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TITLE "Kinetic Studies of the Alkaline Hydrolysis of Some Choline and Carnitine Esters."

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ABSTRACT

KINETIC STUDIES OF THE ALKALINE HYDROLYSIS
OF SOME CHOLINE AND CARNITINE ESTERS

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Master of Science

Youngstown State University, 1978

The rates of alkaline hydrolysis of the butyryl and benzoyl esters of choline, and the butyryl ester of carnitine were measured. Reactions were conducted at 25.0°C in aqueous solutions buffered with sodium glycinate-glycine or sodium carbonate-sodium bicarbonate at ionic strengths varying from 0.2 - 1.2.

Changes were observed in the calculated psuedo-first order rate constants due to production of acid during the run. Second order rate constants were calculated by dividing the first order constant by the hydroxide ion activity. General base catalysis was not observed because the rates of hydrolysis were found to be independent of buffer concentration for all three esters studied. The rates for the choline esters were found to be independent of ionic strength changes within experimental error, but butyrylcarnitine showed an increase in rate with increasing ionic strength.

Average values of the second order constants (benzoylcholine 0.58 sec^{-1} M^{-1}, butyrylcholine 0.73 sec^{-1} M^{-1}, and butyrylcarnitine 0.070 sec^{-1} M^{-1}) are greater than values

reported for analogous neutral esters but less than second order constants reported for the activated p-nitrophenyl esters. Second order constants found using the Sargent-Welch Recording pH Stat confirmed the findings in buffer solutions, though results from this instrument showed greater experimental errors.

The aminolysis reaction of benzoylcholine with cyclohexylamine in dimethylsulfoxide was much slower than hydrolysis of the same ester.

ACKNOWLEDGEMENT

I would like to thank my thesis adviser, Dr. Marvin Lukin, for the advice and encouragement offered during the progress of these experiments. I am appreciative of the numerous techniques and skills acquired while working with him. I also want to thank Dr. James Reeder for suggestions offered during the course of the experimental work and during the writing of this thesis.

TABLE OF CONTENTS

LIST OF SYMBOLS

SYMBOL	DEFINITION	UNITS
A_t	Absorbance at time "t" in a run	none
A_o	Absorbance at start of a run	none
a_{OH_f}	Hydroxide ion activity at end of run	moles/liter
a_{OH_i}	Hydroxide ion activity before addition of sodium chloride	moles/liter
a_{OH_t}	Hydroxide ion activity at time "t" in a run	moles/liter
Δa_{OH}	Change in activity of hydroxide ion	moles/liter
^oC	Temperature	degrees Celsius
$[ester]_t$	Molar concentration of ester at time "t"	moles/liter
$[ester]_o$	Molar concentration of ester at start of a run	moles/liter
g	Mass	grams
K_a	Acid dissociation constant	none
K_B	Rate constant for hydrolysis due to base "B"	$seconds^{-1}$ $moles^{-1}$ liter
kcal	Heat	kilocalories
k_{obsd}	Psuedo-first order rate constant	$seconds^{-1}$
k_2	Second order rate constant	$seconds^{-1}$ $moles^{-1}$ liter
l	Volume	liter
ln	Natural logarithm	none
log	Base 10 logarithm	none
M	Molar concentration	moles/liter

SYMBOL	DEFINITION	UNITS
ml	10^{-3} liter	milliters
mmol	10^{-3} Mole	millimoles
mol	Mole	mole
N	Normality	equivalents/liter
nm	10^{-9} Meter	nanometers
pH	Negative logarithm of hydrogen ion activity	mole/liter
pH_f	pH at end of run	moles/liter
pH_i	pH before addition of sodium chloride	moles/liter
pH_o	pH at start of a run	moles/liter
pK_a	Negative logarithm of an acid dissociation constant	none
pOH	Negative logarithm of hydroxide ion activity	moles/liter
pOH_f	pOH at end of a run	moles/liter
pOH_i	pOH before addition of sodium chloride	moles/liter
pOH_o	pOH at start of a run	moles/liter
sec	Seconds	seconds
S_N1	Substitution nucleophilic unimolecular	none
S_N2	Substitution nucleophilic bimolecular	none
t	Any time in a run	seconds
t_f	Time at end of a run	seconds
t_o	Time at start of a run	seconds
Z_a	Charge on molecule "a"	none
Z_b	Change on molecule "b"	none

SYMBOL	DEFINITION	UNITS
$\Delta\mu$	Change in ionic strength	none
μ	Ionic strength	none
μ_i	Ionic strength before addition of sodium chloride	none
μ_o	Ionic strength at start of a run	none

LIST OF FIGURES

FIGURE PAGE

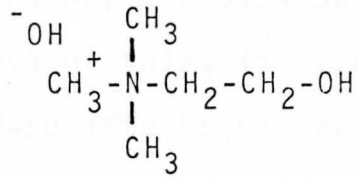
LIST OF TABLES

TABLE PAGE

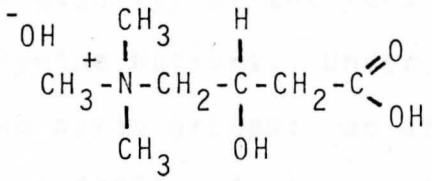
CHAPTER I

INTRODUCTION

Choline and carnitine are naturally occurring compounds which have similar functional groups and hence undergo similar reactions. Nevertheless, they have rather diverse functions in biological systems. Choline (2-hydroxy-N,N,N-



trimethylethanaminium hydroxide) when esterfied with acetic acid to form acetylcholine, functions as a chemical transmitter between nerve endings. It is also a component of several types of lipids. Carnitine (3-carboxy-2-hydroxy-



N,N,N-trimethyl-1-propanaminium hydroxide) functions in β-oxidation of fatty acids by forming esters with these acids and thus permitting their movement across the inner mitochondrial membrane. These two compounds are of interest also because both form esters which yield a relatively large free energy change upon hydrolysis. For example, hydrolysis of acetylcholine at pH 7.0 yields -6.0 kcal/mol. Similarly, acetylcarnitine releases -7.2 kcal/mol.[1] By comparison, ethyl

acetate produces only -1.7 kcal/mol.[1] These values imply that choline and carnitine esters hydrolyze spontaneously and that this hydrolysis proceeds nearly to completion. The large negative free energy changes do not, however, give any information on the rates of reaction of these esters. The purpose of this study is to determine the relative reaction rates of alkaline hydrolysis of some esters of choline and carnitine.

There were several factors which became of interest during our study. As one can see, both choline and carnitine are cations. Because of this, it is possible that ionic interactions may produce effects on the observed rates of hydrolysis. To determine if such effects exist, kinetic runs were conducted under identical conditions except that ionic strengths were varied. Another concern was to determine if the concentration or composition of buffer affected the rates of hydrolysis. The majority of the runs were conducted in sodium hydroxide-glycine buffers. Under alkaline conditions glycine posesses two basic groups: an amino group ($-NH_2$), and a carboxylate group ($-COO^-$). It is possible that these groups may catalyze the hydrolysis reaction. In order to test this possibility, pH and ionic strength were maintained at constant values while buffer concentration and buffer composition were varied.

The esters used in this study were the butryl and benzoyl esters of choline and the butryl ester of carnitine.

Butyrylcarnitine and benzoylcholine were synthesized by proceedures that will be described. The butyrylcholine was purchased.

CHAPTER II

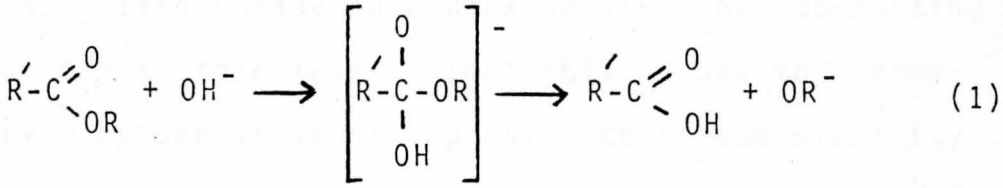
HISTORICAL

Mechanisms of Ester Hydrolysis

Kinetic studies have been an important technique in gaining information concerning the mechanism of a reaction. In addition to measuring the reaction velocity, the order, i.e. the number of molecules participating in a reaction can also be determined. By observing the effects of temperature, ionic strength, solvent polarity, and acidic or basic catalysts on reaction rates further clues about the probable mechanism might be obtained. One can then propose how these molecules react with each other. Nevertheless, kinetic methods in themselves cannot prove that a particular mechanism is applicable. Together. with the results from other types of mechanistic studies (e.g. isotopic labeling, stereochemical changes, and intermediate trapping), a particular mechanism may be confirmed or excluded. Even then, there may still be uncertainty about the details of a reaction.

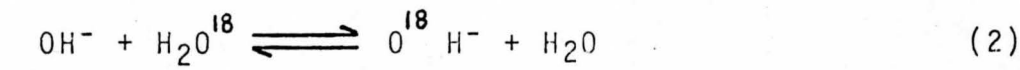
The type of reaction of interest in this study is the hydrolysis of esters. No less than seven different mechanisms have been proposed and observed.[2] Of these, four occur under alkaline or slightly alkaline conditions. Since the author's study has been done under basic conditions, mechanisms proposed for alkaline hydrolysis only will be examined.

The mechanism which has been observed for most esters is the bimolecular cleavage of the molecule between the carbonyl (acyl) carbon and the oxygen of the alcoholic (alkyl) portion of the ester.[3] This reaction proceeds via the following mechanism involving an intermediate anion.



(1)

The evidence for the acyl-oxygen cleavage and for the existence of an intermediate has been obtained from studies with isotopic oxygen (O^{18}). Polanyi and Szabo[4] showed that when water containing isotopic oxygen is used as a solvent, the label occurs only in the acid released in hydrolysis. None became incorporated into the alcohol portion of the ester. The rapid exchange:

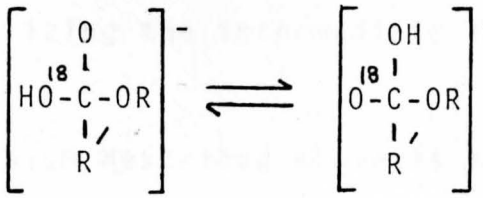


(2)

labels the hydroxide ion which, in subsequent attack on the ester, becomes sustituted into the acyl portion as shown in the above mechanism.

Since an intermediate is relatively stable with respect to an activated complex, it is possible for proton exchange to occur in an intermediate but not in a complex. Studies by M.L. Bender[5,6] on various esters showed that the rate of exchange of isotopic oxygen between the hydroxide ion and the ester was 10-40% of the rate of hydrolysis. This exchange occurs because the intermediate is sufficiently long-lived to allow the acyl oxygens to become equivalent with

respect to the time each spends in the pronated state.



(3)

The latter of the two above intermediate forms can revert to reactants by losing unlabeled hydroxide ion, thus completing exchange of the isotope label. That this occurs to the extent observed by Bender is strong evidence of the stability of the intermediate.

Because the intermediate is an anion, increased rates of reaction have been observed for esters with electron withdrawing, anion stabilizing substituents on either the acyl or alkyl portion of the esters. For example, the accelerating effects of electronegative chlorine atoms can be seen by the relative rates of hydrolysis of the esters[7] of form $RCOCH_3$:

R:	H	CH_3	CH_2Cl	$CHCl_2$
Relative rate:	223	1	761	16,000

It has also been shown that the hydrolysis of esters following this bimolecular mechanism is subject to steric hindrance. By varying substituents on the alkyl part of the molecule, hydrolysis of esters of the form CH_3COR have shown the following relative rates:[8]

R:	CH_3	C_2H_5	$n\text{-}C_3H_7$	$i\text{-}C_3H_2$
Relative rate:	1	0.601	0.549	0.146

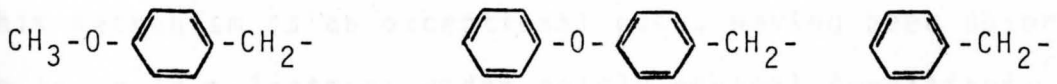
The above decrease in relative rates has been attributed to

the increasingly crowded condition of the intermediate state; which, by destabilizing the intermediate form, reduces the overall rate.

The mechanism described above is applicable to the large majority of esters that have been examined. The three other mechanisms to be described have been observed in only a small number of examples having particular characteristics. Two of them involve cleavage of the bond between oxygen and the alkyl carbon. Of the two, one is a unimolecular process proceeding by an S_N1 mechanism and the other is bimolecular, and possesses S_N2 mechanistic traits. The suggested mechanism for the S_N1 reaction proceeds:

$$R\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}R' \longrightarrow R^+ + {}^-O\text{-}\overset{\overset{O}{\|}}{C}\text{-}R' \longrightarrow R\text{-}OH + HO\text{-}\overset{\overset{O}{\|}}{C}\text{-}R' \qquad (4)$$

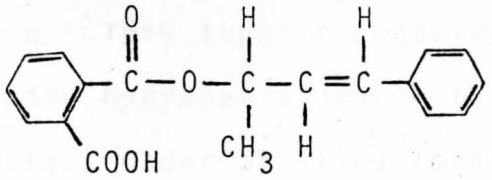
through a carbocation. Esters proceeding by this mechanism must have substituents on the alcohol which can stabilize the cation. Examples of these are the substituent groups:[9]



p-methoxy benzhydryl p-phenoxy benzhydryl benzhydryl

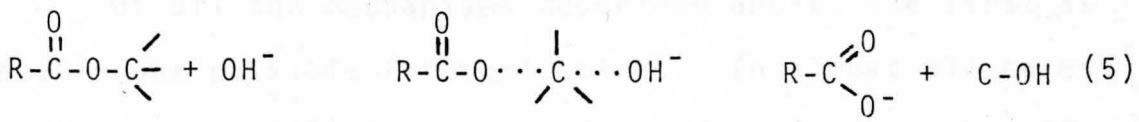
Based on stereochemical observations, esters of this type hydrolyze by two mechanisms. An optically active ester such

as 1-methyl,3-phenyl allyl hydrogen phthalate:



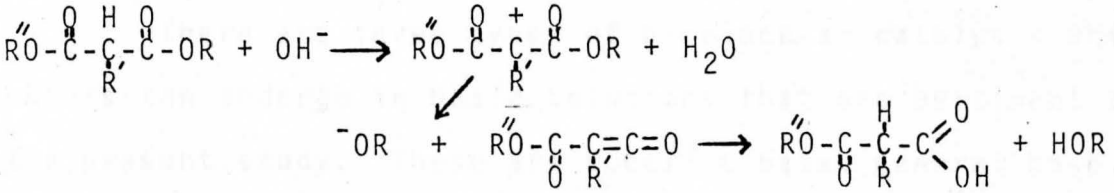
was found to hydrolyze with retention of configuration under stongly alkaline conditions; but hydrolyzed with racemization in more neutral solutions.[10] The reason for this behavior was that the S_N1 reaction was obscured by the faster bimolecular reaction until the hydroxide ion concentration was much decreased. Hydrolysis via the S_N1 mechanism has also been observed for esters of tertiary-alkyl alcohols.[11] These non-aromatic esters can also stabilize a positive charge, thus allowing carbocation intermediates to form.

The second mechanism resulting in alkyl-oxygen scission is the bimolecular substitution reaction at the alcoholic carbon atom.



This mechanism is an exceptional case, having been observed to occur in β-lactones under nearly neutral conditions. Alkyl-oxygen cleavage has been demonstrated by observing inversion of configuration in optically active β-lactones[12] and by the finding of isotopically labeled alcohols upon hydrolysis with $O^{18}H^-$.[13] Like the S_N1 mechanism above, this reaction is masked by the more rapid bimolecular acyl-oxygen fission mechanism when reaction conditions are made more basic.

The final mechanism that has been demonstrated is an elimination reaction. This type of reaction can occur only in esters in which the hydrogen atom α to the carbonyl carbon is able to dissociate. Under alkaline conditions, the base will abstract this proton, leaving the conjugate base of the ester. If the alcoholic portion of the ester is a good leaving group (i.e. if the conjugate base of the alcohol can form a fairly stable anion), then the anion formed upon abstraction of the proton can eliminate an alkoxide ion while itself rearranging to a ketene.



(6)

Evidence for this rather unusual mechanism has been provided by studies showing trapping of the very reactive ketene and by spectrophotometric observation of the carbanion.[14]

Of all the mechanisms described above, the first is the only one possible for most esters. In almost all cases, this mechanism will also supersede the others described if conditions are basic enough.

Catalysis of Ester Hydrolysis

Catalysis is currently defined to be a process in which a molecular species participates in a fundamental way in a reaction mechanism to promote the rate of a reaction. In the overall process the catalyst remains unchanged in structure and concentration.[15] The extent of the dependence

of a reaction on a catalyst can be determined kinetically. In some reactions only a trace of catalyst is required, while in other reactions the concentration of catalyst occurs to the first order or even higher in the rate expressions. Probably the most frequently encountered reactions involving catalysis are those occurring in solution which require the presence of either acid or base. Since this phenomena is very widespread and diverse, the following discussion will concern itself only with the catalysis of ester hydrolysis under alkaline conditions.

There are three types of bimolecular catalysis which esters can undergo in basic solutions that are pertinent to the present study. These are specific base, general base, and nucleophilic catalysis.

The division of base catalysis into specific and general is based on whether the catalytic species is the conjugate base of the solvent or whether it is some other basic substance present in solution. Specific base catalysis occurs when a basic substance (B) reacts with the solvent (SH) to produce the catalyst (S^-).

$$B + SH \longrightarrow BH^+ + S^- \qquad (7)$$

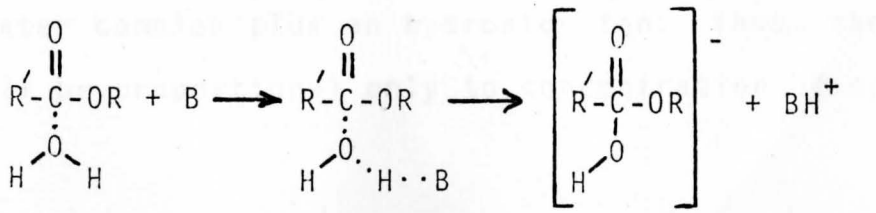
In water, the specific base is the hydroxide ion, and a hydrolysis reaction proceeding with specific base catalysis will go at a rate directly proportional to concentration of hydroxide ion. Chu and Mautner have demonstated specific catalysis for benzoylcholine, one of the esters involved in this study, by showing a linear relationship of pH versus

the logarithm of the psuedo-first order rate constant.[16] The mechanism by which specific catalysis occurs is the direct attack by the hydroxide on the carbonyl carbon to produce the tetrahedral intermediate (see Equation1). Even though (1) shows that the hydroxide ion is consumed in the reaction (which violates the definition of a catalyst) the process is still considered a catalytic one because the hydroxide ion catalyst is actually neutralized by a reaction product. The alkoxide ion (RO^-) from (1) reacts with water to produce hydroxide ion:

$$RO^- + H_2O \longrightarrow ROH + OH^- \qquad (8)$$

The hydroxide reacts immediately with the acid (RCOOH) produced in the hydrolysis reaction, thereby depleting the concentration of catalyst.

General base catalysis occurs when the catalytic species is some basic substance in solution other than the conjugate base of the solvent. General catalysis is demonstrated when the rate of a reaction is shown to be proportional to the total concentration of basic substances present rather than just the concentration of hydroxide ion. General base catalysis of ester hydrolysis operates by increasing the polarity of an oxygen-hydrogen bond of a water molecule which has become aligned at the carbonyl carbon of an ester as shown in Equation (9). This proceeds to form the intermediate anion when the proton is completely removed.[17]



The occurrence of general base catalysis of esters was first definitely demonstrated by Jencks and Carriulo in 1961.[18] They observed that the rates of hydrolysis of the acyl activated esters of ethanol (ethyl diflouroacetate, ethyl dichloroacetate, ethyl chloroacetate, and ethyl trichloroacetate) were proportional to the concentration of conjugate bases of the buffer solutions they employed. Though the occurrence of this phenomena in esters had been suggested as early as 1928,[19, 20] the mechanism had not been proven till 1961 when it was shown that the hydrolytic rates were slower in deuterium oxide (D_2O) than in water.[18] This finding implied that an O — D or an O — H bond was being stretched. Since the bonds in deuterium oxide are stronger than in water, the increased resistance to stretch and hence to polarization resulted in slower rates.

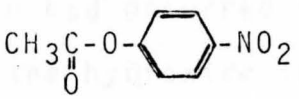
General base catalysis involves a proton transfer from an ester associated water molecule to the general base to form the intermediate (9). If this reaction is fast, and if the subsequent reaction of intermediate into products is slow, an equilibrium concentration of intermediate will develop and then resist any further change with increasing concentration of general base. This is because the strongest base that can exist in water is the hydroxide ion. Any formation of intermediate anion above the equilibrium concentration will react reversibly with water to form the neutral ester-water complex plus an hydroxide ion. Thus, the reaction rate will be proportional only to concentration of specific

base. If, however, the proton transfer from the ester-water complex is slow, and the subsequent decomposition of intermediate to products is fast, the equilibrium concentration will not develop and addition of any more general base will cause an increase in the total rate.[21]

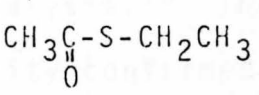
A third type of catalysis that esters may experience is through nucleophilic attack. This type of catalysis does not involve a proton transfer in its rate determining step.[22] Nucleophilic catalysis occurs through the attack of a nucleophile (N) upon the carbonyl center of the ester. Once formed, this intermediate rapidly reacts with a water molecule to produce the catalyst and the acid of the ester.[23]

$$R'\text{-}\overset{\overset{O}{\|}}{C}\text{-}OR + N \longrightarrow R'\text{-}\overset{\overset{O}{\|}}{C}\text{-}N + OR^- \xrightarrow{H_2O} R'\text{-}COOH + ROH + N \quad (10)$$

The requirement for this type of catalysis is that the alcoholic portion of the ester be a good leaving group. This would typically be an alcohol with electron withdrawing substituents. For example, two esters which have been shown to undergo nucleophilic catalysis readily are:[24]



p-nitrophenyl acetate



ethyl thioacetate

This requirement exists because an incoming nucleophile cannot readily displace an alkoxide ion which is a poorer leaving group than itself. Therefore nucleophilic catalysis is not seen in esters having unactivated alcoholic groups.

The rates of general base and nucleophilic catalysis are dependent upon the pK_a of the conjugate acid of the base that produces the catalytic effect. In 1924, Bronsted and Pederson proposed the following relation:[24]

$$\log k_B = \beta pK_a + G_B \qquad (11)$$

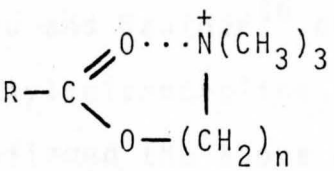
This equation correlates the magnitude of the catalytic rate constant (k_B) to the base strength of the catalyst (pK_a). G_B is a constant for a particular substrate. β is the slope of the line derived from (11) and is a measure of the sensitivity of the reaction to changes in pK_a.[25] For general base catalysis of the ester ethyl dichloroacetate, it has been found that various types of bases with pK_a's ranging from 4-8 (formate, aniline, pyridine, succinate, phosphate, imidazole, and water) all fit on the line. However for stronger bases such as ammonia, hydroxide ion, and tris-(hydroxymethyl) aminomethane, there is a deviation from linearity. The non-linearity of the points for these stronger bases coincided with a change in mechanism which was confirmed by a product analysis showing that nucleophilic substitution had occurred instead of catalysis.[18] The departure of the hydroxide ion from linearity confirmed the difference in mechanism between specific and general base catalysis of esters.

The comparison of diversive nucleophiles does not produce a similar linear relationship. However, comparison of a group of structurally similar nucleophiles does obey the Bronsted catalysis law.[26]

Catalysis of ester hydrolysis can also occur as a unimolecular process in which a functional group on another part of an ester molecule promotes attack by another molecule or ion. The possibilities for the occurrence of intramolecular catalysis for esters used in this study are discussed in the next section.

Intramolecular Catalysis

From a study of the rates of alkaline hydrolysis of various esters, Davis and Ross postulated a mechanism for the intramolecular catalysis of esters containing a quaternary amino group.[27] The formation of a cyclic intermediate which facilitated nucleophilic attack by polarization of the carbonyl bond was suggested.



They found that under basic conditions (0.005 N sodium hydroxide, pH~11.7) the hydrolysis at 50°C in 80% aqueous-acetone of acetylcholine proceeded about 150 times faster than ethyl acetate and about 32 times faster than β-dimethylaminoethyl acetate. The dissociation constant for the dimethylammonium group at 50°C in 80% aqueous-acetone (K_a = 5.2 x 10^{-9}) predicts the ratio of $R\text{-}N(CH_3)_2H^+/R\text{-}N(CH_3)_2$ at pH 11.7 would be about 1:2600.[28] This implies that the unprotonated amino group, acting as a nucleophilic catalyst

is not nearly effective as catalysis through the quaternized nitrogen.

