 illustrates the temperature dependence of the conductivity of hemoglobin with various quantities of adsorbed ammonia. These results

Figure 13. The semiconduction activation energy of hemoglobin in equilibrium with various partial pressures of ammonia. Curves A, B and C are at different, but undetermined, partial pressures of ammonia. Curve D is with no ammonia present.

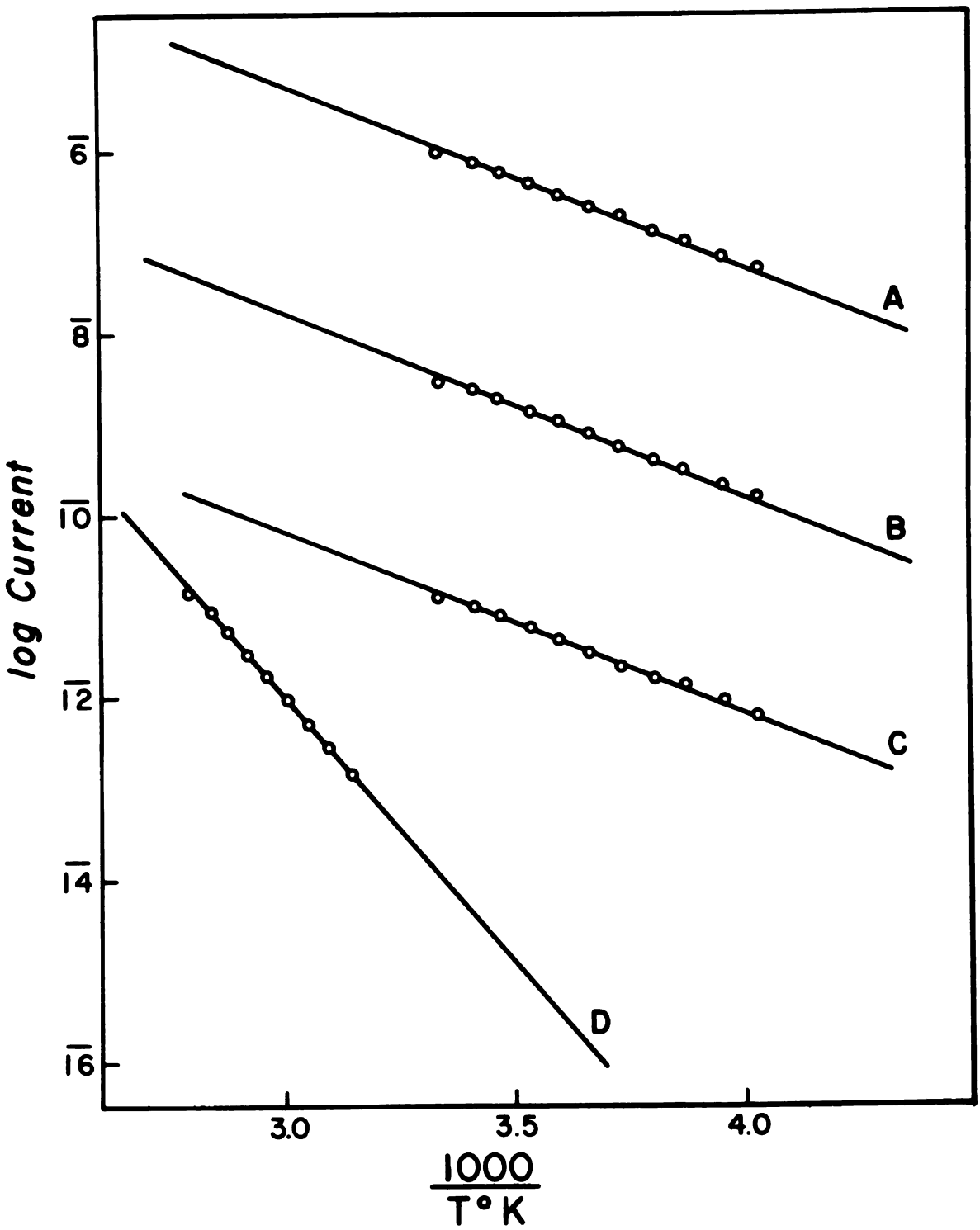

log Current
$\bar{6}$
$\bar{8}$
$\bar{10}$
$\bar{12}$
$\bar{14}$
$\bar{16}$
A
B
C
D
3.0
3.5
4.0
1000
T°K

Figure 13.

are similar to those found in impurity conductivity as discussed in the following section.

Dielectric Studies

Dielectric constants are calculated from measurements of the capacitance according to the formula:

$$\kappa = C_S / C_A \qquad (33)$$

where κ is the dielectric constant of the material, C_S is the capacitance of the sample and C_A is the capacitance of an equal geometry of air. In general the capacitance, and hence the dielectric constant, of a specimen will vary with frequency. Having made direct current conductivity measurements it appeared most appropiate to make dielectric measurements in the low frequency region of the spectrum.

The low frequency dispersion of the dielectric constant of hemoglobin with various quantities of adsorbed water is illustrated in Figure 14. When only a small quantity of water is adsorbed on the hemoglobin the frequency dispersion is flat. However, as the amount of adsorbed water increases a considerable low frequency dispersion in the dielectric constant is found. The low frequency capacitance of a hydrated hemoglobin tablet does not vary inversely with the thickness of the tablet. Electrode polarization, which is independent of sample thickness would account for this result. However, the same results were found when capacitance measurements were made with either an air gap or a sheet of teflon placed above the sample. In the case of air

Figure 14. The frequency dependence of the apparent dielectric constant of hemoglobin with various quantities of adsorbed water (as indicated).

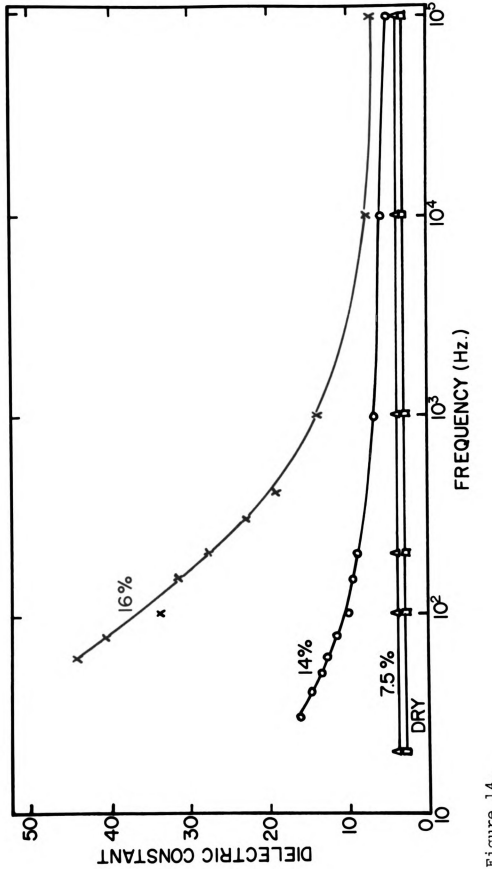

DIELECTRIC CONSTANT
50
40
30
20
10
0
10
10^2
10^3
10^4
10^5
FREQUENCY (Hz.)
16%
14%
7.5%
DRY

Figure 14.

gap measurements the moveable electrode is not in contact with the hemoglobin tablet. The equivalent capacitance of the tablet in series with an air capacitor is measured by the bridge. Measurements are made with a variety of air gaps. The capacitance of the tablet is then obtained by extrapolation to the zero air gap condition. The results of such measurements with and without an air gap are illustrated in Figure 15. An air gap or a sheet of teflon would act as a blocking electrode and should greatly diminish electrode polarization. If electrode polarization is diminished the low frequency dispersion would be altered. As this was not the case it is believed that electrode polarization is not responsible for the low frequency dielectric dispersion in hydrated hemoglobin.

A large positive temperature coefficient is found to be associated with the low frequency dielectric dispersion. This is in agreement with studies of O'Konski, Moser and Shirai (1964) on nucleic acids. At relatively low temperatures ($\sim$ -25° C) the low frequency dielectric dispersion is not found and the capacitance of a hydrated hemoglobin pellet is found to vary inversely with the thickness of the sample.

In Figure 15 it appears that, at low frequencies, the capacitance of hydrated hemoglobin varies inversely as a power of the frequency. However, before this can be verified by plotting the log of both variables it is necessary to demonstrate that setting one variable to zero causes the other variable to be zero simultaneously i.e. the relation between the variables does not contain an additive

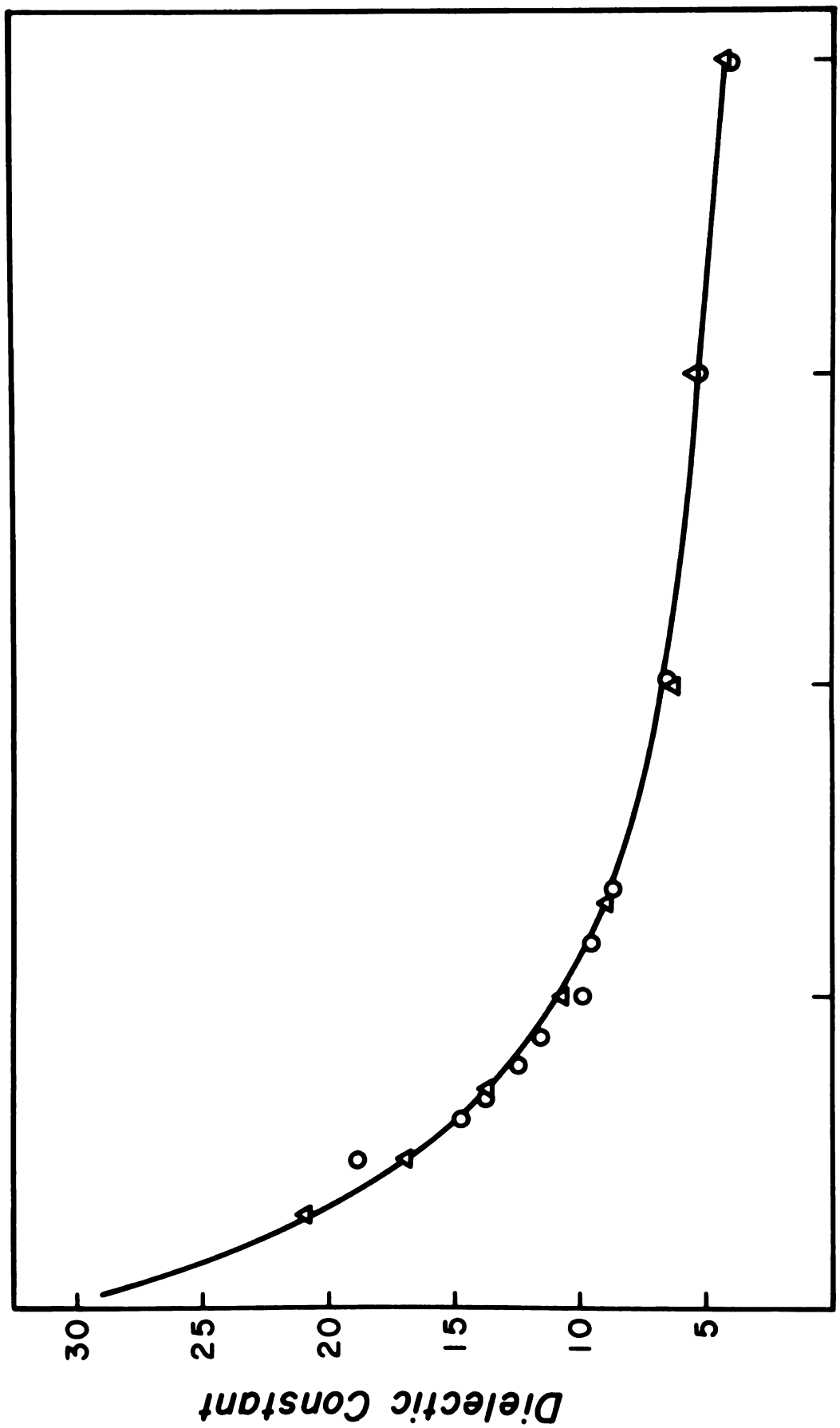


Figure 15. The frequency dependence of the apparent dielectric constant of hydrated hemoglobin as determined by the contact method (circles) and by the air gap method (triangles).

Figure 16. Frequency dependence of the reciprocal of the capacitance of hydrated hemoglobin at very low frequencies.

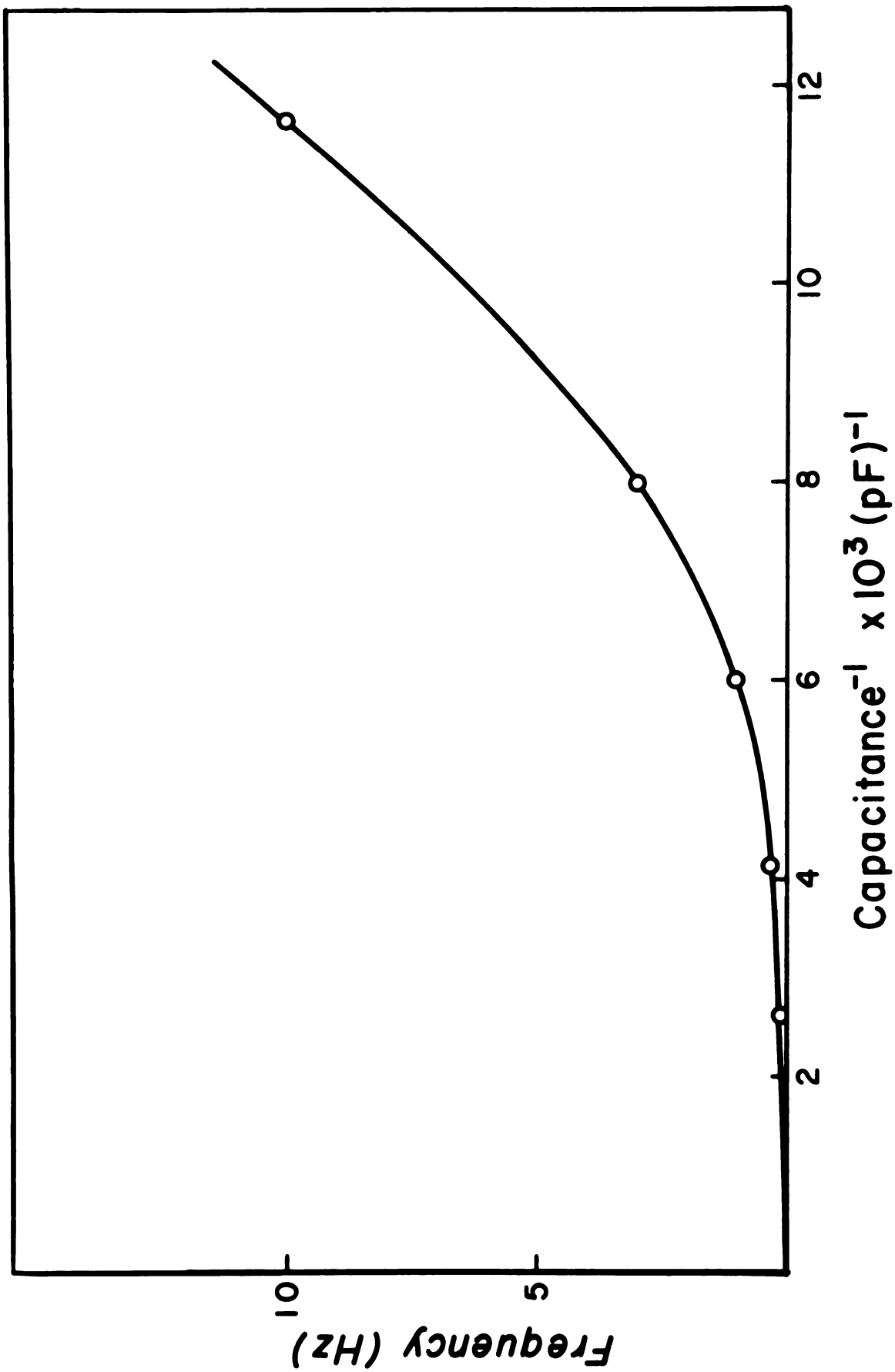


Figure 16.

constant. Low frequency capacitance measurements, obtained using the Ando TR-4 dielectric bridge, of a compacted hemoglobin tablet in equilibrium with an atmosphere of 76% relative humidity, have been plotted against the frequency in Figure 16. The curve intercepts the origin indicating that the data may be displayed on a log-log graph without a change of variable.

