UDC 615.786[612.741.15]-011

145. **Tokuji Suzuki :** Studies on Decomposition and Stabilization of Drugs in Solution. X.*[1] Chemical Kinetic Studies on Aqueous Solution of Succinylcholine Chloride. 2. Overall Velocity Constants for Succinylcholine Chloride Hydrolysis as a Function of pH.

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A number of studies[1~6] have been reported on the hydrolysis of the skeletal muscle relaxant, succinylcholine. It was found that this compound is destroyed rapidly by the pseudo-cholinesterase in the body and it is also hydrolyzed in aqueous solution such as the injection. Therefore, a study of the hydrolysis is of significance both clinically and from the standpoint of preservation. The degradation of succinylcholine in aqueous solution involves the hydrolysis to succinylmonocholine and choline with the subsequent breakdown of succinylmonocholine to succinic acid and choline as shown in the preceding report.[7]

Tammelin[2] reported an extensive work on the hydrolysis of succinylcholine iodide in which the sum of quantities of two esters, succinylcholine iodide and succinylmonocholine iodide, in the buffered solutions was determined with the procedure of Hestrin[8] for photometric determination. The influence of the concentration of the ester, pH, and temperature on the stability of the two ester-linkages was investigated, and the degradation increased with temperature and pH in that study. As compared with succinylcholine iodide for the physiological activity, succinylmonocholine iodide is much less active. Therefore, it is impossible to show the decreasing process of physiological activity of succinylcholine iodide in Tammelin's experiments.

Earles[3] assessed the extent of hydrolysis of succinylcholine chloride injection (unbuffered, pH 3.4) which was sterilized by heating in an autoclave at 10-lb pressure of steam for 30 minutes, by determination of the free acid liberated, and showed almost complete hydrolysis occurred at 37° and about 20% hydrolysis at room temperature after a year's storage. These results were confirmed by paper partition chromatographic and biological tests. Since the stability of succinylcholine chloride in aqueous solution is largely dependent on the pH of the solution, Freese[6] studying in similar unbuffered solution of succinylcholine chloride as Earles, stated that the initial calculated value of the half-life at 37° as an apparent first-order reaction decreased considerably on account of the pH shift of the medium due to the production of hydrogen ion during hydrolysis. In these studies, the percentage hydrolysis of succinylcholine chloride was calculated on the assumption that the acidity of the solution is entirely due to the presence of free succinic acid. Frazer[4] carried out hydrolysis of succinylcholine chloride in various buffered solutions by the biological method using rat diaphragm-phrenic nerve, and showed that the reaction was first-order with respect to succinylcholine chloride.

Although a number of studies have conducted on the stability of succinylcholine

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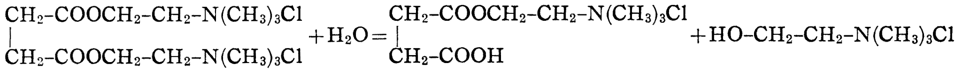
*[1] This work constitutes a part of a series entitled "Studies on Decomposition and Stabilization of Drugs in Solution" by H. Nogami. Part IX : This Bulletin, **10**, 503(1962).

*[2] Hongo, Tokyo (鈴木徳治).

1) V.P. Whittaker : Experientia, **7**, 251 (1951).
2) L.F. Tammelin : Acta. Chem. Scand., **7**, 185 (1953).
3) M.P. Earles, *et al.* : J. Pharm. and Pharmacol., **6**, 773 (1954).
4) P.J. Frazer : Brit. J. Pharmacol., **9**, 429 (1954).
5) L. Larsson, *et al.* : Svenk. Farm. Tidskr., **59**, 229 (1955). [C.A., **49**, 9228h (1955)].
6) C. Freese : Archiv der Rharmazie **290**, 75 (1957).
7) H. Nogami, *et al.* : This Bulletin, **9**, 646 (1961).
8) S. Hestrin : J. Biol. Chem., **180**, 249 (1949).

