

## Effect of Drugs on Human Erythrocytes. I. Morphological Changes and Increase in Fragility of Erythrocytes treated with Drugs<sup>1)</sup>

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To clarify the mechanism of hemolysis of human erythrocytes at higher drug concentrations, the effect of some drugs on osmotic and heat fragility, K<sup>+</sup> efflux from the cells and morphological changes of the cells were studied. As a result of these studies, it was found that chlorpromazine and clemastine at 10<sup>-5</sup>M caused partial swelling, at 10<sup>-4</sup>M changes in cell shape to a sphere and at above 4 × 10<sup>-4</sup>M, at which the hemolysis is initiated, shrinkage and sinking of the cells. The mean cell volume of the cells increased by 4—5% at 5 × 10<sup>-5</sup>M and 8—9% at 10<sup>-4</sup>M. The cell volumes assumed at which concentration the hemolysis is initiated with drugs were 114—115% for these drugs. The initial swelling and subsequent shrinkage of the cells induced with drugs were found to be more predominant in concave portions of the cell membrane than in portions of the rim by scanning electron microscopic studies. The cell exposed to drugs at higher concentrations, at 5 × 10<sup>-5</sup> and 10<sup>-4</sup>M, increased osmotic and heat fragility and the increased fragility was not reduced by washing and subsequent incubation in isotonic NaCl solution, in contrast with their stabilizing effect and their reversibility at the low concentrations. Efflux of K<sup>+</sup> from the cells exposed to drugs was found to increase far more readily than that of hemoglobin.

Studies on the effect of drugs on erythrocytes have been promoted by a number of investigators, and it is known that many kinds of drugs, such as tranquilizers, antihistaminics and anesthetics,<sup>3)</sup> in very low concentrations stabilize erythrocytes against hypotonic hemolysis. Since these compounds cause lysis at higher concentrations,<sup>3j,o,4)</sup> a biphasic effect is observed as a function of drug concentration. The mechanism of this protective effect has been studied<sup>3j,l,o)</sup> and it is explained as the membrane expansion<sup>3j)</sup> or the increase in critical volume by drugs.<sup>3l)</sup> However, the mechanism of hemolysis of the cells at higher drug concentrations has remained obscure.

We had reported that many of the drugs had a hemolytic activity,<sup>5)</sup> and the effect of these drugs at higher concentrations on human erythrocytes was examined in an attempt to clarify the mechanism of hemolysis. In this paper, the increase in osmotic and heat fragility, K<sup>+</sup> efflux from the cells, and morphological changes of the cells with some drugs are described.

1) This work was presented at the 94 Annual Meeting of Pharmaceutical Society of Japan, Sendai, April, 1974.

2) Location: *Mitahora, 492-36, Gifu.*

3) a) H. Chaplin Jr., H. Crawford, M. Cutbush, and P.L. Mollison, *J. Clin. Pathol.*, **5**, 91 (1952); b) P. M. Seeman and H.S. Bialy, *Biochem. Pharmacol.*, **12**, 1181 (1963); c) G. Zografi, D.E. Auslander and P.L. Lytell, *J. Pharm. Sci.*, **53**, 573 (1964); d) L.L.M. van Deenen and R.A. Demel, *Biochim. Biophys. Acta*, **94**, 314 (1965); e) O. Schales, *Proc. Soc. Exptl. Biol. Med.*, **83**, 593 (1953); f) A.R. Freeman and M.A. Spirtes, *Biochem. Pharmacol.*, **11**, 161 (1962); g) A.R. Freeman and M.A. Spirtes, *Biochem. Pharmacol.*, **12**, 47 (1963); h) A.R. Freeman and M.A. Spirtes, *Biochem. Pharmacol.*, **12**, 1235 (1963); i) J.D. Judah, "Drugs and Enzymes, CIBA symposium," ed. by J.L. Mongar and A.V.S. de Reuck, Little Brown, Boston, 1962, p. 359; j) P. Seeman and J. Weinstein, *Biochem. Pharmacol.*, **15**, 1737 (1966); k) S. Roth and P. Seeman, *Biochim. Biophys. Acta*, **255**, 190 (1972); l) J. van Steveninck, W.K. Gjösumt, and H. L. Booiij, *Biochem. Pharmacol.* **16**, 837 (1967); m) S. Roth and P. Seeman, *Nature New Biol.*, **231**, 284 (1971); n) F. Okumura, J. Koh, and I. Ueda, *Masui*, **17**, 186 (1968); o) P. Seeman, *Biochem. Pharmacol.*, **15**, 1753 (1966).

