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Studies on Decomposition and Stabilization of Drugs in Solution. XIX.[1] Critical Micelle Concentration of Acylcholine[2]

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Critical micelle concentrations (CMC) of acylcholines were measured by surface tension, colorimetry of Sky blue FF, fluorophotometry of Eosin and solubilization of Sudan III.

Formation of micelle was not observed on acetylcholine chloride, propionylcholine iodide, butyrylcholine iodide and hexanoylcholine iodide, but observed on octanoylcholine iodide, decanoylcholine iodide, dodecanoylcholine iodide and hexadecanoylcholine iodide.

Relationship between log CMC of octanoylcholine iodide, decanoylcholine iodide and dodecanoylcholine iodide by solubilization of Sudan III at 45° and the number (N) of carbon atoms in the hydrocarbon chain was written in the following manner.

$$\log \mathrm{CMC(M)} = 1.040 - 0.295n$$

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In this series of study XIII and XV,[4] it has already been reported that sodium octyl, lauryl, myristyl and cetyl sulfate (anionic surfactants) in hydrochloric acid solution were stable below their critical micelle concentrations (CMC) and less stable above their CMC.

It was expected that as above phenomena were observed on anionic surfactants, cationic surfactants in acidic solution were more stable above their CMC than below their CMC. For the purpose of this study, compounds with cationic activity and ester linkage were necessary. It was considered that if the carbon number of acyl radical of acetylcholine was increased, the expected compounds were obtained.

In this series of study XIX and XX,[5] acetylcholine chloride (ACC), propionylcholine iodide (PCI), butyrylcholine iodide (BCI), hexanoylcholine iodide (HCI), octanoylcholine iodide (OCI), decanoylcholine iodide (DeCI), dodecanoylcholine iodide (DoCI), and hexadecanoylcholine iodide (HdCI) were studied. Only crystalline form was reported on higher acylcholines[6] and the report except crystalline form was not found. In this study, at first, CMC was investigated.

1) Part XVIII: H. Nogami, J. Hasegawa, *Late* T. Matsuoka and T. Rikihisa, *Chem. Pharm. Bull.* (Tokyo), **16**, 1876 (1968).
2) This work was presented at Meeting of Kanto Branch, Pharmaceutical Society of Japan, Tokyo, January 1965.
3) Location: *a*) *Hongo, Tokyo*; *b*) *Asahi-machi, Niigata.*
4) H. Nogami, S. Awazu and Y. Kanakubo, *Chem. Pharm. Bull.* (Tokyo), **11**, 13 (1963); H. Nogami and Y. Kanakubo, *ibid.*, **11**, 943 (1963).
5) From the thesis of Yoshio Kanakubo for the degree of Doctor of Pharmaceutical Sciences, University of Tokyo, 1967.
6) Cécile Stora, *Compt. Rend.*, **228**, 324 (1949) [*C.A.*, **43**, 7288*a* (1949)]; *idem*, *ibid.*, **230**, 1675 (1950) [*C.A.*, **44**, 10440*g* (1950)]; *idem*, *Bull. Soc. Chim. France*, **1950**, 883 [*C.A.*, **45**, 2284*h* (1951)].

Experimental

Materials——1) ACC: A commercial product of Daiichi Pure Chemical Co., Ltd.
2) PCI, BCI, HCI, OCI, DeCI, DoCI and HdCI were synthesized according to Loury's method.[7] Melting points[8] and results of elementary analyses of these compounds were shown in Table I.

Table I. Melting Points and Elementary Analyses of Acylcholine Iodides

	mp (°C)	Analysis (%) Calcd. C	H	N	Found C	H	N
Propionylcholine iodide	130	33.46	6.32	4.88	33.82	6.51	4.81
Butyrylcholine iodide	89	35.89	6.69	4.65	36.01	6.84	4.33
Hexanoylcholine iodide	126	40.13	7.35	4.25	40.19	7.38	4.29
Octanoylcholine iodide	144	43.70	7.90	3.92	43.78	7.91	4.00
Decanoylcholine iodide	156	46.76	8.37	3.63	47.07	8.55	3.69
Dodecanoylcholine iodide	162	49.39	8.78	3.39	49.41	8.62	3.48
Hexadecanoylcholine iodide	157	53.72	9.45	2.98	54.03	9.54	2.84

