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A MATHEMATICAL ANALYSIS OF AN IRREVERSIBLE CONSECUTIVE FIRST ORDER REACTION USING HYDROCORTISONE SODIUM SUCCINATE AS A MODEL

BY

JOHN W. MAUGER

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACEUTICAL SCIENCES

UNIVERSITY OF RHODE ISLAND

1968

TITLE ABSTRACT

STEROIDAL ESTER DEGRADATION

ABSTRACT

The degradation of hydrocortisone sodium succinate was followed in aqueous systems buffered at pH values of 6.9, 7.2 and 7.6 at 70°C. The decomposition pathway can be described by an irreversible consecutive first order process which followed a two-step sequence: ester $\xrightarrow{k1}$ alcohol $\xrightarrow{k_2}$ degradation products. Hydrolysis of the steroid ester yields the alcohol which in turn degrades to a fraction devoid of the 17-dihydroxyacetone function. For the pH range studied, ester hydrolysis was assumed to occur by way of an intramolecular attack of the anion on the ester carbonyl carbon. A reaction pathway for the decomposition of the steroidal alcohol is proposed, where an etio acid and formic acid are produced by way of an oxidative mechanism. No attempt was made, however, to qualitatively determine the exact form the corticosteroid assumed subsequent to the degradation of the dihydroxyacetone function. Determination of the relevant species at various time intervals involved a spectrophotometric analysis using the blue tetrazolium assay which is specific for the intact 17-dihydroxyacetone chain. The initial and subsequent samples were calculated on the basis of the absorbance reading of the initial sample as 100%. The first rate constants, k_1, at the various pH levels were evaluated by a semilog plot of steroid ester concentration versus time and found to increase with

increasing hydroxyl-ion concentration. Three documented methods which employed differential equations or dimensionless parameters were used to evaluate the second rate constant, k_2. Two additional methods were derived in an attempt to simplify the calculations and were used successfully to calculate the second rate constant for the decomposition of hydrocortisone sodium succinate.

MASTER OF SCIENCE THESIS

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1968

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I. INTRODUCTION

The availability of a quantitative analytical method is essential to the formulator or scientist for the implementation of predictive kinetics. The application of various environmental parameters, such as temperature, permits the rapid approximation of relevant rates and produces data which aid in the mechanistic interpretation of chemical reactions. Thus, it is often found that dosage forms and their physical and chemical properties, such as solubility or buffer content, are predicated upon the nature of the active moieties and the rates at which decomposition occurs.

Studies of the degradation of chemical substances which are specifically used to elicit a physiological response have been many and varied (1, 2) and often beset with difficulties. One of the problems inherent in a specific type, a consecutive irreversible first order reaction, focuses upon the method of determination of the second rate constant, (k_2).

There are several methods available (3-6) for the estimation of k_2, which depend on the use of differential equations or dimensionless parameters derived from the rate expressions describing a consecutive first order reaction. It would seem that any other method of evaluation which is simpler, more rapid and leads to more precise, although not

necessarily more theoretically valid results, would be desirable. It is to this end that this investigation was undertaken using the aforementioned kinetic model.

II. PAST WORK

The hydrolytic pathway of the 21-hydrocortisone hemiester of succinic acid has been investigated under varied environmental conditions, such as pH and temperature (7, 8). The pH profile indicates that hydrolysis is due to specific acid catalysis in the pH range of 1.0 to about 2.5 and specific hydroxyl catalysis from approximately 7.6 to 10.0. The intermediate range being subject to an intramolecular attack of the anion on the ester carbonyl carbon or specific hydroxyl-ion catalysis of the undissociated hemiester.

Steroidal alcohols containing the 17-dihydroxyacetone side chain have been kinetically described under varying conditions of pH, temperature, buffer media, and oxygen deprivation. The rate of disappearance of the dihydroxyacetone function of prednisolone was investigated in solutions of varying hydroxyl-ion concentration, both in the presence and absence of air (9), and resulting data indicated that at least three parallel first order reactions were involved in the decomposition of the steroidal component.

The effect of trace metal content in aqueous solutions of prednisolone has also been studied (10) and shown to significantly increase the production of the steroid devoid of the 17-dihydroxyacetone function.

Sequestering agents were demonstrated to reduce the degradation rate under similar conditions of pH and temperature. Cortisol has also been shown to decompose to fractions devoid of the 17-dihydroxyacetone chain in a phosphate buffer at pH 7.4 and at temperatures of 16°, 25°, and 35° C. (11).

The complete decomposition of fluprednisolone acetate, from the ester, to the free alcohol to further degradation products was followed in an aqueous environment buffered near neutrality (5). The data indicated the kinetic pathway to be consecutive first order.

III. EXPERIMENTAL

Determination of pH for Maximum Partitioning of the Steroid Alcohol - Aqueous solutions of the sodium salt of 21-hydrocortisone hemisuccinate in aqueous solutions of various hydrogen ion concentration were equilibrated with chloroform[1] at room temperature. Subsequent separation of the layers and analysis of the aqueous phase indicated that essentially all of the hemiester remained in the aqueous portion at pH 10, which is consistent with the work of previous investigators (12).

Analytical Methodology, Colorometric Analysis - Maximum color development, using the blue tetrazolium assay which is specific for the intact dihydroxyacetone chain, occurred at 90 minutes and remained stable for at least four hours. Since hydrolysis of the ester must occur prior to color development (13), it was assumed that the steroid alcohol would have also reached its maximum color development during this time period. Hence, all experimental trials were allowed to develop in the dark for 90 minutes, $\pm$ 30 seconds.

---

[1] Fisher Scientific Co., Fair Lawn, New Jersey.

Beer-Lambert Relationship - Alcoholic solutions containing varying concentrations of sodium 21-hydrocortisone hemisuccinate were subjected to the blue tetrazolium assay and spectrophotometrically analyzed at 523 mμ. A plot of absorbance versus concentration of the steroid (calculated as the free alcohol) (Fig. A) indicates that a linear relationship exists up to 22.22 micrograms/milliliter final volume. The slope of this line was calculated by the method of least squares which is described in Appendix A.

Reagents - pH 10 Buffer - Seventy-one grams of anhydrous sodium dibasic phosphate[1] (reagent grade) was dissolved in 1000 milliliters of distilled water and adjusted to pH 10 with 1.0 M sodium hydroxide solution.

Chloroform.[1] Analytical grade.

Dilute Hydrochloric Acid, U.S.P.[2] Reagent grade.

Glacial Acetic Acid.[2] Reagent grade.

Blue Tetrazolium Solution.[1] Fifty milligrams of blue tetrazolium (certified reagent grade, lots 766100 and 764388) were dissolved in 95% alcohol and diluted to 10 milliliters.

Tetramethylammonium Hydroxide Solution.[3] Five milliliters of a

---

[1]Fisher Scientific Company, Fair Lawn, New Jersey.

[2]Allied Chemical Company, Morristown, New Jersey.

[3]Eastman Organic Chemicals, Rochester 3, New York.

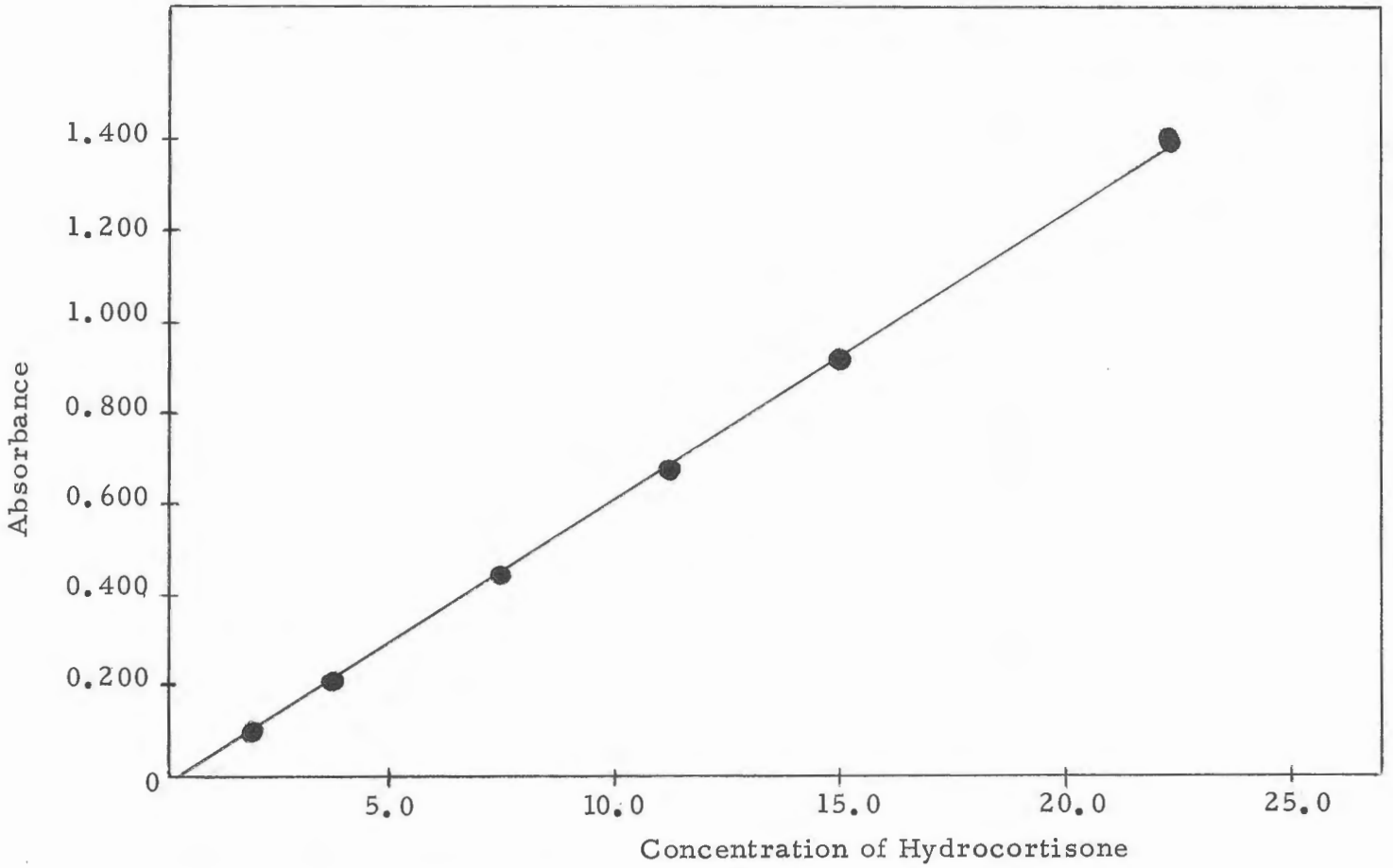


Figure A. A Beer-Lambert plot of absorbance versus the concentration of hydrocortisone (calculated as the free alcohol) in mcg. /ml.

