UDC 615.786[612.741.15]-011

105. Hisashi Nogami and Tokuji Suzuki : Studies on Decomposition and Stabilization of Drugs in Solution. VIII.*1 Chemical Kinetic Studies on Aqueous Solution of Succinylcholine Chloride. (1). Separatory Determination of Succinylcholine Chloride in Hydrolyzed Products.*2

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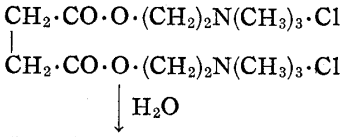
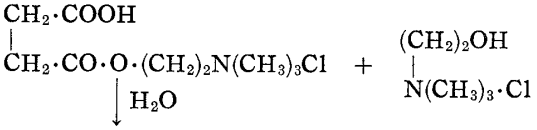
Succinylcholine chloride (I) is a very potent neuromuscular blocking agent. Owing to its rapid destruction by enzymes of the body, it has a short duration of action and freedom from undesirable side-effects. This ester is fairly stable in acidic aqueous solution, but is readily hydrolyzed in the presence of alkali. No systematic work on its degradation has yet been published, because of difficulty of determining (I) in the presence of hydrolyzed products on decomposition in buffered solutions. The purpose of this investigation was to present a reliable chemical separatory method, necessary for the chemical kinetic study of the degradation process of (I), in aqueous solution during its sterilization and storage.

According to Whittaker,[1] (I) may undergo hydrolysis in aqueous solution by the following two steps :

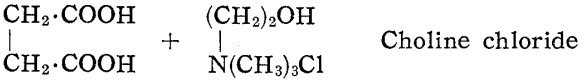
Succinylcholine chloride (I)



Succinylmonocholine chloride (II)



Succinic acid



Choline chloride

Earles, *et al.*[2] carried out separation and identification of the hydrolytic products present in the injection of (I) by paper partition chromatography. The results of their study showed that when hydrolysis was at 25%, a spot which might be due to succinylmonocholine chloride (II) appeared. As hydrolysis proceeded, the choline spot increased in intensity and a spot due to (I) diminished. Therefore, (I), (II), choline chloride, and succinic acid are present together in the solution of partially hydrolyzed (I). These four substances are similar to each other in their chemical structure. (I) and (II) have ester linkage. (I), (II), and choline chloride are quaternary ammonium salts, while (II) and succinic acid have carboxyl group. It is not possible to estimate accurately (I) remaining in the solution without interference by other substances and to determine one substance separately from the others in a buffered solution, because these substances are quite similar to each other in chemical property. (II) is similar to (I) in its curare-like action, but much less active. The ratio of 45:1 on the head-drop dose (H. D.) in rabbits was confirmed

*1 Part VII. H. Nogami, *et al.* : This Bulletin, 8, 1136 (1960).
*2 This work was presented at the 13th Annual Meeting of the Pharmaceutical Society of Japan (1960).
*3 Hongo, Tokyo (野上 寿, 鈴木徳治).
1) V.P. Whittaker : Experientia, **7**, 251 (1951).
2) M.P. Earles, *et al.* : J. Pharm. Pharmacol., **6**, 773 (1954).

by Boved, *et al.*[3] The action of (II) and choline chloride was 1/40 and 1/140, respectively, of (I) on the isolated muscle of frog, according to Matsuno, *et al.*[4] (I) can be determined in a partially hydrolyzed solution by these biological methods. However, they are not suitable for the kinetic study of the hydrolysis of (I), since the procedure of these methods is not easy for frequent determination and the accuracy of this type of method is not sufficient.

The quantities of two substances must be at least measured independently to investigate theoretically the formation or the disappearance of these four substances in aqueous solution. Ion exchanger chromatography method and Hestrin's colorimetric method[5] were applied for the selective determination of (I) from decomposed substances in the present work.

Experimental

Equipment and Materials——A strong acid-type cation exchanger, Zeollex SA, having particles of 200~250 mesh, was converted from its H-form to its Na-form with an excess of 20% NaCl solution. This product was washed with water and poured into a chromatographic tube making a column of 9×60 mm.

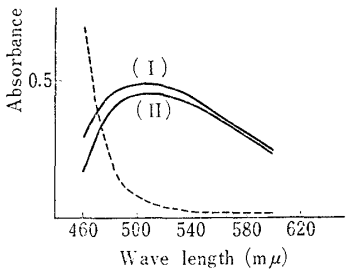
Succinylcholine chloride (I) was recrystallized from EtOH, m.p. 160~162°. *Anal.* Calcd. for $C_{14}H_{30}O_4N_2Cl_2 \cdot 2H_2O$: C, 42.3; H, 8.62; N, 7.05. Found: C, 41.9; H, 8.62; N, 6.74.

