

STUDIES ON DISSOLVED MOLECULAR OXYGEN

IN PURE AND SEA WATER

by

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B.Sc., McGill University, 1958

M.Sc., University of Western Ontario, 1959

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We accept this thesis as conforming to the

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IN PURE AND SEA WATER

ABSTRACT

Dissolved oxygen was studied in distilled water and salt solutions by means of nuclear magnetic resonance. The free induction technique was employed to measure the spin lattice relaxation time, T_1 , for oxygen-free distilled water and for water containing oxygen under one atmosphere of air and one atmosphere of oxygen at temperatures of 1° to 75° C. The same measurements were made for solutions of 0.5 M sodium chloride at temperatures of 1° to 40° C.

The spin lattice relaxation probability, $1/T_{1(c)}$, in the presence of paramagnetic oxygen, was attributed to two relaxation mechanisms: the first a dipole-dipole interaction and the second a hyperfine interaction. The two terms were evaluated from measurements of $T_{1(c)}$ at two different magnetic field strengths at 20° C. Using the theoretical relationship between the dipole-dipole term and η/T , the results were used to evaluate the hyperfine term at different temperatures.

The activation energy obtained from the variation of the dipole-dipole term with temperature was assumed to represent the energy required to break one hydrogen bond between two water molecules. The activation energy found for the hyperfine term was taken as a measure of the breaking of a hydrogen bond between one oxygen molecule and an aggregate of water molecules. The activation energy of the hyperfine term was found to be a function of temperature. Comparison of this quantity with heats of solution of dissolved oxygen in distilled water and salt solution showed a similar pattern of change in both.

Oxygen supersaturation was studied in sea water cultures of Nitzschia closterium and Chlorella strain "A" at a temperature of 12° C. Saturation values up to 200% were reached under illumination with light energy of 9.2×10^{-3} langlies/min.

Nitzschia was found to be more photosynthetically active, under the same culture conditions, than Chlorella.

Oxygen production by Nitzschia was shown to be a function of the difference in photosynthetic pigment concentrations (chlorophyll-a — non-astacin carotenoid), total alkalinity, and the change in catalytic activity of the medium.

Variation of oxygen concentration in Nitzschia cultures under light and dark periods indicated a mechanism by which oxygen may escape as microbubbles to the atmosphere.

The rate of oxygen desupersaturation was measured in water free of organisms. The rate increased with increase of ion content and with the surface to volume ratio of the water column, but was not influenced by addition of siliceous particulate matter to the supersaturated water. Small addition of a surface active agent (heptanoic acid) increased the oxygen desupersaturation rate but further addition decreased the rate.

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PART I
PROTON SPIN RELAXATION BY
PARAMAGNETIC MOLECULAR OXYGEN

INTRODUCTION

The aim in this part is to study the effects of dissolved oxygen on the spin relaxation of protons in distilled water and in salt solutions, at different concentrations of oxygen and different temperatures, by means of Nuclear Magnetic Resonance. It is also the aim to infer from, and correlate with the results any factors that bear on the solubility of oxygen in both distilled and ion-containing water, and to show in particular the close association between the degree of solubility and the tendency of the water solvent to form aggregates and clusters.

The solubility of a gas in a liquid such as oxygen in water, where Henry's law applies (Henry, 1803), is governed by the relation that the partial pressure of the gas in equilibrium with its solution in a liquid is found to be proportional to its concentration in solution:

$$P_2 = KC_2 \quad (1)$$

The proportionality constant, K, depends on the temperature, and on the nature of the solute and solvent, and is independent of pressure.

Notable treatments for this kind of behaviour have been

carried out thermodynamically and kinetically (Moelwyn-Hughes, 1957) on the basis of experimental results obtained from solubilities and their variation with temperature. The thermodynamic approach was used to determine the chemical potential of the solute in a solution of unit concentration, and then further employed to explore the free energies of solutions from which many of their properties are derived. The kinetic treatment, on the other hand, was based on a certain rate of condensation of solute from the gas phase, and on its evaporation from the liquid, all taking place at an uppermost, thin layer in the liquid, and on the probability that the solute and solvent molecules possess a certain energy between them, which if exceeded could eject the solute molecule from that particular surrounding.

The nature of bonding, and the physical picture of the micro-structure of solvent and solute was not dealt with until recently. Heidt and Johnson (1957) advanced a theory of molecular oxygen hydrates in water based on studies of ultraviolet absorption at different temperatures. They postulated the existence of at least two hydrate species existing at equilibrium with each other, and attributed such occurrence to hydrogen bonding. The model suggested was a new kind of a hydrogen bridge which takes place between the diradical form of molecular oxygen and water or paraffin hydrocarbons. In this

bridge the proton would be shared by three electrons, one of which comes from the diradical form of the oxygen molecule and the other two from the original electron pair bond O-H in the case of water and C-H in the case of paraffin hydrocarbons. In this kind of bridge the proton would be nearer to the O or C atom, respectively, of its original electron pair bond than to the oxygen atom of molecular oxygen. The resulting structure would be a ring in the case of 1:1 complex $O_2:H_2O$ and a chain in the 1:2 complex $O_2:2H_2O$. Although this appears to be a feasible approach, nonetheless, the treatment gives an oversimplified picture which excludes effects of other processes taking place in liquid water. These processes include complex formation and the tendency of water to form aggregates, which may have a great influence in determining the extent of oxygen solubility in water.

The present concepts of water aggregates are based mainly on information obtained from x-ray, neutron diffraction, Raman spectra and infra-red studies of ice (Lonsdale, 1958; Ockman and Sutherland, 1958; Frohnsdorff and Kingston, 1958) and water (vanEck et al., 1958; Frank, 1958; Brockhouse, 1958). Hydrogen bonding between water molecules is found to play a leading role in all the interpretations advanced for the structure of liquid water and aqueous solutions. This stems in effect from the structure of a free water molecule which has

its two hydrogens at 0.96\AA from the oxygen atom, with the angle between the O-H bonds $\sim 105^\circ$. In ice, O-H bonds on one molecule point towards the oxygen of adjacent molecules, forming a structure in which four hydrogens are arranged nearly tetrahedrally around each oxygen, two being at 1.01\AA and two at 1.75\AA (Peterson and Levy, 1957).

Thus, water is pictured as a broken-down, ice-like structure which has the capacity to form clusters that are hydrogen bonded to each other. The bonds break and form continuously with the motion of the molecules in the liquid. The continuity of hydrogen bonding, however, is in question. Lennard-Jones and Pople (LJP) (1951), use the term hydrogen bond to refer to any of a wide range of O-H---O configurations, calling the bond "bent" when either the lone pair or the H atom has moved out of the O-O line as a result of rotation of its water molecule. They picture rather extreme bending as being possible without the need to give up the term hydrogen bond. In contrast to this, Frank and Wen (1957) distinguish qualitatively between large and small angles of bend, assigning the former to non-bonded and the latter to bonded categories. A "small" angle of bend means an angle small enough so that the angular requirements for tetrahedral covalent bonding are not transgressed.

From this latter hypothesis liquid water emerges as consisting of flickering clusters of bonded molecules mixed with,

and alternating roles with, non-bonded fluid which encloses them and constitutes the rest of the sample. A (larger or smaller) cluster is pictured as forming when the stage is set by an energy fluctuation which creates a suitably "cold" region, and relaxing when the necessary energy of "melting" becomes available.

This flickering cluster representation receives the support of Wang et al. (1953) since it offers an explanation of the energy of activation in liquid water being 4.6 kcal/mole whether calculated for dielectric relaxation, self-diffusion or viscous flow. This is just what would be expected if the principal requirement for any of these processes in water were the breaking down of a rather rigid structure.

The work of Eigen and DeMaeyer (1958) on the protonic charge transport in water presents a similar picture to that of cluster formation. In this case three water molecules surround a H_3O^+ ion and form a H_9O_4^+ complex. The charge transfer inside the complex is very fast and of the order of 10^{-13} to 10^{-14} sec., while the charge transfer to a molecule outside the complex is governed by the formation of a new hydrogen bond. The average time of liberation of water molecules from the complex and H-bond formation at the complex can be related to the dielectric relaxation times which also involve a mechanism of liberation from the structure and orientation of

the dipole-dipole. The slowest dielectric relaxation process in water is characterized by a time constant of about 10^{-11} sec. and an activation energy of 4.5 kcal/mole (Lane and Saxton, 1952). This process corresponds to the liberation of double H-bonded water molecules. Hindman (1961) in his investigation of NMR effects in aqueous solutions assigns a value for the number of hydrogen bonds being ruptured in liquid water at some temperature from data based on those of Haggis et al. (1952). He estimates 1.82 hydrogen bonds per water molecule at 0°C., corresponding to 9% of the bonds being broken in going from ice to water. This value is between Pauling's (1960) estimate of 15% of the bonds being broken at 0°C. and Pople's (1951) estimate that very few bonds are broken.

In the light of such interpretations, the addition of ionic and non-polar species to water should be examined, and their effects on the clusters or aggregates of water be taken into consideration if any explanation for the difference in oxygen solubility between pure and salt water solutions at different temperatures is to be attempted.

The addition of ionic salts, such as NaCl, has been known (Frank and Wen, 1957) to result in a structure-breaking effect of the clusters on one hand, and on the other hand to form water of hydration which is irrotationally bonded with a specific hydration number for each certain ion. Frank and

Evans (1945) suggest that essentially three regions exist around a dissolved ion: (1) a "frozen" layer of water oriented by the ion, i.e., the hydration shell; (2) an intermediate disorganized region caused by the inability of the hydrated ion to fit into normal water structure; and (3) the region of normal water structure.

For the ion-water complex, or the hydrated shell, two principal models exist. One is based on detailed energy considerations and was advanced by Bernal and Fowler (1933) and the other by Verwey (1942). The Bernal-Fowler model assumes a cation would bind a water molecule in coplanar fashion with the negative end of the water dipole directed toward the ion as shown in Figure I (a), whereas an anion would be preferentially oriented with one hydrogen pointing toward the ions as in Figure I (c) rather than the dipolar position in (d). A water bonded as in Figure I (a) could bond to only two additional water molecules and hence one hydrogen bond must be broken per coordinated molecule, whereas a water bonded as in Figure I (c) could bond to three additional water molecules and hence one bond must be broken for each two coordinated water molecules. Verwey, on the other hand, suggests that univalent cations are attached to the region of negative charge due to the lone-pair electrons of the oxygen in an angular tetrahedral fashion Figure I (b), such that

the coordinated water molecule is able to bond to three additional water molecules. The dielectric constant data, however, gives evidence for difference in the modes of bonding of water around anions and cations (Hindman, 1961). The lowering of the dielectric constant has been interpreted in terms of a model in which water oriented as in Figure I (a) is not free to rotate, while the negligible effect of anion on the dielectric constant has been interpreted as due to free rotation of water molecules bonded as in Figure I (c). Thus the Bernal-Fowler model is considered to be more reasonable than the Verwey one.

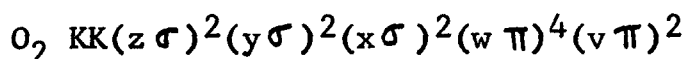
In resume then, addition of ions to water

1. disrupts the water structure and breaks hydrogen bonds;
2. the ionic electric field distorts the electronic structure of water molecules;
3. the water of hydration is dependent on the size of the cation; and
4. three distinct sites for water molecules are provided: near cation, near anion, near other water molecules (Fabricand and Goldberg, 1961).

The addition of non-polar solutes tends to replace the disordered neighbours around the edges of the cluster. The motion of the disordered neighbours provides the mechanism by which the cluster receives the "heat of melting" which

ultimately terminates its existence. If some of these neighbours are replaced by non-polar solute molecules or non-polar groups attached to solute molecules, the cluster boundary will be stabilized, for its new neighbours are only weakly able to transmit displacements and torques (Frank, 1958). Also, it is expected for a solute such as an oxygen molecule to be always found at the extremities of the cluster if bonded to one or more water molecules. The properties of an oxygen molecule hinder its fitting in the middle of the cluster because of its inability to arrange itself in a tetrahedral form in the same manner as water molecules. This also presents the notion that any complex formation, or breaking, taking place between water and oxygen must occur in a jump process where the oxygen molecule will break from the edge of one cluster and hop to the edge of the other when they are approximately at a lattice distance away, or on the "melting" of one cluster the oxygen molecule finds itself sticking to another being formed.

Any postulate of complex formation between oxygen and water molecules should take into account the structure of the oxygen molecule and its capability to participate in hydrogen bond formation. The molecular orbital expression for two oxygen atoms forming an oxygen molecule gives the following electronic arrangement (Cartmell and Fowles, 1961):



Here KK designate the inner (1s) electrons, $(x\sigma)^2$ and $(w\pi)^4$ the sigma and pi bonds formed between the two atoms, $(z\sigma)^2$ and $(y\sigma)^2$ the two unshared pairs of electrons at both ends of the molecule along the bond axis, and $(v\pi)^2$ the two anti-bonding electrons which give the molecule its paramagnetic properties. Since any postulate of hydrogen bonding with any specific orbital will have to take into consideration the hybridization, and delocalization of the orbital, such treatment will not be attempted and will be considered beyond the scope of this work. However, the oxygen molecule as a whole will be considered in terms of polarizability from an electrostatic point of view.

This qualitative approach would account for heats of solution in terms of the electrostatic interaction between the oxygen molecule and the dipole moment of water, taking into account the numbers of bonds broken or formed at different temperatures, and the effect of ions on both solvent and solute. Further electrostatic approach will be examined as a function of the energy of point charge dipoles, and of the distance between the points as well as the dependence on the angle of approach (Pimentel and McClellan, 1960).

The paramagnetic property of the oxygen molecule, and its capability to dissolve in pure and ion-containing water, makes the solution an interesting subject for study by means

of nuclear magnetic resonance. Previous studies of proton relaxation by paramagnetic ions in aqueous solutions (Solomon, 1955; Bloembergen, and Morgan, 1961) provide a lead and a theory for such investigations. Although the solubility of molecular oxygen at ordinary temperature and atmospheric pressure is of the order of 2×10^{17} molecules cm^{-3} , the effect on T_1 , the proton spin-lattice relaxation time, is appreciable and sufficient to produce observable changes from those of oxygen-free water. Guilloto's (1956) work clearly showed changes of T_1 , the spin-lattice relaxation time, at $20^\circ\text{C}.$, with different concentrations of oxygen in solution, and the linear dependence of $1/T_1$ on concentration. This was similar in manner to the linear relaxation effect of paramagnetic concentrations of Ni^{++} and Mn^{++} ions in a range from 2×10^{16} to 2×10^{18} ions cm^{-3} (Hennel et al., 1961).

Earlier studies of paramagnetic impurities in water were interpreted assuming that the relaxation was due only to dipole-dipole interaction between electron and nuclear spins, the fluctuations of these interactions being governed by viscosity and diffusion. Bloembergen et al. (1948) found the addition of paramagnetic ions to water decreased both the spin-lattice and the spin-spin relaxation times, T_1 and T_2 . The additional contribution to the relaxation rate was found to be proportional to the concentration.

Later, Conger (1953) and Morgan et al. (1956) concluded from measurements of relaxation times in water-glycerol solutions of several aquated ions that the observed changes were not entirely attributable to the change in viscosity according to the early theory. It was suggested that the difference observed might well provide an insight into the nature of molecular interactions in those and similar solutions. Morgan suggested that at least part of the difference lay in the presence of the non-exchangeable protons of the glycerol molecules.

Bloembergen (1957) followed with comparison of the ratio of T_1/T_2 in paramagnetic ion solutions finding a deviation from unity for most ions to as high as 7.1 for Mn^{++} . The interpretation advanced emphasized that a spin exchange interaction was taking part in the relaxation mechanism, and this was due to the formation of a hydrated complex between the paramagnetic ions and water molecules. This was supported and extended by Bloembergen and Morgan (1961) to interpret the different effect on relaxation between various paramagnetic ions and the role played by the spins of the ions, the geometry and number of their water of hydrations as well as their relative motions in solution.

A similar approach to the above was employed here for investigating the nature of the solubility of molecular oxygen to provide information on complex formation and hydrogen bonding.

This should be especially informative if the complex included an oxygen molecule and a water aggregate in cluster, since under this condition a spin exchange interaction would take place if the amplitude of the wave function of the unpaired O_2 electrons at the protons was non-zero. The effect on the dipole-dipole interaction on the other hand is related to the tumbling of the water cluster and this in turn is governed by temperature and viscosity. The addition of one oxygen molecule to the cluster will be assumed not to alter the dynamical motions of the complex since the size of the cluster itself is considered to be much larger than the size of an oxygen molecule.

Changes in viscosity may play some role in nuclear spin relaxation and at the same time may be correlated with formation or breaking of water clusters. The addition of non-paramagnetic ions such as Na^+ and Cl^- to water could provide significant relaxation time effects, via a mechanism in which the ions would obtain their hydration spheres and break the water structure without contributing any paramagnetic influence. Since the solubility of oxygen is also decreased by the addition of such ions, the contrasts and similarities between influences of solubilities and influences on relaxation times due to changes in complex formation should become apparent.

THEORY

PROTON SPIN RELAXATION BY PARAMAGNETIC O_2

The proton spin relaxation in presence of molecular oxygen is assumed to be due to two kinds of interaction. The first is a dipole-dipole interaction between the proton and electron spins, and the second a proton-electron spin exchange or hyperfine interaction due to the formation of a complex between water and oxygen molecules. The Hamiltonian for the interaction between the proton on H_2O and the paramagnetic electrons of O_2 could then be described as

$$H = H \text{ dip-dip} + A \vec{I} \cdot \vec{S} \quad (2)$$

where A is a scalar constant; \vec{I} the nuclear spin system; and \vec{S} the electron spin system.

The interaction (2) produces transitions between the nuclear Zeeman levels and the electronic spin Zeeman level and exchanges the Zeeman energy with the energy of the lattice or both. The $(2I+1)$ neighbouring Zeeman levels for a nuclear spin I are separated by $\hbar \omega_I = \gamma_I \hbar H_0$ in an external field H_0 , where γ_I is the nuclear gyromagnetic ratio, while for an electron spin S , the electron spin Zeeman levels are split by $\hbar \omega_S = \gamma_S \hbar H_0$, γ_S being the electron gyromagnetic ratio. For protons $\gamma_I = 2.67 \times 10^4$ while for electron spins on the O_2 molecules $\gamma_S = 1.75 \times 10^7$, so that $\omega_S \gg \omega_I$.

When the nuclear spin system is in equilibrium with the lattice, the distribution of spins in the Zeeman levels is described by a Boltzman distribution for temperature T , the temperature of the lattice. The interest here is with the approach of the nuclear spin temperature towards equilibrium after it has been disturbed by a radio frequency pulse. This approach is governed by a time constant T_1 , the spin-lattice relaxation time.

The contribution of the interaction with the \vec{S} system given by equation (2) to the proton spin relaxation probability $1/T_{1(c)}$ is given by Abragam (1961; equations 88 and 120, Chap. VIII). By evaluating the correlation functions of both terms (Bloom, 1962), it may be noted that the dipole-dipole term and hyperfine term have different correlation times, τ_c and τ_e respectively, associated with them. This is so because the dipole-dipole interaction depends on the vector \vec{r} joining the proton- O_2 pair, while the scalar $\vec{I} \cdot \vec{S}$ interaction is independent of the orientation of this pair. If each O_2 molecule is assumed to be interacting with a complex containing n_h protons which behaves for some time as a rigid unit and if the correlation functions are assumed to have an exponential time dependence, the general expression for $T_{1(c)}$ is as follows:

$$\frac{1}{T_{1(c)}} = \frac{8}{5} \pi \frac{n}{\omega_S} \langle E^2_{dd} \rangle \left\{ \omega_S \tau_c + \frac{7}{3} \frac{\omega_S \tau_c}{1 + \omega_S^2 \tau_c^2} \right\} n_h + \frac{2}{3} S(S+1) \frac{n}{\omega_S} \langle A^2 \rangle \left\{ \frac{\omega_S \tau_e}{1 + \omega_S^2 \tau_e^2} \right\} n_h \quad (3)$$

Where $\langle E^2_{dd} \rangle = \gamma_I^2 \gamma_S^2 \hbar^2 S(S+1) \langle \frac{1}{r^6} \rangle$ (4) is the average value of the dipole-dipole interaction with the protons of the complex; A^2 is the average value of the hyperfine interaction in the complex; n the number of paramagnetic molecules per cm^3 of water; and $\frac{1}{T_{1(c)}} = \frac{1}{T_{1(O_2)}} - \frac{1}{T_{1(\text{pure})}}$

where $T_{1(\text{pure})}$ is the spin-lattice relaxation time in oxygen free water and $T_{1(O_2)}$ is the spin relaxation time in presence of oxygen in water.

Three additional approximations have been made in writing equation (3). (a) $\omega_S \gg \omega_I$, as stated earlier so that $\omega_S \pm \omega_I \simeq \omega_S$ (b) $\omega_I^2 \tau_c^2 \ll 1$. No restriction is made on $\omega_S \tau_c$ or $\omega_S \tau_e$, and, (c) The spin temperature of the \vec{S} system of the electron is taken to be the same as the lattice temperature. That is in the absence of any perturbing r-f at the Larmor frequency ω_S of the \vec{S} system, this condition is fulfilled if $T_{1(S)} \ll T_{1(I)}$; such condition is normally satisfied since $\gamma_S \gg \gamma_I$, so that the electronic spins are very strongly coupled to the lattice by comparison with the nuclear spins.

Equation (3) is identical with the expressions developed for relaxation of protons in aqueous solution of paramagnetic ions (Solomon, 1955; Bloembergen and Morgan, 1961), and which proved to be the most successful form for correlation of experimental results.

The model envisaged in the present work is of an oxygen molecule coming in touch with a water cluster for a time τ_h . The complex formed then rotates and tumbles as a whole in the body of the liquid with a characteristic time τ_r . It should also be noted that if the O_2 spins are relaxed in a time τ_s , the electron spin relaxation time, then the values of τ_c and τ_e are as follows:

$$\frac{1}{\tau_e} = \frac{1}{\tau_h} + \frac{1}{\tau_s} \quad (5)$$

and similarly,
$$\frac{1}{\tau_c} = \frac{1}{\tau_r} + \frac{1}{\tau_s} \quad (6).$$

In general τ_s will be a function of temperature and magnetic field, while τ_r and τ_h will be functions of temperature only.

The relative magnitudes of the three correlation times τ_h , τ_r and τ_s have been investigated for paramagnetic ions in aqueous solutions where it was found that τ_h and τ_s are much larger than τ_r . Bloembergen and Morgan (1961) have suggested that for such a case

$$\tau_c = \tau_c^0 \exp (E_c/RT) \quad (7).$$

In this work it is also assumed that τ_c is mainly dependent on τ_r , and that $\omega_S^2 \tau_c^2 \ll 1$, and $\omega_S^2 \tau_e^2 \gg 1$. That is τ_c is much less than 10^{-11} sec. and τ_e is much larger than 10^{-11} sec. at the value of ω_S at 7000 gauss, the magnetic field used in this experiment where $\omega_S = 1.24 \times 10^{11}$ sec.⁻¹

Equation (3) could then be resolved into the following form:

$$\frac{1}{T_{1(c)}} = C \tau_c + \frac{B}{\omega_S^2 \tau_e} \quad (8)$$

where $C = \frac{8}{5} \pi n \langle E^2_{dd} \rangle \frac{10}{3} n_h$; and $B = \frac{2}{3} S(S+1) n n_h \langle A^2 \rangle$.

EXPERIMENTAL

I. Apparatus

(i) Nuclear Magnetic Resonance by Free Induction Technique:

The apparatus used was a conventional pulsed nuclear magnetic resonance apparatus (Hahn, 1950) and consisted of a magnet with a power source connected to a current stabilizer and operated at 7000 gauss field strength; a pulsed oscillator adjusted to the Larmor precession frequency of 29.80 Mc for the protons; a pulse timer; a trigger; 30 Mc amplifier and detector arranged as in Figure II (a). The signal height was read on an oscilloscope at an output of 2 volts/cm.

(ii) Thermostated Induction Coil Chamber:

A copper coil $\frac{1}{4}$ " diameter was wound in a cylindrical form to a height of 6 cm. and a diameter of 3 cm. This was insulated with aluminum foil on both sides and covered with Scotch electrical tape on the outside, leaving a small opening at the top for the sample entrance. The coil was placed vertically on a wooden board around the induction coil as in Figure II (b). The ends of the copper coil were connected to a circulating water bath with a temperature range from 1°C. to 90°C. and temperature control of $\pm 0.1^\circ\text{C}$. A thermocouple was placed inside the chamber to determine temperature fluctuations at a set temperature. Fluctuations were of the order of

$\pm 0.5^{\circ}\text{C.}$ at low temperatures and $\pm 1.0^{\circ}\text{C.}$ at high temperatures.

(iii) Degassing Instrument:

A one liter bulb with openings at both ends was sealed to a vacuum system on one side, and to the sample tube on the other. The bulb contained a teflon-covered bar magnet 2 cms. in length, and was placed over a magnetic stirrer as in Figure II (c).

(iv) Sample Tubes:

These were of pyrex glass 0.6 cm. I.D. and about 8 cm. in length. Two designs of sample tubes were used, one for the sample of oxygen-free water which was sealed and used permanently, and the other for oxygenated water under either one atmosphere of air or one atmosphere of oxygen (Figure II (d)).

II. Reagents

(i) Double distilled water was used in all samples: oxygen-free, oxygen-concentrated, and salt solutions.

(ii) Compressed Air: "Liquid Air" cylinder with 21% O_2 concentration was used; in addition to O_2 , the gases in mixture were the same as atmospheric gas content.

(iii) Compressed Oxygen: "Liquid Air" cylinder containing 99.5% oxygen was used; the rest of the gas was nitrogen (0.5%).

(iv) Sodium Chloride Crystals: "B & A" reagent grade, which contained metal impurities of 0.0005% as lead, and 0.0002% iron.

III. Procedure

Spin lattice relaxation times were measured for oxygen-free double distilled water, and for water containing oxygen under one atmosphere pressure of air, and one atmosphere pressure of oxygen, at temperatures of 1° to 75°C. The same measurements were carried for solutions of 0.5M sodium chloride at temperatures from 1° to 40°C.

(i) Degassing Method: (a) Double distilled water: 150 ml. of water was placed in the degassing bulb, Figure II (c). Both stopcocks A and B were opened and the mercury diffusion pump, oil pump and magnetic stirrer were switched on. Degassing was carried out for one-half hour, after which stopcock A was closed, then B. The water in the bulb was tilted until three or four ml. gathered in section C; the bulb was then warmed by hand to increase the vapor pressure. Stopcock A was opened and the water was displaced under pressure to the sample tube. The sample was sealed at the constriction and used thereafter in relaxation time measurements.

(b) 0.5M Salt Solution: 28.0 g. of sodium chloride was dissolved in 1000 ml. of water to make approximately 0.48M

solution. The degassing bulb was charged with 250 ml. of the solution and the procedure repeated as before. Estimation of water collected in the trap from evaporation, gave the solution strength as $0.5M^{\pm} 2\%$.

(ii) Oxygen Concentration Method: Two oxygen concentrations were employed throughout the experiment, one was under one atmosphere of air and the other under one atmosphere of oxygen. For any particular temperature, the degassed sample tube plus two others of the type shown in Figure II (d) were placed in the circulating water bath, and the thermoregulator adjusted to the desired temperature. Four mls. of either distilled water or salt solution were placed in the sample tube. A polyethylene tube of 0.2 cm. O.D. connected to the oxygen cylinder was inserted through section A to the bottom of the sample tube. In the same manner, another polyethylene tube was connected to the air cylinder and inserted in another sample tube. Oxygen, or air, was bubbled slowly with stopcock A opened for about 15 minutes. At the end, each stopcock in turn was closed, the polyethylene tube withdrawn carefully, and the opening at A corked. The tube was dried on the outside and placed quickly in the induction coil for relaxation time measurements.

Oxygen concentrations were checked occasionally by the micro-gasometric technique of Scholander et al. (1955) and the

results were $\pm 2.5\%$ of the theoretical concentration at that particular temperature. This is of the same order of magnitude as the usual error in the estimation of oxygen concentration by this method.

(iii) Measurement of Spin-Lattice Relaxation Time T_1 :

In the process of having the three sample tubes equilibrating at the desired temperature and pressure, a glycerine sample tube was placed in the induction coil, and the Larmor frequency of the proton was adjusted to an optimum height signal. Then, the glycerine tube was replaced by a sample tube, and T_1 determined for that particular sample.

T_1 measurements reported here were carried out using a slight modification of the free induction technique pulse method as developed by Hahn (1950). At the Larmor frequency of the protons, transitions between the nuclear Zeeman levels by an r-f magnetic field $2H_1 \cos \omega t$ are applied perpendicular to the polarizing magnetic field H_0 in the form of pulses. H_0 in this experiment was supplied by a permanent magnet. The effect of the pulses is to rotate the nuclear magnetization vector and thus establish components of the macroscopic nuclear magnetization in the plane perpendicular to the polarizing magnetic field. An alternating voltage is thus set up by magnetic induction in the same coil as is used to produce r-f magnetic field, and this voltage is amplified, detected

and applied to the y-axis of an oscilloscope, the sweep of which has been triggered just before application of the pulse. In using a $\pi/2$ - $\pi/2$ pulse sequence the first pulse makes the magnetization wholly transverse ($M_z=0$), and the second pulse, applied at a time τ after the first pulse, is a searching pulse, the amplitude $A(\tau)$ of its induction tail being a measure of the recovery of the z component of magnetization. However, in such cases as relaxation time of water T_1 is long and of the order of seconds and the induction tail amplitude of the second pulse is nearly zero. A modification of the usual pulse technique was then used where the amplitude of the first pulse signal or the echo, which appears at twice the time of separation of the two pulses, could be employed to determine T_1 .

A succession or train of pulses was applied. This train ensured that the magnetization at the end of the train was close to zero. At a time t , later, a similar train of pulses was applied and the height $A(t)$ of the first pulse signal was measured. T_1 was deduced from the usual relation

$$A(t) = A(\infty) [1 - \exp(-t/T_1)] \quad (9)$$

where $A(\infty)$ is the value of $A(t)$ for $t \gg T_1$. Usually t was of the order of $4T_1$ before $A(\infty)$ was obtained. Time was measured by a stopwatch; the train of pulses was operated manually by switch S (Figure II (a)). Figure III shows the

oscilloscope display of the two pulses and the detected r-f signal. The induction tail of the first pulse is shown at $A(\infty)$ and $A(t)$. Figure IV shows evaluation of T_1 from a plot of $\log \Delta A$ [$\Delta A = A(\infty) - A(t)$] as a function of time.

RESULTS AND DISCUSSION

I. (i) Determination of T_1 , the spin lattice relaxation time, for distilled water and 0.5M sodium chloride solution at various temperatures and different concentrations of oxygen.

T_1 values for oxygen-free and for oxygen-containing water under one atmosphere pressure of oxygen and one atmosphere pressure of air were obtained between 1°C. and 75°C. for distilled water and 1°C. to 40°C. for salt solution (Table I). The values were plotted versus temperature and the best lines were drawn by eye through the points as shown for oxygen-free distilled water in Figure VII. The lines were extrapolated to 0°C. and the smoothed values of T_1 were taken at 5°C intervals and listed in Table II for double distilled water and in Table III for 0.5M sodium chloride solution. Figures V and VI show the best curves obtained for T_1 .

The estimated maximum error for T_1 data for the smoothed curves was not more than $\pm 5\%$.

The results of T_1 for oxygen-free distilled water were compared to those of Simpson and Carr (1958) who measured T_1 and the diffusion coefficient D of proton in water at temperatures between 0°C. and 100°C. The results are shown in Figure VII. Agreement at temperatures above 40°C. was very apparent where the difference between the two results was of the order

TABLE I

 T_1 (MEASURED)Distilled Water

Temperature °C.	Oxygen-free T_1 sec.	1 Atm. Air T_1 sec.	1 Atm. O ₂ T_1 sec. ²
1.0	2.5, 2.6	2.0, 2.1	1.1, 1.2
7.0	2.9	2.5	1.4
9.0	3.0	2.4	1.5
12.0	3.2	2.8	1.8
15.0	3.2	-	-
17.0	3.5	3.0	1.9
21.0	3.9	3.2	2.3
25.0	3.9, 4.0	3.6	2.4
28.0	4.2	4.0	2.8
32.0	4.6	4.2	2.9
35.0	4.6	-	-
36.0	4.6	4.3	3.0
40.0	4.6, 4.7	4.5	3.3
44.0	5.4	4.8	3.6
48.0	5.5	5.2	3.7
52.0	6.2, 5.9	5.3, 5.6	4.1, 4.2
55.0	6.4	5.5, 5.8	4.1, 4.2
58.0	6.4, 6.8	5.9, 6.2	4.3, 4.5
64.0	7.1, 7.5	6.4, 6.9	4.8, 4.9
70.0	7.7, 8.0	7.2, 7.5	5.3, 5.5
75.0	8.3, 8.6	7.6, 7.9	5.8, 6.1

0.5M Sodium Chloride Solution

1.0	2.6, 2.7	2.0, 2.1	1.2, 1.3
8.0	3.0	2.7	1.8
13.0	3.3, 3.5	2.8	1.7, 1.9
15.0	3.4	-	-
18.0	3.7	3.0, 3.2	1.9, 2.0
23.0	4.0, 4.2	3.6	2.2
28.0	4.4, 4.6	3.8, 3.9	2.2, 2.4
32.0	5.0	4.1, 4.2	2.5
35.0	5.0, 5.3, 5.2	4.1, 4.4	2.7, 2.8
40.0	5.7, 6.0	5.1, 5.2	3.1, 3.3

TABLE II
VALUES OF T_1 AT DIFFERENT TEMPERATURES AND
CONCENTRATIONS OF OXYGEN AS GIVEN BY SMOOTHED CURVE*

<u>Distilled Water</u>			
Temperature °C.	Oxygen-free T_1 sec.	1 Atm. Air T_1 sec.	1 Atm. O_2 T_1 sec. ²
0	2.53	2.03	1.13
5	2.80	2.31	1.39
10	3.08	2.62	1.67
15	3.37	2.90	1.95
20	3.64	3.19	2.20
25	4.00	3.54	2.48
30	4.30	3.85	2.77
35	4.63	4.17	3.02
40	5.00	4.50	3.28
45	5.38	4.73	3.58
50	5.80	5.29	3.86
55	6.30	5.70	4.17
60	6.85	6.15	4.50
65	7.34	6.76	4.90
70	7.82	7.08	5.40
75	8.40	7.84	8.10

of 1-2%. At lower temperatures the difference was more pronounced, with Simpson and Carr's T_1 being much lower. T_1 's differed by 19% at 20°C. and 39% at 0°C. However, better agreement at lower temperatures was obtained with Guilloto's (1956) work, where the difference was only 4% at 20°C.

* The values of Table II were obtained at 5°C intervals from the smoothed curves of the plots of measured T_1 . These were employed in all subsequent calculations.

TABLE III
VALUES OF T_1 AT DIFFERENT TEMPERATURES AND
CONCENTRATIONS OF OXYGEN AS GIVEN BY SMOOTHED CURVE*

0.5M Sodium Chloride Solution

Temperature °C.	Oxygen-free T_1 sec.	1 Atm. Air T_1 sec.	1 Atm. O_2 T_1 sec. ²
0	2.62	2.10	1.21
5	2.90	2.40	1.43
10	3.20	2.67	1.63
15	3.50	2.94	1.82
20	3.81	3.24	2.04
25	4.20	3.58	2.27
30	4.70	3.98	2.53
35	5.28	4.47	2.80
40	6.00	5.05	3.21

The iron impurities in salt solution were of the order of 10^{15} ions cm^{-3} . Hausser and Laukien (1959) measured the spin relaxation time probability $\frac{1}{T_{1(c)}}$ for solutions of Fe^{++} and Fe^{+++} in aqueous solutions at field strength of 6200 gauss. For concentrations of Fe^{++} of 10^{21} ions cm^{-3} $T_{1(c)}$ was of the order of 10^{-2} sec. with little variation with temperature. For concentrations of Fe^{+++} of 2.6×10^{19} ions cm^{-3} , $T_{1(c)}$ varied between 10^{-2} to 3.5×10^{-2} sec. at temperatures between 0°C . and 90°C . For such concentrations present as impurities in this experiment, the effect of paramagnetic

* The values of Table III were obtained at 5°C intervals from the smoothed curves of the plots of measured T_1 . These were employed in all subsequent calculations.

ions is assumed to be less than the experimental uncertainty of 5% if the iron was in the ferrous state and slightly larger if it was totally in the ferric state.

(ii) Behaviour of $1/T_1$ with oxygen concentration at different temperatures.

The method used to produce two different concentrations of oxygen was simple and accurate to a few per cents of the theoretical values at all experimental temperatures. The only disadvantage was the incapability of attaining constant concentrations at different temperatures which was essential in the observation of oxygen effect on the relaxation time T_1 . It was then the primary concern in data presentation to show the variation of T_1 with concentration and temperature. Tables IV and V and Figures VIII and IX show the behaviour of $1/T_1$ with oxygen concentrations both in distilled water and 0.5M sodium chloride solution. The graphs present clearly the linear relation of oxygen concentration to $\frac{1}{T_1}$.

TABLE IV

VALUES OF $1/T_1$ AT DIFFERENT OXYGEN CONCENTRATIONS

AND DIFFERENT TEMPERATURES - DISTILLED WATER

Temperature °C.	Oxygen free		1 Atm. Air		1 Atm. Oxygen	
	$1/T_1$	O ₂ Conc. mg./l.	$1/T_1$	O ₂ Conc. mg./l.	$1/T_1$	O ₂ Conc. mg./l.
0	.395	-	.492	14.56	.884	69.45
5	.357	-	.432	12.72	.719	60.72
10	.324	-	.382	11.25	.598	53.68
15	.296	-	.344	10.06	.512	48.02
20	.274	-	.313	9.10	.454	43.39
25	.250	-	.282	8.24	.403	39.31
30	.232	-	.260	7.52	.361	35.88
35	.215	-	.240	6.95	.331	33.15
40	.200	-	.222	6.46	.304	30.82
45	.185	-	.211	5.99	.279	28.58
50	.172	-	.189	5.57	.259	26.57
55	.158	-	.175	5.12	.239	24.45
60	.146	-	.162	4.77	.222	22.74
65	.130	-	.148	4.20	.204	20.03
70	.127	-	.141	3.89	.185	18.56
75	.119	-	.127	3.38	.164	16.10

TABLE V

VALUES OF $1/T_1$ AT DIFFERENT OXYGEN CONCENTRATIONS

AND DIFFERENT TEMPERATURES - 0.5M SODIUM CHLORIDE

Temperature °C.	Oxygen free		1 Atm. Air		1 Atm. Oxygen	
	$1/T_1$	O ₂ Conc. mg./l.	$1/T_1$	O ₂ Conc. mg./l.	$1/T_1$	O ₂ Conc. mg./l.
0	.381	-	.476	11.64	.826	55.52
5	.344	-	.416	10.20	.699	48.65
10	.312	-	.374	9.05	.613	43.17
15	.285	-	.340	8.14	.549	38.82
20	.262	-	.308	7.41	.490	35.35
25	.238	-	.279	6.81	.440	32.48
30	.212	-	.251	6.30	.395	30.05
35	.189	-	.223	5.83	.357	27.81
40	.166	-	.198	5.35	.311	25.52

To examine for the linearity, an added test was performed before the final plot was completed. From the best drawn lines for T_1 of oxygen-free water and T_1 for oxygen-concentrated water under 1 atmosphere of oxygen, $1/T_1$ values were obtained and plotted versus oxygen concentrations for different temperatures. A straight line was drawn between the two values at each temperature. For oxygen concentration under one atmosphere of air, $1/T_1$ was read at the concentration of oxygen at that particular temperature and compared with that of $1/T_1$ obtained from the experiment; the two values were within $\pm 1.0\%$ of the smoothed values for all temperatures. Although the individual measurements of T_1 were only accurate to about $\pm 5\%$, presumably the drawing of a smooth curve through the plots of T_1 versus temperature has the effect of averaging out the random errors to some extent.

Another characteristic in Figures VIII and IX is revealed by the change of line slope with change of temperature. This now becomes the most significant aspect of the experiment, for though the linearity of $1/T_1$ with concentration is maintained, the effect of dissolved oxygen on relaxation times is shown to vary as a function of temperature. In addition, a constant concentration line can be obtained from the linearity relation. This will be a measure of the relaxation time effect under the same concentration. A vertical line at 32.00 mg. $O_2/l.$ was

drawn through the slope lines, and the slopes determined and plotted as a function of temperature in Figures X and XI.

The values corrected for the best line on the graph are given in Tables VI and VII. The lines represent $1/T_{1(c)}$ at constant concentration of O_2 of 10^{-3} moles/l. where $1/T_{1(c)} = 1/T_{1(O_2)} - 1/T_{1(pure)}$.

TABLE VI

$1/T_1$ VALUES OF OXYGEN-FREE AND

32.00 MG./L. OXYGEN-CONCENTRATED-DISTILLED WATER

Temp. °C.	$1/T_{1(O_2)}$	$1/T_{1(pure)}$	$1/T_{1(c)}$	$1/T_{1(c)}$ corrected
0	0.607	0.395	0.211	0.211
5	0.547	0.357	0.190	0.186
10	0.487	0.324	0.162	0.162
15	0.440	0.296	0.143	0.143
20	0.407	0.274	0.132	0.132
25	0.374	0.250	0.124	0.123
30	0.348	0.232	0.115	0.116
35	0.326	0.215	0.110	0.110
40	0.307	0.200	0.107	0.107
45	0.290	0.185	0.104	0.104
50	0.276	0.172	0.103	0.103
55	0.263	0.158	0.104	0.103
60	0.250	0.145	0.104	0.102
65	0.239	0.136	0.102	0.102
70	0.231	0.127	0.103	0.102
75	0.210	0.119	0.091	0.102

TABLE VII

$1/T_1$ VALUES FOR OXYGEN-FREE AND
32.00 MG./L. OXYGEN-CONCENTRATED-0.5M SODIUM CHLORIDE SOLUTION

Temp. °C.	$1/T_1(O_2)$	$1/T_1(\text{pure})$	$1/T_1(c)$	$1/T(c)$ corrected
0	0.639	0.381	0.257	0.257
5	0.576	0.344	0.231	0.234
10	0.533	0.312	0.221	0.221
15	0.502	0.285	0.216	0.212
20	0.468	0.262	0.205	0.205
25	0.434	0.238	0.196	0.199
30	0.406	0.212	0.193	0.195
35	0.382	0.189	0.192	0.192
40	0.357	0.166	0.190	0.190

Concentration values of oxygen in 0.5M sodium chloride solution were obtained from the published results of Truesdale et al. (1955) for 30%.S. The values of distilled water, on the other hand, were taken from Handbook of Physics and Chemistry (1960), p. 1706. The use of two different data sources was merely a measure of convenience, since the results of Truesdale et al. were limited to temperatures not exceeding 40°C.

The most apparent effect of oxygen on the proton relaxation time is shown by the curve of $1/T_1(c)$ as a function of temperature for the same concentration of molecular oxygen.

$T_1(c)$ is seen to be larger for distilled water than for NaCl solution at temperatures between 0° and 40°C. If the change in relaxation time is to be considered solely as a function of viscosity or diffusion, then qualitatively the results will be in agreement with the data of oxygen diffusion

in distilled and salt solutions. Diffusion coefficient, D , values for molecular oxygen were obtained by Rotthauwe (1958). By means of a dropping mercury electrode he found the diffusion coefficients of O_2 in air-saturated pure and sea water at $20^\circ C$. to be $2.92 \times 10^{-5} \text{ cm}^2 \text{ sec.}^{-1}$ for distilled water, $2.8 \times 10^{-5} \text{ cm}^2 \text{ sec.}^{-1}$ for 16‰ salinity seawater and $2.72 \times 10^{-5} \text{ cm}^2 \text{ sec.}^{-1}$ for 35‰ salinity seawater. However, as is shown in the next section, viscosity and molecular diffusion play a part in the relaxation mechanism, and although the part played is a major one, other aspects of complex formation enter the picture and contribute to the decrease of $1/T_{1(c)}$ with temperature.

II. Relaxation Terms

If the relaxation time effect $T_{1(c)}$ for the special case of O_2 in water depends on two mechanisms of relaxation, as was assumed in the theoretical part, where one is a dipole-dipole interaction and the other an exchange or hyperfine interaction as in

$$\frac{1}{T_{1(c)}} = c \tau_c + \frac{B}{\omega_s^2 \tau_e} \quad (8),$$

then, the dependency of $1/T_{1(c)}$ on the precession frequency of the electron and hence on the strength of the magnetic field H_0 becomes evident. The form of equation (8) is obtained by the assumptions placed on the correlation times τ_c and τ_e

whose validity depends on the proper evaluation of the different terms and on the understanding of oxygen complex formations.

In such a case, the three essential correlation times τ_s , τ_r and τ_h which are: the relaxation time of the electron, the tumbling relaxation time, and the lifetime of the complex respectively, should be estimated approximately to allow for the conditions where $\tau_c \omega_s \ll 1$ and $\tau_e \omega_s \gg 1$.

The dependence of T_1 in part on the dipolar interaction and hence on temperature is attributed to variation in τ_c where $\frac{1}{\tau_c} = \frac{1}{\tau_r} + \frac{1}{\tau_s}$; thus τ_c will be a function of the tumbling of complex only if τ_r is much smaller than τ_s . In the light of experiments done on paramagnetic ions, the relaxation correlation time of the electron τ_s was found to be between 10^{-9} to 10^{-10} sec. for Mn^{++} , V^{++} , Cu^{++} , Cr^{+++} and Ni^{++} (Bloembergen and Morgan, 1961). On the other hand, τ_r and consequently τ_c become the correlation time for the tumbling of the water oxygen complex. Since the amount of dissolved oxygen is of the order of 10^{-3} moles/l., then probably not more than one oxygen molecule forms a complex with a water cluster at any one time. The presence of oxygen should be of no consequence in regard to viscosity, and hence would allow for the use of viscosity correlation time as related to the tumbling of the cluster. Thus τ_c temperature dependence (Cox and Morgan, 1959), $\tau_c = \tau_c^0 e^{-E_c/RT}$ becomes

that of the water or the salt solution in which the oxygen is dissolved. However, the exponential dependence does not necessarily follow for the spin exchange contribution, nor for the dipolar contribution when τ_c is not primarily determined by the tumbling of the complex, but is also determined by the time dependence of the orientation of the electron spin τ_s . Similarly the argument holds for the exchange interaction where $\frac{1}{\tau_e} = \frac{1}{\tau_s} + \frac{1}{\tau_h}$. Both τ_s and τ_h are temperature dependent, with τ_s increasing with temperature and magnetic field and τ_h decreasing with temperature only.

For paramagnetic ions in general (Bloembergen, 1957; Bloembergen and Morgan, 1961), the relation between the three correlation times is of this order: $\tau_h \gg \tau_s \gg \tau_r$. This order may be similar for oxygen except that in the latter case τ_s may be greater than τ_h , the lifetime of the complex. This follows from the reasoning that the oxygen molecule is non-polar and thus incapable of forming a hydration layer of the same strength or magnitude as that of a charged ion in solution.

Some measure of the relative magnitudes of the three time constants discussed above can be derived from the difference between values of $1/T_1(c)$ at the same temperature and concentration of oxygen but at different magnetic field strength. The work of Guilloto (1956) which was done at a field strength

of 1650 gauss and at 20°C . provided a point of comparison at that temperature as well as means of evaluating equation (8). Furthermore, the use of his results was augmented by the close agreement of T_1 for degassed distilled water reported by him and which differed only by 4% from the value reported here. His value for $1/T_{1(c)}$ at oxygen concentration of 6×10^7 molecules cm^{-3} was 0.354 sec^{-1} in comparison to 0.132 sec^{-1} obtained in this experiment.

It is impossible to fit all the field dependent terms uniquely in equation (3) with values of $1/T_{1(c)}$ at only two different fields at one temperature. However, it may be fruitful to consider two limiting cases.

Case I

If the hyperfine interaction term was assumed to be negligible, i.e. $\langle A^2 \rangle$ was equal to zero, and equation (3) solved from the values of $1/T_{1(c)}$ at the two frequencies, no solution is possible for the $1/T_{1(c)}$ ratios of 2.67 obtained from Guilloto and this work. The largest value that $(1/T_{1(c)})_{\text{H}_2\text{O}} = 1650 / (1/T_{1(c)})_{\text{H}_2\text{O}} = 7000$ can have for $\langle A^2 \rangle = 0$ is 2.1 and this maximum is reached at $\tau_c = 3 \times 10^{-11} \text{ sec}$.

One therefore has to assume that an error of at least 30% exists in the ratio of the two results to be able to solve the equation where the first near ratio to 2.67 occurs for

$$\tau_c = 3 \times 10^{-11} \text{ sec. at } 2.1$$

If τ_c , the correlation time of water, stands for the tumbling of the complex τ_r , then two difficulties present themselves when the above approach is considered. The first is due to the value of τ_c for water which is usually of the order of 10^{-12} sec. From data of dielectric relaxation (Eigen and Maeyer, 1958), neutron diffraction (Brockhouse, 1958), diffusion and viscosity (Wang et al., 1953), it is seen that the largest correlation time τ for pure water at 0°C . is about 10^{-11} sec. The model representing this correlation time is the well known equation of Debye:

$$\tau = \frac{4\pi\eta a^3}{kT} \quad (10)$$

Bloembergen (1961) related τ to τ_c by the equation $\tau = 3\tau_c$, saying that Debye's τ refers only to the orientation of the polar group in space, while for τ_c the relative orientation between the magnetic nuclei must be considered. However, the characteristic time τ of Debye and the correlation time τ_c in the magnetic local field spectrum are proportional in one sample. They both vary in proportion to η/T if the temperature of the water is changed. This leads into the second difficulty where, above the temperature of 45°C ., $1/T_1(c)$ becomes nearly constant. Thus if only the first term of relaxation is considered, it means that τ_c is unchanged between 45° to 75°C . and this is very unlikely.

Further, if the assumption is made that the anomaly discussed above is due to τ_s , the relaxation time of the electron, then correlation with the experimental results at higher temperatures proves difficult. Bernheim et al. (1959) found that τ_s for paramagnetic ions changes linearly with temperature in the fashion $\frac{T-T_0}{T_0}$, increasing with increase in temperature. The increase of τ_s with temperature should further remove it from influencing τ_c , and thus makes τ_c more dependent on τ_r , the tumbling time of the complex.

Case II

When τ_c value is assumed to be sufficiently below 10^{-11} sec. over the entire temperature range that $(\omega_s \tau_c)^2 \ll 1$ and $(\omega_s \tau_e)^2 \gg 1$, equation (8) may be used to interpret the results.

$$\frac{1}{T_{1(c)}} = C \tau_c + \frac{B}{\omega_s^2 \tau_e} \quad (8)$$

The assumption that $\omega_s \tau_e \gg 1$ will be discussed in terms of the behaviour of $1/T_{1(c)}$ curve at higher temperature, showing first that $\langle A^2 \rangle$ is not zero, and secondly $\omega_s^2 \tau_e^2$ is much larger than one.

The coefficients $C \tau_c$ and $\frac{B}{\omega_s^2 \tau_e}$ can be evaluated at 20°C . from the two equations at ω_s equals to $1.24 \times 10^{11} \text{ sec}^{-1}$ used in this work and ω_s equals to $2.92 \times 10^{10} \text{ sec}^{-1}$ for the results of Guilloto. $C \tau_c$ and $\frac{B}{\omega_s^2 \tau_e}$ were obtained as $10.40 \times 10^{-2} \text{ sec}^{-1}$

respectively at the frequency ω_s of $1.24 \times 10^{11} \text{ sec}^{-1}$ the value appropriate to all the temperature dependent measurements of this experiment.

(i) Evaluation of $C \tau_c$ and $\frac{B}{\omega_s^2 \tau_e}$ at different temperatures for distilled water and 0.5M sodium chloride solution.

In order to get information on τ_e as a function of temperature, an assumption has been made about the temperature dependence of τ_c . The assumption was based on two considerations:

1. That oxygen in solution does not alter the size of the complex appreciably nor the water viscosity, and 2. That τ_c is dependent mainly on the tumbling time τ_r of the complex. Guided by other workers (Simpson and Carr, 1958; Bloembergen, 1961, pp.84-91), $C \tau_c$ was considered a function of η/T , and was evaluated at all experimental temperatures.

