THE KINETICS OF AUTOXIDATION OF EPINEPHRINE

A thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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I. INTRODUCTION

Although epinephrine, since its isolation by Abel (1897) and Takamine (1901) and its synthesis by Stolz (1904) and Dakine (1905),[1] has been widely used both clinically and research wise; no serious attempt has been made to study the kinetics of the autoxidation reaction principally responsible for its degradation in solutions exposed to the atmosphere. A considerable number of articles have appeared on the rate of oxidation of epinephrine, but they are full of contradictions on most phases of the problem. This situation is caused by the failure until recently to control some of the many factors which influence the reactions, making it extremely difficult to compare the results of several investigators. Different methods of evaluating experimental data and of following the reaction were used in many cases; in others, the temperature and the concentration of the epinephrine solutions varied; and in some cases no attempts were made to control the pH of the solution. Since the pH changes considerable during the reaction, and since the rate of the reaction is dependent upon the pH, the results of such studies are of questionable value. In most cases, where buffers were used, the types of buffers differed. Furthermore, samples of epinephrine of all grades of purity were used.

An attempt was made in the present work to study the autoxidation reaction systematically by controlling some of the

reaction variables such as pH, temperature, drug concentration and oxygen concentration. In addition to kinetics study on epinephrine, similar investigations were carried out on a number of epinephrine analogs (such as 1-(3,4-dihydroxyphenyl)-1-propanol, 4-acetocatechol, 4-propionocatechol and catechol) to obtain information on the influence of various functional groups on the oxidation rates.

Since the mechanism and general characteristics of reactions responsible for the autoxidation of epinephrine are probably very similar to those of other compounds which have been more widely studied, a general review of the topic of autoxidation is included.

II. AUTOXIDATION

Reactions involving oxidation by molecular oxygen near room temperature are generally called autoxidation reactions, although the true meaning of the term autoxidation (self-oxidation) leaves this usage open to question. The most commonly accepted view concerning the mechanism of autoxidation, proposed simultaneously by Engler and Wild[2] and Bach,[3] is that the addition of molecular oxygen to the substance to be oxidized results in the formation of a highly reactive and unstable peroxide or a cyclic monoxide which might subsequently break down, or hand on its oxygen to other molecules. In many cases such peroxides (hydrogen peroxide, benzoyl hydrogen peroxide) have been isolated. In other cases the formation of the peroxide has been indicated by "induced oxidation" (that is, the oxidation, during an autoxidation, of an added substance which would not be oxidized directly by molecular oxygen).[4] As is quite generally found necessary in explaining

the mechanism of chemical reactions, the freshly formed peroxide is assumed in this theory to retain some of the energy released from the reaction and thus is temporarily more active than the normal peroxide.

Theoretical treatments of the mechanism for the formation of these peroxides have also been presented by Milas[5,6] and by Stephens.[7] Milas states that autoxidation proceeds by a preliminary addition of molecular oxygen to atoms or groups of atoms containing molecular valence electrons comparable to the molecular valence electrons of the various elements, with the subsequent formation of highly metastable peroxides having semi-polar structure which are characterized by high instability and energy content. Owing to their instability, these peroxides may transfer their excess energy to other molecules and thereby initiate new reaction chains, either by reverting instantaneously to ordinary peroxides or by causing intermolecular rearrangements with subsequent splitting of hydrogen peroxides or other peroxides.

Stephens objected to Milas assumption that the first change to take place in any autoxidation was a change in energy level of the molecular valence electrons.[8] He pointed out that since autoxidations in general are thermal reactions any such energetic activation as the electronic activation is sufficient to account for the experimental facts, except in cases where autoxidation is hastened by photochemical means. Then electronic activation must occur. Stephens admits the difference in energy content between the freshly formed peroxide and the normal peroxide, but he feels that there is no existing notation which can depict the structural

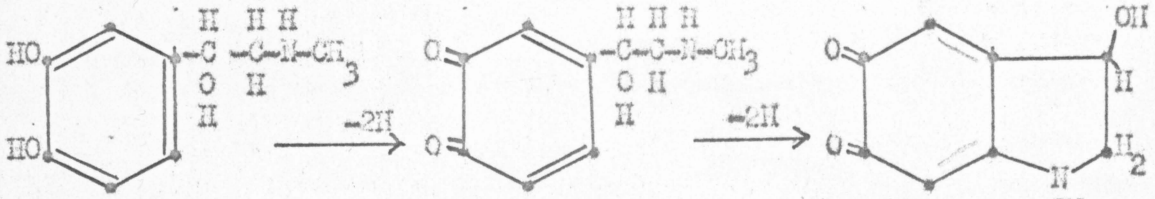
difference. This theory then considers autoxidation to involve vibrational activation of the weakest linkage in the molecule followed by addition of molecular oxygen to form the active peroxide which may undergo a reaction or change to the normal peroxide.

The fact that some experimental results could not be explained by the early intermediate peroxide theory and the fact that many autoxidations involve the removal of hydrogen atoms led Wieland,[9] and later many others, to advance the theory that autoxidations are essentially dehydrogenations in which hydrogen atoms rather than molecular oxygen play the important role. With the realization of the possibility for the temporary existence of a peroxide of higher energy content than that of the isolable peroxides, this idea was in general abandoned.

Luvalle[10] states that two mechanisms are responsible for oxidation of organic systems with oxygen. One is the chain mechanism involving free radicals and the formation of organic peroxides as the bivalently oxidized product.[11,12] The intermediate free radical is assumed to be stabilized to some extent by resonance. A second mechanism, the semiquinone mechanism has been established for the autoxidation of enediols, eneamines and their vinylogues like hydroquinone and p-phenylenediamine.[13,14] The semiquinones free radicals are more stable than the free radicals formed in the chain mechanism. The bivalently oxidized product is assumed to be stabilized by a rearrangement of bonds to form a quinonoidal structure.

III PAST WORK ON EPINEPHRINE

The oxidation reaction of epinephrine which has been reported may be summarized in the following equation:



Epinephrine Adrenaline-quinone Adrenochrome

The pH Dependency of the Reaction

The literature appeared to be incomplete on the effect of pH on the stability of simple epinephrine solutions. Luhr and Rietsche[15] in 1938 suggested that in order to be stable the pH of solutions of epinephrine in HCl should be not higher than 2.9, but they provided no data concerning the exact effect of variation of pH. Vacek[16,17] have reported that below the pH 6.0, more acid solutions were not always stable and that the stability did not increase in proportion to the degree of dissociation of the acid used. This was possible only up to a pH of 6.0.[18] Rowlinson and Underhill[19] made the statement that color of the solution was not an indication of potency, although it was agreed that some decomposition has occurred in all coloured solutions. At a pH of 6.0 there was sudden change in color but not for the keeping quality of the solution, the latter decreasing continuously. Berry and West[20] in 1944, using the frog heart method in determining the loss of activity of epinephrine in HCl and also of epinephrine in HCl and boric acid solution over a pH range from 2 to 5.0, showed that a pH of 4.2 provided optimum stability for storage of solution of

epinephrine in HCl. Chaix [21] claimed the reaction was a function of the pH.

Order of the Reaction

The oxidizibility of epinephrine determined polarographically agreed completely with that determined potentiometrically.[22] The oxidation reaction was second order to about 6.0 and of lower order for pH over 6.0 by the probable formation of a semiquinone.

Rate Dependency upon the Concentration of Epinephrine and Oxygen Concentration

Roest[23] first observed that doubling the epinephrine concentration lead to double the oxygen consumption. Woolfe[24] stated storage in an inert atmosphere produced no increase in stability in the presence of catalytic agents, while Esveld[25] has shown that storage in an inert gas, nitrogen, had little influence on activity. Chaix[21] recently made a report that the autoxidation velocity of epinephrine in the presence of copper catalyst depended upon the partial pressure of the oxygen; in its initial phase the reaction appeared to be a first-order reaction in regard to the oxygen.

Positive Catalysis of the Reaction

Heavy metal ions have been reported to be effective in catalyzing the autoxidation of epinephrine.[26,21] A marked effect by cupric ion has been generally observed. Woolfe[24] found both ferrous and ferric ions had pronounced catalytic effect leading to rapid decomposition of epinephrine in the solution. Chaix[21] claimed that the oxidation of epinephrine depended upon the formation of a

complex-epinephrine-metal (copper, nickel, or manganese). In the case of copper, the maximal velocity of oxygen consumption at pH 7.3 corresponds to a ratio Cu/mol. epinephrine of the order of 1/2. The value of this ratio depended on the pH. Copper was a more powerful catalyst of epinephrine oxidation than nickel or manganese. Iron formed a violet complex with epinephrine that was not autoxidizable under experimental conditions used by Chaix, but manganese did not appear to react. The total oxidative degradation of epinephrine was accompanied by the fixation of three molecules of oxygen per mole of epinephrine when copper was the catalyst, and a fixation of at least four molecules of oxygen per mole of epinephrine when nickel or manganese were used as catalysts.

The Rate of Reaction as Function of Temperature

Esveld[25] has reported that assay of an ampule solution of epinephrine after 14 months at 3°C showed no loss in strength, at 18°C a loss of about 17%, and at 37°C the loss was 45%. Berry and West[20] stated that "The results show two main routes of destruction, one due to oxygen and the other due to heat. pH is an important factor in influencing both rates of destruction". Chaix[21] found that the reaction was a function of the temperature. Its activation energy would be about 25,000 cal/mol.

Light [27,28,29,30,31] has generally been found effective as a catalyst in the autoxidation of epinephrine, so it has been suggested that storage in the dark is preferable to storage in the light. Oxidation in presence of ultra-violet and visible rays was much more intense than that with visible rays only. Oxidation catalyzed by

visible rays only was, however, nearly as intense as by ultra-violet radiation only.(32)

The oxidation product of epinephrine catalyzing the reaction was first demonstrated by Kisch;(33) it acted very definitely in a dilution of 1:10,000,000. Falk(34) recently demonstrated on shaking a solution of epinephrine in air at pH 7.0, followed by measurement of oxygen uptake, that the reaction was typical of an autocatalytic process. In this instance the process may be due to the adrenochrome formed, which acts as a hydrogen carrier and is reduced by epinephrine to leucoadrenochrome, with reoxidation by oxygen.

Methods of Stabilizing Epinephrine

Most of the research on the stabilization of epinephrine has involved the addition of inhibitors or reducing agents to the aqueous solution of epinephrine. Inhibitors such as amino acids(35,36) (d-alanine, tyrosine, cysteine, l-leucine, ornithine, glycine and tryptophane), kynurenine, kynurenic acid,(37) glutathione,(38) HCN and 0.4% chloretone(39) have been commonly suggested. Among those reducing agents used, sodium and potassium metasulfites have generally been recommended.(20,40) For example, Woolfe(41) used 0.1% metabisulfite in injection of procaine and epinephrine solution. However, Moller(42) claimed that sulfites did not increase the stability but only prevented the discoloration.

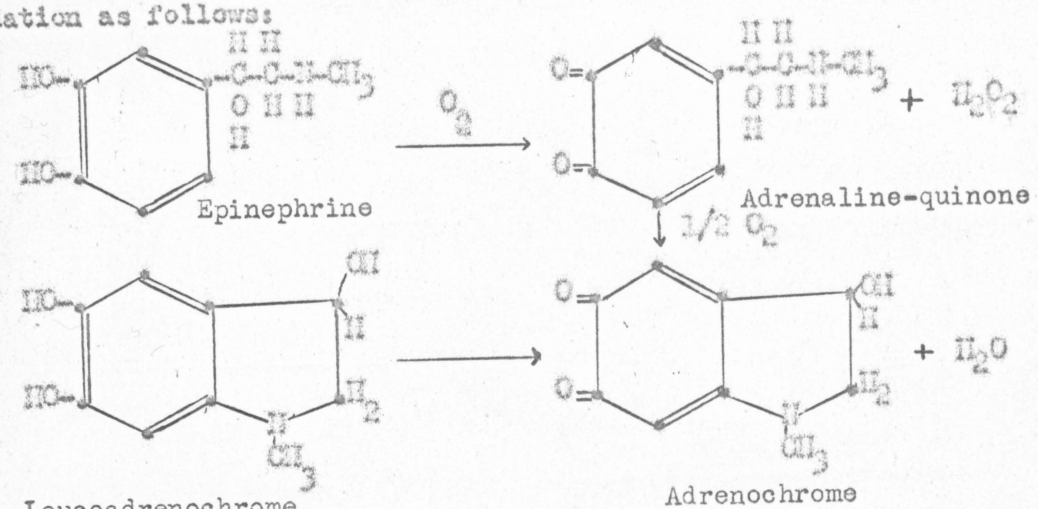
A salt form of epinephrine tartrate was found by West(43) to be a more stable compound than the base or hydrochloride. Donatelli(44) suggested that epinephrine solutions were rendered stable by the

addition of ascorbic acid, sodium ascorbate, sucrose and methyl-p-hydroxyl benzoate, although West claimed that epinephrine ascorbate appeared to possess no advantage over the corresponding tartrate solution.

Mechanism of the Oxidation

Green and Richter[45] obtained a crystalline adrenochrome by oxidation of epinephrine with catechol oxidase or tyrosinase. Veer[46] obtained the same product in 30-35% yield, using silver oxide as the oxidizing agent. Iodoadrenochrome and bromoadrenochrome were also yielded when adrenaline was oxidized with KIO_3 or bromate.[47] The structure of adrenochrome first proposed by Green and Richter has been confirmed. Adrenochrome decomposed at 115-120°C, was reduced by SO_2, H_2S or Pd and H_2 to give leucoform. The intermediate steps in the transformation of epinephrine to adrenochrome have been studied by Rangier(48) through chemical means. When epinephrine HCl (I) was treated with PbO_2 at pH 1.2 it was oxidized to 4-(1-hydroxy-2-methylaminoethyl)-1, 2-benzoquinone (II), which was reduced to (I) by $NaHSO_3$. Neutralization of (II) with Zno caused cyclization to the fluorescent 1-methyl-2, 3-dihydro-3, 6, 7-trihydroxyindole (III). Oxidation of (I) at pH 2-6 gave red adrenochrome (IV), which was reduced by $NaHSO_3$ to (III). Cohen[49] also demonstrated that when epinephrine was dissolved in an excess of HOAC in presence of solid $K_2S_2O_8$, adrenochrome oxidized to form 1-methyl-3, 5, 6-triketoindole, for which Cohen used the name of "oxoadrenochrome". Recently Falk[34] found that adrenochrome

shortened the lag in the oxidation of adrenaline in a simple system containing only epinephrine and adrenochrome at pH 7.0 and 37.5°C. The lag was progressively shortened with increasing amounts of adrenochrome. He also postulated the probable mechanism of the epinephrine oxidation as follows:



Epinephrine

Adrenaline-quinone

Leucoadrenochrome

Adrenochrome

From his experimental results it was claimed that this oxidation was an autocatalytic process which was due to two factors: adrenochrome as it formed catalyzing the oxidation as a hydrogen carrier, and the hydrogen peroxide formed contributing to the peroxidative catalysis. The postulation of adrenochrome as a hydrogen carrier in the oxidation of epinephrine seemed invalid, since it required the reduction of the adrenochrome by the more electropositive adrenaline. However, Ball and Chen(50) measured a thermodynamically reversible step in the oxidation of adrenaline in acid solution, but found that at pH 7.0 the oxidant of the system was extremely unstable. There was little doubt that they were dealing with the system adrenaline⇌adrenaline quinone, the latter undergoing at pH 7.0 a very rapid irreversible change with ring closure to yield adrenochrome by oxidation, either directly

or after rearrangement to leucoadrenochrome or by dismutation. For the latter part of the oxidation process, Cohen believed that adrenochrome oxidized to form oxadrenochrome, this eventually forming the melanine by still unknown reactions probably involving further oxidation and polymerization. A peroxide formation by the addition of water was proposed as one of the intermediate products in the transformation of adrenochrome to melanine.(51)

Oxidation Products

Rozum(52) has made an attempt to separate the oxidation product of epinephrine. He found by chromatographic separation that the red oxidation product called adrenochrome by Green and Richter was not a single compound, but a group consisting of at least seven compounds. The water soluble ether insoluble portion besides the filtered out melanine-like bodies gave six chromatographically differential compounds. Heller(53) recently obtained a flourescent paper chromatogram of oxidation products of epinephrine by exposing the developed and dried chromatogram to ultra-violet light in vapor of ammonia, which produces a green flouresceense, presumably due to leucoadrenochrome. Development with ferricyanide has also been suggested.

IV. EXPERIMENTAL

In the present study an attempt was made to determine the rate of autoxidation of epinephrine as a function of concentration, oxygen tension, hydrogen ion concentration and temperature. The method of purification of reagents and solutions used in the various systems, and procedures employed in these investigations are described in detail. In addition various supplementary experimental techniques used are also described.

Reagents

Purification of Epinephrine:

The epinephrine, which was used in these studies, was obtained from Eastman Kodak Company and Matheson Co. (m.p.214-216°C). The epinephrine was dissolved in 10% hydrochloric acid solution which had been previously made free from oxygen by passing nitrogen through the solution. The hydrochloric acid solution of epinephrine was immediately covered with Skelly A, and ammonia gas passed into the mixture until the epinephrine precipitated. The precipitated epinephrine was suction-filtered, washed thoroughly with absolute ethanol and anhydrous ether. M.p 217-218° (with decomposition).

Preparation of other Reagents:

Water for all purposes was double distilled from an all glass apparatus.

Buffer salts:

Disodium phosphate with twelve molecules of water

from Merck Co. was thrice fractionally crystallized from glass redistilled water and then dried to constant weight over calcium chloride in an desiccator. The dried form had a composition of $Na_2HPO_4.2H_2O$.

Monosodium phosphate ($NaH_2PO_4.H_2O$) from Merck Co. was twice fractionally crystallized from glass redistilled water and dried at 110°C. and 20-30 mm. pressure, the loss of the weight was not more than 0.1%.

Sodium pyrophosphate $Na_4P_2O_7.10H_2O$ reagent grade from Merck Co. was twice recrystallized from redistilled water, and dried at 110°C. to constant weight.

Buffer Mixtures:

For the preparation of the buffer mixtures, separate solutions of the buffer salts were prepared by dissolving the salts in double redistilled water. Concentration of 0.05M. was used. The pH of these buffer mixtures were varied within the prescribed range by changing the ratio of the two salts used for making the buffers. Phosphate buffer mixtures were made of 0.05 M. $Na_2HPO_4.2H_2O$ and 0.05M. $NaH_2PO_4H_2O$. Acetate buffer mixtures were made of 0.05M. sodium acetate and 0.05M. acetic acid. Pyrophosphate buffer mixtures were prepared from 0.05M. sodium pyrophosphate and 0.05M. phosphoric acid.

Apparatus

Oxygen up-take was measured in a Warburg respirometer which simply consisted of a flask attached to a manometer containing a liquid of known density. The flask was immersed in a water bath at a constant temperature, and the system was shaken to promote a

rapid gas exchange between the fluid and the gas phase. The more detailed manometric techniques and principles are described by Umbreit, Burris and Stauffer.(54)

Procedure

Each assembly of respirometer was calibrated to permit conversion of observed pressure changes to the amount of gas utilized in $mm.^3$ or microliter, μl, at 0°C and 760 mm. The procedure as described by Loamis (55) was employed.

General Procedure for the Measurement of Oxygen up-take of Epinephrine Solution:

All equipment was cleaned and rinsed with redistilled water. 5.0×10^{-3}M epinephrine solution was made by dissolving 0.025 g. of purified epinephrine in sufficient amount of 0.05 M phosphate buffer mixture to yield 25 mls. of solution at the desired pH by varying the ratio of the disodium phosphate to the monosodium phosphate buffer. The pH reading of the solution was made with a Beckman type G pH meter.

Ten ml. of the solution was then transferred into a Warburg flask and attached to a manometer. Each run was carried out in duplicate. The blank used for the thermobarometer correction was 0.05 M phosphate buffer having the same pH value as the reaction medium. The flask was immersed into the constant temperature bath and allowed to equilibrate with shaking for ten minutes. Then the manometer fluid was adjusted to 180 mm. on the closed side of manometer with the stopcock open. Immediately following this the

stopcock was closed and readings were taken at definite intervals. The shaking rate was 120 oscillations per minute.

The phosphate buffer was employed for most of the work although check runs were made in some cases with buffered carbonate solutions.

All experiments unless otherwise specified were carried out at 38.0°C (±0.03°). The gas phase was usually air except in the runs made for the determination of the effect of oxygen concentration on the reaction rate, in which cases the gas mixtures other than air were employed.

For each run, the reaction was allowed to proceed to completion or until in some cases to one half completion. The volume of oxygen absorbed was plotted against the time required (time readings were made in general for each 20 /uls. up-take). The oxidation rate at any time was determined as the tangent of the smooth curve at the point in question.

Procedure Employed to Determine the Effect of pH on the Rate of Oxidation of Epinephrine

In this experiment, the pH range was covered from 4.12-8.5. Epinephrine solution of 1.0×10^{-3}M was used, since this concentration corresponded closely to the solubility limit of the compound in alkaline media. Solutions with lower pH from 4.12-5.5 were made by dissolving 0.005 g. of purified epinephrine in sufficient amount of acetate buffer mixture (0.05M) to give 25 mls. of solution with the desired pH.

The solutions with pH 5.5-7.5 were made by dissolving 0.005g. of purified epinephrine in a sufficient amount of phosphate buffer

mixture (0.05 M) to yield 25 mls. of solution with the desired pH.

For the high pH values between pH 7.5-8.5 the solutions were made by dissolving 0.005g. of purified epinephrine in 0.05M pyrophosphate buffer mixture to give a final volume of 25 mls. with the desired pH.

In general, by dissolving 0.025 g. of purified epinephrine in a 0.05 M buffer mixture to give a volume of 25 mls. will increase the pH of the buffer approximately 0.25 unit, e.g. 0.025 g. of purified epinephrine dissolved in 25 mls. of 0.05 M phosphate buffer with an original pH of 6.75 yield a final solution having a pH of 7.0. ($\pm$0.05).

The particular buffers were choosen for different pH ranges investigated because they had maximum buffering capacity over the desired pH range.

The oxygen up-take of the prepared solutions were then determined at constant bath temperature of 38.0°C according to the general procedure described in the previous section (page 14).

After the completion of the run, the hydrogen ion concentration of each solution was always determined on a Beckman type G pH meter.

Procedure Employed to Determine the Effect of Epinephrine Concentration on the Rate of Oxidation

A series of experiments covering various concentrations of epinephrine ranging from 1.70×10^{-4}M, 3.40×10^{-4}, 6.83×10^{-4}, 1.37×10^{-3}, 5.47×10^{-3}, 1.09×10^{-2}, 1.64×10^{-2} to 2.73×10^{-2}M in a buffered solution of pH 6, 7, and 8 at 38.0°C were made for the determination of the rate of oxidation.

pH 6.0 batch -- 0.25 g. purified epinephrine was weighed and

dissolved in sufficient amount of 0.05 M phosphate buffer with pH 5.70 to give 50 ml. of solution (2.73×10^{-2}M). Solutions which were 1.64×10^{-2}M and 1.09×10^{-2}M with respects to epinephrine were made using the same procedure. Series of solutions of lesser concentration were made up by dilution.

pH 7.0 batch -- 0.25 g. purified epinephrine was dissolved in a sufficient amount of 0.05 M phosphate buffer mixture with pH 6.70 to give 50 mls.of solution (2.73×10^{-2}M). Series of solutions of lesser concentration were made up by dilution.

pH 8.0 batch -- 0.25 g. purified epinephrine was weighed and dissolved in a sufficient amount of 0.05 M phosphate buffer with pH 7.68 to give 50 mls.(2.73×10^{-2}M). Series of solutions of lesser concentration were also made up by dilution.

The rate of oxygen up-take at 38.0°C was determined according to the general procedure (page 14).

Procedure Employed to Determine pk_b of Epinephrine

Both 100 mls. of 1.36×10^{-4}M solution of epinephrine in 25% alcohol and 100 mls. of 2.72×10^{-3}M epinephrine in 50% alcohol were potentiometrically titrated with standard 0.1188 N HCl at 26.0°C and type G Beckman pH meter.

Procedure Employed to Determine the Effect of Oxygen Concentration on the Rate of Oxidation of Epinephrine

Measurements of the rate of oxygen uptake by 1.0×10^{-3} M epinephrine solution in 0.05 M phosphate buffer at pH 7.0 and 8.19 were made at varying partial pressure of oxygen. These were produced by making the runs in presence of pure oxygen and at lower

oxygen pressures corresponding to 50% oxygen (in the form of 5
and 50% N_2) 21% oxygen (air) and 10% oxygen (a combination of 50%
N_2 and 50% air).

These gas mixtures were prepared by the displacement method which was simply performed by completely filling bottles with a confining fluid, and allowing the various gases to enter until a given quantity of the fluid has been replaced.

0.02 g. purified epinephrine was dissolved in 100 ml. of 0.05 M phosphate buffer with pH 6.78 to give a final solution with pH of 7.0. The solution with pH of 8.19 was made in the same manner as pH of 7.0 batch using 0.05 M pyrophosphate buffer with pH 7.95 to make up the solution.

Ten ml. of the prepared solution was then transferred into a Warburg flask and attached to the manometer in such a manner that the stopper of the sidearm of the flask was appropriately turned so that gas was vented through the sidearm. The gas mixture or oxygen was passed through the opened stopcock of the closable arm of the manometer for five minutes, and then the stopcock closed. The system was allowed to equilibrate by shaking for ten minutes, then the manometer fluid was adjusted to 180 mm. on closed side of manometer with stopcock open, stopcock closed and readings begun.

A check run of this experiment was made by changing the shaking capacity of Warburg instrument to the maximum to determine, if any, the effect of agitation on the rate of oxygen uptake.

Procedure used to Determine the Temperature Dependency of the Reaction of Epinephrine

A series of oxygen up-take measurements were made on 1.0×10^{-3} M

epinephrine solution in acetate buffer at pH 4.9, in phosphate buffer at pH 7.0 and at pH 8.15 from 38-50.0°C.