in aqueous solution, the experimental results obtained were lacking in theoretical consideration on degradation, since it has not been practicable to estimate accurately succinylcholine in the presence of hydrolyzed products in buffered solution. The purpose of this work, therefore, was to predict the rate of hydrolytic reaction, shown as follows, at various pH values and temperatures. Periodic quantitative determinations of the residual esters were made by the separatory determination method shown in the previous paper.[7]



$$\begin{array}{l}CH_2\text{-}COOCH_2\text{-}CH_2\text{-}N(CH_3)_3Cl\\ |\\ CH_2\text{-}COOCH_2\text{-}CH_2\text{-}N(CH_3)_3Cl\end{array} + H_2O = \begin{array}{l}CH_2\text{-}COOCH_2\text{-}CH_2\text{-}N(CH_3)_3Cl\\ |\\ CH_2\text{-}COOH\end{array} + HO\text{-}CH_2\text{-}CH_2\text{-}N(CH_3)_3Cl$$

Succinylcholine chloride may be present in ionized form in aqueous solution practically all over the ranges of pH because of quaternary ammonium salt. Since the hydrolytic cleavage of ordinary esters is catalyzed by hydrogen and/or hydroxyl ions, it was considered that the ester-linkages in succinylcholine chloride might also be subject to hydrogen or hydroxyl ion catalyzing reaction. Consequently, the catalytic catenary of the hydrolysis is the subject of present investigation. As will be stated later, it was found that the degradation in buffered solutions below pH 2.1 appeared to be specifically catalyzed by hydrogen ion, but the apparent velocity constants were not constant at a fixed pH value, and changed depending on the kind and the concentration of buffer in the case of degradation carried out in buffered solutions above pH 3.6. Thus, hydrogen and hydroxyl ions are not unique for acid-base catalytic reaction in the hydrolytic reaction of succinylcholine chloride, while the hydrolysis of esters such as aspirin,[9] phenobarbital[10] and methantheline bromide[11] (2-diethylaminoethyl xanthene-9-carboxylate methobromide) appears to be catalyzed by hydrogen ion or hydroxyl ion alone (i.e., specific hydrogen or hydroxyl ion catalysis). It may be observed that when kinetic studies are made in buffered systems, the reaction rate increases with buffer concentration, even if pH and other factors such as ionic strength are kept constant. This effect is ascribed to the catalytic effect of general acid or base in the buffered system used. It has been considered that the evidence for the general acid and base catalysis of hydrolysis of ester is doubtful. But, substantial evidences[12~15] for general base were presented recently.

Theoretical Consideration for General Acid-Base Catalyzed Reaction

The rate constant, on the assumption of general acid-base catalysis, may be defined by the sum of the products of concentration and catalytic coefficient for each catalyst in the reaction medium.

Thus,

$$\begin{aligned}k=&k_{H^+}(H^+)+k_{H_2O}(H_2O)+k_{OH^-}(OH^-)+k_{HX}(HX)+k_{X^-}(X^-)\\&+k_{HY}(HY)+k_{Y^-}(Y^-)+\cdots\text{etc.}\end{aligned}\qquad(1)$$

Where HX, HY, etc., are the general acids and X^-, Y^-, etc., are the general bases present in the reaction medium.

Using acetate buffer, k may be expressed by

$$\begin{aligned}k=&k_{H^+}(H^+)+k_{H_2O}(H_2O)+k_{OH^-}(OH^-)+k_{CH_3COO^-}(CH_3COO^-)\\&+k_{CH_3COOH}(CH_3COOH)\end{aligned}\qquad(2)$$

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[9] L.J. Edwards: Trans. Farady Soc., **46**, 723 (1950).

[10] J. Hasegawa, *et al.*: This Bulletin, **6**, 36 (1958).

[11] H. Nogami, *et al.*: *Ibid.*, **6**, 276 (1958).

[12] M.L. Bender, *et al.*: J. Am. Chem. Soc., **79**, 1652, 1656 (1957).

[13] T.C. Bruice, *et al.*: *Ibid.*, **79**, 1663 (1957).

[14] E.R. Garrett: *Ibid.*, **79**, 5206 (1957).

[15] *Idem.*: *Ibid.*, **80**, 4049 (1958).

