**Preparation of (XIII) and (XIV)**——A mixture of 1 g. of (Ⅸ) and 5 ml. of piperidine or pyrrolidine was refluxed for 1 hr., and the excess amine was distilled off under a reduced pressure. The residue was dissolved in water and precipitated by addition of dil. HCl. Yield, 0.6 g.

**Preparation of (XVIII, XIX, XX, and XXI)**——A mixture of 1 g. of (Ⅸ), 0.7 g. of the corresponding amine, 0.5 ml. of conc. HCl and 20 ml. of $H_2O$ was refluxed for 30~60 min. After cool, precipitate was collected and purified by reprecipitation. Yield, 0.7~1.3 g.

***p*-(6-Hydroxy-9*H*-purin-9-yl)benzenearsanilic Acid (XXII)**——A mixture of 0.5 g. of (Ⅸ) and 15 ml. of 90% formic acid was refluxed for 1 hr. The excess formic acid was distilled off under a reduced pressure. The residue was dissolved in NaOH soln., and precipitated by addition of dil. HCl. Yield, 0.3 g.

***p*-(6-Methoxy-9*H*-purin-9-yl)benzenearsonic Acid (XXIII)**——Sodium hydroxide (0.5 g.) and (Ⅸ) (1.0 g.) were dissolved in 40 ml. of MeOH, and the mixture was refluxed for 1 hr. The reaction mixture was evaporated to dryness, and the residue was dissolved by addition of 10 ml. of water. Neutralization with dil. HCl precipitated 0.6 g. of (XXⅢ).

***p*-(6-Phenoxy-9*H*-purin-9-yl)benzenearsonic Acid (XXIV)**——A mixture of (Ⅸ) (0.8 g.), phenol (0.4 g.), and NaOH (0.3 g.) in 7 ml. of water was refluxed for 4 hr. After cool, addition of dil. HCl precipitated (XXIV). Yield, 0.5 g.

**N-(Purin-6-yl)arsanilic Acid (XXV)**——A mixture of arsanilic acid (0.7 g.), 6-chloropurine (0.5 g.), and conc. HCl (0.2 ml.) in 25 ml. of water was refluxed for 20 min. Precipitate formed was collected and purified. Yield, 0.2 g.

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# Tokuji Suzuki and Yuichi Tanimura : A Mehtod for Determining Hydrolytic Rate Constants of Succinylcholine Chloride with Analog Computation.

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The degradation of succinylcholine chloride in aqueous solution followed a two-step sequence, succinylcholinechloride (SCC) → succinylmonocholine chloride (SmCC) → succinic acid.[1] The kinetics of this pathway could be interpreted on the basis of consecutive first-order reactions.[2,3] Both the quantitative ion exchange chromatography and the colorimetry of the ferric-hydroxamic acid complex derivatives of the choline esters by Hestrin's method[4] were used to determine concentration of each of the two esters, SCC and SmCC, at any time in buffer solutions.[5] The rate constants ($k_1$) of the first reaction were calculated from the slopes of the plots of the logarithms of the concentrations of SCC against time at various pH and temperatures, and the rate constants ($k_2$) of the second reaction were measured independently using SmCC under the same conditions.

An absorbance ($E_T$) of degrading SCC solution at any time by the colorimetry is shown as follows :

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[*1] Hongo, Bunkyo-ku, Tokyo (鈴木徳治, 谷村勇一).

1) M. P. Earles, *et al.* : J. Pharm. Pharmacology, **6**, 773 (1954).

2) T. Suzuki : This Bulletin, **10**, 912 (1962).

3) *Idem* : *Ibid.*, **10**, 1017 (1962).

4) S. Hestrin : J. Biol. Chem., **180**, 249 (1949).

5) H. Nogami, T. Suzuki : This Bulletin, **9**, 646 (1961).

$$E_T = [SCC]E_{SCC} + [SmCC]E_{SmCC} \qquad (1)$$

Where, $E_{SCC}$ and $E_{SmCC}$ are the molar extinction coefficients of ferric hydroxamic acid complexes of SCC and SmCC, respectively. Powell showed that the rate constants of two consecutive first-order reactions were obtained from a graphical plot of per cent reaction against log $t$.⁶⁾ A more accurate determination was presented by a time ratio method.⁶⁾ This method is based on a table of time-percentage reaction relations for various relative rate constants ($k_1/k_2$). However, the percentage decomposition of SCC at any time is not estimated only by the colorimetry for the esters in buffered solutions. Because, a ratio of [SCC] to [SmCC] at any time can not be determined without the chromatographic separation.

