

Prediction of Stability in Pharmaceutical Preparations X

Alkaline Hydrolysis of Hydrocortisone Hemisuccinate

By EDWARD R. GARRETT†

The hydrolysis of hydrocortisone 21-hemisuccinate above pH 8 has been studied by constant pH titrations in aqueous solution and appears to involve specific hydroxyl ion catalyzed hydrolysis as the only significant reaction pathway. The apparent heat of activation for specific hydroxyl ion catalyzed hydrolysis when corrected for the heat of ionization of water is unusually low for hemisuccinate esters, 7.0 Kcal./mole. This implicates the configuration and structure of the steroid side chain in the facile hydrolysis of 21-steroid esters. The stability of hydrocortisone 21-hemisuccinate at storage and physiological conditions is predicted.

THE CLINICAL usefulness of hydrocortisone was enhanced by the formation of the water-soluble sodium salt of the 21-hemisuccinate ester (1, 2). The formulation of the water-soluble lyophilized sodium salt of hydrocortisone 21-hemisuccinate¹ permitted facile parenteral administration of hydrocortisone in an homogeneous solution where immediate high levels of circulating hydrocortisone are required.

This paper considers the kinetics of hydrolysis of sodium hydrocortisone hemisuccinate in aqueous solution above pH 7 as studied by constant pH titration. The experimental evidence should permit the prediction of stability of reconstituted solutions and should provide evidence for the understanding of the ester's utilization *in vivo* on parenteral administration.

EXPERIMENTAL

Experimental Procedure for Constant pH Titrations of 0.0165 M Hydrocortisone Hemisuccinate—The Cannon di-functional titrator (3) was standardized before each run with Beckman buffer solution which had been equilibrated for a minimum of 5 minutes in the temperature-controlled beaker of the titrator to be at the same temperature as the hydrocortisone hemisuccinate solution to be studied.

Hydrocortisone hemisuccinate which had been dried at 50° in high vacuum was weighed in a small weighing boat on a Gramatic balance. After the Cannon titrator had been standardized as above, the sample was transferred to the temperature-controlled beaker and 15 ml. of 0.0165 M sodium hydroxide from the constant temperature bath was added. The sodium hydroxide exactly neutralized the hydrocortisone hemisuccinate. A thick rubber band held a silicone covering over the beaker. Inserted into the silicone gasket were the glass-saturated calomel electrodes and a gas duct to

blow nitrogen gently on the surface of the solution in the beaker. No carbon dioxide was absorbed by this system as tested for pH 10 for 1.5 hours.

The starting pH of the solution to be studied was recorded and the titration was then started. In general, the pH of the sodium salt of hydrocortisone hemisuccinate was 6.6. The syringe contained 2.011 N sodium hydroxide. The servomotor was used for the first few seconds to raise the pH quickly to the setting of the pH control and then the synchronous motor was used to maintain the solution at this pH. At the end of the run, the temperature and volume of solution in the beaker were recorded. Typical curves of volume of standard alkali consumed *vs.* time are given in Fig. 1. The controlled pH and observed temperature for the various runs are given in Table I. Solvent evaporation occurred, especially at the low rates encountered at low pH values.

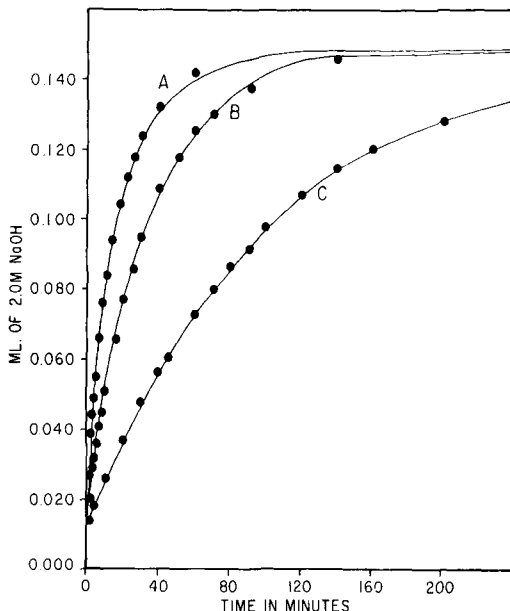


Fig. 1.—Typical curves for the consumption of standard alkali at constant pH by 0.0165 M hydrocortisone hemisuccinate at 48°. Curve A is at pH 10.0; curve B is at pH 9.5, and curve C is at pH 9.0.