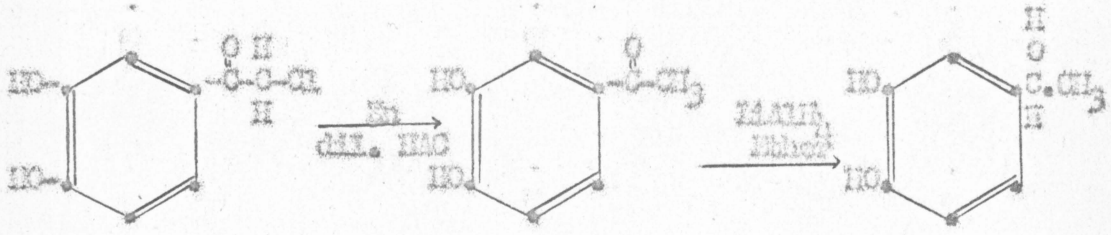
In general, the pH of a buffered solution changes with temperature. For the acetate buffer used in the study, a pH rise of roughly 0.28 unit was noted whenever the solution was measured at the end of the induction period. In case of phosphate buffer used pH was essentially independent of temperature in the range investigated.

Epinephrine solutions with desired pH for this study were prepared according to the previously described procedure (page 14).

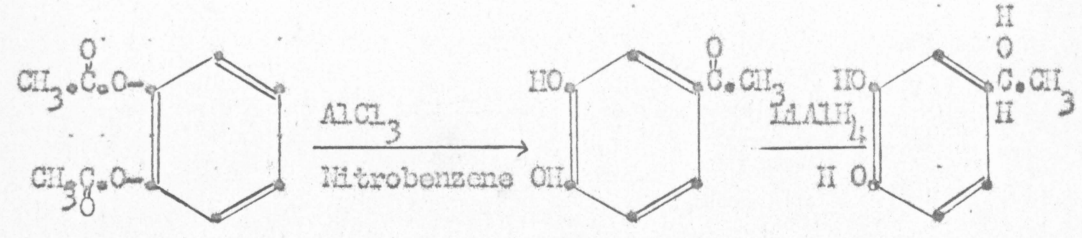
Attempted Synthesis of 1-(3,4-Dihydroxyphenyl)-1-Ethanol

It would be expected that the most convenient method for the preparation of this compound is the reduction of its corresponding ketone. An attempt in this direction is discussed in this section. The plan of synthesis is summarized by the following equation:

(a)

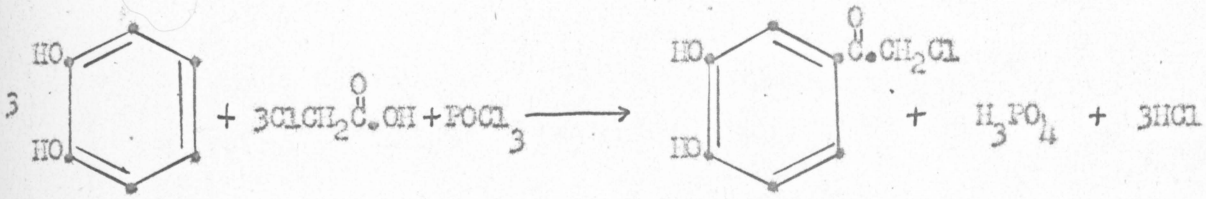


(b)



Ψ-Chloroacetocatechol has been made previously. However by starting with veratrol, chloroacetyl chloride and aluminum chloride in nitrobenzene and following the method of Stephen,[56] the procedure resulted in only a small yield of a dark colored product which could not be purified. Another method of preparation of Ψ -chloroacetocatechol described by Krannichfeldt[57] gave only a small yield of nonidentifiable product. However, when its synthesis from catechol, chloroacetic acid and $POCl_3$ was attempted, a good yield (75%) of the final product was obtained (Mannich and Hahn).

Synthesis of Ψ -Chloroacetocatechol: -- according to the method of Mannich and Hahn[58]



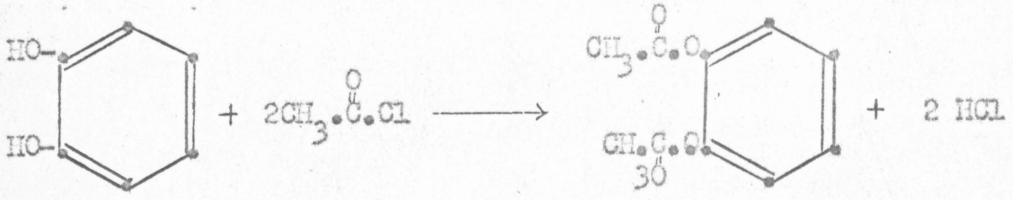
The synthesis was attempted by heating a mixture of 10.0 g. (0.91 mole) of catechol, 10.0 g. (1.05 moles) of chloroacetic acid and 10.0 g. (0.065 mole) of $POCl_3$ in a liter round bottom flask. The mixture was heated on water bath for about one hour until the evolution of hydrochloride gas had ceased, then allowed to stand at room temperature for 1-2 days. At the end of the reaction, the mixture was concentrated on a hot plate and under nitrogen. The reddish purple solid which crystallized out, was dissolved in boiling water and decolorized with Norite while hot. The crystalline mass which

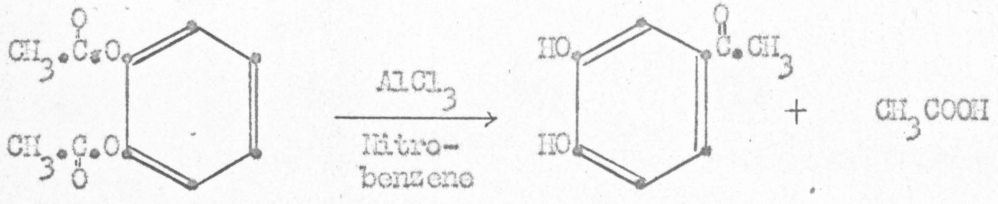
precipitated on cooling, weighed 5.0 g. (yield 30%) and melted at 169-171°C.

Denalogenation of ψ -chloroacetocatechol to 4-acetocatechol: Although the method of Stephen[56] which consisted of reduction of ψ-chloroacetocatechol with zinc dust and dilute acetic acid, was claimed to give fair yields, application of this procedure yielded only a black tarry substance.

Synthesis of 4-acetocatechol: Method of Rosenmund and Lohfert[59]

Since the attempt to prepare 4-acetocatechol through ψ-chloroacetocatechol failed another method was tried. The method of Rosenmund and Lohfert can be summarized by the following equation:





Preparation of Catechol Diacetate:

A mixture of 11 g. (0.1 mole) of catechol and 15.6 g. (0.2 mole) of acetylchloride was heated on a water bath for about one hour until the evolution of hydrochloride gas had ceased. The resulting, slightly yellow, liquid was assumed to be catechol diacetate.

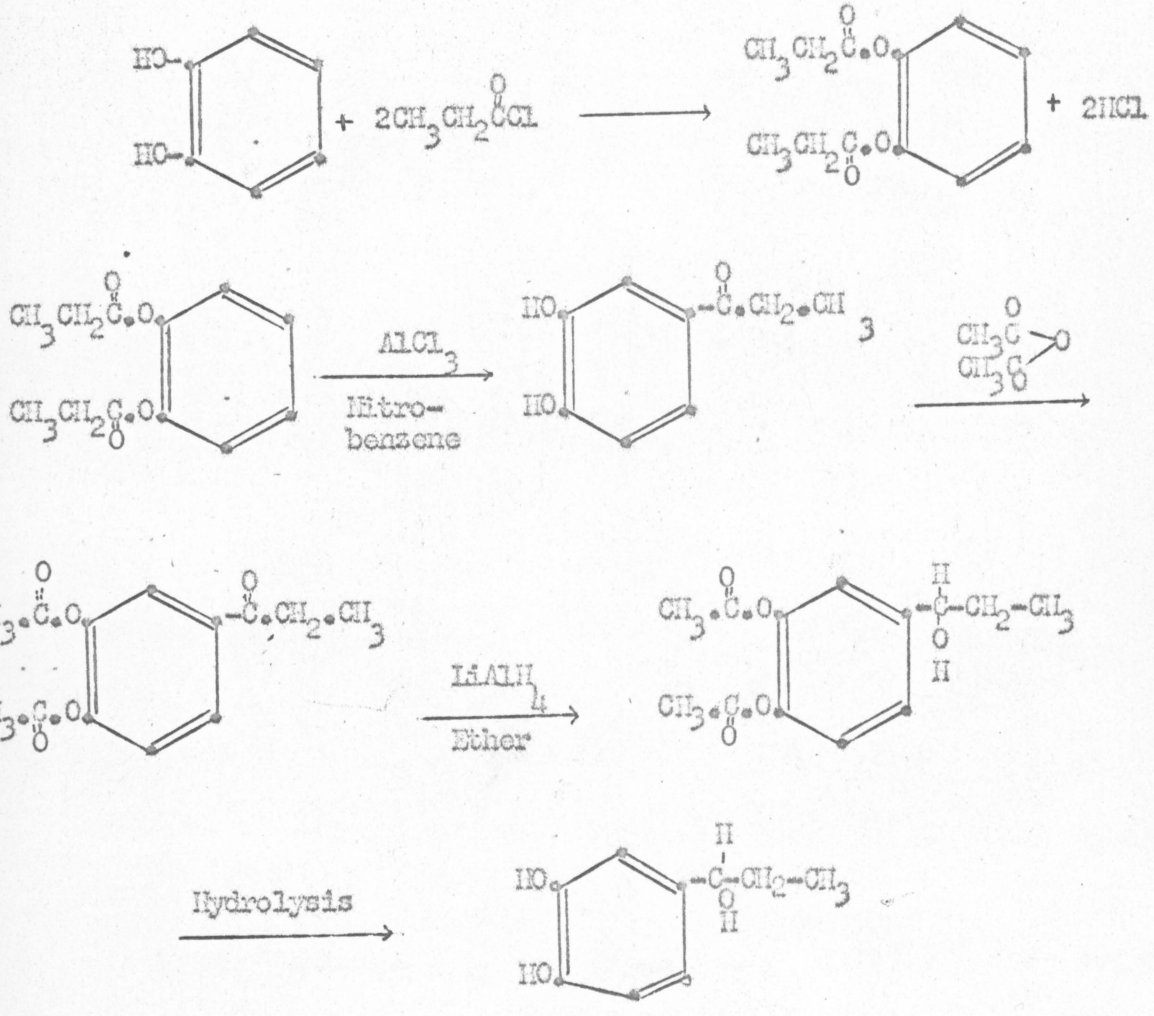
Fries Rearrangement of Catechol Diacetate:

In a liter round bottom flask 5.0 g. (0.025 mole) of catechol diacetate, 7.0 g. (0.05 mole) powdered anhydrous aluminum chloride and 20 g. nitrobenzene were mixed together. After the mixture was heated on water bath at 75°C for two hours, it was treated with ice and 10% hydrochloric acid solution to decompose the aluminum chloride complex. The nitrobenzene was steam distilled off. The resulting brownish-green mixture was extracted with ether in a continuous extraction apparatus. The ether extract was treated with Norite, filtered, dried over anhydrous sodium sulfate, and then concentrated to a thick syrup under reduced pressure. The yellow solid, which crystallized out from the concentrated liquid, melted at 114-116°C. Yield 2.5 g. (21.3%). It was recrystallized from both water and benzene. The use of benzene seemed to give a better yield, melting point 118-119°C.

The reduction of 4-acetocatechol either by means of lithium aluminum hydride and finely divided sodium was tried, but proved unsuccessful, only a small amount of gummy reddish substance was obtained which could not be purified.

The Synthesis of 1-(3,4-Dihydroxyphenyl)-1-Propanol

The synthesis of 1-(3,4-dihydroxyphenyl)-1-propanol through catechol dipropionate, followed by Fries rearrangement was successful. The direct reduction of 4-propionocatechol to the corresponding secondary alcohol resulted in a low yield. Propionocatechol was then acetylated to 4-propionocatechol diacetate which was much more stable than 4-propionocatechol. The reduction of this diacetate by lithium aluminum hydride proved successful.



4-Propionocatechol -- Method of Rosenmund and Lohfert[59]

Catechol dipropionate was made according to the procedure described for catechol diacetate. 6.3 g. (0.025 mole) of catechol dipropionate was dissolved in 25.2 g. (0.112 mole) nitrobenzene together with 6.3 g. (0.047 mole) of powdered anhydrous aluminum chloride. The mixture was stood aside at room temperature for 45 hours, the mixture was then heated on water bath for one hour. The reaction mixture was subjected to the steam distillation to drive off the nitrobenzene. The solution was extracted with ether until the ether layer was no longer colored. The combined ether extract was dried over anhydrous sodium sulfate and then concentrated to a thick syrup under reduced pressure. Light yellow crystals which were obtained were dried on a porous plate. The yield was 2.5 g. (29%) melting at 140-142°C. After recrystallization from benzene the melting point was 146-147°C.

Acetylation of 4-Propionocatechol:

This was done by gently refluxing a solution of 2.0 g. of 4-propionocatechol in 4-5 fold excess of acetic anhydride in the presence of anhydrous sodium acetate for two hours. The reaction mixture was poured into 25 mls. of cold water. The resulting solution was carefully neutralized by an addition of solid sodium carbonate with stirring. The desired compound which was present in form of a precipitate was then recovered by filtration. 4-propionocatechol diacetate was recrystallized from 50% alcohol, which yielded 1.5 g. (83%) of material with a melting point of 71-72°C.

Reduction of 4-Propionocatechol Diacetate with Lithium Aluminum Hydride:

Lithium aluminum hydride solution used for the reduction was prepared by refluxing 4.0 g. of lithium aluminum hydride in 100 ml. of anhydrous ether on a steam bath for 2 to 3 hours and collecting the supernatant ether solution after centrifugation.

The solution was then titrated with a standard solution of one of the lower primary alcohol in benzene to establish the lithium aluminum hydride content (1 ml. $LiAlH_4 \rightleftharpoons$ 0.002 eq.).

One and a half fold excess of a calculated amount of lithium aluminum hydride solution {75 ml. (1 ml. $LiAlH_4 \rightleftharpoons$ 0.002 eq.)} was placed in a three necked flask, equipped with a condenser, a mercury-sealed stirrer and a separatory funnel. Both condenser and separatory funnel were protected from moisture by means of calcium chloride tubes. To the lithium aluminum hydride solution 50 mls. of anhydrous ether and 3.5 g. of acetylated compound of propionocatechol previously dissolved in 30 mls. of anhydrous ether were added. The ether solution was introduced through the separatory funnel in such a manner that a gentle refluxing was maintained. After the addition of the solution, the refluxing was continued for another hour. The solution was then allowed to cool in ice bath. 7 ml. of water was added cautiously to the cooled solution to destroy the excess of lithium aluminum hydride, followed by 20 mls. of 10% sulfuric acid. The ether layer was separated and the aqueous layer was extracted several times with fresh anhydrous ether. The combined ether extract was dried over anhydrous sodium sulfate, and then was concentrated under reduced pressure (0.1 mm.). A light yellow semisolid was obtained. After drying under reduced pressure of 15 mm. Hg.

for several hours, the light yellow solid weighed 1.0 g., corresponding to a yield of 22 per cent. It melted at 126-128°C. A sample of this material after recrystallization from benzene for several times melted at 131-132°C. The compound was found to be quite hygroscopic in nature, and gave negative test for ketone and positive test for phenol. Analysis, calculated: C, 64.29; H, 7.20. Found: C, 65.27; H, 7.43. This compound was not previously described in the literature.

Procedure Employed to Determine the pH Effect on the Rate of Oxidation of 1-(3,4-Dihydroxyphenyl)-1-propanol

0.0084 g. of 1-(3,4-dihydroxyphenyl)-1-propanol was dissolved in a sufficient amount of 0.05 M phosphate buffer with pH 6.7 and 7.2 to give 50 ml. of final solution with pH 6.54 and 7.18 (1.0×10^{-3}M) respectively.

0.0084 g. of 1-(3,4-dihydroxyphenyl)-1-propanol was weighed and dissolved in sufficient amount of 0.05 M pyrophosphate buffer with pH 7.85 and 8.4 to give 50 ml. of final solution with pH 7.74 and 8.31 (1.0×10^{-3}M) respectively.

The measurement of oxygen uptake of the above solutions covering pH range from 6.54 to 8.31 at 38.0°C was carried out by using the same procedure and conditions described previously (page 14). Each run was made in triplicate.

Procedure Employed to Determine the Effect of Concentration on the Rate of Oxidation of 1-(3,4-dihydroxyphenyl)-1-propanol

1.091×10^{-2}M solution of this compound with pH 7.0 was made by dissolving 0.04582 g. of 1-(3,4-dihydroxyphenyl)-1-propanol in 0.05 M phosphate buffer with pH 6.95 to give a volume of 50 ml.

with a final pH of 7.0.

A series of solutions with various concentrations (1.091×10^{-2}, 5.47×10^{-3}, 2.73×10^{-3}, 1.37×10^{-3}, 6.18×10^{-4}, 3.41×10^{-4} M) were prepared from the 1.09×10^{-2} M solution by using the same dilution method employed for epinephrine (page 17).

The rate of oxidation of those prepared solutions (pH 7.0) were determined at 38.0°C by the same techniques used in epinephrine experiment (page 17).

Procedure Employed to Determine the Effect of Oxygen Tension on the Rate of Oxidation of 1-(3,4-dihydroxyphenyl)-1-propanol

0.0168 g. of 1-(3,4-dihydroxyphenyl)-1-propanol was dissolved in a sufficient amount of 0.05 M phosphate buffer of pH 7.02 to yield a final volume of 100 mls. This solution had a pH of 7.0 (1.0×10^{-3} M). The measurements of the rate of oxidation were made at varying partial pressures of oxygen and at 38.0°C as with epinephrine (page 14). The system was flushed with oxygen or other gas mixtures according to the procedure described under oxygen effect on the rate of oxidation of epinephrine (page 17).

Procedure Employed to Determine the Amount of Oxygen Absorbed per mole of Epinephrine

Measurements of the ultimate oxygen uptake of 1.0×10^{-3} M epinephrine at pH of 6.48, 6.79 and 8.01 in 0.05 M phosphate buffer at 38.0°C were made

Fifty ml. of 1.0×10^{-3} M solutions of epinephrine at pH 6.48, 6.79 and 8.01 were prepared according to the procedure described previously (page 14). Ten ml. of each solution was transferred into a Warburg flask and the flask was attached to a manometer. The

The system was flushed with pure oxygen gas for five minutes in a manner as described on page 17, before initiating the oxygen uptake measurement. The reaction was allowed to take place for several days until no appreciable increase in the total oxygen uptake was observed.

Procedure Employed to Determine the Effect of Some Antioxidants on the Rate of Oxidation of Epinephrine

1. A comparison of the rate of oxidation of 5.0×10^{-3}M epinephrine solution with 5.0×10^{-3}M epinephrine solution which were also 2.5×10^{-3}M (1) with respects to 4-acetocatechol, and (2) with respects to 4-propionocatechol was carried out.

Purified epinephrine 0.025 g. was dissolved in 0.05 M phosphate buffer having pH 6.72 to give a final volume of 25 mls. at pH of 6.91. This solution was used as the control.

0.025 g. purified epinephrine and 0.0095 g. of 4-acetocatechol were dissolved in approximately 20 ml of 0.05 M phosphate buffer with pH 6.72. The pH of the solution was adjusted with the disodium phosphate solution and monosodium phosphate solutions (0.05 M) to a pH of 6.91 with a final volume of 25 mls.

The rate of oxidation of these solutions were measured according to the general procedure (page 14).

2. A comparison of the rate of the oxidation of 5.0×10^{-3}M epinephrine solution with 5.0×10^{-3}M epinephrine containing 2.5×10^{-3}M 1-(3,4-dihydroxyphenyl)-1-propanol-3 at pH 6.11 and pH 7.02 at 38.0°C was carried out.

0.025 g. purified epinephrine was dissolved in 0.05 M phosphate buffer having pH 5.62, and pH 6.80 to give a volume of

25 mls. The final solution had a pH of 6.11, and pH of 7.02.

Purified epinephrine 0.025 g. and 1-(3,4-dihydroxyphenyl)-1-propanol 0.0105 g. were dissolved in a sufficient amount of 0.05 M phosphate buffer at pH 5.63, and pH 6.80 to give 25 mls. of solution with a final pH of 6.11, and pH of 7.02 respectively.

The oxygen uptake of these solutions were determined as before (page 14).

3. A comparison of the rate of oxidation of 5.0×10^{-3}M epinephrine solution with a solution 5.0×10^{-3}M with respects to epinephrine and 1.25×10^{-3}M with respects to catechol at pH 7.0 and at 38.0°C was made.

Each individual solution was made by dissolving the calculated amount of the material in 0.05 M phosphate buffer with a pH of 6.75 to give a resulting solution having a pH of 7.0. The oxygen uptake was measured according to the general direction (page 14).

An Attempt to Separate the Oxidized Products of Epinephrine

An attempt was made to separate the oxidized products of epinephrine by paper chromatography as described by Williams.[60] The apparatus used simply consisted of a glass jar containing a pyrex plate at the bottom. 4 µls of the oxidized solution containing 30 µgs. of epinephrine in a phosphate buffer, which was made by previously oxidizing the solution at pH 7.0 at 38.0°C for five hours, was applied at 26.0°C on a sheet of No. 1 Whatman filter paper. After drying the sheet was formed into a cylinder of 4 inches in diameter and 22.5 inches in length, and the edges were stapled together. The paper cylinder was allowed to stand until the

solvent front had nearly reached the top of the paper cylinder. A control one dimensional chromatogram of freshly prepared epinephrine was developed parallel to that of the oxidized sample along one edge of each sheet. The developed cylinder was dried in an oven, and then cut into two strips, one strip of paper was rapidly sprayed with ammoniated silver nitrate solution. The strip was again dried in the oven for five minutes to reveal the positions of bands. One band appeared as dark brown spots on a light brown ground having the R_f value of 0.50. The other strip was examined under ultra-violet light, where it exhibited blue, greenish-yellow and violet fluorescence bands. The strip was then cut into suitable sections according to the respective R_f values. These sections were extracted with methanol or ethanol, and the extracts examined on a Beckman Model DU Quartz spectrophotometer between wave lengths of 200-320 mμ.

RESULTS AND DISCUSSION

The dependency of total oxygen uptake on reaction time is well illustrated by curves shown in Fig. 1. These curves as well as others presented elsewhere in these sections (q.v. Figs. 3, 6, 8, and 16) were readily reproducible under similar experimental conditions. They are characterized by a short induction period and a relatively long period during which the rate of oxygen uptake is essentially constant. As a matter of convenience, the slope of the straight-line portions of these curves have been taken as a measure of the relative rates of the autoxidation process. The effects of variations in hydrogen ion concentration, in epinephrine concentration, in the oxygen tension, and in the temperature of the reaction system on this rate are discussed in the following sections.

Effect of pH on the Rate of the Autoxidation Reaction

The influence of hydrogen ion concentration on the rate of autoxidation of epinephrine is also shown in Fig. I and Table I. The several curves represent the amount of oxygen taken up by the solutions as functions of time at various pH levels. The logarithms of the slopes of the straight line portions of these curves $\left(\text{i.e.} \log \frac{dO_2}{dt}\right)$ are plotted in Figure 2 as a function of pH. It is evident from the latter curve that between pH 5.5 to pH 7.0 the reaction is first order with respect to hydroxyl ion concentration since the logarithmic curve is approximately a straight line over this interval with a slope of approximately one. This would indicate that probably the monovalent ionic form of epinephrine is the species principally responsible for the oxidation

TABLE I

Experimental Data for the Effect of pH on the Oxidation of Epinephrine

Epinephrine Conc. 1.0×10^{-3}M

Temp.: 38.0°C (±0.03)

Time in Minutes	Volume of oxygen up-take in Microliters												
	pH 8.54	pH 8.24	pH 7.97	pH 7.70	pH 7.35	pH 7.19	pH 6.98	pH 6.72	pH 6.33	pH 5.88	pH 5.41	pH 5.12	pH 4.12
20 Min	110	30	12.10	5.00		5.02	*1.030						2.0
25	130	42	17.00	6.00					5.76				
30	165	55	23.00	8.00	5.00	6.00	2.00						
35	175	70	25.00	11.00				6.76	6.00				
40					8.07	7.50	6.00			10.48			
45	228	113	40.65	18.00				10.00					
50						10.00	9.75						
55	260	153	65.75	24.20	13.45			12.20	7.50				
60	295	165	77.00	32.00	15.00	12.50	11.00			11.00			
65	305	182	93.00	38.00									
70						17.50	13.90	12.50	9.60				
75	346	226	122.5	46.00	22.00			15.00		12.75	12.50		
80							15.00						
85		257	156.50	67.00	32.00	25.10		18.35				1.36	
90	387	276	167	76.00	36.00	26.00	20.00						
95								20.00					
100	412	313	200.5	96.25	46.00	33.00	22.50						2.5
105					55.25			21.00					
110	432	340	227	116.5	58.00		25.00	22.50			12.65		
115						46.25							
120	450	375	260	142	73.00		28.50		19.20			4.10	
125					79.20			25.00					
130		399.0	295.0	167.0	87.00	62.00	32.50						
140		417.5	320.0	190.0	101.0	72.50	37.50		21.50	16.00			

Minutes	pH 8.54	pH 8.24	pH 7.97	pH 7.70	pH 7.55	pH 7.19	pH 6.98	pH 6.72	pH 6.33	pH 5.68	pH 5.41	pH 5.12	pH 4.12
145			333.0	202				80.00					
155		448.0	365	225.0			43.25	83.00					
160				240.0	127.5	96.00	46.50		25.00	16.82	13.20		
165			382.5	250.0		99.50		86.00					
170				262.0	145.0	106.0	51.00						
180			399.0	306.0	156.0	116.2	55.00	42.50	27.50	18.50	14.00	5.00	
190				330	174.5	127.0	61.50	48.80					
200			447.0		184.0	136.0	66.00	50.00	30.00				
210					202.0	150.0	72.50	52.50					
220				385.0	212.0	161.0	77.50						
230				402.0	231.0	177.2	82.50	61.75		22.00			
240				422.0	246.0	183.0	88.00	63.50	36.50		16.25	5.90	
250				442.0	255.0	198.0	94.00	68.00					
260					270.0	205		69.60					
270					285		105.0	75.00					
280					306		111.0	77.50	42.50		17.50		
290					311		117.0	81.00		25.50			
295						250.5							
300					322		122.50	82.50	44.00				
310					334	260.0	123.5	88.00				8.00	6.0
330					361		137.00	95.00		26.40			
335						282.2							
355					388	312.0	150.0	105.0	53.00		19.00	9.57	
400					431				62.50				
410											21.00		
425					460.2	386.0	192.50	132.0		35.00		12.00	
455						417.0	208.5		71.50				
465							215.0	145.0					
490								155.0		39.50			
520							239.0	165.0	82.50		27.00	15.00	
540								172	85.00	42.50			
560									88.00				
590									93.00				
610									97.50				