When this data is plotted on a log-log graph, as in Figure 17, it is found that the capacitance varies as $\omega^{-0.35}$. This frequency dependence continues to 200 - 500 Hz for the data at 76% relative humidity. At lower hydration states this effect terminates at lower frequencies. An examination of the dielectric data of Maričić, Pifat and Pravdič (1964b) indicates that the same frequency dependence was found, but not discussed, by these workers. The adsorption of methanol and ethanol on hemoglobin produces similar results.

The apparent low frequency dielectric dispersion of hemoglobin with various adsorbates is believed to be the result of a polarization effect, probably of the Maxwell-Wagner variety. A further discussion of this point will be found in Section V. It can be seen in Figure 14 that in the region 10^4-10^5 Hz, polarization effects are negligible. Therefore, in the following discussion the dielectric constant has been calculated from the capacitance as measured at 10^5 Hz. The dielectric constant, as defined in Equation (31), is corrected for the air filled intersticies found in

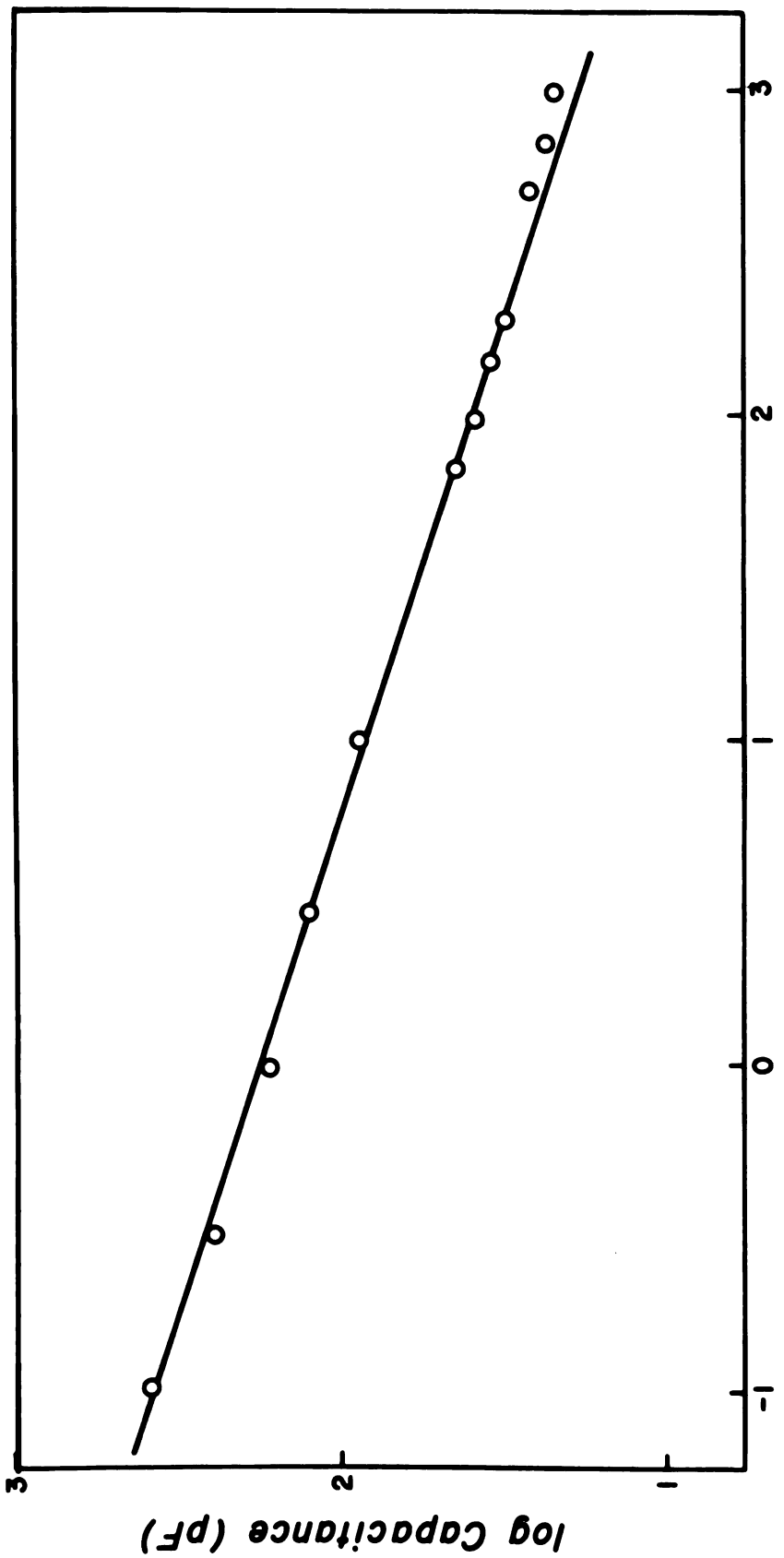


Figure 17. Frequency dependence of the capacitance of hydrated hemoglobin.

Table 5. Dielectric constant and activation energy of hemoglobin with adsorbed water, methanol and ethanol.

Adsorbate	m(mole %)	E (eV)	Dielectric Constant
none	0	2.35	2.3
water	.355	1.95	2.9
	.656	1.77	3.4
	1.01	1.55	4.2
	2.2	1.44	5.5
	-	1.23	7.4
methanol	0.203	1.75	3.2
	0.53	1.45	4.6
	-	1.20	5.9
ethanol	0.073	1.8	3.1
	0.35	1.6	4.5
	-	1.5	4.9

compacted tablets. This is done by extending Böttcher's (1962) treatment of a powder to the case of a compacted tablet. One then obtains for the dielectric constant of the crystallite material:

$$\kappa = \frac{3\kappa_p\delta + 2\kappa_p(\kappa-1)}{3\kappa_p\delta - (\kappa_p-1)} \tag{34}$$

where κ is the dielectric constant of the crystalline material; κ_p is the dielectric constant of the tablet (given as κ in Equation (33)) and δ is the packing fraction, or partial volume of crystalline material in the pellet.

The dielectric constants of hemoglobin with adsorbed water, ethanol and methanol are presented in Table 5. Water, methanol and ethanol, when adsorbed on hemoglobin increase the effective dielectric constant of the sample. Concomitant with this increased dielectric constant a decrease is found in the activation energy for semiconduction. Although the dielectric constant of hemoglobin increases with the adsorption of ammonia these results cannot be correlated with the activation energy or the molar adsorption. They have therefore been omitted at this time.

Adsorption Measurements

The adsorption of water vapor, methanol, ethanol, and ammonia on hemoglobin follows Type II, or BET, isotherm as can be seen in Figure 18, 19, 20 and 21. In each case the data was plotted according to the BET equation and a straight line obtained in the region $p/p_o < 0.3$ as

illustrated in Figures 22, 23, 24, and 25. As discussed above the BET equation contains two constants, V_m the monolayer coverage, and C which is a function of the heat of adsorption of the first layer and of the heat of liquefaction of the vapor. In the case of a compacted hemoglobin tablet, values for V_m and C were calculated from Figures 22, 23, 24 and 25 and are given in Table 6.

Simultaneous with, and under the same conditions as, the adsorption studies, conductivity experiments were conducted on a second sample of hemoglobin. The results of these conductivity experiments are presented in Figures 26, 27, 28, and 29. In order to better compare the effect of these several vapors on the conductivity of hemoglobin the results were redrawn on a mole %, rather than a weight %, basis in Figure 30. An exponential dependence of the conductivity on the quantity of vapor adsorbed, as is indicated in Equation (5), is found to hold in most of the region studied. Values of α, as given in Equation (5), have been calculated from Figure 30 and are presented in Table 7.

Table 6. BET parameters for water, methanol, ethanol and ammonia adsorbed on hemoglobin.

Adsorbate	X_m (gm/100 gm protein)	X_m (moles/100 gm protein) = m	C
Water	8.5	0.47	12.3
Methanol	13.5	0.43	3.5
Ethanol	7.5	0.16	2.0
Ammonia	8.0	0.47	1.7

Table 7. α for water, methanol, ethanol, and ammonia adsorbed on hemoglobin.

Adsorbate	α in (mole %)$^{-1}$
Water	17
Methanol	25
Ethanol	16
Ammonia	14

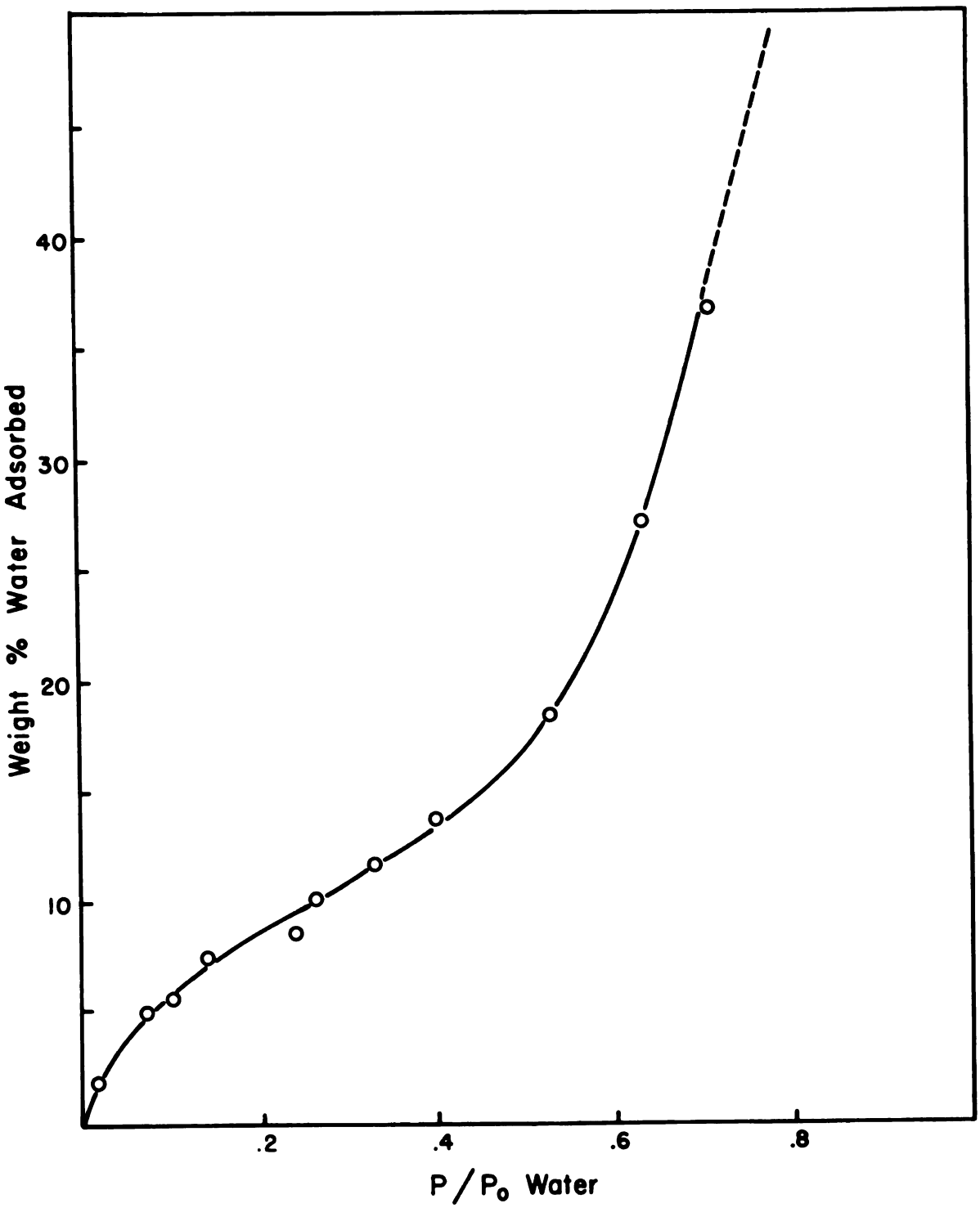


Figure 18. Adsorption isotherm of water on hemoglobin at 24° C.

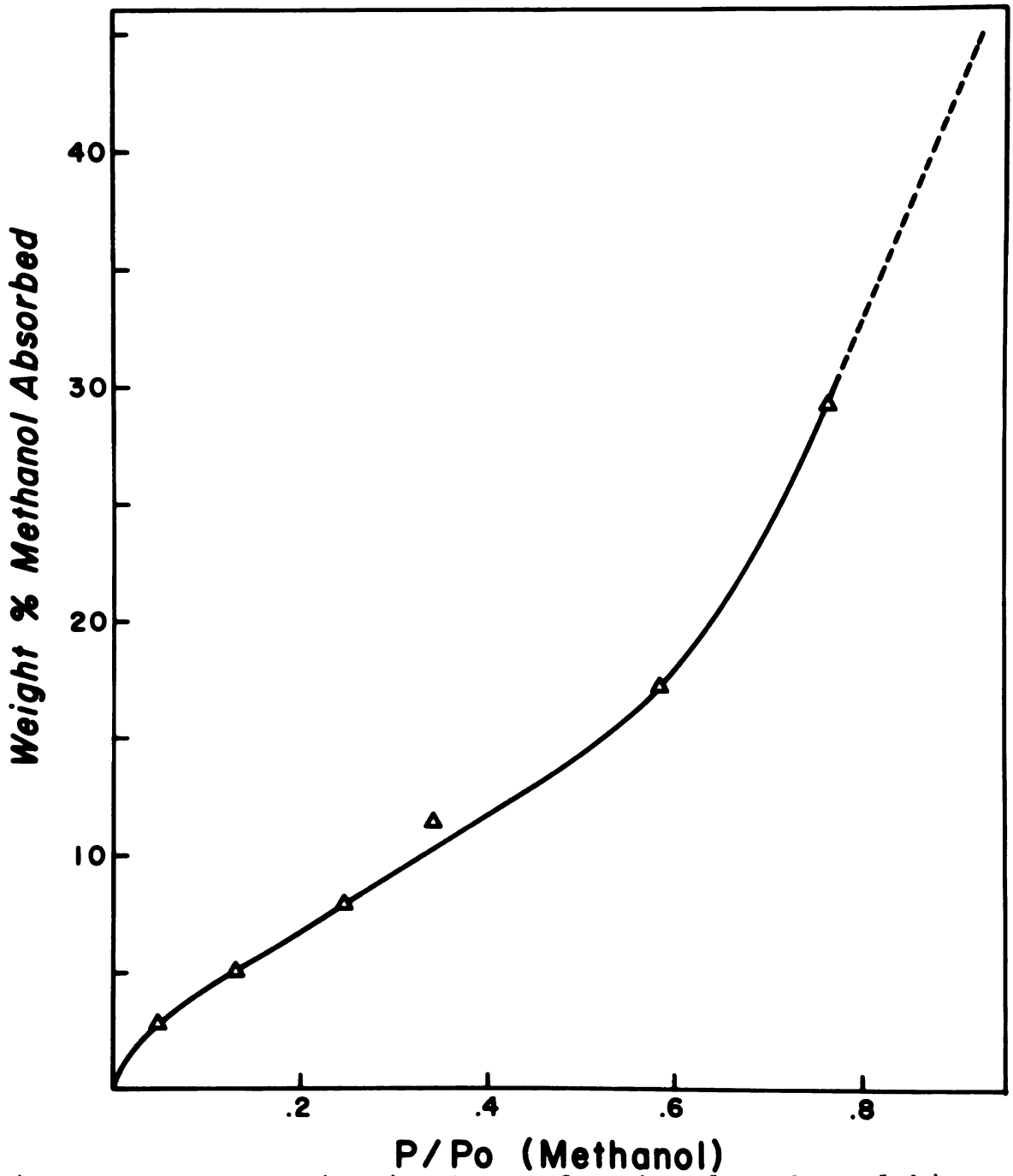


Figure 19. Adsorption isotherm of methanol on hemoglobin at 24° C.