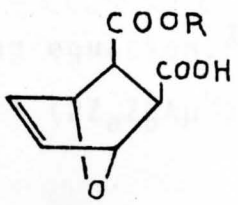
The evidence for this mechanism was supplemented by Zaslowsky and Fischer,[28] who showed using the β-diethylaminoethyl acetate and acetylcholine esters, that under more neutral conditions where the protonated species of the diethylaminoethylamine is predominant, that the rate of hydrolysis of the diethylammonium ester was about 20 times faster than the acetylcholine ester. This apparent reversal in the relative reactivity of the two esters is postulated to occur because the methyl group produces greater shielding of the positive charge compared to hydrogen. Additionally, the protonated diethylammonium species allows closer approach of the positive charge center to the carbonyl oxygen.

A study by Chu and Mautner[16] on benzoylcholine, benzoylthiocholine, benzoylselenocholine, and their dimethylamino analogs has confirmed the above observations. By varying the pH of their kinetic runs, they showed a linear relationship for log k_{obsd} (the psuedo-first order rate constant) versus pH for the trimethylammonium esters but a sigmoid curve was produced when a similar plot was made for the dimethylamino esters. Below a pH of about 9.8, dimethylaminoethyl benzoate underwent hydrolysis faster than benzoylcholine. However, above this pH the opposite was true. As in the other works cited above, the faster rate was observed when the dimethylamino group was protonated. Like choline, carnitine also contains a trimethylammonium group and

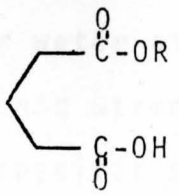
therefore might show a faster rate of hydrolysis than simple esters.

Because the carnitine molecule also posseses a carboxylic acid moiety, it was thought that it might be possible for rate acceleration to occur via intramolecular nucleophilic catalysis. Thanassi and Bruice[29] found that facilitation of hydrolysis by the carboxylate anion occurs in monohydrogen phthalate esters when the pK_a of the conjugate acid of the leaving alcohol anion is less than 13.5. Thus, it was found that esters of phenol and 2,2,2-trifluoroethanol with respective pK_a's of 9.98 and 12.36 were suscentable to carboxylate catalysis while methanol and 2-chloroethanol with pK_a's of 15.5 and 14.3 were catalyzed by the undissociated acid. Though the pK_a of the hydroxyl group of carnitine has not been reported, under the alkaline conditions of the present study, only the possibility of carboxylate anion catalysis exists.

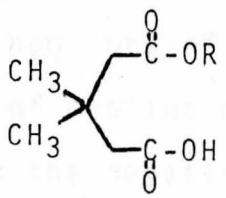
In studying esters containing carboxylate anions, the most effectively catalyzed examples have been those in which the anion is constrained so that it must closely approach the carbonyl center of the ester. This has been very well demonstrated by the 53,000 fold increase in rate of hydrolysis of 3,6-endoxo-Δ^4-tetrahydrophthalate monoester over



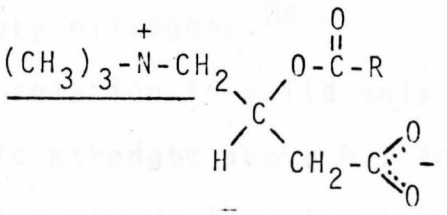
the monoester of glutaric acid.[30,31] In the latter there are



two axes of rotational freedom whereas in the former there are none. In the same study it was also shown that geminal dimethyl groups on the glutaric acid monoester produced a



20 fold rate increase because they constrained rotation. In carnitine the location of the N,N,N-trimethylmethanamminium group



may restrict the rotational freedom and might thus increase the probability of the carboxylate anion being in a catalytically favorable position. At the present, no non-enzymatic rate studies of carnitine esters have been reported.

Ionic Strength Effects

According to the Debye-Hückel theory the effects of ionic strength of rate of reaction between two ions can be described by the following equation.[32]

$$\log k = \log k^{o} + (2Z_a Z_b A\mu^{\frac{1}{2}})/(1 + \mu^{\frac{1}{2}}) + B\mu \qquad (12)$$

Z_a and Z_b are the charges on the reacting ions; A is the Debye-Hückel constant for water at 25°C (0.509 l-mol^{-1}); k^o is the rate at zero ionic strength and B is an empirical constant dependent upon specific salt effects such as chelate and complex formation. k^o and B are determined from experimental data.[33] The above equation predicts an increase in rate for a reaction between ions of like charge, but a decrease in rate when ions of opposite charge react. The alkaline hydrolysis of choline esters is a reaction between a positive ion and the negative hydroxyl ion. The expected decrease in rate with increasing ionic strength has been confirmed by the studies of Asknes and Prue on esters with quaternary nitrogens.[34]

The above relation is valid only for systems near ideality. At ionic strength above 0.1 the Debye-Hückel theory no longer is a good approximation of reality. Theory has been proposed for predicting ionic strength effects at higher salt concentrations[35] for reactions between neutral molecules and ions. However, the use of this theory depends on the evaluation of several experimentally determined coefficients and hence cannot be used to predict a priori, what kind of deviations from ideality would be observed.

At ionic strengths up to $\mu = 5$ it has been demonstrated that both the magnitude and the sign of the change in activity coefficient of a neutral molecule in a reaction are dependent on the particular salt used for ionic strength adjustment.[36] For sodium chloride this study showed that

increasing concentrations of the salt produced a positive effect on the activity coefficient of the substrate molecule. This implies that an increase in rate would be observed in a reaction between an ion and a neutral molecule in an aqueous solution of sodium chloride. This analysis cannot be used for choline esters because they have a net positive charge. However, for carnitine esters, which have no net charge in basis solution, an increase in rate with increasing ionic strength might be anticipated.

CHAPTER III

EXPERIMENTAL

Reagents and Materials

All pH measurements were made with a Sargent-Welch High Temperature-High Alkaline electrode using either an Orion 801 or a Sargent-Welch NX digital pH meter. Some kinetic runs were conducted on a Sargent-Welch Recording pH Stat.

Absorbance measurements were obtained using a Bausch and Lomb Spectronic-20 spectrophotometer.

All chloride analyses were performed by the author on a Buchler-Cotlove chloridometer.

The melting points reported in this investigation are uncorrected. Melting points were determined using a Thomas-Hoover capillary melting point apparatus.

Reagents and their respective purity grades are listed below:

1. Carnitine chloride - Aldrich, 99%
2. Choline chloride - Aldrich, 99%
3. Benzoyl chloride - J. T. Baker, reagent grade
4. Sodium chloride - Fischer Scientific U.S.P.
5. Sodium hydroxide - Fischer Scientific, reagent grade
6. Chloroform - J. T. Baker, reagent grade
7. Glycine - Eastman, reagent grade
8. Butyryl choline chloride - Aldrich, 'Aldrich Analyzed'
9. Ferric chloride hexahydrate - Fischer Scientific, reagent grade
10. Nitric acid (conc) - Mallinckrodt, reagent grade

11. Hydroxylamine hydrochloride - Eastman, reagent grade
12. Standard buffer, pH 9.00 ± 0.02 - Banco
13. Standard buffer, pH 10.00 ± 0.01 - Banco
14. Standard buffer, pH 11.33 ± 0.02 - Banco
15. Anhydrous ether - J. T. Baker, reagent grade
16. Cyclohexyl amine - Eastman, reagent grade
17. t-Butanol - Fischer Scientific, reagent grade
18. Butyryl chloride - Prepared by author[37]
19. Dimethylsulfoxide - J. T. Baker, reagent grade

Preparation of Benzoylcholine Chloride

In a 3-necked, 250-ml, round-bottom flask fitted with a mechanical stirrer, a reflux condenser, and a 50-ml addition funnel, was placed a 100 ml volume of chloroform and a 27.90 g (0.200 mol) sample of choline chloride. The flask was cooled in an ice bath and the mechanical stirrer was started. Over a thirty minute period, 56.20 g (0.400 mol) of benzoyl chloride was slowly added through the addition funnel. The flask was then fitted with a heating mantle and the mixture was refluxed for twelve hours. When the reflux was stopped, the mixture appeared as a suspension of a white, finely divided solid in a clear colorless liquid. The mixture was then washed with a 400 ml volume of diethyl ether and filtered. The product was recrystallized twice from boiling tertiary butyl alcohol and then washed with anhydrous ether to remove traces of tertiary butyl alcohol. The melting range of the crystals was found to be 206.5-207.5°C. This compares with the reported values of 200°C[38] and 205-207°C.[39] The 14.58% chloride determined experimentally agrees with theoretical value of 14.58%.

Preparation of Butyrylcarnitine Chloride[40]

In a 100-ml round-bottom flask were combined a 9.45 g (0.048 mol) sample of carnitine chloride, a 31.5 g (0.3 mol) sample of butyryl chloride and a 15.75-ml volume of trifluoroacetic acid. A hose connected to a water aspirator was placed in the neck of the flask to remove the hydrogen chloride gas that was evolved. The reaction mixture was then heated in a 40°C oil bath with stirring for forty-eight hours. Then, the mixture was poured into 150 ml of dry acetone and stored at 0°C for four hours. Any unreacted carnitine was then filtered off. When the filtrate had warmed to room temperature, anhydrous ether was added to incipient cloudiness. After precipitation had begun, an additional 300 ml of anhydrous ether was added and the covered mixture was set aside overnight at room temperature. The precipitate was then recrystallized twice using the following proceedure. Dissolution of the product (approximately 10 g) in 30 ml of absolute ethanol was followed by addition of 50 ml of dry acetone. Anhydrous ether was added to incipient cloudiness, and the solution was covered and left at room temperature overnight. Because the product is hygroscopic, it must be dried following filtration and stored in a vacuum dessicator. The final product showed a melting range of 144-146°C in a sealed capillary. The reported melting point is 147°C.[41] The 12.9% chloride determined experimentally compares with the theoretical value of 13.25%. A yield of 8 g, representing about 60% of theoretical, was obtained.

Preparation of Buffers

The sodium hydroxide-glycine buffers were prepared according to the method of Sorenson.[42] The amounts of sodium hydroxide and glycine given in Table 1 were dissolved in distilled water up to 1000 ml to produce a pH of 10.80. The ionic strength values reported in the table were derived using a pK_a value of 9.778 for the substituted ammonium group of glycine.[43]

The sodium bicarbonate-sodium hydroxide buffer of pH 10.80 was prepared according to the specifications of Bates and Bower.[44] In order to increase the buffer capacity, the concentrations of the components were increased five fold over the values given in the reference. Ionic strength calculation was based on a pK_a for the bicarbonate ion of 10.33.[45] Total volume of solution is 1000 ml.

Table 1

Buffers Used in Kinetic Runs

NaOH - Glycine Buffers

Grams of NaOH	Molarity of NaOH	Grams of Glycine	Molarity of Glycine	Molarity of Glycine Anion	Molarity of Glycine Zwitterion	Ionic Strength
7.37	0.184	15.02	0.200	0.183	0.017	0.201
14.72	0.368	30.03	0.400	0.365	0.035	0.402
22.08	0.552	45.04	0.600	0.548	0.052	0.602
36.81	0.920	75.07	1.000	0.913	0.087	1.003

NaOH - $NaHCO_3$ Buffer

Grams of NaOH	Molarity of NaOH	Grams of NaOH	Molarity of $NaHCO_3$	Molarity of $(HCO_3)^-$	Molarity of $(CO_3)^{-2}$	Ionic Strength
4.25	0.106	10.501	0.125	0.032	0.093	o.318

Proceedure for Kinetic Assays in Buffer Solutions

Kinetic assays were conducted using the constant temperature platform of a Sargent-Welch Recording pH Stat. The thermistor was immersed in a 100-ml beaker containing 75.0 ml of buffer and a teflon-coated magnetic stirring bar. The bottom of the beaker had been ground thinner with carborundum to permit more efficient temperature regulation. Absorbance of atmospheric carbon dioxide was prevented by positioning a gas purge tube immediately above the surface of the buffer and maintaining a steady flow of nitrogen. A high temperature-high alkaline electrode (Jena type H glass) was also inserted in the buffer to monitor pH. The buffer was then brought to and maintained at 25.0 $\pm$ 0.1^{o}C. The ionic strength of the buffer was then adjusted by adding the appropriate amount of sodium chloride. After the salt had dissolved and the temperature had restabilized, 50.0 ml of solution were withdrawn and added to approximately 0.000175 mol of ester in an identical beaker. The 50.0 ml of ester solution was then placed on the constant temperature platform and brought to 25.0^{o}C. When the temperature had stabilized the pH meter was turned on and a 1.0 ml aliquot was withdrawn for a zero time determination of ester concentration.

The timed removal of 1.0 ml aliquots were assayed for ester concentration by the method of Hestrin[46] with only slight modification: To a 1.0 ml sample of ester solution was added 2.0 ml of 2 M alkaline hydroxylamine. After one minute 1.0 ml of 1:3 nitric acid[47] (4.0 N) solution was added.

This was immediately followed by addition of 1.0 ml of a 0.37 M solution of ferric chloride hexahydrate in 0.1 N nitric acid. The assays were done in eight inch test tubes which were thoroughly mixed on a Vortex Jr. Mixer after each addition in order to prevent the formation of bubbles when poured into the spectrophotometer tubes. A reference blank was also set up for each assay using 1.0 ml of the remaining 25 ml of the buffer-sodium chloride solution. Absorbance at 540 nm was then measured on a Bausch and Lomb Spectronic 20.

Aminolysis of Benzoylcholine

A 3.9 g (0.016 mol) sample of benzoylcholine chloride was dissolved in 50.0 ml of dimethylsulfoxide. This solution was brought to 98°C on a steam bath and a 7.7 g (0.078 mol) sample of cyclohexylamine was then added. Aliquots of 1.0 ml were withdrawn at intervals and diluted up to 50.0 ml in a volumetric flask. After mixing thoroughly, 1.0 ml of this solution was then assayed by the method of Hestrin as described on page 26.

Recording pH Stat Proceedures

Some kinetic runs were conducted at pH of 11.00 ± 0.02 on a Sargent-Welch Recording pH Stat. This instrument automatically maintains a constant pH as ester hydrolysis proceeds. pH was also monitored indepdently of the pH Stat using a high alkaline electrode and an Orion 801 digital pH meter. The instrument and pH meter were calibrated simultaneously with

each day at 25.0°C with buffers at pH of 9.00 ± 0.02 and 11.33 ± 0.02. To conduct a run, about 0.0007 to 0.001 mol of ester was placed in a 100-ml beaker and dissolved in 50.0 ml of distilled water. The solution was then placed on the constant temperature platform of the pH Stat and protected from the atmosphere with a cover which also supported the pH electrode, the gas purge tube, the titrant addition tube, the thermometer, and the temperature sensing thermistor. The solution was stirred with a magnetic stirring bar and flushed with nitrogen gas as it was brought to 25.0°C. The bottom of the beaker had been ground down slightly with carborundum to permit more rapid temperature equilibration. At this point sodium chloride was added in the appropriate runs to adjust the ionic strength to the desired level. When temperature had stabilized at 25.0°C, the instrument was turned on. This activated the chart drive and the pen drive which automatically recorded the amount of 0.1 equiv/l sodium hydroxide solution as it was dispensed into the beaker. The instrument is capable of delivering 10.0 ml of titrant. The kinetic runs were permitted to proceed for the period of two half-lives.

Absorbance of Standard Solutions of Choline and Carnitine Esters

Standard curves of the choline and carnitine esters were produced by assaying distilled water dilutions of stock solutions of the esters. Aliquots ranging from 0.05 ml to 0.9 ml were withdrawn from the stock solutions and diluted to 1.0 ml. A blank containing only 1.0 ml of distilled water

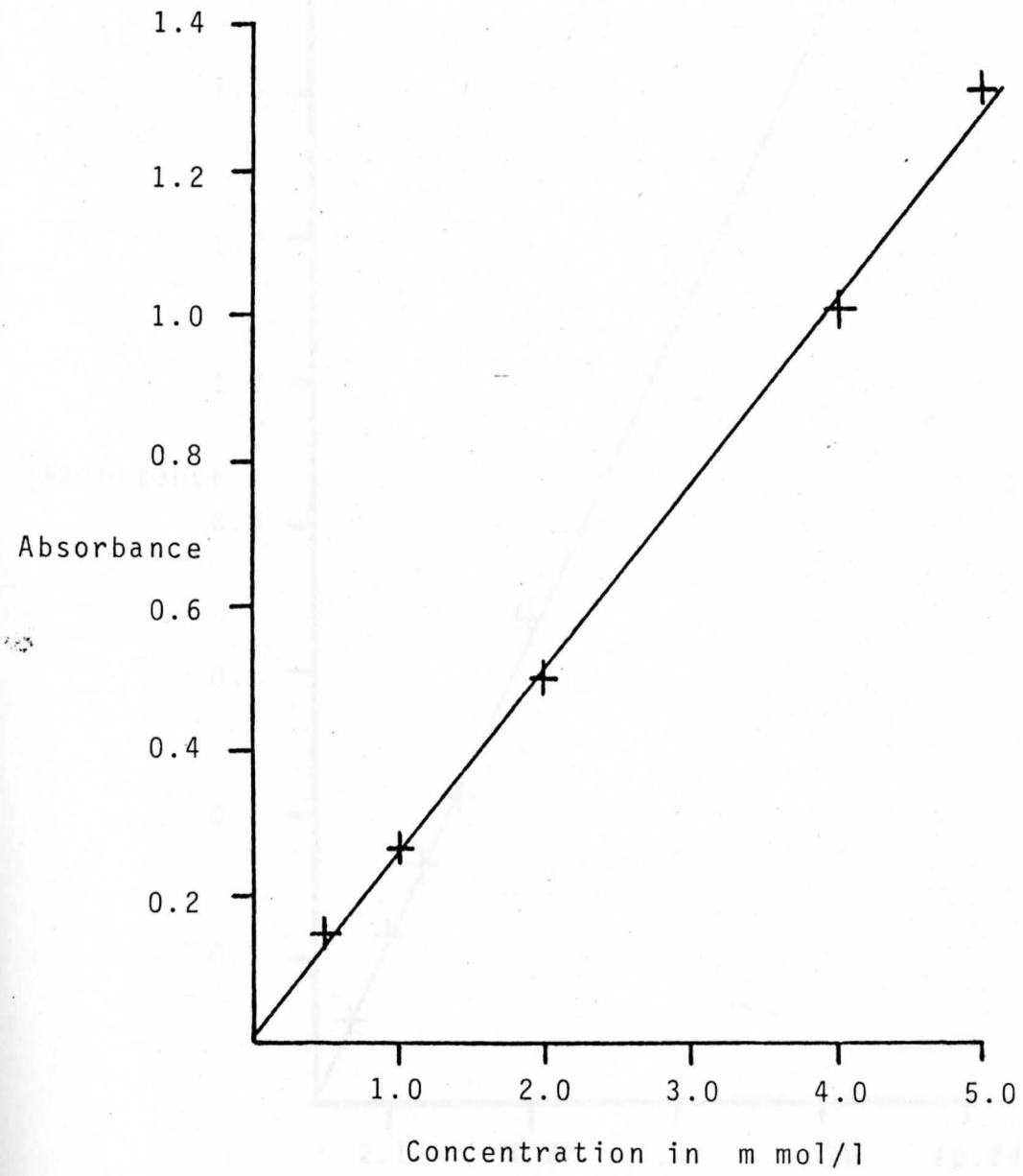


Figure 1 Absorbance of Standard Solutions of Benzoylcholine chloride

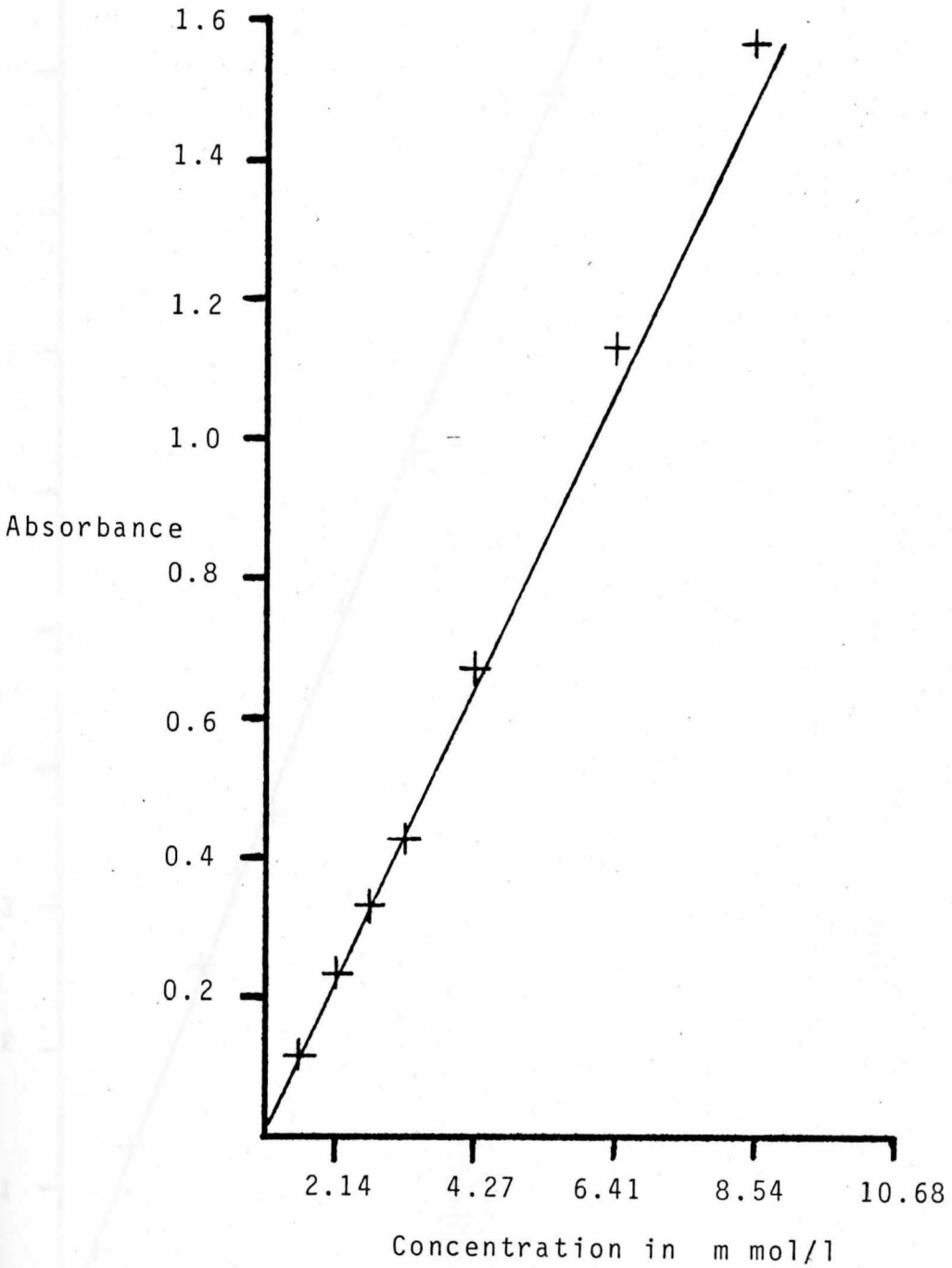


Figure 2 Absorbance of Standard Solutions of butyrylcholine iodide

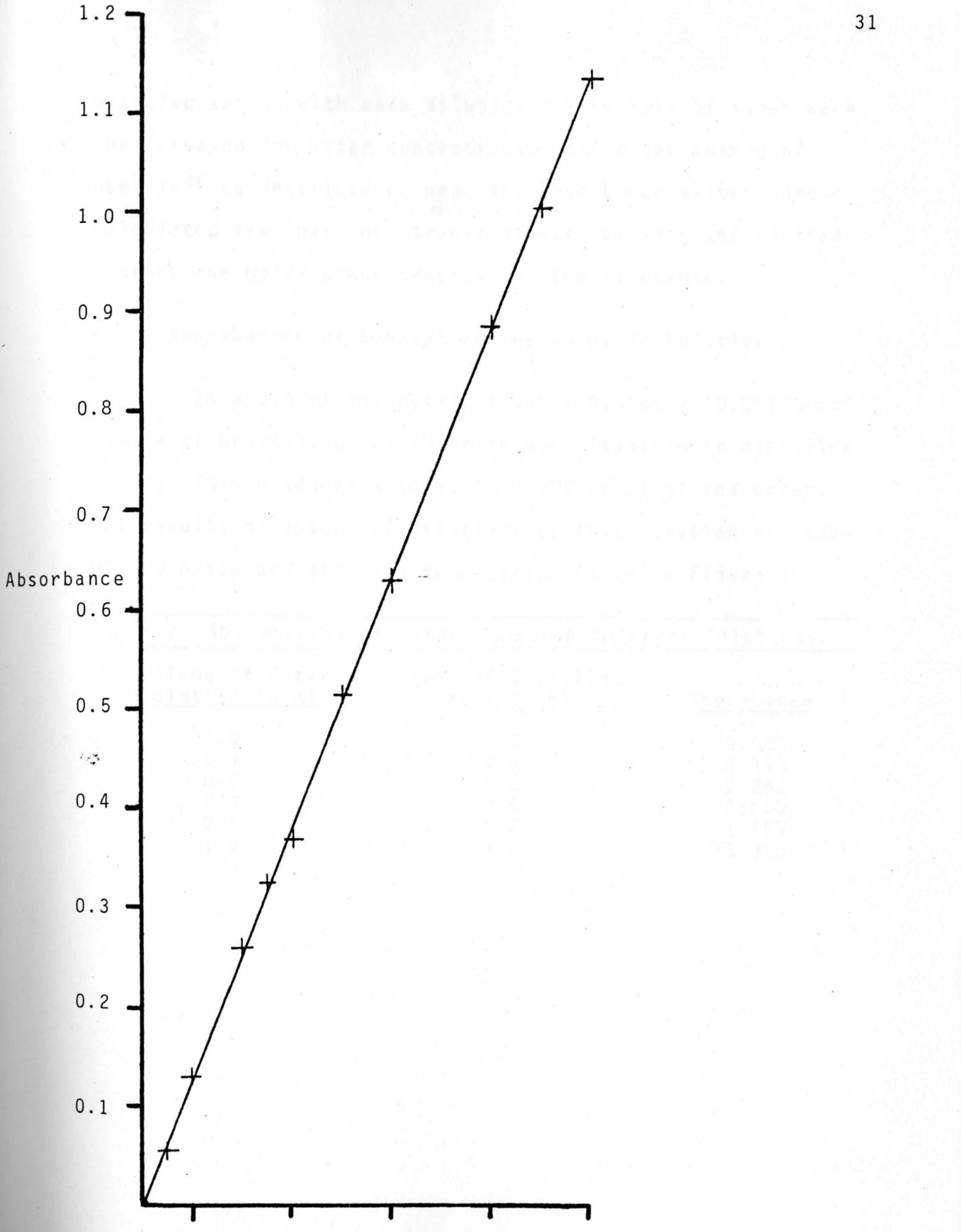


Figure 3 Absorbance of Standard Solutions

was also set up with each dilution. Each pair of tubes were then assayed for ester concentration using the method of Hestrin[46] as described on page 26. Absorbance values were calculated from per cent transmittance readings and plotted against the molar concentrations of the dilutions.