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† Present address: College of Pharmacy, University of Florida, Gainesville.

¹ The trade name of The Upjohn Co. for hydrocortisone sodium succinate is Solu-Cortef.

TABLE I.—RATE CONSTANTS FOR THE APPARENT FIRST-ORDER HYDROLYSIS OF 0.0165 *M* HYDROCORTISONE HEMISUCCINATE AT VARIOUS TEMPERATURES AND pH VALUES

pH	°C.	10 ⁴ <i>k</i> (sec. ⁻¹)		pH	°C.	10 ⁴ <i>k</i> (sec. ⁻¹)		
		Exptl.	Calcd. ^a			Exptl.	Calcd. ^a	
10	65.2	57 ^b	53	10	48.1	11 ^b	11.0	
10	64.0	47 ^b		49.0	9.5	48.1	5.0 ^b	4.0
9.5	65.2	15 ^b	14	9.5	48.0	2.9 ^b	3.46	
9.5	63.7	13 ^b		15.3	9.0	48.0	1.7 ^c	1.2
9.0	65.8	7.6 ^b	5.0	9.0	48.4	0.76 ^b	1.10	
9.0	65.3	4.1 ^b		5.08	8.5	48.0	0.43 ^c	0.35
9.0	63.9	3.3 ^b		4.88	8.0	48.0	0.22 ^c	
8.5	65.3	1.3 ^b		1.6	1.60	10.0	38.4	4.2 ^b
8.5	64.9	2.1 ^b	1.60	8.0	38.2	1.6 ^c	1.27	
8.0	65.1	0.88 ^c	0.86	0.51	9.0	39.2	0.51 ^c	0.42
8.0	64.3	0.84 ^c		0.49	8.5	39.0	0.20 ^c	0.15
7.5	64.4	0.20 ^c	0.16	8.5	38.4	0.09 ^b	0.13	
7.0	65.2	0.090 ^c	0.05	8.5	38.4	0.09 ^b	0.13	
10.0	57.1	25 ^b	25.1	10.0	29.6	1.4 ^b	1.58	
9.5	57.1	8.2 ^b	7.94	9.5	29.2	0.34 ^b	0.491	
9.0	57.5	2.6 ^b	2.7	2.57	9.0	29.8	0.134 ^b	0.159
9.0	57.0	2.9 ^b	2.51	9.0	29.5	0.203 ^b	0.158	
8.5	57.2	0.77 ^c	0.80	0.81				
8.5	57.2	0.82 ^c	0.81					

^a Calculated from $k = k_{\text{OH}^-}[\text{OH}^-]$ where $\log k_{\text{OH}^-} = -1.53 \times 10^{-3}(1/T) + 5.12$ and $[\text{OH}^-] = 10^{-\text{pOH}}$ where $\text{pOH} = \text{pKw} - \text{pH}$ where pKw is given in Table II. ^b Less than 10% loss of volume at 90% hydrolysis. ^c A 10–25% loss of volume at 90% hydrolysis.

The hydrocortisone hemisuccinate, theoretical molecular weight 462, was titrated in 50% ethanol and had a calculated molecular weight of 472, pKa = 5.64, and in 20% ethanol, pKa = 5.10.

CALCULATIONS

The Cannon titrator plots the consumption of standard alkali against time while the solution of hydrocortisone hemisuccinate is maintained at a given pH. Typical curves for the standard alkali consumptions for several pH values at 48° are given in Fig. 1.

The apparent first-order rate constant, *k*, for the hydrolysis of the hemiester was calculated by the Guggenheim method (4).