4) W.O. Kwant and J. van Steveninck, *Biochem. Pharmacol.*, **17**, 2215 (1968).

5) T. Ogiso, M. Watanabe, T. Sato, K. Yamauchi, and Y. Kato, *Nippon Yakurigaku Zasshi*, in press.

## Experimental

**Drugs**—The drugs used were as follows: Chlorpromazine hydrochloride (Nihon Shinyaku), perazine dimalonate (Morishita Pharmaceutical Co.) and carpipramine hydrochloride (Yoshitomi Pharmaceutical Co.) as a tranquilizer; clemastine fumarate (Sankyo Co.) as an antihistaminic; nortriptyline hydrochloride (Dainippon Pharmaceutical Co.) as an antidepressant.

**Preparation of Erythrocyte Suspension**—Human blood was collected from hematologically normal adult donors, utilizing sodium citrate as an anticoagulant. The blood was then centrifuged for 15 min at  $1500 \times g$ . The plasma was carefully removed, including the buffy coat of leucocytes, and washed 3 times with isotonic NaCl solution, pH 7.4. All NaCl solution used were prepared by dilution of the buffered stock solution described by Parpart, *et al.*<sup>6)</sup> Erythrocytes were resuspended in the washing solution to make finally  $40 \pm 1\%$  hematocrit value.

**Potassium Measurement**—To 2 ml of the supernatant obtained in the experiment on drug-induced hemolysis 1 ml of ice-cold 30% trichloroacetic acid was added, the mixture was stood overnight at  $4^\circ$ , and centrifuged at  $2000 \times g$  for 20 min. Potassium released was measured with a Hitachi atomic flame absorption spectrophotometer at  $7665\text{\AA}$ .

**Electron Microscopy**—Erythrocytes, treated with one of the drugs and centrifuged, were fixed with 1.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.2. The cells were washed 3 times with 0.1M phosphate buffer, pH 7.2, and dried with increasing concentrations of acetone (60 to 100%, v/v). The specimens were coated at continuously varying angles with gold and viewed with a Nihon Denshi scanning electron microscope, Model JEM-100B.

**Drug-induced Hemolysis**—To 3 ml of the test solution, 0.3 ml of the erythrocyte suspension (hematocrit value,  $40 \pm 1\%$ ) was added and mixed immediately. The test solution was usually prepared at a drug concentration between  $10^{-5}$  and  $10^{-3}\text{M}$  in diluted NaCl solution. The mixture was incubated for 60 min at  $37^\circ$  and then centrifuged at  $1500 \times g$  for 3 min. The supernatant was collected and the percentage hemolysis was determined by measuring the absorbance of hemoglobin in the supernatant with a Hitachi spectrophotometer Model-101 at 543 m $\mu$ .

**Measurement of Mean Cellular Volume of Erythrocytes**—The cells were exposed to the drug in an isotonic NaCl solution for 30 min and washed with the same buffer. Mean cellular volume was measured by the method described by van Steveninck, *et al.*,<sup>3)</sup> using hematocrit tubes. Trapped medium between cells, centrifuged in Hamburger type tubes, was determined according to the method described by Chaplin and Mollison.<sup>7)</sup>

## Results

### Drug-induced Homolysis

Results from the measurement of percentage hemolysis against drug concentrations are shown in Fig. 1. These drugs were found to show hemolytic action at concentrations ranging from  $4 \times 10^{-4}$  to  $8 \times 10^{-4}\text{M}$ . Clemastine, carpipramine, and chlorpromazine produced the severest lysis. Clemastine and chlorpromazine, therefore, were mainly used for the following experiments.

### Scanning Electron Microscopic Observations and Cell Volume Value of Erythrocytes Treated with Drugs

Fig. 2 shows some typical scanning electron micrographs of erythrocytes treated or untreated with chlorpromazine. The drug induced visual shape changes in erythrocytes. Untreated erythrocytes have a biconcave disk shape with smooth featureless surface. Chlorpromazine at the concentration of  $10^{-5}\text{M}$  caused partial swelling of the cells. At  $10^{-4}\text{M}$ , the cells became an almost smooth sphere. However, at  $4 \times 10^{-4}\text{M}$ , at which the hemolysis is initiated, the cells were shrunk, induced by the release of  $\text{K}^+$  and  $\text{H}_2\text{O}$  from the cells. At the above concentrations, the formation of large pores, through which hemoglobin may be released out, were observed. Fig. 2f) shows a crenated shape of erythrocytes added into 0.35% NaCl solution. Fig. 3 also shows some scanning electron micrographs of erythrocytes treated with clemastine. The sequence of changes can be summarized as follows; 1) at  $10^{-4}\text{M}$  the cells swell and at  $2 \times 10^{-4}\text{M}$  become almost smooth spheres, 2) at  $4 \times 10^{-4}\text{M}$  the cells shrink and sink,

6) A.K. Parpart, P.B. Lorenz, E.R. Parpart, J.R. Gregg, and A.M. Chase, *J. Clin. Invest.*, **26**, 636 (1947).