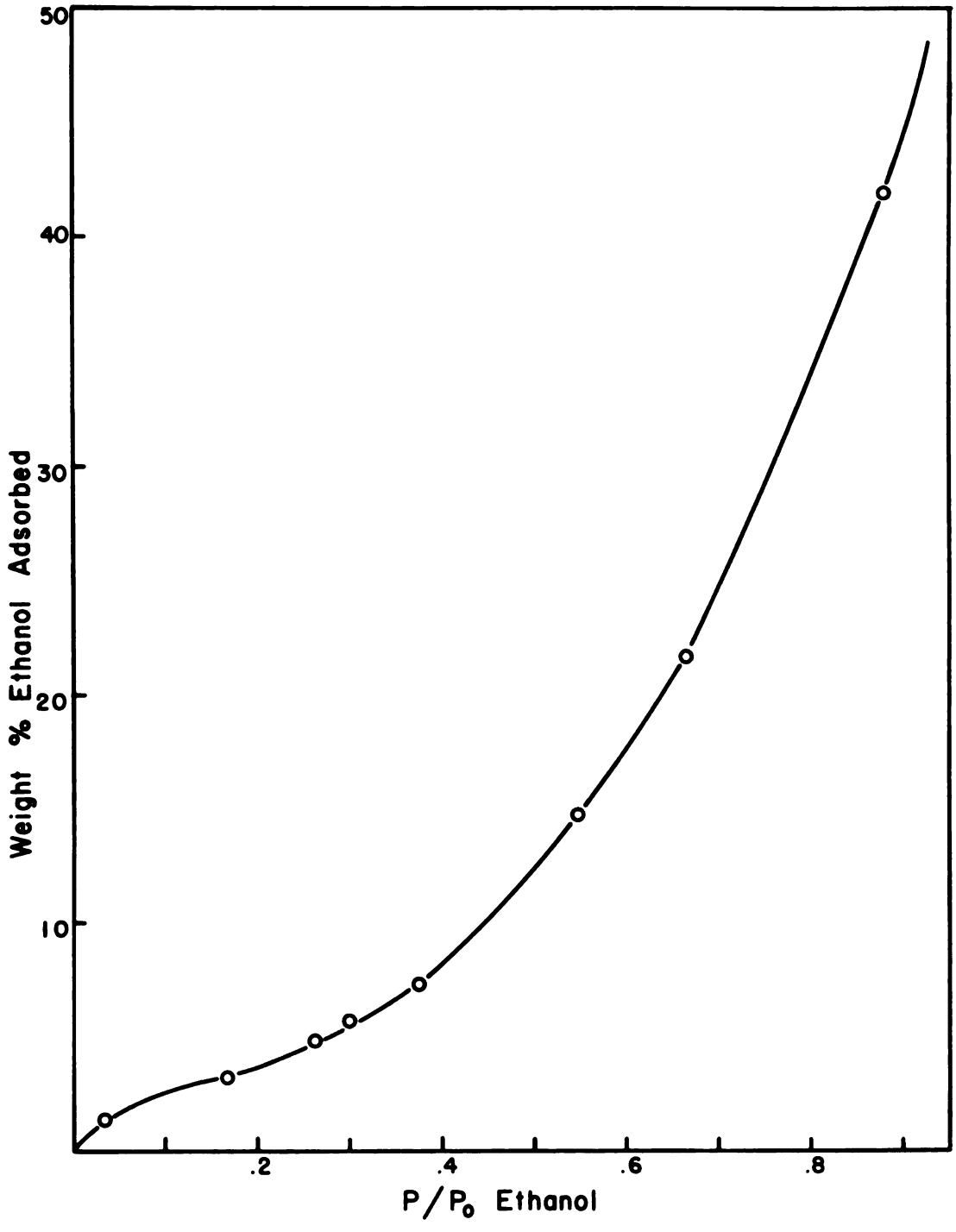


Figure 20. Adsorption isotherm of ethanol on hemoglobin at 24° C.

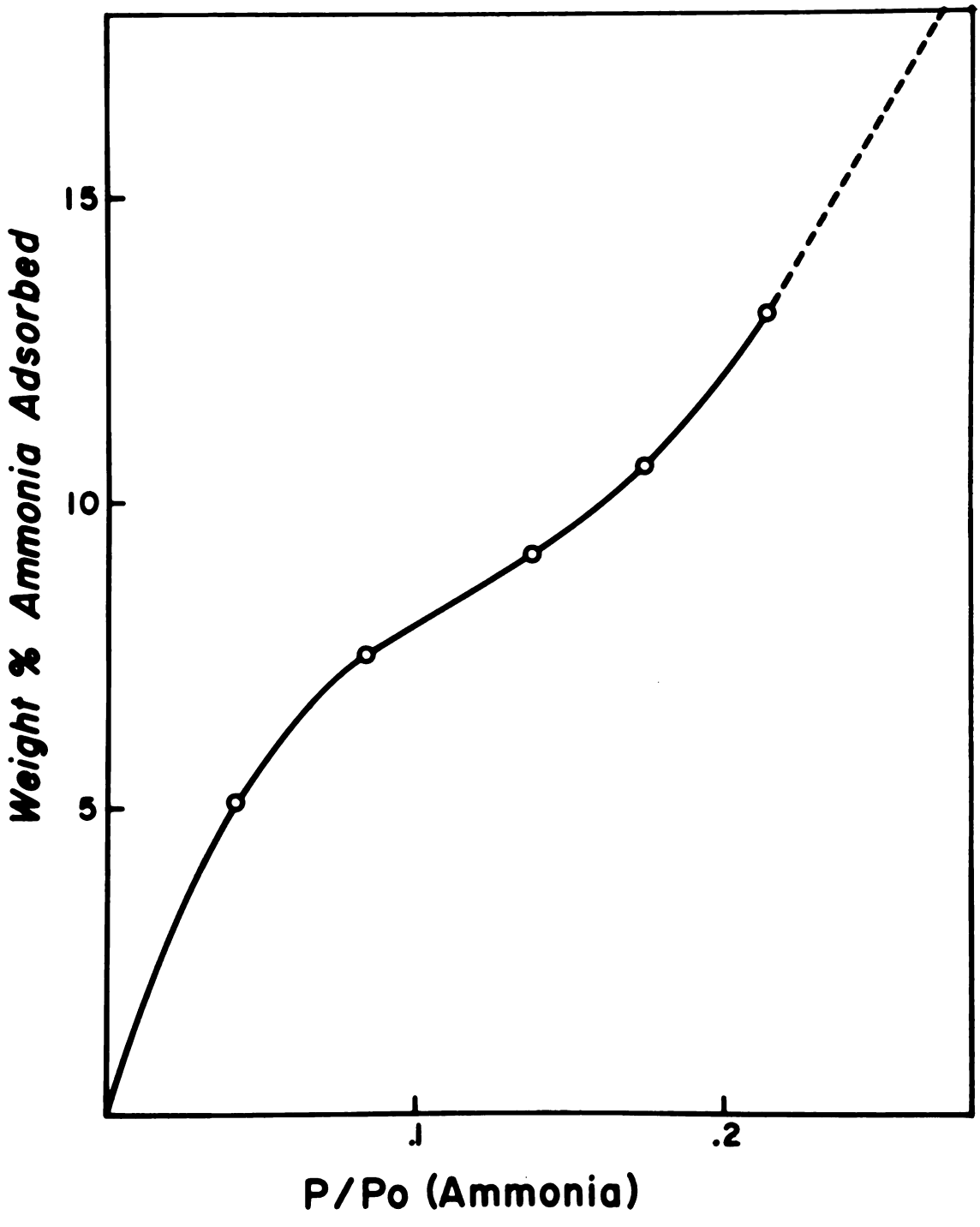


Figure 21. Adsorption isotherm of ammonia on hemoglobin at 0° C.

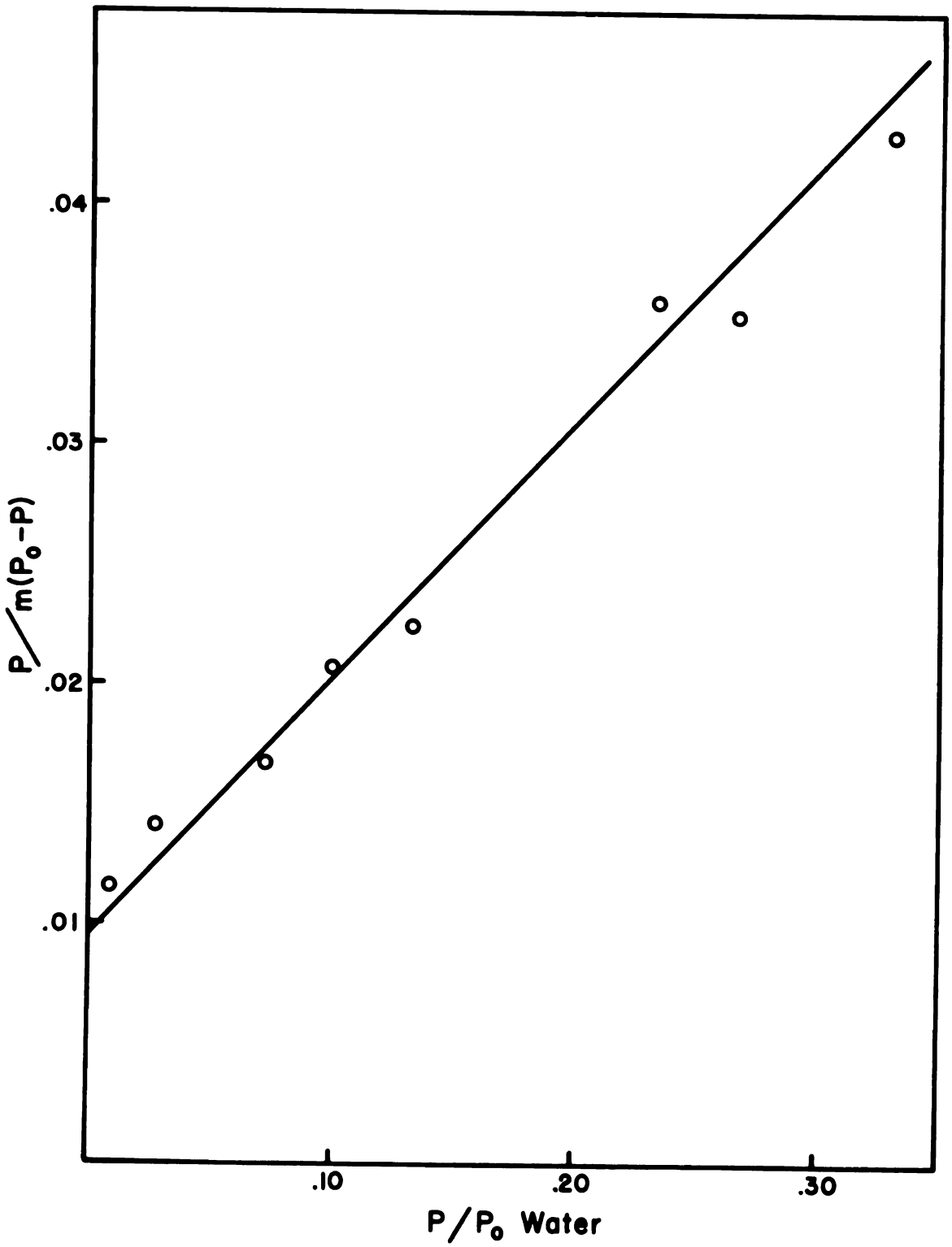


Figure 22. BET curve of water adsorption on hemoglobin.

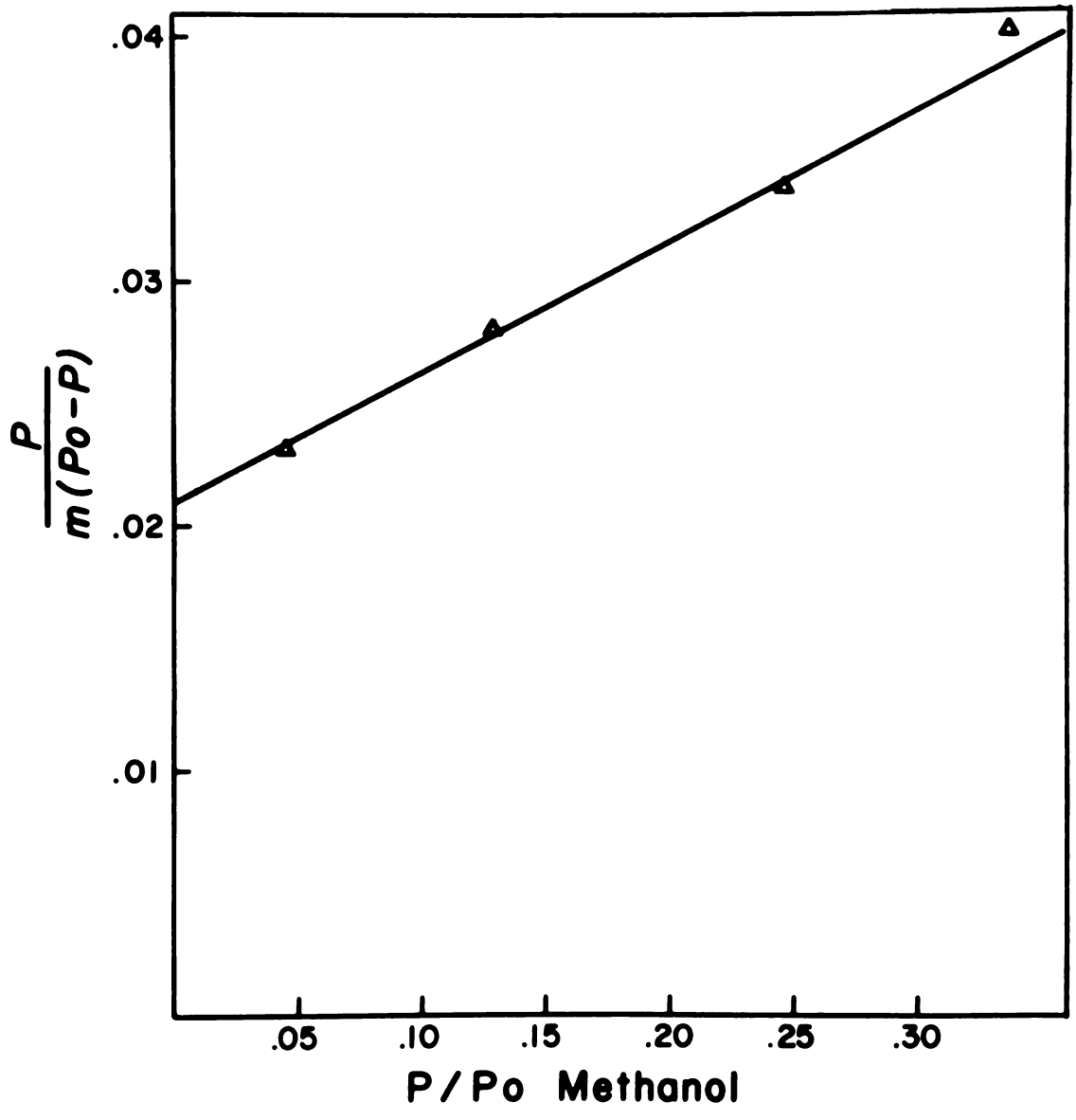


Figure 23. BET curve of methanol adsorption on hemoglobin.