Minutes	pH 8.54	pH 8.24	pH 7.97	pH 7.70	pH 7.35	pH 7.19	pH 6.98	pH 6.72	pH 6.33	pH 5.88	pH 5.41	pH 5.12	pH 4.12
680												18.00	
735												18.22	
900													10.00
1425													13.00
1600													14.00

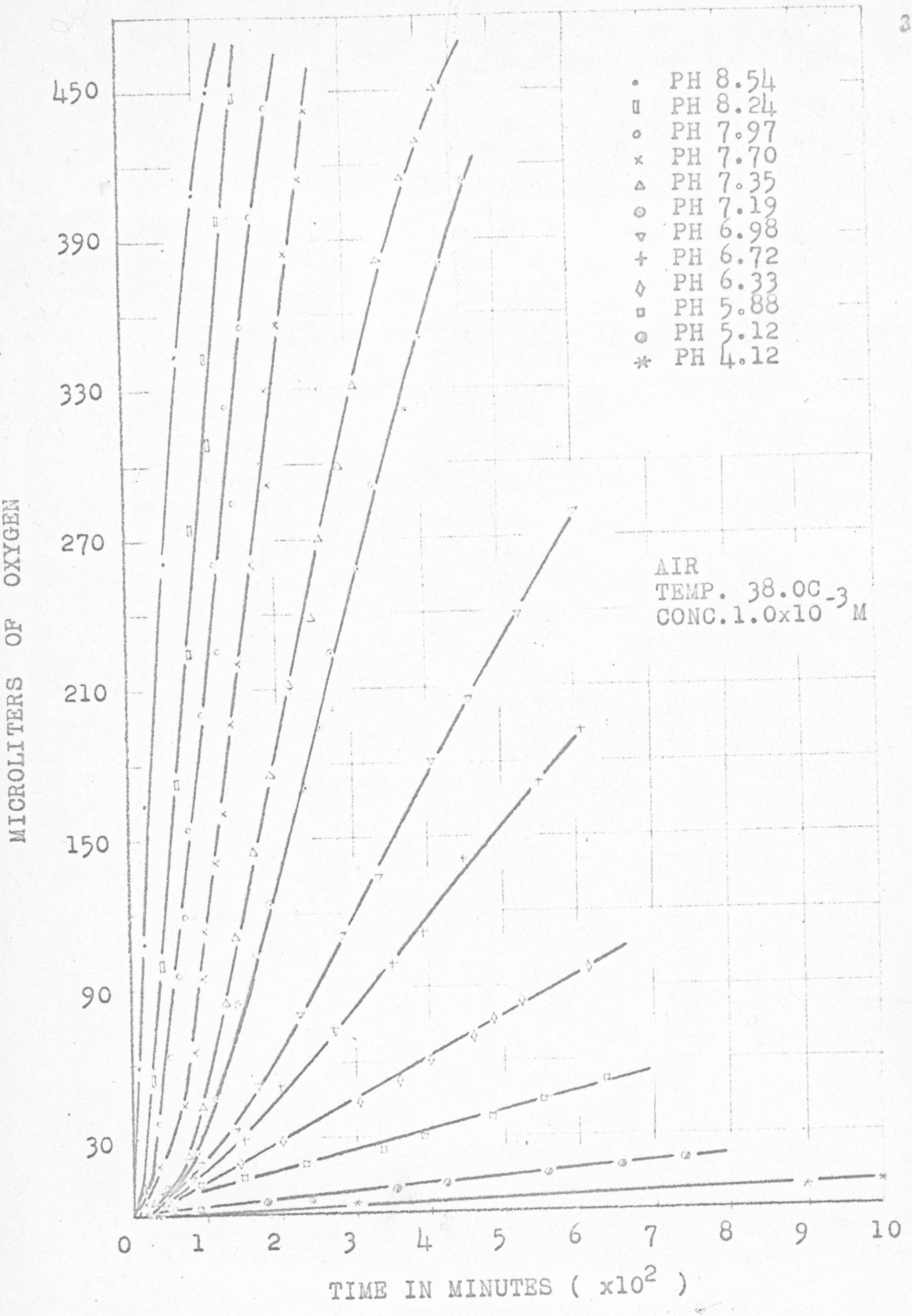


FIG. 1 EFFECT OF PH ON REACTION RATE OF EPINEPHRINE

TABLE II
Summary of Effect of pH
on Reaction Rate of Epinephrine

Epinephrine Conc: 1.0×10^{-3}M Temp: 38.0°C

pH	OH-ion Conc. $\times 10^{-6}$	$-\frac{\Delta O_2}{\Delta t}$	$\text{Log} \frac{\Delta O_2}{\Delta t}$
8.54	8.68×10^{-6}	4.38	0.6415
8.24	4.35×10^{-6}	3.77	0.5763
7.97	2.33×10^{-6}	3.03	0.4814
7.70	1.25×10^{-6}	2.2750	0.3570
7.35	5.61×10^{-7}	1.4120	0.1500
7.19	3.83×10^{-7}	1.104	0.0429
6.98	2.38×10^{-7}	0.526	-0.2790
6.72	1.31×10^{-7}	0.361	-0.4425
6.33	5.34×10^{-8}	0.1565	-0.8055
5.88	1.89×10^{-8}	0.0717	-1.1445
5.41	6.42×10^{-9}	0.0254	-1.5945
5.12	3.30×10^{-9}	0.0197	-1.7051
4.12	3.29×10^{-10}	0.0104	-1.9830

Air Phosphate Buffer 5.0×10^{-2}M

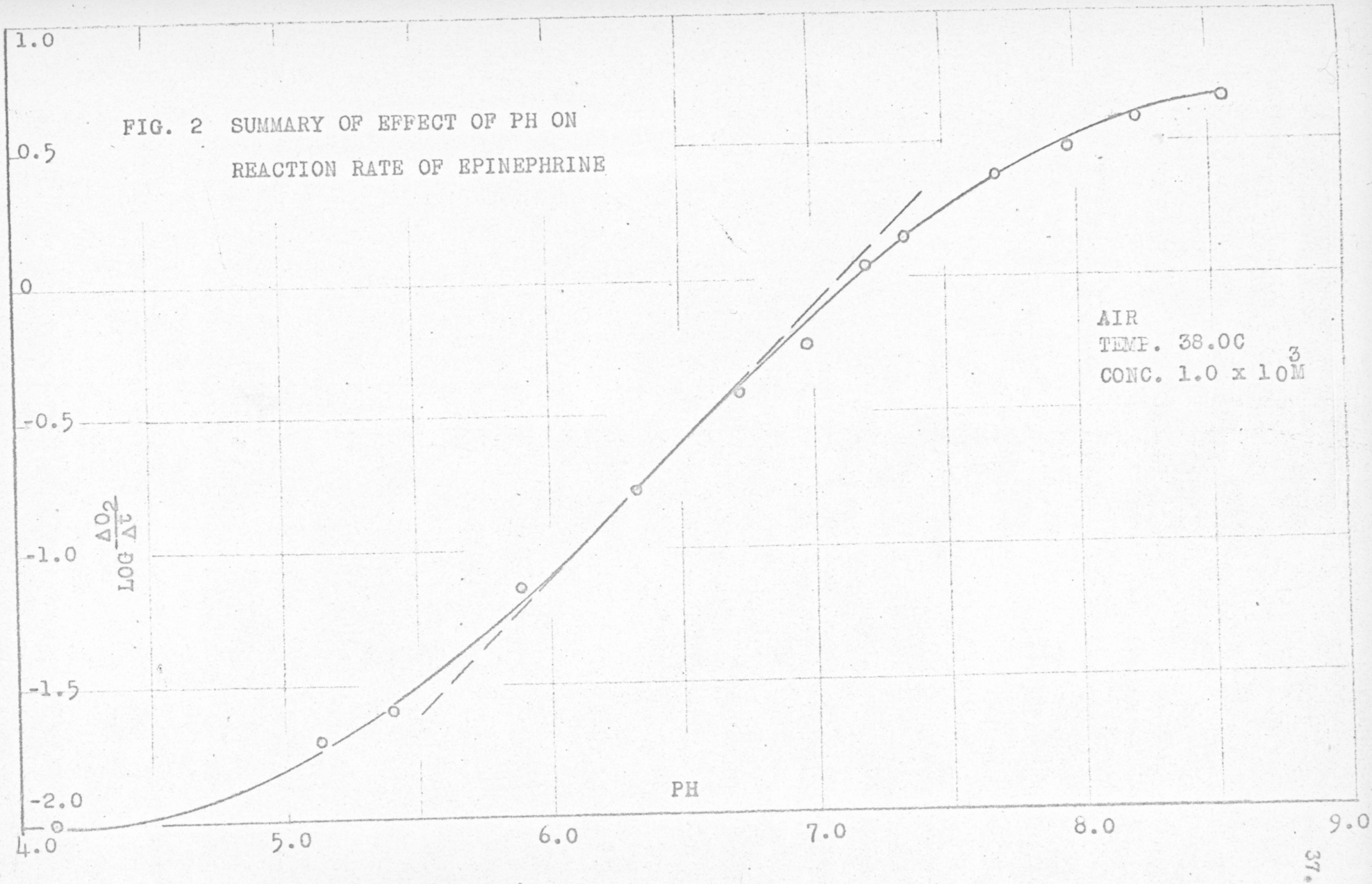


FIG. 2 SUMMARY OF EFFECT OF PH ON REACTION RATE OF EPINEPHRINE

reaction. This is the same conclusion arrived at by both Branch[61] and James[62] for catechol.

An attempt was made to determine the rate of oxidation in the lower pH range below 4.0 but unfortunately because of the extremely slow rate of oxygen uptake requiring more than ten hours to produce detectable pressure change, no reliable rate determination could be made.

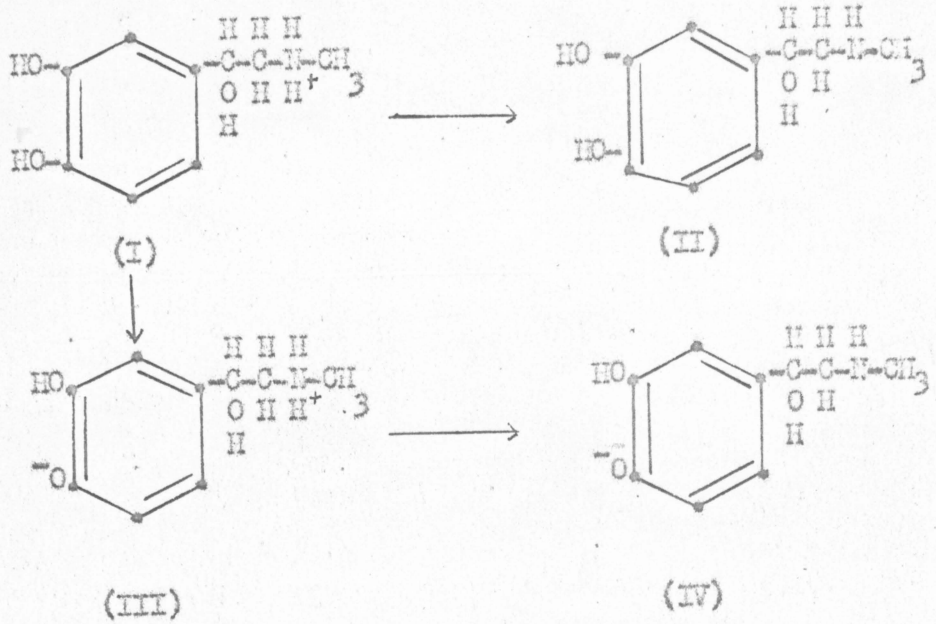
During the course of the autoxidation reaction, a considerable amount of acidic material is evidently produced. This effect is shown in Table III where the pH changes in the reaction mixture during the course of the reaction are listed for a number of runs. The true nature of the acidic product is not known. There has been some evidence that it might be CO_2 since Welch[38] has already claimed that CO_2 is produced during the later stages of the autoxidation of epinephrine.

The nature of the reaction mechanism responsible for the influence of hydrogen ion concentration on the overall rate of oxygen uptake, as illustrated in Figure 2, cannot be completely elucidated on the basis of information available at the moment. This influence, however, probably operates in either of two ways: (1) by affecting the relative concentrations of the several forms of the drug in solution, or (2) by changing the concentration of hydroxyl ions which may participate more directly in some catalytic manner.

Epinephrine can exist in four possible forms in solution in the pH range 4.8. These are:

TABLE III
Data Showing Decrease in
pH of Epinephrine Solution
during Autoxidation

pH of buffer added	Initial pH of Solution	pH of Solution after Oxidation
3.25	3.585	3.30
4.25	4.51	4.25
4.75	4.90	4.68
5.30	5.58	5.60
6.30	6.45	6.30
6.61	6.80	6.68
6.75	7.0	6.72
7.30	7.50	7.22
7.35	7.64	7.28
7.40	7.68	7.50
7.70	8.0	7.68
7.80	8.1	7.80
8.35	8.50	7.94



(I) (II)

(III) (IV)

In the lower pH range the drug exist mainly as form I. At very high pH values considerable amount of it is present as IV. In the intermediate region the remaining types are more important with regards to the oxidative reaction.

A quantitative indication of the relative concentrations of each form can be obtained from the two potentiometric titration curves shown in Fig. 5. Unfortunately because of the insolubility of unionized epinephrine in water, direct titration of the drug was not possible. The two curves shown were obtained by making the determinations in hydroalcoholic solutions containing 25 and 50 volumes of alcohol per 100 volumes of final solution. From inspection of these curves it is evident that the apparent pk_b of epinephrine in 50 per cent alcohol is 5.84 and 5.21 in 25 per cent alcohol. To the first approximation the pk_b of the drug in pure water can be estimated to be 4.6 by assuming linear extrapolation to zero alcohol concentration. In other words

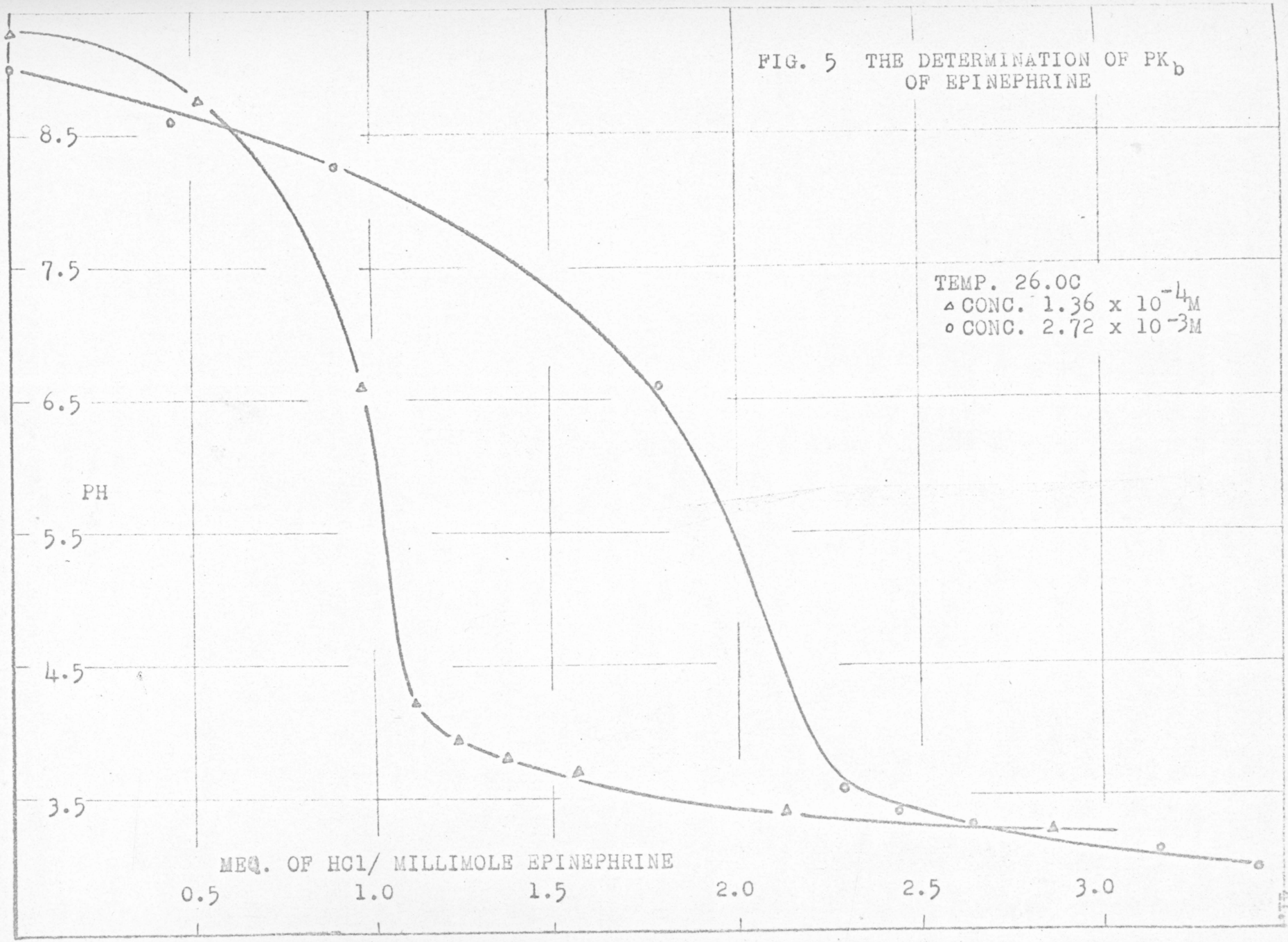


FIG. 5 THE DETERMINATION OF PK_b OF EPINEPHRINE

at pH 9.4 (14.0-4.6) and 25°C approximately one half of the drug will be in the amine salt form.

The Dependency of the Rate of Oxidation of Epinephrine on its Concentration

Data obtained from representative runs made in the course of this study are shown in Table IV and Fig. 3. The influence of the drug concentration on the rate of its autoxidation is summarized in Table V and in Fig. 4. where the logarithms of the initial concentration are plotted against the logarithms of the rates at fixed pH values. The rate values, as previously mentioned, were obtained by determining the slopes of the straight line portion of the respective oxygen uptake versus time curves.

The straight line relationships shown in Fig. 4 indicate that the rate of autoxidation can be related to epinephrine concentration by a simple power equation, i.e.,

$$d(O_2)/dt = K C^a$$

at any given temperature and pH, where C is the concentration of the drug, k, a proportionality constant, and a a constant which is independent of other variables and ranges between 0.66 and 0.72. The simplest fractional expression of a would be 2/3.

Less than a first order dependency of the oxidation rate on the drug substrate concentration indicates that the overall reaction is rather complex, involving two or more reactions occuring both consecutively and concurrently.

TABLE IVa

Effect of Concentration of Epinephrine on the Rate of Oxidation

pH 6.0 — Temp: 33.0°(±0.03°)

Time in Minutes	Volume of oxygen up take in Microliters						
	Conc. 2.73×10^{-2}M	1.64×10^{-2}M	1.09×10^{-2}M	5.47×10^{-5}M	2.73×10^{-3}M	1.37×10^{-3}M	6.83×10^{-4}M
20 Min	20.00	15.00	7.50				
30	25.00	20.00	12.50				
50	36.50	29.05	24.00	5.0			
75	57.50	48.00	42.50	17.50	5.0	7.0	5.0
100	83.60	70.00	60.00	30.00			
135	135.00	100.0	87.25	45.00	12.50	14.00	
155	182.00	125.25	102.50	55.00			10.00
175	235.25	148.00		62.50			
195	292.00	175.00	132.50		27.00	20.00	
215	347.50	203.15		80.00			15.00
240	427.50	240.00	170.00		37.00		
275		290.00	192.50	107.35	48.00	30.05	
300		322.50	210.00	120.00	52.50		
325			230.25	130.25		36.50	22.50
370			263.00	150.00	70.00		
400			285.50	165.50			
425			307.50	175.00	85.00	48.25	31.00
450				187.50			
475					97.50	55.00	36.00
505					105.00		
550						65.00	
600						72.50	46.35
660						107.00	50.00

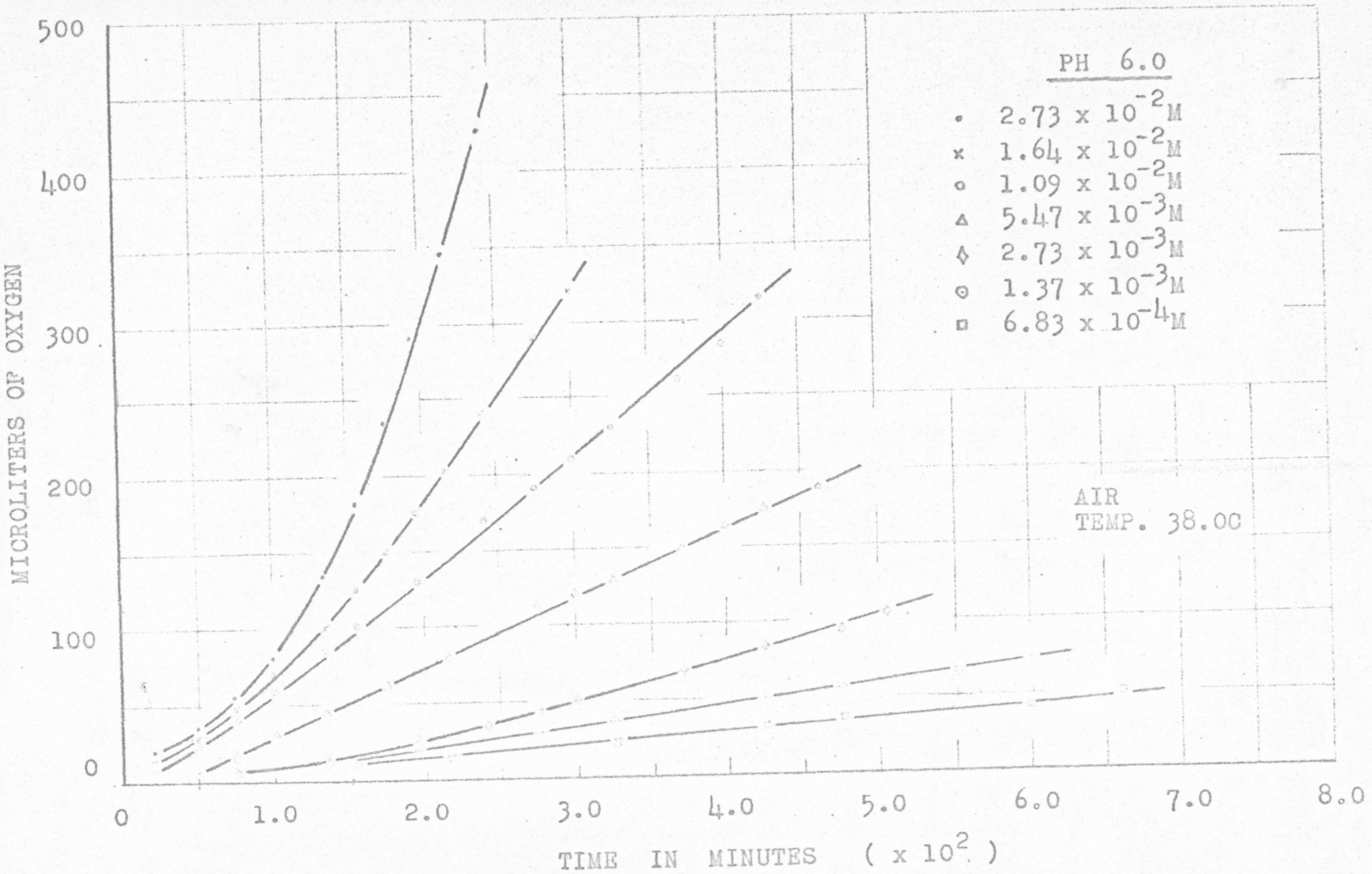


FIG. 3a EFFECT OF EPINEPHRINE CONCENTRATION ON THE RATE OF OXIDATION

TABLE IVb

Effect of Concentration of Epinephrine on the Rate of Oxidation

pH: 7.0 Temp: 38.0°(40.03°)

Time in Minutes	Volume of oxygen up take in Microliters							
	2.73×10^{-2}M	1.64×10^{-2}M	1.09×10^{-2}M	5.47×10^{-3}M	2.73×10^{-3}M	1.37×10^{-3}M	6.83×10^{-4}M	3.41×10^{-4}M
20 Min	20	12.50	5.0	2.5	1.25	2.0		
35	25.20	15.00	8.5	7.20	6.00	6.00		
40							15.00	15.00
50	37.50	22.50	17.50	15.00	12.00	10.00		
70	57.35	32.35	40.00	27.50	25.00	17.50	20.00	22.00
90	97.50	47.50	72.50	45.00	40.00	25.00		
100	112.50	65.00	90.00	56.50			33.00	25.00
110	140.00	85.00	107.50	70.00	62.50	33.50		
120	157.50	100.05	132.50	82.50			42.50	
135	207.20	137.50	170.0	107.5	92.50			37.50
145	260.00	170.00	210.25	130.0		60.00	55.00	
155	322.00	207.50	237.50	152.5	135.0			
165	387.50	250.35	267.00	175.0			62.00	45.00
180	440.00	320.00	313.50	207.50	155.00	85.00	67.25	
185	482.50	345.00		222.00				50.00
190		362.50	340.0		175.25		75.00	
200		415.00		255.00		100.00		
210		455.00	402.50		205.00			
220		485.25	430.00	300.00			87.50	
225			447.5		220	120.00		60.00
240			492.5	345.0	242.50		96.50	
250				370.0		140.25		
260				392.5	265.00		105.00	
270				407.5	280.0	155.00	115.35	71.50
280				430.0				
300				472.0	325.25	178.0	125.00	77.00
315					345.00			87.50

Time in Minutes	2.73×10^{-2}M	1.64×10^{-2}M	1.09×10^{-2}M	5.47×10^{-3}M	2.73×10^{-3}M	1.37×10^{-3}M	6.83×10^{-4}M	3.41×10^{-4}M
					367.50		140.25	
335						222.5	147.50	
355						233.0	157.50	
375						257.5	168.00	105.00
400							177.50	
420							185.00	117.50
455							191.85	
470								

