Table VI—Analysis of Data from Plasma Level Determinations

	Hour 1		Hour 2		Hour 3	
	df	*MS*	*df*	*MS*	*df*	*MS*
Control *vs.* average of others	1	0.31	1	0.63	1	2.89
Capsules	1	8.70	1	8.12	1	34.41[a]
Fillers	1	9.71	1	3.93	1	14.95
Capsules + fillers	1	0.01	1	2.94	1	3.00
Rabbits	8	6.70	8	22.18[b]	8	16.03[a]
Error	32	3.86	32	5.83	32	5.50

[a] Significant at the 5% level. [b] Significant at the 1% level.

two sets of data was considered of little value. A rank order correlation may, however, be observed in that the mean plasma salicylate values, as well as dissolution values, are higher for the more highly compacted No. 4 capsules and in the case of dibasic calcium phosphate. The low plasma salicylate levels may be due to a failure to break down *in vivo*. The *in vitro* agitation intensities resulting from the dissolution medium being forced through the 100-mesh screen were reported by Levy (4) to be far greater than the agitation encountered in the stomach, a fact that could account for the widespread difference in concentration levels between the two studies.

In vitro observations showed that the ASA became moist; over a period of time the gelatin stretched and, together with the mechanical action of striking against the 100-mesh screen, assisted in the breakdown. The stretching effect was not as noticeable in the case of capsules containing excipients.

The low plasma salicylate levels obtained *in vivo* together with the *in vitro* behavior of the capsules would seem to indicate that the gelatin in the hard gelatin capsules had been modified in some manner, either by the acidity of the gastric juice, or by the acidity of the ASA (pKa 3.5) within the microenvironment of the capsule, or by a combination of the two. If due to the weakly acidic drug alone or to a combination of drug and gastric juice, this could be of importance for other drugs of similar properties when encapsulated. If due to the acidity of the gastric juice alone, it would appear that the release of any drug enclosed in hard gelatin capsules could be affected adversely. Gelatin is obtained by heat denaturation of collagen and is built of three strands, which are joined primarily by hydrogen bonding between the strands (8). It is possible that the stretching of these strands accounted for the appearance of the hard gelatin capsules in the *in vitro* tests and perhaps for the low plasma salicylate levels.

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Factors Influencing Solvolysis of Corticosteroid-21-phosphate Esters

G. L. FLYNN and D. J. LAMB

Abstract □ The solvolysis of methylprednisolone-21-phosphate in dilute aqueous solution (<0.005 *M*) is qualitatively similar to that observed for methylphosphate and other simple monoalkyl phosphates, particularly in the pH range 3–8. In more concentrated solutions (>0.02 *M*), however, there is an acceleration of reaction velocities and marked deviation from the expected pH dependency. This change in chemical behavior is attributed to association colloid formation. Support for this mechanism is drawn from hydrolysis-rate data obtained as a function of concentration and independently determined critical micelle concentration values.

Keyphrases □ Corticosteroid-21-phosphate esters—solvolysis, micelle formation □ Solvolysis, corticosteroid-21-PO_4 esters—pH profile, activation energy, aggregation effect □ Critical micelle concentration determination—conductivity, surface-tension methods □ Phosphate, inorganic—analysis

The term phosphate ester is extremely ambiguous because it applies to several distinctly different classes of compounds, each characterized by unique chemical behavior. Furthermore, chemistries in each phosphate ester class can be appreciably different both qualitatively and quantitatively, depending on the types and proximities of neighboring atoms within the molecule. Additional complexity arises from the biformity of the carbon–oxygen–phosphorus linkage. Solvolysis, for instance, may entail carbon–oxygen cleavage, phosphorus–oxygen splitting, or both, depending on the reaction conditions (1).

The hydrolyses of monoalkyl phosphates are characterized by these diversities. The prototype of this phosphate ester class is methylphosphate. It is a relatively simple molecule whose aqueous stability has been extensively studied (2). At pH values below zero, the conjugate acid species predominates, and the solvolysis takes place with both carbon–oxygen and phosphorus–oxygen splitting. The neutral molecule is the principal species in pH range 0–2, and it cleaves exclusively at the carbon–oxygen bond. Above pH 3, the monoanion,

which cleaves exclusively at the phosphorus–oxygen bond, is formed and its concentration determines the reaction velocity well into the basic pH range.

Phosphates are of interest pharmaceutically because they provide a means of making soluble derivatives out of highly insoluble compounds. Additionally, some types of phosphate esters are sufficiently stable to allow the formulation of solutions with practical shelflives. These characteristics translated into biopharmaceutical terms mean instantaneous blood levels and facile parenteral administration. Most important or widely used of the pharmaceutical phosphate esters are the corticosteroid-21-phosphates. This report deals with their chemical behavior in aqueous solution as a function of concentration, pH, and other reaction conditions.

