INTERACTIONS AND CATALYSIS OF ATP AND OTHER ORGANOPHOSPHOROUS ESTERS IN BILE SALT SYSTEMS

by

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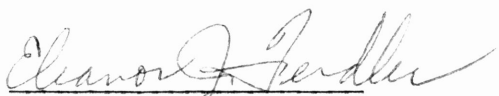

Dr. E. J. Fendler

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Abstract

Adenosinetriphosphate (ATP), bis-p-nitrophenyl phenylphoshonate (bis-PNPP) and tris-p-nitro-phenylphosphate were studied in bile salt systems. ATP showed essentially no solvolysis in dimethyl sulfoxide (DMSO) or water systems with sodium cholate (NaC). Rate constants for the solvolysis of bis-PNPP in DMSO in the presence of aggregated NaC are enhanced by factors of more than 5 million fold and 1.89×10^{-5}-fold with respect to those in the pure bulk solvent and in water systems. The observed rate constant for the formation of p-nitrophenoxide ion exibits a sigmoidal dependence with increasing NaC concentrations reaching a plateau at high NaC concentrations. No kinetic deuterium solvent isotope effects were observed and no reaction occurs in the presence of methyl cholate. A mechanism for the bile salt catalysis is proposed and discussed in terms of both physical and chemical interactions for bis-PNPP. Tris-PNP showed the same trends as bis-PNPP but data was not analyzed completely for this paper.

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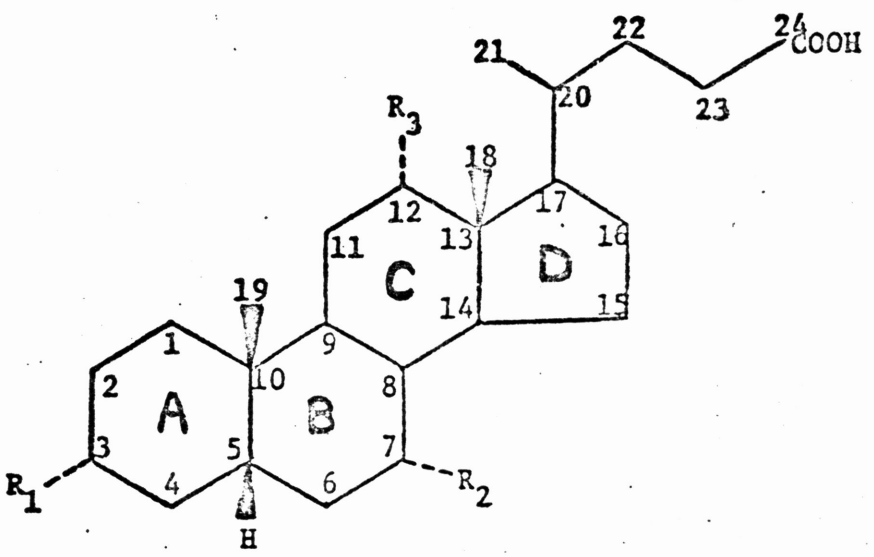
Table of Contents

Tables

Figures

Bile salts are one of the most important groups of naturally occurring surfactants that are found in mammals. In the small intestine bile salts aid in the digestion and absorption of cholesterol, fatty acids, and the vitamins A, D, E and K. The reactions of the bile salts are not yet fully understood, but their importance is clear in human and animal systems.



$R_1 = R_2 = R_3 = H$	Cholanic acid (5β-cholan-24-oic acid)
$R_1 = R_2 = R_3 = OH$	Cholic acid (3α, 7α, 12α-trihydroxy-5β-cholan-24-oic acid)
$R_1 = R_2 = OH$, $R_3 = H$	Chenodeoxycholic acid (3α, 7α-dihydroxy-5β-cholan-24-oic acid)
$R_1 = R_3 = OH$, $R_2 = H$	Deoxycholic acid (3α, 12α-dihydroxy-5β-cholan-24-oic acid)
$R_1 = OH$, $R_2 = R_3 = H$	Lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid)

Of perhaps more interest to the scientific community today is the relationship between bile salts and colon cancer. Persons with colon cancer have elevated levels of bile salts in fecal samples.[1] Population studies show the highest rates of colon cancer are found in Western Europe and the Anglo-Saxon world, while the lowest rates are found in Africa, Asia and South America (except Uruguay and Argentina).[2,3] Rates have also been shown to differ in industrial areas (higher rates)[4] as compared to rural areas as well as between vegetarians (lower rates) and non-vegetarians.[5]

The epidemiological evidence has raised questions as to what dietary factors could be involved in the etiology of colon cancer. Wynder proposed that colon cancer is mainly associated with dietary fat levels.[6] Barkitt points to the decrease in dietary fiber along with the increase of refined carbohydrates as causal factors.[7,8] He also revealed a correlation between color cancer and fecal bulk and fecal passage time. Reddy and Wynder found that populations on a Western diet excreted more bile acids and cholesteral metabolites.[9] Hill reported a correlation between fecal anaerobic bacteria levels and risk to colon cancer.[10] Selikoff has presented evidence that asbestos workers have an increased rate of colon cancer.[11]

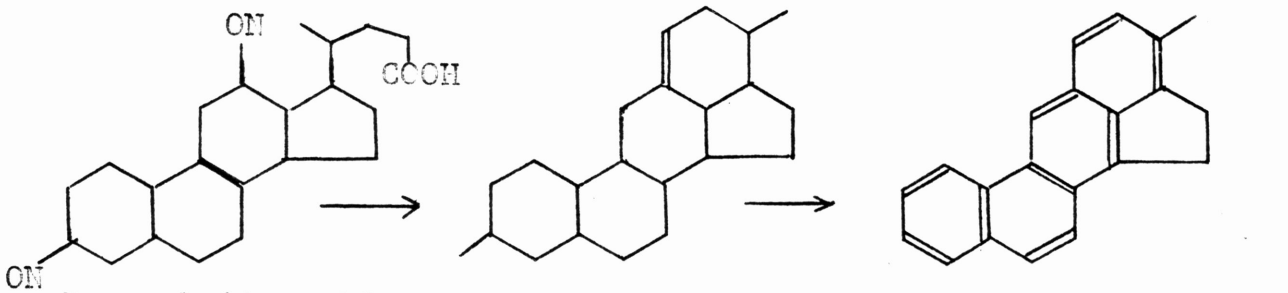
The preceding correlations are open to question in light of some later studies. Wynder and Shigematsu found no relation between constipation and colon cancer.[12] Studies with controlled diets have eliminated possibilities of contamination with carcinogens or co-carcinogens.[13]

Haenszel has found a positive correlation between colon cancer and legumes (which are high in fiber).[14] In Argentina refined carbohydrate consumption is low, while colon cancer rates are relatively high.[15] With these contrasting studies, it can be hypothosized that transit time, stool consistancy, dietary fiber or excess refined carbohydrate intake may be contributing factors in colon cancer but one must look elsewhere for direct causal factors.

It is interesting to note that an increase in dietary fat would also bring about an increase in the bile acid levels. This is important because it has been hypothesized that gut bacteria metabolize bile acids to yield products which are carcinogenic or co-carcinogenic and these reactions would lead to a direct causal relationship.[16] The bile acids themselves are steroidal in nature and by the degradation and aromatization of the acids, carcinogenic compounds have been isolated *in vitro*.[17] Deoxycholic acid can be converted chemically into the very potent carcinogen 20-methylcholanthrene via

dehydronorcholene.[18] It is conceivable that the gut bacteria can perform similar conversions with the various bile acide to form carcinogens and co-carcinogens so understanding the mechanisms of the acids are of extreme importance.