10% aqueous solution of tetramethylammonium hydroxide were diluted to 50 milliliters with 95% alcohol. Both the blue tetrazolium and tetramethylammonium hydroxide solutions were used within twenty-four hours subsequent to preparation and were refrigerated when not in use.

Phosphate Buffer Solutions.[1] Monobasic and dibasic phosphates (Fisher certified reagent grade) were used to prepare 0.02 M buffers of pH 6.9, 7.2 and 7.6. Where necessary, sodium chloride was used to maintain an ionic strength of 0.054. In all cases, the buffer solutions were prepared using freshly boiled glass distilled water.

21-Hydrocortisone Hemisuccinate(Solu-Cortef[2])(supplied through the courtesy of Upjohn Company).

Equipment - Forma Temp Model 2095 constant temperature bath,[3] Leeds Northrup pH meter[4] and Cary 16 Spectrophotometer.[5]

Assay Procedure - A 2 milliliter aliquot of 21-hydrocortisone hemisuccinate containing approximately 133 mg. of the ester was diluted with the appropriate buffer to 200 milliliters. Twenty-five milliliter portions of this solution were pipetted into 60 milliliter screw-top

---

[1]Fisher Scientific Company, Fair Lawn, New Jersey.

[2]The Upjohn Company, Kalamazoo, Michigan.

[3]Forma-Temp Jr. Model 2095, Marietta, Ohio

[4]Leeds and Northrup pH Meter Model 7401, 4901 Stenton Avenue, Philadelphia, Pennsylvania.

[5]Cary 16 Spectrophotometer, 232 Washington Street, Belmont, Massachusetts.

bottles and immediately placed in an ice bath prior to commencing the kinetic run. These samples were then removed to a constant temperature bath set at 70°C. and allowed to attain thermal equilibrium.

One sample designated as the "initial sample" was withheld from the 70° bath and immediately assayed. A ten milliliter quantity of this sample was pipetted into a separatory funnel which contained 10 milliliters of dilute HCl. Four successive extractions were performed with 20 milliliter portions of chloroform. The chloroform phases were drained through a funnel, containing a chloroform wetted pledget of cotton, into a 100 milliliter flask and made to volume with chloroform. Exactly 10 milliliters of this solution were pipetted into a 50 milliliter erlenmeyer flask and the solvent extracted under reduced pressure at 30° C. The residue was dissolved in 20 milliliters of alcohol and 2 milliliters of blue tetrazolium and four milliliters of tetramethylammonium hydroxide solution added. Timing was started and the samples allowed to develop in the dark for 90 minutes. Subsequent to the development period, 1 milliliter of glacial acetic acid was added to stabilize and quench the color. The absorbance was then measured at 523 mμ with a 10 millimeter cell against a blank containing everything except the steroid. All initial and subsequent samples were measured against a blank of this composition at 523 mμ. The pH of the initial sample was also measured.

At suitable time intervals, sample bottles were withdrawn from the temperature bath and immediately placed in an ice bath. After

chilling, a 10 milliliter aliquot was withdrawn and pipetted into a separatory funnel containing 5 milliliters of pH 10 buffer and 5 milliliters distilled water.

Four extractions with 20 milliliter portions of chloroform were performed and the chloroform phases drained through a funnel containing a chloroform wetted pledget of cotton into a 100 milliliter flask. The flask was then made to mark with chloroform.

Another 10 milliliter portion was pipetted from the sample bottle and treated to extraction procedures as were performed with initial sample.

Ten milliliter aliquots of these chloroform solutions from the acid and basic extractions were pipetted into separate 50 milliliter erlenmeyer flasks. These samples were subjected to drying and color development procedures identical to that of the initial sample.

Since the reaction is pH dependent, samples from the bath were measured as the run progressed to insure that the pH remained constant.

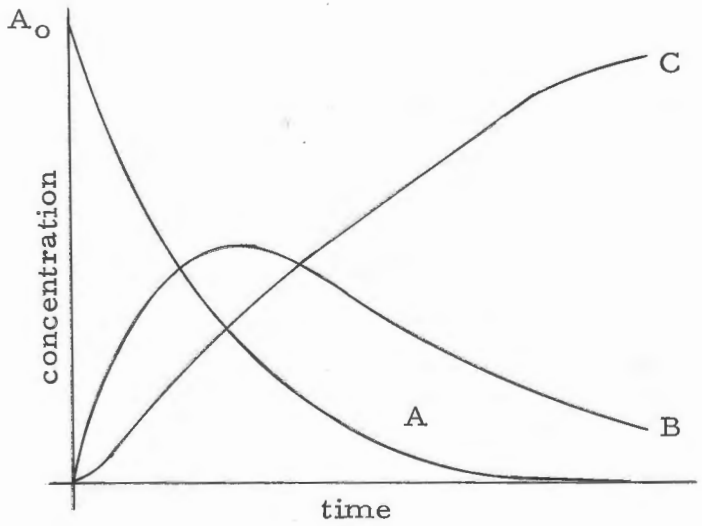
The initial and subsequent samples were calculated on the basis of the absorbance reading of the initial sample as 100%.

IV. THEORETICAL AND CHEMICAL CONSIDERATIONS

The use of reaction rates as predictive tools for evaluating the stability of therapeutically effective drugs in solution is a well known methodology (1, 2). One reaction pathway of particular interest is the consecutive first order type. This pathway is exemplified by the degradation of glucose in acid solutions (3) and by the decomposition of the esters of cortical steroids (5).

A first order reaction of the consecutive type is illustrated below and is followed by a depiction of the time course of content for the relevant species in the reaction.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$



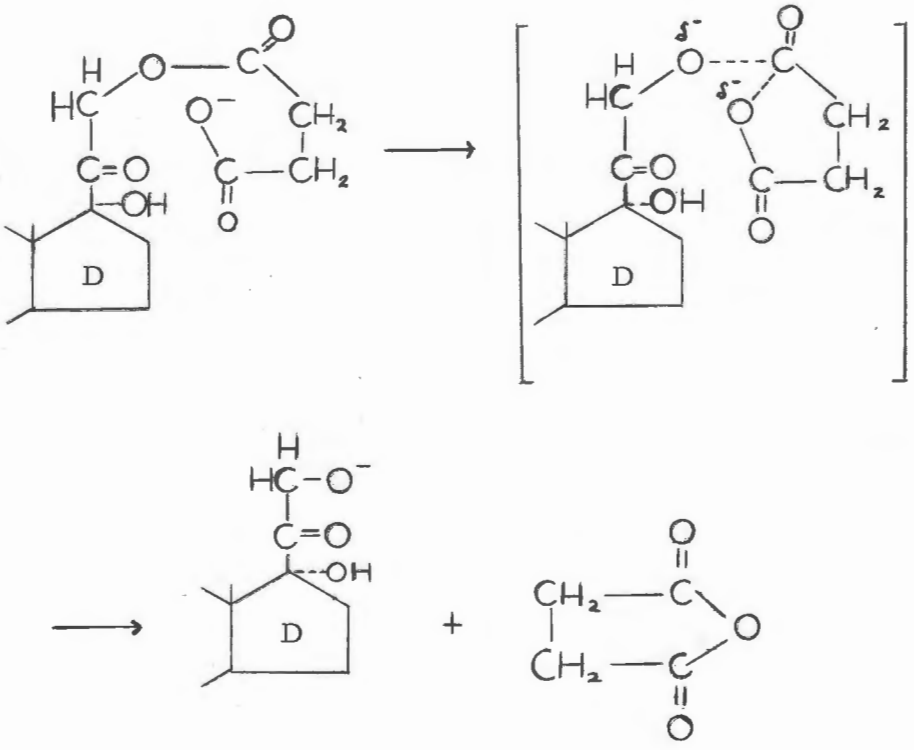
More specifically, a reaction of the above type was studied involving the use of the sodium salt of the steroidal ester, 21-hydrocortisone hemisuccinate.

In this case, the original species, A, would be the ester in the anionic form. The intermediate, B, is the hydrolysis product, i.e., the free alcohol. The further decomposition of B would then cause the formation of other products. These products, which may be characterized as those moieties devoid of the 17-dihydroxyacetone side chain, are gathered together under the symbol C.

As expected, most chemical reactions and the rates at which they occur are environmentally dependent. Thus, the obvious effect of kinetic parameters such as pH, which controls the species and the content of that species in solution, would be of paramount importance in determining the magnitude of the rate as well as the mechanism.

It has been proposed that the decomposition of 21-hydrocortisone hemisuccinate into its hydrolytic alcohol product depends upon an intramolecular attack of the carboxylate anion or by specific hydroxyl ion attack on the undissociated hemiester (7, 8). This would be expected to occur in a narrow pH range, and has been shown to exist at hydrogen ion concentrations near neutrality. Investigation has shown that hydrolysis in this pH region cannot be attributed to general acid-base catalysis (8). Moreover, it is unlikely that specific acid-base catalysis occurs at pH values around neutrality since the decomposition rates exceed those

predicted by specific acid and hydroxyl ion catalyzed hemiester hydrolysis. Since the hydrolytic reactions of substituted phenyl acid succinates proceed by an intramolecular carboxylate attack under favorable pH conditions (14), this pathway would seem to be favored for the hydrocortisone hemiester. The hydroxyl ion catalyzed hydrolysis of the undissociated ester is, however, a recognized alternate mechanistic pathway (8). A scheme of the intramolecular attack is shown below and is felt to be the operative pathway in the pH range close to neutrality.