Succinylmonocholine chloride (II) was synthesized by the Phillips method,[6] m.p. 149~150°. *Anal.* Calcd. for $C_9H_{18}O_4NCl$: C, 45.1; H, 7.57; N, 5.84. Found: C, 44.9; H, 7.42; N, 5.83.

A commercial form of choline chloride was employed. All other chemicals used in this work were of extra pure grade.

Ion Exchange Procedure——Ten cc. of the buffered solution containing about 35 mg. of (I) and about 20 mg. of (II) was passed through the ion exchanger column at the rate of 0.4 cc./cm²/min. The choline esters adsorbed were eluted with NaCl solution at various concentrations at the rate of 0.7 cc./cm²/min. Exactly 10-cc. effluents were collected and each fraction was assayed colorimetrically, as will be described, to detemine the elution process. Efficiency of the eluant was observed by plotting the relationship of the concentration of the fraction to the volume of effluent.

Colorimetry——The colorimetric method used for the determination of (I) and (II) is based on the quantitative reaction of carboxylic ester and hydroxylamine into hydroxamic acid in alkali. These hydroxamic acids form red complexes with Fe^{+3} in acidic solution. Hestrin[5] reported the factors affecting the color intensity in the determination of acetylcholine by this method. The absorption characteristics of the iron-hydroxamic acid complexes of (I) and (II) by Hestrin's procedure are presented in Fig. 1.



------ Ferric chloride blank solution
——— (I) Succinylcholine chloride in 3*M* NaCl,
(II) Succinylmonocholine chloride in 0.6*M* NaCl (caluculated by difference)

Alkaline hydroxylamine reagent is prepared freshly before use by mixing equal volumes of 3.5*M* NaOH and 2*M* hydroxylamine. The mixture keeps for about 3 hours at room temperature. Ten cc. of alkaline hydroxylamine reagent is added to 5 cc. of the solution to be analyzed. After 3 minutes, the pH is brought to 1.2± 0.2 with 5 cc. of HCl solution and 5 cc. of 0.37*M* $FeCl_3$ in 0.1*N* HCl is added.

Fig. 1. Absorption Spectra of the Iron-Hydroxamic Acid Complexes

These complexes exhibit a broad absorption maximum at 510 mμ. From consideration of the absorption of a blank, it was decided to use 540 mμ as in the case of acetylcholine for this work. The standard curves for (I) and (II) are given in Fig. 2. Density of the color was measured 5 min. after the addition of Fe^{3+} to keep the error from fading constant. It can been seen from this figure that the same standard curve was obtained for (I) in 3*M* NaCl and (II) in 0.6*M* NaCl. Aqueous

3) D. Bovet, *et al.*: Arch. intern. pharmacodynamie, **88**, 1 (1951).
4) M. Matsuno, *et al.*: Yakugaku Kenkyu, **29**, 62 (1957).
5) S. Hestrin: J. Biol. Chem., **180**, 249 (1949).
6) A.P. Phillips: J. Am. Chem. Soc., **75**, 4725 (1953).

reineckate reagent was used for determining choline chloride. The reineckate precipitate was dissolved in 10 cc. of Me_2CO and this solution was measured at 524 mμ. The Hitachi EPU-II spectrophotometer was employed to measure the absorbance of the solution.

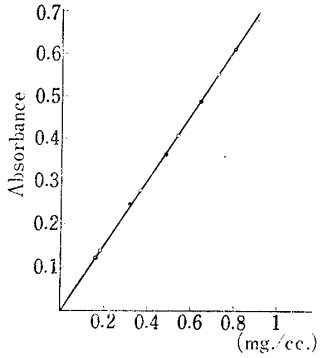
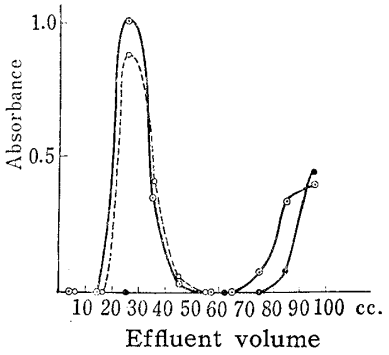


Fig. 2. The Standard Curve

-o——o- Succinylcholine chloride (in 3*M* NaCl)
-•——•- Succinylmonocholine chloride (in 0.6*M* NaCl)

Results

These two esters in various buffered solutions were adsorbed on the resin mostly or completely. Elution curves for (II), (I), and their mixture in the buffered solution (pH 1.22) prepared from 0.2*M* hydrochloric acid and 0.2*M* potassium chloride are shown in Fig. 3.