Chemical conversion of deoxycholic acid to 20-methylcholanthrene via dehydronorcholene.



Deoxycholic acid Dehydronorcholene 20-methylcholanthrene

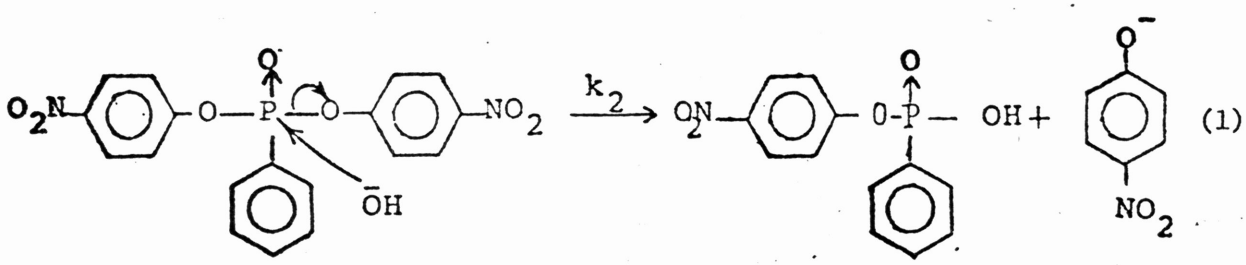
The bile salt itself has hydrophobic and hydrophilic faces (i.e. they possess planar polarity to a striking degree) and the hydroyl and acidic moieties lie on one side of the plane (see fig. 7). Consequently, bile salts form aggregates or micelles when dissolved in water.[19,20] In aqueous solution the shape of the bile salt micelle has been depicted as a cylinder, containing from four to ten or fifteen monomeric units with the hydrophobic side in the interior and the hydrophobic side oriented towards the bulk solvent.[20]

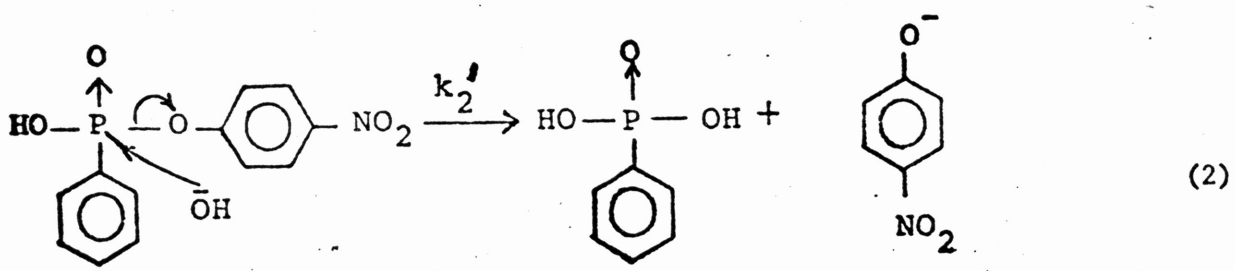
However, studies on ternary systems composed of bile salts, water and n-decanol indicate that two different types of micelles are found, one at low decanol concentrations and one at high decanol concentrations.[21-23] When the amount of n-decanol exceeds 50 mole %, the aggregate structure changes to the reversed or inverted micelle with the hydrophilic side oriented towards the center and the hydrophobic side oriented towards the solvent. Since bile salts form reversed micelles in these ternary systems, it would be expected that they would also form reversed aggregates in non-polar solvents like acetonitrile and dipolar aprodic solvents like dimethyl sulfoxide (DMSO). Indeed the preliminary investigations suggest that bilamellar-type reversed aggregates are formed in both nonpolar and dipolar aprodic solvents.[24] So the study of these important compounds and their systems are essential in further understanding of colon cancer epidimology.

Adenosinetriphosphate (ATP) was one of the compounds picked to study in the bile salt systems. Due to its importance in biological systems and the dirth of basic information on ATP, studies of this compound are both interesting and important. Much work has been done on ATP but the research has been in the area of enzymatic processes and little has been done on its basic reactions, kinctics or other processes

which are not enzyme dependent.

Bis-p-nitrophenyl phenylphosphonate was also one of the substrates chosen for investigation. Its hydrolysis in water is base catalyzed and proceeds with half-lives at 25.0° ranging from greater than 2 hours at ph 8.0 to less than 10 seconds at ph 11.0 (equation 1) where hydrolysis of the monoester (equation 2) becomes apparent, i.e., $k_2 \gg k_2'$. [25,26]

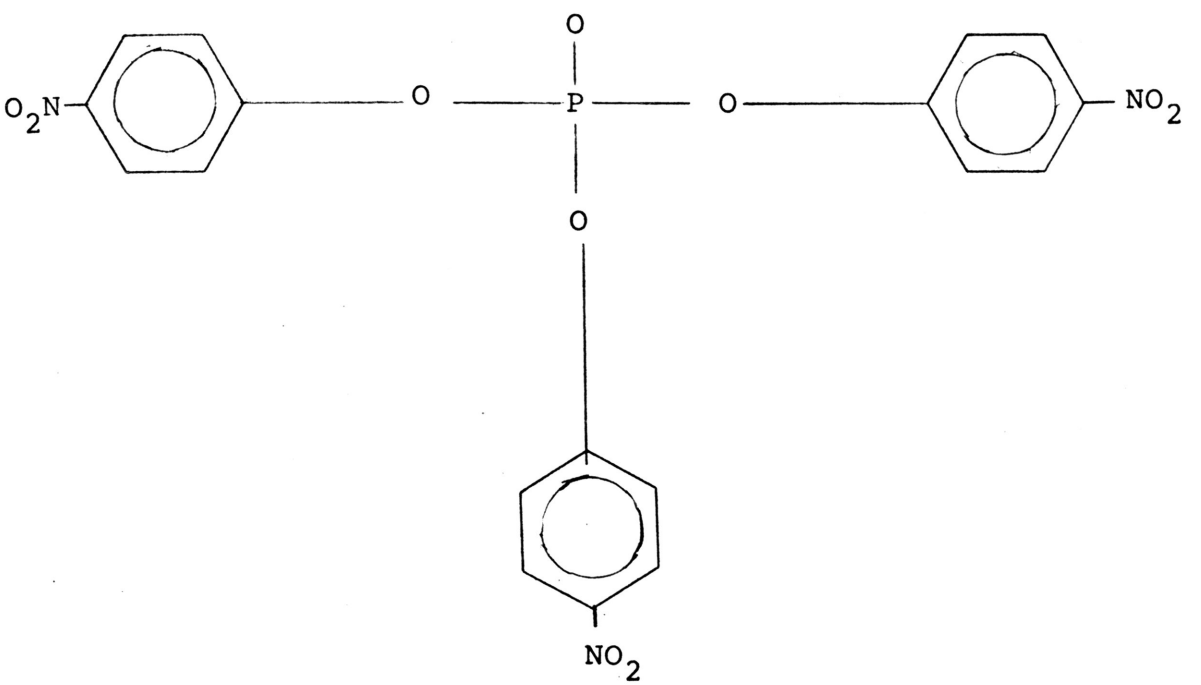




In the presence of synthetic surfactants in aqueous solutions, micellar effects on the first reaction, i.e., on the second order rate constant, k_2 follow the usual trend for anion-molecule reactions: cationic micellar hexadecyltrimethylammonium bromide (CTAB) enhances rates by 260-fold whereas sodium dodecyl sulfate and non ionic polyoxyethylene (20) nonylphenol (Igepal co-850) retard it by factors of ca. 100 and 5 respectively.[25,26] The rate enhancement in CTAB arises from a marked decrease in the activation entrophy. However aqueous micellar sodium cholate and taurocholate (trihydroxy bile salts) both inhibit the reactions.[27]

The last substance to be studied was tris-p-nitrophenyl phosphate. Its reactions have not been studied in bile salt systems but its structural simularities to bis-p-nitrophenyl phenylphoshonate (bis-PNPP) would lead to assumptions that it would act in a simular fashion, (See page 7a).