The basic equation is

$$\log(\lambda_{(t+\tau)} - \lambda_t) = -kt/2.303 + \text{constant} \quad (\text{Eq. 1})$$

where *k* is the apparent first-order rate constant for the hydrolysis of hydrocortisone hemisuccinate at a specific pH, λ_t are the microliters of standard alkali titer consumed, $\lambda_{(t+\tau)}$ are the microliters of standard alkali titer consumed at time *t* + τ or τ time units later than time *t*, and τ is a time interval chosen to permit estimation of the first-order rate constant *k* in sec.⁻¹ without excessively weighting an extrapolated asymptotic value or a value of theoretical alkali consumption. The value τ should exceed the half-life of the reaction.

Typical first-order plots by this Guggenheim method are given in Fig. 2 for several pH values.

The determined rate constants and the pH and temperature of determination are given in Table I.

The rate of alkali consumption, $d[\text{NaOH}]/dt$, can be postulated as equal to the rate of hydrolysis of the hemisuccinate ester, $-d[X]/dt$, and may be proportional to the products of the concentra-

tions of the hydroxyl ion concentration $[\text{OH}^-]$ and the ester concentration $[X]$ so that

$$-d[\text{NaOH}]/dt = -d[X]/dt = -k[X] = -k_{\text{OH}^-}[\text{OH}^-][X] \quad (\text{Eq. 2})$$

where at constant pH

$$k = k_{\text{OH}^-}[\text{OH}^-] \quad (\text{Eq. 3})$$

and the *k* values listed in sec.⁻¹ in Table I are determined from the slopes of the plots of the Guggenheim Eq. 1 as demonstrated in Fig. 2.

The logarithmic expression derived from Eq. 3 is $\log k = \log k_{\text{OH}^-} + \log [\text{OH}^-] = \log k_{\text{OH}^-} - \text{pOH}$ (Eq. 4)

but

$$\text{pOH} + \text{pH} = \text{pKw} \quad (\text{Eq. 5})$$

so that

$$\log k = \log k_{\text{OH}^-} - \text{pKw} + \text{pH} \quad (\text{Eq. 6})$$

where the pKw values at various temperatures are listed in Table II from Harned and Owen (5a).

A plot of $\log k$ against pH should be a straight line with an intercept of $\log k_{\text{OH}^-} - \text{pKw}$. Such plots are given in Fig. 3. The linearity of the plots is in good agreement with the *a priori* bases of Eq. (6).

The plots of Fig. 3 are fitted to a slope of one and permit the determination of the intercepts, $\log k_{\text{OH}^-} - \text{pKw}$, which are given in Table II for the average of the various temperatures studied. The actual temperatures varied among runs by about 0.5° (see Table I) so that the experimental error was actually less than appears from the scatter about the regressions of Fig. 3. Also, in Table I are listed the calculated *k* values as determined by

$$k = k_{\text{OH}^-}[\text{OH}^-] = k_{\text{OH}^-}[10^{-\text{pKw} + \text{pH}}] \quad (\text{Eq. 7})$$

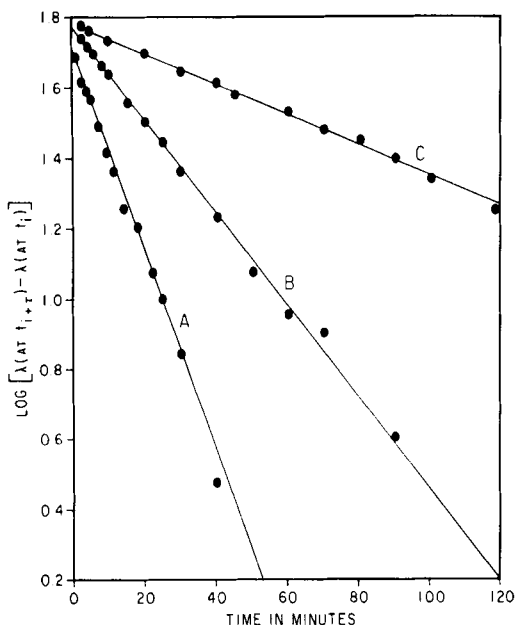


Fig. 2.—Typical first-order plots by the Guggenheim method for the hydrolysis of 0.0165 *M* hydrocortisone hemisuccinate at 48° at constant pH. Curve A is at pH 10.0, curve B is at pH 9.5, and curve C is at pH 9.0. The λ are the numbers of μ l. of 2.0 *M* sodium hydroxide consumed at a particular time, t . τ is a constant time interval that exceeds the half-life of the reaction.