The authors have attempted to determine the $k_1$ value of the first reaction with the aid of an analog computer using the molar extinction coefficients and the $k_2$ value of the second reaction to avoid the time-consuming chromatography. The cocentrations of SCC and SmCC at any time can be expressed in the following differential equations (2) and (3), respectively.

$$-\frac{d[SCC]}{dt} = k_1[SCC] \qquad (2)$$

$$\frac{d[SmCC]}{dt} = k_1[SCC] - k_2[SmCC] \qquad (3)$$

The analog computer program for determining the rate constants of the first reaction from the sum of absorbances of the two esters is given in Fig. 1. The absorbance from spectral reading at any time was plotted on a chart paper. The chart paper was run through at a constant rate of 1 cm. a second. The known rate constant ($k_2$), the molar extinction coefficients of SCC and SmCC which were determined to be 298 and 176, respectively, and an initial concentration of SCC were involved in the computer by setting the potentiometer. The rate constant ($k_1$) of the first reaction was determined by the value giving the best computer fit to the experimental data which were measured directly without the chromatographic separation. The degradation of SCC was studied in four-fold ranges of acetate and pyridine buffer concentrations to evaluate general acid-base catalysis for the hydrolysis of SCC (Table I). A typical fitting of the data in 0.4$M$ acetate buffer at pH 4.68 and 60.0° by the program of Fig. 1 is shown in Fig. 2. The curves of SCC, SmCC and succinic acid in Fig. 2 were obtained from the analog computation by setting the potentiometers at $k_1 = 3.14 \times 10^{-6}$ and $k_2 = 2.87 \times 10^{-6}$ in $\sec^{-1}$ (rate constants obtained in 0.4$M$ acetate buffer at pH 4.68 and 60.0°), and demonstrate the concentrations of each

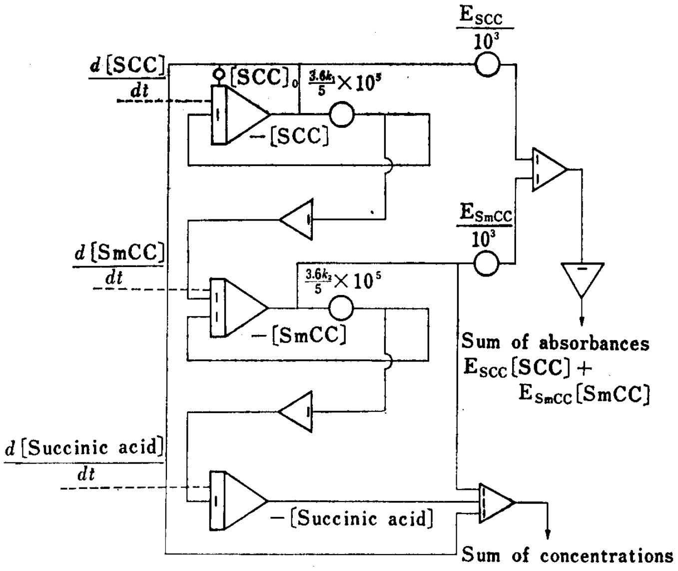

Fig. 1. The Analog Computer Program for Fitting to the Sum of Absorbances of Succinylcholine Chloride and Succinylmonocholine Chloride obtained with the Colorimetry, based on Equations (1) to (3)

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6) Frost and Pearson: Kinetics and Mechanism, 2nd Ed., p. 169~171 (1961). John Wiley & Sons, Inc., New York.

substance against time. Ionic strength of a series of acetate buffers and pyridine buffers with constant buffer ratios at different concentrations was maintained constant (0.2 or 0.5) by addition of sodium chloride. A reasonably good agreement (Table I) was obtained between the computed data and the chromatographic data or the calculated data from the equation reported previously.[2]