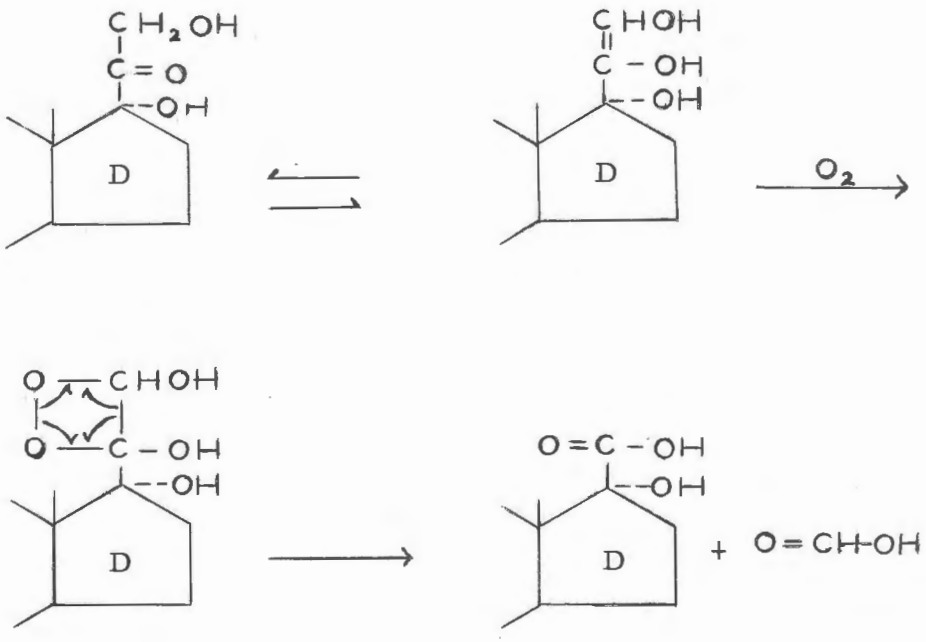
Subsequent to the hemiester hydrolysis and the concomitant production of the free alcohol, degradation of the 17-dihydroxyacetone side chain occurs. The base-catalyzed decomposition of prednisolone, both under oxidative and non-oxidative conditions, has been investigated (9) and the reaction pathway shown to yield steroidal breakdown components that could be characterized broadly as neutral and acidic. In the presence of air, it was presumed that an oxidative cleavage of the 17-dihydroxyacetone chain produced an etianic acid. Other investigations (8) have also suggested that the decomposition of the 17-hydroxyacetone side chain will produce an etio acid and/or formic acid. Shown on the following page is a suggested pathway for this reaction in the presence of air.

Accordingly, cortisol in aqueous solutions near neutrality has been reported to degrade to products devoid of the 17-dihydroxyacetone side chain (11).

An investigation of some of the factors influencing the side chain reaction of the steroidal alcohol in solution was made using prednisolone (10). An apparent metal-catalyzed reaction was observed which was pH independent above pH 7 and below pH 5 and exhibited a first-order-hydroxide-ion dependency in the intermediate range.

The overall pathway of a steroidal ester to its products devoid of the 17-dihydroxyacetone function has been investigated in aqueous solution utilizing fluprednisolone acetate (5), and the data was interpreted on the basis of a consecutive first order reaction. Hence,

the use of the hydrocortisone hemiester as a model for this particular kinetic pathway is substantiated.



Proposed Degradation of the 17-Dihydroxyacetone Function in the Presence of Oxygen

V. DISCUSSION

In the present study, the degradation behavior of the hemisuccinate ester of hydrocortisone was investigated over a narrow pH range. A temperature of 70°C. was chosen in order to accelerate the decomposition rate so that its measurement would be kinetically convenient. It has been assumed that the increased rate is mediated by thermal phenomena.

Since this reaction pathway is of the first order consecutive type, typical concentration-time curves should be observed. In Figure 1, the contents of the relevant species and their time course is shown for a pH value of 6.9. It can be seen that the concentration of the original ester moiety (A) decreases by a first-order rate. The intermediate alcohol product (B) rises to a maximum value at about 18 hours and decreases in an approximately linear fashion to about 17 percent at 64 hours. The concentration of products devoid of the 17-dihydroxyacetone side chain (C), after an induction period of about 1-2 hours, rises rapidly and tails off after about 45 hours due to the near completion of the irreversible reaction. The concentration of the final products, C, is seen to be about 80 percent after 64 hours.

It is also interesting to note that the initial content of the steroidal ester is approximately 5 percent lower than the theoretical concentration of 100 percent. This was felt to be due to a small amount of

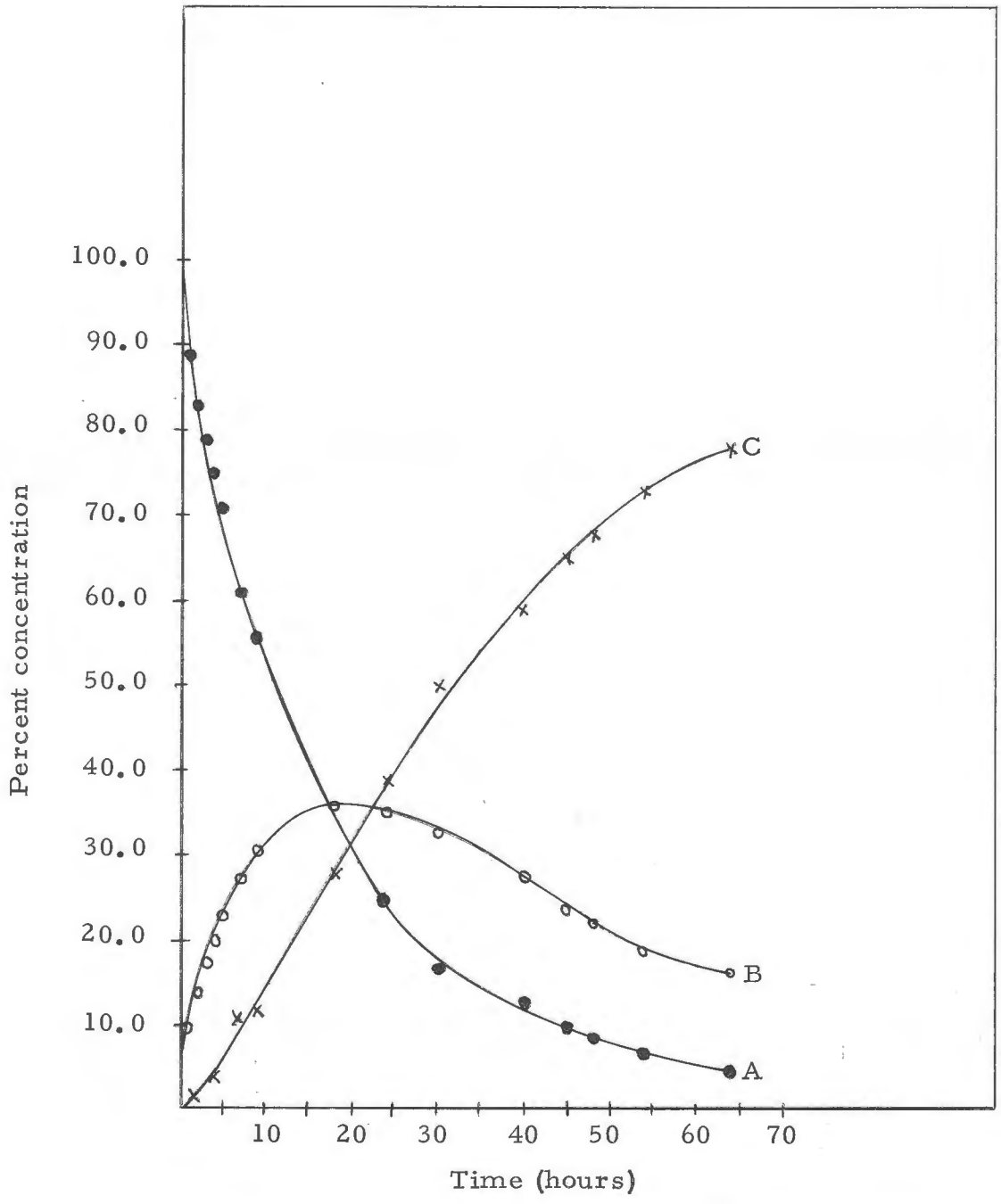


Figure 1. Concentration-time curves for substances A (hydrocortisone hemiester), B (hydrocortisone alcohol), and C (degradation products) at 70°C. and a pH of 6.9.

steroidal alcohol present initially.

Further examination of Figure 1 reveals that A intersects C as B reaches a maximum.

In the above system and those subsequently studied at pH values of 7.2 and 7.6, it should be pointed out that the hydrogen ion concentration was maintained by the use of 0.02 M phosphate buffers at a constant ionic strength of 0.054.

Shown in Figure 2 is a plot of the concentrations of the relevant species, A, B, and C, versus time at a pH value of 7.2.

The concentration of A is seen to asymptotically approach a final value as the reaction proceeds toward completion. At all pH levels investigated, the decrease of A is by a pseudo-first order rate since the solvent, water, is present in large excess. The steroidal alcohol content, B, reaches a maximum after about 13 hours and occurs at a substantially earlier time than with a pH of 6.9.

The concentration of C reaches about 30 percent as B is maximized and examination of the figure shows that A is also intersected at this point.

As previously mentioned, the pH for this and all studies was maintained with 0.02 M phosphate buffers at an ionic strength of 0.054.

Figure 3 is a plot of the concentrations of A, B, and C versus time at a pH of 7.6. It will be noted that this figure parallels those previously shown although A decreases much more rapidly as the pH increases.