TABLE II.—ESTIMATES OF THE BIMOLECULAR RATE CONSTANT k_{OH^-} (1/*M*/sec.) FOR THE HYDROXYL ION CATALYZED HYDROLYSIS OF HYDROCORTISONE HEMISUCCINATE

°C.	log k_{OH^-} - pK_w^a	pK_w^b	log k_{OH^-}	k_{OH^-} (1/ <i>M</i> /sec.)
29	-13.82	13.87	0.05	1.12
38	-13.38	13.60	0.22	1.66
48	-12.96	13.32	0.36	2.29
57	-12.61	13.08	0.47	2.93
65	-12.32	12.89	0.57	3.72

^a Intercept of the plots, Fig. 3, of the logarithm of the first-order rate constants, k (sec.⁻¹), at a given temperature, against the observed pH as per $\log k = pH + (\log k_{OH^-} - pK_w)$. ^b As given in Harned and Owen (5a). ^c Intercept of plot specified in footnote a + pK_w .

where the pK_w 's are given in Table II and the k_{OH^-} is determined for the Arrhenius relation as discussed in the following section. These calculated values show reasonable agreement with the experimental.

Variation of Rate with Temperature.—Arrhenius plots of the logarithm of the apparent first-order rate constant in sec.⁻¹, i.e., $\log k$, against $1/T$ for various controlled pH values are given in Fig. 4. The slopes of these plots are 4.327×10^3 so that the apparent heats of activation, ΔH_a , are 19.8 Kcal./mole as per the expression

$$k = P e^{-\Delta H_a/RT} \quad (\text{Eq. 8})$$

or expressed logarithmically in accordance with the plot of Fig. 4

$$\log k = \log P - (\Delta H_a/2.303R)(1/T) \quad (\text{Eq. 9})$$

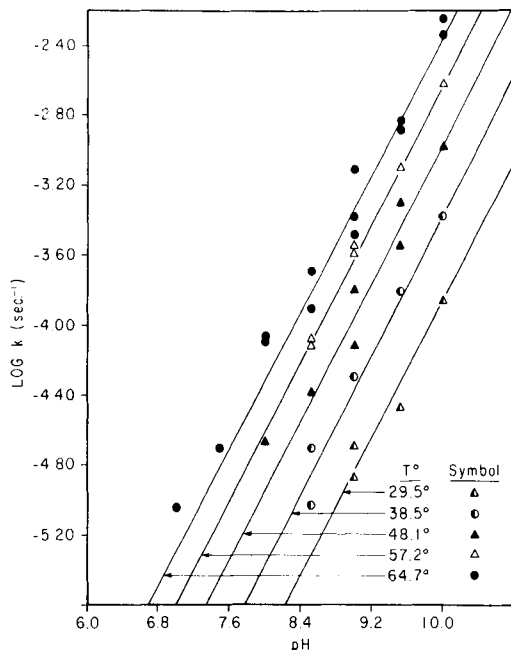


Fig. 3.—Logarithm of apparent first-order rate constants, k in sec.⁻¹, for the constant pH hydrolysis of 0.0165 *M* hydrocortisone hemisuccinate plotted against pH.

TABLE III.—LOG *P* VALUES FOR THE ARRHENIUS RELATION^a BETWEEN TEMPERATURE AND THE LOGARITHMS OF THE APPARENT FIRST-ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF HYDROCORTISONE HEMISUCCINATE AT VARIOUS pH VALUES

pH	log <i>P</i>
10.0	11.50
9.5	10.05
9.0	9.53
8.5	9.03
8.0	8.74

^a $\log k = -\Delta H_a/2.303R(1/T) + \log P$, k in sec.⁻¹.

where T is in absolute degrees. The $\log P$ values for the various controlled pH values are given in Table III.