Absorbances of Benzoylcholine Chloride Solutions

In a 250-ml volumetric flask a 0.3045 g (0.00125 mol) sample of benzoylcholine chloride was dissolved in distilled water. This produced a solution 0.005 mol/l of the ester. The results of assays of dilutions of this solution are tabulated below and are also shown graphically in Figure 1.

Table 2 Absorbances of Benzoylcholine Chloride Solutions

Volume of Ester Solution in ml	Volume of Distilled Water in ml	Absorbance
0.0	1.0	0.000
0.1	0.9	0.148
0.2	0.8	0.263
0.4	0.6	0.500
0.8	0.2	1.092
1.0	0.0	1.310

Absorbances of Butyrylcholine Chloride

In a 50-ml volumetric flask a 0.1300 g (0.000534 mol) sample of butyrylcholine chloride was dissolved in distilled water. This yielded a 0.01068 mol/l solution. The following results are also shown in Figure 2.

Table 3 Absorbances of Butyrylcholine Chloride

Volume of Ester Solution in ml	Volume of Distilled Water in ml	Absorbances
0.05	0.95	0.119
0.10	0.90	0.229
0.15	0.85	0.332
0.20	0.80	0.457
0.30	0.70	0.674
0.50	0.50	1.137
0.70	0.30	1.569

Absorbances of Butyrylcarnitine Chloride Solutions

To a 50-ml volumetric flask was added a 0.161 g (0.000602 mol) sample of butyrylcarnitine chloride and enough distilled water to bring the total to the mark. This resulted in a 0.0120 mol/l solution. See Figure 3 for a graph of the following results.

Table 4 Absorbances of Butyrylcarnitine Chloride Solutions

Volume of Ester Solution in ml	Volume of Distilled Water in ml	Absorbances
0.05	0.95	0.056
0.10	0.90	0.130
0.20	0.80	0.260
0.25	0.75	0.327
0.30	0.70	0.369
0.40	0.60	0.514
0.50	0.50	0.630
0.70	0.30	0.886
0.80	0.20	1.004
0.90	0.10	1.137

By examining Figures 1, 2, and 3, it can be seen that the relationship between ester concentration and the absorbance of the hydroxamic acid complex is a linear one. This implies that the absorbance reading is directly proportional to the concentration of ester.

Calculations of Results of Kinetic Runs in Buffer Solutions

The rates of hydrolysis of the butyrylcholine, butyrylcarnitine, and benzoylcholine esters were measured to determine the relative reactivity of choline esters compared to carnitine esters, to observe any effects due to varying ionic strength, and to test for any catalysis through interaction with glycine ions of the buffer solutions. Ionic strength effects were determined by using a buffer of particular composition and then conducting kinetic runs in solutions of this buffer at varying ionic strengths. Hydrolysis of benzoylcholine for example, was observed in kinetic runs in 0.200 mol/l glycine at ionic strength levels varying from 0.20 to 1.20. The effect of buffer concentration on the rate of reaction was determined by adjusting the ionic strength to a constant value while using buffers of different glycine concentration. Kinetic runs on benzoylcholine were conducted at an ionic strength of 0.66 in buffer solutions 0.200, 0.400, and 0.600 mol/l with respect to glycine. Similar proceedures were followed for butyrylcarnitine and butyrylcholine esters.

The rate of hydrolysis of choline and carnitine esters in buffers at an alkaline pH can be described by first

order rate equations. Even though the actual reaction is bimolecular, because the concentration of one of the reactants, the hydroxide ion, is held close to a constant value, the reaction will proceed as though it were dependent only on the concentration of ester. This is a psuedo-first order reaction and it will obey the first order rate relation:

$$-\frac{d[ester]}{dt} = k_{obsd}\,[ester] \qquad (13)$$

where k_{obsd} is the psuedo-first order rate constant. When the equation is integrated, the expression becomes:

$$k_{obsd} = \frac{1}{t - t_o} \ln \frac{[ester]_o}{[ester]_t} \qquad (14)$$

where t_o and $[ester]_o$ refer to the time and concentration of ester at the start of a run. The values of t and $[ester]_t$ are the time and concentration of ester remaining at that time. This relation also applies for any measurable quantity directly proportional to ester concentration. In particular, it can be seen by the linear relationships in Figures 1, 2, and 3 that absorbances of solutions of hydroxamic acid—ferric chloride complexes produced from ester solutions are directly proportional to ester concentrations. Therefore, the preceding equation can be rewritten as:

$$k_{obsd} = \frac{1}{t - t_o} \ln \frac{A_o}{A_t} \qquad (15)$$

where A_o and A_t are the absorbances at the start of the run and at any later time (t). This is the expression by which the data from the kinetic runs were evaluated. In Table 7

(see Appendix) are shown the results of a typical run which have been calculated using this expression.

It is also possible for kinetic data of this type to be evaluated graphically. By plotting $(t - t_o)$ versus $(\ln A_o - \ln A_t)$, a straight line will be obtained for a first order reaction. The slope of this line will then equal $-1/k_{obsd}$. The results of the kinetic run presented in Table 7 are also shown by this graphic method in Figure 6.

In the above analysis the assumption was made that hydroxide ion activity was constant. Upon calculation of changes in concentration of buffer components a small but significant change was found to have occurred in hydroxide ion activity due to release of acid in the course of ester hydrolysis. Through use of the Henderson-Hasselbalch equation,[48] pH changes due to changing concentrations of buffer components can be accounted for. The Henderson-Hasselbalch relationship is:

$$pH = pK_a - \log \frac{[A^-]}{[HA]} \qquad (16)$$

$[A^-]$ and $[HA]$ represent the dissociated and undissociated forms of an acid. The particular reaction of interest for the buffers employed is the dissociation of the ammonium group in glycine:

$$^+NH_3CH_2COO^- \rightleftharpoons NH_2CH_2COO^- + H^+ \qquad (17)$$

The pK_a for this quaternary ammonium group is 9.778.[43] Since the pH of the buffer is 10.80, the Henderson-Hasselbalch

equation gives:

$$10.80 = 9.778 + \log \frac{[NH_2CH_2COO^-]}{[^+NH_3CH_2COO^-]} \quad (18)$$

This rearranges to give:

$$10.52\ [^+NH_3CH_2COO^-] = [NH_2CH_2COO^-] \quad (19)$$

To solve for either ionic species, another relationship is needed. For demonstration the buffer system 0.200 mol/l in glycine will be used. Since the total glycine concentration is 0.200, then:

$$[^+NH_3CH_2COO^-] + [NH_2CH_2COO^-] = 0.200 \text{ mol/l} \quad (20)$$

By substitution of the appropriate quantity in Equation 19, the concentration of the zwitterionic and anionic species can be determined. This derivation gives the results as:

$$[^+NH_3CH_2COO^-] = 0.0174 \text{ mol/l} \quad (21)$$

$$[NH_2CH_2COO^-] = 0.1826 \text{ mol/l} \quad (22)$$

In a typical run about 0.000175 mol of ester is dissolved in 50.0 ml of buffer producing a 0.00350 mol/l solution. After two half-lives 75% of this would have been hydrolyzed to acid and alcohol. Under the basic conditions of the experiment the acid would immediately react with glycine anion to produce a zwitterionic molecule. Thus, a 0.0035 molar solution of ester would produce a:

$$0.00350 \text{ mol/l} \times 0.75 = 0.00263 \text{ mol/l} \quad (23)$$

increase in the concentration of zwitterionic glycine after two half-lives. The total concentration of this species would then be:

$$0.0174 \text{ mol/l} + 0.00263 \text{ mol/l} = 0.0200 \text{ mol/l} \quad (24)$$

Therefore, the concentration of anionic glycine remaining is:

$$0.200 \text{ mol/l} - 0.0200 \text{ mol/l} = 0.1800 \text{ mol/l} \quad (25)$$

Inserting these values in the Henderson-Hasselbalch equation gives:

$$pH = 9.778 + \log \frac{0.1800}{0.0200} = 10.73. \quad (26)$$

This shows that over the period of this run, a pH change of 0.07 unit occurred; thus raising a question concerning the validity of the assumption of psuedo-first order kinetics. This also suggests that comparison of first order rate constants from kinetic runs in buffers of various strength and composition is invalid because of differences in buffer capacity. It is also to be noted that because the salt effect on hydroxide ion activity (to be described on pages 38 - 42) alters the various buffer solutions to different extents, it could act to produce salt effects on the other reactants in the hydrolysis mechanism. That is, the second order effect occurring when pH drops, might mask the possible first order kinetic changes due to ionic strength effects on the reactants or products. For these reasons, the basis of comparison of the effects of the various buffer and salt solutions on the reaction velocity must be the second order rate constants.

Effect of Sodium Chloride on Hydroxide Ion Activity

It was observed that upon addition of sodium chloride to a buffer solution, a drop in the pH reading occurred. This is due to hydrogen ion activity changes resulting from ionic

strength adjustments. It was also observed that this drop was not precisely reproducible. Since it was desired to compare the various kinetic runs with each other, a number of pH changes resulting from addition of various amounts of sodium chloride were measured. The results of these measurements are shown in Figure 4, for the glycine buffer, and Figure 5, for the bicarbonate buffer. The abscissas give the change in ionic strength ($\Delta\mu$) relative to ionic strength before addition of sodium chloride (μ_i). The ordinates give the change in hydroxide ion activity (Δa_{OH}) relative to the original hydroxide ion activity before the salt addition (a_{OH_i}). The curves drawn through these points were then used to calculate the expected change in hydroxide ion activity upon addition of a particular amount of salt rather than relying on the actual pH drop observed in each run.

An example of how these graphs are used will be given to show the magnitude of the effect on hydroxide ion activity in a typical kinetic run. One of the buffers was 0.400 molar in glycine. The sodium and glycine ions present resulted in an ionic strength of 0.402. If it was desired to adjust the ionic strength to 0.66, a change of 0.258, a 50.0 ml volume of buffer solution would require the addition of 0.75 g of sodium chloride. This gives a $\Delta\mu/\mu_i$ value of 0.642. From Figure 4, the corresponding value of $\Delta a_{OH}/a_{OH_i}$ is 0.138. Since the pH before adding any sodium chloride (pH_i) was 10.80, the pOH before adding the sodium chloride (pOH_i) will be 3.20. This corresponds to a hydroxide ion

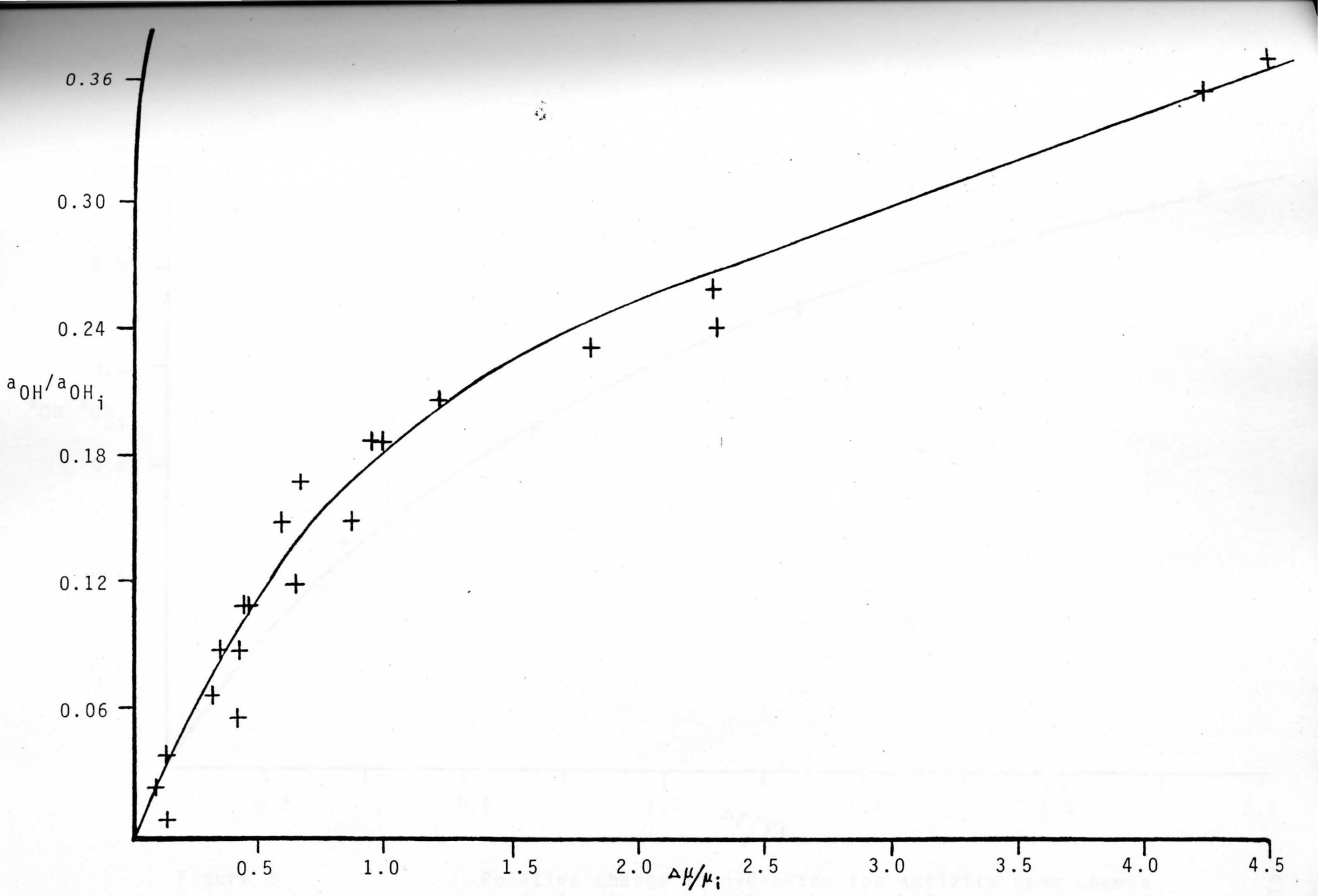


Figure 4 Relative change in hydroxide ion activity upon change in ionic strength of glycine buffer solutions

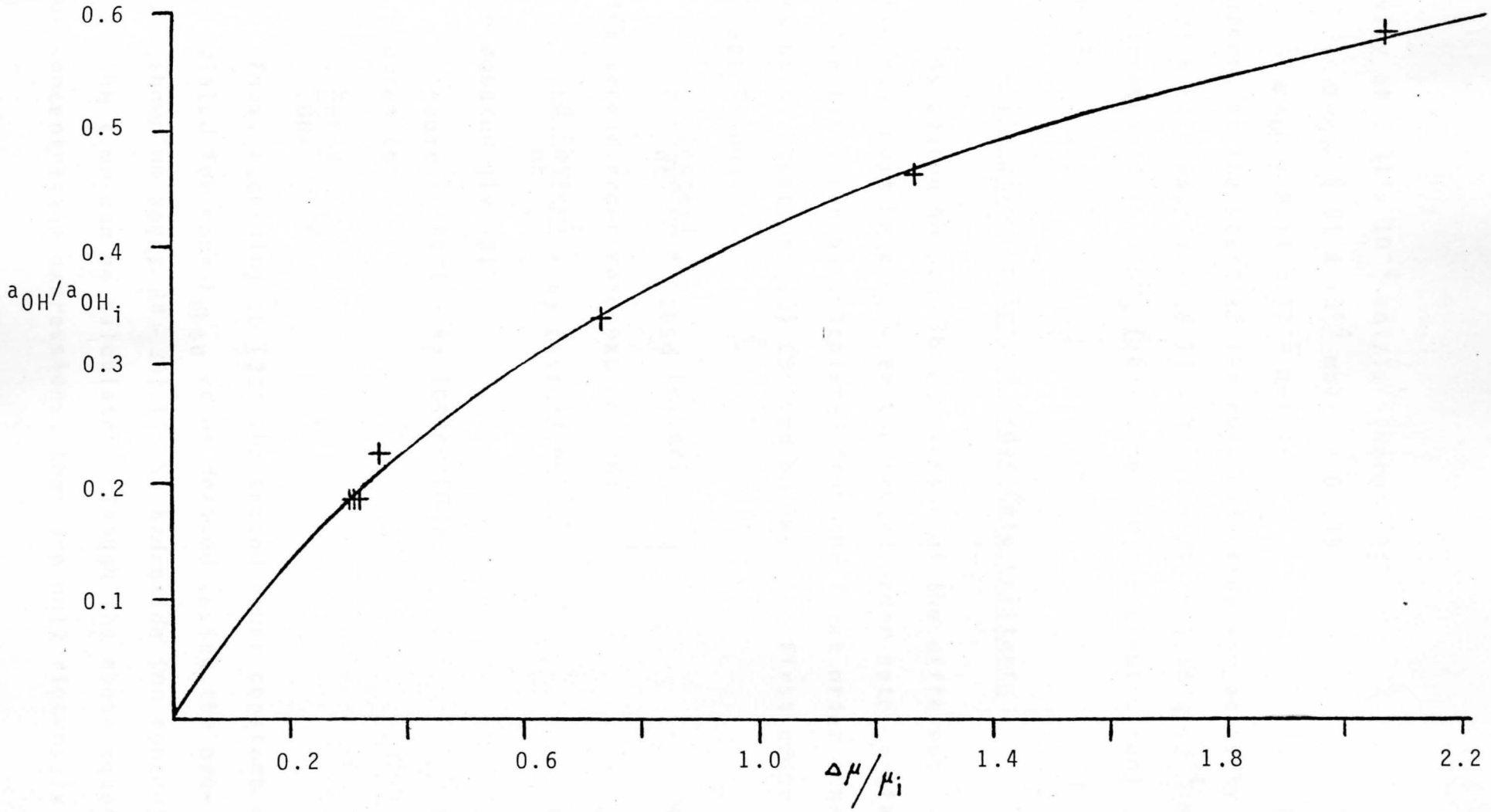


Figure 5 Relative change in hydroxide ion activity upon change in ionic strength of bicarbonate buffer solutions

activity of 6.31×10^{-4} mol/l. Therefore:

$$\Delta a_{OH} / 6.31 \times 10^{-4} \text{ mol/l} = 0.138$$

$$\Delta a_{OH} = 8.71 \times 10^{-5} \text{ mol/l} \quad (27)$$

Therefore, at the start of the run, hydroxide ion activity is:

$$(6.31 \times 10^{-4} \text{ mol/l}) - (8.71 \times 10^{-5} \text{ mol/l}) = 5.44 \times 10^{-4} \text{ mol/l}$$

This corresponds to a pH_o (zero time for the kinetic run) of 10.74.

Calculation of Second Order Rate Constants

As stated on page 38, comparison of the different kinetic runs must be done with the second order rate constants. These constants can be calculated from the first order constants using Equation (20) derived below. The first order rate expression:

$$\frac{-d\,[ester]}{dt} = k_{obsd}\,[ester] \quad (13)$$

and the second order rate expression;

$$\frac{-d\,[ester]}{dt} = k_2\,[ester][OH] \quad (28)$$

can be equated giving;

$$k_{obsd}\,[ester] = k_2\,[ester][OH]. \quad (29)$$

This reduces to:

$$\frac{k_{obsd}}{[OH]} = k_2 \quad (30)$$

Thus, according to (30) the second order constant can be calculated for each k_{obsd} value derived (using the proceedure shown on pages 34 - 36) if the hydroxide ion concentrtion at the time can be calculated. Though the above equations employ concentration expressions, they are only rigorously

true for the corresponding activity values. For this reason, the hydroxide ion activity will be used in the calculation of the second order rate constants.

The uncertainty in the pH readings at the ionic strengths used in this study can be seen by observing the variations in Figure 4. Because of this uncertainty in the electrode response, the hydroxide ion activities used to calculate the second order rate constants are calculated from the amounts of acid produced in the hydrolysis of the esters. An example of the calculations involved in deriving these activity values is presented below.

The following calculations are for the hydrolysis of benzoylcholine in 0.200 mol/l glycine at an ionic strength of 0.660. The original concentration of ester is:

$$[\text{ester}]_o = \frac{0.0331 \text{ g mol}}{(243.5 \text{ g})\ 0.05\ \text{l}} = 0.00272 \text{ mol/l} \qquad (31)$$

Since $\mu_i = 0.201$, adjusting the ionic strength to 0.660 produced a $\Delta\mu = 0.459$. Therefore, $\Delta\mu/\mu_o = 2.25$. From Figure 4, a value of 2.25 corresponds to $\Delta a_{OH}/a_{OH_i} = 0.268$. Since $pH_i = 10.80$, $pOH_i = 3.20$. This is equivalent to a hydroxide ion activity of $10^{-3.20}$ or 6.31×10^{-4} mol/l. Upon adjustment of the ionic strength with sodium chloride the hydroxide ion activity becomes:

$$a_{OH_o} = 6.31 \times 10^{-4} \text{ mol/l} - (6.31 \times 10^{-4} \text{ mol/l} \times 0.268) = 4.62 \times 10^{-4} \text{ mol/l}$$

When a_{OH_o} is 4.62×10^{-4} mol/l, pH_o is 10.665. This is the pH of the solution at the start of the run. The concentration of the glycine zwitterion at this time is determined using the

Henderson-Hasselbalch equation as described on page 36. In 0.200 mol/l glycine buffer, this gives:

$$[^{+}NH_3CH_2COO^{-}] = 0.0230 \text{ mol/l} \quad (32)$$

This run proceeded to 85% completion. At that time the molar concentration of hydrogen ions released by hydrolysis of the ester is given by:

$$0.85 \times [\text{ester}]_o = 0.00231 \text{ mol/l} \quad (33)$$

These hydrogen ions combine with glycine anions to yield a total zwitterion concentration of:

$$0.0230 \text{ mol/l} + 0.00231 \text{ mol/l} = 0.0253 \text{ mol/l} \quad (34)$$

Therefore the pH at the end of the run (pH_f) is:

$$pH_f = 9.778 + \log \frac{0.1747}{0.0253} = 10.617 \quad (35)$$

This corresponds to a hydroxide ion activity of 4.14×10^{-4} mol/l. Since the second order constants are calculated from hydroxide ion activity values, and since the change in the activity of this ion decreases logarithmically as the hydrolysis proceeds, the following expression can be used to relate activity levels to the elapsed time of the run:

$$\ln(a_{OH_t}) = \ln(a_{OH_o}) - \frac{[\ln(a_{OH_o}) - \ln(a_{OH_f})]}{t_f - t_o}(t - t_o) \quad (36)$$

For the first run this equation will read:

$$\ln(a_{OH_t}) = -7.678 - (1.423 \times 10^{-5})(t - t_o). \quad (37)$$

Using this equation, the hydroxide ion activities are calculated for each assay in each kinetic run. Dividing the k_{obsd} for each assay by the corresponding value of a_{OH_t} yields the second order rate constant. There are two other considerations which affected the above calculations in some of the

runs. One was that the butyrylcarnitine ester contains a free carboxylic acid group. Upon solution of this ester in the basic media, the carboxylic acid immediately neutralized an equivalent amount of base. This additional acid acted to lower the initial hydroxide ion activity (a_{OH_o}) of the run. This was accounted for in the calculations in the same manner as the acid produced during hydrolysis, excepting, of course, that this neutralization occurred immediately upon dissolving the ester.

The second consideration was that in calculating the hydroxide ion activity for the bicarbonate buffer, the pK_a value employed was that of the bicarbonate anion, i.e. 10.33.

The results of these calculations are recorded in the Appendix and will be discussed in the following chapter.