Tables VIII and IX give the values for $C \tau_c$ obtained from viscosity (Int. Crit. Tables, 1923; Dorsey, 1940, p. 188) and absolute temperature data for both distilled water and salt solution at a constant concentration of 10^{-3} moles/l. of oxygen. The results are also shown graphically in Figure XII. The term $(C \tau_c)$ was calculated for all temperatures by starting with the determined value from the two frequencies at $20^\circ\text{C}.$, using the following relationship.

$$(C \tau_c)_{Ta} = \frac{\eta_{a/Ta}}{\eta_{b/Tb}} (C \tau_c)_{Tb} \quad (11)$$

TABLE VIII

EVALUATION OF $C \tau_c$ VALUES AT DIFFERENT TEMPERATURES -

DISTILLED WATER

Temperature °C.	η c.p.	$\eta/T \times 10^3$ c.p. deg ⁻¹	$C \tau_c \times 10^2$ sec. ⁻¹
0	1.7921	6.564	20.00
5	1.5188	5.463	16.65
10	1.3077	4.620	14.08
15	1.1404	3.960	12.06
20	1.0050	3.430	10.40
25	0.8937	3.000	9.14
30	0.8007	2.642	8.05
35	0.7225	2.346	7.15
40	0.6560	2.095	6.38
45	0.5988	1.883	5.74
50	0.5494	1.700	5.18
55	0.5064	1.544	4.70
60	0.4688	1.407	4.29
65	0.4355	1.288	3.92
70	0.4061	1.183	3.60
75	0.3799	1.091	3.32

TABLE IX

EVALUATION OF $C \tau_c$ VALUES AT DIFFERENT TEMPERATURES -

0.5M SODIUM CHLORIDE SOLUTION

Temperature °C.	η c.p.	$\eta/T \times 10^3$ c.p. deg ⁻¹	$C \tau_c \times 10^2$ sec. ⁻¹
0	1.8727	6.859	20.90
5	1.5978	5.747	17.51
10	1.3822	4.884	14.88
15	1.2065	4.189	12.76
20	1.0703	3.653	11.08
25	0.9553	3.206	9.77
30	0.8632	2.849	8.68
35	0.7740	2.513	7.66
40	0.7010	2.240	6.82

The term $\frac{B}{\omega_S^2 \tau_e}$ was evaluated at different temperatures from the difference of $1/T_{1(c)}$ and $C \tau_c$ and given in Tables X and XI. Figure XIII shows the variation of $\frac{B}{\omega_S^2 \tau_e}$ with temperature, where for distilled water τ_e decreases linearly, and for the salt solution exponentially. It is also seen that τ_e in salt solution is smaller at all temperatures and decreases faster for the same concentration of oxygen. Behaviour of this nature is consistent with the assumption that $\omega_S \tau_e \gg 1$; the contribution of the second term to $1/T_{1(c)}$ would not increase with increase of temperature if $\omega_S \tau_e$ was either nearly equal to one or less than one. Also, the decrease of τ_e with temperature shows the dependency in this respect to be more on τ_h than on τ_S , since τ_S is also increasing with temperature.

(ii) Comparison of Activation Energies and Heats of Solution.

By identifying the dependence of τ_e on the lifetime of the complex from the above consideration, an exponential temperature dependence may be assigned to τ_e because its variation now is only a function of temperature, independent of the magnetic field. The relationship $\tau_e = \tau_e^{(o)} e^{-E_e/RT}$ would allow for the determination of the activation energy E_e , in the same manner as in $\tau_c = \tau_c^{(o)} e^{-E_c/RT}$. The difference between the two activation energies lies essentially in the

concept treatment of the complex. For τ_c the activation energy will be solely a measure of hydrogen bond breaking between water molecules (Wang et al., 1953), due to the tumbling of the complex, disregarding the size contribution of oxygen. On the other hand, E_e is more a measure of the bond broken between the oxygen molecule and the cluster forming the complex. If the heat of solution of oxygen in water, $-\Delta H$ is actually an indication of the bond formed between the oxygen molecule and water, then, the comparison of $-\Delta H$ and E_e should show a simultaneous change under various conditions.

TABLE X
VALUES OF $\frac{B}{\omega_s^2 \tau_e}$ AND $\frac{B}{\tau_e}$ AT DIFFERENT TEMPERATURES -
DISTILLED WATER

Temp. °C.	$1/T_{1(c)}$ $\times 10^2 \text{ sec}^{-1}$	$\tau_c \times 10^2$ sec^{-1}	$\frac{B}{\omega_s^2 \tau_e} \times 10^2 \text{ sec}^{-1}$	$\frac{B}{\tau_e} \times 10^{-20}$ $\text{sec}.$
0	21.18	20.00	1.18	1.82
5	18.63	16.65	1.98	3.05
10	16.24	14.08	2.16	3.33
15	14.33	12.06	2.27	3.50
20	13.22	10.40	2.82	4.34
25	12.33	9.14	3.19	4.91
30	11.61	8.05	3.56	5.48
35	11.06	7.15	3.91	6.02
40	10.71	6.38	4.33	6.67
45	10.45	5.74	4.71	7.25
50	10.36	5.18	5.18	7.98
55	10.32	4.70	5.62	8.65
60	10.29	4.29	6.00	9.24
65	10.27	3.92	6.35	9.78
70	10.25	3.60	6.65	10.24
75	10.23	3.32	6.91	10.64

TABLE XI
VALUES OF $\frac{B}{\omega_s^2 \tau_e}$ AND $\frac{B}{\tau_e}$ AT DIFFERENT TEMPERATURES -
0.5M SODIUM CHLORIDE SOLUTION

Temp. °C.	$1/T_1(c)$ $\times 10^2 \text{ sec.}^{-1}$	$C \tau_c$ $\times 10^2 \text{ sec.}^{-1}$	$\frac{B}{\omega_s^2 \tau_e}$ $\times 10^2 \text{ sec.}^{-1}$	$\frac{B}{\tau_e}$ $\times 10^{-20} \text{ sec.}$
0	25.74	20.90	4.84	7.45
5	23.47	17.51	5.96	9.18
10	22.18	14.88	7.30	11.24
15	21.22	12.76	8.46	13.03
20	20.50	11.08	9.42	14.51
25	19.92	9.77	10.15	15.63
30	19.50	8.68	10.82	16.60
35	19.22	7.66	11.56	17.80
40	19.03	6.82	12.21	18.80

The activation energy E_c was calculated in Figure XIV on the basis of data obtained from Tables XII and XIII. E_c is shown to be 4.4 kcal. for distilled water and 4.9 kcal. for 0.5M sodium chloride solution. E_e was calculated from Tables XIV and XV and Figures XV and XVI, using the relation $\tau_e = \tau_e(o) e^{-E_e/RT}$.

The heat of solution for oxygen solubility in distilled water and salt solution was obtained by using the constant K of equation (1): $P_{O_2} = KC_2$, where $P_{O_2} = x(P - p_w)$; x = fractional content of O_2 in atmospheres, P = atmospheric pressure; and p_w = water vapor pressure in the van't Hoff equation for enthalpy. The term K was determined at different temperatures under one atmosphere of oxygen as shown in Tables XVI and XVII,

and the results presented graphically in Figure XVII.

Both the plots for E_e and $-\Delta H$ are seen to be non-linear and hence are temperature dependent. Evaluation of E_e and $-\Delta H$ at temperatures of 0° , 15° , 30° and 60°C . showed a decrease with temperature in the same trend and of the same order of magnitude, thus revealing the close association between the two quantities (see Table XVIII).

In general, both E_e and $-\Delta H$ are smaller for salt solution than for distilled water, and their decrease with rise in temperature proceeds at a faster rate. This could be attributed to the weaker nature of the water-oxygen complex in the salt solution and to a higher dependence on temperature change. There is thus an indication that the cluster size determines the strength of the bond formed in the complex between oxygen and water, and hence the solubility. The anomaly at 0° to 15°C . in E_e could be interpreted as a result of subtracting two large terms to obtain a small term. A small percentage error in $C\tau_c$ or $1/T_{1(c)}$ at these temperatures will appear highly magnified in $\frac{B}{\omega_s^2 \tau_e}$. Also the use of four or five significant figures in data tables such as those of viscosity and logarithm values, was simply a matter of straight quotation rather than an indication of the expected accuracy.

TABLE XII
ESTIMATION OF ACTIVATION ENERGY OF τ_c -

DISTILLED WATER

$$C \tau_c = C \tau_c^0 e^{-E/RT}$$

Temperature °C.	$\frac{1000}{T}$ deg ⁻¹ K	τ_c^0 / τ_c	$2.303 \log \tau_c^0 / \tau_c$
0	3.650		
5	3.597	1.201	0.1831
10	3.533	1.420	0.3507
15	3.472	1.658	0.5057
20	3.412	1.923	0.6540
25	3.355	2.188	0.7833
30	3.300	2.484	0.9101
35	3.246	2.797	1.0287
40	3.194	3.135	1.1427
45	3.144	3.484	1.2485
50	3.095	3.861	1.3512
55	3.048	4.255	1.4484
60	3.003	4.662	1.5398
65	2.958	5.102	1.6301
70	2.915	5.556	1.7153
75	2.873	6.024	1.7961

$$E = 4.4 \text{ kcal.}$$

The contribution of the two relaxation terms $C \tau_c$ and $\frac{B}{\omega_s^2 \tau_e}$ is of importance in correlating the experimental results with oxygen solubility in the two solvents. The effective contribution of the term $C \tau_c$ is a function of the viscosity and/or diffusion, and the contribution of the second term $\frac{B}{\omega_s^2 \tau_e}$ a function of the oxygen water complex formation. The ratio of the two terms at various temperatures is obtained from Tables XIV and XV and plotted in Figure XVIII.

TABLE XIII
ESTIMATION OF ACTIVATION ENERGY τ_c -
0.5M SODIUM CHLORIDE SOLUTION

Temperature °C.	$\frac{1000}{T} \text{ deg}^{-1} \text{ K}$	τ_c^0 / τ_c	$2.303 \log \tau_c^0 / \tau_c$
0	3.650		
5	3.597	1.194	0.1773
10	3.533	1.405	0.3399
15	3.472	1.638	0.4938
20	3.412	1.886	0.6347
25	3.355	2.139	0.7605
30	3.300	2.408	0.8788
35	3.246	2.728	1.0039
40	3.174	3.065	1.1202

$$E = 4.9 \text{ kcal.}$$

A striking difference appears between the two ratios when the two terms become equal. For salt solution the equality occurs at 30°C., while for distilled water, it takes place at 50°C. Because of little difference in the $C\tau_c$ terms (Figure XII) between distilled water and salt solution, the anomaly seems to be due largely to τ_e and its dependence on ion content in water, or more correctly on the nature of the cluster formation. The breaking of such water aggregates by either temperature or ions, clearly shows in the variation of τ_e . The close association in this case between cluster and complex formation renders τ_e as a valuable parameter in the evaluation of liquid properties and the extent and nature of bonding of the dissolved substance.

TABLE XIV

RATIO OF $C \tau_c / \omega_s^2 \tau_e^B$ AND ESTIMATION OF ACTIVATION ENERGY OF τ_e -
DISTILLED WATER

Temp. °C.	$\frac{1000}{T}$ deg ⁻¹ K	Ratio $C \tau_c / \omega_s^2 \tau_e^B$	$\frac{\tau_e(o)}{\tau_e}$	$2.303 \log \frac{\tau_e(o)}{\tau_e}$
0	3.650	16.95		
5	3.597	8.41	1.678	0.5177
10	3.533	6.52	1.830	0.6045
15	3.472	5.31	1.924	0.6545
20	3.412	3.64	2.390	0.8715
25	3.355	2.87	2.703	0.9947
30	3.300	2.26	3.017	1.1045
35	3.246	1.83	3.314	1.1983
40	3.194	1.47	3.669	1.3000
45	3.144	1.22	3.992	1.3846
50	3.095	1.00	4.390	1.4797
55	3.048	0.84	4.763	1.5612
60	3.003	0.72	5.085	1.6266
65	2.958	0.62	5.381	1.6833
70	2.915	0.54	5.636	1.7296
75	2.873	0.48	5.856	1.7678

TABLE XV

RATIO OF $C \tau_c / \omega_s^2 \tau_e^B$ AND ESTIMATION OF ACTIVATION ENERGY OF τ_e -
0.5M SODIUM CHLORIDE SOLUTION

Temp. °C.	$\frac{1000}{T}$ deg ⁻¹ K	Ratio $C \tau_c / \omega_s^2 \tau_e^B$	$\frac{\tau_e(o)}{\tau_e}$	$2.303 \log \frac{\tau_e(o)}{\tau_e}$
0	3.650	4.32		
5	3.597	2.94	1.232	0.2087
10	3.533	2.04	1.509	0.4115
15	3.472	1.51	1.749	0.5592
20	3.412	1.18	1.948	0.6669
25	3.355	0.96	2.098	0.7411
30	3.300	0.80	2.236	0.8049
35	3.246	0.66	2.389	0.8712
40	3.194	0.56	2.523	0.9256

TABLE XVI

EVALUATION OF HEATS OF FORMATION FOR SOLUBILITY OF
OXYGEN UNDER ONE ATMOSPHERIC PRESSURE OF O_2 - DISTILLED WATER

Temp. °C.	Water Vapor Pressure mm.Hg.	C_2 :Conc. O_2 moles/l. $\times 10^3$	P_{O_2} Corr. Atm.	$2.303 \log \frac{C_2}{P_{O_2}}$
0	4.6	2.170	0.993	-6.1269
5	6.5	1.897	0.991	-6.2596
10	9.2	1.677	0.988	-6.3800
15	12.8	1.500	0.983	-6.4811
20	17.5	1.355	0.978	-6.5829
25	23.8	1.228	0.968	-6.6711
30	31.8	1.121	0.958	-6.7582
35	42.2	1.035	0.945	-6.8180
40	55.3	0.963	0.928	-6.8719
45	71.9	0.893	0.905	-6.9221
50	92.5	0.830	0.879	-6.9663
55	118.0	0.764	0.845	-7.0099
60	149.4	0.710	0.804	-7.0334
65	187.5	0.625	0.754	-7.0967
70	233.7	0.580	0.692	-7.0856
75	289.1	0.503	0.620	-7.1181

TABLE XVII

EVALUATION OF HEATS OF FORMATION FOR OXYGEN SOLUBILITY UNDER ONE
ATMOSPHERIC PRESSURE OF O_2 - 0.5M SODIUM CHLORIDE SOLUTION

Temp. °C.	Water Vapor Pressure mm.Hg.	C_2 :Conc. O_2 moles/l. $\times 10^3$	P_{O_2} Corr. Atm.	$2.303 \log \frac{C_2}{P_{O_2}}$
0	4.5	1.735	0.994	-6.3517
5	6.2	1.520	0.992	-6.4823
10	7.1	1.349	0.988	-6.5976
15	13.0	1.213	0.983	-6.6987
20	17.3	1.104	0.978	-6.7876
25	23.3	1.015	0.970	-6.8639
30	31.4	0.939	0.959	-6.9300
35	42.3	0.869	0.945	-6.9928
40	54.5	0.797	0.929	-7.0621

TABLE XVIII
HEAT OF SOLUTION AND ACTIVATION ENERGY
OF DISSOLVED OXYGEN IN WATER

Temperature °C.	Distilled Water		0.5M Sodium Chloride Solution	
	<u>E_a</u>	<u>- ΔH</u>	<u>E_a</u>	<u>- ΔH</u>
0	4.4	5.2	6.2	4.8
15	4.4	3.4	3.6	2.9
30	3.6	2.7	2.4	2.4
60	2.8	1.5	-	-

GENERAL DISCUSSION

The case of complex formation in an oxygen-water system is seen to be fundamentally related to hydrogen bond formation in the same manner as that of cluster formation of water molecules. This is evident in the behaviour of oxygen solubility and its dependence on the two important parameters of temperature and ion content. The stability of the hydrogen bond in this aspect may be considered due to either the orientation of the solvent and solute molecules to each other or to the dependence on the size and shape of the water cluster or on both.

The decrease in τ_e with higher temperatures, and additions of ions to water would allow for the increase in diffusion of oxygen molecules because of the more random orientations and the more frequent jumps of oxygen between the clusters. However, a comparison of diffusion coefficients for ion-free and ionized water at the same temperature (Rotthauwe, 1958), shows the diffusion as decreasing with higher salt content, thus making it more a function of the viscosity than of the stability of the complex. Conversely, a lowering of the viscosity by an increase of temperature in ion-free water increases the diffusion coefficient from 1.87 to $2.60 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ between 16° and 25°C . (Millington, 1955; Kolthoff and Miller, 1941). The change of viscosity in ionized solutions is affected by the ions in the neighbourhood of water

molecules. Bernal and Fowler (1933) suggested that the ions form a lattice structure with water molecules, and the structure tightening or loosening is due to extent of hydration of the ions. These structures, however, would be different than the normal clusters formed by ion-free water, and their orientation at the edges are expected to be modified by the field of nearest charged ion.

The model could be pictured then, that an oxygen molecule attaches itself to a water cluster for a time τ_e , this being a function of temperature and ion content, then in short lattice jumps it hops from one cluster to the other within a very small regional environment but does not contribute effectively to diffusion. Thus if each regional environment remains in the same space allotted to it, diffusion then becomes so small as to be of the same order as diffusion in solids. In liquids essentially, the ability to move and exchange regional environments by mass movements of molecules or clusters, becomes the prime factor in determining the magnitude of diffusion.

The energy of activation, E_c , obtained from viscosity and absolute temperature data for distilled water is 4.4 kcal./mole and for 0.5M sodium chloride solution is 4.9 kcal./mole. This is a measure of the energy required for one hydrogen bond breaking between two water molecules (Wang et al., 1953).

A significant characteristic of this quantity is its constancy with temperature. Many experiments were performed by different workers to calculate the enthalpy of a hydrogen bond in water. Their values were 3.4 kcal./mole (Scatchard et al., 1952); 4.4 kcal./mole (Rowlinson, 1949), and 5.7 kcal./mole (Lambert, 1953) at temperatures between -65° to 100°C . Also the activation energy for dielectric absorption in water was found to be 13.2 kcal./mole (Autry and Cole, 1952) and was interpreted as the breaking of three hydrogen bonds each being 4.5 kcal/mole. The temperature independence of hydrogen bond breaking and forming between water molecules distinguishes it from the process occurring between oxygen molecules and water molecules where temperature changes influence the energy of bond forming or breaking.

At temperatures of 0°C . and 15°C . the activation energy E_e is 4.4 kcal./mole and $-\Delta H$ 5.2 and 3.4 kcal. (Table XVIII). For the 0.5M sodium chloride solution E_e varies between 6.2 and 3.6 kcal./mole and $-\Delta H$ between 4.8 and 2.9 kcal/mole. These values of hydrogen bonds formed and broken for an oxygen water complex are of the order of the hydrogen bond strength formed between one water molecule and another at any temperature.

The proximity of the values of E_e to $-\Delta H$ for an oxygen-water system supports the assumptions made in the treatment of the experimental results. This agreement with the values of activation energy and enthalpy for water-water system at

low temperatures ($0^{\circ} - 15^{\circ}\text{C.}$) shows that: 1. Either two bonds are formed between the oxygen molecule and water with each bond being of the order of 2.2 kcal./mole, or 2. only one bond exists between water and oxygen being of the same magnitude as that formed between two water molecules.

The nature of the bond in an oxygen-water system must be different from that formed in a water-water system. The energy involved in an interaction resulting in a hydrogen bond in the case of water molecules is always the same whether the process occurs at -65°C. or in the vapor phase at 100°C. , and is only modified by the presence of ions in solution. Conversely, the decrease of E_e and $-\Delta H$ of oxygen-water bond at higher temperatures indicates that the forces acting on an oxygen molecule, in hydrogen bond formation, are changing continuously. At such temperatures these could be related to the increase in molecular agitation and thus to more distortion of their line of approach where interaction between the dipole moment of water and the induced polarization of the oxygen molecule tends to be less effective. An explanation may be put forward to account for the change in forces as being dependent on either the size and form of the water cluster, or the distance R of the $\text{O-H}\cdots\text{O}$ bond or the angle of approach.

Hughes et al. (1956) interpreted the weakening of a hydrogen bond as it appears in the stretching frequency ν_s in infra-red

as the decrease in the force field on a molecule due to its neighbours. As the temperature rises, the intensity of the force field decreases owing to the greater average distance between molecules. Earlier Cogeshall and Saier (1951) explained the decrease in ν_s in a system of carbon tetrachloride-benzyl alcohol with increase in temperature as being dependent on the following characteristics:

1. Hydrogen bonding systems involve monomeric and usually several polymeric species in rapid equilibrium.

2. Each polymer has a characteristic ν_s , and the higher the polymer the lower is ν_s .

At higher temperatures ν_s is increased, and this is supposed to accompany the decrease in the size of the polymer.

It could be deduced that the stronger hydrogen bonds of oxygen-water are formed in the larger clusters. The decrease of the cluster size by molecular agitation due to an increase in temperature or by the presence of ions lowers the resultant force acting on polarizability of an oxygen molecule.

The effect due to the change in the distance R of the O-H---O bond is dealt with by Pimentel and McLellan (1960, p. 242). The enthalpy ΔH is shown as a function of R for different isolines of electrostatic energy of point charge dipoles. For 0.30 electron charges, ΔH varies by 1 kcal./mole for a change of 0.25\AA in the bond length R . On the other hand,

the angles of approach are significant in the variation of ΔH . Figure XIX shows two ways of approach for two dipolar molecules and the designation of the angles Θ and Φ between them (Pimentel and McClellan, 1960, p. 242).

Although Φ could not be involved in the formation of a hydrogen bond in the case of water-oxygen due to the non-polarity of the oxygen molecule, where the induced polarization is not governed by position, Θ still could contribute to the decrease of $-\Delta H$ and E_e by virtue of orientation of the water dipole molecule whether be it the resultant dipole moment, or simply one of the O-H moments.

CONCLUSION

The results of this work reveal the following new points:

1. The solubility of oxygen in water is related to hydrogen bond formation between oxygen and water molecules.
2. The increase and decrease of solubility depends on the factors governing the building or destroying of clusters of water molecules.
3. Temperature and salt content affect the molecular orientation and the average line of approach of the reactants.
4. The effect of temperature and salt content appears in the activation energy and enthalpy. This is related to bonds broken and bonds formed consecutively.
5. The values of E_e and $-\Delta H$ are a measure of solubility.

SUGGESTIONS FOR FURTHER WORK

1. For supplementing the assumption made for the oxygen water-cluster complex and the dependence of its dipole-dipole interaction correlation time on the viscosity of water, all measurements may be repeated at another frequency and magnetic field strength, and equation (3) solved accordingly.
2. Various other salt concentrations, and different non-paramagnetic salts may be used to examine the change

in heats of solution and activation energies.

3. Another paramagnetic gas, nitric oxide, which contains a permanent dipole moment, may be used in a similar manner and the different values for solubility and relaxation times compared to oxygen for further understanding of the nature of the complex.

PART II

ASPECTS OF OXYGEN SUPERSATURATION IN SEA WATER

INTRODUCTION

In every well mixed water body exposed to and at equilibrium with the atmosphere, the concentration of oxygen at constant temperature depends on Henry's law:

$$P_2 = KC_2 \quad (1).$$

Any deviation from this relationship which displaces the equilibrium, or results in its invalidity, indicates certain physical, chemical or biological processes in action. These processes and their intensities are the prime factors which implement oxygen under- or supersaturation.

"Oxygen Supersaturation" as it applies in this work and as it is commonly referred to (Klots, 1961) in sea, lake, or laboratory system could be defined as follows: A certain quantity of oxygen gas dissolved per unit volume of water in excess of a known saturated value (e.g. that given by Richards and Crowin (1956) for the particular temperature and ion content of the water under one atmosphere of air).

The change of concentration of oxygen in the sea independent of exchange with the atmosphere is governed by depletion

and production. The first may be caused by oxidation of metal ions of lower valency such as iron I and II and manganese II and III; decomposition of organic matter; and respiration of plants and animals. On the other hand, production results from the process of photosynthesis in green plants only, and is limited to the euphotic zone (Richards, 1957).

In natural water bodies oxygen content in the superficial layers often strongly deviates during the whole period of biological activity to either side of 100% saturation. The non-equilibrium content of oxygen in the surface layers under certain conditions may be due to rapid changes of the air temperature near the surface or the mixing of two oxygen-saturated water masses at different temperatures. Also a special but not experimentally demonstrated oxygen supersaturation may be produced at great depths in the ocean if saturated water at the surface sinks at constant temperature and salinity. This results from the increase of Henry's law constant with depth. The reciprocal $1/K$ varies between 1.00 at the surface to 0.88 under 100 atmospheres pressure at 25°C. (Klots, 1961). All these factors cause but a short-lived deflection from equilibrium. However, a prolonged supersaturation of the surface water layers with oxygen points to the predominance within the water basin of processes of synthesis of organic matter (primary production) over processes of their destruction by respiration and oxidation (Vinberg, 1940).

Occurrences of this nature are frequent in the sea, and are more pronounced and varied in the neritic province and at higher latitudes than in the oceanic province and lower latitudes. The influence of land masses in providing more organic and inorganic matter as nutrients, and the more extreme variation in weather conditions of the higher latitudes on these regions, subject them to exhibit characteristics widely different from those of the more homogeneous nature that take place far at sea or in inshore tropical regions. The proximity of land is also believed to render the coastal waters as a whole about fifty times more productive in phytoplankton than the open ocean waters (Sverdrup, Johnson and Fleming, 1942).

Reported oxygen data for several years in the waters of the West Coast of Canada, and particularly for many of the inlets that indent the coast, show oxygen supersaturation to be commonly present in the upper 20 m. layer from spring to early fall (Data Reports, I.O.U.B.C., 1953-61). The values range normally from 110 to 140% with few exceptions where up to 215% (Data Report No. 5, 1955) and 250% (Gilmartin, 1960) were noted. On the basis of the accumulation of data for these inlets, a general description of the locality of oxygen supersaturation in different water basins can be described.

From studies of the general characteristics of British Columbia inlets, Pickard (1961) observed a usual occurrence

of two oxygen maxima. The first, a single surface maximum, was found either near the head or near the mouth of large-runoff inlets, and the second generally appeared below the surface at the mouth or along the length of both large and small-runoff inlets. The latter was usually at a depth of 3 to 8 meters and occasionally as deep as 20 meters, with a location at or below the halocline. Commonly, the surface maximum occurring at the head of the large-runoff inlet displayed no supersaturation while the other maxima, surface and subsurface, showed supersaturation.

The seasonal phenomena and their entailment of large oxygen fluctuations in water initiated the interest for this work. It became the primary concern here to establish the direct relationship between the presence of phytoplankton organisms, their modes of growth and photosynthetic activity, with the extent of oxygen supersaturation that could be reached. It was realized that excellent treatments of prolonged oxygen supersaturation in lakes and inshore waters have already been advanced by Rakestraw (1932), Ricker (1934), Vinberg (1940), Redfield (1948), Hutchinson (1957) and Ramsey (1962). In all these treatments, however, supersaturation was considered to be an outcome of biological activity and photosynthesis without any attempt to relate it to the actual number of organisms present or to their photosynthetic pigment content. The main approach was limited to the study of rates of escape to the

atmosphere and the different paths it follows.

It is the aim, in this second part, to study oxygen supersaturation in sea water in the presence of two common phytoplankton species in terms of (1) their growth and number of organisms produced, (2) the influence of the rate and amount of photosynthetic activity on oxygen production, (3) the influence of their growth and biological activity on the medium, and (4) the dependence of the supersaturation level in water on physical, chemical and biological influences resulting from the presence of these organisms. A review of the background on which these investigations are based is given in order to facilitate the understanding of the reasons which lead to the experimental work, and to the discussion of the results obtained.

The production of marine phytoplankton depends on many factors which account for the variation and abundance of cells at different seasons of the year, in various localities and at different depths in the ocean. Chemical factors influencing production include the presence of phosphate, nitrate, silica, oxygen, carbon dioxide and numerous other secondary elements such as iron, sulfur, calcium and manganese as well as salinity and hydrogen ion concentration. Furthermore, organic requirements of phytoplankton are as important for growth as inorganic substances. Although most of these have been found in the course of investigating the properties of natural substances required to support axenic growth in vitro, nonetheless there

is indication for the need of compounds such as diamine tetra acetic acid (EDTA) (Droop, 1957), vitamin B₁₂ and thiamine (Gran, 1931b). Physical factors involve light, its intensity, quality and duration; temperature; viscosity and density of medium. Circulation, particularly upwelling and vertical mixing, are factors that have a great effect upon the supply of nutritive salts (Harvey, 1955, Chap. V).

The growth and metabolism of phytoplankton have been studied intensively in laboratory cultures. It is possible under these conditions to give a generalized picture of the growth cycle. Following inoculation of an appropriate medium with the algae and exposure to suitable conditions of light and temperature, growth in cell numbers may begin immediately or after a lag period. Growth is usually exponential in the first few days, the cell numbers per unit volume of medium (n_t) at any time (t) being given by the expression (Fogg, 1953) :

$$n_t = n_0 e^{rt} \quad (12)$$

where n_0 is the number of cells per unit volume of medium at zero time, and r the relative growth constant. Following this exponential phase is a period in which the relative growth constant declines continuously and finally a stationary phase is reached in which there is no further increase in cell numbers.

The chemical kinetics underlying the growth sequence in simple algae are presumably similar to those which have been

postulated to occur in bacteria (Hinshelwood, 1946). In material which is not actively growing, enzymes may have been denatured and the concentration of essential metabolites may have fallen so that a period of reconstitution is necessary before exponential growth can begin. In cultures containing a limited volume of medium, exponential growth of algae sooner or later ceases. The factors bringing this about are various, the following being the more important (Fogg, 1953):

1. Exhaustion of nutrient substance or trace element (e.g., Fe) from the medium;
2. Preferential absorption of a particular ion may result in the reaction of the medium becoming inimical to growth;
3. Accumulation of growth-inhibiting products of metabolism; and
4. The increasing density of growth may reduce light penetration into the bulk of the medium so that photosynthesis becomes insufficient to maintain exponential growth.

The assimilation of organic material in phytoplankton and green plants in general is the direct result of the process of photosynthesis. The energy transfer from light into functional chemical energy for the organic build-up is due to green and other accessory pigments present in these plants (Arnon, 1959). These pigments are the green chlorophylls a, b, c, d and e and the various carotenoids, which consist mainly of

β-carotene, xanthophyll and xanthophyll epoxide. The carotenoids are found together with chlorophyll in the chromatophores and are present in either an amorphous or crystalline state (Karrer and Jucker, 1950).

The role of carotenoids in photosynthesis has been established most convincingly in the case of diatoms (Tanada, 1951). The quantum yield has been measured at different wavelengths and compared with the corresponding estimates of the distribution of light absorption among chlorophylls, fucoxanthin and other carotenoids. The comparison of the quantum yield of photosynthesis with the efficiency curve and those for the distribution of absorption among the pigments in vivo indicates that light absorbed by fucoxanthin is utilized in photosynthesis with about the same efficiency as that absorbed by chlorophyll.

When carotenoids sometimes occur elsewhere in the protoplast than in the chromatophores, they are presumably inactive in photosynthesis, and it may be that within the chromatophore spatial arrangements exclude some carotenoid molecules from participating in active light absorption. Another possibility is that certain types of the carotenoids are able to absorb light for use in photosynthesis but that others are quite inactive in this way (Fogg, 1953).

Further information about the part played by accessory pigments in photosynthesis comes from studies of fluorescence. The yield of fluorescence, i.e., the ratio of the energy

emitted to that absorbed, gives information regarding the fate of the excitation energy. The yield of the chlorophyll fluorescence is the same whether it is excited by red light (6000\AA), absorbed exclusively by chlorophyll, or by blue-green light (4700\AA), three-quarters of which is absorbed by carotenoids (Dutton et al., 1943). This evidence of transference of energy from accessory pigments to chlorophyll suggests that light absorbed by the former pigments is not utilized directly in the photochemical reaction. It appears, in fact, that all light energy used in photosynthesis must pass through chlorophyll-a. Thus, in Chlorella only chlorophyll-a fluoresces and its fluorescence is excited by light absorbed by chlorophyll-b (Duysens, 1951). Since chlorophyll-a is the principal and only photosynthetic pigment common to all the algal classes this provides evidence that the photochemical reaction is of the same nature in all algae.

If chlorophyll-a is the essential catalyst for photosynthesis, it might be reasonable to assume that the gross rate of photosynthesis taking place in sea water illuminated by light of a given intensity is a function of chlorophyll-a content of sea water. There is evidence, however, that carotenoids also influence this relationship. Yentsch and Ryther (1957) showed that a linear relationship holds between the ratio of net to gross photosynthesis and the ratio of chlorophyll-a to the total carotenoids in a water sample.

A diurnal fluctuation in the rate of photosynthesis by a plant in its natural surroundings is expected, owing to fluctuation in the light intensity to which it is exposed. In addition there seems to be also a diurnal or similar variation in the intrinsic photosynthetic activity or "vitality" of the plant itself (Strickland, 1960). Rabinovitch (1951), Doty and Oguri (1957) and Ryther (1956) have provided information on the existence of diurnal, geographical and annual influences on rates of photosynthesis. The phenomenon was given the name "Periodicity" for its consistently maintained pattern under various conditions. By contrast, the simple unicellular algae such as found in marine phytoplankton can be grown in culture for many days^a or weeks with no notable daily periodicity in growth rate after an initial adjustment period (Strickland, 1960).

The process of growth of the organisms themselves could cause certain changes in the water or medium in which they live. These changes may act as inhibitive factors for further growth as well as for photosynthesis. For example, carbon dioxide concentration (and pH level) might prove a limiting factor in the supply of reductant material (Arnon, 1959). Talling (1960) indicates that inadequate carbon dioxide supply and inadequate buffering do not affect photosynthesis for short exposures, but might do so after long exposures when culture growth is increasing to high densities. Furthermore, the

release of extracellular products from living, dead or decomposing plankton could inhibit photosynthesis or the growth rate by the different reactions they may undergo in either culture media or at sea. Saunders (1957) sums up these effects in four major points:

1. The extracellular products may supply compounds utilized as energy sources or which contain the basic elements essential to the building of protoplasm.
2. They may supply accessory growth factors which are required for or merely stimulate the growth of organisms.
3. They may be in the form of toxic substances which inhibit activities or cause the death of many aquatic organisms.
4. They may form organic complexes with trace metals, the effect of which may be beneficial or detrimental, depending upon circumstances.

Matsudaira (1950, 1951, 1952a) studied the effect of the catalytic activity of sea water on the decomposition of hydrogen peroxide in the presence of organic matter similar to that extracted from filtered culture water of different age. He found a substantial decrease in the decomposition of hydrogen peroxide in both cases, the catalytic activity decreasing rapidly with higher concentrations of either organic matter or extracellular product. This is equivalent to catalytic inhibition. The versatility of this method in testing for some of the medium influences on growth

recommends it for further use and study in this connection.

The variation of the rate of desupersaturation at sea or in culture water is considered to be influenced by two major factors: the first is a physical-chemical one, which is related to ion content, surface tension, temperature, agitation, surface area of water as related to the "effective" exposed surface, depth of supersaturation column and other factors that contribute to nucleation and spontaneous formation of gas bubbles. The second factor is mainly an outcome of the presence of phytoplankton and other organisms in the supersaturated water. These organisms may act in various ways to reduce the supersaturation level. Chiefly, they may consume oxygen by respiration and oxidation, provide a "wall" effect for nucleation, and even during photosynthesis their average closeness to each other can provide the steps for bubble formation and rapid escape.

By excluding the biological influence on oxygen in water after a rapid and vigorous interval of photosynthetic activity, the mechanisms of desupersaturation can be classified relative to their exchange with the atmosphere in the following manner.

1. For an undisturbed water surface of a quiescent water body, exchange with the atmosphere is governed by an uppermost thin layer (Hutchinson, 1957) which is at equilibrium with the atmosphere above it, any escape or entrance of gas such as oxygen from and to the water body is governed by Fick's diffusion

equation from that layer.

$$\frac{\partial [O_2]}{\partial t} = D \frac{\partial [O_2]_z}{\partial z} \quad (13)$$

Where z indicates depth, t time and D the molecular diffusion coefficient. D at 20°C . is of the order of $2.0 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ and its rate of change with temperature is about $3\% \text{ deg}^{-1} \text{ C}$. (Millington, 1955).

2. For a well mixed body of water where the concentration of dissolved oxygen is uniform through the water at any time, the rate of movement from and to the liquid of oxygen across the surface depends on the difference between the actual concentration and the saturation value. The rate of entrance and escape is given by Bohr's equation (Bohr, 1899).

$$d \frac{O_2}{dt} = a \propto (P - p_t) \quad (14)$$

Where a is the surface area; \propto , is the entrance coefficient; P , partial pressure of gas in the atmosphere; and $p_t = P \frac{[O_2]_t}{[O_2]_s}$ where t designates time and s , saturation value at the temperature and ion content.

A modification of this equation was used by Dorsey (1940, page 564) and Redfield (1948) to interpret the escape rate of oxygen from supersaturated water. This was done by introducing an escape coefficient β which contains $\frac{\propto P}{O_2 s}$. β then is given by the following expression:

$$\beta = - \frac{\bar{Z}}{t} \ln \left(1 - \frac{[\Delta O_2]_t}{[\Delta O_2]_0} \right) \quad (15)$$

where \bar{Z} is average depth of water column; t , time; $[\Delta O_2]$

difference between supersaturation and saturation at time t_0 and t .

The above treatments are advanced essentially for dissolved oxygen molecules assuming that only individual molecules take part in the exchange, where the rate of exchange is believed to follow an exponential time dependence (Findlay and King, 1913).

A more important path of oxygen escape in supersaturated waters, especially pertinent to the sea and other natural water bodies, may occur through formation of gas bubbles. These bubbles rise to the surface and escape to the atmosphere in a relatively short time compared to that taken for molecular exchange. It is believed that microbubbles which act as nuclei for formation of larger gas bubbles may result from the biological activities of photosynthesis and respiration, although there is no experimental proof of this mechanism (Ramsey, 1962). Theoretical treatment for bubble formation has been dealt with by Harvey et al. (1944). They attributed the main contributions to bubble formation to difference between total pressure on the bubble and oxygen tension ($P - p_t$) as defined for Bohr's equation (14). However, P is taken here as the sum of atmospheric and hydrostatic pressure over the bubble. Other contributions include sites for growth of nuclei, diffusion and surface tension of the water. Miyake (1951), Hutchinson (1957) and Ramsey (1962) followed by treatments of conditions

of allowable bubble formation in the sea. Their calculated results were based on ion content, temperature, and total pressure as factors determining the capacity of water for saturation. Earlier, Dean (1944) considered bubble formation and cavitation as a result of movement in liquids. These occur in vortices produced by turbulent motion of the liquids. The vortices could expand bubble nuclei on foreign bodies and dust particles, if they were present, and hence largely contribute to bubble formation and escape. The stability of the bubble in the liquid after its formation depends on pressure and the presence of other gases in solution. The capability of one gas to act as a "seed" for bubble formation renders the bubble a mixture of all the gases found in the liquid. Wyman (1952) related the stability of the bubble to concentration of the different gases, the hydrostatic pressure and the relative diffusion coefficients of each gas. He showed further that the size and the relative concentrations of gases in the bubble is subject to a continuous change which accompanies its ascent to the surface.

In order to establish the direct relationship of oxygen supersaturation in sea water with photosynthetic activity of phytoplankton and its variation with growth characteristics, experiments were set up to study unialgal cultures of two commonly occurring species of marine phytoplankton in the laboratory. Laboratory experiments were preferred to field

investigations because important parameters which influence growth such as light intensity, temperature and nutrient concentration, could be controlled. This was felt to be necessary due to the restriction and limitations which may be imposed on a study of this nature in the sea. The continuous variation of growth-controlling parameters in natural water bodies which include temperature, light, circulation as well as predation by animals are but few of the factors that could interfere in assessment of any obtainable result. Furthermore, the time of investigation is restricted to the time and location of the appearance of a phytoplankton bloom, which is usually not readily detectable without some search.

For the purposes of discussing the results, the proposed experiment is divided into three parts: The first includes continuous observation of oxygen and pH in culture water relative to number of organisms, concentration of photosynthetic pigment and the influence of changes in the medium on growth. The second part deals with fluctuation and extent of oxygen supersaturation under various intervals of light and dark periods. The third takes into consideration the behaviour of organism-free oxygen supersaturated water and the effects of salinity, surface tension, stirring and geometry of the water column on desupersaturation rates.

EXPERIMENTAL

I. Methods and Apparatus

1. Oxygen Determination

(i) Winkler method: (After Strickland and Parsons, 1960).

Concentration of oxygen is reported as ml. O_2 (at STP)/l. of water. The experimental error is assumed at $\pm 0.5\%$.

(ii) Microgasometric technique: (After Scholander et al., 1955). Both oxygen and nitrogen are reported as ml. O_2 or N_2 (at STP)/l. of water. The estimated error for this method is $\pm 3-4\%$.

(iii) Electrode system technique: (After Carrit and Kanwisher, 1959). Determination of dissolved oxygen was made with a "Jarrel-Ash Co. Model No. 26-601 Dissolved Oxygen Analyzer" consisting of a silver-silver oxide reference electrode and platinum indicator electrode. The principle involved the measurement of oxygen diffusion through 0.0005" teflon membrane wrapped around the electrodes with a weak solution of potassium hydroxide placed in between the membrane and the electrodes. Since diffusion to the electrode is a measure of the concentration of oxygen, the stirring of the water was an essential requirement. The concentration of oxygen in water is reported as percentage or as ml. O_2 (at STP)/l. of water. Calibration with the Winkler method indicates

an error of $\pm 3\%$.

2. Salinity Determination: (After Strickland and Parsons, 1960). A 10 ml. sea water sample was titrated with a standardized, approximately 0.2 N silver nitrate solution using potassium chromate as an end point indicator.

3. pH Measurement and Total Alkalinity: (After Strickland and Parsons, 1960). The pH and total alkalinity were determined using a "Beckman Model GS pH Meter". pH was determined on the sample of sea water, and total alkalinity on 100 ml. sample of sea water before and after the addition of an exact quantity of 0.0100 N hydrochloric acid.

4. Light Intensity and Radiation Energy: The light intensity used to illuminate the cultures was determined by a "Photovolt Electronic Photometer - Model 501-M", which gave the intensity of the visible region in foot-candles. This was converted into radiation energy using the factor 1 ft.-cdle. = 2.3×10^{-5} langly/min. (langly/min. = $\text{gram-cal/cm}^2/\text{min.}$) (Strickland, 1958).

5. Cell Count: From a well shaken culture, 1 ml. of medium was taken with a pipette and placed in a "No. 800 Sedgewick-Rafter Counting Chamber". The dimensions of the chamber were 5.0x2.0x0.1 cm. The liquid was covered with a thin cover slip, and left for 15 minutes or until the organisms settled completely to the bottom. The chamber was placed under the microscope, and with the aid of a calibrated

eyepiece, five counts were performed over different regions of the chamber. The counts were averaged and multiplied by the calibration factor. The count is reported as No. of organisms/l. of water.

6. Pigment Concentrations: Chlorophyll-a, b, and c and Carotenoids (After Richards and Thompson, 1952, and Creitz and Richards, 1955). The method consisted of filtering a quantity of a well shaken, homogeneously distributed culture medium through a "millipore HA, 0.45 μ pore size" filter. The filter was placed in 5 ml. of 90% acetone for 20 hours. The liquid was then transferred to a 10 ml. centrifuge-tube and centrifuged for 15 min. Three or four mls. were transferred afterwards to a 1 cm. path-length cell and immediately read in a "Beckman D.U. Spectrophotometer" against a cell containing acetone at 665 $m\mu$, 645 $m\mu$, 630 $m\mu$, 510 $m\mu$ and 480 $m\mu$. Concentrations of chlorophyll-a and b are reported in mg./l. and chlorophyll-c and carotenoids in mspu/l.

7. Rates of Photosynthesis and Respiration: (After Strickland and Parsons, 1960). Part of a well shaken culture sample was transferred into 2 - 150 ml. light B.O.D. bottles and 2 - 150 ml. dark B.O.D. bottles. Oxygen concentration was determined on the other part of the initial sample and on the B.O.D. bottles after one to three hours from incubation under the same light intensity and temperature. Rates of photosynthesis and respiration are reported as mg. C/l./hr.

8. Catalytic Activity of Water on H_2O_2 : (After Matsudaira, 1950). A thirty per cent hydrogen peroxide solution was diluted to 1/100 with double distilled water. This was mixed well and kept in the dark at $12^{\circ}C$. A 5.0 ml. of the stock solution was transferred to 50.0 ml. of sample in a 125 ml. Ehrlenmeyer flask and to 50.0 ml. of double distilled water used as a standard. The flasks were incubated for 24 hours at $12^{\circ}C$. after which they were removed and 10 ml. of each was transferred to a flask containing 10 ml. of distilled water, 1 ml. of (1:3) sulphuric acid, 0.5 g. KI and 1 drop of 10% solution of ammonium molybdate. The flasks were left for 3 minutes and then the liberated iodine was titrated with a 0.009 N sodium thiosulphate solution. A small modification of Matsudaira's method was used by taking the catalytic decomposition as the difference between the distilled water standard and the sample instead of the concentration in the sample before and after incubation. Catalytic activity was reported as K_{cat} . $hr.^{-1}$, the reaction rate constant of a first order decomposition rate.

9. Surface Area of Diatomaceous Earth: (After Brunauer, Emmett and Teller, 1938). Surface area was determined with spectroscopic nitrogen at various pressures and was found to be $1.03 \text{ m}^2 \cdot \text{gm}^{-1}$.

10. Surface Tension Measurements - Capillary Rise

Method: (After Daniels, Mathews et al., 1956, p. 52). The capillary radius was calibrated with double distilled water using 71.80 dynes cm^{-1} . at 25.0°C. All measurements were done in a thermostated bath with a Fisher apparatus No. 14-817 (1961).

11. Enriched Sea Water for Culture Growth: Sea water ranging in salinity between 25.0% to 33‰ was filtered through an HA millipore filter of pore size 0.45 μ . To the filtrate, the following ingredients were added in relative concentrations to one liter of water (Sweeney, 1961):

Sodium Nitrate	0.25 gm.
Potassium Monophosphate	0.02 gm.
Sodium Silicate	0.10 gm.
Trade Element Mixtures - Iron	0.5 mg.
Zinc	0.3 mg.
Manganese, Molybdenum, Boron, Cobalt, Copper	0.1 mg.
Thiamine	0.5 gm.

II. Reagents

1. Phytoplankton Species

(i) Nitzschia closterium: An agar slope of this diatom strain was received in February, 1961 from Scripps Institution of Oceanography. It was subcultured immediately into sterilized 125 ml. Ehrlenmeyer flasks containing 50 ml. of enriched sea water under aseptic conditions.

(ii) Chlorella Strain "A": A broth strain was received in March, 1961 from Woods Hole Oceanographic Institution and subcultured as above. (This strain is toxic to clam and oyster larvae: U. S. Fish and Wildlife Survey Vol. 58, 1958.)

2. Diatomaceous Earth: "J. T. Baker" Reagent grade.

3. Chemicals: All chemicals used were reagent grades except "spectranalyzed" acetone.

III. Procedure

A (i) Observation and measurement of oxygen concentration and pH in cultures of Nitzschia and Chlorella.

Two glass troughs 26x16x20 cm. were cleaned thoroughly with nitric acid, rinsed with distilled water, and then wiped with methyl alcohol and dried. Each trough was filled with 3 liters of sterilized and enriched sea water of salinity 27.01‰. One trough was inoculated with Nitzschia closterium and the other with Chlorella strain "A". The troughs were placed on a shelf of an especially built unit under fluorescent "cool white" light source. Measured radiation energy reaching the water surface was of the order of 9.2×10^{-3} ly./min. By means of an automatic control the light period was adjusted to 16 hours continuous illumination followed by 8 hours of darkness. Measurements of oxygen, nitrogen and pH were made every few days, exactly 6 hours after the beginning of the light period. Oxygen and nitrogen concentrations were deter-

mined by the gasometric technique of Scholander et al. (1955).

A (ii) Estimation of pigment concentration, number of organisms, respiration and photosynthetic rates, pH and total alkalinity in a culture of Nitzschia closterium.

Nine plastic (methyl methacrylate) troughs, 26 cm. in diameter and 9 cm. high, were cleaned as before and each filled with 1 liter of sterilized and enriched sea water of salinity 27.01%. Each trough was inoculated with a 2 ml. suspension from a well mixed subculture strain of Nitzschia. These were incubated at $12 \pm 1^{\circ}\text{C}$. under the same light and dark conditions of A (i). Oxygen, nitrogen and pH were determined every few days, 6 hours after the start of light period. Consecutively, every few days, one trough was taken out and its contents transferred to a 2-liter flask and shaken well to (a) give a homogeneous distribution of organisms in the medium and (b) to bring the oxygen concentration near the saturation level. Part of the sample was used to determine photosynthesis and respiration rates, number of organisms, total alkalinity and salinity. The other part was filtered through a millipore filter and the filter was used to determine pigment content of the sample.

A (iii) Determination of catalytic activity of culture water by rate of decomposition of hydrogen peroxide.

From two troughs as in A (i), one inoculated with Nitzschia and the other with Chlorella and each containing 4 liters of

enriched sea water, salinity 25.5%, two 50.0 ml. samples were taken from each concurrently with oxygen, nitrogen and pH determination. The samples were filtered into extra-clean 125 ml. Ehrlenmeyer flasks, 5.0 ml. of hydrogen peroxide stock solution were added to each, and to a distilled water standard; the samples and distilled water standard were incubated at $12 \pm 1^{\circ}\text{C}$. for 24 hours. At the end of the incubation period, the samples and standard were titrated with thiosulphate solution after addition of potassium iodide and sulphuric acid and the rate of decomposition computed from the difference in concentration between standard and sample. Controls for different pH levels were also made on a sample of the original medium before inoculation, and the variation due to pH was used to correct for the results. The results are reported as $K_{\text{cat.}} \text{ hr.}^{-1}$.

B (i) Study of light and dark period effects on the oxygen level in a heavy culture of Nitzschia closterium.

A plastic (methyl methacrylate) tank 44x24x60 cm. was cleaned and half-filled with 32 liters of enriched and filtered sea water, salinity 30.20%. The water was inoculated with Nitzschia closterium and placed in the incubator under the same light condition as before. After 22, 45, and 90 days, oxygen concentration was measured at different depths by means of glass tubes set at different levels in the tank. The samples were extracted by siphoning the water through

a glass tube into a B.O.D. bottle when the Winkler method was used, or the electrode of the oxygen analyzer was employed by moving it vertically in slow motion at the desired depth. Measurements were taken at the beginning of either a light or dark period and at different time intervals afterwards for a total period of not less than 5 hours.

C (i) Measurement of desupersaturation rate constant in distilled and sea water under quiescent conditions.

In a similar tank as B (i) was placed 32 liters of filtered sea water, salinity 32.00‰. Oxygen gas was bubbled through a diffuser stone in the water at $0.5 \text{ liter min.}^{-1}$ for 4 minutes or until oxygen supersaturation exceeded 150%. The tank was placed in the incubator at temperature of $12 \pm 1^\circ\text{C}$. Oxygen concentration was determined at various depths by means of the Winkler method at intervals of 7 to 8 hours. The procedure was repeated for smaller quantities of water in the tank, and also for distilled water. Relative humidity ranged from 84% to 95% in the thermostated room. The desupersaturation rate constant was calculated from the integrated first order rate equation and reported as $K_{des} \cdot \text{hr.}^{-1}$

C (ii) Measurement of desupersaturation rate constant for waters of different salinities under stirred conditions.

One liter of water of salinity 32.80‰ was placed in a 2-liter glass beaker containing a teflon-covered magnet bar. The water was supersaturated by bubbling oxygen gas through it

as in C (i) to a level not exceeding 150%. The oxygen analyzer electrode was placed in the middle of the beaker 2 cm. below the surface, and the magnetic stirrer operated at about 800 r.p.m. Oxygen concentration was read on a recorder chart at different intervals of time. The rate constant, $K_{des.} \text{ hr.}^{-1}$ was computed as before. The same procedure was repeated for water ranging in salinity between 0.00 and 32.80%.

C (iii) Measurement of desupersaturation rate constant in presence of various quantities of diatomaceous earth or different concentrations of surface-active agent (heptanoic acid) under stirred conditions.

To the same tank used in C (i) containing 32 liters of sea water, salinity 27.70% and two magnet bars operated by two stirrers at 800 r.p.m. was added a weighed amount of diatomaceous earth. The water was supersaturated by bubbling oxygen gas through it, and the rate of desupersaturation was measured at different time intervals totalling not less than 7 hours. The same procedure was repeated for 32 liters of sea water salinity 33.01% with addition of different quantities of heptanoic acid. The surface tension was measured after each such addition in a thermostated bath at 25.0°C . The desupersaturation rate constant $K_{des.} \text{ hr.}^{-1}$ is reported for the total surface area of diatomaceous earth, and for variation in surface tension.