Now the apparent first-order rate constant, k , is the product of the specific hydroxyl ion rate constant, k_{OH^-} , and the hydroxyl ion concentration as shown in Eq. 3. Each of these factors can be expected to vary with temperature as expressed in Arrhenius relations

$$k_{OH^-} = P_1 e^{-\Delta H_1/RT} \quad (\text{Eq. 10})$$

$$[OH^-] = K_w/[H^+] = P_2 e^{-\Delta H_2/RT}/[H^+] \quad (\text{Eq. 11})$$

where ΔH_2 is the heat of ionization of water, 12.8 Kcal./mole at 40° (5b). Thus

$$k = k_{OH^-}[OH^-] = P_1 P_2 e^{-(\Delta H_1 + \Delta H_2)/RT}/[H^+] \quad (\text{Eq. 12})$$

and logarithmically

$$\log k = \log P_1 P_2 + pH - \{(\Delta H_1 + \Delta H_2)/2.303R\} (1/T) \quad (\text{Eq. 13a})$$

$$= \log P - \{\Delta H_a/2.303R\} (1/T) \quad (\text{Eq. 13b})$$

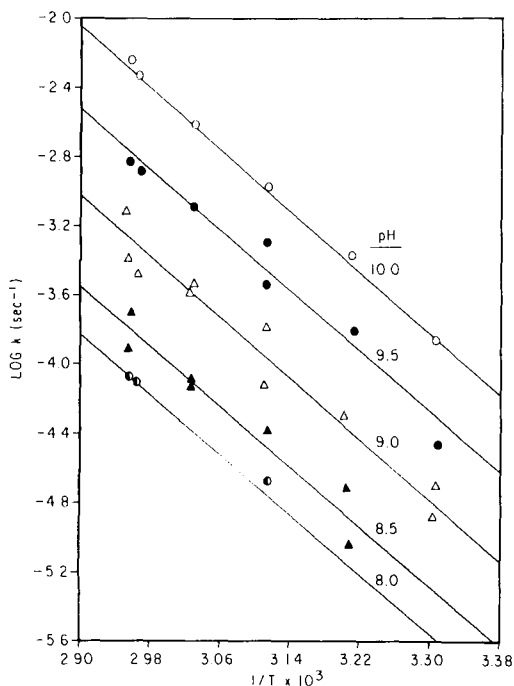


Fig. 4.—Arrhenius plot for the apparent first-order hydrolysis of 0.0165 *M* hydrocortisone hemisuccinate by constant pH titrations.

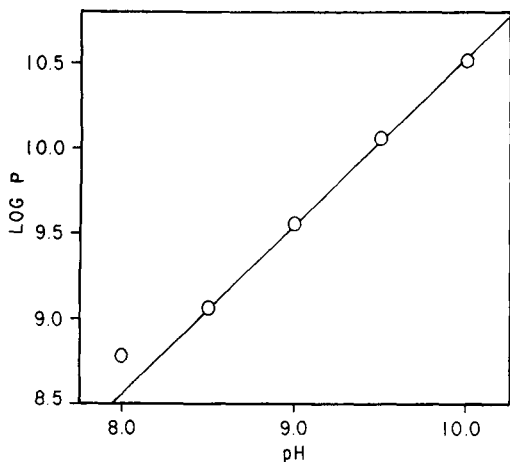


Fig. 5.—Plot of the intercept, $\log P$, of the Arrhenius plots, $\log k$ vs. $1/T$, for the hydrolysis of hydrocortisone hemisuccinate against the pH of hydrolysis.