Since a dipolar aprodic DMSO system would more closely resemble the enviornment near a membrain surface and a variety of other <u>in vivo</u> situations than would normal or reversed micellar systems in water or nonpolar solvents (e.g. benzene) all reactions (with exceptions of some ATP experiments) will be followed in this solvent.



tris-p-nitrophenylphosphate

EXPERIMENTAL SECTION

Certified spectranalyzed DMSO (Fisher) stored over freshly activated Linde-type 4A molecular sieves was used in all experiments. Reagent grade dioxane (Fisher) was purified by refluxing with sodium metal followed by fractional distallation and was stored over freshly activated Linde-type molecular sieves.

Bis-p-nitrophenyl phenylphosphonate (bis-PNPP) was prepared using a modified procedure (patent)[28] of Tolkmith for bis-p-nitrophenyl methylphosphonate. Attempted preparation using p-nitrophenol, phenylphosphonic dichloride and triethylamine in carbon tetrachloride resulted in an oil which solidified with evolution of HCl. p-Nitrophenol (8.20 gm, 0.059 mole) and dry phridine (5.62 ml, 0.0695 mole) dissolved in 70 ml of anhydrous ether was added slowly to a solution of 5.60 gm (0.0287 mole) of phenyl phosphonic dichloride (Stauffee Chemical Co.) in 50 ml of anhydrous ether. The reaction mixture was then refluxed for 3½ hours and filtered using vacuum followed by washing with anhydrous ether. The filtrate was rotary evaporated to dryness in vacuo and dried in vacuo over phosphorus pentoxide yielding a crude solid (m.p. 65-68°C). Purification was accomplished by dissolving the crude product in dry acetonitrile, additon of neutral alumina (Woelm), rapid

filtration under nitrogen, and rotary evaporation to dryness. This procedure was then repeated. The resulting product which melted at 94-97°C was established to be at least 98% pure using ultraviolet-visible and infrared spectrophotometry and 1_H nmr spectroscopy.

Sodium cholate (Sigma) was dried *in vacuo* over phosphorus pentoxide prior to use. Its impurities were checked using infrared and UV spectrophotometry in addition to ^1H and ^{13}C NMR spectroscopy.

ATP (Sigma) was obtained and stored at 0° (desiccated). Magnesium Chloride (Fisher) was certified A.C.S. at 99.4% pure.

Tris-p-nitrophenyl phosphate (Sigma) was obtained and stored at 0° (desiccated).

Methyl cholate was prepared by the method of Hofmann.[29] Cholic acid (10.0 gm, 0.024 mole) was dissolved in 30 ml (0.741 mole) of anhydrous methanol and 1 ml of concentrated hydrochloric acid was added. This solution was refluxed in a covered beaker for 15 min. and then allowed to stand overnight in a refrigerator at *ca.* 0°C, the solution turning golden brown. The mother liquor was removed by filtration and the product was recrystallized from methanol and dried *in vacuo* over phosphorus pentoxide giving pure product in *ca.* 95%

yield with m.p. 155° - 156°C (lit. 155° - 156°C). The purity of this compound was also verified by the absence of detectible impurities at high amplitude in its ^{1}H and ^{13}C nmr spectra.

Buffer solutions were prepared by weight and diluted to volume using double distilled water. Sodium tetraborate (0.01 M) was used in the pH range of 8.0 - 10.8. Acids and bases were prepared in double distilled water using 1.0 M and 0.10 M standard CVS ampoules of sodium hydroxide and hydrochloric acid (BDH) and were used for pH adjustment of the buffer solutions. The pH was monitored on a Radiometer PHM-26 pH meter at ca. 25°C using a Sargent combination micro electrode.

Kinetic Procedures

ATP and Mg^{++} ion were mixed on a 1:1 basis to activate the molocule. All solutions were prepared freshly after two days. The solvolyses of ATP was followed by phosphate appearance using the Vanadomolybdophosphoric acid colorimetric method. Since the bile acids are quite insoluble in acidic solutions, the procedure was modified. First the test aliquot was acidified in .1N HCl (4 ml:1ml, HCl:ATP) then centrafuged and a 1 ml aliquot of this solution was then added to the Vanadomolydate test solution (concentrations were varied accordingly). Ten minutes was allowed for the solution to

reach full color then absorption was measured on a cary 118 spectrophotometer.

It was also noted that over time (i.e. one day) the solution appeared to be changing hue. A separate study was initiated and ATP by itself and ATP in a one to one mixture with magnesium were studied.

The solvolyses of bis-p-nitrophenyl phenylphosphonate were followed by measuring the increase in absorbance of p-nitrophenoxide ion at 403 nm in the thermostated cell compartments of an appropriate recording spectrophotometer. Since the rates of these solvolyses varied considerably, experimental procedures varied depending on whether the rate of the reaction in question was very fast ($t_{\frac{1}{2}} \sim 1$ sec), very slow ($t_{\frac{1}{2}} \sim$ several hours), or intermediate.

The basic solvolyses of bis-PNPP with bile salt micelles in dipolar aprotic DMSO containing aggregated bile salts were the most rapid. Kinetic runs were carried out by use of a Durrum-Gibson Model D-110 stopped-flow spectrophotometer. Results were stored on an oscilloscope and photographed with a Polaroid camera for future reference using established stopped-flow procedures.[34-36] Slower reactions were carried out on a Cary 118-C spectrophotometer by injection of an appropriate amount of bis-PNPP in dioxane to the thermally

equilibrated solution in the cell compartment through a small bore in the Teflon stopper. The desired temperatures were carried out under pseudo-first-order conditions and the rate constants, k_{ψ}, were calculated from plots of log $(A - A_t)$ *vs.* time. The error in individual rate constants were generally not more than 2%.

The solvolyses of Tris-p-nitrophenyl phosphate was observed also with the increase in absorbance of p-nitrophenoxide ion in the same experimental conditions as the bis-PNPP.

RESULTS AND DISCUSSION

Due to the amount of information gathered the results will be divided into three parts.

ATP-Mg

Solubility studies were not available for the ATP-Mg complex in dipolar aprotic DMSO and NaC. The complex was found to be $<<.2$ mg/l which is the lowest concentration detectable by the vanadomolybdophosphoric colormetric method. Further test with other nonpolar (e.g. benzene) produced similar results. Also it should be noted, for future reference, that while increases of DMSO with the standard solution showed typical Michaelis-Menton effects (Fig. 1), in solutions greater than 40% volumn, DMSO in water inhibited the Vanodomolybdophosphoric complex. It was therefore decided to study the ATP-Mg complex in water and NaC systems.

Results indicate that the solvolysis of ATP is an extremely slow reaction in water and NaC systems. After 19 days at 50^{o} the phosphate concentration was less than .5% of the theoretically calculated possible reaction of the primary phosphate. Furthermore concentrations of NaC were varied (0.2M, ..., 0.0M) and no significant changes were noted with respect to phosphate levels.

The observation of the color change as a function of time in the Vanodomolybdate (VM) solution led to a review

of the literature for the catalytic effects of the VM complex. The kinetics with molybdate had been studied[31] but no studies with VM could be located. It was found that $K_1=5.25 \times 10^{-6}$ for 1.0×10^{-3} M ATP in the MV solution at 25°(MV was from the stock solution)[30] while the ATP-Mg complex showed no effects in the same experimental conditions. This suggests that the magnesium when complexed with the ATP inhibited the primary phosphate as a leaving group.