EXPERIMENTAL

Sample Preparation—In the kinetic studies, solutions were prepared at predetermined concentrations using distilled water and appropriate amounts of buffering agents and organic phosphate. In the pH 3–6 region, these were adjusted to pH at room temperature using either a concentrated solution of sodium hydroxide or concentrated hydrochloric acid. In the very low and high pH ranges, the samples were adjusted to pH at the temperature of the run. Initial concentrations were then determined by the Porter–Silber procedure (3, 4). After pH adjustment, the samples were filled in appropriately sized glass vials (usually 5 ml.) and placed in constant-temperature oil baths maintained within ±0.1° of the indicated temperatures. These were removed and analyzed on a suitable, predetermined schedule.

In the surface-tension determinations of critical micelle concentrations (CMC), a concentrated solution of the drug in distilled water was prepared and then diluted by 50 or 60% successively to levels far below the expected CMC. These were analyzed for surface tension as a function of concentration. Conductimetric studies were carried out on solutions prepared in a tight range around the expected CMC values (1.5×10^{-3} *M* to 3×10^{-2} *M*).

Equipment—Spectrophotometric determinations were performed on a Cary 11 recording spectrophotometer. The pH adjustments were monitored on either a Beckman model GS or a Corning expanded scale meter. The surface-tension measurements were done on a Cenco DuNoüy 70545 tensiometer and conductivity measurements on a Serfass conductivity bridge model RCM 15B1.

Inorganic Phosphate Procedure—*Chemicals*—All of the following chemicals were used without further purification: methylene chloride (distilled in glass); hydrochloric acid, reagent grade; potassium phosphate, monobasic, crystals A.R.; *N,N*-dimethylformamide (distilled in glass); ammonium molybdate A.R.; glacial acetic acid, reagent grade; cupric sulfate (anhydrous) A.R.; ascorbic acid USP; sodium acetate A.R., granular; stannous chloride, crystals A.R.; and sodium phosphate, dibasic (anhydrous) A.R.

Hydrocortisone-21-phosphate (disodium salt), prednisolone-21-phosphate (disodium salt), and dexamethasone-21-phosphate (disodium salt) were used as received.[1] Methylprednisolone-21-phosphate (M-21-P) (disodium salt) (Upjohn) was purified *via* continuous methylene chloride extraction for micellar kinetic studies. Otherwise, it was used as received.

Reagents—Originally the Mokrasch (5) procedure was employed. On later runs, a modified procedure with the following reagents was used:

1. Ammonium molybdate—2.0% $(NH_4)_6Mo_7 \cdot 4H_2O$ in double-distilled water.
2. Acetate buffer—310 ml. of glacial acetic acid, 49 g. of potassium acetate, and 48 mg. of cupric sulfate dissolved in double-distilled water and diluted to 1000 ml.
3. Ascorbic acid—2% ascorbic acid in distilled water.
4. Stannous chloride—20% stannous chloride in hydrochloric acid; 1 ml. diluted to 100 ml. with distilled water prior to use.
5. Standard inorganic phosphate solution—300 mg. (accurately weighed) of Na_2HPO_4 (dried 2 hr. at 110°) in 100 ml. of distilled water; 10 ml. diluted to 200 ml. with distilled water (approximately 2×10^{-4} *M*). When buffer systems were used, the standard was prepared in them.

Procedure—Total sample was removed from the ampuls, placed in a 60-ml. separator, and extracted with two 25-ml. portions of methylene chloride to remove formed sterol. An aliquot of the extracted sample was then appropriately diluted. To separate 100-ml. volumetric flasks, the following were added: (*a*) 2 ml. of standard, q.s. to 10 ml.; (*b*) 2 ml. of standard and 2–5 ml. of sample q.s. to 10 ml.; (*c*) 2–5 ml. of sample [appropriate size varied from concentration to concentration but same amount of sample used in (*b*) and (*c*) in any case for given run]; and (*d*) 10 ml. distilled water (blank).

This was followed immediately for each solution with 50 ml. of acetate buffer, 5 ml. of ammonium molybdate solution, 5 ml. of ascorbic acid solution, and 5 ml. of stannous chloride solution, mixing well after each addition. These were then diluted to volume with acetate buffer and allowed to develop for 30 min. The absorbance of the samples at 740 mμ against the blank was determined within 3 hr. using 1-cm. cells.