-⊙—⊙- Succinylcholine chloride and succinylmonocholine chloride
-•——•- Succinylcholine chloride alone
-o- - -o- Succinylmonocholine chloride alone
Column : Zeollex SA, R-Na form, 200~250 mesh, 9×60 mm.
Flow rate : 0.7 cc./cm²/min.
Solution : 0.6 *M* NaCl

Fig. 3. Chromatographic Separation of Succinylcholine Chloride and Succinylmonocholine Chloride in Buffer (pH 1.22)

From this graph, following facts are noted.

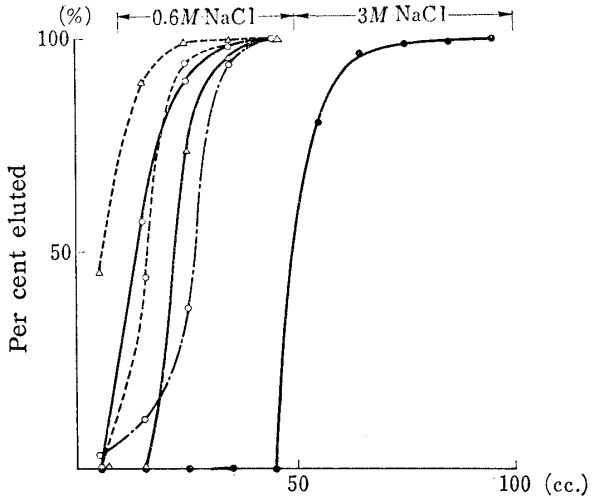
1) All of (II) and a part of (I) are eluted by 100 cc. of 0.6*M* sodium chloride solution.

2) (I) has a stronger affinity to the resin than (II). (II) is eluted by the fifth 10-cc. fraction and (I), after the eighth 10-cc. fraction.

3) The difference of elution curves between each of the esters alone and their mixture is not significant.

When 0.5*M* and 0.7*M* sodium chloride were used as the eluant, a similar curve was observed as with 0.6*M* sodium chloride. Above the concentration of 0.8*M*, the elution curve of each ester overlapped and the separatory determination became difficult.

Integral elution curves of each ester in various buffered solutions are presented in Fig. 4. (II) was eluted by 0.6*M* sodium chloride. (I) was eluted continuously by 3*M* sodium chloride after elution with 50 cc. of 0.6 *M* sodium chloride. Fig. 4 shows that (II) is eluted by the fifth 10-cc. fraction and (I) from sixth to tenth 10-cc. fractions in the buffered and unbuffered solutions. The recovery in various solutions is given in. Table I. Each ester was eluted individually in the same way as shown in Fig. 4. (II) was determined with initial 50-cc. effluent of 0.6*M* sodium chloride. (I) was determined with 50-cc.



Succinylmonocholine chloride
-△---△- 0.5*M* AcONa+0.5*M* AcOH (pH 5.5)
-o——o- 0.5*M* NaOH+0.5*M* potassium hydrogenphthalate (pH 4.4)
-o---o- 0.2*M* AcONa+0.2*M* AcOH (pH 4.7)
-△——△- 0.2*M* HCl+0.2*M* KCl (pH 1.7)
-o---o- H_2O
Succinylcholine chloride
-•——•- 0.57*M* AcONa+0.57*M* AcOH (pH 5.1) or H_2O
Column : Zeollex SA, R-Na form, 200~250 mesh, 9×60 mm.
Flow rate : 0.7 cc./cm²/min.