Calculation for Kinetic Runs on pH Stat

The pH stat maintains a constant hydroxide ion activity and simultaneously produces a recording of the amount of base added to retain this constant activity. Because the hydroxide level is constant, the reaction will follow first order kinetics even though the actual mechanism is bimolecular. The first-order rate expression:

$$k_{obsd} = \frac{1}{(t - t_o)} \ln \frac{[ester]_o}{[ester]_t} \qquad (38)$$

can be readily evaluated because the amount of base added is known from the recording. Therefore, the amount of ester at

any time can be calculated by subtracting the moles of base added from the original number of moles of ester. Since the titrant used was 0.1000 normal sodium hydroxide, addition of 1.0 ml of basic solution implied that 100.0 μmol of ester had been hydrolyzed.

In a typical run, approximately 800 μmol of ester were dissolved in 50.0 ml of distilled water. This produced $[ester]_0$ of 16.0 mmol/l. For convenience, the rate constant was determined at time intervals corresponding to the hydrolysis of 100.0 μmol of ester. Thus, the first calculated constant would be:

$$k_{obsd} = 1/t \ln \frac{800/50.0}{(800-700)/51}$$

$$k_{obsd} = 1/t \ 2.10$$

CHAPTER IV

RESULTS AND DISCUSSION

Results of Kinetic Runs in Buffer Solution

The results of the kinetic runs in buffer solutions are given in the Appendix by a graph and a table for each run. A tabular summary of the second order rate constants for these runs is shown on the following page.

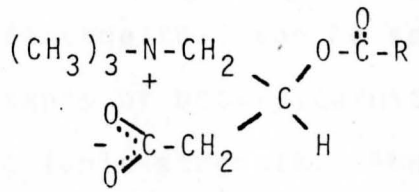
The average of 0.58 sec^{-1} M^{-1} for the basic hydrolysis of benzoylcholine compares with the value of 1.11 sec^{-1} M^{-1} determined by Chu and Mautner.[16] Rate constants for the butyric acid esters of choline and carnitine were not available for comparison with the results of this study. The cause of the difference between the values determined in this study and that found by Chu and Mautner, is unknown. Both studies were done at a temperature of 25°C and an ionic strength of 0.66. Also, both showed that there was no effect due to the buffers employed (phosphate buffer by Chu and Mautner; sodium hydroxide-glycine by the author). The reactions done in phosphate buffer were measured in a spectrophotometer for disappearance of absorption at 230 nm. The author's study employed the colorimetric method of Hestrin for determining the amount of ester remaining. These were the only differences in the two studies.

TABLE 5

SUMMARY OF KINETIC RUNS IN BUFFER SOLUTIONS

Buffer	Ionic Strength	k_2 sec^{-1} M^{-1}
Benzoylcholine		
0.200 Molar Glycine	0.201	0.52
"	0.454	0.57
"	0.454	0.56
"	0.660	0.57
"	1.20	0.62
0.400 Molar Glycine	0.660	0.60
"	0.660	0.56
0.600 Molar Glycine	0.660	0.61
"	0.660	0.61
"	0.660	0.57
0.125 Molar Bicarbonate	0.660	0.58
Butyrylcholine		
0.200 Molar Glycine	0.454	0.74
"	0.660	0.73
0.400 Molar Glycine	0.660	0.73
"	0.660	0.74
0.600 Molar Glycine	0.660	0.73
"	0.660	0.73
0.125 Molar Bicarbonate	0.660	0.73
Butyrylcarnitine		
0.200 Molar Glycine	0.201	0.059
"	0.660	0.071
"	1.20	0.097
0.600 Molar Glycine	0.660	0.069
"	0.660	0.071
1.000 Molar Glycine	0.997	0.085
0.125 Molar Bicarbonate	0.660	0.065

In the present study the greatest difference in rates are observed when comparing the rate constants of the choline esters with those of the carnitine ester. Under identical conditions butyrylcarnitine hydrolyzes 8-10 times more slowly than butyrylcholine. Several factors may be responsible for this. The interaction of the carboxylate anion with the quaternary ammonium to form an inner salt may result in the reduction of the catalytic effect of the positive charge



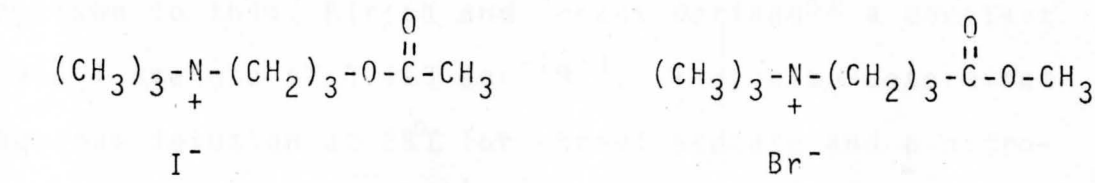
described in CHAPTER II. It is also possible that there may be some steric resistance due to the carboxymethyl substituent. The substitution of this group for a hydrogen atom is the difference between butyrylcholine and butyrylcarnitine. The decrease in rate obtained for esters with bulkier substituents was described on page 6.

Another obvious difference in the rate constants is that observed in comparing the butyryl ester of choline with the benzoyl ester. There is approximately a 25% increase in the rate of hydrolysis of butyryl ester over benzoyl ester. The dissociation constants of the benzoic and butyric acids are about 6.5×10^{-5} and 1.5×10^{-5} respectively.[49] These values would imply that the benzoate anion is slightly more stable than the butyrate anion; thus, further implying that it could better stabilize the intermediate anion formed in ester hydrolysis. Since this small difference is not reflected

in the rate constants, the other factor to be considered, i.e. steric hindrance, seems to dictate the relative rates. In an earlier study,[50] it was shown that the rate of ethyl butyrate hydrolysis was slightly faster than that for ethyl pentanoate, and over twice as fast as the rates for ethyl isobutyrate and ethyl benzoate. Though the magnitude of the difference does not agree with the present study, it does agree in the direction of change.

A third difference that can be seen is that of the change in rate constants of butyrylcarnitine hydrolysis that occurs with changing ionic strength. The value of the rate constant undergoes a 60% increase upon a 6-fold increase in ionic strength. The reasons for this rate increase cannot be attributed to any particular mechanistic phenomena. Nevertheless, as mentioned in CHAPTER II, a neutral molecule will respond to increasing ionic strength by showing an increased rate of reaction. If a zwitterion can be considered neutral, this prediction is in agreement with the present study.

In studies by Asknes and Prue[34] using some esters with quaternary nitrogens (3 - acetoxypropyl trimethylammonium iodide and 3 - methoxycarbonylethyl trimethylammonium bromide:



a decrease in the rate of alkaline hydrolysis was observed with increasing ionic strength. According to equation 12, which is applicable only to solutions of low ionic strengths ($\mu < 0.1$); this is the predicted result for a reaction between oppositely charged ions. However, of the ionic strengths used in the present study, (0.2 - 1.2) no significant salt effect can be detected in the rate constants in Table 5 for butyrylcholine and benzoylcholine. There is no theoretical means of predicting any possible salt effects at these higher ionic strengths for the reactions of these choline esters. Thus, whether the results of these experiments are in error, or whether no salt effect in fact exists, cannot be determined.

It is of interest to compare the rates of hydrolysis of the esters used in this study with the rate constants of other esters. The problem with such comparisons is that solvent systems other than water are generally employed because of the insolubility of many esters in water. The different solvent effects, however, introduce uncertainties in such comparisons. Tarbell found[51] that ethyl acetate, which is soluble in water, yielded a second order rate constant of alkaline hydrolysis at 20°C of 0.029 $sec^{-1}M^{-1}$. In comparison to this, Kirsch and Jencks derived[52] a constant for ethyl acetate of 0.113 $sec^{-1}M^{-1}$. They also determined in aqueous solution at 25°C for phenyl acetate and p-nitrophenyl acetate of 1.26 $sec^{-1}M^{-1}$ and 9.5 $sec^{-1}M^{-1}$ respectively. Considering the second order values determined in the present

study in water: 0.58 $sec^{-1}M^{-1}$, 0.73 $^{-1}M^{-1}$, and 0.070 $sec^{-1}M^{-1}$ for benzoylcholine, butyrylcholine, and butyrylcarnitine respectively, it can be seen that these esters hydrolyze at a rate intermediate to those cited for the neutral esters.

In another study at 25°C a second order constant of 0.6 $sec^{-1}M^{-1}$ for p-nitrophenyl benzoate in 33% acetonitrile was found by Caplow and Jencks.[53] It is interesting to note that this ester does not hydrolyze any more rapidly than the choline ester at benzoic acid (that is, if it is assumed the 33% acetonitrile did not greatly inhibit the rate.) p-nitrophenyl esters have been used extensively in peptide synthesis because they readily undergo aminolysis. Perhaps choline esters may undergo rapid aminolysis if the proper conditions can be found.

In some other studies in mixed solvents at 25°C rate constants of 0.0029 $sec^{-1}M^{-1}$ for ethyl benzoate in 40% water - 60% acetone[54] and 0.0018 $sec^{-1}M^{-1}$ for the alkaline hydrolysis of ethyl butyrate in 85% aqueous ethanol[55] were found. The esters used in the present study differ from the two cited above only by the presence of the trimethylammonium group. The greater than 100-fold increase in the rate constants for the benzoyl and butyryl choline esters is probably attributable to a combination of both solvent and substituent group effects.

It can also be seen in Table 5 that there was no change in the rate of hydrolysis upon changing the concentration of buffer. The buffer concentration was varied to

determine if general base catalysis was occurring. As described in CHAPTER II, the presence of basic substances (here the amino group and carboxylate anion of glycine and the bicarbonate anion) can act to increase the rate of ester hydrolysis above that specifically due to the hydroxide ion. That no increase was observed is evidence that hydrolysis is by specific catalysis alone. This implies that the rate of formation of the tetrahedral intermediate is faster than the dissociation of intermediate into the products. This suggests that the enzymatic hydrolysis of these esters may not occur with participation of general base. However, the validity of this idea would require corroboration by investigations into the specific mechanism through which the enzymes act.

Results of Kinetic Runs from the pH Stat

The values of the rate constants determined with this instrument showed a steady increase as each run progressed. The differences between the original constants and the constants calculated at two half-lives were in the range of 10-15%. Reproducibility of any particular run could not be certain by more than 10-15%. The reasons for this uncertainty could not be discovered by this author; even after extensive examination of instrument and proceedures. Because of the large degree of uncertainty in the results, only a summary will be tabulated on following page.

Table 6

KINETIC RUNS ON THE pH STAT

Ester	μ	number of runs	Approximate average k_{obsd}	k_2 at pH 11.00 $sec^{-1}M^{-1}$
Butyrylcholine	~0.00	2	8.4×10^{-4} sec^{-1}	0.84
"	0.66	7	9.0×10^{-4} sec^{-1}	0.90
Benzoylcholine	~0.00	4	7.0×10^{-4} sec^{-1}	0.70
"	0.66	5	6.2×10^{-4} sec^{-1}	0.62
Butyrylcarnitine	~0.00	3	6.5×10^{-5} sec^{-1}	0.065
"	0.66	3	1.1×10^{-4} sec^{-1}	0.11

The results above show a fair agreement with those obtained by colorimetric method. The rate constants for different ionic strength show an increase in the rate of hydrolysis of butyrylcholine with increasing ionic strength, but a decrease in the rate of hydrolysis of benzoylcholine. The magnitude of this difference can be explained by the experimental uncertainty. However, for the carnitine ester a change of about 75% is observed as ionic strength is increased. This compares with the 60% increase found for the reactions run in buffers.

Aminolysis of Benzoylcholine

A kinetic run was conducted in dimethylsulfoxide to determine the rate of aminolysis of benzoylcholine in the absence of water. The proceedure described on page 27 produced the following results:

Time (minutes)	Absorbance
1	1.16
32	0.89
64	1.12
950	0.81
2510	0.32
5540	0.11

If the original absorbance is taken to be about 1.10, the reaction was about 90% complete only after approximately four days. This was at 98°C. This contrasts with the 80% completion of the hydrolysis of benzoylcholine in water at 25°C in two hours. It also agrees with the finding of Chu and Mautner,[16] who observed no aminolysis of benzoylcholine by butylamine in water at 25°C. The slow rate observed in the author's study may be partly attributed to the poor solvation of anions by dimethylsulfoxide. This would reduce the stability of intermediate and hence slow the overall rate of the reaction. The slow rates of aminolysis noted above discourages the use of choline esters as reagents for peptide synthesis. However, increased reactivity may be obtained in a different solvent under different reaction conditions.

APPENDIX

On the following pages are graphs and tables of the results of the kinetic runs performed on the esters: benzoylcholine, butyrylcholine, and butyrylcarnitine. They are arranged so that the table containing the data for a particular run is immediately followed by a graph of the same data.

The mean values of k_2 were calculated using all the k_2 values derived except those marked by an asterisk. The values with an asterisk are more than 4% different (an arbitrarily selected cut-off point) from all other k_2 values found for that particular run. The mean k_2 value given at the end of each table is expressed as plus or minus two standard deviations. Most of the discarded values were also excluded from the graphs of the kinetic runs.

TABLE 7

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 1

Buffer: 0.200 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.665

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.678 - (1.423 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
410	0.094	2.30	0.50*
680	0.157	2.31	0.50*
970	0.242	2.50	0.55
1435	0.354	2.47	0.55
1765	0.445	2.53	0.56
2060	0.544	2.64	0.59
2745	0.700	2.55	0.57
3590	0.893	2.49	0.57
5295	1.295	2.46	0.57
7740	1.899	2.45	0.59

Mean k_2: 0.57 ± 0.03 sec^{-1} M^{-1}

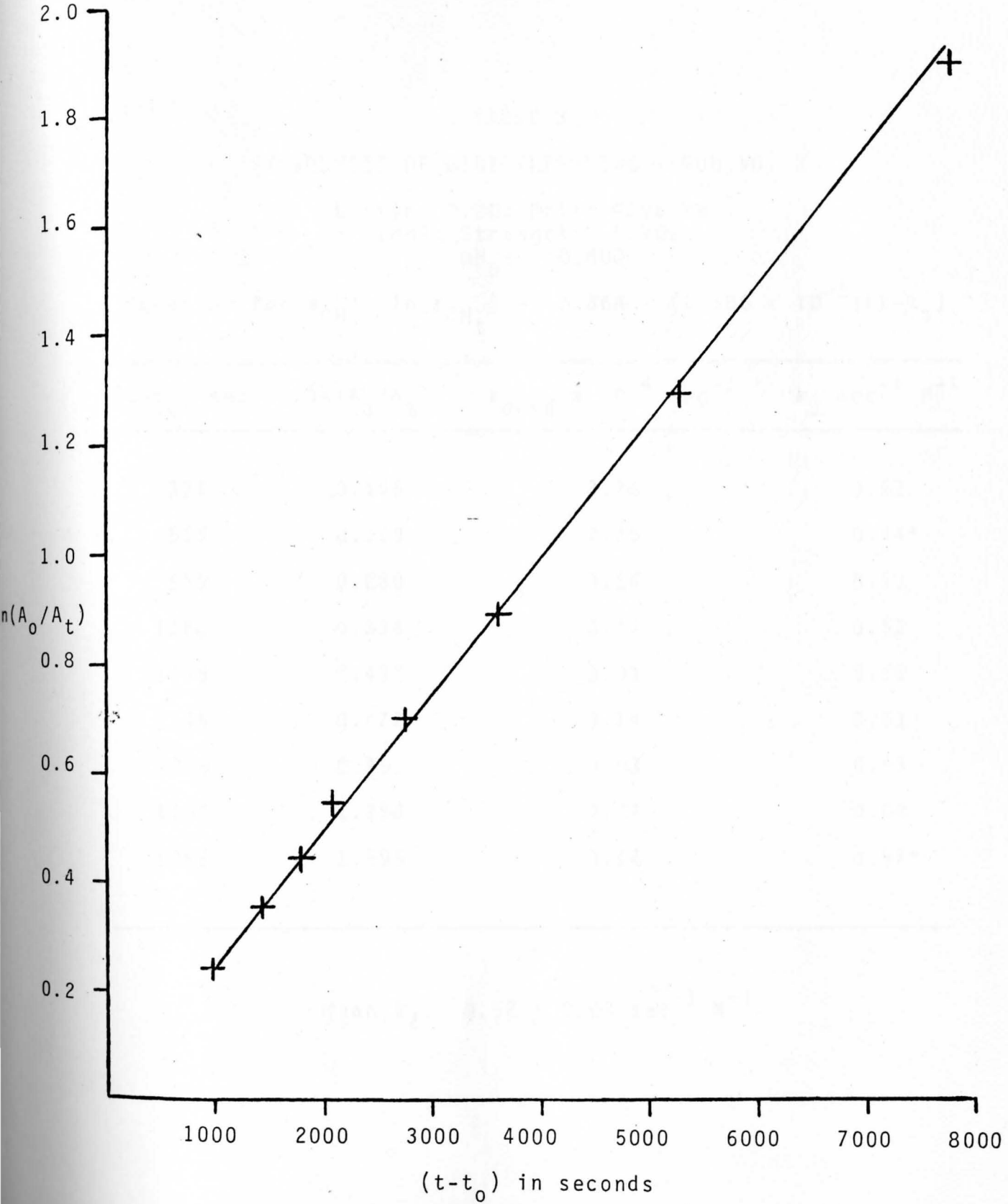


Figure 6 Hydrolysis of benzoylcholine in 0.200 molar glycine buffer at ionic strength of 0.660

TABLE 8

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 2

Buffer: 0.200 Molar Glycine
Ionic Strength: 0.201
pH_o: 10.800

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.368 - (2.586 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
325	0.106	3.26	0.52
615	0.169	2.75	0.44*
855	0.280	3.26	0.53
1060	0.336	3.17	0.52
1435	0.432	3.01	0.50
2140	0.672	3.14	0.53
3085	0.950	3.08	0.53
4100	1.258	3.07	0.54
5085	1.599	3.14	0.57*

Mean k_2: 0.52 ± 0.03 sec^{-1} M^{-1}

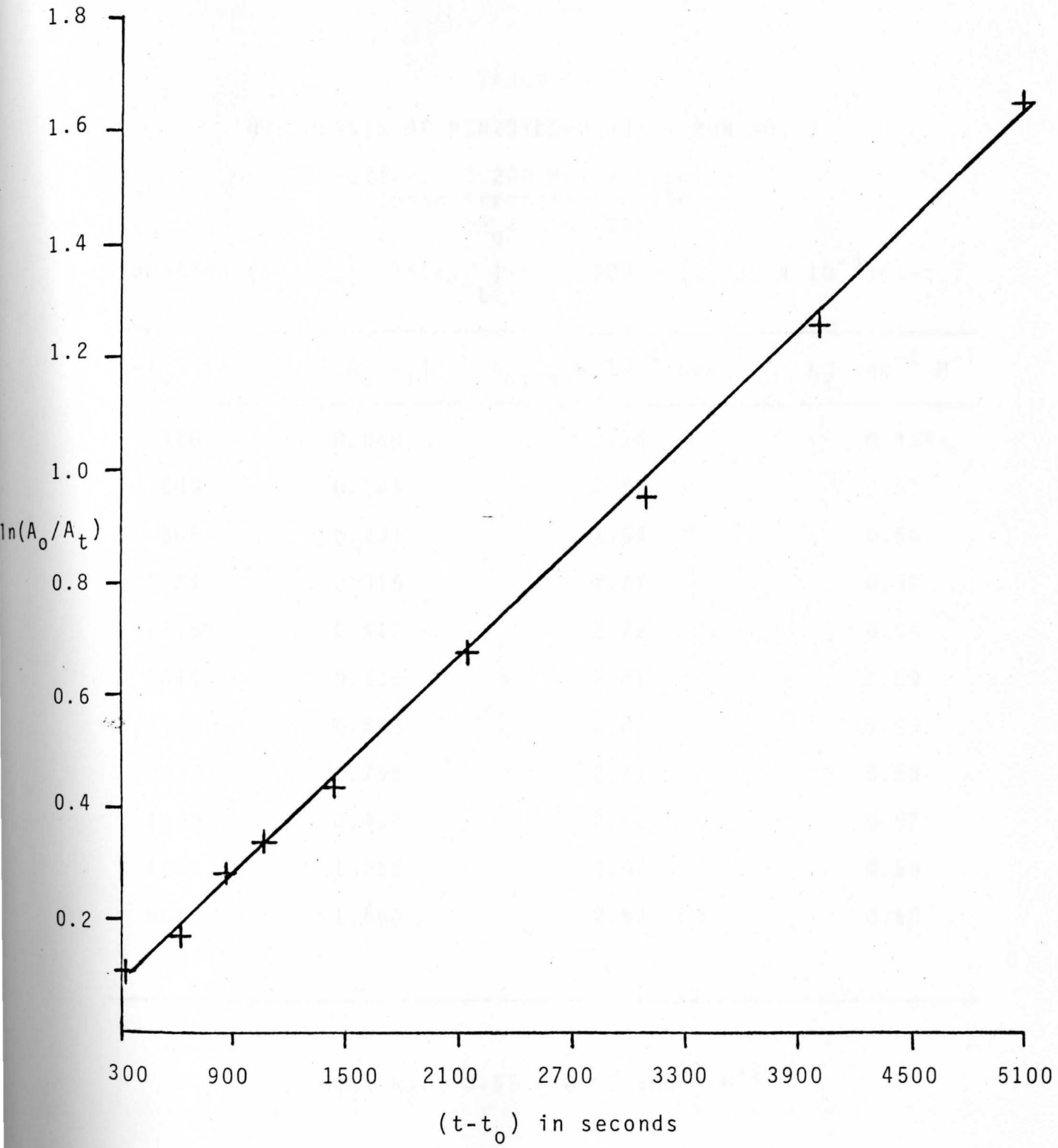


Figure 7 Hydrolysis of benzoylcholine in 0.200 molar glycine buffer at ionic strength of 0.201

TABLE 9

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 3

Buffer: 0.200 Molar Glycine
Ionic Strength: 0.454
pH_o: 10.696

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.608 - (1.930 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
320	0.068	2.14	0.43*
600	0.155	2.58	0.53
885	0.233	2.64	0.54
1175	0.315	2.77	0.57
1515	0.412	2.72	0.56
1845	0.519	2.81	0.59
2115	0.596	2.82	0.59
2945	0.798	2.71	0.58
3690	0.967	2.62	0.57
4695	1.255	2.67	0.59
5610	1.500	2.67	0.60

Mean k_2: 0.57 ± 0.05 sec^{-1} M^{-1}

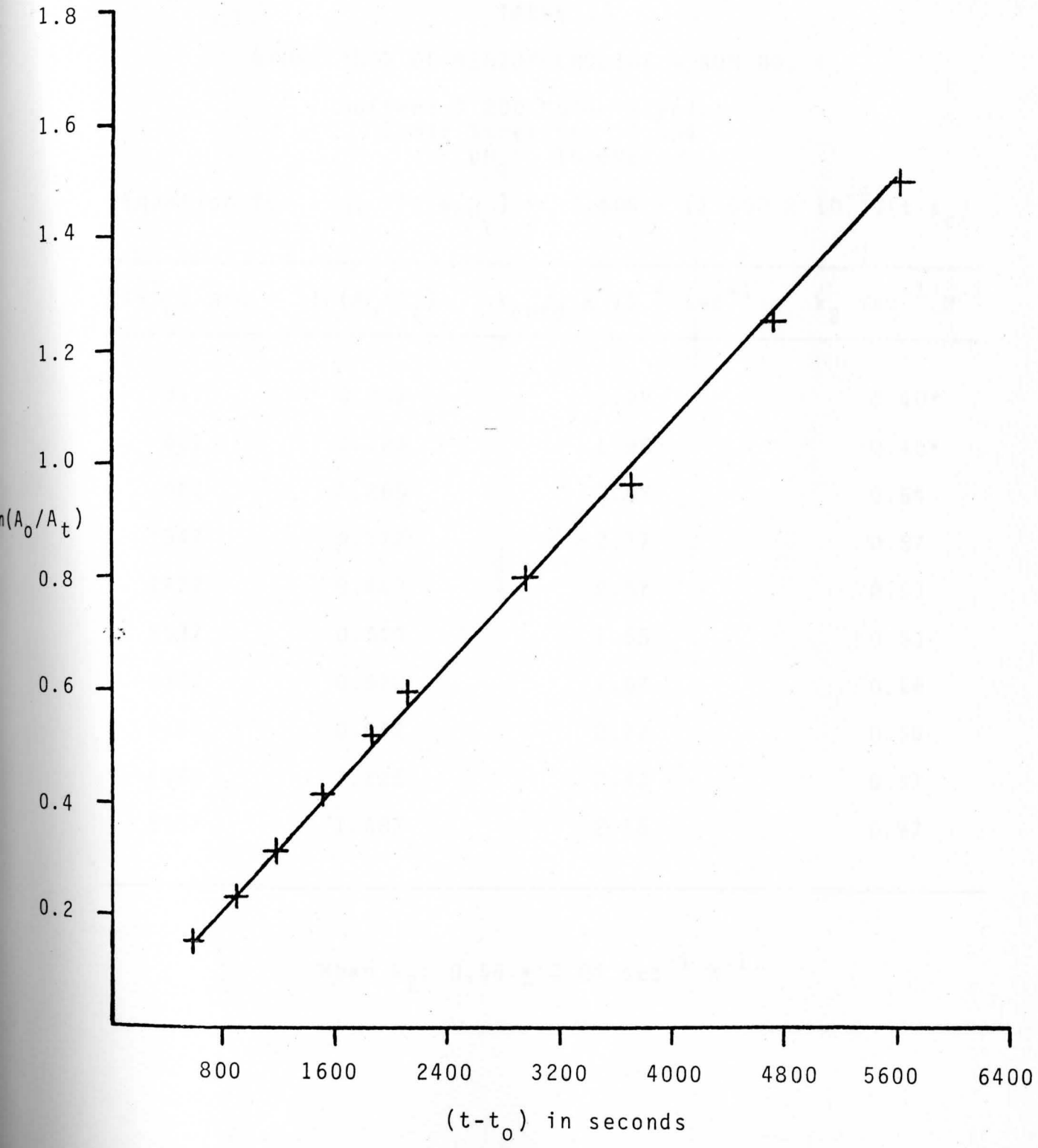


Figure 8 Hydrolysis of benzoylcholine in 0.200 molar glycine buffer at ionic strength of 0.454