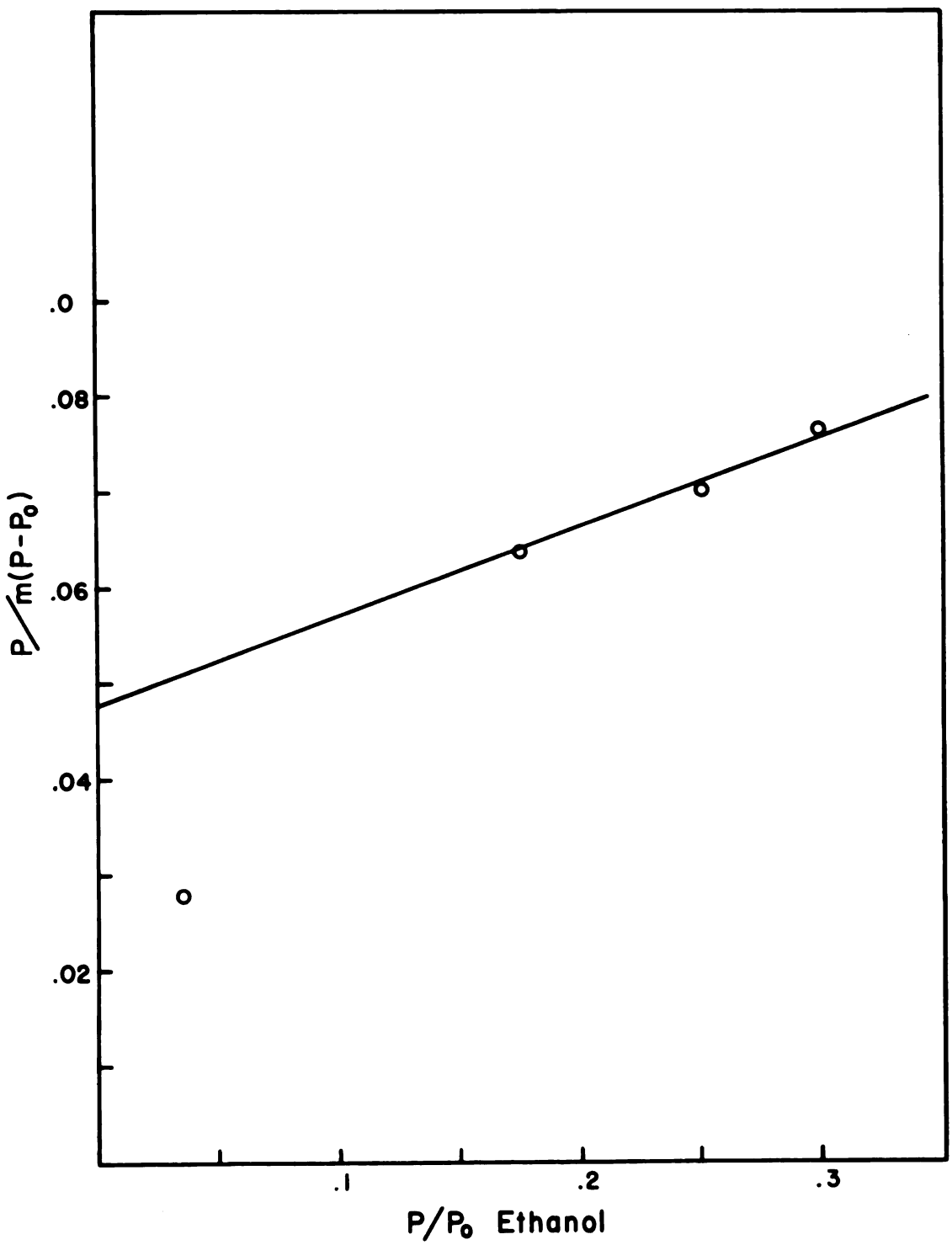


Figure 24. BET curve of ethanol adsorption on hemoglobin.

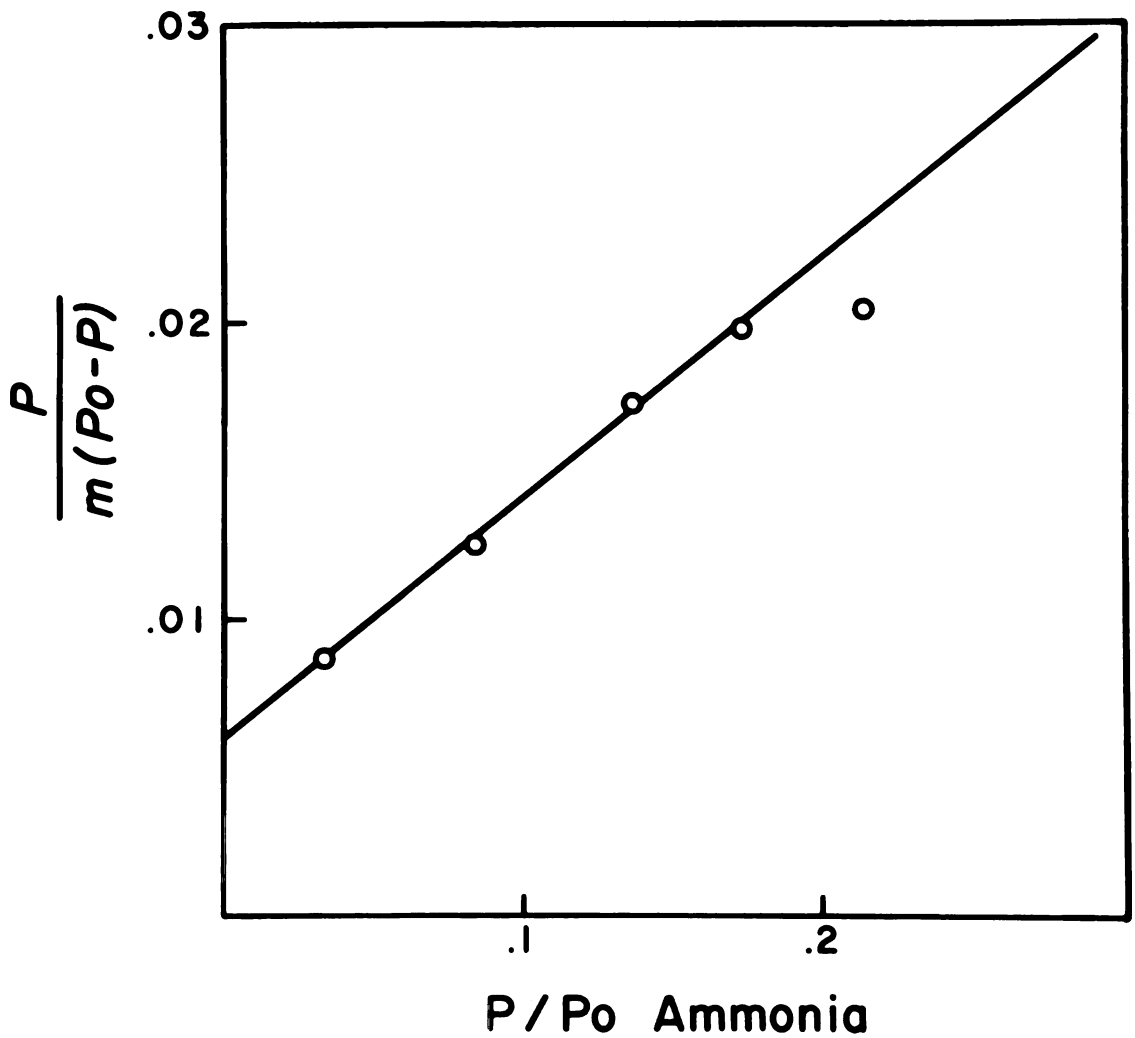


Figure 25. BET curve of ammonia adsorption on hemoglobin.

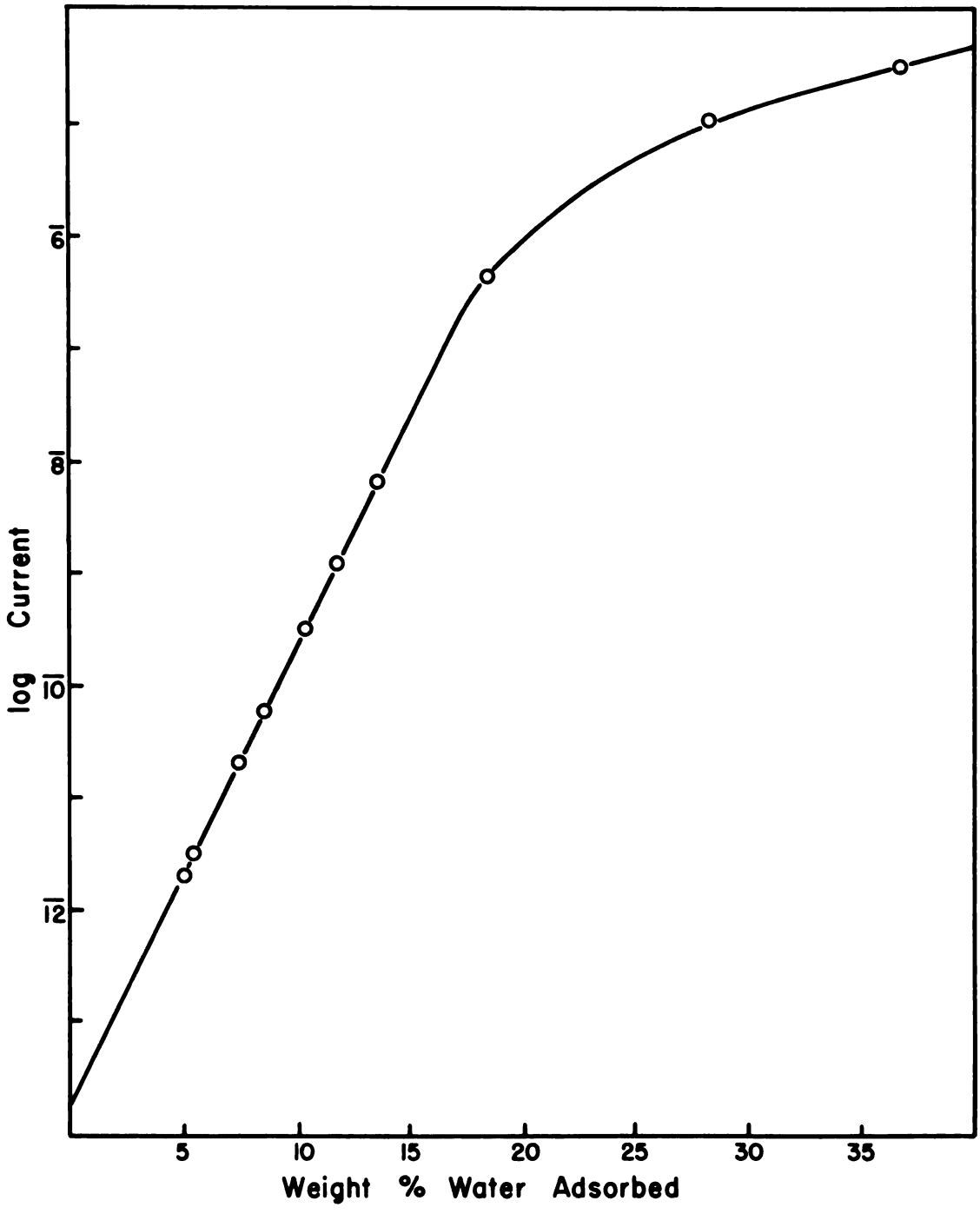


Figure 26. Conductivity of hemoglobin as a function of water adsorbed.

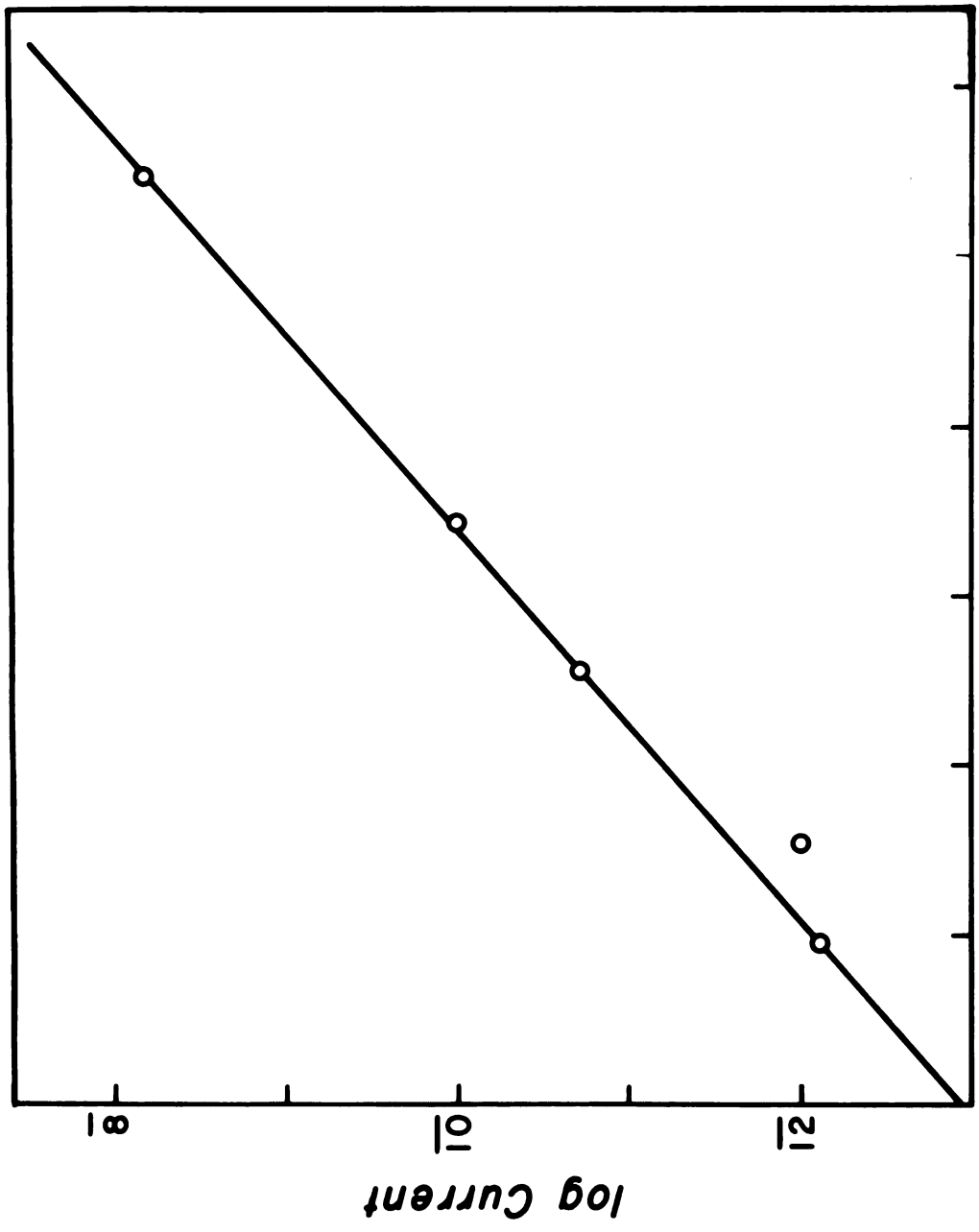


Figure 27. Conductivity of hemoglobin as a function of methanol adsorbed.

