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201. Hisashi Nogami,*1 Shoji Awazu,*2 and Motoharu Iwatsuru*1 : Studies on Decomposition and Stabilization of Drugs in Solution. XIV.*3 Stabilization of Methantheline Bromide and Choline Derivatives in Aqueous Solution by Surface-active Agents.

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The stabilization of methantheline bromide (quaternary ammonium salt with ester linkage) in aqueous solution by sodium lauryl sulfate (anionic surfactant) was studied in the previous report,[1] in which it was found that the effect of stabilization was dependent on the concentration of sodium lauryl sulfate.

Nakajima reported[2] that the stabilization of acetyl salicylic acid with non-ionic surfactants was in parallel with the degree of solubilization by them. The solubilized system of quaternary ammonium salt with anionic surfactant is not so simple.

A quaternary ammonium salt would form complex compound with surfactant in anionic surfactant solution, and the complex compound would be solubilized into the micelle formed with residual surfactant. The equilibria would occur in the stage of complex formation and between the complex compound in and out of the micelle. If the stabilization is the result of solubilization into the micelle as mentioned before,[1~4] the stability would be affected by chain length of surfactants and solubilizates, respectively, since it is well known fact[5] that the chain length of them has relation to the degree of solubilization.

In the first place the effect of the alkyl chain length of surfactants was studied, and found that the longer the length of alkyl chain, the more methantheline bromide was stabilized.

Secondly the length of acyl chain of the hydrolyzate was examined. It was so troublesome to modify the acyl chain of methantheline bromide that acetylcholine chloride and its derivatives were chosen as the simplified forms. Acyl cholinesters used were acetylcholine chloride, propionylcholine iodide, butyrylcholine iodide, hexanoylcholine iodide and octanoylcholine iodide. Only sodium lauryl sulfate was used because of its properties, *e.g.* solubility, Krafft point.

As expected the surfactant enhanced the stability of hydrolyzates and the cholinesters having long acyl chain were remarkably stabilized by the addition of surfactant.

Experimental

Materials——1) Methantheline bromide and ethylene dichloride were same as reported previously.[6] 2) Sodium lauryl sulfate, sodium octyl sulfate, sodium tetradecyl sulfate, sodium cetyl sulfate : A

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3) S. Riegelman : J. Am. Pharm. Assoc., **49**, 339 (1960).
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commercial products of Nikko Co., Ltd., recrystallized from EtOH after extraction of higher alcohols with Et_2O.

3) Acetylcholine chloride: A commercial product of Daiichi Pure Chemicals Co., Ltd.

4) Propionylcholine iodide, butyrylcholine iodide, hexanoylcholine iodide, octanoylcholine iodide were synthesized according to Loury's method.[7,8]

5) Buffer solutions used were

Na_2HPO_4 0.0614 M+KH_2PO_4 0.00534*M*

$Na_2B_4O_7$ 0.010 M+H_3BO_3 0.160*M*

pH values were determined with the Beckman Model G pH-meter.

Phosphate buffer was used in methantheline bromide, and borate buffer used in cholinesters to avoid the obstruction by phosphate ion at the colorimetry.

Depression of pH value was almost within the range of 0.05 throughout a run and this influence was negligible.

All reagents used were of extra pure grade.

Kinetic Procedure——Fifty mg. methantheline bromide and accurately weighed additive were placed in a volumetric flask and made to 50 ml. with the buffer solution. The volumetric flask was immersed in a thermostatically controlled water bath maintained at 70°±0.1°.

Samples were taken from the flask at given intervals and methantheline bromide was determined. In the cases of alkylcholine acetylcholine chloride 50 mg., propionylcholine iodide 79 mg., butyrylcholine iodide 83 mg., hexanoylcholine iodide 91 mg., octanoylcholine iodide 98 mg. were weighed respectively,*4 and other procedures were same as methantheline bromide.

Determination——1) Methantheline bromide: Ten ml. of buffer solution (pH 8, 0.5 ml. of 2*M*/15 KH_2PO_4+9.5 ml. of 2*M*/15 Na_2HPO_4), 20 ml. of ethylene dichloride, and 2 ml. of sample were pipetted into a glass-stoppered centrifugal bottle. The bottle was shaken for 10 min. and centrifuged for 15 min. The ethylene dichloride layer was assayed spectrophotometrically at 283 mμ.