TABLE 10

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 4

Buffer: 0.200 Molar Glycine
Ionic Strength: 0.454
pH_o: 10.696

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.608 - (1.660 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
317	0.063	1.99	0.40*
627	0.123	1.96	0.40*
987	0.265	2.68	0.54
1347	0.372	2.77	0.57
1722	0.443	2.57	0.53
2037	0.519	2.55	0.53
2327	0.620	2.67	0.56
3167	0.862	2.72	0.58
4662	1.225	2.63	0.57
6557	1.667	2.56	0.57

Mean k_2: 0.56 ± 0.04 sec^{-1} M^{-1}

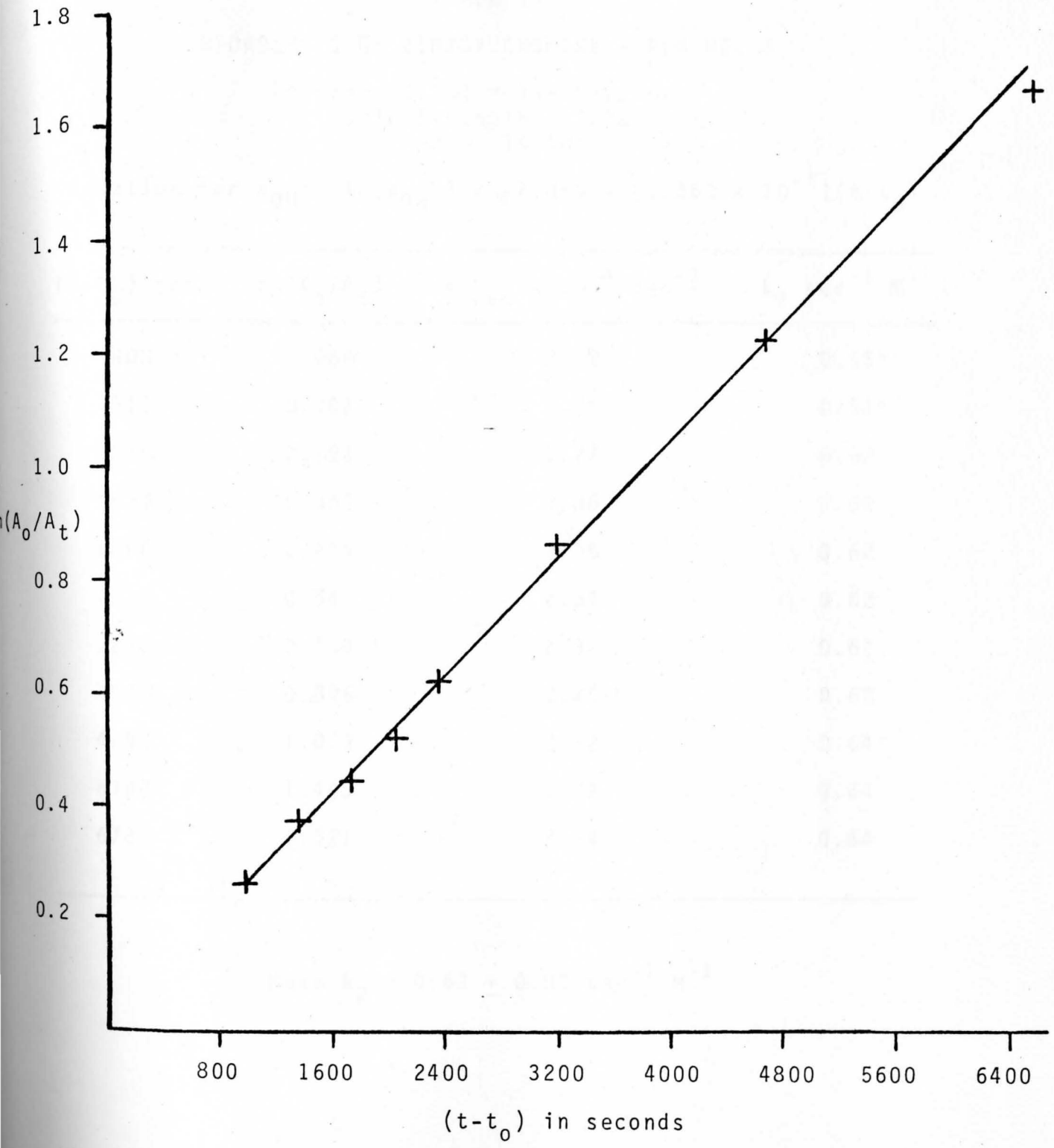


Figure 9 Hydrolysis of benzoylcholine in 0.200 molar glycine buffer at ionic strength of 0.454

TABLE 11

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 5

Buffer: 0.200 Molar Glycine
Ionic Strength: 1.20
pH_o: 10.587

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.859 - (1.350 \times 10^{-5})(t-t_0)$

$(t-t_0)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
405	0.086	2.12	0.55*
915	0.207	2.26	0.57*
1230	0.291	2.37	0.62
1530	0.362	2.36	0.62
1860	0.438	2.35	0.62
2310	0.541	2.34	0.62
2830	0.658	2.32	0.62
3520	0.806	2.29	0.62
4630	1.073	2.32	0.64
6360	1.443	2.27	0.64
6780	1.521	2.24	0.64

Mean k_2: 0.62 ± 0.02 sec^{-1} M^{-1}

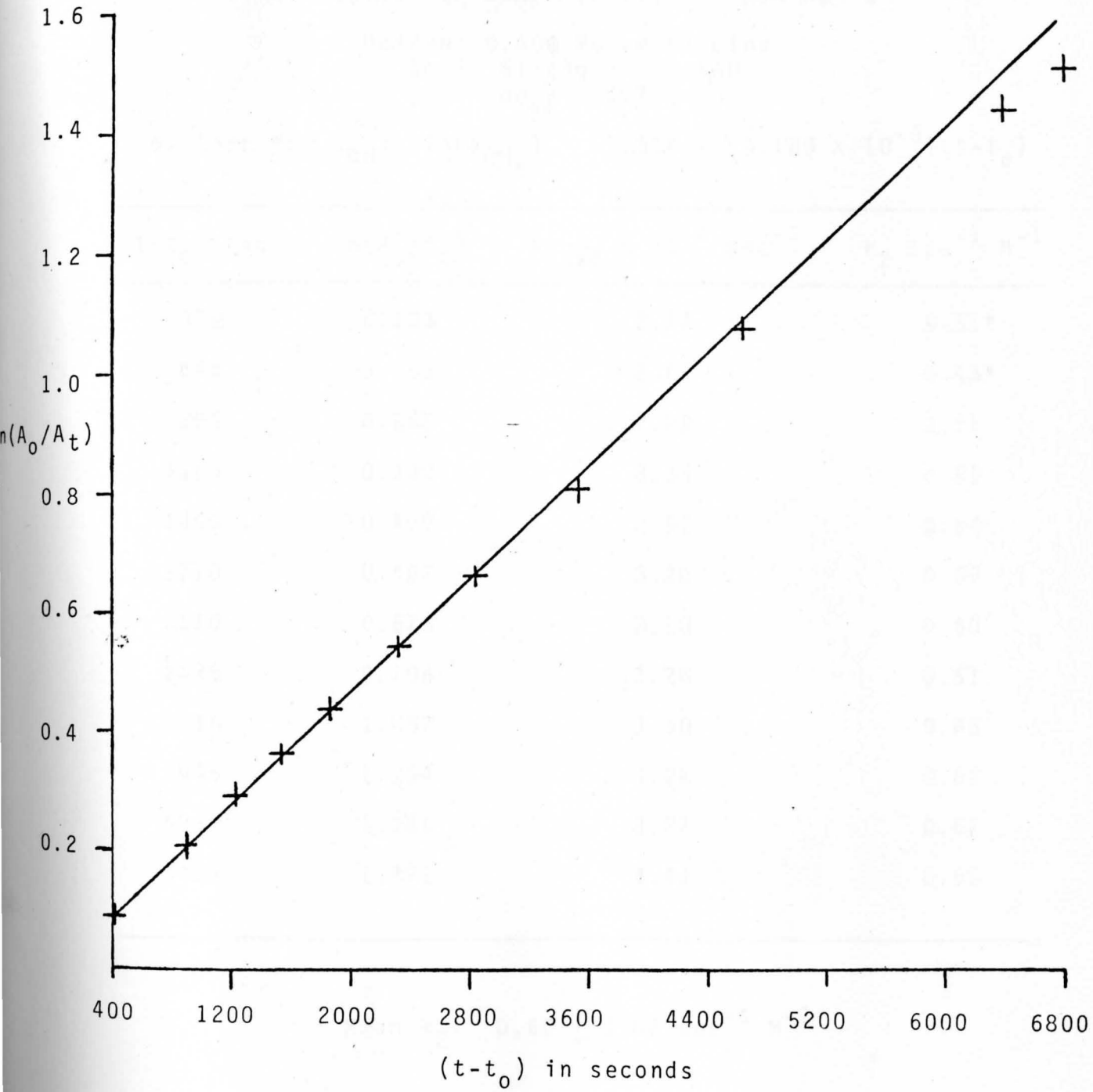


Figure 10 Hydrolysis of benzoylcholine in 0.200 molar glycine buffer at ionic strength of 1.20

TABLE 12

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 6

Buffer: 0.400 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.735

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.518 - (5.120 \times 10^{-6})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
370	0.103	2.77	0.51*
645	0.169	2.62	0.48*
895	0.268	2.99	0.55
1165	0.390	3.35	0.62
1400	0.409	2.92	0.54
1770	0.567	3.20	0.59
2110	0.675	3.20	0.60
2425	0.796	3.28	0.61
3015	1.057	3.50	0.65
3945	1.294	3.28	0.62
4235	1.386	3.27	0.62
5485	1.871	3.41	0.65

Mean k_2: 0.60 ± 0.07 sec^{-1} M^{-1}

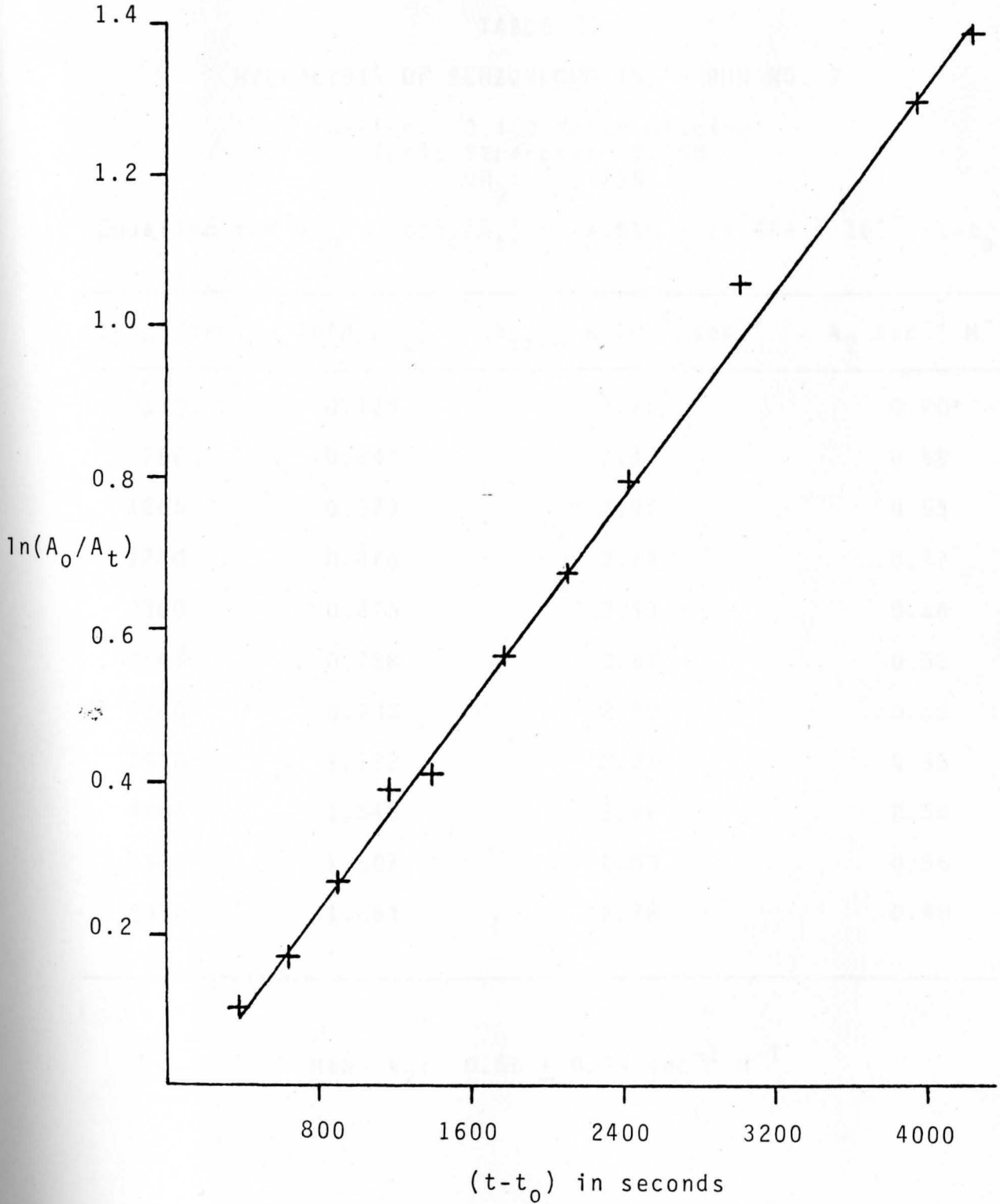


Figure 11 Hydrolysis of benzoylcholine in 0.400 molar glycine buffer at ionic strength of 0.660

TABLE 13

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 7

Buffer: 0.400 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.735

Equation for a_{OH}: $\ln(A_o/A_t) = -7.518 - (1.484 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
330	0.125	3.79	0.70*
760	0.242	3.18	0.59
1265	0.373	2.95	0.55
1740	0.476	2.74	0.52
2300	0.675	2.93	0.56
2745	0.788	2.87	0.55
3240	0.933	2.89	0.56
3910	1.122	2.87	0.56
4705	1.346	2.86	0.56
5380	1.507	2.80	0.56
5970	1.653	2.78	0.56

Mean k_2: 0.56 ± 0.03 sec^{-1} M^{-1}

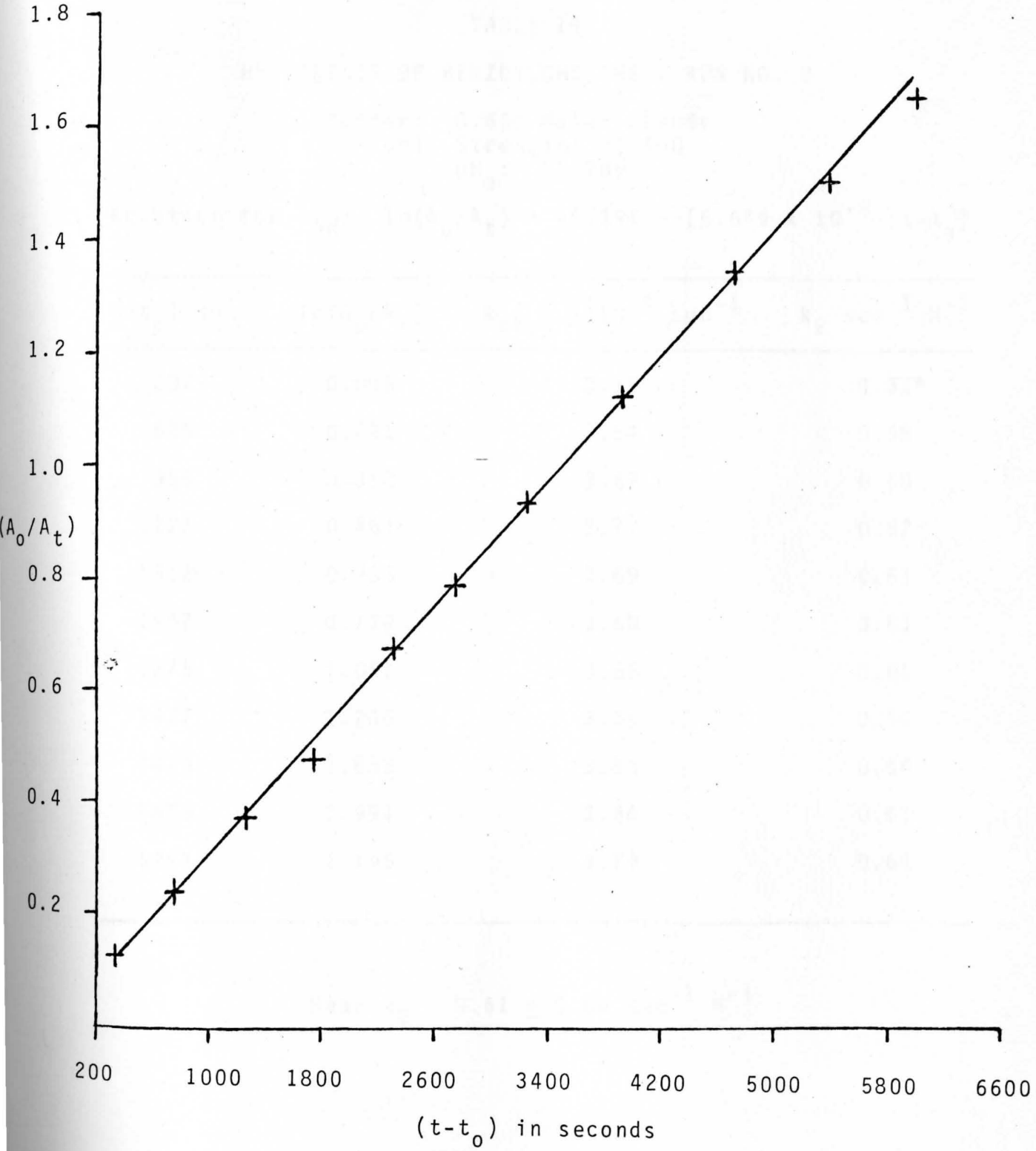


Figure 12 Hydrolysis of benzoylcholine in 0.400 molar glycine buffer at ionic strength of 0.660

TABLE 14

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 8

Buffer: 0.600 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.789

Equation for a_{OH}: $\ln(A_o/A_t) = -7.394 - (6.679 \times 10^{-6})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
297	0.095	3.19	0.52*
625	0.221	3.54	0.58
955	0.350	3.67	0.60
1227	0.463	3.77	0.62
1512	0.558	3.69	0.61
1957	0.720	3.68	0.61
2875	1.051	3.66	0.61
3477	1.236	3.56	0.59
4425	1.682	3.80	0.64
5475	1.994	3.64	0.61
5797	2.195	3.79	0.64

Mean k_2: 0.61 ± 0.04 sec^{-1} M^{-1}

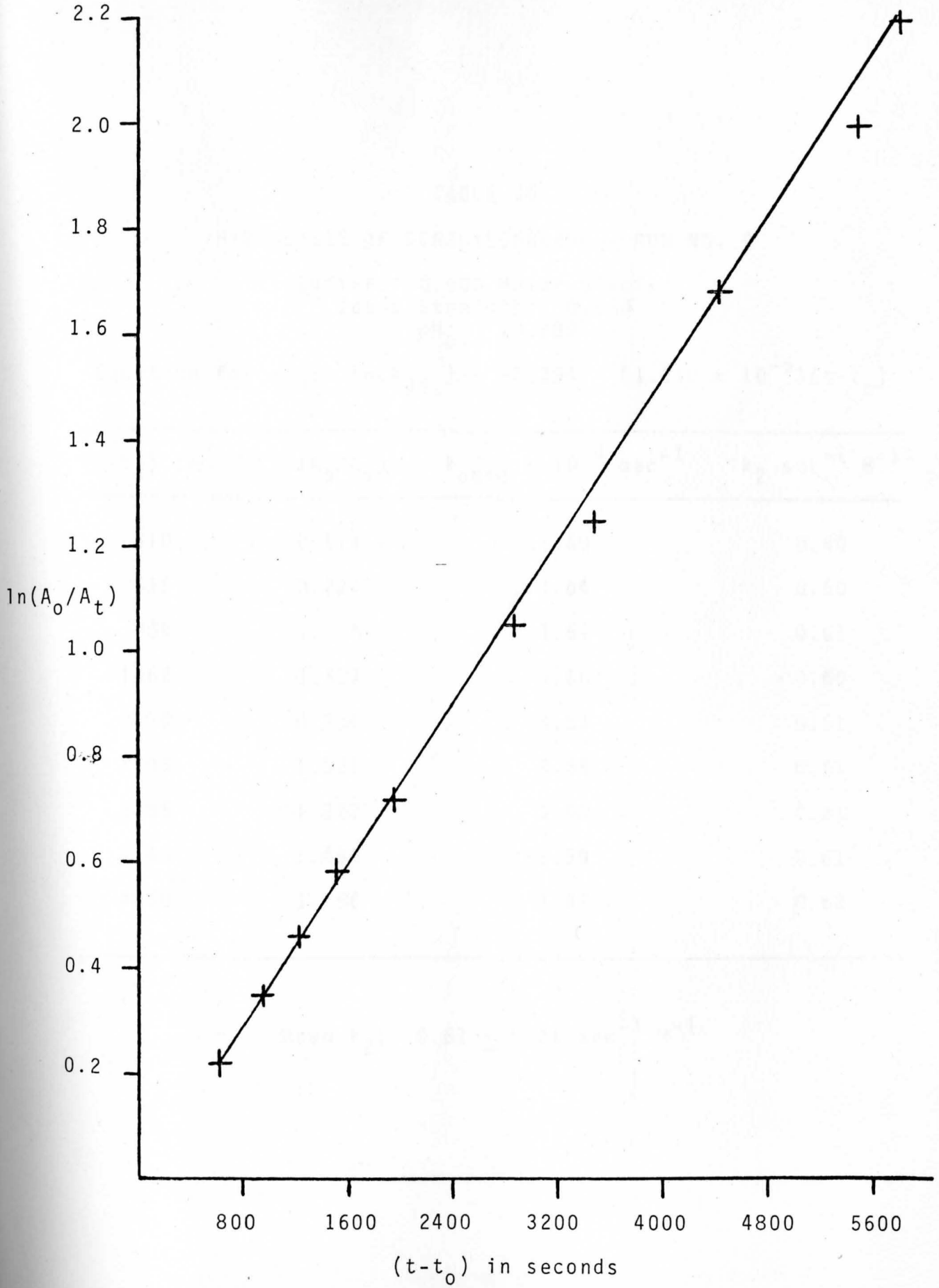


Figure 13 Hydrolysis of benzoylcholine in 0.600 molar

TABLE 15

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 9

Buffer: 0.600 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.789

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.394 - (1.210 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
310	0.114	3.68	0.60
615	0.224	3.64	0.60
965	0.356	3.69	0.61
1465	0.527	3.60	0.60
2080	0.754	3.63	0.61
2795	1.021	3.65	0.61
3395	1.222	3.60	0.61
4165	1.496	3.59	0.61
4850	1.756	3.62	0.62