7) H. Chaplin Jr. and P.L. Mollison, *Blood*, **7**, 1227 (1952).

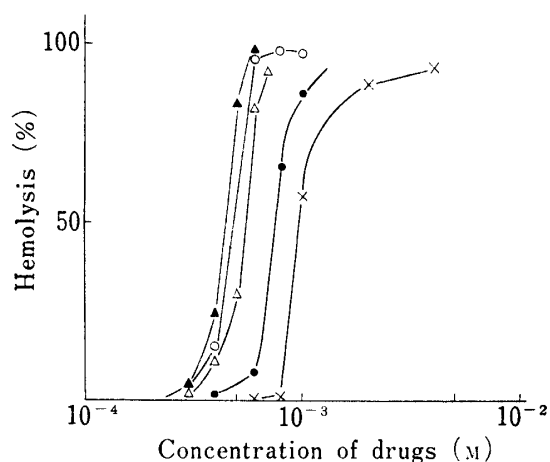


Fig. 1. Lytic Effect of Various Drugs on Erythrocytes

Experimental conditions are described in text. ▲, clemastine; △, chlorpromazine; ●, perazine; ×, nortriptyline; ○, caripramine.

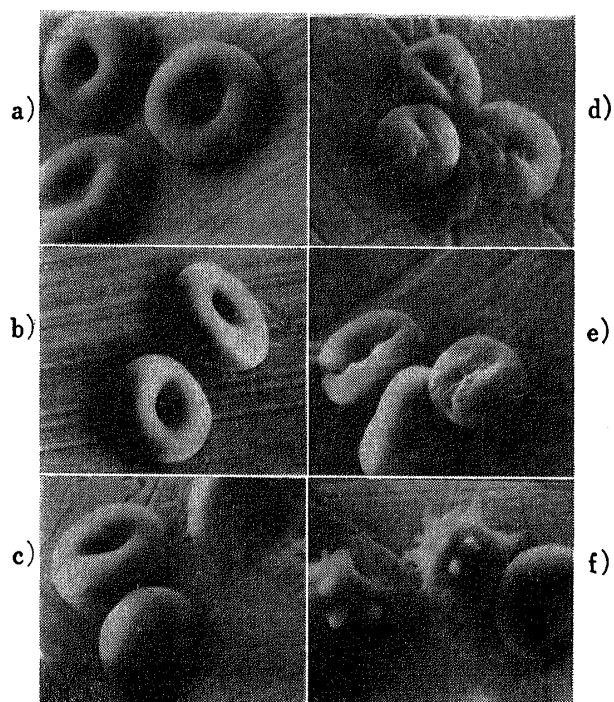


Fig. 2. Scanning Electron Micrographs of Erythrocytes Treated with Chlorpromazine

a) no drug treatment; b) 10<sup>-6</sup> M; c) 10<sup>-4</sup> M; d) 4 × 10<sup>-4</sup> M; e) 6 × 10<sup>-4</sup> M; f) 0.35% NaCl. Magnification 10000 ×.

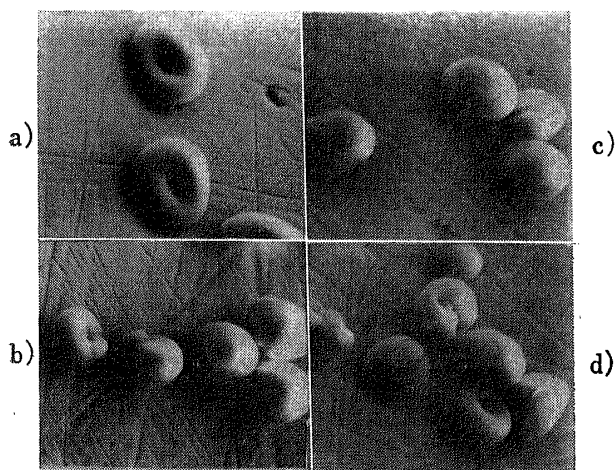


Fig. 3. Scanning Electron Micrographs of Erythrocytes treated with Clemastine

a) no drug treatment; b) 10<sup>-4</sup> M; c) 2 × 10<sup>-4</sup> M; d) 4 × 10<sup>-4</sup> M. Magnification 6920 ×.