The intercepts of $\log k$ vs. $1/T$ in Fig. 4 for a given pH are $\log P = \log P_1 P_2 + \text{pH}$, so that if these intercept values as given in Table III are plotted against pH, the result should be a straight line of unit slope and intercept $\log P_1 P_2$. Figure 5 demonstrates the validity of this rationale and permits an estimate of

$$\log P_1 P_2 = \log P_1 + \log P_2 = 0.53 \quad (\text{Eq. 14})$$

A plot of $\log K_w$ vs. $1/T$ from the data in the literature (5a) gives an apparent ΔH_2 as 12.8 Kcal./

mole and $\log P_2$ as -4.61 . Thus $\log P_1$ should be 5.14 from Eq. 14. Since ΔH_a for $\log k$ vs. $1/T$ for all pH values is 19.8 Kcal./mole, $\Delta H_1 = \Delta H_a - \Delta H_2$ should be 7.0 Kcal./mole for the bimolecular rate constant, k_{OH^-} .

The ΔH_1 and $\log P_1$ can be determined directly from the k_{OH^-} values listed in Table II and plotted as per the logarithmic form of Eq. 10 in Fig. 6. The resultant expression is

$$\log k_{\text{OH}^-} = -1.53 \times 10^3(1/T) + 5.12 \quad (\text{Eq. 15})$$

where $\Delta H_1 = 7.00$ Kcal./mole in agreement with the $\Delta H_1 = 7.0$ derived from the relations expressed in the Eqs. 13a and 13b, and $\log P_1 = 5.12$ in agreement with the $\log P_1 = 5.14$ derived from Eq. 14.

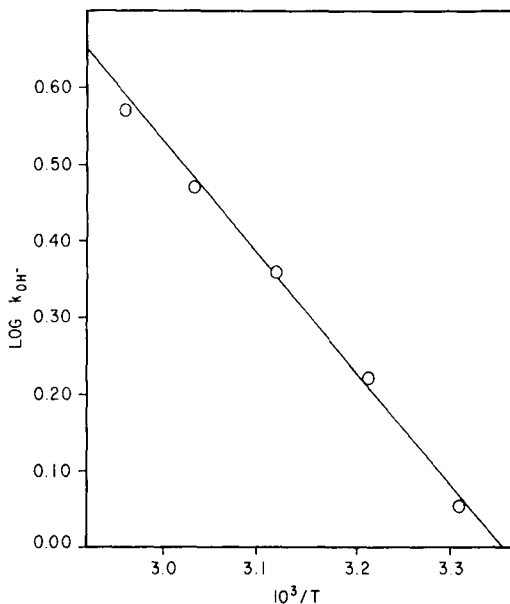


Fig. 6.—Arrhenius plot of the bimolecular rate constant, k_{OH^-} in L./M./sec., for the hydroxyl ion catalyzed hydrolysis of hydrocortisone hemisuccinate.

DISCUSSION

The rates of hydrolysis of hydrocortisone hemisuccinate, $[X]$, can be calculated from the following expressions

$$dX/dt = -k[X] \quad (\text{Eq. 16})$$

or

$$\log X/X_0 = -(k/2.303)t \quad (\text{Eq. 17})$$

where

$$k = k_{\text{OH}^-}[\text{OH}^-] \quad (\text{Eq. 18})$$

and

$$\log k_{\text{OH}^-} = -1.53 \times 10^3(1/T) + 5.12 \quad (\text{Eq. 19})$$

and

$$[\text{OH}^-] = 10^{-\text{pOH}} \quad (\text{Eq. 20})$$

where

$$\text{pOH} = \text{pK}_w + \text{pH} \quad (\text{Eq. 21})$$

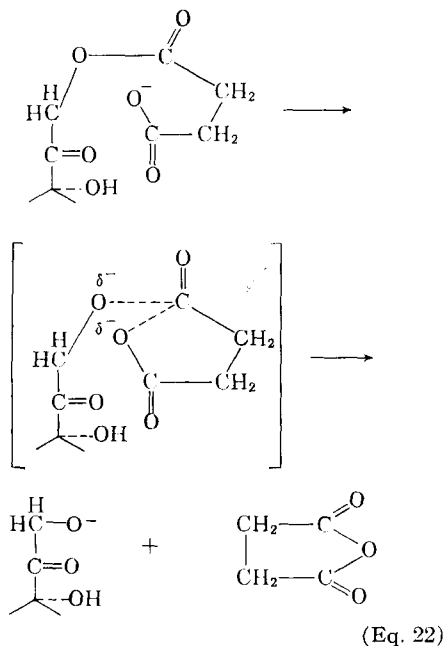
where pH is experimentally observed and $pK_w = 2.8 \times 10^{-3}(1/T) + 4.61$ (5a).