Mean k_2: 0.61 ± 0.01 sec^{-1} M^{-1}

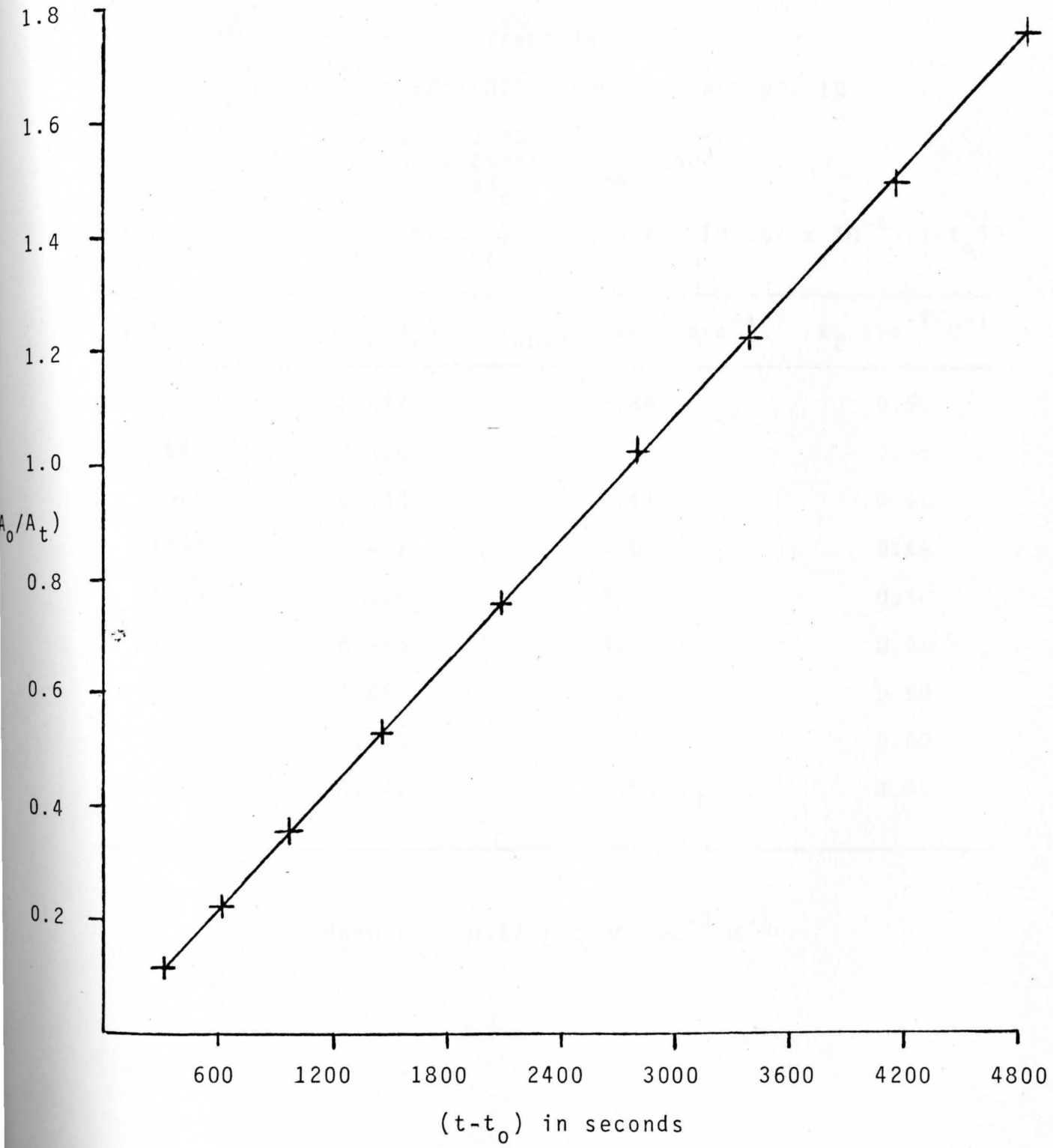


Figure 14 Hydrolysis of benzoylcholine in 0.600 molar glycine buffer at ionic strength of 0.660

TABLE 16

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 10

Buffer: 0.600 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.789

Equation for a_{OH}: $\ln(a_{OH_t}) = - 7.394 - (8.167 \times 10^{-6})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
290	0.097	3.36	0.55
625	0.210	3.37	0.55
975	0.335	3.43	0.55
1390	0.497	3.58	0.59
1705	0.616	3.61	0.58
2167	0.786	3.63	0.58
2810	1.037	3.69	0.59
3123	1.160	3.72	0.60
3665	1.348	3.68	0.58

Mean k_2: 0.57 ± 0.04 sec^{-1} M^{-1}

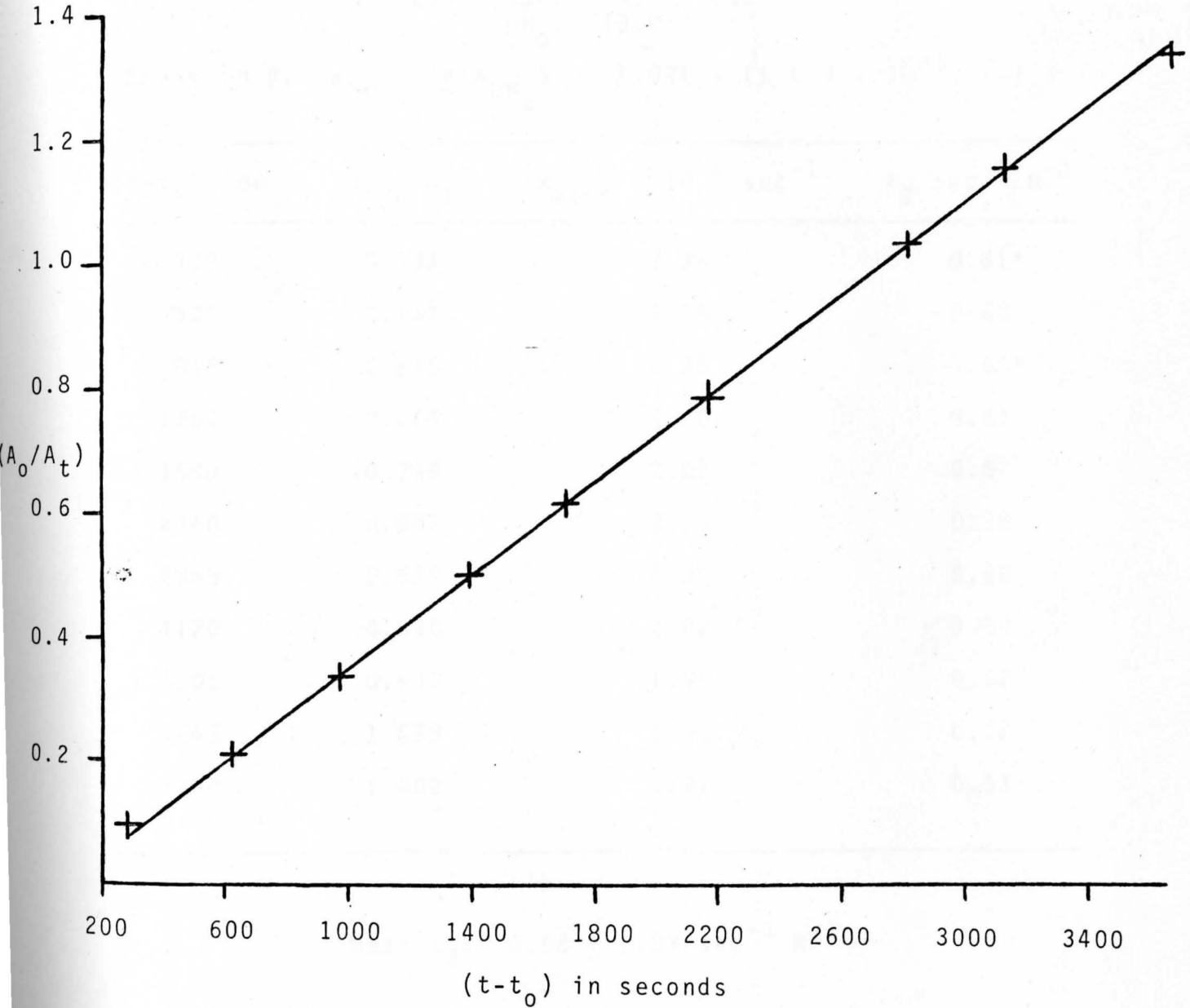


Figure 15 Hydrolysis of benzoylcholine in 0.600 molar glycine buffer at ionic strength of 0.660

TABLE 17

HYDROLYSIS OF BENZOYLCHOLINE - RUN NO. 11

Buffer: 0.125 Molar Bicarbonate
Ionic Strength: 0.660
pH_o: 10.560

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.921 - (1.009 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
330	0.097	2.94	0.81*
630	0.137	2.18	0.60
915	0.215	2.35	0.65*
1200	0.264	2.20	0.61
1580	0.319	2.02	0.57
2360	0.483	2.05	0.58
2965	0.619	2.09	0.59
4120	0.830	2.02	0.58
5105	0.993	1.95	0.57
5645	1.099	1.95	0.57
7300	1.402	1.92	0.57

Mean k_2: 0.58 ± 0.03 sec^{-1} M^{-1}

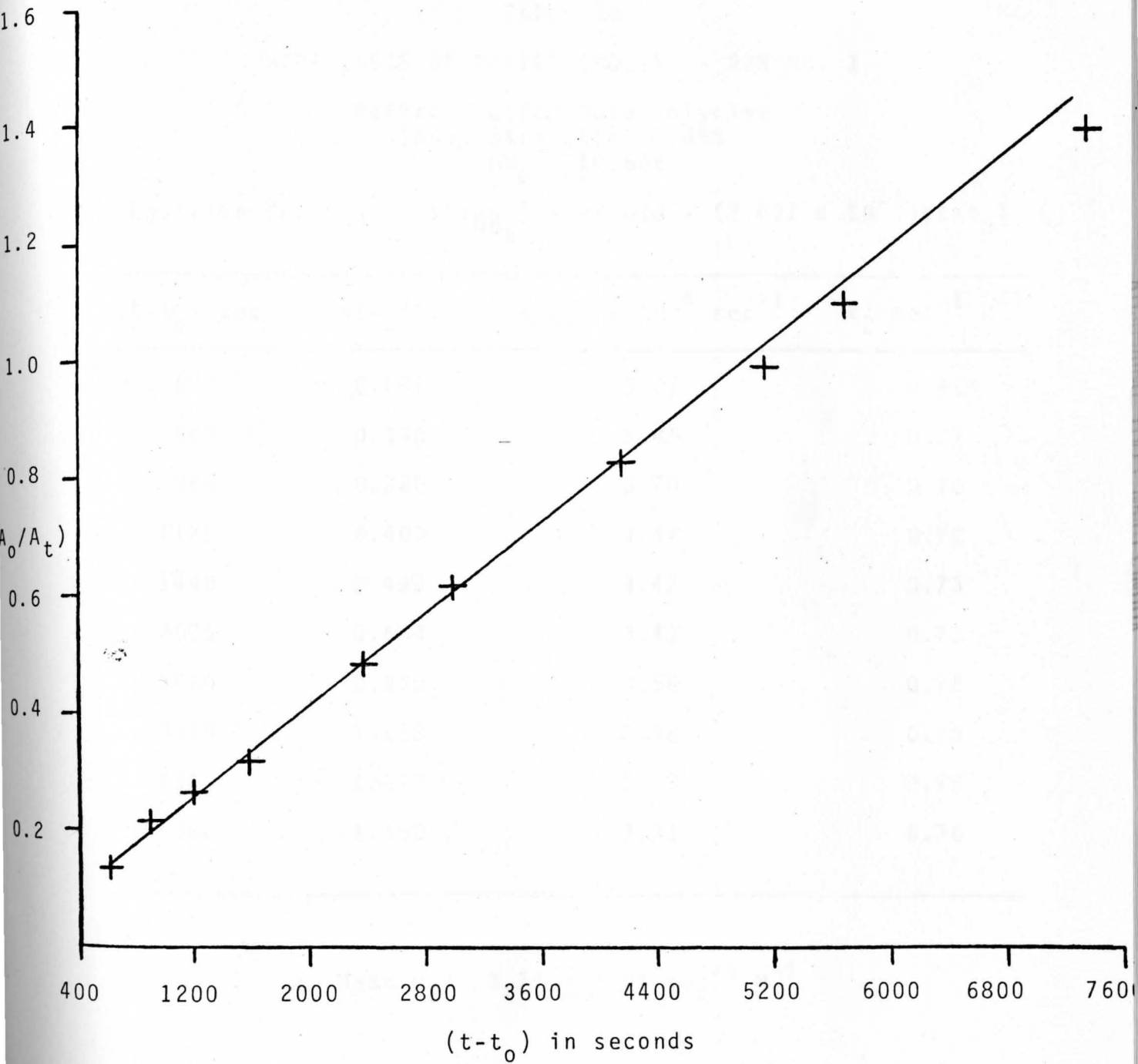


Figure 16 Hydrolysis of benzoylcholine in 0.125 molar bicarbonate buffer at ionic strength of 0.660

TABLE 18

HYDROLYSIS OF BUTYRYLCHOLINE - RUN NO. 1

Buffer: 0.200 Molar Glycine
Ionic Strength: 0.454
pH_o: 10.696

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.608 - (2.837 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
270	0.087	3.22	0.65*
550	0.190	3.45	0.71
865	0.320	3.70	0.76
1175	0.405	3.45	0.72
1440	0.499	3.47	0.73
2005	0.684	3.41	0.73
2660	0.930	3.50	0.76
3310	1.118	3.38	0.75
4310	1.412	3.28	0.75
4680	1.550	3.31	0.76

Mean k_2: 0.74 ± 0.04 sec^{-1} M^{-1}

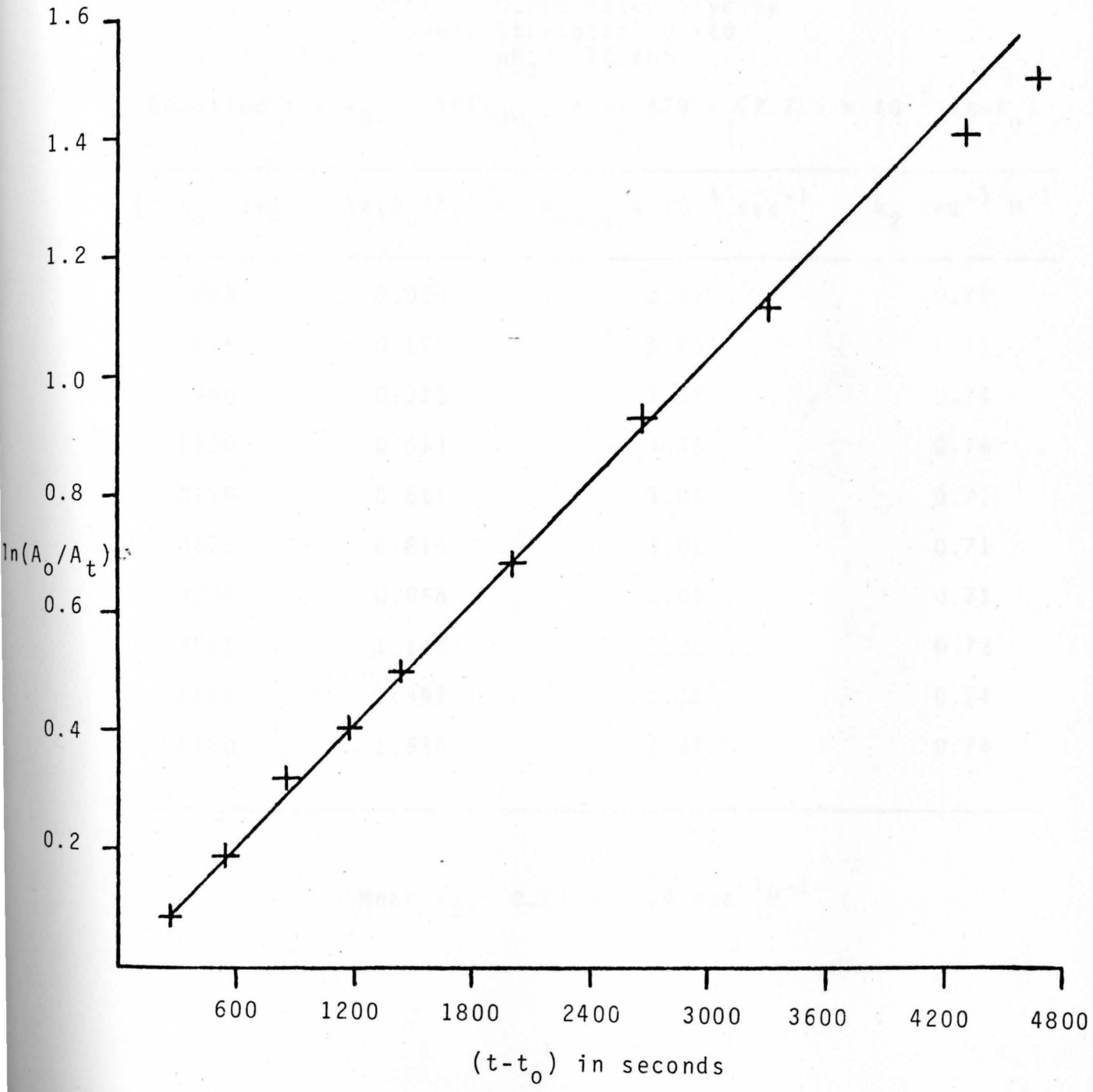


Figure 17 Hydrolysis of butyrylcholine in 0.200 molar glycine buffer at ionic strength of 0.454

TABLE 19

HYDROLYSIS OF BUTYRYLCHOLINE - RUN NO. 2

Buffer: 0.200 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.665

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.679 - (2.715 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
295	0.098	3.31	0.72
605	0.195	3.23	0.71
950	0.315	3.32	0.74
1530	0.513	3.35	0.76
2115	0.651	3.08	0.71
2675	0.816	3.05	0.71
3205	0.968	3.02	0.71
3825	1.149	3.00	0.72
4645	1.392	3.00	0.74
5180	1.538	2.97	0.74

Mean k_2: 0.73 ± 0.04 $sec^{-1}M^{-1}$

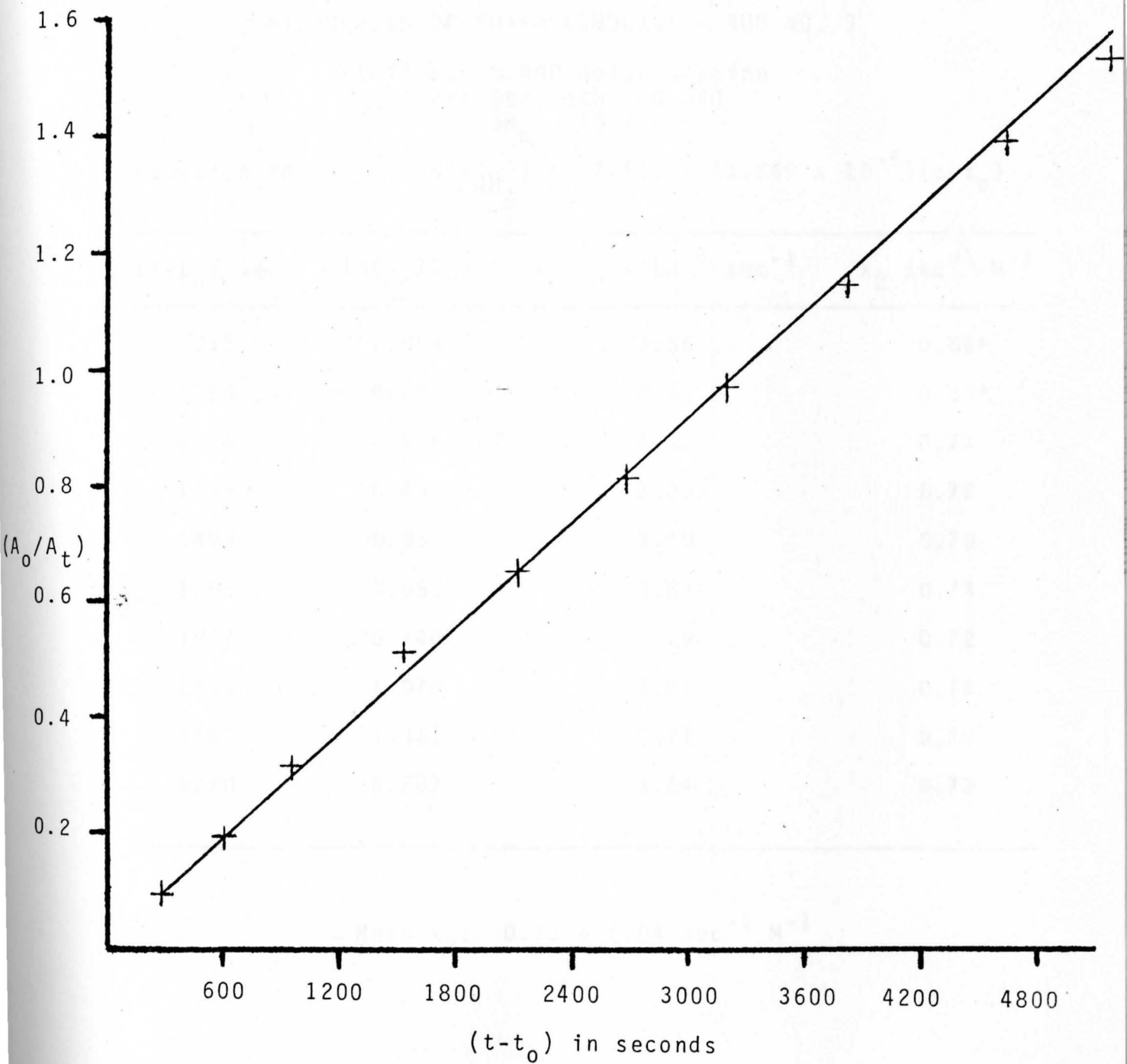


Figure 18 Hydrolysis of butyrylcholine in 0.200 molar glycine buffer at ionic strength of 0.660

TABLE 20

HYDROLYSIS OF BUTYRYLCHOLINE - RUN NO. 3

Buffer: 0.400 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.737

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.513 - (1.869 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
235	0.084	3.56	0.66*
790	0.276	3.49	0.65*
1055	0.434	4.11	0.77
1285	0.491	3.82	0.72
1495	0.552	3.69	0.70
1698	0.651	3.84	0.73
1952	0.740	3.79	0.72
2830	1.078	3.81	0.74
3607	1.361	3.77	0.74
4770	1.737	3.64	0.73

Mean k_2: 0.73 ± 0.04 sec^{-1} M^{-1}

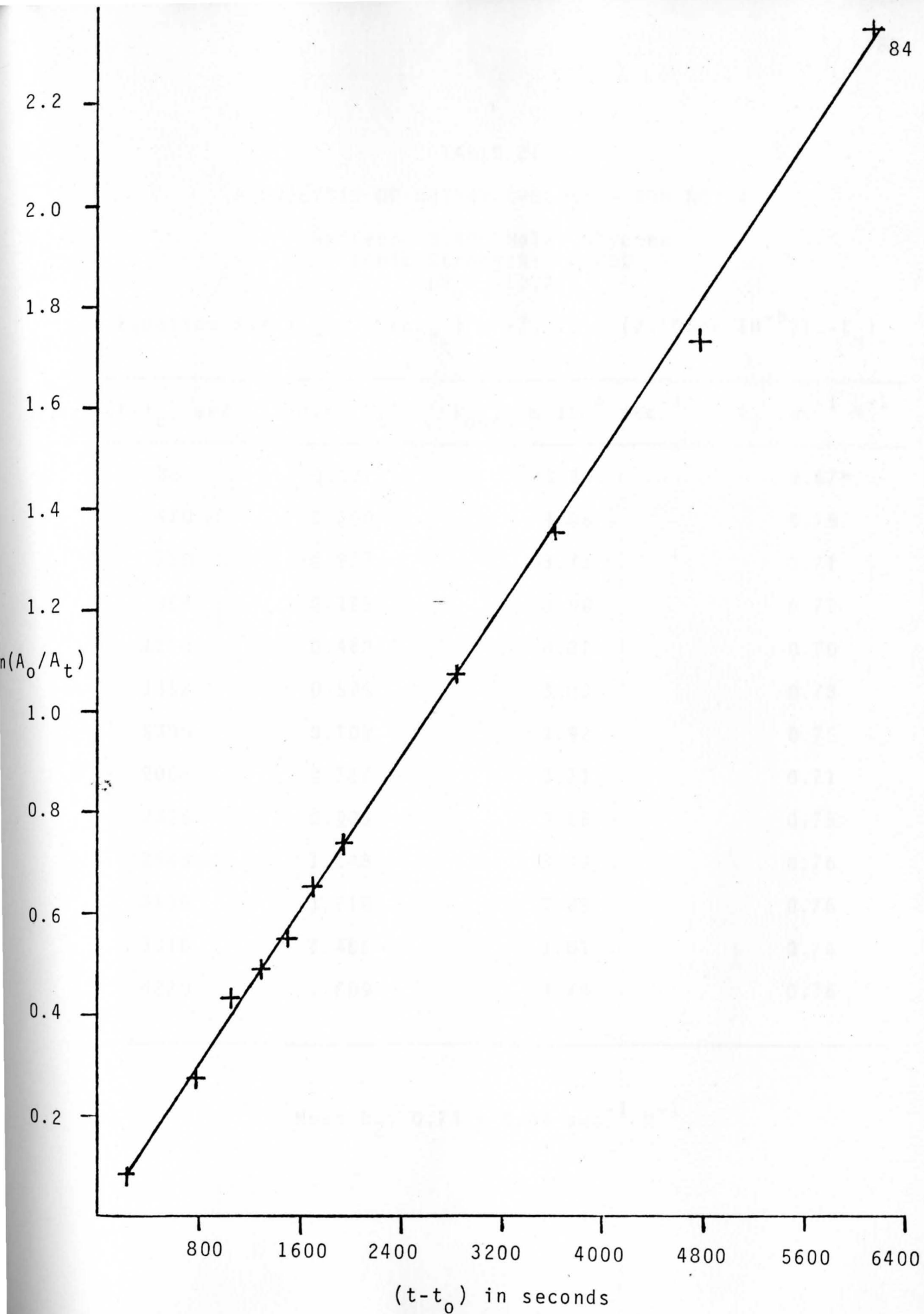


Figure 19. Hydrolysis of butynylchali...