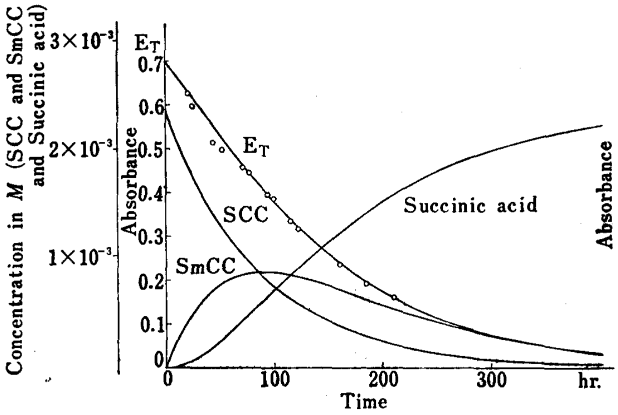

Fig. 2. Typical Analog Computer Fit of the Data obtained Spectrophotometrically from the Hydrolytic Decomposition of Succinylcholine Chloride in 0.4$M$ Acetate Buffer at pH 4.68 and 60.0°.

Molar extinction coefficients, $E_{SCC}$ and $E_{SmCC}$ are 298 and 176, respectively. Curves of SCC (succinylcholine chloride), SmCC (succinylmonocholine chloride) and succinic acid were obtained by setting the potentiometers at $k_1=3.14\times10^{-6}$, $k_2=2.87\times10^{-6}$ in sec$^{-1}$ and the initial concentration ($2.34\times10^{-3}M$) of SCC.

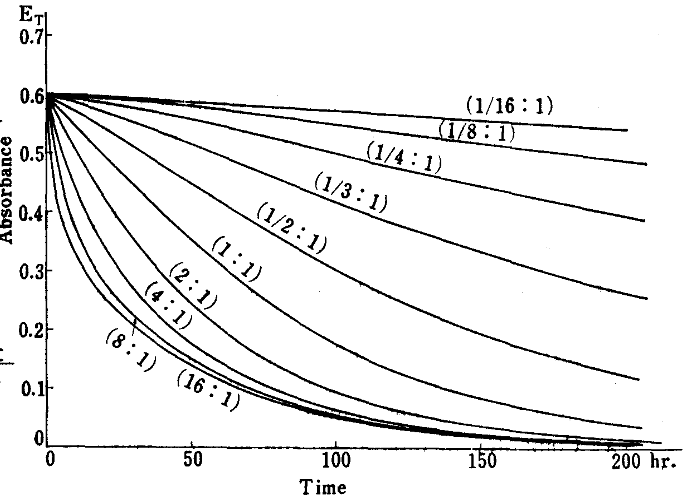

Fig. 3. $E_T$–time Curves computed for Varying Ratios of $k_1$ to $k_2$

Molar extinction coefficients ($E_{SCC}=298$ and $E_{SmCC}=176$), the initial concentration ($2.01\times10^{-3}M$) of succinylcholine chloride and $k_2$ ($2.87\times10^{-6}$ sec$^{-1}$) were used for the computation based on Eqs. (1) to (3). Each curve is labeled as to the ratio of $k_1$ to $k_2$.

Table I. Rates ($k_1$ and $k_2$ in sec$^{-1}$) for Succinylcholine Choline Hydrolysis at Varying Buffer Concentrations in Acetate and Pyridine Buffers

| pH[a] | | Buffer Composition ($M$) | | | $10^6k_1$ | | $10^6k_2$ |
|---|---|---|---|---|---|---|---|
| | | [$CH_3COOH$] | [$CH_3COONa$] | [NaCl] | Computed | Chromatographic | |
| 4.68 | 4.68 | 0.200 | 0.200 | 0.000 | 3.14 | 3.14[b] | 2.87 |
| 4.68 | | 0.150 | 0.150 | 0.050 | 2.50 | 2.53[b] | 2.51 |
| 4.67 | | 0.100 | 0.100 | 0.100 | 1.75 | 1.74[b] | 2.23 |
| 4.67 | | 0.050 | 0.050 | 0.150 | 1.00 | 1.05[b] | 1.99 |
| 5.28 | 5.29 | 0.050 | 0.200 | 0.000 | 4.37 | 3.97[c] | 2.89 |
| 5.29 | | 0.038 | 0.150 | 0.050 | 3.47 | 3.22[c] | 2.67 |
| 5.30 | | 0.025 | 0.100 | 0.100 | 2.75 | 2.52[c] | 2.52 |
| 5.29 | | 0.013 | 0.050 | 0.150 | 1.97 | 1.83[c] | 2.34 |
| | | [Pyridine HCl] | [Pyridine] | | | | |
| 4.90 | 4.92 | 0.500 | 0.500 | 0.000 | 3.47 | —[d] | 3.22 |
| 4.91 | | 0.300 | 0.300 | 0.200 | 2.75 | —[d] | 2.82 |
| 4.95 | | 0.125 | 0.125 | 0.375 | 1.76 | 1.73 | 2.42 |