3) Sky blue FF: A commercial product of Aikuma Co., Ltd.
4) Eosin: A commercial product of Koso Chemical Co., Ltd.
5) Sudan III: A commercial product of Tokushu Chemical Co., Ltd.
Samples were prepared with purified water of electric conductivity below 2×10^{-7} ohm^{-1} cm.$^{-1}$
Determination of CMC——1) Measurement of Surface Tension: Surface tension was measured with a du Noüy apparatus and a platinum ring was washed and heated before use.
2) Colorimetry of Sky blue FF: Sky blue FF concentration was 0.02 mM and absorbance was measured at 630 mμ.
3) Fluorophotometry of Eosin: Eosin concentration was 0.1 mM and intensity of fluorescence was measured at 565 mμ, regarding that intensity of fluorescence of 100 mM OCI and that of 20 mM DeCI were 100%, respectively.
4) Solubilization of Sudan III: Twenty ml of various concentrations of acylcholine and about 50 mg of Sudan III were sealed in an ampoule and shaken in a thermostatically controlled water bath maintained at 45°±0.2° for given intervals (negligible time for hydrolysis[9]). But as HdCI was slightly soluble at 45°, the experiment was carried out at 55° and 60°. Excess of Sudan III was removed by suction filtration and absolute ethanol was added. The solubilized Sudan III was measured at 490 mμ. The spectrophotometer used in above experiments was a Shimadzu QB-50 spectrophotometer.

Results and Discussion

1. Surface Tension

Relationship between surface tension and acylcholine concentration (log C) was shown in Fig. 1.

A lowering of surface tension was not observed on ACC and PCI. A slight lowering was observed on BCI and a considerable lowering on HCI. A break point was not observed in the concentration range of this experiment (<150 mM) on these compounds, but observed on OCI and DeCI. The break point of OCI was 60 mM and that of DeCI 13 mM, respectively. As DoCI was slightly soluble and HdCI was insoluble in water at room temperature, the experiment was not carried out.

7) R. Schneider and A.R. Timms, *Brit. J. Pharmacol.*, **12**, 30 (1957).
8) Melting points were uncorrected.
9) Shaking time was as follows.

ACC	2 hr	BCI	4 hr	OCI	8 hr	DoCI	8 hr
PCI	4 hr	HCI	4 hr	DeCI	8 hr	HdCI	9 hr

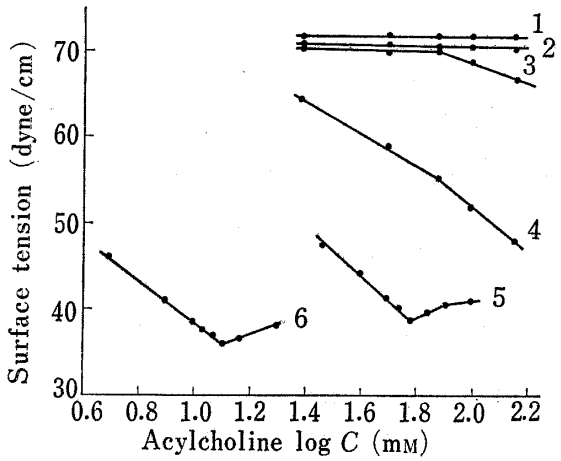

Fig. 1. Surface Tension of Acylcholine Solution at Room Temperature

1: acetylcholine chloride
2: propionylcholine iodide
3: butyrylcholine iodide
4: hexanoylcholine iodide
5: octanoylcholine ioidide
6: decanoylcholine iodide