Rate constants calculated on these premises are compared with the experimental values in Table I and are reasonably consistent. The determination of alkaline hydrolysis rates by measurement of alkali consumption at constant pH in these studies is obviously not as precise as would be liked. The experimental setup was such that solvent evaporation was significant, especially at higher temperatures and for the lower rates at the lower pH values. However, the Guggenheim procedure for the evaluation of first-order reaction rate constants served to minimize the error due to the experimental variation of concentration for prolonged reaction times. Another possible source of error is anomalous pH reading in unbuffered solutions. The internal consistency of the kinetic and thermodynamic equations and the better agreement of the calculated rate with the averages of replicated studies shows that the technique is useful.

Great accuracy is obtained with faster reaction and, in the case of solutions of low ionic concentrations, at higher pH values. The obtained data describe the hydrolysis of hydrocortisone hemisuccinate as being specifically catalyzed by hydroxyl ion above a pH of 8.

Below a pH of 8, the apparent rates of hemisuccinate hydrolysis may be greater than the extrapolation of the specific hydroxyl ion catalysis rate expressions would predict. The values that can be calculated on the basis of these expressions should only be considered as the minimum estimate of the rates at pH values below 8.

Certain chemical phenomena that may produce rates below pH 8 that exceed prediction may be (a) the increase in the relative importance of spontaneous hydrolysis or intramolecular attack of the anion on the ester carbonyl carbon (6-10)



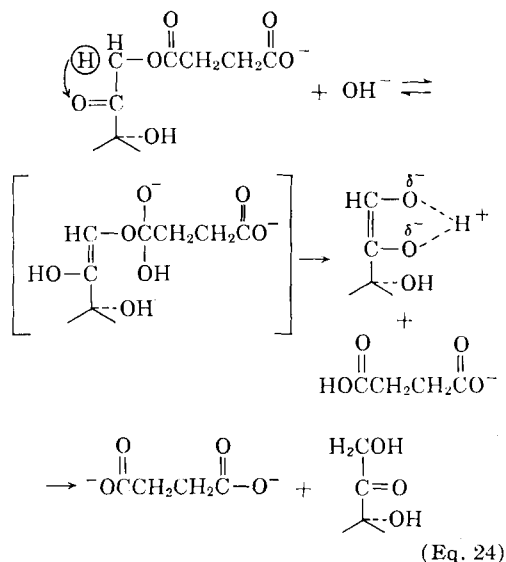
or (b) a significant contribution of hydronium ion catalysis to hemisuccinate hydrolysis at pH values

below 8 since this would involve a reaction accelerated by the attraction of opposite charges



The heat of activation for the specific hydroxyl ion catalysis of hydrocortisone hemisuccinate is 7.0 Kcal./mole and is significantly less than that found for the alkaline hydrolysis of hemisuccinic acid ester of secondary alcohols, i.e., 9-14 Kcal./mole (11-13). The rate constants for hydrocortisone hemisuccinate hydrolysis are also much higher than for other succinate esters. For example, at 40° the rate constant is *ca.* 200 times greater than that for the hydrolysis of cyclohexanol hemisuccinate (12).