a) Measured at 60.0°.
b) Data of the previous report.[2]
c) Calculated from the following derived equation ($k$ in hr.$^{-1}$) in the previous report[2]:
$k=6.47\times10^{-1}[H^+]+5.01\times10^{-6}[H_2O]+2.04\times10^{5}[OH]+5.01\times10^{-2}[CH_3COO^-]$
d) This pyridine buffer interfered with the chromatographic method and the rate constant was not obtained.

This computation method is applicable for general consecutive two first-order reactions, in cases in which both A and B at the reaction, A→B→C, are measurable with same quantitative assay procedure. The estimation of $k_1$ from this fitting will be more accurate, when the shift of $E_T$-time curves resulted from variation of $k_1$ at the specified $k_2$, $E_{SCC}$ and $E_{SmCC}$ and at the specified initial concentration of SCC is larger. The $E_T$-time curves for various ratios, $k_1$ to $k_2$, at $k_2=2.87\times10^{-6}\ \mathrm{sec}^{-1}$, $E_{SCC}=298$, $E_{SmCC}=176$ and the initial concentration ($2.01\times10^{-3}M$) of SCC are shown in Fig. 3. The largest shift was found at about 1:1. The $E_T$-time curves for the various ratios at any other $k_2$ value and at $E_{SCC}=298$, $E_{SmCC}=176$ and the initial concentration ($2.01\times10^{-3}M$) of SCC are the same curves as those in Fig. 3, if the time scale in Fig. 3 is appropriately altered. Therefore, it may be said that this computation procedure can be utilized for the consecutive reactions with ratios of the rates between about 10:1 and about 1/10:1 at $E_{SCC}=298$ and $E_{SmCC}=176$, judging qualitatively from Fig. 3.

## Experimental

**Material**——Succinylcholine chloride (SCC) and succinylmonocholine chloride (SmCC) were the same as those reported previously.[5]

**Kinetic Procedure**——The rate constans ($k_2$) of the second reaction, SmCC to succinic acid, were determined according to the same procedure as the previous paper.[3] The rate constants ($k_1$) of the first reaction, SCC to SmCC, were determined by the following methods.

a) Computer method : All the buffers shown in Table I were equilibrated in a constant temperature bath (60.0°) maintained within ± 0.05°. A 93.0 mg. of SCC was weighed into a 100 ml. volumetric flask, and it was made up to volume with the buffer. Five-milliliter aliquots of the sample solution were taken out immediately and periodically at appropriate time intervals. The total absorbance of the esters in the buffer was determined according to the colorimetry in the previous paper.[5] The absorbances with time were plotted on a graph paper of a recorder, and the $k_1$ values were obtained from curve-fitting by the programmed analog computer.

b) Ion exchange chromatographic method[3] : A 93.0 mg. of SCC was weighed into a 100 ml. volumetric flask, and it was made up to volume with $0.2M$ pyridine buffer solution at pH 4.92. The solution was equilibrated at 60.0° in the constant temperature bath and an appropriate quantity of the sample solution was taken out periodically. A 10 ml. of its solution was run through a column of a strong cation exchange resin (Zeollex SA) in the Na-form to separate SmCC formed by the hydrolysis of SCC. The SCC absorbed on the resin was eluted with 50 ml. of $3M$ NaCl after the elution with 50 ml. of $0.5M$ NaCl in advance, and the absorbance for SCC by the Hestrin's colorimetry[4] was measured by 50 mm. cells on a Hitachi EPU-II spectrophotometer.

**The Analog Computer**——The rate constants ($k_1$) of the degradation, SCC to SmCC, in acetate and pyridine buffers were computed by a Hitachi ALM-502T analog computer. The Unicorder Model U-100M (Nippon Denkikikai Co., LTD., Tokyo) was used as a recorder to fit the data.