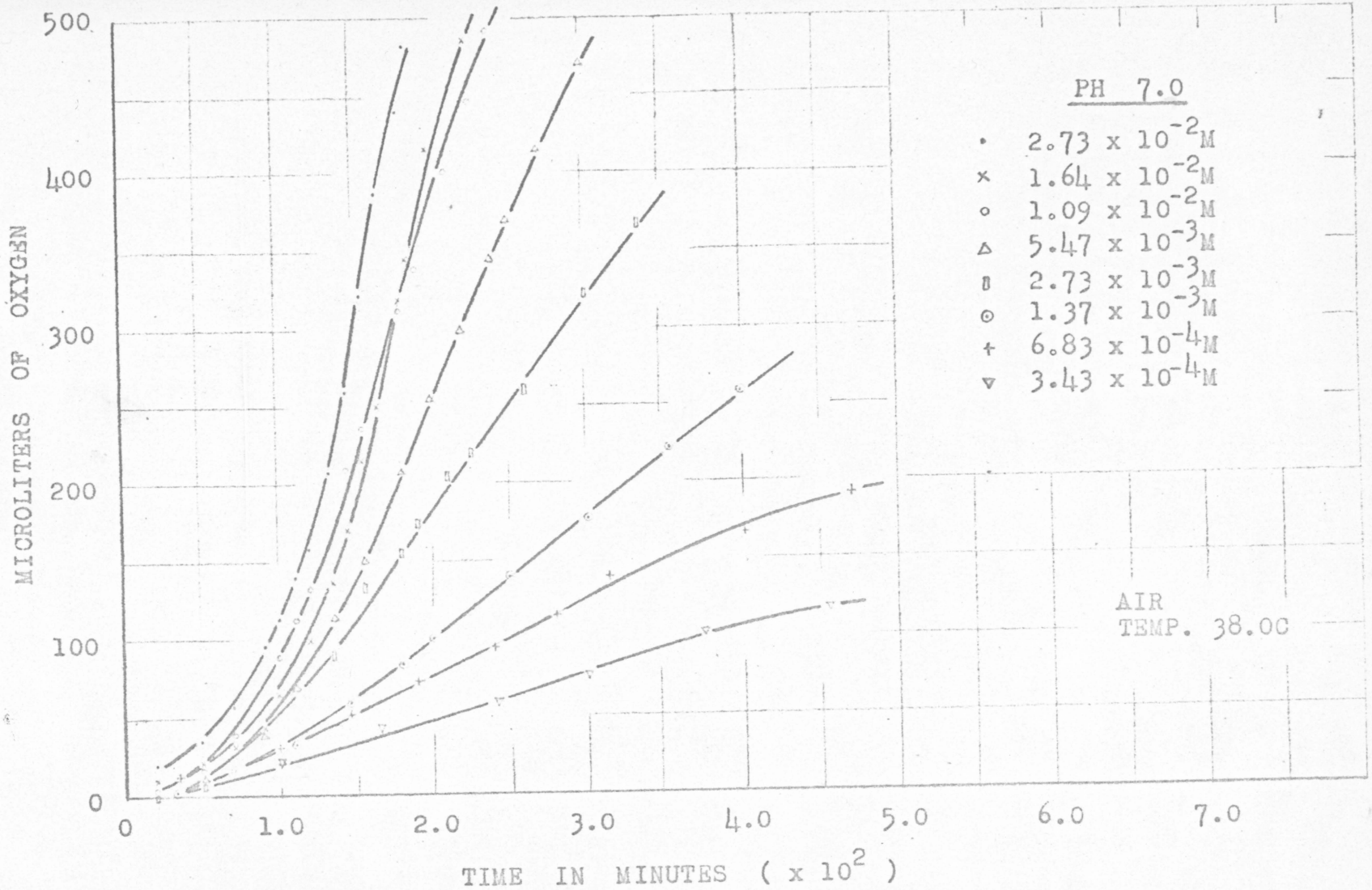


FIG. 3b EFFECT OF EPINEPHRINE CONCENTRATION ON THE RATE OF OXIDATION

TABLE IVc

Effect of Concentration of Epinephrine on the Rate of Oxidation

pH: 8.0 Temp: 38.0°(±0.03°)

Time in Minutes	Volume of oxygen up take in Microliters					
	Conc. 5.47×10^{-3}M	2.75×10^{-3}M	1.37×10^{-3}M	6.83×10^{-4}M	3.41×10^{-4}M	1.71×10^{-4}M
15 Min	14.00	45.00	0		6.50	
40	28.00	8.00	2.5	5.0	0.50	1.0
65	80.00	31.50	15.00	10.00	7.0	
80	124.50	52.50	25.00	17.50	15.50	12.50
100	185.00	95.00	58.50	40.00	27.50	25.00
125	280.00	160.25	100.00	67.50		
135	312.50	185.00	115.00		55.00	40.00
155	375.00	235.50		100.00	72.50	
160	395.00	257.50	157.50			52.00
175	442.50	287.00	180.00	120.25		
190		333.00	202.00		100.00	
200		352.50		147.50		62.50
220		400.00	255.50		117.50	
230		425.00		132.00		
240		445.00	287.50			80.00
260			320.00	215.10	145.00	
280			365.25	245.00	165.00	100.00
325			415.00	270.00		
300				297.5	187.30	
415				337.5	200.00	110.0
450				350.00		
505				367.50	207.50	115.0
555				377.50		
620				387.50	218.00	118.00

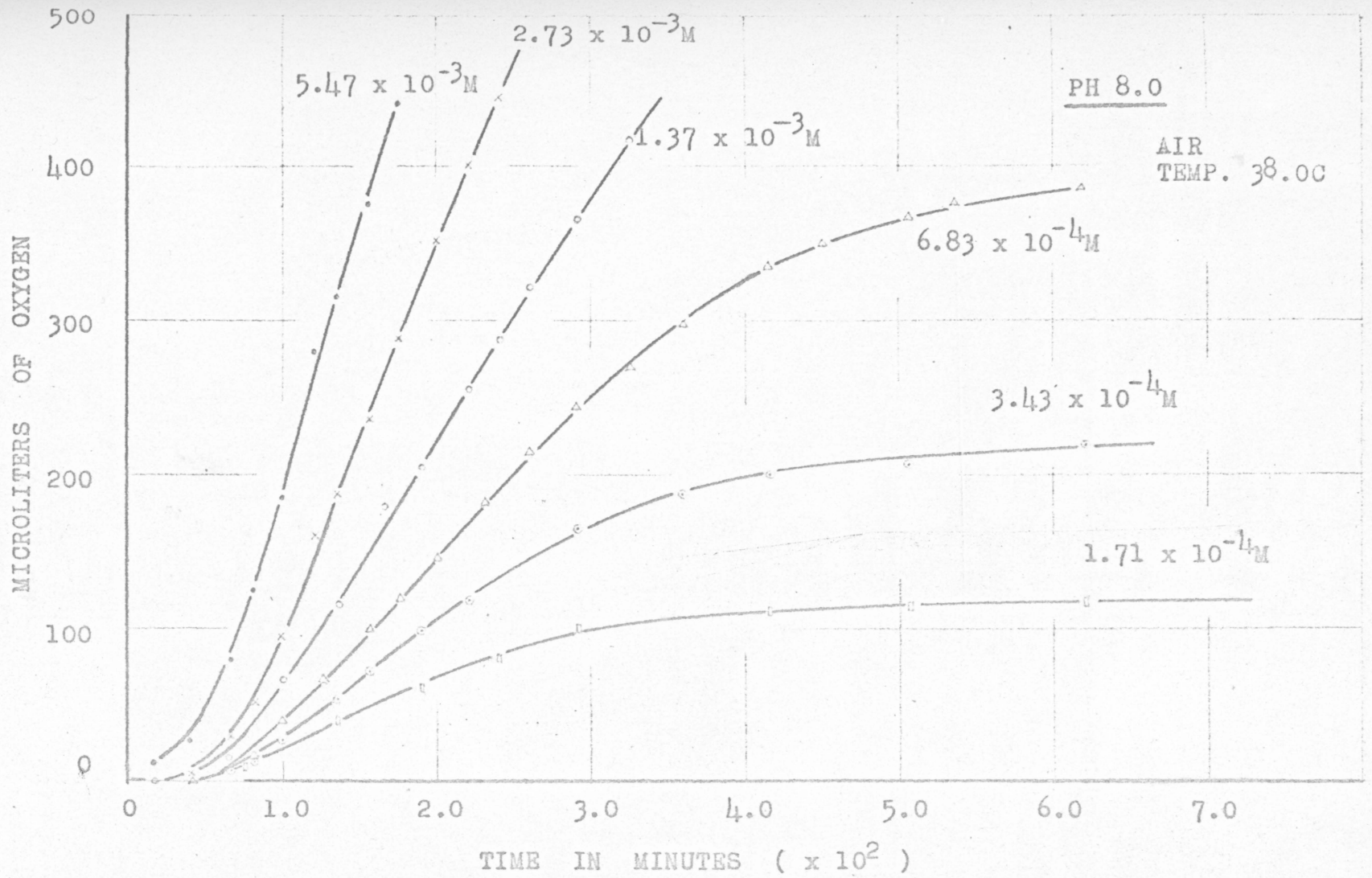


FIG. 3c EFFECT OF EPINEPHRINE CONCENTRATION ON THE RATE OF OXIDATION

TABLE V

Summary of Effect of Epinephrine Concentration on the Reaction Rate

Air — Temp: 38.0°C

Conc. M	$-\frac{\Delta O_2}{\Delta t}$			$\text{Log} -\frac{\Delta O_2}{\Delta t}$		
	pH 6.0	7.0	8.0	pH 6.0	pH 7.0	pH 8.0
2.73×10^{-2}M	2.00	5.5		0.3010	0.7404	
1.64×10^{-2}M	1.28	4.44		0.1072	0.6474	
1.09×10^{-2}M	0.750	3.08		-0.1249	0.4857	
5.47×10^{-3}M	0.446	2.22	3.33	-0.3507	0.3464	0.5224
2.73×10^{-3}M	0.265	1.38	2.53	-0.5768	0.1399	0.4031
1.37×10^{-3}M	0.15	0.78	1.62	-0.8239	-0.1079	0.2095
6.83×10^{-4}M	0.095	0.46	1.05	-1.0223	-0.3372	0.0212
3.41×10^{-4}M	0.046	0.29	0.64	-1.3372	-0.5376	-0.2676
1.71×10^{-4}M			0.409			-0.3883

FIG. 4b SUMMARY OF EFFECT OF EPINEPHRINE CONCENTRATION OF THE RATE OF OXIDATION

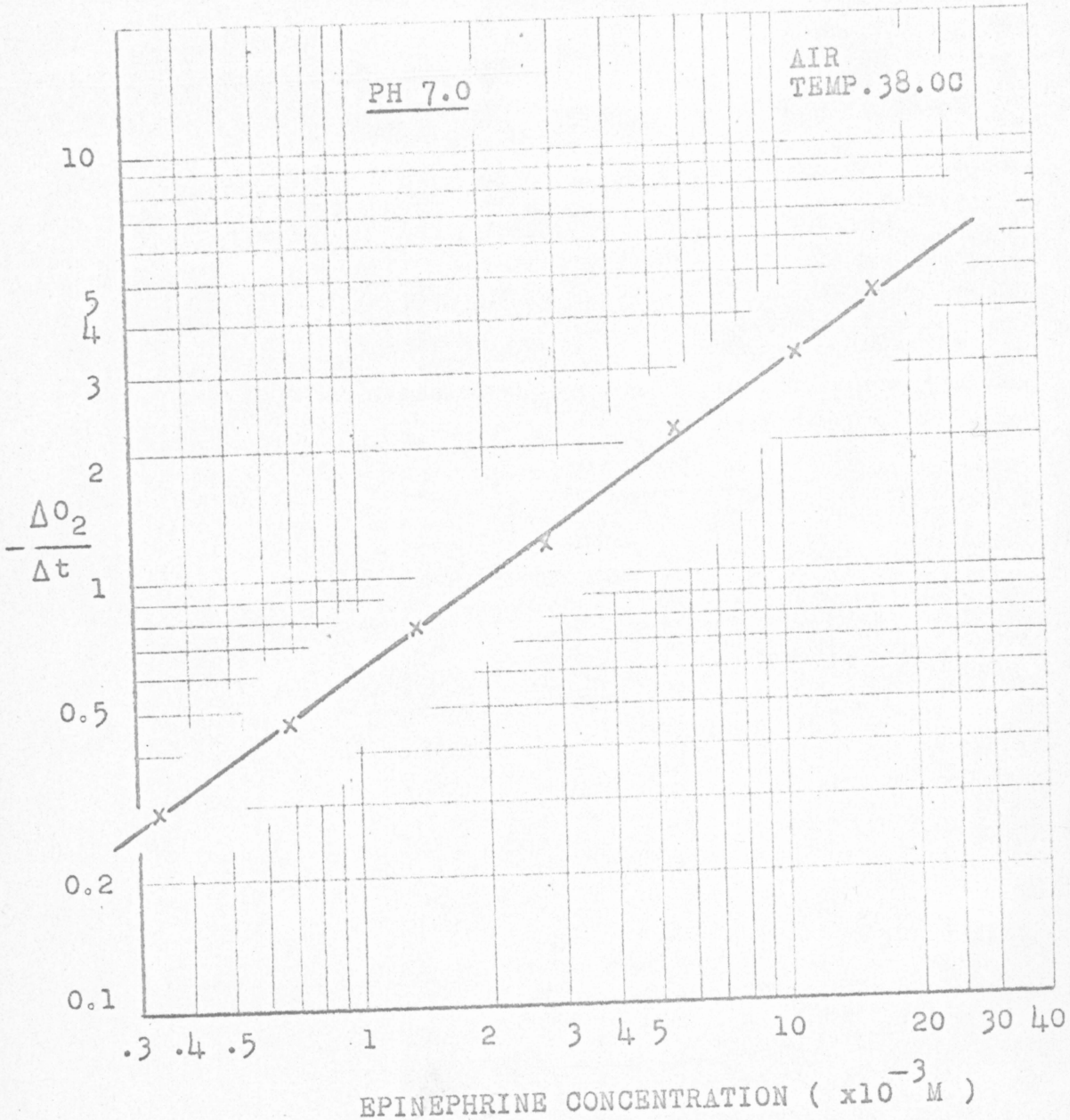
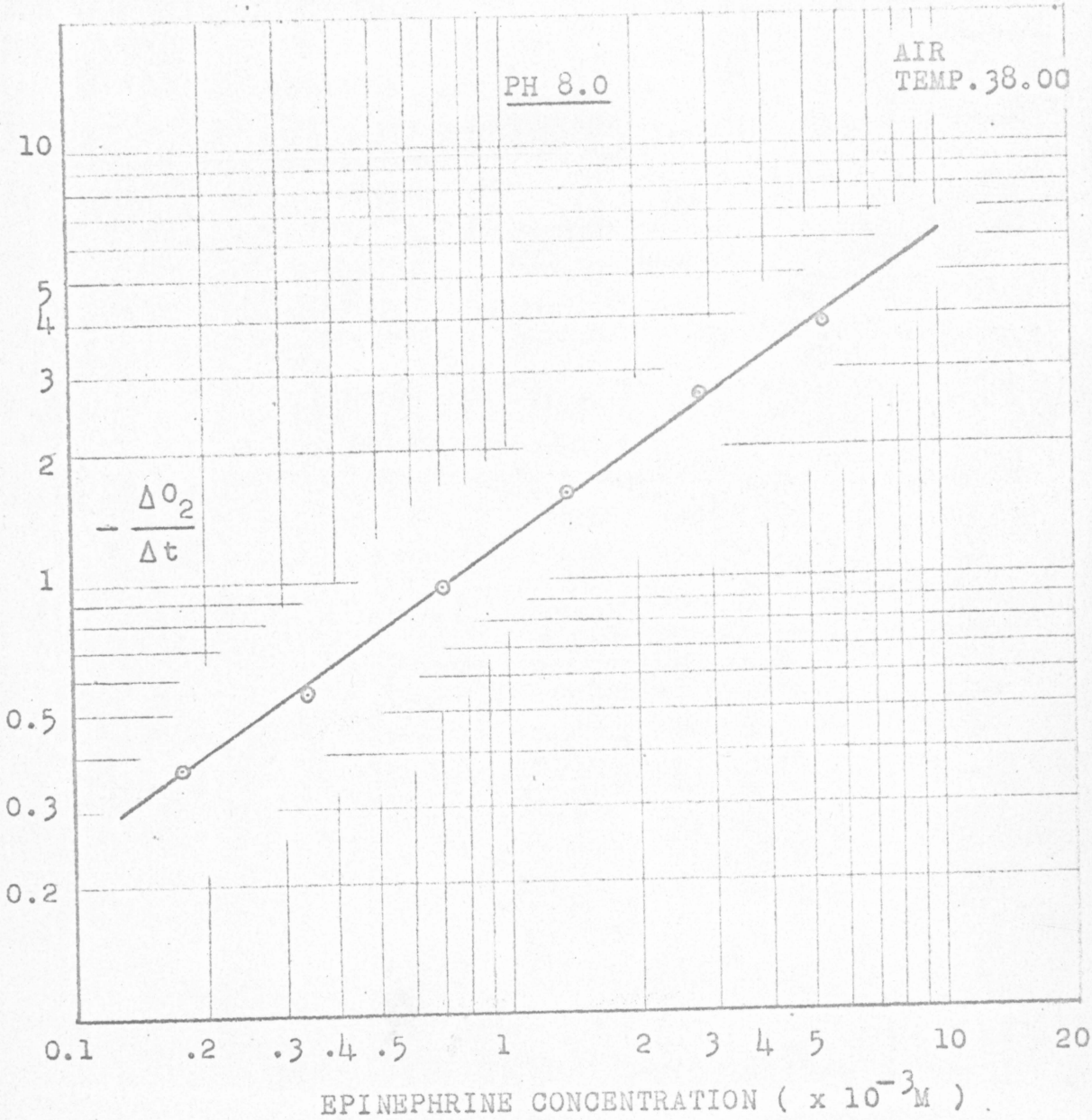


FIG. 4c SUMMARY OF EFFECT OF EPINEPHRINE CONCENTRATION ON THE RATE OF OXIDATION



The Order of the Autoxidation Reaction of Epinephrine with Respects to the Oxygen Tension

Experimental data indicates that the rate of autoxidation of epinephrine is not directly proportional to the partial pressure of oxygen over the solution. This is evident in the several oxygen uptake versus time runs made at different pressures, the data for which are given in Table VI and shown graphically in Fig. 6. The nature of the relationship between oxygen tension and the rate is more readily seen in Fig. 7. where the rates derived from these data have been plotted against the corresponding oxygen pressure. The form of the last curve can be closely approximately by the following empirical relationship,

$$\text{Rate} = \frac{k\,p}{a + p}$$

where p is the pressure of oxygen over the system and k and a are constant. If p is expressed in atmosphere, the curve shown in Fig. 7 can be fitted by setting k equal to 96 and a to 2.5 as shown in Table VII.

The type of dependency of the oxidation rate on oxygen pressure, similar to that given above, can be expected if there is a gradual transition of the rate determining step from an oxygen dependent reaction to an oxygen independent reaction. A possible rate determining step which may be directly dependent on the oxygen pressure is the transfer of oxygen from the gas phase to the solution phase. The possibility that this may be the rate determining step was discarded when check results were obtained with threefold difference in the amplitude of shaking.

TABLE VI

Effect of Oxygen Concentration on the Reaction Rate of Epinephrine

pH: 7.0 Temp: 38.0°C

Time in Minutes	Volume of oxygen up take in Microliters			
	po_2 (atm) 1.0	po_2 (atm) 0.5	po_2 (atm) 0.21	po_2 (atm.) 0.10
20	9.17	11.12		6.02
70			0	
75	95.0	56.50	13.55	23.25
80	114.0	125.90	54.60	
120	215.0			24.10
140	327.0	161.80	67.20	
165		204.0	67.20	42.20
215	433.0	284.0	105.00	60.30
245	492.0	327.0	128.00	73.20
270	536.5	370.0	151.5	82.20
295	576.5	410.0	173.0	91.65

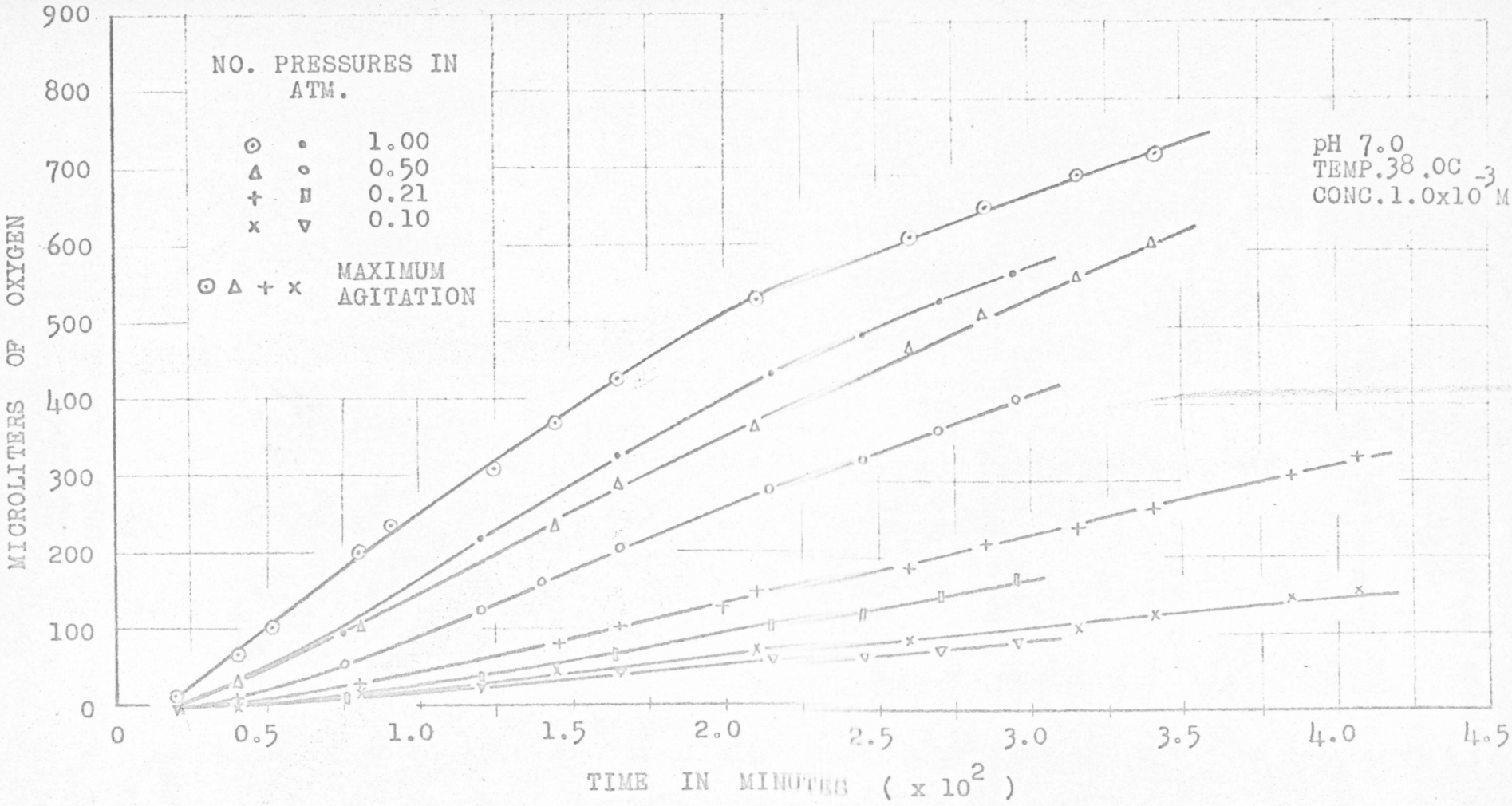


FIG. 6 EFFECT OF OXYGEN CONCENTRATION ON THE RATE OF OXIDATION OF EPINEPHRINE

TABLE VII

Summary of Effect of Oxygen Tension
on Reaction Rate of Epinephrine

Temp. 38.0°C pH 7.0 Conc. 1.0×10^{-3} M

	p_{O_2} (atm.)	$-\frac{\Delta O_2}{\Delta t}$ Experimental	$-\frac{\Delta O_2}{\Delta t}$ *Predicated
1.	1.00	25.40	27.5
2.	0.50	15.90	15.4
3.	0.21	8.00	7.5
4.	0.10	4.00	3.7

* Rate = $\frac{K\,p}{p + a}$

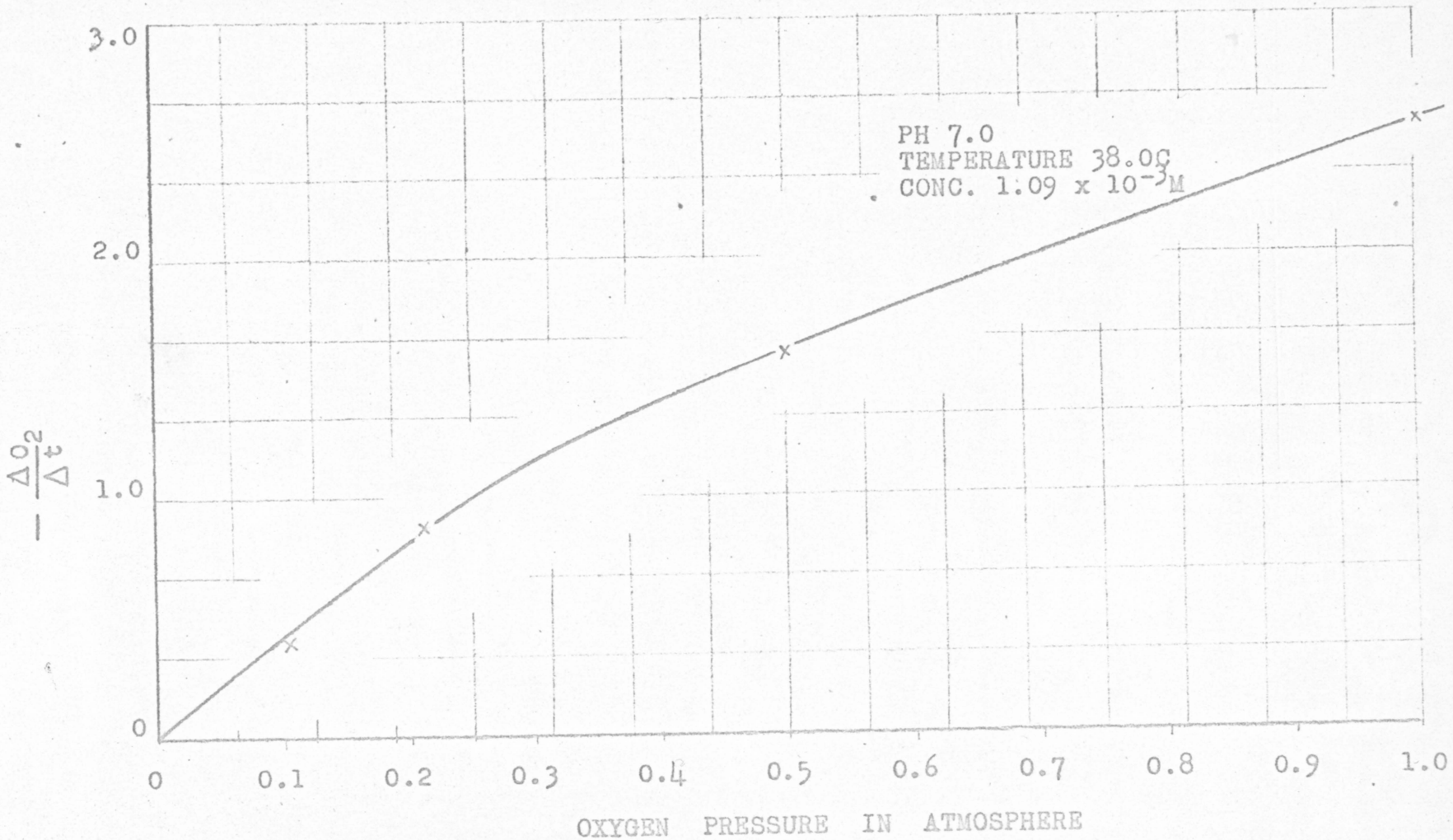


FIG. 7 SUMMARY OF EFFECT OF OXYGEN CONCENTRATION ON REACTION RATE OF EPINEPHRINE

Temperature Dependency of Rate of Autoxidation of Epinephrine

The influence of temperature in increasing the rate of autoxidation of epinephrine is evident in the data shown in Table VIII and Fig. 8. The adherence of the system to the Arrhenius equation is shown in Fig. 9a, 9b, and 9c, where the logarithms of the rates of oxygen uptake have been plotted against the reciprocals of the absolute temperatures of the runs. In each case a rather good linear relationship is evident. The calculated apparent heats of activation at the three pH settings are 23 kilocalories at pH 4.9, 17.2 kilocalories at pH 7.0, and 14.3 kilocalories per mole at pH 8.13.