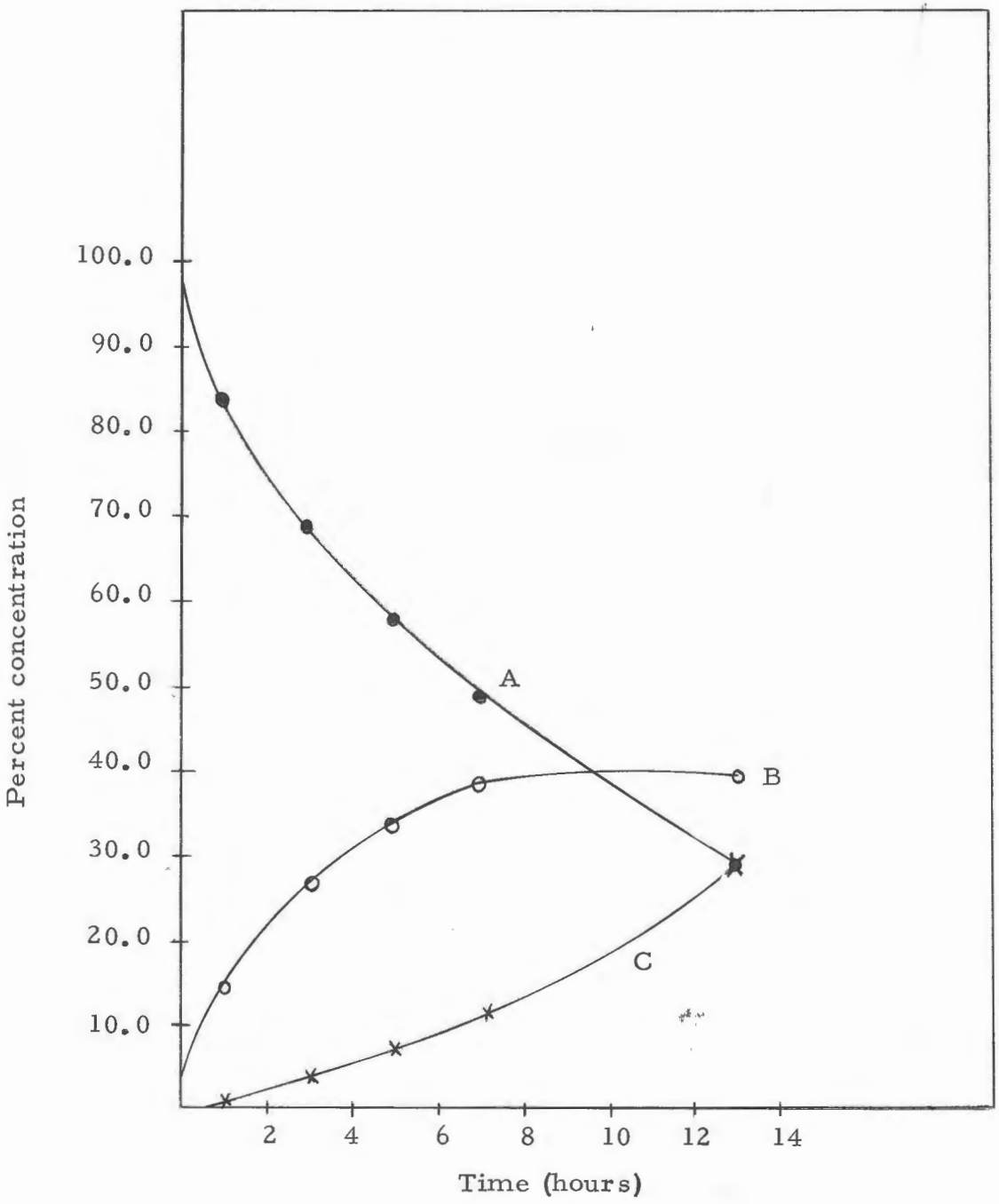


Figure 2. Concentration-time curves for substances A (hydrocortisone hemiester), B (hydrocortisone alcohol), and C (degradation products) at 70°C. and a pH of 7.2.

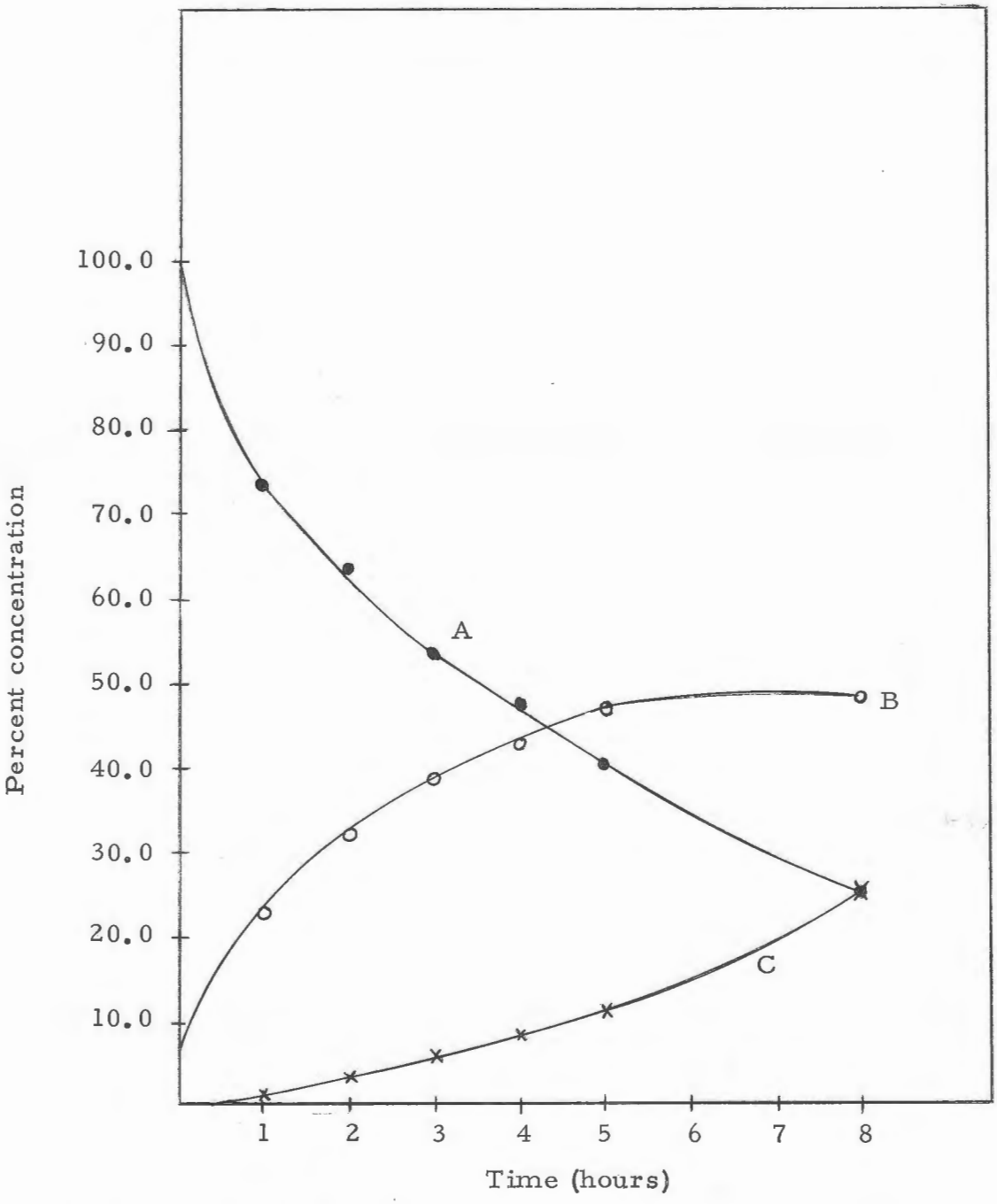


Figure 3. Concentration-time curves for substances A (hydrocortisone hemiester), B (hydrocortisone alcohol), and C (degradation products) at 70°C. and a pH of 7.6.

Here, the content of B reaches a maximum after only 8 hours. It must be mentioned, however, that the second rate constant does not soley depend upon the time at which B is maximized, but also the concentration at this point. Examination of the plot shows that B reaches its maximum at a concentration of about 48 percent compared to a maximum value of approximately 40 percent from Figure 2. This indicates that actually less C has been formed at a pH of 7.6, although the maximum has occurred earlier. The concentration of products devoid of the 17-dihydroxyacetone side chain is about 25 percent at this point.

Consistent with the data from previous figures, is the fact that C intersects A as B reaches a maximum concentration.

It will be noted from Figures 1, 2 and 3 that the concentration versus time plots all have the same pattern, indicating a consecutive first order reaction in all cases studied. The data for the concentration of each species for all pH levels investigated is shown in Tables I-III.

TABLE I

PERCENT CONCENTRATIONS OF THE STEROID ESTER (A), STEROID ALCOHOL (B) AND PRODUCTS DEVOID OF THE 17-DIHYDROXYACETONE SIDE CHAIN (C) AND THEIR TIME COURSE AT pH VALUE OF 6.9

Time (hours)	%A	%B	%C
1	89.5	10.1	0.4
2	83.2	14.5	2.3
3	79.4	17.9	2.7
4	75.7	20.1	4.2
5	71.5	23.5	5.0
7	61.1	27.9	11.1
9	56.1	31.1	12.7
18	35.4	36.0	28.5
24	25.4	35.2	39.4
30	17.0	33.4	49.6
40	13.1	28.4	58.6
45	10.4	24.1	65.5
48	9.2	22.8	68.0
54	7.4	19.5	73.1
64	5.2	16.7	78.1

TABLE II

PERCENT CONCENTRATIONS OF THE STEROID ESTER (A), STEROID ALCOHOL (B) AND PRODUCTS DEVOID OF THE 17-DIHYDROXYACETONE SIDE CHAIN (C) AND THEIR TIME COURSE AT pH VALUE OF 7.2

Time (hours)	%A	%B	%C
1	83.8	14.9	1.3
3	69.1	27.1	3.8
5	58.4	34.1	7.5
7	48.5	39.0	12.5
13	29.9	40.9	29.2

TABLE III.

PERCENT CONCENTRATIONS OF THE STEROID ESTER (A), STEROID ALCOHOL (B) AND PRODUCTS DEVOID OF THE 17-DIHYDROXYACETONE SIDE CHAIN (C) AND THEIR TIME COURSE AT pH VALUE OF 7.6

Time (hours)	%A	%B	%C
1	74.4	23.4	2.2
2	63.9	32.6	3.5
3	54.4	39.4	6.2
4	47.6	43.4	9.0
5	40.7	47.5	11.9
8	25.6	48.7	25.9

The equations describing a first order consecutive reaction were first integrated by Harcourt and Esson (15) (Appendix B) and predict that the rate of hydrolysis of the steroid ester may be evaluated by a semilog plot of the concentration of the ester versus time. Plots of this type for pH values of 6.9, 7.2 and 7.6 are shown in Figure 4.

Examination of the figure indicates that the rates of hydrolysis of the steroidal ester increases with an increase in pH. This is consistent with the results of previous work (7, 8) done with 21-hydrocortisone hemisuccinate in this pH area. It can be seen that the hydrolysis rate at a value of pH 7.6 is approximately three times greater than at pH 6.9.

The calculated initial amount of steroidal ester is seen from Figure 4 to deviate from the theoretical concentration of 100 percent. As mentioned previously, this is felt to be due to a small amount of steroid alcohol initially present.

Good linearity is observed at each pH level indicating that the disappearance of 21-hydrocortisone hemisuccinate is by a pseudo-first order rate. The data for these plots were treated by the method of least squares (Appendix A) in order to evaluate the slopes. The derived first order rate constants are shown in Table IV.