Calculations—

$$[\text{M-21-P}]_t = [\text{M-21-P}]^0 - [IP]_t \quad \text{(Eq. 1)}$$

$$= [\text{M-21-P}]^0 - [S] \times \frac{A_{mp}}{A_s} \times \text{D.F.} \times \frac{\Delta A_{mp}{}^t}{\Delta A_{mp}{}^0} \quad \text{(Eq. 2)}$$

where:

$[\text{M-21-P}]_t$ = molar concentration of methylprednisolone-21-phosphate with respect to time
$[\text{M-21-P}]^0$ = initial molar concentration of methylprednisolone-21-phosphate (independently determined); alternately, the inorganic phosphate infinity value was used as material balance was good
$[IP]_t$ = inorganic phosphate molar concentration with respect to time
$[S]$ = molar concentration of phosphate standard
A_{mp} = absorbance of sample
A_s = absorbance of standard
D.F. = dilution factor
$\Delta A_{mp}{}^t$ = absolute difference in the spiked and nonspiked sample at time, *t*
$\Delta A_{mp}{}^0$ = absolute difference in the spiked and nonspiked sample initially; equal to A_s

CMC Determinations—*1. Surface-Tension Measurements*—These were made on the tensiometer at room temperature. Each dilution was read three times and the values were averaged. It was found that pH was relatively invariant at pH = 7.5, and no adjustments were made. Experiments run at lower pH were done on solutions that had been accurately pH adjusted.

2. Conductimetric and Spectrophotometric Determinations—These were run on solutions diluted to between 0.75 and 15 mg./ml. corresponding to 1.5×10^{-3} *M* and 3×10^{-2} *M*, respectively. The conductimetric measurements were carried out in a jacketed-glass beaker in which the temperature was maintained within 0.1° of that indicated. The cell constant of the conductance probe was 1.0.

pKa Determinations—Solutions of M-21-P were prepared to contain 1.33 mg./ml. NaCl was added to give an ionic strength of 0.15. Titration curves were run on a radiometer titrator at the indicated temperature using 0.09823 *N* HCl as the titrant. The pKa_2 values were read directly from the graph at the half-neutralization point. The pKa_1 values were estimated from the one and one-half neutralization point and were corrected for water content of the phosphate sample. Equation 3 was used:

$$pKa_1 = pH_1 - \log \frac{[A^- + H^+]}{[HA - H^+]} \quad \text{(Eq. 3)}$$

THEORETICAL CONSIDERATIONS

From the beginning of these studies, it was realized that spurious, unquenchable reactions would be occurring simultaneously with the solvolytic reaction in the pH range of interest. To avoid the potentially serious complications resulting from these secondary decompositions, a method of monitoring the reaction, which was either sensitive to the presence of intact organic phosphate or capable of assessing formed inorganic phosphate, was sought. Two established analytical procedures, the Porter–Silber (3, 4) procedure

[1] Supplied by Merck & Co., Rahway, N. J.

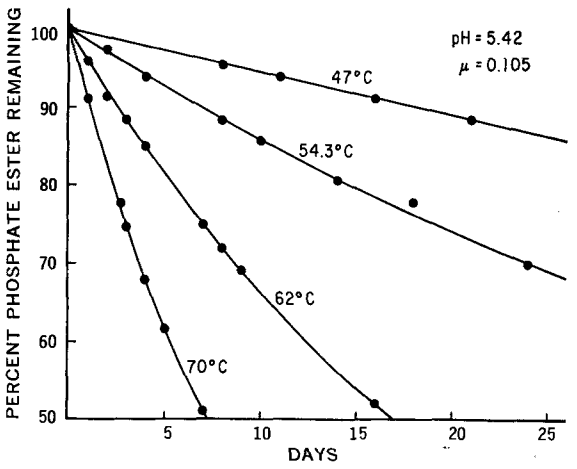


Figure 1—*Typical curves for the disappearance of M-21-P from aqueous media as a function of temperature at pH 5.42 and an ionic strength of 0.105.*

and the Mokrasch (5) inorganic phosphate procedure, were deemed suitable. After some experimentation with each, the inorganic phosphate procedure was chosen. This procedure is based on the reduction of phosphomolybdate to form a colored product. It was developed expressly for the determination of inorganic phosphate in the presence of labile organic phosphate and thus was particularly suited to the investigation.

The removal of formed neutral species was found to be a necessary condition to the successful application of this technique. Otherwise, color development was erratic. In addition, each sample was assayed with a counterpart spiked with a known amount of inorganic phosphate. The procedure is similar to that used in scintillation counting where counting efficiency is determined by adding an exact quantity of unstable isotope (^3H, ^{14}C, *etc.*) to the sample after it has been counted for its unknown concentration. In both cases the known concentration corrects the value of the unknown for variances in the sensitivity of the assay. Thus, any tendency for color development to drift was offset by including the drift factor, $\Delta A_{mp}{}^t/\Delta A_{mp}{}^0$, in the calculations (Eq. 2). With these precautions the infinity time values by the inorganic phosphate method were within experimental error of the independently determined initial concentrations.