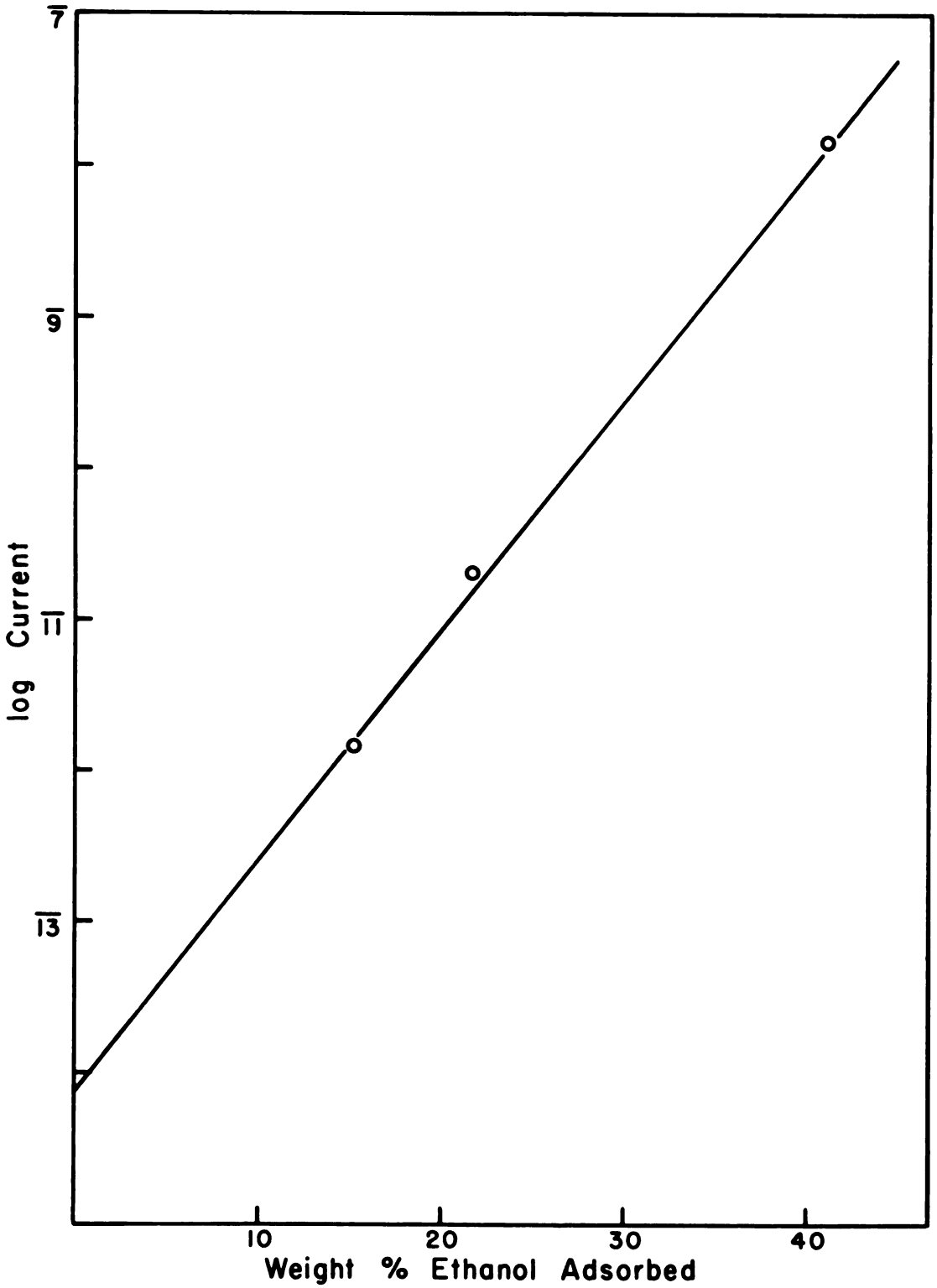


Figure 28. Conductivity of hemoglobin as a function of ethanol adsorbed.

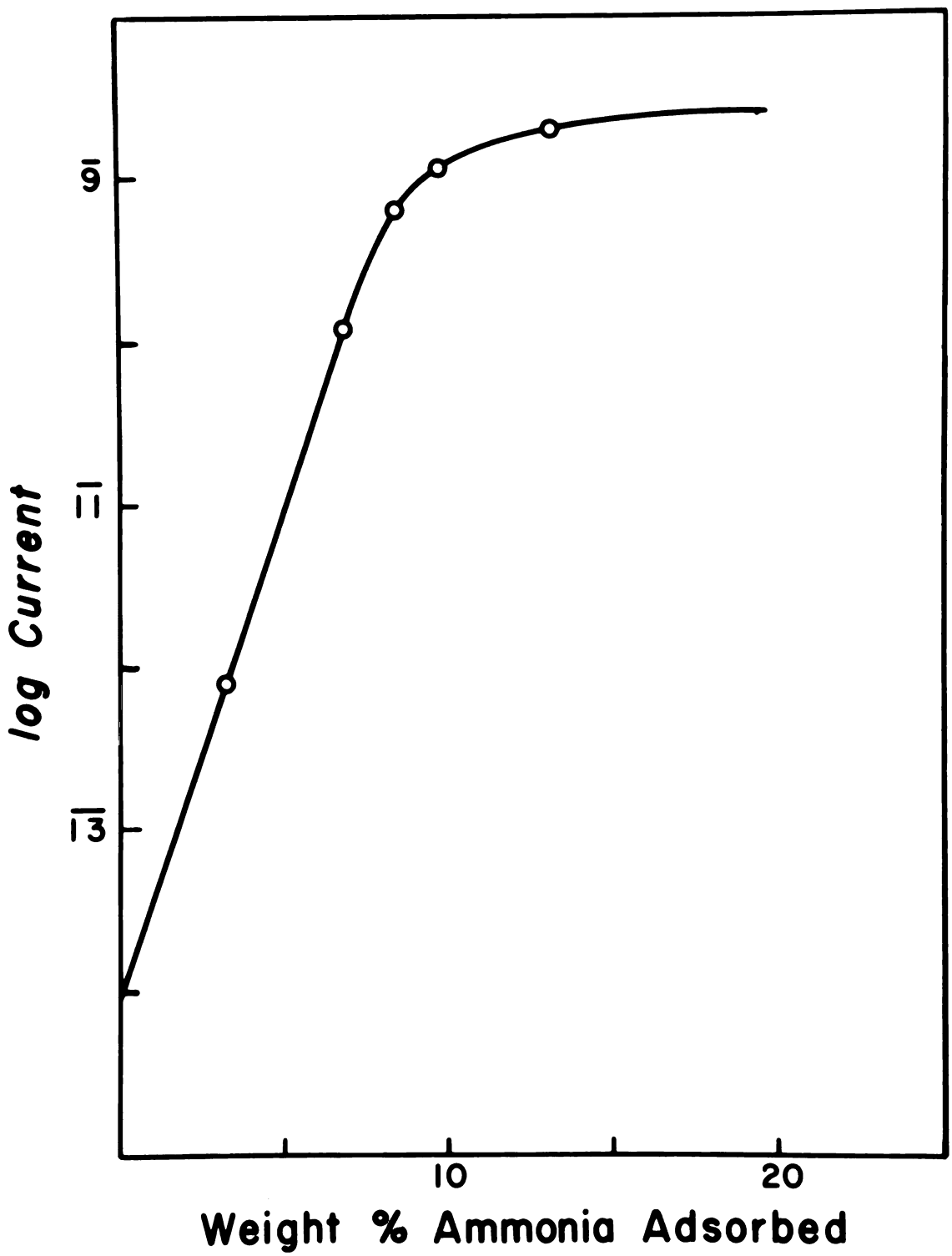


Figure 29. Conductivity of hemoglobin as a function of ammonia adsorbed.

Figure 30. Conductivity of hemoglobin as a function of mole % adsorbate. Ammonia adsorption is illustrated in curve A, methanol adsorption in curve B, water adsorption in curve C and ethanol adsorption in curve D. The vertical line indicates monolayer coverage (see Section V).

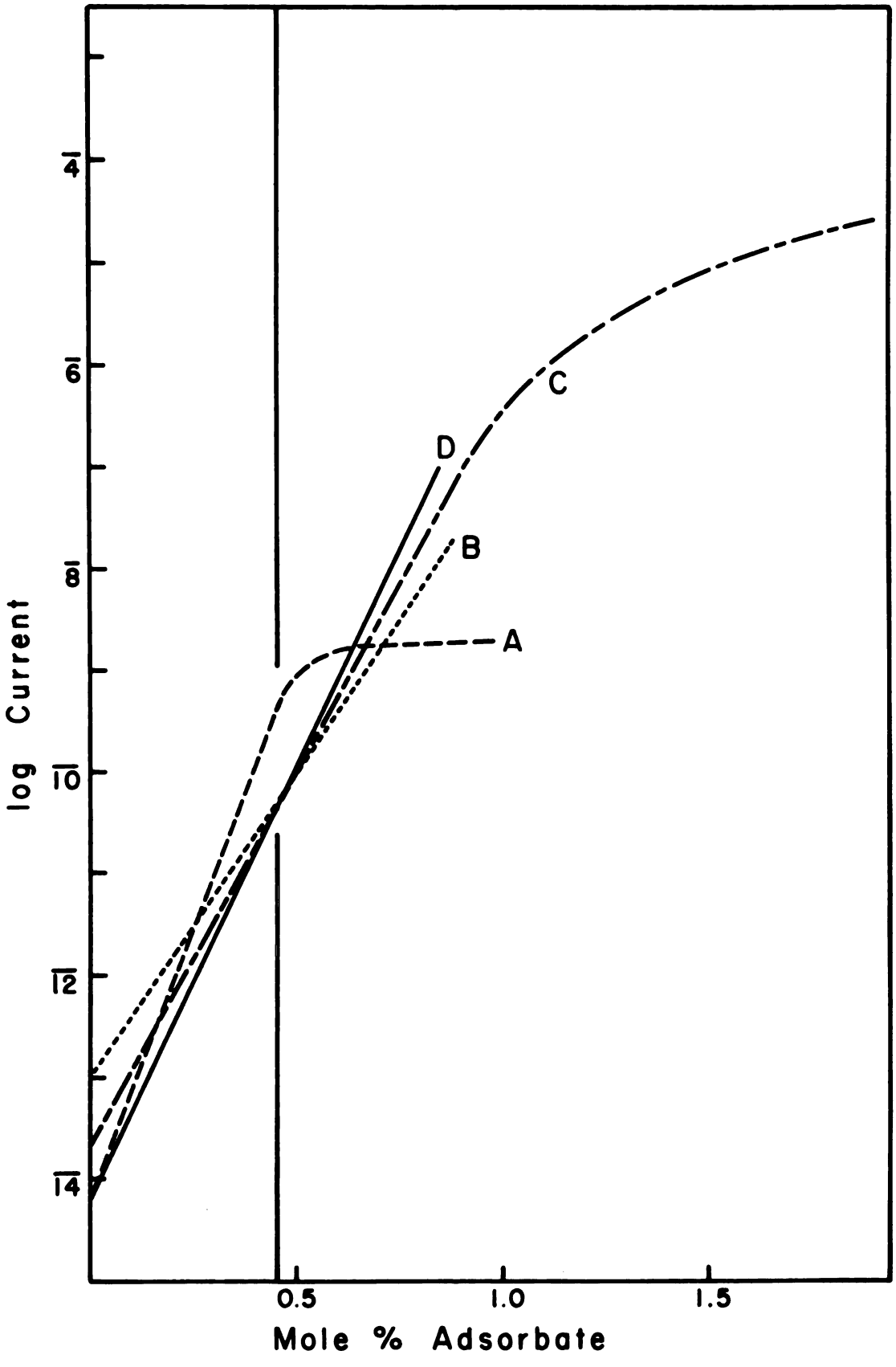


Figure 30.

V. DISCUSSION

Impurities in Biological Semiconductors

Some inorganic semiconductors are of "electronic grade" purity, i.e. less than one impurity molecule for each 10^{10} substrate molecules. Although a few organic crystals can be greatly purified by zone refining techniques, biological substances are not obtainable at purity levels even approaching "electronic grade." "Electronic grade" biochemicals, particularly those of the polymeric variety, are not possible in the forseeable future. The cell contains many "impurities." Therefore, a relative insensitivity of the conductivity mechanism to general impurities is demanded of semiconductive models postulated in biological systems.

The difference in sensitivity to impurities is not between inorganic and biochemical semiconductors but between covalent crystalline semiconductors on the one hand and molecular crystals on the other. Tauc (1967) has reported that the incorporation of impurities, at low concentration levels, influences the conductivity of amorphous germanium but very little. This is in contradistinction to crystalline germanium where low concentration impurities dominate the semiconductive process. Covalent crystals possess a long range order which may extend over a distance as great as 1000

unit cells. This provides for a long range interaction not found in amorphous materials, polymers, or molecular crystals. In covalent crystals, any alteration of the long range order, such as the introduction of an impurity, will have widespread ramifications. The same is not the case for a system possessing only short-range order. In such a system impurities, until their concentration becomes rather high, exhibit only local effects which do not statistically affect the short range order of the system (Gutmann and Lyons, 1967).

The experimental evidence presented in Figures 7, 9 and 11 indicates that hemoglobin with adsorbed water, methanol or ethanol is not an impurity dominated process. In the case of hemoglobin with adsorbed ammonia, however, an impurity mechanism could well explain the results of Figure 13. A classic example of inorganic impurity semiconductivity is given in Figure 31A. Each region of the curve can be described by an equation of the same form as Equation (1). However, both the activation energy for semiconduction and the pre-exponential factor differ in the two regions. The temperature dependence of the conductivity can therefore be described by an equation of the form:

$$\sigma = \sigma_1 \exp(-E_1/2kT) + \sigma_2 \exp(-E_2/2kT) \qquad (35)$$

The pre-exponential factor in the impurity conductivity term is a function of the concentration of impurities.

At low temperatures, and hence low conductivity, impurity conductivity will dominate because of the lower

Figure 31. A. Schematic diagram of classical impurity semiconductivity. B. Schematic diagram of semiconductivity found in hemoglobin-adsorbate systems.

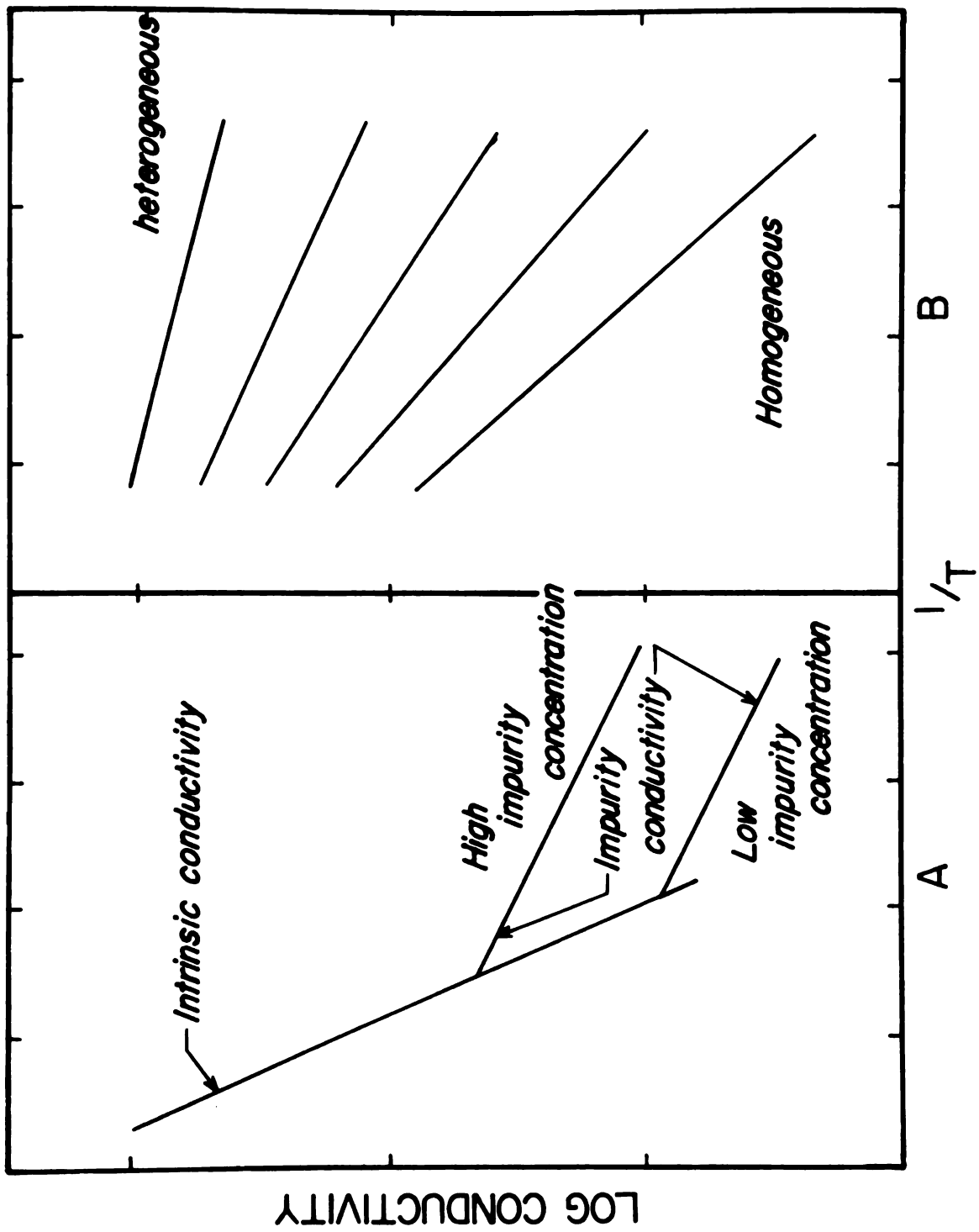

heterogeneous
Homogeneous
B
A
1/T
Intrinsic conductivity
High impurity concentration
Impurity conductivity
Low impurity concentration
LOG CONDUCTIVITY

Figure 31.

activation energy needed to promote the charge to the conduction band from the impurity center, while at higher temperatures the intrinsic conductivity of the substrate material will dominate because of its higher activation energy and hence its faster increase with temperature. As the impurity concentration is increased the dominance of impurity conductivity is extended to a higher temperature region. Alteration of the impurity concentration does not change the activation energy of impurity conductivity because only the number of carriers is affected by such a change. (If impurity band conduction results from an increased impurity concentration, then a concomitant change will be found in the semiconduction activation energy.) Varying the impurity concentration yields parallel curves, indicating a changing pre-exponential factor but constant activation energy. The semiconduction curve for hemoglobin with adsorbed ammonia may be of this form. However, the conductivity data for hemoglobin with adsorbed water, ethanol and methanol are not of this form. In the latter cases increased adsorbate concentration alters the activation energy of the system but does not affect the pre-exponential factor. Thus, a compensation effect, as discussed below, is not found in hemoglobin. A linear relation is found between the activation energy and the amount of adsorbate present in the system. This indicates that the adsorbate is not behaving as an impurity but is in some way altering the intrinsic semiconductive properties of the hemoglobin. The property which

is altered is the magnitude of the polarization relaxation of the matrix.