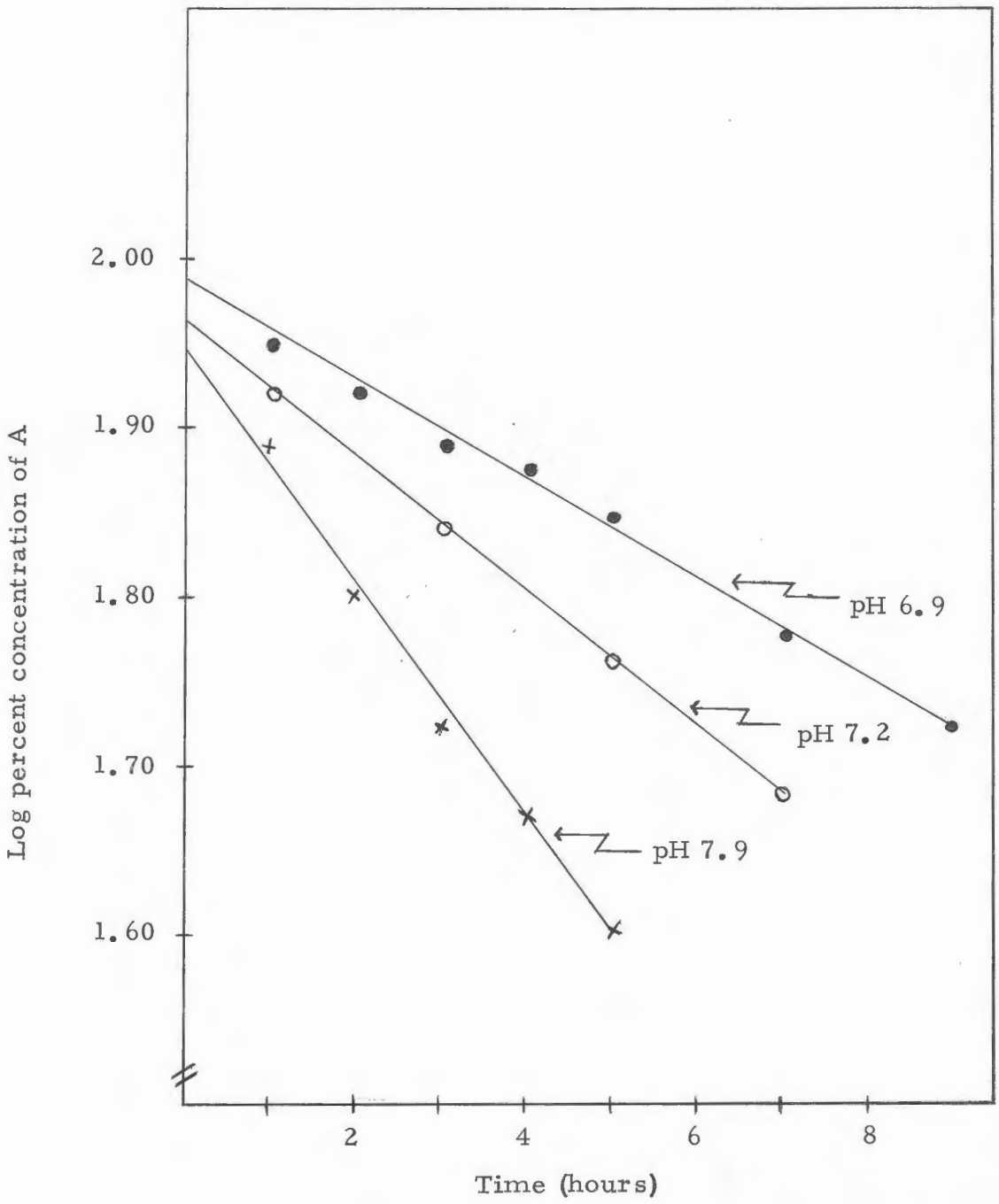


Figure 4. Plots of the log of hydrocortisone sodium succinate concentration versus time at pH values of 6.9, 7.2 and 7.6 at 70°C.

TABLE IV

FIRST ORDER RATE CONSTANTS

FOR

HYDROLYSIS OF 21-HYDROCORTISONE HEMISUCCINATE

IN BUFFERED AQUEOUS MEDIA AT 70°C.

pH	rate constant (k_1) per hr.
6.9	0.055
7.2	0.085
7.6	0.151

Methods of Evaluating k_2 -

In the kinetic model described previously, it was implied that the time course content for the intermediate or final species could be described by appropriate overall rate constants.

The importance of the relative magnitude of these two rate constants would be seen in describing a reaction as occurring or not occurring by a rate limiting process. That is, a large value for k_1 relative to k_2 or vice versa indicates that for the scheme of $A \xrightarrow{k_1} B \xrightarrow{k_2} C$, C is practically nonexistent in the first case, and, in the second, B is depleted to a large extent at a small expense of A. Only when the two rate constants are of similar magnitudes does the intermediate species assume a role contributing to the significance of k_2. Thus, the total interpretation of any model must include all the relevant rates and their concurrent magnitudes. An irreversible first order reaction of the consecutive type which is limited to two steps is rather complex relative to obtaining the second rate constant (k_2). This is attested to by the availability of various methods of estimation of k_2 in the literature (3-6). These methods have been described below, and each has been applied to the presently obtained data and summarized in an appropriately referenced table shown in a latter portion of this section.

Method I - Dimensionless Parameters:

Frost and Pearson (4) have described equations relating the maximum occurring with the intermediate component, B, and its relationship

to k_2 by the use of various analytical expressions which have been defined and are given in Appendix C. Jensen and Lamb (5) have successfully applied this method to their data where $\mathfrak{B}$ maximum was obtained by plotting $\mathfrak{B}$ versus $(1-\alpha)$ and evaluating the maximum from the graph. The symbol α is defined as the fraction of ester (A_o) decomposed. It will be noted from equation 4 of Appendix C that the ratio of k_2/k_1, i.e., $\mathfrak{K}$, is related to $\mathfrak{B}$ maximum and subsequent to the calculation of $\mathfrak{K}$, k_2 may easily be evaluated since k_1 is already known. The data in Tables I-III were evaluated in terms of the dimensionless parameters and plots of $\mathfrak{B}$ versus $(1-\alpha)$ are shown in Figures 5, 6, 7 for the three pH values used. Examination of the plots indicates that $\mathfrak{B}$ maximum is increasing in systems of increasing basicity.

In Table V the results of this method relative to the $\mathfrak{B}$ maximum and $\mathfrak{K}$ values are given. It can easily be seen that the $\mathfrak{K}$ values are decreasing with increasing $\mathfrak{B}$ maximum values. Hence, since k_2 is the product of k_1 and $\mathfrak{K}$, k_2 depends not only on the magnitude of k_1, but also the point at which $\mathfrak{B}$ is maximized.

The limitation of this method is the calculation of $\mathfrak{K}$, which in practice becomes tedious. Other workers (5) have suggested that this value may be obtained conveniently with the aid of a computer.

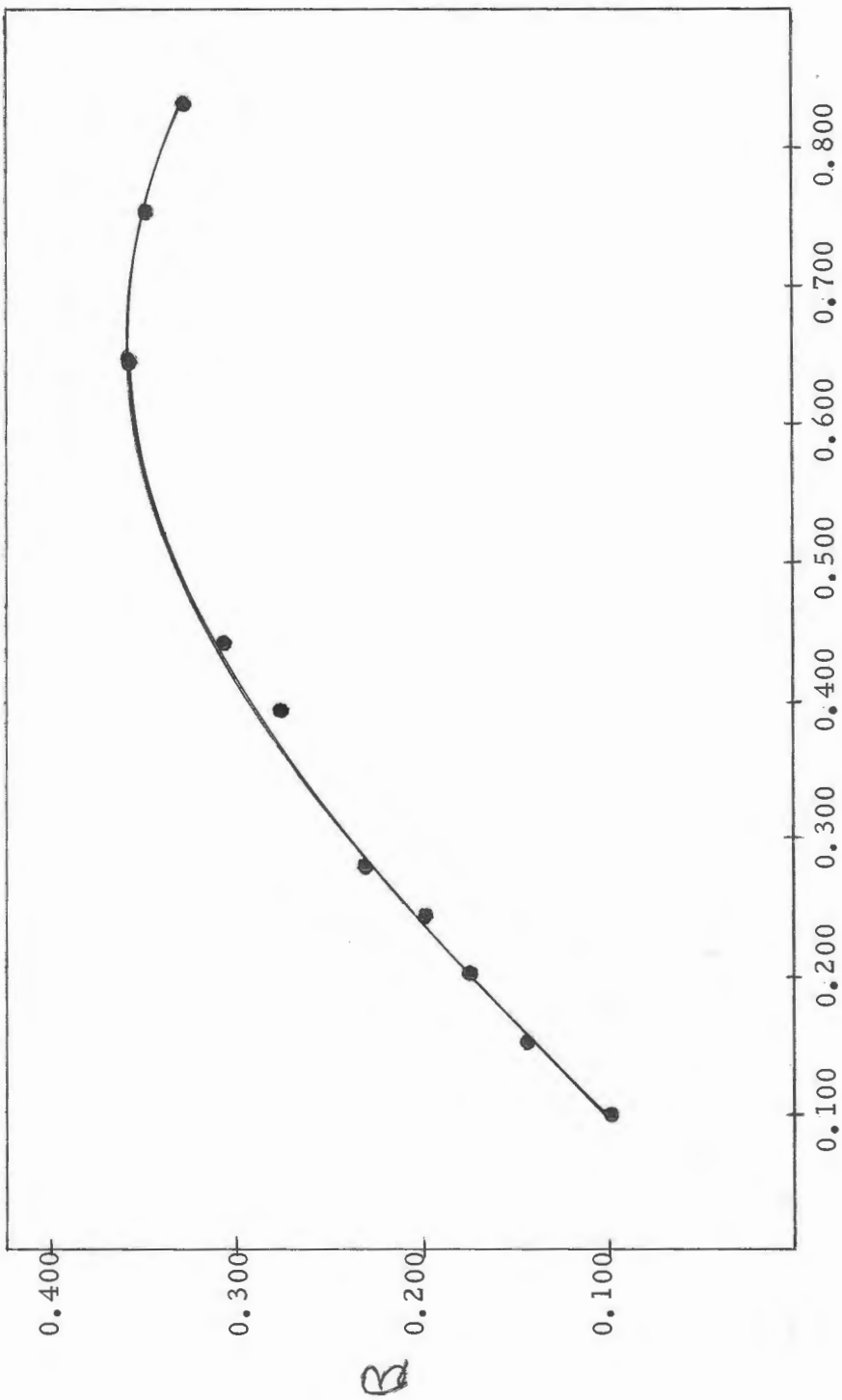


Figure 5. A plot of β versus (1 - ∝) at a pH value of 6.9.