To conclude, the interactions of ATP-Mg in bile salt - water systems has been found to be extremely slow. Magnesium inhibits the solvolysis of ATP in the presence of the vanodomolybdate complex. Unfortunately time restraints prevented further study of noncomplexed ATP.

Bis-p-Nitrophenyl Phenylphosphonate (bis-PNPP)

The observed rate constants for the formation of p-nitrophenoxide ion from bis-PNPP in the presence of NaC in dipolar aprodic DMSO have been determined at three (Table I) different concentrations of added water and a typical sigmoidal dependence of K on surfactant concentration is evident in each case (Fig. 3). At high surfactant concentrations the curves tend to approach a phateau. At 0.147M NaC in DMSO the rate constant for the solvolysis of the phosphonate is 1.89×10^5-fold greater than that in water at pH 7 ($K_2=83.5$ $1.mole^{-1}$ sec^{-1} at 25.0°).[26,27] . Addition of water in the plateau region results in a curvalinear decrease in the observed rate constant for solvolysis with increasing water

TABLE I

SOLVOLYSIS OF BIS-PNPP
IN DMSO SOLUTIONS OF NcC
AT 25.0°C[a]

10^3[NaC],M	k_ψ, sec^{-1}	$k_{[H_2O]}$, sec^{-1} M	
	0.0055	0.011	0.165
4.30	0.354		0.226
8.60	0.412		0.318
11.0		0.411	
17.3	0.771		0.400
23.8		0.605	
30.0		0.700	
34.7	0.944		0.558
44.1	1.02		0.674
88.3	1.24		0.778
108.0		1.28	
124.0	1.54		0.833
144.0		1.24	
149.0	1.58		0.878

[a][Bis-PNPP]=6.0×10^{-5} M

concentration (Fig. 4).

It is apparent from the rate constant-surfactant concentration profile (Fig. 3) at each water concentration that the data in Table 1 can be treated reasonably in terms of simple substrate-micelle (or aggregate) association. Assuming that the micelle-substrate association is a one to one stoichiometric relationship and that the substrate does not complex with the monomeric surfactant, then the rate constant for solvolysis in the bulk dipolar aprotic solvent, k_o, and that in the polar micellar pseudo-phase, k_m, can be expressed by equation 3 where M, S, and MS represent the aggregated NaC, the phosphonate diester and the complexed phosphonate diester-NaC species, respectively. K is the binding or association constant and P

$$\begin{array}{ccc} M + S & \overset{K}{\rightleftharpoons} & MS \\ \downarrow k_o & & \downarrow k_m \\ P & & P \end{array} \qquad (3)$$

is the p-nitrophenoxide ion and phosphonate monoester products. The observed pseudo-first-order rate constant, k_ψ, is given by

$$k_\psi = \frac{k_o \; k_m \; K(M)}{1 \; K(M)} \qquad (4)$$

where (M) is the concentration of micelles. Assuming that

the concentration of monomers remains constant above the CMC, the concentration of micelles can be expressed by

$$(M) = \frac{C_D - CMC}{N} \qquad (5)$$

where C_D is the stoichiometric concentration of the surfactant, CMC is the critical micelle concentration, and N is the aggregation number, or number of monomers per micelle. Combination of equations 4 and 5 and rearrangement gives

$$\frac{1}{k_o - k_\psi} = \frac{1}{k_o - k_m} + \frac{1}{k_o - k_m}\left(\frac{1}{C_D - CMC}\right)\frac{N}{K} \qquad (6)$$

or

$$\frac{k_\psi - k_o}{k_m - k_\psi} = -\frac{K}{N}(C_D - CMC) \qquad (7)$$

Plots of the left-hand side of equation 7 vs. C_D should give straight lines with slopes of K/N and intercepts of $\frac{K}{N}$ (CMC) from which the CMC can be calculated. Treatment of the kinetic data using equation 7 (Table II) results in remarkably linear behavior from which $\frac{K}{N}$ and the CMC have been determined. The results are given in Table III and a typical binding constant plot is shown in Fig. 5.

Since no product formation could be detected in more than 3 months in solutions containing 6.0×10^{-5} M bis-PNPP in DMSO ($(H_2O) = 1.1 \times 10^{-2}$ M) in the absence of surfactant, k_o must be very much less than 10^{-7} sec $^{-1}$. This very slow

TABLE II

BINDING CONSTANT DATA[a]

10^3[NaC], M	$\frac{k_\psi - k_o}{k_m - k_\psi}$ [H_2O], M		
	0.0055	0.011	0.165
4.30	0.29		0.35
8.60	0.35		0.57
11.0		0.41	
17.3	0.96		0.84
23.8		0.74	
30.0		0.97	
34.7	1.50		1.75
44.1	1.82		3.31
88.3	3.77		7.83
108.0		9.36	
124.0	38.37		18.77
144.0			
149.0			

[a]Calculated from Table I according to equation 7; k_m = 1.575, 1.420, 0.878 sec $^{-1}$ for the respective water concentrations and $k_o = 10^{-7}$ sec $^{-1}$

TABLE III

RELATIVE BINDING (ASSOCIATION) CONSTANTS AND CRITICAL MICELLE CONCENTRATIONS FOR BIS-PNPP AND NaC IN THE PRESENCE OF WATER AT 25°C[a]

$[H_2O]$,M	$\frac{K}{N}$	CMC,M
0.0055	40.8	3.06×10^{-3}
0.011	30.0	2.70×10^{-3}
0.165	46.4	2.70×10^{-3}

[a]Calculated from Figure 5 and analogous plots, according to equation 7.

rate of reaction is quite reasonable considering the low concentration of water and the pseudo-first order rate constant for hydrolysis in pure water at pH 7.0 and 25.0°C (k_{ψ} = 8.35 x 10^{-6} sec $^{-1}$). [25, 26]. The critical micelle concentration (2.7-3.1 x 10^{-3} M) is quite similar to those found for a wide variety of ionic and non-ionic surfactants which aggregate appreciably in both aqueous and non-aqueous solvants [32] as well as for sodium cholate in water and aqueous salt solutions.[20] Additionally, the constancy of the CMC values calculated for NaC with 0.0055, 0.011 and 0.165 M added water indicates that the aggregate structure does not change over this concentration range.

Although the CMC and hence aggregate structure apparently do not vary with increasing water concentration, the observed rate constants in the plateau region decrease linearly as functions of both increasing water and deuterium oxide concentration in the range of 5.5 x 10^{-2} to 0.275 M water (Table IV and Figure 4). It is apparent from comparison of the data in Table IV that there is no kinetic deuterium solvent isotope effect over the concentration range of water employed. Therefore, in terms of the reaction mechanism, water is neither acting as a nucleophile nor is it covalently bonded in the transition state for the rate limiting step. It is probable, however, that it is hydrogen bonded strongly

TABLE IV

SOLVOLYSIS OF BIS-PNPP IN

0.147 M NaC IN DMSO CONTAINING

WATER AND D_2O at 25°C[a]

$[H_2O]$,M[a]	$[D_2O]$,M[b,c]	k_ψ, sec^{-1}		
		H_2O	D_2O	K_{H_2O}
0.055	0.055	1.006	1.006	1
0.110	0.110	.9406	.9506	0.9894
0.165	0.165	.8722	.8782	0.9931
0.220	0.220	.8199	.8199	1
0.275	0.275	.7500	.7500	1

[a] [Bis-PNPP] = 6.0×10^{-5} M

[b] Concentration of water added to DMSO solutions containing 0.055 M H_2O.