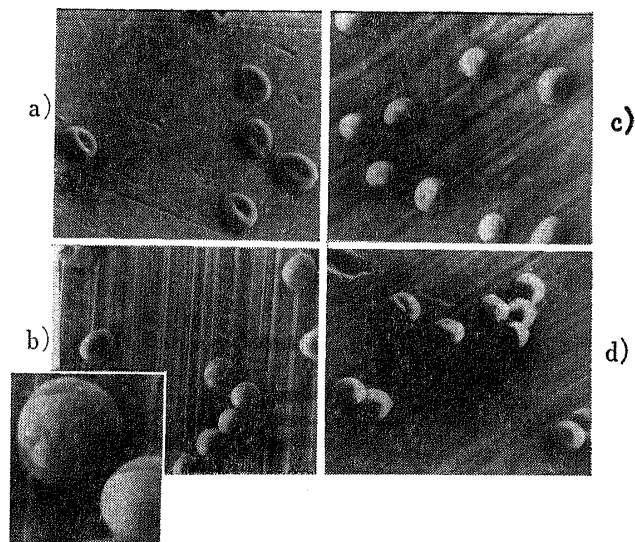


Fig. 4. Scanning Electron Micrographs of Erythrocytes treated with Various Drugs

The drug concentration was 10<sup>-4</sup> M for all. a) no drug treatment; b) caripramine; c) nortriptyline; d) perazine. Magnification 3000 ×.

3) at above 4 × 10<sup>-4</sup> M, the formation of large pores is observed. These changes were similar to those observed upon treatment with chlorpromazine. The cells treated with other drugs at the concentration of 10<sup>-4</sup> M are shown in Fig. 4. They are transformed to smooth spheres and these changes closely resemble to those produced by chlorpromazine and clemastine. As the concentrations increased further the cell shape changed similar to that by chlorpromazine. It was found from these observations that the initial swelling and subsequent shrinkage of the cells induced with drugs predominantly occurred in concave portions on the cells.

Since it is known that anesthetics and tranquilizers increase the volumes of cells<sup>3i,4)</sup> and induce the membrane expansion,<sup>3k,o,8)</sup> the mean cell volume of the erythrocytes treated with chlorpromazine and clemastine was measured. As a result, the mean cell volume of the cells increased by 4—5% at  $5 \times 10^{-5}\text{M}$  and 8—9% at  $10^{-4}\text{M}$  of the drugs. Nortriptyline, which causes lysis at concentrations above  $8 \times 10^{-4}\text{M}$ , also increased the mean cell volume by 8% at  $10^{-4}\text{M}$  and by 9% at  $2 \times 10^{-4}\text{M}$ , although in all experiments tested the volume of trapped NaCl solution was decreased with rising concentration of the drugs, as shown in Table I, indicating that the cell volume increases with rising their concentrations. This observation is almost consistent with the data reported by van Steveninck, *et al.*<sup>3j)</sup> that chlorpromazine at the optimal concentration caused a 10% increase in the mean cellular volume. Logarithmic plots of drug concentrations against cell volumes, which were an almost linear, showed that the cell volumes assumed at which concentration the hemolysis is initiated with drugs under the conditions tested were both 114% for chlorpromazine and clemastine and 115% for nortriptyline. This suggests that the maximal (or critical) cell volume was about 114—115% under the conditions.

TABLE I. Mean Cell Volume and Trapped Medium of Erythrocytes treated with Drugs

Drug concentration (M)	Chlorpromazine		Clemastine		Nortriptyline	
	Trapped medium(%)	Mean cell volume(%)	Trapped medium(%)	Mean cell volume(%)	Trapped medium(%)	Mean cell volume(%)
None	4.2	100.0	4.1	100.0	4.3	100.0
$2 \times 10^{-5}$	4.0	100.1 ± 0.8	4.0	100.1 ± 1.8		
$5 \times 10^{-5}$	3.2	104.8 ± 1.6	3.5	103.5 ± 1.3	4.0	100.9 ± 0.5
$10^{-4}$	2.6	108.1 ± 0.8	3.0	108.7 ± 0.8	3.3	107.9 ± 2.0
$2 \times 10^{-4}$					3.1	108.9 ± 1.4

Values are means ± SE of 3 experiments.