TABLE 21

HYDROLYSIS OF BUTYRYLCHOLINE - RUN NO. 4

Buffer: 0.400 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.737

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.513 - (2.105 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
267	0.097	3.64	0.67*
470	0.190	4.05	0.75
730	0.277	3.79	0.71
988	0.385	3.90	0.73
1210	0.463	3.82	0.70
1552	0.595	3.83	0.73
1805	0.708	3.92	0.75
2068	0.767	3.71	0.71
2325	0.903	3.88	0.75
2945	1.145	3.89	0.76
3130	1.215	3.88	0.76
3910	1.489	3.81	0.76
4270	1.609	3.79	0.76

Mean k_2: 0.74 ± 0.04 sec^{-1} M^{-1}

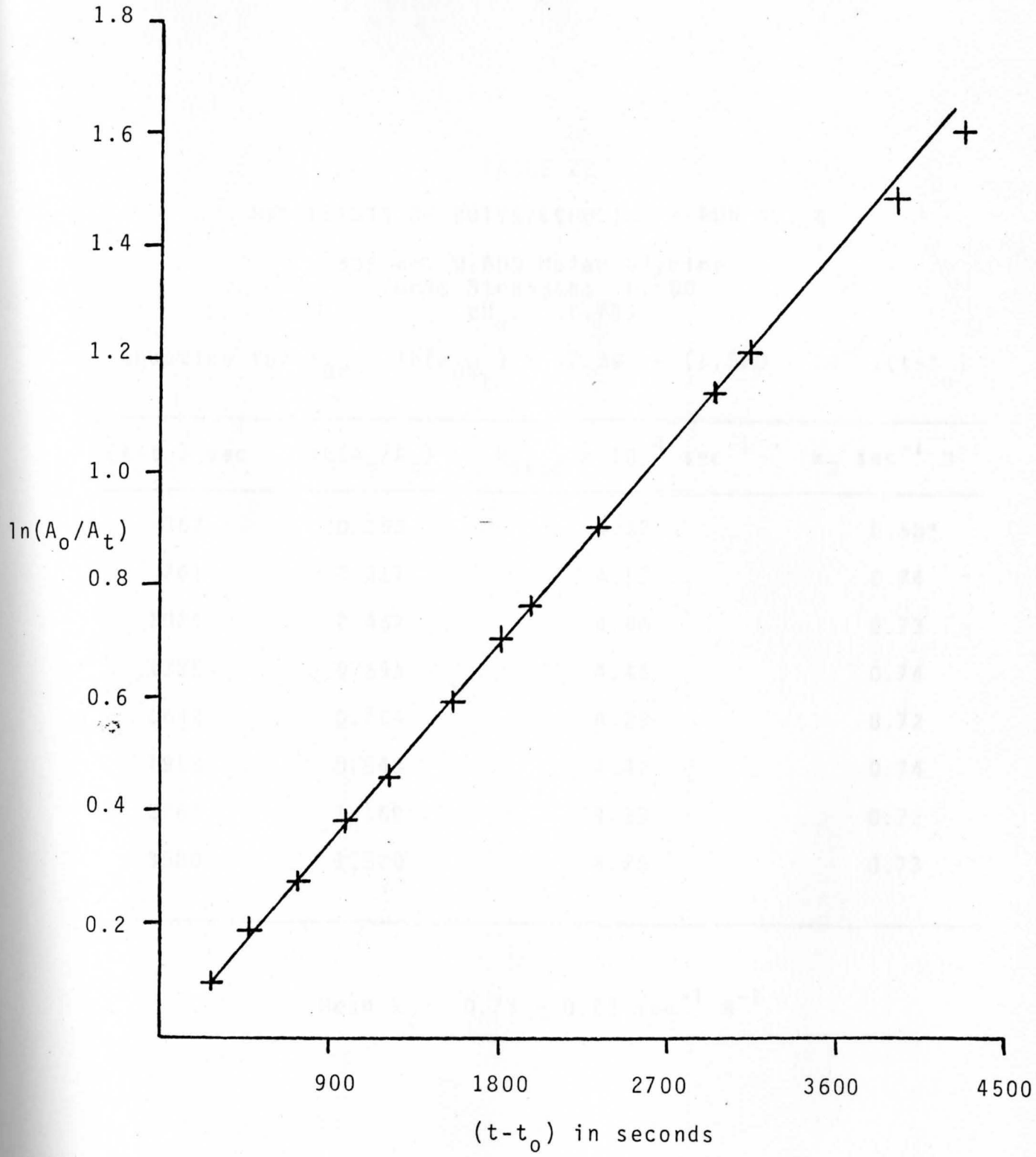


Figure 20 Hydrolysis of butyrylcholine in 0.400 molar glycine buffer at ionic strength of 0.660

TABLE 22

HYDROLYSIS OF BUTYRYLCHOLINE - RUN NO. 6

Buffer: 0.600 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.789

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.394 - (1.520 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
367	0.153	4.17	0.68*
701	0.317	4.52	0.74
1025	0.457	4.45	0.73
1325	0.591	4.46	0.74
1642	0.704	4.29	0.72
1919	0.848	4.42	0.74
2765	1.169	4.23	0.72
3580	1.520	4.25	0.73

Mean k_2: 0.73 ± 0.02 sec^{-1} M^{-1}

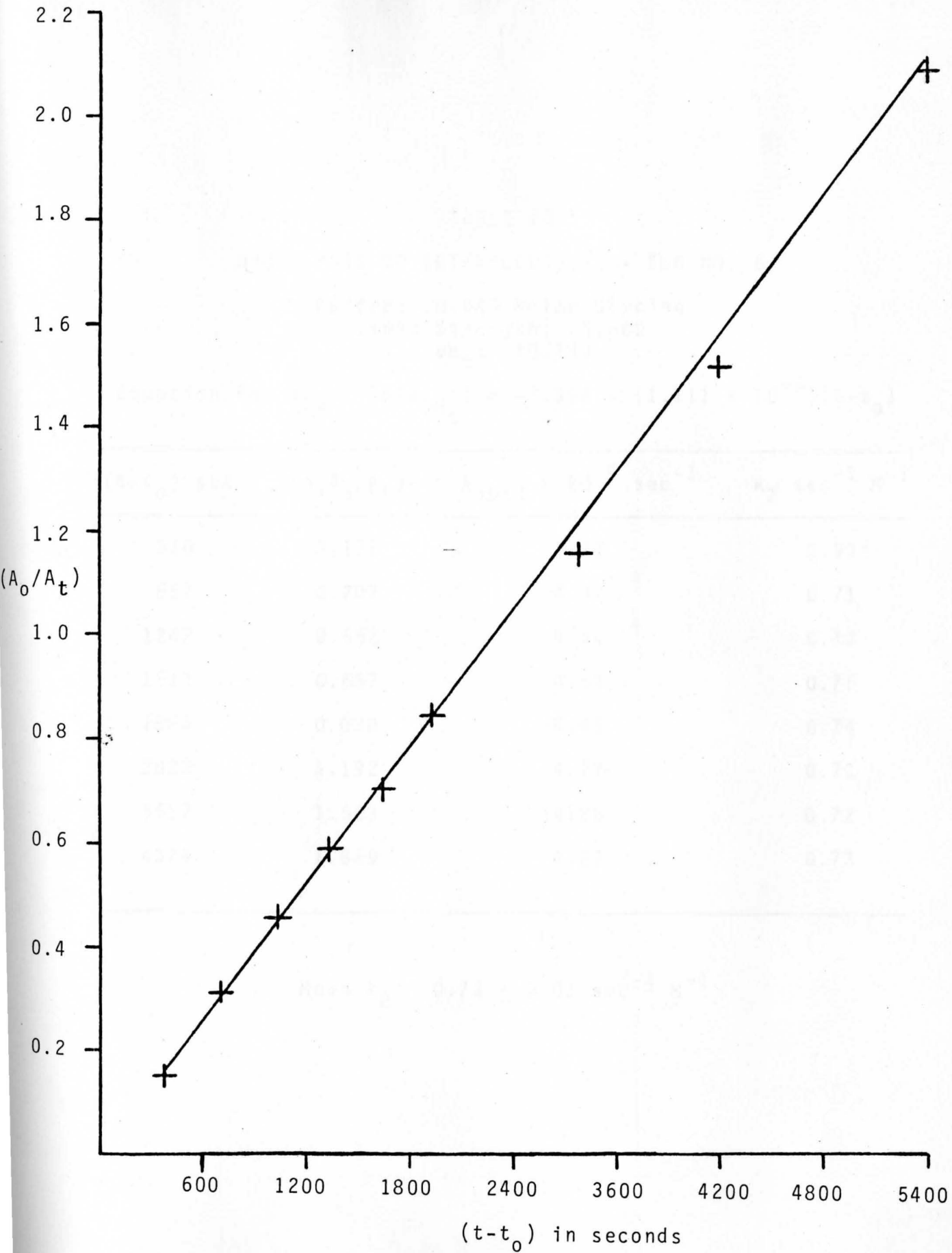


Figure 21 Hydrolysis of butyrylcholine in 0.600 molar

TABLE 23

HYDROLYSIS OF BUTYRYLCHOLINE - RUN NO. 5

Buffer: 0.600 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.789

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.394 - (1.111 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4$ sec^{-1}	k_2 sec^{-1} M^{-1}
310	0.177	5.71	0.93*
657	0.287	4.37	0.71
1242	0.552	4.44	0.73
1513	0.687	4.54	0.75
1884	0.838	4.45	0.74
2822	1.192	4.23	0.71
3532	1.503	4.26	0.72
4374	1.869	4.27	0.73

Mean k_2: 0.73 ± 0.03 sec^{-1} M^{-1}

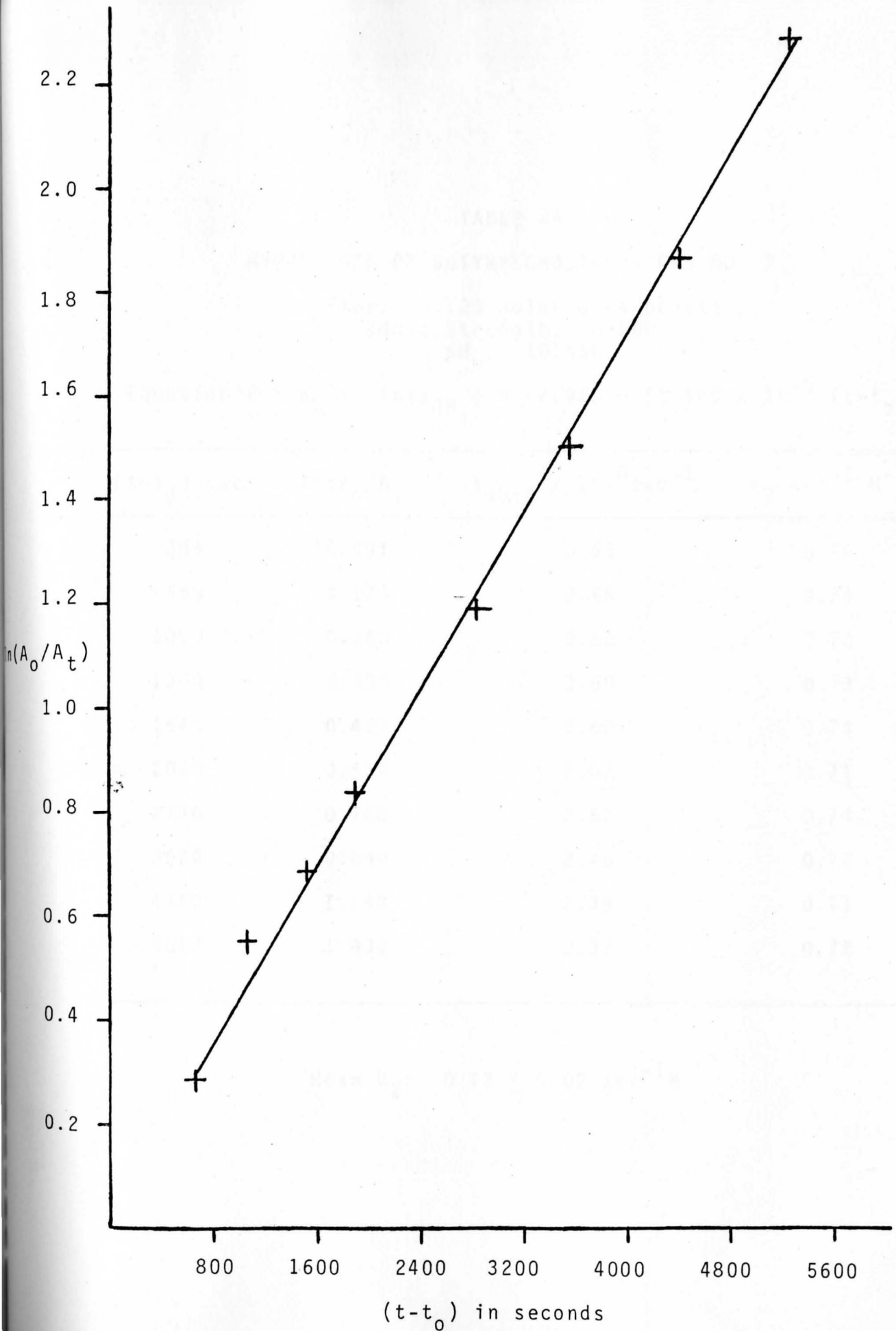


Figure 22. Hydrolysis of butyrylcholine in 0.600 molar

TABLE 24

HYDROLYSIS OF BUTYRYLCHOLINE - RUN NO. 7

Buffer: 0.125 Molar Bicarbonate
Ionic Strength: 0.660
pH_o: 10.558

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.925 - (1.560 \times 10^{-5})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^4 sec^{-1}$	k_2 sec^{-1} M^{-1}
345	0.091	2.65	0.74
650	0.173	2.66	0.74
1000	0.260	2.60	0.73
1300	0.339	2.60	0.73
1645	0.427	2.60	0.74
2060	0.509	2.47	0.71
2730	0.700	2.57	0.74
3620	0.890	2.46	0.72
4390	1.048	2.39	0.71
6050	1.432	2.37	0.72

Mean k_2: 0.73 ± 0.02 $sec^{-1}M^{-1}$

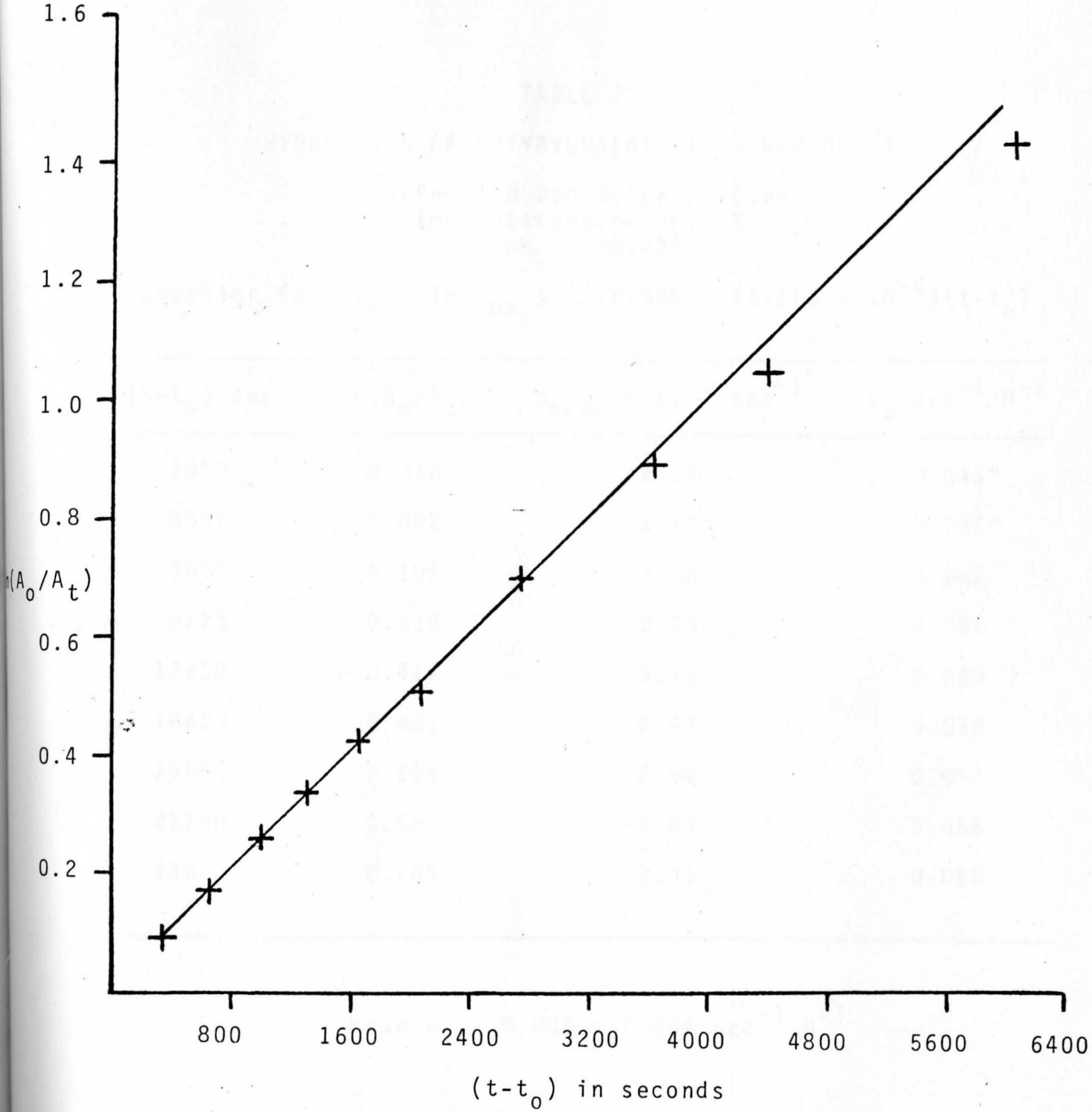


Figure 23 Hydrolysis of butyrylcholine in 0.125 molar bicarbonate buffer at ionic strength of 0.660

TABLE 25

HYDROLYSIS OF BUTYRYLCARNITINE - RUN NO. 1

Buffer: 0.200 Molar Glycine
Ionic Strength: 0.201
pH_0: 10.739

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.508 - (2.752 \times 10^{-6})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^5$ sec^{-1}	k_2 sec^{-1} M^{-1}
1980	0.046	2.33	0.043*
3390	0.092	2.73	0.050*
5855	0.197	3.36	0.062
9725	0.316	3.25	0.061
13210	0.417	3.16	0.060
16420	0.481	2.93	0.056
19055	0.564	2.96	0.057
22760	0.683	3.00	0.058
23670	0.708	2.99	0.058

Mean k_2: 0.059 ± 0.004 sec^{-1} M^{-1}

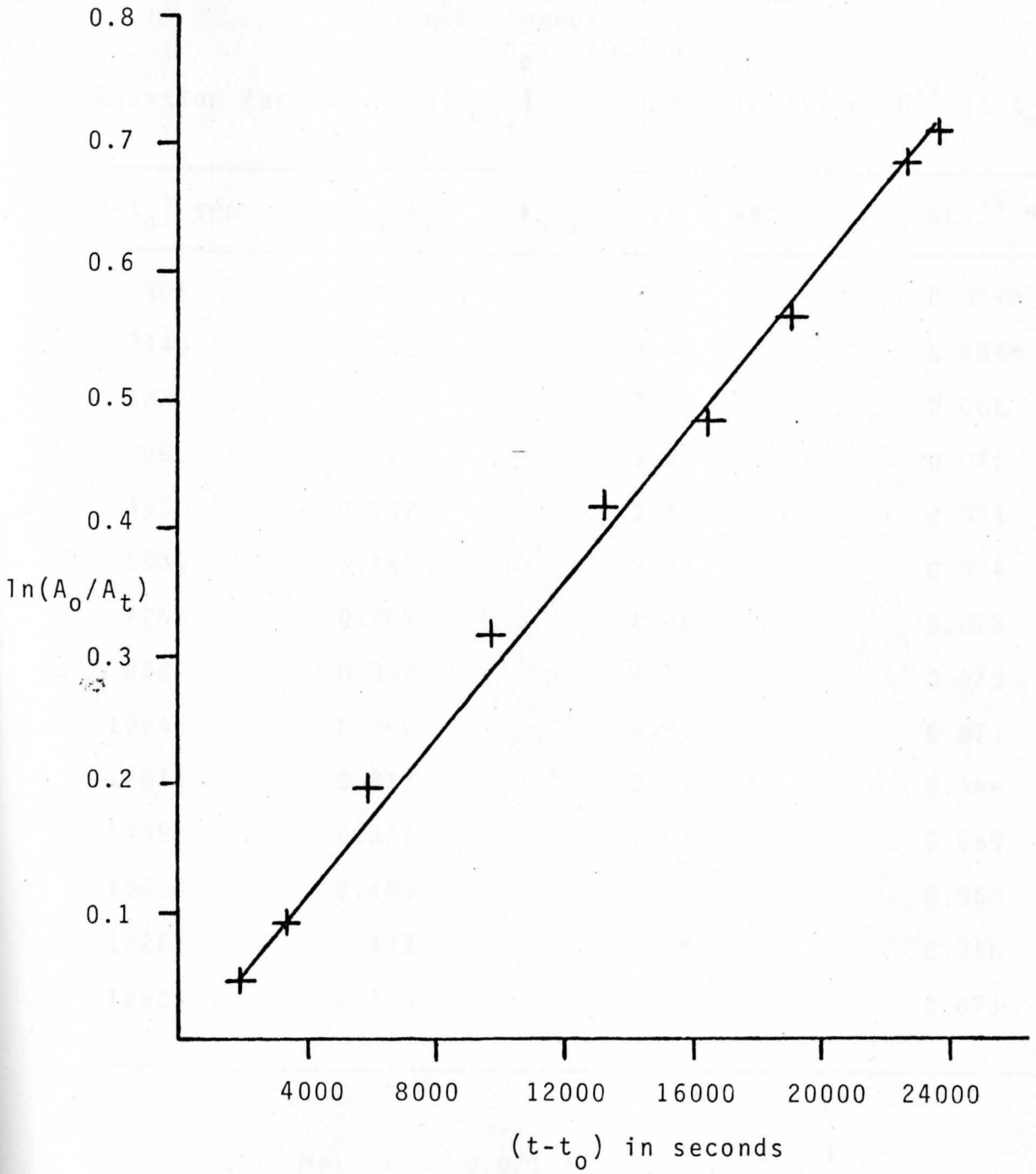


Figure 24 Hydrolysis of butyrylcarnitine in 0.200 molar glycine buffer at ionic strength of 0.201

TABLE 26

HYDROLYSIS OF BUTYRYLCARNITINE - RUN NO. 2

Buffer: 0.200 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.600

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.829 - (2.902 \times 10^{-6})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^5$ sec^{-1}	k_2 sec^{-1} M^{-1}
905	0.036	3.94	0.099*
2440	0.083	3.38	0.086*
3275	0.086	2.62	0.066
3985	0.120	3.00	0.076
4950	0.142	2.87	0.073
5630	0.163	2.89	0.074
7265	0.208	2.86	0.073
8585	0.232	2.70	0.070
10945	0.298	2.73	0.071
12275	0.312	2.54	0.066
14355	0.377	2.63	0.069
15400	0.406	2.64	0.069
17265	0.445	2.58	0.068
18605	0.500	2.69	0.071