RESULTS AND DISCUSSION

A (i) Variation of oxygen concentration and pH in cultures of Nitzschia and Chlorella.

Oxygen concentration and pH were determined concurrently at the fixed time of 6 hours after the beginning of the light period in order to relate photosynthetic activity to changes in these quantities in a sufficient and given time. It is well known, however, that better growth occurs under aeration and shaking of cultures. This is presumably due to the acceleration of carbon dioxide diffusion into the water which buffers the medium and lowers the pH. Nonetheless, an aerated or shaken culture could neither represent a meta-stable system of a dissolved gas where concentration is a function of influx and escape, nor define the limitation of pH as an inhibitive factor. It is assumed, therefore, in maintaining quiescent conditions for these cultures, that at least the influence of outside sources is minimized.

Oxygen, nitrogen and pH values for the two cultures are given in Table XIX and shown graphically in Figure XX for Nitzschia and Figure XXI for Chlorella. It could be seen from these presentations that oxygen and pH follow the same pattern of change, indicating that both are the result of photosynthetic activity and carbon assimilation. The values

TABLE XIX

VARIATION OF O_2 , N_2 AND pH IN CULTURES OF NITZSCHIA AND CHLORELLA

Exp. A (i), Temp. $12 \pm 1^\circ C.$, $S\% = 27.01$, 6 hours light
 Oxygen Saturation Level = 6.17 ml./l.

Initial

Concentration:

Time in Days	Nitzschia 10^6 org./l.			Chlorella 4×10^6 org./l.		
	O_2 ml/l	N_2 ml/l	pH	O_2 ml/l	N_2 ml/l	pH
1	5.75	10.75	8.19	6.25	11.00	8.05
3	6.75	11.13	8.23	6.50	10.88	8.25
5	9.00	11.75	8.60	6.88	11.50	8.32
7	11.00	11.13	9.10	7.87	11.00	8.50
9	12.63	11.38	9.55	8.38	11.50	8.75
10	11.38	11.00	9.63	8.68	11.25	8.77
12	10.88	11.00	9.45	9.63	11.00	8.85
14	11.00	11.00	9.65	9.50	11.13	9.22
15	11.75	11.00	9.70	9.13	11.00	9.25
19	11.25	11.00	9.62	9.00	11.00	9.48
21	11.75	11.00	9.87	9.00	11.13	9.63
23	13.00	11.37	9.65	10.50	11.37	9.49
26	11.00	11.38	10.00	9.00	11.88	9.35
28	10.25	11.38	9.85	9.25	11.63	9.50
30	11.75	11.63	10.05	10.25	11.63	9.40
33	10.38	11.50	9.70	10.00	11.63	9.50
35	10.25	11.63	9.60	10.50	11.63	9.40
37	9.25	11.63	9.55	9.50	11.63	9.50
40	9.75	11.25	9.60	10.50	11.63	9.55
42	9.38	11.63	9.45	10.38	11.63	9.55
44	8.50	11.63	9.45	9.75	11.63	9.60

Oxygen and nitrogen measured by micro-gasometric technique

increase rapidly in the first few days, after which they fluctuate about a maximum level for a period of time reaching over 30 days. Then both values start showing signs of decline in Nitzschia, but not in Chlorella. Nitzschia was by far the more active of the two species, producing oxygen concentrations in water in excess of 200% saturation and pH up to 9.00.

In comparison, the values of oxygen and pH in the Chlorella culture were about 30% and 15% less, respectively.

Estimation and measurement of nitrogen in a photosynthetic medium serves many purposes. On the whole, nitrogen in the sea does not participate in any reaction that could noticeably alter its concentration. Furthermore, it is quite distinct from either carbon dioxide or oxygen, because of its inability to enter into any photosynthetic process. This rather inert nature of nitrogen was conveniently put to use by oceanographers as an indicator of the extent of gas exchange between atmosphere and hydrosphere. The observed concentrations of nitrogen in both cultures, Figures XX and XXI, are seen to assume a relatively constant level with time. However, on the whole, these concentrations were about 1.0 to 1.5 ml/l less than the saturation values for that particular salinity and temperature (Hamm et al., 1941). The rapid production of oxygen under these conditions is expected to displace and "flush" nitrogen out of the medium in the course of its escape to the atmosphere. It was assumed by Redfield (1948) that in localities of oxygen supersaturation in the sea, two-fifths of the total oxygen exchange with the atmosphere is due to oxygen produced by photosynthesis. Hence, use of nitrogen as an indicator for such exchange could not be totally applicable in these regions, at least not during the seasonal occurrence of oxygen supersaturation.

Similar results to those found here had been obtained at sea. In their study of the dynamics of a phytoplankton bloom, Ryther et al. (1958) reported that both oxygen and pH followed a similar course of change during periods of photosynthetic activity. pH varied between 8.5 early in the morning to 9.5 in the afternoon. They assumed that at its maximum no free carbon dioxide was available and any further photosynthesis was dependent upon bicarbonate and carbonate ions. The water was approximately 90% saturated with oxygen at daybreak, after which it increased steadily till it reached a maximum in the early afternoon. One such maximum was reported to have reached 270%.

A (ii) Relation of growth and photosynthetic activity to oxygen supersaturation in Nitzschia.

a. Number of organisms

Frequent determinations of oxygen, nitrogen and pH were carried out on nine separate cultures of Nitzschia incubated at a fixed time interval of illumination in order to account for any deviation which might take place in any one culture apart from the rest. Table XX shows that oxygen and pH were similar at any day's measurement for all cultures within a few per cent of the average values. This agreement allows for treatment of all these separate cultures, being under the same conditions, as one system; further, it permits the termination and study of one certain culture at a time without

TABLE XX

SERIES CULTURE OF NITZSCHIATemp. $12\pm 1^{\circ}\text{C.}$, S‰ = 27.01, 6 hours lightInitial Concentration: 58×10^6 organisms/l.

Time Days	<u>B₁</u>			<u>B₂</u>		
	O ₂ ml/l	N ₂ ml/l	pH	O ₂ ml/l	N ₂ ml/l	pH
1	7.13	12.00	8.55	7.13	12.00	8.50
2	7.50	12.50	8.55	7.13	12.00	8.50
4				8.50	11.63	9.00
6				9.38	11.88	10.10

Time Days	<u>B₃</u>			<u>B₄</u>		
	O ₂ ml/l	N ₂ ml/l	pH	O ₂ ml/l	N ₂ ml/l	pH
1	7.00	11.88	8.50	7.13	12.00	8.63
2	7.75	12.37	8.50	7.25	12.13	8.50
4	9.28	11.88	9.10	8.75	11.88	9.23
6	8.33	11.50	10.38	8.50	12.00	10.50
8	8.75	11.63	10.50	8.33	11.63	10.40
10				8.00	11.50	10.50
13				7.50	11.87	10.50

Time Days	<u>B₅</u>			<u>B₆</u>		
	O ₂ ml/l	N ₂ ml/l	pH	O ₂ ml/l	N ₂ ml/l	pH
1	6.88	12.00	8.55	7.13	12.00	8.65
2	7.38	12.13	8.50	7.25	12.00	8.42
4	8.00	12.00	8.85	7.63	12.00	8.68
6	7.38	11.75	9.22	7.25	11.63	8.82
8	8.25	11.63	9.40	7.00	11.63	8.70
10	8.13	11.63	9.73	8.00	11.88	9.00
13	7.37	11.75	9.80	7.63	11.63	9.00
15	6.88	11.75	9.75	6.88	12.00	9.02
17				7.25	11.63	9.15

(continued)

TABLE XX (Continued)

Time Days	B ₇			B ₈		
	O ₂ ml/l	N ₂ ml/l	pH	O ₂ ml/l	N ₂ ml/l	pH
1	7.25	11.88	8.70	7.13	11.50	8.75
2	7.63	12.00	8.70	7.50	11.63	8.78
4	9.00	11.88	9.47	8.88	11.75	9.47
6	8.37	11.88	9.60	9.00	11.88	9.85
8	8.38	11.63	9.45	8.63	11.75	10.20
10	8.50	12.13	9.40	7.75	11.88	10.27
13	7.50	11.88	9.70	7.25	11.88	10.20
15	7.00	11.88	9.85	7.50	11.88	9.95
17	7.50	11.63	10.45	7.50	11.75	10.10
20	6.38	11.50	10.35	7.75	11.63	10.35
22				7.00	11.63	10.25

Time Days	B ₉		
	O ₂ ml/l	N ₂ ml/l	pH
1	7.13	11.25	8.58
2	7.50	11.63	8.48
4	7.63	11.50	8.88
6	7.00	12.38	9.10
8	7.63	11.75	9.20
10	8.00	11.88	9.40
13	7.75	11.88	9.35
15	7.38	12.00	9.22
17	7.50	11.63	9.30
20	7.13	11.63	9.25
22	6.20	11.38	9.25

Oxygen and nitrogen measured by micro-gasometric technique

disturbing the system as a whole. All essential quantities that were anticipated to influence or control oxygen supersaturation in these cultures are given in Table XXI. These include number of organisms, photosynthetic pigment content, rates of photosynthesis and respiration and total alkalinity.

TABLE XXI

QUANTITIES INFLUENCING OXYGEN PRODUCTION IN A NITZSCHIA CULTURE

Sample No.	B ₁	B ₂	B ₃	B ₄	B ₅	B ₆	B ₇	B ₈	B ₉
Age after incubation	2	6	8	13	15	17	20	22	23
Chlorophyll-a mg/l	34	184	112	121	76	73	130	80	163
Chlorophyll-c mspu/l	28	86	130	117	181	93	209	134	174
Carotenoids non-astacin mspu/l	15	62	61	67	54	57	100	74	116
Number of organisms x10 ⁻⁶ /l	171	622	524	446	915	865	1063	809	1076
Total alkalinity	2.13	2.52	2.34	3.07	2.83	2.69	2.96	2.96	3.07
Rate of photosynthesis mg C/l/hr	0.290	0.912	0.358	0.031	0.156	0.072	0.201	0.040	0.192
Rate of respiration mg C/l/hr	0.059	0.209	0.247	0.237	0.402	0.178	0.230	0.316	0.284

(continued)

TABLE XXI (Continued)

Sample No.	B ₁	B ₂	B ₃	B ₄	B ₅	B ₆	B ₇	B ₈	B ₉
% Saturation of oxygen	121	152	142	122	111	117	103	113	100
<u>Net</u> Gross photosynthesis	0.83	0.84	0.64	0.14	0.32	0.33	0.66	0.13	0.44
<u>Chlorophyll-a</u> Carotenoids	2.29	2.95	1.84	1.81	1.39	1.27	1.30	1.08	1.40
(Chlorophyll-a - Carotenoids)	19.6	98.1	51.0	54.4	21.8	15.0	30.1	5.9	46.5

The total number of organisms reached in each culture had been expected to be an indication of the growth rate and, hence, to some extent, related to the degree of biosynthesis. However, Figures XXII and XXIII show that the number of organisms is not directly proportional to photosynthetic pigment concentration nor to rates of photosynthesis and respiration. On this evidence, cell counts may then be dismissed as indices to the relative degree of biosynthesis in a given sample or to growth rate after the stationary phase of growth is reached. A similar conclusion to this effect was reached by Graham (1943) on the basis of the usually extreme difference in skeleton size or skeleton weight found among the organisms. It is generally believed, however, that chlorophyll and auxiliary pigment assays should afford a more reliable measure of the potential organic synthesis in any given sample or region.

There are many indications that chlorophyll concentration as well as carotenoids vary diurnally for a certain number of organisms, depending on the light intensity (Yentsch and Ryther, 1957; Ryther et al., 1958; Yentsch and Scagel, 1958) and other growth factors. This is independent of new and dead cells variation, but more a change of the stationary phase. Though the organisms may not be actively growing they are not necessarily either dying or perishing in great numbers.

b. Photosynthetic pigment concentration.

It was logical to relate oxygen supersaturation to the magnitude and duration of net photosynthesis in a medium, where net photosynthesis was the excess oxygen produced in presence of respiration and oxidation. The rate of net photosynthesis in turn should be dependent on the concentration or the activity of photosynthetic pigment under constant conditions of light temperature and sufficient nutrient concentrations. Since chlorophyll-a was considered to be the chief agent for conversion of light energy into chemical energy, the primary step was an attempt to compare concentration of chlorophyll-a to rate of net photosynthesis. The relation was non-linear pointing to the possibility of yet another factor which might enter and influence oxygen production. By correlating carotenoid concentration, which is assumed to be a polyfunctional pigment with chlorophyll-a, a linear relation was obtained by plotting the difference of the two pigment concentrations (chlorophyll-a - non-astacin carotenoid) versus rate of net photosynthesis, Figure XXIV. An approximately similar result was obtained by Yentsch and Ryther (1957) and Ryther et al. (1958) by employing the following relationship

$$\frac{\text{chlorophyll-a}}{\text{carotenoids}} = K_1 + \frac{\text{gross photosynthesis}}{\text{net photosynthesis}} \times K_2 \quad (16)$$

Comparison of Figure XXIV to the relationship applied by Yentsch and Ryther, Figure XXV shows that both relationships

behave approximately in a similar manner. Although it appears that the application of difference in pigment concentrations fits the results with less point scatter than the ratio of pigment concentrations, nevertheless, the influence of carotenoids on net photosynthesis cannot be neglected.

Carotenoid pigments have been assumed to play multiple and various roles in the cell, chiefly among these is the transfer of light energy to chlorophyll in diatoms at about 4700Å (Tanada, 1951; Dutton et al., 1943) when the pigments are in the chromatophore. Otherwise certain of these carotenoids are inactive either because of some chemical change in their composition or because of their occurrence outside the chromatophore (Fogg, 1953). In this latter case they most probably act as light scavengers and shields to light penetration to the chromatophores. The carotenoids also contain a large number of double bonds which form conjugated systems giving rise to the possibility of a large number of cis-trans isomers, many of which have been isolated. Retrovsky (quoted by Goodwin, 1953) suggested that fucoxanthin together with violaxanthin and zeaxanthin play a part in redox system in algae. This latter suggestion is shown to apply here by the comparison of the rate of respiration to concentration of carotenoids, Figure XXVI. Further clarification of the complex roles of carotenoids is made by Tanada (1951) who showed that only light absorbed by fucoxanthin, which is one of five

xanthophyll pigments in diatoms is utilized by photosynthesis in the same efficiency as light absorbed by chlorophyll. In the case of the remaining carotenoids, however, light absorbed by them appears not to be used in the photosynthetic process. Pace (1941) found that in Nitzschia culture the average ratio of chlorophyll to carotenoids was 3.77 and that of xanthophylls to carotene to be 8.35. Assuming that fucoxanthin is one-third of the xanthophylls (Strickland, 1960), then one-fourth of the carotenoids could participate actively in photosynthesis. The linear relation of difference in chlorophyll-a and carotenoids to rate of photosynthesis indicates the possibility that the rest of the carotenoids may act as a light shield for the chlorophyll.

Light intensity, furthermore, has a direct influence on photosynthesis not only by the light quanta offered to the pigments but also by the manner it conditions both chlorophyll and carotenoids concentrations in the cell. There is evidence that light intensity and duration directly influence pigment build-up in the sea. Diurnal investigation of pigment concentration relative to light intensity by Ryther et al. (1958) in Senix Creek showed chlorophyll concentration to have increased gradually during the day until it reached a peak at sunset, after which it decreased rapidly throughout the night until daybreak. Also, Emerson (1929), Fleisher (1936), Sargent (1940) and Myers (1946) reported chlorophyll concentration to

have increased with light intensities from 2 to 12-fold range in Chlorella cells. Similarly plant carotenoid pigments increased during the day and decreased at night, (Ryther et al., 1958). The ratio of chlorophyll-a:carotenoid decreased throughout the daylight periods from a maximum observed at sunrise. The change in chlorophyll is usually much larger than in carotenoids. Spoehr and Milner (1949) observed a 10-fold change in chlorophyll to the magnitude of change in carotenoids for Chlorella at high cellular yield.

No correlation of chlorophyll-c to any apparent activity seemed feasible under the experimental conditions, although theoretically chlorophyll-c is supposed to transmit light energy to chlorophyll-a acting as an accessory pigment. Yentsch and Seagel (1958) assume on the basis of Granick's work (1949) that chlorophyll-c is a precursor pigment of chlorophyll-a. The actual interpretation of chlorophyll-c is left, however, for further studies and investigation.

Because of the constant conditions of light intensity, duration and temperature employed in this experiment, the rate of net photosynthesis and hence the difference in pigment concentration was expected to be proportional to the amount of oxygen saturation found in the culture medium at the time of rate determination. Figure XXVII shows per cent saturation to be independent of rate of respiration but in a manner related to rate of photosynthesis. The pattern of the increase in both

quantities is apparent but not to the expected degree. This, however, is not surprising when it is remembered that the conditions under which rates of photosynthesis and respiration were determined were slightly different from conditions in the culture. There are two effects which could be said to have a bearing on the determination and on the obtained results, namely, total alkalinity and bacterial contamination.

c. Total alkalinity

The deviation from linear dependence of oxygen supersaturation in the culture on rate of net photosynthesis may be attributed to the shaking of the culture prior to rate measurement. Shaking thus could introduce an amount of carbon dioxide from the air to the culture changing the condition under which photosynthesis was taking place. This influence may be observed in Figure XXVIII, where the level of oxygen supersaturation is seen to be inversely proportional to total alkalinity.

The highest concentration of Nitzschia closterium in these cultures ranged between 10^8 to 10^9 cells/liter, in agreement with the maximum obtained by other workers (Ketchum et al., 1949; Ketchum and Redfield, 1949). At these concentrations periodic fluctuations in the number of organisms occur regularly involving birth of new organisms and decomposition of dead ones. Stanbury (1931) attributes many of these fluctuations and periodic decrease in number to the siliceous shells of the diatoms dissolving in solution at high pH.

Aleem (1949) used the method of determining the amount of silica in the diatom frustules as a measure of their photosynthetic periodicity. He expressed in terms of unit weight per volume the amount of silica to indicate the amount of active phytoplankton in a sample of diatoms. It appears, therefore, that close connection exists between the level of pH or total alkalinity and metabolic activity of phytoplankton. This photosynthetic inhibition appears to be more dependent on the effect of the chemical action on the siliceous frustules than on the supply of carbon dioxide. The evidence for this last statement comes from Talling (1960), who demonstrated this point by supplying bicarbonate to culture water at high pH. He thus showed the maintenance of growth under buffered conditions with the utilization of bicarbonate ions for carbon fixation instead of carbon dioxide. It is reasonable to suggest then, that at sea or in laboratory cultures, photosynthesis may proceed as long as carbonate or bicarbonate ions are supplied in the medium; and inhibition due to pH may occur only when the level reaches a degree where it could become harmful to the chemical composition of the organisms themselves.

d. Bacterial contamination

The presence of bacteria in a unialgal culture is of certain significance in the conditioning of the mode of growth of phytoplankton. In the stationary phase of growth bacteria

may accelerate to a large extent the rapid breakdown of the dead organisms and hence supply basic needed requirements to the living cells. This rapid breakdown acts in an opposite manner to photosynthetic activity by lowering the pH through the release of carbon dioxide. On the other hand the oxygen demand for any rate of bacterial growth under both light and dark periods may alter the apparent rates of photosynthesis and respiration. Errors of this nature and their observed influence on measurements and growth have been discussed by Pratt and Berkson (1959). They pointed out for example that during measurement of respiration rate at temperatures ranging between 11-21°C. in two-day light and dark bottle experiment, bacteria were responsible for 42.5% to 62.5% of the total respiration customarily attributed to phytoplankton.

In this work, one particular culture, sample no. B₄, showed a large concentration of bacteria which appeared under the microscope during cell count. The sample results used in Figures XXIV, XXV, and XXVIII are designated differently from the rest to contrast the marked deviation from the other points. Although bacterial contamination is not ruled out completely for the other samples, no further comments in this connection are possible for lack of sufficient observation.

A (iii) Relation of catalytic activity to oxygen production in Chlorella and Nitzschia cultures.

The release of cellular compounds into the waters in which phytoplankton grow (Allen, 1914; Gran, 1931a; Matsudaira, 1939; Harvey, 1925 and 1940) has been of prime interest regarding their growth stimulative or inhibitive action. Certain properties were found by Pratt (1942) for Chlorellin, the growth inhibitor formed by Chlorella vulgaris. In some cases these substances were found to be thermolabile. Jorgensen (1956) found that Nitzschia could form autotoxic substances while Asterionella species formed substances which accelerated the growth of Asterionella cells and those of Nitzschia, in some cases. Furthermore, he suggested that growth inhibiting substances formed by algae could account for the unialgal nature of a bloom occurring in any natural body of water, where rarely any other species is ever apparent in the vicinity.

For detectable and identifiable compounds in cultures of many organisms, it has been shown that secretion of extracellular products to the medium parallels that of cell multiplication. Guillard and Wangersky (1958) reported parallel increase in number of seven species of phytoplankton to concentration of carbohydrates secreted. It was also demonstrated earlier by Lewin (1956) that production of polysaccharides in C. parvula cultures appeared to be concomitant with growth and proportional to number of organisms.

The close association between secretion of extracellular products, phytoplankton growth and variation with catalytic activity was demonstrated successfully by Matsudaira (1950, 1951, 1952a). By noting that the aging effect of cultures, as well as the addition of organic materials similar to those secreted by the organisms, simultaneously inhibited the catalytic activity of water, he was able to conclude that inhibition of catalytic activity is also accompanied by growth inhibition. Variation of catalytic activity for Chlorella and Nitzschia are given here in Tables XXII and XXIII and plotted in Figures XXIX and XXX. A comparison is made with the level of oxygen concentration reached in the culture six hours after the beginning of light period. The parallelism of both quantities with time of culture for both species is unmistakable. It may prove fruitful to compare Figure XXIX with that obtained by Guillard and Wangersky (1958) for the production of extracellular carbohydrates relative to number of organisms with time - Figure XXXI. Although the diagram is for the flagellate Isochrysis galbana, a similar relationship was found to hold for Nitzschia, where a reported maximum carbohydrate in solution reached 26 mg./l. The close association of supersaturation, increase of catalytic activity and production of extracellular carbohydrate to the increase in number of organisms in the exponential stage of growth reveals that decomposition of hydrogen peroxide is proportional to extracellular product

TABLE XXII
VARIATION OF OXYGEN CONCENTRATION WITH
CATALYTIC ACTIVITY - NITZSCHIA

Time Days	O ₂ ml./l.	pH of sample	a (distilled water) as ml. Thio- sulphate	a-x (sample corrected for pH and S%) as ml. Thio- sulphate	K _{cat.} hr. ⁻¹ x10 ³
4	7.63	8.10	18.65	17.70	2.19
6	9.50	8.45	18.40	15.30	7.70
11	12.00	8.95	18.13	14.25	10.04
14	15.00	9.20	18.00	13.68	11.44
19	14.50	9.10	17.50	13.95	9.43
23	15.75	9.17	17.32	13.60	10.09

TABLE XXIII
VARIATION OF OXYGEN CONCENTRATION WITH
CATALYTIC ACTIVITY - CHLORELLA

Time Days	O ₂ ml./l.	pH of sample	a (distilled water) as ml. Thio- sulphate	a-x (sample corrected for pH and S%) as ml. Thio- sulphate	K _{cat.} hr. ⁻¹ x10 ³
4	7.13	7.95	18.65	17.80	1.96
6	7.50	8.00	18.45	17.05	3.28
11	8.00	8.40	18.15	16.40	4.23
14	9.50	8.70	17.95	15.70	5.57
19	9.50	9.00	17.45	14.75	7.00
23	12.75	9.25	17.25	14.25	7.97

Oxygen measured by micro-gasometric technique

secretion and concomitant with growth.

The decomposition of hydrogen peroxide in a water medium is dependent, beside other factors, on the chlorinity and pH of the sample, with decomposition increasing with increase of

either chlorinity or pH over 7.0. By correcting for these two quantities there remain the action of metal hydroxide ions on the H_2O_2 decomposition: Cu (Matsudaira, 1952b), Cr, Fe, Ni, Os, Mn and Co, which are but few of the ions that can participate in the catalytic reaction (Schumb et al., 1955). Catalytic action on H_2O_2 from biological compounds may be due to many products of the cell; most important among them is the peroxidase enzyme which occurs widely in plants (Eliot, 1932). Other substances such as carotene also have been reported to exhibit peroxidase-like activity (Schumb et al., 1955).

In order to present a general picture in this work to represent the various reactions of the catalytic activity and mainly to explain the increase of activity with increase of growth and its decrease with culture aging and decay, the following points are suggested:

1. The catalytic activity of a sea water sample depends on chlorinity, pH and metal ions in solution in absence of organic compounds or biological growth.

2. When phytoplankton start growing in the sample, the secretion of extracellular products, or the decomposition of dead cells, produce enzymes which may raise the catalytic activity. The main biological enzymes may be peroxidase and carotene-like substances.

3. The presence of extracellular products may act to enhance growth by providing ready-made complex organic compounds,

or may inhibit their own growth and other species' growth with semioxidized compounds or denatured enzymes.

4. With further secretion of organic material further inhibition or stimulation may be possible; however, organic compounds may react with metal ions at a certain pH to form chelate compounds which may precipitate as particulate matter. This step could deprive the organisms of their trace metal ions and inhibit their growth and at the same time prevent the metal ions from acting as catalysts, thus inhibiting the decomposition rate.

B. Effect of light and dark periods on oxygen concentration in a heavy culture of Nitzschia closterium

The dependence of oxygen concentration on photosynthetic activity, and consequently on duration of light and dark periods as well as on the age of culture, was examined here to account for variation in the saturation level. Table XXIV shows the different concentrations of oxygen at different depths in the culture medium, at three different culture ages, ranging from 22 to 91 days, and their change with light and dark periods under which the culture was incubated. Each age period demonstrated characteristics sharply different from the others, hence allowing for the discussion of three separate cases:

TABLE XXIV

VARIATION OF OXYGEN CONCENTRATION UNDER LIGHT AND DARK PERIODS

IN NITZSCHIA CULTURE

(Oxygen Measured by Winkler and Polarographic Methods)

<u>22 days after inoculation</u>					<u>23 days after inoculation</u>				
<u>Initial</u>		<u>Dark</u>			<u>Initial</u>		<u>Light</u>		
Depth		2	4	6		2	4	6	8
cm.	%O ₂	hrs.	hrs.	hrs.	%O ₂	hrs.	hrs.	hrs.	hrs.
		%O ₂	%O ₂	%O ₂		%O ₂	%O ₂	%O ₂	%O ₂
2	123	117	113	110	120	121	122	126	128
16	121	115	111	108	118	119	121	124	127
30	119	113	109	106	116	118	121	123	126

Surface Tension 72.8 dynes cm.⁻¹

<u>44 days after inoculation</u>			<u>45 days after inoculation</u>		
<u>Initial</u>		<u>Dark</u>	<u>Initial</u>		<u>Light</u>
Depth		5			5
cm.	%O ₂	hrs.	%O ₂	%O ₂	hrs.
		%O ₂			%O ₂
2	148	145	137		142
12	150	145	137		142
22	151	147	138		140
30	150	145	137		140

Surface Tension 72.6 dynes cm.⁻¹

<u>90 days after inoculation</u>			<u>91 days after inoculation</u>		
<u>Initial</u>		<u>Dark</u>	<u>Initial</u>		<u>Light</u>
Depth		5			5
cm.	%O ₂	hrs.	%O ₂	%O ₂	hrs.
		%O ₂			%O ₂
2	78	76	71		72
12	83	78	70		75
22	78	76	70		75
30	82	67	74		74

Surface tension 71.5 dynes cm.⁻¹

Case I

At the age of 22-23 days from inoculation, the culture was assumed to have just reached the stationary phase of growth. All organisms were at the bottom of the container leaving a clear medium for light penetration. No gas bubbles or impurities were noticed either in the body or at the surface of the liquid. Average saturation between dark and light periods was of the order of 121% during the two-day experiment. Variation in oxygen concentrations at different depths in the tank with dark and light periods followed an exponential decrease and increase (Figures XXXII and XXXIII), with a particular oxygen gradient that accompanied all measurements. This gradient, and the fact that oxygen supersaturation was far less than expected in comparison to experiment A (i), indicates a special mechanism for oxygen escape.

If in a young culture most of the organisms are expected to be photosynthetically active, and their activity continuous; then the suggestion that microbubbles may result from such activity may be applicable in this case (Ramsey, 1962). The rapid production of oxygen in a closely packed culture produces a strong tendency for the emitted molecular oxygen to coalesce. When this occurs, rapid diffusion into and out of the microbubble is possible. The microbubble, being buoyant, starts an ascent to the surface. An oxygen concentration gradient may possibly develop under these conditions if the aggregate size

distribution was a function of the depth of the water column. Otherwise, a concentration gradient may form by the action of diffusion and solubility of the different gases participating in microbubble formation. The studies on gas bubbles' stability in liquids by Wyman et al. (1952) suggest that as bubbles rise and hydrostatic pressure decreases more diffusion of gases could take place from bubble to liquid. Since both oxygen and nitrogen have nearly the same diffusion coefficients, their relative escape from bubble to liquid depends more on their solubilities. Oxygen is nearly twice as soluble as nitrogen, thus oxygen diffusion is more rapid to the liquid as the bubble rises than nitrogen. After a certain time, however, the concentration in the liquid becomes limiting for oxygen diffusion and a constant concentration gradient is formed.

Case II

At the age of 44-45 days from inoculation, the oxygen concentration gradient disappeared, and the average saturation in the culture during the two-day measurements became of the order of 145% saturation. The culture medium became slightly cloudy. The escape mechanism of oxygen is assumed to have reverted to molecular exchange with the atmosphere. Probably, because at this stage oxygen production is more sporadic and intermittent than before, the chance for coalescence due to rapid production is decreased, eliminating the effect of

buoyancy and hydrostatic pressure. This allows molecular oxygen to be primarily influenced by molecular and convectional diffusion, and hence remains in the water for a longer time.

Case III

When the culture reached the age of 90-91 days, the medium became very cloudy, and the brown colour in the organisms became more dominant. The surface of the liquid supported a thin layer of impurities composed mainly of bacteria. Surface tension dropped from 72.8 to 71.5 dynes cm^{-1} , and the average saturation for the two-day measurements fell to 75%. It is expected at this time that oxygen production may have been very sporadic and highly dependent, on light penetration through the medium, besides rapid breakdown of organisms and lowering of pH. Bacterial utilization of oxygen, and a high demand in the oxidative process of decomposing substances may have been the prime factors in bringing down the oxygen concentration to well below the saturation level.

C (i) Influence of water column geometry on rate of desupersaturation

To examine for the influence of differently shaped containers, used in previous cultures, on the attained level of supersaturation, the rate of desupersaturation of oxygen was measured for different surface to volume ratios of oxygen supersaturated water. The rate constant K_{des} was a measure of the rate of oxygen escape from physically induced super-

saturation in a certain time interval (Findlay and King, 1913).

$$K_{des.} = \frac{2.303}{t} \log \frac{a}{a-x} \quad (17)$$

Where a is the difference between saturation and supersaturation in ml./l. at time zero, and $a-x$ the difference at time t .

The results are given in Tables XXV and XXVI for both sea water, salinity 30.20%, and distilled water, and also shown graphically in Figure XXXIV. The value $K_{des.} \text{ hr.}^{-1}$ is seen to increase exponentially with increase of surface to volume ratio in both sea and distilled water.

Comparison of the rate constant $K_{des.}$ for saline water with rate of decrease of oxygen under dark periods in experiment B for the same surface:volume ratio, gives further evidence to the proposed mechanisms in Cases I and II. The calculated $K_{des.}$ (biologically active) for the 22-day-old culture is $.107 \text{ hr.}^{-1}$, and $.0190 \text{ hr.}^{-1}$ for the 45-day-old culture. While the calculated $K_{des.}$ (organisms-free) for the same surface:volume ratio of 0.0323 cm.^{-1} gives $.0186 \text{ hr.}^{-1}$. This is an indication that organism consumption of oxygen in the first case, i.e., in the younger culture, and its proposed escape by microbubble formation exceeds that of molecular escape by about six times. On the other hand, most of the oxygen loss in the older culture (45 days) is due to molecular escape through the surface.

TABLE XXV

EFFECT OF SURFACE TO VOLUME RATIO ON
DESUPERSATURATION RATE CONSTANT

$$t = 12 \pm 1^{\circ}\text{C.} \quad S = 30.2\%$$

Surface: Volume cm. ⁻¹	a O ₂ ml./l.	a-x O ₂ ml./l.	t hrs.	<u>Kdes.</u> hr. ⁻¹
0.0322	6.15	5.30	8.00	0.0186
0.0417	5.27	4.12	7.00	0.0352
0.0541	7.30	5.79	7.00	0.0331
0.0690	7.74	5.80	7.00	0.0412
0.1053	7.08	4.55	7.50	0.0590
0.2000	6.94	3.69	7.00	0.0902
0.2500	7.50	3.40	8.00	0.0986

TABLE XXVI

EFFECT OF SURFACE TO VOLUME RATIO ON
DESUPERSATURATION RATE CONSTANT

$$t = 12 \pm 1^{\circ}\text{C.} \quad \text{Distilled Water}$$

Surface: Volume cm. ⁻¹	a O ₂ ml./l.	a-x O ₂ ml./l.	t hrs.	<u>Kdes.</u> hr. ⁻¹
0.0322	3.70	3.28	7.00	0.0172
0.0400	3.54	3.15	7.25	0.0161
0.0500	4.30	3.43	7.50	0.0302
0.0714	4.52	3.57	7.00	0.0337
0.1111	3.90	2.73	7.00	0.0505
0.2000	4.10	2.25	7.50	0.800
0.2500	3.67	1.82	7.50	0.935

C (ii) Influence of salinity on rate of escape of oxygen under stirred conditions

The escape of oxygen from stirred or quiescent water is governed by a molecular layer at the surface which is usually at complete equilibrium with the atmosphere above it. The escape is governed in quiescent waters by the magnitude of the concentration gradient that forms subject to molecular or convectional diffusion. The effect of stirring normally is to decrease the thickness of the surface layer thus increasing the rate of escape, or to increase the concentration gradient resulting in the same effect. Further extreme conditioning in the surface layer, such as rapid evaporation or breaking of the surface enhances escape of gas to the atmosphere.

The oxygen desupersaturation rate constant was measured under stirred conditions for water ranging in salinity between 0 and 32.82‰. The rate constant $K_{des.}$ is shown in Table XXVII and Figure XXXV to increase with salinity. These results are in accordance with investigations of oxygen solubility in distilled and ion-containing water in the first part of this work. The tendency in ion-containing water is to achieve a more rapid equilibrium with the dissolved gas than in ion-free water due to the weaker bond formation with the ion-containing water.

TABLE XXVII

VARIATION OF DESUPERSATURATION RATE CONSTANT WITH
SALINITY UNDER STIRRED CONDITIONS

S‰	a % O ₂	a-x % O ₂	t hrs.	<u>Kdes.</u> hr. ⁻¹
0.00	48.6	18.8	2.0	.475
4.10	45.3	15.4	2.0	.561
8.20	46.7	9.5	2.5	.637
16.41	42.1	14.4	1.5	.715
21.30	44.9	9.4	2.0	.782
24.61	47.3	10.2	2.0	.767
32.82	41.2	11.2	1.5	.869

Oxygen measured by polarographic method

C (iii) Influence of diatomaceous earth and surface tension on desupersaturation rate constant

The use of diatomaceous earth as a means to examine for nucleation and microbubble formation of gases in the presence of photosynthetic organisms was necessitated by the difficulty of employing living or dead organisms. This is understandable when photosynthesis, respiration and oxidation are considered in such systems. The errors which may be introduced by these side reactions could completely cover any apparent influence resulting from nucleation. The nearest similar substance that may act in the capacity of diatoms, and provide some information would be the aged deposits of diatoms themselves. Table XXVIII gives values of Kdes. for the surface area of diatomaceous earth added to water under stirred conditions.

TABLE XXVIII

INFLUENCE OF DIATOMACEOUS EARTH WALL EFFECT ON K_{des} .

- Stirred -

 $S\% = 27.70$ Temp. = $12 \pm 1^\circ\text{C}$.

Diatomaceous Earth gm./l.	Surface Area m^2	a O_2 ml./l.	a-x O_2 ml./l.	t hrs.	K_{des} . hr.^{-1}
0.000	0.00	7.45	5.55	7.00	.0420
0.031	1.03	5.75	4.05	7.60	.0468
0.062	2.06	7.40	5.50	7.00	.0423
0.124	4.12	6.47	4.65	7.50	.0440
0.186	6.18	5.85	4.05	8.00	.0459
0.248	8.24	6.55	4.80	7.00	.0444
0.372	12.36	6.55	4.75	7.00	.0459

TABLE XXIX

INFLUENCE OF SURFACE TENSION ON K_{des} .

- Stirred -

 $S\% = 33.01$ Temp. = $12 \pm 1^\circ\text{C}$.

Conc. of Heptanoic Acid mg./l.	Surface Tension dynes cm.^{-1}	a O_2 ml./l.	a-x O_2 ml./l.	t hrs.	K_{des} . hr.^{-1}
	72.9	6.55	4.30	7.00	.0601
50	72.6	7.15	4.65	7.00	.0615
100	72.2	8.00	5.25	7.00	.0601
150	71.6	6.00	3.85	7.00	.0633
200	70.9	4.65	2.80	7.50	.0677
250	68.2	5.97	4.07	7.00	.0640
300	66.8	4.60	3.00	7.00	.0610
350	64.1	4.00	2.70	7.00	.0561
400	61.3	4.30	3.10	7.00	.0546

Oxygen measured by the Winkler method

However, as shown in Figure XXXVI, no apparent effect is observable. The results may mean that either these aged organisms lack certain surface substances which could originally have induced bubble formation, or actual bubble formation results only from a highly active photosynthetic region in the presence of the live organisms.

Addition of surface active agents, however, proved more fruitful. The desupersaturation rate constant increased with decreased surface tension upon the addition of small quantities of heptanoic acid then decreased with further addition, Table XXIX. Figure XXXVII shows the change in the rate of desupersaturation with surface tension. These results are similar to those obtained by Eckenfelder and Barnhart (1961) for the influence of surface tension on the coefficient of transfer of a gas, such as oxygen, to the liquid from a gas bubble. They found that the transfer coefficient decreased rapidly with decrease of surface tension, then increased or remained constant on further addition of substances such as peptone and heptanoic acid.

The decrease in surface tension on addition of surface active compounds, results from the concentration of these molecules at the surface of the liquid. With small additions, the surface forces decrease and allow for a more rapid escape of the supersaturated gas to the atmosphere. However, further addition produces a monolayer film at the surface of the liquid

which may act as a shield for further escape of gas. A large proportion of the gas which escapes from the uppermost thin layer is also dependent on the rate of evaporation of the liquid. The presence of the organic monolayer film may cut evaporation to about 0.01 of its value in pure water (Bikerman, 1958, p. 83). Therefore, more addition of surface active agents results in either a constant or a decreased rate of escape.

CONCLUSION

Experimental results in this work have confirmed the following, previously known points:

1. Oxygen supersaturation in sea water is directly related to photosynthetic organisms.
2. Oxygen consumption due to respiration is related to the concentrations of carotenoid pigments in the organisms.
3. Oxygen production and photosynthesis in diatoms are influenced by total alkalinity and pH of the medium.

On the other hand, the following new relations are deduced from experimental results:

1. The biological activity of Nitzschia closterium in saline water ranging between 25‰ and 31‰, and at temperature of 12°C., exceeds that of Chlorella strain "A".
2. Oxygen production may be related to the difference in concentration between chlorophyll-a and carotenoids under the same light intensity and duration and at constant temperature.
3. Extracellular production of organic compounds and the catalytic activity are concomitant with growth.
4. In new and highly active cultures, microbubble formation may be one of the oxygen paths of escape to the atmosphere.
5. Depth of the water column, stirring and salinity influence the rate of escape of oxygen from the supersaturated

water to the air.

6. No observable effect on the rate of oxygen escape can be attributed to the presence of siliceous substances, as demonstrated by addition of diatomaceous earth to the supersaturated water.

7. A small decrease in surface tension on addition of organic, surface active agents increases the rate of escape of oxygen from the supersaturated water. Further addition of the organic substance decreases surface tension and also decreases the rate of oxygen escape.

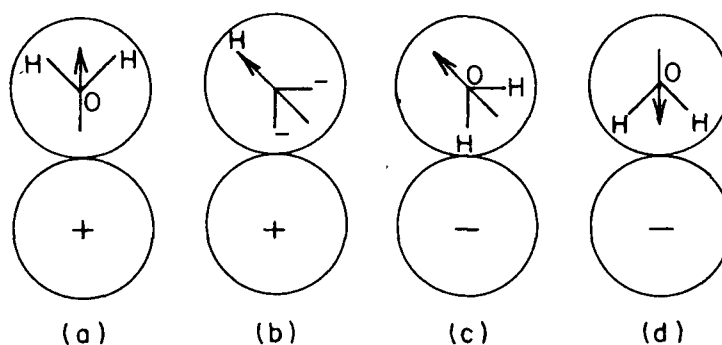


Figure I. Proposed Dipole Orientation of a Water Molecule in Presence of Ions (From Hindman, 1961)

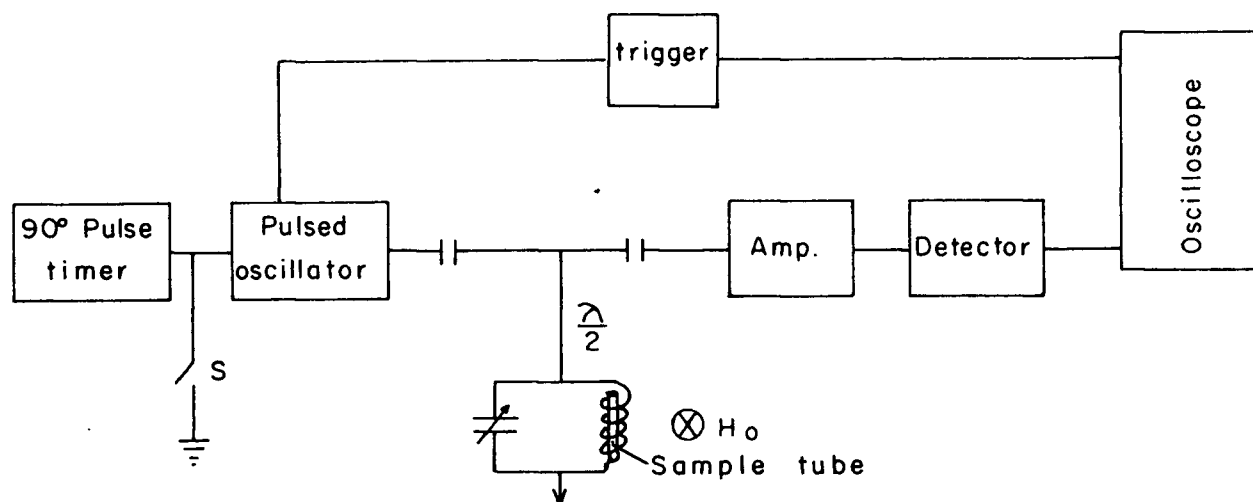


Figure II (a). NMR Assembly

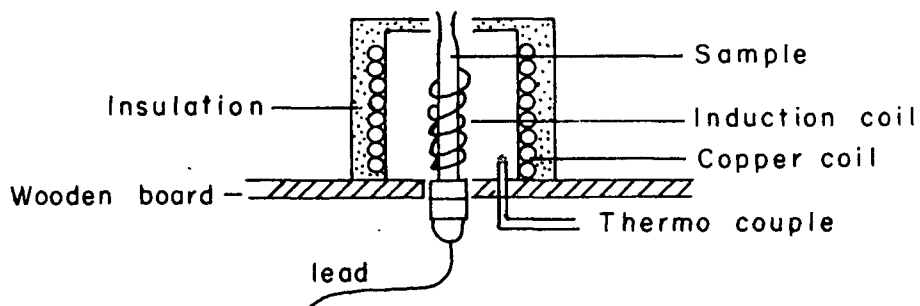


Figure II (b). Induction Coil Chamber

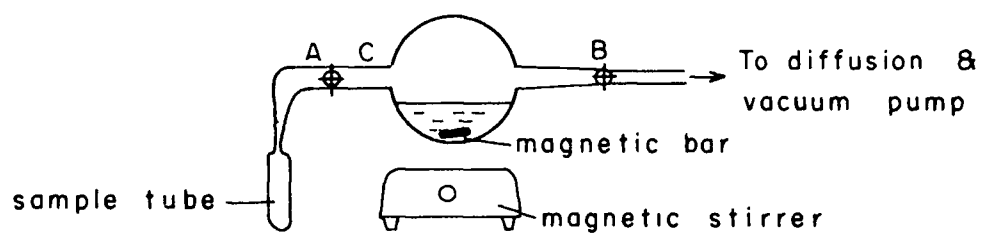


Figure II (c). Degassing Instrument

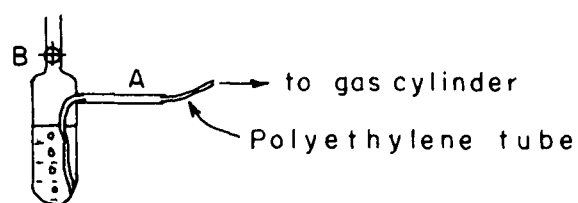


Figure II (d). Sample Tube

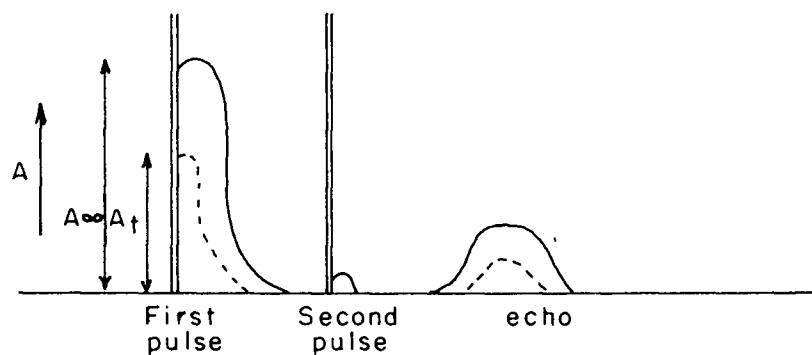


Figure III. Oscilloscope Display of the Two Pulses and the Detected r-f Signal

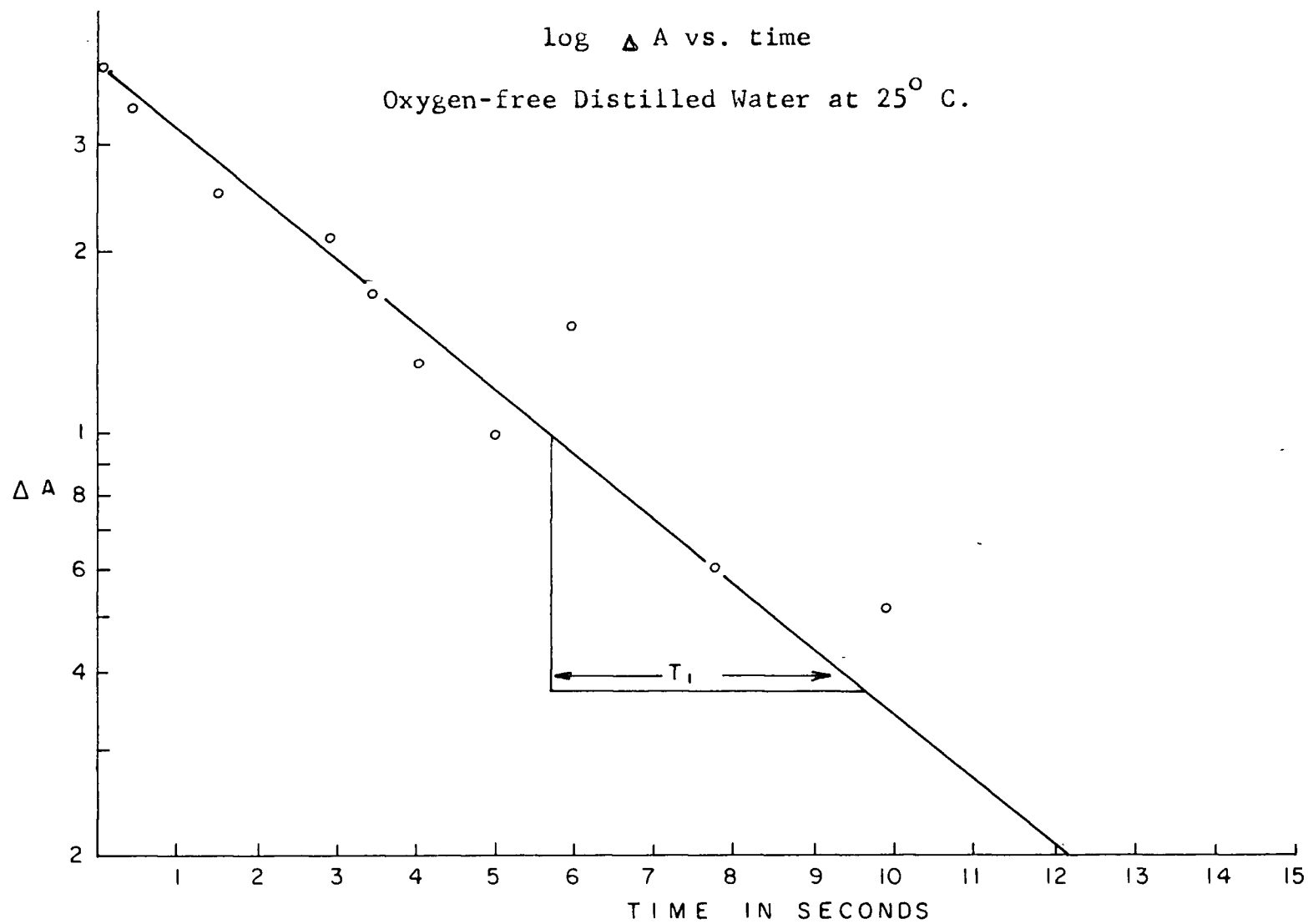


Figure IV. Determination of T_1

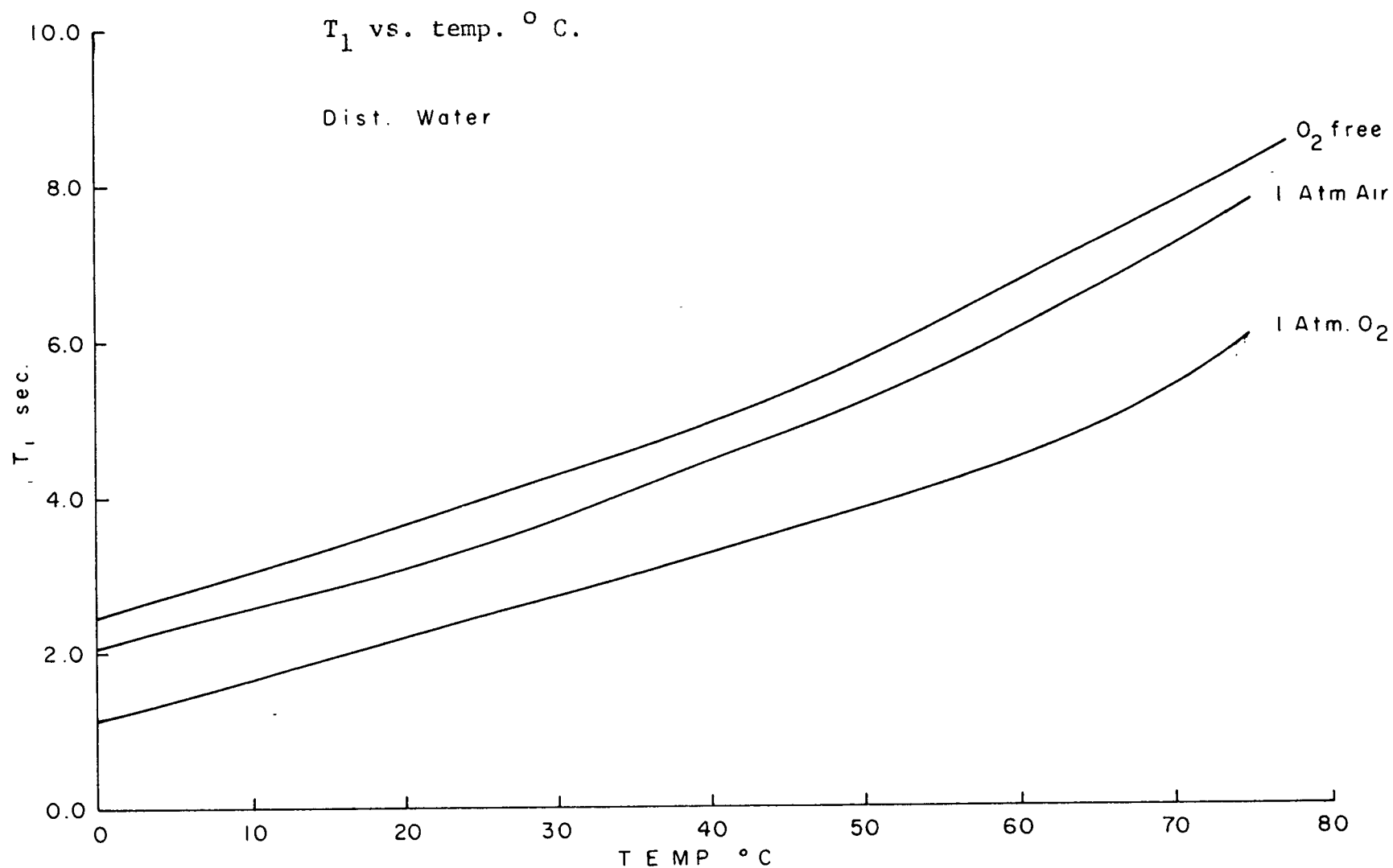


Figure V. Smoothed Curves of T_1 Values at Different Temperatures (Distilled Water)