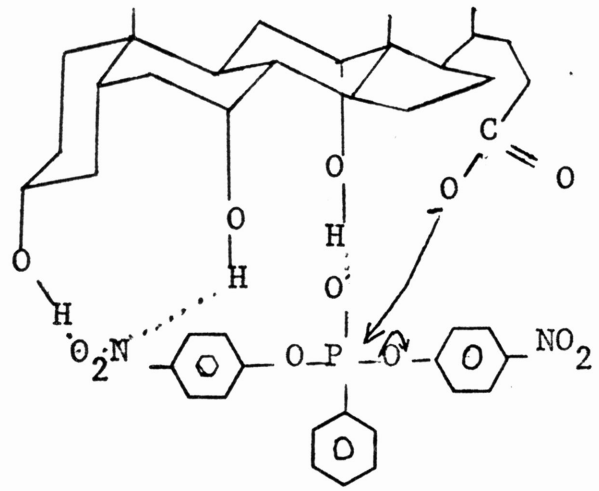
[c] D_2O used was greater than 99.8% deuterium.

to the carboxylate ion of sodium cholate and to a lesser extent to the phosphoryl oxygen atom and the solvent DMSO.

The magnitude of the catalysis as well as the role of water are compatible with a bifunctional type of catalytic mechanism in which the phosphonate binds to the hydroxyl groups of the aggregated cholate ion in an appropriate configuration for nucleophilic attack by the carboxylate ion on phosphorus. An unusually strong binding of the phosphonyl and p-nitrophenoxy groups of the substrate with a bile salt is not without precedent; a number of polyfunctional phenolic compounds have been found to associate strongly with bile acid and derivatives, the extent of intermolecular association depending primarily on the stereochemical orientation of the molecules involved.[33]

Intramolecular facilitation of organophosphate and phosphonate ester hydrolyses by carboxylate ion are well-documented.[34-36] It is probable that the phosphonyl oxygen atom hydrogen bonds to the C-12 hydroxyl hydrogen atom of the cholate while the C-3 and C-7 hydroxyl groups interact with at least one of the substrate p-nitrophenoxy groups as shown schematically in Figure 6, thereby binding the phosphonate in the appropriate orientation for nucleophilic attack by the carboxylate anion of the aggregated solium cholate on phosphorus with concerted or subsequent explusion

of a p-nitrophenoxide ion:



The decrease in the rate constant as a function of increasing water concentration and the lack of a solvent deuterium isotope effect are explicable in terms of this proposed mechanism. It is highly probable that, in addition to the solvent DMSO, added water would preferentially solvate the most polar and highest charge density entity, the carboxylate ion of the catalyst, and secondly the phosphonyl group of the substrate. In both cases, formation of hydration spheres would result in a decrease in the reaction rate, the former being the consequence of decreased nycleophilicity and the latter of an increase in electron density on phosphorus due to hydrogen bonding by water to the phosphonyl oxygen atom. However, addition of relatively low concentrations of water (H_2O or D_2O) would neither alter the aggregate structure appreciably (which is apparent from the constancy of the obtained CMC values) nor significantly effect the

binding of the phosphonate to the cholate hydroxyl groups (which is indicated by the invariance of the $\overline{K}_N$ values).

In order to further elucidate this proposed mechanism, the solvolysis of bis-PNPP was investigated in dry DMSO ($[H_2O] = 5.5 \times 10^{-4}$ M) in the presence of 0.20 M methyl cholate. If the carboxylate ion of the aggregated or micellar sodium cholate was the nucleophile then the observed rate constant would predictably be quite small in the presence of this methyl ester while the binding or association behavior would not be altered appreciably. Not surprisingly no reaction could be detected spectrophotometrically in this solution in more than 1 month at ca. 25°C, i.e. $k_\psi << 10^{-7} sec^{-1}$.

The activation parameters, given in Table V, for the solvolysis in the plateau region (0.15 M NaC) as a function of water concentration are quite compatible both with the proposed mechanism and with appreciable hydration of the carboxylate ion and phosphonyl group. With the exception of solutions containing the highest concentration of added water (0.110 M), the activation energy and enthalpy are considerably more favorable than those for the base catalyzed hydrolysis both in the absence and the presence of micellar cationic CTAB, anionic NaLS, and anionic NaTC.[25,26] In the presence of 0.110 M water the activation energy and entropy are ca. 1 kcal./mole greater than those in water but

TABLE V

ACTIVATION PARAMETERS FOR THE SOLVOLYSIS

OF BIS-PNPP IN NaC-DMSO SYSTEMS

AT 25.0°C[a]

$[H_2O]$, M	E_a, kcal mole^{-1}	$\Delta H^{\neq}$, kcal mole^{-1}	Log A	$\Delta S^{\neq}$,
0.0055	9.34	8.74	0.418	27.3
0.055	9.44	8.84	0.367	27.1
0.110	11.3	10.7	0.358	20.9

[a] [Bis-PNPP] = 6.0×10^{-5} M, [NaC] = 0.15 M

markedly less than those in aqueous micellar CTAB, NaLS, and NaTC solutions. As in the case of the mutarotation of a- and b-d-glucose in the presence of micellar dodecylammonium propionate in dipolar aprotic N,N-dimethylacetamide, [37] both the energy and entropy of activation contribute to the observed catalysis.

Tris-p-Nitrophenyl Phosphate (tris-PNP)

Experimentation with tris-PNP was started too late to compile and analyze the data for this paper. General trends that have been observed suggest that in DMSO with low concentrations of water ($<10^{-2}$ M) the reaction is faster than the bis-PNPP. This is based on the reaction being too fast to be recorded on the Durrum-Gibson stopped flow recorder. Reaction time has been shown to be effected by increasing water concentrations but, as of now, the data is still being processed and a complete anaylsis will appear at a later date.

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FIGURE LEGENDS

Figure 1 Plot of standard phosphate with increasing DMSO of 1ml (- - -) and 2ml (⊷⊷⊷).

Figure 2 - Solvolysis of ATP (——) and ATP-Mg (---) in Vanadomolybdate solution with $K_1 = 5.25 \times 10^{-6}$ as a function of time.

Figure 3 - Observed rate constants, k_ψ, for solvolysis of bis-PNPP in DMSO containing 5.5×10^{-3} (□), 1.1×10^{-2} M (○) and 1.65×10^{-1} M (△), H_2O in the presence of NaC at 25.0°C.

Figure 4 - Observed rate constants K , for solvolysis of bis-PNPP in the presence of o.15 M NaC as functions of the molar concentrations of DMSO and of water in DMSO/H_2O systems at 25°C.

Figure 5 - Binding constant plot for reaction 1 in DMSO containing 5.50×10^{-3} M H_2O in the presence of NaC at 25.0°C.

Figure 6 - Generalized mechanistic representation of the NaC catalyzed reaction of bis-PNPP.