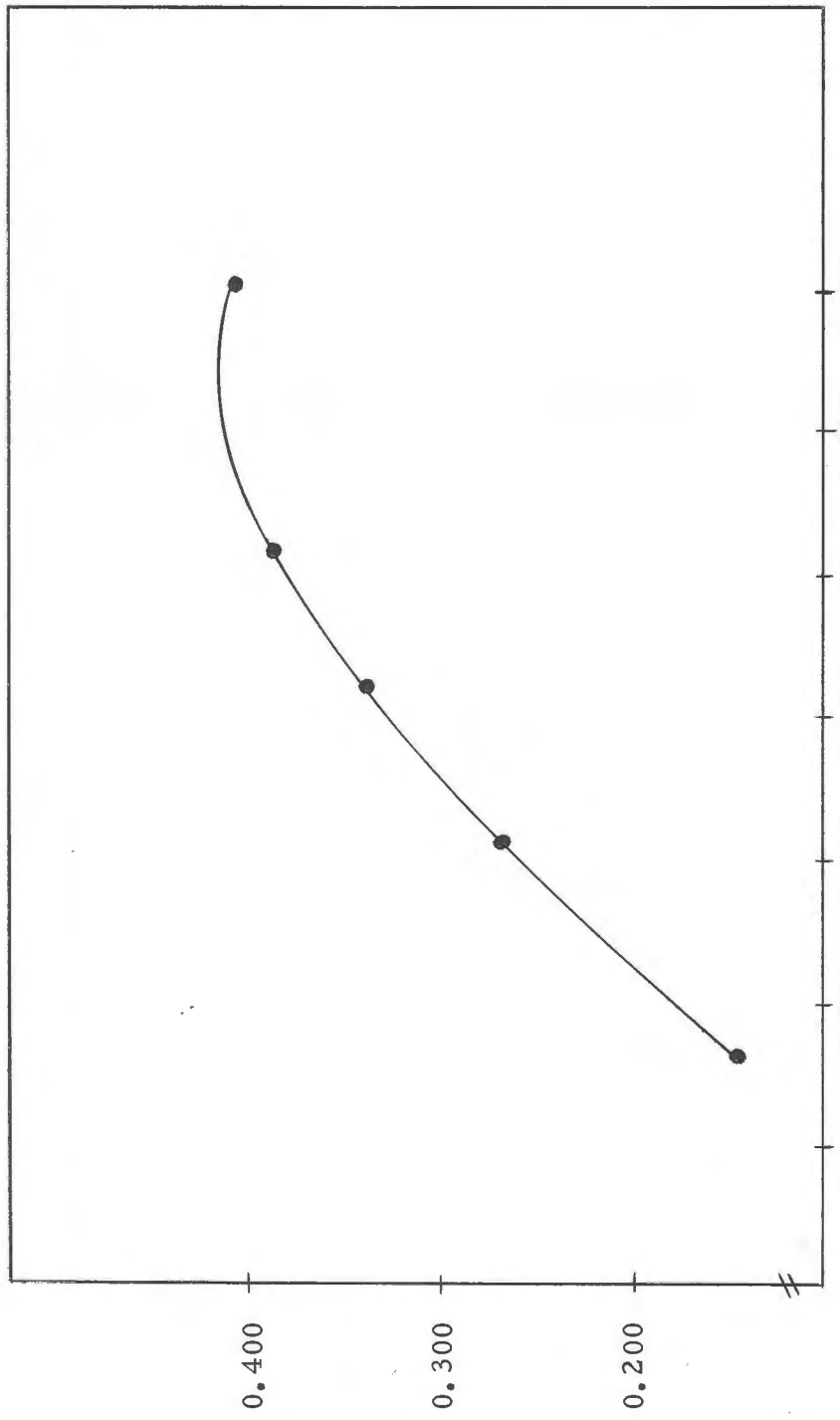


Figure 6. A plot of β versus (1 - ϰ) at a pH value of 7.2.

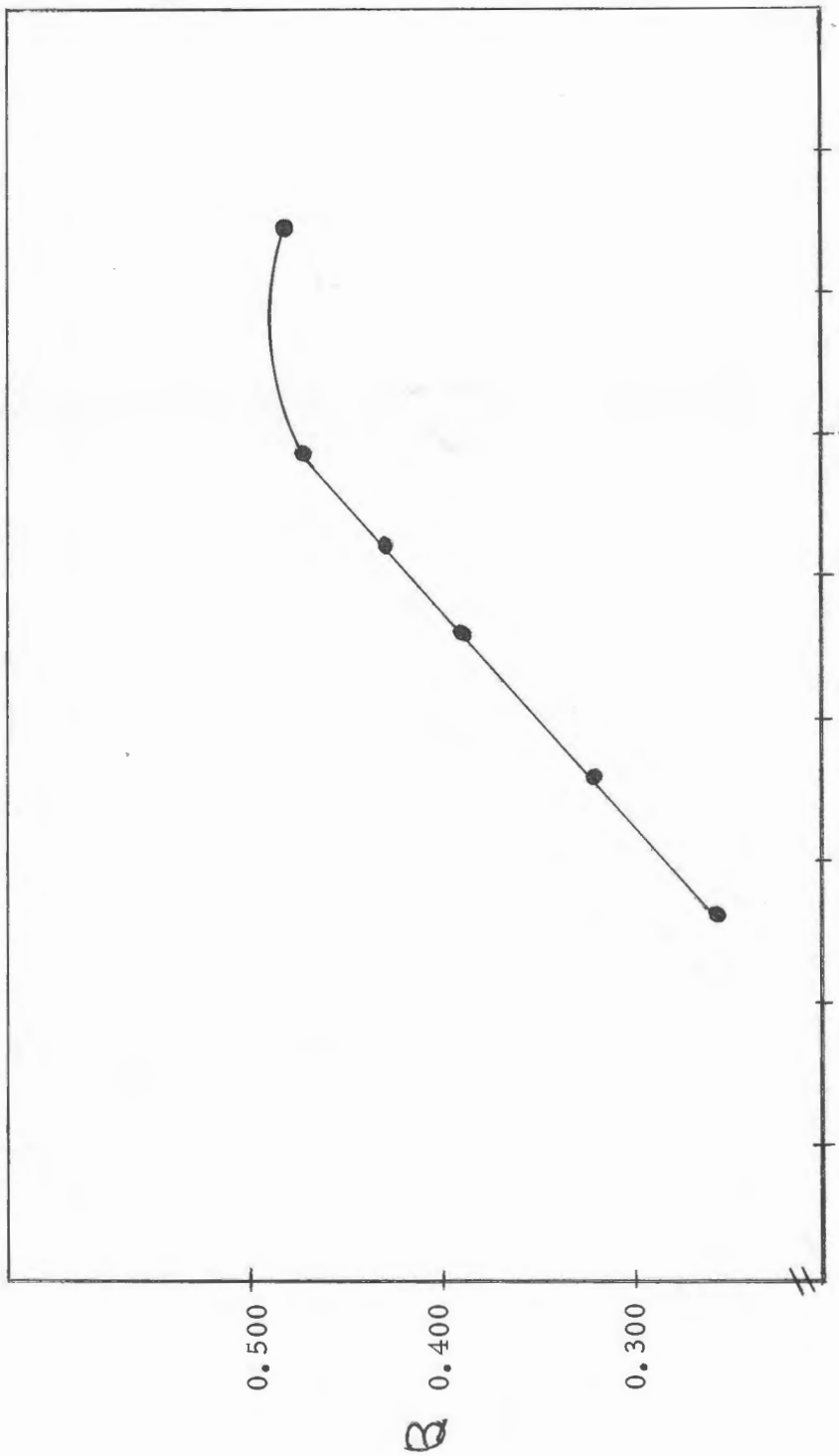


Figure 7. A plot of β versus (1 - ∝) at a pH value of 7.6.

TABLE V

VALUES OF K AND B MAXIMUM

AT pH VALUES OF 6.9, 7.2 AND 7.6

pH	B maximum	K
6.9	0.363	0.91
7.2	0.415	0.79
7.6	0.490	0.53

Method II - Time Ratio Method

Swain (6) first described a method whereby a time-percentage relationship may be utilized to calculate k_2. Frost and Pearson (4) have used this method with the further application of dimensionless parameters (Appendix D) and give the time-percentage reaction relationship for various values of k_2/k_1 in tabular form. A plot of $\mathcal{S}$, (i.e., $\mathcal{B} + 2\mathcal{Y}$) versus time for the data from pH 6.9 trial is shown in Figure 8.

Examination of Figure 8 shows that $\mathcal{S}$ increases with time and from the plot, the times for 15 percent and 35 percent reaction were chosen (i.e., where $\mathcal{S} = 0.3$ and 0.7 respectively).

It was found that the time corresponding to an ordinate value of 0.7 was 10.7 hours and 4.2 hours at 0.3. The log of the ratio of times for 35 and 15 percent reaction was subsequently found to be 0.395. This value may be used to evaluate k_2/k_1 from tables relating k_2/k_1, to the log t_{35}/t_{15} (4). In this case, k_2/k_1 was found to be 0.90 and since k_1 has previously been calculated, k_2 may be evaluated.

The results obtained by the Swain method should be verified by using additional pairs of times for other various combinations of percentage reactions (6). Hence, it is necessary to carry the reaction towards virtual completion in order to achieve several time pairs. This in practice may not be convenient or in some cases quantitatively possible.