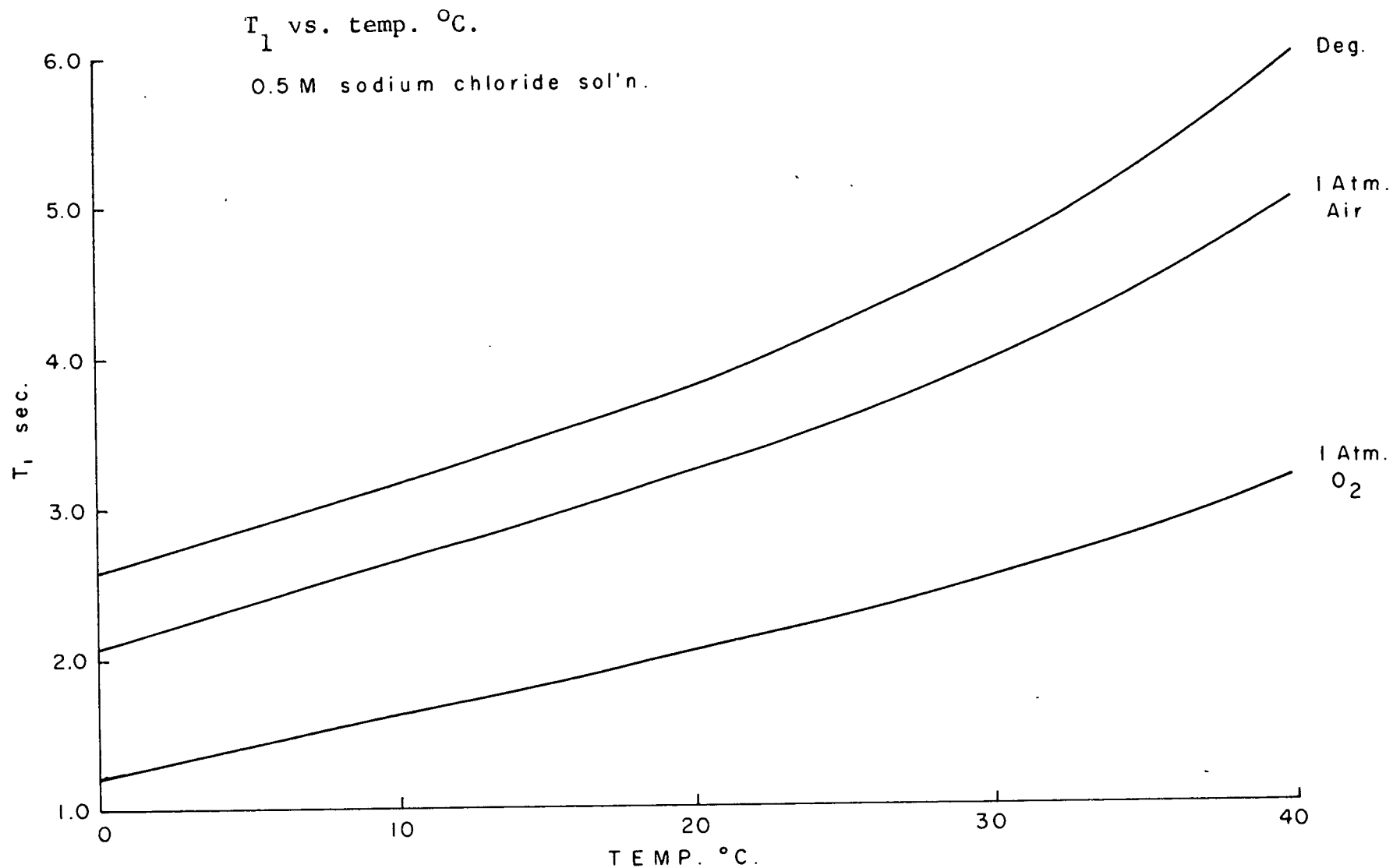


Figure VI. Smoothed Curves of T_1 Values at Different Temperatures (Salt Solution)

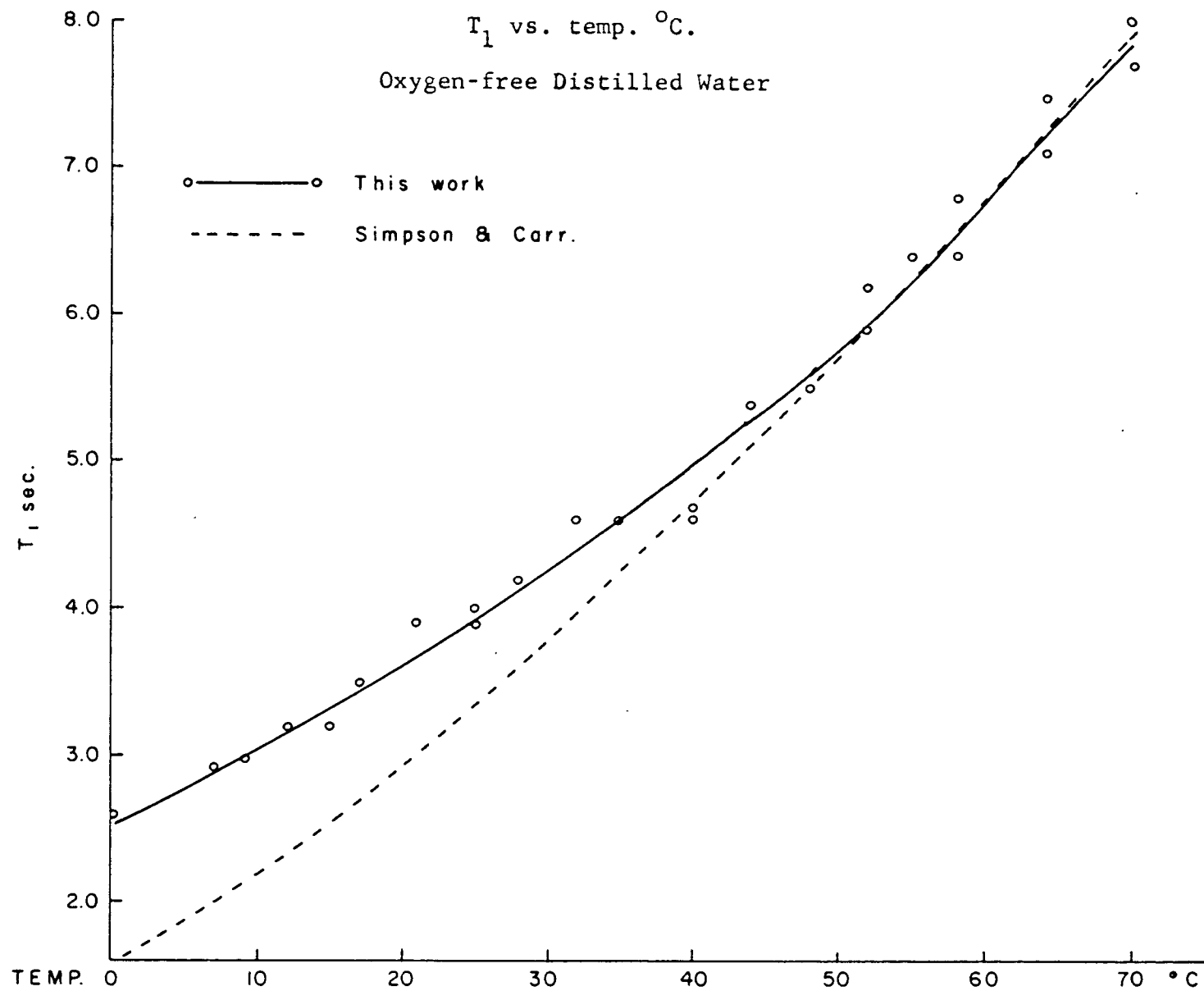


Figure VII. Comparison of T_1 Values for Oxygen-free Distilled Water

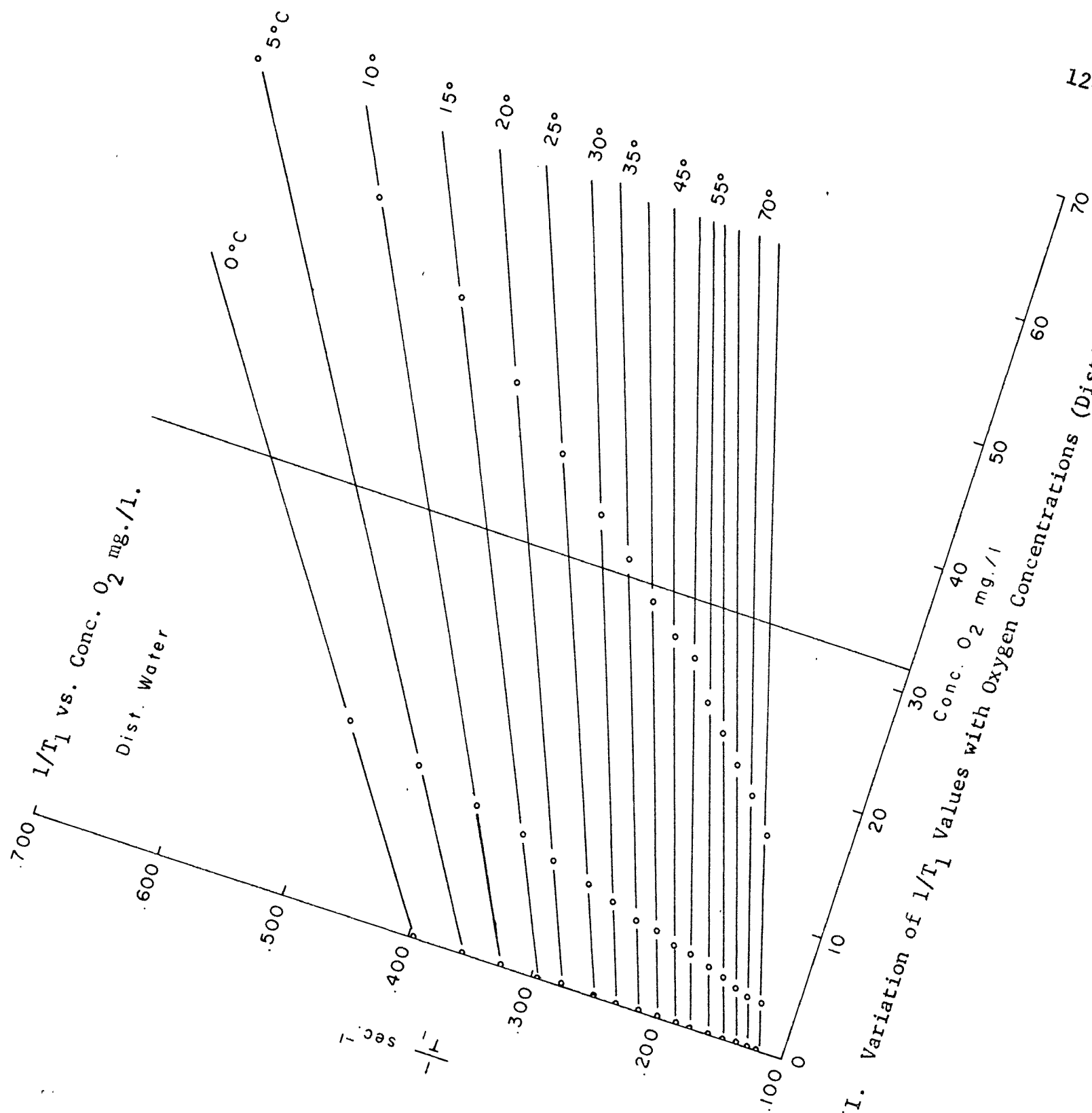


Figure VIII. Variation of $1/T_1$ Values with Oxygen Concentrations (Distilled Water)

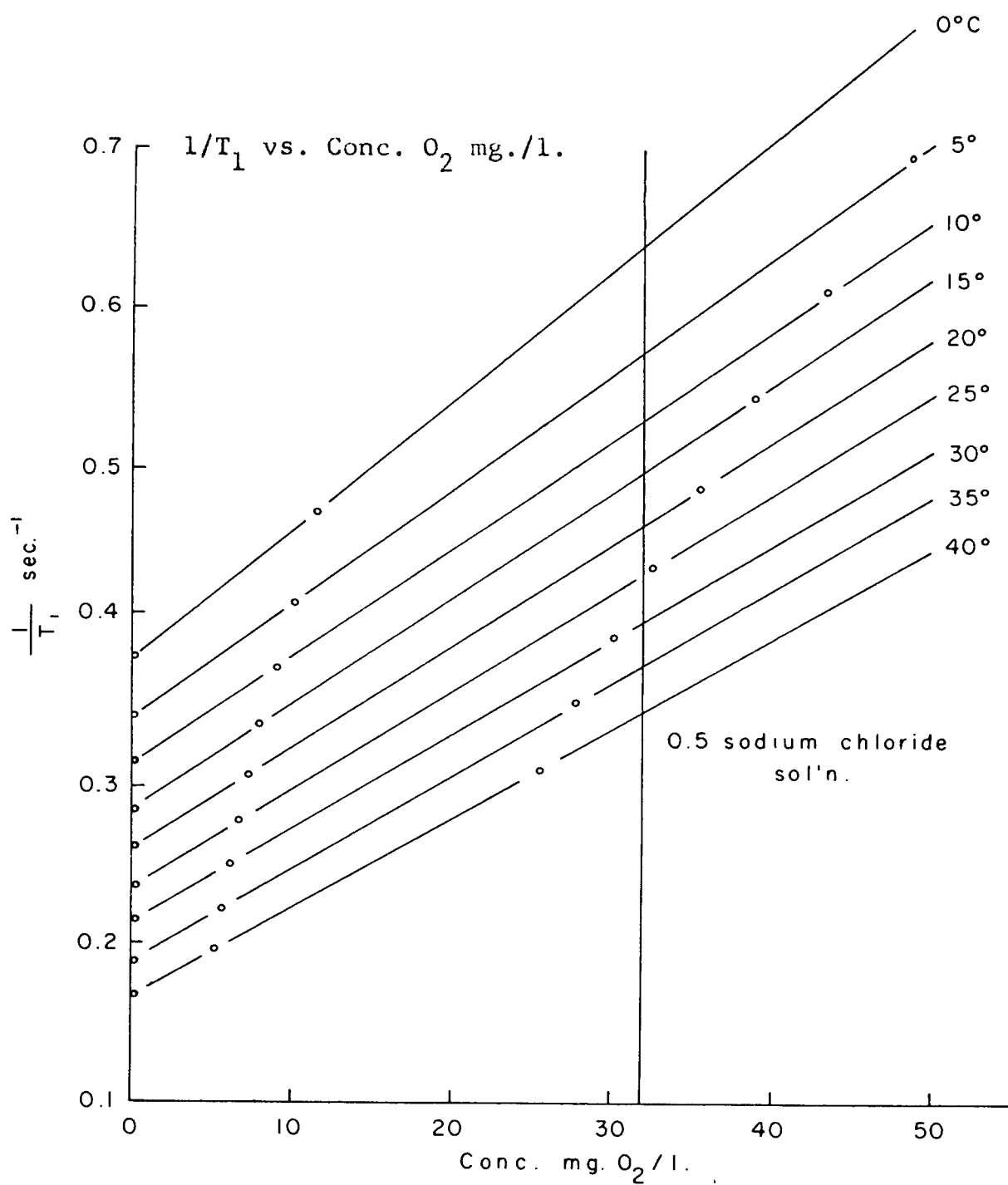


Figure IX. Variation of 1/T₁ Values with Oxygen Concentrations (Salt Solution)

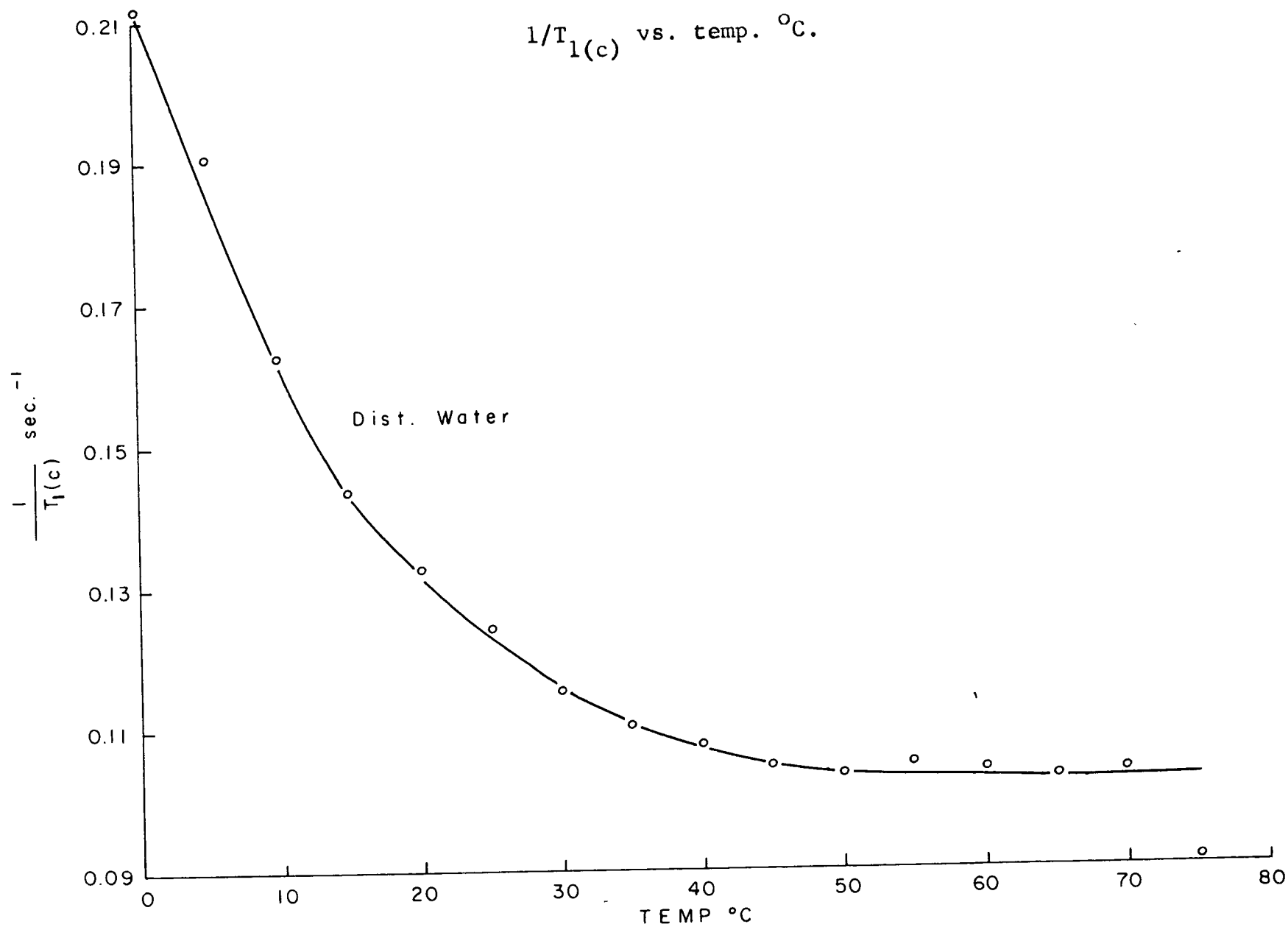


Figure-X. Behaviour of $1/T_{1(c)}$ with Temperature (Distilled Water)

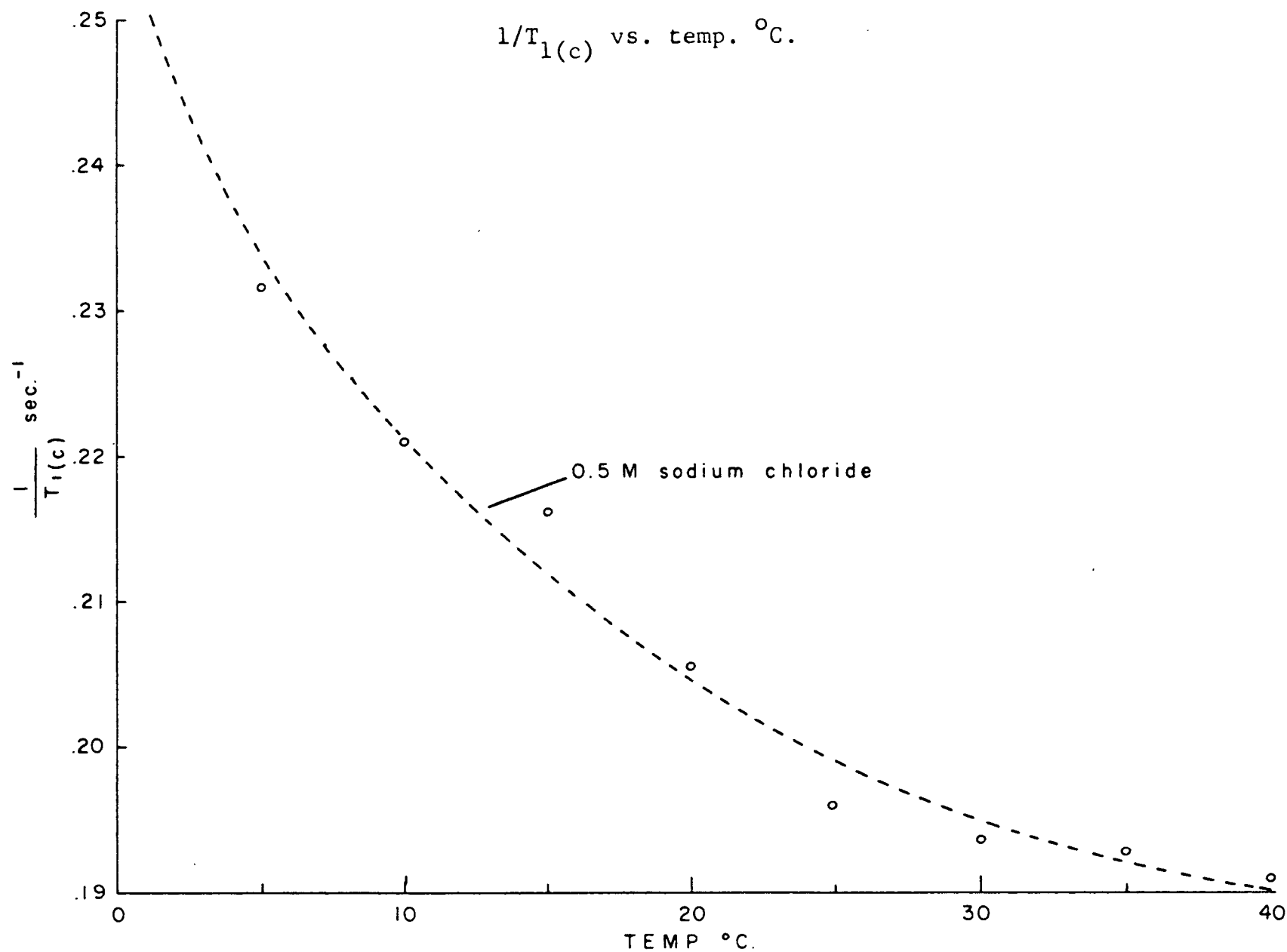


Figure XI. Behaviour of $1/T_{1(c)}$ with Temperature (Salt Solution)

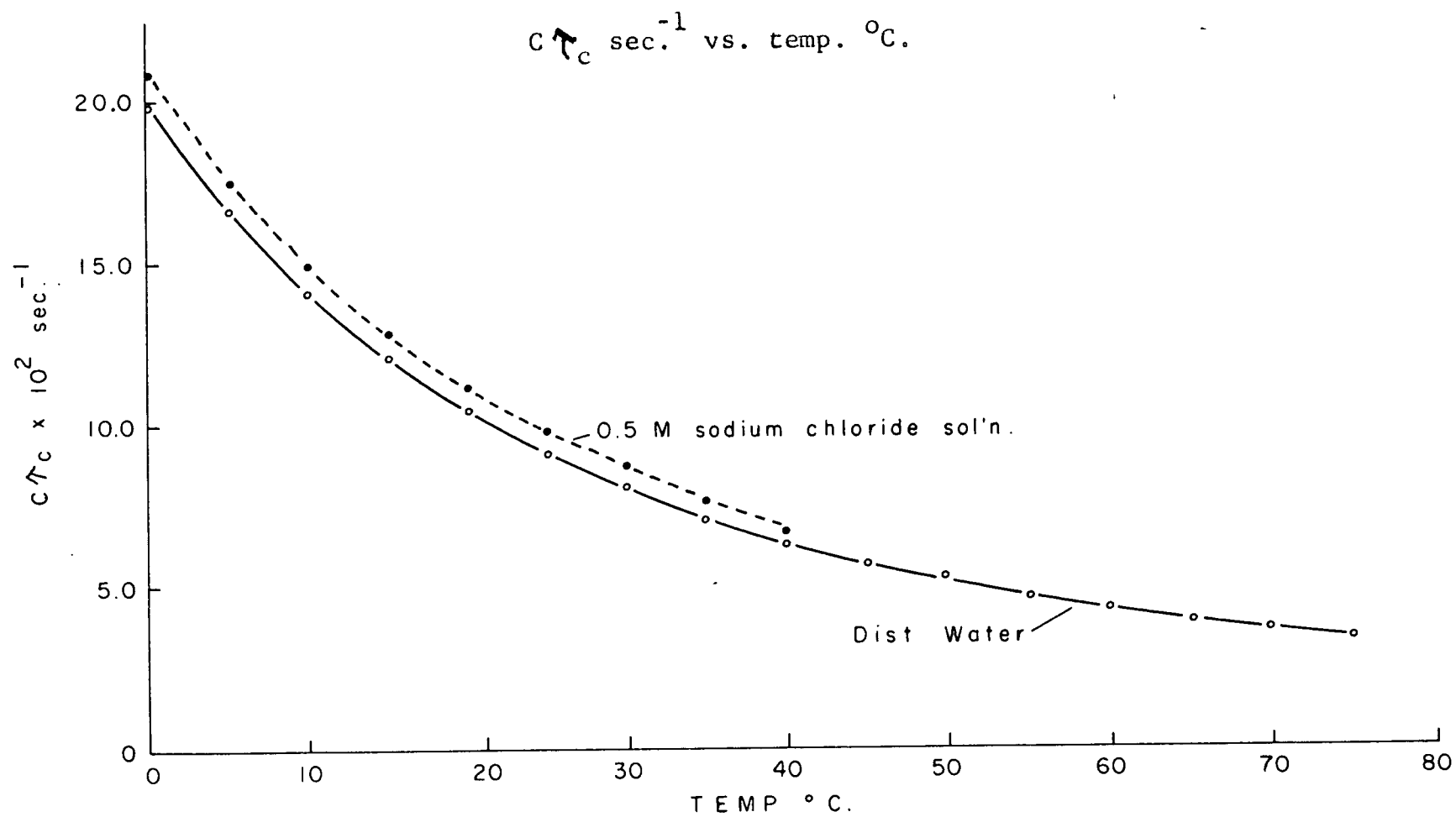


Figure XII. Change of $C \tau_c$ Term with Temperature

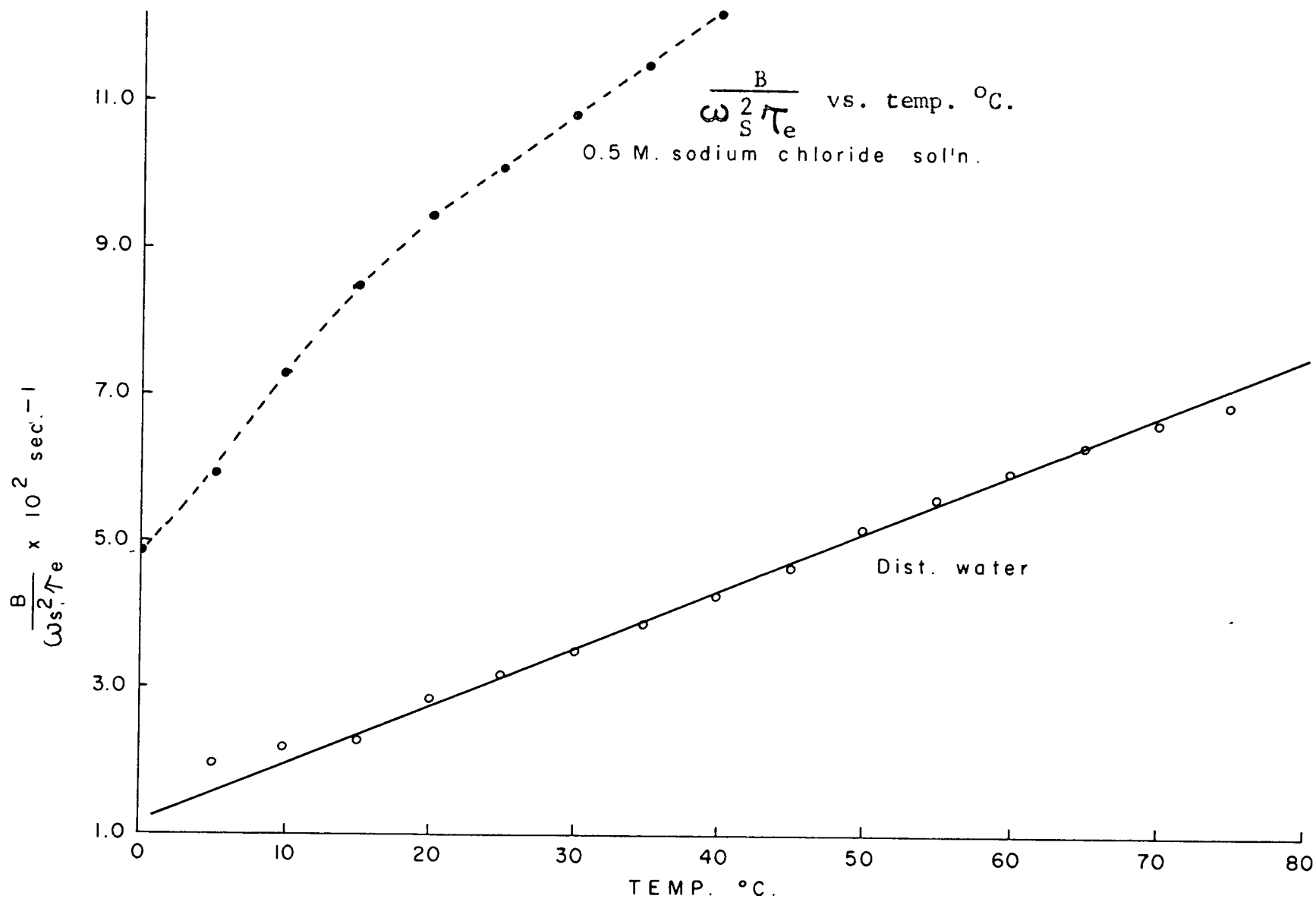


Figure XIII. Change of $\frac{B}{\omega_s^2 \tau_e}$ Term with Temperature

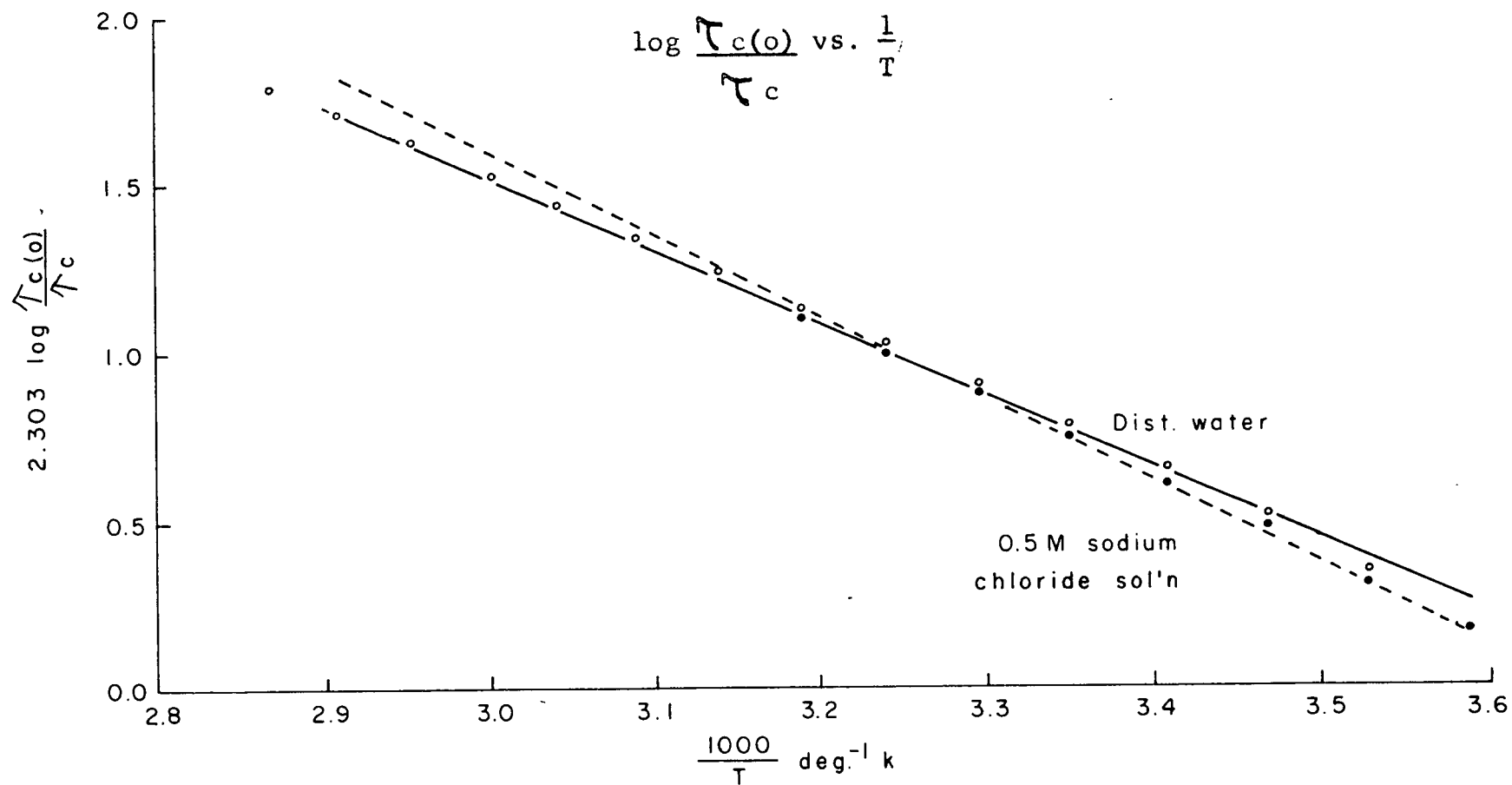


Figure XIV. Determination of the Activation Energy E_c

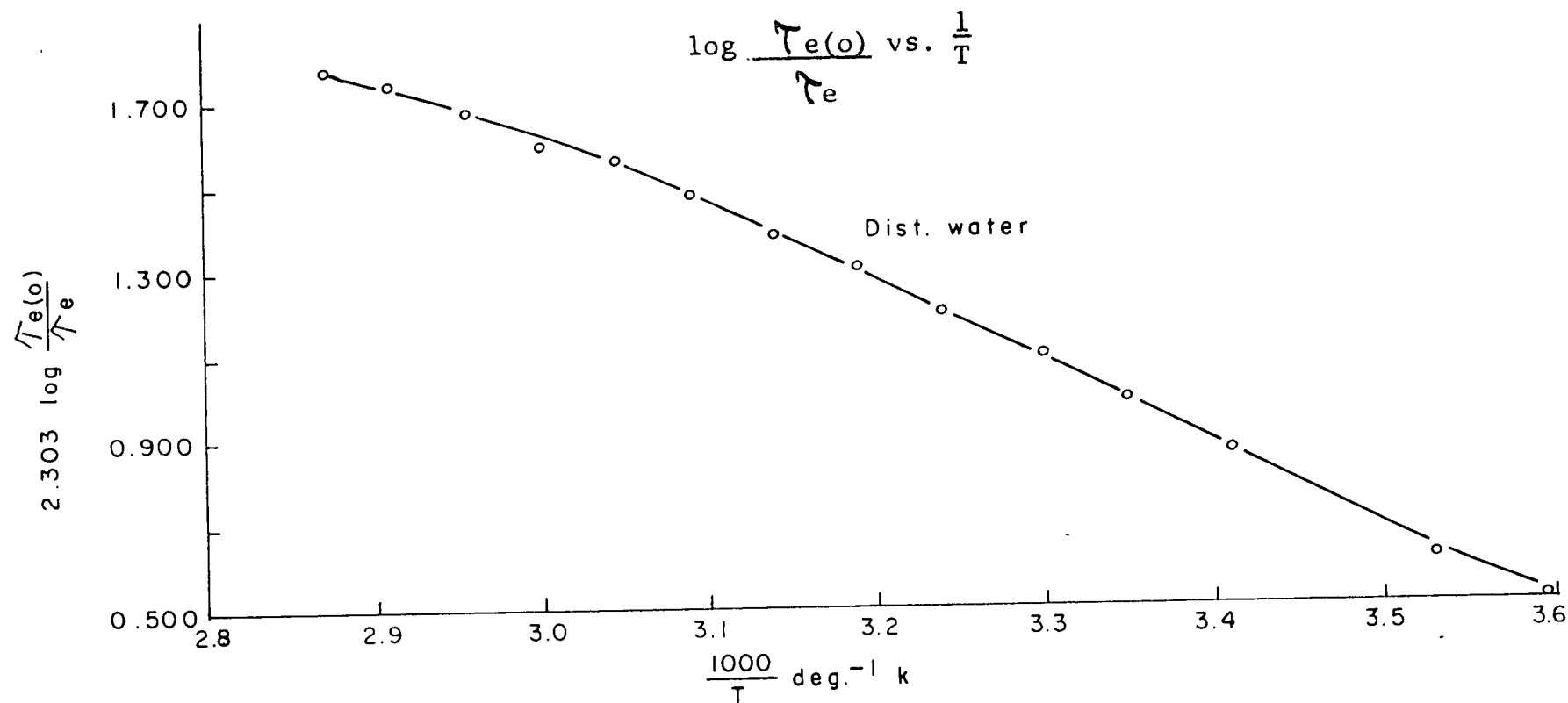


Figure XV. Determination of the Activation Energy E_e
at Different Temperatures (Distilled Water)

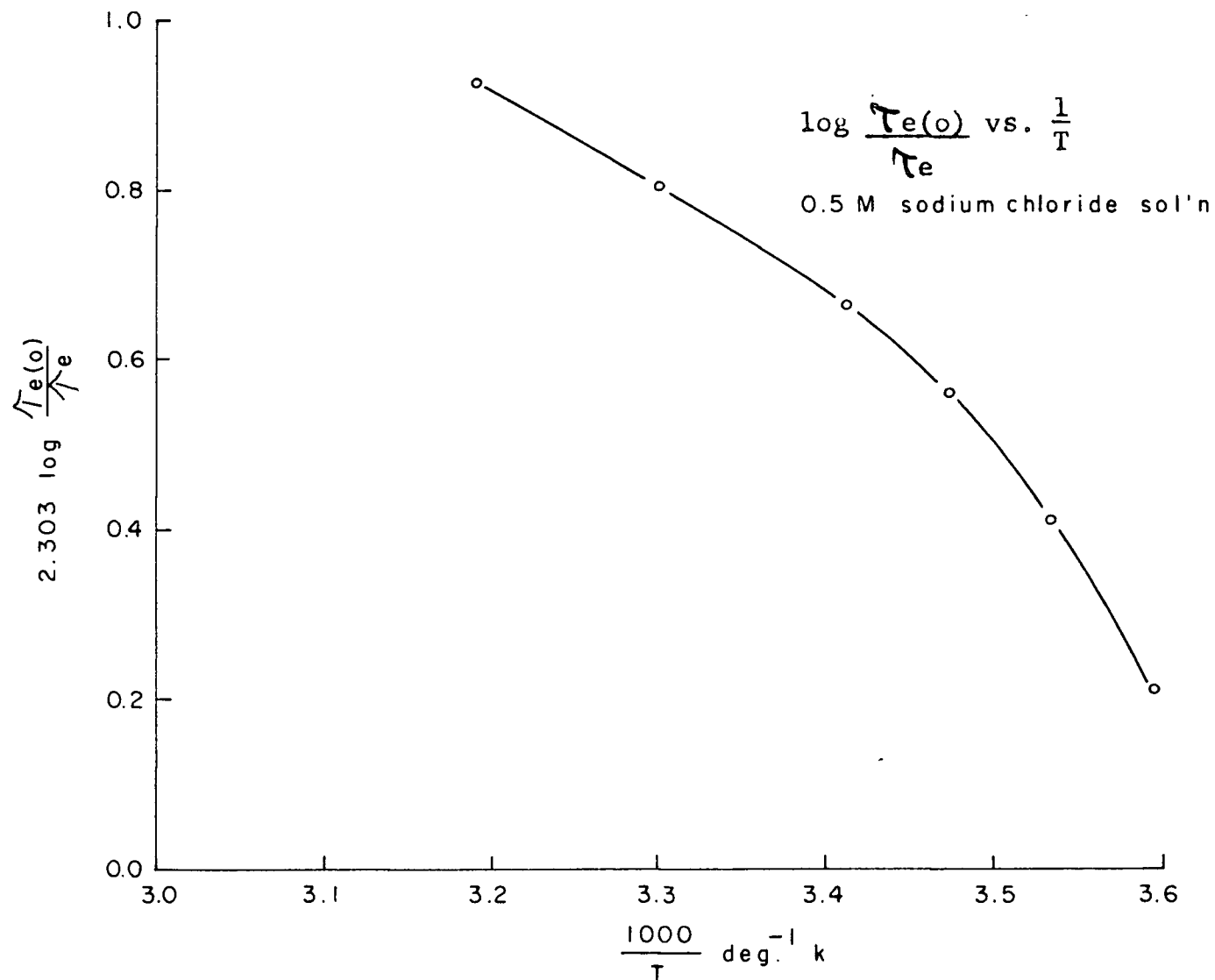


Figure XVI. Determination of the Activation Energy E_e at Different Temperatures (Salt Solution)

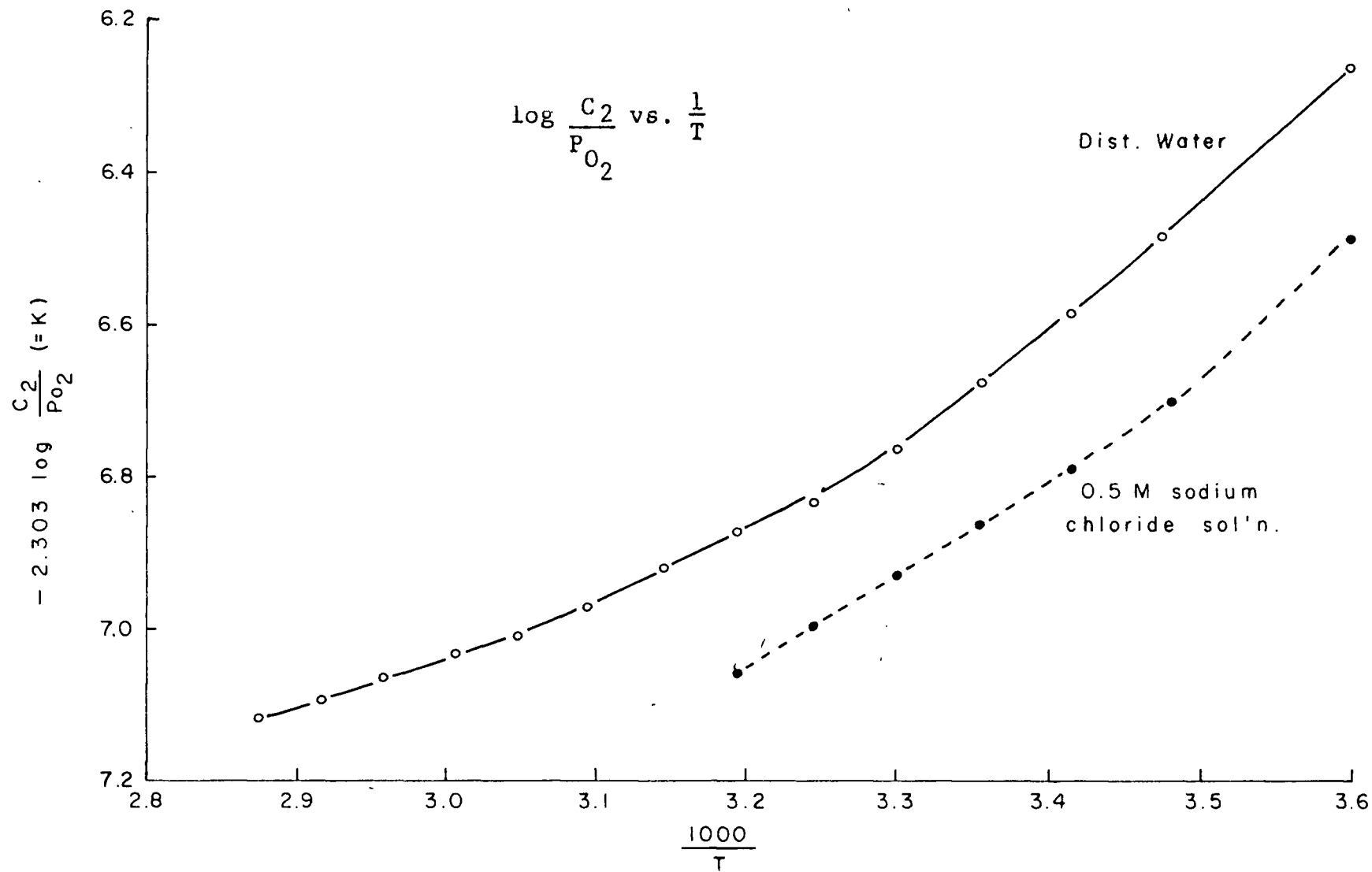


Figure XVII. Determination of the Heat of Solution $-\Delta H$ for Oxygen in Water under 1 Atm. Pressure

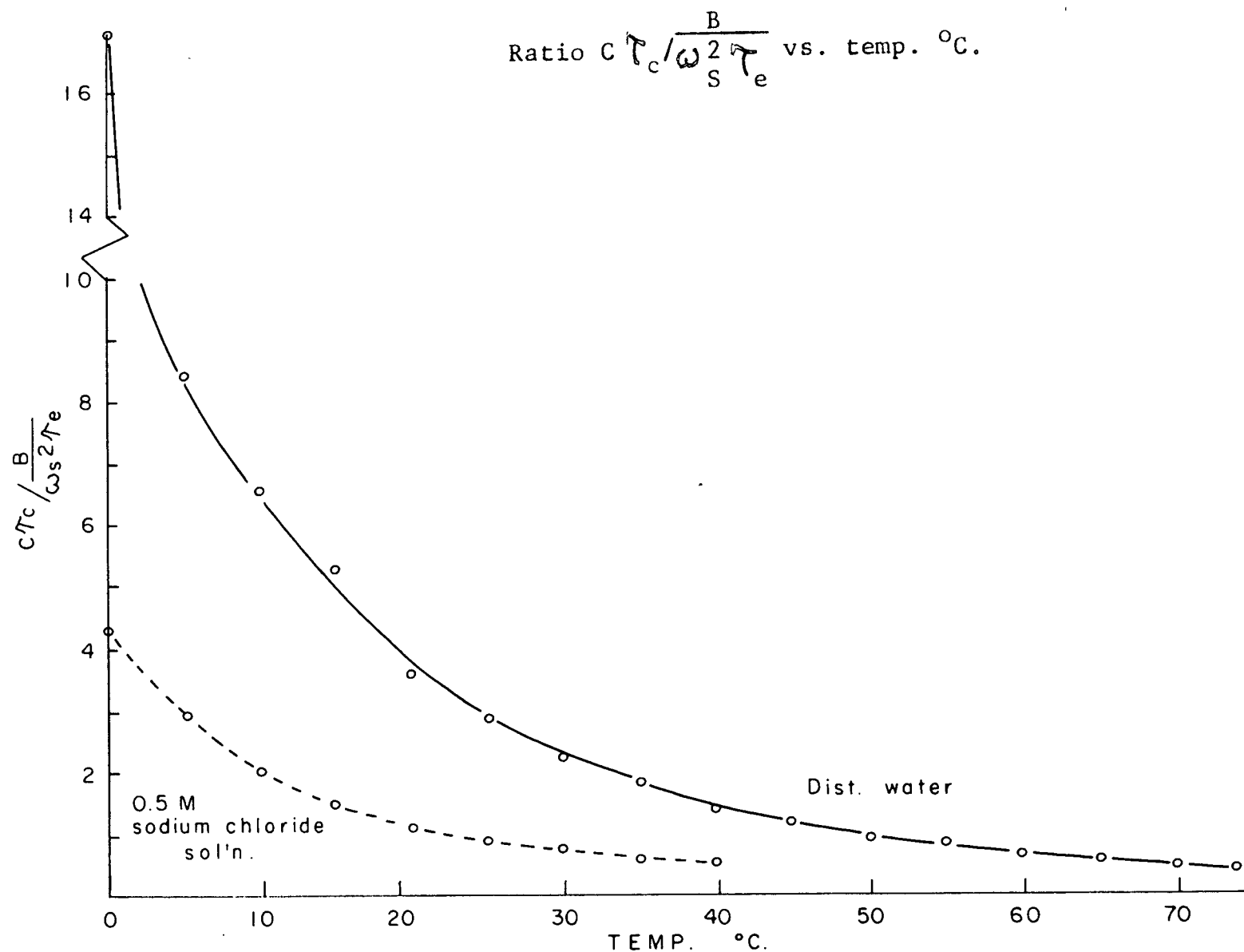


Figure XVIII. Variation of the Ratio of the Two Relaxation Terms
- Equation (8) - with Temperature

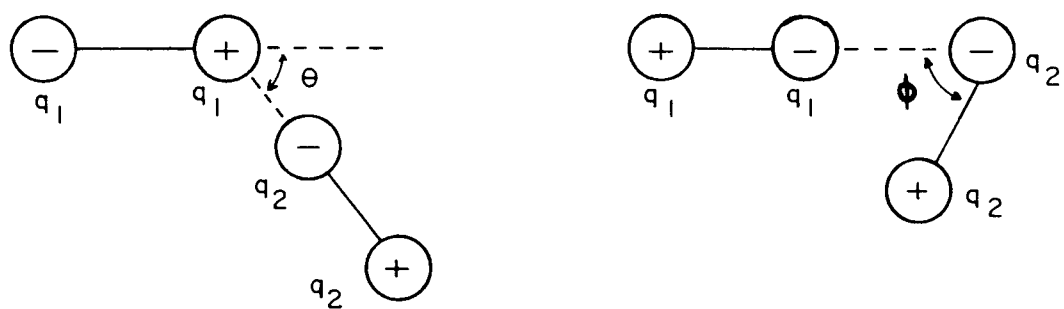


Figure XIX.