The solvolysis of M-21-P between pH 0 and 8 was expected to be pseudo-first-order based on literature reports (6, 7) for similar compounds:

$$\frac{d[\text{M-21-P}]}{dt} = -k_{obs.}\,[\text{M-21-P}] \qquad \text{(Eq. 4)}$$

where [M-21-P] stands for the instantaneous concentration of M-21-P and $k_{obs.}$ is the observed pseudo-first-order rate constant at a given pH, temperature, ionic strength, *etc.* The integrated form of this equation becomes the familiar

$$[\text{M-21-P}]_t = [\text{M-21-P}]^0\, e^{-k_{obs.}t} \qquad \text{(Eq. 5)}$$

Since $[\text{M-21-P}]_t = [\text{M-21-P}]^0 - [\text{inorganic phosphate}]$, then

$$[\text{M-21-P}]^0 - [\text{inorganic phosphate}] = [\text{M-21-P}]^0\, e^{-k_{obs.}t} \qquad \text{(Eq. 6)}$$

or, on a percentage basis:

$$\frac{[\text{M-21-P}]^0 - [\text{inorganic phosphate}]}{[\text{M-21-P}]^0} \times 100 = 100\, e^{-k_{obs.}t} \qquad \text{(Eq. 7)}$$

Semilogarithmic plots of either intact M-21-P concentration or percent M-21-P remaining *versus* time should yield straight lines intercepting at log $[\text{M-21-P}]^0$ (Eq. 6) or log 100 (Eq. 7), respectively, with slopes of $k_{obs.}/2.303$.

RESULTS

The disappearance of M-21-P in reaction systems typical of those considered here is shown as a function of time and pH in Fig. 1.

Table I—Buffers Used in M-21-P Kinetic Studies

pH	Buffer
0.02	1.0 *M* $HClO_4$
0.72	0.2 *M* $HClO_4$
1.24	0.0645 *M* HCl, 0.05 *M* KCl
1.61	0.0263 *M* HCl, 0.05 *M* KCl
2.50	0.05 *M* Phthalate, 0.396 *M* HCl
3–8	Either 0.02 *M* citrate or 0.02 *M* bisulfite, 0.02 *M* citrate

Table II—Rate Constants at pH = 4.06 at 70° for Solvolysis of Several Corticosteroid-21-phosphate Esters

	$k^{70°}$ (days^{-1})
Methylprednisolone	0.151
Dexamethasone	0.148
Prednisolone	0.156
Hydrocortisone	0.148

This figure shows the residual percentage of the initial M-21-P in solutions consisting initially of 2×10^{-3} *M* M-21-P at several temperatures and pH = 5.42. The buffers used in the studies are from a paper by Bunton *et al.* (2) and are outlined in Table I.

As would be expected on the basis of Eq. 7, semilogarithmic plots of the residual percentage of M-21-P against time are linear. Typical pseudo-first-order curves are shown in Fig. 2 for the reaction at several temperatures and pH = 5.32. The solvolyses of prednisolone-21-phosphate, hydrocortisone-21-phosphate, and dexamethasone-21-phosphate proceeded in a similar fashion as shown by a single pH and temperature study summarized in Table II. These rates are indistinguishable within experimental error from one another.

The pH dependency of the reaction velocity is shown in Fig. 3. These data are for the reaction of solutions initially of 2×10^{-3} *M* concentration at 70°. Included with these data for later consideration are the 70° literature data of Marcus (8) for the solvolysis of hydrocortisone-21-phosphate and 101° data extrapolated to 70° for methylphosphate (2) using an activation energy of 30.6 kcal./mole.

The temperature dependency of the reaction was determined at multiple points above pH = 3. As predicted by the Arrhenius equation, plots of the log of the observed rate constant against the reciprocal of the absolute temperature yielded straight lines (Fig. 4). The lines representing different values of pH were also parallel. The activation energy values calculated from the slopes of these lines are tabulated in Table III. The average of these values, 27.5 kcal./mole, is assumed to be the best estimate of the activation energy.