### Fragility of Erythrocytes treated with Drugs

Many investigators reported that human erythrocytes are protected or stabilized against hypotonic and mechanical hemolysis in the presence of a low concentration of tranquilizers and antihistaminics.<sup>3j,4)</sup> To compare fragility of the cells treated with drugs at the relatively high concentrations with that at the low concentrations, the cells were incubated with chlorpromazine or clemastine at  $2 \times 10^{-5}$ ,  $5 \times 10^{-5}$  and  $10^{-4}\text{M}$ .

**A) Osmotic Fragility**—As shown in Fig. 5, osmotic fragility of the cells treated with drugs at  $5 \times 10^{-5}$  and  $10^{-4}\text{M}$  was increased. Especially, the cells treated with  $10^{-4}\text{M}$  of the drugs underwent hemolysis even in 0.8% NaCl solution, while  $2 \times 10^{-5}\text{M}$  of drugs had little effect on fragility.

**B) Heat Fragility**—Drug-treated cells were exposed to heat ( $40^{\circ}$ — $55^{\circ}$ ) for 10 min and the hemolytic percentage was measured. The result is shown in Fig. 6. Fragility of the cells treated with  $10^{-4}\text{M}$  drugs increased extremely, while at  $2 \times 10^{-5}\text{M}$ , their fragility remained at the control levels.

**C) Fragility during Incubation**—Drug-treated cells were incubated at  $20^{\circ}$ . As can be seen from Fig. 7, the percentage hemolysis enhanced relatively fast in the cells treated with both drugs at  $10^{-4}\text{M}$ . After 71 hr, the cells treated with chlorpromazine and clemastine showed 48% and 51% hemolysis, respectively, while, at  $2 \times 10^{-5}\text{M}$  the protecting effect against hemolysis was observed.

These tests on cellular fragility indicated that erythrocytes treated with drugs at relatively high concentrations increased the fragility and accordingly the lytic sensitivity.

8) P. Seeman, W.O. Kwant, and T. Sauks, *Biochim. Biophys. Acta*, **183**, 499 (1969).

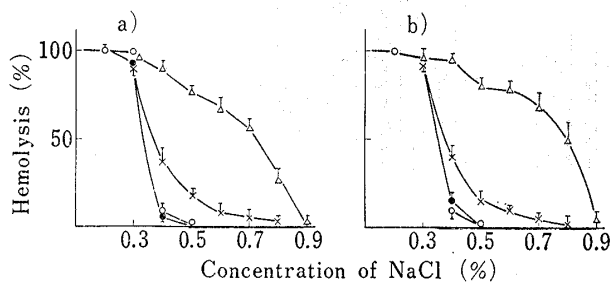


Fig. 5. Osmotic Fragility of Erythrocytes treated with Drugs

The cells were incubated in the drug solution at indicated concentration for 1 hr at 37°, and washed twice with isotonic NaCl solution. 0.3 ml of the cell suspension (hematocrit value, about 40%) was added to 3 ml of hypotonic NaCl solution. The mixture was incubated for 1 hr at 25° and after centrifuging was subjected to spectroscopic measurements at 543 m $\mu$ . Points represent the mean  $\pm$  SE of 3 experiments. a) chlorpromazine; b) clemastine.  $\circ$ , control;  $\bullet$ ,  $2 \times 10^{-5}$  M;  $\times$ ,  $5 \times 10^{-5}$  M;  $\Delta$ ,  $10^{-4}$  M.

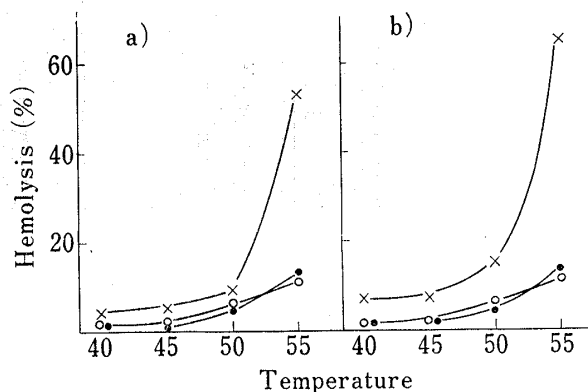


Fig. 6. Effect of Temperature on Hemolysis of Erythrocytes treated with Drugs

The cells were treated as described in Fig. 5 and 0.3 ml of the cell suspension was added to 3 ml of isotonic NaCl solution. The suspension was heated for 10 min at indicated temperature and the percentage of hemolysis was estimated. Points are means of values in 2 experiments. a) chlorpromazine; b) clemastine.  $\circ$ , control;  $\bullet$ ,  $2 \times 10^{-5}$  M;  $\times$ ,  $10^{-4}$  M.