The observed differences in the determined heats of activation at the several pH values are to be expected since they correspond to different regions in the pH versus rate curve shown in Fig. 2 (page 37). At pH 4.9 the rate determining step is practically independent of hydroxide ion concentration, at pH 7.0 the rate is nearly directly proportional to the hydroxide ion concentration. At the highest pH region studied, 8.13, the rate dependency on hydroxide ion is again less than first order.

For reactions which are first order with respects to hydroxide ion concentration and which are buffered to constant pH values, it has been shown that the heat of ionization of water must be subtracted from the overall heats of activation.(63) This would reduce the energy requirement at pH 7.0 from 17.2 to 5.2 kilocalories per mole. Since this correction would be less at pH 8.13 because of the lower order of hydroxyl ion dependency, the net heat of activation at this pH would roughly be the same. The activation energy at pH 4.9, however, would not

TABLE VIIIa

Temperature Effect on the Reaction Rate of Epinephrine

pH8.13

Time in Minutes	Volume of oxygen up take in Microliters			
	Temp. 50.0°C(±0.03?)	46.0°C	42.0°C	38.0°C
10	63.40	50.50		
20	147.2		13.72	10.00
25		144.20		
30		185.5		22.00
35	266.2	220.2		
45		290.0		52.00
50	391.0			
60		392.0	116.2	75.00
75				150.00
84			240.0	190.00
107			364.0	
110			374.0	
120				327.5
125			448.0	
130				350
150				425.00

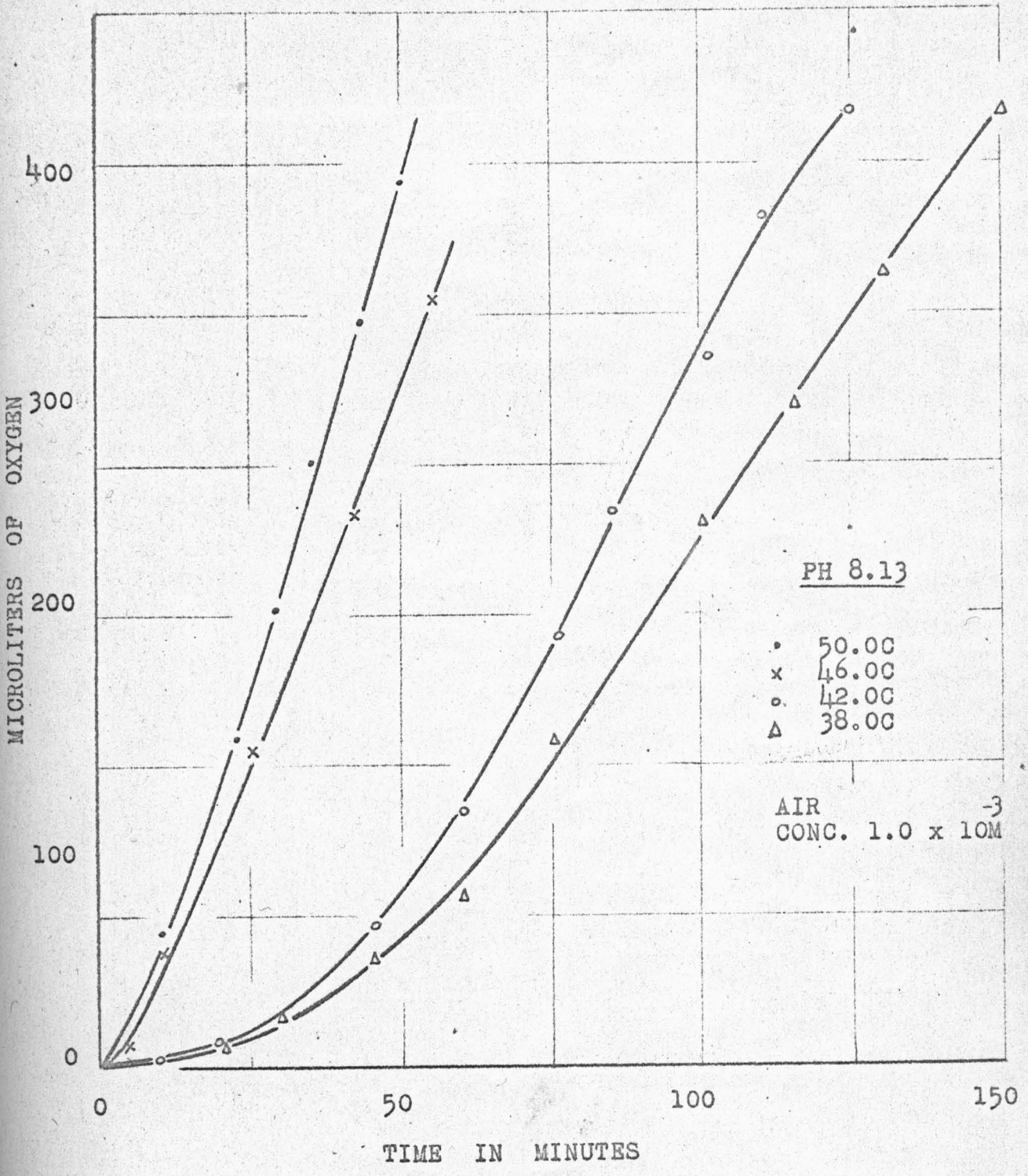


FIG. 8a EFFECT OF TEMPERATURE ON REACTION RATE OF EPINEPHRINE

TABLE VIIIb

Temperature Effect on the Reaction Rate of Epinephrine

pH 7.0

Time in Minutes	Volume of oxygen up take in Microliters			
	Temp. 50.0°C(±0.03°)	46.0°C	42.0°C	38.0°C
20	16.68			4.78
30			12.72	
40		23.60		
45				11.82
55		48.65		
65	75.50		38.62	
80		82.80		
85				
100	136.50			
105		125.0		
110	152.5			
150			136.00	
170	254.0			91.20
185		259.0		
195	290.0			
200			182.00	
220	325.0			131.20
225		319.0		
240	354.0			
245				151.20
250		352.2		
260			255.5	
270	394.0			
275				175.69
285		425.0		
290			283.0	189.20
305	428.0			

Time in Minutes	Temp. 50.0°C(±0.03°)	46.0°C	42.0°C	38.0°C
345			332.0	
360				245.0
380				258.5
400				274.0
410				280.0
440			412.0	
460				318.0

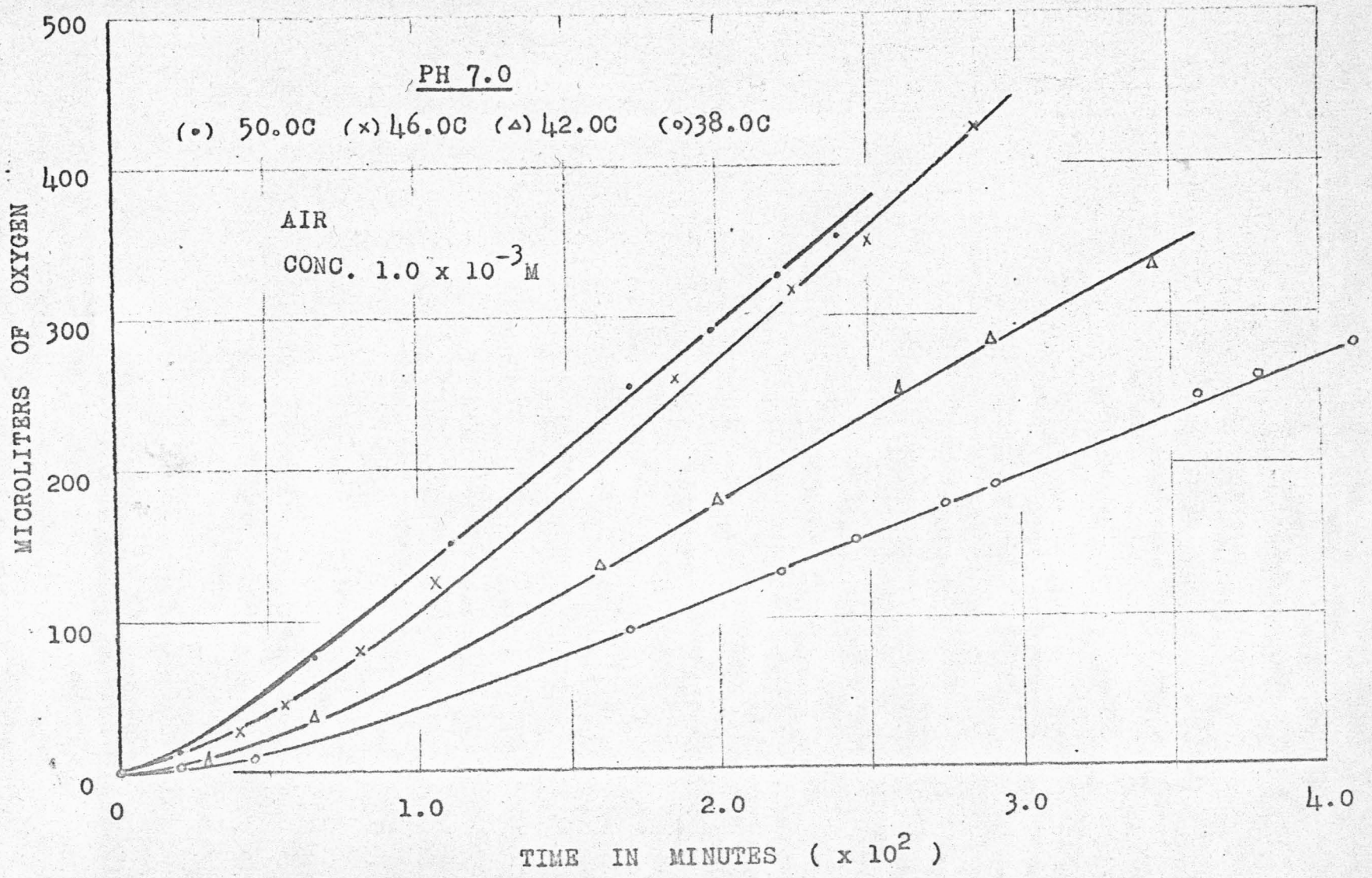


FIG. 8b EFFECT OF TEMPERATURE ON REACTION RATE OF EPINEPHRINE

TABLE VIII c

Temperature Effect on the Reaction Rate of Epinephrine

pH 4.91

Time in Minutes	Volume of oxygen up take in Microliters			
	Temperature 50°C	46°C	42°C	38°C
20 Min			7.00	
40	22.82	20.00		
50			5.37	2.00
90		26.15		
100	45.65			5.00
110			11.90	
150	69.55	40.00		9.00
155			19.20	
180	84.20	42.00		
215			26.50	
220	99.00			14.00
240	105.8	56.00		
255	114.2		35.00	
280	152.2	75.00		18.00
305			40.75	
320			42.80	
360	154.2	80.00	49.20	25.00
370		90.00		
397			53.00	
427		98.00		28.00
500			68.40	
540	214.0	110.0		35.00
580	224.5			
585		130.0	82.20	
610			93.50	38.00
717		140.5		

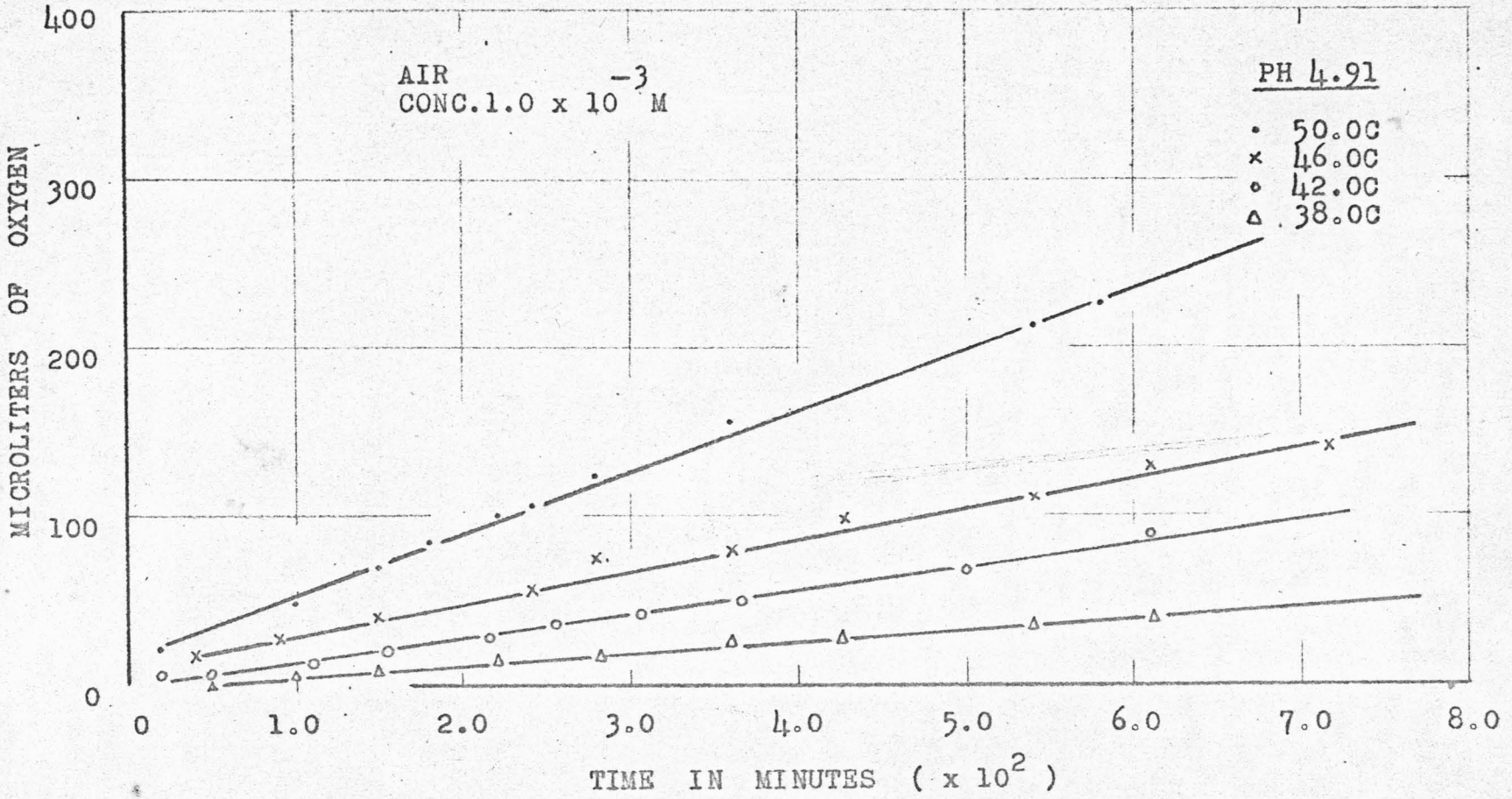


FIG. 8c EFFECT OF TEMPERATURE ON REACTION RATE OF EPINEPHRINE

TABLE IX
Summary of Temperature Effect on
the Reaction Rate of Epinephrine

Conc.: 1.0×10^{-3}M — Air

Temperature t°C	$1/T \times 10^5$	$-\frac{\Delta O_2}{\Delta t}$ *	$-\frac{\Delta O_2}{\Delta t}$ **	$-\frac{\Delta O_2}{\Delta t}$ ***	Log $-\frac{\Delta O_2}{\Delta t}$ *	Log $-\frac{\Delta O_2}{\Delta t}$ **	Log $-\frac{\Delta O_2}{\Delta t}$ ***
50°	309	0.393	2.291	8.12	-0.4055	0.360	0.9230
46°	313	0.217	1.571	6.72	-0.6630	0.1962	0.7960
42°	317	0.141	1.063	4.866	-0.8500	0.0265	0.6750
38°	321	0.0759	0.792	3.51	-1.1200	-0.1013	0.5450

*pH 4.91
**pH 7.0
***pH 8.13

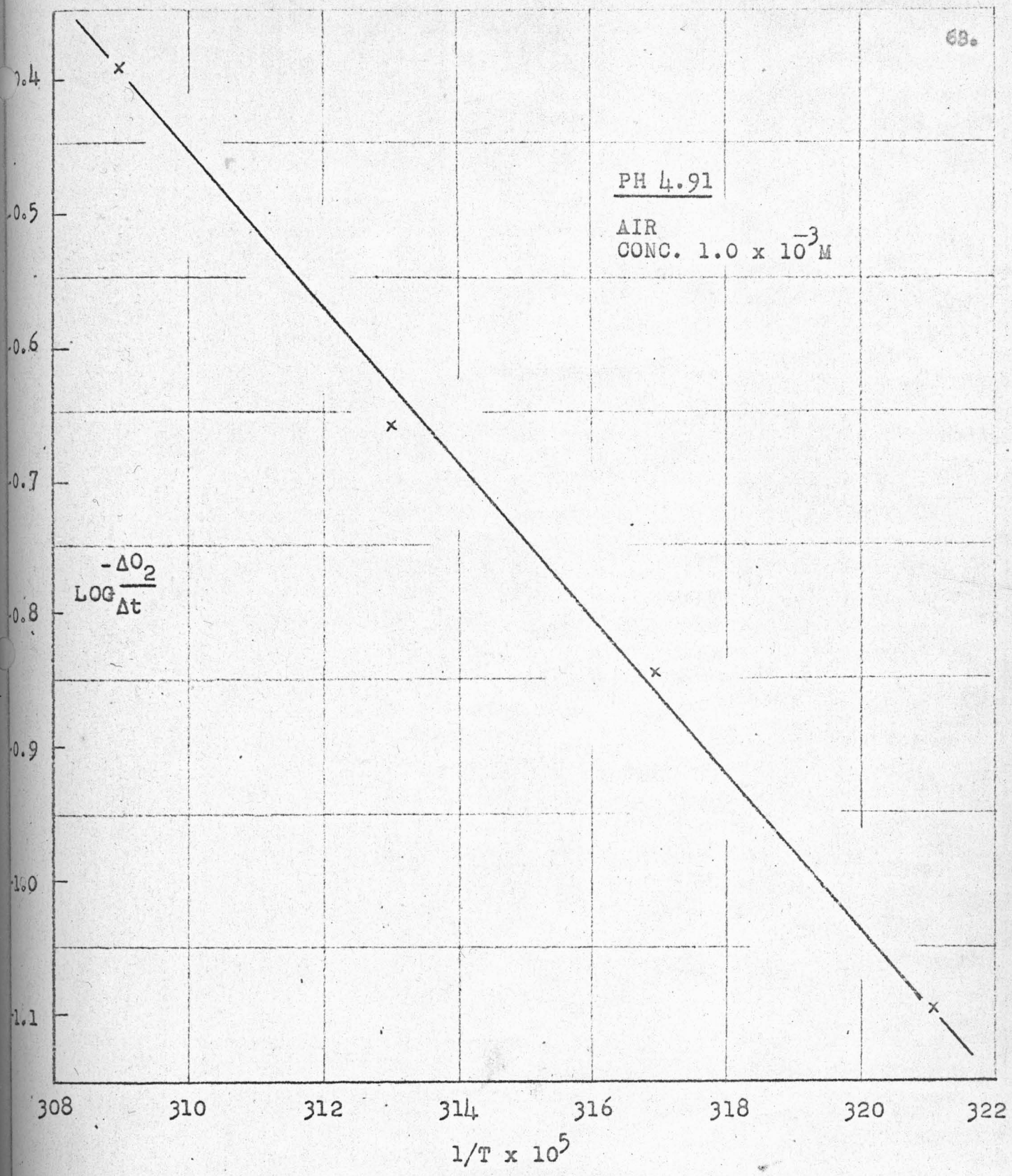


FIG. 9a SUMMARY OF EFFECT OF TEMPERATURE ON REACTION RATE OF EPINEPHRINE

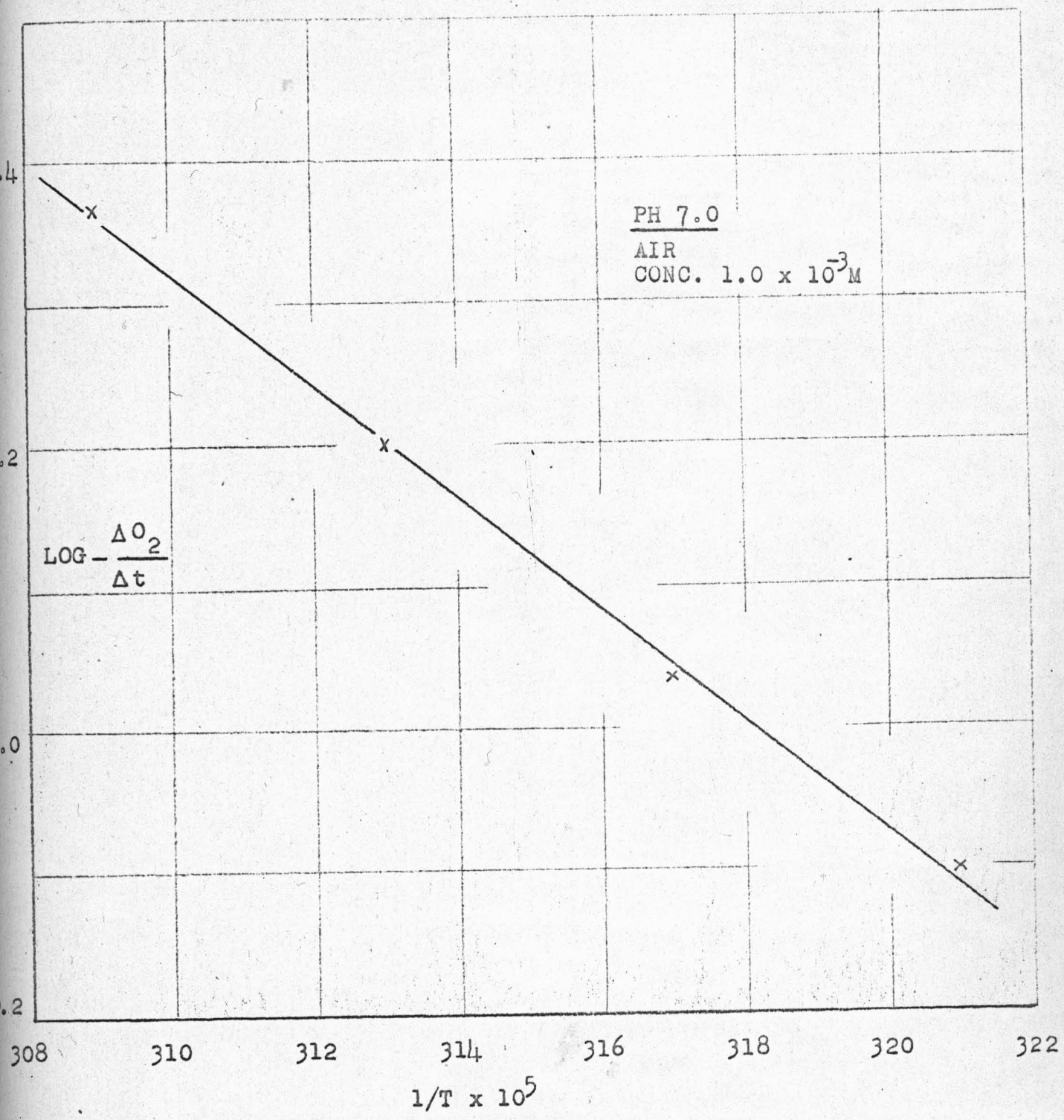
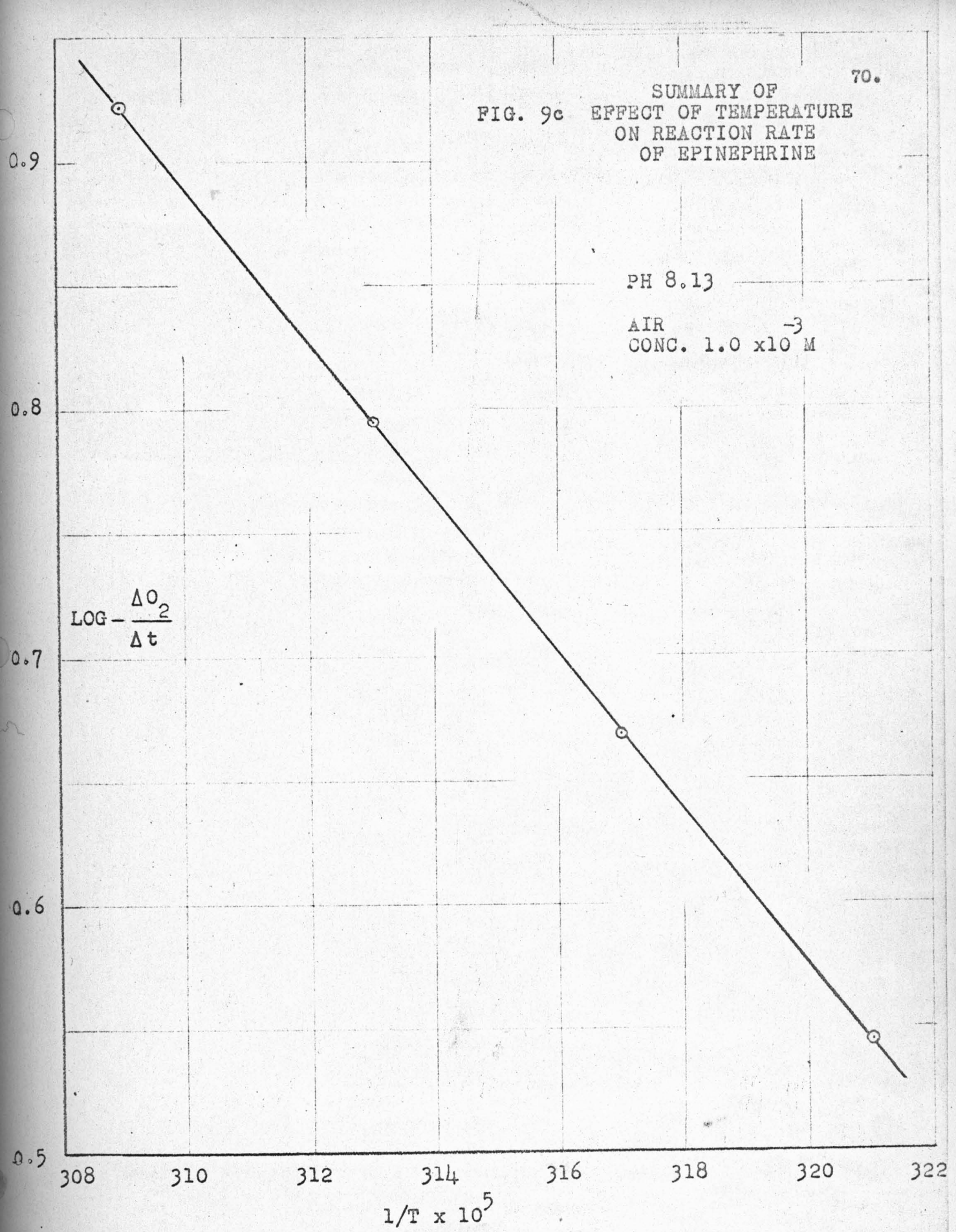


FIG. 9b SUMMARY OF EFFECT OF TEMPERATURE ON REACTION RATE OF EPINEPHRINE

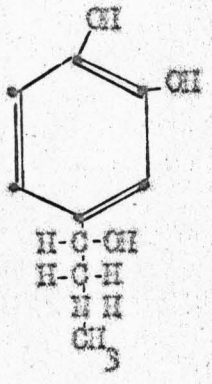
SUMMARY OF
FIG. 9c EFFECT OF TEMPERATURE
ON REACTION RATE
OF EPINEPHRINE



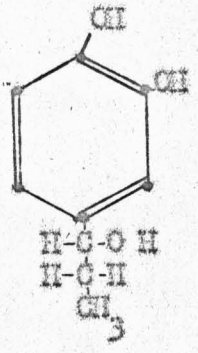
be reduced since the rate in this instance is nearly independent of pH. It is probably reasonable to assume, therefore, that the rate determining step at the two higher pH is the same, whereas the one in the acdic region must be quite different.