Two Different Orientations of Two Dipole Molecules

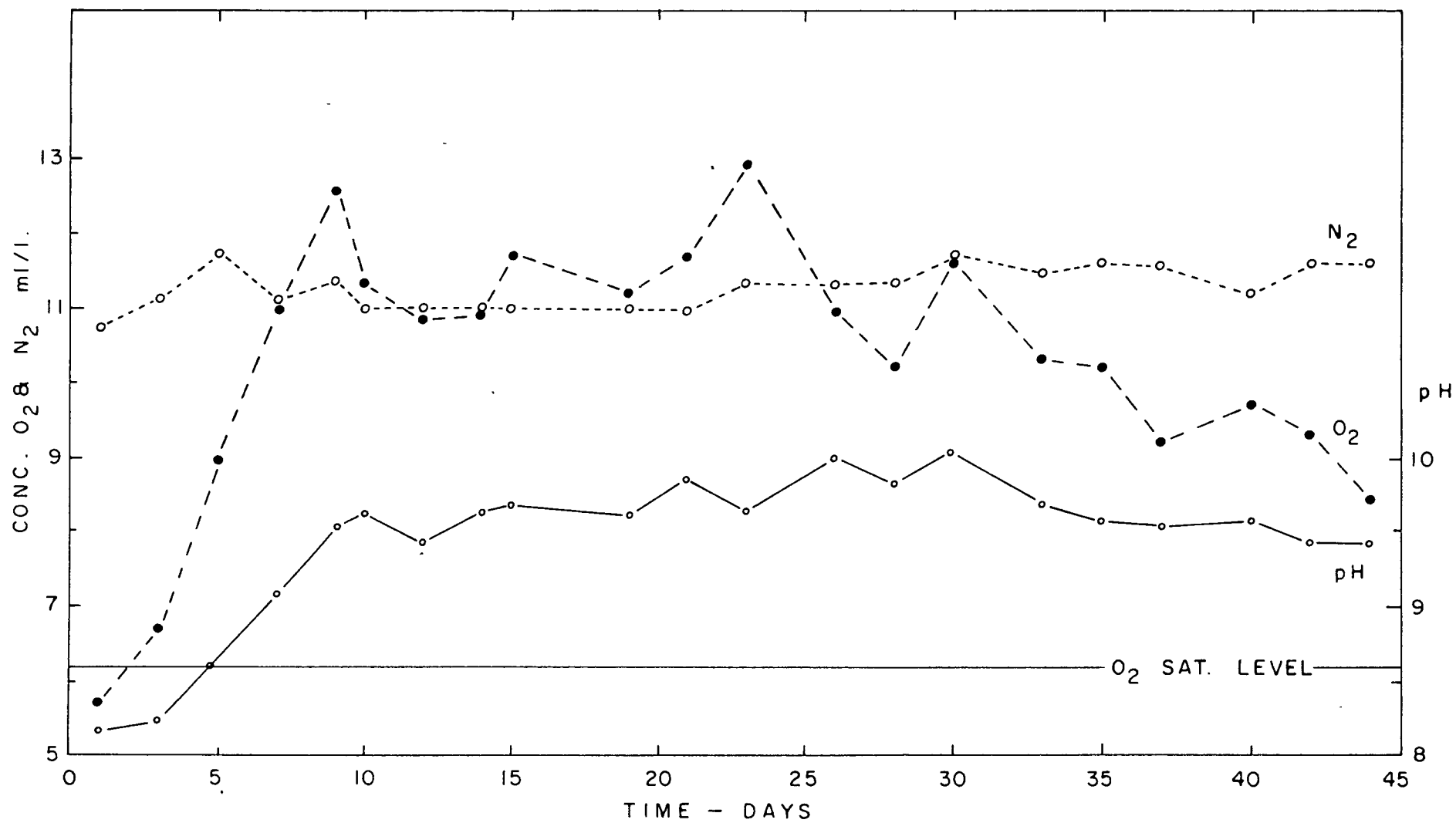


Figure XX. Variation of Oxygen and pH in *Nitzschia* Culture with Time

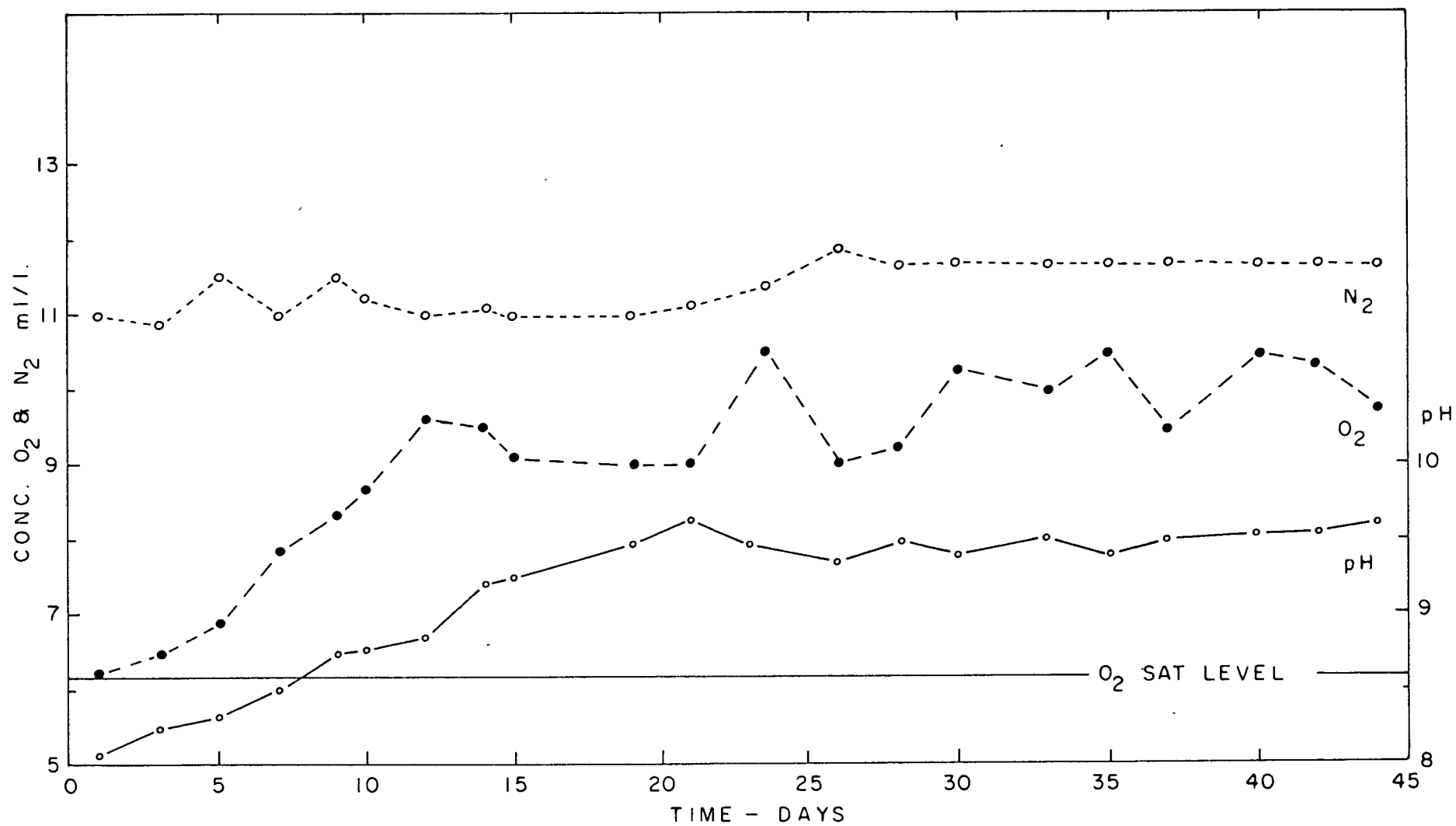


Figure XXI. Variation of Oxygen and pH in *Chlorella* Culture with Time

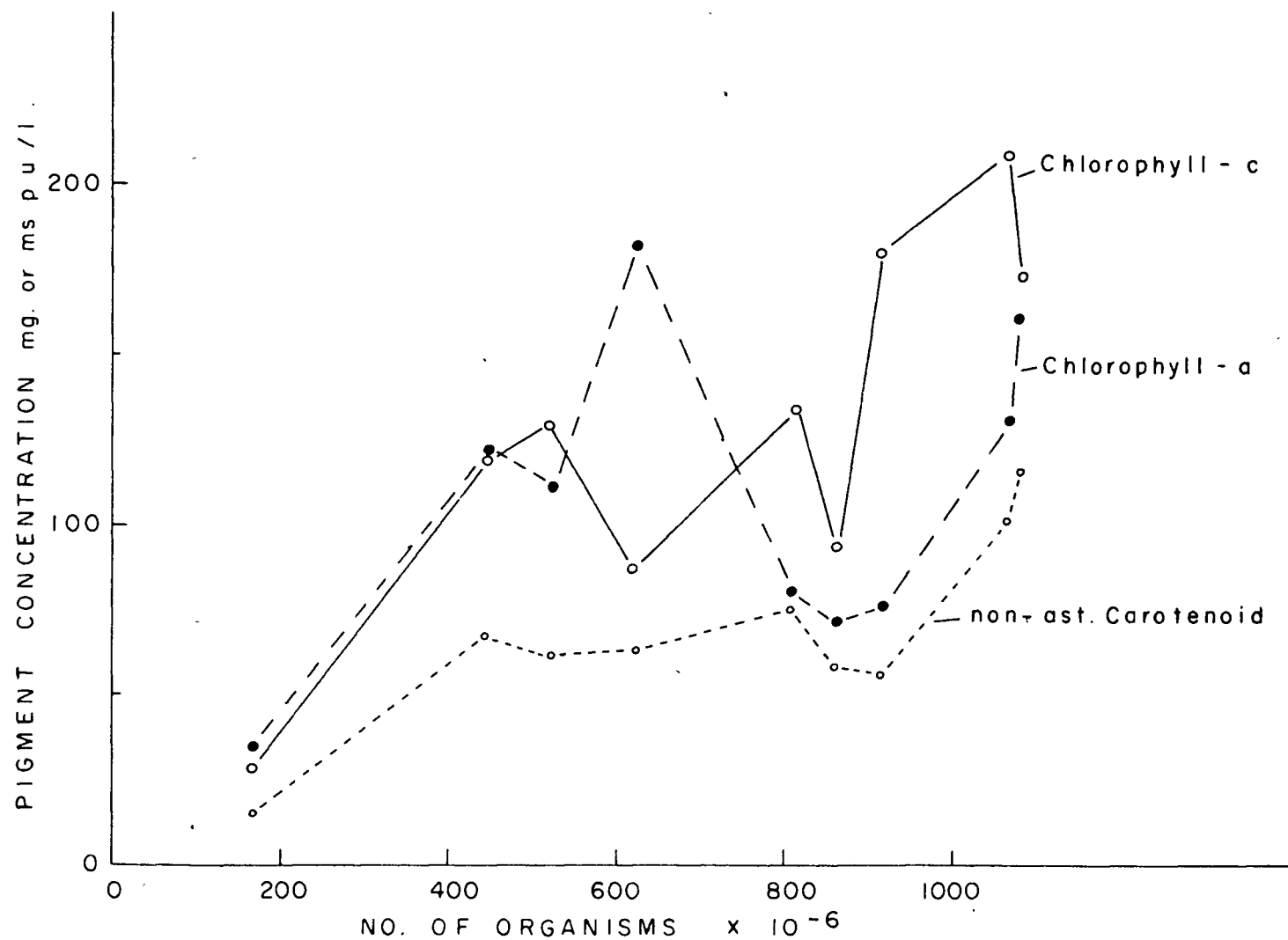


Figure XXII. Comparison of Number of Organisms in a Nitzschia Culture with Photosynthetic Pigment Concentration

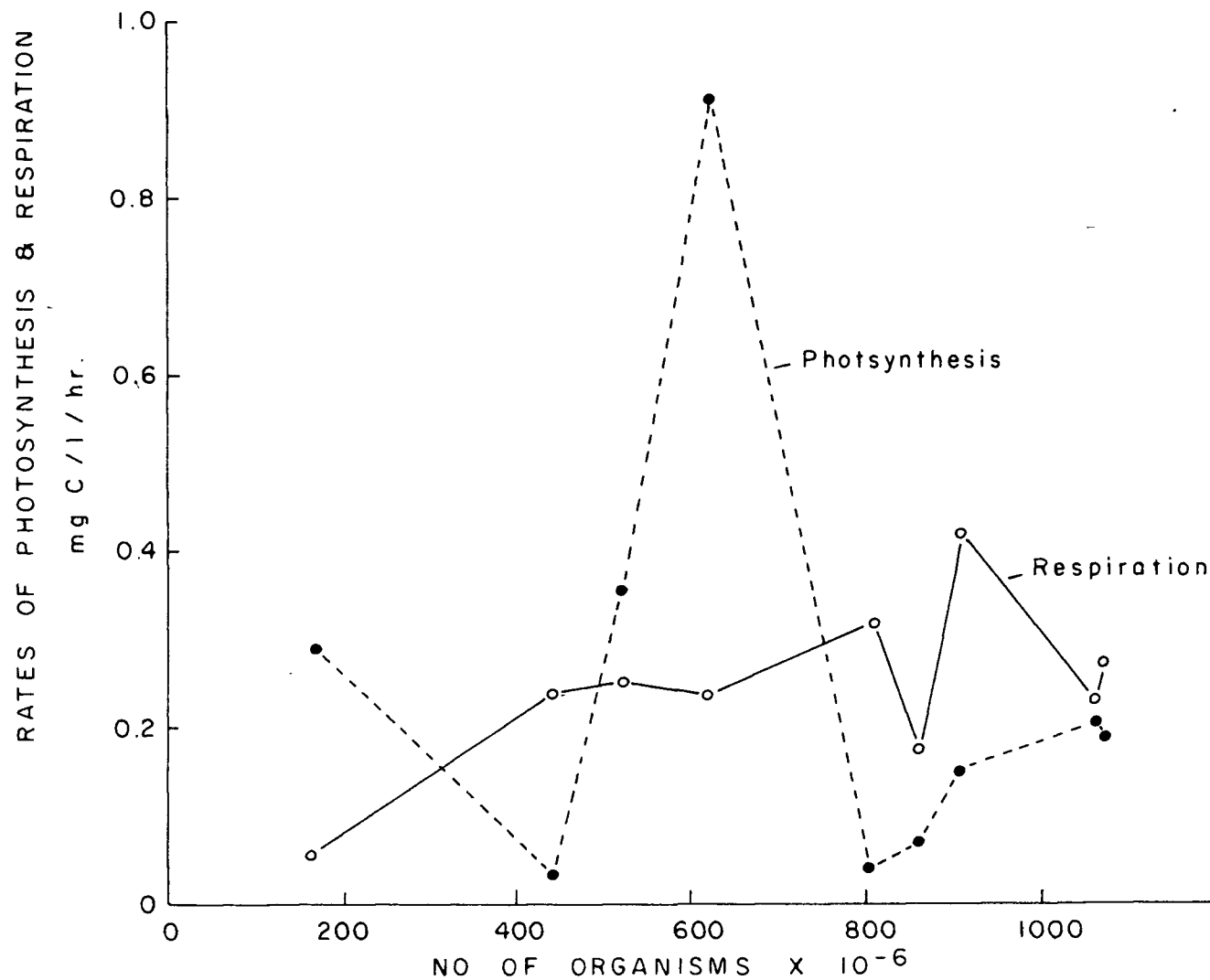


Figure XXIII. Comparison of Number of Organisms in a *Nitzschia* Culture with Rates of Photosynthesis and Respiration

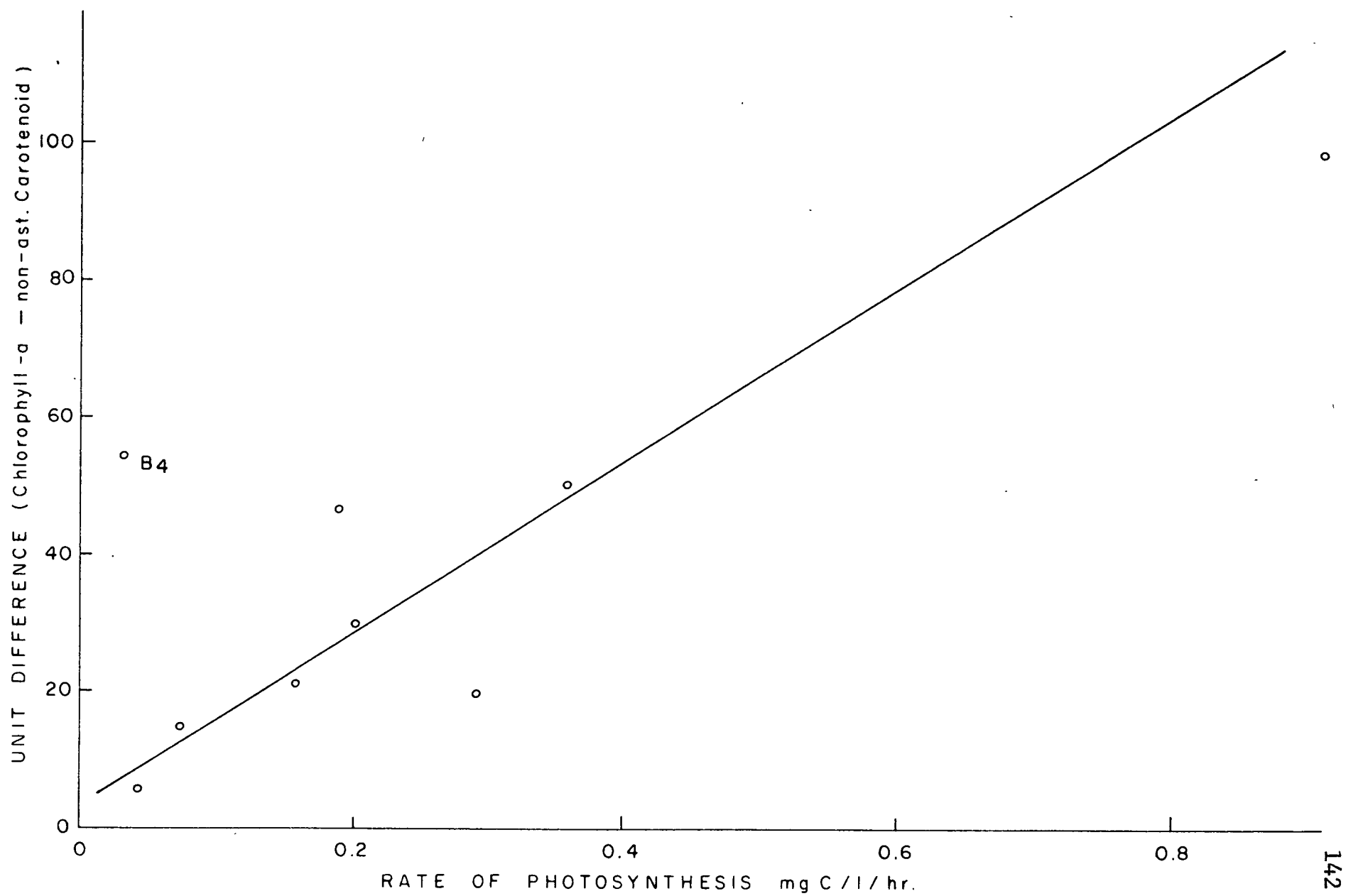


Figure XXIV. Behaviour of Photosynthetic Rate in Nitzschia with the Difference of Pigment Content

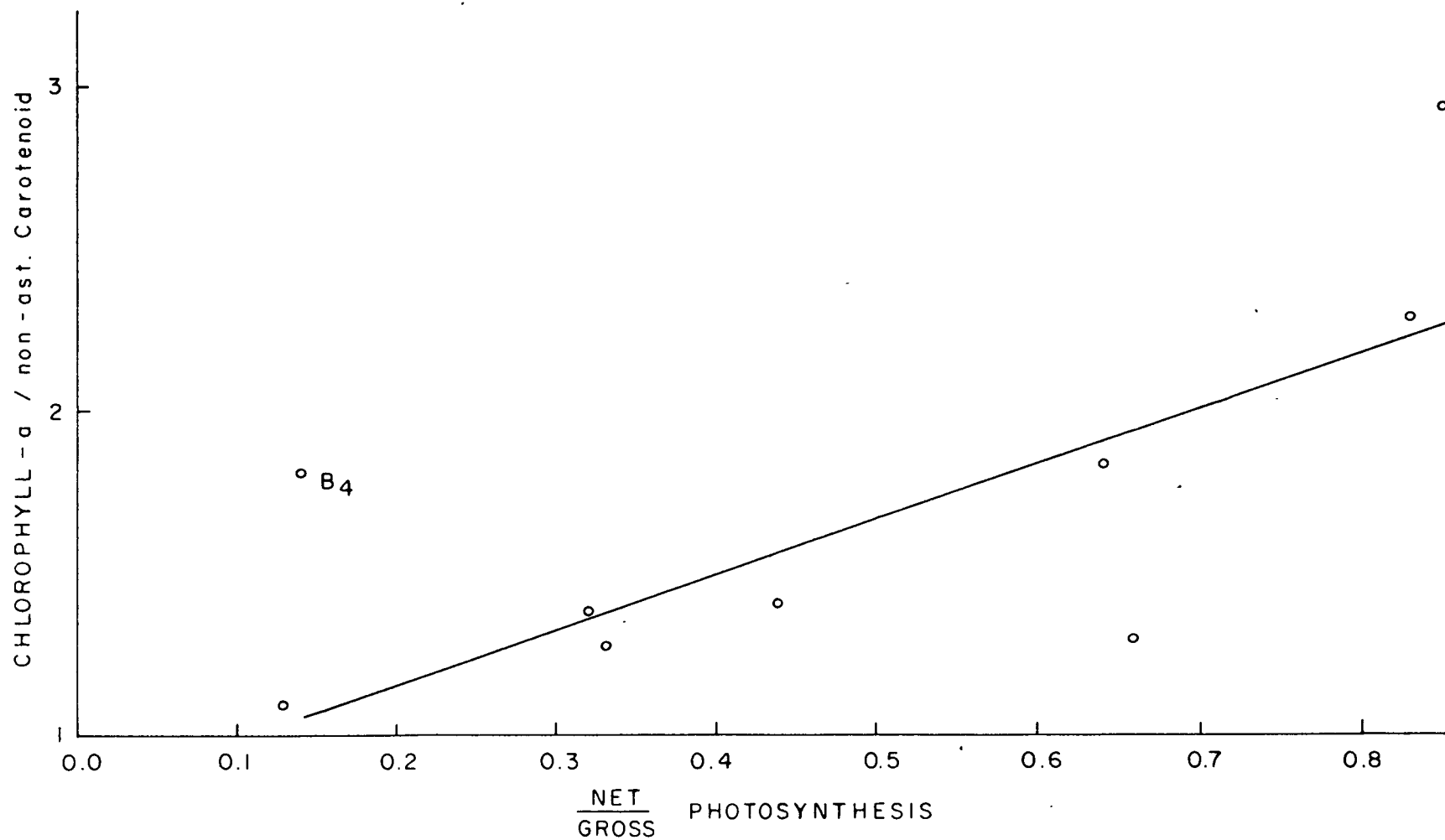


Figure XXV. Behaviour of Net:Gross Photosynthetic Rates in Nitzschia with Ratio of Pigment Content

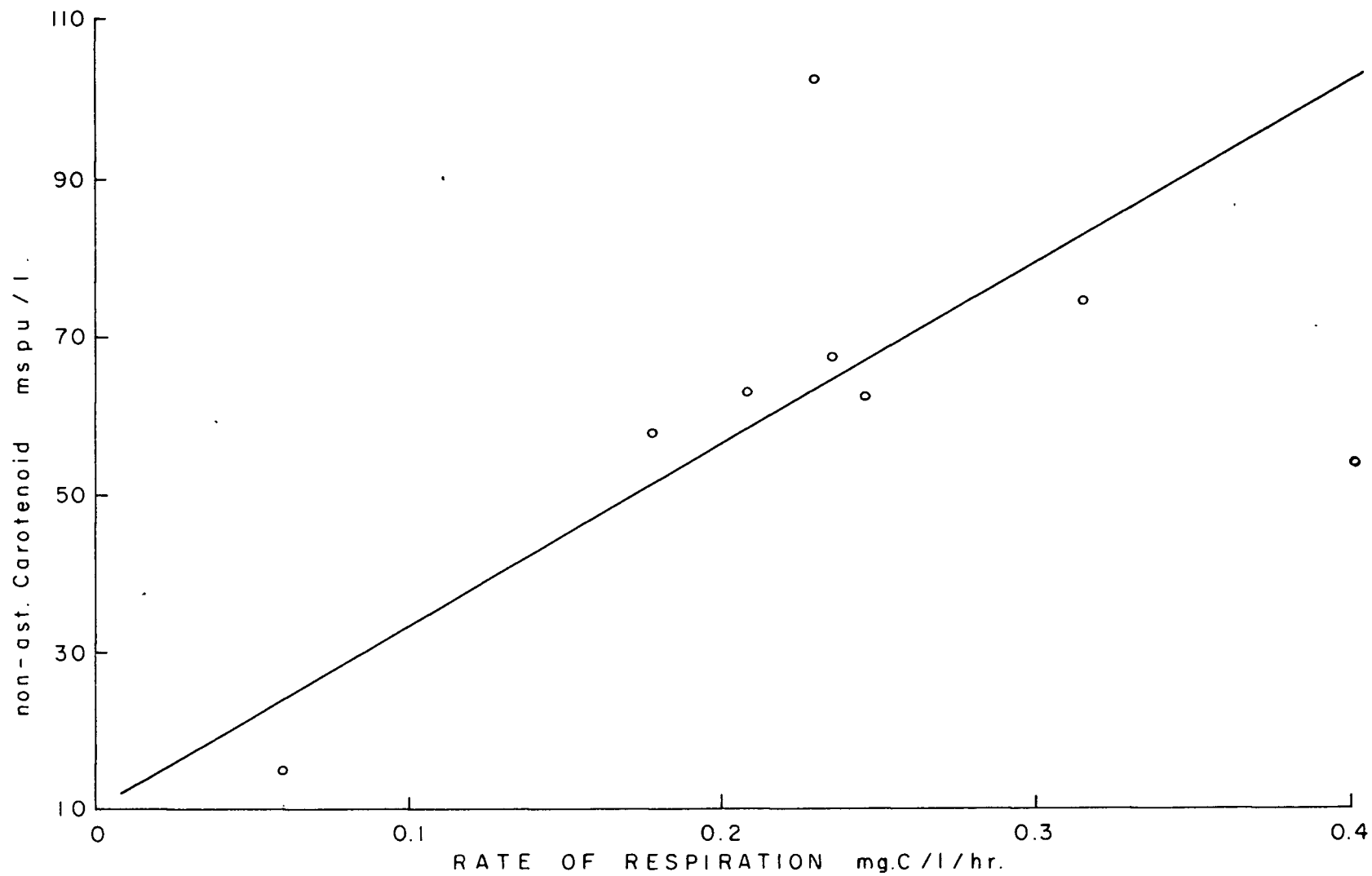


Figure XXVI. Dependence of Respiration Rate in Nitzschia on Carotenoid Pigment Concentration

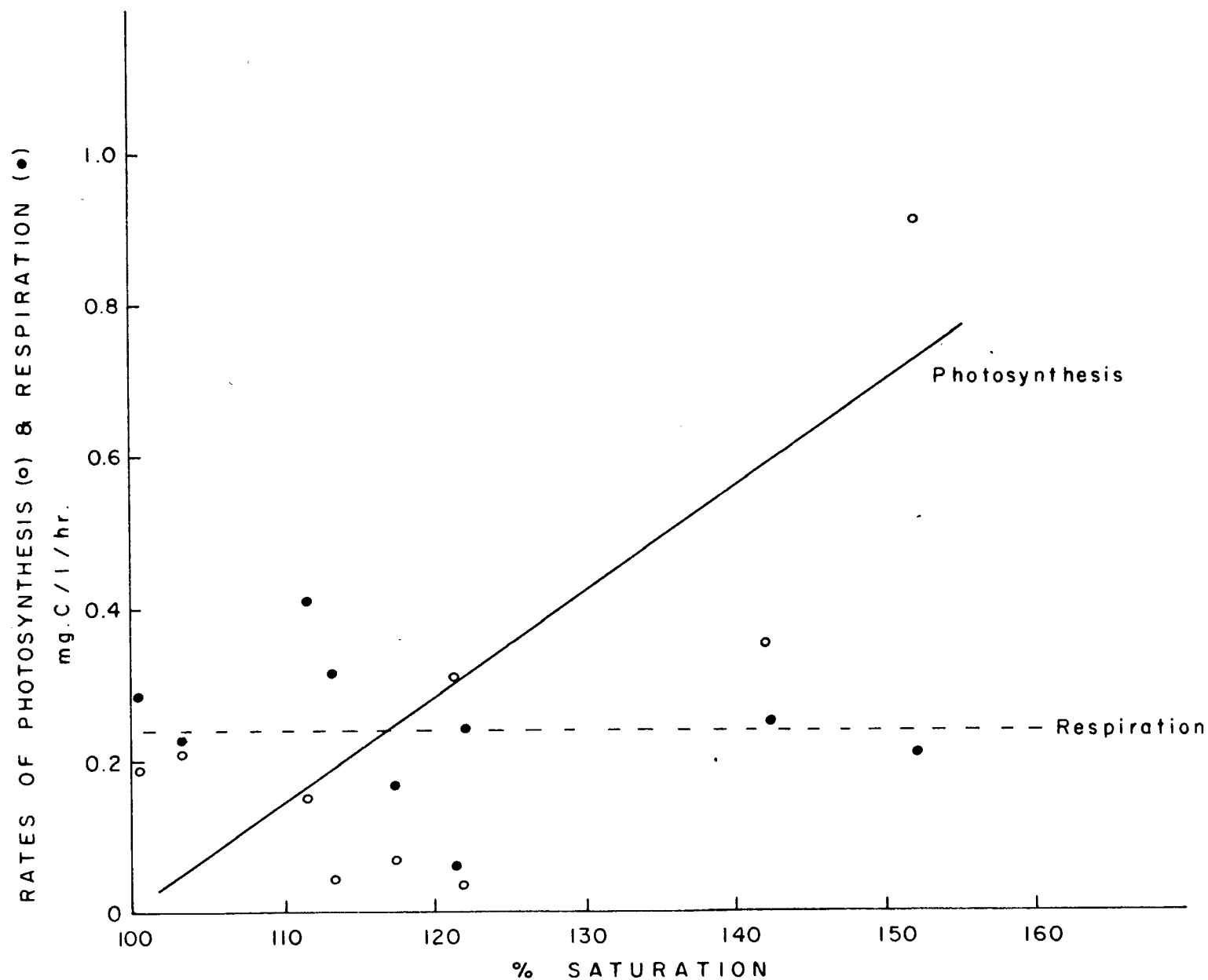


Figure XXVII. Relation of Per Cent Saturation to Rates of Photosynthesis and Respiration in *Nitzschia*

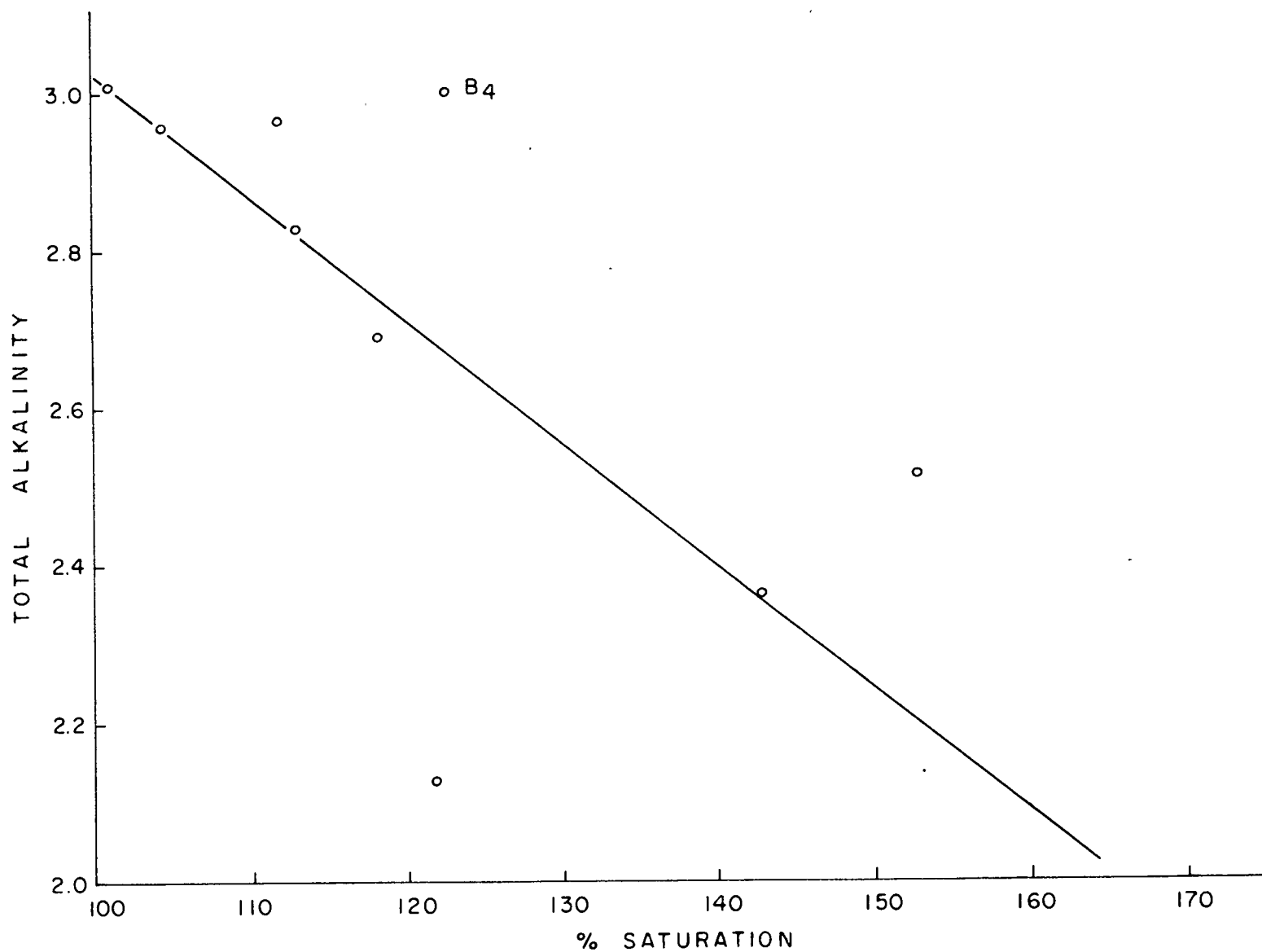


Figure XXVIII. Influence of Total Alkalinity on the Saturation Level
in a Nitzschia Culture

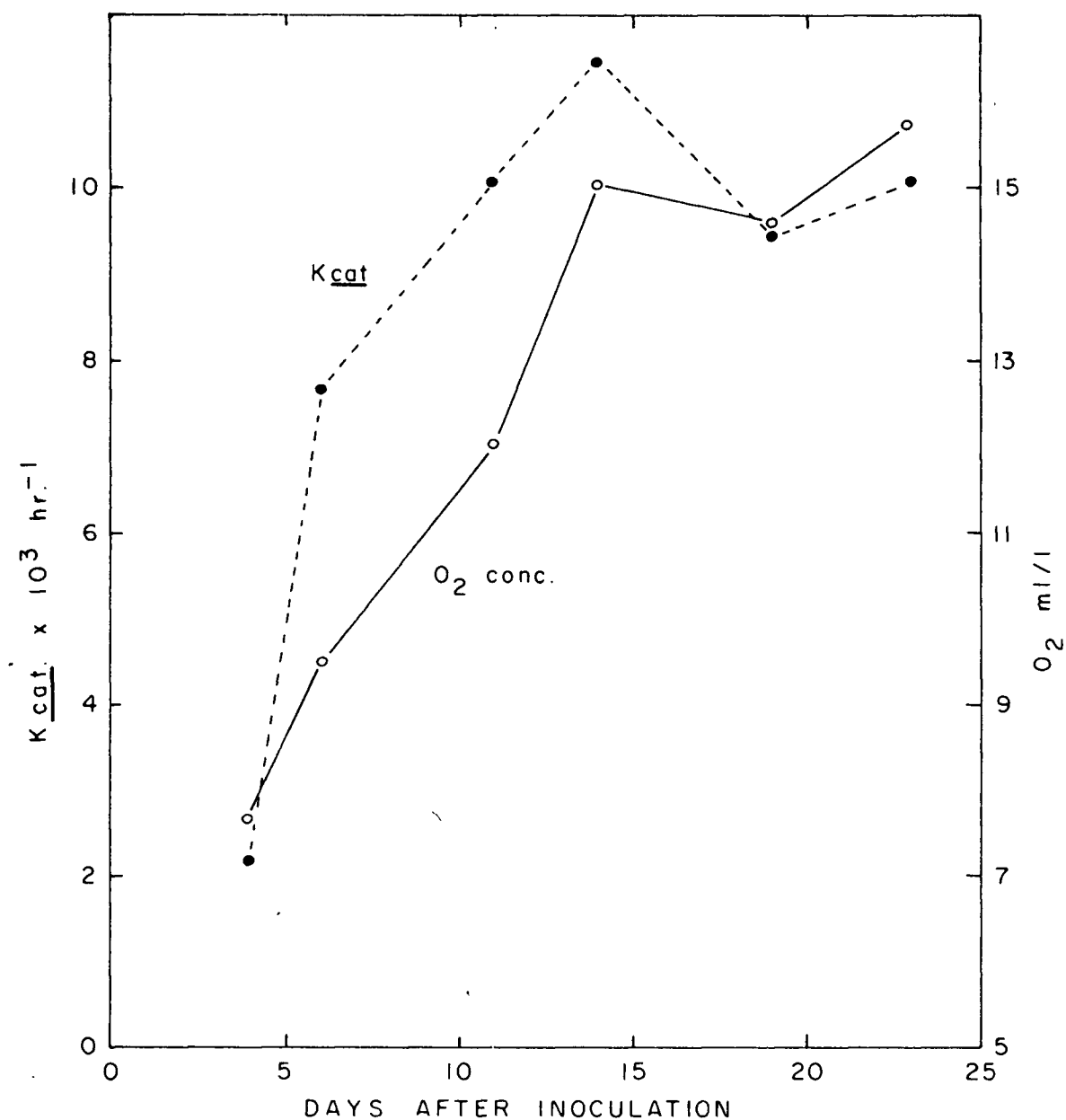


Figure XXIX. Variation of Oxygen and Catalytic Activity in a Nitzschia Culture with Time

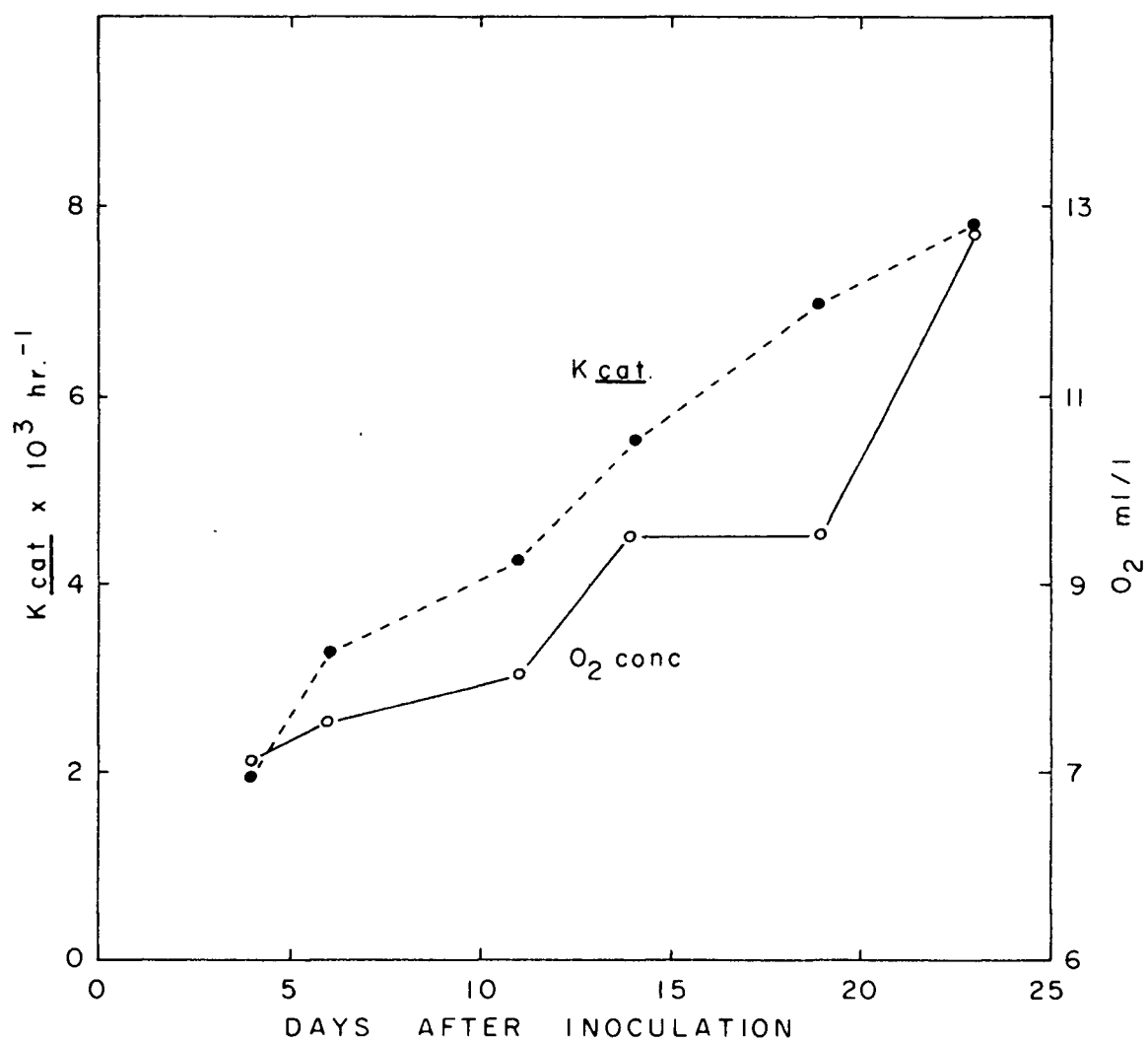


Figure XXX. Variation of Oxygen and Catalytic Activity in a Chlorella Culture with Time

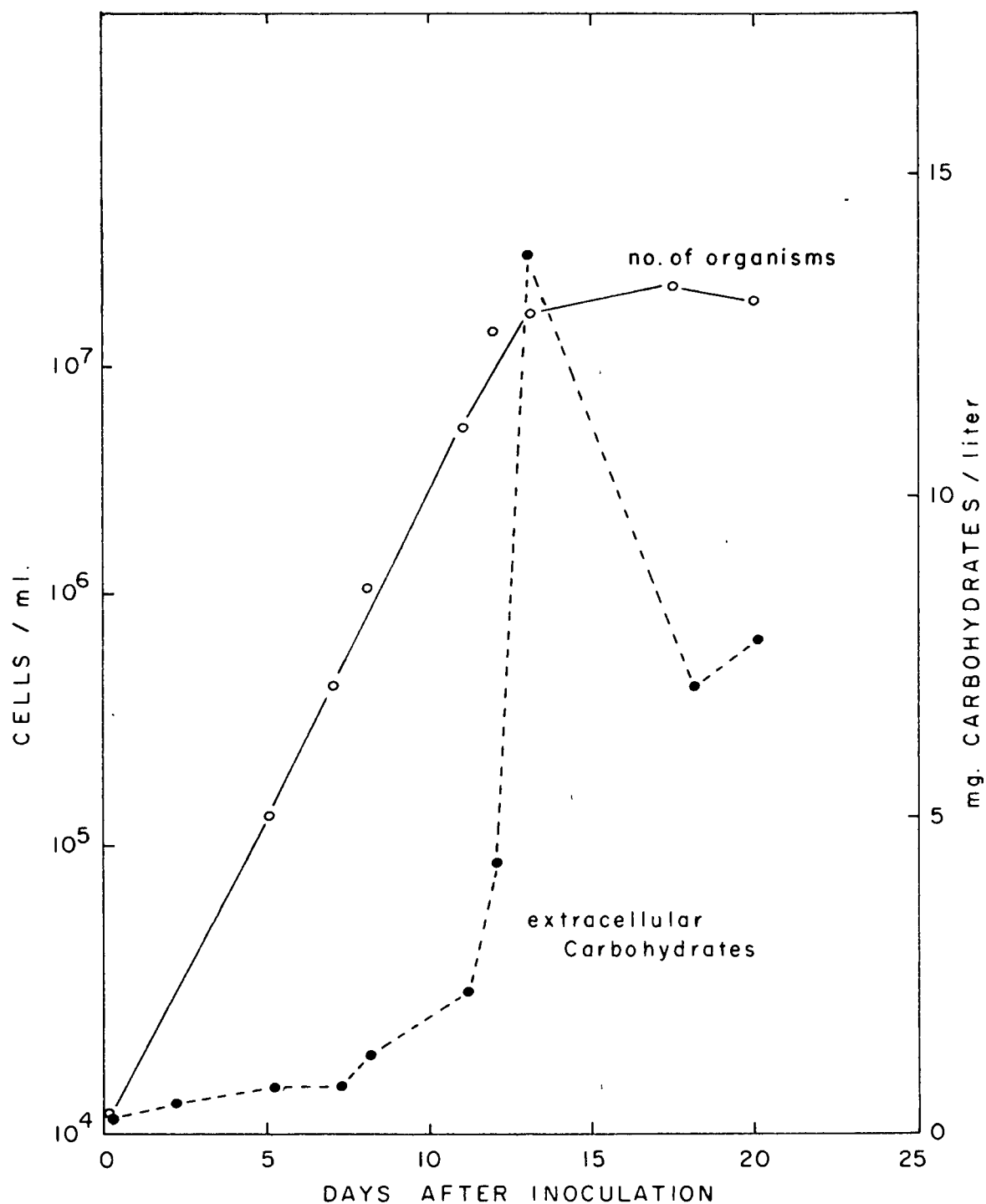


Figure XXXI. Extracellular Carbohydrate Relative to Number of Organisms for the Flagellate *Isochrysis galbana* (after Guillard, 1958)

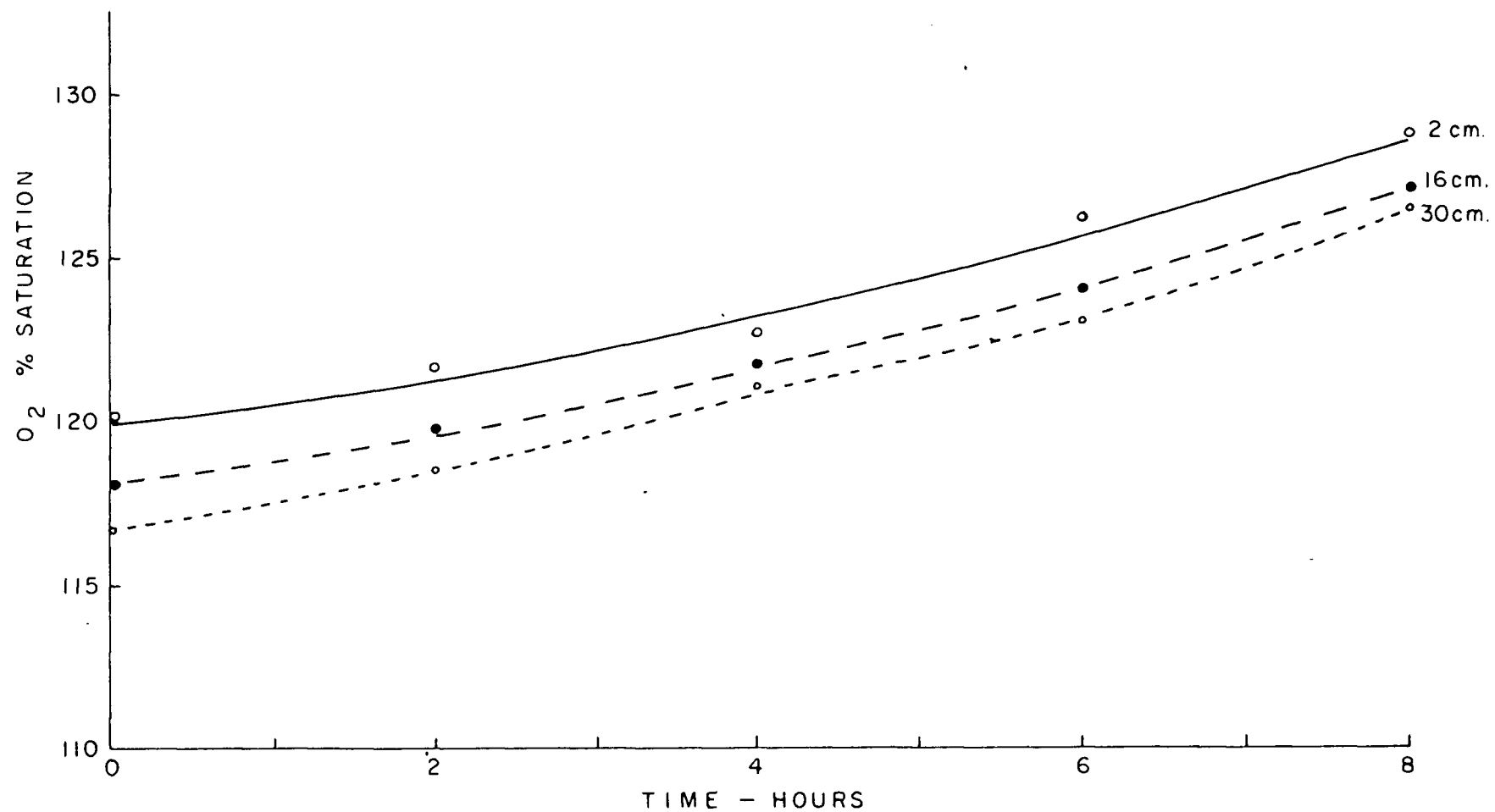


Figure XXXII. Change of Oxygen Concentration under Illumination in a Nitzschia Culture at Different Depths

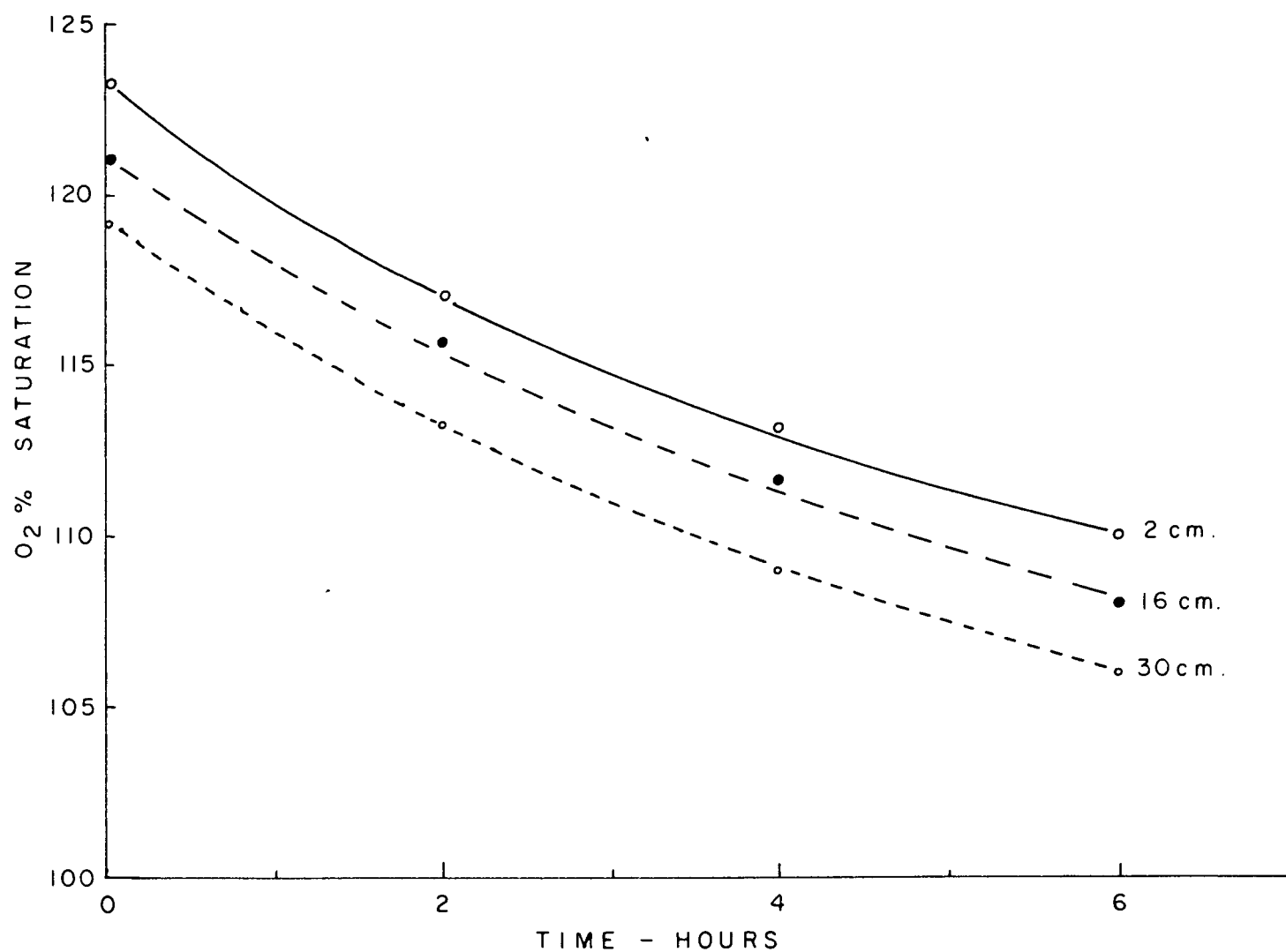


Figure XXXIII. Change of Oxygen Concentration under Dark in
a Nitzschia Culture at Different Depths