The rate constant, k_0, catalyzed by H^+, H_2O, and OH^- may be given by equation (3).

$$k_0 = k_{H^+}(H^+) + k_{H_2O}(H_2O) + k_{OH^-}(OH^-) \qquad (3)$$

Substituting equation (3) into equation (2), equation (2) reduces to

$$\begin{aligned} k &= k_0 + k_{CH_3COO^-}(CH_3COO^-) + k_{CH_3COOH}(CH_3COOH) \\ &= k_0 + \left[k_{CH_3COO^-} + \frac{k_{CH_3COOH}}{K}(H^+)\right](CH_3COO^-) \qquad (4) \end{aligned}$$

Where, K is the dissociation constant of acetic acid, and k_0 is constant at a constant pH.

Substituting α for $k_{CH_3COO^-} + \frac{k_{CH_3COOH}}{K}(H^+)$ in equation (4), equation (5) is obtained.

$$k = k_0 + \alpha(CH_3COO^-) \qquad (5)$$

The rate constant, k, is a function of hydrogen and acetate ion concentrations. The α values and the hydrogen ion concentrations observed for two cases at constant ionic strength permit the evaluation of $k_{CH_3COO^-}$ and k_{CH_3COOH} by equation (5).

Experimental

Material——Succinylcholine chloride was the same as reported previously.[7]

Buffer solution——Buffer solutions were employed to maintain the pH of the solution for method I stated later. Twice concentrated Clark-Lubs' buffers (0.2*M*) of pH 0.90~2.09 at 50°, 60°, and 70°; 0.5*M* acetate buffers of pH 3.60, 4.41, 4.96 and 5.58 at 60°; Acetate buffers (containing 0.2*M* AcONa) of pH 5.08, 5.50, and 5.90 at 60°; Sörensen's phosphate buffers of pH 6.42 (0.71*M*), 7.13 (0.58*M*), and 7.50 (0.55*M*) at 60°. The determination of pH value was carried out with the Towa Denpa Kōgyō Co. pH meter Model HM-5.

Kinetic Procedure——a) Method I. : A 350 mg. sample of succinylcholine chloride was dissolved in 100 cc. of the buffered solution to be examined. The solution was poured into 10 cc. ampules. These ampules were kept in a thermostatically controlled water bath adjusted to 50°, 60°, and 70°, accurate to within ±0.1°. The ampules were taken out periodically at given intervals and cooled immediately in ice water. Ten cc. of its content was run through a column of a strong cation exchange resin (Zeollex SA) in the Na-form to separate succinylmonocholine chloride formed by the hydrolysis of succinylcholine chloride, and succinylcholine chloride was determined colorimetrically according to the previous paper.[7]

b) Method II. : The reaction vessel as shown in Fig. 1 was employed. The jacketed vessel fitted with a plastic cover with inlets for the electrodes from the pH meter and for the microburette. There is also an opening for removal of samples. Water from a thermostatted water bath is circulated through the cell jacket, and the temperature of the sample solution in the vessel is kept constant. A 105 mg. sample of succinylcholine chloride was dissolved in 150 cc. of 0.22*M* NaCl and the solution was poured into this vessel. The solution was brought to the desired pH by titrating with 0.05*M* NaOH from microburette, after the solution reached the desired temperature. A 10 cc. sample was taken from the vessel for assay at appropriate intervals, and the reaction was quenched by addition of 2 cc. of 0.01*N* HCl. Throughout the experiment, the hydrogen ions produced during hydrolysis of succinylcholine chloride were neutralized with 0.05*M* NaOH and the pH of the solution

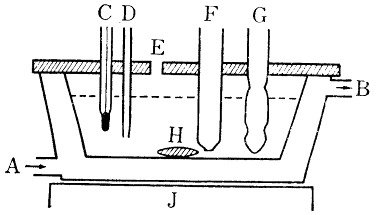

Fig. 1. Reaction Vessel used at Method II

A : water inlet
B : water outlet
C : thermometer
D : burette
E : sample removal
F : calomel electrode
G : glass electrode
H : stirring bar
J : magnetic stirrer

was maintained at the initial value. The withdrawn samples were assayed for residual succinylcholine chloride content in the same way as with the Method I, but in this case, 50 mm. cells were employed for photometric determination. Succinylmonocholine chloride also was determined colorimetrically in Methods I and II according to the previous paper.[7]

Results and Discussions

Hydrolytic Reaction Catalyzed by Hydrogen Ion

Velocity constants for succinylcholine chloride were determined at 50°, 60°, and 70° in the buffered solutions (pH 0.90~2.09) prepared from 0.2*M* hydrochloric acid and 0.2*M* potassium chloride (twice concentrated Clark-Lubs' buffers). At constant temperature and pH, the observed rate of hydrolysis of succinylcholine chloride wes considered as first-order with respect to the ester concentration. The typical results were illustrated in the previous paper.[7] The velocity constant of the decomposition at each temperature and pH value is shown in Table I.