Fig. 4. Integral Elution Curves of Succinylcholine Chloride and Succinylmonocholine Chloride

Table I. Recovery Test for Succinylmonocholine Chloride and Succinylcholine Chloride of Known Quantity

	pH	Buffer	Taken (mg.)	Recovered (mg.)	Recovery rate (%)
Succinylmonocholine Chloride	4.74	0.2*M* AcONa + 0.2*M* AcOH	26.3	26.4	100.5
	1.69	0.2*M* HCl + 0.2*M* KCl	27.3	27.2	99.5
		H_2O	20.1	20.2	100.7
Succinylcholine Chloride	5.10	0.57*M* AcONa + 0.57*M* AcOH	34.8	34.6	99.4
	5.10	0.57*M* AcONa + 0.57*M* AcOH	17.4	17.5	100.8
	1.69	0.2*M* HCl + 0.2*M* KCl	30.7	31.2	101.7
		H_2O	34.6	34.4	99.4
		H_2O	17.3	17.2	99.2

effluent of 3*M* sodium chloride after elution with 50 cc. of 0.6*M* sodium chloride in which (I) and (II) due to hydrolysis of (I) were not found in either case. The results shown in Table I indicate that the recovery of choline esters was within 2% of the theoretical value. These choline esters were not hydrolyzed during the elution in this exchanger resin, although an ion exchange resin generally accelerates hydrolysis of an ester.

The single elution curve of choline chloride by 0.6*M* sodium chloride solution is given in Fig. 5. It is considered that the elution process of choline chloride is not affected by the presence of esters in the quantity described above. Therefore, choline chloride will be found in the fraction of (II) at the elution of the partially hydrolyzed products.

In order to verify that the decomposition of (I) in the buffered solutions could be investigated by the ion exchanger method, (I) was dissolved to make 0.35% solution in a buffered solution prepared from 0.2*M* hydrochloric acid and 0.2*M* potassium chloride. The solution was poured into 10-cc ampules, these ampules were stored in a thermostatically

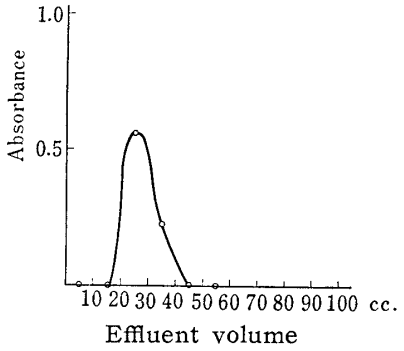


Fig. 5. Elution Curve of Choline Chloride in Buffer (pH 1.70)

Choline Chloride 21.3 mg. in 10 cc. Buffer prepared from 0.2 *M* HCl and 0.2*M* KCl
Column : Zeollex SA, R-Na form, 200～250 mesh, 9×60 mm.
Flow rate : 0.7 cc./cm²/min.
Solution : 0.6*M* NaCl

controlled water bath, and were taken out periodically to determine the amount of (I) by the above method. Fig. 6 illustrates semi-log plots of the fraction of unhydrolyzed ester as a function of time. Fig. 6 shows that the degradation is a first-order reaction with respect to (I) at these constant hydrogen ion concentrations and that it is possible to apply this ion exchanger method to examine of hydrolysis of (I).

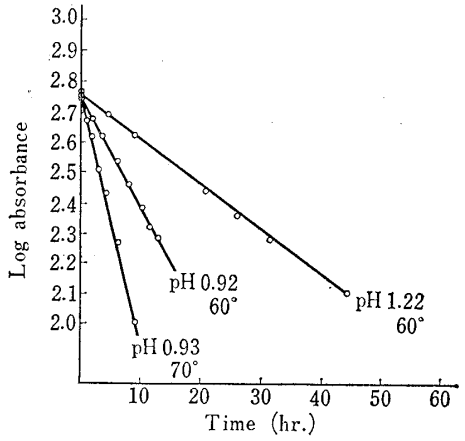


Fig. 6. First-order Disappearance of Succinylcholine Chloride

Succeeding work on the decomposition of these esters in various pH will be described in a later report.

The authors are very grateful to Dr. T. Yoshino (Government Chemical Industrial Research Institute, Osaka) for kind advices in this work and express their gratitude to Yamanouchi Pharmaceutical Co. for the supply of succinylcholine chloride.

Summary

Separation of partially hydrolyzed products of succinylcholine chloride in various buffered solutions by ion exchanger chromatography was attempted using a strong acid-type cation exchanger (R-Na form). It was concluded that succinylcholine chloride could be satisfactorily separated from the degradation products. Succinylcholine chloride and succinylmonocholine chloride were not hydrolyzed during elution. The recovery of these choline esters was within 2% of the theoretical values. It was ascertained that the decomposition of succinylcholine chloride in various buffered solutions could be investigated by this separatory method from the standpoint of chemical kinetics.

(Received December 8, 1960)