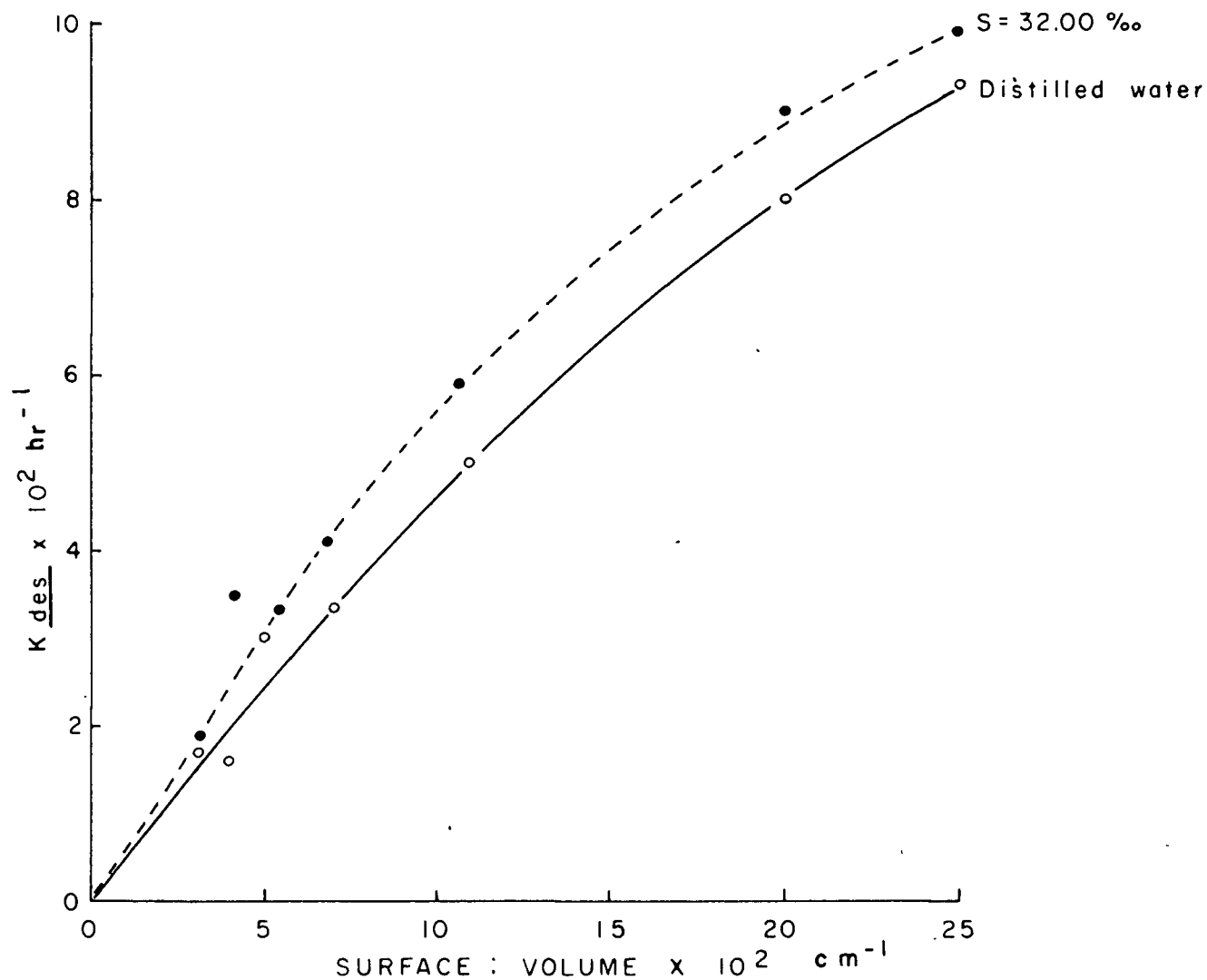


Figure XXXIV. Variation of Oxygen Desupersaturation Rate Constant with Surface to Volume Ratio of a Water Column

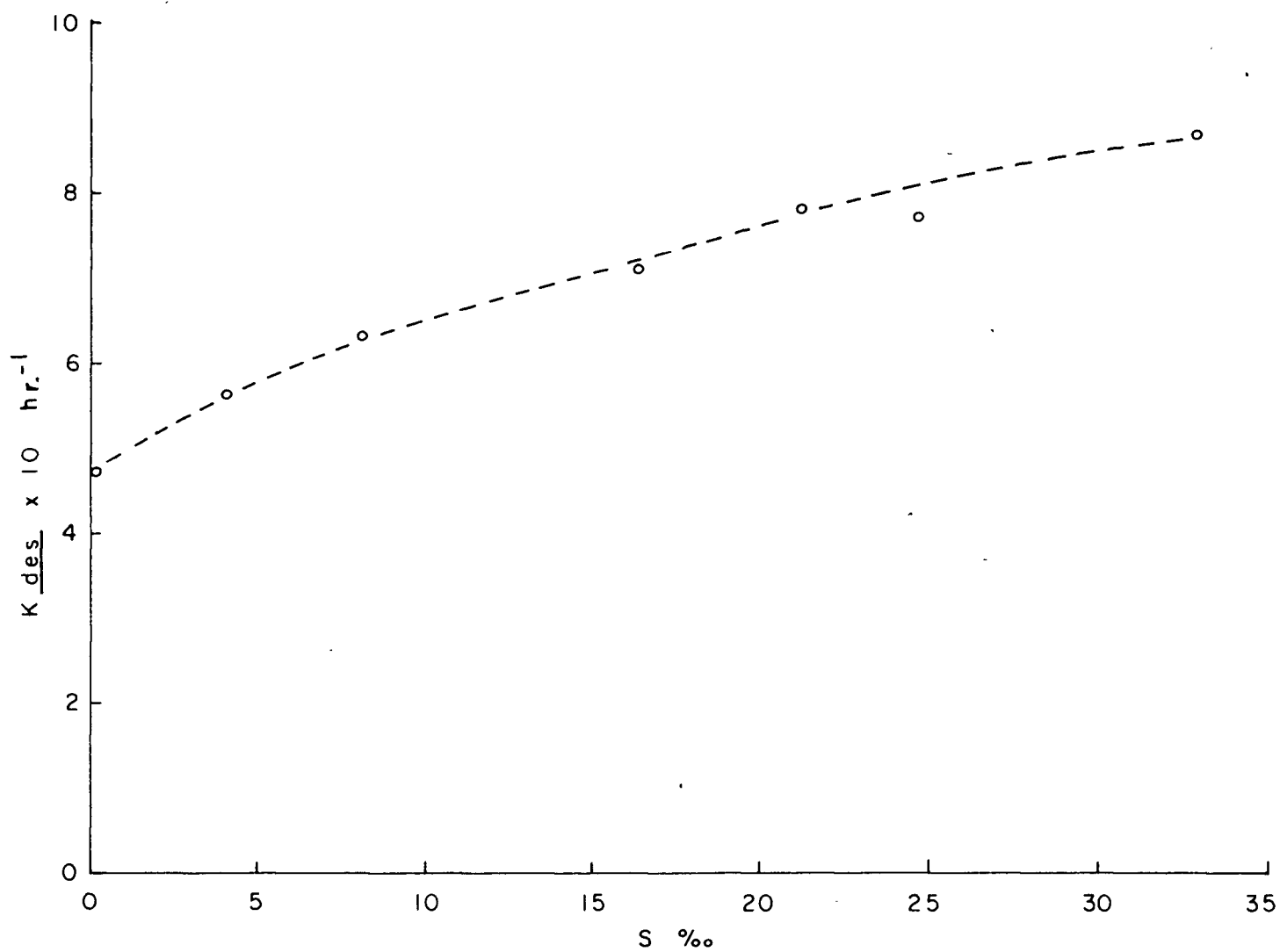


Figure XXXV. Variation of Oxygen Desupersaturation Rate Constant with Salinity under Stirred Conditions

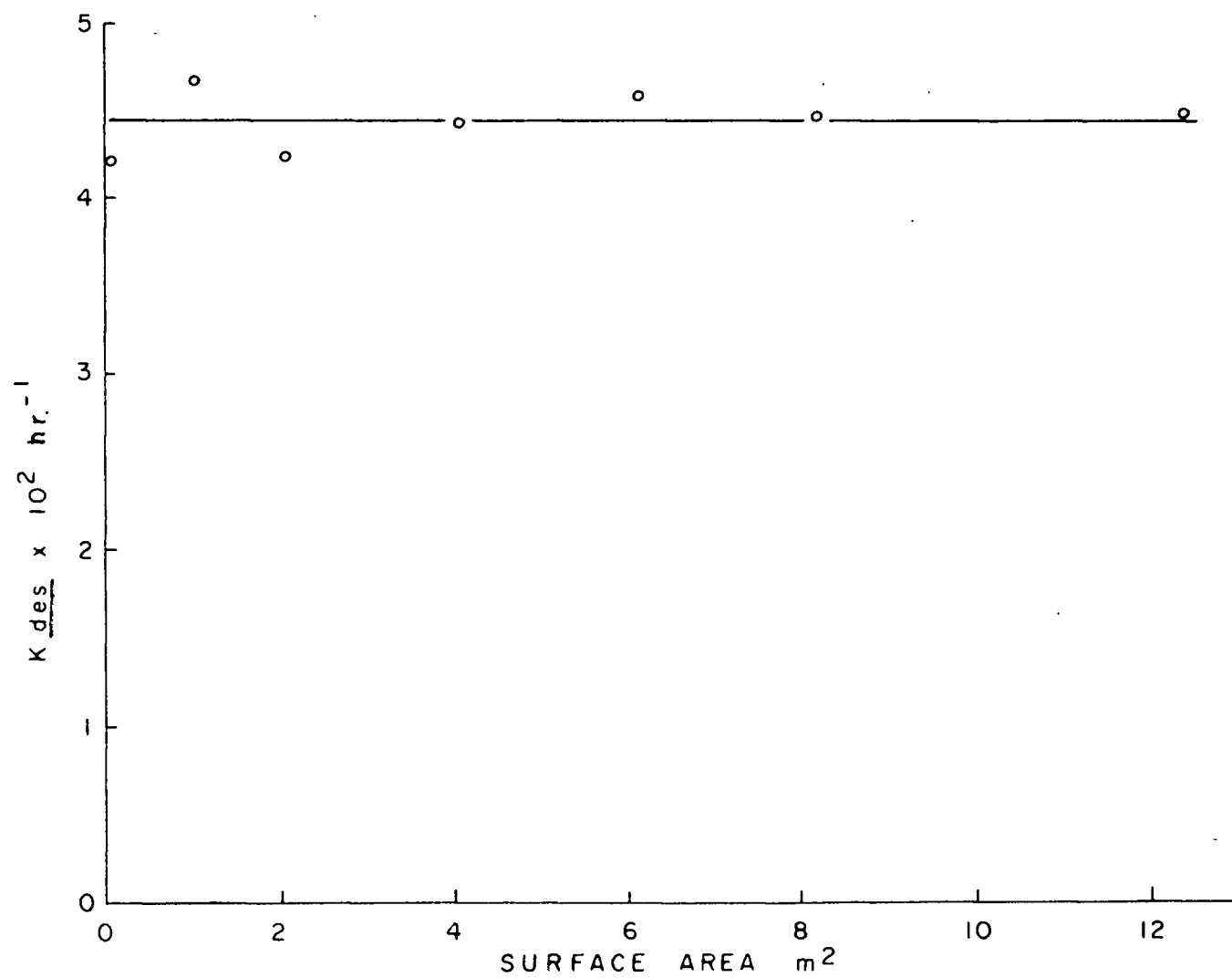


Figure XXXVI. Behaviour of Siliceous Surface Area with
Oxygen Desupersaturation Rate Constant

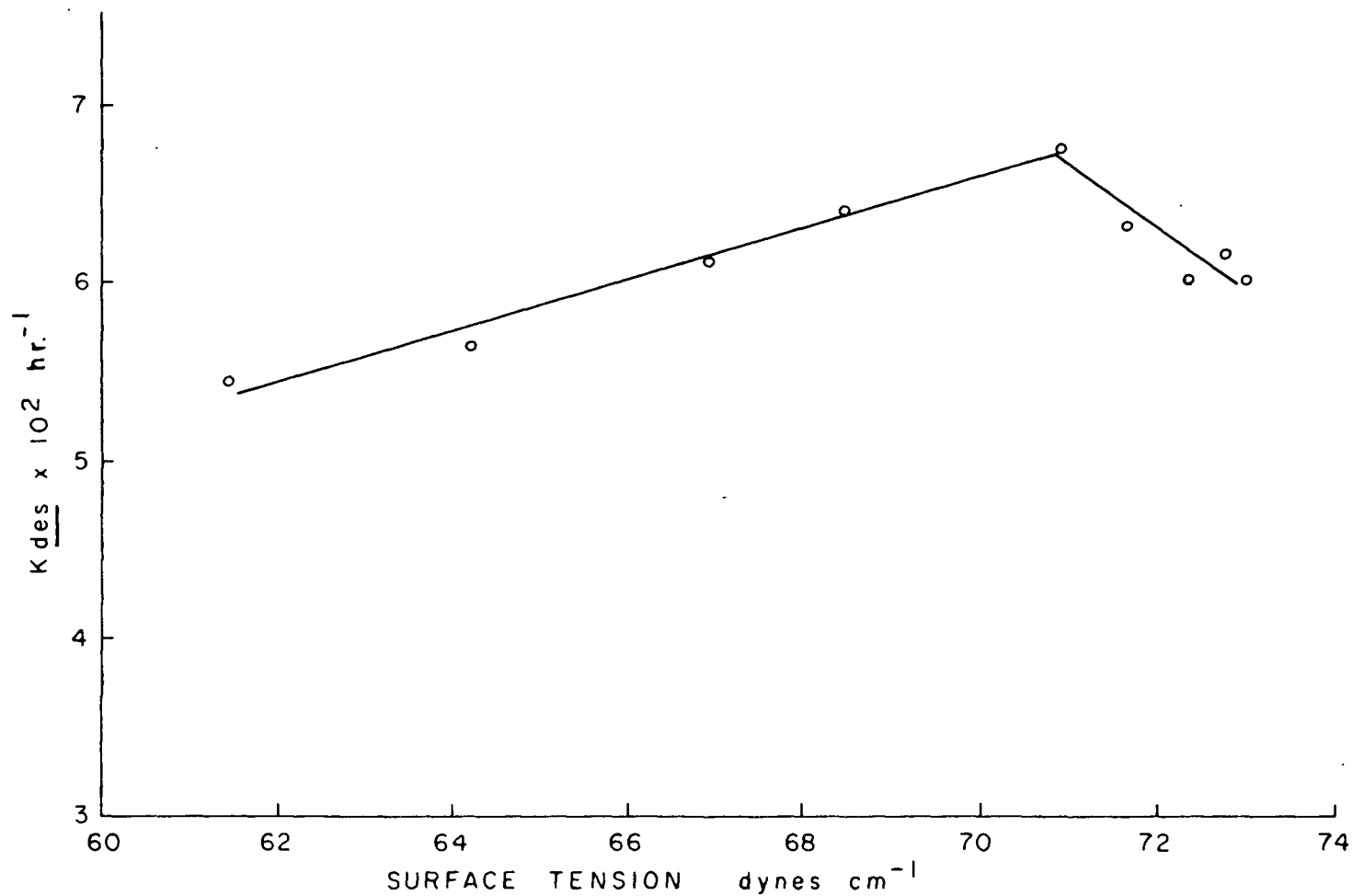


Figure XXXVII. Influence of Heptanoic Acid Addition on Oxygen Desupersaturation Rate Constant

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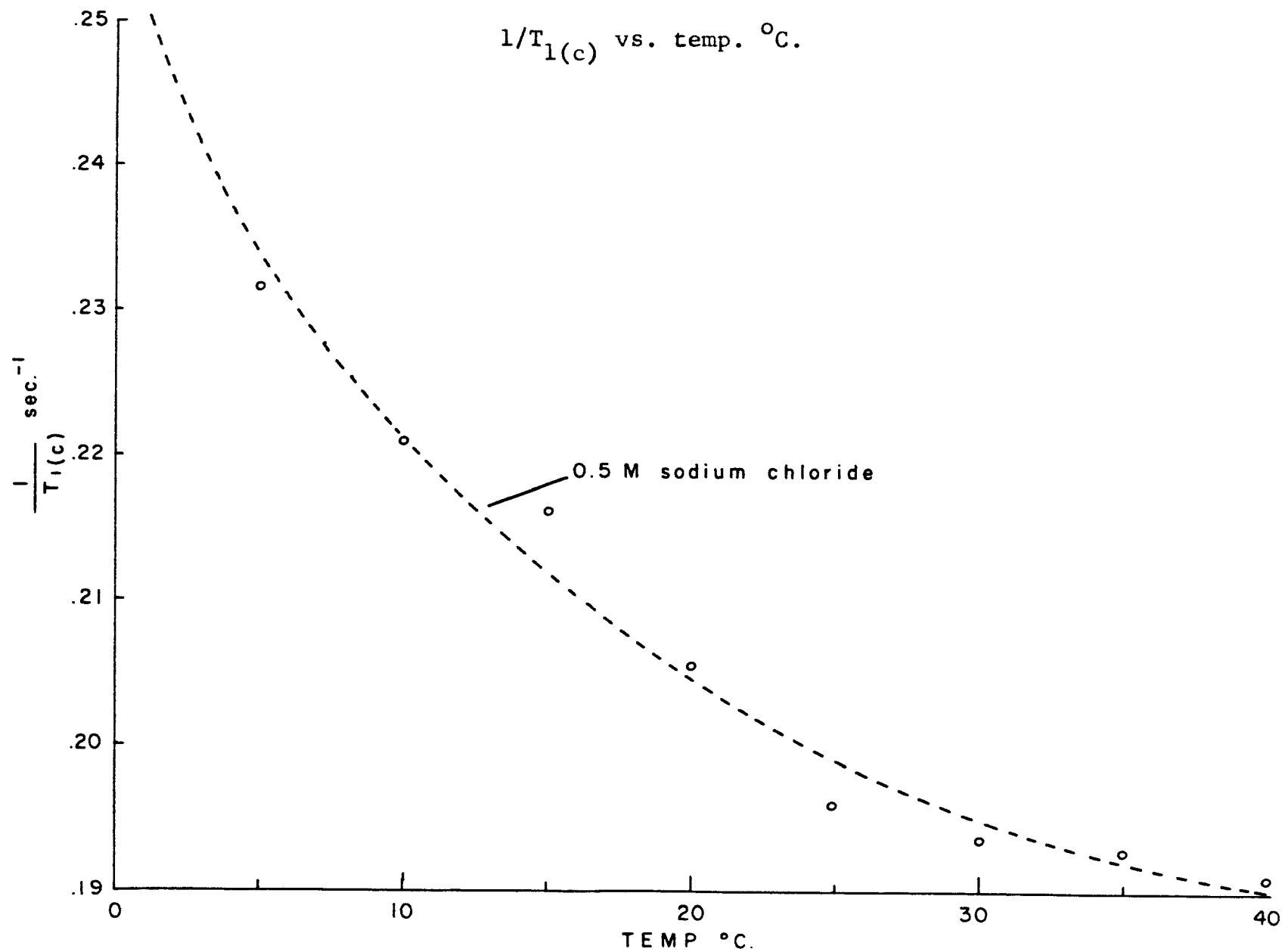


Figure XI. Behaviour of $1/T_{1(c)}$ with Temperature (Salt Solution)

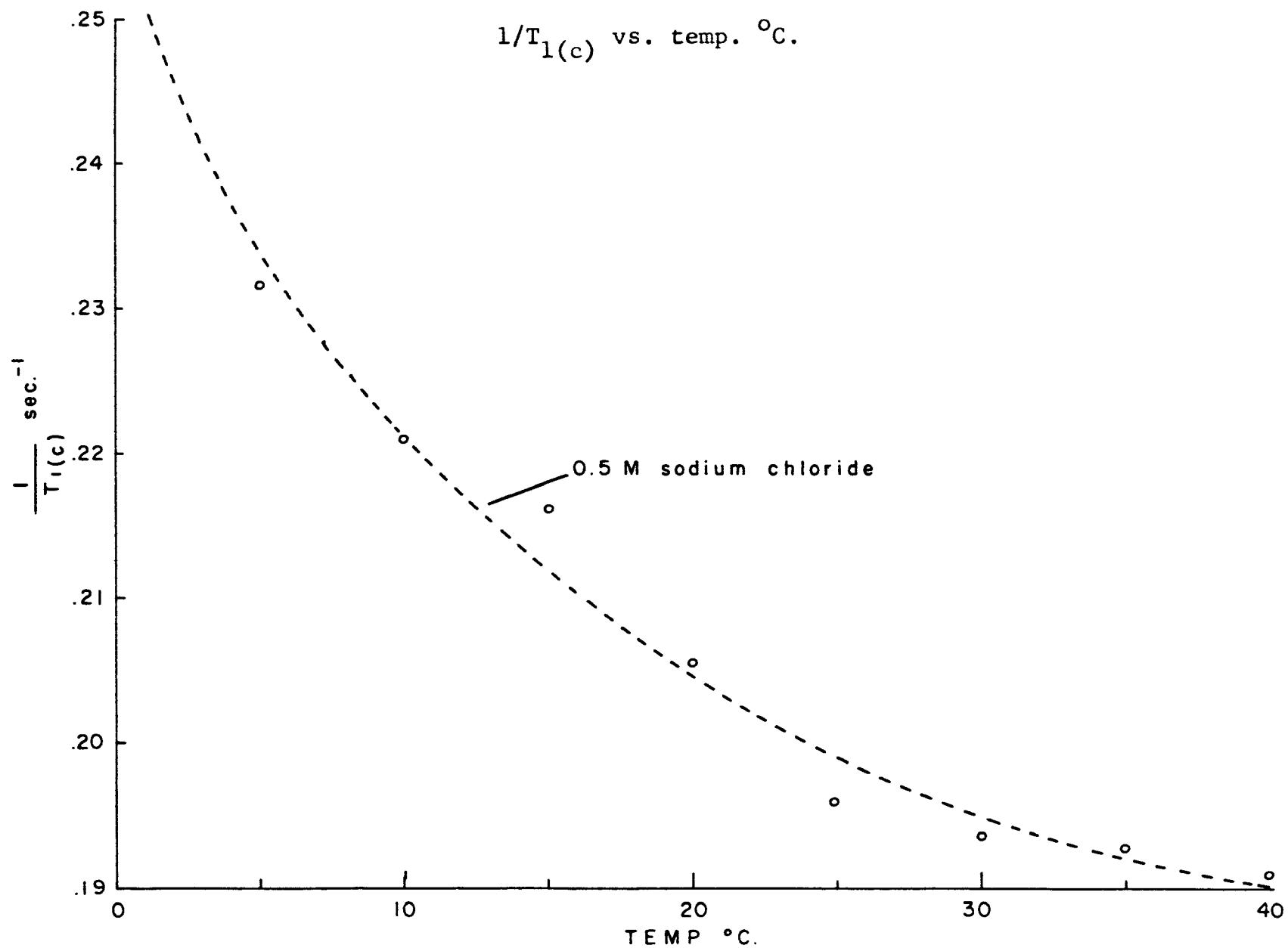


Figure XI. Behaviour of $1/T_{1(c)}$ with Temperature (Salt Solution)

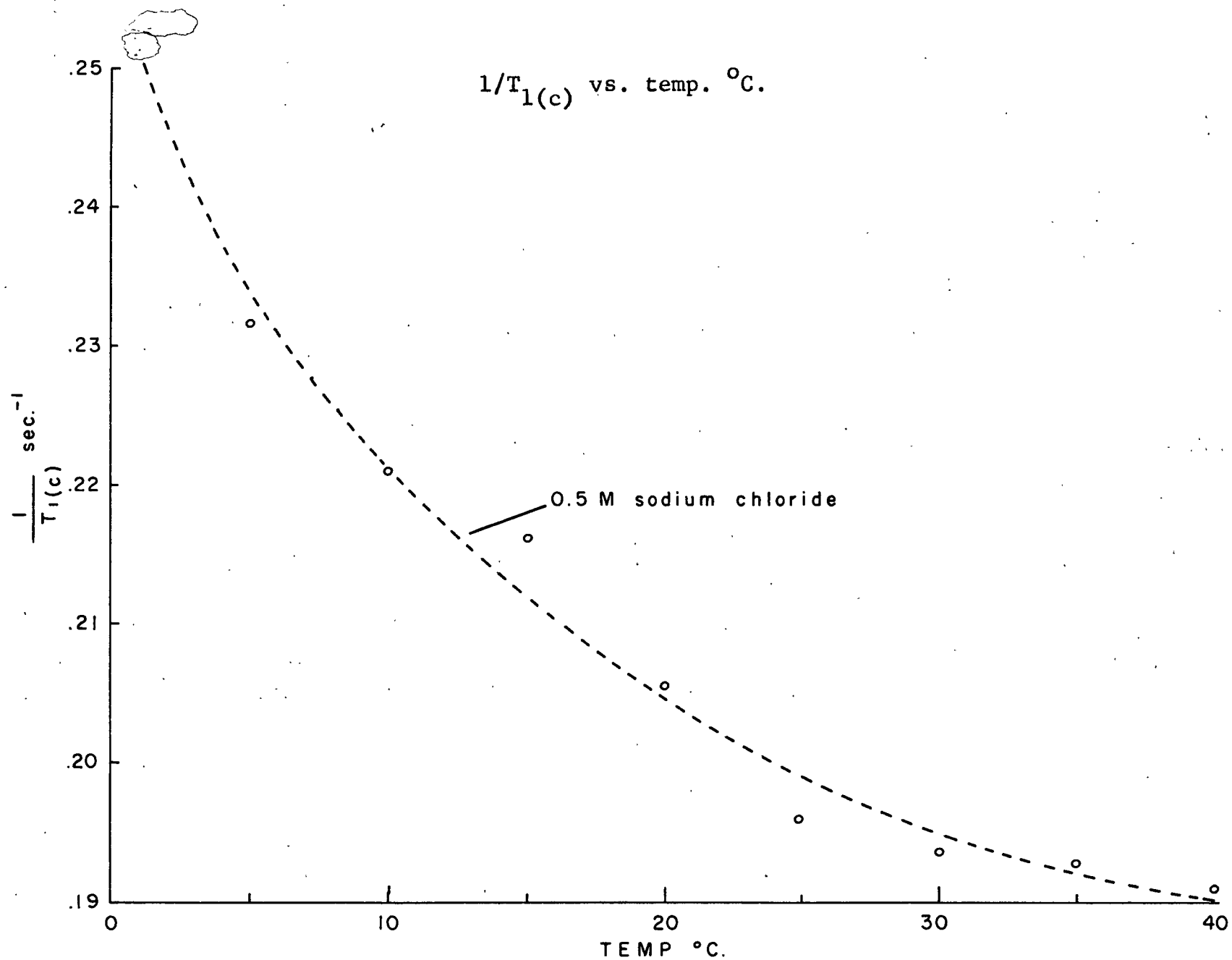


Figure XI. Behaviour of $1/T_{1(c)}$ with Temperature (Salt Solution)

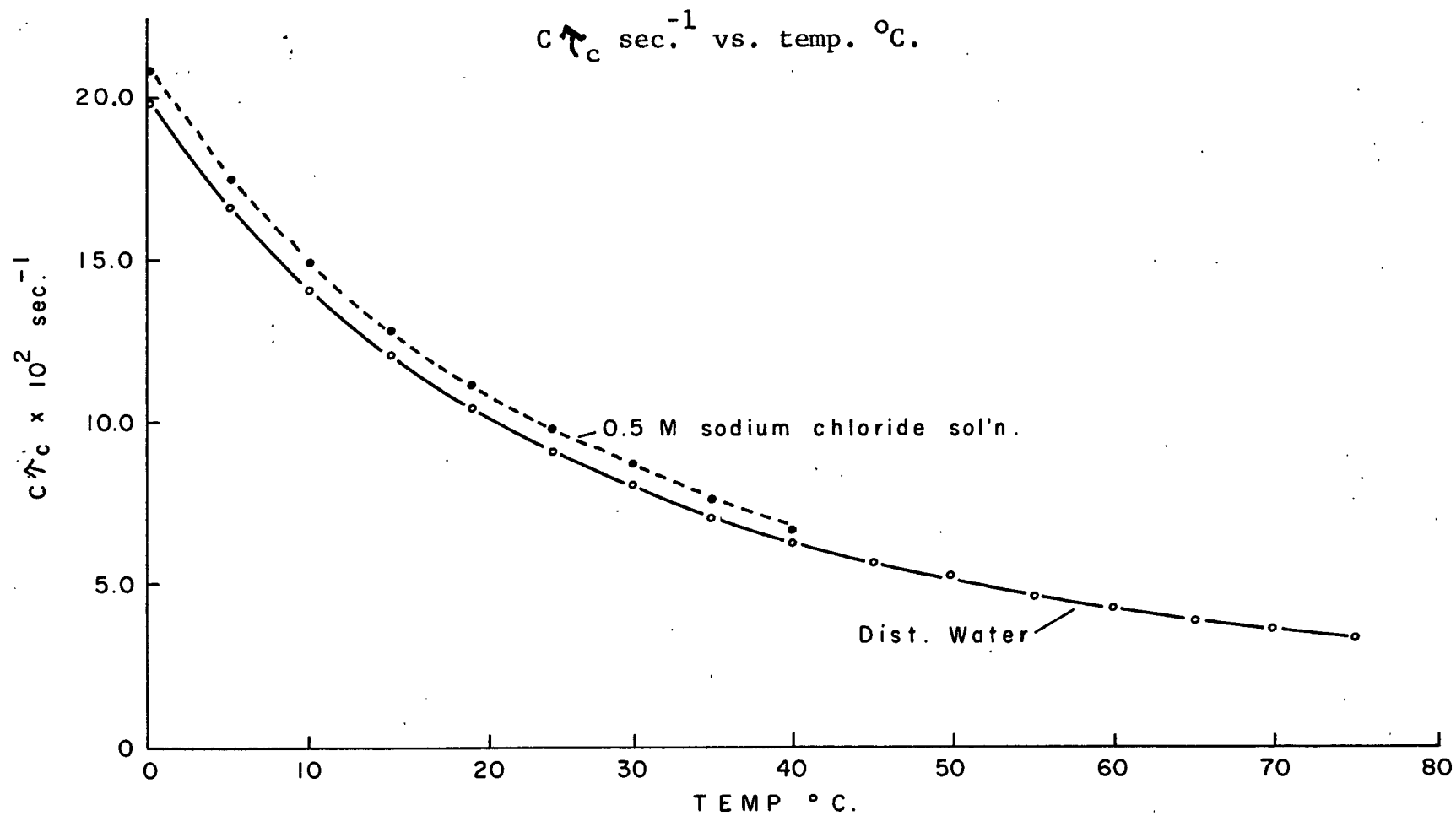


Figure XII. Change of $C \tau_c$ Term with Temperature

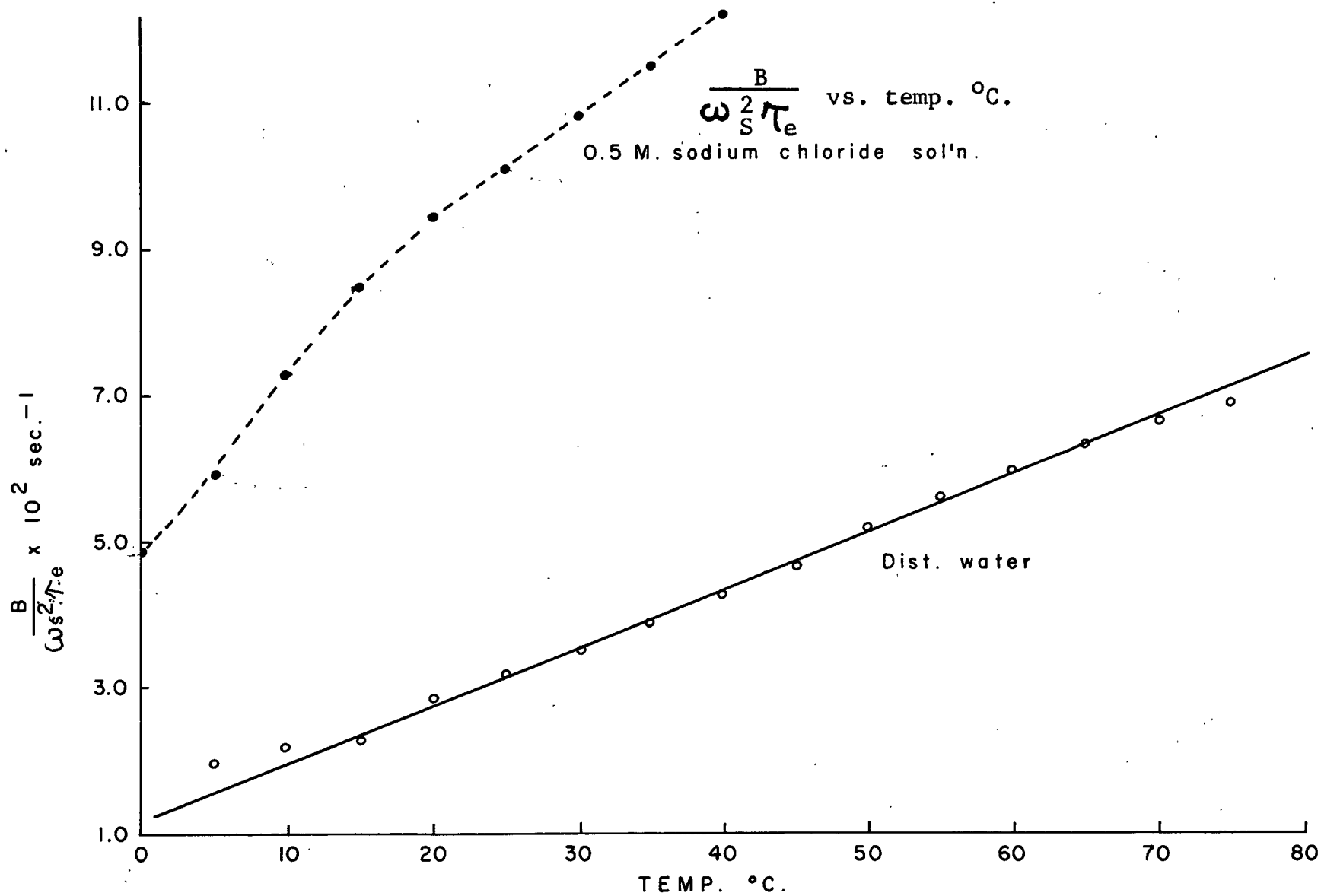


Figure XIII. Change of $\frac{B}{\omega_s^2 \tau_e}$ Term with Temperature

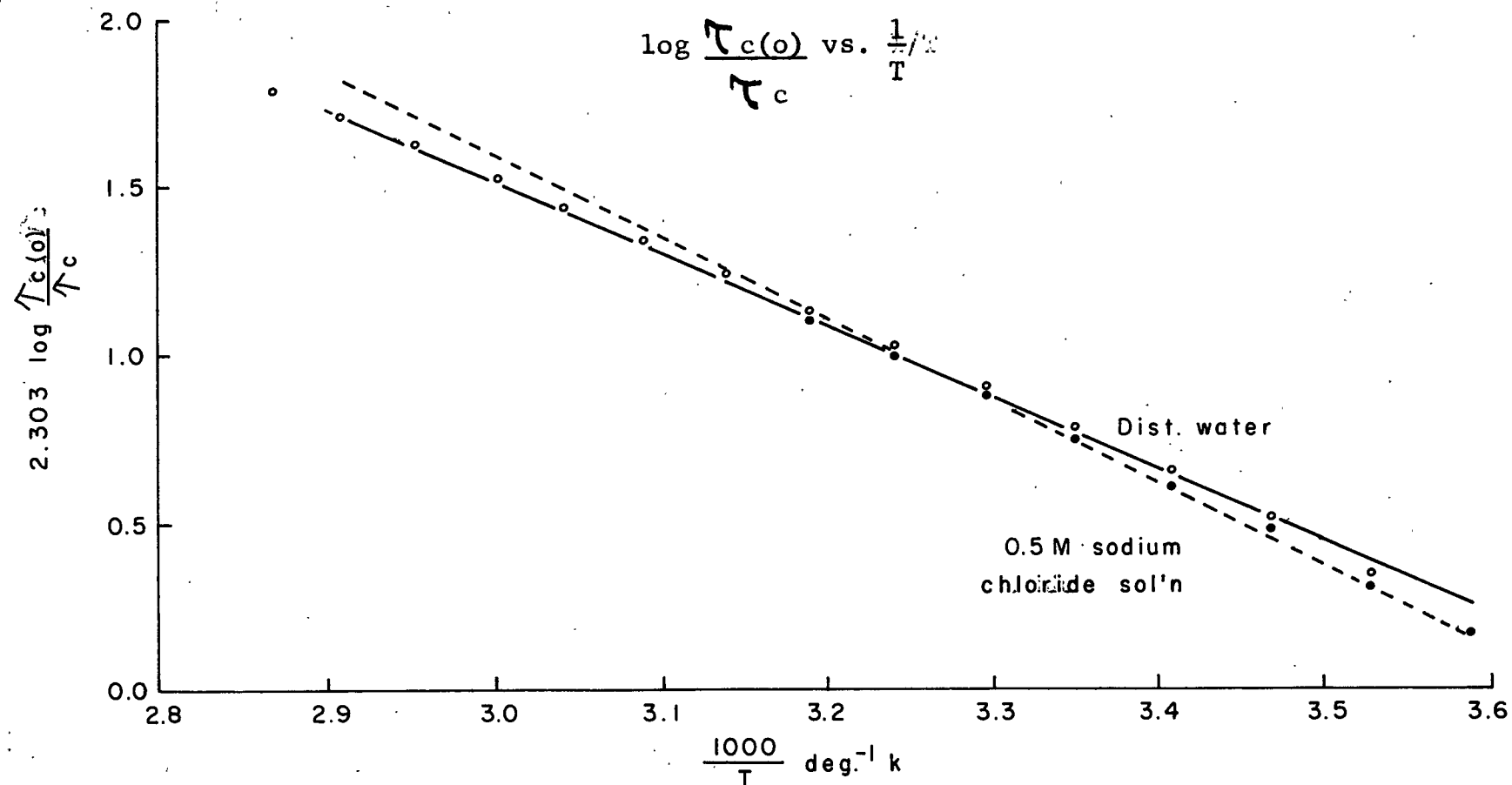


Figure XIV. Determination of the Activation Energy E_c

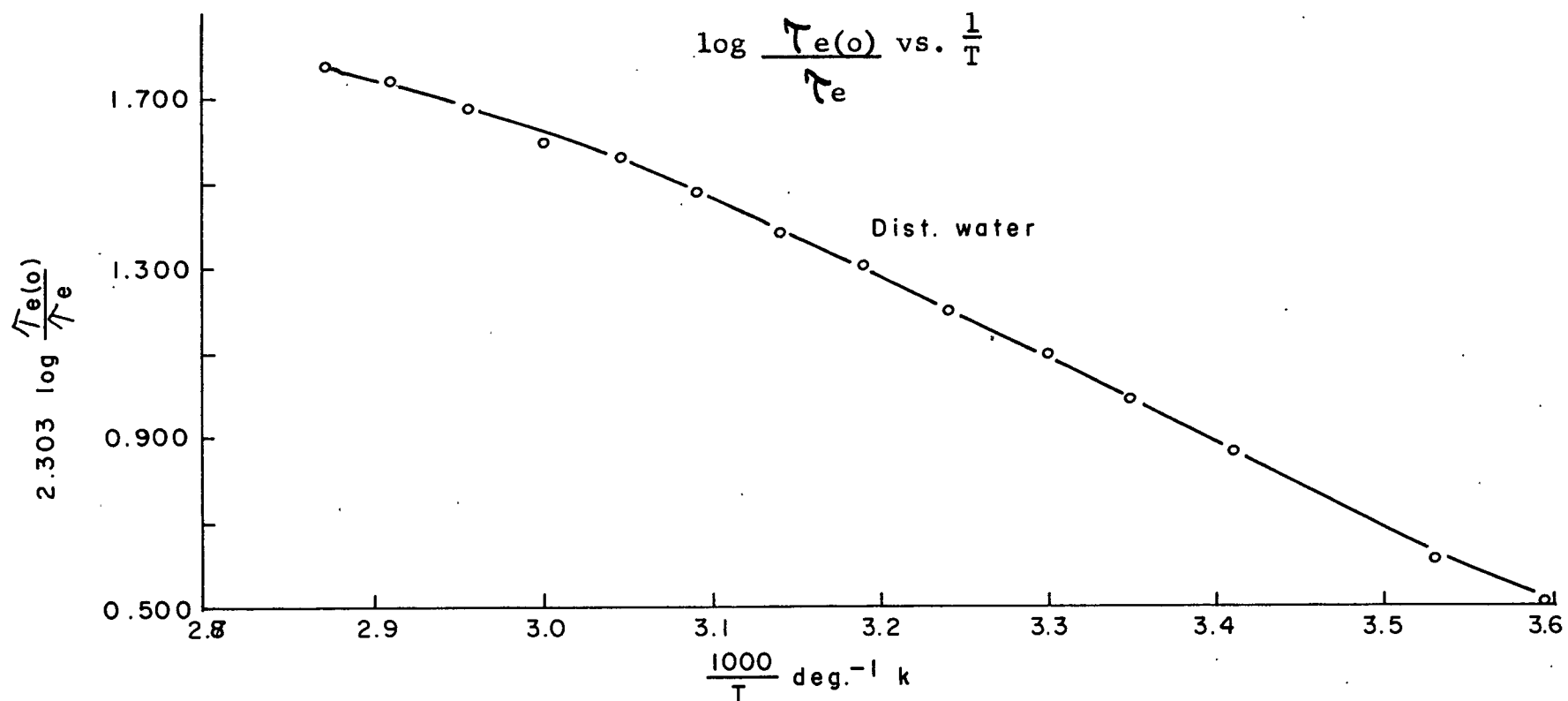


Figure XV. Determination of the Activation Energy E_e
at Different Temperatures (Distilled Water)

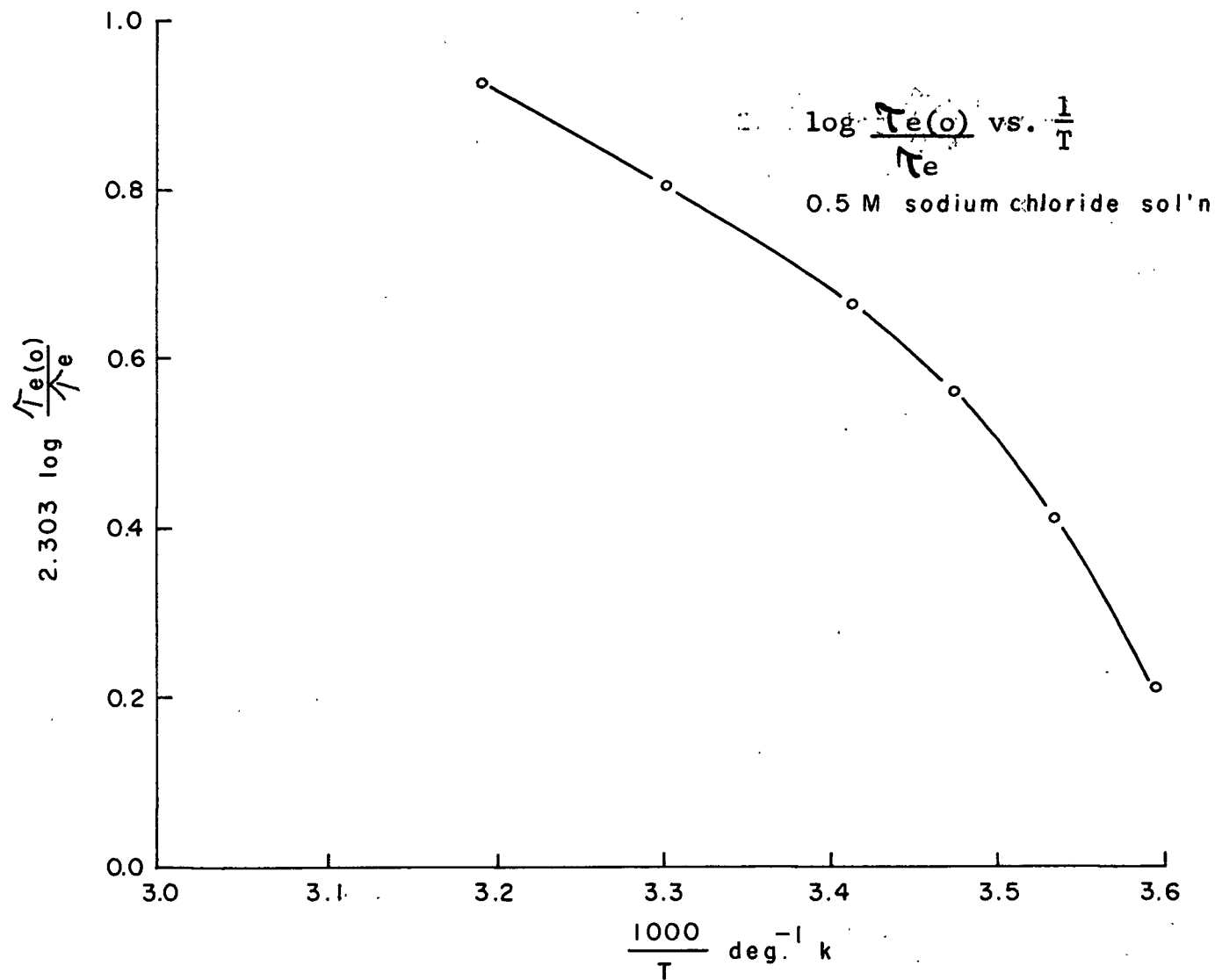


Figure XVI. Determination of the Activation Energy E_e at Different Temperatures (Salt Solution)

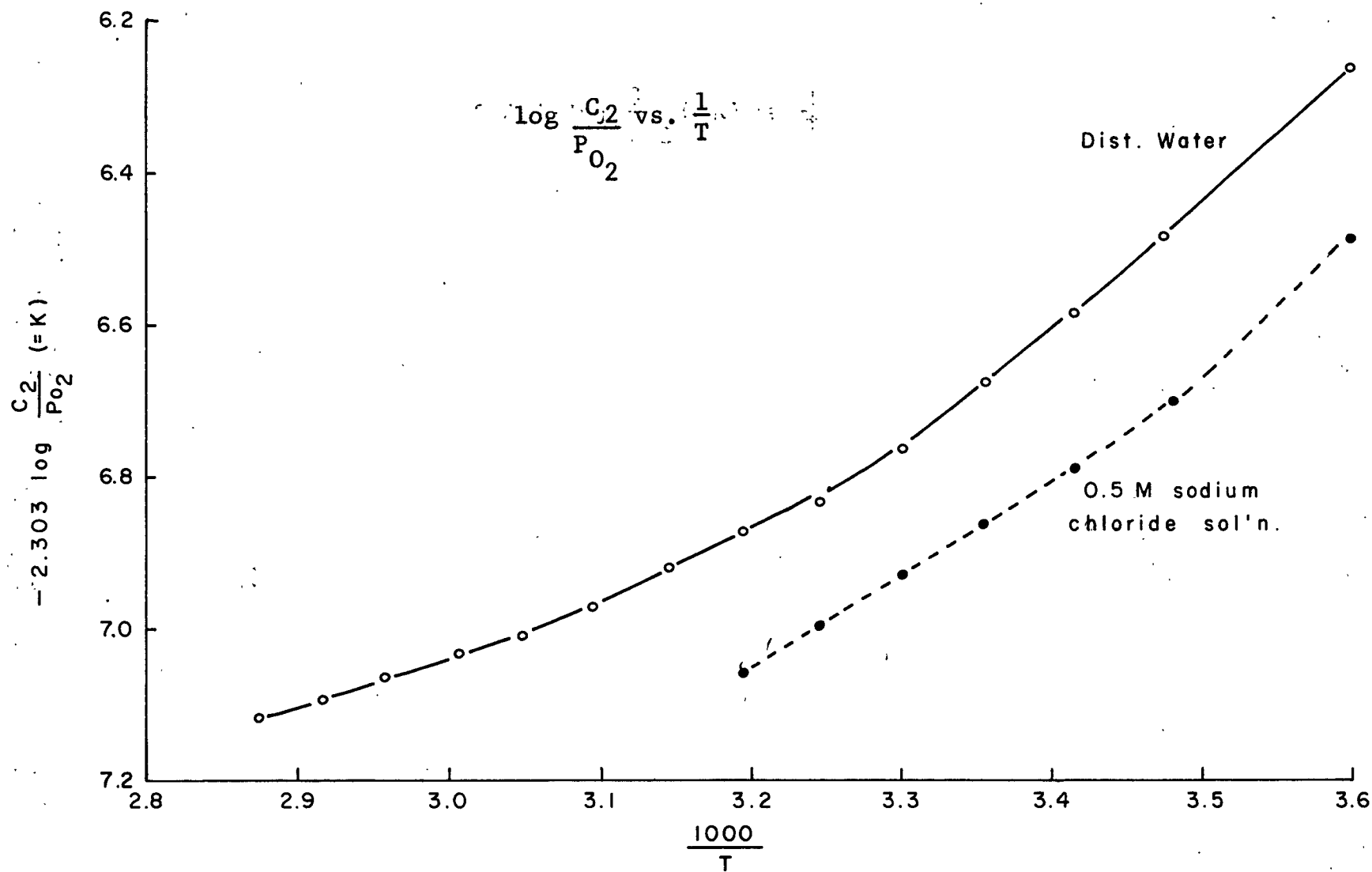


Figure XVII. Determination of the Heat of Solution $-\Delta H$ for Oxygen in Water under 1 Atm. Pressure