Table I. pH and Velocity Constnts at 50°, 60°, and 70°

Molarity of bufler components	pH 50°	pH 60°	pH 70°	k (hr.$^{-1}$) 50°	k (hr.$^{-1}$) 60°	k (hr.$^{-1}$) 70°
0.150*M* HCl 0.050*M* KCl	0.90	0.92	0.93	4.06×10^{-2}	8.38×10^{-2}	1.79×10^{-1}
0.070*M* HCl 0.130*M* KCl	1.20	1.22	1.26	1.92×10^{-2}	3.51×10^{-2}	7.96×10^{-2}
0.027*M* HCl 0.173*M* KCl	1.66	1.68	1.70	6.82×10^{-3}	1.32×10^{-2}	2.99×10^{-2}
0.010*M* HCl 0.190*M* KCl	2.07	2.08	2.09	2.17×10^{-3}	5.82×10^{-3}	1.10×10^{-2}

The log k is plotted against pH at the left side in Fig. 2 in which the relationship between pH and log k is a straight line with a slope of -1.

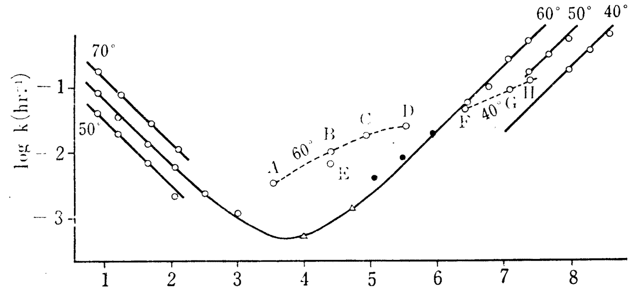

Fig. 2. Overall Velocity Constants for Succinylcholine Chloride as a Function of pH

A, B, C, D : 0.50*M* Acetate buffer
E : 0.25*M* 〃
F : 0.71*M* Phosphate buffer
G : 0.58*M* 〃
H : 0.55*M* 〃

Therefore, the hydrolysis may be specifically hydrogen ion catalyzed reaction in these regions, and these results are in accord with equation (6).

$$k = k_{H^+}(H^+) \qquad (6)$$

Where, k=apparent velocity constant, k_{H^+}=the second-order hydrogen ion constant.

The values of k_{H^+} obtained at each temperature are given in Table II.

Table II. Second-order Velocity Constants (L.·mol.$^{-1}$·hr.$^{-1}$) of Each Species at Each Temperature

Temp. (°C)	k_{H^+}	k_{OH^-}	$k_{CH_3COO^-}$
40		5.76×10^{4}	
50	2.95×10^{-1}	1.05×10^{5}	
60	6.46×10^{-1}	2.04×10^{5}	5.01×10^{-2}
70	1.45		

The linear relationship was obtained between $\log k_{H^+}$ and the reciprocal of absolute temperature as shown in Fig. 3. The activation energy E_A and the frequency factor A were calculated using the Arrhenius equation, $k=A\exp\left(\frac{-E_A}{RT}\right)$, and k_{H^+} was expressed as follows :

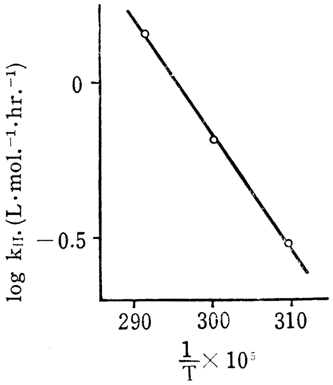

Fig. 3. Arrhenius Plot of the Hydrogen Ion Catalyzed Hydrolysis of Succinylcholine Chloride

$$k_{H^+}=1.36\times10^{11}\exp\left(\frac{-17,230}{RT}\right) \qquad (7)$$

Hydrolytic Reaction Catalyzed by Base

In order to obtain the catalytic constant of hydroxyl ion for succinylcholine chloride, acetate and phosphate buffers were employed, The results in these buffered solutions are shown by the plots of A, B, C, D, E at 60°, and F, G, H at 40° in Fig. 2. Although the buffer concentration of E is a half of D and both are of same pH value,