The Effect of Inter-granular Spaces on Conductivity

A micro-crystalline powder was compacted to form tablets which contain inter-granular spaces. The capacitance of inter-granular spaces often causes the dc resistance to be significantly greater than the resistance which is measured at high frequencies. High frequencies short out the capacitance of the inter-crystalline barrier. The activation energy was determined from measurements of dc conductivity. It is therefore important to investigate the role of inter-granular effects on the activation energy determinations.

Siemons, Bierstedt and Kepler (1963) have compared the semiconduction activation energy for single crystals and compressed tablets of the highly conducting charge-transfer complex $Cs_2(TCNQ)_3$. Compressed tablets exhibit an activation energy for semiconduction of 0.07 eV while the activation energy of single crystals is 0.01 eV. In the case of the $Cs_2(TCNQ)_3$ compressed tablet nearly all of the activation energy results from inter-granular impedances. If this value, 0.07 eV, is generally indicative of the activation energy resulting from inter-granular impedance, then the activation energy measurements reported herein, 1.2 - 2.4 eV, are indicative of processes occurring in the bulk of the material.

The Pre-exponential Factor in Hemoglobin Conductivity

The solid state electrical conductivity of organic substances is generally thought of as having a temperature dependence given by Equation (1). For many polynuclear hydrocarbons there exists a correlation between the activation energy and the pre-exponential factor (Many, Harnik and Gerlach, 1955). The data can be seen (Gutmann and Lyons, 1967) to fit roughly the relationship:

$$\log\sigma_o = \alpha E + \beta \qquad (36)$$

where α and β are constants for all of the compounds considered.

Recently Rosenberg et al. (1968) have shown that a compensation effect is exhibited by several biological compounds when they are treated in different ways. Several organic semiconductors exhibit a compensation effect as well (Eley, Fawcett and Willis, 1968). These results are similar to the Meyer-Neldel (1937) rule for inorganic semiconductors. In both cases a single compound is capable of exhibiting a variety of activation energies depending on its method of preparation or pretreatment. The activation energy and the pre-exponential factor vary as described in Equation (36). Among the biological substances which exhibit a compensation effect are oxidized cholesterol, nucleic acids and retinal. Melanin with adsorbed water, hemoglobin with several adsorbed vapors and bovine plasma albumen complexed with various carcinogens (Snart, 1968) do not follow the compensation relationship.

If it is assumed that most semiconductive materials exhibit a compensation effect, then it is of interest to investigate the implications of a given substance not following such a relationship. We may rewrite Equation (1) as:

$$\log\sigma = \log\sigma_o - E/2kT \tag{37}$$

Substituting Equation (36) we obtain:

$$\log\sigma = E/2kT_o + \beta - E/2kT \tag{38}$$

where we have replaced α in Equation (36) by $1/2kT_o$. T_o is then that temperature at which two samples which have different activation energies will exhibit the same conductivity. In the case of hemoglobin with various adsorbed vapors this temperature is infinity.

The most general interpretation of Equation (38) is in terms of activated complex theory (Eley, 1967). The exponential terms then take on thermodynamic significance as an analogy is drawn between Equation (38) and:

$$k_F = (kT/h)\exp(\Delta S/R) \cdot \exp-(\Delta H/RT) \tag{39}$$

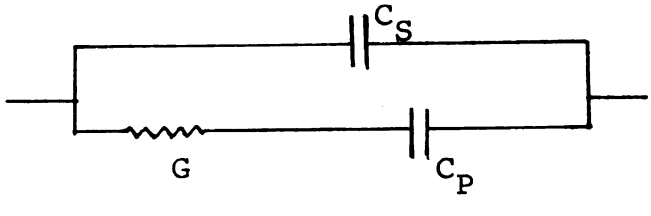
where k_F is a rate constant; ΔS and ΔH are the entropy and enthalpy of activation respectively. If this analogy is drawn we are confronted with the perplexing statement that conductivity in hemoglobin conserves entropy but conductivity in DNA does not.

Another interpretation of Equation (38) is that the process measured, i.e. conductivity, is a two step process, only one of the steps being thermally activated. The step which is not thermally activated may be, for example,

intermolecular quantum tunneling. In the case of protein conductivity the probability of the second step is so high that its effective rate constant is unity. If indeed the second step is intermolecular tunneling, as suggested by Kemeny (1968), then the intermolecular barrier in proteins would be very low.

Apparent Low Frequency Dielectric Dispersion

Low frequency dielectric dispersions attributed to electrode polarization, in aqueous solution measurements, possesses a frequency dependence which is very different from that found for hydrated compacted tablets. Electrode polarization, in the former case, varies as the -1.5 to -1.7 power of the frequency. Employing Scheider's (1962) model of a lumped polarization capacitance in series with the specimen conductance, which is in turn in parallel with the specimen capacitance:



The admittance of the network is given by:

$$Y = G + i\omega C_S + \left\{ \frac{1}{1 + \frac{\omega^2 C_P^2}{G^2}} \right\} (-G + i\omega C_P) \qquad (40)$$

where G is the sample conductance, C_S is the sample capacitance, C_P is the polarization and ω is the frequency of the impressed voltage. The real part of the admittance is the

conductance and the imaginary part is the capacitance. In the case of measurements of biomolecules in aqueous solutions it is assumed that:

$$\frac{\omega^2 C_P^2}{G^2} >> 1 \tag{41}$$

Under this condition the apparent parallel polarization capacitance, C_A, the second imaginary term in Equation (40), is:

$$C_A = \frac{G^2}{\omega^2 C_P} \tag{42}$$

According to Wolff's (1936) direct measurement of low frequency polarization capacitance in aqueous solutions C_P varies as the -0.5 to -0.3 power of the frequency. C_A, the apparent polarization capacitance should then vary as the -1.5 to -1.7 power of the frequency which is found to be the case in the measurement of the capacitance of aqueous solutions of biomolecules. However, in the case of hydrated hemoglobin tablets typical low frequency values would be $C = 10^{-10}$ farad and $G = 10^{-7}$ mho at $\omega = 100$Hz. Therefore, it is more reasonable to assume:

$$\frac{\omega^2 C_P^2}{G^2} << 1 \tag{43}$$

in which case:

$$Y = i\omega(C_S + C_P) \tag{44}$$

The measured capacitance should then be the arithmetic sum of the specimen capacitance and the polarization capacitance.

If, in the low frequency region the sample capacitance does not exhibit a disperison, the frequency region dependence of the measured capacitance will be that of the polarization capacitance. This is the experimental result which is found, i.e.:

$$C \propto \omega^{-1/3} \tag{45}$$

as illustrated in Figure 17. When these polarization effects are subtracted the low frequency dielectric dispersion in hydrated hemoglobin tablets disappears.

Low frequency dielectric dispersion is most probably the result of a Maxwell-Wagner type of process. This type of polarization arises at the boundary between two materials which do not possess the same conductivity to dielectric constant ratio. If a specimen composed of two such materials, is initially in the completely uncharged state, then when a potential is instantaneously impressed across it the dielectric displacement, D, at the first moment, will be constant throughout the specimen. The charges have not yet penetrated into the sample. However, current density is determined by:

$$j = \sigma E = \frac{\sigma}{\kappa} D \tag{46}$$

where j is the current density and E is the field strength. An accumulation of charges at boundaries which separate regions of different σ/κ must then occur. The buildup of charge at the boundary will continue until j is constant. This building up process requires time because it depends upon a finite conduction through the media on both sides of

the boundary considered. It is then a process which may be characterized by a relaxation time (Schwan, 1957). This boundary polarization may be associated either with the interface between the electrode and the hydrated pellet or with the interface between the crystallites and the intersticies.

Comparison of Results with Dielectric Theory

The adsorption of water, ethanol or methanol alters the conductivity of hemoglobin in such a way that the conductivity increases while the semiconduction activation energy decreases. The pre-exponential factor in the conductivity equation is unchanged by the adsorption process. These results can be interpreted in terms of the theory developed in Section II.

It appears that the adsorption of ammonia has a different effect on the conductivity of hemoglobin. In this case the results resemble classical impurity semiconductivity. The increased conductivity of hemoglobin with ammonia cannot be accurately correlated with the quantity of ammonia adsorbed because the partial pressure of the gas in the conductivity chamber could not be determined. However, as the rate of flow of ammonia, relative to that of nitrogen, is increased the conductivity of the specimen is increased indicating dependence of the conductivity on the quantity of ammonia adsorbed. The results of Figure 13 can be understood either if ammonia is acting as an impurity donating charge

carriers to the hemoglobin or if the charge carriers both originate from and move through the ammonia, i.e. conductivity of ammonia on a hemoglobin substrate. In the latter case the activation energy measured, 0.8 eV, should be that of ammonia. From the data of Cuelleron and Chariet (1954) the semiconduction activation energy of liquid ammonia can be calculated. A value of 0.1 eV is obtained. At present, therefore, it appears that ammonia is behaving as an impurity in the classical sense. The concentration of ammonia in the hemoglobin tablet is, however, much greater than concentrations of impurities in inorganic semiconductors. The impurity nature of the conductivity could be verified by following the temperature dependence of the conductivity of hemoglobin with adsorbed ammonia in order to determine if at higher temperatures intrinsic conductivity, i.e. the conductivity of hemoglobin with an activation energy of 2.35 eV, is found. But at higher temperatures the ammonia desorbes producing anomalous results. Very small quantities of adsorbed ammonia must be used which will decrease the temperature at which intrinsic conductivity will dominate. However, hemoglobin with small quantities of adsorbed ammonia has a relatively low conductivity and the present apparatus can not be used for these measurements. A vibrating reed electrometer, and therefore static measurements, must be used to investigate this conductivity region.

When water, ethanol or methanol is adsorbed on hemoglobin the conductivity increases and the semiconduction activation energy decreases in a manner inconsistent with

classical impurity semiconductivity. These solvents do not affect the value of the pre-exponential factor. The theory developed in Section II predicts a linear dependence of activation energy on the reciprocal of the effective dielectric constant. Since the log of the conductivity varies linearly with activation energy, a similar relation should exist between the log of the conductivity and dielectric constant.

In Figure 32 the reciprocal of the dielectric constant is plotted against the log of the conductivity. Data for water, ethanol and methanol all fit the same straight line. Interpreted in terms of the theory outlined in Section II this indicates that, according to Equation (18), R, the polarization radius, is independent of the adsorbate. The reciprocal of the dielectric constant is plotted against the activation energy in Figure 33. This is according to Equation (16) and has the same significance as Figure 32. From these figures a value of R may be calculated. In both cases R = 4.3 Å. The relaxation of the dielectric medium within a region of radius 4.3 Å is responsible for the decreased activation energy and hence higher conductivity of hemoglobin samples with adsorbed water, ethanol or methanol.

The effective dielectric constant of hemoglobin with the same mole percent of the various solvents adsorbed does not vary as the dielectric constant of the solvent. Since R is the same constant for water, ethanol and methanol adsorption, it can be seen from Equation (17) that α does not

Figure 32. Variation of conductivity with dielectric constant. The circles indicate water, the triangles ethanol and the filled circles methanol adsorption on hemoglobin respectively.

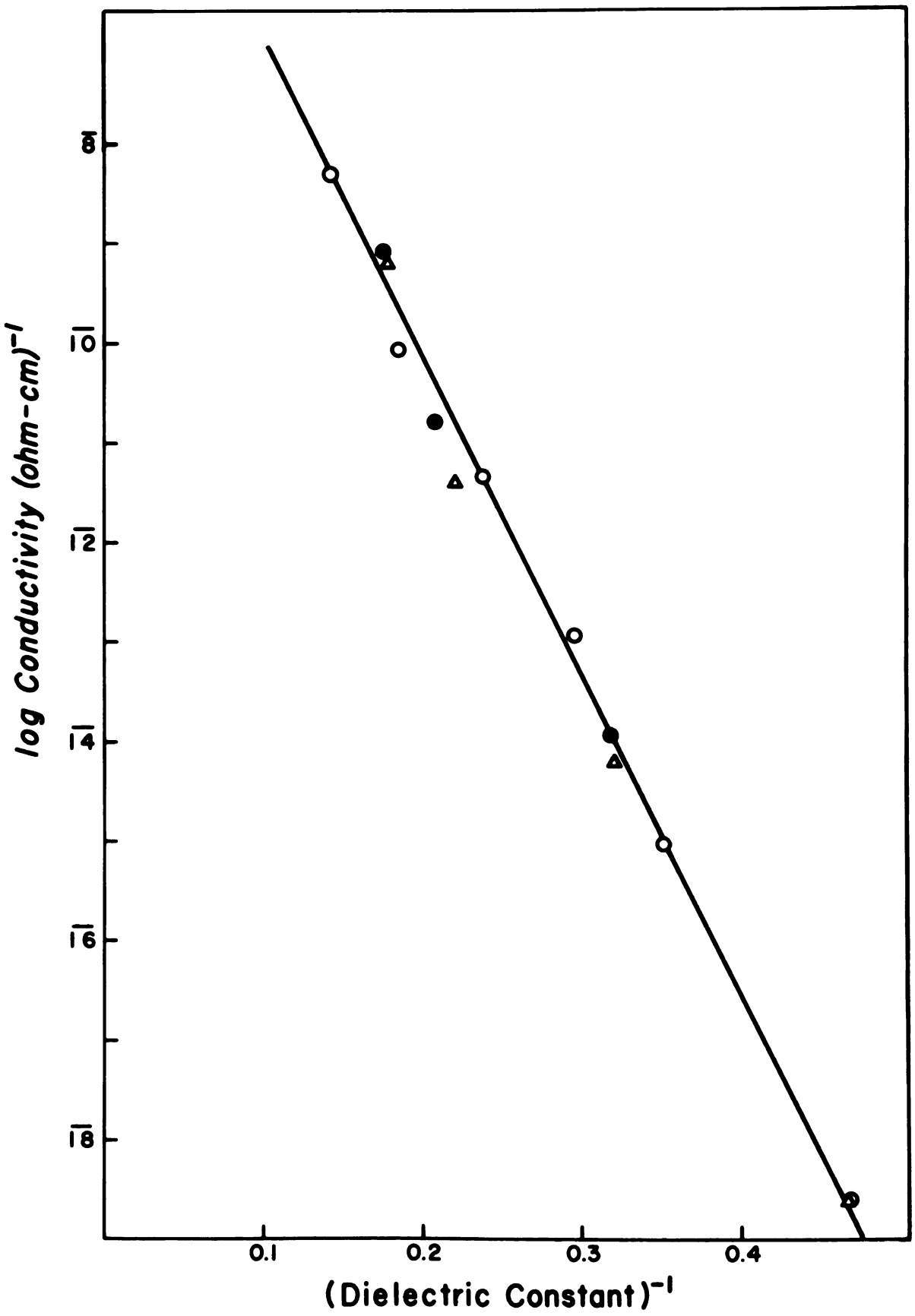

log Conductivity (ohm-cm)⁻¹
-8
-10
-12
-14
-16
-18
0.1
0.2
0.3
0.4
(Dielectric Constant)⁻¹

Figure 32.