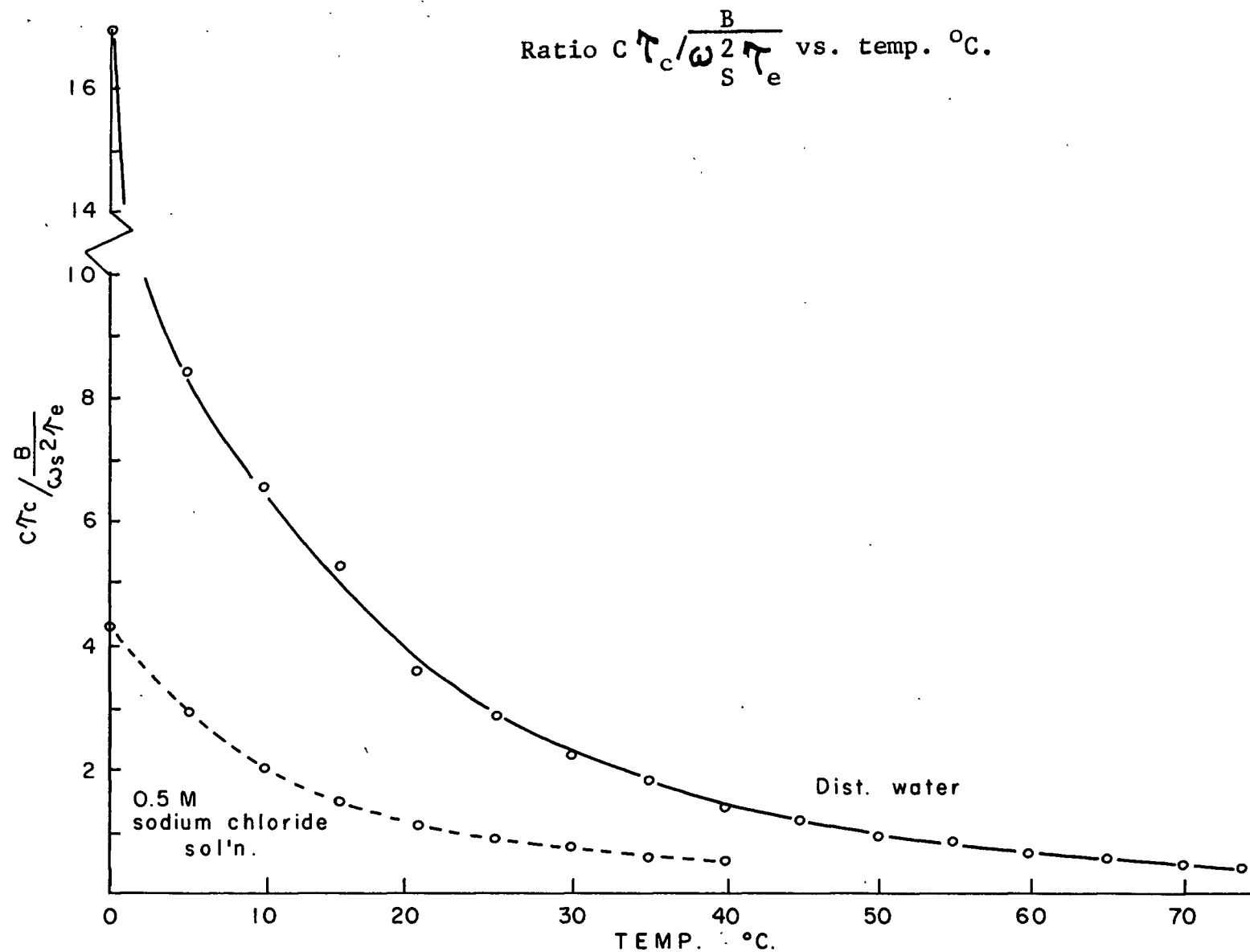


Figure XVIII. Variation of the Ratio of the Two Relaxation Terms
- Equation (8) - with Temperature

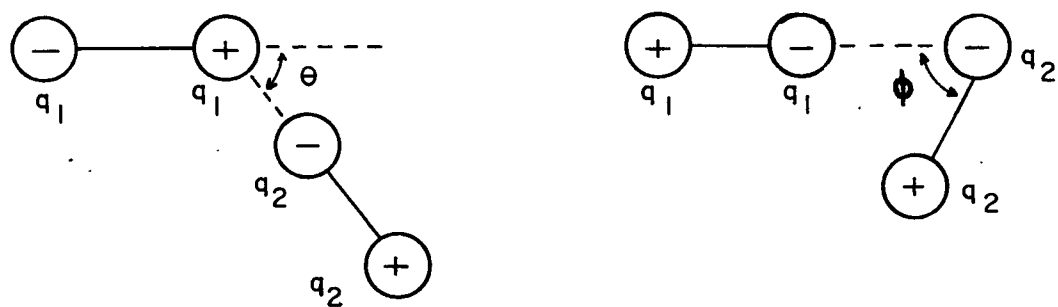


Figure XIX.
Two Different Orientations of Two Dipole Molecules

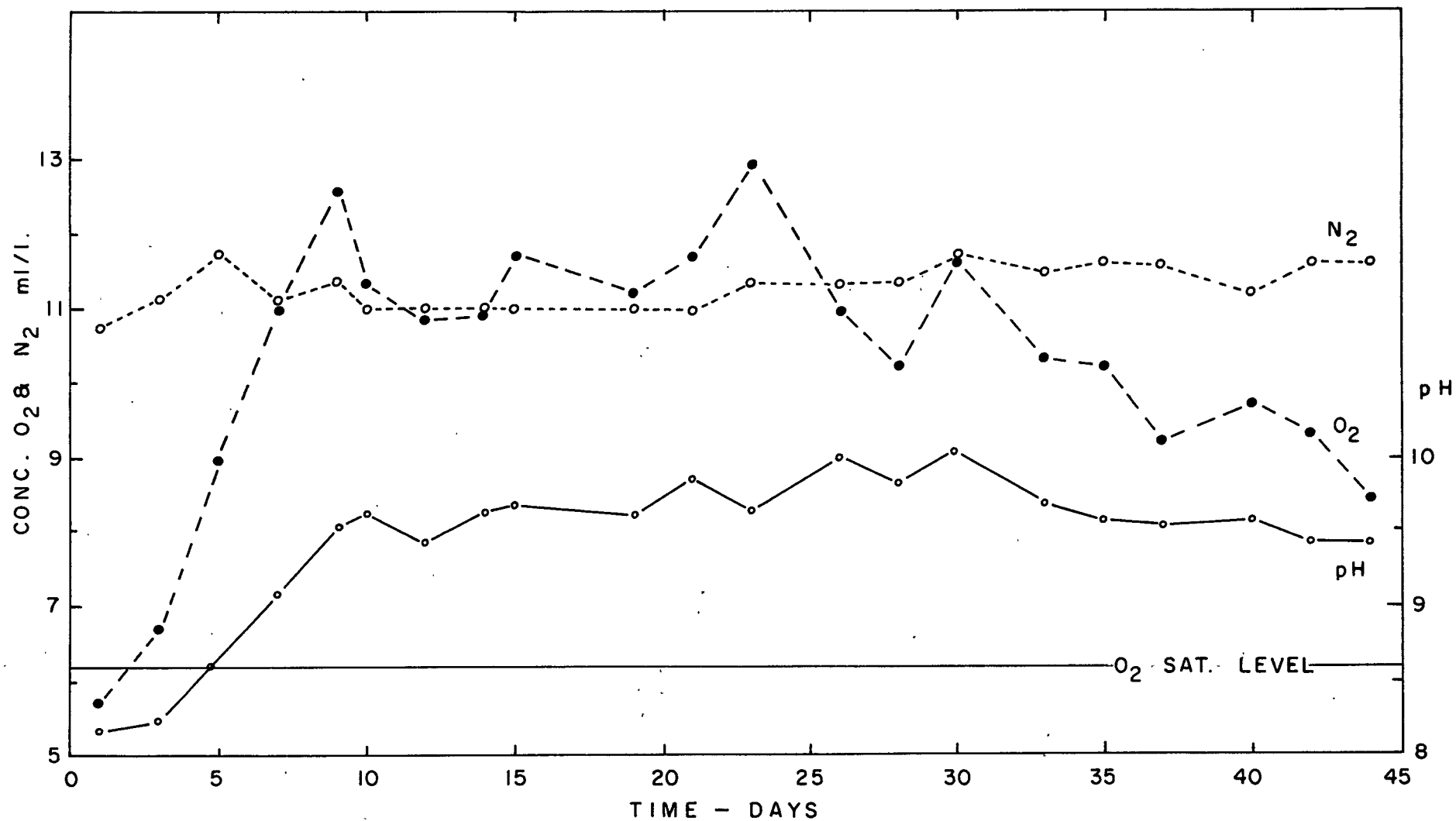


Figure XX. Variation of Oxygen and pH in Nitzschia Culture with Time

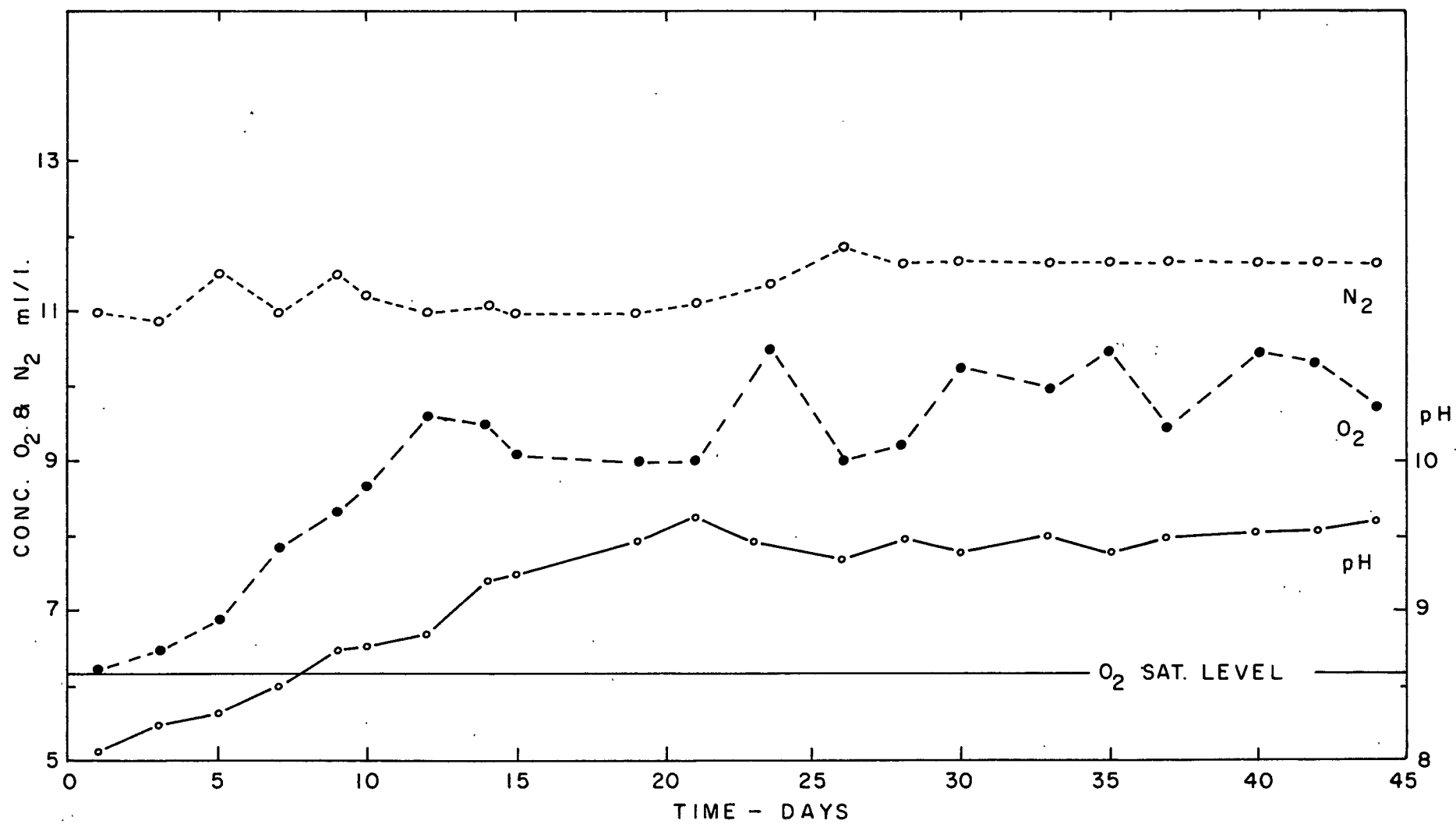


Figure XXI. Variation of Oxygen and pH in Chlorella Culture with Time

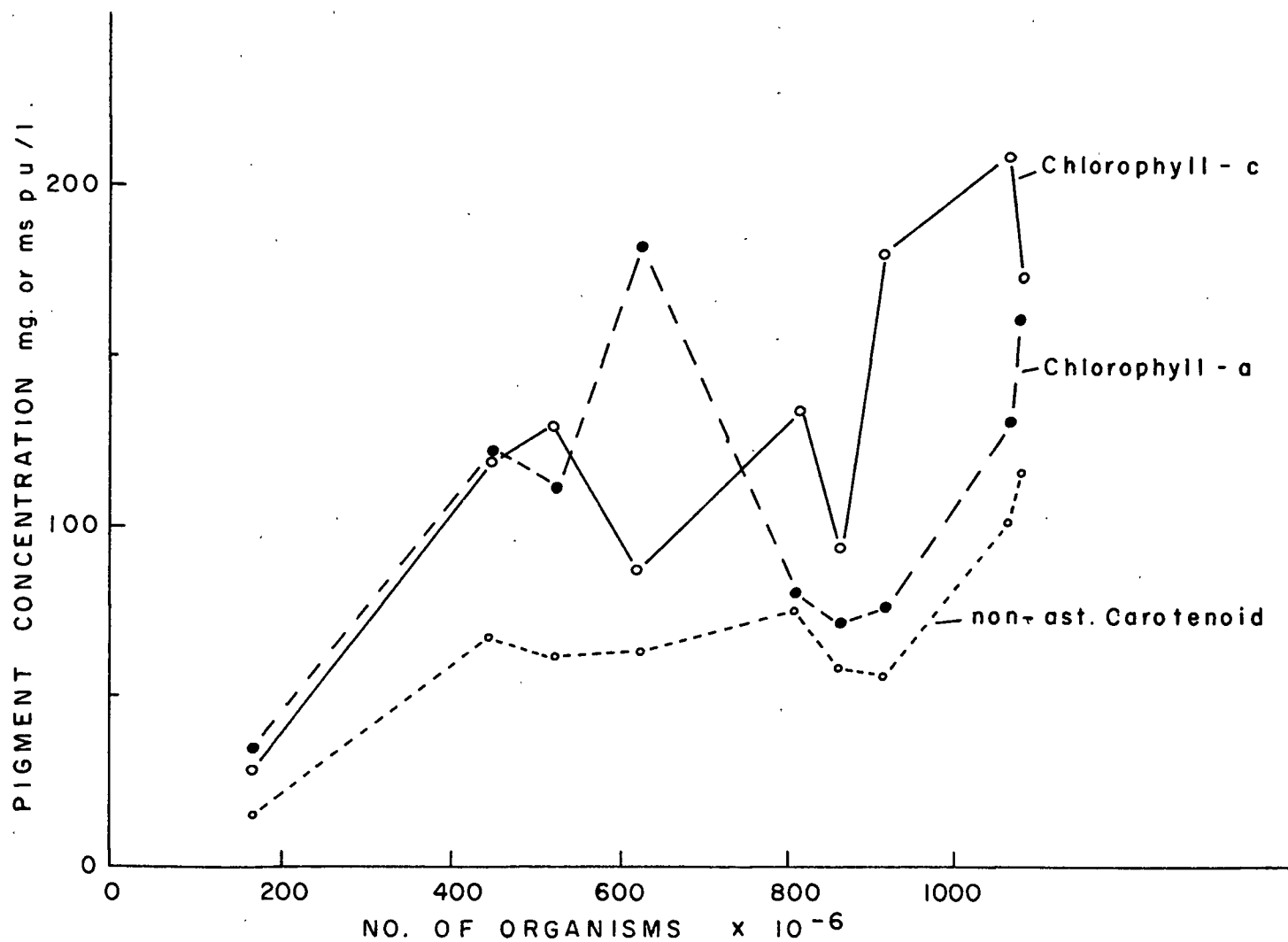


Figure XXII. Comparison of Number of Organisms in a Nitzschia Culture with Photosynthetic Pigment Concentration

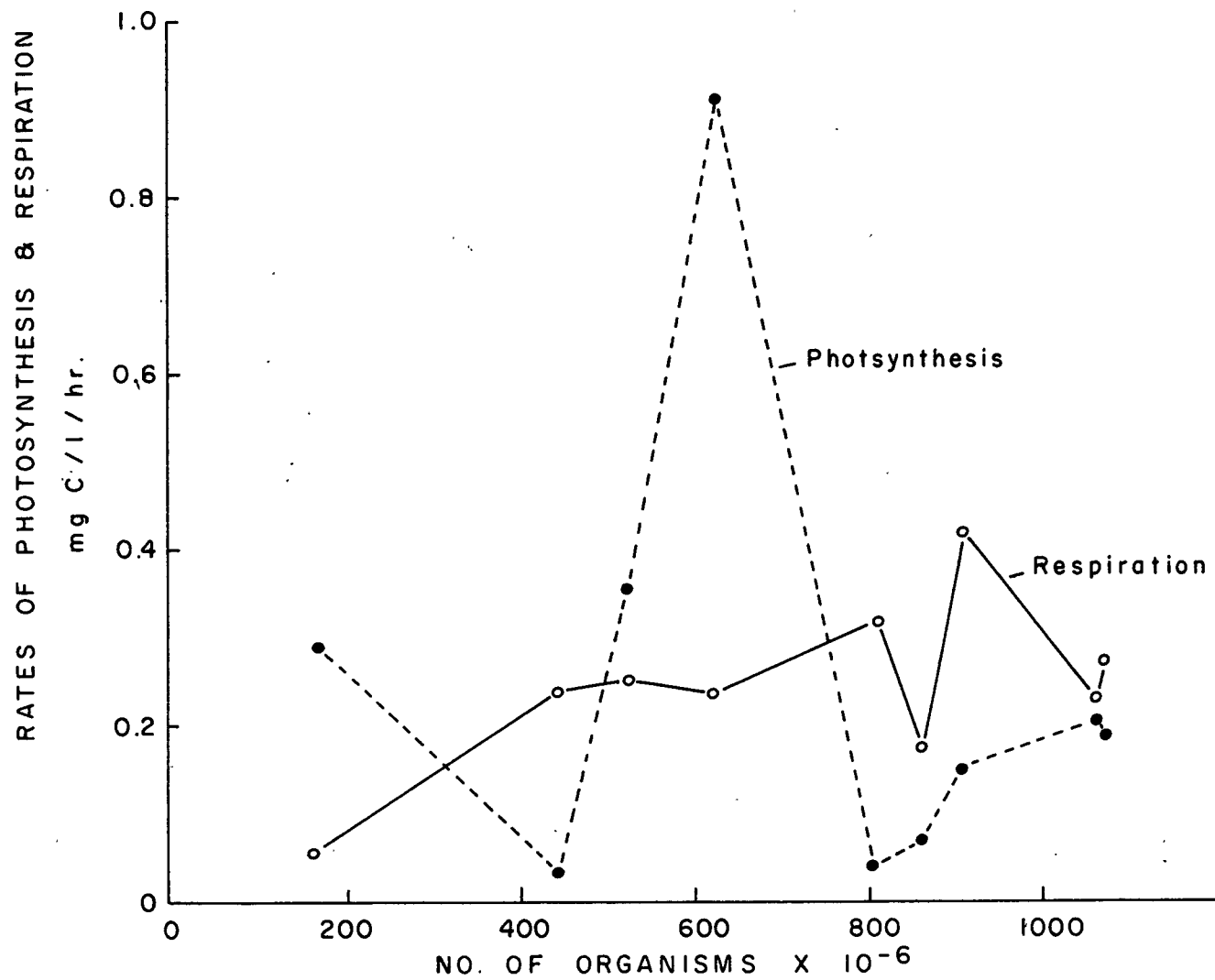


Figure XXIII. Comparison of Number of Organisms in a *Nitzschia* Culture with Rates of Photosynthesis and Respiration

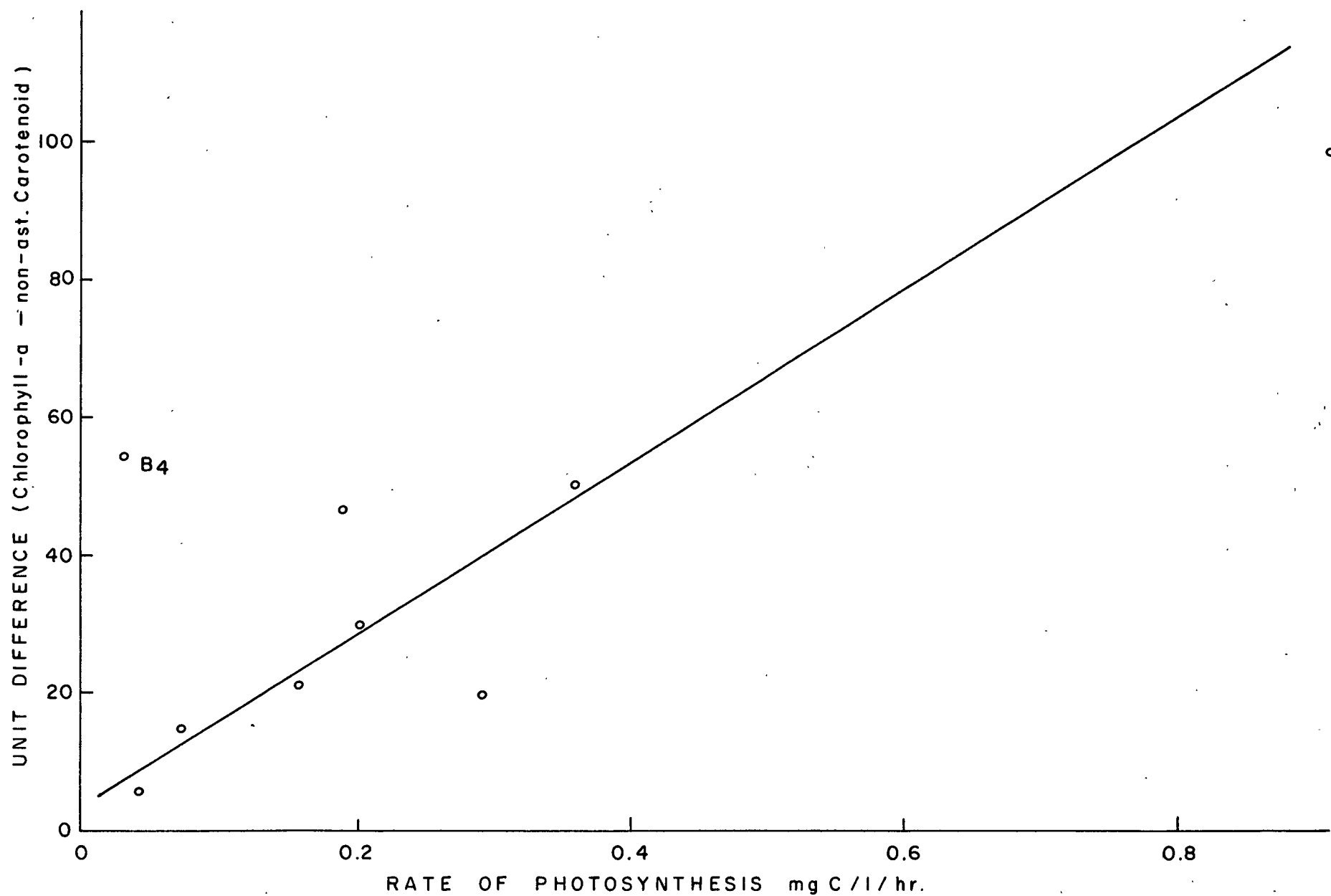


Figure XXIV. Behaviour of Photosynthetic Rate in *Nitzschia* with the Difference of Pigment Content

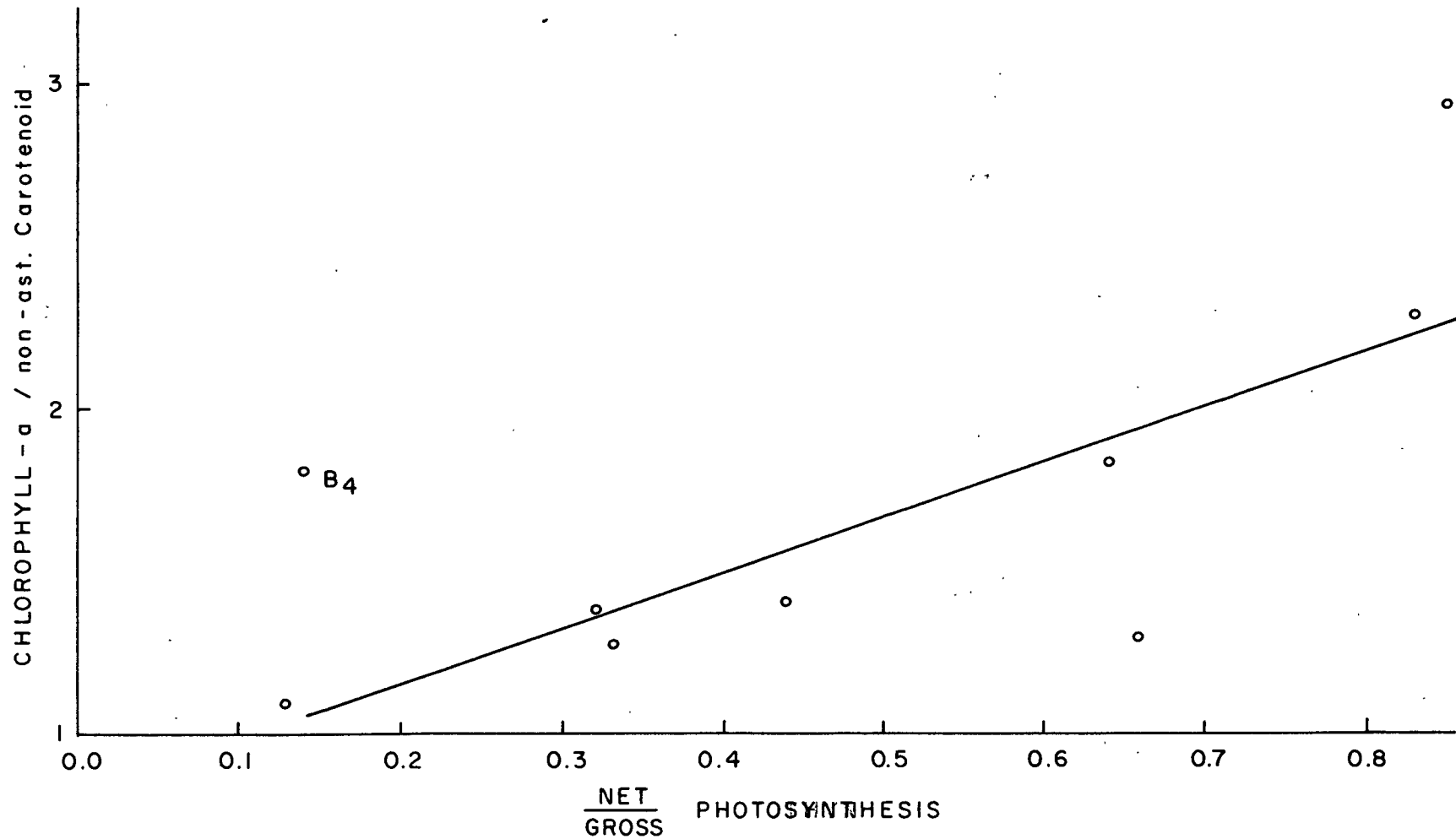


Figure XXV. Behaviour of Net:Gross Photosynthetic Rates in Nitzschia with Ratio of Pigment Content

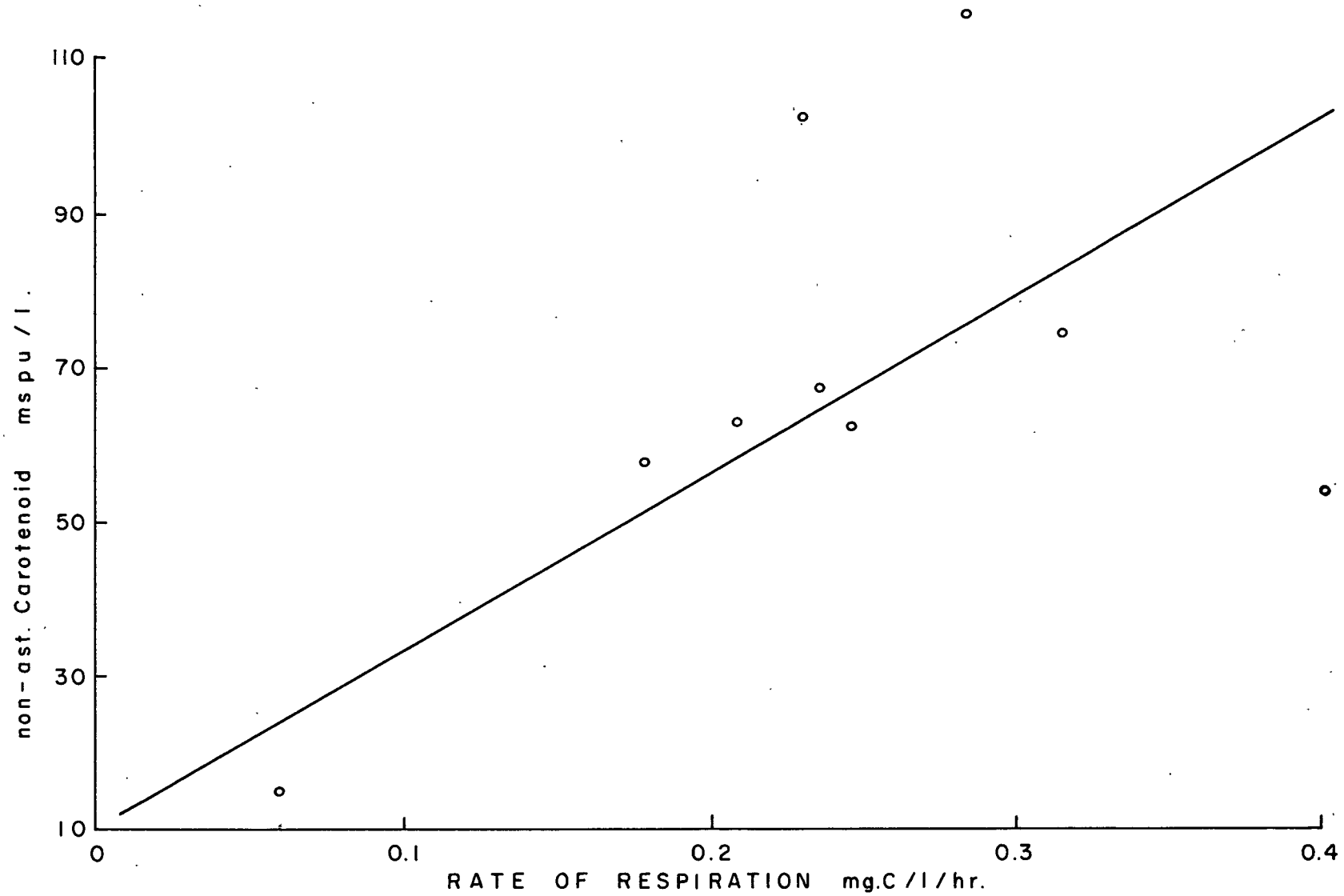


Figure XXVI. Dependence of Respiration Rate in Nitzschia on Carotenoid Pigment Concentration

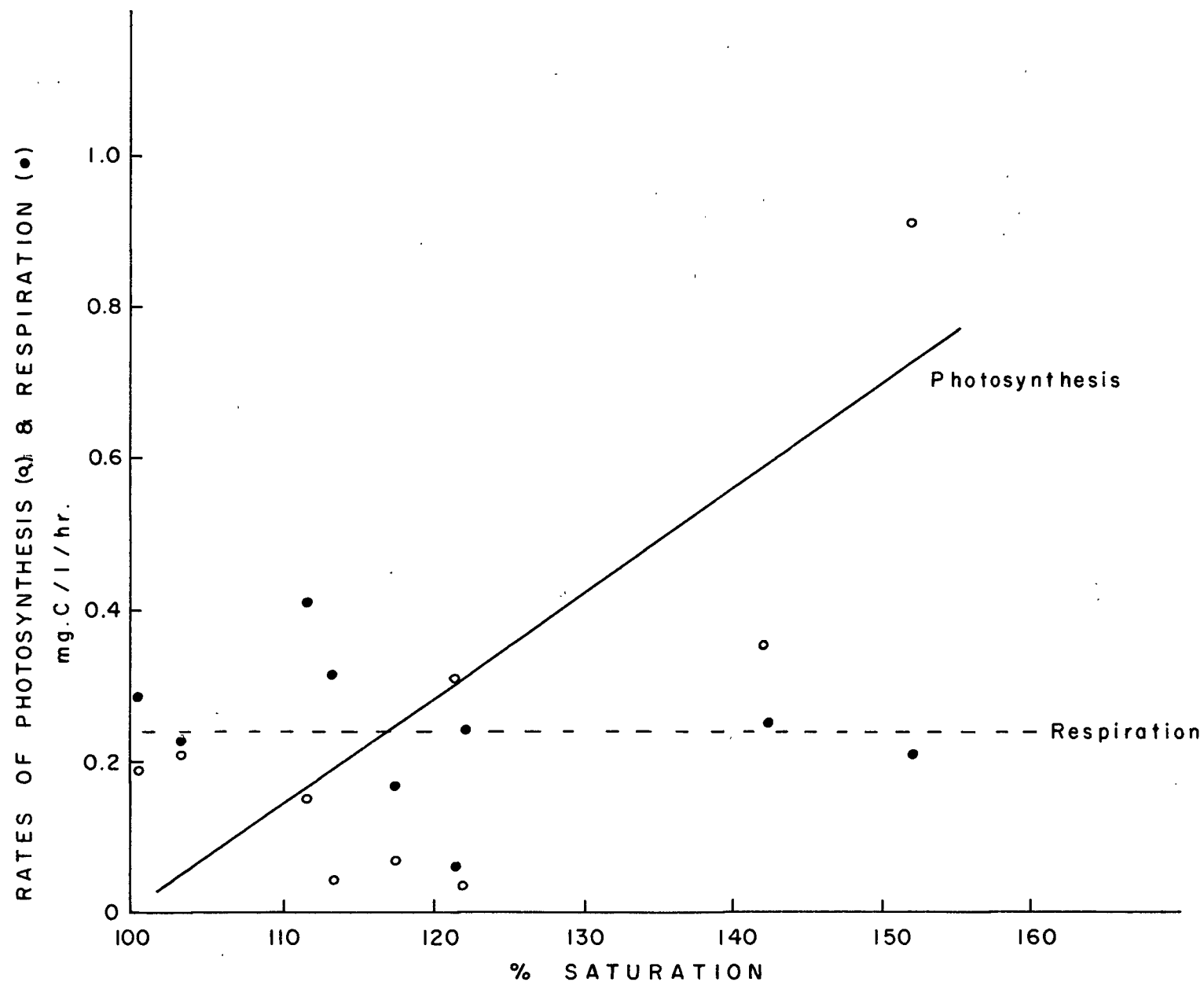


Figure XXVII. Relation of Per Cent Saturation to Rates of Photosynthesis and Respiration in Nitzschia

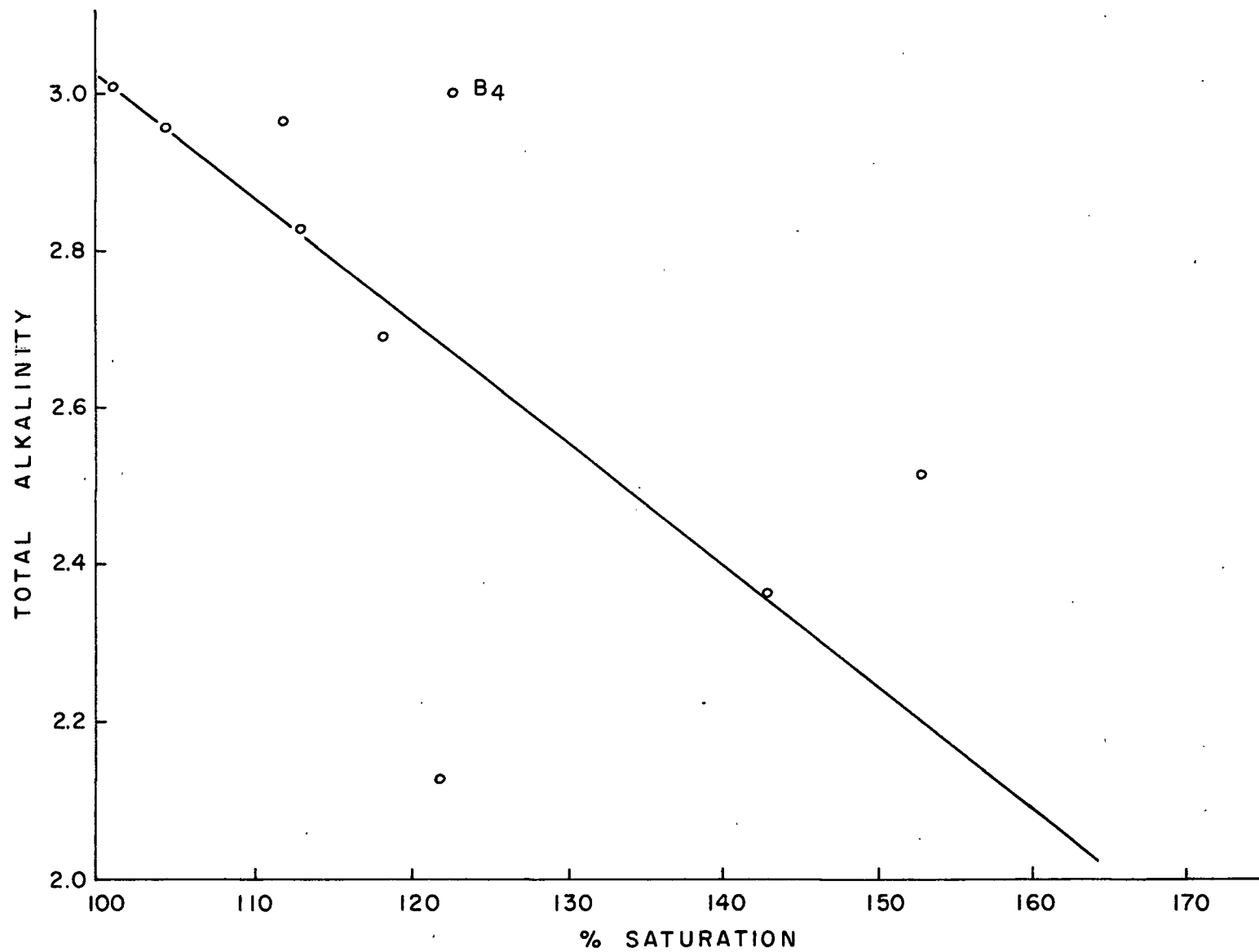


Figure XXVIII. Influence of Total Alkalinity on the Saturation Level
in a Nitzschia Culture

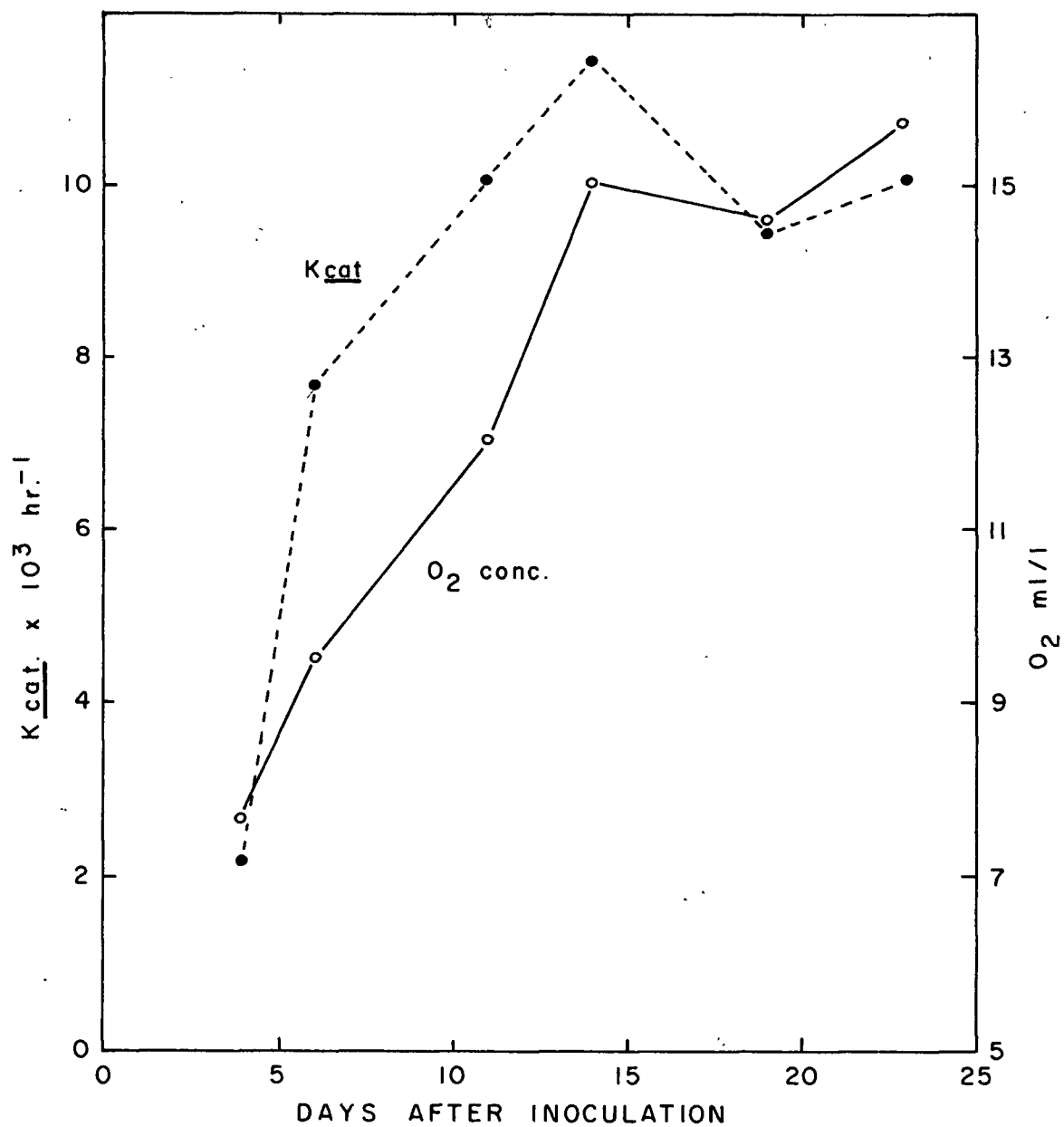


Figure XXIX. Variation of Oxygen and Catalytic Activity in a Nitzschia Culture with Time

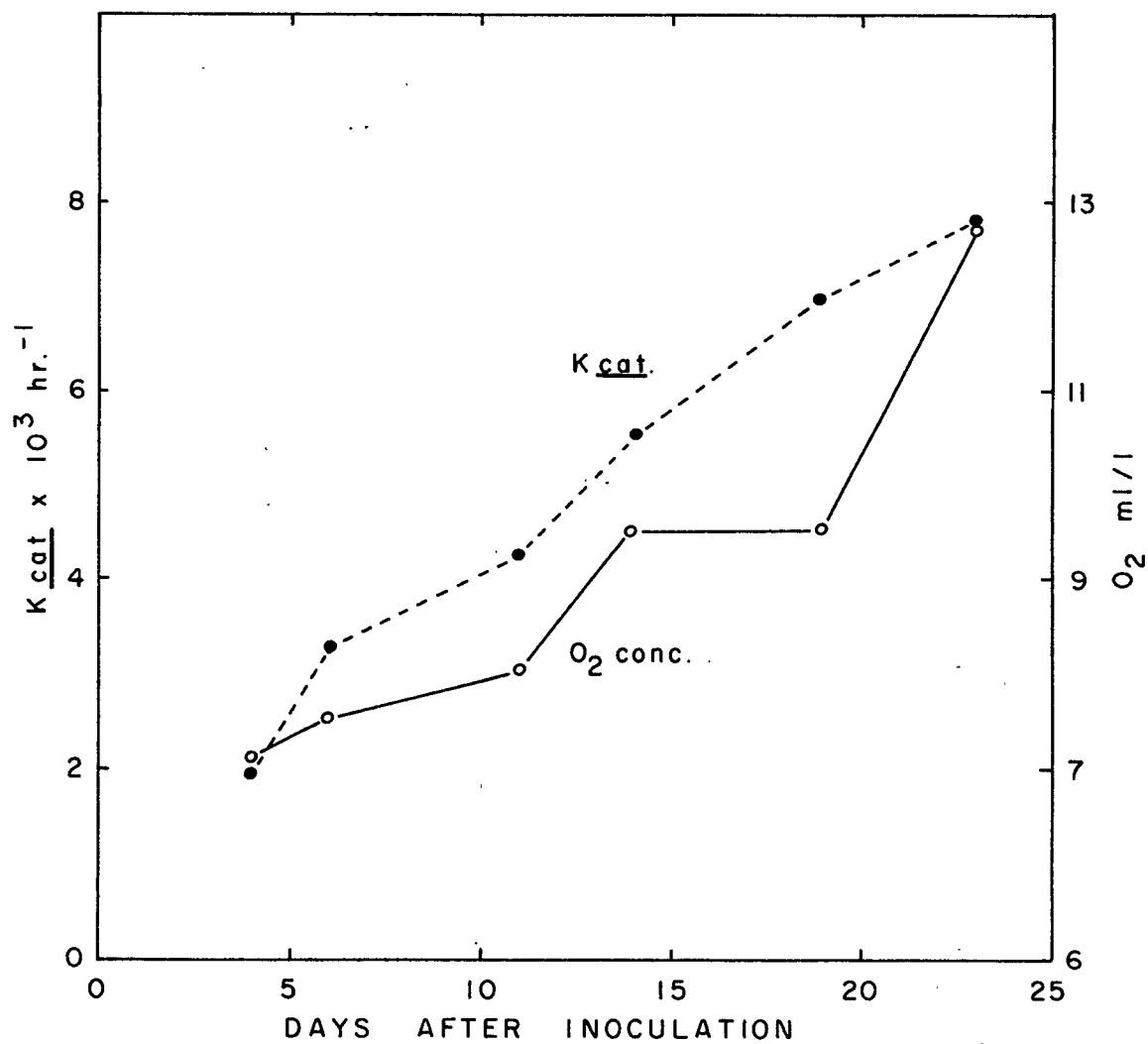


Figure XXX. Variation of Oxygen and Catalytic Activity in a Chlorella Culture with Time

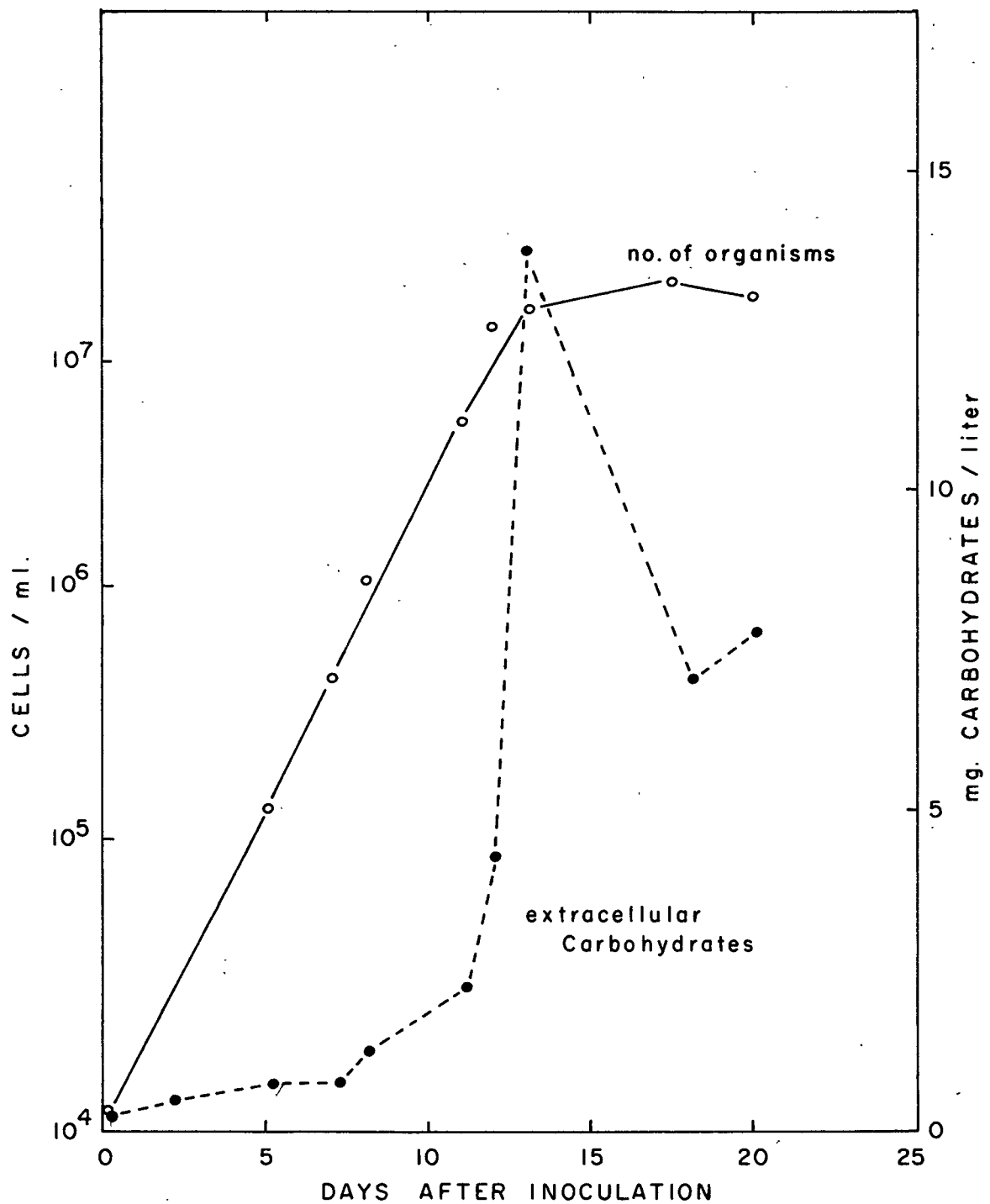


Figure XXXI. Extracellular Carbohydrate Relative to Number of Organisms for the Flagellate Isochrysis galbana (after Guillard, 1958)

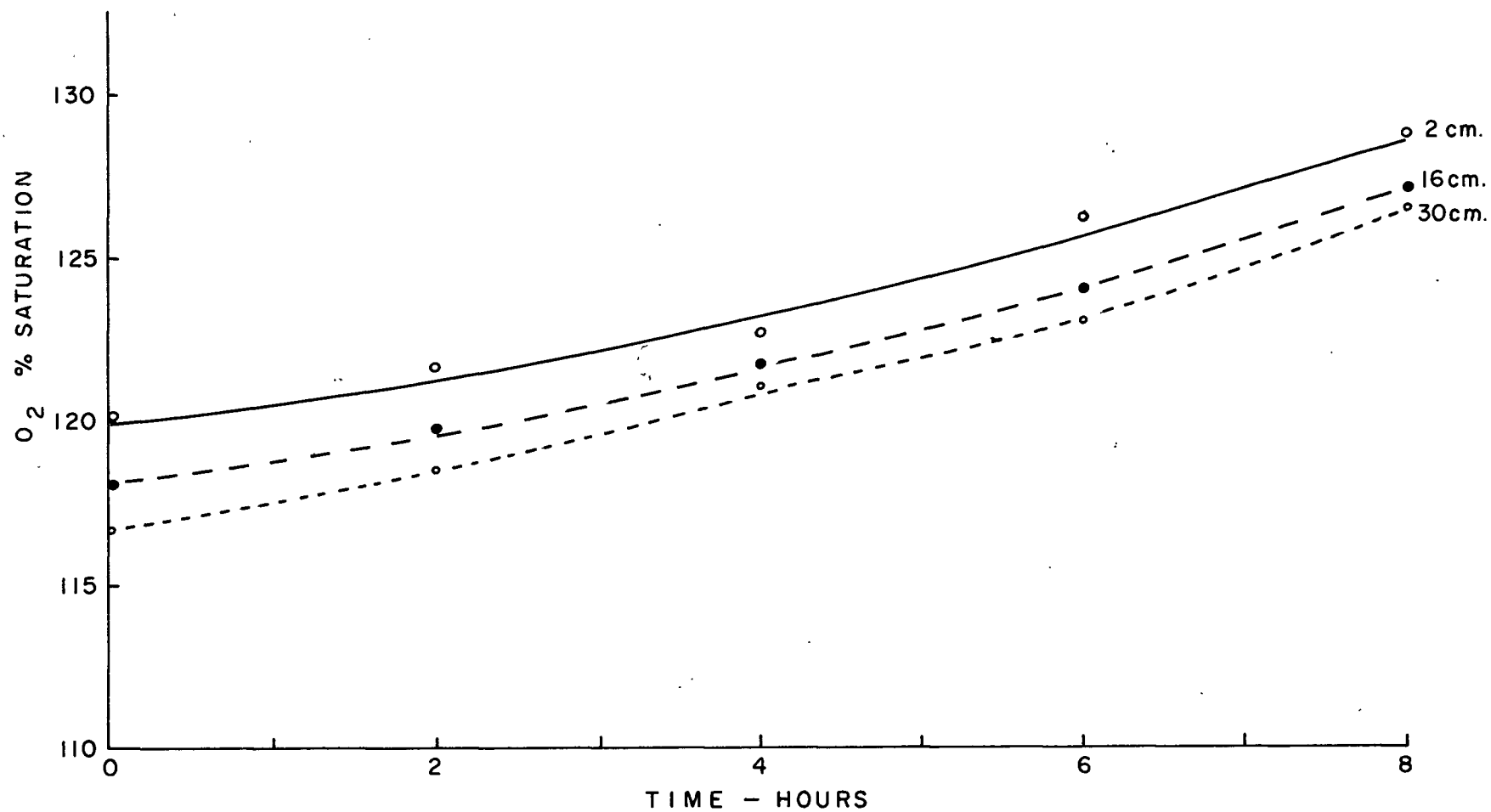


Figure XXXII. Change of Oxygen Concentration under Illumination in a Nitzschia Culture at Different Depths