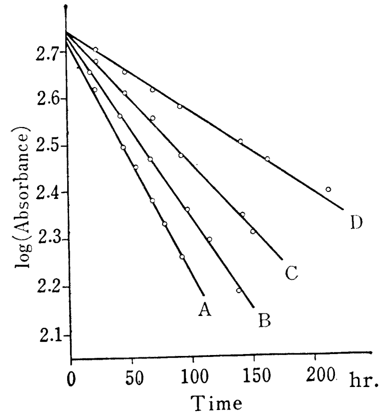

Fig. 4. First-order Disappearence of Succinylcholine Chloride in Various Acetate Buffers at pH 4.68 (μ=0.2), 60°

A : 0.2*M* CH_3COOH, 0.2*M* CH_3COONa; B : 1.5 Times Dilution of A with 0.2*M* NaCl; C : Twice Dilution of A with 0.2*M* NaCl; D : 2.5 Times Dilution of A with 0.2*M* NaCl.

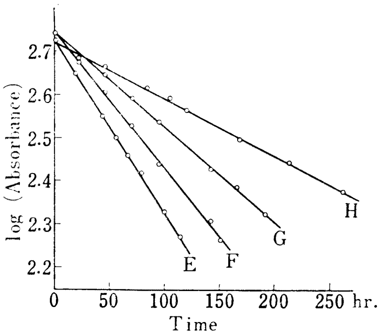

Fig. 5. First-order Disappearence of Succinylcholine Chloride in Various Acetate Buffers at pH 3.98 (μ=0.2), 60°

E : 0.82*M* CH_3COOH, 0.18*M* CH_3COONa, 0.02 *M* NaCl; F : 1.5 Times Dilution of E with 0.2*M* NaCl; G : Twice Dilution of E with 0.2*M* NaCl; H : 2.5 Times Dilution of E with 0.2*M* NaCl.

the velocity constant of E is 60% of D. It may not be considered that succinylcholine chloride in aqueous solution was subject to specific hydroxyl ion catalyzed hydrolysis in these pH regions, since the velocity constant was affected by the buffer concentration even in the same pH. In Figs. 4 and 5, the relations between the logarithm of concentration of succinylcholine chloride remaining in acetate buffers at various concentrations and the time of storage are shown. In Fig. 6, the observed rate constants were plotted against varying acetate concentration for two different pH values (4.68, 3.98) at 60°. Ionic strength of buffer solution was maintained constant ($\mu=0.2$) by addition of sodium chloride.

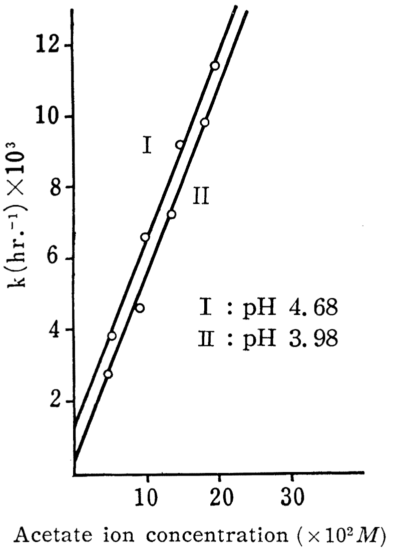

Fig. 6. Effect of Varying Acetate Ion Concentration at Constant Ionic Strength at 60° ($\mu=0.2$)

The observed first-order rate constants depend on acetate concentration, and the linearity of the these plots conformed the relation at equation (5). The slopes, α, and intercepts, k_0, of these lines are given in Table III. Calculation using these values indicated that $k_{CH_3COO^-}$ was 5.01×10^{-2} (L.·mol.$^{-1}$·hr.$^{-1}$) at 60° and the effect of k_{CH_3COOH} was negligible.