Because of the striking diversity in these results and previous results on hydrocortisone-21-phosphate and the obvious structural

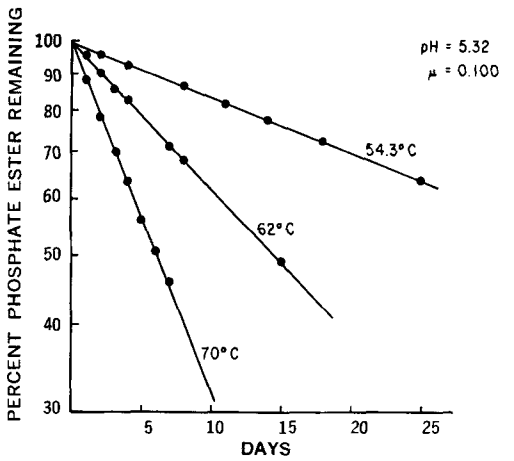


Figure 2—*Semilogarithmic plots showing the disappearance of M-21-P as a function of temperature at pH 5.32 and an ionic strength of 0.100.*

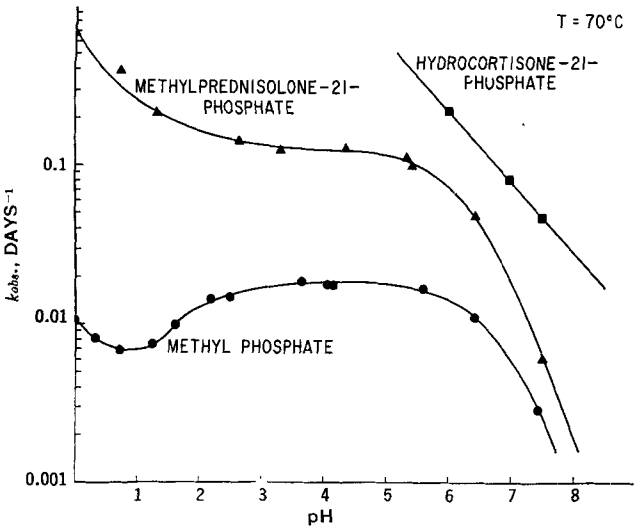
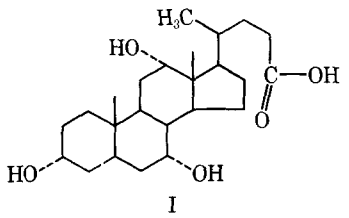


Figure 3—*The 70° pH profiles for methylphosphate, M-21-P, and hydrocortisone-21-phosphate.*

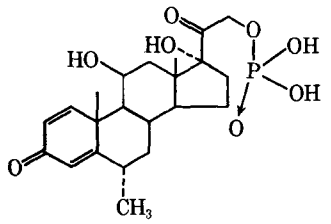
semblance between the corticosteroid phosphates and the surface-active bile acids (Structures I and II), studies attempting to relate the

mol. wt. = 409



I

mol. wt. = 454



II

I—cholic acid II—methylprednisolone-21-phosphate

observed differences to molecular aggregation effects were initiated. Typical results of surface-tension measurements of serially diluted solutions of M-21-P at room temperature and pH = 7.5 are shown in Fig. 5. These are plotted in the usual fashion as the surface tension *versus* the log of the molar concentration. Distinct breaks in the curves at approximately 0.02 *M* were observed. Because of the tedium of the procedure and interest in expanding the data to include surface-tension measurements as a function of pH and temperature, conductimetric studies supplanted surface-tension experiments. The data from a representative temperature experiment at pH = 7.5 are graphically presented in Fig. 6. It appears from this plot that there is a slight increase in the CMC with increasing temperature. CMC values fall between 0.01 and 0.02 *M*, which is in good agreement with the surface-tension studies. Neither method yielded sharply defined CMC's. Conductimetric studies were also performed at 25° and several pH values. At low pH, where the free acid predominates, the solubility of the compound was too low to make measurements. CMC's measured above pH = 3 were not distinguishable from one another.

Table III—Apparent Activation Energy as a Function of pH for the Solvolysis of M-21-P

pH	E_a
7.52	27.0
6.45	27.3
5.42	26.3
5.32	28.9
4.35	29.4
3.32	27.6

With the amphiphilic character of the compound definitely established, studies were conducted to assess the effect of association colloid formation on the reaction. Unfortunately, in the pH range of maximum interest, the data gathered were only crudely quantitative due to an inability to control pH in bufferless systems. Therefore, the results will only be reported here in qualitative terms. The reaction rate was found to be invariant with concentration at pH = 4 in solutions ranging from 0.005 to 0.16 *M* (0.005, 0.01, 0.02, 0.04, 0.08, and 0.16 *M*). In these studies, pH drifts were found to be no more than several hundredths of a unit throughout the concentration range. However, on reactions run at pH = 7 and 7.5, a different behavior was observed for series of solutions of the same concentrations. For the dilute solutions, pH shifts to more acidic conditions exceeded a full pH unit. As the concentration increased, the pH drift narrowed to slightly over half a unit. The downward shift in pH would tend to accelerate the reaction. Despite this, the reaction in 0.16 *M* solutions was consistently observed to be 2–3 times more rapid than in 0.08 *M* solutions. There appeared to be little difference between 0.08 and 0.04 *M* solutions. Below 0.04 *M*, the pH shifts became exaggerated and there was an apparent increase in reaction rate.