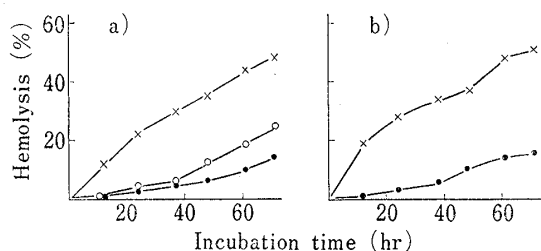


Fig. 7. Effect of Incubation Time on Hemolysis of Erythrocytes treated with Drugs

The cells were treated as described in Fig. 5 and 0.3 ml of the cell suspension was added to 3 ml of isotonic NaCl solution. The suspension was incubated for indicated time at 20°. Points are means of values in 3 experiments. a) chlorpromazine; b) clemastine.  $\circ$ , control;  $\bullet$ ,  $2 \times 10^{-5}$  M;  $\times$ ,  $10^{-4}$  M.

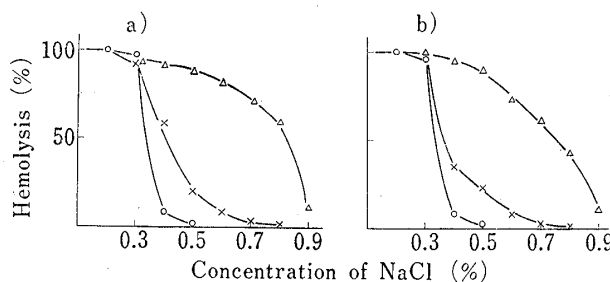


Fig. 8. Osmotic Fragility of Erythrocytes Incubated for 3 hr in Isotonic NaCl Solution after Treatment with Drugs

The cells were treated with drugs as described in Fig. 5. The washed cells were incubated in isotonic NaCl solution for 3 hr at 37° and then 0.3 ml of the cell suspension was added to 3 ml of hypotonic NaCl solution. The mixture was followed by spectroscopic measurements. Points are means of values in 3 experiments. a) chlorpromazine; b) clemastine.  $\circ$ , control;  $\times$ ,  $5 \times 10^{-5}$  M;  $\Delta$ ,  $10^{-4}$  M.

### Irreversibility of Fragility increased with Drugs

Erythrocytes treated with drugs were washed twice with isotonic NaCl solution and then incubated at 37° for 3 hr in the same solution. The cell suspensions were tested for stabilization-lysis with drugs. Aliquots of the suspensions were added to the hypotonic NaCl (0.2—0.9%) solution and the osmotic fragility was measured. The results revealed that the osmotic fragility was little reduced by washing and subsequent incubation without drugs, as shown in Fig. 8. This result is contrary to the observation described by Seeman and Weinstein<sup>3j)</sup> that the membrane stabilization was rapidly reversible at low concentrations of drugs.

The scanning electric micrographs of the drug swollen cells after incubation in the isotonic NaCl solution for 3 hr are shown in Fig. 9. The cell shape was an almost smooth sphere and did not return to their original, indicating that the drugs incorporated into the cells may not desorb off the membrane with great ease or that the structure of the membrane changed by drugs may not return to its original condition.

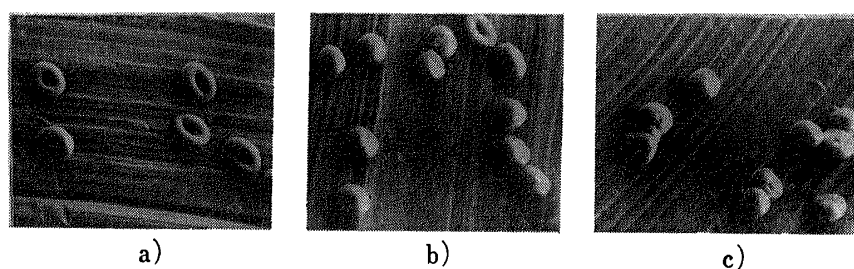


Fig. 9. Scanning Electron Micrographs of Erythrocytes incubated for 3 hr in Isotonic NaCl solution after Treatment with Drugs  
a) no drug treatment; b)  $10^{-4}\text{M}$  chlorpromazine; c)  $10^{-4}\text{M}$  clemastine. Magnification 3000 X.