TABLE III. Dependency of Pseudo First-order Rate Constants (k in hr.$^{-1}$) of Succinylcholine Chloride at 60° on Acetate Ion

pH	Ionic strength of buffer	$\alpha\times10^2$	$k_0\times10^4$
3.98	0.2	5.12	4.9
4.68	0.2	5.03	13.7

The effect of buffering agent must be taken away to get exactly the catalytic constant of hydroxyl ion for succinylcholine chloride. In order to maintain the pH of the solution constant without buffering agent, the apparatus as shown in Fig. 1 was used. This apparatus is a thermostatically controlled reaction vessel connected with pH meter. The general expression for the first-order reaction is

$$\ln C_0 - \ln C = kt \qquad (8)$$

where, C_0= the initial concentration of succinylcholine chloride, C= the concentration at any time, t. When pH-shift due to the production of hydrogen ion during hydrolysis in unbuffered solution is maintained at the initial pH value by the addition of sodium hydroxide solution, the decreasing rate of concentration of succinylcholine chloride consists of the rates of degradation and dilution.

$$\frac{dC}{dt}=\left(\frac{\partial C}{\partial t}\right)_m+\left(\frac{\partial C}{\partial t}\right)_v \tag{9}$$

Where, m=amount of succinylcholine chloride
v=volume of the solution

Since the degradation is the first-order,

$$\left(\frac{\partial C}{\partial t}\right)_v=-kC \tag{10}$$

and

$$\left(\frac{\partial C}{\partial t}\right)_m=\left(\frac{\partial\frac{m}{v}}{\partial t}\right)_m=-\frac{C}{v}\frac{dv}{dt} \tag{11}$$

Substituting equations (10) and (11) into equation (9), equation (12) is obtained.

$$\frac{dC}{dt}=-C\left(k+\frac{1}{v}\frac{dv}{dt}\right) \tag{12}$$

Equation (12) is integrated and equation (13) is obtained.

$$\ln C_0-\ln C\frac{v}{v_0}=kt \tag{13}$$

When the apparatus in Fig. 1 is used, the decrease of the volume of solution by sampling must be considered. From the time of the n-th sampling to the time of the $(n+1)$-th sampling, equation (14) can be applicable.

$$\ln C_n-\ln C_{n+1}=k(t_{n+1}-t_n)+\ln\left(\frac{v_{n+1}+s}{v_n}\right) \tag{14}$$

Where, t_n=time of n-th sampling
s=sampling volume
C_n=concentration at time of the n-th sampling
v_n=volume immediately after the n-th sampling

Summing up n equations which are obtained by substituting n=1, 2, 3, ...n at equation (14).

$$\ln C_1-\ln\left[C_n\times\frac{\Pi(v_{n+1}+s)}{\Pi v_n}\right]=kt \tag{15}$$

Thus, if the concentration, C_n, at the n-th sampling is correct as $\left(C_n\times\frac{\Pi(v_{n+1}+s)}{\Pi v_n}\right)$, the relation of the first-order is obtained, and from the slope, a velocity constant is calculated.

The relationship between log k obtained by equation (15) and pH was a straight line as shown at the right side in Fig. 3 which represents the plots of log k vs. pH at 40°, 50°, and 60°, and the degradation of succinylcholine chloride appears to be catalyzed by hydroxyl ion alone. These results are in accord with equation (16).

$$k=k_{OH^-}(OH^-) \tag{16}$$

Where, k_{OH^-} is the second-order hydroxyl ion constant. Velocity constant at each pH and temperature is shown in Table IV.

Table IV. pH and Velocity Constants at 40°, 50°, and 60°

pH			k (hr.$^{-1}$)		
40°	50°	60°	40°	50°	60°
8.00	7.40	6.50	1.81×10^{-1}	1.31×10^{-1}	5.57×10^{-2}
8.30	7.70	6.80	3.32×10^{-1}	2.96×10^{-1}	1.23×10^{-1}
8.60	8.00	7.10	5.92×10^{-1}	5.15×10^{-1}	2.65×10^{-1}
		7.40			4.95×10^{-1}