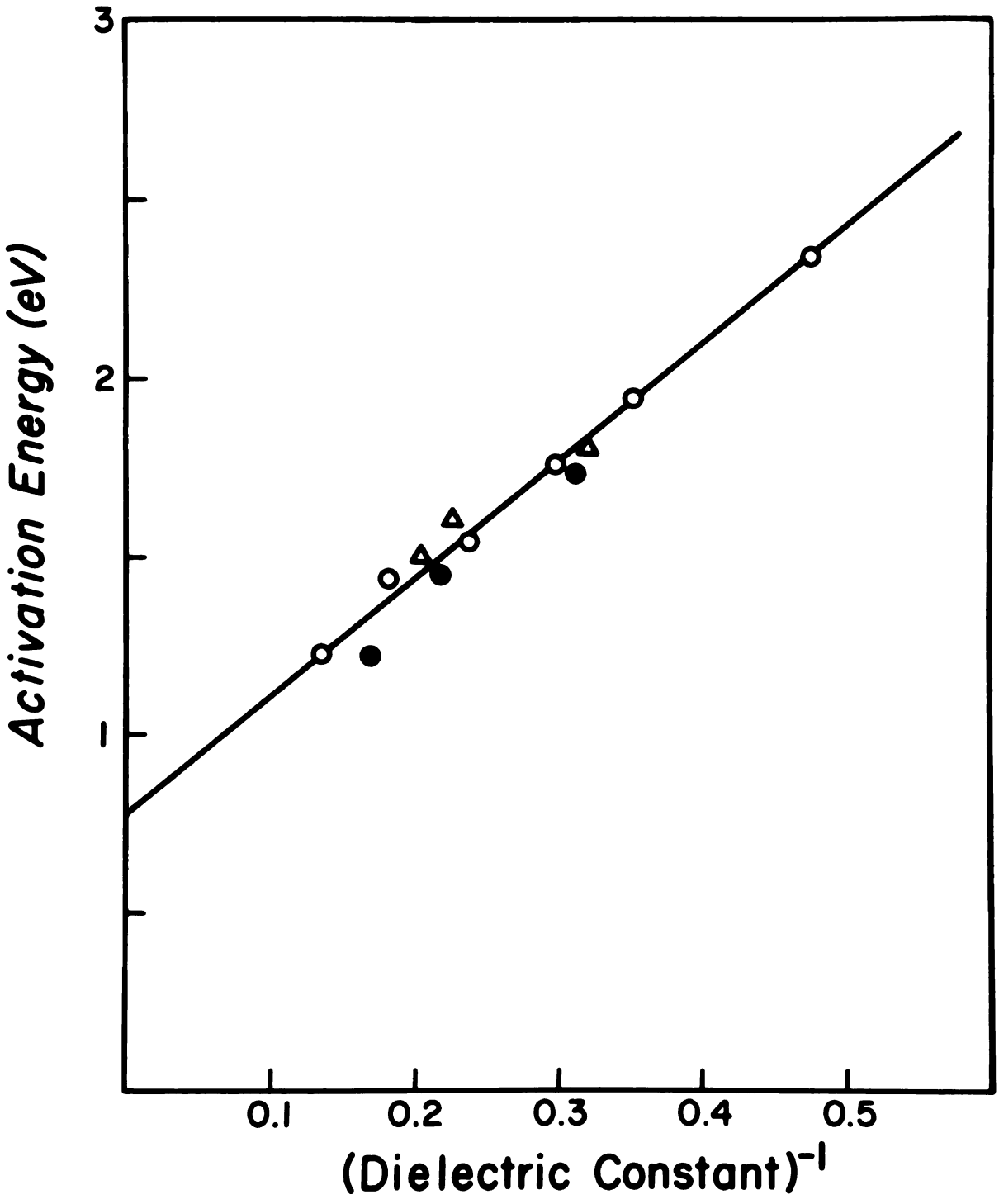


Figure 33. Variation of activation energy with dielectric constant. The circles indicate water adsorption, the triangles indicate ethanol adsorption and the filled circles indicate methanol adsorption.

vary directly with the dielectric constant of the adsorbed phase.

Equation (17) specifies a linear relation between the quantity of vapor adsorbed on the hemoglobin and the reciprocal of the effective dielectric constant of the hemoglobin-adsorbate system. The data for the adsorption of water on hemoglobin is shown in Figure 34. In the region of hydration where the conductivity is believed to be electronic, i.e. <2BET monolayers of adsorbed water, this relation is seen to hold. When hemoglobin is further hydrated deviation from this linearity is found. From Equation (17) and a knowledge of α, which was determined in Section IV, R, the radius of polarization, can be calculated. A value of 3.7Å is obtained. This is in close agreement with the value of 4.3Å calculated above. The data for ethanol and methanol adsorption are insufficient to draw any conclusions regarding the variation of dielectric constant with the quantity of vapor adsorbed.

In both the theoretical development of Section II and the experimental presentation no assumptions were made concerning either the nature of the charge carriers or the mechanism of conduction. (Activation energies have been calculated on a 1/2kT basis which does indicate the conceptual framework of a band theory. The elimination of the troublesome 2 will, however, only halve the activation energy values and double the polarization radius. Neither of these changes negate any of the preceeding discussion.) Neither the experimental nor the theoretical considerations

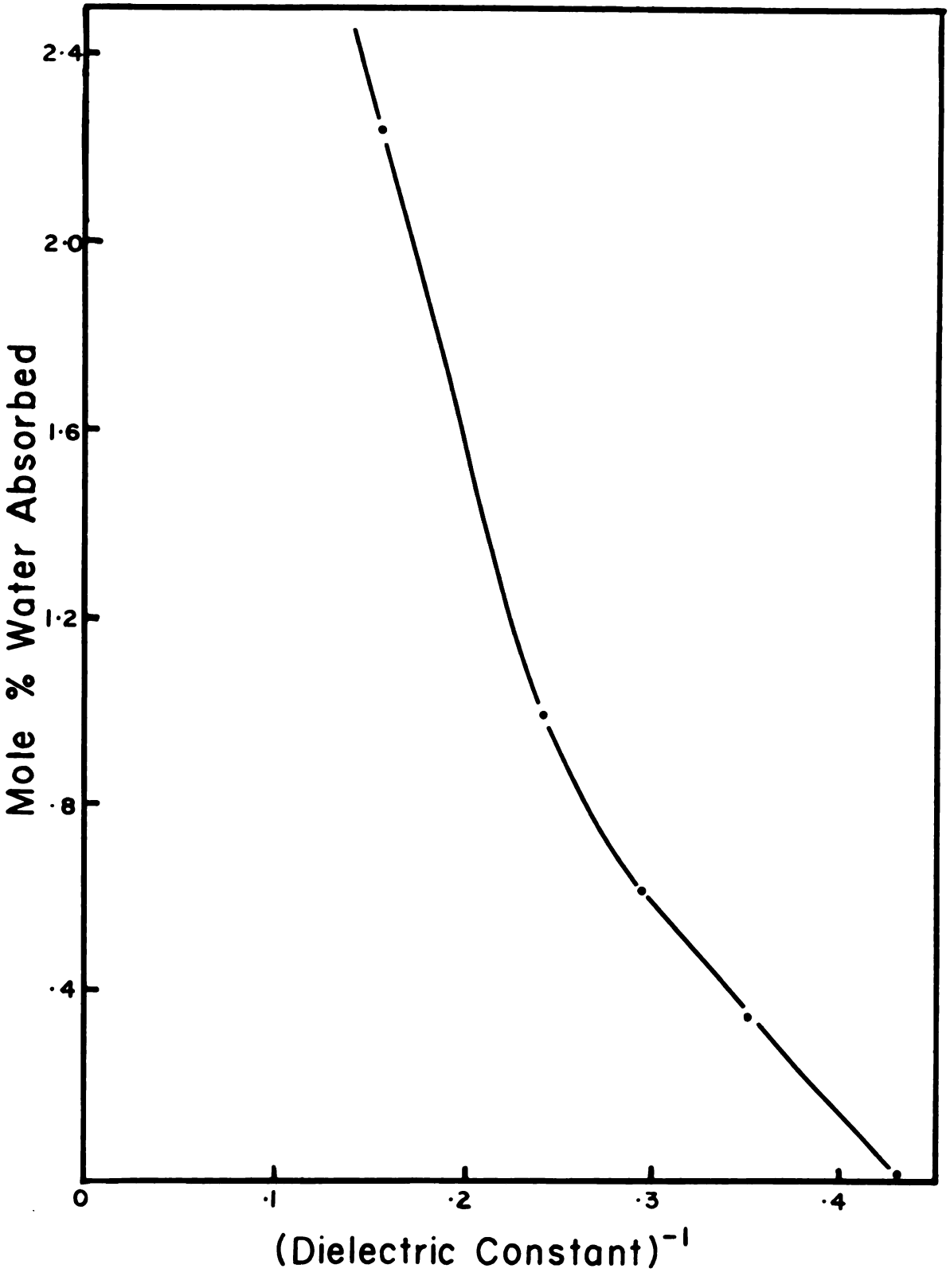


Figure 34. Variation of dielectric constant with the adsorption of water on hemoglobin.

presented herein can distinguish from amongst the several possibilities indicated in Section I. However, both the theoretical and experimental discussions (except for the anomalous results with ammonia adsorption) indicate a constancy of mechanism over the range of environments considered.

Comparison of Quantum Calculations with Experimental Results

A number of theorectical calculations have attempted to evaluate the possible band structures of energy levels in a protein system. In all such cases the protein has been treated as a repeating structure linked by hydrogen bonds across the peptide chains. The π electrons are considered to be delocalized across the hydrogen bonds. This is essentially the method used by Evans and Gergely (1949) who employed Hückel LCAO molecular orbital calculations. Yomosa (1964) extended the analysis to HMO-SCF and ASMO-SCF calculations. A further modification was made by Suard *et al.* (1961) to include the oxygen lone pair electrons as well. Suard-Sender (1965) has extended the calculation to include an infinite two dimensional network. Using the results of this latter calculation, a semiconductivity band gap of 5 eV was calculated (as compared with 3 eV calculated by Evans and Gergely). It was stated that this band gap is too large for intrinsic electronic conductivity to be significant and, therefore, measured band gaps of 2.4 eV must refer to some extrinsic processes.

A value of the ionization potential minus the electron affinity for a protein can be calculated. Rewriting Equation (14) we obtain:

$$I_g - A_g = E_D + \frac{e^2}{R}(1 - 1/\kappa) \qquad (47)$$

In Section III we have obtained values of the dry state activation energy (2.35 eV) and dry state dielectric constant (2.3) of hemoglobin. Furthermore, earlier in this section a value of R, the cavity radius, has been calculated as 4.3 Å. Substituting this value in Equation (47) we obtain $I_g - A_g = 4.3$ eV.

It is difficult to specify exactly what this means, but presumably it refers to vertical ionization and electron attachment processes in a small isolated polypeptide region of the protein molecule, i.e. a gas of peptide bonds. This would correspond to the general usage of I_g and A_g as gas state values. Simpson (1964) has estimated that the ionization potential (I_g) of an isolated amide group is 8.5 eV and that the electron affinities are usually 1 - 2 eV. This value of I_g is close to values of I_g listed by Gutmann and Lyons (1967) for N, N-diethylacetamide (8.60 eV) and N-methylacetamide (8.9 eV). It is believed that these compounds more closely resemble a peptide bond than does formamide (10.25 eV). Suard et al. suggest that in going from a monopeptide linkage to a polypeptide linkage the value of I_g would decrease by 1 eV and A_g would increase by 0.6 eV. From these rough estimates a value for $(I_g - A_g)_{protein}$ of 4.9 - 5.9 eV is calculated. This is in better agreement with the experimental value of 4.3 eV.

From band theory, if such a model is applicable, it is expected that the semiconduction band gap, E, is equal to $I_c - A_c$ where I_c and A_c are the solid state values of the ionization energies and electron affinities. The difference between the gas values and the solid state values are the result of polarization stabilization energy, P, arising from relaxation of the lattice around the new charge centers. Thus, $I_c = I_g - P$ and $A_c = A_g + P$ or:

$$E = I_c - A_c = I_g - A_g - 2P \tag{48}$$

as in Equation (12). In the case of protein this relaxation may take place in secondary, tertiary or quaternary structure.

Quantum calculations are inherently vapor state calculations (Kasha, 1962). Therefore, the calculations of Evans and Gergely, Yomosa, Suard, et al. and of Suard-Sender do not explicitly take into account polarization of the medium when charges are separated. Quantum calculations are for a medium with dielectric constant of 1. The experiments, however, are executed in a medium of dry state dielectric constant 2.3. It is, therefore, suggested that the value of 5 eV calculated by Suard-Sender should be compared with the experimental value of $I_g - A_g$ and that when a proper accounting is made of the dielectric constant of the medium the discrepancies between theory and experiment are diminished.

As far as optical transitions are concerned the quantum calculations are not questioned by the experimental results herein under discussion. That is, an optical transition is not expected at 2.35 eV (band to band transitions),

or at even lower energies in the case of hydrated proteins, which is the measured activation energy. It would then appear that the excitation states of a protein lie at higher energies than the conducting states. This is not an unusual condition for organic molecules.

Adsorption Studies

Determinations of V_m, the monolayer coverage, have been presented in Table 6. These values are somewhat larger than 5.76 gm water per 100 gm of ox hemoglobin reported by Cardew and Eley (1958). The differences between the values herein reported and those of Cardew and Eley cannot be attributed to protein denaturation. Cardew and Eley, as well as Eley and Leslie (1966) have shown that denaturation has a small effect on the value of V_m. Differences between the amino acid composition or tertiary structure of ox and bovine hemoglobins could not be great enough to account for these differences in V_m.

The values of V_m reported herein, 430 - 470 moles/10^5 gm protein are very close to the number of polar side chains, including proline, found for hemoglobin, 435 moles/10^5 gm protein (Tristam, 1949). It would appear that water, methanol and ammonia are adsorbed predominantly on the polar sites of the hemoglobin molecule. The majority of the polar side chains of amino acids are on exposed portions of the molecule, as discussed in Section I, and form the bulk of the adsorption sites. One BET layer is completed when a molecule of vapor is adsorbed on each of these polar sites.

An apparent anomaly exists in the case of ethanol adsorption. Because of the larger size of the ethanol molecule, as compared to water or ammonia, the diffusion of ethanol into the sample is at a much slower rate than the diffusion of the other vapors discussed. This decreases the accuracy with which the adsorption isotherm can be obtained but could hardly explain a 300% variation in the determination of a BET adsorption.