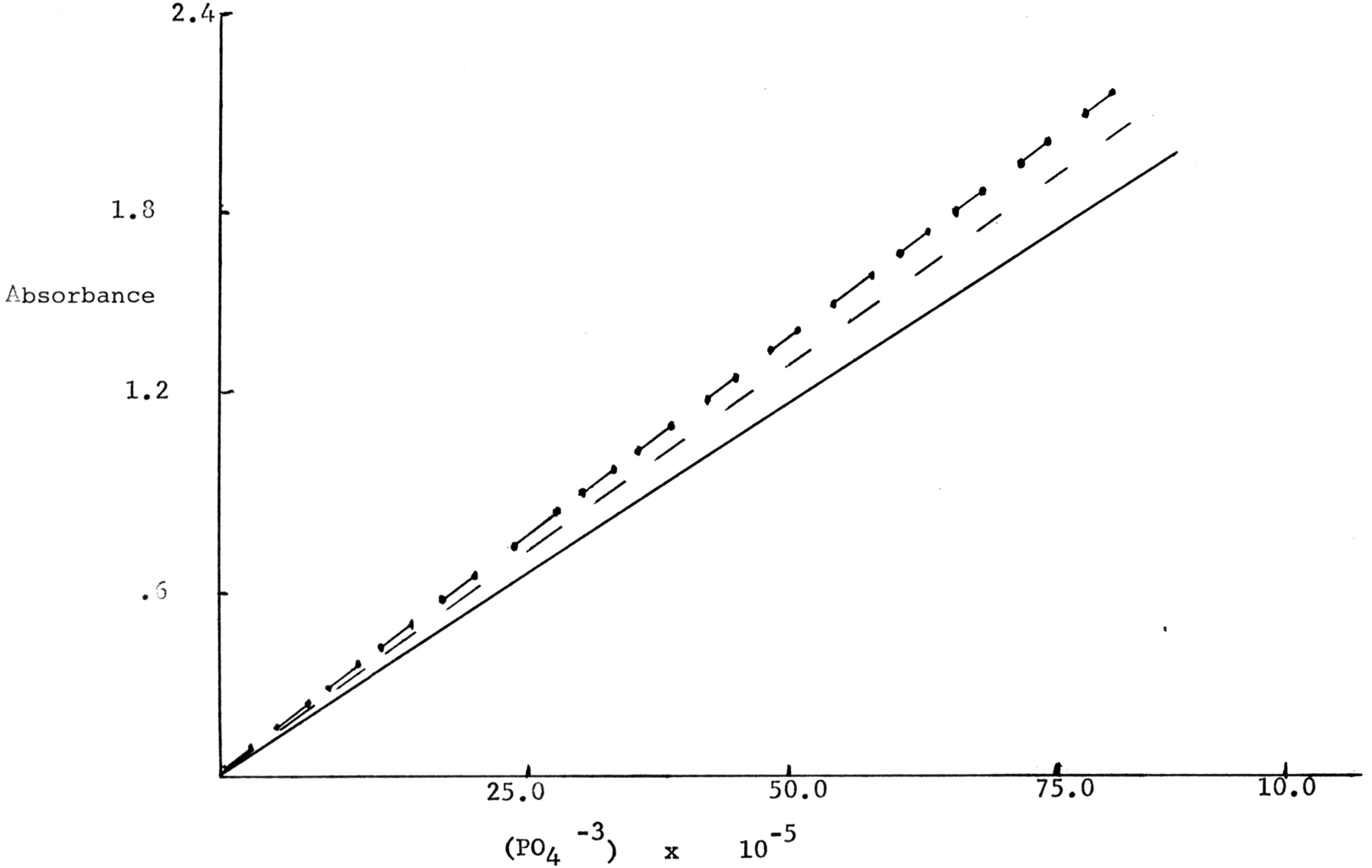


FIGURE I

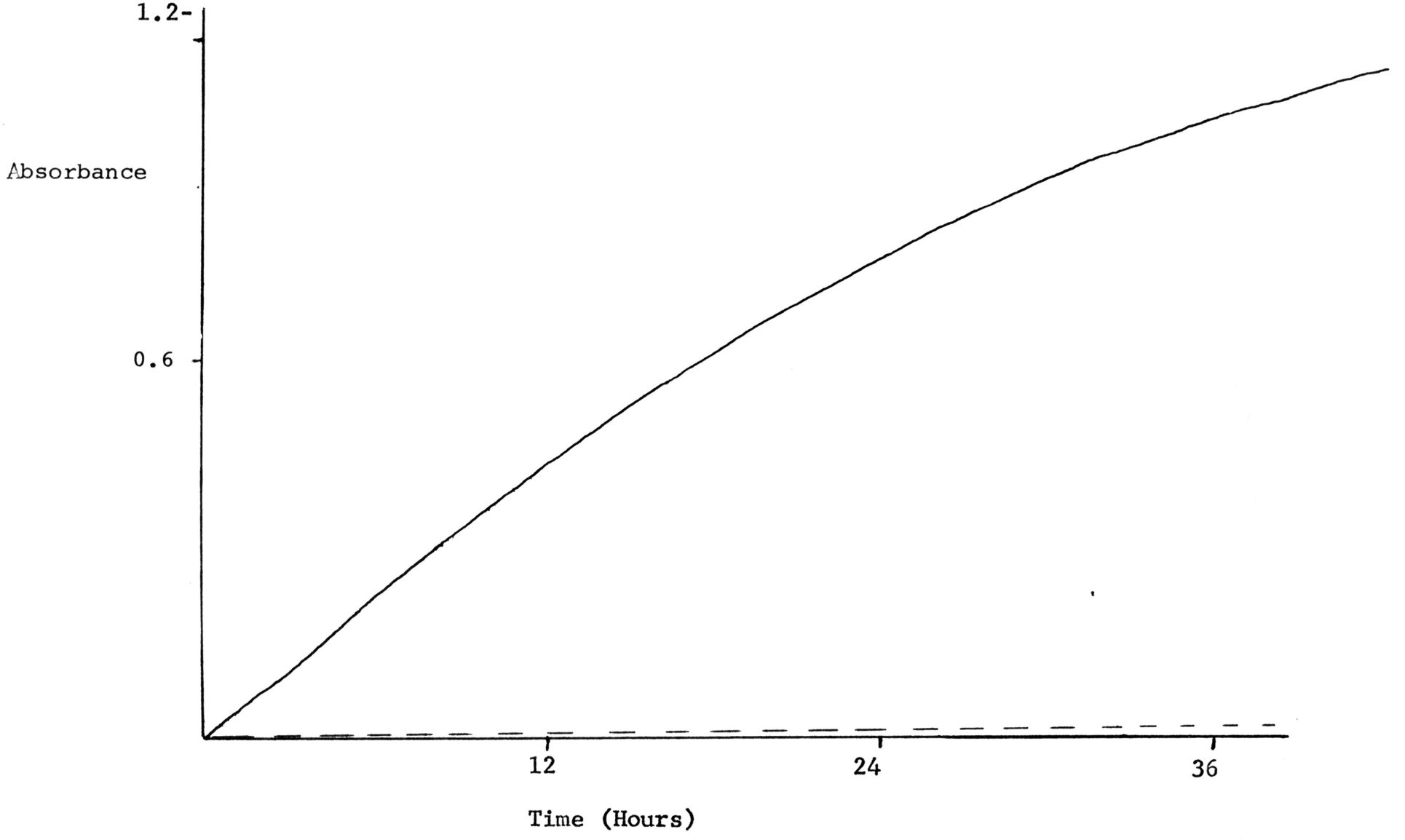