DISCUSSION

Analysis of the pH–Rate Profile—The pH profile for all alkyl phosphates is a composite of the individual species profiles, and its shape is determined by their relative concentrations and rates (1). Four species are possible: the conjugate acid, the free acid, the monoanion, and the dianion. The conjugate acid is formed in highly acidic solution (pH $\ll$ 1.0) and thus is only of marginal concern for the steroid-21-phosphates. In pH range 1–8, the instability of these compounds can be explained on the basis of the independent reactivities of the free acid and monoanion. An M-21-P species profile as a function of pH is given in Table IV. These data are calculated using averaged, independently determined pK_{a_1} and pK_{a_2} values of 2.55 and 6.04, respectively, and Eqs. 8–10:

$$C_N = \frac{[H^+]^2}{K_{a_1}K_{a_2} + K_{a_1}[H^+] + [H^+]^2} \quad \text{(Eq. 8)}$$

$$C_M = \frac{K_{a_1}[H^+]}{K_{a_1}K_{a_2} + K_{a_1}[H^+] + [H^+]^2} \quad \text{(Eq. 9)}$$

$$C_D = 1 - [C_N + C_M] = \frac{K_{a_1}K_{a_2}}{K_{a_1}K_{a_2} + K_{a_1}[H^+] + [H^+]^2} \quad \text{(Eq. 10)}$$

where C is the fractional concentration and subscripts N, M, and D refer to the neutral species, monoanion, and dianion, respectively.

Comparison of the pH profiles of methylphosphate and M-21-P between pH 0 and 2, where the neutral species prevails, indicates that the trough found for methylphosphate is leveled out for its corticosteroid counterpart (Fig. 3). Reaction rates at pH = 1.0 differ by approximately two orders of magnitude. Similar plateauing is observed in the profiles of α-D-glucose-1-phosphate (9) and monobenzyl phosphate (10). Unlike these two cases, the increased reactivity of the neutral species cannot be attributed to a change from an S_N2 solvolytic displacement to an S_N1 carbonium-ion mechanism. The carbonium ion of M-21-P would be even less energetically favored than that for methylphosphate due to the electron-withdrawing α-carbonyl. A probable explanation for the increased reactivity is stabilization of the transition stated (S_N2 mechanism with sp^2 hybridization) due to π-orbital overlap with the electron-rich neighboring oxygen atom (11). A similar effect of similar or greater magnitude for α-carbonyls has been noted in other solvolytic displacements (12). The plateau in the M-21-P profile suggests that the specific second-order rate constants for the cleavage of the neutral species and monoanion are of the same order of magnitude.