The second-order hydroxyl ion rate constants, k_{OH^-}, obtained at each temperature are tabulated in Table II. Arrhenius plot of the hydroxyl ion catalyzed hydrolysis is shown in Fig. 7, and k_{OH^-} was expressed as follows :

$$k_{OH^-}=7.88\times10^{13}\exp\left(\frac{-13,091}{RT}\right) \tag{17}$$

The second-order hydroxyl ion rate constants were calculated as $k\frac{(H^+)}{k_w}$, and k_w values were calculated from equation (18)[16]

$$\log k_w=\frac{-4470.99}{T}+6.0875-0.017060T \tag{18}$$

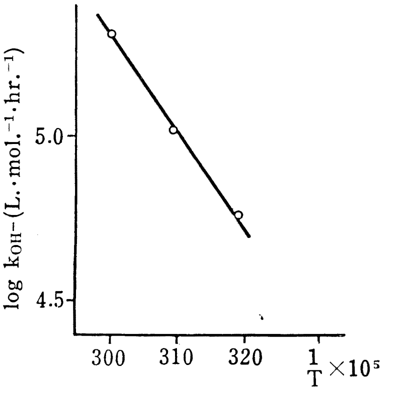

Fig. 7. Arrhenius Plot of the Hydroxyl Ion Catalyzed Hydrolysis of Succinylcholine Chloride

Catalytic Catenary of Hydrolysis of Succinylcholine Chloride

In Fig. 2, △ plots represent the values obtained by extrapolation to zero buffer concentration at pH 4.68 and 3.98 shown in Fig. 6, and solid circles represent the values obtained in acetate buffer solutions containing 0.2*M* sodium acetate minus the value due to acetate ion. The velocity constants at pH 2.5 and 3.0 are the values obtained in 0.6*M* and 0.06*M* acetic acid. The first-order rate constant of the hydrolytic reaction which is specifically catalyzed by the hydrogen and hydroxyl ion, is given by equation (3). Judging from these results, this pH-rate curve which passes through these plots and the linear plots catalyzed by hydrogen ion and the linear plots catalyzed by hydroxyl ion at 60° shows the catenary curve, assuming that the hydrolytic reaction of succinylcholine chloride is catalyzed specifically by hydrogen and hydroxyl ions. In practice, the rate constants may shift upper or lower side of this catenary curve as shown in Fig. 3 in

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[16] Harned and Owens : The Physical Chemistry of Electrolytic Solutions 3rd Ed. p. 645 (1958). Reinhold Publishing Corp., New York.

the case of the hydrolytic reaction in an optional buffer according to the effect of general acid-base catalysis and ionic strength. Equation (19) expresses the apparent rate constant in acetate buffers ($\mu=0.2$) at 60°.

$$k=6.47\times10^{-1}(\mathrm{H}^+)+5.01\times10^{-6}(\mathrm{H_2O})+2.04\times10^{5}(\mathrm{OH}^-)+5.01\times10^{-2}(\mathrm{CH_3COO}^-) \qquad (19)$$

The pH value, $(\mathrm{pH})_m$, of minimum velocity constant which is obtained by differentiating k with respect to (H^+) in equation (3) and equating to zero is expressed as follows:

$$(\mathrm{pH})_m=-\frac{1}{2}[\log k_w+\log k_{\mathrm{OH}^-}-\log k_{\mathrm{H}^+}] \qquad (20)$$

Substituting equation (7), (17), and (18) into equation (20), the values of $(\mathrm{pH})_m$ were calculated which are shown in Table V. The values of $(\mathrm{pH})_m$ calculated and observed at 60° were in good agreement.

TABLE V. Calculated pH at Minimum Velocity

Temp (°C)	$(\mathrm{pH})_m$	Temp (°C)	$(\mathrm{pH})_m$
100	3.54	30	4.05
60	3.76	10	4.30

The pH of aqueous solution of succinylcholine at various concentrations and the pH of succinic acid solution occurred from the decomposition of succinylcholine chloride at each corresponding concentration are shown in Table VI.