2) Choline derivatives: Determined at 520 mμ according to Hestrin's method.[9]

Results and Discussion

Effects of Sodium Cetyl Sulfate Concentration on the Stabilization of Methantheline Bromide

The decomposition of methantheline bromide in 0.1, 0.2, 0.3, 0.4, and 0.5% sodium cetyl sulfate solution are illustrated in Fig. 1. The results agreed with that of sodium lauryl sulfate and the effect of repression increased with the elevation of sodium cetyl sulfate concentration as reported previously.[1]

The decomposition does not follow a pseudo-first order course as mentioned previously.[1] As the rate constants could not be calculated, the magnitude of stabilizations was compared by half lives and one-fourth reaction times which are tabulated in Table I.

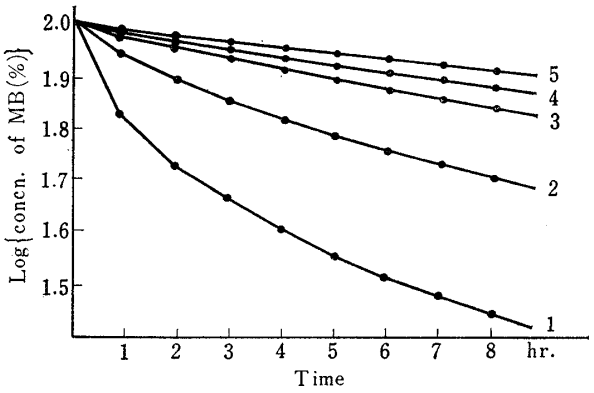

Fig. 1. Decomposition of Methantheline Bromide in Sodium Cetyl Sulfate Solution at 70°

1: 0.1% SCS soln.
2: 0.2% SCS soln.
3: 0.3% SCS soln.
4: 0.4% SCS soln.
5: 0.5% SCS soln.
SCS: sodium cetyl sulfate
MB: methantheline bromide

*4 They are of equimolalconcentration (5.5 m*M*).

7) M. Loury: C. R. Acad. Sci. Paris, **209**, 682 (1939).

8) R. Schneider, A. R. Timms: Brit. J. Pharmacol., **12**, 30 (1957).

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Table I. Half Lives and One-fourth Reaction Times of Methantheline Bromide in Sodium Cetyl Sulfate (SCS) Solution at 70°

Concn. of SCS (%)	0.1	0.2	0.3	0.4	0.5
Half life (min.)	140	740			
One-fourth reaction time (min.)		150	400	>540	>780
pH	7.76	7.80	7.75	7.72	7.75

Effect of Alkyl Chain Length of Surfactant on the Decomposition of Methantheline Bromide

The decomposition of methantheline bromide at 70° in 0.5% anionic surfactant are illustrated in Fig. 2. Half lives are tabulated in Table II.

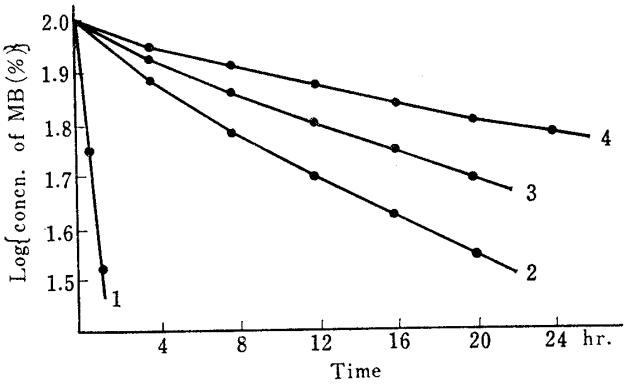

Fig. 2. Effect of Alkylchain Length of Surfactants on the Decomposition of Methantheline Bromide at 70°

1 : 0.5% sodium octyl sulfate
2 : 0.5% sodium lauryl sulfate
3 : 0.5% sodium tetradecyl sulfate
4 : 0.5% sodium cetyl sulfate
MB : methantheline bromide

Table II. Half Lives of Methantheline Bromide in Anionic Surfactant Solutions at 70° (Concn. of Surfactant 0.5%)

Surfactant	SOS	SLS	STS	SCS
Number of C-atoms	8	12	14	16
Half life (hr.)	0.6	12.2	19	>32
pH	7.71	7.76	7.77	7.73

SOS : Sodium octyl sulfate
SLS : Sodium lauryl sullate
STS : Sodium tetradecyl sulfate
SCS : Sodium cetyl sulfate

Sodium octyl sulfate shows little validity to stabilize methantheline bromide. It may be deduced from the hardness of formation of sodium octyl sulfate micelles, and micelle formation seems to play an important role in stabilization of methantheline bromide.