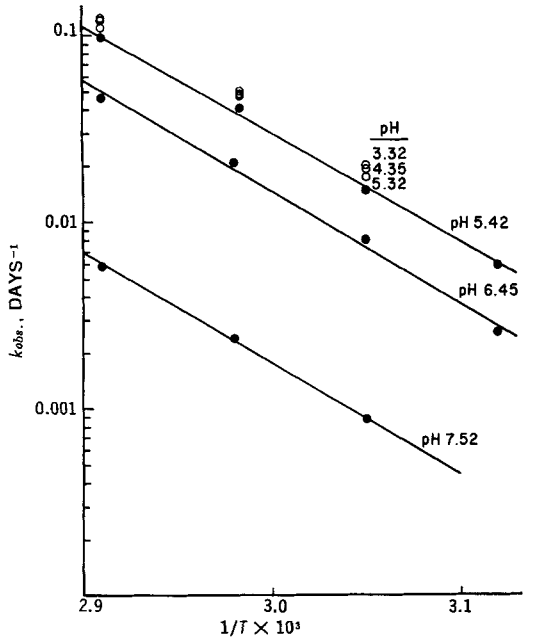


Figure 4—*Arrhenius plots for the hydrolysis of M-21-P at several values of pH.*

In the pH region of pharmaceutical interest, the principal degradative route for phosphate monoesters is the cleavage of the monoanion. The qualitative similarity of the reaction for M-21-P and methylphosphate above pH = 3 is readily apparent from Fig. 3. The reaction proceeds with P—O splitting. The specific requirements for the hydrolysis of the monoanion led Butcher and Westheimer (13) to postulate a mechanism in which an unstable monomeric metaphosphate-ion intermediate is formed. This mechanism is consistent with the relative rapidity of the hydrolysis, the requirement of protonation of one of the phosphate oxygens, P—O splitting, and the marked insensitivity of the hydrolytic velocity to the nature of the leaving group. At 70° the monoanion rate constant for M-21-P is just over seven times that found for methylphosphate. Because of an approximately 3-kcal./mole difference in observed activation energies, this factor shrinks as the temperature is lowered. Regardless, this difference is significant and likely attributable to electronic effects of the α-carbonyl on the strength of the P—O bond. An interesting fact discovered in these studies is that, in dilute solution, the nature of the steroid nucleus has little effect on the specific second-order monoanion instability constant for the corticosteroid-21-phosphates, at least insofar as hydrocortisone, methylprednisolone, dexamethasone, and prednisolone are different. This suggests that the overriding influence on the observed rate is the adjacent carbonyl and that the M-21-P data can be considered representative of the stability of related steroid phosphates.

Throughout the course of the kinetic studies at premicellar concentrations, there was no noticeable effect of buffers on the reaction rate. Buffers could be interchanged or changed in concentration without a measurable reaction velocity change. This is not too surprising because at low pH, the buffer species present are extremely poor nucleophiles. At high pH the mechanism precludes buffer involvement but, neglecting this, the reaction requirements are such that two negatively charged species must be brought together and this is energetically unfeasible.

Because of doubts regarding the relative validity of the available methods for following the progress of the reaction, the disappearance of M-21-P was also followed directly using the Porter–Silber procedure (3). Results by this procedure were comparable with those obtained following the appearance of inorganic phosphate. For instance, at 54.3° and pH = 5.4, the inorganic phosphate procedure yielded a value for $k_{obs.}$ of 1.45×10^{-2} day^{-1} while the value by the Porter–Silber procedure was 1.83×10^{-2} day^{-1}. These results were obtained on ampuls from the same set. It was implicit in these studies that the formation of spurious products would have negligible effect on the principal reaction. In other words, it was assumed that changes in the molecule prior to solvolysis, if any, would not appreciably affect processes at the phosphate moiety. Products were assumed to be inert. The dominance of the solvolysis as the major instability, the linearity of plots, and the reproducibility of data by both of these methods attest to the credibility of these assumptions.

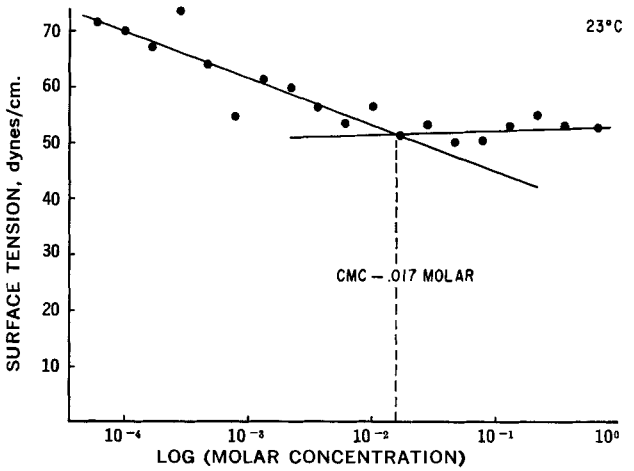


Figure 5—*Determination of the CMC of M-21-P by surface-tension measurement. Each point is an average of three determinations.*

The dianion of M-21-P is virtually unreactive. Negative linear dependency on the pH is observed far into the high pH range, and this dependency parallels the concentration of the monoanion. Presumably, the concentration of the monoanion should eventually become negligible, resulting in a plateau in the pH profile. This point, if it is reached at all, is well above pH = 8 based on the observations in this study.

Micellization and Its Probable Influence on Reaction Rate—Strong evidence has already been presented for the formation of association colloids in these systems. In addition, slight but unmistakable deviations from Beer's law were observed above the CMC. Similarly, the solubility of methylprednisolone in solutions of M-21-P increases sharply as the CMC is exceeded.