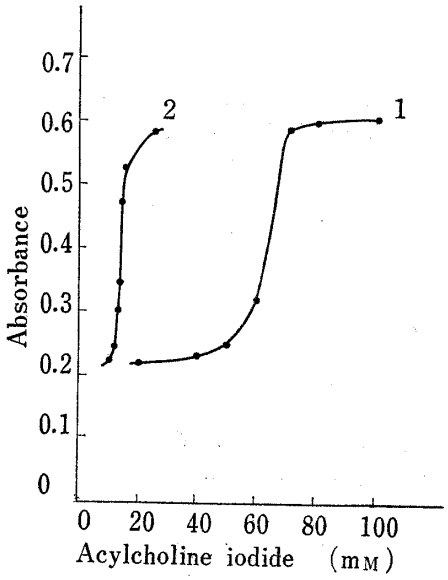

Fig. 2. Absorbance of Sky blue FF in Acylcholine Iodide Solution at Room Temperature

1: octanoylcholine iodide
2: decanoylcholine iodide

2. Colorimetry of Sky blue FF

When small amount of OCI, DeCI, DoCI and HdCI was dissolved in Sky blue FF solution, respectively, color of their solutions became reddish and when large amount of them was dissolved, color became blue. But when small amount of ACC, PCI, BCI and HCI was dissolved, color remained blue and change of color was not observed. Accordingly, the experiment was carried out on OCI, DeCI and DoCI. This result was shown in Fig. 2.

As the mean value of the concentration of sudden change of absorbance is CMC, the CMC of OCI was 60 mM, DeCI 13 mM and DoCI about 3.5 mM, respectively.

3. Fluorophotometry of Eosin

Regarding that intensity of fluorescence of 100 mM OCI and that of 20 mM DeCI were 100%, respectively, samples were measured. This result was shown in Fig. 3. The CMC of OCI was 72 mM and DeCI 14 mM, respectively.

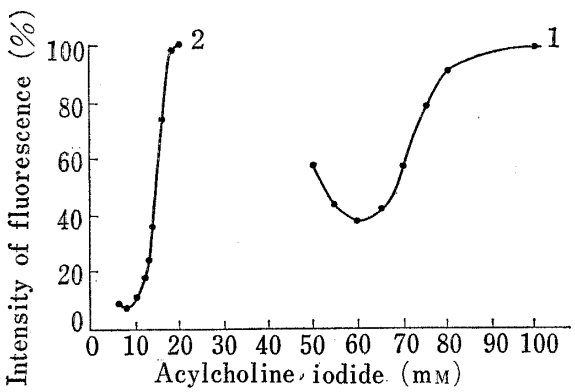

Fig. 3. Intensity of Fluorescence of Eosin in Acylcholine Iodide Solution at Room Temperature

1: octanoylcholine iodide
2: decanoylcholine iodide

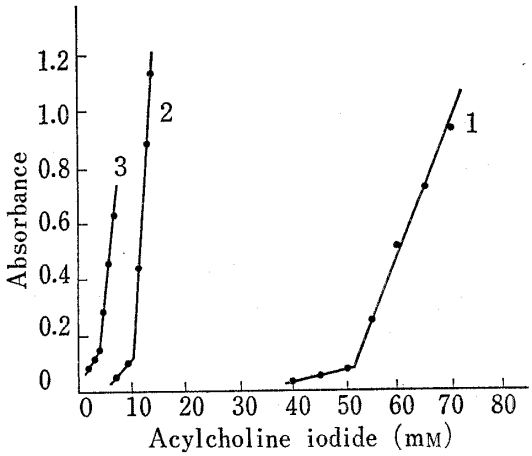

Fig. 4. Solubilization of Sudan III in Acylcholine Iodide Solution at 45°

1: octanoylcholine iodide
2: decanoylcholine iodide
3: dodecanoylcholine iodide

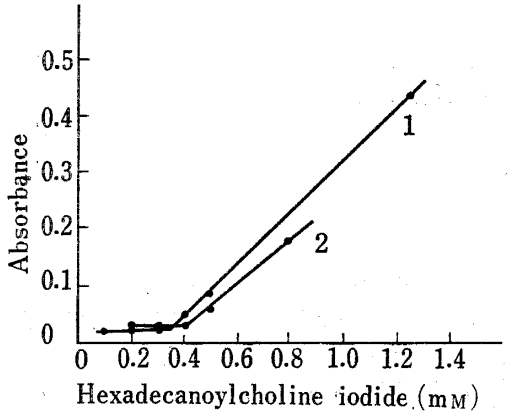

Fig. 5. Solubilization of Sudan III in Hexadecanoylcholine Iodide Solution at 55° (1) and at 60° (2)