Effect of Acyl Chain Length of Choline Derivatives

Hydrolyses of acetylcholine chloride, propionylcholine iodide, butyrylcholine iodide, hexanoylcholine iodide and octanoylcholine iodide at 70° with or without 0.5% sodium lauryl sulfate are illustrated in Figs. 3, 4, 5, 6, and 7, respectively.

In these cases, too, as in methantheline bromide, the decompositions do not obey pseudo-first order course. Half lives are tabulated in Table III.

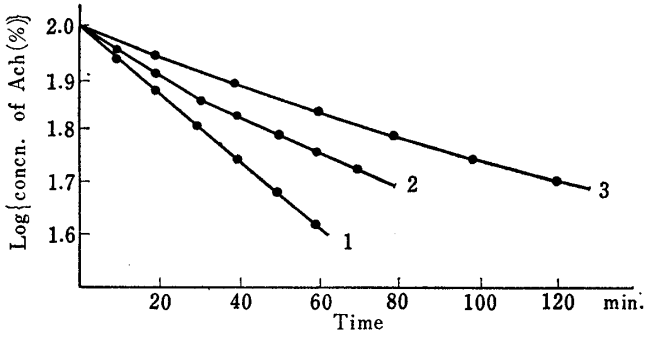

Fig. 3. Hydrolysis of Acetylcholine Chloride in Sodium Lauryl Sulfate Solution at 70°

1 : control
2 : 0.5% sodium lauryl sulfate
3 : 1.15% sodium lauryl sulfate
Ach : acetylcholine chloride

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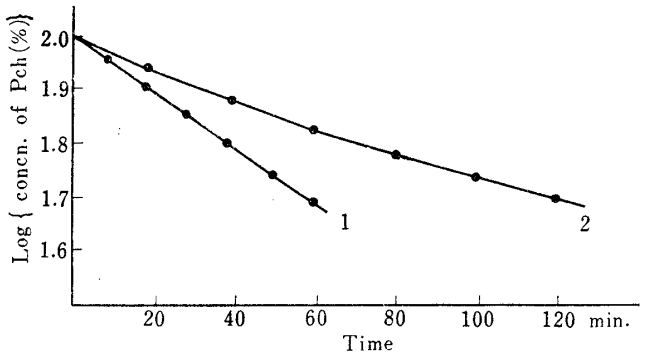

Fig. 4. Hydrolysis of Propionylcholine Iodide in Sodium Lauryl Sulfate Solution at 70°

1 : control
2 : 0.5% sodium lauryl sulfate
Pch : propionylcholine iodide

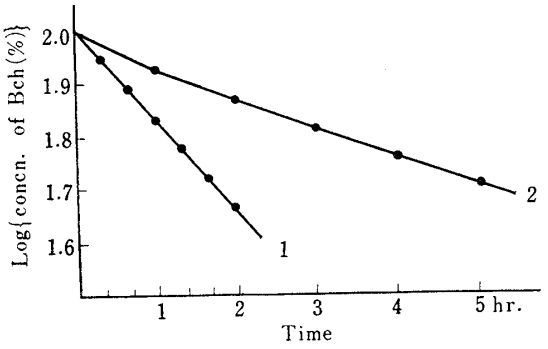

Fig. 5. Hydrolysis of Butyrylcholine Iodide in Sodium Lauryl Sulfate Solution at 70°

1 : control
2 : 0.5% sodium lauryl sulfate
Bch : butyrylcholine iodide

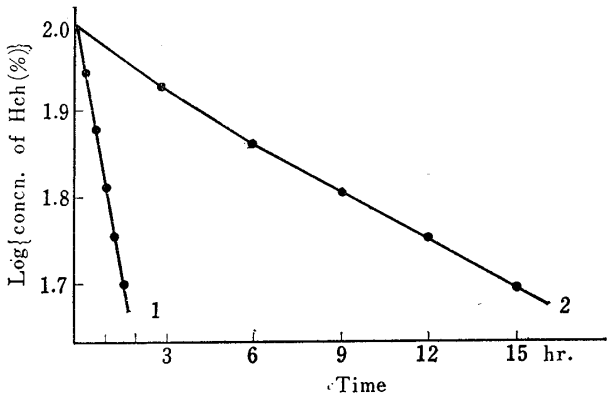

Fig. 6. Hydrolysis of Hexanoylcholine Iodide in Sodium Lauryl Sulfate Solution at 70°