The enthalpy for the formation of the micelles, ΔH_m, is calculable from the available data and Eq. 11:

$$\Delta H_m = -RT^2 \left(\frac{d \ln \text{CMC}}{dT}\right) \qquad \text{(Eq. 11)}$$

and was found to be approximately −1.0 kcal./mole at 40°. This is entirely consistent with the behavior of other systems (14). The significance of this value is that it indicates the marked temperature insensitivity of micellization. Over a narrow temperature range,

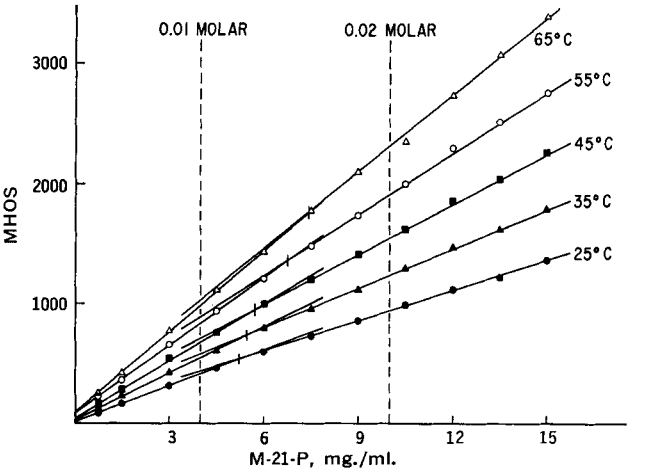


Figure 6—*Determination of the CMC of M-21-P by conductance measurement. Each line represents a different temperature. In all cases, the apparent CMC fell between 0.01 and 0.02* M.

Table IV—M-21-P Species Concentrations of $f(pH)^a$

pH	Free Acid (N)	Monoanion (M)	Dianion (D)
0	0.997	0.003	Negligible
1	0.973	0.027	Negligible
2	0.78	0.12	Negligible
3	0.262	0.738	Negligible
4	0.034	0.956	0.008
5	0.003	0.914	0.083
6	Negligible	0.523	0.477
7	Negligible	0.099	0.901
8	Negligible	0.011	0.989
9	Negligible	Negligible	0.998
10	Negligible	Negligible	1.000

[a] M-21-P total = 1.0.

the fraction of molecules participating in micelles to total surfactant molecules remains relatively constant. In other words, temperature effects parallel those of chemical equilibria rather than chemical reactions.

Another factor to be kept in mind is the salt effect or, more properly, the gegenion effect on micellization. Many investigators working with diverse systems have observed that the logarithm of the CMC changes linearly with the logarithm of the concentration of gegenion (14):

$$\ln \text{CMC} = -K \ln Cg + \text{constant} \qquad \text{(Eq. 12)}$$

In practical terms, this contraindicates the use of buffers in well-designed studies on micellar kinetics. It also suggests that the hydrocortisone-21-phosphate reaction systems of Marcus (8) were of significant micellar character. His working phosphate concentration was 0.02 M, slightly above the CMC, and his studies were performed in 0.20 M phosphate buffer providing as little as 0.2 M and up to 0.4 M concentration of positively charged ions (gegenions), depending on pH. The activation energy reported by Marcus for the solvolysis of hydrocortisone-21-phosphate is 17.0 kcal./mole, fully 10 kcal./mole less than that found for M-21-P and 13 kcal./mole less than the average for this entire class of compounds (1). A shifting micellar fraction with temperature can account for much of these substantial differences.

Controlling variables in micellar kinetic studies can be a difficult job. Not only do the usual parameters such as temperature and pH have to be controlled, but also gegenion concentration and impurities. Buffers can have a multiplicity of effects. Therefore, several studies at pH $\simeq$ 4 and pH > 7.0 were initiated in bufferless systems. There was definitely no micellar facilitation of the reaction at low pH. At high pH, it appeared as if the reactions were accelerated above the CMC, particularly when changing pH effects, which ran counter to the micellar effects, were taken into account. The insensitivity to micellization at pH $\simeq$ 4 is explicable when the peculiar requirements and mechanism of the phosphate monoanion decomposition are considered. The rate-determining step, the production of a metaphosphate anion, is a unimolecular reaction reflecting the intrinsic instability of a given molecule. With the exception of factors influencing protonation, the presence of neighboring molecules would not play a major role in the reaction. It is implicit here that the pH at the micellar surface is not appreciably different from the bulk pH. This is not necessarily true at higher pH. The surface potential, ψ, is becoming increasingly negative. If the hydrogen ions are present in a Boltzman distribution represented by Eq. 13:

$$[H^+]_s = [H^+]_b \exp. - (|e|\psi/kt) \qquad \text{(Eq. 13)}$$

where $[H^+]_s$ and $[H^+]_b$ are the surface and bulk hydrogen-ion concentrations, respectively, then the pH at the micellar surface is expected to lag behind that of the bulk solution. This will change the ratio of phosphate monoanions to dianions relative to the bulk and, therefore, produce an apparent increase in rate. This factor and the gegenion effect together could help produce the rather unusual pH and temperature dependency observed by Marcus (8) in the pH range 5–8.

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