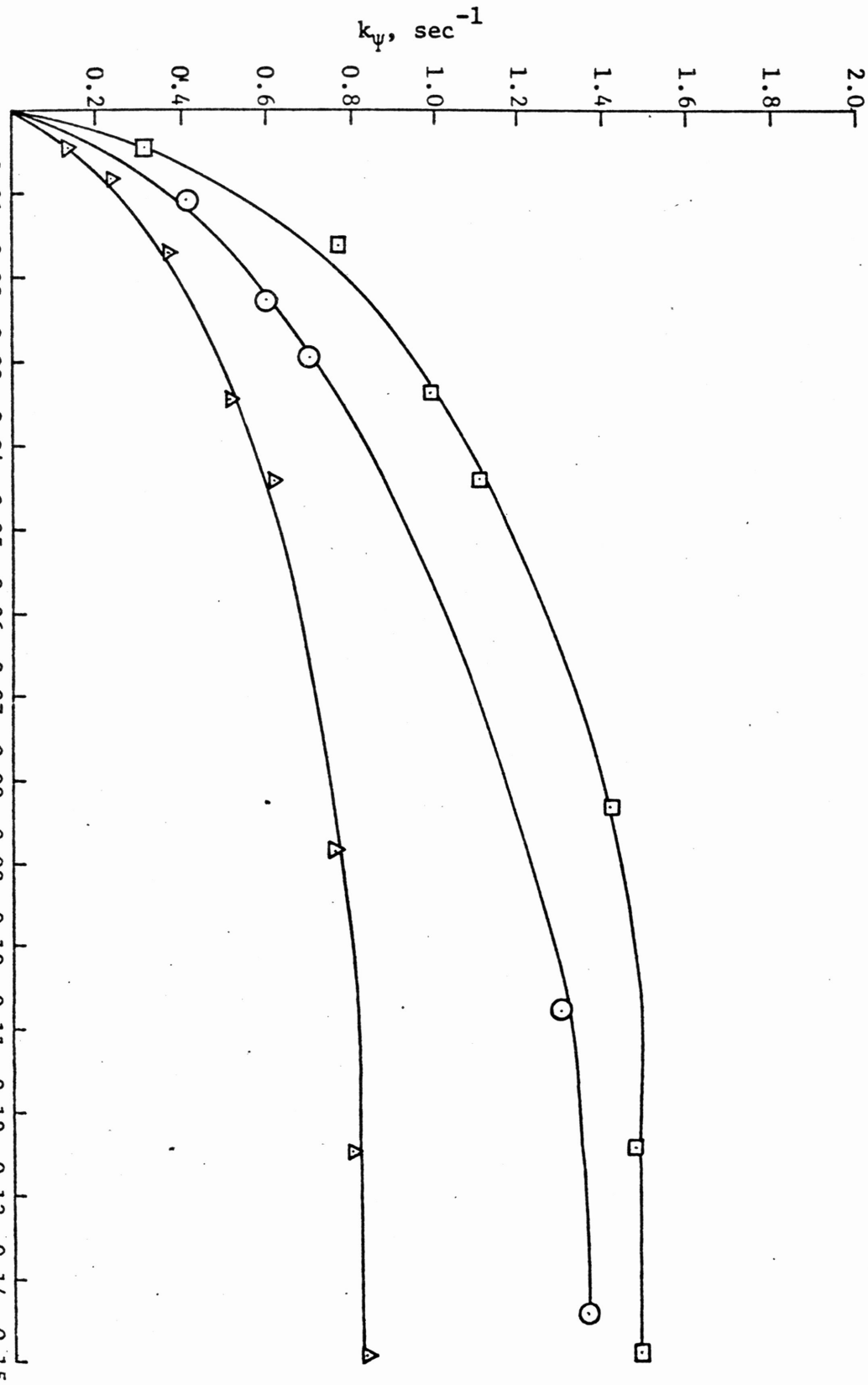
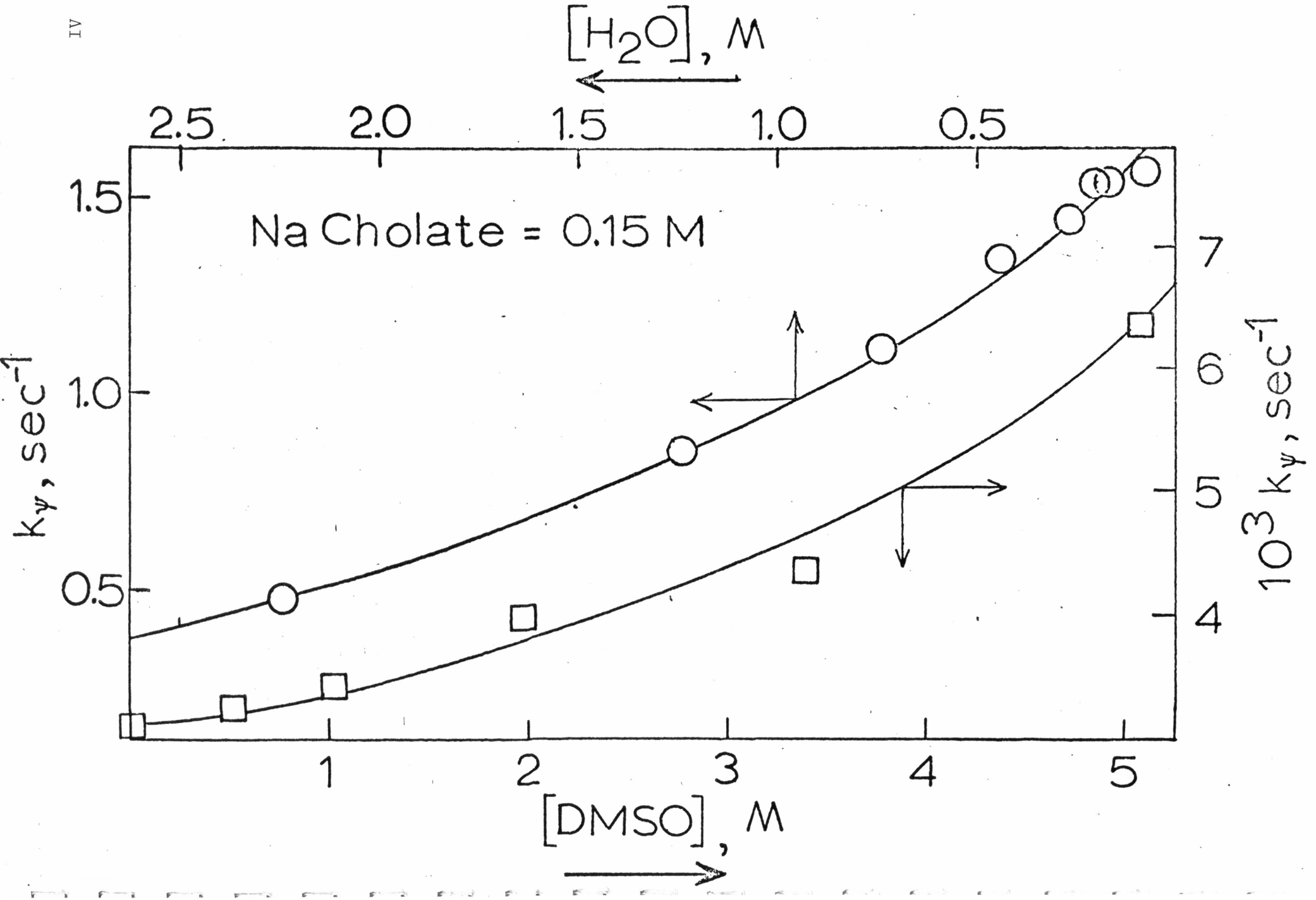
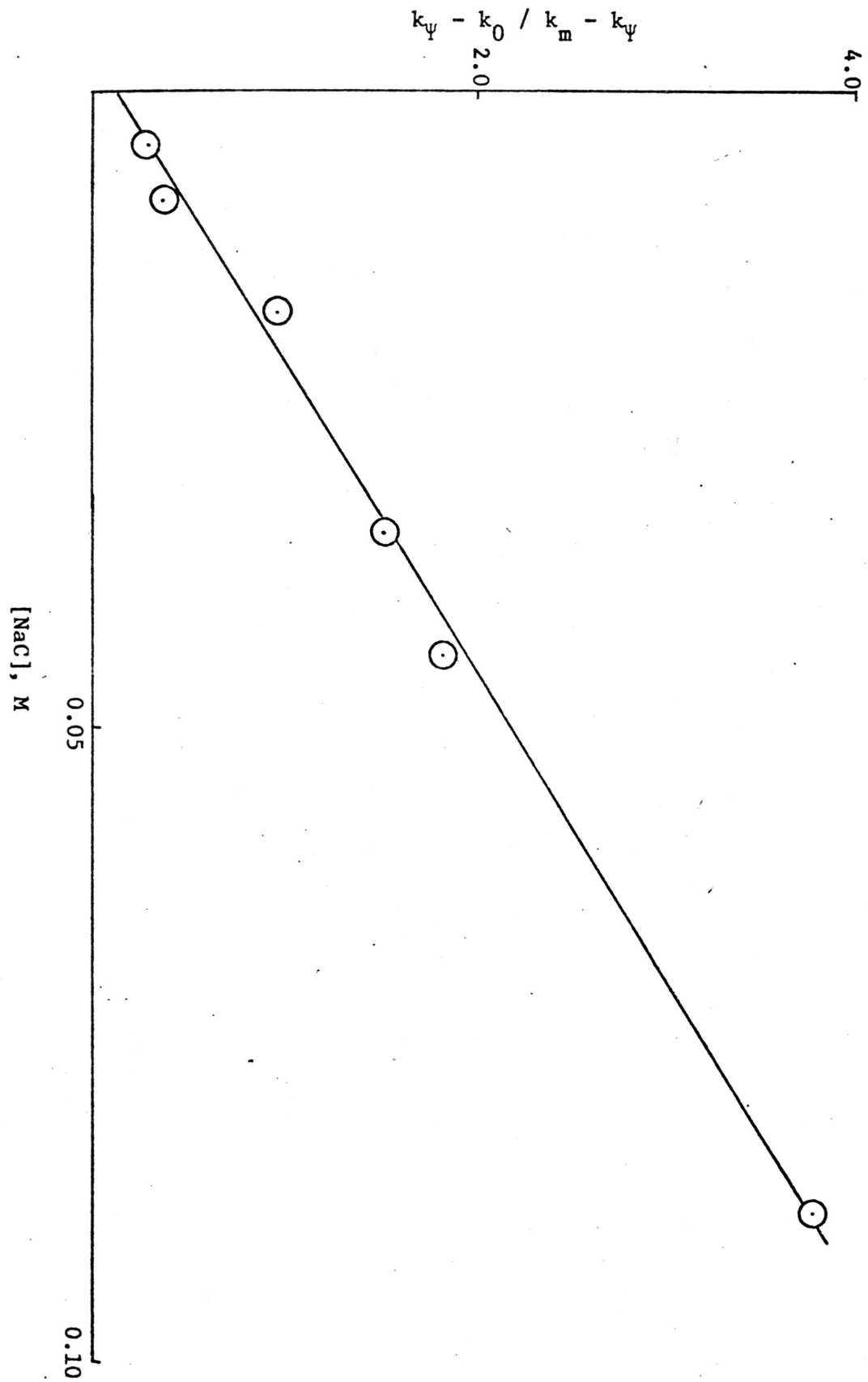