If the cross-sectional areas of adsorbed water and ammonia are taken as 10.8 $\mathring{A}^2$ and 14.6 $\mathring{A}^2$ respectively (Livingstone, 1949) and the surface area of a dry spheroid of hemoglobin is taken as 8,350 $\mathring{A}^2$ (Bragg, Howells and Perutz, 1954), then at a coverage of one BET monolayer of water 39% of the surface area of the protein molecule is covered. The coverage for one BET monolayer of ammonia is 53%. The cross-sectional areas of methanol and of ethanol may be calculated from the formula given by Emmett and Brunauer (1953):

$$\text{Area} = 1.09 \left[\frac{M}{\rho N}\right]^{2/3} \tag{49}$$

where M is the molecular weight of the vapor; ρ is the density of the vapor and N is Avogadro's number. This yields a cross-sectional area of 14 $\mathring{A}^2$ for methanol, and 18 $\mathring{A}^2$ for ethanol. One BET monolayer of methanol then occupies 46% of the surface area of the hemoglobin molecule. If it is assumed that one BET monolayer of ethanol is adsorbed at 0.16 moles/100 gms of hemoglobin, then 22% of the surface of the hemoglobin molecule is covered at the monolayer. However, if

one molecule of ethanol is adsorbed at each polar site the coverage is about 65%. It may be argued that all of the polar sites are not occupied by ethanol molecules at the apparent BET monolayer because the polar sites are on the average clumped and the adsorption of an ethanol molecule at each site is sterically impossible. The difference between the cross-sections of methanol and ethanol is not, however, large enough to explain the large difference in the BET calculations. A convincing explanation of the apparent anomaly escapes the present author.

If the polar sites are distributed uniformly on the surface of the hemoglobin, then each site will have associated with it 29 $\mathring{A}^2$. When one BET monolayer of water is adsorbed, the average center-to-center distance between nearest neighbor water molecules will be $\sim$ 6 $\mathring{A}$. The diameter of the water molecule is $\sim$ 3.8 $\mathring{A}$; therefore, a single molecule of water can, on the average, bridge the gap between nearest neighbor water molecules. When this gap is bridged, and a contiguous path of water molecules can be found, it is expected that protonic conductivity, through these water molecules, will dominate the conductive process. This may occur when less than two BET layers of water are adsorbed, which is consistent with the results of Maricic and Pifat (1966) who found 90% ionic conductivity in hemoglobin with 15% adsorbed water.

Values for the constant C calculated from the BET equation are given in Table 6. Eley and Leslie (1966)

Pg 72

report a value of 8 to 12 for water adsorbed on hemoglobin. The value of 12.3 reported herein is in close agreement with the published results. They, however, obtain a value of 22 for methanol adsorbed on bovine plasma albumen compared with 3.5 found for the adsorption of the same vapor on bovine hemoglobin. The value of C is very sensitive to small errors in the adsorption isotherm and should only be taken as an indication of the difference between the heat of liquefaction of the first BET monolayer and the heat of liquefaction of the bulk.

Equation (30) defines C which may be rewritten as:

$$RT \log C = E_1 - L \qquad (50)$$

Calculations of $(E_1 - L)$, along with accepted values of L, are given in Table 8. In all cases the heat of liquefaction in the first BET layer differs only slightly from the heat of liquefaction in the bulk.

Table 8. Heat of vaporization calculations of water, methanol, ethanol and ammonia.

Adsorbate	C	L(cal/mole)	E_1 - L(cal/mole)
Water	12.3	10,500	2,820
Methanol	3.5	8,400	735
Ethanol	1.7	9,400	287
Ammonia	2.0	5,100	374

The parameter, α, is defined by Equation (5) and computed in Table 7. This parameter is a measure of the effectiveness of the adsorbate in increasing the conductivity of the substrate, in this case hemoglobin. Although it has been shown, see Figure 32, that adsorption induced conductivity increments are related to the effective dielectric constant of the system, it is interesting to note that the efficiency with which an adsorbate increases the conductivity is not a simple function of its dielectric constant.

If the tablet is assumed to consist of spherical particles of hemoglobin and spherical adsorbate particles then a relation can be derived which relates the measured dielectric constant of the tablet, κ_P, the dielectric constant of hemoglobin, κ_H, the dielectric constant of the adsorbate, κ_A, the partial volume of hemoglobin, δ_H, and the partial volume of the adsorbate, δ_A. The measured dielectric constant of the tablet is considered to be a continuous property of the tablet, then the electric field inside the hemoglobin, E_H, and the electric field inside the adsorbate, E_A, are given by:

$$E_H = \frac{3\kappa_P}{2\kappa_P + \kappa_H} E \tag{51}$$

$$E_A = \frac{3\kappa_P}{2\kappa_P + \kappa_A} E \tag{52}$$

The polarization of the tablet is limited to the polarization of hemoglobin and the polarization of the adsorbate. The polarization of the tablet (per cm^3) is then:

$$P = \frac{\kappa_P - 1}{4\pi} E = \frac{\delta_H(\kappa_H - 1)}{4\pi} E_H + \frac{\delta_A(\kappa_A - 1)}{4\pi} E_A \qquad (53)$$

Eliminating E_H and E_A between Equations (51), (52) and (53) we obtain:

$$\frac{\kappa_P - 1}{3\kappa_P} = \frac{\kappa_H - 1}{\kappa_H + 2\kappa_P} \delta_H + \frac{\kappa_A - 1}{\kappa_A - 2\kappa_P} \delta_A \qquad (54)$$

If the bound nature of the adsorbate is considered, then the measured dielectric constant of the tablet, κ_P, may be consistant with Equation (54). In this case adsorbate-adsorbent interactions need not necessarily be postulated.

VI. SOME THOUGHTS ON THE BIOLOGICAL RELEVANCE OF SOLID STATE SEMICONDUCTION

The semiconductive properties of biologically important molecules may be related to their functions. The transport of charge over relatively long distances is an important process in biology. Several tentative models which utilize the semiconductive properties of biological molecules have been proposed. These models are most often hypothesized in organelles where electron microscopy or x-ray diffraction studies have indicated extensive order on the molecular level.

Some years ago Arnold and Sherwood (1957) proposed a solid state model for the action of chloroplast grana in the photosynthetic apparatus. It was proposed that free electrons and holes are generated by the adsorption of light in the chlorophyll. The electrons and holes then move independently to different trapping centers where the dark chemistry of photosynthesis proceeds.

The visual pigments in the highly organized lamellar structures of the outer segments of rods and cones is another system. Rosenberg (1962b) has proposed that photoexcitation leads to the creation a free charge carrier (probably electronic in nature) which is injected from the chromophore into the attached protein moiety. The drifting of free charges

in the protein constitutes an electric current which can change the potential across a neural membrane. This model establishes a causal chain from the adsorption of a light quanta to the initiation of the neural response.

The semiconductive nature of lipids has been demonstrated by Leslie et al. (1967) and by Rosenberg and Jendrasiak (1968). The temperature dependence of the conductivity of lipid bilayers, 50 - 100 Å thick, follows Equation (1) and hence they are semiconductors. It has been proposed (Rosenberg and Bhowmik, 1968) that lipid bilayers are electronic semiconductors. This opens up an entirely new area of semiconductive models. The membrane mediated oxidation-reduction scheme of Mitchell (1961) may then proceed via electronic carriers. Solid state models of neural transmission, e.g. the model of Wei (1967), must now be viewed in a new context.

All of the above systems are characterized by an intermolecular transport of charge carriers. Semiconductive models can also be employed in systems where the movement of charge is within a single macromolecule. In enzymes which involve the transfer of an electron the protein molecule may act as the path of the electronic charge carrier between the sites of oxidation and reduction.

Attempting to test the feasibility of this model Cardew and Eley (1959) calculated the conductivity expected on a molecular level. The conductivity parameters of dry hemoglobin were used. For a sea urchin egg the respiration rate would be equivalent to a current of 1.68×10^{-10} amp. If this current, as assumed by Cardew and Eley, flows through

a 1 micron fiber of 50 Å diameter under a tension of 1 V, then the conductivity of hemoglobin is 16 orders of magnitude too small to explain the oxidation mechanism. The activation energy of dry hemoglobin (data from Cardew and Eley on a 1/kT basis) is 1.4 eV while the activation energy for respiration is about 0.5 eV. Therefore, they conclude that a semiconductive model can not explain biological respiratory processes.

Rosenberg and Postow (1968) have amended this calculation by employing the conductivity parameters of hydrated hemoglobin. They assumed intra-molecular electron transport and therefore considered a conductor of 25 Å diameter and 36 Å length, the approximate dimensions of a cytochrome c molecule. The resistivity was taken as that of hydrated hemoglobin, i.e. 10^8 ohm-cm. For a 1 V redox potential a current of 10^{-15} amp/molecule is expected. This would require a concentration of 10^5 molecules of cytochrome per sea urchin egg which is a reasonable figure. The conductivity corresponds to a maximum turnover rate of 10^4 electrons/sec/molecule which is above that typical of most enzymes. The activation energy of hydrated protein, 0.6 eV (on a 1/kT basis) is close to the activation energy of respiration. It must therefore be concluded that the assumption of electronic conductivity in protein molecules as the rate limiting step in electron transfer processes is consistant with biological data.

The kinetics of electronic conduction through a particle has been examined by Cope (1965). The kinetics

predicted under the assumption of electronic conductivity are consistent with those observed in the cytochrome oxidase system.

Anomalous Arrhenius plots of enzymatic activity have recently been reported for several different enzymes shown in Table 9. The transition temperatures range from 13° C to 24° C for the different enzymes. Activation energies for enzymatic activity calculated at temperatures above the transition temperature are in all cases smaller than those calculated below the transition point. The activation energies calculated from Arrhenius plots (on a 1/2kT basis), shown in Table 9, are very similar to those which would be expected for dry (or partially hydrated) and hydrated proteins. Optical rotatory dispersion, ultra violet difference spectra, flourescence studies and visible spectra all indicate a conformational change at the transition temperature.

If it is hypothesized that, for these several enzymes, the rate limiting step for enzymatic activity is intramolecular electronic transport, then the extant data can be explained with the aid of Figure 35. The conformational change may so alter one portion of the protein (site A in Figure 35) as to permit its extensive hydration in one configuration while demanding a hydrophobic environment in the other configuration. When site A is in a hydrophobic environment, low dielectric region, the activation energy for semiconduction is high. In a hydrophilic environment, high dielectric medium, after the conformational change, the activation energy is decreased.

Table 9. Activation energies (1/2kT basis) calculated from Arrhenius plots of maximum turnover rates of enzymes showing configurational transitions.

Enzyme	Transition Temperature (°C)	E(eV)		Reference
		low temperature	high temperature	
Myosin	16	2.1	1.1	Levy et al.(1959)
d-amino acid oxidase (d-alanine substrate)	14	1.5	0.9	Massey et al.(1966)
d-amino acid oxidase (d methione substrate)	24	2.1	1.1	Massey et al.(1966)
glycogen phosphorylase b	13	4.0(?)	1.6	Graves et al.(1965)
rabbit muscle pyrurate kinase	14	1.7	1.0	Kayne (1966)

Figure 35. Illustration of the semiconduction model of enzyme activity. When site A is in a hydrophobic environment the activation energy for semiconduction will be high. If the protein conformation changes so as to place site A in a hydrophilic environment the activation energy for semiconduction will be lowered.

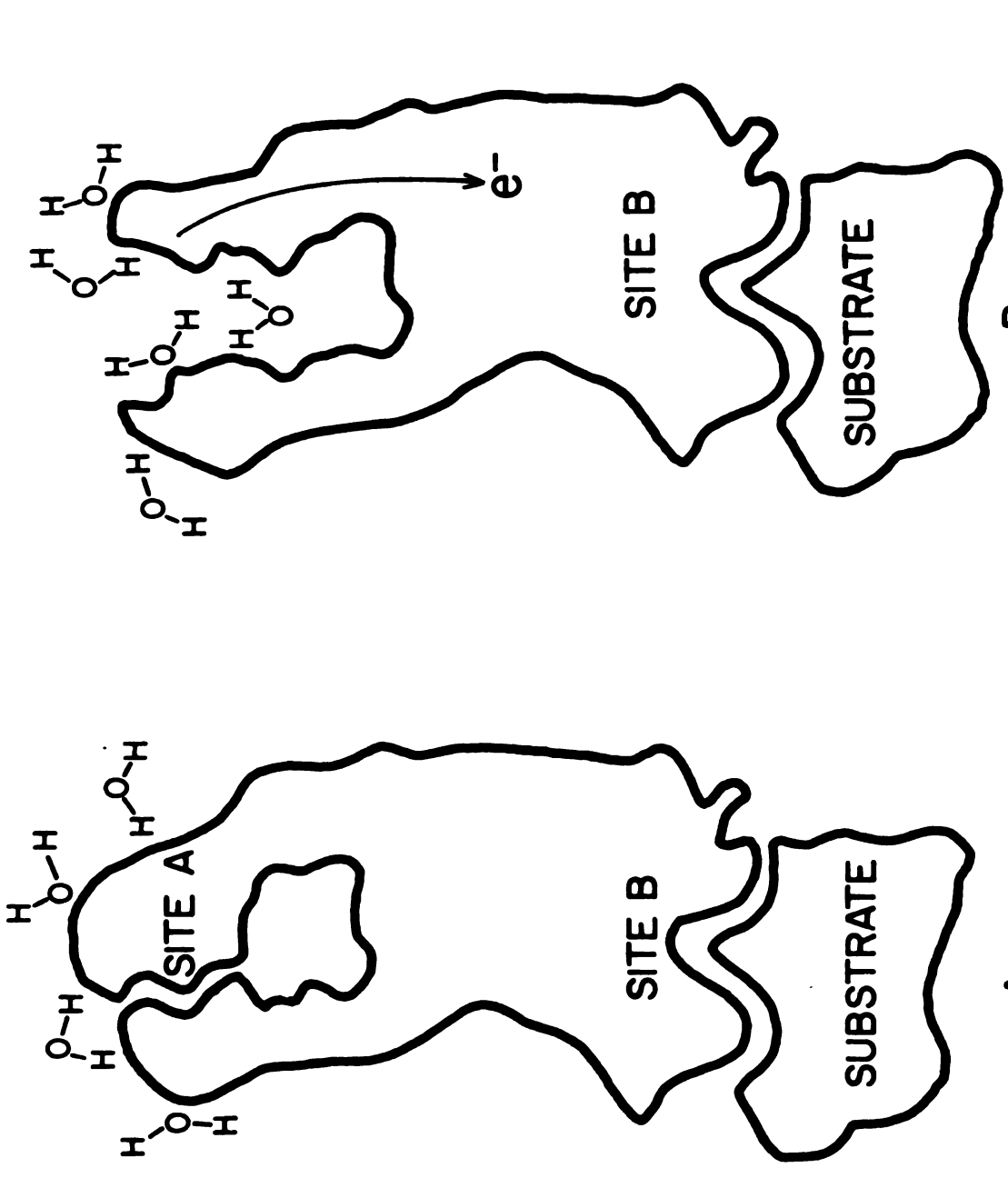

SITE A
SITE B
SUBSTRATE
A
HIGH ACTIVATION ENERGY
e⁻
SITE B
SUBSTRATE
B
LOW ACTIVATION ENERGY

Figure 35.

Conductivity measurements are by nature bulk determinations. These measurements alone cannot, therefore, provide information about specific sites on the enzyme molecule. However, support for the semiconductive model of enzymatic function will accrue if alteration of enzymatic function changes the solid state conductive properties of the protein. A suitable system for experimentation would be an enzyme which could be crystallized with an inhibitor. The inhibitor should be located at the site of charge generation, but as the sign of the dominant charge carriers in protein is not known this site can not be specified. If the attachment of an inhibitor to an enzyme molecule decreases its conductivity and increases its activation energy for semiconduction, then the semiconductive model would be supported. If this is not the case the model cannot be eliminated entirely because several other factors (e.g. the site of charge carrier generation may not be the one inhibited, the inhibition may permit charge carrier generation but alter activity in some other way, or the system chosen for experimentation may be one in which the model is not applicable) could cause negative results.

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