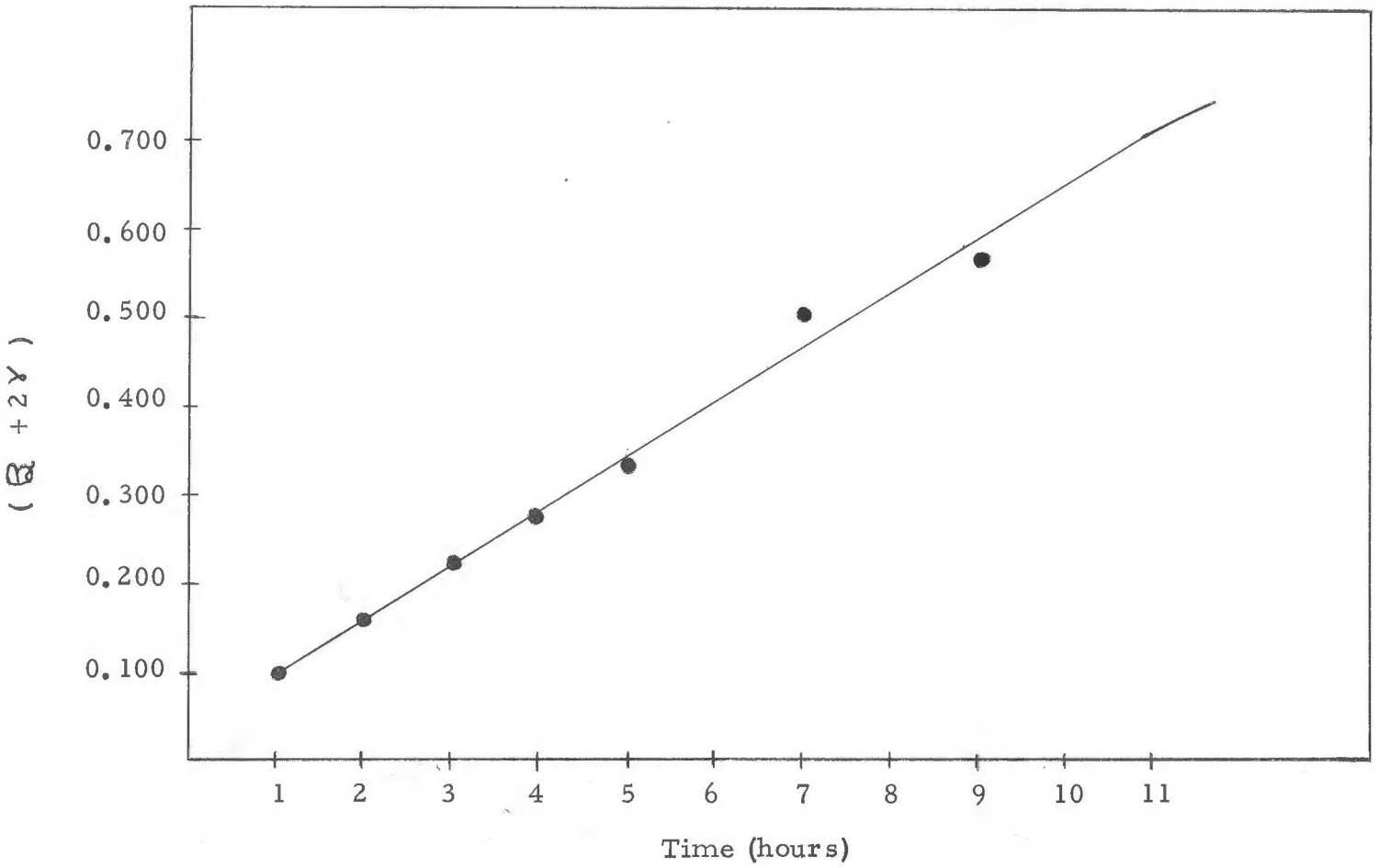


Figure 8. A plot of (β + 2γ) versus time at a pH value of 6.9.

Method III - Differential Equations

The rate of change of the intermediate, B, with time and its relationship with k_1 and k_2 has been used to evaluate k_2 (3). Equation 3 of Appendix B indicates that a plot of dB/dt versus B should yield a straight line with a y-intercept of k_1A and a negative slope corresponding to k_2. The data from pH 7.6 was plotted in this manner and is shown in Figure 9.

Examination of this figure indicates that fairly good linearity was found for the points plotted. A negative slope of 0.320 was calculated from the graph and the value for k_1A was evaluated by extrapolation to the y-intercept. It was found that A at this point corresponds to a value slightly greater than 100 percent.

The disadvantages of this method are twofold since: 1) A as given in the differential equation is not a constant, but assumes the value of A_o when plotted in this manner and 2) dB/dt is difficult to evaluate and the differential equation is extremely sensitive to rate changes.

The method of determining the dB/dt values from a plot of B versus time has been described (3) and utilizes a mirror and tangents taken from the curve. Values measured in this manner were evaluated by three operators and shown in Table VI. The reproducibility of these values would seem to be fairly consistent, but the sensitivity of the differential equation and the ultimate effect any point will have on the slope limits its use.

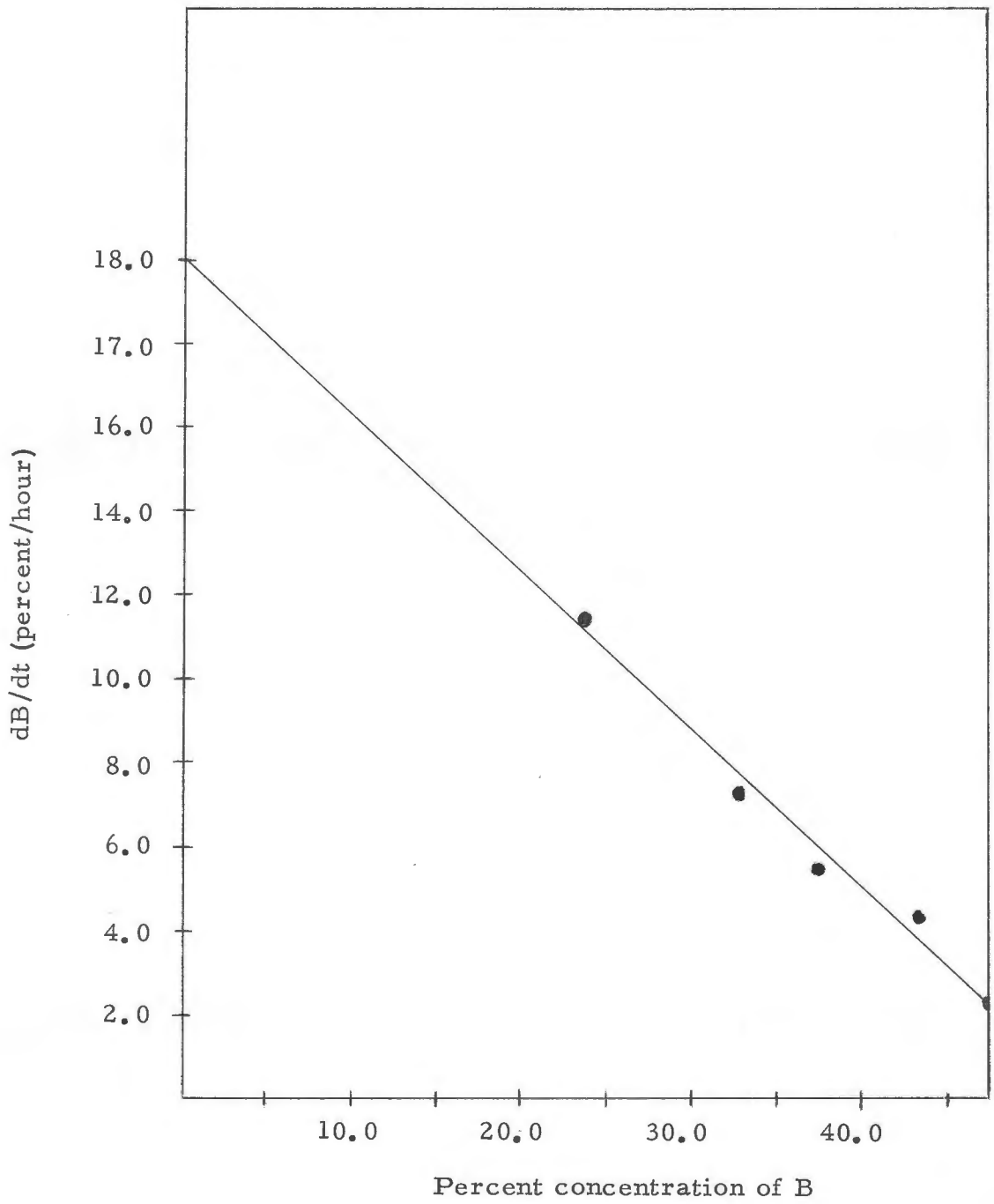


Figure 9. The rate of formation of hydrocortisone alcohol (dB/dt) versus the concentration of hydrocortisone alcohol (B) at 70°C. and a pH of 7.6.

TABLE VI

COMPARISON OF dB/dt VALUES BY MIRROR METHOD

FOR THE pH 7.6 TRIAL

	dB/dt (percent per hour)		
Time (hour)	Operator I	Operator II	Operator III
1	10.0	13.5	11.0
2	6.2	7.0	8.5
3	5.9	6.0	4.6
4	4.4	4.5	3.6
5	2.6	2.5	2.8

Method IV - Intersection of A and C

From Figures 1-3, it may be seen that curve A intersects curve C as B reaches a maximum. This fortuitous relationship was realized by the authors as a method of evaluating k_2.

The equations relating to this method are shown in Appendix E. It is apparent that as B is maximized, the ratio of A/B equals k_2/k_1.

As A intersects C, their ratio becomes one and if the values of A and C can be conveniently ascertained, then B may be calculated from the stoichiometric relationship where $A_o = A + B + C$. The ratio of A/B then becomes k_2/k_1 and k_2 may be evaluated since k_1 is known.

An empirical relationship was found to exist between the concentration of steroidal ester, A, and the log of the ratio of the ester and products devoid of the 17-dihydroxyacetone chain, A/C, and plots of this type for pH values of 6.9, 7.2 and 7.6 are shown in Figure 10.