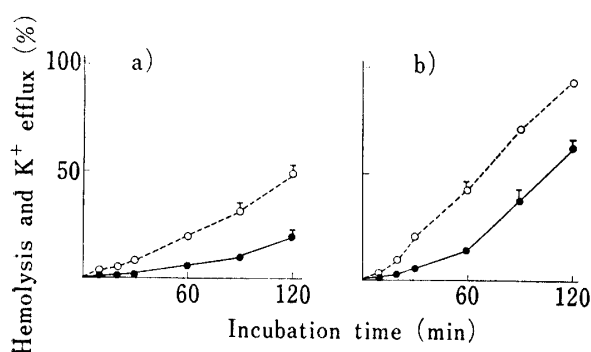


Fig. 10. Time Course of Hemolysis and Release of  $\text{K}^+$  from Erythrocytes in the Presence of Drugs

Experimental conditions are described in text. Points represent the mean  $\pm$  SE of 3 experiments. The drug concentration was  $4 \times 10^{-4}\text{M}$  for all. a) chlorpromazine; b) clemastine. ●, hemolysis; ○, release of  $\text{K}^+$ .

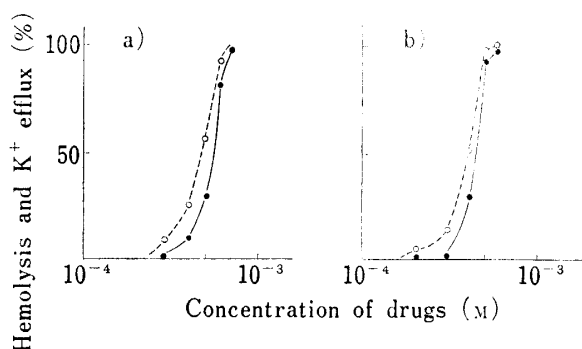


Fig. 11. Effect of Drug concentration of  $\text{K}^+$  Efflux and Hemolysis

Points are means of values in 3 experiments. a) chlorpromazine; b) clemastine. ○,  $\text{K}^+$  efflux; ●, hemolysis.

### Effect of Drugs on $\text{K}^+$ Efflux

Efflux of  $\text{K}^+$  from erythrocytes after exposure of different duration to  $10^{-4}\text{M}$  drugs was found to increase far more readily than that in hemoglobin, as shown in Fig. 10. The release of  $\text{K}^+$  from the cells in the presence of different concentrations of drugs exceeds hemoglobin liberation, over the whole range from 0 to 100% hemolysis as shown in Fig. 11. These results agreed well the data in many papers.<sup>3j,k,4)</sup> Thus,  $\text{K}^+$  release may be related to changes in cell shape, which were demonstrated by electron microscopy.

### Discussion

Knowledge about adverse reactions to drug therapy has arisen largely from many reports by physicians who observed these events in their patients. Several types of blood dyscrasia are shown to cause by use of certain drugs.<sup>9)</sup> It is observed that the high local concentration of a drug occasionally gives rise to partial hemolysis. Additionally, some drugs at high concentrations have a lytic action on lysosomes.<sup>10)</sup> Thus, we have been studying the mechanism of drug-induced hemolysis in an attempt to prevent such adverse reaction of drugs.

It is known that a variety of surface-active compounds (alcohol, anesthetics, fatty acids, steroids, detergents, and lipid-soluble vitamins) at a low concentration cause stabilization

9) M.C. MacDonald and B.R. MacKay, *J. Am. Med. Assoc.*, **190**, 1071 (1964).

10) D.A. Lewis, *J. Pharm. Pharmacol.*, **22**, 909 (1970); D.A. Lewis, A.M. Symons, and R.J. Ancill, *J. Pharm. Pharmacol.*, **22**, 902 (1970).

of many types of membranes.<sup>11)</sup> Drugs which are surface active, such as tranquilizers and antihistaminics, are no exception to this general finding.<sup>3j-o,4)</sup> The mechanism of the protection was also reported by many investigators.<sup>3j,l,o)</sup> One possible explanation is that the erythrocyte membrane is expanded with the drugs somewhat in the same way that lipid monolayers are penetrated by these compounds.<sup>3d,j)</sup> The cells with higher area/volume ratio are associated with a lower osmotic fragility<sup>3j,o,12)</sup> and a drug-induced increase in critical volume of the cells is responsible for the decreased osmotic fragility.<sup>3i)</sup> However, relatively little is known about the mechanism for drug-induced hemolysis at higher concentration of drugs.