TABLE VI. pH of Succinylcholine Chloride Solution and pH of Solution of Succinic Acid occurred from Decomposition of Succinylcholine Chloride at Each Corresponding Concentration

pH (Succinylcholine chloride)	pH (Succinic acid)
4.12 (20 mg./cc.)	2.78
4.23 (10 mg./cc.)	2.95
5.11 (2 mg./cc.)	3.31

Assuming that the degradation at pH 5 in unbuffered aqueous solution is catalyzed by hydroxyl ion alone, the apparent first-order rate constants and the percentage residual succinylcholine chloride under various conditions of storage are calculated as shown in Table VII.

TABLE VII. Predicted Stability of Succinylcholine Chloride Solution, assuming that pH of the Solution is kept Constant and the Degradation is catalyzed by Hydroxyl Ion alone

Temy. (°C)	k (hr.$^{-1}$)	Percentage Hydrolysis
100	9.18×10^{-2}	95.3[a]
30	4.09×10^{-5}	69.9[b]
10	1.75×10^{-6}	98.7[b]

a) after 30 minutes *b*) after 1 year

Strictly, velocity constant is not dependent on hydroxyl ion alone. As temperature decreases, influence of other catalysts must be considered. Moreover, it may be considered that if not buffered, the pH of the medium is apt to be lowered due to the production of hydrogen ion during hydrolysis. Because of this lowering of pH, the rate constant changes in unbuffered solution toward larger or smaller value, depending upon the concentration of succinylcholine chloride and temperature.

Many workers have studied the hydrolysis of succinylcholine chloride under various cenditions of storage and analysis as mentioned in the introduction. Tammelin[2)] reported succinylcholine iodide solution to be most stable at pH 4 to 5. Larsson⁵⁾ reported the pH value at the lowest rate of hydrolysis of succinylcholine chloride as 3.6. Nevertheless, these values can not be compared directly with the values as found in this work. In conclusion, from the results obtained in this work, it was recognized that the injection of succinylcholine chloride should be stored at the lowest temperature possible and the use of buffering agent is not always recommended because the components of buffers considerably accelerate hydrolysis of succinylcholine chloride.

The author wishes to express the thanks and appreciation to Prof. Dr. H. Nogami of the University of Tokyo for his guidance and encouragement throughout this study, and also to Dr. M. Horioka, Mr. F. Nakagawa, and Dr. K. Umemura for the discussions, and Yamanouchi Pharmaceutical Co., Ltd. for the suppiy of succinylcholine chloride.

Summary

1) The degradative reaction of succinylcholine chloride was investigated over the pH range of 0.9 to 8.5 by ion exchange chromatographic method from the standpoint of chemical kinetics. The decomposition was recognized as a first-order reaction at any given pH value with respect to succinylcholine chloride. From the data obtained at the lower pH regions, the reaction constants (L.·mol.$^{-1}$·hr.$^{-1}$) catalyzed by hydrogen ion were determined as follows :

$$k_{H^+}=1.36\times10^{11}\exp\left(\frac{-17,230}{RT}\right)$$

2) From the data obtained at higher pH regions, the presence of catalysis of general base besides hydroxyl ion was shown. It was recognized that the velocity constant was affected by a buffer concentration even in the same pH and the same ionic strength.

3) From the observed rate constants against varying acetate ion concentrations in the acetate buffers ($\mu=0.2$) for two different pH values (4.69, 3.98) at 60°, the second-order rate constant, $k_{CH_3COO^-}$, was determined as 5.01×10^{-2} (L.·mol.$^{-1}$·hr.$^{-1}$).

4) The catalytic constant (L.·mol.$^{-1}$·hr.$^{-1}$) of hydroxyl ion, k_{OH^-}, for succinylcholine chloride was obtained, maintaining at the initial pH value without buffering agent. The reaction constants catalyzed by hydroxyl ion were determined as follows :

$$k_{OH^-}=7.88\times10^{13}\exp\left(\frac{-13,091}{RT}\right)$$

5) The rate constant (hr.$^{-1}$) in an acetate buffer ($\mu=0.2$) at an optional concentration was expressed as follows :

$$k=6.47\times10^{-1}(H^+)+5.01\times10^{-6}(H_2O)+2.04\times10^{5}(OH^-)+5.01\times10^{-2}(CH_3COO^-)$$

It was concluded that the use of buffering agent is not always recommended for the storage of succinylcholine chloride solution, because it accelerates hydrolysis of succinylcholine chloride.

(Received June 28, 1961)