As indicated by the above linear relationship, the y-intercept occurs as the log of A/C approaches zero (i.e., where the ratio is one or the point where A equals C). It was realized that the point where A intercepts C could be chosen from time course content curves such as Figures 1-3, but the above method offers two distinct advantages. First of all, the linearity provides a mathematical method of presumably predicting the y-intercept as the log of A/C approaches zero. Thus, the reaction would not need to be carried to the point where it is maximized in order to calculate k_2. Secondly, it will be noted from the figure that

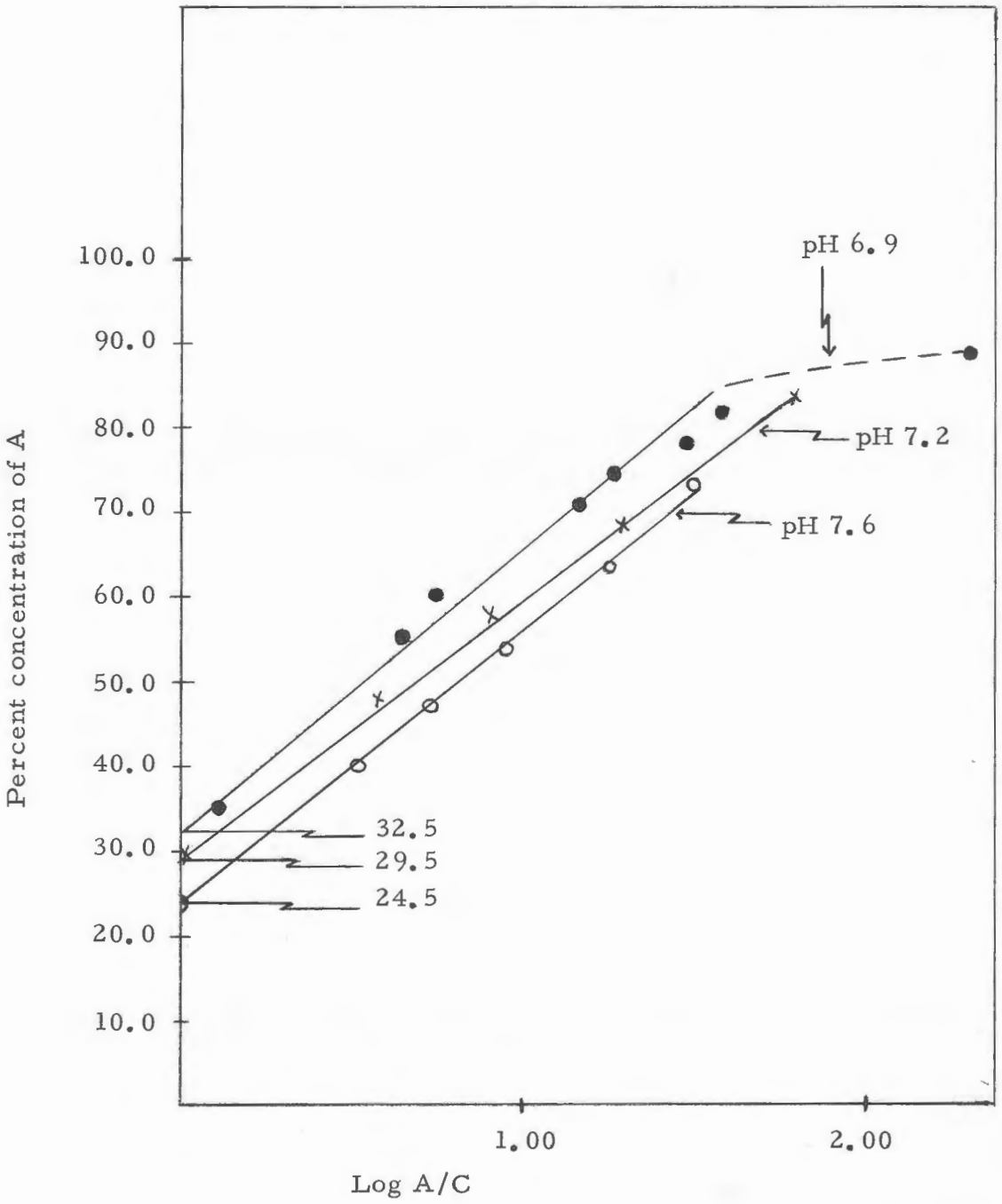


Figure 10. A plot of percent hydrocortisone sodium succinate (A) versus the log of the ratio of (A) and degradation products (C) for pH values of 6.9, 7.2 and 7.6.

the earlier portions of the plots for pH values of 6.9 and 7.2 are not linear indicating the time period when C has not formed to any appreciable extent.

Shown in Table VII are the values of the relevant species A, B and C where log of A/C is zero for each pH level.

The major limitation of the method would seem to be when k_2 is much larger than k_1. This is exemplified by the data of Heimlich and Martin (3) where B reaches a maximum after A has degraded to only a small extent. That is, A cannot possibly intersect C in this instance.

Method V - Approximation

The second rate constant may be very rapidly and easily approximated by gross examination of Figures 1-3 where each species of the reaction is plotted versus time. From equation 3 of Appendix B, it is seen that when the rate of change of B is zero, then k_1A equals k_2B. It is evident that this relationship is true only as B reaches its maximum.

Returning to Figures 1-3, it is possible to estimate from each graph where B is at a maximum and the corresponding value of A at this point. Hence, the product of the first rate constant k_1, and the ratio of A/B yields k_2.

Values of A/B calculated in the above manner are shown in Table VIII.

TABLE VII

VALUES OF THE STEROID ESTER (A), FREE ALCOHOL (B)

AND PRODUCTS NOT CONTAINING THE

17-DIHYDROXYACETONE CHAIN (C)

WHERE THE LOG OF A/C IS ZERO

pH	%A	%B	%C
6.9	32.5	35.0	32.5
7.2	29.5	41.0	29.5
7.6	24.5	51.0	24.5

TABLE VIII

RATIOS OF A/B

TAKEN FROM FIGURES 1-3

Figure	pH	A/B
1	6.9	0.87
2	7.2	0.75
3	7.6	0.53

It is interesting to note that with the exception of the method utilizing a plot of dB/dt versus B, all other methods depend on the evaluation of the ratio k_2/k_1, as a prerequisite to the calculation of the second rate constant, k_2. Shown in Table IX is a correlation of the second rate constants and the ratios of k_2/k_1 for the various methods discussed. Also indicated are the concentrations of B at its maximum where relevant to the method.

TABLE IX

COMPARISON OF k_2, k_2/k_1 AND B AT ITS MAXIMUM AS OBTAINED BY THE VARIOUS METHODS

Method	k_2/hour	k_2/k_1	B maximum (%)
A. pH 6.9			
I. Dimensionless Parameters	0.050	0.91	36.3
II. Time Ratio	0.050	0.90	-
IV. Intersection	0.051	0.93	35.0
V. Approximation	0.048	0.87	36.0
B. pH 7.2			
I. Dimensionless Parameters	0.067	0.79	41.5
IV. Intersection	0.061	0.72	41.0
V. Approximation	0.064	0.75	40.0
C. pH 7.6			
I. Dimensionless Parameters	0.080	0.53	49.0
III. Differential Equation	0.320	2.12	-
IV. Intersection	0.072	0.48	51.0
V. Approximation	0.080	0.53	48.0

Each method of evaluating k_2 showed excellent correlation, with the exception of method III where deviation is presumed due to factors previously discussed. It should also be mentioned that the method of approximation yielded excellent results as compared to the more complex methods.

The reaction pathway for the 21-hemiester of hydrocortisone follows an irreversible first order consecutive pathway which implies that the 17-dihydroxyacetone chain may only be degraded subsequent to the formation of the free alcohol. Each method of calculating k_2 indicates consistently that for the pH range of 6.9 to 7.6, the decomposition of the alcohol increases with increasing hydroxyl ion concentration.

VI. SUMMARY

1. The degradation of 21-hydrocortisone hemisuccinate was followed at 70°C. in aqueous solutions buffered at pH values of 6.9, 7.2 and 7.6. Resultant assay data indicated that the overall kinetic pathway at each pH level could be interpreted as consecutive first order.

2. Ester hydrolysis is presumed to be by an intramolecular attack of the anion on the ester carbonyl carbon or specific hydroxyl-ion catalysis of the undissociated hemiester.

3. A mechanism for the decomposition of the steroidal alcohol is proposed, where an etio acid and formic acid are produced by an oxidative mechanism.

4. Several methods of evaluating the second rate constant were applied to the data and the results compared. Correlation of k_2 from each method was achieved with the exception of the use of the differential equation (i.e., method III).

5. A method of calculating k_2 is proposed by the authors and offers the advantage of predicting the rate constant without carrying the reaction to completion. Its usefulness is limited to cases where the ratio of k_2/k_1 is approximately 0.1 to 0.9.

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VIII. APPENDIX

VIII. APPENDIX

A. Method of Least Squares

1. $y = a + b(x - \bar{x})$ where
   - y is the ordinate value
   - a is the intercept of the ordinate axis
   - b is the slope
   - x is the absissa value
   - $\bar{x}$ is the arithmetic mean of the absissa values

2. $\bar{y} = a$ where
   - $\bar{y}$ is the arithmetic mean of the ordinate values

3. $$B = \frac{\sum[(x - \bar{x})(y - \bar{y})]}{\sum[(x - \bar{x})^2]}$$

4. Sample calculation using data from Beer-Lambert plot

   a. $$b = \frac{18.44}{286.26} = 6.44 \times 10^{-2}$$

   b. $$y = 6.44 \cdot 10^{-2}x$$

B. Expressions Describing Consecutive First Order Reaction

1. $A \xrightarrow{k_1} B \xrightarrow{k_2} C$
   where A is the steroid ester, B the steroid alcohol and C is the product devoid of the 17-dihydroxyacetone side chain.

2. The rate of change of A is given by $dA/dt = -k_1A$ or $A = A_oe^{-k1t}$ where A_o is original concentration of steroid ester.

3. The rate of change of B is $dB/dt = k_1A - k_2B$

4. The rate of change of C is $dC/dt = k_2B$.

C. Dimensionless Parameters and Evaluation of β maximum.

1. $\beta = B/A_o$
2. $\alpha = A/A_o$
3. $K = k_2/k_1$
4. β maximum $= K^{K/1-K}$

D. Dimensionless Parameters and Time Percentage Relationship (Time Ratio Method)

1. $\beta = B/A_o$
2. $\gamma = C/A_o$
3. $\delta = \beta + 2\gamma$

E. Intersection Method

1. The rate of change of B is $dB/dt = k_1A - k_2B$ which may be arranged to the linear form

$$A/B = \frac{dB/dt}{B\ k_1} + k_2/k_1$$

2. When $dB/dt \longrightarrow O$, then $A/B = k_2/k_1$

3. As $dB/dt \longrightarrow O$, $A/C = 1$

4. The stoichiometric relationship of A_o, A, B and C is
   $A_o = A + B + C$
   When $A/C = 1$, B at its maximum is
   $A_o - A - C = B$ maximum

5. The ratio of A over B when B is maximized is $A/B = k_2/k_1$