The erythrocytes were expanded with chlorpromazine and clemastine at lower concentrations below  $2 \times 10^{-4}M$  (Fig. 2—4), as indicated by many investigators,<sup>3j,l,4)</sup> and the cells were shrunk at concentrations above  $4 \times 10^{-4}M$ . The cells exposed to either drug at  $5 \times 10^{-4}$  or  $10^{-4}M$  were found to increase osmotic and heat fragility, and fragility in a prolonged incubation, while the cells exposed to  $2 \times 10^{-5}M$  drugs were partially decreased the fragility (Fig. 7). Swelling of the cells, therefore, caused with the drugs at high concentrations may be associated with the increased fragility of the cells, because an increase in the swelling paralleled the increased fragility in concentrations ranging from  $2 \times 10^{-5}$  to  $10^{-4}M$ . Our studies on morphologic changes of the cells (Fig. 2—4) showed that the initial swelling and subsequent shrinkage of the cells induced with drugs were more predominant in their concave portions than in portions of the rim. This indicates that the wall thickness and the extensional stiffness of the cell membrane vary in different portions of the membrane and the concave portions are fragile in the stiffness. Fung and Tong<sup>13)</sup> presented a similar view from a rigorous mathematical solution of sphering of a red blood cell.

It is noted that the mean cell volume in isotonic NaCl solution increased by about 8—9% with both drugs at  $10^{-4}M$ , in accord with the data that the critical volume in the presence of  $10^{-4}M$  chlorpromazine increased by 10% ( $110.19 \pm 4.3$ ).<sup>3i)</sup> The maximal cell volumes obtained from logarithmic plots of drug concentrations against cell volumes were 114—115% for these drugs. This suggests that the maximal increase of the cell volume may be about 115% in isotonic buffer and much more expansion on the cell membrane may lead to hemolysis.

Seeman and Weinstein<sup>3j)</sup> reported that the membrane stabilization by tranquilizers and antihistaminics was rapidly reversible and lowering the extracellular drug concentration or photo-oxidizing the adsorbed drug caused the membrane to return to its original condition of fragility. The present study, however, showed irreversibility of the fragility under the conditions tested, indicating that when erythrocytes were exposed to the drugs at higher concentrations, the drugs incorporated into the membrane or the cell appear not to be desorbed with great ease or that the structure of membrane changed by drugs will not return easily to its original condition. Presumably the cell membrane exposed to drugs at higher concentrations may contain a relatively large amount of drugs, since at chlorpromazine concentrations higher than  $3 \times 10^{-5}M$ , the membrane concentrations are shown to increase very steeply, much higher than 0.066 mole/liter wet membrane.<sup>14)</sup> The drugs penetrating into the membrane may be bound to the hydrophobic regions of the protein and lipid layers. This view is supported by

- 11) H. Beaufay and C. de Duve, *Biochem. J.*, **73**, 604 (1959); J.D. Judah, *Biochim. Biophys. Acta*, **53**, 375 (1961); H. Zalkin, A.L. Tappel, I. Desai, K. Caldwell, and D.W. Peterson, *Federation Proc.*, **20**, 303 (1961); M.A. Spirtes and P.S. Guth, *Biochem. Pharmacol.*, **12**, 37 (1963); L. Elmer, *Federation Proc.*, **22**, 2780 (1963); E.E. Smith, C. Watanabe, J. Louie, W.J. Jones, H. Hoyt, and F.E. Hunter Jr., *Biochem. Pharmacol.*, **13**, 643 (1964); H. Weil-Malherbe and H.S. Posner, *Biochem. Pharmacol.*, **13**, 685 (1964); L.J. Ignarro, *J. Pharmacol. Exptl. Therap.*, **182**, 179 (1972); A. Haksar and F.G. R eron, *Biochim. Biophys. Acta*, **264**, 548 (1972).
- 12) R.P. Rand and A.C. Barton, *J. Cell Comp. Physiol.*, **61**, 245 (1963).
- 13) Y.C.B. Fung and P. Tong, *Biophys. J.*, **8**, 175 (1968).
- 14) W.O. Kwant and P. Seeman, *Biochim. Biophys. Acta*, **183**, 530 (1969).

the fact that the chlorpromazine-membrane interaction is hydrophobic.<sup>14)</sup> Consequently, the membrane-drug interactions may induce a transition or a conformational change in the lipid lamellar structures and the protein structures, and the cells may be hemolyzed.

The other possibility to explain the drug-induced hemolysis is that drugs induce the disruption of a balance between active and passive transport of  $\text{Na}^+$  and  $\text{K}^+$  by directly altering the permeability of the membrane.

Further study on the structure and the permeability of the cells are under way.

**Acknowledgement** We thank Pharmaceutical Co. for supply of drugs.