These phenomena imply that the structure of the steroid side chain is such that it promotes 21 ester hydrolysis over and above possible repulsion of the attacking hydroxyl ion by a negative charge on the molecule. A possible explanation is that a leaving group is stabilized by resonance, a group such as an enediol anion



The major conclusion is that a 21-steroid ester is highly susceptible to alkaline hydrolysis by virtue of the unique structure of the steroid side chain.²

The stability of hydrocortisone hemisuccinate can be predicted at 25° by application of Eq. 15 where k_{OH^-} at 25° = 1.0 and $pK_w = 14$. The predictions of half-life are given in Table IV.

TABLE IV.—PREDICTED HALF-LIVES OF HYDROCORTISONE HEMISUCCINATE AT VARIOUS pH VALUES AT 25°

pH	$t_{1/2}$
7	80 days
8	8 days
9	19 hours
10	1.9 hours

² A possible alternative mechanism to postulate is one where the 17 α -hydroxyl group is involved as an assisting group. Dr. S. Morris Kupchan of The University of Wisconsin suggests that this is consistent with his studies.

Recent studies on blood levels from parenterally administered hydrocortisone-21-hemisuccinate (14, 15) have shown that the steroid administered in this form is quickly available to the tissues. It was also shown that levels of free steroid after injections of the 21-phosphate ester were higher than those obtained after the 21-hemisuccinate esters were injected. Since comparative studies on biological activities are lacking, no conclusion as to medical significance could be drawn (15).

However, it was speculated (14) that a portion of the succinate ester was metabolically transformed without prior hydrolysis. Contrarily, no significant differences were observed between the phosphate and succinate esters studied in terms of percentage of injected steroid excreted in the urine.

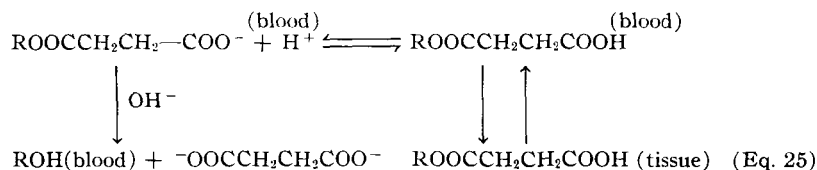
A reasonable explanation for the blood level difference in free steroid is the greater deposition of hydrocortisone hemisuccinate in the tissues over that of the phosphate. Hydrocortisone hemisuccinate has a pK_a ca. 5.0, and at the neutral pH of the blood, the sodium salt is in equilibrium with the free uncharged hemiester. In general, uncharged molecules exhibit higher lipoidal or fat solubility than charged molecules and penetrate cell membranes more rapidly

On the basis of Marcus' equation relating hydrolysis rate with pH and temperature (18), the apparent first-order rate constant for the production of free steroid from the phosphate ester is predicted as $2.5 \times 10^{-4} \text{ hr.}^{-1}$ at 38° and pH 7.0. On the basis of the experimental data in this paper, and by use of Eq. 15 with $pK_w = 13.60$ at 38° so that $[\text{OH}^-] = 2.51 \times 10^{-7}$ at pH 7, the apparent first-order rate constant for the hydrolysis of hemisuccinate ester is predicted to be $1.3 \times 10^{-3} \text{ hr.}^{-1}$ at 38° and pH 7.0. It follows that, on the basis of solvolysis alone, free steroid should be produced from the succinate ester ca. five times faster than from the phosphate ester.

This fact is inconsistent with an assumption that 21-phosphate esters more readily produce free steroid in the blood than the 21-hemisuccinate esters by solvolytic mechanisms. This fact strongly validates the model proposed in Eq. 25.

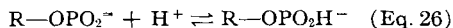
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This fact has been well discussed in the literature (16, 17).

The doubly charged steroid phosphate is the major form of hydrocortisone phosphate at physiological pH values and has two pK_a 's, 6.2 and ca. 2 (18). At neutral pH the doubly charged phosphate anion is in equilibrium with the singly charged phosphate anion



Thus, uncharged phosphate ester would be available for diffusion into lipid-like membranes only if the pH were decreased to ca. 4 and this is not physiologically feasible.

It follows, therefore, that increased tissue permeability of the hemisuccinate ester over the phosphate ester could account for the lower blood levels of free alcohol from the former.

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