Mean k_2: 0.071 ± 0.006 sec^{-1} M^{-1}

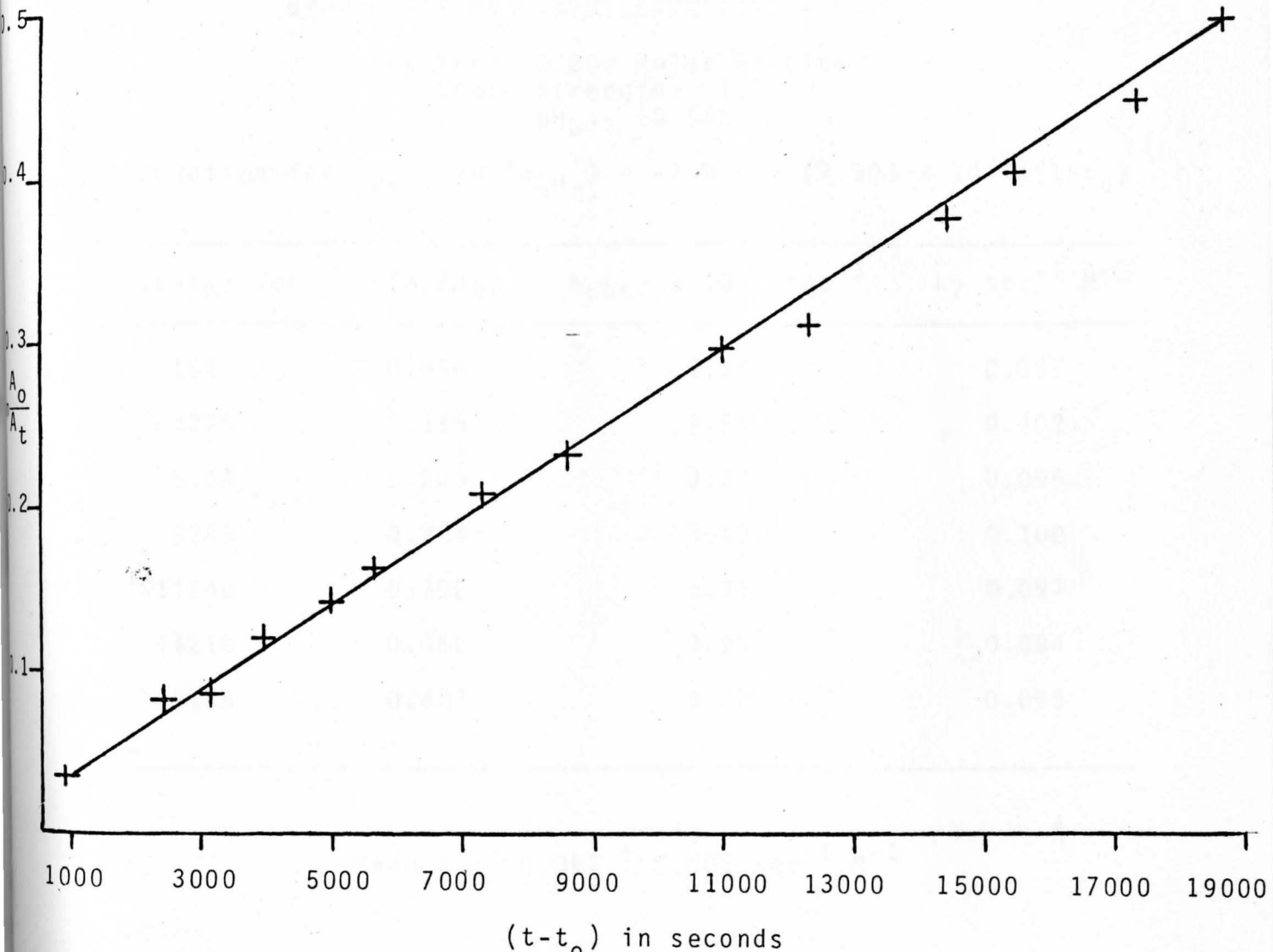


Figure 25 Hydrolysis of butyrylcarnitine in 0.200 molar glycine buffer at ionic strength of 0.660

TABLE 27

HYDROLYSIS OF BUTYRYLCARNITINE - NO. 3

Buffer: 0.200 Molar Glycine
Ionic Strength: 1.20
pH_o: 10.545

Equation for a_{OH}: $\ln (a_{OH_t}) = -7.955 - (2.393 \times 10^{-6})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^5$ sec^{-1}	k_2 sec^{-1} M^{-1}
1645	0.056	3.38	0.097
3235	0.115	3.54	0.102
6110	0.203	3.32	0.096
8285	0.284	3.43	0.100
11840	0.392	3.31	0.097
14210	0.455	3.20	0.094
15115	0.487	3.22	0.095

Mean k_2: 0.097 ± 0.006 sec^{-1} M^{-1}

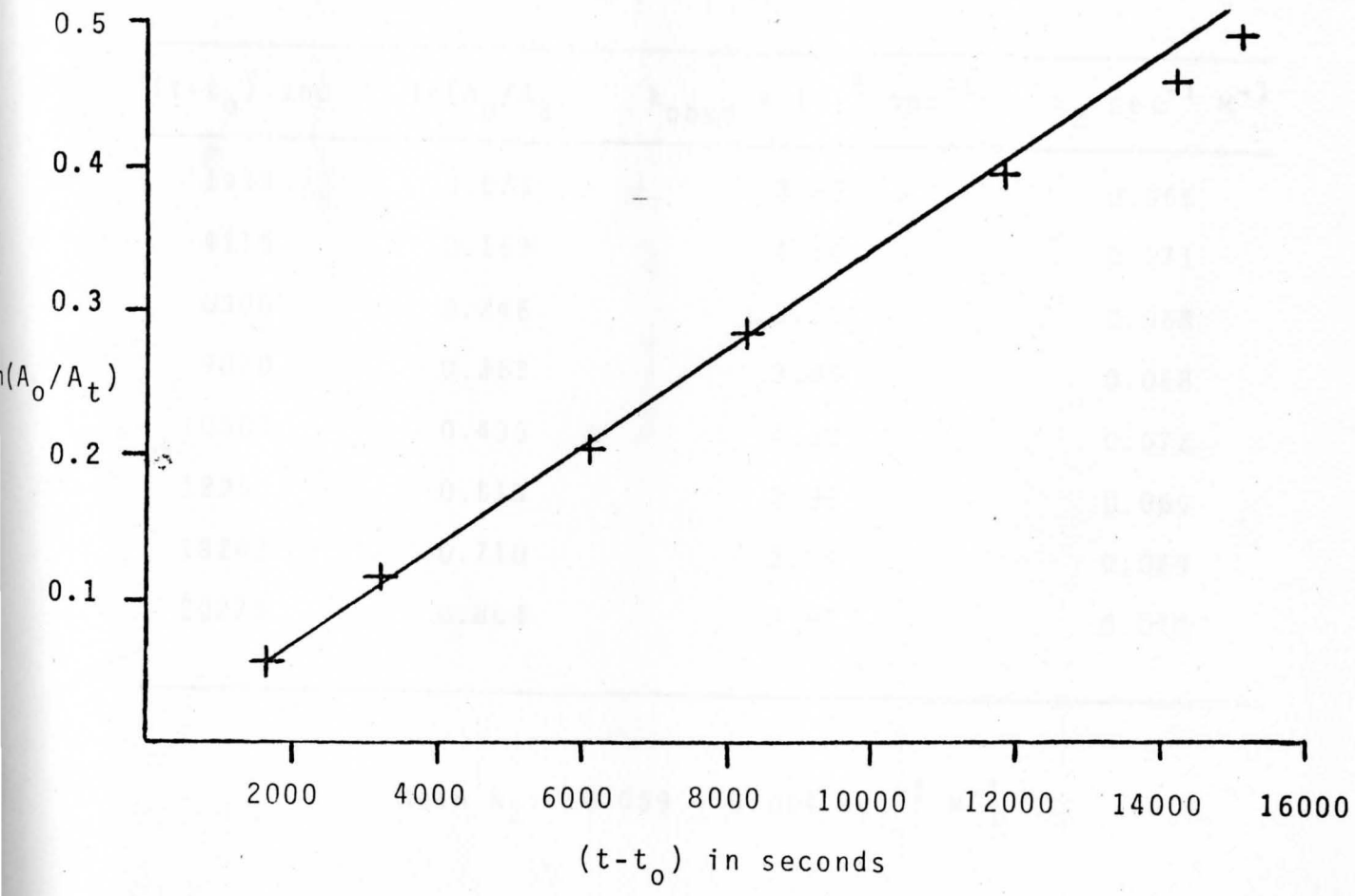


Figure 26 Hydrolysis of butyrylcarnitine in 0.200 molar glycine buffer at ionic strength of 1.20

TABLE 28

HYDROLYSIS OF BUTYRYLCARNITINE - RUN NO. 4

Buffer: 0.600 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.765

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.449 - (1.457 \times 10^{-6})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^5$ sec^{-1}	k_2 sec^{-1} M^{-1}
1953	0.075	3.83	0.066
4115	0.169	4.10	0.071
6305	0.246	3.90	0.068
9070	0.353	3.89	0.068
10560	0.435	4.12	0.072
12950	0.513	3.96	0.069
18243	0.710	3.89	0.069
20275	0.804	3.97	0.070

Mean k_2: 0.069 ± 0.004 sec^{-1} M^{-1}

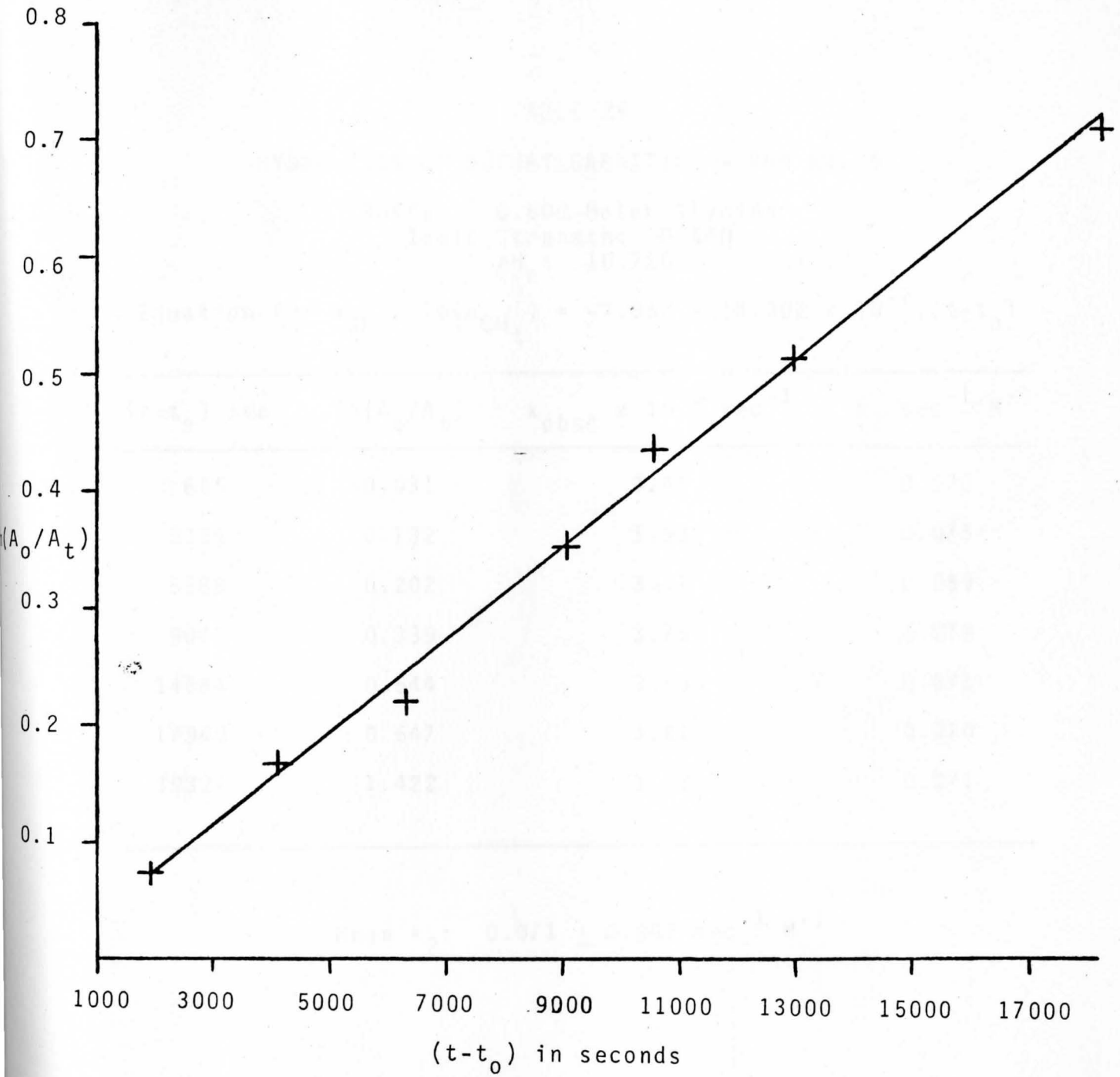


Figure 27 Hydrolysis of butyrylcarnitine in 0.600 molar glycine buffer at ionic strength of 0.660

TABLE 29

HYDROLYSIS OF BUTURYLCARNITINE - RUN NO. 5

Buffer: 0.600 Molar Glycine
Ionic Strength: 0.660
pH_o: 10.720

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.552 - (8.302 \times 10^{-7})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^5$ sec^{-1}	k_2 sec^{-1} M^{-1}
855	0.031	3.65	0.070
3361	0.132	3.93	0.075*
5588	0.202	3.61	0.069
9048	0.339	3.75	0.072
14684	0.544	3.70	0.071
17943	0.647	3.61	0.070
39324	1.422	3.62	0.071

Mean k_2: 0.071 ± 0.002 sec^{-1} M^{-1}

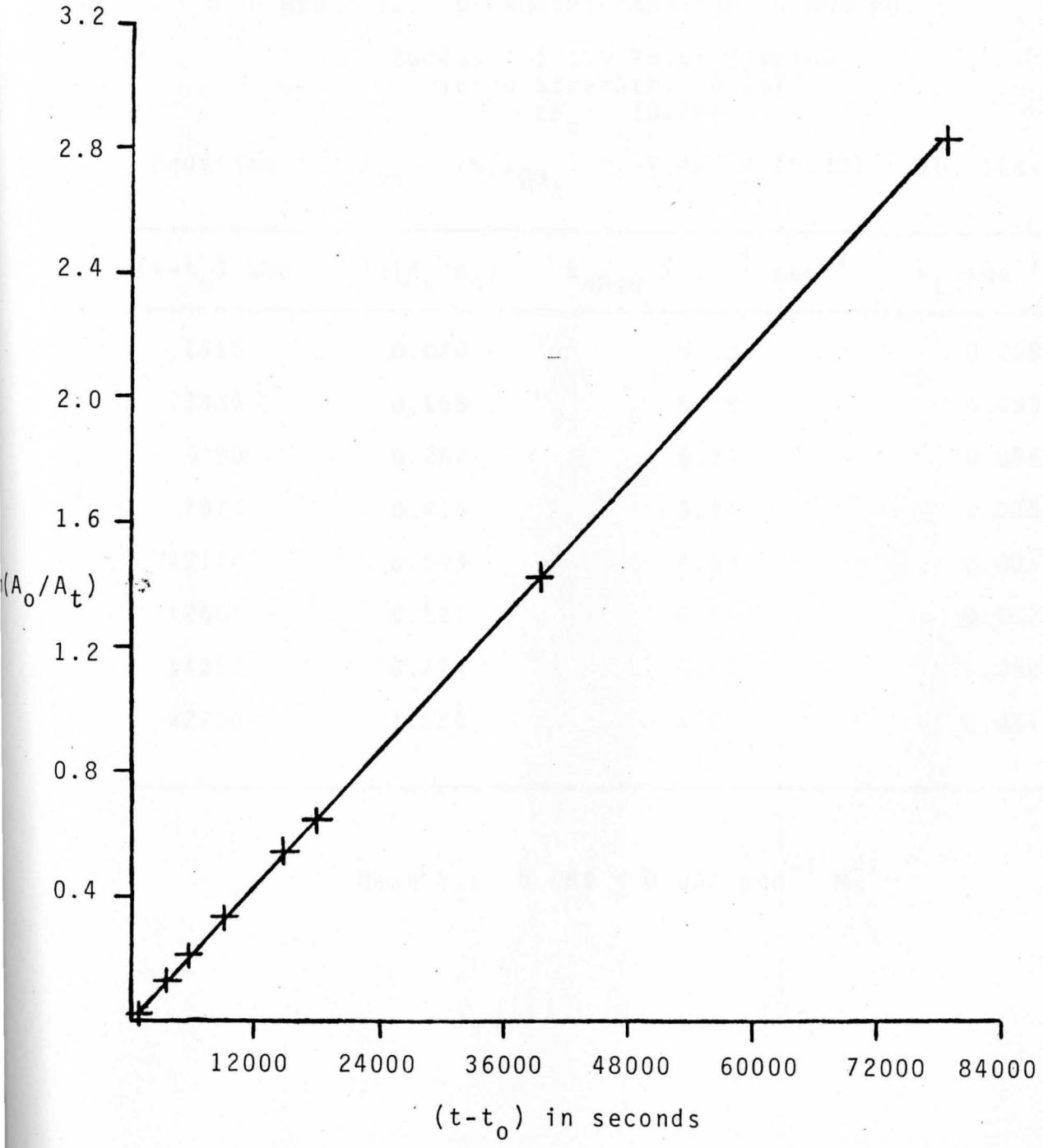


Figure 28 Hydrolysis of butyrylcarnitine in 0.600 molar glycine buffer at ionic strength of 0.660

TABLE 30

HYDROLYSIS OF BUTYRYLCARNITINE - RUN NO. 6

Buffer: 1.000 Molar Glycine
Ionic Strength: 0.997
pH_o: 10.784

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.405 - (9.231 \times 10^{-7})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^5$ sec^{-1}	k_2 sec^{-1} M^{-1}
1315	0.070	5.32	0.088
3335	0.168	5.02	0.083
5095	0.267	5.23	0.086
7975	0.410	5.14	0.085
12110	0.593	4.89	0.081
12600	0.621	4.93	0.082
14350	0.774	5.40	0.090
22200	1.075	4.84	0.081

Mean k_2: 0.085 ± 0.006 sec^{-1} M^{-1}

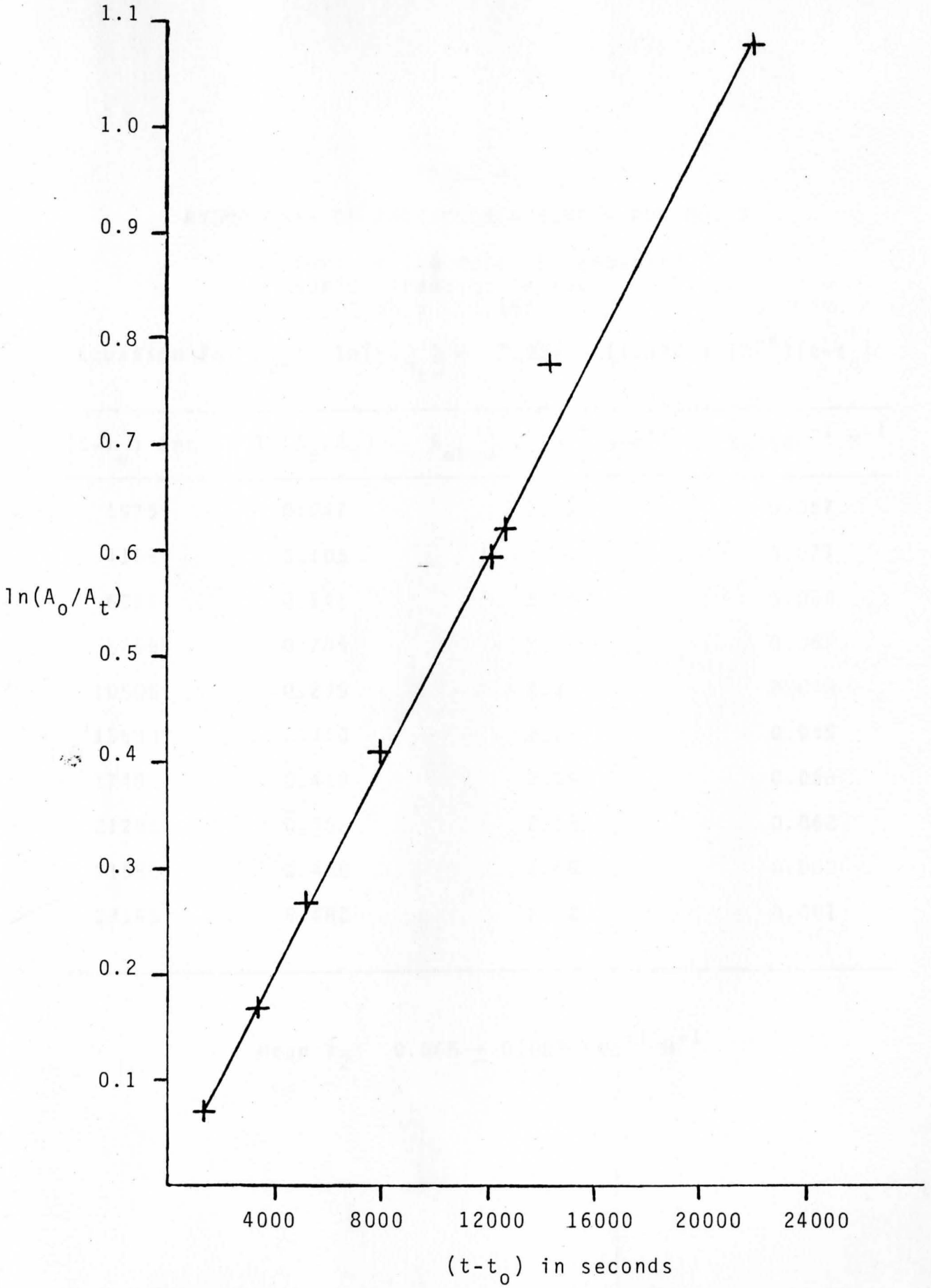


Figure 29 Hydrolysis of butyrylcarnitine in 1.00 molar

TABLE 31

HYDROLYSIS OF BUTYRYLCARNITINE - RUN NO. 7

Buffer: 0.125 Molar Bicarbonate
Ionic Strength: 0.660
pH_o: 10.553

Equation for a_{OH}: $\ln(a_{OH_t}) = -7.983 - (1.322 \times 10^{-6})(t-t_o)$

$(t-t_o)$ sec	$\ln(A_o/A_t)$	$k_{obsd} \times 10^5$ sec^{-1}	k_2 sec^{-1} M^{-1}
1975	0.047	2.38	0.067
4155	0.105	2.54	0.071
6235	0.141	2.26	0.064
8705	0.205	2.36	0.067
10505	0.239	2.27	0.065
13680	0.310	2.27	0.066
17480	0.418	2.39	0.068
21295	0.457	2.15	0.062
21580	0.450	2.09	0.060
23145	0.480	2.12	0.061

Mean k_2: 0.065 ± 0.007 sec^{-1} M^{-1}

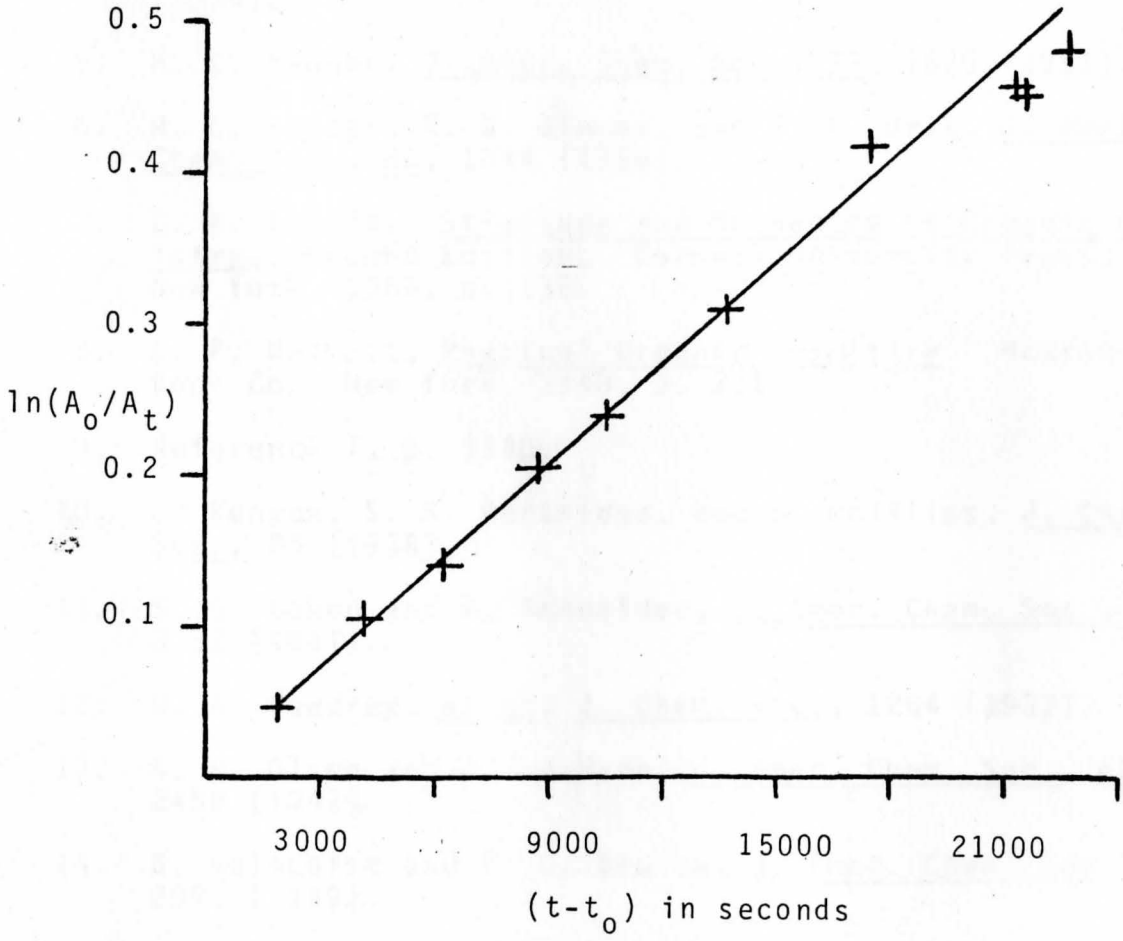


Figure 30 Hydrolysis of butyrylcarnitine in 0.125 molar bicarbonate buffer at ionic strength of 0.660

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