Influence of Some Variables on the Autoxidation Rates of Some Analogues of Epinephrine

It is exceedingly doubtful that the intact and unchanged epinephrine is necessary to give all the reaction characteristics of the autoxidation reaction. The reaction rate and several of the features of autoxidation reaction of catechol and certain catechol derivatives are at least outwardly similar to those of epinephrine. In the present study a particular attention was paid to the oxidation reaction of 1-(3,4-dihydroxyphenyl)-1-propanol which is structurally very similar to epinephrine:



Epinephrine



1-(3,4-Dihydroxyphenyl)-1-Propanol

In Table X and Fig. 10 are presented data showing the dependency of the rate of autoxidation of 1-(3,4-dihydroxyphenyl)-1-propanol as a

TABLE X

Effect of pH on the Reaction Rate of
1-(3,4-Dihydroxyphenyl)-1-Propanol

Time in Minutes	Volume of oxygen up take in Microliters pH 6.54	pH 7.18	pH 7.74	pH 8.31
25		3.08		
35			14.25	
45				62.60
60	5.67			
85			31.20	116.0
115				141.5
125		10.05	49.20	
145				169.5
155			57.50	
160	11.65			
170		16.62		
185			68.20	
210		20.50		
220				-222.5
260			92.50	
320		31.50		
325				275.5
355	14.4			
365			120.5	
485		45.50		341.0
520	21.60			
525			156.20	
655		62.60		
690	24.70			
886		77.0		
901	27.30			
1155		95.0		
1190	36.00			

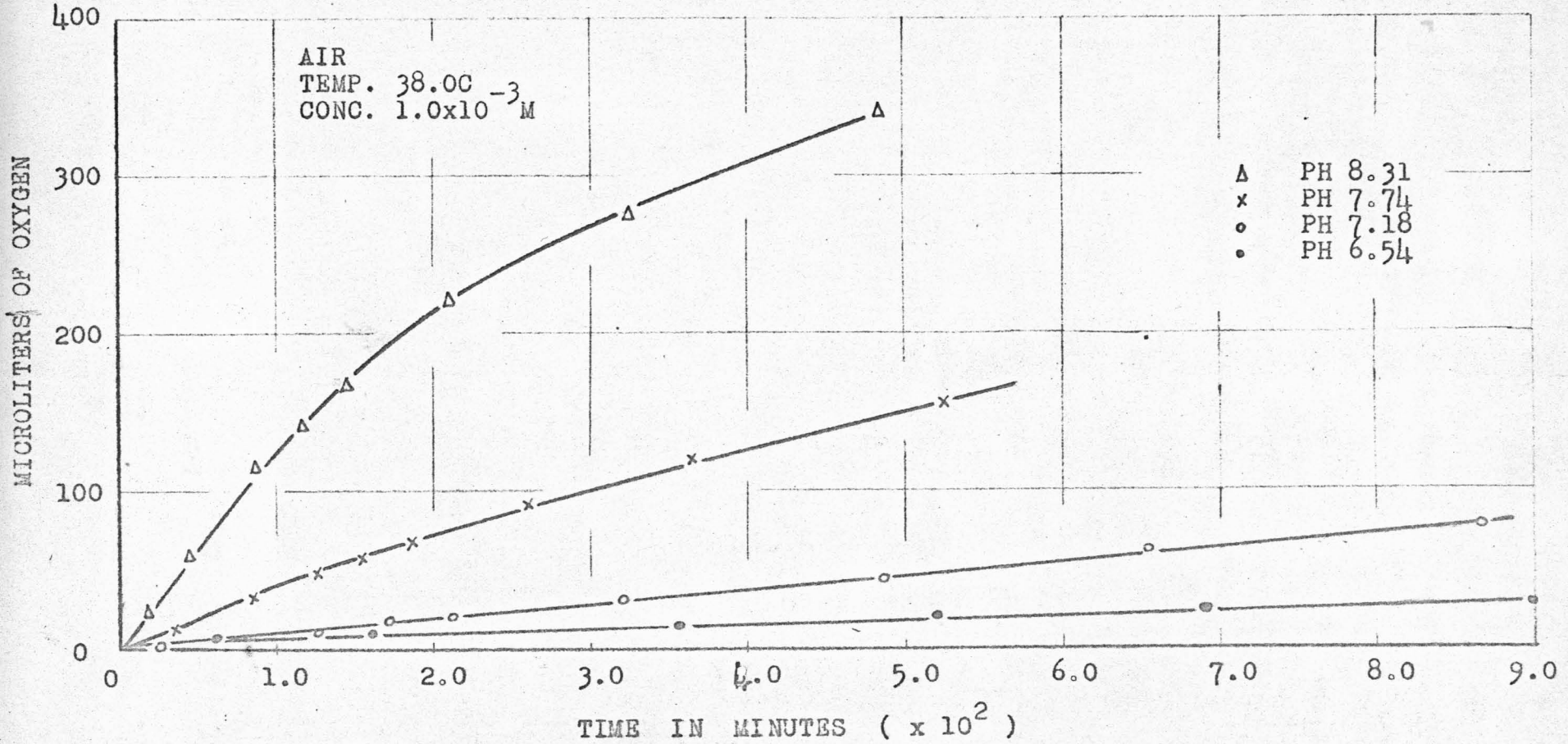


FIG. 10a EFFECT OF PH ON REACTION RATE OF 1-(3,4-DIHYDROXYPHENYL)-1-PROPANOL

FIG. 10b EFFECT OF PH ON THE REACTION RATE OF
1-(3,4-DIHYDROXYPHENYL)-1-PROPANOL

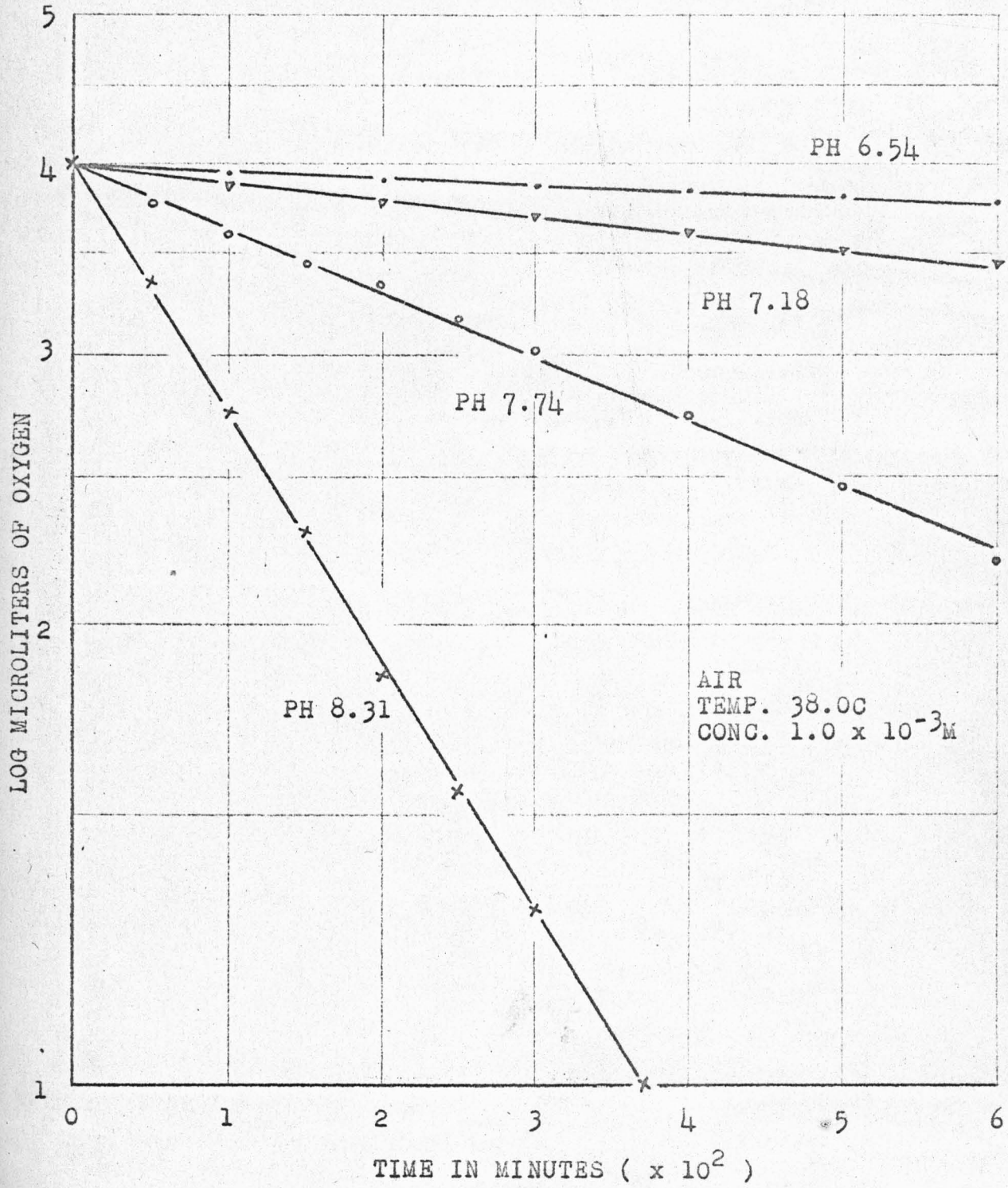


TABLE XI

Summary of Effect of pH on Reaction
Rate of 1-(3,4-Dihydroxyphenyl)-1-Propanol

Conc. 1.0×10^{-3}M Temp. 38.0^{0}C

pH	$-\frac{\Delta O_2}{\Delta t}$ Initial	Log $-\frac{\Delta O_2}{\Delta t}$ Initial
6.54	0.0375	-1.4260
7.18	0.0860	-1.0655
7.74	0.4265	-0.3701
8.31	1.59	0.2014

Air

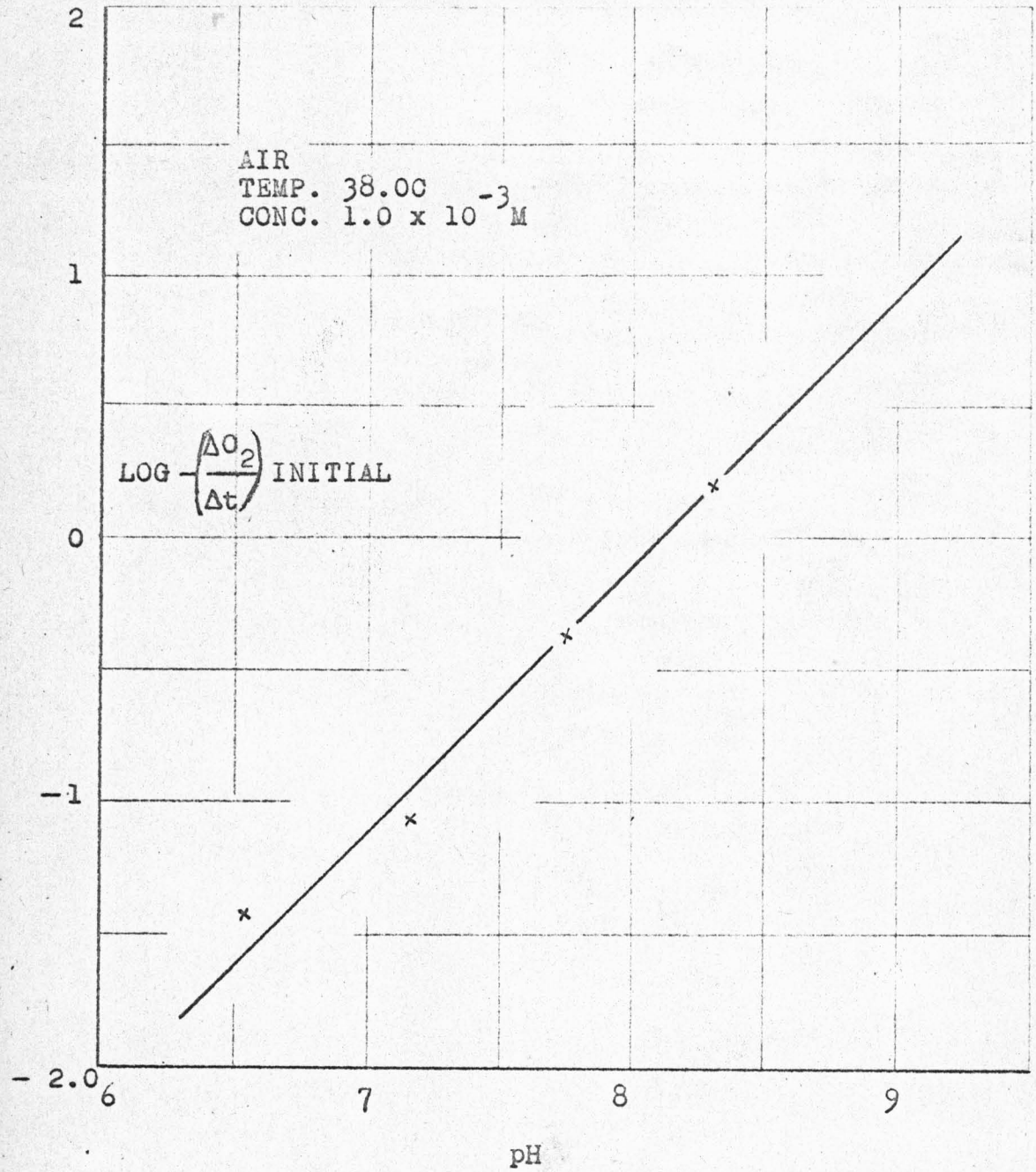


FIG. 11 SUMMARY OF EFFECT OF PH ON REACTION RATE OF 1-(3,4-DIHYDROXYPHENYL)-1-PROPANOL

function of pH. The curves shown exhibit certain essential difference from those of epinephrine. The most noticeable difference is in the absence of the induction period, each curve extrapolating through the origin at zero time. The curves, furthermore, do not have straight-line portion but rather appear to follow the usual first order disappearance relationship. This is confirmed by Fig. 10b where the data on Fig. 10, when plotted on a logarithmic scale by assuming that the total oxygen uptake for the system is 400 µls. per flask, (a value which corresponds to exactly 1.50 moles of oxygen per mole of 1-(3,4-dihydroxyphenyl)-1-propanol taken) yielded straight lines. If the logarithms of the slopes of these straight lines are plotted against the corresponding pH values, another straight line is obtained (as shown in Table XI and Fig. 11) with a slope of approximately one.

Further evidence supporting the acceptance of the reaction as being first order with respects to the substrate is presented in Table XII and Fig. 12. These data, which are summarized in a logarithmic form in Table XIII and Fig. 13, prove conclusively that the reaction is first order, the slope of the straight line in Fig. 13 being approximately one, 1.1 to be exact. Apparently the absence of the amino group in this compound is responsible for the increase in the order of the reaction, 2/3 in the case of epinephrine, to one in the present case.

The influence of oxygen tension on the first order rate constant of the autoxidation reaction of 1-(3,4-dihydroxyphenyl)-1-propanol is shown in Tables XIV and XV and Figs. 14 and 15. As compared to epinephrine, the rate of autoxidation of the present compound is more directly proportional to the oxygen pressure, showing however a small drop off in the rate at higher pressures.

TABLE XII

Effect of concentration on the Reaction Rate of 1-(3,4-Dihydroxyphenyl)-1-Propanol

Time in Minutes	Volume of oxygen up take in Microliters				
	Conc. 1.09×10^{-2}M	5.47×10^{-3}M	2.73×10^{-3}M	1.37×10^{-3}M	6.18×10^{-4}M
40 Min	70.0	10.0	8.0	+6.0	+2.5
70		20.0			
100	87.0	30.0	20.57	+2.5	2.5
140	122.0	50.0			
170	142.0	60.0	37.87	2.5	
205	175.0	72.0	40.25	5.0	8.0
250		92.0			
275	235.0	100.0	58.23	12.50	12.50
325	272.0	123.0	67.23	15.00	
390	322.0	140.0	80.00	20.0	15.00
415	360.0	162.0	88.60	25.0	20.00
520	415.0	183.0	100.0	30.0	
555					22.00
630					26.50

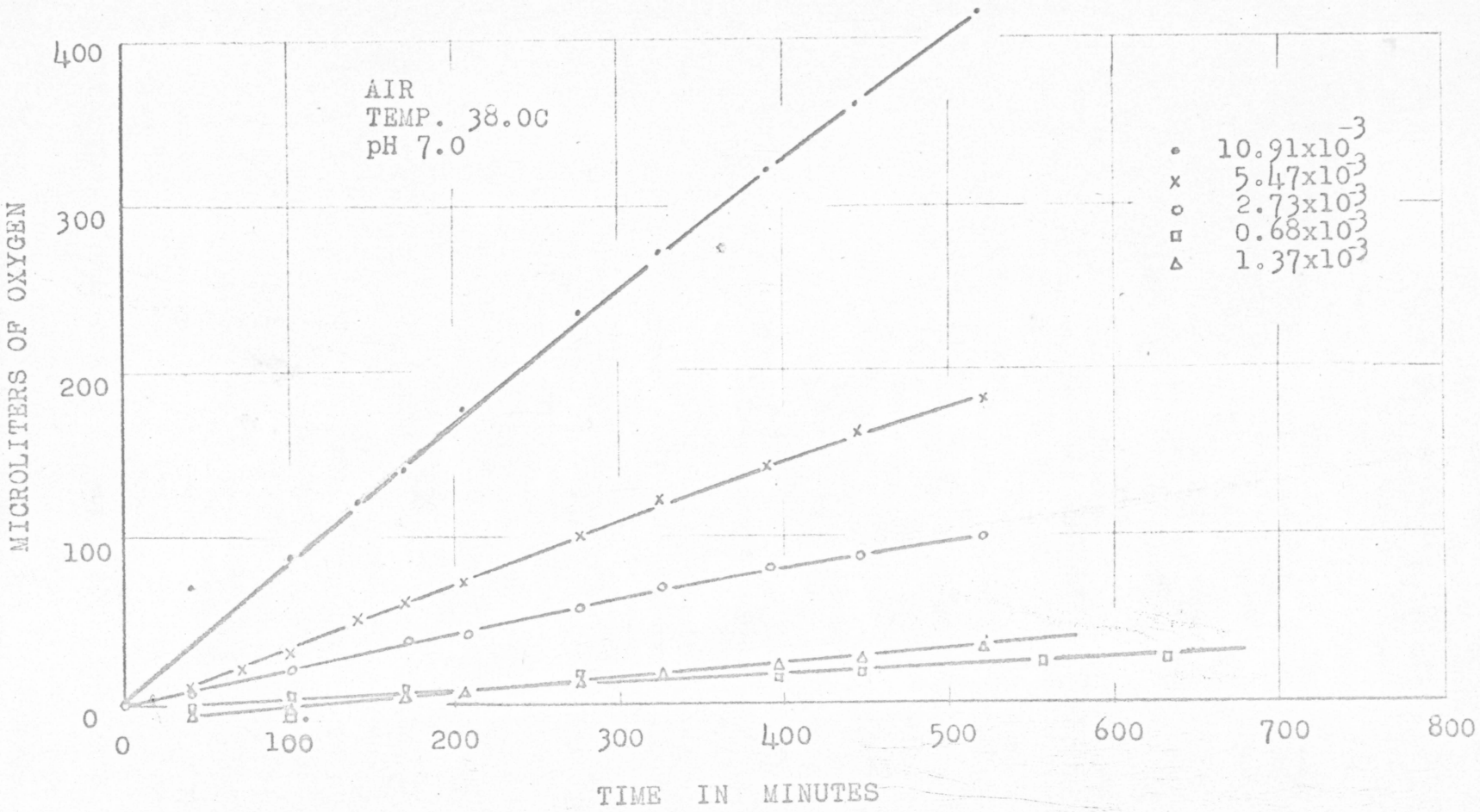


FIG. 12 EFFECT OF CONCENTRATION ON REACTION RATE OF 1-(3,4-DIHYDROXYPHENYL)-1-PROPANOL

TABLE XIII
Summary of Effect of Concentration
on the Reaction Rate of
1-(3,4-Dihydroxyphenyl)-1-Propanol

pH 7.0 Temp. 38.0°C Air

Conc.	Log Conc.	$-\frac{\Delta O_2}{\Delta t}$	$\text{Log} -\frac{\Delta O_2}{\Delta t}$
1.09×10^{-2}M	1.0374	0.8365	-0.078
5.47×10^{-3}M	0.7376	0.422	-0.378
2.73×10^{-3}M	0.4362	0.202	-0.692
1.37×10^{-3}M	0.1351	0.0813	-1.050
6.18×10^{-4}M	-1.8341	0.0428	-1.3686

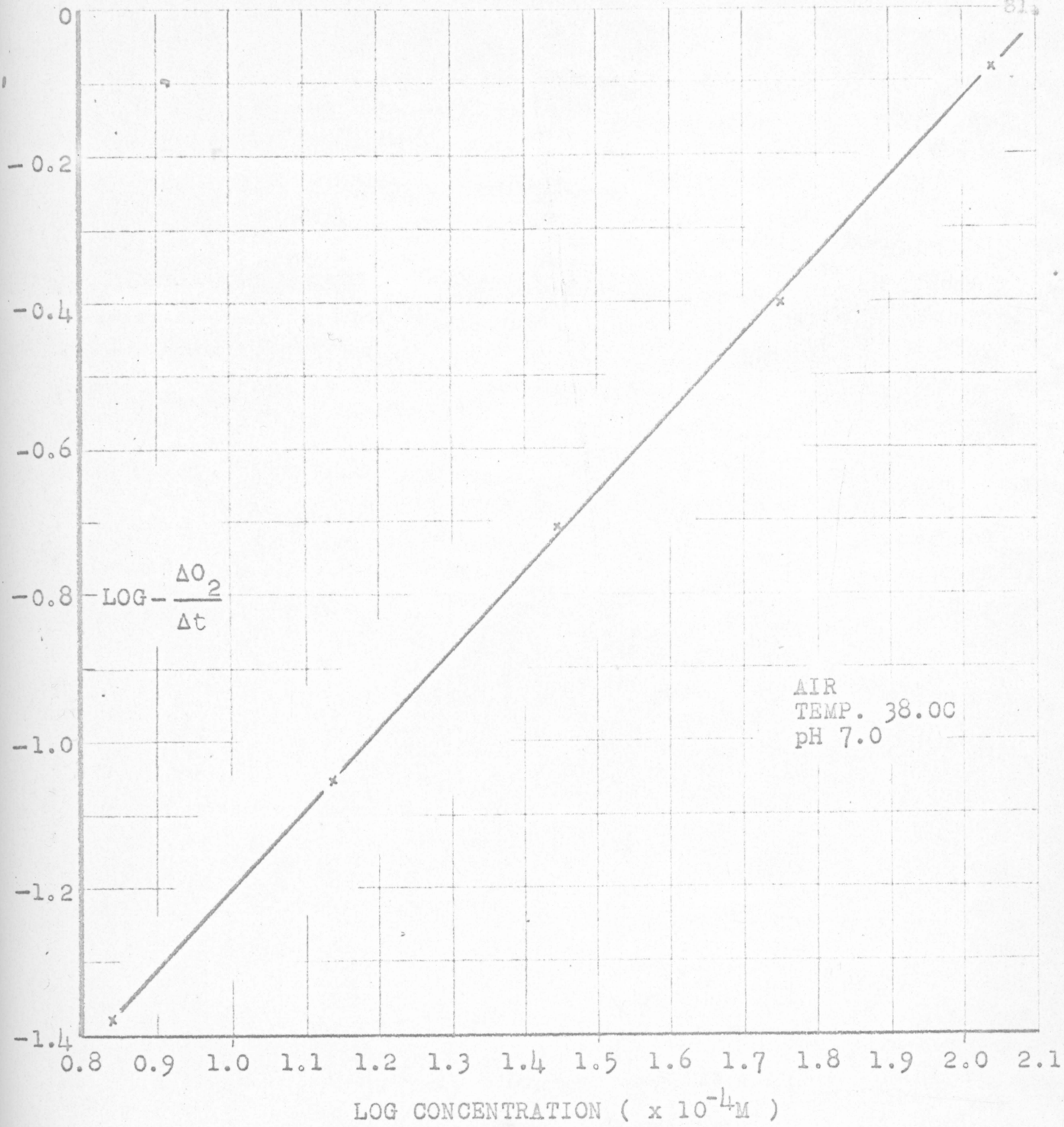


FIG. 13 SUMMARY OF CONCENTRATION EFFECT ON THE REACTION RATE OF 1-(3,4-DIHYDROXYPHENYL)-1-PROPANOL

TABLE XIV

Effect of Oxygen Concentration on the Reaction Rate of 1-(3,4-Dihydroxyphenyl)-1-Propanol