1 : control
2 : 0.5% sodium lauryl sulfate
Hch : hexanoylcholine iodide

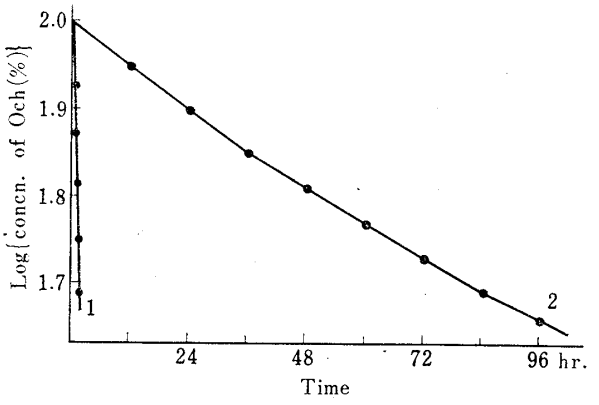

Fig. 7. Hydrolysis of Octanoylcholine Iodide in Sodium Lauryl Sulfate Solution at 70°

1 : control
2 : 0.5% sodium lauryl sulfate
Och : octanoylcholine iodide

Table III. Half Lives of Cholinesters in Sodium Lauryl Sulfate Solutions at 70°

Cholinester (5.5 mM)	Concn. of Sodium Lauryl Sulfate				(B)/(A)
	0% (A)	(min.) (pH)	0.5% (B)	(min.) (pH)	
Acetylcholine chloride	48	(7.79)	80	(7.76)	1.7
Propionylcholine iodide	60	(7.77)	120	(7.79)	2.0
Butyrylcholine iodide	104	(7.69)	305	(7.79)	2.9
Hexanoylcholine iodide	100	(7.75)	894	(7.73)	8.9
Octanoylcholine iodide	100	(7.73)	5040	(7.74)	50.4

Table III shows that as the chain was prolonged, the degree of the stabilization rate (B/A) increased remarkably. Without surfactant, as Larsson reported,[10] the inclination was observed that the longer the length of acyl chain, the more the stabilities of alkylcholines were increased, as far as butyrylcholine iodide.

Butyrylcholine iodide, hexanoylcholine iodide, and octanoylcholine iodide showed about the same stability.

10) L. Larsson : Acta Chem. Scand., 8, 1017 (1954).

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Summarizing this study, alkyl sulfate type surfactant repressed the hydrolysis of quaternary ammonium salt with ester linkage, and the longer the alkyl chain of surfactant or the acyl chain of the hydrolyzate, the more the degree of repression was enhanced.

The authors express their thanks to Dainippon Pharmaceutical Co., Ltd. for the supply of methantheline bromide and to Nikko Co., Ltd. for the supply of anionic surfactants, and also to Miss Hamada for her technical assistance.

Summary

1) The decomposition of methantheline bromide was repressed by the addition of alkyl sulfate (anionic surfactant). The longer the alkyl chain length of alkyl sulfate, the greater the effect was.

2) The addition of surfactant enhanced the stability of cholinesters, and the effect increased with the acyl chain length.

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202. Tsutomu Unemoto: Studies on polyamines. II.*1 Metabolism of Spermidine and spermine by Amine Oxidase in Beef Serum.

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Amine oxidase which attacks spermidine and spermine was first described by Hirsch[1] and it was purified from beef plasma some 150 to 200-fold by Tabor, *et al.*[2] Recently this oxidase has been crystallized by Yamada and Yasunobu.[3] In spite of such successful purification of this enzyme, the stoichiometry of the degradation of these polyamines by the oxidase still remaines unsolved. In connection with the finding of Hirsch[1] that some of the oxidation products of these polyamines inhibit the growth of tubercle bacilli, an attempt has been made to clarify the reaction mechanism of this enzyme.

Quantitative studies on the uptake of oxygen and the liberation of ammonia from spermidine and spermine by the action of oxidase have already been reported by other workers. Using unfractionated sheep serum as an enzyme preparation, Hirsch[1] demonstrated that the oxidation of one mole each of spermidine and spermine resulted in the consumption of 2 and 4 atoms of oxygen, respectively. By contrast, our experiments showed that when an unfractionated beef or goat serum was used as an enzyme preparation, the values of oxygen consumption fluctuated from one experiment to the other, say from 1.4 to 1.8 and from 2.6 to 3.4 atoms of oxygen per mole of spermidine and spermine, respectively. Such a fluctuation seemed to be caused by the contamination

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