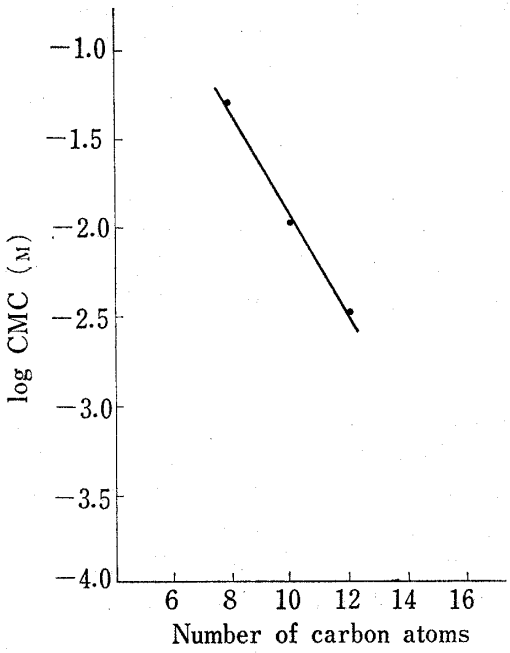

Fig. 6. Variation of Critical Micelle Concentration of Acylcholine Iodide with Number of Carbon Atoms of Acyl Radical

4. Solubilization of Sudan III

The sudden increase of solubility of Sudan III was not observed on ACC, PCI, BCI and HCI in the concentration range of this experiment (<150 mM), but observed on OCI, DeCI, DoCI and HdCI. The CMC of OCI was 53 mM, DeCI 10 mM, DoCI 3.5 mM and HdCI 0.32 mM (55°) and 0.4 mM (60°). These results were shown in Fig. 4, 5.

Above results were summarized in Table II.

Table II. Critical Micelle Concentration of Acylcholines (mM)

	Method			
Acylcholines	1	2	3	4
Acetylcholine chloride	—a)	—	—	—
Propionylcholine iodide	—	—	—	—
Butyrylcholine iodide	—	—	—	—
Hexanoylcholine iodide	—	—	—	—
Octanoylcholine iodide	60	60	72	53 (45°)
Decanoylcholine iodide	13	13	14	10 (45°)
Dodecanoylcholine iodide		*ca.* 3.5		3.5 (45°)
Hexadecanoylcholine iodide				0.32 (55°)
				0.4 (60°)

1: surface tension
2: colorimetry of Sky blue FF
3: fluorophotometry of Eosin
4: solubilization of Sudan III
a) : The minus(−) sign indicated no micelle formation.

A CMC has been shown to be satisfied by an equation which may be simplified and written in the following manner.[10]

$$\log \mathrm{CMC} = A - BN \qquad (1)$$

10) H.B. Klevens, *J. Am. Oil Chemist's Soc.*, **30**, 74 (1953).

where N: number of carbon atoms in the chain,
B: an empirical constant (may be taken to be log 2 with sufficient accuracy),
and A: a constant for the particular temperature and homologous series, which may be determined from a known value of CMC for one member of the series.

It was assumed that equation (1) might be established for acylcholines. Relationship between log CMC of OCI, DeCI and DoCI by solubilization of Sudan III at 45° and the number (N) of carbon atoms in acyl radicals of these compounds was shown in Fig. 6. An empirical equation was written in the following manner.

$$\log \mathrm{CMC(M)} = 1.040 - 0.295N \qquad (2)$$

Assuming that N was 16 (HdCI) in equation (2), the CMC was 0.21 mM. As HdCI was slightly soluble in water at 45°, the experiment could not be carried out. The CMC of HdCI was 0.32 mM (55°) and 0.4 mM (60°), respectively. If the CMC would exist at 45°, it was considered that the CMC might be approximately coincident with above calculated value. Next, assuming that N was 6 (HCI), the CMC was 187 mM. In this study, the maximum concentration of HCI was 150 mM and formation of micelle was not observed.

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