Time in Minutes	Volume of oxygen up take in Microliters			
	po_2(atm.) 1.0	po_2(atm.) 0.5	po_2(atm.) 0.21	po_2(atm.) 0.10
35	2.29	5.24	5.00	4.275
100	4.25	15.05	11.35	8.99
155	11.12	21.82	15.40	11.10
330	31.05		25.00	16.68
355	36.00	35.60		17.10
400	40.20	40.80	27.12	
470	48.40	46.20	30.00	21.40
590	60.80	55.75	32.70	22.20
990	100.00	72.75	43.88	24.80
1105		82.75	45.00	
1240	113.0	87.50	48.00	
1405	141.0	99.50	60.58	35.50

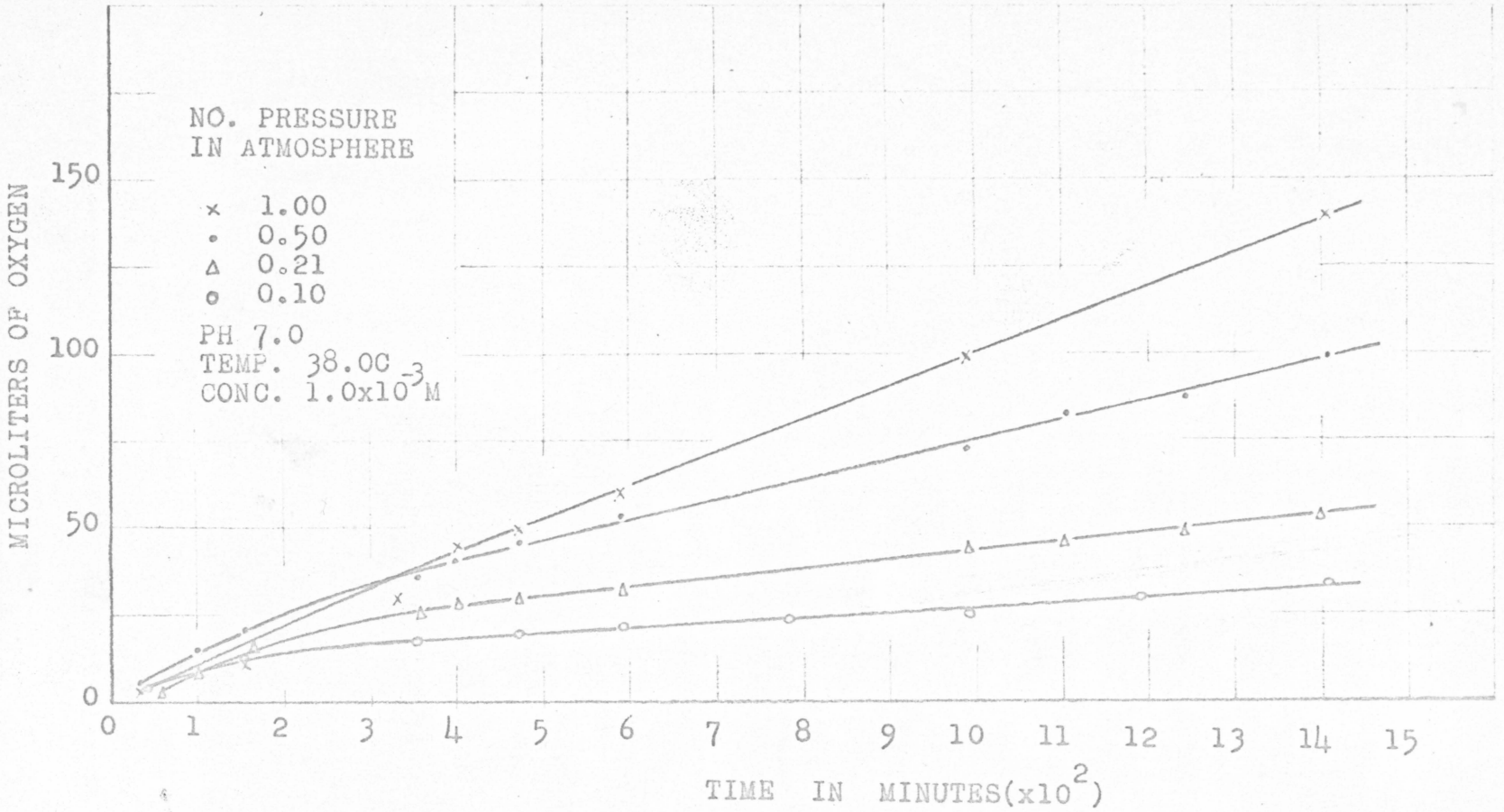


FIG. 14 EFFECT OF OXYGEN CONCENTRATION ON REACTION RATE OF 1-(3,4-DIHYDROXYPHENYL)-1-PROPANOL

TABLE XV
Summary of Effect of Oxygen Concentration
on the Reaction Rate of
1-(3,4-Dihydroxyphenyl)-1-Propanol

Conc. 1.0×10^{-3}M pH 7.0 Temp. 38.0°C

Oxygen Pressure in atmosphere	$-\frac{\Delta O_2}{\Delta t}$
1.0	1.505
0.5	0.924
0.21	0.440
0.10	0.240

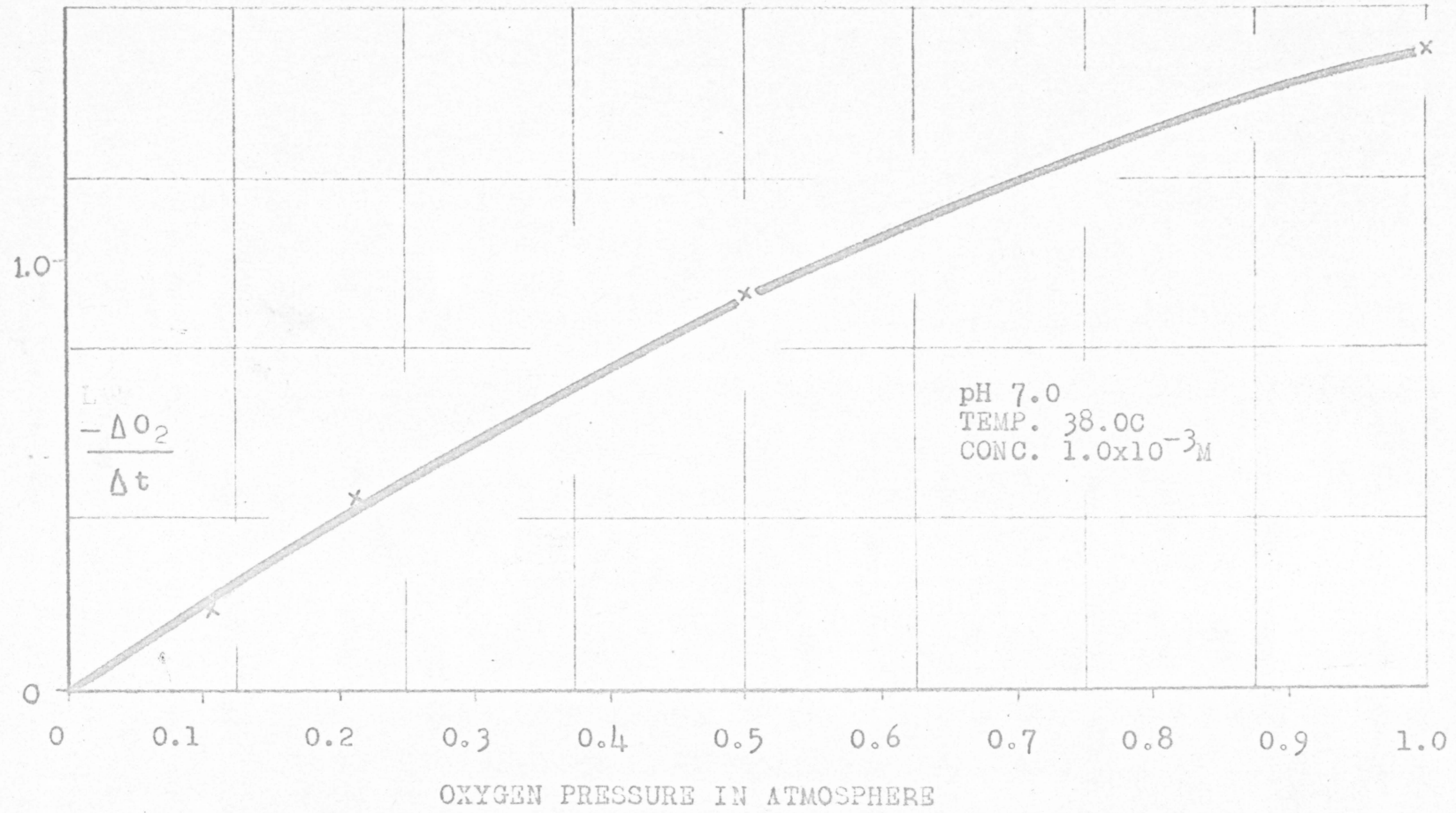


FIG. 15 SUMMARY OF OXYGEN CONCENTRATION EFFECT ON REACTION RATE OF 1-(3,4-DIHYDROXYPHENYL)-1-PROPANOL

The chemical kinetics of autoxidation of catechol has been investigated by Joselyn and Branch.[62] As may be expected, the dependencies of this reaction on oxygen, substrate, and hydrogen ion concentrations are much closer to those of 1-(3,4-dihydroxyphenyl)-1-propanol than to those of epinephrine. The facts are summarized in Table XVI.

Since there was some likelihood that the secondary alcohol group adjacent to the aromatic ring may be involved in the oxidative scheme, the rate of autoxidation of two keto compounds were studied. The conversion of the secondary alcohol to the keto form apparently has a strongly stabilizing effect on the catechol nucleous as both 4-acetocatechol and 4-propionocatechol take up oxygen at extremely slow rate even at pH 7.7. For some unexplained reason, the extension of the side chain by a methyl group, i.e. from aceto to propiono results in roughly two fold decrease in the rate, the total oxygen uptake by the propiono derivative being less than 40 µls over eight hour period at pH 7.7 at 38°C as shown in Fig. 19. This study indicates, therefore, that the chances that the keto form may be an active intermediate are rather small.

Ultimate Oxygen Uptake During the Autoxidation of Epinephrine

The limiting amount of oxygen taken up per mole of epinephrine was determined as described on page 27 at pH 8.01, 6.79 and 6.49. The oxygen uptake versus time curves for these runs are shown in Figure 16. Calculations are given below.

At pH 8.01 the total amount of oxygen taken up was 800 uls. Then the amount of oxygen taken up by a mole of epinephrine =

TABLE XVI

Summary of Comparative Reaction Characteristic of
Epinephrine, Catechol and 1-(3,4-Dihydroxyphenyl)-1-Propanol

	Epinephrine	Catechol	1-(3,4-Dihydroxyphenyl) -1-Propanol
1. Induction Period	Yes	No	No
2. Limiting Oxygen Uptake	3 Moles/ Mole	1/2-1 (Enzymatic)	1.5
3. Order respects to Compound	Approx. 2/3	1	1
4. (OH-)	1	1	1
5. Oxygen Tension	$\frac{p}{p + a}$	p	p
6. Comparative Rate	Fast (Roughly ten times as fast as Catechol)	Slow	Slow - Same order as Catechol
7. Influence from Shape of Oxygen Uptake vs. Time Curve	Autocatalytic type	Appears to be uncatalyzed by reaction product	Appears to be uncatalyzed by reaction product

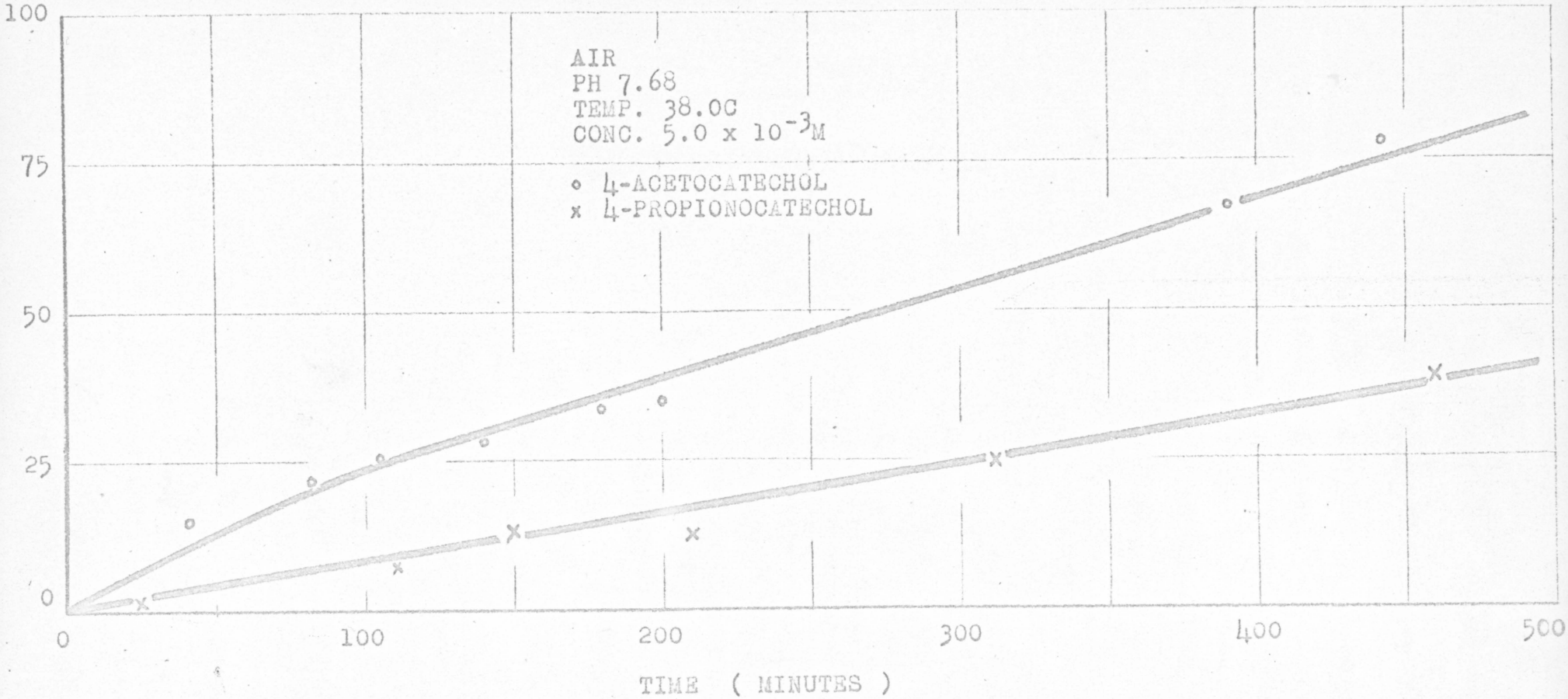


FIG. 19 THE REACTION RATE OF 4-ACETOCATECHOL AND 4-PROPIONOCATECHOL

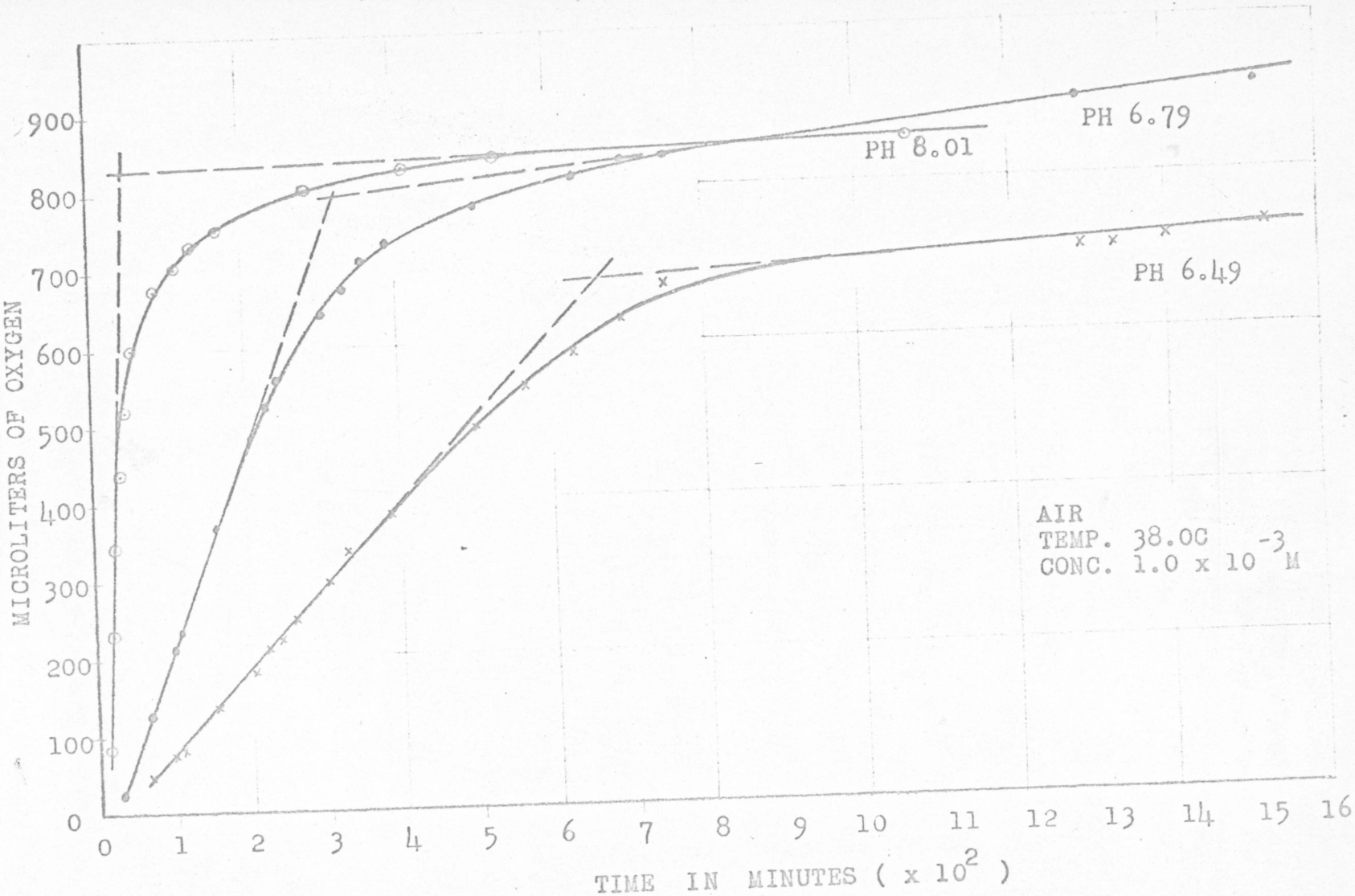


FIG. 16 AMOUNT OF OXYGEN ABSORBED PER MOLE OF EPINEPHRINE

$$\frac{\text{total amount of } O_2}{22.4 \times \text{umole of epinephrine}} = \frac{800 \times 10^{-6}}{22.4 \times 1.0 \times 10^{-5}} = 3.5 \text{ mole.}$$

At pH 6.79 the total amount of oxygen taken up was 775 uls. Then the amount of oxygen taken up by a mole of epinephrine =

$$\frac{775 \times 10^{-6}}{22.4 \times 1.0 \times 10^{-5}} = 3.46 \text{ mole.}$$

At pH 6.49 the total amount of oxygen taken up was 710 uls. Then the amount of oxygen taken up by a mole of epinephrine =

$$\frac{710 \times 10^{-6}}{22.4 \times 1.0 \times 10^{-5}} = 3.17 \text{ mole.}$$

Apparently at the two higher pH values slightly more total oxygen is consumed than at pH 6.49. It is possible that at much lower pH values the ratio may be a good deal less.

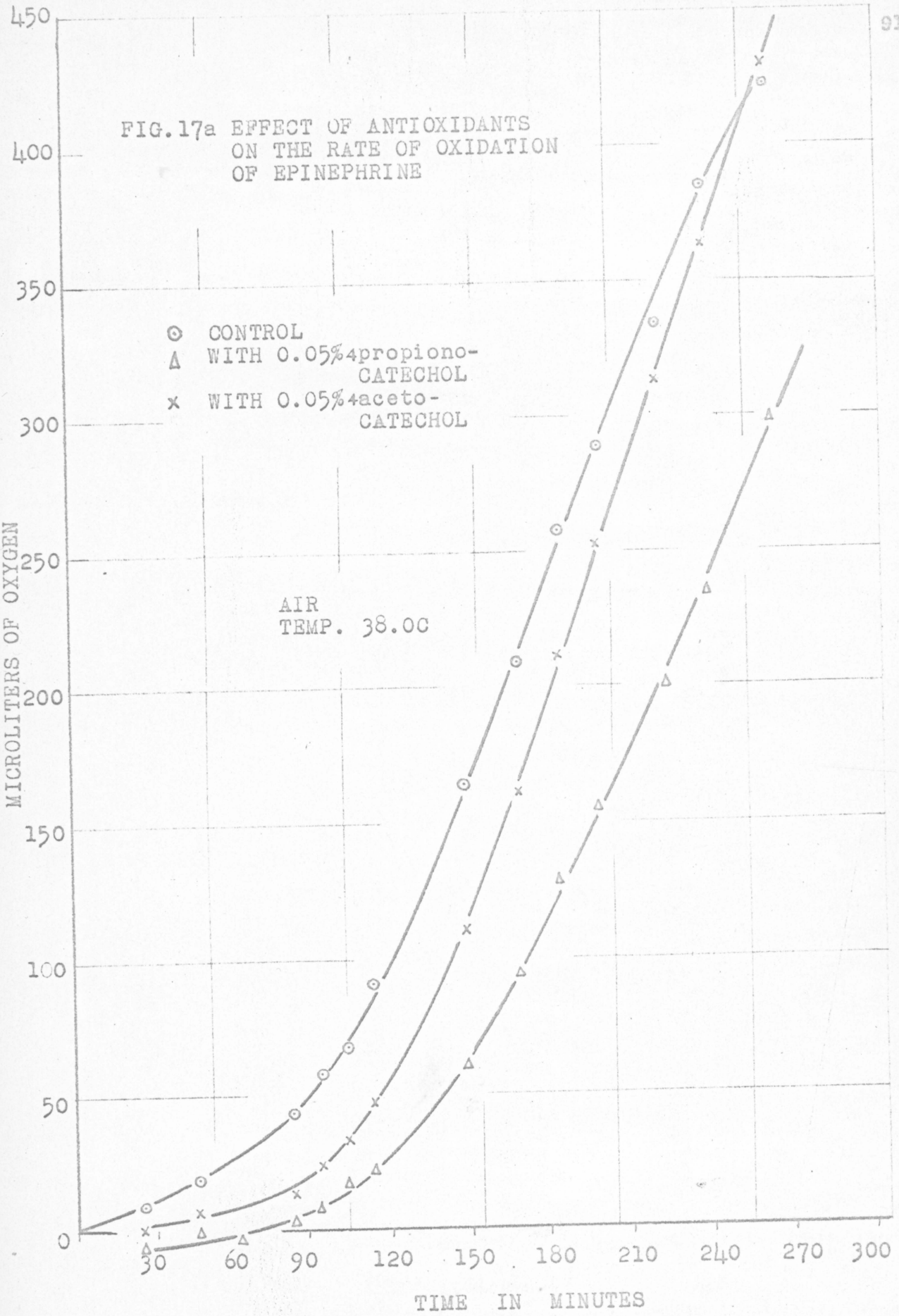
A Study of Some Catechol Derivatives as Possible Antioxidant for Epinephrine

The influence of catechol, 4-acetocatechol, 4-propionocatechol, and 1-(3,4-dihydroxyphenyl)-1-propanol on the rate of autoxidation of epinephrine was studied according to the procedures described previously (page 28). The results obtained are shown in Fig. 17. Catechol, 1-(3,4-dihydroxyphenyl)-1-propanol, and the 4-acetocatechol appear to be ineffective as possible inhibitors. The 4-propionocatechol, on the other hand, appears to possess some inhibitory action but no retarding action beyond the inhibited period.