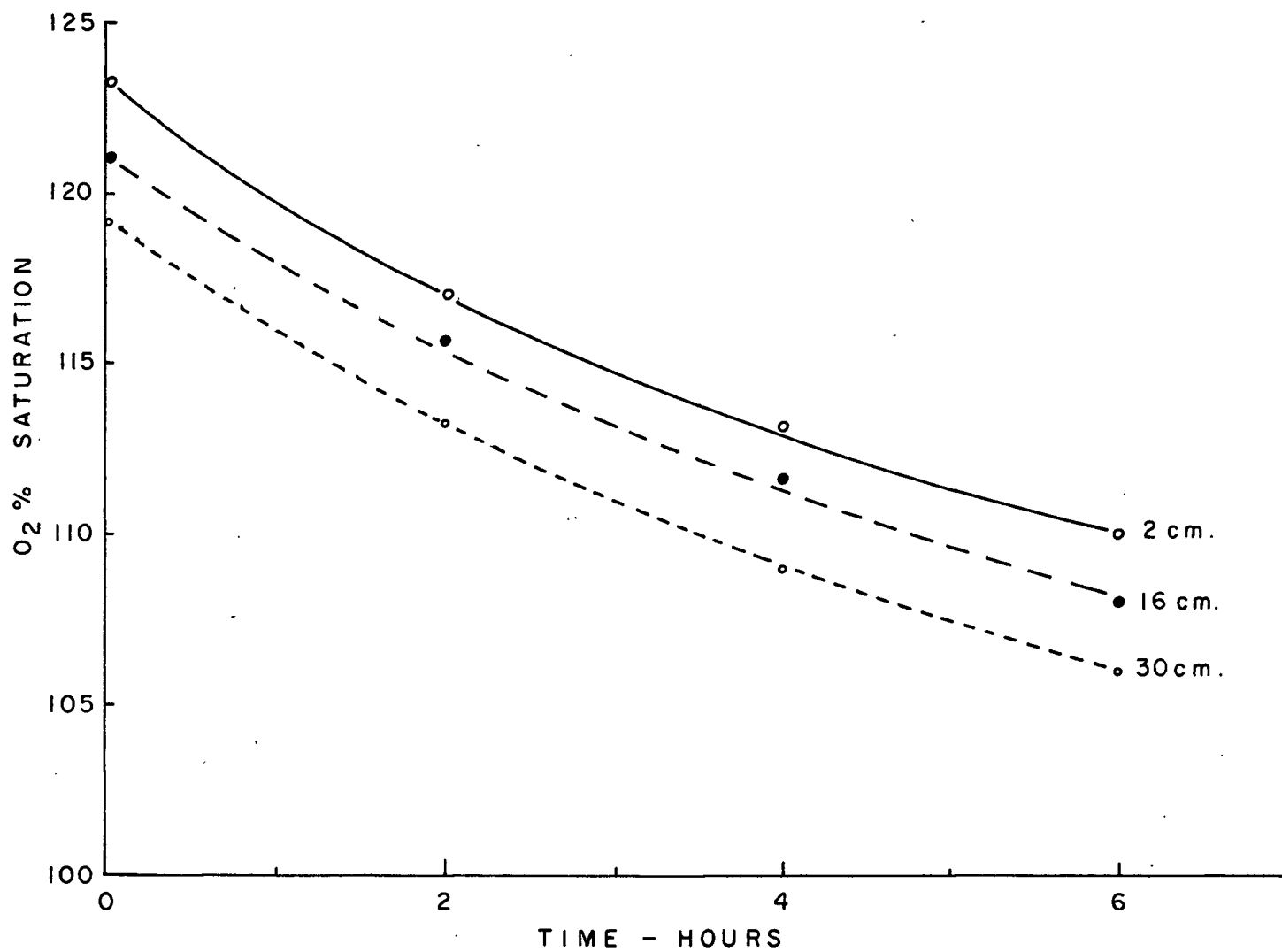


Figure XXXIII. Change of Oxygen Concentration under Dark in a Nitzschia Culture at Different Depths

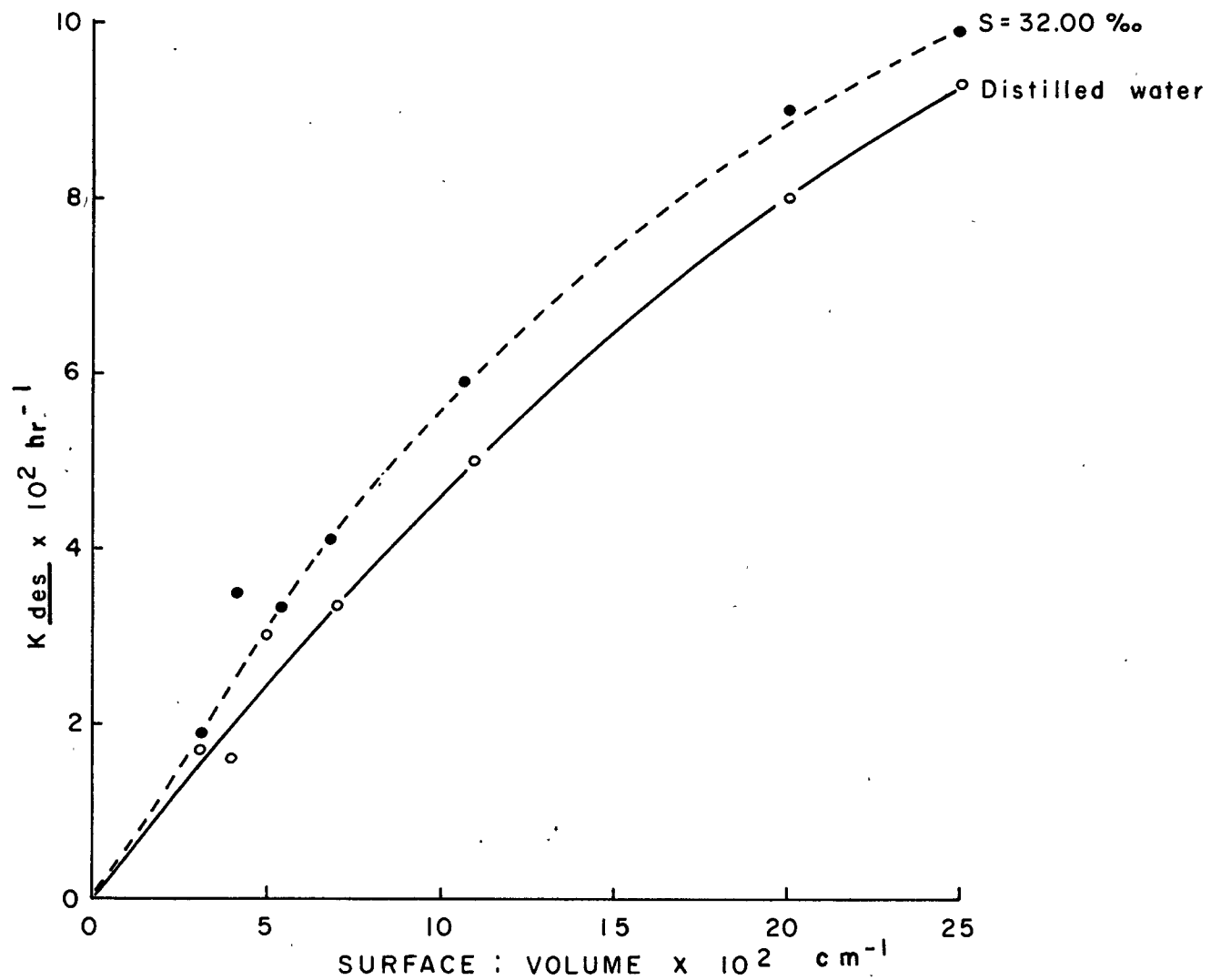


Figure XXXIV. Variation of Oxygen Desupersaturation Rate Constant with Surface to Volume Ratio of a Water Column

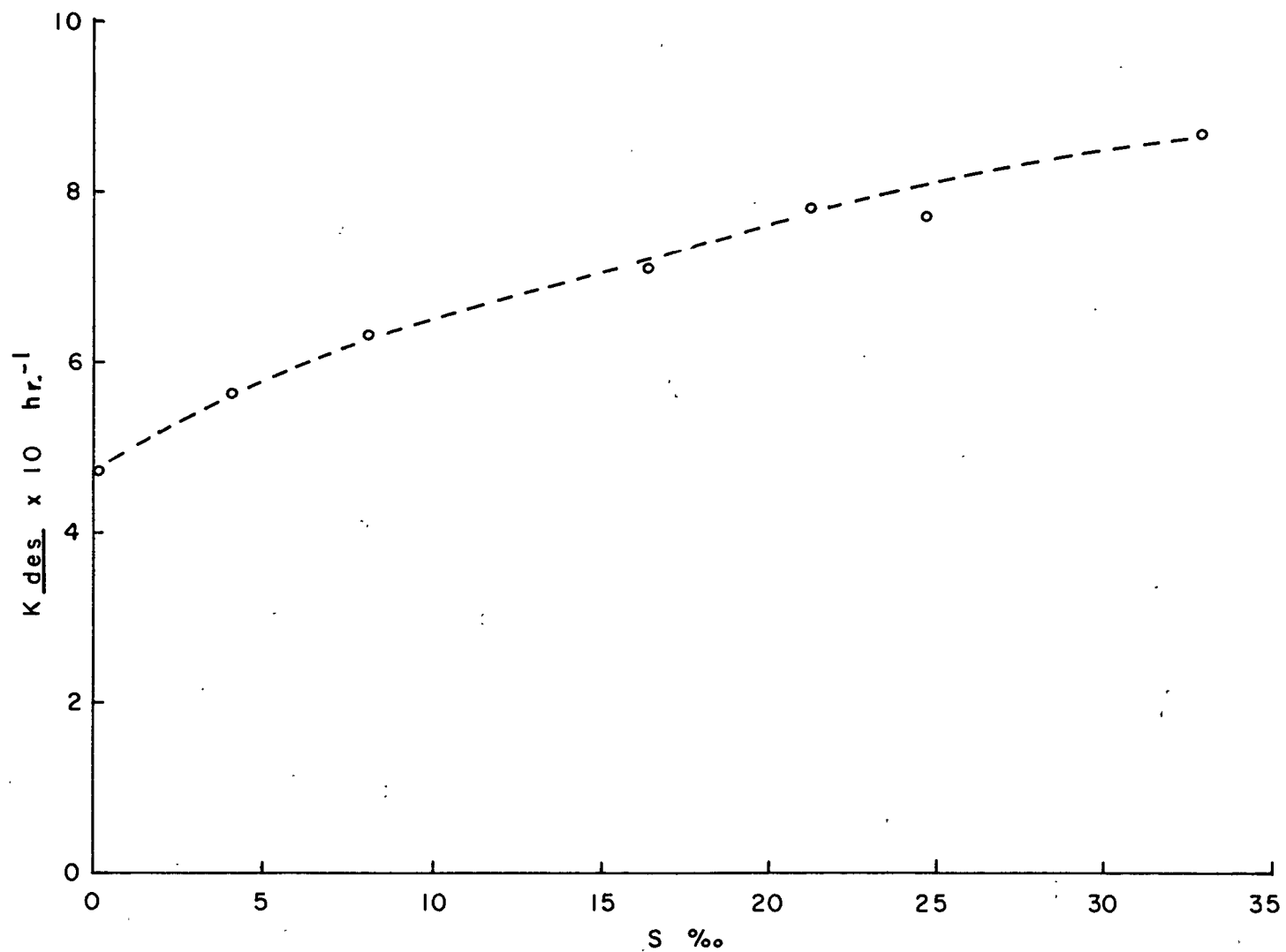


Figure XXXV. Variation of Oxygen Desupersaturation Rate Constant with Salinity under Stirred Conditions

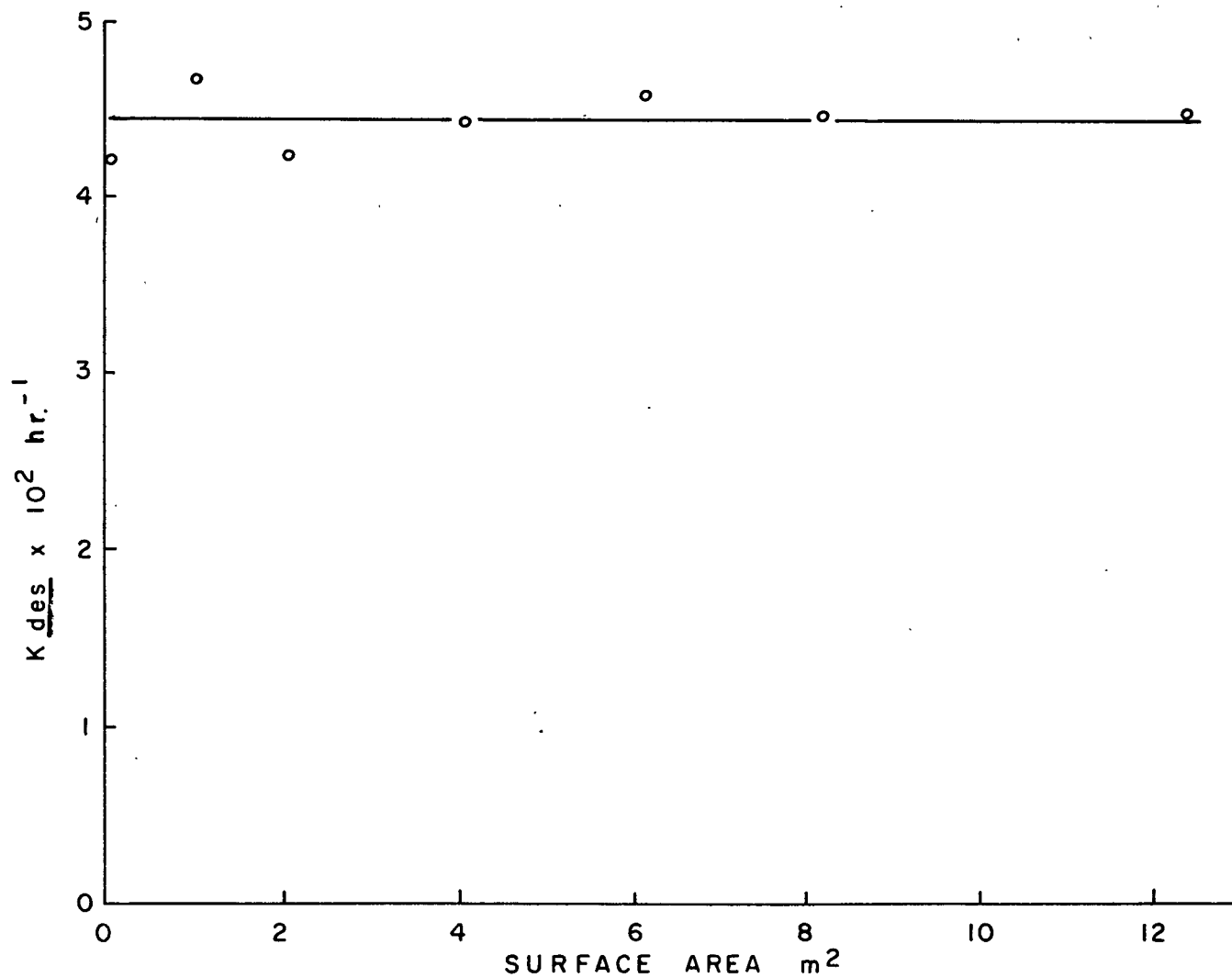


Figure XXXVI. Behaviour of Siliceous Surface Area with
Oxygen Desupersaturation Rate Constant

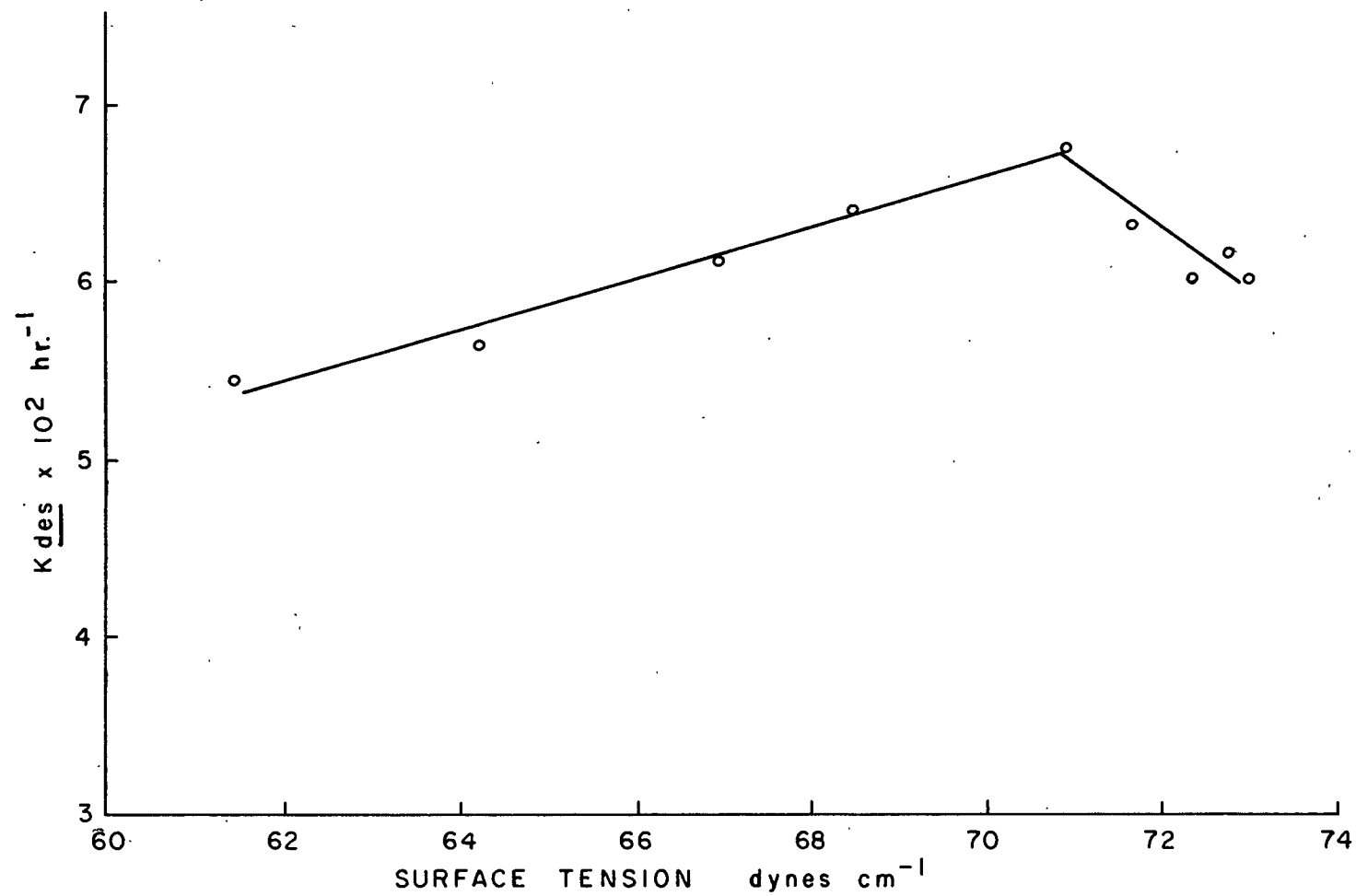


Figure XXXVII. Influence of Heptanoic Acid Addition on Oxygen Desupersaturation Rate Constant

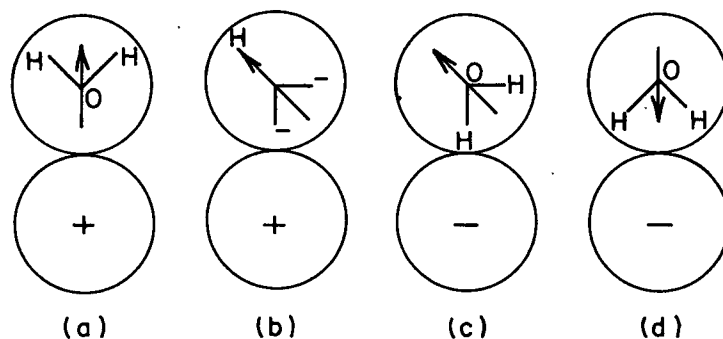


Figure I. Proposed Dipole Orientation of a Water Molecule in Presence of Ions (From Hindman, 1961)

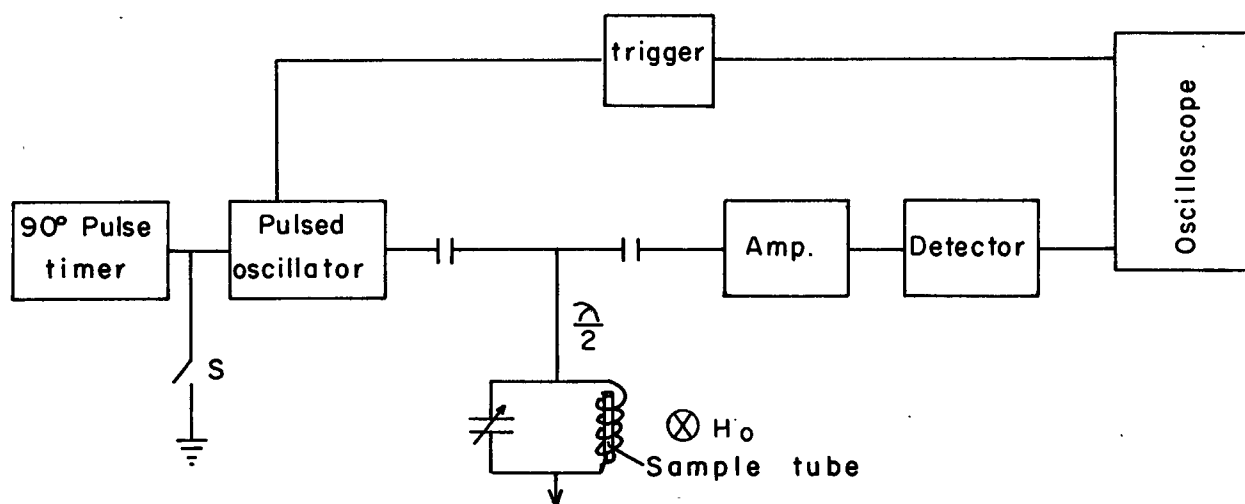


Figure II (a). NMR Assembly

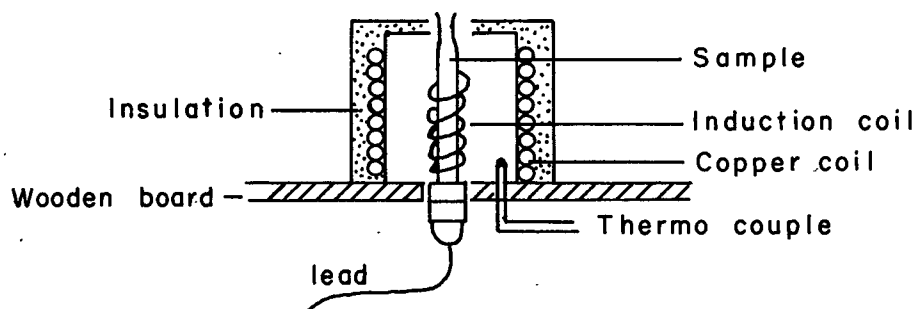


Figure II (b). Induction Coil Chamber

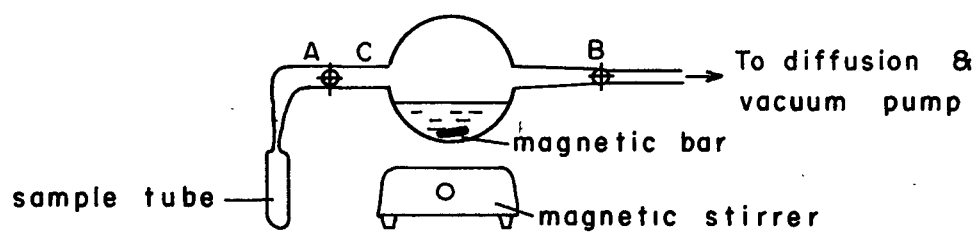


Figure II (c). Degassing Instrument

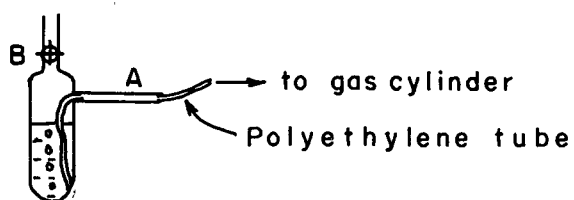


Figure II (d). Sample Tube

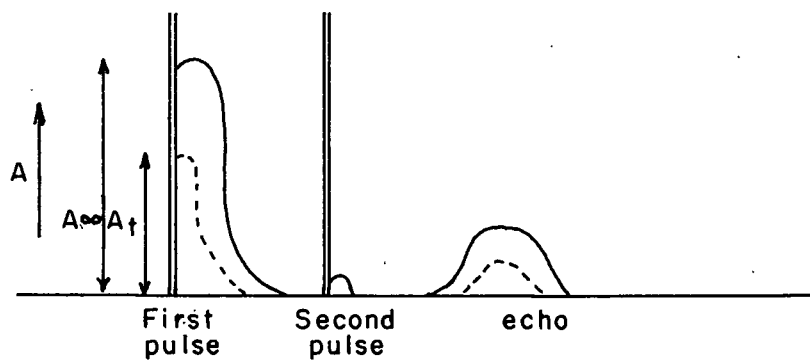


Figure III. Oscilloscope Display of the Two Pulses and the Detected r-f Signal

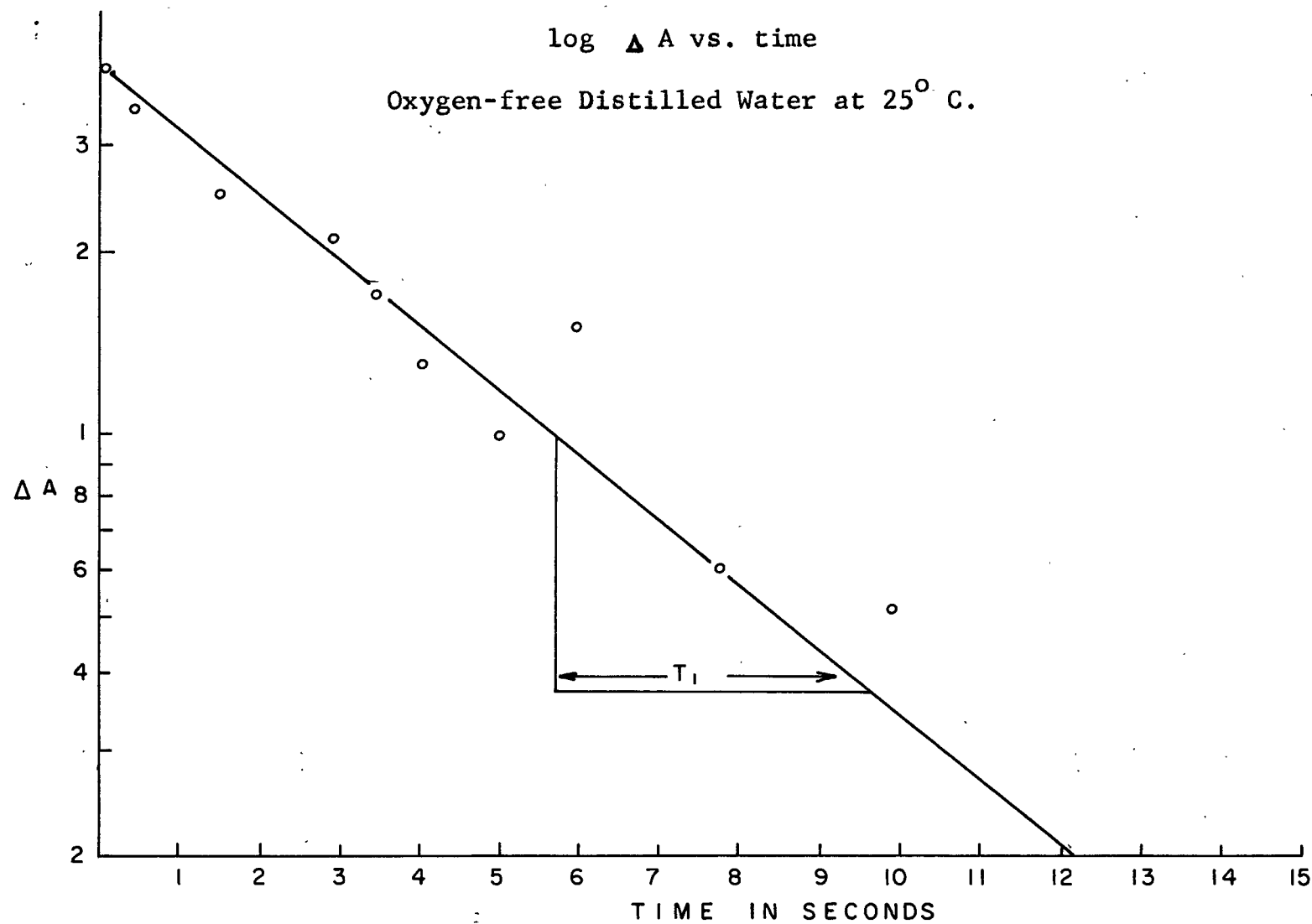


Figure IV. Determination of T_1

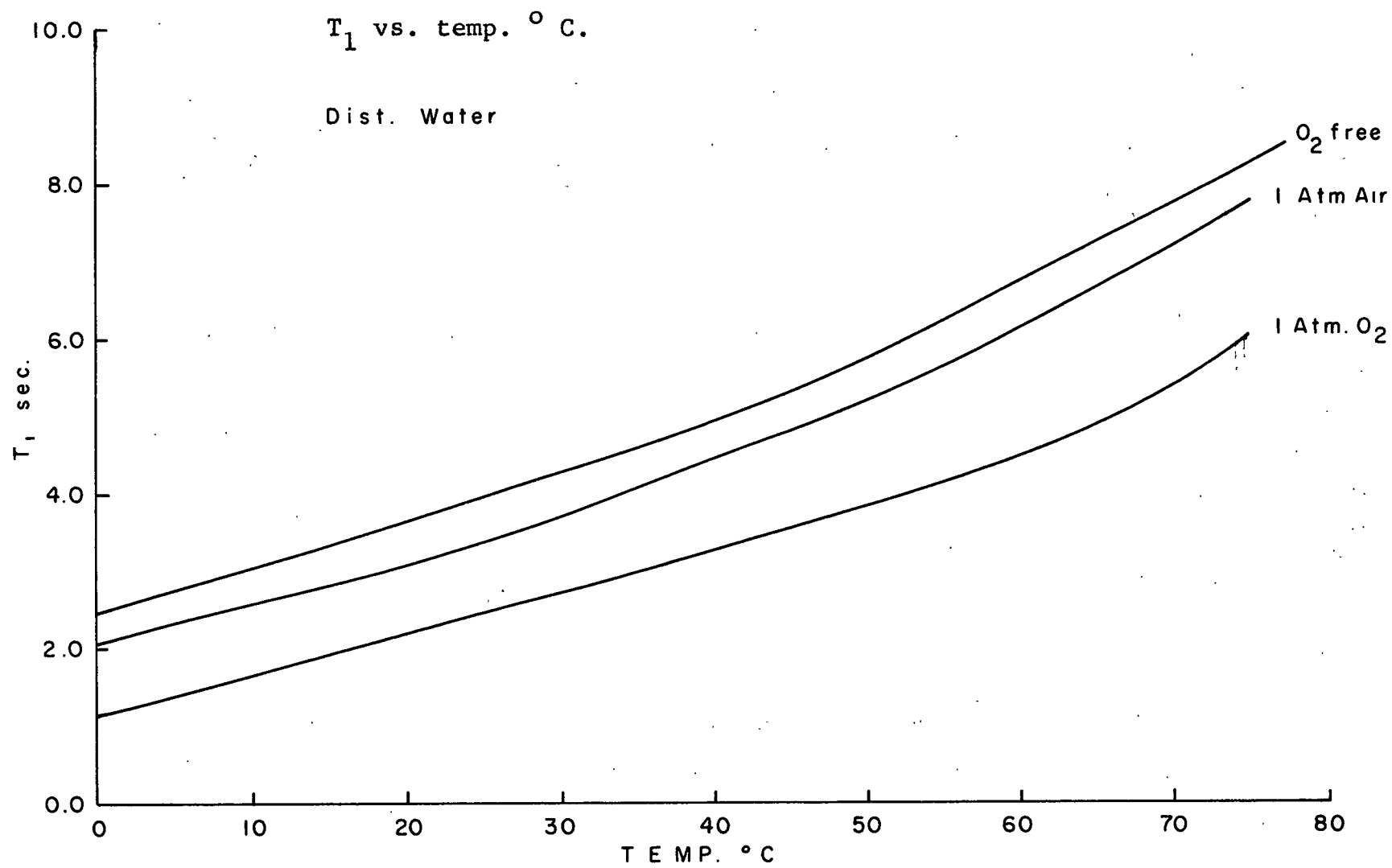


Figure V. Smoothed Curves of T_1 Values at Different Temperatures (Distilled Water).

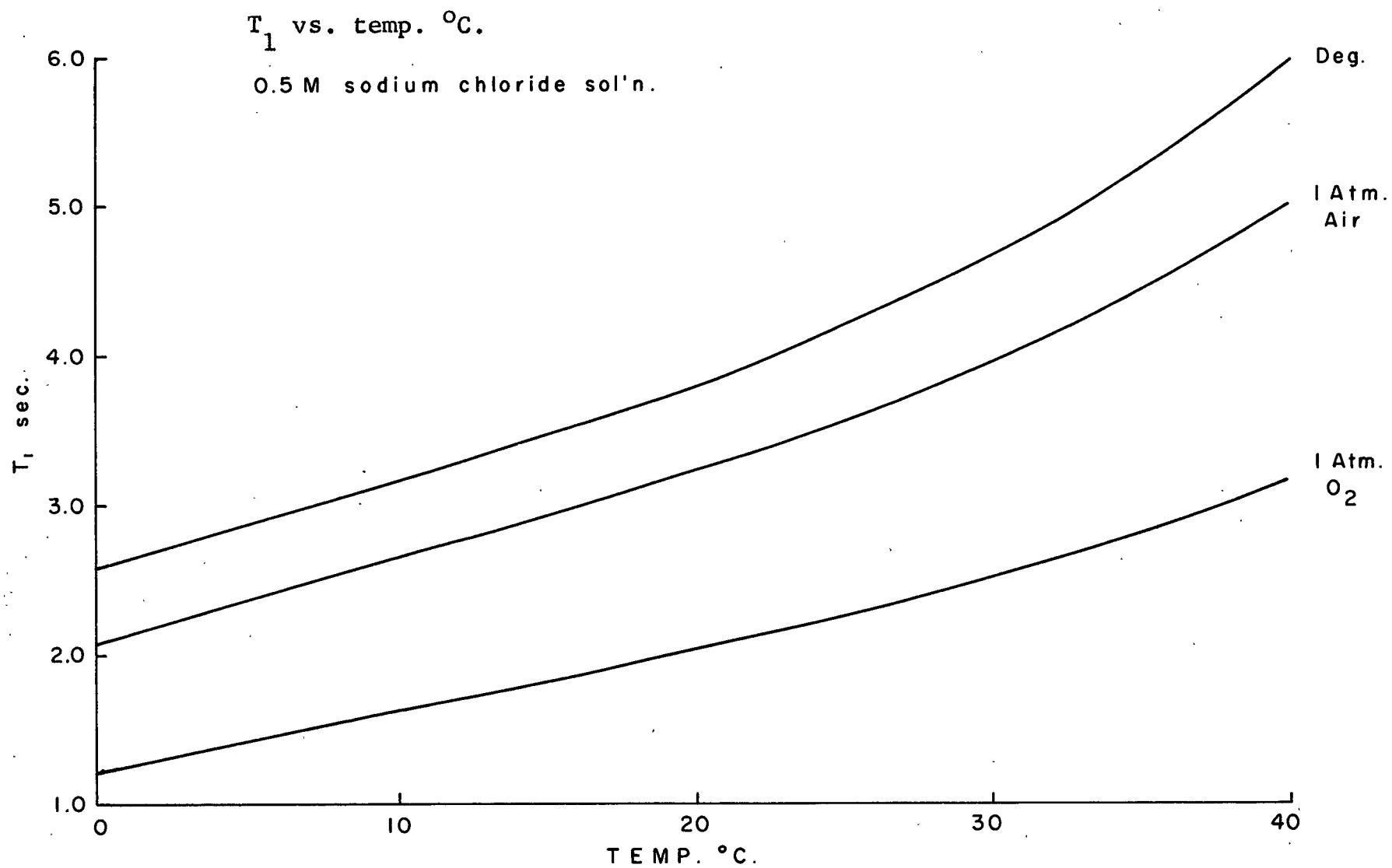


Figure VI. Smoothed Curves of T_1 Values at Different Temperatures (Salt Solution)

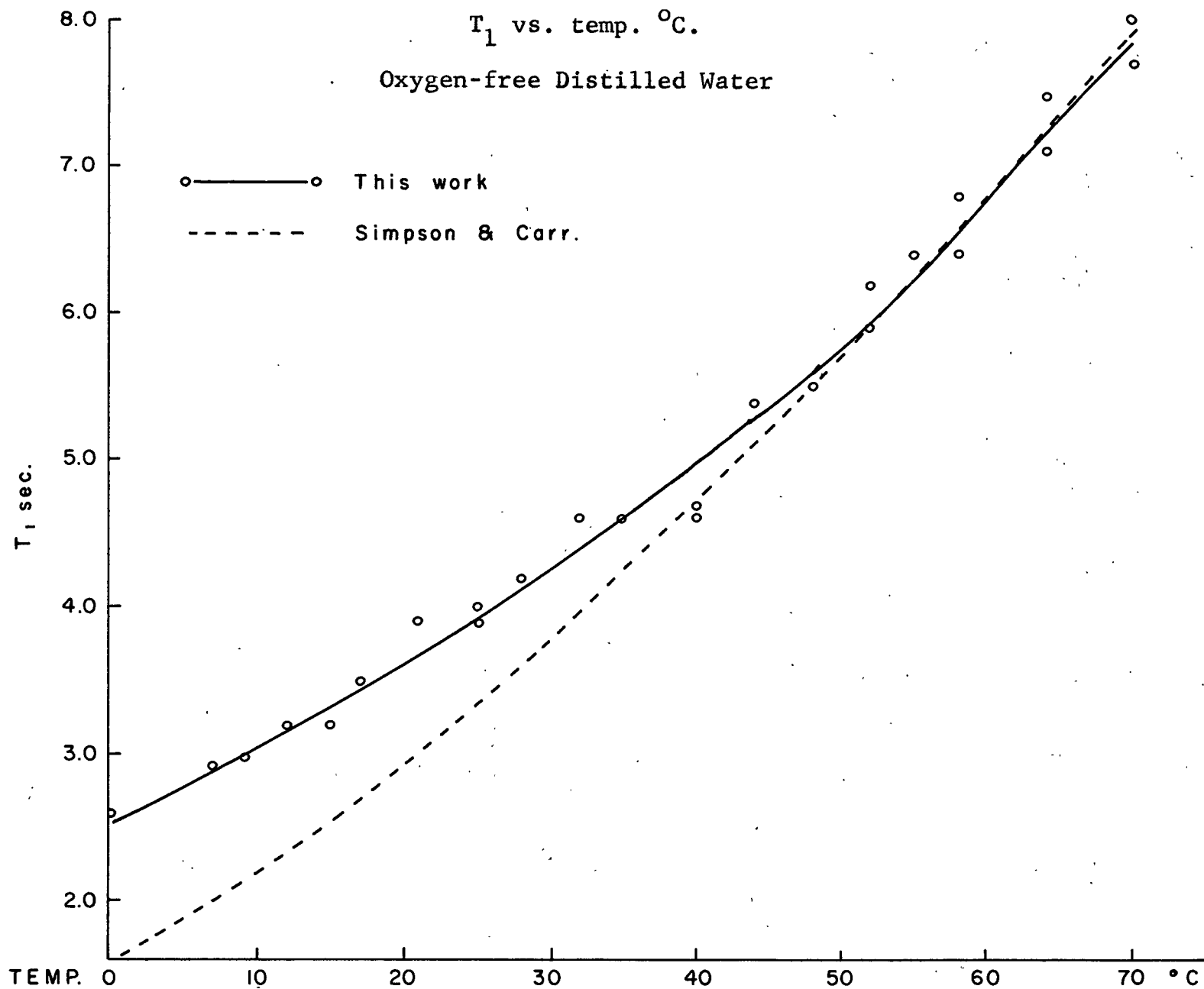
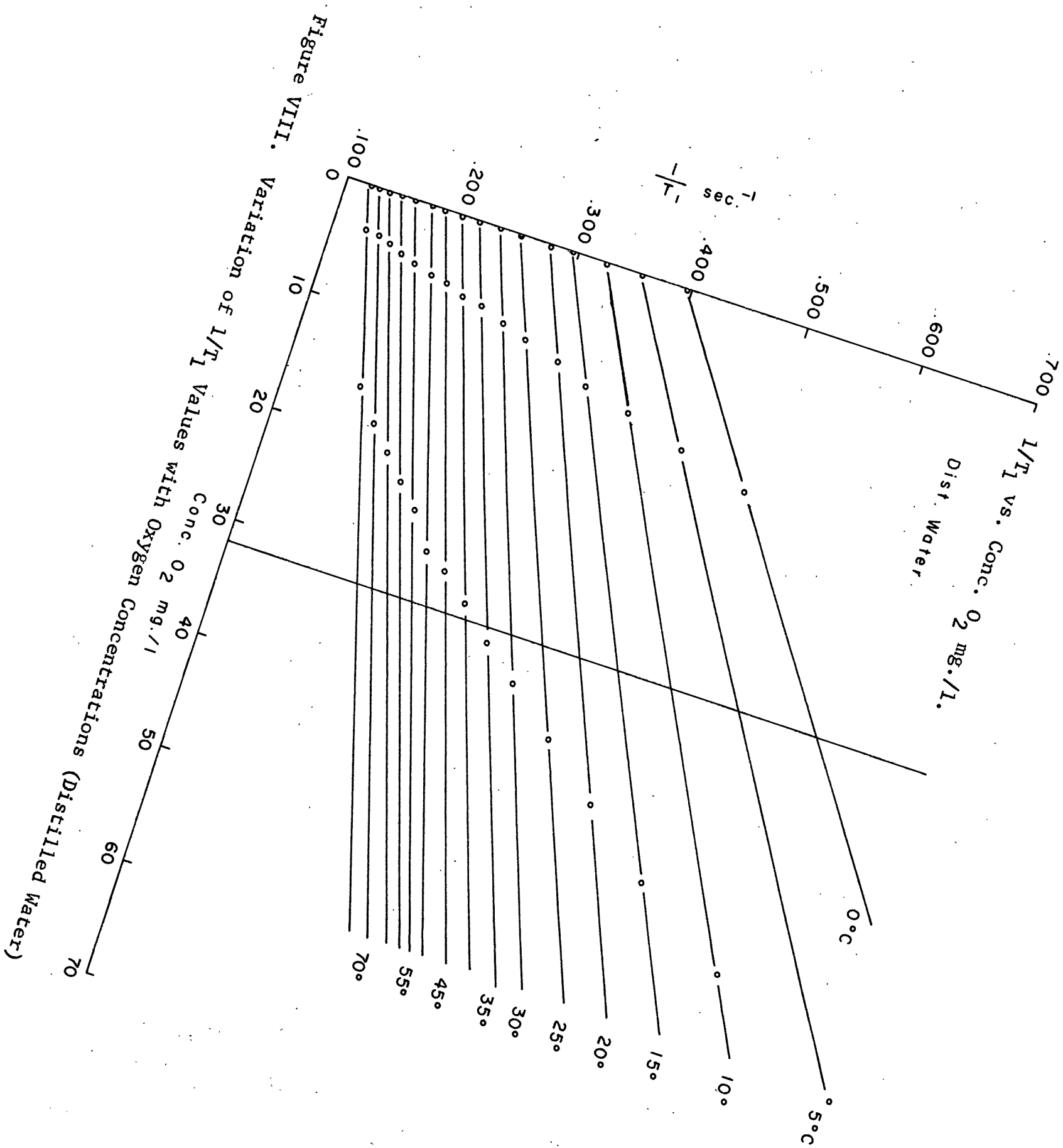


Figure VII. Comparison of T_1 Values for Oxygen-free Distilled Water



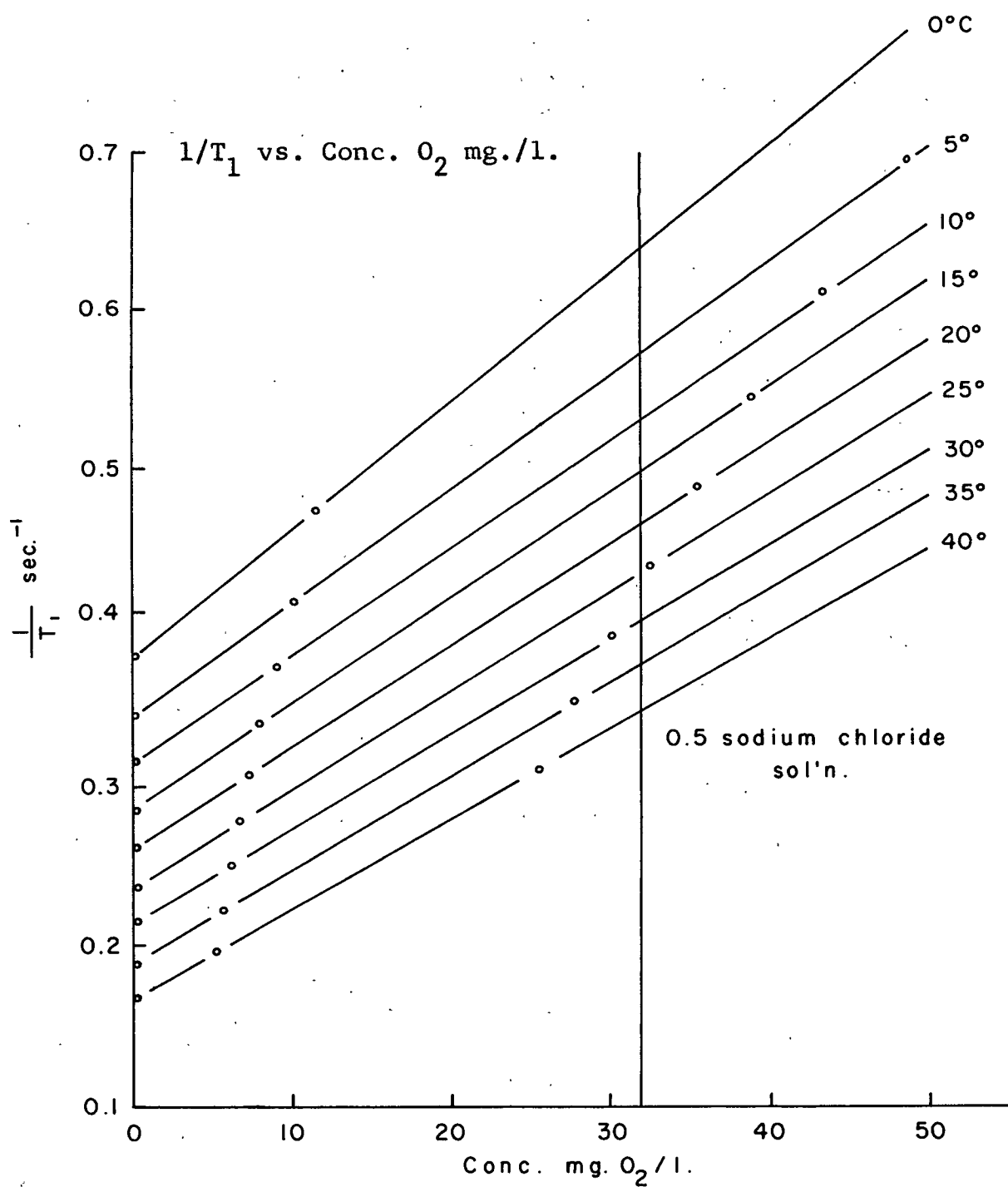


Figure IX. Variation of 1/T₁ Values with Oxygen Concentrations
(Salt Solution)

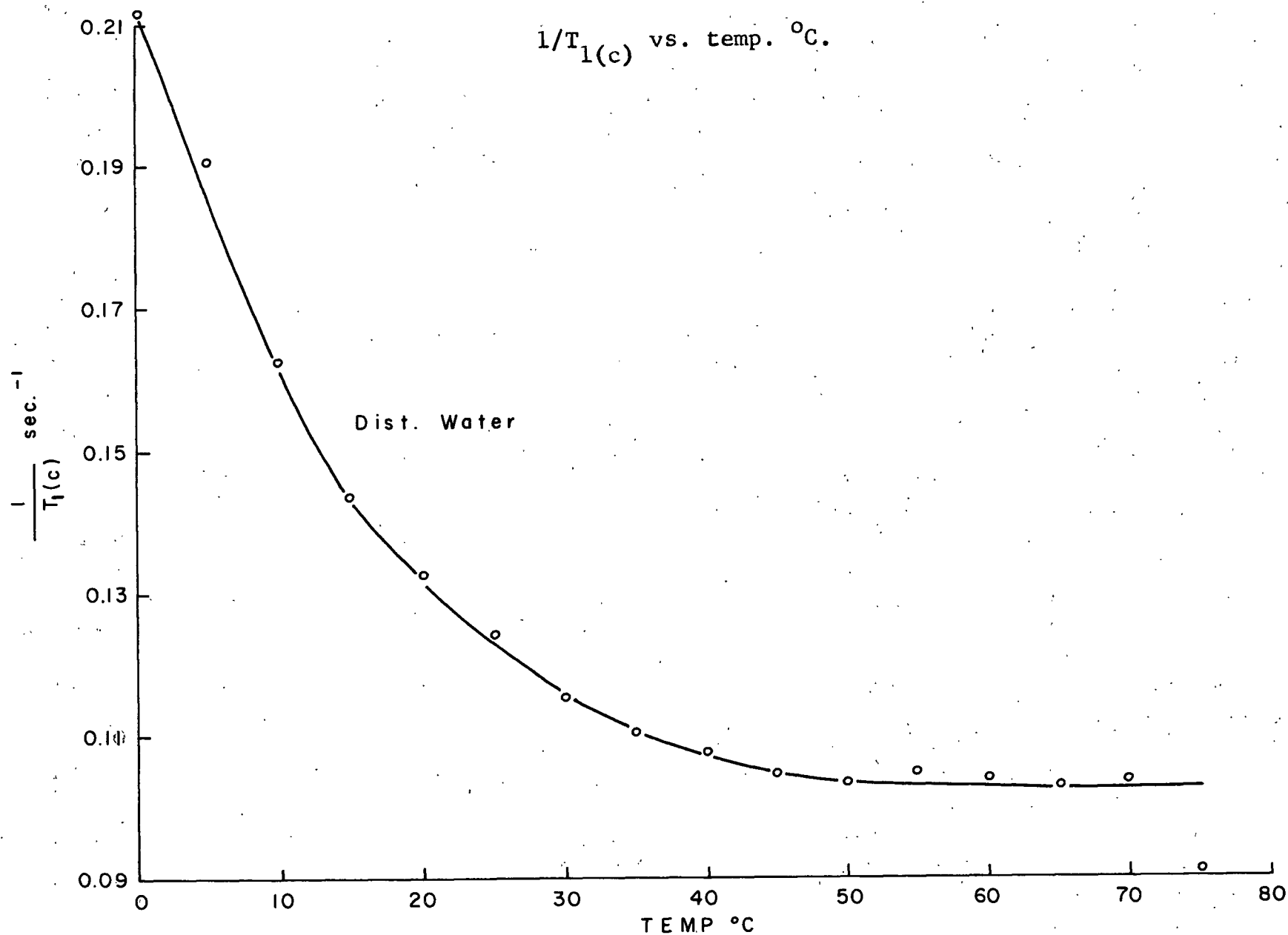


Figure X. Behaviour of $1/T_{1(c)}$ with Temperature (Distilled Water)