FIGURE 2


k_ψ, sec^{-1}
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08 0.09 0.10 0.11 0.12 0.13 0.14 0.15
[NaCl], M

Solvolysis of Bis-p-nitrophenyl Phenylphosphonate




$k_\psi - k_0 / k_m - k_\psi$
4.0
2.0
0.05
0.10
[NaC], M

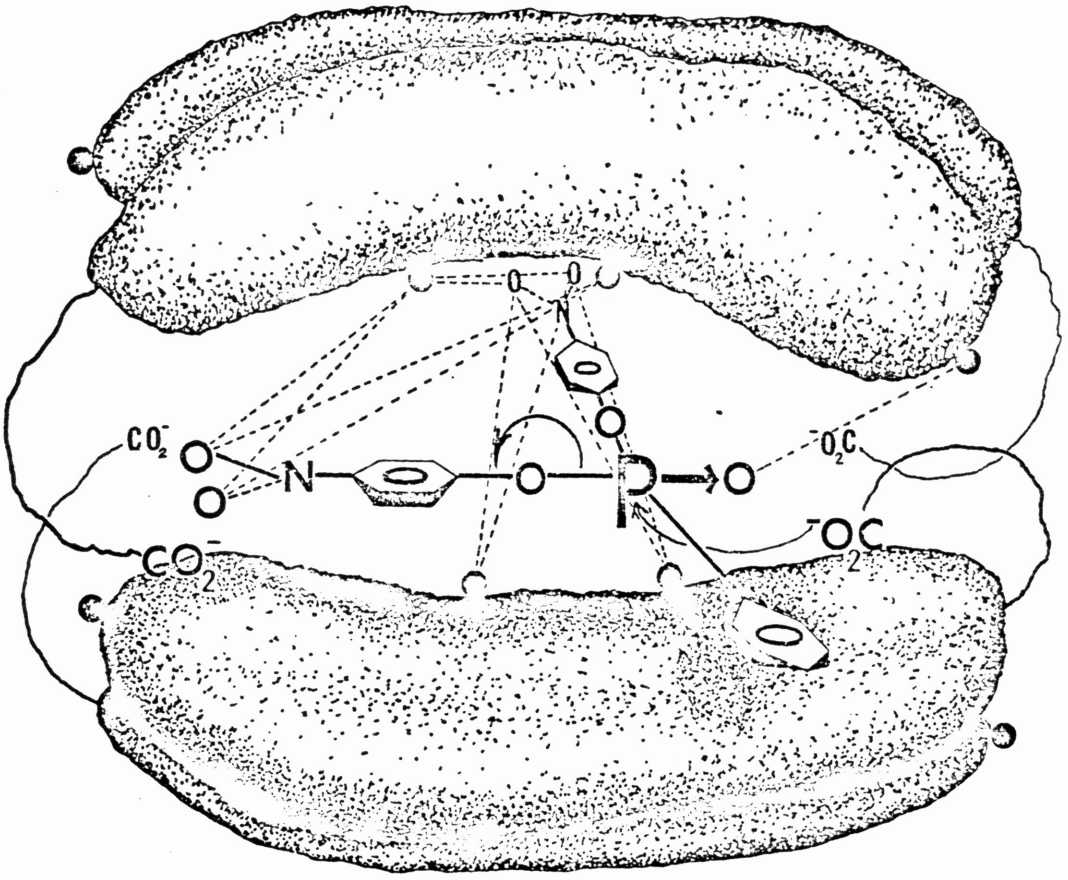
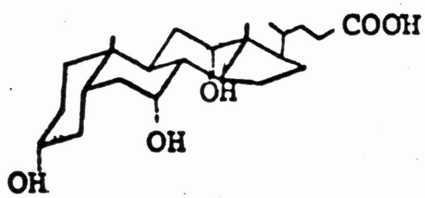
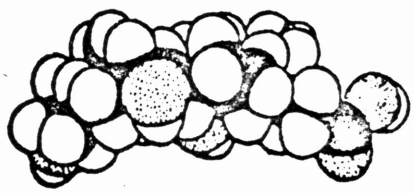
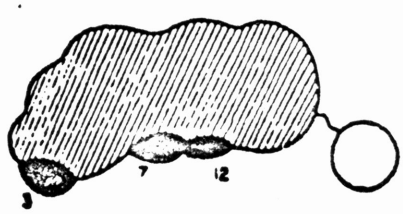


FIGURE 6







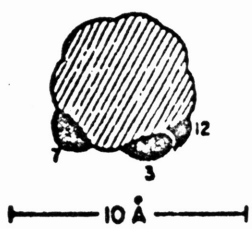


FIGURE 7