Results of Chromatographic Attempts to Isolate Reaction Intermediates

Attempts to isolate reaction products and intermediates by paper chromatography as described on page 29, yielded only indefinite results. The spectrophotometric absorption curves of various fractions

FIG. 17a EFFECT OF ANTIOXIDANTS ON THE RATE OF OXIDATION OF EPINEPHRINE



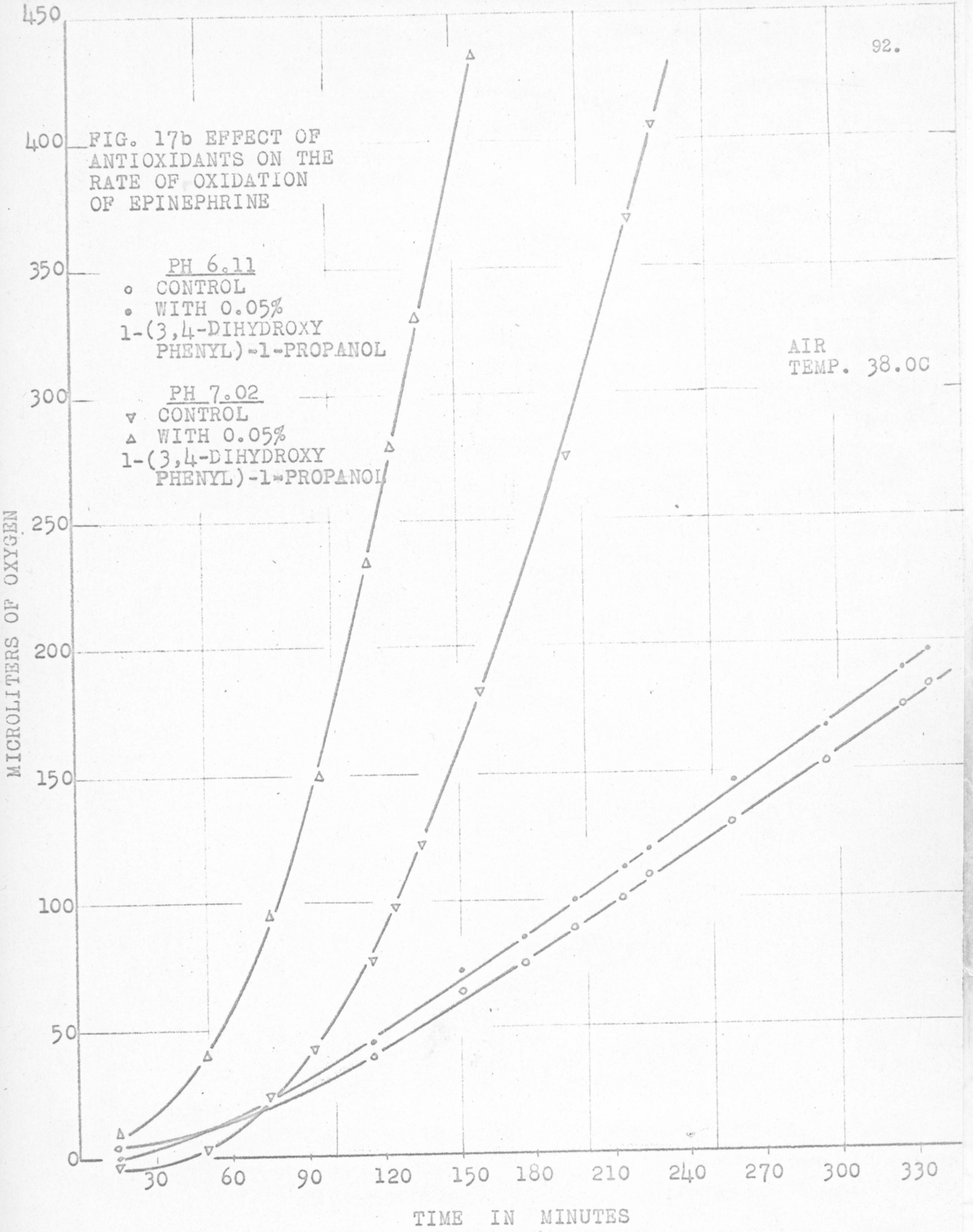
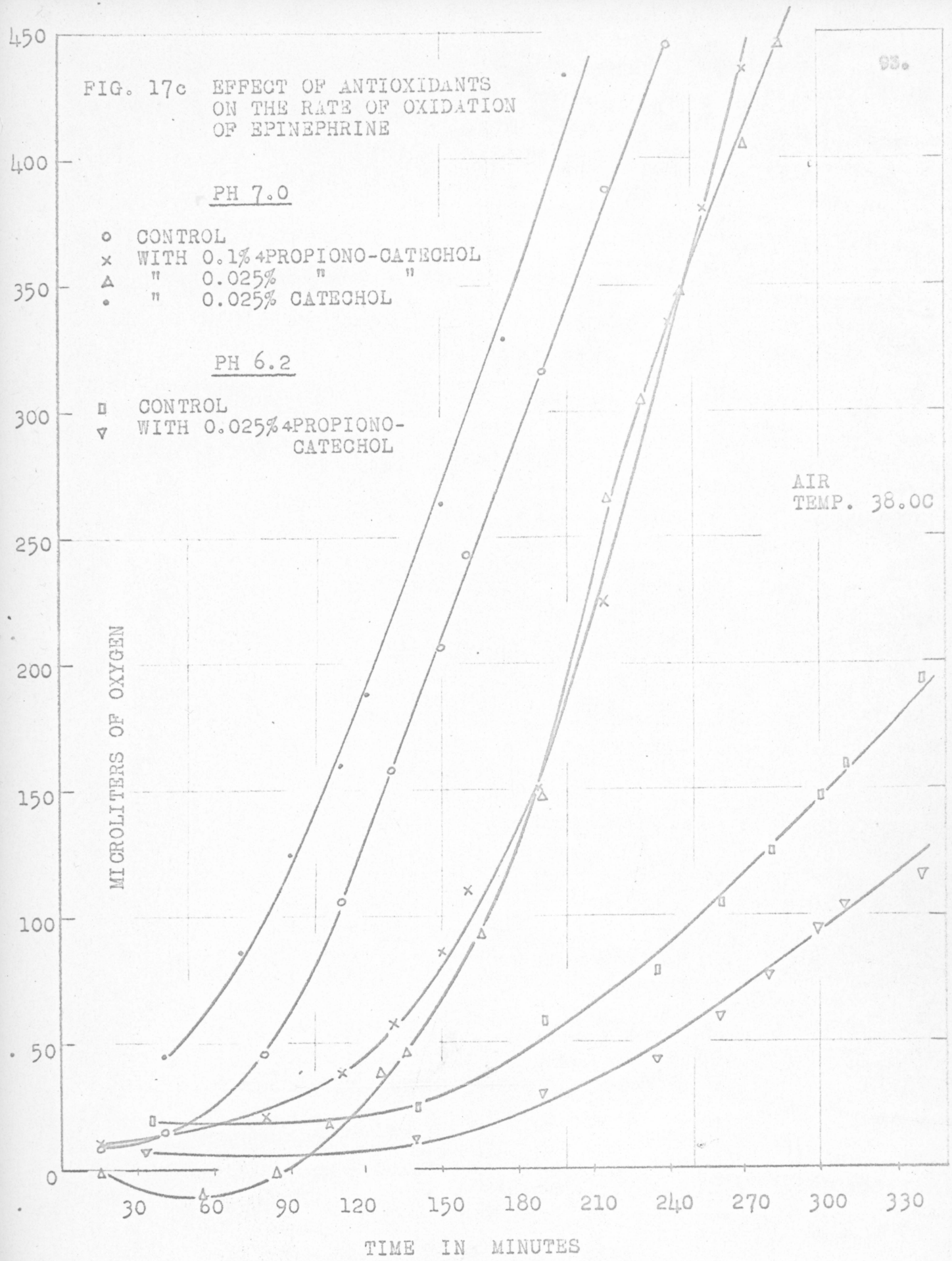


FIG. 17b EFFECT OF ANTIOXIDANTS ON THE RATE OF OXIDATION OF EPINEPHRINE
PH 6.11
CONTROL
WITH 0.05% 1-(3,4-DIHYDROXY PHENYL)-1-PROPANOL
PH 7.02
CONTROL
WITH 0.05% 1-(3,4-DIHYDROXY PHENYL)-1-PROPANOL
AIR TEMP. 38.0C
MICROLITERS OF OXYGEN
TIME IN MINUTES
0
50
100
150
200
250
300
350
400
450
30
60
90
120
150
180
210
240
270
300
330

FIG. 17c EFFECT OF ANTIOXIDANTS ON THE RATE OF OXIDATION OF EPINEPHRINE



eluted from different sections of the paper chromatograms are shown in Fig. 18. Although a section corresponding to a R_f value of 0.5 which fluoresced with a greenish yellow color under an ultra violet source appear to be epinephrine, no other definite compound could be located among the several fractions. No particular evidence of presence of "adrenochrome" was found.

FIG. 18a ULTRAVIOLET ABSORPTION CURVE OF COMPONENTS SEPARATED ON PAPER CHROMATOGRAM OF REACTION MIXTURE

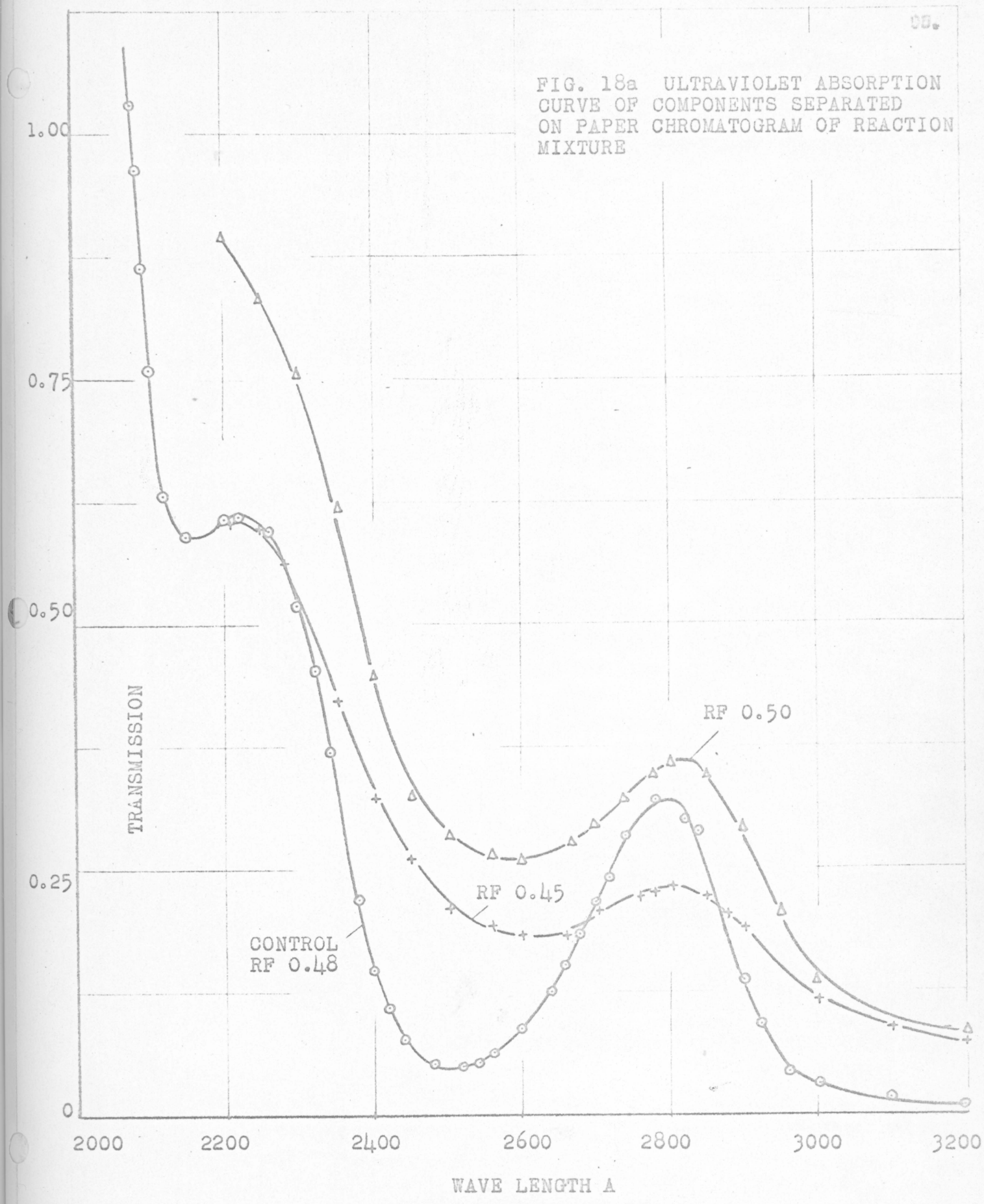


FIG. 18b ULTRAVIOLET ABSORPTION CURVE OF COMPONENTS SEPARATED ON PAPER CHROMATOGRAM OF REACTION MIXTURE

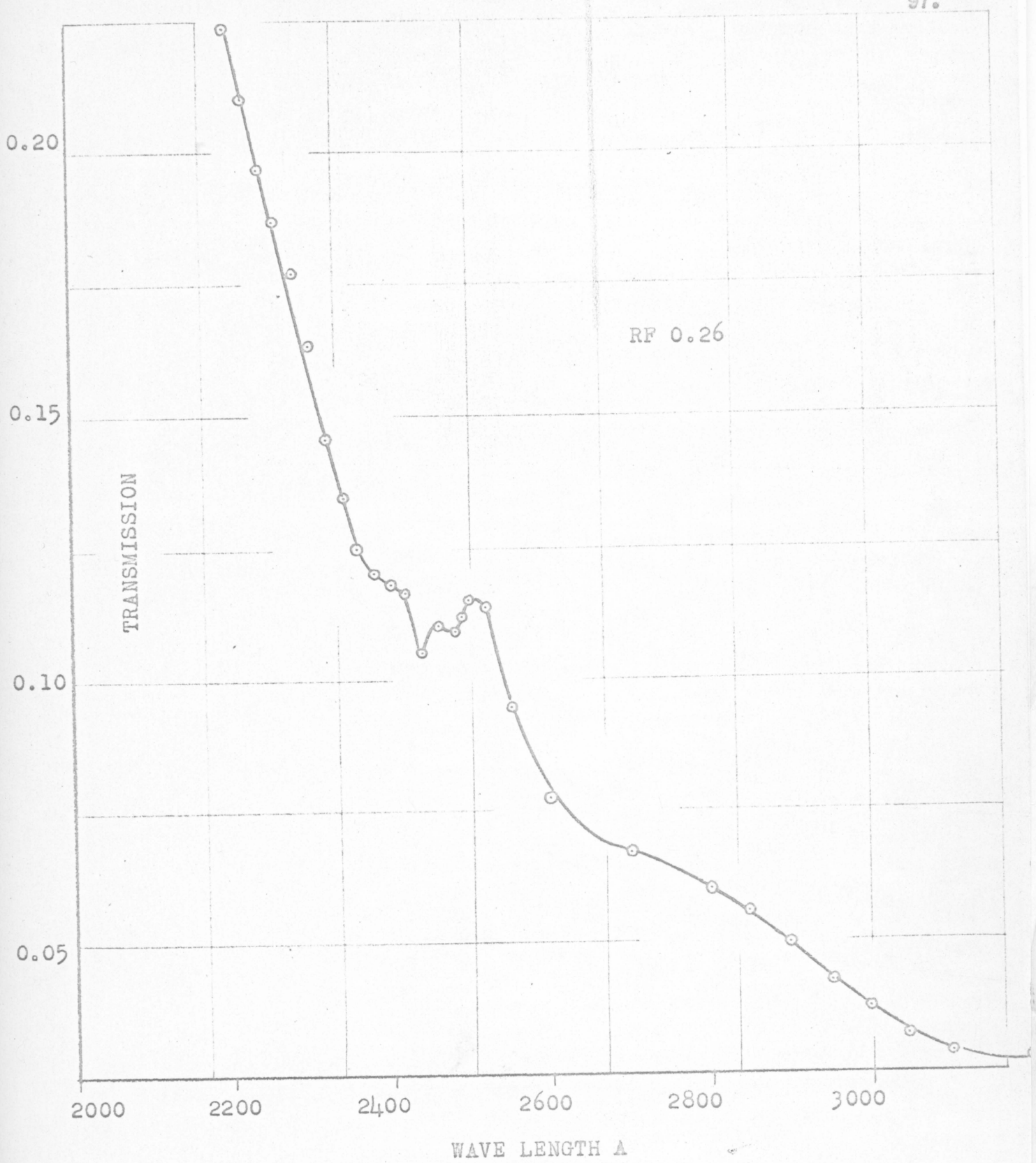


FIG. 18c ULTRAVIOLET ABSORPTION CURVE OF COMPONENTS SEPARATED ON PAPER CHROMATOGRAM OF REACTION MIXTURE

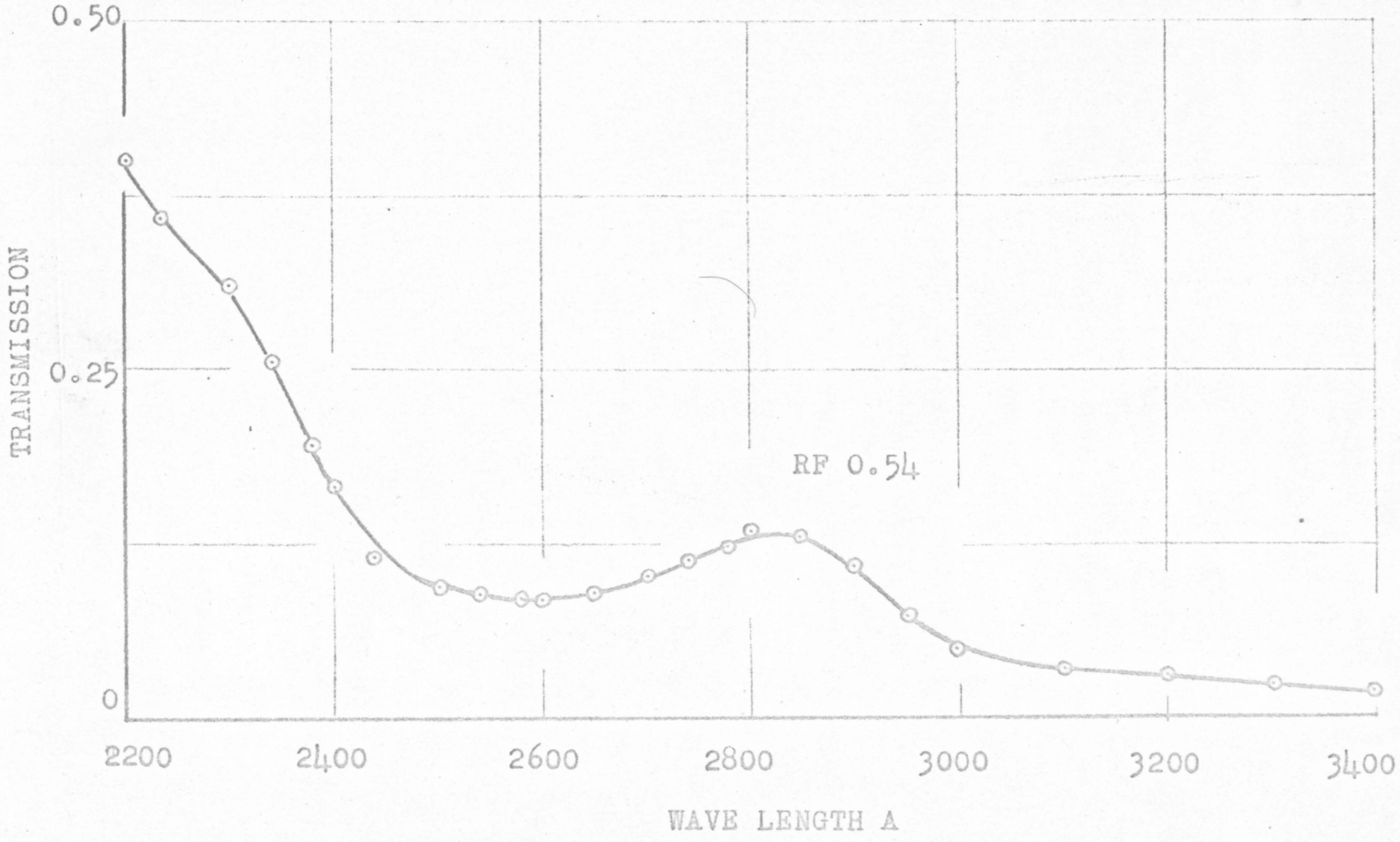
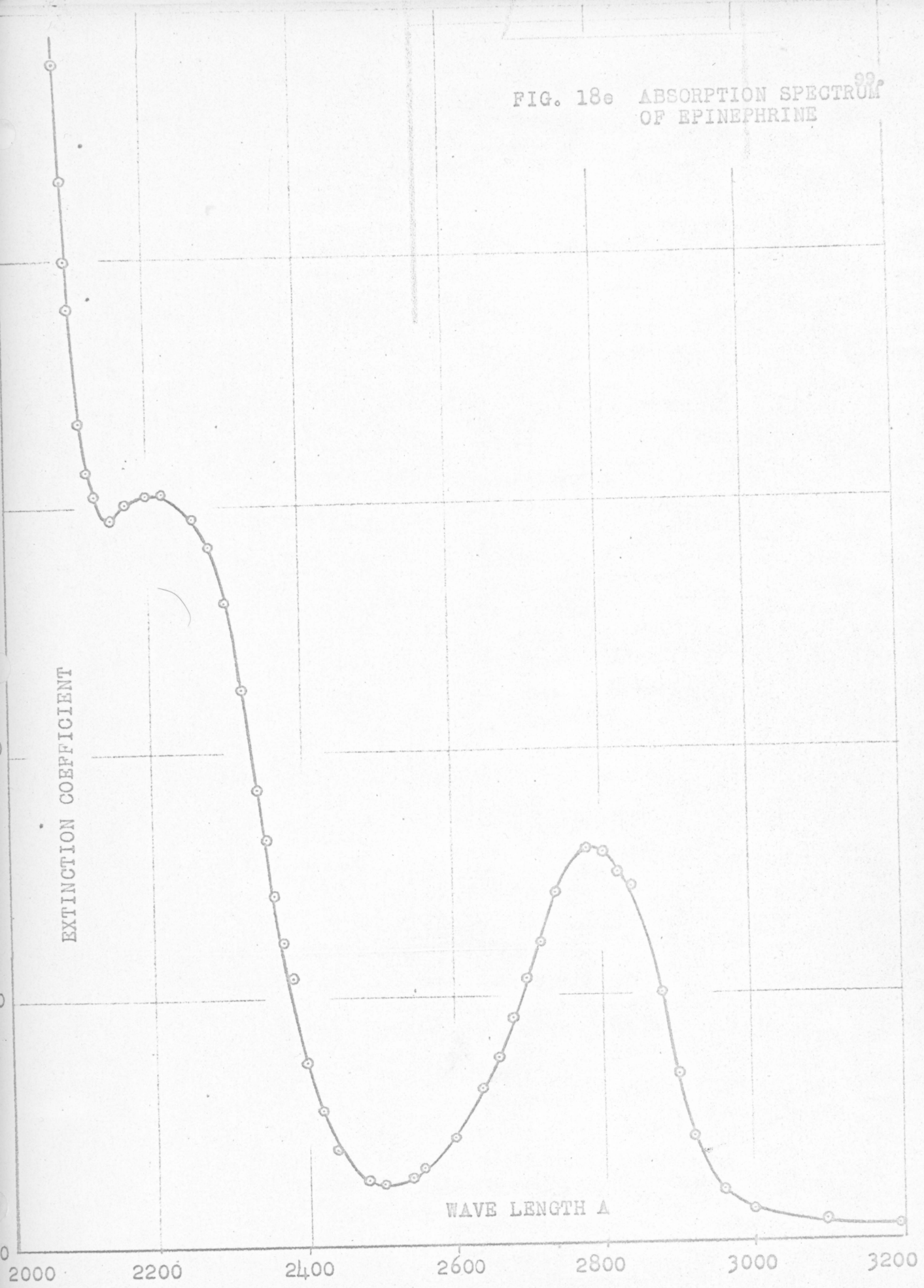


FIG. 18d ULTRAVIOLET ABSORPTION CURVE OF COMPONENTS SEPARATED ON PAPER CHROMATOGRAM OF REACTION MIXTURE

FIG. 18e ABSORPTION SPECTRUM OF EPINEPHRINE



CONCLUSION

In the preceding sections experimental data have been evaluated to yield separate relationship between the reaction rate and the several variables:

1. Hydrogen ion concentration: -

The rate is inversely proportional to the hydrogen ion concentration or, equally, directly proportional to the hydroxyl ion concentration.

2. Drug concentration: -

The reaction was found to have a less than a first order, approximately two thirds, dependency indicating that the overall reaction is rather complex, involving two or more reactions occurring both consecutively and concurrently.

3. Oxygen tension: -

The dependency of the reaction on oxygen tension, at pH = 7.0, fitted the empirical formula

$$\text{Rate} = \frac{k\,p}{a+p}$$

There appears to be, therefore, two limiting reactions, one at relatively low oxygen pressure which is dependent on oxygen pressure, and another at high oxygen pressure which is oxygen independent.

4. The heat of activation was approximately 6 kilocalories at pH 6.4 or above and 23 kilocalories at approximately pH 4.9.

By combining these factors, it is possible to formulate an overall kinetics expression which is valid over widely differing experimental conditions.

With the evidence available at the moment, it is not possible to propose an unique mechanism which would satisfy the experimental data. A comparison of chemical kinetics data of compounds related to epinephrine with epinephrine indicates however that the amino group in epinephrine plays an important role in the autoxidation reaction.

SUMMARY

An attempt was made in the present work to study the autoxidation reaction of epinephrine systematically by controlling some of the reaction variables such as pH, temperature, drug concentration and oxygen concentration.

The following relationship between the reaction rate and the several variables have been found experimentally:

1. Hydrogen ion concentration: -

The rate is inversely proportional to the hydrogen ion concentration or, equally, directly proportional to the hydroxyl ion concentration.

2. On the drug concentration: -

The reaction was found to have a less than a first order, approximately two thirds, indicating that the overall reaction is rather complex, involving two or more reactions occurring both consecutively and concurrently.

3. The dependency of the reaction on oxygen tension fitted the empirical formula: -

$$\text{Rate} = \frac{k\,p}{a+p}$$

There appears to be, therefore, two limiting reactions, one at relatively low oxygen pressure which is dependent on oxygen pressure, and another at high oxygen pressure which is oxygen independent.

4. The heat of activation was approximately 6 kilocalories

at pH 6.4 or above and 23 kilocalories at approximately pH 4.9.

By combining these factors, it is possible to formulate an overall kinetics expression which is valid over widely differing experimental conditions.

Chemical kinetics data on autoxidation of a number of other compounds closely related to epinephrine were also obtained. The reaction characteristics of catechol and 1-(3,4-dihydroxyphenyl)-1-propanol were found to be similar to each other but differed considerably from those of epinephrine. They are compared in Table XVI. (page 87)

In addition a number of catechol derivatives were tested as possible stabilizer for epinephrine. These included 4-acetocatechol, 4-propionocatechol, catechol, 1-(3,4-dihydroxyphenyl)-1-propanol. Only 4-propionocatechol showed any promise.

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